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Synthesis of novel fused heterocyclic pyrazolo-pyridazine derivatives as antimicrobial agents

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Abstract

A series of pyrazolo-pyridazine derivatives (**4a-j**) were synthesized and evaluated for their antimicrobial activities with an aim to obtain promising antimicrobial agents. In the first step, 6-substituted phenyl-2,3,4,5-tetrahydropyridazin-3-one (**2a,b**) was prepared by reacting 4-(4-methylphenyl)-4-oxobutanoic acid (**1a)** and 4-(4-chlorophenyl)-4-oxobutanoic acid (**1b**) with hydrazine hydrate. Then, aryl-aldehydes were reacted with **2** to furnish pyridazinones (**3a-j**). Finally, pyridazinones (**3a-j**) were reacted with hydrazine hydrate to furnish the title compounds (**4a-j**). The newly synthesized compounds were evaluated for their *in vitro* antibacterial and antifungal activities against six microbial strains. Compound **4i,** 5-(4-Hydroxy-3-methoxyphenyl)-3-(4-chlorophenyl)-3,3a,4,7-tetrahydro-2*H*-pyrazolo [3,4-c] pyridazine, was found to have significant action against Gram positive and Gram negative bacteria, whereas compound **4g,** 3-(4-Bromophenyl)-5-(4-chlorophenyl)-3,3a,4,7-tetrahydro-2*H*-pyrazolo [3,4-c] pyridazine, exhibited potential antifungal activity.

Keywords: Pyridazine, Pyrazole, Antibacterial, Antifungal

Introduction

Pyridazine ring is a part of the structures of a number of drugs available in the market [1] such as hydralazine, minaprine, cefozopran, and pipofezine. Pyridazine derivatives have been reported to possess various pharmacological activities including antibacterial [2-5], antifungal [3-5], anti-tubercular [6-8], anticonvulsant [8-10], antihypertensive [11], analgesic [12, 13] and anti-inflammatory [12, 13]. Similarly, pyrazole (five-membered nitrogen containing heterocyclic ring) derivatives also showed potential biological activities including antimicrobial actions [14, 15].

In recent years, the incidence of systemic bacterial and fungal infections is on rise due to an increase in the number of immune-compromised hosts [16]. Immunosuppression due to malignancy, immune-suppressive therapies, HIV-infection, broad-spectrum antimicrobial treatment and age, as well as invasive procedures and mucosal barriers places patients at risk for microbial infections. The increasing incidence of resistance to a large number of antibacterial agents is

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becoming a major concern [17]. Currently, a small number of antifungal agents are available, and all have some drawbacks regarding their spectrum, toxicity, tissue distribution, and high cost [18]. These observations place new emphasis on the need of as well as search for alternative new and more effective antimicrobial agents with a broad spectrum activity.

In view of the antimicrobial activities exhibited by nitrogen containing heterocyclic such as pyridazines and pyrazoles, it was thought worthwhile to study their fused derivatives having pyrazole and pyridazine in the same structure as potential antibacterial and antifungal agents. We have previously reported the antibacterial and antifungal activity of some pryazolo-pyridazine derivatives [19] possessing an unsubstituted phenyl and a meta or para substituted phenyl group that respectively adjacent to pyridazine and pyrazole moiety of the fused bicyclic ring system. It was observed that the compounds which were bearing electron donating groups showed potent antibacterial activity, while compounds having electron withdrawing groups exhibited excellent antifungal activity. Therefore, we thought to synthesize a library of pyrazolo-pyridazine derivatives (**4a-j**) bearing different substituents on the two phenyl rings in order to study the structure activity relationship by evaluating their antibacterial and antifungal activities against some selected pathogenic microbes.

Materials and Methods

Chemistry

Melting points were determined in open capillary tubes and are uncorrected. All the solvents were purified before use. Purity of compounds was checked by TLC on silica gel-G layer, using benzene: acetone (8:2, v/v) as the solvent system. Infrared (IR) spectra were recorded on Bruker alpha T Spectrophotometer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker 400 MHz instrument in $CDCl_3$/DMSO, using tetramethylsilane [$(CH_3)_4Si$] (TMS) as internal standard. Mass spectra were recorded on Jeol JMS-D300 instrument. Elemental analyses were performed on a Perkin-Elmer 240 analyzer and were found in range of ± 0.4 % for each element analyzed (C, H, N). The synthesis of intermediates, **2a, b** and **3a-j**, and target compounds **4a-j** was achieved following **Figure 1**.

Compound **2a** and **2b** were synthesized by reported method [20] with slight modifications. Their synthesis involved the reaction of 4-(4-methylphenyl)-4-oxobutanoic acid (**1a)** and 4-(4-chlorophenyl)-4-oxobutanoic acid (**1b**) with hydrazine hydrate. Compound **2a** and **2b** were used for the preparation of compounds **3a-j**; 4-substituted-benzylidene-6-substituted-phenyl-4,5-dihydropyridazin-3(2*H*)-ones. Finally compounds **3a-j** were treated with hydrazine hydrate to get the desired compounds **4a-j**; 3,5-disubstituted-phenyl-3,3a,4,7-tetrahydro-2*H*-pyrazolo[3,4-*c*]pyridazines.

