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Synthesis of Some Bioactive Sulfonamide and Amide Derivatives of Piperazine Incorporating Imidazo[1,2-B]Pyridazine Moiety

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Abstract

Some new sulfonamide and amide derivatives containing piperazine ring and imidazo[1,2-b]pyridazine moiety have been synthesized by the reaction of 6-chloro-2-substituted aryl(or alkyl)imidazo[1,2-b]pyridazine derivatives [obtained by the reaction of 3-amino-6-chloro pyridazine with 2-bromo-1-substituted aryl(or alkyl)ethanone] with homopiperazine in NMP and followed by reaction with alkyl (or substituted aryl) acid chloride or sulfonyl chloride in presence of triethyl amine and dichloromethane. All the synthesized compounds were characterized by elemental analysis, ^{1}H NMR and LCMS. These were screened for *in vitro* antimicrobial activity against two gram positive (*Bacillus subtilis* and *Staphylococcus aureus*) and two gram negative bacteria (*Pseudomonas fluorescens* and *Escherichia coli*), as well as for antifungal and antimalarial activity.

Keywords: Imidazo[1,2-b]pyridazine; Homopiperazine; Antimicrobial and antimalarial activity

Introduction

Sulfonamide and amide derivatives comprise an important class of drugs with diverse biological applications. Sulfonamides are widely used as antimicrobial [1,2], anticancer [3,4], anti-inflammatory [5] and antiviral agents as well as HIV protease inhibitors [6]. Sulfonamides were the first effective chemotherapeutic agents to be utilized efficiently to prevent and cure the bacterial infection in human beings [7-10].

The piperazine moiety appears in many drugs encompassing a broad range of activities (e.g., Oxatomide, Almitrine, Hydroxyzine, Buclizine, Lomerizine [11]. Nitrogen in piperazine ring plays an important role in exerting biological effects. The basicity of piperazine nitrogen plays an important role in selectivity and potency towards the biological targets. This moiety (monoaryl and diarylpiperazine) also found in drug candidates displaying anti-allergenic [12], antibacterial [13], anti-anxiety [14], anti-emetic [15], antimigraine [16] and platelet anti-aggregatory activities [17]. In addition, piperazine moiety is present in many cardiovascular drugs [18] (e.g., Manidipine, Doxazosin, Trimetazidine, Flunarizine, Prazosin) and drug candidates [19,20]. Piperazine and their derivatives also possess antimalarial activity [21], antioxidative activity [22] and antifungal activity [23] and found in many drug molecules such as Meclizine (motion sickness drug), Cyclizine (antiemetic and antihistamine), Clozapine (antipsychotic drug), Imatinib (leukemia drug), Befuraline (stimulant and antidepressant), Antrafenine (analgesic), Trazodone (sedating antidepressant) and Niaprazine (sedating antihistamine) etc.

Piperazine and sulfonamide derivatives represent a category of pharmacologically interesting compounds having diverse biological activities. Intensive research has been carried out on the synthesis and analysis of pharmacological activities of these derivatives. Substituted sulfonamide derivatives are important category of pharmacophores that have a wide spectrum of pharmaceutical accomplishments: as antimalarial [24], anti-microbial [25], anti-bacterial [26,27], anti-cancer [28] anti-fungal [29], anti-oxidant [30], anti-HIV [31], antiplasmodial [32], anti-neoplastic [33], anti-proliferative [34] activities and additionally known to act as 5-HT6, 5-HT7 receptor antagonists [35,36], A2B and CXCR3 antagonists [37,38],11β-HSD [39], histone deacetylase (HDAC) inhibitors [40], β-secretase (BACE1) inhibitors [41] and dual PI3K/mTOR inhibitors [42].

The chemistry of pyridazines and their fused heterocyclic derivatives has received considerable attention owing to their synthetic and effective biological importance. Pyridazines have been reported to possess antimicrobial [43-45], antituberculosis [46-48], antifungal [49], anticancer [50], anti-hypertensive [51], herbicidal [52], anti-inflammatory [53] activities and protein tyrosine phosphatise 1B(PTP1B) inhibitors [54]. They also have an immense potential in agricultural science as plant growth regulators and crop protection agents [55]. The incorporation of two moieties increases biological activity of both and thus it was of value to synthesize some new heterocyclic derivatives having two moieties in the same molecules.

Several imidazo [1,2-b] pyridazine derivative have demonstrated biological activity including inhibitors of the central nervous system [56], antipyritic and hypothermal activity [57], anticonvulsant activity, analgesic and antispasmotic activity [58-60].

Looking at the importance of these heterocyclic nuclei, it is thought of interest to accommodate sulphonamide and amide of piperazine with imidazo[1,2-b] pyridazine moieties in single molecular framework and screen them for their various biological activities.

