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Chemical Biology LETTERS

Synthesis of novel Imidazo [1,2-b] pyridazine derivatives and study of their biomedicinal efficacy

Ashish Bhatt,[1] Rajesh Kumar Singh,[2] Ravi Kant[1*]

[1]*Department of Chemistry,* [2]*Department of Pharmacy, Mewar university, Chittorgarh (Rajasthan),312901, India*

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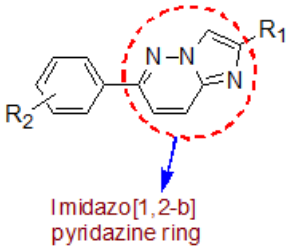
ABSTRACT



Structure	Antibacterial Activity (MIC) µ g/ml	Antimalarial Activity(IC 50) µg/ml
2f	62.5 (E.Coli)	0.64
2g	62.5 (P.Aeruginosa)	0.70
Ampicillin	100 (E.Coli, P.Aeruginosa)	0.268 (Quinine standard)
Chloramphenicol	50 (E.Coli, P.Aeruginosa)	

R_1 = 4-CF_3 Phenyl, R_2 = 4-CF_3 **(2f)**
R_1 = 4-CF_3 Phenyl, R_2 = 4-Cl **(2g)**

Novel 2-Substituted aryl (or alkyl)-6-(substituted aryl) imidazo[1,2-b]pyridazine derivatives have been synthesized by the suzuki reaction of 6-chloro-2-substituted aryl(or alkyl) imidazo[1,2-b]pyridazine [obtained by the reaction of 3-amino-6-chloro pyridazine with 2-bromo-1-substituted aryl (or alkyl) ethanone] with substituted aryl boronic acid in presence of bis(triphenylphosphine)palladium(ii) chloride and potassium carbonate in dimethyl formamide. These were screened for *in-vitro* antimicrobial activity against two gram positive (*Streptococcus pyogenes and Staphylococcus aureus*) and two gram negative bacteria (*Pseudomonas aeruginosa and Escherichia coli*) as well as for antifungal and antimalarial activity against *Plasmodium falciparum* strain. Compound **2f** and **2g** exhibited good antimicrobial and antimalarial activity.

Keywords: Imidazo[1,2-b]pyridazine, Antimicrobial, Antimalarial activity

INTRODUCTION

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,[1-3] antituberculosis,[4-6] antifungal,[7] anticancer,[8] antihypertensive,[9] herbicidal,[10] anti-inflammatory[11] activities, and protein tyrosine phosphatase 1B (PTP1B) inhibitors.[12] They also have an immense potential in agricultural science as plant growth regulators and crop protection agents.[13]

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 derivatives of pyridazine incorporating 1,2,4-triazole, imidazole, isoxazole, and triazine rings have been shown to display a wide spectrum in biological and therapeutic areas.[14-18]

Currently, imidazopyridazines are in the focus of attention of researchers due to their diverse biological activity. Anticancer,[19] anticonvulsant (antiepileptic),[20] antimalarial[21] effects as well as stimulating activity on soluble guanylate cyclase (relief of angina pectoris),[22] activity against human immunodeficiency virus[23] and influenza[24] have been identified for these compounds. Ponatinib, a third-generation tyrosine kinase inhibitor has been implemented in clinical practice for the treatment of chronic myeloid leukemia. Among the possible alternatives of systems where imidazole and pyridazine cycles

Dr. Ravi Kant
Tel: 91-7742845501
Email: itsbhatt2007@yahoo.co.in

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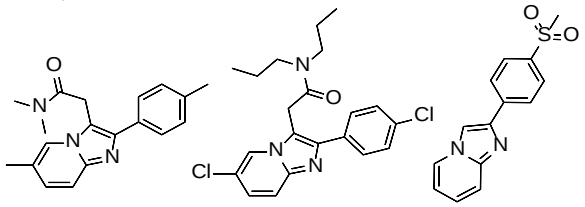
are fused of greatest interest in terms of biological activity are imidazo[1,2-b] pyridazine[25,26] and imidazo[4,5-d] pyridazine[27,28] heterocyclic systems.

Recently, imidazo [1,2-a] pyridines have significant importance in the pharmaceutical industry[29] owing to their various interesting biological activity displayed over a broad range of therapeutic classes; these molecules exhibit antiviral (anticytomegalo-zoster and antivaricella-zoster virus),[30] antiinflammatory,[31] analgesic, antipyretic, antiulcer, and antibacterial[32] properties. They are also β-amyloid formation inhibitors, GABA and benzodiazepine receptor agonists[33] and cardiotonic agents.[34] Drug formulations containing imidazo[1,2-a]pyridine that are currently available on the market include alpidem (anxiolytic),[35] zolpidem (hypnotic)[36] and zolimidine (antiulcer).[37]



Zolpidem Alpidem Zolimidine

Inspired by these above drug formulation, we decided that introduction of additional heteroatom into the pyridine ring of the imidazo[1,2-a] pyridine system should be undertaken and this work should lead to the identification of imidazo[1,2-b] pyridazine ring as a pharmaceutically active moiety. Several imidazo[1,2-b] pyridazines have demonstrated biological activity including inhibitors of the central nervous system,[38] antipyretic and hypothermal activity,[39] anticonvulsant activity, analgesic and antispasmotic activity.[40-42]

Therefore looking at the importance of these heterocyclic nuclei, it is of interest to synthesize new 2,6-substituted imidazo[1,2-b] pyridazine derivatives and to evaluate these derivatives for antimicrobial and antimalarial activity against plasmodium falciparum strain.

