SYNTHESIS AND ANTIMICROBIAL ACTIVITIES OF NEW PYRAZOLO-PYRIDAZINE DERIVATIVES

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ABSTRACT:

Several new pryrazolo-pyridazine derivatives (**4a-h**) were synthesized through multi-step synthesis and evaluated for their antimicrobial activities. In the first step, 6-phenyl-2,3,4,5-tetrahydropyridazin-3-one (**2**) was prepared by reacting 4-(4-chlorophenyl)-4-oxobutanoic acid (**1**) with hydrazine hydrate. Then, aryl-aldehydes were reacted with **2** to furnish pyridazinone derivatives (**3a-g**). Finally, pyridazinones (**3a-h**) were reacted with hydrazine hydrate to furnish the title compounds (**4a-h**). The newly synthesized compounds were evaluated for their *in vitro* antibacterial and antifungal activities against six microbial strains. Compounds **4d, 4e** and **4f** exhibited significant antibacterial action, whereas compounds **4c** and **4d** showed potential antifungal activity. Compound **4d**, 5-(4-Chlorophenyl)-3-(4-fluorophenyl)-3,3a,4,7-tetrahydro-2H-pyrazolo[3,4-c]pyridazine, emerged as lead compound having broad spectrum of antimicrobial action.

Keywords: Pyridazinone, pyrazole, fused, antibacterial, antifungal

INTRODUCTION:

Pyridazine, a six membered nitrogen containing heterocyclic ring, plays an important role in pharmaceuticals particularly in the field of medicinal chemistry. Pyridazine ring is a part of the structures of a number of drugs available in the market[1] like cadralazine, minaprine, hydralazine, pipofezine, etc (**Figure 1**). Pyridazine derivatives found to possess important biological activities[2-9] including antibacterial, antifungal, anti-tubercular, anticonvulsant, antihypertensive, analgesic and anti-inflammatory. Another heterocycle, pyrazole, is an example of five-membered nitrogen containing heterocyclic ring systems. Pyrazole derivatives have also been reported to possess potential biological activities including antibacterial and antifungal actions[10,11].