Materials and Methods

General procedures

Reagent grade chemicals were used without further purification. All the melting points were taken in open capillaries and are uncorrected. The purity and mass of the synthesized compounds was checked by LCMS. 1H NMR spectral was recorded in $CDCl_3$ /DMSO with tetramethylsilane (TMS) as the internal standard at 400 MHz on a Bruker DRTX-400 spectrophotometer. The chemical shifts are reported as parts per million (ppm). Elemental analysis was performed using a (EURO EA 3000 instrument). Acme silica gel-G and Merck silica gel (100 to 200, 60 to 120 meshes) were used for analytical TLC and Column chromatography respectively

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Chemistry

We have prepared some novel sulfonamide or amide derivatives of piperazine ring incorporating imidazo[1,2-b]pyridazine moiety in three steps, using 3-amino-6-chloro pyridazine, 2-bromo-1-substituted aryl(or alkyl)ethanone, homopiperazine and alkyl (or substituted aryl) acid chloride or sulfonyl chloride as the starting materials. 3-amino-6-chloro pyridazine were treated with 2-bromo-1-substituted aryl(or alkyl)ethanone in ethanol to obtain 6-chloro-2-substituted aryl(or alkyl)imidazo[1,2-b]pyridazine 1(a-d) which on reaction with homopiperazine in NMP results 6-(piperazin-1-yl)-2-substituted aryl(or alkyl)imidazo[1,2-b]pyridazine 2(a-d) and further by reacting with alkyl (or substituted aryl) acid chloride or sulfonyl chloride in presence of triethyl amine in Dichloromethane results the desired sulfonamide or amide derivatives. The clear procedure for the preparation of desired sulfonamide or amide derivatives are given below.

Preparation of novel sulfonamide and amide derivatives of piperazine incorporating imidazo[1,2-b]pyridazine moiety

General procedure for the synthesis of 6-chloro-2-substituted aryl(or alkyl)imidazo[1,2-b]pyridazine: To a solution of 3-amino-6-chloro pyridazine (0.01 mole) in ethanol (10 mL) was added 2-bromo-1-substituted aryl (or alkyl) ethanone at room temperature. Then the reaction mixture was refluxed at 80°C for 4 hrs. The reaction mixture was then cooled and poured into ice-cold water. The resulting precipitate was filtered, washed several times with water, dried and recrystallized from ethanol.

Spectral data of intermediate

6-chloro-2-(trifluoromethyl)imidazo[1,2-b]pyridazine 1(a): ^{1}H-NMR (400 MHz, $CDCl_3$): 9.05 (s, 1H, Imidazo-H), 8.36 (d, J=9.6 Hz,1H, pyridazine-H), 7.59 (d, J=9.6 Hz,1H, pyridazine-H). MS: 222.3 (M^+). Anal. Calcd for $C_7H_3ClF_3N_3$: C- 37.95%, H- 1.36%, Cl-16.00%, F-25.72%, N-18.96. Found: C- 37.85%, H- 1.33%, Cl-15.96%, F-25.70%, N-18.92.

6-chloro-2-p-tolylimidazo[1,2-b]pyridazine 1(b): ^{1}H-NMR (400 MHz, $CDCl_3$): 8.85 (s, 1H, Imidazo-H), 8.20 (d, J=9.6 Hz,1H, pyridazine-H), 7.93 (d, J=8.4 Hz, 2H, Ar-H), 7.35 (d, J=9.6 Hz, 1H, pyridazine-H), 7.28 (d, J=8.0 Hz,2H, Ar-H), 2.34 (s,3H, Ar-CH_3). MS: 244.4 (M^+). Anal. Calcd for $C_{13}H_{10}ClN_3$: C- 64.07%, H- 4.14%, Cl-14.55%, N-17.24. Found: C- 64.04%, H- 4.11%, Cl-14.52%, N-17.21%.

6-chloro-2-(4-(trifluoromethyl)phenyl)imidazo[1,2-b]pyridazine 1(c): ^{1}H-NMR (400 MHz, $CDCl_3$): 9.05 (s, 1H, Imidazo-H), 8.26-8.23 (m, 3H), 7.83 (d, J=8.0 Hz, 2H, Ar-H), 7.41 (d, J=9.6 Hz, 1H, pyridazine-H). MS: 298.2 (M^+). Anal. Calcd for $C_{13}H_7ClF_3N_3$: C-52.45%, H- 2.37%, Cl-11.91%, F-19.15%, N-14.12%. Found: C- 52.42%, H- 2.33%, Cl-11.89%, F-19.14%, N-14.09%.

6-chloro-2-(2,5-dichlorophenyl)imidazo[1,2-b]pyridazine 1(d): ^{1}H-NMR (400 MHz, $CDCl_3$): 8.92 (s, 1H, Imidazo-H), 8.30 (d, J=9.2 Hz,1H, pyridazine-H), 8.21-8.20 (m, 1H, Ar-H), 7.64 (d, J=8.8 Hz, 1H, Ar-H), 7.51-7.46 (m, 2H). MS: 298.4 (M^+). Anal. Calcd for $C_{12}H_6Cl_3N_3$: C- 48.28%, H- 2.03%, Cl-35.62%, N-14.07%. Found: C- 48.25%, H-1.99%, Cl-35.60%, N-14.03%.