EXPERIMENTAL SECTION

Material and methods

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 were checked by LCMS . IR spectra (KBr) and (^{1}H & ^{13}C) NMR spectra ($CDCl_3$/DMSO-d_6) were recorded on FTIR RXI Perkin-Elmer 1800 spectrophotometer and Bruker DRTX-400 spectrophotometer (400 MHz) using TMS as internal standard, respectively. 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.

Chemistry

We have prepared some 2,6-substituted imidazo[1,2-b] pyridazine derivative in two steps, using 3-amino-6-chloro pyridazine, 2-bromo-1-substituted aryl(or alkyl) ethanone, and substituted phenyl boronic acid 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 Suzuki coupling reaction with substituted phenyl boronic acid in presence bis(triphenylphosphine)palladium(ii) chloride as a catalyst and potassium carbonate in DMF results 2- substituted aryl (or alkyl)-6-(substituted aryl) imidazo[1,2-b]pyridazine derivative. The clear procedure for the preparation of desired 2,6-substituted imidazo[1,2-b] pyridazine derivatives are given below.

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

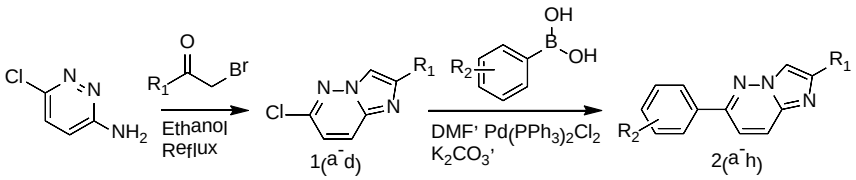
A mixture of 3-amino-6-chloro pyridazine (0.01 mole) and 2-bromo-1-substituted aryl (or alkyl) ethanone (0.012 mole) in ethanol (10 mL) was refluxed at 80 ^{O}C for 4 hrs. The reaction mixture was then cooled and poured into ice-cold water. The resulting precipitate was filtered, washed several times with water, dried and recrystallized from ethanol.

6-chloro-2-methylimidazo[1,2-b]pyridazine 1(a):

Off white solid, 72.4% yield, m.p. 110-112 ^{o}C. ^{1}H-NMR (400MHz, DMSO-d_6): 8.62 (s, 1H, Imidazo-H), 8.28 (d, J = 9.6 Hz,1H, pyridazine-H), 7.85 (d, J = 9.6 Hz,1H,pyridazine-H), 2.28(s, 3H). ^{13}C NMR (DMSO-d_6): 153.2, 142.4, 141.5, 134.1, 129.4, 128.1, 18.1. LC-MS calcd. for $C_7H_6ClN_3$ 167.03; found: m/z 168.3 (M^++1). Elemental analysis calcd for $C_7H_6ClN_3$: C-50.17%, H-3.61%, Cl-21.15%, N-25.07; found: C-50.15%, H-3.58%, Cl-21.11%, N-24.98%.

6-chloro-2-p-tolylimidazo[1,2-b]pyridazine 1(b):

Off white solid, 65.2% yield, m.p. 127-129 ^{o}C. ^{1}H-NMR (400MHz, DMSO-d_6): 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). ^{13}C NMR (DMSO-d_6): 155.1, 143.7,



2a: R1 = CH_3, R2 = 4-CF_3
2b: R1 = 4-CH_3 phenyl, R2 = 4-CF_3
2c: R1 = CF_3, R2 = 4-CF_3
2d: R1 = CF_3, R2 = 4-Cl,3-CF_3
2e: R1 = CF_3, R2 = 4-F
2f: R1 = 4-CF_3 phenyl, R2 = 4-CF_3
2g: R1 = 4-CF_3 phenyl, R2 = 4-Cl
2h: R1 = CF_3, R2 = 2,4-DiChloro

Scheme 1. Chemical synthesis of Imidazo[1,2-b] pyridazine derivative

142.3, 135.5, 134.8, 133.4, 130.1, 127.2, 126.3, 22.3. LC-MS calcd for $C_{13}H_{10}ClN_3$ 243.06; found: m/z 244.4 (M^++1). Elemental analysis 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-(trifluoromethyl)imidazo[1,2-b]pyridazine 1(c):

Brown solid, 44.7% yield, m.p. 115-117 ^oC. ^1H-NMR (400MHz, DMSO-d6): 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). ^{13}C NMR (DMSO-d_6): 157.2, 140.2, 135.4, 131.2, 128.5, 127.1, 124.5. LC-MS calcd for $C_7H_3ClF_3N_3$ 221.01; found: m/z 222.3 (M^++1). Elemental analysis 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-(4-(trifluoromethyl)phenyl)imidazo[1,2-b]pyridazine 1(d):

Off white solid, 58.2% yield, m.p. 125-127 ^oC.^1H-NMR (400MHz, DMSO-d_6): 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). ^{13}C NMR (DMSO-d_6): 154.2, 144.1, 142.7, 139.2, 136.6, 132.3, 130.4, 129.1, 127.3, 125.7. LC-MS calcd for $C_{13}H_7ClF_3N_3$ 297.04; found: m/z 298.2 (M^++1). Elemental analysis 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%.