General procedure for synthesis of 4-substituted -benzylidene-6-(4-chloro/methyl-phenyl)-4,5-dihydropyridazin-3(2H)-one (**3a-j**): A mixture of compound **2a or 2b** (0.01 mol) and appropriate aryl-aldehyde (0.01 mol) in ethanol (20 mL) was taken in a round bottom flask. To this, piperidine (1 mL) was added with stirring and then the reaction mixture was refluxed for 5-8 h, intermittently the progress of reaction was monitored by TLC using benzene: acetone (8:2, v/v) as the solvent system. On completion of reaction, the contents were cooled and then poured onto crushed ice. A solid mass separated out, which was filtered, washed with water, dried, and recrystallized from methanol.

(4E)-4-(3-bromobenzylidene)-6-(4-methylphenyl)-4,5-dihydropyridazin-3(2H)-one (**3a**): R=4-CH_3, R′=3-Br; Yield: 52 %; M.p. (°C): 221-223; R_f (benzene : acetone, 8:2): 0.87; IR (cm^{-1}, KBr): 3282 (NH), 1670 (CO); ^{1}H NMR (δ, ppm): 2.40 (s, 3H, CH_3), 2.94 (s, 2H, CH_2), 7.38-7.44 (m, 4H, H-3,5 and H-4′,5′), 7.52 (s, 1H, H-2′), 7.66 (d, 2H, *J*=8.4Hz, H-2,6) 7.74 (d, 2H, *J*=8.0Hz, H-6′), 7.86 (s, 1H, arylidene), 10.82 (bs, 1H, CONH); Mass [$C_{18}H_{15}BrN_2O$]: *m/z* 356 (M^+), 358 (M^++2).

(4E)-4-(4-bromobenzylidene)-6-(4-methylphenyl)-4,5-dihydropyridazin-3(2H)-one (**3b**): R=4-CH_3, R′=4-Br; Yield: 48 %; M.p. (°C): 227-229; R_f (benzene : acetone, 8:2): 0.86; IR (cm^{-1}, KBr): 3295 (NH), 1683 (CO); ^{1}H NMR (δ, ppm): 2.38 (3H, CH_3), 2.96 (s, 2H, CH_2), 7.40 (d, 2H, *J*=8.4Hz, H-3,5), 7.56 (d, 2H, *J*=8.0Hz, H-3′,5′), 7.70 (d, 2H, *J*=8.4Hz, H-2,6), 7.78 (d, 2H, J=8.0Hz, H-2′,6′), 7.90 (s, 1H, arylidene), 10.86 (bs, 1H, CONH); Mass [$C_{18}H_{15}BrN_2O$]: *m/z* 356 (M^+), 358 (M^++2).

(4E)-4-(3,4-dimethoxybenzylidene)-6-(4-methylphenyl)-4,5-dihydropyridazin-3(2H)-one (**3c**): R = 4-CH_3, R′=3,4-OCH_3; Yield: 61 %; M.p. (°C): 205-207; R_f (benzene : acetone, 8:2): 0.83; IR (cm^{-1}, KBr): 3290 (NH), 1679 (CO); ^{1}H NMR (δ, ppm): 2.38 (s, 3H, CH_3), 2.98 (s, 2H, CH_2), 3.84 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 6.9 (d, 1H, *J*=8.4Hz, H-5′),7.32 (d, 2H, *J*=8.8Hz, H-3,5), 7.48 (s, 1H, H-2′), 7.58 (d, 1H, *J*=8.4Hz, H-6′), 7.68 (d, 2H, *J*=8.8Hz, H-2,6), 7.90 (s, 1H, arylidene), 10.74 (bs, 1H, CONH); Mass [$C_{20}H_{20}N_2O_3$]: *m/z* 337 (M^+).

(4E)-4-(4-hydroxy-3-methoxybenzylidene)-6-(4-methylphenyl)-4,5-dihydropyridazin-3(2H)-one (**3d**): R= 4-CH_3, R′=3-OCH_3, 4-OH; Yield: 57 %; M.p. (°C): 238-240; R_f (benzene : acetone, 8:2): 0.68; IR (cm^{-1}, KBr): 3295 (NH), 1686 (CO); ^{1}H NMR (δ, ppm): 2.40 (s, 3H, CH_3), 2.98 (s, 2H, CH_2), 3.80 (s, 3H, OCH_3), 6.90 (d, 1H, *J*=8.4Hz, H-5′), 7.34-7.40 (m, 3H, H-3,5 and H-2′), 7.56 (d, 1H, *J*=8.4Hz, H-6′), 7.68 (d, 2H, *J*=8.4Hz, H-2,6), 7.90 (s, 1H, arylidene), 9.62 (bs, 1H, OH), 10.86 (bs, 1H, CONH); Mass [$C_{19}H_{18}N_2O_3$]: *m/z* 323 (M^+).

(4E)-4-[4-(dimethylamino)benzylidene]-6-(4-methylphenyl)-4,5-dihydropyridazin-3(2H)-one (**3e**): R = 4-CH_3, R′ = 4-N$(CH_3)_2$; Yield: 69 %; M.p. (°C): 166-168; R_f (benzene:acetone, 8:2): 0.82; IR (cm^{-1}, KBr): 3297(NH), 1683 (CO); ^{1}H NMR (δ, ppm): 2.42 (s, 3H, CH_3), 3.12 (s, 3H, CH_3), 3.14 (s, 3H, CH_3), 6.84 (d, 2H, *J*=8.4Hz, H-3′,5′), 7.34 (d, 2H, *J*=8.4Hz, H-3,5), 7.62-7.68 (m, 4H, H-2,6 and H-2′,6′), 7.90 (s, 1H, arylidene), 10.70 (bs, 1H, CONH); Mass [$C_{20}H_{21}N_3O$]: *m/z* 320 (M^+).