General procedure for the synthesis of 6-(piperazin-1-yl)-2-substituted aryl(or alkyl)imidazo[1,2-b]pyridazine

The mixture of 6-chloro-2-substituted aryl(or alkyl)imidazo[1,2-b] pyridazine (0.01 mole) and homopiperazine (0.05 mole) in NMP (5 mL) was heated at 150°C for 1 hr. The reaction mixture was diluted with water and extracted with ethyl acetate. The organic layer was washed with water, brine and dried over Na_2SO_4. The solvent was evaporated and crude compound was used as such for next step without any purification.

Spectral data of intermediate

6-(piperazin-1-yl)-2-(trifluoromethyl)imidazo[1,2-b]pyridazine 2(a): ^{1}H-NMR (400 MHz, $CDCl_3$): 8.50 (s,1H, Imidazo-H), 7.91 (d, J=10.0 Hz,2H, pyridazine-H), 7.37 (d, J=10.0 Hz,2H, pyridazine-H), 3.43-3.41 (m, 4H, piperazine-H), 3.34 (bs, 1H, NH), 2.80-2.78(m, 4H, piperazine-H), MS:272.6 (M^+). Anal. Calcd for $C_{11}H_{12}F_3N_5$: C- 48.71%, H- 4.46%, F-21.01%, N-25.82%. Found: C-48.67%, H- 4.42%, F-20.98%, N-25.79%.

6-(piperazin-1-yl)-2-p-tolylimidazo[1,2-b]pyridazine 2(b): ^{1}H-NMR (400 MHz, $CDCl_3$): 8.40 (s,1H, Imidazo-H), 7.83-7.80 (m,3H), 7.21 (d, J=8.0 Hz,2H, Ar-H), 7.15 (d, J=10.0 Hz,1H, pyridazine-H), 3.39-3.37 (m, 4H, piperazine-H), 3.34 (bs, 1H, NH), 2.82-2.79(m,4H, piperazine-H), 2.31 (s,3H, Ar-H). MS: 294.4 (M^+). Anal. Calcd for $C_{17}H_{19}N_5$: C- 69.60%, H- 6.53%, N-23.87%. Found: C- 69.58%, H-6.51%, N-23.83%.

6-(piperazin-1-yl)-2-(4-(trifluoromethyl)phenyl)imidazo[1,2-b]pyridazine 2(c): ^{1}H-NMR (400 MHz, $CDCl_3$): 8.02 (s,1H, Imidazo-H), 7.97 (d, J=8.4 Hz,2H, Ar-H), 7.80 (d, J=10.0 Hz,1H, pyridazine-H), 7.62 (d, J=8.4 Hz, 2H, Ar-H), 7.30 (d, J=10.0 Hz,1H, pyridazine-H), 3.40-3.37 (m, 4H, piperazine-H), 3.34 (bs, 1H, NH), 2.81-2.78(m,4H, piperazine-H). MS: 348.1 (M^+). Anal. Calcd for $C_{17}H_{16}F_3N_5$: C- 58.78%, H- 4.64%, F-16.41%, N-20.16%. Found: C- 58.76%, H- 4.61%, F-16.37%, N-20.13%.