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

A mixture of 6-chloro-2-substituted aryl(or alkyl) imidazo[1,2-b]pyridazine (0.01mole) and potassium carbonate (0.015mole) was dissolved in 10 mL DMF. Then to it was added bis(triphenylphosphine)palladium(ii) chloride (0.001 mole) followed by substituted phenyl boronic acid (0.015 mole) under nitrogen atmosphere at room temperature and the reaction mixture was heated at 80 ^OC for 2hrs. The reaction mixture was diluted with ethyl acetate and washed with water. The organic layer was dried over Na_2SO_4 and evaporated. The crude compound was purified by using column chromatography with 100-200 silica gel to give compound 2(a-h) Scheme 1.

Spectral data of Imidazo[1,2-b] pyridazine derivative:

2-methyl-6-(4-(trifluoromethyl)phenyl)imidazo[1,2-b]pyridazine (2a) :

Brown solid, 39.6% yield, m.p. 110-111 ^oC. IR(cm^{-1}): 3887.5, 3839.1, 3740.5, 3612.4, 2341.3, 1745.1, 1698.7, 1542.0, 1321.3, 1115.4, 802.9, 713.7. ^1H-NMR (400MHz, DMSO-d_6): 8.26 (d, J = 8.0 Hz, 2H, Ar-H), 8.16 (s,1H, Imidazo-H), 8.14 (d, J = 9.6 Hz, 1H, Pyridazine-H), 7.91 (d, J = 8.4 Hz,2H, Ar-H), 7.81 (d, J = 9.6 Hz,1H, Pyridazine-H), 2.42(s,3H, CH_3). ^{13}C NMR (DMSO-d_6): 152.7, 138.7, 137.2, 137.1, 131.9, 130.5, 128.2, 126.2, 126.1, 125.6, 125.4, 124.2, 123.8, 16.2. LC-MS found: m/z 278.1 (M^++1) and calcd for $C_{14}H_{10}F_3N_3$ is 277.06. LCMS Purity: 98.15 %, Elemental analysis calcd for $C_{14}H_{10}F_3N_3$: C-60.65%, H- 3.64%, F-20.56%, N-15.16% and experimentally found : C- 60.63%, H- 3.61%, F-20.53%, N-15.14%.

2-p-tolyl-6-(4-(trifluoromethyl)phenyl)imidazo[1,2-b]pyridazine (2b) :

Offwhite solid, 31.2% yield, m.p. 150-152 ^oC. IR(cm^{-1}): 3860.6, 3811.6, 3742.9, 3668.3, 3619.2, 2358.1, 1741.6, 1693.9, 1540.1, 1316.5, 1125.1, 1064.1, 812.3, 745.9. ^1H-NMR (400MHz, DMSO-d_6): 8.93 (s,1H, Imidazo-H), 8.30 (d, J = 8.0 Hz, 2H, Ar-H), 8.27 (d, J = 9.6 Hz, 1H, Pyridazine-H), 7.98-7.93 (m,4H), 7.90 (d, J = 9.6 Hz, 1H, Pyridazine-H), 7.29 (d, J = 8.4 Hz,2H, Ar-H), 2.35 (s,3H). ^{13}C NMR (DMSO-d_6): 150.2, 147.1, 139.2, 138.8, 138.7, 131.9, 130.5, 129.7, 127.4, 126.1, 125.3, 115.8, 112.8, 21.5. LC-MS found: m/z 354.1 (M^++1); calcd for $C_{20}H_{14}F_3N_3$ 353.1. LCMS Purity: 96.26%. Elemental analysis calcd for $C_{20}H_{14}F_3N_3$: C- 67.98%, H- 3.99%, F-16.13%, N-11.89%; found: C-67.96%, H- 3.97%, F-16.11%, N-11.88%.