(4E)-4-(3-bromobenzylidene)-6-(4-chlorophenyl)-4,5-dihydropyridazin-3(2H)-one (**3f**): R = 4-Cl, R′ = 3-Br; Yield: 56 %; M.p. (°C): 203-205; R_f (benzene : acetone,

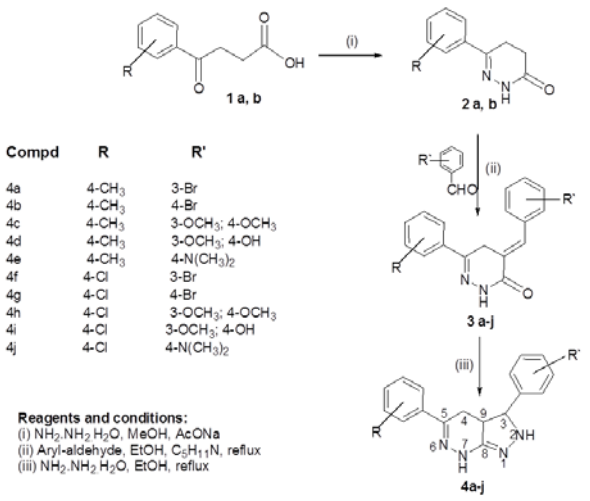

Figure 1 Protocol for synthesis of title compounds (**4a-j**)

8:2): 0.82; IR (cm^{-1}, KBr): 3292 (NH), 1674 (CO); ^{1}H NMR (δ, ppm): 2.98 (s, 2H, CH_2), 7.48-7.52 (m, 4H, H-4′,5′ and H-3,5), 7.62 (s, 1H, H-2′), 7.66 (d, 1H, *J*=8.4Hz, H-6′), 7.70 (d, 2H, *J*=8.4Hz, H-2′,6′), 7.92 (s, 1H, arylidene), 10.84 (bs, 1H, CONH). Mass [$C_{17}H_{12}BrClN_2O$]: *m/z* 377 (M^+), 379 (M^++2).

(4E)-4-(4-bromobenzylidene)-6-(4-chlorophenyl)-4,5-dihydropyridazin-3(2H)-one (**3g**): R = 4-Cl, R′ = 4-Br; Yield: 47 %; M.p. (°C): 197-199; R_f (benzene : acetone, 8:2): 0.81; IR (cm^{-1}, KBr): 3297 (NH), 1684 (CO); ^{1}H NMR (δ, ppm): 2.96 (s, 2H, CH_2), 7.52-7.56 (m, 4H, H-3,5 and H-3′,5′), 7.68 (d, 2H, *J*=8.4Hz, H-2′, 6′), 7.72 (d, 2H, *J*=8.4Hz, H-2,6), 7.94 (s, 1H, arylidene), 10.80 (bs, 1H, CONH); Mass [$C_{17}H_{12}BrClN_2O$]: *m/z* 377 (M^+), 379 (M^++2).

(4E)-4-(3,4-dimethoxybenzylidene)-6-(4-chlorophenyl)-4,5-dihydropyridazin-3(2H)-one (**3h**): R = 4-Cl, R′ = 3,4-OCH_3; Yield: 61 %; M.p. (°C): 223-225; R_f (benzene : acetone, 8:2): 0.82; IR (cm^{-1}, KBr): 3289 (NH), 1672 (CO); ^{1}H NMR (δ, ppm): 2.98 (s, 2H, CH_2), 3.08 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 6.98 (d, 1H, *J*=8.4Hz, H-5′), 7.50-7.54 (m, 3H, H-3,5 and H-2′), 7.60 (d, 1H, *J*=8.4Hz, H-6′), 7.68 (d,2H, *J*=8.4Hz, H-2,6), 7.88 (s, 1H, arylidene), 10.80 (bs, 1H, CONH); Mass [$C_{19}H_{17}ClN_2O_3$]: *m/z* 357 (M^+), 359 (M^++2).

(4E)-4-(4-hydroxy-3-methoxybenzylidene)-6-(4-chlorophenyl)-4,5-dihydropyridazin-3(2H)-one (**3i**): R=4-Cl, R′=3-OCH_3, 4-OH; Yield: 65 %; M.p. (°C): 201-203; R_f (benzene : acetone, 8:2): 0.85; IR (cm^{-1}, KBr): 3292(NH), 1681 (CO); ^{1}H NMR (δ, ppm): 2.96 (s, 2H, CH_2), 3.84 (s, 3H, OCH_3), 6.94 (d, *J*=8.4Hz, H-5′), 7.36 (s, 1H, H-2′), 7.52 (d, 2H, *J*=8.4Hz, H-3,5), 7.56 (d, 1H, *J*=8.4Hz, H-6′), 7.68 (d, 2H, *J*=8.4Hz, H-2, 6), 7.92 (s, 1H, arylidene), 10.82 (bs, 1H, CONH); Mass [$C_{18}H_{15}ClN_2O_3$]: *m/z* 344 (M^+), 346 (M^++2).