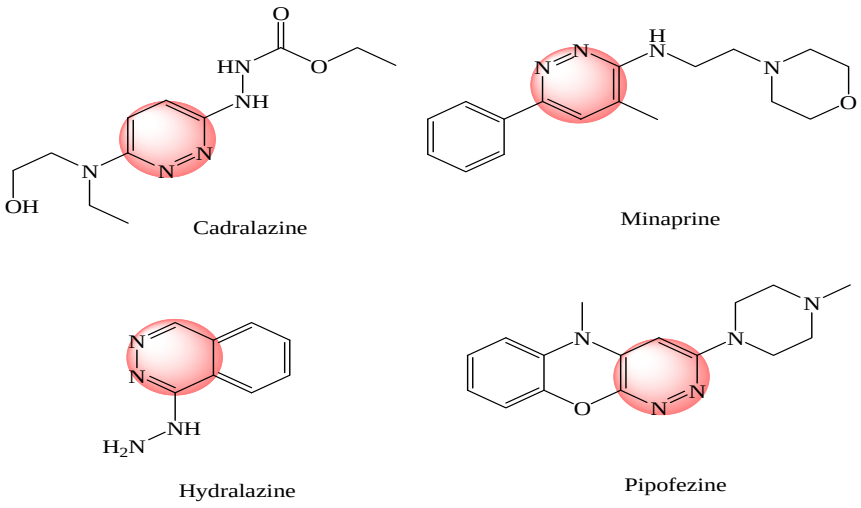
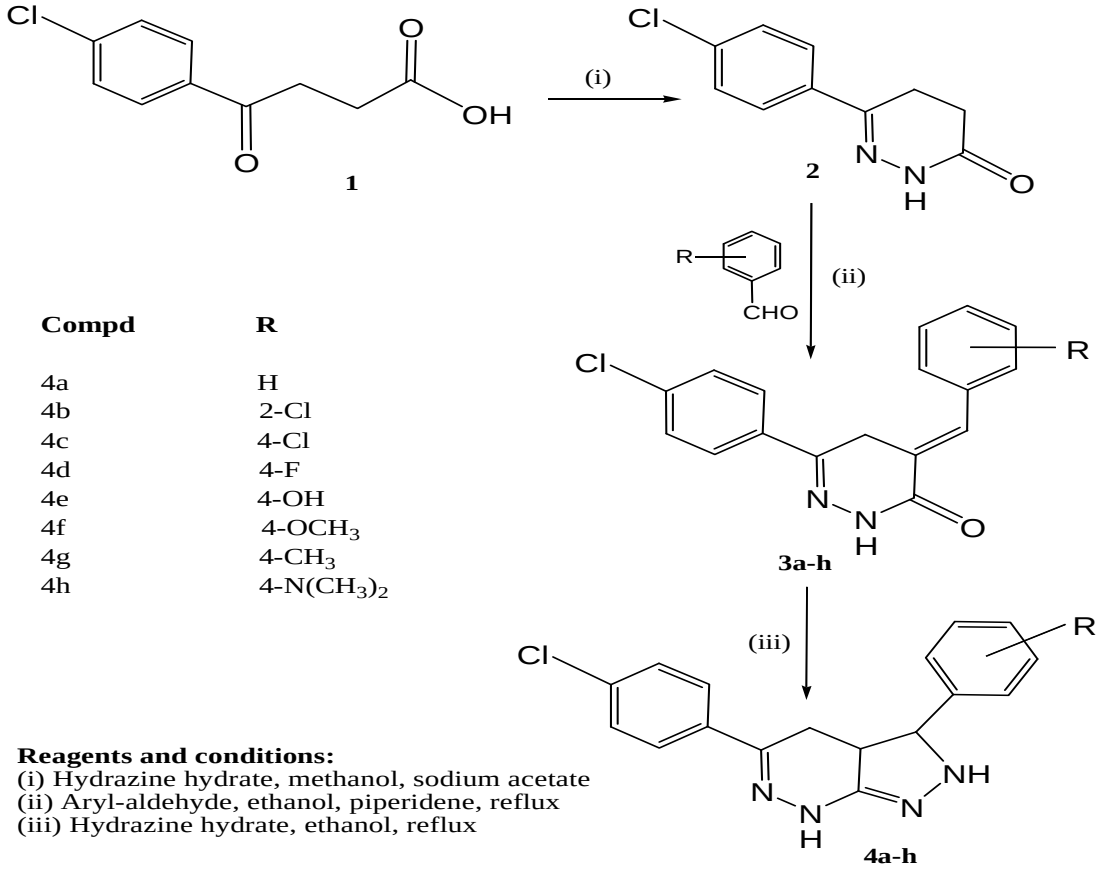


Figure 1: Pyridazine Bearing Drugs

In view of the antimicrobial activities associated with pyridazine and pyrazole derivatives, it was thought worthwhile to study their fused derivatives as antibacterial and antifungal agents. Thus, several pryrazolo-pyridazine derivatives (**4a-h**) have been synthesized and evaluated for their antibacterial and antifungal actions against six microbial strains.

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 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, **2** and **3a-h**, and target compounds **4a-h** was achieved following **Scheme 1**.



Compd	R
4a	H
4b	2-Cl
4c	4-Cl
4d	4-F
4e	4-OH
4f	4-OCH_3
4g	4-CH_3
4h	4-$N(CH_3)_2$

Reagents and conditions:
(i) Hydrazine hydrate, methanol, sodium acetate
(ii) Aryl-aldehyde, ethanol, piperidene, reflux
(iii) Hydrazine hydrate, ethanol, reflux

Scheme 1: Protocol for synthesis of title compounds (4a-h).