2-(trifluoromethyl)-6-(4-(trifluoromethyl)phenyl)imidazo [1,2-b]pyridazine (2c) :

Offwhite solid, 37.6% yield, m.p. 120-122 ^oC. IR(cm^{-1}): 3841.6, 3738.4, 3053.3, 2919.6, 2851.2, 2361.3, 1723.2, 1614.5, 1547.0, 1461.3, 1318.7, 1215.7, 1102.3, 1062.3, 802.1, 715.2. ^1H-NMR (400MHz, DMSO-d_6): 9.10 (s,1H, Imidazo-H), 8.43 (d, J = 10.0 Hz, 1H, Pyridazine-H), 8.31 (d, J = 8.0 Hz, 2H, Ar-H), 8.10 (d, J = 9.6 Hz, 1H, Pyridazine-H), 7.97 (d, J = 9.6 Hz, 2H, Ar-H). ^{13}C NMR (DMSO-d_6): 151.8, 138.5, 138.2, 134.1, 130.5, 128.0, 127.3, 126.0, 125.3, 123.0, 122.6, 120.3, 119.3, 117.2. LC-MS found : m/z 332.3 (M^++1) and calcd for $C_{14}H_7F_6N_3$ is 331.05. LCMS Purity: 98.15 %, Elemental analysis calcd for $C_{14}H_7F_6N_3$: C- 50.77%, H- 2.13%, F-34.42%, N-12.69% and experimentally found : C- 50.75%, H- 2.11%, F-34.40%, N-12.67%.

6-(4-chloro-3-(trifluoromethyl)phenyl)-2-(trifluoromethyl)imidazo[1,2-b]pyridazine (2d) :

Pale yellow solid, 44.1% yield, m.p. 163-165 ^oC. IR(cm^{-1}): 3741.7, 3049.2, 2357.6, 1547.3, 1481.7, 1369.3, 1328.7, 1170.5, 1128.5, 950.6, 907.8, 822.6, 774.0, 706.7 . ^1H-NMR (400MHz, DMSO-d_6): 9.12 (s,1H, Imidazo-H), 8.49 (d, J = 2.0 Hz, 1H, Ar-H), 8.43 (d, J = 10.0 Hz, 1H, Pyridazine-H), 8.40 (d, J = 2.0 Hz, 1H, Ar-H), 8.16 (d, J = 9.6 Hz, 1H, Pyridazine-H), 7.98 (d, J = 8.4 Hz, 1H, Ar-H). ^{13}C NMR (DMSO-d_6): 151.2, 138.5, 136.4, 135.0, 133.6, 132.5, 131.3, 129.4, 127.4, 126.3, 124.0, 122.7, 117.7, 116.3. LC-MS found : m/z 366.1 (M^++1) and calcd for $C_{14}H_6ClF_6N_3$ is 365.02. LCMS Purity: 97.02 %, Elemental analysis calcd for $C_{14}H_6ClF_6N_3$: C- 45.99%, H- 1.65%, Cl-9.70%, F-31.17%, N-11.49% and experimentally found : C-45.97%, H- 1.63%, Cl- 9.68%, F-31.15%, N-11.47%.

6-(4-fluorophenyl)-2-(trifluoromethyl)imidazo[1,2-b]pyridazine (2e):

Light brown solid, 38.6% yield, m.p. 162-164 ^oC. IR(cm^{-1}): 3896.0, 3859.5, 3743.7, 3619.2, 3564.6, 3157.2, 3055.6, 2912.1, 2847.3, 2359.3, 1596.0, 1546.9, 1501.4, 1202.2, 1158.9, 1128.2, 1097.5, 942.4, 816.3, 768.7, 724.6, 702.2. ^1H-NMR (400MHz, DMSO-d_6): 9.03 (s,1H, Imidazo-H), 8.36 (d, J = 9.6 Hz, 1H, Pyridazine-H), 8.17-8.14 (m, 2H, Ar-H), 8.03 (d, J = 9.6 Hz, 1H, Pyridazine-H), 7.46-7.41 (m, 2H, Ar-H). ^{13}C NMR (DMSO-d_6): 165.7, 163.2, 152.6, 138.5, 136.1, 131.1, 129.3, 129.2, 126.9, 118.4, 116.5, 116.3, 116.2. LC-MS found : m/z 282.2 (M^++1) and calcd for $C_{13}H_7F_4N_3$ is 281.06. LCMS

Purity: 98.9%, Elemental analysis calcd for $C_{13}H_7F_4N_3$: C- 55.52%, H- 2.51%, F-27.02%, N-14.94%; found : C- 55.50%, H- 2.50%, F-26.99%, N-14.92%.

2,6-bis(4-(trifluoromethyl)phenyl)imidazo[1,2-b] pyridazine (2f) : Offwhite solid, 25.5% yield, m.p. 145-147 ^oC.IR(cm^{-1}): 3855.5, 3815.3, 3735.7, 3624.4, 2712.3, 2343.3, 1749.2, 1658.1, 1521.0, 1471.2, 1354.7, 1189.3, 1095.3, 1010.6, 816.2, 765.2; ^{1}H-NMR (400MHz, DMSO-d_6): 9.13 (s,1H, Imidazo-H), 8.33-8.27 (m, 5H), 7.95-7.93 (m, 3H), 7.83 (d, J = 8.4 Hz, 2H, Ar-H); ^{13}C NMR (DMSO-d_6): 150.8, 145.2, 139.1, 138.8, 136.8, 132.0, 130.5, 127.5, 126.3, 126.1, 126.0, 125.6, 116.6, 114.0. LC-MS found : m/z 408.1 (M^++1) and calcd for $C_{20}H_{11}F_6N_3$ is 407.09. LCMS Purity: 89.70%. Elemental analysis calcd for $C_{20}H_{11}F_6N_3$: C- 58.98%, H-2.72%, F-27.99%, N-10.32%, Found : C- 58.96%, H- 2.70%, F-27.97%, N-10.30%.