(4E)-4-[4-(dimethylamino)benzylidene]-6-(4-chlorophenyl)-4,5-dihydropyridazin-3(2H)-one (**3j**): R = 4-Cl, R′ = 4-N(CH_3)$_2$; Yield: 69 %; M.p.(°C): 185-187; R_f (benzene : acetone, 8:2): 0.89; IR (cm^{-1}, KBr): 3299(NH), 1681 (CO); ^{1}H NMR (δ, ppm): 2.96 (s, 2H, CH_2), 3.06 (s, 3H, CH_3), 3.08 (s, 3H, CH_3), 6.86 (d, 2H, *J*=8.8Hz, H-3′, 5′), 7.52-7.58 (m, 4H, H-3, 5 and H-2′,6′), 7.70 (d, 2H, *J*=8.4Hz, H-2, 6), 7.88 (s, 1H, arylidene), 10.84 (bs, 1H, CONH); Mass [$C_{19}H_{18}ClN_3O$]: *m/z* 341 (M^+), 243 (M^++2).

General procedure for synthesis of 3-substituted-phenyl-5-(4-chloro/methyl-phenyl)-3,3a,4,7-tetrahydro-2H-pyrazolo[3,4-c]pyridazine (**4a-j**): Ethanolic solution of compounds **3a-j** (0.01 mol) was taken in a round bottom flask. To this, hydrazine hydrate (0.02 mol) was added and the resulting reaction mixture was refluxed on steam bath for 8-10 h. Progress of the reaction was monitored by TLC using benzene : acetone (8:2, v/v) as the solvent system. After completion of reaction, the contents were concentrated, cooled and poured onto crushed ice. The separated solid was filtered, washed with water, dried, and recrystallized from methanol.

3-(3-Bromophenyl)-5-(4-methylphenyl)-3,3a,4,7-tetrahydro-2H-pyrazolo[3,4-c]pyridazine (**4a**): R = 4-CH_3, R′=3-Br; Yield: 67 %; M.p. (°C): 198-200; R_f (benzene : acetone, 8:2): 0.87; IR (KBr, cm^{-1}): 3320 (NH), 3292 (NH); ^{1}H NMR (δ, ppm): 2.28-2.34 (m, 3H, H_b and H_c), 2.38 (s, 3H, CH_3), 3.08 (d, 1H, *J*=11.2Hz, H_a), 6.56 (bs, 1H, NH), 7.34 (d, 2H, *J*=8.4Hz, H-3, 5), 7.46-7.50 (m, 2H, H-4′,5′), 7.56 (s, 1H, H-2′), 7.62 (d, 1H, *J*=8.4Hz, H-6′),7.70 (d, 2H, *J*=8.4Hz, H-2, 6), 8.80 (bs, 1H, NH); ^{13}C NMR (δ, ppm): 21.5 (-CH_3), 22.4 (-CH_2), 44.6 (CH, C-9), 49.8 (CH, C-3), 121.8-147.1 (phenyl-C), 156.7 (C=N, C-5, 8); Mass [$C_{18}H_{17}BrN_4$]: *m/z* 370 (M^+), 372 (M^++2).

3-(4-Bromophenyl)-5-(4-methylphenyl)-3,3a,4,7-tetrahydro-2H-pyrazolo[3,4-c]pyridazine (**4b**): R = 4-CH_3, R′=4-Br; Yield: 61 %; M.p. (°C): 203-205; R_f (benzene : acetone, 8:2): 0.91; IR (KBr, cm^{-1}): 3326 (NH), 3302 (NH); ^{1}H NMR (δ, ppm): 2.30-2.34 (m, 3H, H_b and H_c), 2.40 (s, 3H, CH_3), 3.10 (d, 1H, *J*=11.2Hz, H_a), 6.56 (bs, 1H, NH), 7.32 (d, 2H, *J*=8.4Hz, H-3,5), 7.50 (d, 2H, *J*=8.4Hz, H-3′,5′), 7.58-7.74 (m, 4H, H-2,6 and H-2′,6′), 8.82 (bs, 1H, NH); ^{13}C NMR (δ, ppm): 21.3 (-CH_3), 22.6 (-CH_2), 44.4 (CH, C-9), 49.9 (CH, C-3), 120.8-146.1 (phenyl-C), 155.3 (C=N, C-5, 8); Mass [$C_{18}H_{17}BrN_4$]: *m/z* 370 (M^+), 372 (M^++2).

5-(3,4-Dimethoxyphenyl)-3-(4-methylphenyl)-3,3a,4,7-tetrahydro-2H-pyrazolo[3,4-c]pyridazine (**4c**): R=4-CH_3, R′ = 3,4 -OCH_3; Yield: 49 %; M.p. (°C): 228 - 230; R_f (benzene : acetone, 8:2): 0.74; IR (KBr, cm^{-1}): 3342 (NH), 3290 (NH); ^{1}H NMR (δ, ppm): 2.30-2.34 (m, 3H, H_b and H_c), 2.40 (s, 3H, CH_3), 3.10 (d, 1H, *J*=11.2Hz, H_a), 3.82 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 6.54 (bs, 1H, NH), 6.90 (d, 1H, *J*=8.8Hz, H-5), 7.34 (d, 2H, *J*=8.4Hz, H-3,5), 7.50 (s, 1H, H-2′), 7.56 (d, 1H, *J*=8.8Hz, H-6′), 7.66 (d, 2H, *J*=8.4Hz, H-2,6), 8.84 (bs, 1H, NH); ^{13}C NMR (δ, ppm): 21.3 (-CH_3), 23.1 (-CH_2), 45.1 (CH, C-9), 48.8 (CH, C-3), 59.2 (-O**C**H_3), 115.8-143.1 (phenyl -C), 155.1 (C=N, C-5, 8); Mass [$C_{20}H_{22}N_4O_2$]: *m/z* 351(M^+).