6-(4-Chlorophenyl)-2,3,4,5-tetrahydropyridazin-3-one (**2**), was synthesized by reported method[12] with slight modifications. The reaction of 4-(4-chlorophenyl)-4-oxobutanoic acid (**1**) with hydrazine hydrate furnished compound **2**. It was used for the preparation of compounds **3a-h**; 4-substituted-benzylidene-6-(4-chlorophenyl-4,5-dihydropyridazin-3(2*H*)-ones. In the final step, compounds **3a-h** were treated with hydrazine hydrate to get the title compounds; 3-substituted-phenyl-5-(4-chlorophenyl)-3,3a, 4,7-tetrahydro-2*H*-pyrazolo[3,4-c]pyridazine (**4a-h**).

General procedure for synthesis of 4-substituted-benzylidene-6-(4-chlorophenyl)-4,5-dihydropyridazin-3(2*H*)-one (3a-h): A mixture of compound **2** (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-benzylidene-6-(4-chlorophenyl)-4,5-dihydropyridazin-3(2*H*)-one **(3a)**:* IR (cm^{-1}, KBr): 3286 (NH), 1672 (CO); ^{1}H NMR (δ, *ppm*): 2.98, s, 2H, CH_2; 7.36-7.40, m, 3H, H-3', 4',5'; 7.54, d, 2H, J=8.0Hz, H-3,5; 7.60, d, 2H, J=8.4Hz, H-2',6'; 7.70, d, 2H, J=8.0Hz, H-2,6; 7.92, s, 1H, arylidene; 10.88, bs, 1H, CONH; Mass: *m/z* 296 (M^+), 298 (M^++2).

*(4E)-4-(2-chlorobenzylidene)-6-(4-chlorophenyl)-4,5-dihydropyridazin-3(2*H*)-one* ***(4b)*****:** IR (cm^{-1}, KBr): 3294 (NH), 1672 (CO); ^{1}H NMR (δ, *ppm*): 2.96, s, 2H, CH_2; 7.42-7.48, m, 3H, H-3', 4',5'; 7.56, d, 2H, J=8.0Hz, H-3,5; 7.66, d, 1H, J=8.4Hz, H-6'; 7.70, d, 2H, J=8.0Hz, H-2,6; 7.90, s, 1H, arylidene; 10.84, bs, 1H, CONH; Mass: *m/z* 330 (M^+), 332 (M^++2).

*(4E)-4-(4-chlorobenzylidene)-6-(4-chlorophenyl)-4,5-dihydropyridazin-3(2*H*)-one* ***(3c)*****:** IR (cm^{-1}, KBr): 3279 (NH), 1663 (CO); ^{1}H NMR (δ, *ppm*): 3.02, s, 2H, CH_2; 7.52-7.56, m, 4H, H-3,5 & H-3',5'; 7.64, d, 2H, J=8.4Hz, H-H-2',6'; 7.72, d, 2H, J=8.0Hz, H-2,6; 7.94, s, 1H, arylidene; 10.80, bs, 1H, CONH; *m/z* 330 (M^+), 332 (M^++2).

*(4E)-4-(4-fluorobenzylidene)-6(4-chlorophenyl)-4,5-dihydropyridazin-3(2*H*)-one* ***(3d)*****:** IR (cm^{-1}, KBr): 3284 (NH), 1672 (CO); ^{1}H NMR (δ, *ppm*): 2.98, s, 2H, CH_2; 7.22, t, 2H, J=7.6Hz, H-3',5'; 7.52, d, 2H, J=8.4Hz, H-3,5; 7.62, m, 2H, H-2',6'; 7.70, d, 2H, J=8.4Hz, H-2,6; 7.90, s, 1H, arylidene; 10.78, bs, 1H, CONH; *m/z* 314 (M^+), 316 (M^++2).

