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Phenylsulfonyl piperazine bridged [1,3]dioxolo[4,5-*g*]chromenones as promising antiproliferative and antioxidant agents

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ABSTRACT

Two series of sulfonylpiperazines linked [1,3]dioxolo[4,5-*g*]chromenones were synthesized featuring phenyl (**7a-k**) and chalcone (**12a-k**) bridge representing flavones or homoisoflavonoids core. New molecules are synthesized utilizing aldol condensation to inspect as antioxidants against DPPH$^{\cdot}$ and ABTS$^{\cdot+}$ and antiproliferative agents toward selected human cancer cell lines. Cytotoxicity of new compounds was confirmed using SRB assay against non-cancer MDCK cell line. The results concluded that both individual structures of **7** and **12** were vital for modulating pharmacological potencies and presence of different electron withdrawing and electron donating functional group(s) on the phenylsulfonyl entity yielded varied biological effects. Substituent **h** (OCF_3) and **j**, **k** (OCH_3) were found to play a crucial role scavenging DPPH$^{\cdot}$ and ABTS$^{\cdot+}$ as well as inhibiting cancer cell lines SK-OV-3 and HT-29. Moreover, molecules bearing halogen atom(s) such as substituent **b-g** expressed excellent inhibitory potential against HeLa and A-549 cancerous cell lines. Bioassay data displayed some interesting structure-activity relationships which are discussed in this paper. The results justified that tested derivatives are promising antioxidants and cytotoxic agents and warrant further structural optimization and bioassay studies. Spectroscopic techniques such as FT-IR, ^{1}H NMR, ^{13}C NMR and elemental analysis (CHN) were carried out to confirm the final structures.

1. Introduction

Cancer is a life-threating ailment that is manifested by out-of-control irregular cell development with the chance of invading other cells and/or spread to other organs. Melanoma could impact almost any aspect of the body and has many anatomic and molecular subtypes that each need particular control techniques. It is the major cause of loss of life worldwide and is approximated to account for 9.6 million loss of life in 2018. Cancers such as Liver, prostate, colorectal and lung cancer as well as breast, cervix and thyroid cancer are the most common among men and women, respectively [1]. Despite all the treatment techniques such as radiation, hormone therapy, surgery and chemotherapy, the use of a perfect and outright cure is a major challenge and there is an urgent need to build up more potential anticancer agents [2]. Cancer control can be handled by various systems targeted at different levels of carcinogenesis like initiation, promotion and progression. The desirable options of destroying carcinogenesis include modulation of biotransformation of carcinogen, gene expression manipulation involved in signaling pathways, free radical scavenging as well as cell proliferation and differentiation [3,4]. Moreover, human's exposure to toxins present in the environment as well as their lifestyle factors significantly impact the existing homeostatic balance which results in a diminished antioxidant capacity that triggers production of reactive oxygen species (ROS). This phenomenon is termed as oxidative stress which then causes serious medical conditions like cancer and DNA damage [5,6]. There are enzymatic and non-enzymatic antioxidants those reduce oxidative stress within important biological cells utilizing various mechanisms. In recent years, chemical modification of natural products has attracted great attention of many analysis categories these days, with the aim to improve their unique scientific activities as well as to deliver potencies against one or more targets [7].

Chromanones are the most important heterocyclic substances, which is a frequent and important function of a wide range of natural products, medicinal agents and modification possibilities of their framework provide a high level of benefits to further rationalize potential molecular libraries. They are an essential category of oxygen-containing

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heterocyclic substances with a benzoannelated γ-pyrone ring and are a crucial feature of the flavonoid natural product class [8]. Chromanone and its analogs, abundant in nature, are essential pharmacophores and key structures in drug discovery research and can be seen as the main core in several clinically used drugs [9,10], specifically, as anticancer molecules [11–14]. Furthermore, various chromenes moiety-containing substances which are architectural analogs of chromanones were also revealed with great anticancer action [15,16]. Furthermore, homoisoflavonoids, a flavonoid class, have been identified to possess a broad range of bioactivities like anti-microbial, anti-mutagenic, anti-oxidant, immunomodulatory, anti-diabetic, cytotoxic, anti-angiogenic, vasorelaxant, and anti-inflammatory effects [17,18]. They mainly consist of a chromanone, chromone, or chromane skeleton and are ubiquitous in plants. Therefore, homoisoflavonoids might have a huge prospective for further research of their bioactivities to be able to recognize important leads. Moreover, Chalcones, α-β-unsaturated ketones, are valuable compounds of therapeutic significance due to existence of the reactive double bond in conjugation with carbonyl functionality, reflecting the flavonoid family [19]. Numerous reports highlighting the synthesis and therapeutic importance of chalcone derivatives have already been recorded in the literary concluding them as anti-proliferative, anti-malarial, anti-microbial and anti-HIV agents [20]. In a view of aforementioned points, we have attempted to construct two different types of molecular series featuring chromanone, chalcones and homoisoflavonoid rings. In addition, organic molecules endowed with sulfone entity found to have promising potential as the biologically active agents [21], whereas piperazine analgoues [22] have been proposed to be key factor enhancing biological effects of the bearing molecules. Finally, we have already prepared some flavonoid based therapeutic agents targeting different cancer cell lines (HeLa, CaSki, SK-OV-3) and free radicals (DPPH$^{\cdot}$ and ABTS$^{\cdot+}$) and results obtained in those studies prompted us to further optimize the flavone type ring systems [23,24]. More importantly, compounds holding antiproliferative and antioxidant effects are of key interest and relation of antioxidants with cancer is well studied as antioxidants exercise crucial roles in the maintenance of cellular integrity and therefore are critical in maintaining the homeostasis of the host immune system as it is studied that some flavonoids which exhibit anticarcinogenic properties in part via their antioxidant activities [25]. While such free radicals are utilized as an intrinsic mechanism of host immunity to resist towards extracellular pathogens, an amplified generation outcomes into imbalance in cellular redox potential causes discrepancy in signaling pathways and neocarcinogenesis [26]. When compared to the normal healthy cells, melanoma tissues normally carry higher basal oxidative stress and it is believed that several antioxidant systems play essential positions in counteracting the destructive results of improved ROS [27]. Moreover, one of the repercussions of chemotherapy and radiotherapy treatment is the generation of ROS which then results in aberrations in several cell signaling pathways which lead to the therapy-induced cell death. Thus, new agents with anticancer efficacies are most welcomed which are also helps potentiate chemotherapy/radiotherapy–mediated antitumor reactions required to induce long-term benefits in cancer patients [28]. There are studies suggesting that some antioxidants are found to positively influence cancer treatments and helps inhibiting cancer cells while decreasing cytotoxic impacts on normal cells [29]. Therefore, we have integrated essential entities into the base chromanones in order to obtain new molecules bearing both cytotoxic and free radical scavenging potencies.

2. Materials and methods

All commercial chemicals and solvents are of reagent grade and were used without further purification. Melting points are uncorrected and recorded on Stuart SMP3 melting point apparatus. The thin layer chromatography was performed on Merck pre-coated silica gel 60 F_{254} plates, with visualization under UV light. IR spectra (KBr) were recorded on an FT-IR 200 spectrophotometer (ύ, cm^{-1}). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AVANCE III 400 instrument spectrometer and *J* values are in Hertz, and chemical shifts (d) are reported in ppm relative to internal tetramethylsilane. Elemental analysis was carried out using C,H,N,S analyzer.

2.1. Synthesis of 3-(benzo[d][1,3]dioxol-5-yl)-1-(4-fluoro-2-hydroxyphenyl) prop-2-en-1-one (3)

To a solution of piperonal (**1**, mmol) in 20 mL of ethanol with 4′-Fluoro-2′-hydroxyacetophenone (**2**, 10 mmol), 10 mL of 20% KOH was added and the reaction mixture was allowed to react at room temperature for 24 h. After the completion of the reaction monitored by TLC, the reaction mass was quenched by 30 mL of water and extracted with 3 × 30 mL of chloroform followed by drying of the organic layer using anhydrous sodium sulfate. The mass was then concentrated in vacuum and purified by column chromatography with petroleum ether:ethyl acetate to give **3** in 69% of yield. Yellowish brown solid, m.p. 167–169 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.71 (s, 1H), 7.83 (d, J = 15.1 Hz, 1H), 7.67–7.44 (m, 6H), 7.39 (d, J = 15.3 Hz, 1H), 6.05 (s, 2H, CH_2).

2.2. Synthesis of 6-(4-fluorophenyl)-8H-[1,3]dioxolo[4,5-g]chromen-8-one (4)

Catalytic amount of I_2 (0.1 equiv) was added to a solution of **3** in DMSO and the mass was refluxed for more than 10 h while monitoring reaction with TLC. After the completion of the reaction, the resulting reaction mass was cooled to room temperature and poured onto saturated aqueous sodium thiosulfate solution and resulting precipitates were filtered, washed with chilled water and recrystallization from dichloromethane-methanol yielded crystalline **4** in 62%. Yellow solid, m.p. 198–200 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.68–7.47 (m, 4H), 7.39 (s, 1H, chroman), 7.11 (s, 1H, chroman), 6.48 (s, 1H, chroman), 5.96 (s, 2H, CH_2).

2.3. Synthesis of 6-(4-(piperazin-1-yl)phenyl)-8H-[1,3]dioxolo[4,5-g] chromen-8-one (5)

To a solution of **4** in DMF (35 mL), equivalent ratio of piperazine hexahydrate was added and reaction was allowed to heat at 110 °C for 12 h in the presence of Cs2CO3. After the completion of the reaction as monitored by the TLC, the reaction mass was poured onto crushed ice, filtered, dried in vacuum and recrystallized from dichloromethane-methanol to result **5** in 72%. Yellow solid, m.p. 198–200 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.74–7.49 (m, 4H), 7.33 (s, 1H, chroman), 7.02 (s, 1H, chroman), 6.42 (s, 1H, chroman), 6.04 (s, 2H, CH_2), 3.45 (t, J = 4.9 Hz, 4H, CH_2), 2.71 (t, J = 4.9 Hz, 4H, CH_2).

2.4. General procedure for the preparation of sulfonylpiperazine based chrysin derivatives (7a-k)

To a solution of 0.5 mL of pyridine and 0.5 mmol of intermediate **5**, 20 mL of dried dichloromethane was added followed by 1 mmol of selected substituted sulfonyl chlorides (**a-k**). The resulting reaction mixture was refluxed for 12–17 h and reaction was monitored by TLC. After completion, the reaction mass was quenched using 20 mL of 10% NaOH followed by the extraction with chloroform with quantities 3 × 15 mL. The organic portion was then passed through anhydrous sodium sulfate, concentrated in vacuo and purified by column chromatography (DCM) to afford **7a-k**. For example, ***6-(4-(4-(phenylsulfonyl)piperazin-1-yl) phenyl)-8H-[1,3]dioxolo[4,5-g]chromen-8-one (7a)***: Yield: 52%. m.p. 247–249 °C; IR (KBr) cm^{-1}: 3066, 3034, 2933, 2855, 1669, 1613, 1590, 1479, 1379, 1344, 1266, 1154, 1039; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.81–7.70 (m, 4H), 7.52–7.38 (m, 5H), 7.34 (s, 1H, chroman), 7.03 (s, 1H, chroman), 6.44 (s, 1H, chroman), 6.05 (s, 2H, CH_2), 3.53 (t,

J = 4.87 Hz, 4H, CH_2), 2.78 (t, J = 4.85 Hz, 4H, CH_2). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 181.1, 156.2, 150.9, 144.7, 139.1–118.0, 112.6, 105.6, 102.1, 97.5, 52.8, 44.4. Anal. Calcd. for $C_{26}H_{22}N_2O_6S$: C, 63.66; H, 4.52; N, 5.71. Found: C, 63.55; H, 4.72; N, 5.89.

*2.5. 3-(Benzo[d][1,3]dioxol-5-yloxy)propanoic acid 9 and 6,7-dihydro-8H-[1,3]dioxolo[4,5-g]chromen-8-one 10 were prepared following the literature procedure [30]. Compounds of **12a-k** were synthesized as per the procedure described for **7a-k**. For example, **7-((4-(phenylsulfonyl) piperazin-1-yl)methylene)-6H-[1,3]dioxolo[4,5-g]chromen-8(7H)-one (12a)***

Yield: 49%. m.p. 222–224 °C; IR (KBr) cm^{-1}: 3066, 3034, 2933, 2855, 1669, 1613, 1590, 1479, 1371, 1344, 1266, 1154, 1039; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.81 (s, 1H, benzylidene), 7.63–7.41 (m, 5H, ArH), 7.31 (s, 1H, chroman), 7.09 (s, 1H, chroman), 6.05 (s, 2H, CH_2), 5.41 (d, 2H, chroman, J = 1.9 Hz), 3.55 (t, J = 4.87 Hz, 4H, CH_2), 2.71 (t, J = 4.85 Hz, 4H, CH_2). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 179.1, 154.5, 152.4, 142.9, 142.2, 138.4–116.5, 110.6, 101.3, 96.3, 64.5, 51.1, 44.2. Anal. Calcd. for $C_{21}H_{20}N_2O_6S$: C, 58.87; H, 4.70; N, 6.54. Found: C, 58.77; H, 4.91; N, 6.67.

*2.6. Synthesis of 7-(piperazin-1-ylmethylene)-6H-[1,3]dioxolo[4,5-g] chromen-8(7H)-one (**11**)*

A solution of 0.7 mmol of 1-formyl piperazine and 6,7-dihydro-8H-[1,3]dioxolo[4,5-*g*]chromen-8-one **10** (0.5 mmol) was ice cooled and dry HCl was passed through it in the presence of 5 mL of absolute ethanol for 5 min. The resulting reaction mass was then allowed to stand for two days at room temperature so that precipitation occurs when were then filtered, dried in vacuum and recrystallized from ethanol and water to yield **11**. Yield: 41%. m.p. 189–191 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.87 (s, 1H, benzylidene), 7.36 (s, 1H, chroman), 7.05 (s, 1H, chroman), 6.01 (s, 2H, CH_2), 5.48 (d, 2H, chroman, J = 1.8 Hz), 3.51(t, J = 4.9 Hz, 4H, CH_2), 2.77 (t, J = 4.9 Hz, 4H, CH_2), 2.14 (s, 1H).

3. Biological screening

3.1. Evaluation of antioxidant capacity by using the DPPH assay

In vitro free radical scavenging potential of the final derivatives was quantitatively measured by DPPH method [31,32]. In brief, 20 µL of tested compounds (0.1, 1, 10, 100 µL) were added to a 96-well microplate, to which 180 µL of DPPH was added. Methanol (20 µL) was used as the blank, and after incubation for 30 min, the optical density at 517 nm was calculated. Ascorbic acid was used as the reference compound and all determinations were carried out in triplicate.

The DPPH$^{\cdot}$ scavenging activity was calculated by using the equation provided by Mensor et al. [33].

$$\%Scavenging = \frac{Absorbance\ of\ blank - Absorbance\ of\ test}{Absorbance\ of\ blank} \times 100$$

A plot of concentration of test compounds and % scavenging activity showed half-maximal inhibitory concentrations (IC_{50s}) in the presence of ascorbic acid as the standard.

3.2. Evaluation of antioxidant capacity of the ABTS assay

All the final compounds were screened for ABTS$^{\cdot+}$ radical cation scavenging assay [31,34]. In brief, Different concentrations (0.1, 1, 10, 100 µL) of tested derivatives (20 µL) were added to a 96-well microplate, than added 180 µL of ABTS solution followed by 10 min of incubation under dark condition. The absorbance was read at 734 nm. Ascorbic acid was used as the reference compound and all determinations were carried out in triplicate. ABTS scavenging effect was calculated as percentage of ABTS$^{\cdot+}$ scavenging using the following equation:

$$\%Scavenging = \frac{Absorbance\ of\ blank - Absorbance\ of\ test}{Absorbance\ of\ blank} \times 100$$

A plot of concentration of test compounds and % scavenging activity showed half-maximal inhibitory concentrations (IC_{50s}) in the presence of ascorbic acid as the standard.

3.3. In vitro cytotoxicity bioassay

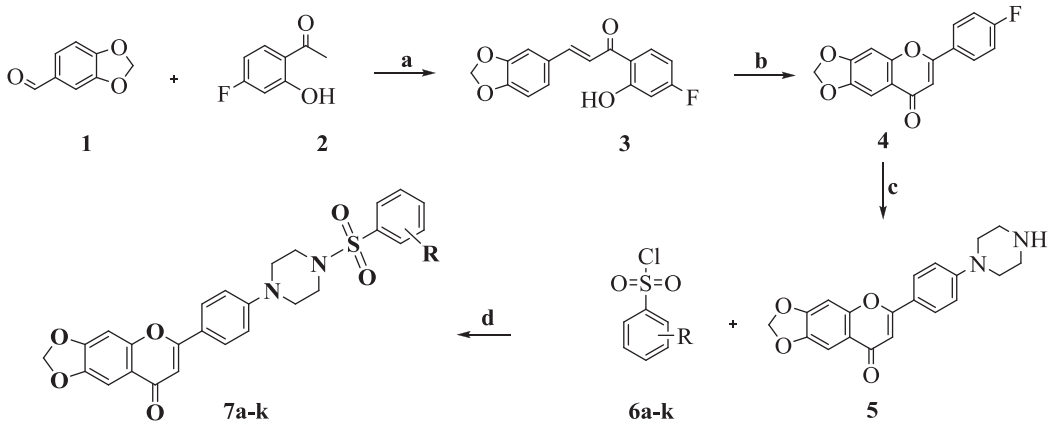
In vitro cytotoxicity bioassay of the synthesized compounds was carried out using the SRB assay method [31,35]. In briefly, all the cell lines were well maintained in Dulbecco's Modified Eagle's Medium (DMEM) and RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic-antimycotic solution (100X) in a humidified cell culture incubator in the presence of 5% of CO_2 at 37 °C. HeLa, SK-OV-3, A-549, HT-29 and Madin-Darby canine kidney non-cancer (MDCK) cells were seeded into 96-well plates at the density of 2×10^4 cells per well plate. The synthesized compounds were dissolved in DMSO and treated with the cells after 24 h and diluted in RPMI or DMEM medium giving risen to four concentrations comprising 0.1, 1, 10, and 100 µL. The infected plates were then incubated in a CO_2 incubator for 48 h after the addition of the compounds, 100 µL of SRB (0.4 mg/L) was added to each well and incubated for overnight. After that, 70% of cold acetone was added to each well to fix the viable cells washed, dried, and dyed by 100 µL of SRB (0.4 mg/L) followed by SRB removal and three washes with 1% acetic acid. The unbounded dye was separated, while the protein-bound dye was extracted with 10 mM Tris base and incubated overnight. Multi-well spectrophotometric data were recorded at 510 nm to calculate the IC_{50} and the 50% cytotoxic concentration (CC_{50}).

4. Results and discussion

4.1. Chemistry

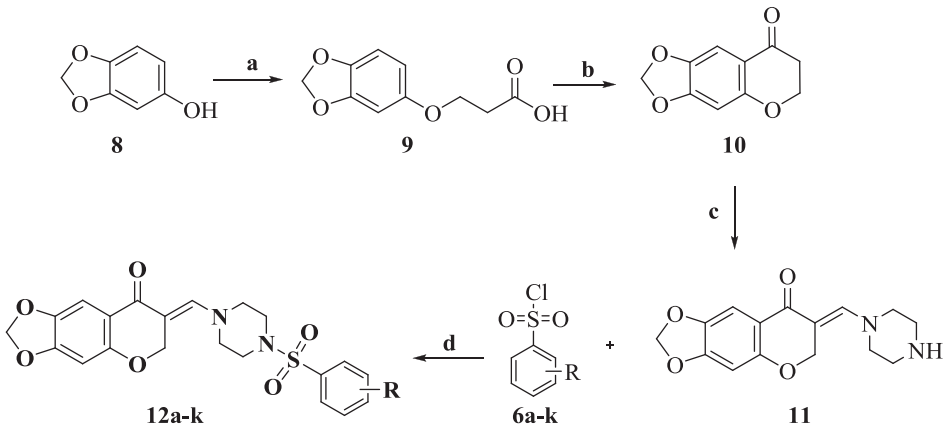
Synthetic steps adopted to furnish final compounds **7a-k** and **12a-k** are drawn in scheme 1. Treatment of commercially available piperonal (**1**) with 4′-Fluoro-2′-hydroxyacetophenone (**2**) gave an intermediate chalcone **3** in the presence of KOH utilizing aldol condensation. Cyclization of **3** in the DMSO using catalytic amount of I_2 followed by treatment with saturated aqueous sodium thiosulfate solution lead to the formation of a key intermediate 6-(4-fluorophenyl)-8H-[1,3]dioxolo [4,5-*g*]chromen-8-one (**4**). Subsequently, the final intermediate (**5**) was prepared by the nucleophilic substitution of fluorine atom of **4** with piperazine following the literature procedure [36,37]. Finally, in the last step, refluxing **5** with different substituted benzenesulfonyl chlorides (**a-k**) in the presence of pyridine in DCM solvent followed by the treatment with 10% NaOH furnished final molecules **7a-k** in reasonable yields. Furthermore, for the initiation of the synthesis of **12a-k**, benzo [d][1,3]dioxol-5-ol (**8**) was allowed to react with 3-bromopropanoic acid in basic conditions using sodium hydroxide produced compound **9** which was then further treated with oxalyl chloride in benzene solvent in the presence of Tin tetrachloride to give intermediate **10**. Chalcone moiety (**11**) was constructed utilizing aldol condensation of **10** with 1-formyl piperazine in the presence of HCl gas. In the last step, final intermediate **11** was refluxed with different substituted benzenesulfonyl chlorides (**a-k**) in the presence of pyridine in DCM solvent followed by the treatment with 10% NaOH furnished final molecules in reasonable yields [38,39]. FT-IR, ^{1}H NMR and ^{13}C NMR data were in the accordance of the proposed structures. All compounds gave C, H and N analyses within limits from the theoretical values.

Series 1



Reagents and conditions: **(a)** 20% KOH, EtOH, rt, 24h; **(b)** I_2, DMSO, reflux, 11 h; **(c)** piperazine hexahydrate, Cs_2CO_3, DMF, 110°C, 12h; **(d)** pyridine, DCM, reflux, 12-17 h;

Series 2



Reagents and conditions: (**a**) NaOH, Na_2CO_3, $Br(CH_2)_2COOH$, H_2O, reflux; (**b**) oxalyl chloride, $SnCl_4$, benzene; (**c**) 1-formyl piperazine, HCl (g), 0°C; (**d**) pyridine, DCM, reflux, 8-21h;

R= **a**: H; **b**: 4-Cl; **c**: 2,4-diCl; **d**: 4-Br; **e**: 2,4-diBr; **f**: 4-F; **g**: 2,4-diF; **h**: 4-OCF_3; **i**: 4-NO_2; **j**: 4-OCH_3; **k**: 2,4-diOCH_3

Scheme 1. Synthesis of phenylsulfonyl piperazine bridged [1,3]dioxolo[4,5-*g*]chromenones **7a-k** and **12a-k**.

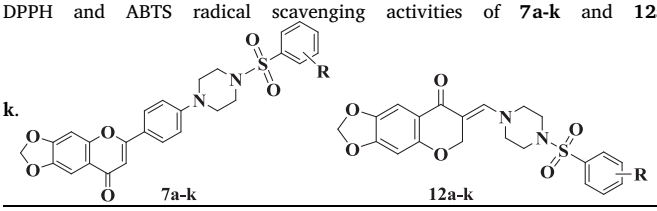
4.2. Pharmacology

4.2.1. Antioxidant activities

Newly synthesized analogues **7a-k** and **12a-k** were subjected to evaluate their *in vitro* antioxidant (DPPH˙ and $ABTS^{\cdot+}$ scavenging) and antiproliferative (human ovarian cancer SK-OV-3, cervical cancer HeLa, human colon adenocarcinoma HT-29, and human non-small-cell lung carcinoma A549) activities. The results of bioassay screenings adopting DPPH, ABTS and SRB assay are furnished in Tables 1 and 2. Along with cytotoxicity of new compounds against cancer cell lines, they were also inspected for their tolerable cytotoxic properties against non-cancer MDCK cells and results are noted down it Table 2. Overall, tested compounds were found to have promising pharmacological potential and results delivered some interesting structure-activity relationships. For example, in some bioassay cases, overall structure found to be responsible to express specific bioactivities, while for the other cases, placing an appropriate substituent was a key to anticipated potency. Moreover, presence of electron withdrawing (EWD) or electron donating (ED) functional group exercised different activity levels against different targets. Likewise, amongst EWD and ED, presence of single or more than one groups had a substantial impact on the biological activities of the resultant molecules.

DPPH antioxidant assay revealed that chalcone hybrids **12a-K** were more powerful in scavenging DPPH˙ than **7a-k**. Hence, it would suffice to mention here that overall structure of the designed molecule was essential to deliver anti- DPPH˙ activity. However, there was an exception in case of an analogue bearing nitro group (**7i**) from **7a-k** family showing 28.79 ± 0.94 μg/mL of IC_{50} when compared to that of **12i** with 33.45 ± 1.09 μg/mL. Amongst the most potent derivatives, those from **12a-k** set with 2,4-diOCH_3 (**12k**) and OCF_3 (**12h**) functionalities showed most potent action against DPPH˙ with 8.21 ± 0.83 μg/mL and 8.98 ± 0.56 μg/mL of IC_{50s}, respectively. Overall, amongst both sets of compounds, those bearing OCF_3 and OCH_3 functional groups were found to have better DPPH˙ scavenging efficacies that those holding halo atom (s). For example, within **7a-k** and **12a-k** groups, derivatives **7j**, **7k**, **7h** and **12j** expressed 9.34–10.39 μg/mL of IC_{50s} in comparison to those

Table 1
DPPH and ABTS radical scavenging activities of **7a-k** and **12a-k**.



No.	R	IC_{50}[a] μg/mL ± SD	
		DPPH[b]	ABTS[c]
7a	H,	39.19 ± 0.74	24.25 ± 1.48
7b	4-Cl	23.81 ± 1.15	11.29 ± 1.22
7c	2,4-diCl	21.26 ± 0.51	9.18 ± 1.02
7d	4-Br	25.98 ± 0.88	10.55 ± 0.66
7e	2,4-diBr	25.10 ± 0.96	10.92 ± 0.41
7f	4-F	19.04 ± 1.03	9.11 ± 1.29
7g	2,4-diF	17.91 ± 0.59	8.13 ± 0.19
7h	4-OCF_3	8.98 ± 0.43	5.47 ± 0.93
7i	4-NO_2	28.79 ± 0.94	18.99 ± 1.20
7j	4-OCH_3	10.39 ± 0.79	5.66 ± 1.11
7k	2,4-diOCH_3	9.34 ± 1.03	4.74 ± 0.49
12a	H	37.04 ± 0.90	27.90 ± 0.39
12b	4-Cl	14.57 ± 0.35	7.91 ± 1.09
12c	2,4-diCl	12.59 ± 0.69	6.97 ± 0.33
12d	4-Br	15.10 ± 1.13	10.94 ± 1.17
12e	2,4-diBr	12.99 ± 0.21	11.73 ± 0.59
12f	4-F	12.11 ± 1.09	10.18 ± 0.91
12g	2,4-diF	11.24 ± 0.89	9.01 ± 0.55
12h	4-OCF_3	9.41 ± 0.56	5.22 ± 1.07
12i	4-NO_2	33.45 ± 1.09	22.78 ± 0.79
12j	4-OCH_3	9.82 ± 0.36	6.04 ± 0.41
12k	2,4-diOCH_3	8.21 ± 0.83	4.82 ± 0.77
Ascorbic acid		12.72 ± 0.274	5.0925 ± 0.2090

[a] Antioxidant activities are shown as IC_{50} values in μg/mL. All assays were carried out in triplicate, and the results are expressed as an average ± standard deviation.
[b] DPPH = 2,2-diphenyl-1-picrylhydrazyl.
[c] ABTS = 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid.

(**7b-7g** and **12b-12g**) endowed with halogen atom(s) with 11.24 ± 0.89–25.98 ± 0.88 μg/mL of IC_{50s}. These results suggested that although overall structure of chalcones was crucial for anti- $DPPH^{\cdot}$ activity, but amongst the active set of compounds, presence of optimum substituent was an influential factor. The statement was further supported by the activity data observed in case of compounds bearing halo functional groups. For instance, all derivatives with dihalo groups acted as better DPPH radical scavengers than their single halogenated precursors. For example, **12e** with 2,4-diBr group showed 12.99 ± 0.21 μg/mL of IC_{50} and **12d** with 4-Br expressed 15.10 ± 1.13 μg/mL of IC_{50}, whereas **7c** (2,4-diCl) and **7b** (4-Cl) expressed 21.26 ± 0.51 μg/mL and 23.81 ± 1.15 μg/mL of IC_{50s}, respectively. Importantly, within halogenated analogues, those presenting fluorine atom(s) such as **12f** and **12g** showed most potent anti- $DPPH^{\cdot}$ action than others observing 12.11 ± 1.09 μg/mL and 11.24 ± 0.89 μg/mL of IC_{50s}, respectively furnishing activity orders of F > Cl > Br. Absence of any substituent lead to the compound with poorer $DPPH^{\cdot}$ scavenging activity as found from the IC_{50} data of **7a** and **12a** above 37 μg/mL. It is suffice to state that almost all derivatives from **12a-k** set as well as some from **7a-k** group presented better $DPPH^{\cdot}$ scavenging potencies then control drug ascorbic acid with 12.72 ± 0.274 μg/mL of IC_{50}. Thus, the study suggested the importance of bridging suitable pharmacophores to a main core.