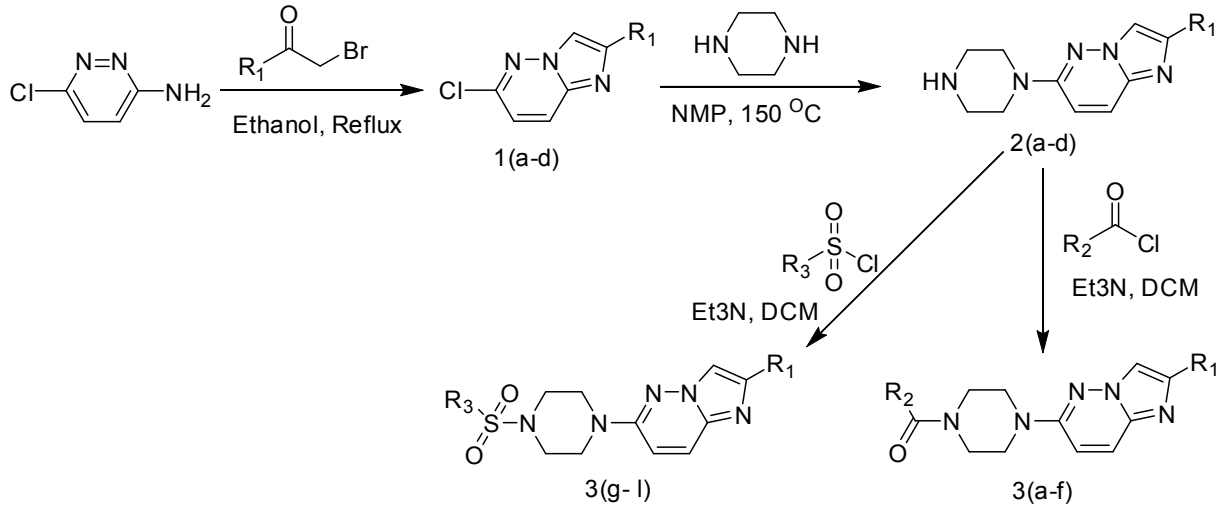
2-(2,5-dichlorophenyl)-6-(piperazin-1-yl)imidazo[1,2-b]pyridazine 2(d): ^{1}H-NMR (400 MHz, $CDCl_3$): 8.46 (s,1H, Imidazo-H), 8.20 (d, J=2.4 Hz,1H, Ar-H), 7.89 (d, J=10.0 Hz,1H, pyridazine-H), 7.57 (d, J=8.8 Hz,1H, Ar-H), 7.39 (dd, J1=8.6 Hz, J2=2.6Hz,1H, Ar-H), 7.27 (d, J=10.0 Hz,1H, pyridazine-H), 3.43-3.40 (m, 4H, piperazine-H), 3.33 (bs, 1H, NH), 2.82-2.79(m,4H, piperazine-H). MS: 348.5 (M^+). Anal. Calcd for $C_{16}H_{15}Cl_2N_5$: C- 55.19%, H- 4.34%, Cl-20.36%, N-20.11%. Found: C- 55.16%, H- 4.31%, Cl-20.33%, N-20.09%.

General procedure for the conversion of 6-(piperazin-1-yl)-2-substituted aryl(or alkyl)imidazo[1,2-b]pyridazine into alkyl (or substituted aryl) sulfonamide or amide 3(a-j)

To a solution of 6-(piperazin-1-yl)-2-substituted aryl(or alkyl) imidazo[1,2-b]pyridazine (0.01 mole) in Dichloromethane was added triethyl amine (0.015 mole) followed by alkyl (or substituted aryl) acid chloride or sulfonyl chloride (0.013 mole) at 0°C and stirred the reaction mixture at room temperature for 2 hrs. The reaction mixture was diluted with water and extracted with Dichloromethne. The organic layer was washed with water, brine and dried over Na_2SO_4. The solvent was evaporated and crude compound was purified by using column chromatography with 100-200 silica gel to give compound 3(a-j) (Scheme 1).

Spectral data of Desired sulfonamide and amide derivatives

1-(4-(2-(trifluoromethyl)imidazo[1,2-b]pyridazin-6-yl)piperazin-1-yl)propan-1-one 3(a): ^{1}H-NMR (400 MHz, $CDCl_3$): 8.54 (s,1H, Imidazo-H), 7.97 (d, J=10.0Hz,1H, pyridazine-H), 7.41(d, J=10.0 Hz,1H, pyridazine-H), 3.58-3.51 (m, 8H, piperazine-H), 2.37 (q, J=7.4 Hz, 2H, -$COCH_2$), 1.00 (t, J=7.4 Hz,3H,-CH_3). LCMS: 328.4 (M^+), Purity-97.5%. Anal. Calcd for $C_{14}H_{16}F_3N_5O$: C- 51.37%, H-4.93%, F-17.41%, N-21.40%, O-4.89%. Found: C- 51.33%, H- 4.91%, F-17.39%, N-21.37%, O-4.87%.



R_1 = CF_3, $4CH_3$- phenyl, 4-CF_3 phenyl, 2,4-Dichloro phenyl

R_2 = Ethyl, p- phenyl, 4-CF_3 phenyl

Scheme 1

p-tolyl(4-(2-(trifluoromethyl)imidazo[1,2-b]pyridazin-6-yl) piperazin-1-yl)methanone 3(b): ^{1}H-NMR (400 MHz, $CDCl_3$): 7.93 (s,1H, Imidazo-H), 7.78 (d, J=10.0 Hz,1H, pyridazine-H), 7.35 (d, J=8.0 Hz, 2H, Ar-H), 7.24 (d, J=8.0 Hz,2H, Ar-H), 6.95(d, J=10.0 Hz,1H, pyridazine-H), 3.88-3.56 (m, 8H, piperazine-H), 2.40 (s,3H, Ar-CH_3). LCMS: 390.2 (M^+), Purity-98.2%. Anal. Calcd for $C_{19}H_{18}F_3N_5O$: C- 58.61%, H- 4.66%, F-14.64%, N-17.99%, O-4.11%, Found: C- 58.58%, H- 4.63%, F-14.61%, N-17.96%, O-4.09%.