*(4E)-4-(4-hydroxybenzylidene)-6-(4-chloropheny)l-4,5-dihydropyridazin-3(2*H*)-one* ***(3e):*** IR (cm^{-1}, KBr): 3290 (NH), 1675 (CO); ^{1}H NMR (δ, *ppm*): 3.08, s, 2H, CH_2; 6.94, d, m, J=8.8Hz, H-3',5'; 7.52, d, m, J=8.4Hz, H-3,5; 7.62, d, m, J=8.8Hz, H-2',6'; 7.68, d, m, J=8.4Hz, H-2,6; 7.88, s, 1H, arylidene; 10.82, bs, 1H, CONH; *m/z* 312 (M^+), 314 (M^++2).

*(4E)-4-(4-methoxybenzylidene)-6-(4-chlorophenyl)-4,5-dihydropyridazin-3(2*H*)-one* ***(3f):*** IR (cm^{-1}, KBr): 3298 (NH), 1681 (CO); ^{1}H NMR (δ, *ppm*): 3.02, s, 2H, CH_2; 3.84, s, 3H, CH_3; 6.98, d,2H, J=8.8Hz, H-3',5'; 7.50, d, 2H, J=8.4Hz, H-3,5; 7.64, d, m, J=8.8Hz, H-2',6'; 7.70, d, m, J=8.4Hz, H-2,6; 7.90, s, 1H, arylidene; 10.84, bs, 1H, CONH; *m/z* 326 (M^+), 328 (M^++2).

*(4E)-4-(4-methylbenzylidene)-6-(4-chlorophenyl)-4,5-dihydropyridazin-3(2*H*)-one* ***(3g):*** IR (cm^{-1}, KBr): 3287(NH), 1669 (CO); ^{1}H NMR (δ, *ppm*):): 2.42, s, 3H, CH_3; 2.94, s, 2H, CH_2; 7.30, d, 2H, J=8.4Hz, H-3',5'; 7.50, d, 2H, J=8.4Hz, H-3,5; 7.58, d, 2H, J=8.4Hz, H-2',6'; 7.70, d, 2H, J=8.4Hz, H-2,6; 7.90, s, 1H, arylidene; 10.80, bs, 1H, CONH; *m/z* 310 (M^+), 312 (M^++2).

*(4E)-4[4-(dimethylamino)benzylidene]-6-(4-chlorophenyl)-4,5-dihydropyridazin-3(2*H*)-one* ***(3h):*** 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 & H-2',6'; 7.70, d, 2H, J=8.4Hz, H-2,6; 7.88, s, 1H, arylidene; 10.84, bs, 1H, CONH; *m/z* 339 (M^+), 341 (M^++2).

General procedure for synthesis of 3-substituted-phenyl-5-(4-chlorophenyl)-3,3a, 4,7-tetrahydro-2*H*-pyrazolo[3,4-c]pyridazine (4a-h): Ethanolic solution of compounds **3a-h** (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.

*5-(4-Chlorophenyl)-3-phenyl-3,3a,4,7-tetrahydro-2*H*-pyrazolo[3,4-c]pyridazine* ***(4a):*** IR (cm^{-1}): 3348 (NH), 3302 (NH); ^{1}H NMR (δ, *ppm*): 2.32-2.36, m, 3H, $H_{b\&}H_c$; 3.10, d, 1H, J=11.2Hz, H_a; 7.30-7.36, m, 3H, H-3',4',5'; 6.62, bs, 1H, NH; 7.48, d, 2H, J=8.0Hz, H-3,5; 7.56, d, 2H, J=8.4Hz, H-2',6'; 7.72, d, 2H, J=8.0Hz, H-2,6; 9.02, bs, 1H, NH; Mass: *m/z* 310 (M^+), 312 (M^++2).