Bioassay results observed in ABTS assay suggested that both structure and substituents were crucial to effectively scavenge $ABTS^{\cdot+}$. Regarding bioassay data, however, the mixed trend was noticed for **7a-k** and **12a-k** families but presence of three F atoms as well as more OCH_3 groups demonstrated excellent anti-$ABTS^{\cdot+}$ action. For instance, the most potent compounds in ABTS assay were **7k** and **12k** with 2,4-diOCH_3 group with 4.74 ± 0.49 μg/mL and 4.82 ± 0.77 μg/mL of IC_{50s}, respectively, which were even better than control ascorbic acid with 5.0925 ± 0.2090 μg/mL of IC_{50}. Furthermore, **7h** and **12h** with OCF_3 gave 5.47 ± 0.93 μg/mL and 5.22 ± 1.07 μg/mL of IC_{50s}, respectively, which were comparable to the control ascorbic acid. Amongst halogenated analogues, in case of Cl and F containing molecules (**7c**, **7g**, **12c** and **12g**), those with two atoms showed higher $ABTS^{\cdot+}$ scavenging effects than their single halogenated counterparts (**7b**, **7b**, **12f** and **12f**). To mention, **7c** carrying 2,4-diCl showed 9.18 ± 1.02 μg/mL of IC_{50}, while **7b** (4-Cl) displayed 11.29 ± 1.22 μg/mL of IC_{50}. Opposite t it, in case of brominated compounds, single Br bearing analogues (**7d**: 10.55 ± 0.66 μg/mL, **12d**: 10.94 ± 1.17 μg/mL) showed powerful action in terms of IC_{50s} in scavenging $ABTS^{\cdot+}$ than di-Br analogues (**7e**: 10.92 ± 0.41 μg/mL, **12e**: 11.73 ± 0.59 μg/mL) hence establishing the activity order of Cl > F > Br within halogenated subjects. However, to conclude ABTS assay in terms of halogenated compounds, set of **7a-k** exercised better in case of Cl substitution, while **12a-k** chalcones acted well with Br and F substituents. Overall structure of the rationalized molecules had an influential action in ABTS assay as unsubstituted **7a** had greater anti-$ABTS^{\cdot+}$ impact with 24.25 ± 1.48 μg/mL of IC_{50} than **12a** showing 27.90 ± 0.39 μg/mL of IC_{50}. Moreover, it can be stated that in case of nitro bearing substituents **7a-k** were better antioxidants than chalcones **12a-k**, because in ABTS assay **7i** exerted 18.99 ± 1.20 μg/mL of IC_{50} and **12i** furnished 22.78 ± 0.79 μg/mL of IC_{50}. Most importantly, from the above discussion it can be concluded that both two sets of compounds are more effective in scavenging ABTS radical than DPPH radical as shown from the IC_{50} data. Overall, when compared to the data documented for the control in both the antioxidant assay, it can be surely stated that the substances developed in the present research provides exciting and appealing free radical scavenging potential when compared to the control drug and can a tool for creating further related molecules with considerably higher effects.

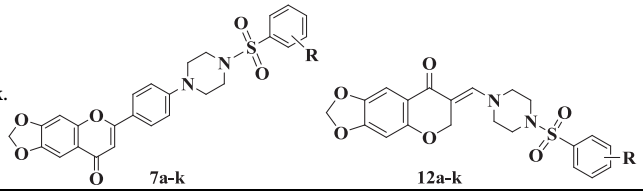
4.2.2. Antiproliferative activities

Cytotoxic potential of **7a-k** and **12a-k** was investigated against four human cancer cell lines namely human ovarian cancer (SK-OV-3), cervical cancer (HeLa), human colon adenocarcinoma (HT-29) and human non-small-cell lung carcinoma (A549) cell lines as well as non-cancer MDCK cells. In general, it can be stated that both sets of compounds were fruitful to deliver anticipated antiproliferative action against all four cancer cell lines and thus, the present work of designing and rationalizing these two different compact systems has been justified. Likewise antioxidant bioassay data, in SRB assay evaluations, structure and selected substituents placed an equal importance while delivering promising structure-activity relationships which will be discussed below.

New derivatives **7a-k** and **12a-k** demonstrated strong inhibitory potential of human ovarian cancer cell line SK-OV-3 with IC_{50s} ranging from 11.35 ± 2.10–58.93 ± 1.18 μg/mL. The mixed IC_{50} trends suggested that both structures were of a great impact on SK-OV-3 with the most influence came from variable substituents. Presence of three F atoms as well as two OCH_3 groups demonstrated excellent anti-ovarian cancer effects as analogues **7h** (4-OCF_3) and **12k** (2,4-diOCH_3) displayed 11.35 ± 2.10 μg/mL and 11.48 ± 0.79 μg/mL of IC_{50s}, respectively followed by 12.68 ± 1.09 and 13.04 ± 0.98 μg/mL of IC_{50s} of compounds **12h** (4-OCF_3) and **7k** (2,4-diOCH_3), respectively. Again, compounds with EWD halo atom(s) revealed interesting structure-activity relationship as in **7a-k** set those with single halo atom inhibited SK-OV-3 well than their dihalo counterparts, whereas, within chalcones family (**12a-k**) a case with fluorine was in line with what observed in **7a-k**, and in case of Cl and Br, those with two atoms were strong inhibitory agents of SK-OV-3 than their single halogenated counterparts. For an example, **7d** with 4-Br, **7e** bearing 2,4-diBr, **12c** holding 2,4-diCl and **12b** carrying 4-Cl had 19.34 ± 1.17 μg/mL, 28.08 ± 0.88 μg/mL, 24.79 ± 0.72 μg/mL and 29.13 ± 0.53 μg/mL of IC_{50s}, respectively.

Table 2

Antiproliferative activities of **7a-k** and **12a-k**.



No.	R	[a]IC_{50} μg/mL ± SD				[b]CC_{50} μg/mL ± SD
		SK-OV-3	HeLa	A-549	HT-29	MDCK
7a	H	58.93 ± 1.18	38.47 ± 1.41	47.11 ± 0.56	56.90 ± 1.09	321.73 ± 1.74
7b	4-Cl	26.10 ± 0.92	7.78 ± 0.91	21.24 ± 0.70	21.23 ± 1.48	289.64 ± 1.42
7c	2,4-diCl	28.90 ± 0.33	7.80 ± 0.59	20.79 ± 1.43	19.12 ± 0.47	277.21 ± 0.64
7d	4-Br	19.34 ± 1.17	8.33 ± 0.99	19.57 ± 1.32	34.08 ± 1.75	265.92 ± 1.25
7e	2,4-diBr	28.08 ± 0.88	7.90 ± 0.46	19.98 ± 0.65	36.72 ± 0.46	247.37 ± 1.01
7f	4-F	23.16 ± 1.01	7.81 ± 0.88	18.33 ± 0.32	15.84 ± 85	272.10 ± 0.53
7g	2,4-diF	34.17 ± 0.62	8.01 ± 1.00	19.56 ± 1.23	16.90 ± 0.56	257.11 ± 1.74
7h	4-OCF_3	11.35 ± 2.10	17.88 ± 1.20	26.47 ± 1.33	17.04 ± 0.37	244.57 ± 0.83
7i	4-NO_2	48.52 ± 0.97	43.19 ± 0.89	36.01 ± 1.11	47.11 ± 0.95	212.90 ± 0.39
7j	4-OCH_3	18.19 ± 0.92	14.88 ± 0.57	29.57 ± 1.13	34.51 ± 1.53	254.12 ± 1.81
7k	2,4-diOCH_3	13.04 ± 0.98	21.22 ± 1.04	33.41 ± 0.43	32.98 ± 1.95	231.19 ± 0.66
12a	H	55.12 ± 0.91	33.10 ± 0.81	56.10 ± 0.85	63.01 ± 0.47	298.81 ± 1.42
12b	4-Cl	29.13 ± 0.53	6.87 ± 1.27	25.19 ± 1.32	24.72 ± 0.76	257.82 ± 0.89
12c	2,4-diCl	24.79 ± 0.72	4.97 ± 0.39	26.22 ± 0.88	22.98 ± 0.35	251.21 ± 0.38
12d	4-Br	33.18 ± 1.24	7.88 ± 0.33	23.04 ± 0.75	27.33 ± 0.54	243.10 ± 0.55
12e	2,4-diBr	31.09 ± 0.67	7.67 ± 1.11	24.81 ± 1.37	36.09 ± 1.86	221.54 ± 0.74
12f	4-F	24.17 ± 0.79	6.98 ± 0.77	22.21 ± 0.84	17.66 ± 0.57	238.76 ± 1.59
12g	2,4-diF	30.44 ± 0.99	4.55 ± 0.44	22.99 ± 1.37	14.43 ± 1.26	231.29 ± 1.84
12h	4-OCF_3	12.68 ± 1.09	16.19 ± 0.36	27.58 ± 0.28	13.39 ± 0.36	247.27 ± 0.36
12i	4-NO_2	47.21 ± 0.82	40.13 ± 0.47	40.19 ± 0.84	50.19 ± 1.90	227.18 ± 0.50
12j	4-OCH_3	17.89 ± 1.01	13.97 ± 0.47	32.67 ± 0.85	32.10 ± 1.75	236.19 ± 1.22
12k	2,4-diOCH_3	11.48 ± 0.79	18.34 ± 0.96	33.13 ± 1.39	29.27 ± 1.65	252.72 ± 0.89

SK-OV-3: human ovarian cancer cell line, HeLa: human cervical cancer cell line, HT-29: human colon adenocarcinoma cell line, A-549: human non-small cell lung carcinoma cell line.

[a] Cytotoxicity is shown as IC_{50} values in μg/mL. All assays were carried out in triplicate, and the results are expressed as an average ± standard deviation.

[b] CC_{50} – cytotoxicity concentration of 50%.

Analogues **7j** and **12j** expressed 18.19 ± 0.92 μg/mL and 17.89 ± 1.01 μg/mL of IC_{50s}, respectively, which were better than any other halogenated analogues (19.34 ± 1.17–33.18 ± 1.24 μg/mL) thereby placing an importance of methoxy functional groups to inhibit ovarian cancer cells than halo-substituted compounds. Moreover, it was noticed that presence of appropriate substituent on the phenylpiperazinyl ring is essential to gain activity against SK-OV-3 as all analogues along with those with nitro substituent (**7i**: 48.52 ± 0.97 μg/mL and **12i**: 47.21 ± 0.82 μg/mL) displayed reasonably higher activities than unsubstituted derivatives **7a** (58.93 ± 1.18 μg/mL) and **12a** (55.12 ± 0.91 μg/mL) in terms of IC_{50s}. However, this fact could not discourage the construction of unsubstituted sulfonylpiperazine based molecules because anti-MDCK activity data showed that analogue **7a** and **12a** presented most potent and tolerable behavior with 321.73 ± 1.74 and 298.81 ± 1.42 μg/mL of CC_{50s}, respectively meaning that the presented designs of compounds are safer to use as an anticancer agents with the further development option on the other position of the base core. In case of inhibitory potential of cervical cancer cell line HeLa, the overall structure of the designed compounds was a key as **12a-k** (4.55 ± 0.44–40.13 ± 0.47 μg/mL) were found more potent than **7a-k** (7.78 ± 0.91–43.19 ± 0.89 μg/mL). The most potent analogous were from **12a-k** set as **12g** (2,4-diF) had 4.55 ± 0.44 μg/mL and **12c** (2,4-diCl) showed 4.97 ± 0.39 μg/mL of IC_{50s} while concluding that all the dihalo analogues were more active than their single halogenated counterparts. For example, **12e** and **7e** with 2,4-diBr substituent displayed 7.67 ± 1.11 and 7.90 ± 0.46 μg/mL of IC_{50s}, respectively, while **12d** and **7d** with 4-diBr substituent furnished 7.88 ± 0.33 and 8.33 ± 0.99 μg/mL of IC_{50s}, respectively. Amongst compound bearing halo groups, the overall activity order against HeLa observed Cl > F > Br. Moving forward, observing anti-HeLa activity of molecules with ED groups, those with single methoxy functional group had better activity than dual OCH_3 containing analogues. Such as, **7j** with 4-OCH_3 presented 14.88 ± 0.57 μg/mL of IC_{50} and **7k** with 2,4-diOCH_3 had 21.22 ± 1.04 μg/mL of IC_{50}. Noticing antiproliferative potential of derivatives bearing NO_2 group and derivatives without any substitution, HeLa assay results were an exception because unsubstituted **7a** and **12a** had better IC_{50s} when compared to **7i** and **12i** with NO_2 functionality with 38.47 ± 1.41 μg/mL, 33.10 ± 0.81 μg/mL, 43.19 ± 0.89 μg/mL and 40.13 ± 0.47 μg/mL, respectively. Furthermore, activity data observed against A-549 cell line suggested the importance of the overall structure of **7a-k** analogues and thereby justified the rationale of the current work about optimizing a specific position of a base core for the suitable substitution. As mentioned, **7a-k** were more active against **A-549** than chalcones **12a-k**, wherein, single halo bearing compounds were identified as promisingly active then their dihalo precursors. There was just one exception as **12k** with 2,4-diOCH3 group was slightly more active than its counterpart **7k** having 33.13 ± 1.39 and 33.41 ± 0.43 μg/mL of IC_{50s}, respectively. The most active derivatives noticed were belonged to F and Br group amongst **7a-k**, as **7d** (4-Br), **7e** (2,4-diBr), **7f** (4-F) and **7g** (2,4-diF) showed 18.33 ± 0.32–19.98 ± 0.65 μg/mL of IC_{50s} being 7f as the most active one. The halo and dihalo activity pattern followed in the same manner in **12a-k** set, for example **12b** involving 4-Cl substitution had 25.19 ± 1.32 μg/mL of IC_{50} and **12c** (2,4-diCl) had 26.22 ± 0.88 μg/mL of IC_{50} which showed the activity order for halo compounds as F > Br > Cl. As anticipated from the data set of tested derivatives against A549, those bearing OCF_3 group were more active then molecules endowed with OCH_3 functionality. For an instance, **7h** and **12h** revealed 26.47 ± 1.33 μg/mL and 27.58 ± 0.28 μg/mL of IC_{50s}, respectively, whereas, **7j** and **12j** had 29.57 ± 1.13 μg/mL and 32.67 ± 0.85 μg/mL of IC_{50s}, respectively. In regard with NO_2 bearing analogues versus unsubstituted ones, as usual NO_2 appeared with

higher activity (IC_{50s}: 36.01 ± 1.11–40.19 ± 0.84 μg/mL) then the later ones (47.11 ± 0.56–56.10 ± 0.85 μg/mL). It seemed that presence of a single functional group was the key to receive good anti-A549 potency as molecules with single OCH_3 groups showed better IC_{50s} than those with 2,4-diOCH_3 functionality. For example, **7j** having 4-OCH_3 showed 29.57 ± 1.13 μg/mL of IC_{50}, while **7k** (2,4-diOCH_3) presented 33.41 ± 0.43 μg/mL of IC_{50} and the similar trend has been recorded in case of **12j** and **12k**. The optimized insertion of fluorine atom(s) into a rationalized molecules can uniquely alter the membrane permeability, pK_a, metabolic pathways, binding interactions, intrinsic potency, selective reactivities, pharmacokinetic properties and molecular conformation [40,41]. The stated importance of F has been proved from the data obtained against HT-29 cell lines as all the fluorinated analogues were of high potency than others. For example, compound **12h** bearing 4-OCF_3 group exhibited 13.39 ± 0.36 μg/mL of IC_{50} and was the most potent one amongst others. However, there was an interesting SAR observed within two sets as single F bearing analogue (**7f**, 15.84 ± 85 μg/mL) from **7a-k** was more active than **12f** with 17.66 ± 0.57 μg/mL of IC_{50}, whereas in terms of two or more F bearing analogues those from **12a-k** set were more efficacious than their **7a-k** counterparts. For example, **12g** having 2,4-diF showed 14.43 ± 1.26 μg/mL of IC_{50} and **7g** holding 2,4-diF expressed 16.90 ± 0.56 μg/mL of IC_{50}. These facts, places an equal importance of deriving two different schemes whereas special emphasis was found placed on the fluorine substitution within both the sets. Interestingly, amongst halogenated analogues within both sets, all the Cl bearing molecules from **7a-k** were more active than chlorinated **12a-k**, where diCl bearing derivatives found to have greater inhibitory effects against HT-29 than single Cl holding compounds within both sets. For example, **7b** (4-Cl) and **7c** (2,4-diCl) showed 21.23 ± 1.48 μg/mL and 19.12 ± 0.47 μg/mL of IC_{50s}, respectively, and **12b** (4-Cl) and **12c** (2,4-diCl) exerted 24.72 ± 0.76 μg/mL and 22.98 ± 0.35 μg/mL of IC_{50s}, respectively. Opposite to it, in regard with brominated compounds, chalcones **12a-k** were effective against HT-29 than **7a-k**. Again, on the contrary to the chlorinated compounds, single Br bearing analogues were active than double Br containing molecules. For example, **12d** (4-Br), **12e** (2,4-diBr), **7d** (4-Br) and and **7e** (2,4-diBr) indicated 27.33 ± 0.54 μg/mL, 36.09 ± 1.86 μg/mL, 34.08 ± 1.75 μg/mL and 36.72 ± 0.46 μg/mL of IC_{50s}, respectively. Clearly, methoxy functionality seemed unsuitable to inhibit HT-29 as all the molecules bearing OCH_3 group appeared to have at least more than 29.27 ± 1.65 μg/mL of IC_{50}, where in fact, **12a-k** worked well than **7a-k** such as, **12j** and **7j** with 4-OCH_3 had 32.10 ± 1.75 μg/mL and 34.51 ± 1.5365 μg/mL of IC_{50s}, respectively. In addition, it was observed that placing of two methoxy groups was beneficial to inhibit HT-29 as IC_{50s} of **12k** and **12j** were 29.27 ± 1.65 μg/mL and 32.10 ± 1.75 μg/mL respectively the same was observed in case of **7j** and **7k**. As observed from the other data, again the substitution of at least one functional group on the piperazinylphenyl entity was crucial as analogues with NO_2 group had higher inhibitory effects against HT-29 than unsubstituted ones. The data facts, revealed the key features of the present study comparing two different heterocyclic systems and linked pharmacophores as a sulfonylpiperazine moiety featuring single and di substitution of the specific functional group(s). As data suggested that all the tested derivatives were fruitful in case of inhibiting cancer cell lines, however, their cytotoxic nature towards non-cancer or healthy cells should be investigated to conclude them as drug-like candidates. Therefore, we have screened all the final compounds against non-cancer MDCK cells where surprisingly, unsubstituted analogues had the highest CC_{50s} followed by those involving substitution of Cl atom(s) as the CC_{50s} order falls as Cl > OCF_3 > Br > F amongst halogenated molecules. Where, single halo bearing compounds were more tolerable by MDCK than their dihalo counterparts. In fact, halogenated molecules from **7a-k** set showed higher CC_{50s} than their **12a-k** counterparts. Finally, methoxy holding molecules revealed mixed trend in terms of CC_{50s} concluding that they were reasonably tolerable too. In addition, chalcones bearing OCH3 functionality had greater CC_{50s} observed than their **7a-k** counterparts. Overall, all the tested derivatives from both the sets demonstrated tremendous inhibitory potential against all the four cancer cell line studied and can be of a great deal of attraction for future optimization.

5. Conclusion

Two series of [1,3]dioxolo[4,5-*g*]chromenones featuring phenylsulfonylpiperazines liked to the chroman core through phenyl (**7a-k**) and chalcone (**12-k**) function has been rationalized, synthesized and investigated adopting antioxidant and anticancer assays. Free radicals ($DPPH^{\cdot}$ and $ABTS^{\cdot+}$) scavenging potencies were inspected along with cancerous cell inhibitory efficacies against human ovarian cancer (SK-OV-3), cervical cancer (HeLa), colon adenocarcinoma (HT-29) and non-small-cell lung carcinoma (A549) cell lines. Results from bioassay investigations concluded that selected molecules provided appealing medicinal potential and exciting structure-activity connections. For example, in some bioassay situations, overall framework discovered to be crucial to convey specific bioactivities, while for the other situations, putting an appropriate substituent(s) was a key to expected efficiency. Moreover, existence of EWD and ED group worked out different action levels against different objectives. Furthermore, amongst EWD and ED, presence of single or more than one groups had significant effect on the scientific activities of the resulting elements. For example, in regard with the importance of the overall structure, analgoues from **7a-k** group presented promising potential against non-small-cell lung carcinoma (A549) cell line, whereas molecules from chalcone series **12a-k** worked well with HeLa, HT-29 and $DPPH^{\cdot}$. Moreover, both sets were equally importance to receive anticipated potencies against $ABTS^{\cdot+}$ and SK-OV-3. Furthermore, single halogenated compounds from both the sets showed promising pharmacological potential against $ABTS^{\cdot+}$, A-549 whereas, dihalo substitution was a key to inhibit $DPPH^{\cdot}$, SK-OV-3 and HeLa. In regard with the OCF_3 and OCH_3 substitutions, all derivatives bearing them showed tremendous potential against both $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ as well as against SK-OV-3 and HT-29. Double OCH_3 substitution was beneficial to inhibit both free radicals as well as SK-OV-3 and HT-29, whereas, single OCH_3 carrying analogues showed higher efficacies against HeLa and A-549. Lowest IC50s observed in case of unsubstituted derivatives from both the set suggested that placing a suitable functional group would worth the research attempt against free radicals and cancer cell lines, but higher CC_{50s} observed in case of these unsubstituted analogues denotes further structural optimizations in the future studies. Thus, the results suggested that there was an equal importance of both features, as a structure as well as placement of functional group(s) to gain enhanced and anticipated potencies.

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Conflict of interest

The authors report no conflicts of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.03.002.

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Pluronics®-Cyclodextrin Inclusion Complex for Targeted Drug Delivery System

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Abstract

'Host–Guest' inclusion complexes have in Pluronics® and Cyclodextrins (CDs) have an enormous research attraction as drug vehicles. In addition, CDs in Pluronics® solutions has captured much attention for the researchers to develop supramolecular biomaterials by improving the solubility and stability of drugs in aqueous and gel medium by Pluronics®-CDs inclusion complexation.

Keywords: *Supramolecular, Pluronics®, Cyclodextrins (CDs), Inclusion complex.*

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INTRODUCTION

Pluronics® or Poloxamer are the trade names of Block copolymer based on poly (ethyleneoxide)-poly (propyleneoxide)-poly (ethyleneoxide) (PEO–PPO-PEO). Blocks or chains are covalently linked together (Figure 1a). As the two blocks PEO and PPO in Pluronics® are incompatible with one another, they undergo phase segregation, thereby forming a wide range of nanostructure assemblies with intricate morphologies. In addition to the typical and preliminary spherical micelles in Pluronics®, vesicles have grabbed significant attention due to their hollow structures in drug delivery applications. However, such self-assemblies rely on the co-operation of van der Waals, electrostatic, hydrogen bonding, and hydrophobic interactions. [1, 2]

These amphiphilic Pluronics® aggregate to form micelles at the critical micelle concentration (CMC) in an aqueous solution where the hydrophobic chains tend to collapse, while the hydrophilic chain show affinity towards solvent and gets solvated. Here at a very low concentrations, the block copolymers exist as individual micelles or unimers. When the CMC exceeds, these unimers undergo aggregation to form micelles where the insoluble core-domain gets surrounded by a soluble "corona" or "shell" better known as spherical "core-shell" micelle. This system exhibits a sol-gel transition below or close to the physiological temperature which may be due to the 3-dimensional packing of the micelles due to the hydrophilic-hydrophobic balance (HLB), increasing micellar packaging during gelation. However, this balance can be tuned by incorporating the side chains with hydrophilic/hydrophobic segments.

Amongst several surface-active block polymers known, Pluronics® F-127 is used widely as food additives, drug delivery carriers, in pharmaceuticals, tissue engineering processes, etc. because it is non-toxic and has been FDA and EPA approved (average molecular weight of 11500 g/mol). Reverse thermal gelation is one of the characteristics of this compound. 20-25% F-127 gels are fluid at refrigerator temperature (4-5°C) but are highly viscous gels at room temperature. These aqueous clear gels appear to have a potential as topical drug delivery system. [3, 4]

Cyclodextrins (CDs) are cyclic oligosaccharides consisting of six, seven, or eight glucopyranose units (α, β, and γ-CDs) linked by 1, 4-R-glycosidic bonds (Figure 2) and are mostly used for "host-guest" interactions in particular class of materials.

CDs have been extensively used as drug delivery carriers through host–guest interactions, due to their merits in increasing drug solubility [5, 6], controlling drug release profiles [2], alleviating systemic toxicity [7],

and improving the permeability of biological barriers [8]. PEO has been widely applied in biomedical fields due to its solubility in water and its biocompatibility [9, 10]. Among PEO chains, those with low molecular weights exhibit its excellent chain flexibility, while those with high molecular weights possess high mechanical strength and toughness. Moreover, the hydrogels of PEO are also extensively researched for their promising potential as drug delivery vehicles [11]. Recently, CD/PEO-based poly(pseudo)rotaxanes (PRTx), whose inclusion complexes are composed of multiple CD rings that were threaded onto a polymer chain with (or without) bulky end-caps, vesicles, or supramolecular hydrogels, have led to an exciting interesting development in the biomaterials field. While some of the PEO-based polymers that consist solely of a PEO chain, many of the polymers described are block copolymers.

Considering the terminal hydrolysis–triggered drug incorporation into the CDs cavity, they have hydrophobic cavity with toroidal shape which can easily make the inclusion complexes through non-covalent interactions with a hydrophobic drug molecular as a guest that can fit into their cavity or could be threaded onto long polymeric chains, forming so-called "(*pseudo*)-polyrotaxanes (PRTx)". The outer rims are hydrophilic, and the inside cavities are hydrophobic that render CDs to form stable inclusion complexes in presence of organic additives (drugs, pigments, flavours, and vitamins). This CDs have been extensively used due to their high improving and enhancing drug solubility and controlling drug release profiles. [5, 6] CDs based PRTx derivatives are well reported where the polymeric cores could be polyesters, polyamides, *poly* (ethylene glycol) (PEG), *poly* (propylene glycol) (PPG), and many di- and triblock copolymers (Figure 3). Studies have shown the hydrophobic interaction between these polymers and CDs cavities, hydrogen-bonding among the rims of the neighbouring CDs on the same polymer chain as the driving forces resulting in various nano-structures self-assemblies like growing micelle, hollow sphere, rod-like and platelet structures. [6-8]

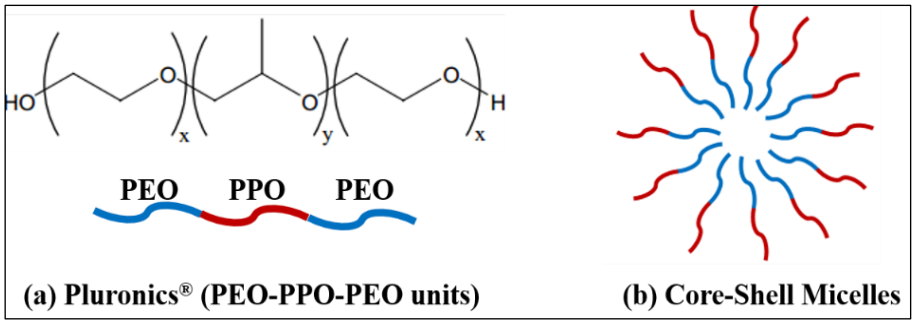

Fig. 1: *(a) Chemical Structure and (b) Micellar structure of Pluronics®.*

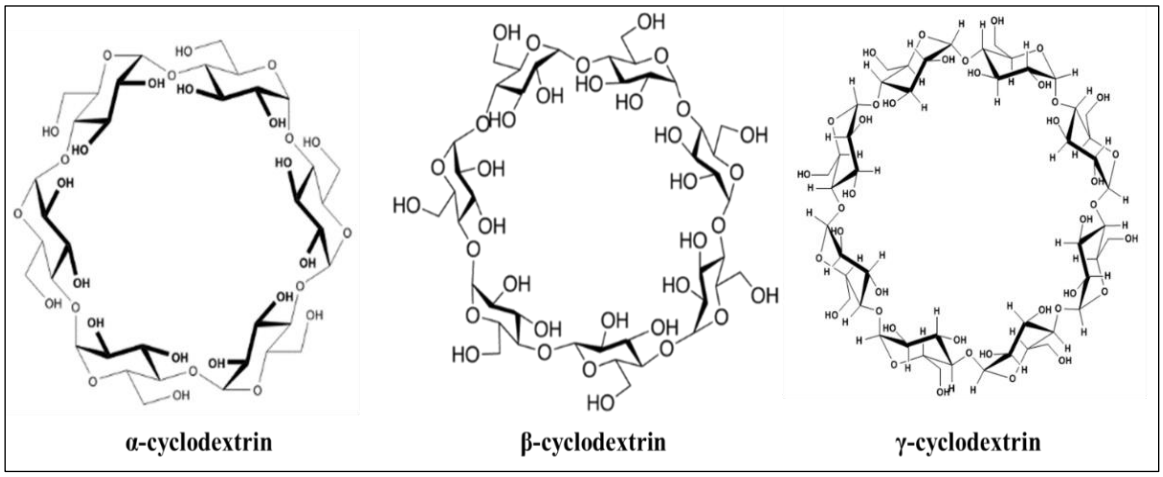

Fig. 2: *Chemical Structure of CDs.*

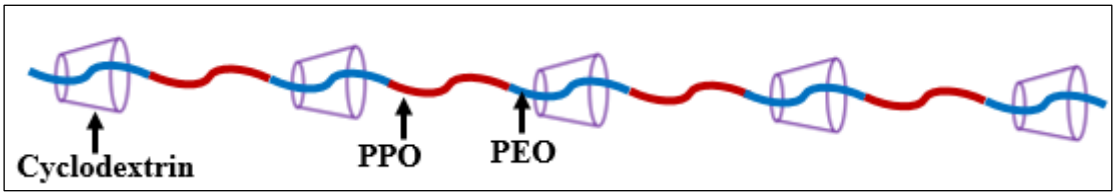

Fig. 3: *Supramolecular assemblies of Pluronics®-CDs inclusion complexation.*

Pharmacological activity of a drug is the result of affinity and interaction with the biological target, but also of optimum exposure at the target site. It is therefore necessary for the drug to reach the site of action following administration (i.e., oral, intravenous, transdermal, etc.) at sufficient concentration, avoiding nonspecific uptake and rapid clearance from the blood stream. Cyclodextrin (CD)-based drug carriers may be a versatile approach for materializing these aims. Among the many strategies explored to enhance drug solubility and stability and regulate release rate, and thus bioavailability, formation of inclusion complexes with CDs has been extensively applied. In fact, the use of CDs as individualized entities already has a long history in pharmacy. More recently, the design of novel systems in which the CDs act cooperatively to further exploit the host-guest interactions, mimicking the relatively weak but redundant interactions of molecular recognition in Nature, is opening an unexpectedly wide range of advanced applications.