(4-(2-(trifluoromethyl)imidazo[1,2-b]pyridazin-6-yl)piperazin-1-yl)(4-(trifluoromethyl) phenyl) methanone (3c): ^{1}H-NMR (400 MHz, $CDCl_3$): 7.94 (s,1H, Imidazo-H), 7.81 (d, J=10.0 Hz,1H, pyridazine-H), 7.72 (d, J=8.0 Hz, 2H, Ar-H), 7.57 (d, J=8.0Hz,2H, Ar-H), 6.94 (d, J=10.0 Hz,1H, pyridazine-H), 3.95-3.56 (m, 8H, piperazine-H). LCMS: 444.3 (M^+), Purity-95.7%. Anal. Calcd for $C_{19}H_{15}F_6N_5O$: C- 51.47%, H- 3.41%, F-25.71%, N-15.80%, O-3.61%, Found: C- 51.44%, H- 3.39%, F-25.68%, N-15.77%, O-3.57%.

1-(4-(2-p-tolylimidazo[1,2-b]pyridazin-6-yl)piperazin-1-yl)propan-1-one (3d): ^{1}H-NMR (400 MHz, $CDCl_3$): 7.97 (s,1H, Imidazo-H), 7.80 (d, J=8.0 Hz,2H, Ar-H), 7.75 (d, J=10.0 Hz,1H, pyridazine-H), 7.24 (d, J=8.4 Hz, 2H, Ar-H), 6.79 (d, J=9.6 Hz,1H, pyridazine-H), 3.80-3.71 (m,2H, piperazine-H), 3.64-3.62 (m,2H, piperazine-H), 3.55-3.53 (m,2H, piperazine-H), 3.49-3.47 (m,2H, piperazine-H), 2.41 (q, J=7.4 Hz, 2H,-$COCH_2$), 2.38 (s,3H, Ar-CH_3), 1.19 (t, J=7.4 Hz, 3H,-CH_3). LCMS: 350.3 (M^+), Purity-96.4%. Anal. Calcd for $C_{20}H_{23}N_5O$: C- 68.47%, H- 6.63%, N-20.04%, O-4.58%, Found: C-68.45%, H- 6.60%, N-20.01%, O-4.54%.

1-(4-(2-(4-(trifluoromethyl)phenyl)imidazo[1,2-b]pyridazin-6-yl)piperazin-1-yl)propan-1-one (3e): ^{1}H-NMR (400 MHz, $CDCl_3$): 8.05 (s,1H, Imidazo-H), 8.01 (d, J=8.4 Hz,2H, Ar-H), 7.77 (d, J=10.0 Hz,1H, pyridazine-H), 7.67 (d, J=8.4 Hz, 2H, Ar-H), 6.85 (d, J=9.6 Hz,1H, pyridazine-H), 3.81-3.78 (m,2H, piperazine-H), 3.64-3.63 (m,2H, piperazine-H), 3.57-3.55 (m,2H, piperazine-H), 3.52-3.49 (m, 2H, piperazine-H), 2.41 (q, J=7.4 Hz, 2H,-$COCH_2$), 1.19 (t, J=7.4 Hz, 3H,-CH_3). LCMS: 404.2 (M^+), Purity-98.4%, Anal. Calcd for $C_{20}H_{20}F_3N_5O$: C- 59.55%, H- 5.00%, F-14.13%, N-17.36%, O-3.97%, Found: C-59.53%, H- 4.97%, F-14.11%, N-17.33%, O-3.94%.

1-(4-(2-(2,5-dichlorophenyl)imidazo[1,2-b]pyridazin-6-yl) piperazin-1-yl)propan-1-one (3f): ^{1}H-NMR (400 MHz, $CDCl_3$): 8.44 (s,1H, Imidazo-H), 8.26 (d, J=2.4 Hz,1H, Ar-H), 7.77 (d, J=10.0 Hz,1H, pyridazine-H), 7.38 (d, J=8.4 Hz, 1H, Ar-H), 7.20 (dd, J1=8.4 Hz, J2=2.4 Hz,1H, Ar-H), 6.87 (d, J=10.0Hz,1H, pyridazine-H),3.81-3.78 (m,2H, piperazine-H), 3.64-3.62 (m,2H, piperazine-H), 3.58-3.56 (m,2H, piperazine-H), 3.52-3.49 (m,2H, piperazine-H), 2.41 (q, J=7.4 Hz, 2H,-$COCH_2$), 1.19 (t, J=7.4 Hz, 3H,-CH_3). LCMS: 404.3 (M^+), Purity-99.07%, Anal. Calcd for $C_{19}H_{19}Cl_2N_5O$: C- 56.45%, H- 4.74%, Cl-17.54%, N-17.32%, O-3.96%, Found: C-56.42%, H- 4.71%, Cl-17.52%, N-17.29%, O-3.93%