*3-(2-Chlorophenyl)-5-(4-chlorophenyl)-3,3a,4,7-tetrahydro-2*H*-pyrazolo[3,4-c]pyridazine* ***(4b):*** IR (KBr, cm^{-1}): 3338 (NH), 3292 (NH); ^{1}H NMR (δ, *ppm*): 2.32-2.36, m, 3H, $H_{b\&}H_c$; 3.10, d, 1H, J=11.2Hz, H_a; 6.60, bs, 1H, NH; 7.38-7.44, m, 3H, H-3',4',5'; 7.50, d, 2H, J=8.4Hz, H-3,5; 7.58, d, 2H, J=8.4Hz, H-2',6'; 7.72, d, 2H, J=8.4Hz, H-2,6; 8.96, bs, 1H, NH; Mass: *m/z* 344 (M^+), 346 (M^++ 2).

*3,5-bis(4-Chlorophenyl)-3,3a,4,7-tetrahydro-2*H*-pyrazolo[3,4-c]pyridazine* ***(4c):*** IR (KBr, cm^{-1}): 3328 (NH), 3288 (NH); ^{1}H NMR (δ, *ppm*): 2.34-2.38, m, 3H, $H_{b\&}H_c$; 3.08, d, 1H, J=11.2Hz, H_a; 6.64, bs, 1H, NH; 7.48-7.52, m, 4H, H-3,5 & H-3',5'; 7.62, d, 2H, J=8.4Hz, H-2',6'; 7.70, d, 2H, J=8.4Hz, H-2,6; 8.96, bs, 1H, NH; Mass: *m/z* 344 (M^+), 346 (M^++ 2).

*5-(4-Chlorophenyl)-3-(4-fluorophenyl)-3,3a,4,7-tetrahydro-2*H*-pyrazolo[3,4-c]pyridazine* ***(4d):*** IR (KBr, cm^{-1}): 3336 (NH), 3304 (NH); ^{1}H NMR (δ, *ppm*): 2.34-2.38, m, 3H, $H_{b\&}H_c$; 3.10, d, 1H, J=11.2Hz, H_a; 6.62, bs, 1H, NH; 7.20, t, 2H, J=7.6Hz, H-3',5'; 7.52, d, 2H, J=8.4Hz, H-3,5; 7.60, m, 2H, H-2',6'; 7.72, d, 2H, J=8.4Hz, H-2,6; 8.80, bs, 1H, NH; Mass: *m/z* 328 (M^+), 330 (M^++ 2).

*4-{5-(4-Chlorophenyl)-3,3a,4,7-tetrahydro-2*H*-pyrazolo[3,4-c]pyridazin-3-yl}phenol* ***(4e):*** IR (KBr, cm^{-1}): 3330 (NH), 3296 (NH); ^{1}H NMR (δ, ppm): 2.30-2.34, m, 3H, $H_{b\&}H_c$; 3.08, d, 1H, J=11.2Hz, H_a; 6.60, bs, 1H, NH; 6.88, d, 2H, J=8.8Hz, H-3',5'; 7.54, d, 2H, J=8.4Hz, H-3,5; 7.60, d, 2H, J=8.8Hz, H-2',6'; 7.72, d, 2H, J=8.4Hz, H-2,6; 8.76, bs, 1H, NH; 10.74, bs, 1H, OH; Mass: *m/z* 326 (M^+), 328 (M^++2).

*5-(4-Chlorophenyl)-3-(4-methoxyphenyl)-3,3a,4,7-tetrahydro-2*H*-pyrazolo[3,4-c]pyridazine* ***(4f):*** IR (KBr, cm^{-1}): 3338 (NH), 3290 (NH); ^{1}H NMR (δ, *ppm*): 2.32-2.36, m, 3H, $H_{b\&}H_c$; 3.10, d, 1H, J=11.2Hz, H_a; 3.84, s, 3H,

OCH_3; 6.68, bs, 1H, NH; 6.96, d, 2H, J=8.8Hz, H-3',5'; 7.50, d, 2H, J=8.4Hz, H-3,5; 7.58, d, 2H, J=8.8Hz, H-2',6'; 7.72, d, 2H, J=8.4Hz, H-2,6; 8.80, bs, 1H, NH; Mass: *m/z* 340 (M^+), 342 (M^++2).