6-(4-chlorophenyl)-2-(4-(trifluoromethyl)phenyl)imidazo [1,2-b]pyridazine (2g):

Brown solid, 28.7% yield, m.p. 139-140 ^oC.IR(cm^{-1}): 3894.5, 3835.6, 3739.5, 3677.8, 3617.5, 3039.2, 2361.9, 1743.6, 1700.5, 1605.2, 1536.6, 1490.1, 1317.4, 1154.8, 1105.5, 1051.4, 1002.3, 840.1, 804.2, 746.9, 723.9, 689.1. ^{1}H-NMR (400MHz, DMSO-d_6): 9.08 (s,1H, Imidazo-H), 8.29-8.26 (m, 3H), 8.12 (d, J = 8.8Hz, 2H, Ar-H), 7.88 (d, J = 9.6 Hz, 1H, Pyridazine-H), 7.84 (d, J = 8.0 Hz, 2H, Ar-H), 7.65 (d, J = 8.4 Hz,2H, Ar-H). ^{13}C NMR (DMSO-d_6): 151.1, 144.8, 139.0, 136.9, 136.4, 133.9, 130.1, 129.5, 128.3, 126.2, 125.9, 125.6, 116.6, 113.9. LC-MS found : m/z 374.1 (M^++1) and calcd for $C_{19}H_{11}ClF_3N_3$ is 373.06. LCMS Purity: 99.83%. Elemental analysis calcd for $C_{19}H_{11}ClF_3N_3$: C- 61.06%, H- 2.97%, Cl-9.49%, F-15.25%, N-11.24% and experimentally found: C- 60.98%, H- 2.95%, Cl-9.46%, F-15.23%, N-11.22%.

6-(2,4-dichlorophenyl)-2-(trifluoromethyl)imidazo[1,2-b]pyridazine (2h) :

Offwhite solid, 59.5% yield, m.p. 143-144 ^oC. IR(cm^{-1}): 3862.1, 3807.6, 3745.5, 3679.0, 3621.5, 3142.7, 3091.2, 2334.1, 1707.5, 1585.2, 1547.8, 1477.4, 1449.3, 1376.0, 1297.5, 1268.6, 1216.3, 1172.3, 1107.5, 1022.0, 943.8, 869.8, 832.3, 805.7, 762.6, 730.3, 700.6, 674.3. ^{1}H-NMR (400MHz, DMSO-d_6): 9.09 (s,1H, Imidazo-H), 8.38 (d, J = 10.0 Hz,1H, Pyridazine-H), 7.90 (d, J = 1.6 Hz, 1H, Ar-H), 7.72-7.64 (m, 3H). ^{13}C NMR (DMSO-d_6): 152.8, 138.3, 136.9, 136.3, 133.5, 133.1, 132.2, 130.4, 127.9, 125.8, 121.7, 116.1. LC-MS found : m/z 332.1 (M^++1) and calcd for $C_{13}H_6Cl_2F_3N_3$ is 330.9. LCMS Purity: 97.8%. Elemental analysis calcd for $C_{13}H_6Cl_2F_3N_3$: C-47.01%, H-1.82%, Cl-21.35%, F-17.16%, N-12.65% and experimentally found: C-46.98%, H-1.80%, Cl-21.32%, F-17.13%, N-12.62%.

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[43-46] 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 & 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 dilution were prepared in primary and secondary screening. In primary screening 1000 µg/ml, 500 µg/ml, and 250 µg/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 µ l 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 5mg/ml of each of the test samples was prepared in DMSO and subsequent dilutions were prepared with culture medium. The diluted samples in 20 µ l volume were added to the test wells so as to obtain final concentrations (at five fold dilutions) ranging between 0.4 µ g/ml to 100 µ g/ml in duplicate well containing parasitized cell preparation. The culture plates were incubated at 37^{o}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.

RESULT 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 Suzuki coupling reaction with substituted phenyl boronic acid in presence of bis(triphenylphosphine)palladium(ii) chloride and potassium

carbonate in DMF results 2- substituted aryl (or alkyl)-6-(substituted aryl) imidazo[1,2-b]pyridazine derivative 2(a-h).

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* compounds **2b**, **2d**, **2f** and **2g** exhibit good activity while **2a**, **2c**, **2e** and **2h** show moderate activity. In case of *S. pyogenus* compound **2f** exhibit good activity while rest of the compounds show moderate activity. In case of *E. coli* Compound **2f** shows higher activity and **2g** shows good activity while rest of the compounds possess less activity. In case of *P. aeruginosa* compound **2g** shows higher activity and compound **2f** shows good activity while rest of the compound possess moderate activity. The results are given in Table 1.