5-(4-Hydroxy-3-methoxyphenyl)-3-(4-methyl-phenyl)-3,3a,4,7-tetrahydro-2H-pyrazolo[3,4-c]pyrida-zine (**4d**): R=4-CH_3, R′=3-OCH_3, 4-OH; Yield: 55 %; M.p. (°C): 217-219; R_f (benzene:acetone, 8:2): 0.92; IR (KBr, cm^{-1}): 3334 (NH), 3284 (NH); ^{1}H NMR (δ, ppm): 2.30-2.36 (m, 3H, H_b and H_c), 2.42 (s, 3H, CH_3), 3.08 (d, 1H, *J*=11.2Hz, H_a), 3.80 (s, 3H, OCH_3), 6.58 (bs, 1H, NH), 6.92 (d, 1H, *J*=8.8Hz, H-5′), 7.32 (d, 2H, *J*=8.4Hz, 7.46 (s, 1H, H-2′), 7.58 (d, 1H, *J*=8.4Hz, H-6′), 7.66 (d, 2H, *J*=8.4Hz, H-2,6),

8.68 (bs, 1H, NH); ^{13}C NMR (δ, ppm): 22.1 (-CH_3), 22.9 (-CH_2), 44.8 (CH, C-9), 50.3 (CH, C-3), 58.5 (-O**C**H_3), 114.1 - 144.8 (phenyl -C), 159.1 (C=N, C-5, 8); Mass [$C_{19}H_{20}N_4O_2$]: *m/z* 337 (M^+).

*4-(5-(4-Methylphenyl)-3,3a,4,7-tetrahydro-2*H*-pyrazolo[3,4-c]pyridazin-3-yl)*-N,N-*dimethylbenzenamine* (**4e**) : R=4-CH_3, R′=4-N(CH_3)$_2$; Yield: 59 %; M.p. (°C): 196-198; R_f (benzene : acetone, 8:2): 0.89; IR (KBr, cm^{-1}): 3344 (NH), 3296 (NH); ^{1}H NMR (δ, ppm): 2.28-2.34 (m, 3H, H_b and H_c), 2.38 (s, 3H, CH_3), 3.08 (d, 1H, *J*=11.2Hz, H_a), 3.12 (s, 3H, CH_3), 3.14 (s, 3H, CH_3), 6.68 (bs, 1H, NH), 6.86 (d, 2H, *J*=8.4Hz, H-3′,5′), 7.32 (d, 2H, *J*=8.4Hz, H-3,5), 7.48 (d, 2H, *J*=8.4Hz, H-2′,6′), 7.62 (d, 2H, *J*=8.4Hz, H-2,6), 8.74 (bs, 1H, NH); ^{13}C NMR (δ, ppm): 21.3 (-CH_3), 22.4 (-CH_2), 43.8 (CH, C-9), 46.2 (N-**C**H_3), 49.6 (CH, C-3), 118.1-148.2 (phenyl -C), 157.4 (C=N, C-5, 8); Mass [$C_{20}H_{23}N_5$]: *m/z* 334 (M^+).

3-(3-Bromophenyl)-5-(4-chlorophenyl)-3,3a,4,7-tetrahydro-2H-pyrazolo[3,4-c]pyridazine (**4f**): R = 4-Cl, R′= 3-Br; Yield: 70 %; M.p. (°C): 168-170; R_f (benzene: acetone, 8:2): 0.77; IR (KBr, cm^{-1}): 3340 (NH), 3294 (NH); ^{1}H NMR (δ, ppm): 2.32-2.36 (m, 3H, H_b and H_c), 3.08 (d, 1H, *J*=11.2Hz, H_a), 6.60 (bs, 1H, NH), 7.50-7.56 (m, 4H, H-3,5 and H-4′,5′), 7.60 (s, 1H, H-2′),7.68-7.70 (m, 3H, H-2,6 and H-6′), 8.92 (bs, 1H, NH); 22.7 (-CH_2), 44.3 (CH, C-9), 49.1 (CH, C-3), 119.3-143.5 (phenyl-C), 155.1 (C=N, C-5, 8); Mass [$C_{17}H_{14}$Br ClN_4]: *m/z* 433 (M^+), 435 (M^++2).

3-(4-Bromophenyl)-5-(4-chlorophenyl)-3,3a,4,7-tetrahydro-2H-pyrazolo[3,4-c]pyridazine (**4g**): R = 4-Cl, R′= 4-Br; Yield: 63 %; M.p. (°C): 165-167; R_f (benzene: acetone, 8:2): 0.73; IR (KBr, cm^{-1}): 3342 (NH), 3298 (NH); ^{1}H NMR (δ, ppm): 2.30-2.34 (m, 3H, H_b and H_c), 3.10 (d, 1H, *J*=11.2Hz, H_a), 6.64 (bs, 1H, NH), 7.50 (d, 2H, *J*=8.4Hz, H-3′,5′), 7.54 (d, 2H, *J*=8.4Hz, H-3,5), 7.64 (d, 2H, *J*=8.4Hz, H-2′,6′), 7.72 (d, 2H, *J*=8.4Hz, H-2,6), 8.86 (bs, 1H, NH); ^{13}C NMR (δ, ppm): 23.5 (-CH_2), 45.3 (CH, C-9), 50.1 (CH, C-3), 113.3-141.6 (phenyl-C), 158.4 (C=N, C-5, 8); Mass [$C_{17}H_{14}BrClN_4$]: *m/z* 433 (M^+), 435 (M^++2)