*5-(4-Chlorophenyl)-3-(4-methylphenyl)-3,3a,4,7-tetrahydro-2*H-*pyrazolo[3,4-c]pyridazine* ***(4g):*** IR (KBr, cm^{-1}): 3342 (NH), 3296 (NH); ^{1}H NMR (δ, *ppm*):): 2.32-2.36, m, 3H, $H_{b\&}H_c$; 2.40, s, 3H, CH_3; 3.08, d, 1H, J=11.2Hz, H_a; 6.52, bs, 1H, NH; 7.30, d, 2H, J=8.4Hz, H-3',5'; 7.52-7.56, m, 4H, H-3,5 & H-2',6'; 7.72, d, 2H, J=8.4Hz, H-2,6; 8.72, bs, 1H, NH; Mass: *m/z* 324 (M^+), 326 (M^++2).

*4-{5-(4-Chlorophenyl)-3,3a,4,7-tetrahydro-2*H-*pyrazolo[3,4-c]pyridazine-3-yl}*-N,N-*dimethylbenzenamine* ***(4h):*** IR (KBr, cm^{-1}): 3338 (NH), 3304 (NH); ^{1}H NMR (δ, *ppm*): 2.30-2.34, m, 3H, $H_{b\&}H_c$; 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; Mass: *m/z* 353 (M^+), 355 (M^++2).

Table 1: Physical parameters of the synthesized compounds (3a-h & 4a-h).

Compd.	R	M.p. (°C)	Yield %	R_f values	Molecular Formula	M .Wt.
3a	H	178-180	72	0.83	$C_{17}H_{13}ClN_2O$	296
3b	2-Chloro	192-193	75	0.79	$C_{17}H_{12}Cl_2N_2O$	330
3c	4-Chloro	184-185	71	0.87	$C_{17}H_{12}Cl_2N_2O$	330
3d	4-Fluoro	165-167	58	0.68	$C_{17}H_{12}ClFN_2O$	314
3e	4-Hydroxy	220-222	76	0.88	$C_{17}H_{13}ClN_2O_2$	312
3f	4-Methoxy	238-240	67	0.77	$C_{18}H_{15}ClN_2O_2$	326
3g	4-Methyl	163-164	75	0.91	$C_{18}H_{15}ClN_2O$	310
3h	4-Dimethyl amino	186-188	69	0.89	$C_{19}H_{18}ClN_2O$	339
4a	H	195-197	62	0.79	$C_{17}H_{15}ClN_4$	310
4b	2-Chloro	191-193	58	0.94	$C_{17}H_{14}Cl_2N_4$	344
4c	4-Chloro	185-186	54	0.91	$C_{17}H_{14}Cl_2N_4$	344
4d	4-Fluoro	208-210	61	0.84	$C_{17}H_{14}ClFN_4$	328
4e	4-Hydroxy	216-217	72	0.93	$C_{17}H_{15}ClN_4O$	326
4f	4-Methoxy	221-223	68	0.79	$C_{18}H_{17}ClN_4O$	340
4g	4-Methyl	246-247	60	0.90	$C_{18}H_{17}ClN_4$	324
4h	4-Dimethyl amino	253-254	52	0.85	$C_{19}H_{20}ClN_5$	353

Solvent of recrystallization: Methanol; R_f values were determined in (Benzene: Acetone (8:2)

Microbiology:

Antibacterial activity[13]

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 (35mL/Petri dish) was transferred into the Petri dishes (8Petri dishes/sample) and allowed the medium to solidify. Then, the 200µL-standardized culture (99 mL Nutrient broth media + 1mL 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) atthree different concentrations (50, 100, and 200µg/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° C. 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*). Antibacterial activity of the synthesized compounds is reported in **Table 2**.