Moreover, the supramolecular hosts have attracted much attention because they provide the precursors for novel biomedical materials and can use as drug delivery vehicles. Recent studies have shown a great importance of Pluronic®-CDs based PRTx as they are attracted architectures of micelles widely applicable in the biomaterials field. Such evolution of the fine structures of self-assembled PRTx in Pluronic® solutions containing dilute to highly concentrated *β*-CDs was first illustrated by Kuo Chih Shih *et al.* [6]

The ability of Pluronic® F127 to form supramolecular gels in the presence of α-CD has been explored as a way to design syringeable gel formulations able to sustain drug release of vancomycin by Tan *et al.* [7] Li *et al.* formed thermo-sensitive supramolecular hydrogels with high mechanical strength, viscous gel precursors block-selected inclusion complexation between β-cyclodextrin (β-CD) and Pluronic F68/poly (ε-caprolactone) block copolymer terminated with acryloyl groups in aqueous media, and subsequently in situ photo-crosslinking was employed in the presence of a photo-initiator. [8] Wanga *et al.* designed the inclusion complex of CD-based supramolecular Nano systems with detailed in vitro and in vivo characterization of the special focus on the advances materialized in the last five years, including clinical trials. [9] Yadav *et al.* the inclusion between carboxylated cyclodextrin (MAH-CD) and block polymer F127 forms a column-like structure, which may be close-packed to produce a crystal flake. With the help of anchoring cyclodextrin through the carboxyl group on Ag-Fe_3O_4, a flower-like gel morphology could be built by the directionally arranged flakes. Without carboxyl, however, the inclusions with normal β-CD could only result in a gel with a bulk solid morphology. [2, 3] Christopher J. Collins *et al.* used Pluronics® based *β*-CDs PRTx for treatment of Niemann-Pick Type C (NPC) disease. [11]

CONCLUSION

However, the hydrogels of Pluronics® and Cyclodextrins (CDs) are widely studied because of their outstanding characteristics in biological drug delivery systems and these biomaterials based on CD and Pluronics® are used for controlled drug delivery systems. The inclusion of CDs into these supramolecular structures can optimize their performance as drug carriers, such as their biocompatibility, flexibility, and stability. The already long experience in the use of individualized CDs as components of medicines is undoubtedly paving the way to the clinical trials of some developed prototypes, which have already provided results that clearly demonstrate the advantages they may offer in therapeutics. Nevertheless, deeper insight into efficacy but also safety in human beings is still required, knowledge about stability of the

supramolecular entities after administration through a systemic route, performance regarding drug targeting and release, and subsequent clearance is mandatory for the correct design and further optimization of the CD-based carriers. Further exploit the host-guest interactions, mimicking the relatively weak but redundant interactions of molecular recognition in Nature is opening an unexpectedly wide range of advanced applications.

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Original article

Investigation of anticancer potencies of newly generated Schiff base imidazolylphenylheterocyclic-2-ylmethylenethiazole-2-amines

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ABSTRACT

A new series of multi-heterocyclic Schiff base was constructed starting from 4′-(imidazol-1-yl)-acetophenone which was converted to its 2-bromoethanone precursor which on cyclic condensation with thiourea yielded final thiazol-2-amine intermediate (**3**) to be reacted with substituted aldehydes to generate final imidazolylphenylheterocyclic-2-ylmethylenethiazole-2-amines (**4a–4i**). New Schiff base was investigated for their *in vitro* cytotoxic efficacies against a panel of three human cancer cell lines namely, MCF7 (human breast cancer), HCT116 (human colon cancer), and DU145 (human prostate cancer) and one normal skin fibroblast (SF). Most of these synthetic derivatives shown important cytotoxic actions against individual carcinoma cell line collections, but weak actions against SF, which is as anticipated. Observations of SAR suggested that the difference in the characteristics of substituents attached to the Schiff base function leads to the interesting variations within pharmacological effects of resultant molecular systems. Structural analysis performed using FT-IR, ^{1}H NMR, ^{13}C NMR spectroscopy and CHN analysis for final potent anticancer Schiff base, which warrant further investigations.

1. Introduction

Schiff bases, named after Hugo Schiff and their very first formation was accomplished in the nineteenth millennium [1]. They are known as azomethine or imine as nitrogen analogue an aldehyde or ketone structurally, where imine or azomethine group found to replace the carbonyl group [2]. Schiff base is very widely used and the most appreciated organic building blocks to have a diverse range of pharmacological importance as well as a versatile tool to explore in many other fields as biological, inorganic and analytical chemistry. They hold a spectrum of biological importance as antioxidant, anthelmintic, antitubercular, anti-inflammatory, anticancer, antimicrobial, anticonvulsant and so forth [3]. The active centers of cell constituents are supposed to get interacted with azomethine's nitrogen atom *via* forming a hydrogen bond which interferes with normal cell processes [4] and results in the destruction of enzymatic activity of cancerous cells, thereby presents Schiff base as a potential target to discover anticancer chemotherapeutics. Hence, in the current research, we were directed to construct Schiff base derivatives involving the presence of three different types of heterocycles as imidazole, thiazole, and other heteroaromatics. Imidazole ring, highly polar heterocycle, is an important five-membered aromatic heterocycle widely present in natural products and synthetic molecules. It readily binds with different targeted enzymes in a biological system *via* ion–dipole, coordination, van der Waals forces, π–π stacking, hydrophobic effects, cation–π, and so on, because of its unique electronrich characteristic which establishes diverse weak interactions, thereby exhibiting broad bioactivities [5]. Hence, we have selected imidazole as a starting heterocyclic core to build the desire Schiff bases with intermediate thiazole entity which represents a versatile tool to generate anticancer chemotherapeutics [6]. Finally, different N, S and O-based heterocycles were

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attached to the Schiff base function because, such heterocyclic compounds have a considerable active role as antibacterial [7,8], anti-viral [9], anti-fungal [10], anti-inflammatory [11], and anti-tumor drugs [12–14].

Heterocycles represent a common architectural device of most promoted drugs and hence, we effort here to produce multi-heterocyclic systems to be examined for their *in vitro* anticancer results, because cancer provides being a major health issue in developed along with developing countries. With >100 types of cancer exist, 8.2 million people die each year from cancer, an estimated 13% of all deaths worldwide and 70% the increase in new cases of cancer expected over the next 2 decades [15]. In the attempts to distinguish a variety of chemical substances that may provide as leads for creating novel anticancer agents, nitrogen- and sulfur- that contains heterocycles are of specific attention [16–18] and the fact prompted us to generate anticancer multi-hetero-aromatic systems as imidazolylphenylheterocyclic-2-ylmethylene-thiazole-2-amines. Recently, we have observed many structures those represent similar features as ours with Schiff bases function linked to 2-aminothiazole nucleus presenting interesting pharmacological effects [19–22]. Hence, we attempt here to generate Schiff base derivatives holding all three other building block as diverse heterocycles which anticipated delivering significant anticancer effects.

2. Results and discussion

2.1. Chemistry

Scheme 1 represents the chemical steps followed to obtain desired Schiff base **4a–4i**. 4′-(Imidazol-1-yl)acetophenone was selected as a starting material to be treated with aqueous HBr with glacial acetic acid as well as bromine to give the first intermediate derivative 1-(4-(1*H*-imidazol-1-yl)phenyl)-2-bromoethanone (**2**). Intermediate **2** was subsequently reacted with thiourea in the presence base to generate final thiazole-2-amine intermediate **3**. FT-IR spectra of intermediate **2** revealed aromatic stretching at 2976 cm^{-1}, carbonyl group characteristic peak at 1697 cm^{-1} as well as C-Br peak at 691 cm^{-1} which ensure its correct formation. In addition, FT-TR solid state spectrum obtained for intermediate **3** revealed NH_2 characteristic peak at 3438 cm^{-1} and thiazole ring peaks were observed as 1523 for C=N as well as 691 as C—S—C linkage. ^1H NMR spectra for intermediate **2** revealed peak for $COCH_2Br$ linkage as a singlet 4.80 ppm, whereas, a peak at 7.71 ppm was assigned to thiazole-H and such data confirmed the correct formation of both intermediates. In further steps, intermediate **3** was reacted with various desired heterocyclic aldehyde entities (**a–i**) in the presence of organocatalyst piperidine with molecular sieve (4A) to furnish final thiazolyl Schiff bases **4a–4i** [24]. All FT-IR and ^1H NMR data were in accurate accordance with the proposed structural features of the final compounds and ^1H NMR for compound **4a** as a representative analogue; N=CH peak was observed at 8.91 ppm which apparently confirms the accurate synthesis. Mass spectral data were presented as M+1 molecular ion peaks and which were also in accordance with the molecular weights for the **4a–4i** and elemental analysis data were in a considerable range acceptable to confirm the correct formation of anticipated structures.

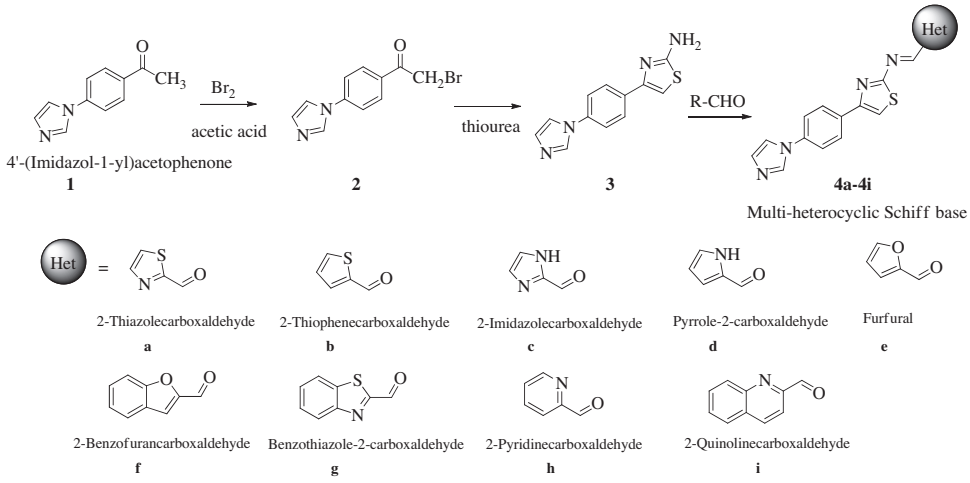
Table 1
Results of anticancer activity investigation for final Schiff base **4a–4i**.

Entry	MTT assay (IC_{50} μmol/L ± SD)[a]			
	MCF7[b]	HCT116[c]	DU145[d]	SF[e]
4a	38.28 ± 2.03	72.10 ± 0.92	40.32 ± 2.80	>50
4b	54.90 ± 3.24	58.99 ± 2.04	49.87 ± 1.82	>50
4c	38.32 ± 1.89	46.56 ± 2.37	64.23 ± 1.78	>50
4d	67.34 ± 1.85	42.76 ± 3.21	70.12 ± 2.08	>50
4e	96.45 ± 3.56	82.96 ± 1.22	50.09 ± 0.41	>50
4f	86.67 ± 2.67	93.21 ± 2.07	27.54 ± 2.76	>50
4g	27.76 ± 2.14	82.45 ± 2.31	25.78 ± 1.65	>50
4h	56.98 ± 0.87	44.45 ± 1.09	57.23 ± 3.01	>50
4i	61.26 ± 2.19	36.34 ± 2.57	26.90 ± 0.84	>50
Doxorubicin	11.12 ± 0.56	5.02 ± 0.34	4.68 ± 0.50	>50

[a] Each value is the mean of three independent experiments.
[b] Human breast cancer.
[c] Human colon cancer.
[d] Human prostate cancer.
[e] Normal skin fibroblast cell.

2.2. Pharmacology

The newly yielded multi-heterocomponent Schiff base **4a–4i** were subjected to the screening of their *in vitro* anticancer potencies against a panel of three cancerous cell lines as MCF7 (human breast cancer), HCT116 (human colon cancer), and DU145 (human prostate cancer) as well as one non-cancer normal skin fibroblast (SF) to inspect the cytotoxic character of the said molecules toward the healthy cell line. The results of anticancer screening are presented in Table 1 in terms of IC_{50}s in μmol/L, and the bioassay data suggested that these new Schiff base displayed a variable degree of anticancer activity with a broad scope of SAR



Scheme 1. Schematic presentation for the synthesis of imidazolylphenyl-heterocyclic-2-ylmethylene-thiazole-2-amines (**4a–4i**).

studies leading to a tool which can facilitate further rationale in drug designing. It was noticed that primary imidazole and intermediate thiazole rings were having an essential role to provide promising cancerous cell inhibitory effects, whereas, varying the type of coupled heterocyclic entity to the Schiff base function has a key role in determining the final anticancer potency of resultant molecule.

A molecule with a benzothiazole entity (**4g**) connected to the azomethine function showed the highest anticancer activity against MCF-7 cell line with IC_{50} of 27.76 ± 2.14 μmol/L which can be comparable to that of control drug doxorubicin with 11.12 ± 0.56 μmol/L of IC_{50}. Moreover, the presence of two heteroatoms as S and N was found beneficial to gain activity against MCF-7 as compounds **4a** with thiazole ring and **4c** with imidazole moiety presented 38.28 ± 2.03 μmol/L and 38.32 ± 1.89 μmol/L of IC_{50}s, respectively. All these three derivatives indicated low cytotoxic character toward normal skin fibroblast cell with >50 μmol/L of IC_{50}. It will be suffice to mention here that S heteroatom has a good role to play for the anticancer potency against MCF-7 because a compound with S-heterocyclic entity in terms of thiophene (**4b**) demonstrated good anticancer action with 54.90 ± 3.24 μmol/L of IC_{50}, whereas, compounds (**4e** and **4f**) bearing oxygenated heterocycles showed poor anticancer potencies with IC_{50}s >85 μmol/L. Finally, molecules holding single N atom as **4d**, **4h** and **4i** appeared to have moderate anticancer effects with IC_{50}s, 67.34 ± 1.85 μmol/L, 56.98 ± 0.87 μmol/L and 61.26 ± 2.19 μmol/L, respectively. Furthermore, quinolone ring (**4i**) had the most influence on HCT116 cell line with 36.34 ± 2.57 μmol/L of the IC_{50} level and >50 μmol/L of IC_{50} against non-cancer skin fibroblast cell being the most active analogue of the series against the mentioned cancerous cell line. Similar to the activity results observed against MCF-7, nitrogen-based heterocycles were found remarkably active against HCT116 cell line as compounds **4c**, **4d**, and **4h** with imidazole, pyrrole and pyridine rings demonstrated 46.56 ± 2.37 μmol/L, 42.76 ± 3.21 μmol/L and 44.45 ± 1.09 μmol/L, respectively, however their cytotoxicity towards SF was observed beyond 50 μmol/L, thus suggesting these *N*-heterocyclic candidates as potentially active anticancer agent against human colon cancer. The above data suggested that heterocycle with single *N* atom were active against human colon cancer than those carrying two *N* atoms. In fact, the presence of an additional heteroatom in the form of S lead to the reduced activity of the molecule as **4g** and **4a** showed >70 μmol/L of IC_{50}s against HCT116 cell line, respectively. However, molecules bearing only a single sulfur atom as compound **4b** observed to have moderate anticancer activity against HCT116 cell line with IC_{50} of 58.99 ± 2.04 μmol/L. Lastly, with O-based heterocycles, similar to activity recorded against MCF-7, compounds **4e** and **4f** were found least sensitive against HCT116 cell line with >80 μmol/L of IC_{50}s. Presence of an additional aromatic ring along with a heterocyclic core in terms of benzofuran (**4f**), benzothiazole (**4g**) and quinolone (**4i**) was a key to have successive anticancer action against DU-145 prostate cancer cell line with IC_{50}s, 27.54 ± 2.76 μmol/L, 25.78 ± 1.65 μmol/L and 26.90 ± 0.84 μmol/L, respectively and it was found that heterocyclic with two different heteroatoms (**4g**) was most active. Similarly, further data recorded against DU-145 cell line proved that S has an excellent role to play as molecules with thiazole (**4a**) and thiophene (**4b**) entities showed 40.32 ± 2.80 μmol/L and 49.87 ± 1.82 μmol/L of IC_{50}s, respectively; and >50 of IC_{50}s toward SF cells presenting themselves as lead anticancer candidates against prostate cancer. However, with an exception to the activity data for MCF-7 and HCT116, a compound with O heteroatom in the form of furan (**4e**) was as active as that with thiophene ring towards DU-145 cell line with 50.09 ± 0.41 μmol/L of IC_{50}. Finally, a derivative with a pyridine (**4h**) ring has good action than imidazole (**4c**: IC_{50}, 64.23 ± 1.78 μmol/L) and pyrrole (**4d**: 70.12 ± 2.08 μmol/L) with 57.23 ± 3.01 of IC_{50} against DU-145 cell line. Overall, many of the final Schiff base derivatives showed <50 μmol/L of IC_{50}s against all three cancerous cell lines and >50 μmol/L of IC_{50}s against non-cancer cells which present them as leading candidates for anticancer drug discovery.

3. Conclusion

To conclude, a successful attempt has been made to furnish a new series holding multi-hetero component resides with tremendous anticancer action against three different cancerous cell lines. Imidazole and thiazole core were kept constant in all final molecules to get a regular anticancer data with a variable degree of efficacy observed while varying heterocyclic moieties connected to the Schiff base link. The correct formation of the desired molecules was ensured utilizing different analytical techniques followed by the inspection of their *in vitro* anticancer effects, in which, all compounds presented <100 μmol/L of IC_{50s}. Core imidazole liked to the thiazole ring was very beneficial, as final results also displayed anticipated potencies with thiazole core lined to the Schiff base function. The presence of particular heteroatom, as well as presence of an additional benzene ring, had a significant effect against MCF-7 and DU-145, and heterocycles with S atoms were more active, whereas, against HCT116, *N*-heterocycles played a major role. Overall, **4a**, **4g,** and **4i** showed tremendous activity against all cancerous cell lines. In addition, all compounds presented >50 μmol/L of IC_{50s} against non-cancer normal skin fibroblast (SF) cells which indicated tolerable cytotoxic nature of these Schiff bases toward healthy cells. As some substituent were found inactive, for example, those with O heteroatom, more studies are obviously warranted to replace basic core from imidazole and thiazole to other heteroatom-containing rings to examine their overall influence on anticancer effects.

4. Experimental

Reichert Thermover instrument was utilized to record melting points of the final compounds which were uncorrected. FT-IR spectra were recorded using KBr on Perkin Elmer RXI spectrometer. Proton and carbon NMR data were recorded on Bruker AVANCE III 400 instrument spectrometer (^{1}H NMR, 400 MHz; ^{13}C NMR, 100 MHz) in the presence of TMS internal standard and with DMSO. Mass spectra have been registered on JEOL-Accu TOF JMS-T100LC DART-MS spectrometer. Microanalytical data were collected using Carlo Erba analyzer model 1108. TLC plates were used to monitor the reaction as well as to identify the purity of the prepared products.

*4.1. Synthetic procedure for 1-(4-(1H-imidazol-1-yl)phenyl)-2-bromoethanone **(2)***

In a flask charged with 4′-(imidazol-1-yl)acetophenone (0.01 mol) with glacial acetic acid (20 mL) and aqueous hydrobromic acid (HBr) [48% (w/w)]. The resulting mixture was immersed in an ice–salt mixture (0–5 °C), and bromine (0.01 mol) was added, and temperature of the reaction mixture does not exceed 5 °C. After completion of addition, it was stirred at room temperature for 2–3 h. The progress of the reaction was monitored by using TLC. After the completion of the reaction, the suspension was poured onto crushed ice. The colorless precipitate was filtered, repeatedly washed with water and dried at room temperature. Yield: 55%–57%, m.p: 147–149 °C; FT-IR (KBr) cm^{-1}: 2976 (Ar-stretching), 1697 (C=O), 691 (C—Br); ^{1}H NMR (400 MHz, DMSO–d_6): δ 8.38 (s, 1H), 8.13 (d, 1H, J = 7.6 Hz,), 8.02 – 7.96 (m, 2H), 7.94 – 7.87 (m, 2H), 7.84 (d, 1H, J = 7.5 Hz,), 4.80 (s, 2H). ^{13}C NMR

(100 MHz, DMSO–d_6): δ 181.34, 150.12, 148.34, 137.6, 134.2, 132.7, 129.9, 125.4, 123.7, 122.5, 34.23.

4.2. Synthetic procedure for 4-(4-(1H-imidazol-1-yl)phenyl)thiazol-2-amine (**3**)

In a flask charged with compound 2 (0.01 mol), thiourea (0.01 mol) and triethylamine (0.01 mol) and the reaction mixture was refluxed for 10–12 h in 30 mL acetonitrile until the complete consumption of starting material as detected by TLC. The reaction mixture was then cooled, poured onto ice. The precipitate was filtered, dried and recrystallised from ethanol. Yield: 45%–53%, m. p: 160–163 °C; FT-IR (KBr) cm^{-1}: 3438 (NH_2), 1577 (aromatic C=C stretch), 1523 (C=N) thiazole, 1154 (C—N), 691 (C—S—C) thiazole; ^{1}H NMR (400 MHz, DMSO–d_6): δ 5.08 (s, 1H, NH_2), 8.13 (d, 1H, J = 7.5 Hz,), 7.98–7.91 (m, 2H), 7.84 (d, 1H, J = 7.5 Hz,), 7.71 (s, 1H), 7.61 (s, 2H), 7.48–7.42 (m, 2H). ^{13}C NMR (100 MHz, DMSO–d_6): δ 167.52, 153.71, 147.89, 146.21, 138.7, 134.6, 131.8, 128.7, 125.6, 124.6, 122.8, 121.9. Anal Calcd. for $C_{22}H_{22}N_2O_3$: C, 72.91; H, 6.12; N, 7.73; Found: C, 72.76; H, 6.29; N, 7.62.

4.3. General method for the preparation of imidazolylphenyl-heterocyclic-2-ylmethylene-thiazole-2-amines (**4a–4i**)

A mixture of substituted compound 3 (0.01 mol), substituted heterocyclic aldehyde (0.01 mol) and piperidine (0.01 mol) was refluxed in 30 mL ethanol for 6–8 h. The reaction mixture was poured into an ice–water mixture to get a product, which was filtered, dried, and recrystallized using ethanol to give the final compounds **4a–4i**, for example, 4-(4-(1*H*-imidazol-1-yl)phenyl)-*N*-(thiazol-2-ylmethylene)thiazol-2-amine (**4a**): Yield 65%. m.p. 213–214 °C; FT-IR (KBr) cm^{-1}: 3067 (aromatic C—H stretch), 1608 (N=CH stretch, azomethine), 1572 (aromatic C=C stretch), 1509 (C=N) thiazole, 1147 (C—N), 685 (C—S—C) thiazole; ^{1}H NMR (400 MHz, DMSO–d_6): δ 8.91 (s, 1H, N=CH); 8.38 (s, 1H), 8.13 (d, 1H, J = 7.5 Hz), 8.05 (d, 1H, J = 7.5 Hz), 7.99–7.92 (m, 2H), 7.84 (d, 1H, J = 7.5 Hz), 7.75 (s, 1H), 7.55 (d, 1H, J = 7.5 Hz), 7.49–7.43 (m, 2H). ^{13}C NMR (100 MHz, DMSO–d_6): δ 164.71, 164.03, 152.22, 147.72, 146.81, 144.11, 135.26, 131.25, 130.19, 125.67, 123.88, 122.45, 121.37, 118.30, 113.17, 112.61. EI-MS m/z (M^+): 338.74; Anal Calcd. for $C_{16}H_{11}N_5S_2$: C, 56.95; H, 3.29; N, 20.76; Found: C, 56.88; H, 3.35; N, 20.64.

4.4. In vitro evaluation of anticancer activity

Human cancer cell lines namely, MCF7 (human breast cancer), HCT116 (human colon cancer), and DU145 (human prostate cancer) and one normal skin fibroblast (SF), MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide], DMEM, trypsin–EDTA, RPMI and 96-well flat bottom tissue culture plates were obtained from the commercial suppliers. Cancerous were grown in DMEM medium, and SF cells were grown in RPMI medium supplemented with 10% fetal bovine serum, 200 lg/mL streptomycin, 100 l g/mL penicillin, 2 mmol/L L-glutamine, and culture was maintained with 5% CO_2. A stock solution of for compounds to be tested was prepared in DMSO with sterile PBS to get required concentration. A standard MTT colorimetric assay was performed for investigating the anticancer potential of **4a–4i** because MTT is a photosensitive compound and is consumed by living organisms and a mitochondrial dehydrogenase enzyme performs its reduction to a purple formazan product that is impermeable to the cell membrane. DMSO solubilization of test compounds yields liberation and resulting formazan product with purple color is directly related to the cell viability. Cells placed in 96-well plates were incubated with test compounds and a controlled drug (doxorubicin) with variable dilutions at 37 °C for 24 h in DMEM medium carrying 10% FBS medium. After that, 90 μL of fresh serum free media and 10 μL of MTT reagent (5 mg/mL) was placed in the place of old media followed by the incubation at 37 °C for 4 h and lastly, this media was changed to 200 μL of DMSO followed by last incubation at 37 °C for 10 min. The absorbance at 570 nm was calculated on a spectrophotometer (Spectra Max, Molecular devices) IC_{50} principles were identified from plot: % cell viability (from control) *versus* concentration [23].

Conflicts of interest

The authors report no conflicts of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cclet.2016.10.021.

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Surface-Active Properties and Antimicrobial Study of Conventional Cationic and Synthesized Symmetrical Gemini Surfactants

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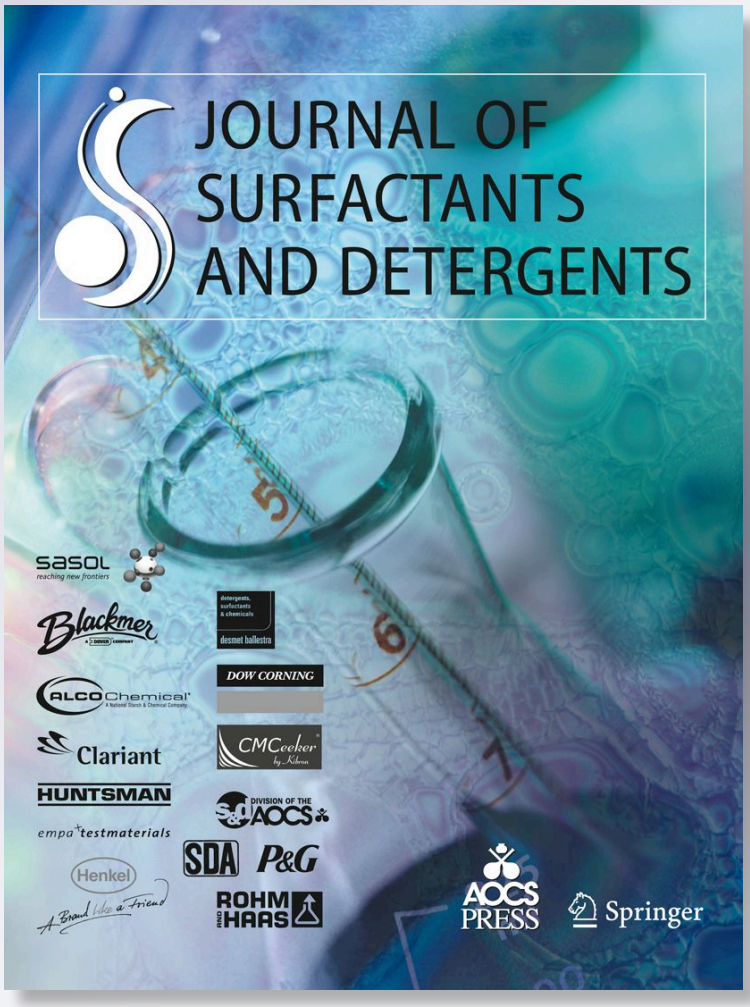

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ORIGINAL ARTICLE

Surface-Active Properties and Antimicrobial Study of Conventional Cationic and Synthesized Symmetrical Gemini Surfactants

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Abstract Symmetrical gemini surfactants of cationic series α,ω-alkanediyl bis (dimethyl ammonium bromide) commonly referred as "*m–s–m*" have been synthesized. Spectral analysis was performed to confirm compound structures and purity. Conductivity and surface tension measurements provide better understanding of the micellization process. Their self-assembly behavior in aqueous solution is also discussed in detail. The antimicrobial efficacy was measured by bacterial and fungal growth inhibition expressed as minimal inhibitory concentration values against five strains of a representative group of microorganisms viz. *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Salmonella paratyphi B* and *Aspergillus niger*. All of the synthesized surfactants showed antimicrobial activity against them, but at different levels depending on their structures. The surfactants possessing longer alkyl chains (more hydrophobic environment) demonstrated better antimicrobial functionality. The antimicrobial potency was found to be dependent on the representative target microorganism (Gram-positive bacteria > fungi > Gram-negative bacteria), as well as on the ionic nature of the surfactant (cationic), alkyl chain length (m = 12, 16) and spacer length (s = 2, 4, 6) of the synthesized compounds. Gemini surfactants such as 12-2-12 and 12-4-12 were found to be weakly active whereas 16-2-16 and 16-4-16 compounds proved to be the most potent antimicrobial surface-active agents among the synthesized gemini homologues.