6-(4-(ethylsulfonyl)piperazin-1-yl)-2-(trifluoromethyl) imidazo[1,2-b]pyridazine (3g): ^{1}H-NMR (400 MHz, $CDCl_3$): 7.95 (s,1H, Imidazo-H), 7.79 (d, J=10.0 Hz,1H, pyridazine-H), 6.93(d, J=10.0 Hz,1H, pyridazine-H), 3.65-3.62 (m, 4H, piperazine-H), 3.46-3.43 (m, 4H, piperazine-H), 3.00 (q, J=7.4 Hz, 2H, -SO_2CH_2), 1.40 (t, J=7.4 Hz,3H,-CH_3). LCMS: 364.2 (M^+), Purity-97.39%. Anal. Calcd for $C_{13}H_{16}F_3N_5O_2S$: C- 42.97%, H- 4.44%, F-15.69%, N-19.27%, O-8.81%, S-8.82%. Found: C-42.93%, H- 4.41%, F-15.66%, N-19.25%, O-8.79%, S-8.78%

6-(4-tosylpiperazin-1-yl)-2-(trifluoromethyl)imidazo[1,2-b] pyridazine (3h): ^{1}H-NMR (400 MHz, $CDCl_3$): 7.90 (s,1H, Imidazo-H), 7.73 (d, J=10.0 Hz,1H, pyridazine-H), 7.66 (d, J=8.0 Hz, 2H, Ar-H), 7.34 (d, J=8.0 Hz,2H, Ar-H), 6.84 (d, J=10.0 Hz,1H, pyridazine-H), 3.64-3.61 (m, 4H, piperazine-H), 3.15-3.12 (m, 4H, piperazine-H), 2.42 (s,3H, Ar-CH_3). LCMS: 426.2 (M^+), Purity-99.73%. Anal. Calcd for $C_{18}H_{18}F_3N_5O_2S$: C- 50.82%, H- 4.26%, F-13.40%, N-16.46%, O-7.52%, S-7.54%, Found: C- 50.79%, H- 4.23%, F-13.37%, N-16.43%, O-7.49%, S-7.51%.

2-(trifluoromethyl)-6-(4-(4-(trifluoromethyl)phenylsulfonyl) piperazin-1-yl)imidazo[1,2-b] pyridazine (3i): ^{1}H-NMR (400 MHz, $CDCl_3$): 7.93-7.91 (m,3H), 7.83 (d, J=8.0 Hz, 2H, Ar-H), 7.75 (d, J=10.0 Hz,1H, pyridazine-H), 6.84 (d, J=10.0 Hz,1H, pyridazine-H), 3.66-3.64 (m, 4H, piperazine-H), 3.21-3.19 (m, 4H, piperazine-H). LCMS: 480.3 (M^+), Purity-94.54%. Anal. Calcd for $C_{18}H_{15}F_6N_5O_2S$: C- 45.10%, H- 3.15%, F-23.78%, N-14.61%, O-6.67%, S-6.69%. Found: C- 45.09%, H- 3.13%, F-23.75%, N-14.59%, O-6.65%, S-6.66%.

6-(4-(ethylsulfonyl)piperazin-1-yl)-2-p-tolylimidazo[1,2-b] pyridazine (3j): ^{1}H-NMR (400 MHz, $CDCl_3$): 7.97 (s,1H, Imidazo-H), 7.80 (d, J=8.0 Hz,2H, Ar-H), 7.75 (d, J=10.0 Hz,1H, pyridazine-H), 7.24 (d, J=8.4 Hz, 2H, Ar-H), 6.78 (d, J=10.0 Hz,1H, pyridazine-H), 3.62-3.60 (m,4H, piperazine-H), 3.46-3.43 (m,4H, piperazine-H), 3.00 (q, J=7.4 Hz, 2H,-SO_2CH_2), 2.38 (s,3H, Ar-CH_3), 1.40 (t, J=7.4 Hz, 3H,-CH_3). LCMS: 386.3 (M^+), Purity-96.27%. Anal. Calcd for $C_{19}H_{23}N_5O_2S$: C- 59.20%, H- 6.01%, N-18.17%, O-8.30%, S-8.32%, Found: C-59.18%, H- 6.00%, N-18.14%, O-8.28%, S-8.29%.

6-(4-(ethylsulfonyl)piperazin-1-yl)-2-(4-(trifluoromethyl) phenyl)imidazo[1,2-b]pyridazine (3k):

^{1}H-NMR (400 MHz, $CDCl_3$): 8.06 (s,1H, Imidazo-H), 8.00 (d, J=8.0 Hz,2H, Ar-H), 7.78 (d, J=10.0 Hz,1H, pyridazine-H), 7.67 (d, J=8.0 Hz, 2H, Ar-H), 6.84 (d, J=10.0 Hz,1H, pyridazine-H), 3.63-3.62 (m,4H, piperazine-H), 3.47-3.45 (m,4H, piperazine-H), 3.00 (q, J=7.2 Hz, 2H,-SO_2CH_2), 1.41 (t, J=7.4 Hz, 3H,-CH_3). LCMS: 440.3 (M^+), Purity-92.91% Anal. Calcd for $C_{19}H_{20}F_3N_5O_2S$: C- 51.93%, H- 4.59%, F-12.97%, N-15.94%, O-7.28%, S-7.30%, Found: C-51.91%, H- 4.55%, F-12.94%, N-15.91%, O-7.25%, S-7.28%.