Table 2: Antibacterial activity of the title compounds (4a-g).

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

-shows no antibacterial activity

Antifungal activity [14]

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 a 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 of approximately 6mm in diameter were made in the Sabouraud agar medium using sterilized cup borer under aseptic conditions. Then 0.1mL of each standard (100µg/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±2º C and then growth and zones of inhibition (in mm) were recorded. The antifungal activity of synthesized compounds is tabulated in **Table 3**.

Table 3: Antifungal activity of the title compounds (4a-g).

Compd	**Concentration (µg/mL)**	**Zone of inhibition (in mm)**	
		C. albicans	***C. neoformans***
4a	100	-	-
	250	7	5
	500	9	7
4b	100	-	-
	250	13	10
	500	16	14
4c	100	8	9
	250	13	12
	500	18	20
4d	100	7	9
	250	14	16
	500	19	20
4e	100	-	-
	250	8	10
	500	13	15
4f	100	-	-
	250	9	6
	500	10	9
4g	100	-	-
	250	10	8
	500	11	12
4h	100	-	-
	250	8	8
	500	10	12
Fluconazole	100	24	28
Griseofulvin	100	23	26

-shows no antifungal activity

RESULTS AND DISCUSSION:

The starting material, 4-(4-chlorophenyl)-4-oxobutanoic acid (**1**), was reacted with hydrazine hydrate to get 6-(4-chlorophenyl)-2,3,4,5-tetrahydropyridazin-3-one (**2**). Aryl-aldehydes were reacted with **2** in ethanol in presence of piperidene to furnish (4*E*)-4-substituted-benzylidene-6-(4-chlorophenyl)-4,5-dihydropyridazin-3(2*H*)-ones (**3a-h**). In the final step, compounds **3a-h** were treated with hydrazine hydrate to obtain the title compounds **4a-h**; 3-substituted-phenyl-5-(4-chlorophenyl)-3,3a,4,7-tetrahydro-2*H*-pyrazolo[3,4-c]pyridazines. The structures of the compounds were established on the basis of IR, ^{1}HNMR and Mass analysis data results.

The title compounds (**4a-h**) 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 2**). DMSO was used as the control, and Ampicillin and chloramphenicol as standard drugs for comparison. It is evident from the results of antibacterial evaluation, that most of the compounds have comparable activity against the tested bacterial strains. Compounds **4d, 4e** and **4f** were found to be the highly active against both *Gram positive* and *Gram negative* bacterial strains. Compounds bearing fluoro, hydroxyl or methoxy group exhibited very good activity against all the four strains. 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 3**). The zone of inhibition (mm) of each compound was determined and compared with the standard drug, fluconazole. The results indicated that compounds bearing electron withdrawing group at *para*-position (**4c** & **4d**) exhibited better activity against both the strains. Rests of

the compounds were moderate in their antifungal action. Further, compound bearing fluorine group (**4d**) was found to be the most active antibacterial and antifungal agent.

CONCLUSION:

In conclusion, seven new fused heterocyclic derivatives, 3-substituted-phenyl-5-(4-chlorophenyl)-3,3a,4,7-tetrahydro-2*H*-pyrazolo[3,4-c]pyridazine (**4a-h**), were synthesized and evaluated for their antimicrobial actions against four bacterial and two fungal strains. From the antimicrobial data, it could be concluded that compounds bearing fluoro, hydroxyl or methoxy group (**4d, 4e** and **4f**) were highly active against all the four bacterial strains, whereas compound bearing electron withdrawing group, chloro or fluoro **(4c** & **4d)** exhibited potential antifungal activity. Compound **4d** emerged as lead compound. The data of antimicrobial screening showed the antibacterial and antifungal potential of the pyrazolo-pyridazine derivatives.

Conflict of interest: Authors declare no conflict of interest.

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