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Keywords Gemini surfactants · Surface tension · CMC · Antimicrobial activity

Introduction

Conventional quaternized ammonium surfactants (QAS) normally contain one hydrophilic group and one hydrophobic chain. Due to their ability to self-assemble in aqueous solution they exhibit superior micellar morphologies such as spherical micelles, vesicles, lamellar structures, etc. and attract a keen research interest [1, 2]. Such aggregates once formed create sharp polarity gradients at the interface and define clear hydrophobic regions. In recent years, interest has emerged for a new class of amphiphilic molecules with novel structures from which new aggregates and physical properties may be expected. One of these amphiphilic classes is the "gemini" or "dimeric surfactant", which has two hydrophobic and hydrophilic groups per molecule (m), separated by a covalently bonded spacer (s). A Spacer can be of variable length—short (with 2 methylene groups) or long (with 12 methylene groups), rigid or flexible, polar or nonpolar [3, 4].

Gemini surfactants enhance the adsorption and aggregation properties by strengthening the intra- or intermolecular hydrophobic interactions and therefore appear to be better than the corresponding single-chain conventional surfactants. They tend to have much lower critical micellar concentrations (CMC) and surface tension values than conventional surfactants at the same molar or mass concentrations [5–8]. In addition to these, an important feature of these surfactants is their ability to design and tune the physico-chemical properties, supramolecular structure, and biodegradability by altering the nature and size of the

spacer and alkyl chains [9]. Consequently they possess specific rheological, aggregation, wetting and better solubilizing properties. They are widely used as effective emulsifiers, dispersants, antifoaming agents, detergents in soil remediation, in enhanced oil recovery, in the construction of high-porosity materials and for drug entrapment and release. Thus, greater efficiency and effectiveness of gemini surfactants make them more applicable as well as more environmentally suitable [10, 11].

Amongst the various gemini surfactants known, the symmetrical cationic dimeric (bifunctional structure) surfactants of the bis quaternary ammonium type, i.e., the homologous series of bis (quaternary alkyl ammonium) surfactants given by general formula $[C_mH_{2m+1}(CH_3)_2N^+ - (CH_2)_s - N^+(CH_3)_2\ C_mH_{2m+1}]\ Br_2$ are the most investigated surfactants. Such dimeric surfactants are abbreviated as "*m*–*s*–*m*", where *m* is the number of carbon atoms in the single-surfactant chain and *s* indicate the number of carbon atoms in the spacer. Here the spacer is covalently attached with the nitrogen atoms of the individual conventional surfactant [12–15].

Amphiphilic compounds having dimeric moieties are well-known and effective antimicrobial agents. The efficacy of such agents is conditioned by the amphiphilic nature of the surfactant molecule, surface-active properties and aggregation patterns. Several contributions have reported synthetic antimicrobial agents having new active moieties such as heteroatoms, aromatic and non-aromatic cyclic substituents, and perfluorinated chains, among others. The pathogenic bacterial cell membrane is predominantly negatively charged as compared with eukaryotic cells. These synthesized gemini surfactants possess properties such as attraction for negatively charged surfaces like bacteria and facilitates their interactions with the bacterial membrane. Such quaternary ammonium salts kill microorganisms by damaging cytoplasmic membrane of cell and affect the permeability properties of cells, different from antibiotics that inhibit certain reproductive enzymes. Thus, the antimicrobial action of cationic surfactants is based on their ability to disrupt the integral bacterial membrane by a combined hydrophobic and electrostatic adsorption phenomenon at the membrane-water interface to create disorganization [16–20].

The present study offers a better understanding of the synthesized cationic gemini surfactants, their unique behavior in aqueous media and their chemical–biological relationships. Although there is lot of current interest in the synthesized dimeric micelles having flexible, hydrophobic spacer and two alkyl chains, the present communication reports the results of surface active characterization of these gemini homologues. Their antimicrobial performance and biological activity are also thoroughly discussed.

Materials and Methods

Materials

Conventional cationic surfactants viz., dodecyltrimethylammonium bromide (DTAB) and hexadecyltrimethylammonium bromide (CTAB) were purchased from Aldrich, USA. Various organic solvents viz., α,ω-dibromoalkane (α = 1, ω = 4 and 6), *N*, *N*, *N*′, *N*′ tetramethyl ethylenediamine, *N*,*N*′-dimethyldodecylamine (≥98%), *N*,*N*′-dimethylhexadecylamine (≥98%), 1-bromododecane, 1-bromotetradecane, 1-bromohexadecane, were purchased from Fluka, Switzerland and used without further purification. Milli-Q grade distilled water for (surface tension 72.8 mN m^{-1}) and D_2O (^{1}H-NMR measurements) at ambient temperature was used to prepare all the necessary solutions.

Synthesis

Scheme 1

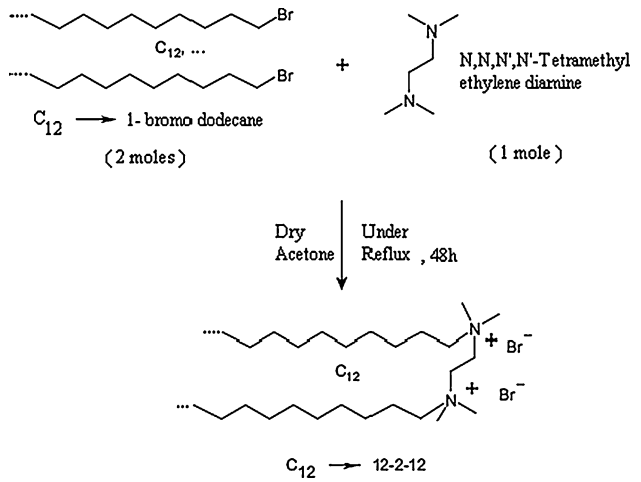
Gemini surfactants referred to as *m*–2–*m*, where *m* = 12 and 16 were synthesized as reported in the literature [12, 13, 15, 20]. Mixture of *N*,*N*,*N*′,*N*′ tetramethyl-ethylenediamine and 1-bromoalkanes in a molar ratio of 1:2 respectively in dry acetone (30 mL) was refluxed until the entire volume of diamine was consumed (approx. 48 h).

After removal of the solvent by evaporation, the crude surfactants were recrystallized twice in dry acetone. The resulting product was dissolved in the minimum volume of absolute ethanol and subsequent extractions with hexane/ethyl acetate (50/50) to eliminate the excess of alkyl bromide. A white product was finally obtained by filtration under vacuum pressure. The product was dried and stored in desiccators to prevent moisture absorption. The preparation of 12–2–12 compound is presented in Scheme 1. Similar synthesis route was followed for 16-2-16.

Scheme 2

The compounds with spacer = 4 and 6 were prepared by the action of the corresponding *N*,*N*′-dimethylhexadecylamine, respectively, on 1, 4-dibromobutane and 1, 6-dibromohexane. This mixture was refluxed for 48 h in dry acetone until the alkyl amine was totally consumed. The addition product thus formed was purified in the similar way as mentioned above.

Gemini surfactant 12-4-12 was synthesized similarly by refluxing *N*,*N*- dimethyldodecylamine with 1, 4-dibromobutane. Scheme 2 presented the synthesis route for *m*–4–*m* and *m*–6–*m* compounds and the compounds synthesized are: 12-4-12, 16-4-16 and 16-6-16.



Scheme 1 Synthesis of gemini cationic surfactant *m*-2-*m* ($m = 12$ and 16)

Media

Antibacterial and antifungal activities of the gemini surfactants were evaluated using a protocol adapted from the European [21] and ASTM norms [22]. Their antimicrobial evaluations were conducted using MIC (Minimal Inhibitory Concentration) measurements against five strains of a representative group of microorganisms: two Gram positive bacteria: *Bacillus subtilis (BS)* (ATCC 6633), *Staphylococcus aureus (SA)* (ATCC 6538), two Gram negative bacteria: *Klebsiella pneumonia (Kleb)* (ATCC 10031), *Salmonella paratyphi B (SPB)* (ATCC 19940) and one fungus: *Aspergillus niger (A. niger)* (ATCC 16404).

Nutrient agar medium was used to assess the biocidal activity. It was composed of (g L^{-1}) 5.0 peptone, 1.0 meat extract; 2.0 yeast extract; 5.0 g sodium chloride and 15.0 g agar. Similarly, molt extract agar in this medium was used in the analytical biological assay test for *A. niger*. It was composed of (g L^{-1}) 30.0 molt agar; 15.0 agar and 5.0 peptone. This is a specific medium for fungi. The pH was adjusted to 7.5 throughout.

Gradient plate technique was followed where 15.0 mL of the agar inoculum medium was poured, while hot, into an inclined Petri dish (diameter 10 cm) to from a wedge. After it had solidified, the Petri dish was replaced in a horizontal position and another 15.0 mL medium containing the tested substance was added.

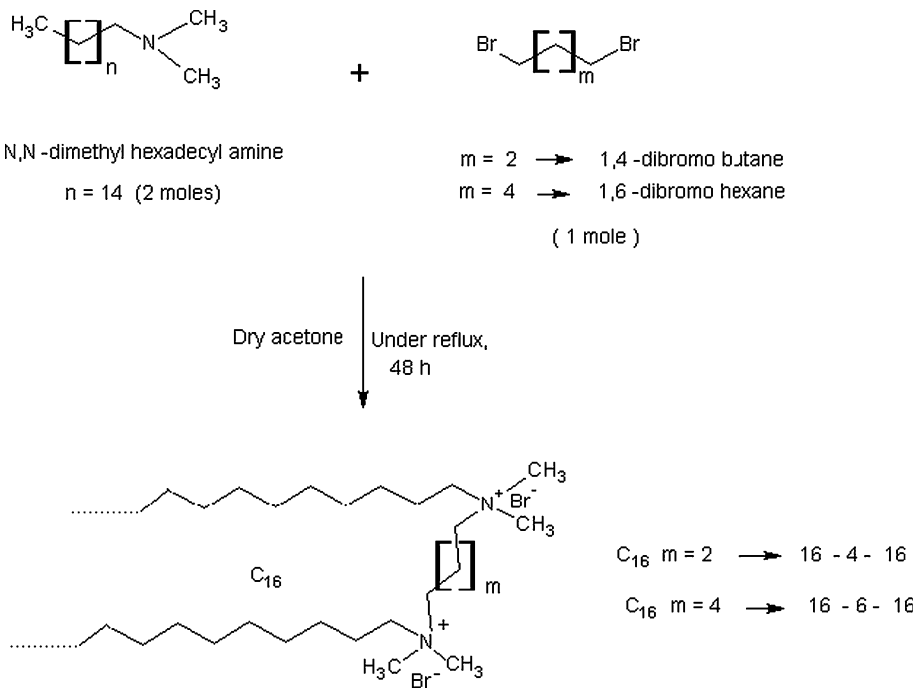
Synthesized gemini surfactants were tested at the concentration of 300 ppm in aqueous medium. A gradient of concentrations of the test materials along the diameter of the petri dish was thus obtained. Accordingly, the concentration of the substance at one end of this diameter line can be considered as maximal. The plate was then streaked with the enriched cultures along the axis of gradient and the plate was then incubated at 30 °C for 24 h [23, 24].

Methods

^{1}H NMR Analysis

^{1}H-NMR measurements were performed using BRUKER AVANCE-II with a proton frequency of 400 MHz. Spectral information for the synthesized gemini surfactants are given below:

Scheme 2 Gemini cationic surfactants of type 16-*s*-16 ($s = 4$ and 6)



bis(hexadecyldimethylammonium)ethane (16-2-16): White product, ^{1}H-NMR (400 MHz, D_20) δ 0.87 (*t*, 6H, alkyl chain 2 × 1 CH_3), 1.12-1.84 (*m*, 56H, alkyl chain 2 × 14 CH_2), 3.25 (*s*, 12H, 2 × 2 N^+ CH_2), 3.40 (*m*, 4H, alkyl chain 2 × 1 CH_2 N^+), 3.85 (*m*, 4H, spacer chain 1× $N^+CH_2CH_2$ N^+). C (62.79%), H (11.37%), N (3.85%).
bis(hexadecyldimethyl ammonium)butane (16-4-16): White product, ^{1}H-NMR (400 MHz, D_20) δ 0.88 (*t*, 6H, alkyl chain 2 × 1 CH_3), 1.25–1.98 (*m*, 56H, alkyl chain 2 × 14 CH_2), 2.20 (*m*, 4H, spacer chain 1× CH_2CH_2), 3.30 (*s*, 12H, 2 × 2 N^+ CH_2), 3.41–3.50 (*m*, 4H, alkyl chain 2 × 1 CH_2 N^+), 3.98 (*s*, 4H, spacer chain 2 × 1 N^+CH_2). C (63.22%), H (11.43%), N (3.78%).
bis(hexadecyldimethyl ammonium)hexane (16-6-16): White product, ^{1}H-NMR (400 MHz, D_20) δ 0.88 (*t*, 6H, alkyl chain 2 × 1 CH_3), 1.15–1.85 (*m*, 56H, alkyl chain 2 × 14 CH_2), 2.20 (*m*, 4H, spacer chain 1× CH_2CH_2), 3.25 (*s*, 12H, 2 × 2 N^+ CH_2), 3.40 (*m*, 4H, alkyl chain 2 × 1 CH_2 N^+), 3.85 (*m*, 4H, spacer chain 1× $N^+CH_2CH_2$ N^+). C (64.04%), H (11.53%), N (3.64%).
bis(dodecyldimethyl ammonium)ethane (12-2-12): White product, ^{1}H-NMR (400 MHz, D_20) δ 0.87 (*t*, 6H, alkyl chain 2 × 1 CH_3), 1.12–1.84 (*m*, 52H, alkyl chain 2 × 13 CH_2), 3.25 (*s*, 12H, 2 × 2 N^+ CH_2), 3.40 (*m*, 4H, alkyl chain 2 × 1 CH_2 N^+), 3.85 (*m*, 4H, spacer chain 1× $N^+CH_2CH_2$ N^+). C (58.62%), H (10.82%), N (4.56%).
bis(dodecyldimethyl ammonium)butane (12-4-12): White product, ^{1}H-NMR (400 MHz, D_20) δ 0.87 (*t*, 6H, alkyl chain 2 × 1 CH_3), 1.12–1.84 (*m*, 52H, alkyl chain 2 × 13 CH_2), 3.25 (*s*, 12H, 2 × 2 N^+ CH_2), 3.40 (*m*, 4H, alkyl chain 2 × 1 CH_2 N^+), 3.85 (*m*, 4H, spacer chain 1× $N^+CH_2CH_2$ N^+). C (60.88%), H (11.12%), N (4.18%).

Surface Tension Measurements

Surface tension (S.T.) measurements were measured using a Du Nouy tensiometer (Kruss type 8451) for various concentrations of the synthesized surfactants at 30 °C. To ensure removal of surface-active contaminants, properly cleaned and rinsed glassware was used. Samples were prepared by weighing the appropriate amount of surfactant that is dissolved in the appropriate volume of Millipore water. Sometimes a series of dilutions were made for concentrations in a narrow range. Experiments were carried out in triplicate to determine any change with time and to obtain an average value [25, 26].

Conductivity Measurements

An ESICO microprocessor-based conductivity bridge, Model 1601, and a dip-type cell made of platinum black were used for conductivity measurements. KCl solution of known concentrations was used for calibration. The conductivity measurement was done by successive addition of pure water to a concentrated surfactant solution. Temperature equilibrium was maintained after thorough mixing. About 30–40 conductivity data were measured in each set of experiment for different gemini surfactants as a function of concentration. The CMC values were calculated from the break points in the specific conductivity versus concentration (plot not shown) whereas the degree of dissociation (β) was determined as the ratio of slopes in post-micellar region to pre-micellar region. Data above and below the accent point were linearly fitted, with correlation coefficients greater than 0.998 [1, 27].

Diffusion Disc Method

A sterilized disc of filter paper saturated with a measured quantity of the sample is placed on a plate containing a solid bacterial medium of nutrient agar broth which has been heavily seeded with the spore suspension of the tested organism. After incubation, the diameter of the clear zone of inhibition surrounding the sample is taken as a measure of the inhibitory power of the sample against the particular test organism [17, 23, 24, 28].

Minimum Inhibitory Concentration Method

The lowest concentration of compounds inhibiting the development of visible growth after 24 h of incubation at 37 °C is expressed as Minimum Inhibitory Concentration (MIC). It is a measure of bacteriostatic activity and provides some idea of the effectiveness of a chemotherapeutic agent against microorganisms [29].

The MICs of the gemini assays were carried out as described by Clause with minor modifications [30]. Antibacterial activities of the bacterial strains were carried out in Muller–Hinton broth (Difco) medium, at pH 6.9, with an inoculums of (1–2) × 10^3 cells mL^{-1} by the spectrophotometric method and an aliquot of 0.1 mL was added to each tube of the serial dilution. The MIC of the chemical compounds were recorded as the lowest concentration of each chemical compounds in the tubes with no growth (i.e. no turbidity) of inoculated bacteria.

Results and Discussion

Evaluation Method of Surface Active Properties

Various concentrations of aqueous solution of the prepared Gemini surfactants were plotted versus the corresponding

surface tension as shown in Fig. 1. Surface parameters such as C_{20}, Γ_{max}, π_{CMC} and A_{min} are reported in Table 1.

Maximum Surface Excess

The maximum surface excess (Γ_{max}) of surfactant molecules at the air/solution interface was calculated using the Gibbs equation:

$$\Gamma_{max} = \frac{1}{2.303nRT}\left(\frac{\partial\gamma}{\partial \log c}\right)_{T,P} \quad (1)$$

where Γ_{max} is the saturation adsorbed amount in mol cm^{-2}, γ is the surface tension in mN m^{-1}, R is the gas constant (8.314 $Jmol^{-1}$ K^{-1}), T is the absolute temperature, c is the surfactant concentration and $\partial\gamma/\partial \log c$ is the slope in the surface tension isotherm when the concentration is near the CMC at 30 °C.

The value of n is taken as 2 for the conventional surfactant and 3 for a dimeric surfactant (a divalent surfactant ion and two univalent counterions in the absence of a swamping electrolyte [1, 2, 26].

Effectiveness

Effectiveness (π_{CMC}) is the difference between the surface tension of the pure water (γ_o) and the surface tension of the surfactant solution (γ) at the critical micelle concentration

$$\pi_{CMC} = \gamma_o - \gamma \quad (2)$$

The most efficient surfactant is that which gives the largest reduction of the surface tension at the CMC. According to the results of effectiveness C_{16} was found to be more efficient; it achieved the maximum reduction of the surface tension at CMC.

Efficiency

Efficiency (pC_{20}) is defined as the degree of greatest lowering in surface tension for a CMC and is determined by the concentration (mol L^{-1}) of the surfactant solutions needed to suppress the surface tension by 20 dyne cm^{-1}. The values of the efficiency of the synthesized surfactants are shown in Table 1. It is obvious that efficiency of these surfactants decrease in the following order $C_{16} < C_{12}$ supporting the idea that efficiency depends mainly on the hydrophobic characters of the aliphatic chains in gemini surfactant [23].

Minimum Surface Area

The optimal cross-section surface area (A_{min}) occupied by each molecule adsorbed on the interface is given by

$$A_{min} = (N_A \times \Gamma_{max})^{-1} \times 10^{14} \quad (3)$$

where N_A is Avogadro's number and A_{min} is in nm^2. Both the Γ_{max} and A_{min} values are reported in Table 1. Results indicate that the minimum area per molecule at the aqueous solution/air interface for the prepared surfactants increases with the increase in molar ratio of methylene units (hydrophobic chain length) [13].

A comparison of CMC data amongst various gemini homologues (from Table 1) demonstrated that increasing the hydrocarbon or spacer chain length, degree of dissociation (β) increases. Also the tendency of lowering the CMC was found to increase due to the conformational changes occurring in spacer. For short spacer length $s = 2$ it was observed that due to such short distance the hydrophobic interaction enhances. Consequently the hydrophobic hydration surrounding the tail region is restricted and electrostatic repulsion between them decreases resulting in lowering of CMC. While for $s = 4$–6, the spacer chain tends to remain in an extended conformation where the spacer comes in contact with bulk water leading to increase the hydrophobic hydration and delays the micellization thereby increasing CMC. Thus due to such enhanced hydrophobic interaction the gemini surfactants with $s = 2$ facilitate better germicidal activity and proton-binding ability of QAS [11, 17].

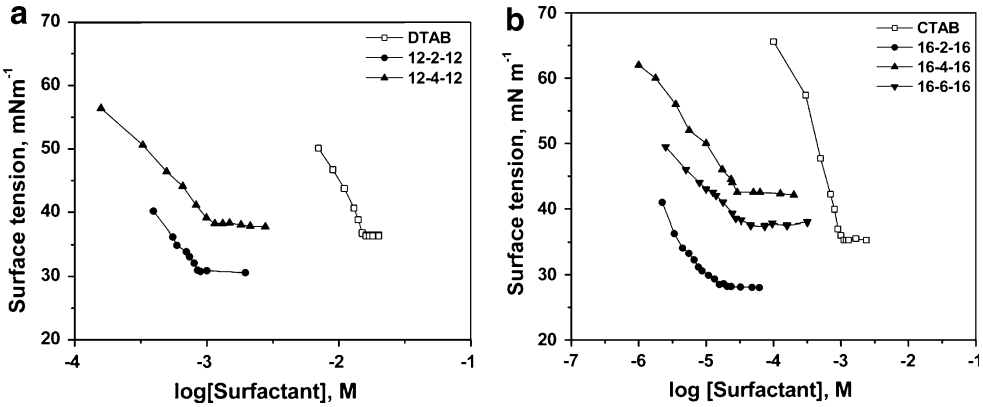


Fig. 1 Surface tension plots of (**a**) 12-*s*-12 and (**b**) 16-*s*-16 in aqueous solution at 30 °C

Table 1 Physico-chemical properties for various conventional and symmetrical Gemini surfactants in aqueous solution at temperature 30 ± 0.5 °C and pH = 7 *(Experimental conditions)*

Surfactants	CMC (mM) measurements		π_{CMC}	C_{20} mM	γ_{CMC} mN m^{-1}	pC_{20}	β	$\Gamma_{max} \times 10^{-10}$ mol cm^{-2}	A_{min} nm^2 per molecule
	S.T.	Cond.							
Conventional									
DTAB	15.1	15.4	36.4	5.25	36.4	2.28	0.22	3.77	0.44
CTAB	0.98	0.92	37.5	0.35	35.3	3.42	0.28	3.55	0.48
Gemini									
12-2-12	0.82	0.85	42.2	0.16	30.6	3.78	0.21	1.89	0.88
12-4-12	1.25	1.20	34.6	0.26	38.2	3.59	0.29	1.41	1.17
16-2-16	0.021	0.030	44.3	2.6×10^{-4}	28.5	6.58	0.33	0.78	2.14
16-4-16	0.037	0.039	29.8	5.3×10^{-3}	43.0	5.28	0.35	0.77	2.25
16-6-16	0.048	0.051	35.8	1.4×10^{-3}	37.0	5.87	0.41	0.69	2.41

Error in determining degree of counterion dissociation (β), concentration required to reduce the surface tension of the solvent by 20 mN m^{-1} (C_{20}), maximum surface excess concentration at the air/solution interface (Γ_{max}), and minimum area per surfactant molecule (A_{min}) is up to 6.2%

CMC data given in Table 1 are measured by surface tension (S.T.) and conductance (Cond.) methods. These data are in well agreement with the values reported in literature [32–35]

Similar enhanced micellar aggregation and growth was observed in 12-2-12 and 16-2-16 due to shorter spacer unit, which most likely increases the geometrical constraints in the formation of aggregates with decreasing length of the spacer unit. As a consequence, they form more dense micelles in their respective surfactant homologues than the other gemini surfactants. Such similar trend is followed by the conventional surfactants [21, 29].

Antimicrobial Test

Several lipids (*building units*) and the protein layers in bilayer fashion constitute the cell membrane of microorganisms which imparts the hydrophobic character to them. The permeability of such a lipoprotein membrane represents the main function, namely controlling the biological reactions occurring in the cell. Any factor which influences the selective permeability in the cell membrane causes great damage to the microorganisms. Cationic surfactants have a unique ability to adsorb at the interface of the cell membrane which is predominantly negatively charged. On comparing the single-chain surfactants viz., DTAB and CTAB, with corresponding geminis, the latter ones contributes more positive charge thereby facilitating more electrostatic interaction at the membrane. Such adsorption at the cell membrane interface increases the hydrophobicity and the permeability of the membrane. This causes disturbance in the biochemical reactions within the cell cytoplasm and as a consequence, the synthesized gemini surfactants exhibit better surface active properties and more efficient biocidal activity towards microorganisms than single chain conventional surfactants [18, 31].

In vitro antibacterial and antifungal activity results against pathogenic bacteria and fungi are reported in Table 2 for various microbes *Bacillus subtilis* (*BS*), *Staphylococcus aureus* (*SA*), *Klebsiella pneumonia* (*Kleb*), *Salmonella paratyphi B* (*SPB*) *and Aspergillus niger* (*A. Niger*) indicated that all the synthesized QAS possess antimicrobial activity. The difference in their biological activity (*zone of growth inhibition*) depends on the interfacial properties of the prepared gemini surfactants in their aqueous solution.

It was inferred that the compounds attributed excellent in vitro antibacterial activity against *BS* and antifungal activity against *A. niger*. A variable effect of antimicrobial activity was seen against Gram negative strains. As expected, the Gram-negative bacteria were found to be less sensitive than the Gram-positive bacteria as their outer membranes are less permeable to such amphiphilic

Table 2 Antimicrobial in vitro activity of gemini surfactants against pathogenic bacteria and fungus using the disc-diffusion method

Microorganism	Gemini surfactant				
	16-2-16	16-4-16	16-6-16	12-2-12	12-4-12
B. subtilis (ATCC 6633)	+++	+++	++	+	+
S. aureus (ATCC 6538)	++	++	+	+	+
Kleb (ATCC 10031)	++	++	+	+	+
SPB (ATCC 19940)	+	−	−	+	+
A. niger (ATCC 16404)	+++	+++	++	++	+++

Zones of growth inhibition in: −: 0–6 mm; +: 7–12 mm; ++: 13–20 mm; +++>21 mm

Table 3 Activity table showing minimum inhibitory concentration (with an error of ±0.01 mM)

Gemini compound no.	Minimum Inhibitory concentration (MIC)				
	Microorganisms				
	Antibacterial strains				Antifungal strains
	Gram positive		Gram negative		
	B. subtilis (ATCC 6633)	*S. aureus* (ATCC 6538)	*Kleb* (ATCC 10031)	*SPB* (ATCC 19940)	*A. niger* (ATCC 16404)
16-2-16	0.13	0.20	0.20	0.28	0.10
16-4-16	0.14	0.19	0.19	0.53	0.10
16-6-16	0.19	0.32	0.32	0.51	0.19
12-2-12	0.40	0.33	0.40	0.33	0.24
12-4-12	0.39	0.31	0.39	0.31	0.16
Ampicillin	0.27	0.13	0.20	0.13	–
Ciprofloxacin	0.25	0.19	0.19	0.12	–
Fluconazole	–	–	–	–	0.35

compounds. It was also observed that with an increase in alkyl chain length of the gemini surfactants, the compounds exhibit a stronger antimicrobial effect due to a more hydrophobic environment and a lower CMC. These findings agree well with the existing reports that if the alkyl chain of quaternary ammonium compounds is shorter, the greater will be the CMC [13, 19, 26]. As a consequence, the antimicrobial power of 12-2-12 and 12-4-12 proves to be much weaker than the 16-*s*-16 series [24].

Minimum Inhibitory Concentration

All prepared compounds were assayed quantitatively by determining the Minimum Inhibitory Concentrations (MICs) for each microorganism. Different standard drugs viz., ampicillin trihydrate, ciprofloxacin (*antibacterial agent*) and fluconazole (*antifungal agent*) were used as reference. In this method, the gemini surfactants and reference drugs were prepared by dissolving them in DMF at a concentration of 30 mg mL^{-1} (a nutrient medium). Further, they were inoculated with the organism followed by incubation on a rotary shaker at 37 °C for 24 h at 150 rpm. The twofold dilutions of the compounds were prepared (25, 20, 15, 10 and 05) mg mL^{-1}.

In addition to the antibacterial study of these gemini surfactants against various biological strains, a relationship between the MIC and CMC was also found, and a relationship between chain length and antibacterial activity was determined. Table 3 shows the MIC measurements of the gemini compounds recorded at the lowest concentration with no growth (i.e. no turbidity) of inoculated bacteria and after nullifying the effect of the diluent (DMF). It was found that the homologous of gemini surfactant with greater hydrophobic environment adsorb well at the cell membrane, thereby exhibiting much better antimicrobial activity than the reference drugs. Our findings from Table 3 concluded that the lower the MIC for the tested gemini compounds, the higher its antibacterial activity.

It was found that upon dispersion in water, the hydrocarbon tail of the surfactant tends to minimize the water exposure. As a consequence they undergo self aggregation (micellization) which is completely an entropy driven phenomenon. While these hydrocarbon chains compactly organize themselves, the polar head groups with identical charge repel each other due to electrostatic repulsion and extensive head group hydration. It is also observed that gemini surfactants with short spacer ($s \leq 4$) tends to remain in as much extended rigid conformation to minimize the electrostatic repulsion. This relatively lowers the head-group surface area which can lead to a more closely packed arrangement yielding more effective self-assembly at lower CMC and exhibiting excellent antimicrobial activity. On the contrary, longer spacer ($s \geq 6$) tend to loop into the micelle interior in order to avoid its exposure to water, leading to slow micellization, and higher CMC. As a result they exhibit moderate or poor antimicrobial potency. Indeed, when the spacer becomes too long and flexible ($s \geq 10$), it becomes too hydrophobic to remain in contact with water and moves to the air side of the interface, adopting a folded, wicket-like conformation. It partly penetrates into the hydrophobic core of the micelles, restricting the hydration of the alkyl chains in the micelles and as a result the head groups approach closer to each other and thus area of the surfactant molecule decreases upon increasing spacer length [13, 14, 17, 20]. Our results follow the similar trend as reported in the literature for oligomeric surfactants and indicate that all synthesized symmetrical cationic dimeric compounds

satisfy the primary requirement of being good surfactants [23, 29, 31].