5-(3,4-Dimethoxyphenyl)-3-(4-chlorophenyl)-3,3a,4,7-tetrahydro-2H-pyrazolo[3,4-c]pyridazine (**4h**): R = 4-Cl, R′=3,4 -OCH_3; Yield: 65 %; M.p. (°C): 216-218; R_f (benzene : acetone, 8:2): 0.82; IR (KBr, cm^{-1}): 3344 (NH), 3288 (NH); ^{1}H NMR (δ, ppm): 2.30-2.34 (m, 3H, H_b and H_c), 3.08 (d, 1H, *J*=11.2Hz, H_a), 3.80 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 6.58 (bs, 1H, NH), 6.94 (d, 1H, *J*=8.8Hz, H-5′), 7.44 (s, 1H, H-2′), 7.50 (d, 1H, *J*=8.8Hz, H-6′), 7.54 (d, 2H, *J*=8.4Hz, H-3,5), 7.70 (d, 2H, *J*=8.4Hz, H-2,6), 8.76 (bs, 1H, NH); ^{13}C NMR (δ, ppm): 22.2 (-CH_2), 44.8 (CH, C-9), 49.3 (CH, C-3), 57.5 (-O***C***H_3), 117.8-145.9 (phenyl -C), 158.4 (C=N, C-5, 8); Mass [$C_{19}H_{19}ClN_4O_2$]: *m/z* 372 (M^+), 374 (M^++2).

5-(4-Hydroxy-3-methoxyphenyl)-3-(4-chlorophenyl)-3,3a,4,7-tetrahydro-2H-pyrazolo[3,4-c]pyridazine (**4i**): R =4-Cl, R′=3-OCH_3, 4-OH; Yield: 55 %; M.p. (°C): 225-227; R_f (benzene : acetone, 8:2): 0.86; IR (KBr, cm^{-1}): 3336 (NH), 3290 (NH); ^{1}H NMR (δ, ppm): 2.32-2.36 (m, 3H, H_b and H_c), 3.10 (d, 1H, *J*=11.2Hz, H_a), 3.84 (s, 3H, OCH_3), 6.62 (bs, 1H, NH), 6.92 (d, 1H, *J*=8.8Hz, H-5′), 7.42 (s, 1H, H-2′), 7.52 (d, 1H, *J*=8.8Hz, H-6), 7.56 (d, 2H, *J*=8.4Hz, H-3,5), 7.72 (d, 2H, *J*=8.4Hz, H-2,6), 8.78 (bs, 1H, NH), 10.46 (bs, 1H, OH); ^{13}C NMR (δ, ppm): 21.4 (-CH_2), 43.2 (CH, C-9), 49.7 (CH, C-3), 55.6 (-O**C**H_3), 116.6-143.1 (phenyl -C), 155.7 (C=N, C-5, 8); Mass [$C_{18}H_{17}ClN_4O_2$]: *m/z* 358 (M^+), 360 (M^++2).

4-(5-(4-Chlorophenyl)-3,3a,4,7-tetrahydro-2H-pyrazolo[3,4-c]pyridazin-3-yl)-N,N-dimethylbenzenamine (**4j**): R = 4-Cl, R′ = 4-N(CH_3)$_2$; Yield: 52 %; M.p. (°C): 253-255; R_f (benzene : acetone, 8:2): 0.85; IR (KBr, cm^{-1}): 3338 (NH), 3304 (NH); ^{1}H NMR (δ, ppm): 2.30-2.34 (m, 3H, H_b and H_c), 3.08 (d, 1H, *J*=11.2Hz, H_a), 3.12 (s, 3H, CH_3), 3.14 (s, 3H, CH_3), 6.55 (bs, 1H, NH), 6.86 (d, 2H, *J*=8.8Hz, H-3′,5′), 7.48 (d, 2H, *J*=8.8Hz, H-2′,6′), 7.54 (d, 2H, *J*=8.4Hz, H-3,5), 7.72 (d, 2H, *J*=8.4Hz, H-2,6), 8.76 (bs, 1H, NH); ^{13}C NMR (δ, ppm): 22.3 (-CH_2), 43.5 (CH, C-9), 46.8 (N-***C***H_3), 49.2 (CH, C-3), 115.1-145.7 (phenyl -C), 159.3 (C=N, C-5, 8); Mass [$C_{19}H_{20}ClN_5$]: *m/z* 335 (M^+), 337 (M^++2).

Microbiology

Antibacterial activity [21]: Antibacterial activity of the compounds was determined by adopting cup plate method. In this method, sample solution diffuses from a vertical cylinder or a cavity through the solidified agar layer of a Petri dish in a manner that growth of the added microorganism is prevented entirely in a circular area or a zone around the cylinder or cavity containing a solution of the sample if the added sample possesses antibacterial activity. For determining antibacterial activity, freshly prepared liquid agar medium (35 mL/Petri dish) was transferred into the Petri dishes (8 Petri dishes/sample) and allowed the medium to solidify. Then, the 200 µL-standardized culture (99 mL Nutrient broth media + 1 mL culture) of organism was spread on each Petri dish by L-shaped spreader. With the help of the borer (5 mm), three bores were made in each plate. The synthetic compounds diluted with dimethyl sulfoxide (DMSO) at three different concentrations (50, 100, and 200 µg/mL) were added to each well separately. The Petri dishes were kept aseptically for approximately 4 to 5 h for diffusion of the sample. Following diffusion, all the Petri dishes were incubated for 24 h at a temperature of 37 ºC. After the stipulated period of 24 h, the activity of compounds in terms of zone of inhibition was observed against two Gram-positive: *Staphylococcus aureus* (*S. aureus*) and *Micrococcus luteus* (*M. luteus*), and two Gram-negative microbial strains; *Escherichia coli* (*E. coli)* and *Klebsiella pneumoniae* (*K. pneumoniae*). Ampicillin and chloramphenicol antibiotics were used as positive control for the comparison purpose. Antibacterial activity of the synthesized compounds is reported in **Table 1**.