2-(2,5-dichlorophenyl)-6-(4-(ethylsulfonyl)piperazin-1-yl) imidazo[1,2-b]pyridazine (3l):

^{1}H-NMR (400 MHz, $CDCl_3$): 8.44 (s,1H, Imidazo-H), 8.26 (d, J=2.80 Hz,1H, Ar-H), 7.78 (d, J=10.0 Hz,1H, pyridazine-H), 7.38 (d, J=8.8 Hz, 1H, Ar-H), 7.20 (dd, J1=8.8 Hz, J2=2.4 Hz,1H, Ar-H), 6.85 (d, J=10.0 Hz,1H, pyridazine-H),3.65-3.62 (m,4H, piperazine-H), 3.46-3.44 (m,4H, piperazine-H), 3.00 (q, J=7.4 Hz, 2H,-SO_2CH_2), 1.40 (t, J=7.4 Hz, 3H,-CH_3). LCMS: 440.5 (M^+), Purity-98.9%, Anal. Calcd for $C_{18}H_{19}Cl_2N_5O_2S$: C- 49.10%, H- 4.35%, Cl-16.10%, N-15.90%, O-7.27%, S-7.28%, Found: C-49.08%, H- 4.32%, Cl-16.07%, N-15.88%, O-7.24%, S-7.25%.

Antimicrobial activity

All the synthesized compounds were tested against two gram positive bacteria (*Staphylococcus aureus*, *Streptococcus pyogenes*) and two gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa*) using micro broth dilution method [61-63] for the determination of minimal inhibition concentration. For the antifungal activity the common standard strains that were used, are *C. albicans*, *A. niger* and *A. clavatus*. Muller Hinton broth (Microcare laboratory and Tuberculosis Research Centre, Surat-3, India) was used as nutrient medium to grow and dilute the drug suspension for the test bacteria. Inoculum Size for Test Strain was adjust to 10^8 Cfu [Colony Forming Unit] per milliliter by comparing the turbidity. DMSO was used as diluents / vehicle to get desired concentration of drugs to test upon Standard bacterial strains. Serial dilutions were prepared in primary and secondary screening. In primary screening 1000 micro/ml, 500 micro/ml, and 250 micro/ml concentrations of the synthesized compounds were taken. The active synthesized compounds found in this primary screening were further tested in a second set of dilution against all microorganisms. The highest dilution showing at least 99% inhibition zone is taken as MIC. The test mixture should contain 10^8 organism/ml. Standard drugs Ampicillin and Chloramphenicol were used as antibacterial for comparison. Standard drugs Nystatin and Greseofulvin were used as antifungal for comparison.

Antimalarial activity

The *in vitro* antimalarial assay was carried out in 96 well microtitre plates according to the microassay protocol reference. The cultures of *P. falciparum* strain were maintained in medium RPMI 1640 supplemented with 25 mM HEPES, 1% D-glucose, 0.23% sodium bicarbonate and 10% heat inactivated human serum. The asynchronous parasites of *P. falciparum* were synchronized after 5% D-sorbitol treatment to obtain only the ring stage parasitized cells. For carrying out the assay, an initial ring stage parasitaemia of 0.8 to 1.5% at 3% haematocrit in a total volume of 200 µl of medium RPMI-1640 was determined by Jaswant Singh Bhattacharya (JSB) staining to assess the percent parasitaemia (rings) and uniformally maintained with 50% RBCs (O^+). A stock solution of 5 mg/ml of each of the test samples was prepared in DMSO and subsequent dilutions were prepared with culture medium. The diluted samples in 20 µl volume were added to the test wells so as to obtain final concentrations (at five fold dilutions) ranging between 0.4 µg/ml to 100 µg/ml in duplicate well containing parasitized cell preparation. The culture plates were incubated at 37°C in a candle jar. After 36 to 40 h incubation, thin blood smears from each well were prepared and stained with JSB stain. The slides were microscopically observed to record maturation of ring stage parasites into trophozoites and schizonts in presence of different concentrations of the test agents. The test concentration which inhibited the complete maturation into schizonts was recorded as the minimum inhibitory concentrations (MIC). Quinine was taken as the reference drug.