Conclusion

Surface characterization of gemini homologues indicates their good surface active and self-aggregation properties. Our study revealed the possible relationship between the alkyl chain and spacer chain length with the biological activity. The antimicrobial potency showed the sequence (Gram-positive bacteria > Fungi > Gram-negative bacteria) on the targeted microorganisms. Results successfully predicted the possible dependence of antimicrobial activity of compounds on CMC. These features make the gemini (dimeric) surfactants important for understanding their micellization phenomenon and micellar transitions which encourage the optimization of various formulations with compatible oppositely charged/neutral surfactants for making the antimicrobial systems much more robust and applicable for extensive commercial utilization.

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Research Article

Synthesis and *In vitro* Anti-Mycobacterial Activity Evaluation of Some Quinazolinyl Thioureido Scaffolds

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- Thioureido linkage
- Minimum Inhibitory Concentration (MIC)
- Antitubercular activity

Abstract

The current study reports design and diversity oriented synthesis of novel heterocycles incorporating of thioureido linkage on position no C-4 which not yet been explored and could find new applications in biology. Employing reagent based skeletal diversity approach; a facile synthesis of heterocycles with thioureido linkage at C-4 position of the quinazoline moiety has been accomplished. All the newly synthesized compounds were subjected to in vitro screening against various mycobacterial, bacterial and fungal strains. The bioassay results indicate some compounds could be emerged as the most promising anti tubercular and antimicrobial agents. All the intermediates and products have been isolated and fully characterized by IR, ^{1}H NMR and ^{13}C NMR.

INTRODUCTION

Discovery is the identification of novel active chemical compounds, often called "hits", which are typically found by assay of compounds for a desired biological activity. Initial hits can come from repurposing existing agents toward new pathologic processes. Tuberculosis (TB) has been a leading cause of death since time immemorial and it continues to cause immense human misery even today. Tuberculosis control requires new drugs that act at novel drug targets to help combat resistant forms of Mycobacterium tuberculosis and reduce treatment duration. In view of the fact there is need for the discovery of new, selective and promising inhibitor with an improved safety and efficacy profile has stimulated us to present an attractive approach towards design and development of new antimycobacterial scaffolds. Literature study reveals that more effectual new chemical scaffolds can be envisaged by incorporating two cyclic systems into a single molecule via linkage. With proven pharmacological significance, quinazolines have become a favourite field for many investigators and their efforts are quite significant in literature. According to recent data, quinazoline nucleus has attracted the attention of medicinal chemists due to its well known antifungal, anticancer & antitumor activity [1,2]. Quinazoline derivatives also known to show antibacterial and antitubercular activity [3,4]. This wide range of biological activities displayed by quinazolines is conferred by the diversity of the substituents that can be combined on the C-2,C-3 and C-6 centres. It is therefore of great interest to explore new type of substituent on the quinazoline ring in which incorporating of thioureido linkage on position C-4 which not yet been explored and could find new applications in biology.

Thioureas are of novel class of molecules found in many natural products. Their synthesis becomes a significant aspect in these days as many surrogates or substituents on the thiourea linkage may enhance its activity. In addition, thiourea compounds are associated with series of biological activities [5,6]. Thiourea are mimicking the urease in many functions; with growing application and versatile activity as active motif leads the chemists to make such thioureido [7] moieties. For these reasons synthesis of quinazoline moiety incorporated with thioureido linkage is of high interest. These novel compounds open up new perspectives in drug design by providing an entire range of highly specific, selective and non-toxic pharmaceuticals. Considering the advantage of biocompatibility and structural diversity of thiourea residues with the biological system, currently there is huge tendency of conjugating thiourea residues with bioactive heterocyclic motifs in the field of biomedical research. So here we designed synthetic route wherein the central key Quinazoline and different cyclic moieties built at C_4 position employing diversity oriented synthesis.

R = **7a** 4-NO_2, **7b** 3-NO_2, **7c** 2-NO_2, **7d** 4-F, **7e** 3-CH_3, **7f** 2-Cl: 3-Cl, **7g** 4-CH_3, **7h** 4-OCH_3, **7i** 2-Cl, **7j** 3-Cl

Scheme 1. Reagents: a) $HCONH_2$, 150°C b) PCl_5, $POCl_3$, 115-118°C c) IPA, K_2CO_3, NH_2CH_2COOH, reflux 5-6 hrs. d) $SOCl_2$ e) Acetone, NH_4NCS f) Acetone, sub. amine.

RESULTS AND DISCUSSION

Chemistry

The synthesis of the quinazolinyl thioureido unreported title compounds is as outlined in Scheme 1.In general, the basic strategy was the same as was used to make the lead structure; the combination of aromatic electrophilic and aromatic nucleophilic moieties corresponding to the target analogues. Hence in continuation of our work on quinazolines, we have incorporated thioureido linkage with quinazoline moiety. In this work, the lead structure, (6-nitroquinazolin-4(3h)-one) compound **2** was synthesized using the Niementowski cyclization according to the literature [8]. Compound **2** on reaction with phosphorous pentachloride and phosphorous oxychloride gave compound **3** which was synthesized according to literature [9]. Compound **3** on reaction with glycine gave quinazolinyl glycine derivative compound **4.** The latter was then treated with thionyl chloride to produce compound **5** which on subsequent treatment with ammonium thiocyanate according to literature [10] gave compound **6** which on treatment with various aryl amines in acetone gave **7a-j.** All the synthesized compounds were fully characterized by IR, ^{1}H-NMR, ^{13}C NMR spectroscopy and elemental analysis.

The titled compounds were prepared through the reaction sequences depicted in Scheme 1. Compound **2** showed the presence of >NH group in IR (3170 cm^{-1}) and ^{1}H NMR spectra which showed a broad signal around δ 7.17 (NH). The IR spectrum of compound **3** showed peak at 760 cm^{-1} for C-Cl**.** The IR spectrum of titled compound **7a** showed peak at 1211 cm^{-1} (thioureido CS group) and its ^{1}H NMR spectrum showed a singlet at δ 8.62 (NHCSNH). In addition to that, the absence of a peak at 760 cm^{-1} for chloro group and presence of a peak at 1211 cm^{-1} (thioureido CS group) in compound **7a** reveals that p-nitro phenyl thioureido linkage is present as a linker between phenyl ring and quinazoline moiety. The ^{13}C NMR spectrum of **7a** reveals carbon signals at δ 180.25, 169.29, 159.11 and 155.90 assigned to C=S, C=O, C_2 and C_4, respectively.

Biological Activity

Antibacterial Activity:

1. Compounds

Test compounds were dissolved in DMF at an initial concentration of 40 mg\ ml and then were serially diluted in culture medium.

2. Cells

Bacterial strains: Staphylococcus aureus, Bacillus subtillis, Salmonella typhi, Klebsiella pneumonia

Fungal strain: Aspergillus niger

The minimum inhibitory concentrations (MICs) of the chemical compounds assays were carried out as described by Clause [11] with minor modifications. References viz., ampicillin trihydrate and ciprofloxacin (antibacterial agent) and fluconazole (antifungal agent) were used. Solutions of the test compounds and reference drugs were dissolved in DMF at a conc. of 20 mg ml^{-1}. The two fold dilution of the compounds and reference drugs were prepared (40, 30, 20, 10, 05, etc.) mg ml^{-1}. Antibacterial activities of the bacterial strains were carried out in Muller–Hinton broth (Difco) medium, at pH 6.9, with an inoculum of (1–2) × 10^3 cells ml^{-1} by the spectrophotometric method and an aliquot of 100 µl was added to each tube of the serial dilution. The chemical compounds–broth medium serial tube dilutions inoculated with each bacterium were incubated on a rotary shaker at 37°C for 24 h at 150 rpm. The MICs of the chemical compounds were recorded as the lowest concentration of each chemical compounds in the tubes with no growth (i.e.,no turbidity) of inoculated bacteria. The results obtained is summarized in Table 1.

Table 1 represents the antibacterial effect of the substituted quinazoline-4(3H)-ones and their thioanalogues. Two compounds of the obtained series owed high *in vitro* antimicrobial activity. Amongst the entire tested compounds, **7c** showed excellent activity against S.Typhi and Kleb; **7f** showed good activity against Bacillus subtillis and displayed excellent activity against fungus Aspergilus Niger.

Antitubercular Activity: All compounds 7(a-j) were screened at 62.5 µg/ml for their in vitro antimycobacterial activity against M. tuberculosis $H_{37}R_v$ strain using Lowenstein-Jensen medium method [12]. Positive and negative growth controls were run in each experiment and Rifampicin was used as standard drug.

The antitubercular effects of new synthesized compounds **7a-j** were investigated against M. tuberculosis $H_{37}R_v$ strain and the results are shown in Figure 1. The compounds **7c** (2-NO_2)**, 7d** (4-F) and **7i** (2-Cl) possess elevated activity against M. tuberculosis $H_{37}R_v$ strain while all the remaining compounds possess moderate to poor efficiency.

CONCLUSION

A series of quinazoline moiety incorporated with thioureido linkage were successfully synthesized and screened for antimicrobial and antitubercular activity. It is seen from the biological screening result that the several quinazoline moiety incorporated with thioureido linkage were interestingly found to be more active than their corresponding precursors. The probable reason for such behaviour with Gram –ve is the ortho position of nitro group on the aromatic ring compared to Meta and para position. In addition to this the presence of more than one electron-donating group on the aromatic ring in general influences the antifungal activity compared to compounds with electron withdrawing groups. Besides this, presence and the position of thioureido (–NHCSNH-) group as the connecting linker between the aromatic ring and quinazoline ring seem to be very significant for antimicrobial effect. The tested compounds were found to be active against S.typhi, Kleb and A.niger as paralleled to standards. Antitubercular activity of some compounds was found good against M. tuberculosis $H_{37}R_v$ as compared to that of Rifampicin.

Table 1: Antimicrobial Activity.

Compound No.	MIC in mM Antibacterial Strains		MIC in mM Antibacterial Strains		Antifungal Strains *A. niger*
	B. subtilis	*S. aureus*	*S. typhi*	*Kleb*	
7a	0.70	0.70	0.70	0.47	0.70
7b	0.70	0.70	0.70	0.70	0.47
7c	0.70	0.70	**0.23**	**0.18**	0.70
7d	0.74	0.50	0.50	0.75	0.50
7e	0.50	0.76	0.76	0.50	0.50
7f	**0.44**	0.66	0.66	0.44	**0.44**
7g	0.81	0.81	0.81	0.54	0.81
7h	0.72	0.72	0.72	0.73	0.72
7i	0.72	0.72	0.48	0.48	0.71
7j	0.72	0.72	0.48	0.72	0.71
Ampicillin	0.27	0.13	0.20	0.13	-
Ciprofloxacin	0.25	0.19	0.19	0.12	-
Fluconazole	-	-	-	-	0.35

Abbreviations: B. Subtilis-Bacillus Subtilis; S. Aureus-Staphylococcus Aureus; S. Typhi-Salmonella Typhi; Kleb- Klebsiella Pneumonia; A. Niger-Aspergillus Niger

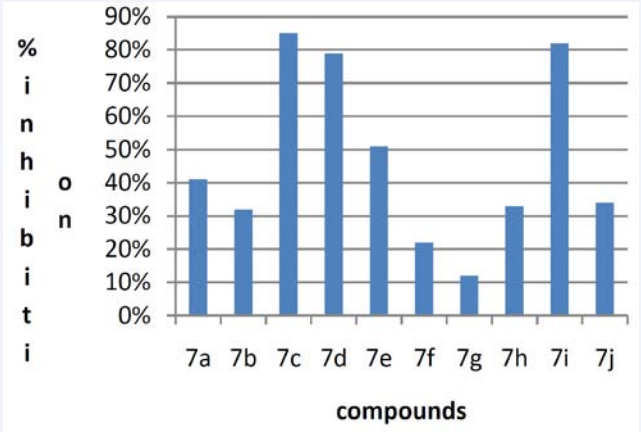

Figure 1 Antitubercular Activity of synthesized compounds 7(a-j).

EXPERIMENTAL

Reagents, Instrumentation and Measurements

All chemicals and solvents were of analytical grade and used directly. All the melting points were determined in open capillaries and are uncorrected. TLC analysis was done using pre-coated silica gel plates and visualization was done using iodine.

Apparatus

IR spectra (υmax in cm^{-1}) were recorded on Nicolet is10 FTIR spectrophotometer using KBr pellets. ^{1}H NMR and ^{13}C NMR spectra were recorded at 300 MHz and 75.5 MHz, respectively (Bruker Avance II), using DMSO as solvent and TMS as internal reference (chemical shifts in δ, ppm).The elemental analysis (C, H, N) of compounds were performed on Carlo Erba–1108 elemental analyzer. Their results were found to be in good agreement with the calculated values.

Synthesis of 6-nitroquinazolin-4(3h)-one (Compound 2)

A mixture of 5-Nitroanthranilic acid (0.01 mole) and formamide (20 ml) were heated for 8-10 hrs at 150-160°C under stirring with a magnetic stirrer. The mixture was then allowed to cool, and the precipitates were filtered off and dried at 80°C to give 6-nitro quinazolin-4(3H)-one (M.P.283-285°C).

Synthesis of 4-chloro-6-nitroquinazoline (Compound 3)

A mixture of 6-Nitro quinazoline-4(3H)-one (0.01mole), phosphorus pentachloride (0.05mole) and phosphorus oxychloride (16 ml) were heated and stirred under reflux for 8 hr at 115-118°C. Excess of phosphorus oxychloride was then removed by distillation. The residue obtained was acidified with sodium bicarbonate solution (5%w/v). The resulting precipitate was collected by filtration, washed with water, and dried to give 4-chloro-6-nitroquinazoline (M.P.220-223°C).

Synthesis of [(6-nitroquinazolin-4-yl) amino] acetic acid (compound 4)

A mixture of 4-Chloro-6-nitroquinazoline (0.01mole) and anhydrous K_2CO_3 (0.02mole) were taken in isopropyl alcohol. To this drop wise addition of glycine (0.01mole) in IPA with continuous stirring was done for half an hour. Then the reaction mixture was refluxed for 5-6 hrs. After the completion of reaction (checked by TLC), reaction mixture was then poured over crushed ice. The resulting precipitate was neutralized by conc. HCl. The product was collected by filtration, washed with water and dried to give [(6-nitro quinazoline-4-yl) amino] acetic acid. (M.P.157-159°C).

Synthesis of n-[(substituted phenyl) carbamothioyl]-2-[6-nitroquinazolin-4-yl) amino] acetamide (Compound 5,6 &7)

A mixture of [(6-nitroquinazolin-4-yl) amino] acetic acid

(0.01mole) and thionyl chloride (0.015mole) were refluxed for 3 hrs. Excess of thionyl chloride was distilled off. The product was then cooled and was dissolved in acetone (40 ml). Ammonium thiocyanate in acetone was then added slowly with constant stirring at room temperature. The reaction mixture was kept under reflux condition for 1 hr. Afterwards the solution of amine (0.01mole) in acetone was added slowly with constant stirring at room temperature. The reaction mixture was then refluxed for 3-4 hrs. The solution was poured into ice-cold water and the above product was recrystallized by using ethanol.

Spectral data of Compounds 7(a-j)

Compound (7a). Yield 67%, brown; mp 150 °C; Anal. Calcd for $C_{17}H_{13}N_7O_5S$ (427.39): C, 47.77%; H, 3.07%; N, 22.94%. found: C, 47.54%; H, 2.58 %; N, 22.87%; **IR (KBr)/cm^{-1}:** 3172 cm^{-1} (-NH-), 1680 cm^{-1} (>C=O), 1615 cm^{-1} (-C=N-), 1211 cm^{-1} (C=S), 1339 cm^{-1} (-C-N), 1557,1283 cm^{-1} (-NO_2); **^{1}H NMR CDCl$_3$**: 4.28 (s, 2H, CH_2), 7.11 (s, 1H, Ar-NH), 8.62 (s, 1H, CSNH), 9.08 (s, 1H, CONH), 7.30-8.11 (m, 8H, Ar-H);**^{13}C NMR** (75.5 MHz, $CDCl_3$): δ 180.25 (C=S), 169.29 (C=O), 159.11 (C=N, C_2), 155.90 (C=N, C_4), 151.54 (CHAr), 137.31 (CHAr), 134.58 (CHAr), 131.12 (CHAr), 129.84 (CHAr), 128.68 (CHAr), 127.79 (CHAr), 120.60 (CHAr), 118.79 (CHAr), 115.62 (CHAr), 112.58 (CHAr), 48.27 (NCH_2).

Compound (7b). Yield 64%, yellow; mp 170 °C; Anal. Calcd for $C_{17}H_{13}N_7O_5S$ (427.39): C, 47.77%; H, 3.07%; N, 22.94%. found: C, 47.57%; H, 2.86 %; N, 22.78%;**IR (KBr)/cm^{-1}:** 3175 cm^{-1} (-NH-), 1676 cm^{-1} (>C=O), 1612 cm^{-1} (-C=N-), 1210 cm^{-1} (C=S), 1334 cm^{-1} (-C-N), 1555,1281 cm^{-1} (-NO_2); **^{1}H NMR CDCl$_3$**: 4.31 (s, 2H, CH_2), 7.09 (s, 1H, Ar-NH), 8.59 (s, 1H, CSNH), 9.09 (s, 1H, CONH), 7.28-8.12 (m, 8H, Ar-H); **^{13}C NMR** (75.5 MHz,CDCl3): δ 180.23 (C=S), 170.01 (C=O), 159.08 (C=N, C_2), 155.87 (C=N, C_4), 152.34 (CHAr), 137.05 (CHAr), 133.28 (CHAr), 130.08 (CHAr), 128.82 (CHAr),127.88 (CHAr), 126.19 (CHAr), 120.56 (CHAr), 118.58 (CHAr), 115.31 (CHAr), 113.80 (CHAr), 48.22 (NCH_2).

Compound (7c). Yield 65%, yellow; mp 146 °C; Anal. Calcd for $C_{17}H_{13}N_7O_5S$ (427.39): C, 47.77%; H, 3.07%; N, 22.94%. found: C 47.46%; H,2.86 %; N,22.75%; **IR (KBr)/cm^{-1}:** 3198 cm^{-1} (-NH-), 1684 cm^{-1} (>C=O), 1610 cm^{-1} (-C=N-), 1208 cm^{-1} (C=S), 1339 cm^{-1} (-C-N), 1560,1283 cm^{-1} (-NO_2); **^{1}H NMR CDCl$_3$**: 4.28 (s, 2H, CH_2), 7.09 (s, 1H, Ar-NH), 8.63 (s, 1H, CSNH), 9.13 (s, 1H, CONH), 7.15-8.08 (m, 8H, Ar-H); **^{13}C NMR** (75.5 MHz,$CDCl_3$): δ 180.15 (C=S), 169.75 (C=O), 159.16 (C=N, C_2), 154.87 (C=N, C_4), 152.56 (CHAr), 138.31 (CHAr), 134.28 (CHAr), 133.02 (CHAr), 130.62 (CHAr),129.68 (CHAr), 127.45 (CHAr), 121.60 (CHAr), 119.61 (CHAr), 114.62 (CHAr), 112.24 (CHAr), 48.25 (NCH_2).

Compound (7d). Yield 68%, brown; mp 200 °C; Anal. Calcd for $C_{17}H_{13}FN_6O_3S$ (400.38): C, 51.00%; H, 3.27%; N, 20.99%. found: C, 50.82%; H,3.11 %; N, 20.56%; **IR(KBr)/cm^{-1}:** 3169 cm^{-1} (-NH-), 1684 cm^{-1} (>C=O), 1617 cm^{-1} (-C=N-), 1211 cm^{-1} (C=S), 1337 cm^{-1} (-C-N), 1559,1281 cm^{-1} (-NO_2), 1132 cm^{-1} (C-F); **^{1}H NMR CDCl$_3$**: 4.30 (s, 2H, CH_2), 7.10 (s, 1H, Ar-NH), 8.60 (s, 1H, CSNH), 9.08 (s, 1H, CONH), 7.30-8.10 (m, 8H, Ar-H); **^{13}C NMR** (75.5 MHz, $CDCl_3$): δ 180.14 (C=S), 169.62 (C=O), 158.43 (C=N, C_2), 155.75 (C=N, C_4), 152.04 (CHAr), 137.89 (CHAr), 135.56 (CHAr), 132.52 (CHAr), 130.64 (CHAr),128.68 (CHAr), 127.21 (CHAr), 121.93 (CHAr), 119.34 (CHAr), 115.12 (CHAr), 113.87 (CHAr), 48.19 (NCH_2).

Compound (7e). Yield 70%, white; mp 238 °C; Anal. Calcd for $C_{18}H_{16}N_6O_3S$ (396.42): C, 54.54%; H, 4.07%; N, 21.20%. found: C, 54.26%; H,3.85 %; N, 21.01%; **IR (KBr)/cm^{-1}:** 3185 cm^{-1} (-NH-), 1661 cm^{-1} (>C=O), 1615 cm^{-1} (-C=N-), 1217 cm^{-1} (C=S), 1337 cm^{-1} (-C-N), 1558,1287 cm^{-1} (-NO_2); **^{1}H NMR CDCl$_3$**: 4.28 (s, 2H, CH_2), 7.07 (s, 1H, Ar-NH), 8.54 (s, 1H, CSNH), 9.10 (s, 1H, CONH), 7.15-8.22 (m, 8H, Ar-H);**^{13}C NMR** (75.5 MHz, $CDCl_3$): δ 181.05 (C=S), 170.29 (C=O), 158.11 (C=N, C_2), 156.90 (C=N, C_4), 152.60 (CHAr), 138.51 (CHAr), 135.42 (CHAr), 132.02 (CHAr), 131.32 (CHAr),127.55 (CHAr), 126.79 (CHAr), 125.30 (CHAr), 120.52 (CHAr), 119.12 (CHAr), 113.70 (CHAr), 48.22 (NCH_2).

Compound (7f). Yield 75%, white; mp 210 °C; Anal. Calcd for $C_{17}H_{12}Cl_2N_6O_3S$ (451.28): C, 45.24%; H, 2.68%; N, 18.62%. found: C, 45.21%; H,2.55 %; N, 18.52%; **IR (KBr)/cm^{-1}:** 3177 cm^{-1} (-NH-), 1672 cm^{-1} (>C=O), 1616 cm^{-1} (-C=N-), 1217 cm^{-1} (C=S), 1340 cm^{-1} (-C-N), 1557,1282 cm^{-1} (-NO_2), 757 cm^{-1} (C-Cl); **^{1}H NMR CDCl$_3$**: 4.31 (s, 2H, CH_2), 7.08 (s, 1H, Ar-NH), 8.55 (s, 1H, CSNH), 9.05 (s, 1H, CONH), 7.11-8.27 (m, 7H, Ar-H); **^{13}C NMR** (75.5 MHz, $CDCl_3$): δ 181.23 (C=S), 170.15 (C=O), 159.20 (C=N, C_2), 155.45 (C=N, C_4), 152.34 (CHAr), 137.92 (CHAr), 135.02 (CHAr), 132.15 (CHAr), 130.65(CHAr), 129.84 (CHAr), 127.79 (CHAr), 121.85 (CHAr), 120.58 (CHAr), 117.35 (CHAr), 112.58 (CHAr), 47.86 (NCH_2).

Compound (7g). Yield 67%, white; mp 190 °C; Anal. Calcd for $C_{18}H_{16}N_6O_3S$ (396.42): C, 54.54%; H, 4.07%; N, 21.20%. found: C, 54.40%; H,3.89 %; N, 21.02%; **IR (KBr)/cm^{-1}:** 3195 cm^{-1} (-NH-), 1676 cm^{-1} (>C=O), 1614 cm^{-1} (-C=N-), 1205 cm^{-1} (C=S), 1328 cm^{-1} (-C-N), 1568,1277 cm^{-1} (-NO_2); **^{1}H NMR CDCl$_3$**: 4.30 (s, 2H, CH_2), 7.09 (s, 1H, Ar-NH), 8.54 (s, 1H, CSNH), 9.11 (s, 1H, CONH), 7.21-8.34 (m, 8H, Ar-H); **^{13}C NMR** (75.5 MHz, $CDCl_3$): δ 181.25 (C=S), 170.19 (C=O), 160.31 (C=N, C_2), 156.54 (C=N, C_4), 151.54 (CHAr), 138.01 (CHAr), 135.32 (CHAr), 133.54 (CHAr), 130.84 (CHAr), 129.68 (CHAr), 128.79 (CHAr), 122.34 (CHAr), 120.67 (CHAr), 119.19 (CHAr), 113.51 (CHAr), 48.07 (NCH_2).

Compound (7h). Yield 71%, white; mp 215 °C; Anal. Calcd for $C_{18}H_{16}N_6O_4S$ (412.42): C, 52.42%; H, 3.91%; N, 20.38%. found: C, 52.25%; H,3.67 %; N, 20.16%; **IR (KBr)/cm^{-1}:** 3184 cm^{-1} (-NH-), 1672 cm^{-1} (>C=O), 1616 cm^{-1} (-C=N-), 1206 cm^{-1} (C=S), 1330 cm^{-1} (-C-N), 1568,1277 cm^{-1} (-NO_2); **^{1}H NMR CDCl$_3$**: 4.30 (s, 2H, CH_2), 7.09 (s, 1H, Ar-NH), 8.53 (s, 1H, CSNH), 9.11 (s, 1H, CONH), 7.21-8.24 (m, 8H, Ar-H); **^{13}C NMR** (75.5 MHz, $CDCl_3$): δ 180.31 (C=S), 170.62 (C=O), 160.45 (C=N, C_2), 155.90 (C=N, C_4), 152.32 (CHAr), 139.33 (CHAr), 135.53 (CHAr), 132.12 (CHAr), 130.52 (CHAr),128.40 (CHAr), 127.61 (CHAr), 119.60 (CHAr), 118.53 (CHAr), 114.62 (CHAr), 113.58 (CHAr), 48.12 (NCH_2).

Compound (7i). Yield 68%, yellow; mp 160 °C; Anal. Calcd for $C_{17}H_{13}ClN_6O_3S$ (416.84): C, 48.98%; H, 3.14%; N, 20.16%. found: C, 48.74%; H,3.01 %; N, 20.03%; **IR (KBr)/cm^{-1}:** 3186 cm^{-1} (-NH-), 1679 cm^{-1} (>C=O), 1621 cm^{-1} (-C=N-), 1208 cm^{-1} (C=S), 1328 cm^{-1} (-C-N), 1570,1281 cm^{-1} (-NO_2), 760 cm^{-1} (C-Cl); **^{1}H NMR CDCl$_3$**: 4.32 (s, 2H, CH_2), 7.13 (s, 1H, Ar-NH), 8.54 (s, 1H, CSNH), 9.12 (s, 1H, CONH), 7.17-8.38 (m, 8H, Ar-H),**^{13}C NMR** (75.5 MHz, $CDCl_3$): δ 180.27 (C=S), 169.65 (C=O), 160.21 (C=N, C_2), 156.55 (C=N, C_4), 152.54 (CHAr), 137.31 (CHAr), 135.46 (CHAr), 131.80 (CHAr), 129.84 (CHAr), 128.32 (CHAr), 127.60 (CHAr), 119.25 (CHAr), 118.90 (CHAr), 114.62 (CHAr), 112.58 (CHAr), 48.20 (NCH_2).

Compound (7j). Yield 70%, yellow; mp 200 °C; Anal. Calcd for $C_{17}H_{13}ClN_6O_3S$ (416.84): C, 48.98%; H, 3.14%; N, 20.16%. found: C,48.81%; H,2.89 %; N, 20.01%; **IR(KBr)/cm^{-1}:** 3189 cm^{-1} (-NH-), 1675 cm^{-1} (>C=O), 1618 cm^{-1} (-C=N-), 1210 cm^{-1} (C=S), 1325 cm^{-1} (-C-N), 1573,1287 cm^{-1} (-NO_2), 762 cm^{-1} (C-Cl); **^{1}H NMR CDCl$_3$**: 4.29 (s, 2H, CH_2), 7.10 (s, 1H, Ar-NH), 8.50 (s, 1H, CSNH), 9.08 (s, 1H, CONH), 7.13-8.27 (m, 8H, Ar-H),**^{13}C NMR** (75.5 MHz, $CDCl_3$): δ 181.22 (C=S), 169.32 (C=O), 160.62 (C=N, C_2), 156.20 (C=N, C_4), 152.90 (CHAr), 138.35 (CHAr), 135.18 (CHAr), 133.02 (CHAr), 128.86 (CHAr), 128.20 (CHAr), 126.19 (CHAr), 121.35 (CHAr), 120.58 (CHAr), 119.79 (CHAr), 113.70 (CHAr), 48.36 (NCH_2).

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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design, synthesis of some 2,6,9-trisubstituted purinyl thioureido derivatives and evaluation of antimicrobial activity

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Abstract Some new trisubstituted purinyl thioureido were synthesized and evaluated for their in vitro antimicrobial activity against Gram positive and Gram negative strains and antifungal strain using a micro dilution procedure. Synthesized compounds **6a–j** prove to be effective with minimum inhibitory concentration (MIC) (mg ml^{-1}), among them **6a**, **6d**, and **6e** showed excellent activity against a panel of microorganisms. The newly synthesized compounds were characterized using IR, ^{1}H-NMR, and elemental analysis.