Antifungal activity [22]: The Sabouraud agar medium (dextrose 4 %, peptone 1 %, and agar 1.5 %) was used for determining antifungal activity of the compounds. The medium was prepared and sterilized in an autoclave for 15 min at 15 psi. Then, it was aseptically transferred into sterilized Petri plates. After duration of 2 h, the two fungal strains; *Candida albicans* (*C. albicans*) and *Cryptococcus neoformans* (*C. neoformans*) were inoculated on the surface of Petri plates separately. Following this, the cups

Table 1 Antibacterial activity of the title compounds (**4a-j**)

Compound	Concentration (µg/mL)	Zone of inhibition (in mm)			
		Gram positive		Gram negative	
		S. aureus	***M. luteus***	***E. coli***	***K. pneumoniae***
4a	50	-	-	-	-
	100	6	-	-	6
	200	9	-	8	9
4b	50	-	-	-	-
	100	-	-	7	8
	200	9	-	10	11
4c	50	-	-	-	-
	100	-	11	-	11
	200	13	14	14	16
4d	50	-	-	-	-
	100	-	-	-	-
	200	15	14	13	-
4e	50	-	-	-	-
	100	11	-	12	-
	200	14	15	17	-
4f	50	-	-	-	-
	100	-	13	-	14
	200	18	15	14	15
4g	50	-	-	-	-
	100	-	15	17	15
	200	16	17	15	16
4h	50	-	-	-	-
	100	19	-	-	18
	200	20	19	20	20
4i	50	-	-	-	-
	100	16	-	-	17
	200	20	21	20	21
4j	50	-	-	-	-
	100	-	-	19	18
	200	15	18	20	19
Ampicillin	50	24	22	27	24
Chloramphenicol	50	23	27	25	22

"-" refers to no antibacterial activity

of approximately 6 mm in diameter were made in the Sabouraud agar medium using sterilized cup borer under aseptic conditions. Then 0.1 mL of each standard (100 µg/mL) and test compounds (100, 250 and 500 µg/mL) prepared by dissolving in DMSO was added into cups. Following addition of solutions, these Petri plates were incubated for 48 h at a temperature of 28 ± 2 ºC and then growth and zones of inhibition (in mm) were recorded. Fluconazole and griseofulvin drugs were used as positive control for the comparison purpose. The antifungal activity of synthesized compounds is tabulated in **Table 2**.

Results and Discussion

The reaction of 4-(4-methylphenyl)-4-oxobutanoic acid (**1a)** and 4-(4-chlorophenyl)-4-oxobutanoic acid (**1b**) with hydrazine hydrate gave 6-(4-methylphenyl)-2,3,4,5-tetrahydropyridazin-3-one (**2a**) and 6-(4-chlorophenyl)-2,3,4,5-tetrahydropyridazin-3-one (**2b**), respectively. Aryl-aldehydes were reacted with ethanolic solution of **2a** or **2b** in presence of piperidine to obtain (4*E*)-4-substituted-benzylidene-6-(4-chlorophenyl)-4,5-dihydro-pyridazin-3(2*H*)-ones (**3a-j**). Finally, compounds **3a-j** were treated with hydrazine hydrate to get the desired compounds **4a-j**; 3-substituted-phenyl-5-(4-chlorophenyl) -3,3a,4,7-tetrahydro-2*H*-pyrazolo[3,4-c]pyridazines. The structure of the compounds were established on the basis of modern analytical techniques; IR, ^{13}C-NMR, Mass and elemental analysis data results. The spectral and analytical data are in full agreement with the proposed structures. IR spectra of all title compounds (**4a-j**) showed distinct bands in the region 3290-3344 cm^{-1} indicating the presence of NH group. Also no peak was observed for the carbonyl group of pyridazin3-one (**3a-j**) in the final compounds, which confirm the formation of pyrazole ring as a result of reaction between carbonyl group of pyridazinone and amino group of hydrazine hydrate. In the ^{1}H NMR of compounds **4a-e**, a singlet of three protons was observed at δ2.38-2.42, which was assigned to methyl protons of phenyl ring while this peak was missing in compounds **4f-j**, as methyl group was replaced by 4-

Table 2 Antifungal activity of the title compounds (4a-j)

Compound	Concentration (µg/mL)	Zone of inhibition (in mm)	
		C. albicans	*C. neoformans*
4a	100	-	-
	250	8	11
	500	11	14
4b	100	5	8
	250	7	12
	500	14	18
4c	100	-	-
	250	6	8
	500	9	11
4d	100	-	-
	250	6	9
	500	9	12
4e	100	-	-
	250	6	5
	500	9	7
4f	100	6	-
	250	9	11
	500	15	12
4g	100	12	13
	250	15	17
	500	20	21
4h	100	-	-
	250	10	8
	500	12	11
4i	100	-	-
	250	9	9
	500	12	10
4j	100	-	-
	250	10	8
	500	11	12
Fluconazole	100	24	28
Griseofulvin	100	23	26