Table 1: Antibacterial activity (MIC in µg/mL)

Compound	*S. aureus*	*S. pyogenus*	*E. coli*	*P. aeruginosa*
2a	200	250	500	200
2b	100	200	250	250
2c	500	250	125	200
2d	125	125	200	200
2e	200	200	200	125
2f	125	100	62.5	100
2g	100	125	100	62.5
2h	200	125	125	200
Ampicillin	250	100	100	100
Chloramphenicol	50	50	50	50

Table 2: Antifungal activity (MIC in µg/mL)

Compound	*C. albicans*	*A. niger*	*A. clavatus*
2a	1000	>1000	>1000
2b	1000	>1000	>1000
2c	1000	>1000	>1000
2d	500	1000	1000
2e	500	500	500
2f	500	250	500
2g	500	250	250
2h	500	250	500
Nystatin	100	100	100
Greseofulvin	500	100	100

Table 3: Antimalarial activity

Compound	Mean IC50 (µg/ml)
2a	0.75
2b	1.09
2c	0.85
2d	0.9
2e	0.76
2f	0.64
2g	0.70
2h	0.85
Quinine	0.268

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* compounds **2d**, **2e**, **2f**, **2g** and **2h** exhibit good activity while rest of the compounds possess less activity. In case of *A. niger* and *A. clavatus* all the compounds possess less activity. The results are given in Table 2.

ANTIMALARIAL ACTIVITY

For antimalarial activity, Compound **2f** and **2g** exhibit good activity closer to reference compound Quinine while rest of the compounds possess less activity against *Plasmodium falciparum* strain.. The results are given in Table 3.

CONCLUSION

In conclusion, we have designed and synthesized new substituted imidazo[1,2-b] pyridazine derivatives with different substitution at 2 & 6 position and characterized through elemental and spectral analysis. . These derivatives were evaluated for antimicrobial and antimalarial activity. It can be concluded from antibacterial screening (Table-1) that compound **2f** and **2g** were found to be active against *Escherichia coli* and *Pseudomonas aeruginosa*, respectively compared to the rest of the compounds. However, the activities of the tested compounds are much less than those of standard agents used. These compounds **2f** & **2g** also showed good antimalarial activity but not superior to the standard.

Further synthetic modification is required to enhance the potency of imidazo[1,2-b] pyridazine derivatives by changing molecular configuration,which is in progress at our laboratory. The present study throws light on the identification of this new structural class as antimicrobials and antimalarial, which can be of interest for further detailed preclinical investigations.

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REFERENCES AND NOTES

1. S.N. Ahmed Al-Kamali, A.Ahmed Al-Hazmi, Mohammed H.M. Alhousami, Mokhtar A. Al-Masany. Synthesis and antibacterial activity of some novel thieno[2,3-c]pyridazines using 3-amino-5-phenyl-2-ethoxycarbonylthieno[2,3-c]pyridazine as a starting material. *Arabian J. Chem.*, **2014**, 7(5), 775-780.
2. Roxana-Angela Tucaliuc, Valeriu V. Cotea, Marius Niculaua, Cristina Tuchilus, Dorina Mantu, Ionel I. Mangalagiu. New pyridazine–fluorine derivatives: Synthesis, chemistry and biological activity. Part II. *Eur.J.Med.Chem.*, **2013**, 67, 367-372.
3. A. Benmoussa, J. El harti, M.Ansar et al., Synthesis and antimicrobial properties of some pyridazin-3-thiones derivatives, *Int. J. PharmTech Res.*, **2012**, 4(4), 1591-1594.
4. I. I. Mangalagiu, Recent achievements in the chemistry of 1,2-diazines, *Curr. Org. Chem.*, **2011**, 15(5), 730-752.
5. D. Mantu, M. C. Luca, C. Moldoveanu, G. Zbancioc, I. I. Mangalagiu. Synthesis and antituberculosis activity of some new pyridazine derivatives. Part II, *Eur. J. Med. Chem.*, **2010**, 45(11), 5164-5168.