Keywords Purine · Thioureido linkage · Minimum inhibitory concentration (MIC)

Introduction

A great variety of di-, tri-, or tetra substituted purines so far described in the literature have been found to be potent inhibitors of chaperone HSP90, protein kinases (MAP, Src, and Cdk), sulfotransferases, phosphodiesterases, and microtubule assembly, inducers of interferon and (de) differentiation, antagonists of adenosine receptors, and corticotropin-releasing hormone receptors (Legraverend and Grierson, 2006). This wide range of biological activities displayed by purines is conferred by the diversity of the substituents that can be combined on the C-2, C-6, C-8, and N-9 centers. It is therefore of great interest to explore new types of substituents on the purine ring of which introduction of an amide function at positions 2 and/or 6 is particularly pertinent since known inhibitors of various protein kinases have been improved in this way (Furet *et al.*, 2003; Yue *et al.*, 2004). Whereas many 2,6-diaminopurine derivatives (roscovitine, purvalanol) exhibit potent biological activities, 6-amido-2-aminopurines have not yet been explored and, therefore, could find new applications in biology.

Purine derivatives also known to show antibacterial activity (Paoli *et al.*, 2004; Zhao *et al.*, 2007). The thioamide moiety including the thioureido group is a versatile building block in the synthesis of heterocycles. Thioureido group forms a component in a number of useful drugs and is associated with many biological pharmaceutical and therapeutical activities (Vig *et al.*, 1998; Venkatachalam *et al.*, 2004; Fathalla and Pazdera, 2002). The goal of the present work was to synthesize trisubstituted purine derivatives bearing the alternative combination of an amide at the 6-position, an amine at position 2, and sulfonyl group at the position 9 from a readily available 2-amino, 6-chloro purine (Scheme 1).

Experimental

Reagents, instrumentation, and measurements

All reagents and solvents were of analytical grade and used directly. All the melting points were determined in open capillaries and are uncorrected. The purity of compounds was checked routinely by TLC (0.5 mm thickness) using silica gel-G coated Al-plates (Merck), and spots were visualized by exposing the dry plates in iodine vapors.

IR spectra (υ_{max} in cm^{-1}) were recorded on a Perkin Elmer spectrum BX series FT-IR spectrometer using KBr or Nujol technique. ^{1}H NMR spectra were recorded on a Bruker WM 400 FT MHz NMR instrument, using $CDCl_3$

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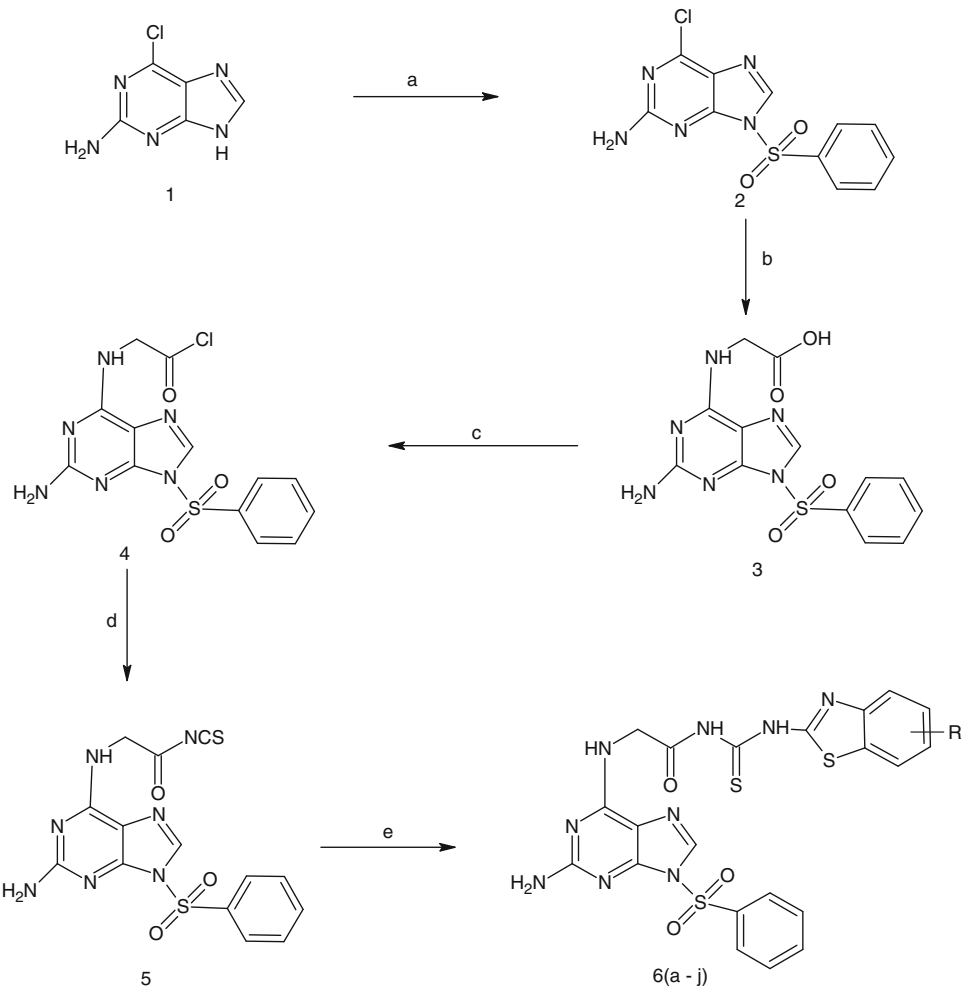

Scheme 1 Reagents: (a) Benzene sulfonyl chloride, DMF, pyridine, (b) IPA, K_2CO_3, NH_2CH_2COOH, reflux 5–6 h, (c) $SOCl_2$, (d) acetone, NH_4NCS, (e) acetone, sub. benzothiazole. R = **6a** 4-Cl, **6b** 5-NO_2, **6c** 4-NO_2, **6d** 6-F, **6e** 6-OCH_3, **6f** 6-Cl, **6g** 6-CH_3, **6h** 6-NO_2, **6i** 5-OCH_3, **6j** H

as solvent and TMS as internal reference (chemical shifts in δ, ppm). The elemental analysis (C, H, N) of compounds was performed on Carlo Erba-1108 elemental analyzer. Their results were found to be in good agreement with the calculated values.

*Synthesis of 6-chloro-9-(phenylsulfonyl)-9h-purin-2-amine (compound **2**)*

In a 250-ml conical flask, a mixture of 2-amino, 6-chloro purine (0.01 mol) in DMF, a few drops of pyridine and benzene sulfonyl chloride (0.01 mol) were stirred for 3 h. After the completion of reaction (checked by TLC), the resulting precipitates were collected by filtration and dried to give **2** (mp 121–124°C).

*Synthesis of {[2-amino-9-(phenylsulfonyl)-9h-purin 6-yl] amino} acetic acid (compound **3**)*

In a 250-ml round bottom flask, 6-chloro-9-(phenylsulfonyl)-9H-purin-2-amine (0.01 mol) and anhydrous K_2CO_3 (0.02 mol) were taken in isopropyl alcohol. To this, dropwise addition of glycine (0.01 mol) in iso propyl alcohol with continuous stirring was done for half an hour. Then the mixture was stirred at reflux for 10–12 h. After the completion of reaction (checked by TLC), reaction mixture was poured into crushed ice. The resulting precipitate was neutralized by conc. HCl. The product was collected by filtration, washed with water, and dried to give **3** (mp 167–169°C).

*Synthesis of preparation of 2-[(2-amino-9-phenylsulfonyl-9h-purin-6-yl) amino]-n-[(substituted-1,3-benzothiazol-2yl) carbamo thioyl] acetamide (compounds **4**, **5**, and **6**)*

In a 250-ml round bottom flask, {[2-amino-9-(phenyl sulfonyl)-9H-purin 6-yl] amino} acetic acid (0.01 mol) and thionyl chloride (0.015 mol) were refluxed for 3 h. Excess of thionyl chloride was distilled off. The product was then cooled and was dissolved in acetone (40 ml). Ammonium thiocyanate in acetone was then added slowly with constant stirring at room temperature. The reaction mixture was kept under reflux condition for 1 h. Afterward the solution of substituted benzothiazol (0.01 mol) in acetone was

added slowly with constant stirring at room temperature. The reaction mixture was then refluxed for 3–4 h. The solution was poured into ice-cold water, and the product was recrystallized by using ethanol.

Spectral data of compounds **6(a–j)**

*Compound **6a***

Yield 70%, brown; mp 186°C; Anal. Calcd for $C_{21}H_{16}ClN_9O_3S_3$ (574.05): C, 43.94%; H, 2.96%; N, 21.80%. found: C, 43.77%; H, 2.65%; N, 21.80%; NMR $CDCl_3$: 4.26 (s, 2H, CH_2), 7.12 (s, 1H, Ar–NH), 8.59 (s, 1H, CSNH), 9.07 (s, 1H, CONH), 7.40–8.10 (m, 8H, Ar–H), 6.10 (s, 2H, Ar–NH_2), IR (KBr)/cm^{-1}: 3320 cm^{-1} (–NH_2–), 3173 cm^{-1} (–NH–), 1682 cm^{-1} (>C=O), 1625 cm^{-1} (–C=N–), 1210 cm^{-1} (C=S), 1339 cm^{-1} (–C–N), 1368,1150 cm^{-1} (–SO_2), 759 cm^{-1} (–Cl).

*Compound **6b***

Yield 68%, yellow; mp 146°C; Anal. Calcd for $C_{21}H_{16}N_{10}O_5S_3$ (584.61): C, 43.14%; H, 2.76%; N, 23.96%. found: C, 43.01%; H, 2.59%; N, 23.82%; NMR $CDCl_3$: 4.27 (s, 2H, CH_2), 7.11 (s, 1H, Ar–NH), 8.60 (s, 1H, CSNH), 9.06 (s, 1H, CONH), 7.36–8.11 (m, 8H, Ar–H), 6.11 (s, 2H, Ar–NH_2), IR (KBr)/cm^{-1}: 3311 cm^{-1} (–NH_2–), 3172 cm^{-1} (–NH–), 1680 cm^{-1} (>C=O), 1617 cm^{-1} (–C=N–), 1205 cm^{-1} (C=S), 1340 cm^{-1} (–C–N), 1367, 1152 cm^{-1} (–SO_2), 1570, 1281 cm^{-1} (–NO_2).

*Compound **6c***

Yield 72%, light yellow; mp 169°C; Anal. Calcd for $C_{21}H_{16}N_{10}O_5S_3$ (584.61): C, 43.14%; H, 2.76%; N, 23.96%. found: C, 43.01%; H, 2.61%; N, 23.72%; NMR $CDCl_3$: 4.25 (s, 2H, CH_2), 7.10 (s, 1H, Ar–NH), 8.62 (s, 1H, CSNH), 9.08 (s, 1H, CONH), 7.38–8.15 (m, 8H, Ar–H), 6.15 (s, 2H, Ar–NH_2), IR (KBr)/cm^{-1}: 3314 cm^{-1} (–NH_2–), 3165 cm^{-1} (–NH–), 1684 cm^{-1} (>C=O), 1615 cm^{-1} (–C=N–), 1211 cm^{-1} (C=S), 1337 cm^{-1} (–C–N), 1365, 1148 cm^{-1} (–SO_2), 1568, 1277 cm^{-1} (–NO_2).

*Compound **6d***

Yield 73%, gray; mp 182°C; Anal. Calcd for $C_{21}H_{16}FN_9O_3S_3$ (557.60): C, 45.23%; H, 2.89%; N, 22.61%. found: C, 45.05%; H, 2.67%; N, 22.47%; NMR $CDCl_3$: 4.25 (s, 2H, CH_2), 7.21 (s, 1H, Ar–NH), 8.59 (s, 1H, CSNH), 9.05 (s, 1H, CONH), 7.32–8.16 (m, 8H, Ar–H), 6.13 (s, 2H, Ar–NH_2), IR (KBr)/cm^{-1}: 3321 cm^{-1} (–NH_2–), 3168 cm^{-1} (–NH–), 1685 cm^{-1} (>C=O), 1612 cm^{-1} (–C=N–), 1215 cm^{-1} (C=S), 1342 cm^{-1} (–C–N), 1371, 1142 cm^{-1} (–SO_2), 1132 cm^{-1} (–F).

*Compound **6e***

Yield 70%, white; mp 224°C; Anal. Calcd for $C_{22}H_{19}N_9O_4S_3$ (569.63): C, 46.39%; H, 3.36%; N, 22.13%. found: C, 46.17%; H, 3.14%; N, 22.01%; NMR $CDCl_3$: 4.28 (s, 2H, CH_2), 7.11 (s, 1H, Ar–NH), 8.62 (s, 1H, CSNH), 9.08 (s, 1H, CONH), 7.42–8.21 (m, 8H, Ar–H), 6.11 (s, 2H, Ar–NH_2), IR (KBr)/cm^{-1}: 3318 cm^{-1} (–NH_2–), 3163 cm^{-1} (–NH–), 1682 cm^{-1} (>C=O), 1613 cm^{-1} (–C=N–), 1210 cm^{-1} (C=S), 1343 cm^{-1} (–C–N), 1357, 1150 cm^{-1} (–SO_2).

*Compound **6f***

Yield 69%, brown; mp 169°C; Anal. Calcd for $C_{21}H_{16}ClN_9O_3S_3$ (574.05): C, 43.94%; H, 2.81%; N, 21.96%. found: C, 43.77%; H, 2.62%; N, 21.72%; NMR $CDCl_3$: 4.00 (s, 2H, CH_2), 7.30 (s, 1H, Ar–NH), 8.45 (s, 1H, CSNH), 9.09 (s, 1H, CONH), 7.80–8.20 (m, 9H, Ar–H), 6.10 (s, 2H, Ar–NH_2), IR (KBr)/cm^{-1}: 3310 cm^{-1} (–NH_2–), 3198 cm^{-1} (–NH–), 1696 cm^{-1} (>C=O), 1637 cm^{-1} (–C=N–), 1203 cm^{-1} (C=S), 757 cm^{-1} (–Cl), 1367, 1152 cm^{-1} (–SO_2).

*Compound **6g***

Yield 67%, green; mp 180°C; Anal. Calcd for $C_{22}H_{19}N_9O_3S_3$ (553.63): C, 47.73%; H, 3.46%; N, 22.77%. found: C, 47.54%; H, 3.29%; N, 22.63%; NMR $CDCl_3$: 4.12 (s, 2H, CH_2), 7.11 (s, 1H, Ar–NH), 8.62 (s, 1H, CSNH), 9.04 (s, 1H, CONH), 7.50–8.11 (m, 8H, Ar–H), 6.14 (s, 2H, Ar–NH_2), IR (KBr)/cm^{-1}: 3315 cm^{-1} (–NH_2–), 3182 cm^{-1} (–NH–), 1689 cm^{-1} (>C=O), 1622 cm^{-1} (–C=N–), 1216 cm^{-1} (C=S), 1339 cm^{-1} (–C–N), 1368, 1152 cm^{-1} (–SO_2).

*Compound **6h***

Yield 70%, light yellow; mp 152°C; Anal. Calcd for $C_{21}H_{16}N_{10}O_5S_3$ (584.61): C, 43.14%; H, 2.76%; N, 23.96%. found: C, 43.01%; H, 2.61%; N, 23.76%; NMR $CDCl_3$: 4.17 (s, 2H, CH_2), 7.12 (s, 1H, Ar–NH), 8.52 (s, 1H, CSNH), 9.13 (s, 1H, CONH), 7.32–8.15 (m, 8H, Ar–H), 6.12 (s, 2H, Ar–NH_2), IR (KBr)/cm^{-1}: 3316 cm^{-1} (–NH_2–), 3183 cm^{-1} (–NH–), 1687 cm^{-1} (>C=O), 1615 cm^{-1} (–C=N–), 1211 cm^{-1} (C=S), 1330 cm^{-1} (–C–N), 1362, 1153 cm^{-1} (–SO_2), 1558, 1287 cm^{-1} (–NO_2).

*Compound **6i***

Yield 68%, brown; mp 196°C; Anal. Calcd for $C_{22}H_{19}N_9O_4S_2$ (569.63): C, 46.39%; H, 3.36%; N, 22.13%. found: C, 46.18%; H, 3.18%; N, 22.01%; NMR $CDCl_3$: 4.18 (s, 2H,

CH_2), 7.05 (s, 1H, Ar–NH), 8.42 (s, 1H, CSNH), 9.13 (s, 1H, CONH), 7.33–8.19 (m, 8H, Ar–H), 6.11 (s, 2H, Ar–NH_2), IR (KBr)/cm^{-1}: 3318 cm^{-1} (–NH_2–), 3192 cm^{-1} (–NH–), 1683 cm^{-1} (>C=O), 1613 cm^{-1} (–C=N–), 1201 cm^{-1} (C=S), 1335 cm^{-1} (–C–N), 1374, 1142 cm^{-1} (–SO_2).

Compound ***6j***

Yield 70%, white; mp 183°C; Anal. Calcd for $C_{21}H_{17}N_9O_3S_3$ (539.61): C, 46.74%; H, 3.18%; N, 23.36%. found: C, 46.51%; H, 3.01%; N, 23.15%; NMR $CDCl_3$: 4.27 (s, 2H, CH_2), 7.11 (s, 1H, Ar–NH), 8.64 (s, 1H, CSNH), 9.06 (s, 1H, CONH), 7.20–8.05 (m, 8H, Ar–H), 6.12 (s, 2H, Ar–NH_2), IR (KBr)/cm^{-1}: 3310 cm^{-1} (–NH_2–), 3172 cm^{-1} (–NH–), 1686 cm^{-1} (>C=O), 1616 cm^{-1} (–C=N–), 1215 cm^{-1} (C=S), 1333 cm^{-1} (–C–N), 1372, 1150 cm^{-1} (–SO_2).

Results and discussion

The goal of the present work was to synthesize purine derivatives bearing the alternative combination of an amide at the position 6 and an amine at position 2 from a readily available 2-amino, 6-chloro purine (Scheme 1). However, according to the literature (Zareef *et al.*, 2008) starting compound **1** reacted directly with sulfonyl chlorides in presence of pyridine and DMF, to get novel purine sulfonyl derivative (compound **2**). Compound **2** on reaction with glycine gave purinyl glycine derivative compound **3**. The product **3** was then treated with thionyl chloride to produce compound **4** which on subsequent treatment with ammonium thiocyanate according to literature (Mohd and Kumar 2004) gave compound **5** which on treatment with various benzothiazoles in acetone gave **6a–j**. Here the absence of a peak at 740 cm^{-1} for chloro group and the presence of the peak at 1200–1220 cm^{-1} for thioureido CS group in titled compounds and further evidenced by the signal for CSNH at 8.4–8.7 δ ppm. All the synthesized compounds were fully characterized by IR, ^{1}H-NMR spectroscopy, and elemental analysis.

Biological assays

Compounds

Test compounds were dissolved in DMF at an initial concentration of 40 mg ml^{-1} and then were serially diluted in culture medium.

Cells

Bacterial strains: *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumonia*. Fungal strain: *Aspergillus niger*.

Antibacterial assays

The minimum inhibitory concentrations (MICs) of the chemical compounds assays were carried out as described by Clause (1989) with minor modifications. References viz., ampicillin trihydrate and ciprofloxacin (antibacterial agent) and fluconazole (antifungal agent) were used. Solutions of the test compounds and reference drugs were dissolved in DMF at a conc. of 20 mg ml^{-1}. The twofold dilution of the compounds and reference drugs were prepared (40, 30, 20, 10, 05, etc.) mg ml^{-1}. Antibacterial activities of the bacterial strains were carried out in Muller–Hinton broth (Difco) medium, at pH 6.9, with an inoculum of (1–2) × 10^3 cells ml^{-1} by the spectrophotometric method and an aliquot of 100 µl was added to each tube of the serial dilution. The chemical compounds–broth medium serial tube dilutions inoculated with each bacterium were incubated on a rotary shaker at 37°C for 24 h at 150 rpm. The MICs of the chemical compounds were recorded as the lowest concentration of each chemical compounds in the tubes with no growth (i.e., no turbidity) of inoculated bacteria.

The results of the compounds of preliminary antibacterial testing shown in Table 1 revealed that three compounds of the obtained series owed high in vitro antimicrobial activity. Among the entire tested compounds, **6a** and **6d** showed excellent activity against *Kleb*; **6e** showed significant activity against *Bacillus subtilis* and displayed excellent activity against *fungus Aspergilus Niger*. Compound **6e** exhibited MIC value even less than that of standard drugs.

Table 1 MIC value of the synthesized compounds **6(a–j)**

Compound no.	MIC in mM				
	Antibacterial strains				Antifungal strains
	B. Subtilis	*S. Aureus*	*S. Typhi*	*Kleb*	*A. Niger*
6a	0.35	0.35	0.35	**0.17**	0.35
6b	0.51	0.51	0.51	0.51	0.51
6c	0.51	0.51	0.51	0.51	0.51
6d	0.36	0.53	0.36	**0.18**	0.36
6e	**0.18**	0.35	0.54	0.35	**0.18**
6f	0.35	0.52	0.53	0.35	0.35
6g	0.54	0.54	0.53	0.54	0.54
6h	0.51	0.51	0.51	0.51	0.51
6i	0.35	0.53	0.35	0.53	0.35
6j	1.29	0.56	0.56	0.55	0.55
Ampicillin	0.27	0.13	0.20	0.13	–
Ciprofloxacin	0.25	0.19	0.19	0.12	–
Fluconazole	–	–	–	–	0.35

Compounds in bold value have excellent activity against microorganism

The MIC values were determined as the lowest concentration that completely inhibited visible growth of the microorganisms. The probable reason for such behavior is the presence of electron-donating group specially halogenated and methoxy group on the benzothiazole ring in general which influences the antibacterial and antifungal activity compared to compounds with electron-withdrawing groups. Besides this, the position and presence of –NHCSNH– group in the connecting linker between the benzothiazole ring and purine ring seem to be very significant for antimicrobial effect.

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Mini Review

A Review on Synthesis of Silver NPS from Natural Source and their Applications

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Abstract

Silver Nanoparticles have gained an increasing attention especially in the field of bio medicinal applications, sensor and catalysis. Many techniques are available for synthesis of silver Nanoparticles like chemical reduction, ion sputtering etc., but many of the Nanoparticles synthesis methods involve use of hazardous chemicals or high energy requirements. Hence the popularity of 'Green' environment friendly synthesis of silver Nanoparticles is increasing. Greener synthesis of Nanoparticles also provides advancement over other methods as they are simple, one-step, cost-effective, environment friendly and relatively reproducible and often results in more stable materials. Thus, in the present review we critically assess the role of plants in synthesis of silver Nanoparticles.

Keywords: Silver nanoparticles; Green synthesis; Cost-effective

Introduction

Materials in the nano dimensions (1-100nm) have remarkable difference in the properties compared to the same material in the bulk. With advancement in nanotechnology, a large number of Nanomaterials are appearing with unique properties, opening spectrum of applications and research opportunities. The use of silver as an antibacterial agent is not new, but the application of silver as silver nanoparticles (AgNPs) against various microbes is new. The main reason is their high reactivity due to the large surface to volume ratio. Various methods have been used for the biosynthesis of inorganic material, especially metal Nanoparticles using microorganisms and plants. Silver Nanoparticles have many important applications.

AgNPs have been synthesized by physio-chemical techniques such as chemical reduction, gamma ray radiation, micro emulsion, electrochemical method, laser ablation, autoclave, microwave and photochemical reduction. These methods have effective yield, but they are associated with the limitations like use of toxic chemicals and high operational cost and energy needs. Considering the drawbacks of physio-chemical methods, cost-effective and energy efficient new alternative for AgNPs synthesis using microorganisms, plant extracts and natural polymers as reducing and capping agents are emerging very fast.

In comparison to microorganisms, the application of plant extracts for the synthesis of AgNPs is more advantageous in terms of resource availability, security, reaction rate and convenience, and feasibility of large-scale synthesis [1,2]. It has been proved that many plant extracts are suitable for the phyto-synthesis of AgNPs [3]. Several factors including pH, dosage of plant extract, dosage of silver ions, reaction temperature and time affect the phyto-synthesis of AgNPs [4,5]. The reduction rate of silver ions is associated with the species of plants and the key active components of plant extracts.

It was studied the green synthesis of silver nanoparticles (AgNPs) from the reduction of a silver nitrate solution (1 and 10mM) in the presence of an extract of basil leaves. The synthesized nanoparticles were characterized by UV visible spectroscopy (UV-vis), evidencing absorbance at wavelengths of 417nm (AgNPs-1) and 414nm (AgNPs10), which are characteristic peaks of this metallic nanoparticles. Scanning Electron Microscopy (SEM) was used to determine the size of the synthesized nanoparticles [6,7].

Conclusion

This review proved that the phytochemicals components present in the basil leaves/natural source extract can promote the formation of silver nanoparticles at room temperature with a uniform size distribution. This methodology is characterized by its easy performance and its low cost, which contributes to the environmental wealth that is achieved when no toxic compounds are used during the synthesis stage. Finally, the biosynthesised AgNPs were characterised by UV-visible absorption spectroscopy, SEM and TEM.

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SYNTHESIS OF NOVEL 1,2,4-TRIAZOLES AND THEIR SCHIFF BASE DERIVATIVES AND EVALUATING THEIR ANTIMICROBIAL ACTIVITY

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ABSTRACT

An efficient synthesis was carried out for synthesizing novel schiff base derivatives of 1,2,4-triazoles in a good yield from the reaction of 4-amino-5-(3-bromo-4-methoxyphenyl)-4,5-dihydro-3*H*-1,2,4-triazole-3-thiol with substituted aldehydes in methanol in presence of conc. HCl. The structures of these compounds were confirmed by IR, ^{1}H NMR & ^{13}C spectral analysis. The compounds were assayed by the disk diffusion method and broth dilution technique against B.Subtilis (*Gram +ve) (ATCC-6633)*,E.Coli (*Gram* -ve*) (ATCC-6538)* & C.Albicans *(ATCC-64550)*.

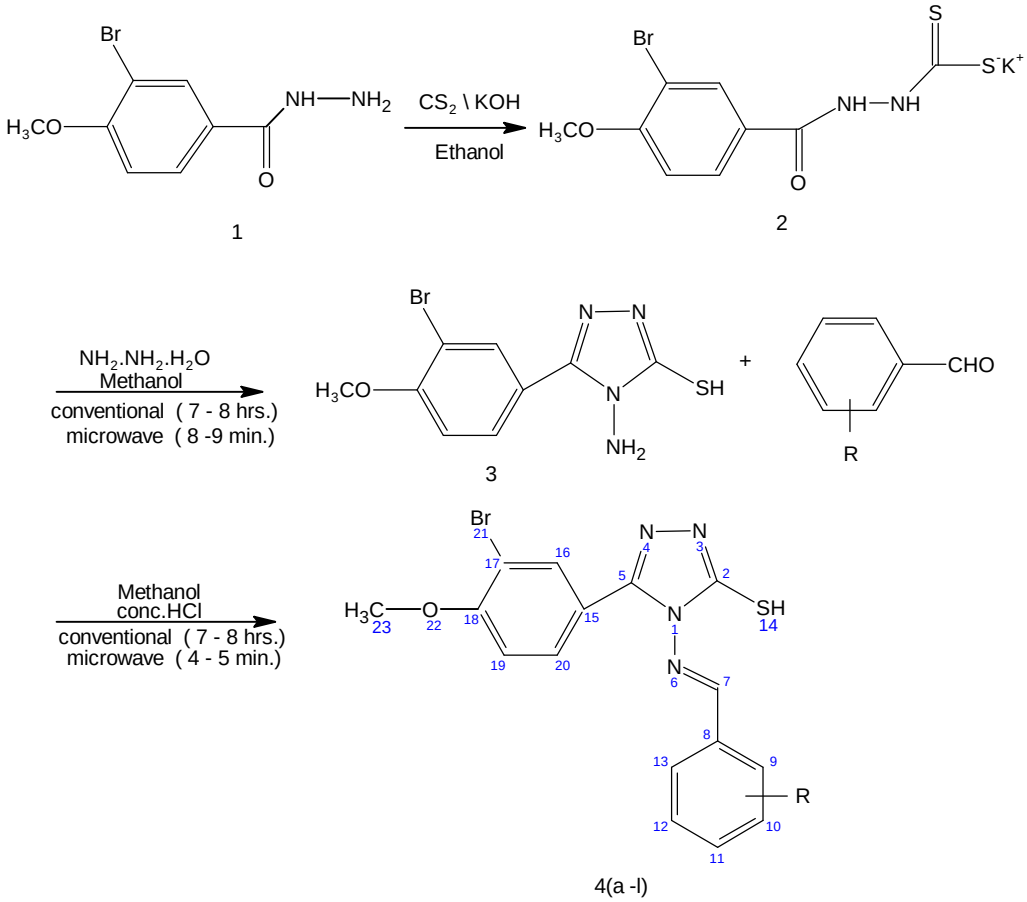
Keywords: Triazole, Schiff base, Minimum Inhibitory Concentration (MIC).

INTRODUCTION

3,5-Disubstituted 1,2,4-triazoles are found in several pharmacologically active compounds. Recent examples include the selective adenosine A_{2A} receptor antagonist [1] and the phosphodiesterase V inhibitor [2]. In particular, triazoles and their heterocyclic derivatives have been reported to be used as drugs and to have considerable biological activities (analgesic, anthelmintic, antitubercular, plant growth regulating, antiviral, antifungal and anticancer properties) [3-8].It is evident that in Schiff base motifs the –C=N- linkage is an essential structural requirement for the biological activity. Schiff base motifs are known to possess various important biological properties. Schiff base motifs documented as

antibacterial[9], antifungal[10], anticonvulsant[11], anti-cancer[12-13], antiviral[14-15] and various of pharmacological activity.