Results and Discussion

Chemistry

3-amino-6-chloro pyridazine on reaction with 2-bromo-1-substituted aryl(or alkyl)ethanone in ethanol gives 6-chloro-2-substituted aryl(or alkyl)imidazo[1,2-b]pyridazine 1(a-d). The obtained compound (1) on reaction with homopiperazine in NMP results 6-(piperazin-1-yl)-2-substituted aryl(or alkyl)imidazo[1,2-b] pyridazine 2(a-d) which on further reaction with alkyl (or substituted aryl) acid chloride or sulfonyl chloride gives sulfonamide or amide derivatives of piperazine ring incorporating imidazo[1,2-b]pyridazine moiety 3(a-l). The list of synthesized compound are represented by Table 1.

Antibacterial activity

The antibacterial activity of all the synthesized compounds were tested *in-vitro* against pathogenic *E. coli*, *P. aeruginosa*, *S. aureus* and *S. pyogenus* and the results were compared with standard drugs (Ampicillin and Chloramphenicol). In case of *S. aureus*, Compound 3b shows higher activity and compounds 3a, 3c, 3e, 3f, 3j, and 3l exhibit good activity while 3d, 3g, 3h, 3i and 3k show moderate activity. In case of *S. pyogenus* compounds 3c, 3d and 3f exhibit good activity and compound 3b show moderate activity while 3a, 3b, 3e, 3g, 3h, 3i, 3j, 3k and 3l possess less activity. In case of *E. coli* Compound 3d exhibit higher activity and compounds 3f, 3j show moderate activity while rest of the compounds possess less activity. In case of *P. aeruginosa* compounds 3e, 3k and 3l exhibit good activity than the rest of the compounds. The results are given in Table 2.

Antifungal activity

The antifungal activity of all the synthesized compounds were tested *in-vitro* against fungi *C. albicans*, *A. niger* and *A. clavatus* and the results were compared with standard drugs (Nystatin and Greseofulvin). In case of *C. albicans* compound 3a exhibit higher activity and 3c, 3e, 3h, 3i, and 3l, show good activity while compounds rest of the compoun possess less activity. In case of *A. niger* and *A. clavatus* all the compounds possess less activity. The results are given in Table 3.

Antimalarial activity

For antimalarial activity, Compounds 3a, 3c and 3k exhibit good activity closer to reference compound Quinine while rest of the compounds possess less activity. The results are given in Table 4.

Compound	R1	R2	M.P	Yield (%)
3a	CF3	C2H5	117	46.2
3b	CF3	4CH3- phenyl	156	52.3
3c	CF3	4CF3- phenyl	124	49.5
3d	4CH3- phenyl	C2H5	172	58.3
3e	4CF3- phenyl	C2H5	228	55.7
3f	2,5- Dichloro phenyl	C2H5	152	65.3
3g	CF3	C2H5	196	63.4
3h	CF3	4CH3- phenyl	188	58.4
3i	CF3	4CF3- phenyl	207	48.9
3j	4CH3- phenyl	C2H5	218	40.2
3k	4CF3- phenyl	C2H5	202	61.3
3l	2,5- Dichloro phenyl	C2H5	221	39.4

Table 1: List of Synthesized compound.

Compound	*S. aureus*	*S. pyogenus*	*E. coli*	*P. aeruginosa*
3a	100	200	200	200
3b	62.5	125	200	250
3c	100	100	200	200
3d	250	100	62.5	125
3e	125	200	125	100
3f	100	100	100	200
3g	250	200	125	250
3h	250	200	250	500
3i	200	200	250	250
3j	100	250	100	250
3k	250	200	250	100
3l	100	200	250	100
Ampicillin	250	100	100	100
Chloramphenicol	50	50	50	50

Table 2: Antibacterial activity of sulfonamide and amide derivatives (In MIC).

Compound	*C. albicans*	*A. niger*	*A. clavatus*
3a	250	>1000	>1000
3b	1000	1000	1000
3c	500	250	500
3d	1000	>1000	>1000
3e	500	>1000	>1000
3f	1000	500	1000
3g	1000	>1000	>1000
3h	500	500	250
3i	500	500	250
3j	1000	1000	1000
3k	>1000	500	500
3l	500	>1000	>1000
Nystatin	100	100	100
Greseofulvin	500	100	100

Table 3: Antifungal activity (in MIC).

Conclusion

All the newly synthesized compounds were screened for antibacterial, antifungal and antimalarial activity. The data in the Tables 2 and 3 indicate that among the synthesized compound 3a, 3b and 3c possesses good antimicrobial activity. However, the activities of the tested compounds are much less than those of standard agents used. These compounds also show potent antimalarial activity. From the results of various biological activities it is clear that these compounds would be of better use in drug development to combat bacterial infections and as antimalarial agents in the future.

Compound	Mean IC_{50} (Microgram/ml)
3a	0.77
3b	0.97
3c	0.79
3d	0.80
3e	0.89
3f	0.98
3g	1.01
3h	1.12
3i	0.98
3j	1.12
3k	0.79
3l	0.96
Quinine	0.268

Table 4: Antimalarial activity.

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