6. R. M. Butnariu , I. I. Mangalagiu. New pyridazine derivatives: synthesis, chemistry and biological activity, *Bioorg. Med. Chem.*, **2009**, 17(7), 2823-2829.
7. Esvet Akbas, Ismet Berber. Antibacterial and antifungal activities of new pyrazolo[3,4-d]pyridazin derivatives. *Eur. J. Med. Chem.*, **2005**, 40(4), 401-405.
8. R. M. Butnariu, M. D. Caprosu, V. Bejan et al., Pyridazine and phthalazine derivatives with potential antimicrobial activity, *J. Heterocyclic Chem.*, **2007**, 44(5), 1149-1152.
9. Ravinesh Mishra, Anees A. Siddiqui, Asif Husain, Mohd Rashid, Sameya Anjum. Acute and repeated dose toxicity studies of novel pyridazine substituted s-triazin-2-imine/one/thione derivatives as new class of antihypertensive agent, *Toxicol. Lett.*, **2013**, 221, S88-S89.
10. X. Han, Y. H. Hong, Z. H. U. You Quan, Z. X. Mao LIU Bim, H. U. Fang Zhong, Y. H. Zheng, Synthesis and herbicidal activities of novel 4-(3-trifluoromethylphenyl)-2H-pyridazin-3-one derivatives, *Sci. China Chem.*, **2010**, 53(1), 157-166.
11. Nadia Abdalla Khalil, Eman Mohamed Ahmed, Khaled Omar Mohamed, Yassin Mohammed Nissan, Sawsan Abo-Bakr Zaitone, Synthesis and biological evaluation of new pyrazolone–pyridazine conjugates as anti-inflammatory and analgesic agents, *Bioorg. Med. Chem.*, **2014**, 22(7), 2080-2089.
12. Pramod C. Nair, M. Elizabeth Sobhia. CoMFA based de novo design of pyridazine analogs as PTP1B inhibitors, *J. Mol. Graph. Model.*, **2007**, 26(1), 117-123..
13. R. B. Tucaliuc, I. M. Risca, G. Drochioiu, I. Mangalagiu, Biological effect of some new pyridazine derivatives on wheat in germination experiments, *Rom. Biotechnol. Lett.*, **2008**, 13(4), 3837-3842.
14. T. Ishikawa, Y. Iizawa, K. Okonogi, A. Miyake. Studies on Anti-MRSA parenteral cephalosporins I. Synthesis and antibacterial activity of 7, *J. Antibiot.*, **2000**, 53(10), 1053-1070.
15. C. Almansa, A. F. De Arriba, F. L. Cavalcanti et al., Synthesis and SAR of a new series of COX-2-selective inhibitors: pyrazolo[1,5-a]pyrimidines, *J. Med. Chem.*, **2001**, 44(3), 350-361.
16. G. B. Barlin, L. P. Davies, P. W. Harrison. Imidazo [1,2-b]pyridazines. XXI. Syntheses of some 3-acylaminomethyl-6-(chloro and iodo)-2- (substituted phenyl)- imidazo[1,2-b] pyridazines and imidazo[1,2-a]pyridines and their interaction with central and mitochondrial benzodiazepine, *Aust. J. Chem.*, **1997**, 50(1), 61-68.
17. H. M. Faidallah, K. A. Khan, M. S. I.Makki. Synthesis and biological evaluation of new fused isoxazolo[4,5-d]pyridazine derivatives, *J. Chin. Chem. Soc.*, **2011**, 58(2), 191-198.
18. N. F. Abd El-Ghaffar, M. K. Mohamed, M. S. Kadah, A. M.Radwan, G. H. Said, S. N. Abd el Al. Synthesis and antitumoractivities of some new pyridazinones containing the 2-phenyl-1H-indolyl moiety, *J. Chem. Pharma. Res.*, **2011**, 3(3), 248-259.
19. Ken-ichi Kusakabe, Nobuyuki Ide, Yataro Daigo, Takeshi Itoh, Takahiko Yamamoto et al. Discovery of Imidazo[1,2-b]pyridazine Derivatives: Selective and Orally Available Mps1 (TTK) Kinase Inhibitors Exhibiting Remarkable Antiproliferative Activity. *J. Med. Chem.*, **2015**, 58 (4), 1760–1775.
20. S. Moreau, P. Coudert, C. Rubat et al. Synthesis and anticonvulsant properties of triazolo- and imidazopyridazinyl carboxamides and carboxylic acids. *Bioorg. Med. Chem.*, **1998**, 6(7), 983-991.
21. N. Bouloc, J.M. Large, E. Smiljanic et al. Synthesis and in vitro evaluation of imidazopyridazines as novel inhibitors of the malarial kinase PfPK7. *Bioorg. Med. Chem. Lett.*, **2008**, 18(19), 5294-5298.
22. Starke, Ingemar. Imidazo(1,2-a)(pyridazines Or Pyrazines) For Treatment Of Diseases Related To Bone Loss .WO **1989**/003833 A.
23. J. Singh, B.S. Chhikara. Comparative global epidemiology of HIV infections and status of current progress in treatment. *Chem. Biol. Lett.*, **2013**, 1(1), 14-32.
24. Knight, J. David, I. Scopes, C. David. Imidazopyridayine derivatives having antiviral activity. US Patent **1987**/4690917.
25. Maria Grazia Rimoli, Emilio Russo, Mauro Cataldi, Rita Citraro, Paolo Ambrosino. T-type channel blocking properties and antiabsence activity of two imidazo[1,2-b]pyridazine derivatives structurally related to indomethacin. *Neuropharmacol.*, **2009**, 56(3), 637-646.