Attempt is made here to synthesize novel triazoles and their Schiff base derivatives (4a-l) and to adjudge their antimicrobial activities (Scheme 1)



	R		R
a	H	*g*	4-OCH_3
b	2-Cl	*h*	2-OH
c	3-OCH_3,4-OH	*i*	3-Cl
d	4-COOH	*j*	4-Cl
e	2-OH,4-$N(C_2H_5)_2$	*k*	3-F
f	4-NO_2	*l*	2-OCH_3

Scheme 1

MATERIALS AND METHODS

All reagents and solvents were of analytical grade and used directly. All the melting points were determined in open capillaries and are uncorrected. The purity of compounds was checked routinely by TLC (0.5 mm thickness) using silica gel–G coated Al–plates (Merck)

and spots were visualized by exposing the dry plates in iodine vapors. IR spectra (υ_{max} in cm^{-1}) were recorded on a Perkin Elmer spectrum BX series FT-IR spectrometer using KBr or Nujol technique. ^{1}H NMR spectra were recorded on a Bruker WM 400 FT MHz NMR instrument, using $CDCl_3$ or DMSO–d_6 as solvent and TMS as internal reference (chemical shifts in δ, ppm). ^{13}C NMR spectra were recorded on a Varian AMX 400 (100 MHz) spectrometer as solutions in $CDCl_3$. The elemental analysis (C, H, N) of compounds were performed on Carlo Erba–1108 elemental analyzer. Their results were found to be in good agreement with the calculated values.

Synthesis of 3-bromo-4-methoxy benzoic acid hydrazide: (1)

A mixture of 3-bromo-4-methoxy benzoyl chloride (0.01 mole, 2.49 g) in methanol (20 ml) and hydrazine hydrate (0.01mole,0.9 ml) were refluxed for 5-6 hrs. The excess solvent was distilled off and the solid product was filtered, dried and crystallized from ethanol. Yield 82 %, M.P. 175-180^{0}C.

Synthesis of Potassium 3-bromo-4-methoxy benzoyl dithiocarbamate: (2)

A mixture of potassium hydroxide (0.015 mole, 0.84 g) in absolute ethanol (25 ml), 3-bromo-4-methoxy benzoic acid hydrazide (0.01 mole, 2.45 g) and carbon disulfide (1.14 ml, 0.015 mole) were stirred for 10-12 hrs. The product was precipitated by adding diethyl ether (50 ml). The solid product was filtered, dried and crystallized from ethanol .Yield 75 %, M.P.153-156^{0}C: IR υ [cm^{-1}, KBr]: 3310 (N-H), 1639 (C=O), 1270 (C=S), 2845 (-OCH_3),763 (C-Br).

Synthesis of 4-amino-5-(3-bromo-4-methoxyphenyl)-4,5-dihydro-3H-1,2,4-triazole-3-thiol

A suspension of potassium 3-bromo-4-methoxy benzoyl di thiocarbamate (0.01 mole,3.59 g), hydrazine hydrate (95 %,0.02 mole) in methanol were refluxed with stirring for 8 hrs. The content was cooled and acidified with glacial acetic acid to get the product.The solvent was removed and solid product was recrystallised from absolute alcohol to get compound 3.Yield 78%,M.P.198-202^{0}C: IR [ν, cm^{-1}, KBr]: 3338 (N-H), 2542 (SH),1598 (C=N in triazole), 1298 (C-N in triazole), 1016 (N-N in triazole), 2845, 765 ; ^{1}H NMR [400MHz, δ, ppm, DMSO-d_6]: 5.01 (s,2H,NH_2), 7.06-7.36 (m,3H,Ar-H), 2.76 (s,1H,-SH), 3.96 (s, 3H,-OCH_3). Calcd. for: $C_9H_9BrN_4OS$ (301.16);(%):C,35.89; H,3.01; N,18.60; Found (%): C, 35.84; H, 2.96; N, 18.54.

Synthesis of 4-[(E)-benzylideneamino]-5-(3-bromo-4-methoxyphenyl)-4,5-dihydro-3H-1,2,4-triazole-3-thiol

In a 250 ml R.B.F., 4-amino-5-(3-bromo-4-methoxyphenyl)-4,5-dihydro-3*H*-1,2,4-triazole-3-thiol (0.01mole,3.01 g), benzaldehyde (0.01mole,1.06 ml) and few drops of conc.HCl were refluxed for 7-8 hrs. in methanol using Dean-Stark apparatus.After the completion of reaction,methanol was removed by distillation to give solid.The solid obtained was filtered, dried and crystallized from ethanol. Yellowish powder, Yield 65%,M.P.152-156^{o}C; Anal: Calcd. for: $C_{16}H_{13}BrN_4OS$ (389.26);(%):C,49.37; H,3.37; N,14.39; Found (%): C, 49.35; H, 3.35; N, 14.37; IR [ν, cm^{-1}, KBr]: 1670 (C=N of schiff base), 2541 (-SH), 1585 (C=N), 1295 (C-N) ,1016 (N-N) ,763 (C-Br),2842 (-OCH_3) ; ^{1}H NMR [400MHz, δ, ppm, DMSO-d_6]: 2.76 (s,1H,-SH), 7.00-7.62 (m,5H,Ar-H), 10.14 (s,1H,N=CH OF schiff base), 3.96 (s, 3H,-OCH_3);^{13}CNMR[100MHz,δ,ppm,DMSO-$d_6$or $CDCl_3$]: 112.7-157.0(C_1,C_2,C_3,C_4,C_5,C_6, aromatic), 148 (C_7&C_8), 128.9-133.8(C_9,C_{10},C_{11} C_{12},C_{13},C_{14}),156.9(C_{15} of Schiff base),55.1 (C_{16}),

RESULTS

4-[(*E*)-benzylideneamino]-5-(3-bromo-4-methoxyphenyl)-4,5-dihydro-3*H*-1,2,4-triazole-3-thiol (4a): Yellowish powder,M.P.152-156^{o}C; Anal. Calcd. for: $C_{16}H_{13}BrN_4OS$ (389.26);(%):C,49.37; H,3.37; N,14.39; Found (%): C, 49.35; H, 3.35; N, 14.37; IR [ν, cm^{-1}, KBr]: 1670 (C=N of schiff base), 2541 (-SH), 1585 (C=N), 1295 (C-N) ,1016 (N-N) ,763 (C-Br) ,2842 (-OCH_3) ; ^{1}H NMR [400MHz,δ,ppm, DMSO-d_6]: 2.76 (s,1H,-SH), 7.00-7.62 (m,5H,Ar-H), 10.14 (s,1H,N=CH of schiff base), 3.96 (s, 3H,-OCH_3); ^{13}C NMR [100 MHz, δ, ppm, DMSO-d_6 or $CDCl_3$]: 112.4-157.3 (C_1,C_2,C_3,C_4,C_5,C_6,aromatic), 148 (C_7&C_8), 128.9-133.8 (C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}), 156.9 (C_{15} of schiff base), 55.1 (C_{16}).

5-(3-bromo-4-methoxyphenyl)-4-[(*E*)-(2-chloro benzylidene)amino]-4,5-dihydro-3*H*-1,2,4-triazole-3-thiol (4b): Brown powder,M.P.162-163^{o}C; Anal. Calcd.for: $C_{16}H_{12}BrClN_4OS$ (423.71); (%):C,45.35; H,2.85; N,13.22; Found (%): C, 45.32; H, 2.84; N, 13.20; IR [ν, cm^{-1}, KBr]: 1672 (C=N of schiff base), 2542 (-SH), 1598 (C=N), 1298 (C-N) ,1017 (N-N) ,763 (C-Br), 728 (C-Cl) ,2845 (-OCH_3) ; ^{1}H NMR [400MHz, δ, ppm, DMSO-d_6]: 2.78 (s,1H,-SH), 7.00-7.64 (m,5H,Ar-H), 10.14 (s,1H,N=CH of schiff base), 3.95 (s,3H,-OCH_3); ^{13}C NMR [100 MHz,δ,ppm,DMSO-d_6 or $CDCl_3$]: 112.1-157.5 (C_1,C_2,C_3,C_4,C_5,C_6,aromatic), 148.1 (C_7&C_8), 127.2-133.6 (C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}), 156.9 (C_{15} of schiff base), 55.1 (C_{16}).

Table I Antimicrobial Inhibition Zone values

Table II Antimicrobial activities of compounds 4a-l (Zone of Inhibition)			
	Antibacterial activity in (μg/ml)		Antifungal activity in (μg/ml)
	Gram positive (+)	Gram positive (-)	
Compound No.	*B.s.* *(ATCC-6633)*	*E. c* *(ATCC-6538)*	*C.a* *(ATCC-64550)*
4a	+++	+++	++
4b	++++	++++	+++
4c	++	+++	+++
4d	+++	++	+++
4e	+++	+++	-
4f	+++	++	+++
4g	++++	+++	+++
4h	++	+++	+++
4i	+++	++++	+++
4j	+++	++++	+++
4k	++++	+++	+++
4l	++	++++	+++
Zone of Inhibition of Standard Drugs (μg/ml)			
Ampicillin	++++	++++	-
Fluconazole	-	-	++++
The inhibition diameter in mm: (-) < 6, (+) 7-9, (+ +) 10-15, (+ + +) 16-22, (+ + + +) 23-28.			
B. s – Bacillus substilis*; *E. c - Escherichia coli*; *C. a- Candida albicans.			

4-[(*E*)-{[3-(3-bromo-4-methoxyphenyl)-5-sulfanyl-3,5-dihydro-4*H*-1,2,4-triazol-4-yl]imino}methyl]-2-methoxyphenol (4c): Light yellow powder, M.P.158-161^{o}C; Anal. Calcd. for: $C_{17}H_{15}BrN_4O_3S$ (435.29);(%):C,46.91; H,3.47; N,12.87; Found (%): C, 46.89; H, 3.45; N, 12.86; IR [ν, cm^{-1}, KBr]: 1669 (C=N of schiff base), 2542 (-SH), 1595 (C=N), 1297 (C-N) ,1017 (N-N) ,767 (C-Br), 3425 (C-OH), 2845 (-OCH_3) ; ^{1}H NMR [400MHz, δ, ppm, DMSO-d_6]: 2.75 (s,1H,-SH), 7.00-7.62 (m,5H,Ar-H), 10.12 (s,1H,N=CH of schiff base), 5.5 (s,1H,OH), 3.96 (s, 3H,-OCH_3); ^{13}C NMR [100 MHz, δ, ppm, DMSO-d_6 or $CDCl_3$]: 112.7-156.8 (C_1,C_2,C_3,C_4,C_5,C_6,aromatic), 147.5 (C_7&C_8), 114.7-151.8 (C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}), 156.8 (C_{15} of schiff base), 55.1 (C_{16}), 56.2 (C_{17}).

4-[(*E*)-{[3-(3-bromo-4-methoxyphenyl)-5-sulfanyl-3,5-dihydro-4*H*-1,2,4-triazol-4-yl]imino}methyl]benzoic acid (4d): White powder,M.P.102-103^{o}C; Anal.Calcd. for: $C_{17}H_{13}BrN_4O_3S$ (433.27) ;(%):C,47.12; H,3.02; N,12.93; Found (%): C, 47.10; H, 3.01; N, 12.91; IR [ν, cm^{-1}, KBr]: 1671 (C=N of schiff base), 2540 (-SH), 1593 (C=N), 1298 (C-N)

,1016 (N-N) ,767 (C-Br), 2843 (-OCH_3);^{1}H NMR [400MHz, δ, ppm, DMSO-d_6]: 2.76 (s,1H,-SH), 7.03-7.68 (m,5H,Ar-H), 10.11 (s,1H,N=CH of schiff base), 3.95 (s, 3H,-OCH_3) ,12.75 (s,1H,COOH);^{13}C NMR [100MHz,δ,ppm,DMSO-d_6 or $CDCl_3$]:111.9-156.2 (C_1,C_2,C_3,C_4,C_5,C_6, aromatic), 148.2 (C_7&C_8), 129.2-139.0 (C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}), 156.5 (C_{15} of schiff base), 55.3 (C_{16}),169.3(C_{17}).

Table II MIC results of Schiff base derivatives.

Compound No.	MIC in mM		Antifungal Strains
	Antibacterial Strains		
	B. Subtilis	***E.Coli***	***C.Albicans***
4a	1.02	1.28	1.28
4b	0.47	0.70	1.04
4c	1.14	0.46	0.91
4d	0.92	1.15	0.92
4e	1.04	0.83	-
4f	0.92	1.15	0.70
4g	0.48	0.95	0.81
4h	1.23	0.98	1.18
4i	0.47	0.70	1.15
4j	0.47	0.70	0.94
4k	0.73	0.98	0.98
4l	0.48	0.71	0.71
Ampicillin	0.53	0.27	-
Ciprofloxacin	0.52	0.64	-
Fluconazole	-	-	0.35

2-[(*E*)-{[3-(3-bromo-4-methoxyphenyl)-5-sulfanyl-3,5-dihydro-4*H*-1,2,4-triazol-4-yl]imino}methyl]-5-(diethylamino)phenol (4e): Dark brown powder, M.P.124-125^{o}C ; Anal. Calcd. for: $C_{20}H_{22}BrN_5O_2S$(476.38);(%):C,50.42;H,4.65;N,14.70; Found(%) :C,50.39;H,4.62;N,14.67. IR [ν, cm^{-1}, KBr]: 1670 (C=N of schiff base), 2542 (-SH), 1592 (C=N), 1298 (C-N) ,1018 (N-N) ,765 (C-Br), 3430 (C-OH), 2845 (-OCH_3) ; ^{1}H NMR [400MHz, δ, ppm, DMSO-d_6]: 2.78 (s,1H,-SH), 7.00-7.74 (m,5H,Ar-H), 10.12 (s,1H,N=CH of schiff base), 5.4 (s,1H,OH),3.95(s,3H,-OCH_3),1.14(q,2H,2×CH_2),3.2(t,3H,2×CH_3);^{13}C NMR [100MHz,δ, ppm ,DMSO -d_6 or $CDCl_3$] :112.6-156.3 (C_1,C_2,C_3,C_4,C_5,C_6 ,aromatic), 148.5 (C_7&C_8), 109.2-169.1 (C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}), 156.9 (C_{15} of schiff base), 55.4 (C_{16}), 44.5 (C_{17} & C_{19}), 12.7 (C_{18} & C_{20}).

5-(3-bromo-4-methoxyphenyl)-4-[(*E*)-(4-nitrobenzylidene)amino]-4,5-dihydro-3*H*-1,2,4-triazole-3-thiol (4f): Yellow powder, M.P.138-140^{o}C; Anal. Calcd. for: $C_{16}H_{12}BrN_5O_3S$(434.26); (%) : C,44.25; H,2.79; N,16.13; Found (%): C, 44.23; H, 2.77; N,

16.11;IR [ν, cm^{-1}, KBr]: 1670 (C=N of schiff base), 2552 (-SH), 1592 (C=N), 1285 (C-N) , 1012 (N-N) ,762 (C-Br), 2845 ($-OCH_3$),1345($-NO_2$); ^{1}H NMR [400MHz, δ, ppm, DMSO-d_6]: 2.77 (s,1H,-SH), 7.05-7.64 (m,5H,Ar-H),10.10(s,1H,N=CH of schiff base),3.96 (s,3H,-OCH_3);^{13}C NMR[100MHz,δ, ppm,DMSO-d_6 or $CDCl_3$] :112.5-156.7 (C_1,C_2,C_3,C_4,C_5,C_6 ,aromatic), 148.4 (C_7&C_8), 124.2-150.3 (C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}), 156.7 (C_{15} of schiff base), 55.2 (C_{16}).

5-(3-bromo-4-methoxyphenyl)-4-[(*E*)-(4-methoxybenzylidene)amino]-4,5-dihydro-3*H*-1,2,4-triazole-3-thiol (4g): White powder,M.P.176-180^{o}C; Anal.Calcd. for: $C_{17}H_{15}BrN_4O_2S$ (419.29);(%):C,48.70; H,3.61; N,13.36; Found (%): C, 48.67; H, 3.58; N, 13.33; IR [ν, cm^{-1}, KBr]: 1662 (C=N of schiff base), 2554 (-SH), 1598 (C=N), 1297 (C-N) ,1016 (N-N) ,763 (C-Br), 2845 ($-OCH_3$) ; ^{1}H NMR [400MHz, δ, ppm, DMSO-d_6]: 2.75 (s,1H,-SH), 7.03-7.72 (m,5H,Ar-H), 10.11 (s,1H,N=CH of schiff base), 3.96 (s,3H,-OCH_3);^{13}C NMR [100MHz,δ,ppm,DMSO-d_6 or $CDCl_3$] :111.9-156.2 (C_1,C_2,C_3,C_4,C_5,C_6 ,aromatic), 148.2 (C_7&C_8),114.2-162.8(C_9,C_{10},C_{11},C_{12},C_{13},C_{14}),156.5(C_{15} of schiff base),55.3 (C_{16}),55.8(C_{17}).

2-[(*E*)-{[3-(3-bromo-4-methoxyphenyl)-5-sulfanyl-3,5-dihydro-4*H*-1,2,4-triazol-4-yl]imino}methyl]phenol (4h): Light yellow powder,M.P.143-145^{o}C; Anal. Calcd. for: $C_{16}H_{15}BrN_4O_2S$ (405.26);(%):C,47.42; H,3.23; N,13.82; Found (%): C, 47.41; H, 3.21; N, 13.80 ; IR [ν, cm^{-1}, KBr]: 1664 (C=N of schiff base), 2550 (-SH), 1592 (C=N), 1289 (C-N) , 1015 (N-N) ,764 (C-Br), 2845 ($-OCH_3$), 3421 (-OH) ; ^{1}H NMR [400MHz, δ, ppm, DMSO-d_6]: 2.76 (s,1H,-SH), 7.03-7.70 (m,5H,Ar-H), 10.12 (s,1H,N=CH of schiff base), 3.96 (s,3H,-OCH_3), 5.3 (s,1H,-OH);^{13}CNMR[100MHz,δ,ppm,DMSO-$d_6$or $CDCl_3$] :112.6-156.5 (C_1,C_2,C_3,C_4,C_5,C_6 ,aromatic), 148.4 (C_7&C_8), 116.3-161.7 (C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}), 156.9 (C_{15} of schiff base), 55.1 (C_{16}).

5-(3-bromo-4-methoxyphenyl)-4-[(*E*)-(3-chlorobenzylidene)amino]-4,5-dihydro-3*H*-1,2,4-triazole-3-thiol (4i): Yellow powder,M.P.154-155^{o}C; Anal. Calcd for: $C_{16}H_{15}BrClN_4OS$ (423.71);(%):C,45.35; H,2.85; N,13.22; Found (%): C, 45.33; H, 2.82; N, 13.21; IR [ν, cm^{-1}, KBr]: 1662 (C=N of schiff base), 2548 (-SH), 1590 (C=N), 1292 (C-N) , 1012 (N-N) ,764 (C-Br), 2855 ($-OCH_3$), 730 (C-Cl) ; ^{1}H NMR [400MHz, δ, ppm, DMSO-d_6]: 2.75 (s,1H,-SH), 7.04-7.69 (m,5H,Ar-H), 10.13 (s,1H,N=CH of schiff base), 3.95 (s,3H,-OCH_3); ^{13}C NMR [100MHz,δ,ppm,DMSO-d_6 or $CDCl_3$] :112.7-156.9 (C_1,C_2,C_3,C_4,C_5,C_6 , aromatic), 147.9 (C_7&C_8), 127.2-135.6 (C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}), 156.5 (C_{15} of schiff base), 55.3 (C_{16}).

5-(3-bromo-4-methoxyphenyl)-4-[(*E*)-(4-chlorobenzylidene)amino]-4,5-dihydro-3*H*-1,2,4-triazole-3-thiol (4j): White powder,M.P.131-135^{o}C; Anal. Calcd. for: $C_{16}H_{12}BrClN_4OS$ (423.71);(%):C,45.35; H,2.85; N,13.22; Found (%): C, 45.34; H, 2.81; N, 13.19; IR [ν, cm^{-1}, KBr]: 1661 (C=N of schiff base), 2548 (-SH), 1592 (C=N), 1293 (C-N) , 1014 (N-N) ,763 (C-Br), 2845 (-OCH_3), 738 (C-Cl) ; ^{1}H NMR [400MHz, δ, ppm, DMSO-d_6]: 2.79 (s,1H,-SH), 7.00-7.62 (m,5H,Ar-H), 10.14 (s,1H,N=CH of schiff base), 3.96 (s,3H,-OCH_3); ^{13}C NMR [100MHz,δ,ppm,DMSO-d_6 or $CDCl_3$] :112.9-156.2 (C_1,C_2,C_3,C_4,C_5,C_6 , aromatic), 148.5 (C_7&C_8), 128.7-137.2 (C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}), 156.6 (C_{15} of Schiff base), 55.1 (C_{16}).

5-(3-bromo-4-methoxyphenyl)-4-[(*E*)-(3-fluorobenzylidene)amino]-4,5-dihydro-3*H*-1,2,4-triazole-3-thiol (4k):Light green powder,M.P.123-125^{o}C; Anal. Calcd. for: $C_{16}H_{12}BrFN_4OS$ (407.26);(%):C,47.19; H,2.97; N,13.76; Found (%): C, 47.17; H, 2.95; N, 13.75.; IR [ν, cm^{-1}, KBr]: 1660 (C=N of schiff base), 2552 (-SH), 1593 (C=N), 1289 (C-N) , 1013 (N-N) ,764 (C-Br), 2845 (-OCH_3), 1126 (C-F) ; ^{1}H NMR [400MHz, δ, ppm, DMSO-d_6]: 2.78 (s,1H,-SH), 7.01-7.72 (m,5H,Ar-H), 10.13 (s,1H,N=CH of schiff base), 3.96 (s,3H,-OCH_3); ^{13}C NMR [100MHz,δ,ppm,DMSO-d_6 or $CDCl_3$] :112.3-156.6 (C_1,C_2,C_3,C_4,C_5,C_6 , aromatic), 148.2 (C_7&C_8), 114.2-163.4 (C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}), 156.5 (C_{15} of schiff base), 55.2 (C_{16}).

5-(3-bromo-4-methoxyphenyl)-4-[(*E*)-(2-methoxybenzylidene)amino]-4,5-dihydro-3*H*-1,2,4-triazole-3-thiol(4l): White powder,M.P.138-140^{o}C; Anal. Calcd. for: $C_{17}H_{15}BrN_4O_2S$ (419.29);(%):C,48.70; H,3.61; N,13.36; Found (%): C, 48.68; H, 3.59; N, 13.34. IR [ν, cm^{-1}, KBr]: 1660 (C=N of schiff base), 2550 (-SH), 1593 (C=N), 1290(C-N) ,1014 (N-N) ,763 (C-Br), 2845 (-OCH_3) ; ^{1}H NMR [400MHz, δ, ppm, DMSO-d_6]: 2.78 (s,1H,-SH), 7.01-7.73 (m,5H,Ar-H), 10.13 (s,1H,N=CH of schiff base), 3.95 (s,3H,-OCH_3); ^{13}C NMR [100MHz, δ,ppm,DMSO-d_6 or $CDCl_3$] :112.7-156.2 (C_1,C_2,C_3,C_4,C_5,C_6 ,aromatic), 148.5 (C_7&C_8), 114.2-160.1 (C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}), 156.9 (C_{15} of schiff base), 55.3 (C_{16}),55.8(C_{17}).

DISCUSSION

The titled compounds were prepared through the reaction sequences depicted in Scheme 1.Initial compound was prepared from available 3-bromo-4-methoxy benzoic acid.3-bromo-4-methoxy benzoyl chloride on reaction with hydrazine hydrate gave 4-methoxy benzoic acid hydrazide **(1).**Compound **1** on reaction with carbon disulfide in ethanolic potassium hydroxide gave potassium 3-bromo-4-methoxy benzoyl dithiocarbamate **(2).**Compound 2

was evidenced by appearance of bands at 3310,1639 & 1270 cm^{-1} for NH,C=O & C=S groups respectively**.** Compound 2 on reaction with hydrazine hydrate gave 4, N-amino-5-(3-bromo-4-methoxy phenyl)-4H-1,2,4-triazole-3-thiol **(3).** The S-H & NH_2 proton of compound 3 was observed at 2.76 & 5.01 ppm respectively which confirmed the formation of compound 3.Titled compounds were obtained from the reaction of compound 3 and various substituted aldehydes with hydrazine hydrate under reflux condition with methanol solution.The Schiff base derivatives **4(a-l)** were characterized by the presence of the methine protons (N=CH) at 10.10-10.14 ppm and in ^{13}C NMR spectra, signals were observed at 156.5-156.9 ppm, due to –CH=N– group also confirmed the formation of titled compounds**.**The data of all the compounds are given in the Experimental section.

Antimicrobial activity

The newly synthesized compounds were screened for their antibacterial activity against locally isolated ***Escherichia coli*** *(ATCC-6538)* and ***Bacillus Subtilis*** *(ATCC-6633)* bacterial strains and ***Candida albicans*** *(ATCC-64550)* , a fungal strain by the disc diffusion method [16]. Overnight incubated cultures of these bacteria were introduced onto the surface of sterile agar plates, and a sterile glass spreader was used for even distribution of the inoculum. The discs measuring 6.25 mm in diameter were prepared from Whatman No. 1 filter paper and sterilized by dry heat at 140 °C for an hour. The sterile discs previously soaked by the test compound dissolved in DMSO were placed on the inoculated nutrient agar medium. The plates were inverted and incubated for one day at 37 °C .**Ciprofloxacin, Ampicillin & Fluconazole** were used as standard drugs. Growth inhibition zones were measured and compared with the controls. The bacterial inhibition zone values are summarized in Table I.
Minimum inhibitory concentrations (**MIC**) were also determined by the broth dilution technique [17]. The Nutrient Broth, which contained logarithmic serially two fold diluted amount of test compound and controls, were inoculated with approx. 5 x 105 c.f.u. of actively dividing bacterial cells. The cultures were incubated for 24 h at 37 °C, and the growth was monitored visually and spectrophotometrically. The MIC are given in Table II.

Studies on the biological activity of compounds led to the fact that halogenated and methoxy substituted compounds have shown more activity compared to standard drugs in terms of MIC.Compounds **4b, 4g, 4i , 4j** & **4l** exhibit in general growth inhibition activity more relevant than that of the standard drug against *Bacilus subtilis,* while compounds **4i, 4j** & **4l** exhibit significant antibacterial activity against *E.Coli* compared to standard drug. Compound

4c exhibited the highest degree of inhibition against *E.Coli* which is higher than the standard drug ciprofloxacin. Compounds **4f** & **4l** showed highest activity against *Candida albicans.*

CONCLUSION

Data reveals that halogenated compounds have shown more activity compared to Ampicillin and Ciprofloxacin. Besides, when methoxy group is at meta position in compounds shown more activity compared to standard drug. By visualizing antifungal data seems that some of the compounds possess good activity against species. In general these compounds are found to possess more antibacterial activity than antifungal activity.

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Synthesis and Characterization of Polyurethane in Powder Form

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ABSTRACT

Polyurethanes (PUs) are defined as a class of versatile materials with great potential for use in different applications, especially based on their structure-property relationships. Their specific mechanical, physical, biological, and chemical properties are attracting significant research to tailor made PUs for various applications. Enhancement of the properties and performance of PU-based materials are achieved through altering the production process or the raw materials used in their fabrication or via the use of advanced characterization techniques. The present research article reports briefly general features of Polyurethane chemistry, production and applications. The article also presents synthesis and characterization of Powder Polyurethane using suspension and dispersion polymerization.

Keywords: powder polyurethane, isocyanates, polyol, suspension polymerization, dispersion polymerization,

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INTRODUCTION

Polyurethanes are polymers, which contains urethane groups in the main polymer chain. The polymers known as polyurethanes also include materials that incorporate the carbamate group. The name polyurethane originated from ethyl carbamate known as urethane. Polyurethanes are produced by the reaction of a poly-functional isocyanate with polyol, or other reactants containing two or more groups reactive with isocyanates.[6]

Typically polyurethane is produced by reaction of polyols and isocyanates and DABCO & DBTDL is generally used as catalyst. Paraffin oil is used as reaction medium. Generally Paraffin oil is used as a medium to solve the problem of heat dissipation. Improper heat dissipation may lead to run-away or may form gel like or rigid material. [3]

By selecting the proper isocyanate and polyols, or combination of isocyanates and combination of polyols, tailored structure can be obtained to achieve desired properties. It is therefore, necessary to know the relationship between the structure and properties. The flexibility to tailor the structure during processing itself is one of the biggest advantages of polyurethanes over other polymers.[4] Urethane groups are capable of forming strong hydrogen bonds among themselves and with different substrates. These strong intermolecular bonds make Polyurethanes useful for diverse applications in adhesives and coatings segments and also in elastomers and foams. Other great advantages of polyurethanes arise from the high reactivity of isocyanates, which readily reacts with a number of different functional groups. This allows polymerization reaction at relatively low temperatures and in short times (within several minutes) [7].

BRIEF HISTORY OF POLYURETHANES

Polyurethanes were invented in the 1930s by Professor Dr. Otto Bayer). There are various classes of polyurethanes, which have look and feel very different from each other. They are used in numerous and vast products, from coatings and adhesives to shoe soles, mattresses and foam insulation. However, the basic chemistry of each type is same.

Wide spread use of polyurethanes was initially seen during World War II, when they were utilized as a replacement for rubber, which was expensive and hard to obtain. During the war, other applications of Polyurethanes were also developed, largely involving coatings of different kinds, from Aircraft finishes to resistant clothing.

Till the 1950s, polyurethanes were mainly being used in adhesives, elastomers and rigid foams and, in the latter part of the same decade, flexible cushioning foams similar to those used today.

Subsequent decades saw rapid developments in Polyurethane usage and today we are surrounded by polyurethane applications in every aspect of our lives. Actually polyurethane is a product that most people are not familiar with, as it is 'hidden' behind covers or surfaces of other materials. However, it would be hard to imagine life without polyurethanes.

WHY POLYURETHANE?

- Polyurethane is available in different forms such as film, foam, powder, resins.
- They can be easily synthesized as soft as well as rigid foams.
- They can be modified as per the stoichiometric ratio of monomer or type of monomers.
- Production is economically feasible with great utility.
- It can be studied for encapsulation studies.
- Polyurethane has vast useful properties like hardness, tensile, flex etc. and used in numerous applications.
- Polyurethane is mostly replaced leather in hardwearing shoe soles, with excellent long-term mechanical properties and keep water out, and no way limiting design potential.