"-" refers to no antifungal activity

chloro group. A doublet of one proton at δ 3.08-3.10 (J=11.2 Hz) was attributed to the CH_a of pyrazole ring. However, two protons at C-4 and one proton at C-9 position of pyrazole-pyridazine ring (**Figure 1**), a multiplet of three protons was observed in the region δ 2.28-2.36. In addition to this, compound **4c**, **4d**, **4h** and **4i** showed additional singlets downfield due to the presence of methoxy group(s). ^{13}C-NMR spectra further supported the chemical structure of synthesized compounds. The molecular ion peak (M^+) for all the synthesized compounds was also obtained in mass spectra and was of good intensity. The mass spectra of all compounds barring **4c**, **4d** and **4e** also showed M^++2 isotopic peak, which is due to the presence of chlorine or bromine atom in the molecule. The elemental analysis for C, H, and N was also within the ± 0.4 % range of theoretical values.

The newly synthesized compounds (**4a-j**) were screened for their antibacterial activity against *S. aureus*, *M. luteus*, *E. coli* and *K. pneumonia* by cup plate technique in nutrient agar at concentrations of 50, 100 and 200 µg/mL (**Table 1**). DMSO was used as the control, and ampicillin and chloramphenicol as standard drugs for comparison. These pathogenic microbes are common cause of bacterial infections in humans affecting skin, lungs, nose, mouth, GIT and other organs. They are also routinely detected in very young, very old and people suffering from some other diseases such as cancer [23]. *K. pneumoniae* is the most common cause of hospital acquired respiratory tract and premature intensive care infections. *S. aureus* and *E. coli* can be grown and cultured easily and inexpensively in a laboratory setting, and has been intensively investigated for many decades. Both ampicillin and chloramphenicol showed broad spectrum of antibacterial activity against all the four microbes at the dose of 50 µg/mL. The zone of inhibition was observed to be in the range of 22-27 mm, however, none of the tested synthesized compounds was found to be active in inhibiting the growth of micro-organisms at this concentration. All the title compounds except **4d** exhibited moderate to good antibacterial activity at a concentration of 100 µg/mL. It is evident from the results of antibacterial evaluation, that most of the compounds have comparable activity against the tested bacterial strains. The maximum inhibition of microbial growth was noted at 200 µg/mL concentration and compounds **4i** and **4h** were found to be the highly active against both Gram-positive and Gram-negaitive bacterial strains. Compound **4i,** in which 4-chlorophenyl is attached to pyridazine ring and 4-hydroxy, 3-methoxy phenyl substituent is connected to pyrazole core, exhibited the best activity against all the four strains. Compounds **4j** and **4f** showed moderate type of activity against the bacteria. The zone of inhibition against *S. aureus*, *M. luteus*, *E. coli* and *K. pneumonia* was observed to be 6 - 20 mm, 11 - 21mm, 7 - 20 mm and 6-21 mm, respectively. It was quite surprising that compounds **4a**, **4b** did not show any activity against *M. luteus* even at the highest concentration. Similarly, *K. pneumonia* was found to be resistant to **4d** and **4e** at a concentration of 200 µg/mL concentration. The compounds **4a**, **4b**, **4d** and **4e** have common structural feature i.e., these compounds bear electron donating groups on both phenyl rings, however, the most active compounds **4i** and **4h** have an electron withdrawing group (-Cl) on phenyl ring attached to pyridazine nucleus. Interestingly, compound **4f** and **4g** which contain electron withdrawing groups (–Cl, –Br) on both phenyl rings are less active than the other compounds in series (**4h-j**), which have one electron withdrawing group and one electron donating group on phenyl rings. Thus it could be concluded that presence of electron donating group such as methyl on phenyl ring at C-5 position of fused bicyclic ring system leads to decrease in antibacterial activity.

The title compounds were also evaluated for their antifungal activity against *C. albicans* and *C. neoformans* using cup-plate method in the sabouraud agar medium at concentrations of 100, 250 and 500 µg/mL (**Table 2**). The zone of inhibition (mm) of each compound was determined and compared with the standard drug, fluconazole and griseofulvin. A concentration dependent anti-fungal activity was noted for the tested compounds. All the compounds were inactive against the fungal strains at the lowest concentration of 100 µg/mL barring **4b** and **4f.** The result clearly indicated that compounds bearing electron donating groups (**4b** and **4f**) exhibited better activity against both the fungal strains. Further, compound

bearing two electron withdrawing groups (**4g**) was found to be the most active antifungal agent and its activity was comparable with the standard drugs. The rest of the compounds were moderate in their antifungal action.

Conclusion

In conclusion, 3-substituted-phenyl-5-(4-chloro phenyl)-3,3a,4,7-tetrahydro-2*H*-pyrazolo[3,4-c]pyridazine (**4a-j**) were successfully synthesized through multistep synthesis. Microbiological screening of these compounds showed the antimicrobial potential of the pyrazolo-pyridazine derivatives. From the antimicrobial data, it could be concluded that compound bearing both electron withdrawing (–Cl) and donating (–OH, $–OCH_3$) groups **(4i)** appeared to be the most active against *Gram-positive* and *Gram-negaitive* bacteria, whereas compound bearing only electron donating groups **(4g)** exhibited potential antifungal activity. These compounds could be further derivatized to get even better antimicrobial agents.

Conflict of interest

Authors declare no conflict of interest.

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