26. Enza Palazzo, Maria Grazia Rimoli, Maria De Chiaro, Francesca Guida, Daniela Melisi et al. Intra-periaqueductal grey microinjections of an imidazo[1,2-b]pyridazine derivative, DM2, affects rostral ventromedial medulla cell activity and shows antinociceptive effect. *Neuropharmacol.*, **2010**, 58(3), 660-667.
27. Peter Borowski, Melanie Lang, Annemarie Haag, Herbert Schmitz. Characterization of Imidazo[4,5-d]Pyridazine Nucleosides as Modulators of Unwinding Reaction Mediated by West Nile Virus Nucleoside Triphosphatase/Helicase: Evidence for Activity on the Level of Substrate and/or Enzyme. *Antimicrob. Agents Chemother*. **2002** ,46(5), 1231-1239.
28. Ravi K. Ujjinamatada, Robin L. Paulman, Roger G. Ptak, Ramachandra S. Hosmane. Nucleosides with self-complementary hydrogen-bonding motifs: Synthesis and base-pairing studies of two nucleosides containing the imidazo[4,5-d] pyridazine ring system. *Bioorg. Med. Chem.*, **2006**, 14(18), 6359-6367.
29. S.M. Hanson, E.V. Morlock, K.A.Satyshur, C.Czajkowski. Structural requirements for eszopiclone and zolpidem binding to the gamma-aminobutyric acid type-A (GABAA) receptor are different, *J. Med. Chem.*, **2008**, 51(22), 7243-52.
30. S. Mavel, J.L Renou, C. Galtier, R. Snoeck, G. Andrei, J. Balzarini, E. De Clercq , A. Gueiffier, One-potSynthesis of 3-Aminoimidazo[1,2-a]pyridines Catalyzed by Heteropolyacids, *Arzneim.-Forsch*, **2001**, 51, 304.
31. R.B. Lacerda, C.K. De Lima, L.L Da Silva, Discovery of novel analgesic and anti-inflammatory 3-aryl amine imidazo[1,2-a]pyridine symbiotic prototypes. *Bioorg. Med.Chem. Lett.*, **2009**, 17, 74.
32. P.S. Bhale , S.B. Dongare, Synthesis and antimicrobial screening of Schiff's bases of imidazo [1,2-a] pyridine. *Int. J. Chem.Sci*.,**2013**, 11, 1563.
33. A.C Humphries, E. Gancia, M.T. Gilligan, S. Goodacre, D. Hallett, K.J. Marchant S.R Thomas, 8-Fluoroimidazo [1,2-a]pyridine: synthesis, physicochemical properties and evaluation as a bioisosteric replacement forimidazo[1,2-a]pyrimidine in an allosteric modulator ligand of the GABA A receptor, *Bioorg. Med. Chem. Lett*.,**2006**, 16,1518.
34. D. Dvey, P.W. Erhardt, W.C. Lumma, J. Jr.Wiggins,; M. Sullivan, D. Pang, E. Cantor, Novel 8-aryl substituted imidazo[1,2-a]- and -[1,5-a]pyridines and imidazo [1,5-a] pyridinones as potential positive inotropicagents, *J. Med. Chem*., **1987**, 30, 1337.
35. S.Z. Langer, S. Arbilla. Benavides, Zolpidem and alpidem:two imidazopyridines with selectivity for omega 1- andomega 3-receptor subtypes, *J. Adv. Biochem. Psychopharmacol.*, **1990**, 46, 61.
36. T.S. Harrison, G.M. Keating .Zolpidem: a review of its use in the management of insomnia CNS Drugs, **2005**, 19, 65.
37. A.P Green, J.E Lander, D.H. Turner. The effect of stress and indomethacin, carbenoxolone, salbutamol, metiamide, zolimidine and CF19415 on rat gastric mucus. *J Pharm Pharmacol*. **1981**,33(6),348-352.
38. B. Stanovnik, M. Tisler, Synthesis of pyridazine derivatives - 13. Formation of some substituted imidazo(1,2-b) pyridazines, *Tetrahedron*, **1967**, 23, 2739.
39. L. Almirante, L. Polo, A. Mugnaini, E. Provinciali, P. Rugarli, A. Gamba, A. Olivi, W. Murmann, Derivatives of imidazole II. Synthesis and reaction of imidazo[1,2-alpha] pyrimidines and other bi- and tricyclic imidazo derivatives with analgesic, anti-inflammatory, antipyretic and anticonvulsant activity. *J. Med. Chem*, **1966**, 9, 29.
40. Japanese Patent 22,264 (**1965**); Chem. Abstr., 64, 3566 (**1966**).
41. Japanese Patent 22,265 (**1965**); Chem. Abstr., 64, 3566 (**1966**).
42. Japanese Patent 22,263 (**1965**); Chem. Abstr., 64, 3566 (**1966**).
43. Clinical microbiology procedure handbook, vol II, chapter 5 page no. 5.0.1 Henry d. Isenberg,2nd edition.
44. N.C Desai,P.N Shihora , D.L. Moradia, Synthesis and characterization of new quinazolines as potential antimicrobial agents . *Indian J. Chem.*, **2007**, 46B, 550-553.
45. S. Shadomy. In Manual of Clinical Microbiology; Albert,B., Ed.; ASM Press: Washington, DC, **1991**; p 1173.
46. A. Rattan. Antimicrobials in Laboratory Medicine; BIChurchill Livingstone: India, **2000**; p 85.