STRUCTURE OF POLYURETHANE

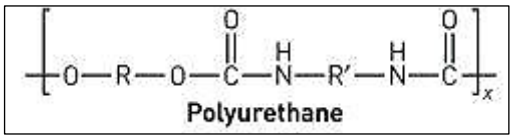


Fig. 1. *General Structure of polyurethane.*

BRIEF LITERATURE REVIEW

Witkiewicz and Andrzej Zieliński[14] have reported laboratory investigations made on the PU foams. These foams are considered to be used as a core material in production of the light barges for shallow waters. Elida BH [5], reported that the compatibilizing effect of poly(hexamethylene oxide) (PHMO) in the synthesis of polyurethanes based on α, ω-bis(6-hydroxyethoxypropyl) and poly(dimethylsiloxane)(PDMS). The hard segments of the polyurethanes were based on 4, 4'-methylenediphenyl diisocyanate (MDI) and 1, 4-butanediol. The main objective of this study was to develop PDMS macro-diol-based polyurethanes with mechanical properties comparable to conventional polyurethanes.

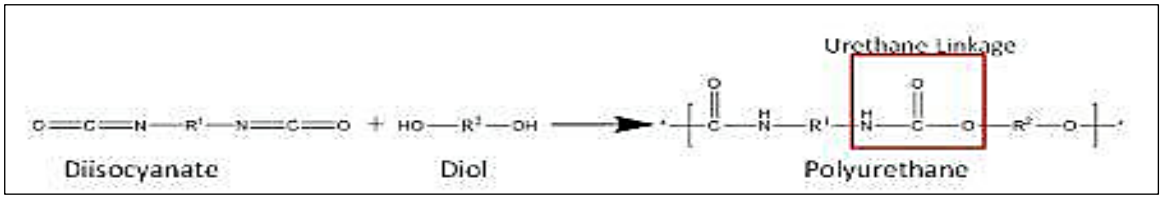


Fig. 2. *Reaction of polyurethane.*

NJ Sangeetha et al. [9] reported the recent methods and synthesis of polyurethanes derived from natural resources such as natural oil, natural fillers, natural fibers and other natural resources..

An article by C. Ciobanu et al. [2] reported variation of the stress–strain characteristics with lignin content and crosshead speed reveals different behavioral patterns. In all cases, the mechanical properties of blends are increased as com-pared with those of the pure polyurethane homo-polymer. The better properties obtained at 4.2% lignin concentration. However, the mechanical behavior of LPU blends indicates that lignin improves the polyurethane properties only when is incorporated in limited extent (no more than 10%, and preferably up to 5%).

Krishna V. Baratha et al. [8] , in a research article reported about the PU films which were prepared from a liquid Hydroxyl Telechelic Natural Rubber (HTNR) which was obtained through a controlled degradation. The mass percentage of the chain extenders added in the PU film was varied from 1 to 8%. The films were analyzed using FTIR and Raman spectroscopy. A Raman mapping was used to observe the phosphorus distribution in the PU film. It was concluded by the results that only with shorter chain lengths (n=13), a homogeneous distribution was observed.

Vincent Besseet et. al[13], presented synthesis of isocyanate free polyurethanes which is nowadays a major concern. The paper reported the synthesis of new bio base disosorbidedicyclocarbonates from isosorbide. Then polyhydroxyurethanes (PHUs) were synthesized by a cyclocarbonate–amine step growth poly-addition with four commercial diamines (e.g. jeffamine D-400, 1, 10 diaminodecane, diethylenetriamine and isophoronediamine). These products were characterized by H-NMR, FTIR, DSC and TGA analyses.

Albert Lee [1] reported the synthesis route for the preparation of polyurethane using 100% sustainable materials. Lignin was used as one raw material, while the other was soybean oil. The reaction occurs in 3 steps and it was done in 2 different pot reactions. Results indicated that the highest tensile strength achieved was 1.4 MPa, which is slightly below the typical tensile strengths of processible polyurethane.

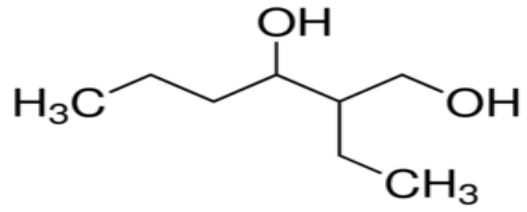
In an research article S.S. Ramanathan [12] reported a novel poly-condensable macro-diol with a long hydrophobic acrylate ester moiety and an amphiphilic block copolymer were successfully used in the particle forming polymerization of di-isocyanate and diol. The performance of the macro-diol and the block copolymer as stabilizers was found to be dependent both on its molecular weight & concentration. The particle size decreases as the concentration of the stabilizer increases and nearly monodisperse polyurethane microspheres are formed in nanometer size range in the case of macro-diol stabilizer. Further studies are in progress .

Zhang Li-Wei[15] in a review presented general routes for synthesis and important mechanical properties of pre-polymer of poly-(e-capro-lactone-co-L-lactic acid)-poly(ethylene glycol)-poly-(e-caprolac-tone-co-L-lactic acid) (PCLL-PEG-PCLL) which was synthesized via bulk ring-opening polymerization with PEG600 as an initiator and L-lactide (L-LA), e-caprolactone (CL) as monomers. This pre-polymer was chain-extended with an isocyanate-terminated urethane triblock to obtain the PUs. PU films containing long uniform-size hard segments had higher tensile strength than PU films extended with HDI and were expected to be more suitable for biomedical applications.

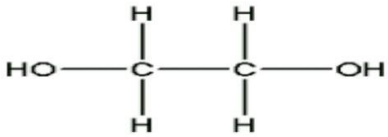
We now report the synthesis of Polyurethane in powder form. Following are the details of Raw Materials, Experimental set up, and processing conditions used.

MATERIALS AND APPARATUS REQUIRED FOR THE SYNTHESIS OF POLYURETHANE POWDER

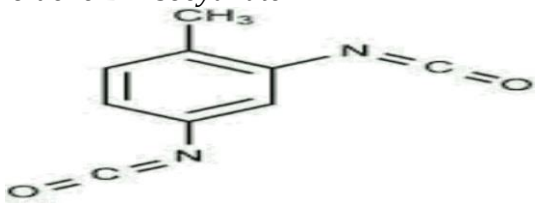
2-ethyl 1,3-Hexanediol



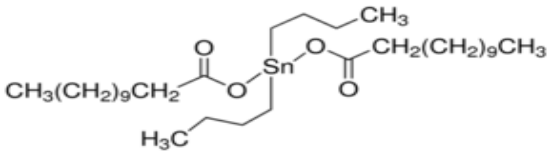
Ethylene Glycol



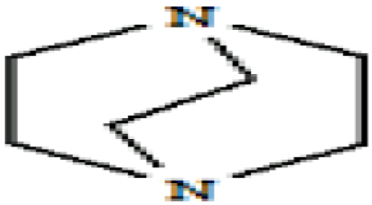
Toluene Di-isocyanate



Di-butyl tin di-laurate (DBTDL)



Diazo bicycle -2,2,2- octane (DABCO)



Paraffin oil: Molecular formula= C_nH_{2n+2} n=16~24

Experimental set up used following items for conducting the polymerization are enlisted as below:

1) Four neck flask with holding stand
2) Electrical Stirrer
3) Oil bath
4) Condenser
5) Nitrogen Cylinder for purging reaction kettle
6) Thermometer
7) Glass wares (measuring cylinder, beaker, rod etc.)

Synthesis of Powder Polyurethane using Dispersion and Suspension Polymerization

Polyurethanes (PUs) usually exhibit good mechanical properties and desirable blood compatibility, which are considered to be important characteristics for biomedical materials. For these advantages, PUs have been widely used in meniscal reconstruction, hemostatic sponge, vascular prosthesis and artificial skin.[11]

Activities in this phase include poly-condensation process by which PU powder or foam can be produce. Isocyanate and polyol on reaction with each other can form foam and powder.

The powder polyurethanes may find application in automobile industry as a PU sponge.

In laboratory PU powder was synthesized using suspension polymerization at 500 rpm to achieve desired particle size and granules of PU.

Intended use of this powder PU was in automobiles.

Effect of Various Ingredients on Yield of Powder PU

Effect of catalyst: It was concluded lower catalyst leads to longer reaction cycle time and in some cases powder did not form

and end products were crumbs or viscous liquid.

Effect of paraffin oil: Paraffin oil is used as a medium in synthesis of PU powder

Optimized recipes used to study the effect of reaction ingredients on the Yield % are tabulated in Table 1.

Table 1. *Data of recipe used for suspension polymerization for synthesis of powder polyurethane.*

Batch no.	TDI	Diol	Catalyst(1% DABCO)	Paraffin Oil	Yield
1	10.6 g	3 g	0.5 g	25 g	56%
2	10.6 g	3 g	0.5 g	40 g	74%
3	10.6 g	3 g	0.5 g	50 g	82%
4	10.6 g	3 g	0.5 g	60 g	86%

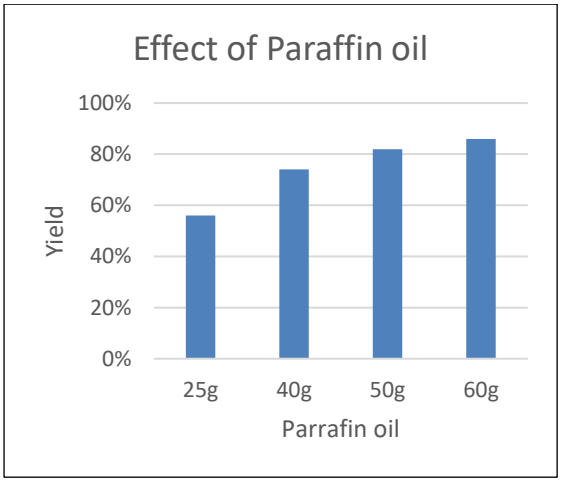


Fig. 3. *Effect of paraffinic oil concentration on yield% (suspension polymerization)*

Table 2. *Data of recipe used for dispersion polymerization for the synthesis of powder polyurethane.*

Synthesis no.	TDI	EHG	Catalyst(1% DBTDL)	Paraffin Oil	Yield
1	9.2 g	6 g	1 g	25 g	38%
2	9.2 g	6 g	1 g	40 g	54%
3	9.2 g	6g	1 g	50 g	66%
4	9.2 g	6g	1 g	60 g	78%
5	9.2 g	6g	1 g	75 g	81%
6	9.2 g	6g	1 g	100 g	82%

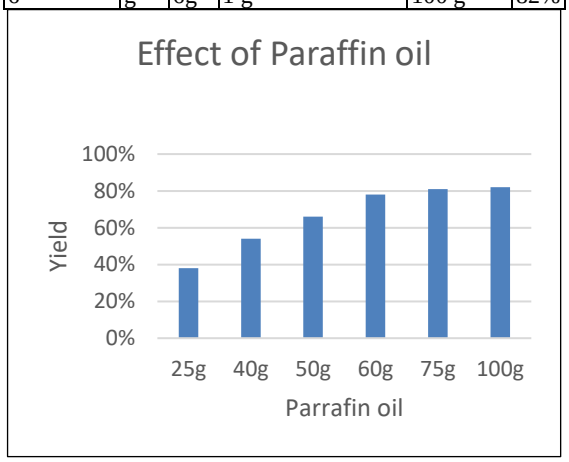


Fig. 4. *Effect of paraffinic oil on yield% (dispersion polymerization)*

Evaluation of Mechanical properties Powder PU Synthesized using Suspension and Dispersion Polymerization

Tensile strength of the various samples were determined on a UTM Machine and results are summarized Table 3.

Table 3.*Tensil Strength Values of PU Samples*

Sample No.	Tensile strength (MPa)
1	4.1
2	4.5
3	4.83
4	6
5	6.12
6	6.5

Melting point of Polyurethane powders

(1) Dispersion polymerization: 147ºC
(2) Suspension Polymerization: 158ºC

CONCLUSION

Polyurethanes synthesized in powder form using Dispersion and Suspension Polymerization techniques may find applications in Automobile, Printing Rollers, Solid PU plastic, TPU, Sealant, binders and Fiber Coating Etc.

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— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*
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(54) Title: PROCESS FOR RECOVERY OF SILVER CHLORIDE AND MERCURY SULPHIDE NANOPARTICLES FROM CHEMICAL OXYGEN DEMAND TEST WASTEWATER

(57) Abstract: The invention discloses a process for recovering silver chloride and mercury sulphide nanoparticles from a laboratory wastewater specifically chemical oxygen demand (COD) test wastewater

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"PROCESS FOR RECOVERY OF SILVER CHLORIDE AND MERCURY SULPHIDE NANOPARTICLES FROM CHEMICAL OXYGEN DEMAND TEST WASTEWATER"

Technical filed:

The invention relates to a process for recovering silver chloride and mercury sulphide nanoparticles from a laboratory wastewater. More particularly, the invention relates to a process for recovering silver chloride and mercury sulphide nanoparticles from chemical oxygen demand (COD) test wastewater.

Background and prior art:

The chemical oxygen demand (COD) test is commonly used to indirectly measure the amount of organic compounds in water. The COD test is performed to evaluate the biochemical oxygen requirement of organic compounds present in water to convert into CO_2 and H_2O. The COD test is applied to determine the amount of organic pollutants found in surface and ground waters thereby making the COD test method useful for the measurement of quality of water in any given sample that includes domestic and industrial samples. COD test method also determines the amount of oxygen required for the chemical oxidation of pollutants and also used to measure the amount of oxygen in water consumed for chemical oxidation of organic pollutants.

The basic principle of COD test is to completely oxidize the organic matter present in the waste water. There are different reagents added to the test solution viz., potassium dichromate is added as oxidizing agent, silver sulphate is added as a catalyst and mercury sulphate is added to reduce the interference of chloride ions. The method includes refluxing the sample with a known amount of potassium dichromate in presence of sulfuric acid, silver sulphate and mercury sulphate. After digestion of the test sample, the sample is analyzed and titrated with ferrous ammonium sulphate to measure unreacted dichromate. The dichromate consumed by the sample is considered as equivalent to the amount of oxygen required to

oxidize the organic matter. The wastewater generated from this test generally contains silver sulphate, mercury chloride and unreacted mercury sulphate. However, the prior art fails to address the recovery of these essential and expensive reagents from COD waste water, which can be recycled and reused for any other industrial purpose.

Therefore, the present inventors felt a need to address this issue by recovering the silver and mercury from the COD test waste water in the present invention, for which protection is sought.

Summary of the invention:

In line with the above objective, the present invention provides a cost-effective process for recovering silver chloride and mercury sulphide nanoparticles from a laboratory wastewater, specifically, chemical oxygen demand (COD) test waste water which comprises;

a) Treating the COD test waste water with sodium chloride solution to precipitate silver chloride; and
b) Isolating the silver chloride precipitate prior to treating the silver removed waste water with sodium sulphide solution to precipitate mercury sulphide.

Brief Description of drawing:

Figure 1 describes flow sheet of the process of the recovery of the silver chloride and mercury sulphide nanoparticles from chemical oxygen demand test wastewater
Figure 2 depicts SEM image of silver chloride nanparticles
Figure 3 depicts SEM image of mercury sulphide nanoparticles.

Detailed description of the invention:

The invention will now be described in detail in connection with certain preferred and optional embodiments, so that various aspects thereof may be fully understood and appreciated.

The COD waste water generally comprises silver sulphate, mercury chloride and mercury sulphate in addition to other metal ions such as iron and chromium.
It is observed by the present inventors that one liter of COD waste water comprises an amount of 3100 mg/l of silver and 2600 mg/l of mercury along with iron 500 mg/l and 450 mg/l chromium.

According to the process of the present invention, silver present in wastewater is precipitated in the first stage as fine grain powder in the form of silver chloride spherical nanoparticles by employing reagent A. After recovering silver chloride, the mercury present in the left over waste water is precipitated in the form of mercury sulphide nanoparticles, by employing reagent B.

In a preferred embodiment, the reagent A is sodium chloride and the reagent B is sodium sulphide, both are easily available and cheaper reagents.

Accordingly, the present invention provides a cost-effective process for recovering silver chloride and mercury sulphide nanoparticles from a laboratory wastewater specifically chemical oxygen demand (COD) test wastewater which comprises;

a) Treating the COD test waste water with sodium chloride solution to precipitate silver chloride; and
b) Isolating the silver chloride precipitate prior to treating the silver removed waste water with sodium sulphide solution to precipitate mercury sulphide.

In a preferred embodiment, COD waste water is treated with 1N solution of sodium chloride in the first stage to precipitate silver chloride in the form of nanoparticles. Accordingly, 1 N solution of NaCl is prepared and 1 liter of COD waste water is treated with 40 ml of 1N NaCl solution to recover 99.9 % of silver from the COD waste water in the form of silver chloride.

After the recovery of the precipitated silver chloride by filtration, the left over COD waste water is treated with Sodium sulphide. Accordingly, 1 N solution of Sodium

sulphide is prepared and used to precipitate mercury sulphide in the form of nanoparticles. 4.7 gm of mercury sulphide per litre of COD waste water was recovered which is equivalent to 99.9% mercury sulphide removal from wastewater.

The treatment of waste water according to the invention is conducted at ambient temperature.

In a specific embodiment, the treatment of both the stages is conducted at ambient temperature. Ambient temperature for the purpose the present invention may be considered as the temperature in the range of 25 to 35°C.

In a specific embodiment, the treatment is conducted under stirring at 100 to 500 rpm.

The size of the silver chloride and mercury sulphide nanoparticles are in the range of 50-80nm and 30-50 nm, respectively. The size of the particles is confirmed with SEM images of the particles as shown in figures 2 and 3 respectively.

Other features and embodiments of the invention will become apparent by the following examples which are given for illustration of the invention rather than limiting its intended scope. Various changes and modifications to the disclosed embodiments will be apparent to those skilled in the art.

Examples

Example: 1

Silver chloride recovery

To recover silver chloride, 1 N solution of NaCl was prepared and 40 ml of NaCl was added to 1 liter of waste water under stirring at 200 rpm at room temperature to recover 99.9 % of silver in the form of silver chloride nano particles.

The amount of NaCl present in 40ml of 1N solution was about 2.32 gm, which is used to recover 99.9% silver present in one liter of COD wastewater. The chemical equation is shown below:

$$Ag_2SO_4 + 2NaCl \longrightarrow 2AgCl + Na_2SO_4$$

Silver Sulphate Sodium chloride Silver chloride Sodium sulphate

By mole balance 1 mole of silver sulphate reacts with 2 moles of sodium chloride and produces 2 moles of silver chloride. 1 mole of silver sulphate has 311.79 gm and will react with 2 moles of sodium chloride (116.88gm) and will produce (286 gm) 2 moles of silver chloride. This is a theoretical calculation.

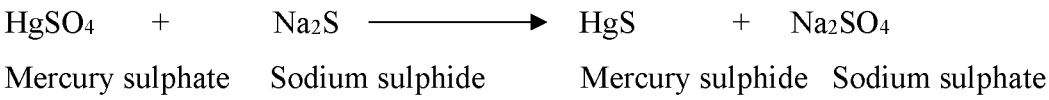
In the current experiment, 2.32 gm of NaCl was used in one liter of wastewater and 2.6 gm of silver chloride was recovered. It is quite in agreement with theoretical analysis.

Mercury sulphide recovery

After recovery of silver chloride, the wastewater was treated for mercury sulphide recovery. Sodium sulphide of 1 N solution was prepared and used to precipitate mercury sulphide. 40 ml of 1 N solution of sodium sulphide was used to precipitate mercury sulphide in one liter of wastewaterunder stirring at 200 rpm at room temperature to recover 99.9 % of mercury in the form of mercury sulphide nano particles.

The amount of sodium sulphide present in 40 ml of 1 N solution is about 3.2gm. The treatment of 1 liter COD waste water with 40 ml of 1 N solution of sodium sulphide results in precipitation of 4.7 gm of mercury sulphide and by quantitative analysis it is 99.9 % recovery.

4.7gm of mercury sulphide per litre was recovered that results in 99.9% mercury removal from wastewater.



According to chemical reaction 1 mole of mercury sulphate reacts with 1 mole of sodium sulphide and equal mole of mercury sulphide is generated. 1 mole of mercury sulphate carries 296 gm of mercury sulphate and makes 232 gm of mercury sulphide with 78 gm of sodium sulphide in equal moles. The experimental result are in agreement with theoretical prediction.

Recovery of both Reagents was carried out at room temperature under 200 rpm stirring. The pH of the wastewater was observed 0.01before and after recovery of reagents. COD of the wastewater was 3000 mg/l before recovery and 1300 mg/l after recovery of reagents.

After precipitation, both the reagents were washed with distilled water to remove excess acidic reagent and dried separately in air and further dried in oven at 60 ^{o}C for 2 hrs.

Industrial advantage of the present invention

The process for recovery of silver chloride and mercury sulphide from chemical oxygen demand test generated wastewater to recover silver chloride and mercury sulphide is simple and cost-effective. The recovery of these two valuable laboratory reagents provides great industrial advantage as both the reagents are expensive and can be put to reuse.

We claim,

1. A cost-effective process for recovering silver chloride and mercury sulphide nanoparticles from a laboratory wastewater specifically chemical oxygen demand (COD) test wastewater comprising;
   c) Treating the COD test waste water with sodium chloride solution to precipitate silver chloride; and
   d) Isolating the silver chloride prior to treating the silver removed waste water with sodium sulphide solution to precipitate mercury sulphide.

2. The process according to claim 1, wherein, the treatment of waste water in step a) is conducted at ambient temperature.

3. The process according to claim 1, wherein, the treatment of waste water in step a) is conducted at ambient temperature.

4. The process according to claim 1, wherein, thetreatment is conducted under stirring at 100 to 500 rpm.

5. The process according to claim 1, wherein, the silver chloride is obtained in spherical nanoparticles.

6. The process according to claim 1, wherein, the mercury sulphide is obtained in nanoparticles.

7. The process according to claim 1, wherein, the size of the silver chloride and mercury sulphide nanoparticles are in the range of 50-80nm and 30-50 nm, respectively.

8. The process according to claim 1, wherein, the solution of sodium chloride and sodium sulphide is used as 1N solution.

9. The process according to claim 1, wherein, the pH of the waste water is the same before and after recovery of sodium chloride and mercury sulphide.

10. The process according to any one of the preceding claims, COD test waste water comprises silver sulphate, mercury chloride and mercury sulphate.

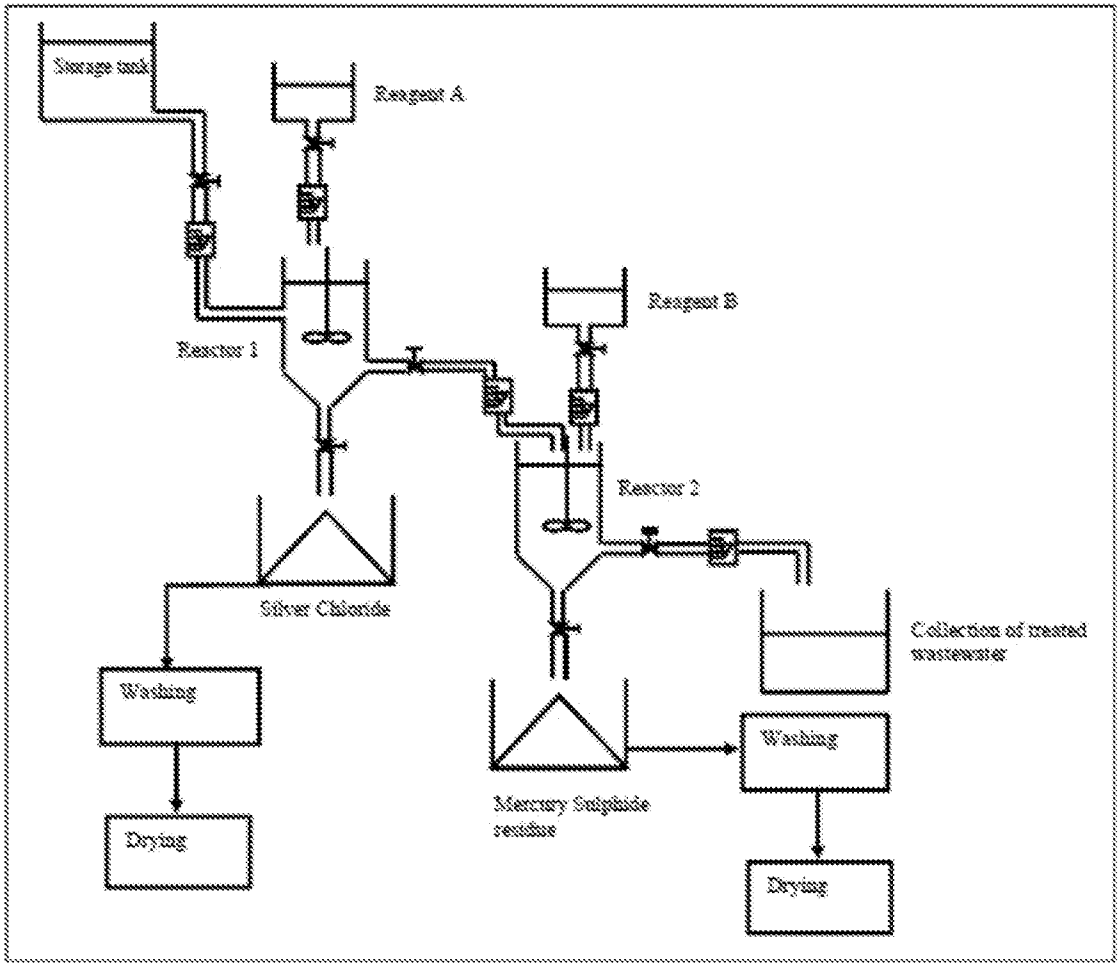

Storage tank
Reagent A
Reagent B
Reactor 1
Reactor 2
Silver Chloride
Washing
Drying
Mercury Sulphide residue
Collection of treated wastewater
Washing
Drying

Figure 1

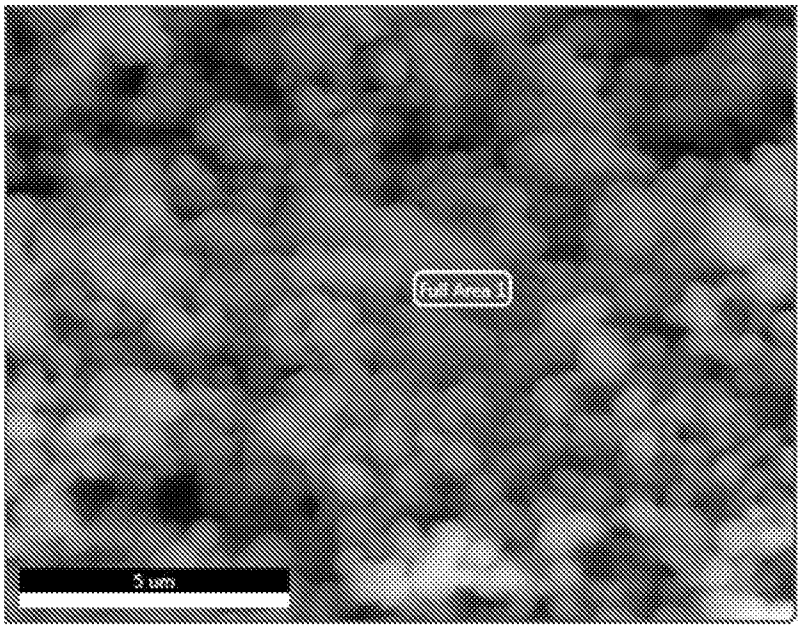


Figure 2

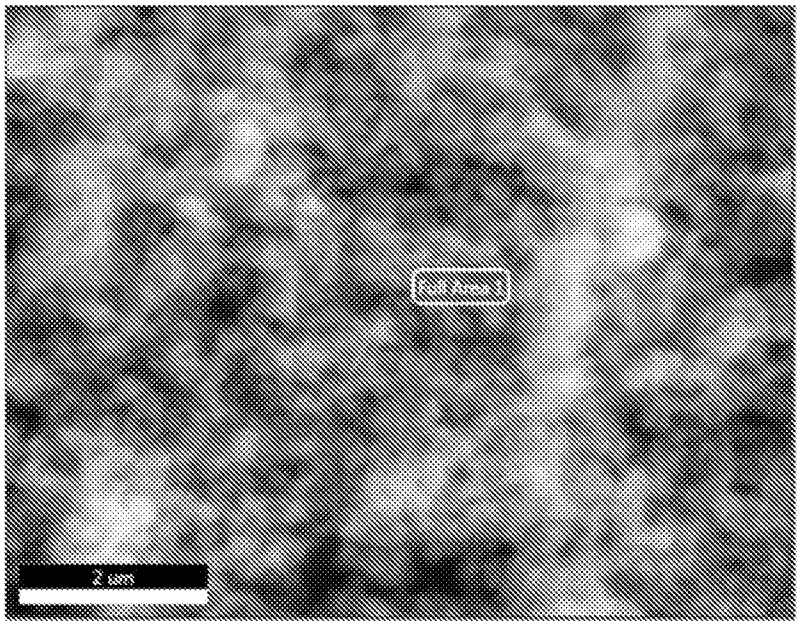


Figure 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IN2018/050650

A. CLASSIFICATION OF SUBJECT MATTER
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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C02F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

TotalPatent One, IPO Internal Database

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CN104211243 A (SICHUAN YUECHENG ENVIRONMENTAL PROT ENERGY SAVING SCIENCE & TECHNOLOGY CO LTD) 17 DECEMBER 2014 (17-12-2014) abstract, para [0029]- [0031]	1-10

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
07-01-2019	07-01-2019
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INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
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CN 104211243 A	17-12-2014	CN 104211243 B	01-06-2016
		CN 201410517278	30-09-2014