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SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SOME NOVEL OXADIAZOLE DERIVATIVES

MOJAHIDUL ISLAM*[1], ANEES A SIDDIQUI[1], RAMADOSS RAJESH[1], AFROZ BAKHT[1] and SUNIL GOYAL[2]

[1] Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Jamia Hamdard, Hamdard Nagar, New Delhi-110062 (India)
[2] Rajendra Institute of Science and Technology, Sirsa, Haryana, (India)

Abstract: A series of 5-{3'-oxo-6'-(substituted aryl)-2',3',4',5'-tetrahydropyridazin-2'-ylmethyl}-2-substituted 1,3,4-oxadiazole has been synthesized. Appropriate aromatic hydrocarbon reacts with succinic anhydride in the presence of $AlCl_3$ to yield β-aroyl propionic acid (**1a**). The corresponding acid is cyclized with hydrazine hydrate to give 6-(substituted aryl)-2,3,4,5-tetrahydro-3-pyridazinone (**1b**).This ntermediate after reaction with ethyl bromoacetate, was hydrazinolyzed into 3-oxo-6-(substituted aryl)-2, 3, 4, 5-tetrahydropyridazinyl acetohydrazide (**1c**).The resulting product was converted into 5-[3'-oxo-6'-(substituted aryl)-2',3',4',5'-tetrahydropyridazin-2'-ylmethyl]-2-substituted 1,3,4-oxadiazole (Scheme 1). All the final compounds were structurally elucidated on the basis of IR, ^{1}H-NMR, MS data and elemental analysis and screened for antibacterial, antifungal and antitubercular activity. All the compounds are evaluated for their antibacterial activity against *E. coli, S. aureus, Micrococcus luteus* and *Klebsiella pneumoniae* by using cup plate technique in the nutrient agar at 100 μg/mL concentration. Antitubercular activity was determined using the BACTEC 460 system. Stock solutions of test compounds were prepared in DMSO. MIC of rifampin was calculated by established procedures.All the synthesized compounds were screened at 6.25 μg/mL against *M. tuberculosis* H37 Rv comparable with that of standard rifampicin and isoniazid. All the final compounds were evaluated for antifungal activity against *C. albicans* and *C. neoformans* by using cup-plate method in the Sabouraud agar media The zone of inhibition (mm) of each compound was determined and compared with standard drug fluconazole.

Keywords: β-aroyl propionic acid, acetohydrazide, 1,3,4-oxadiazole, antimicrobial activity

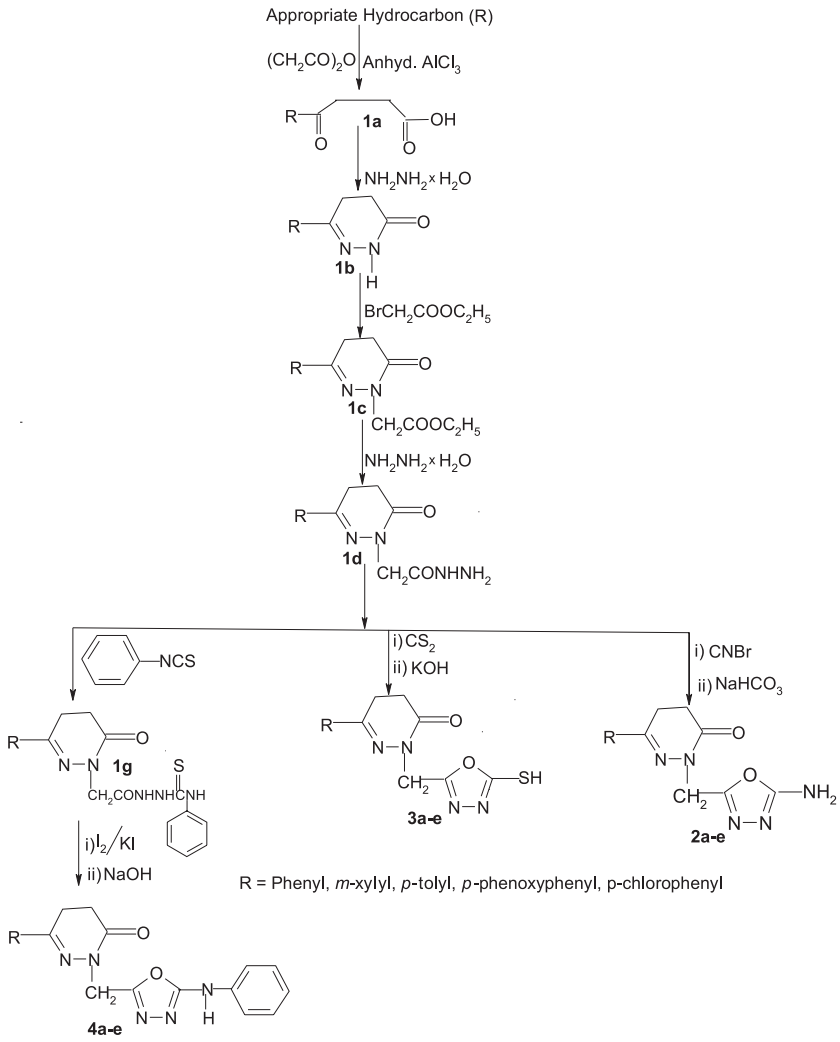
The 1,3,4-oxadiazoles are known to exhibit diverse pharmacological activities like antimicrobial (1-3), antihistaminic (4), anticancerous (5-6), anti-inflammatory (7-9) and antihypertensive activities (10). Hence, some new 1,3,4-oxadiazoles are synthesized according to reaction sequence outlined in Scheme 1. Friedel-Crafts acylation of appropriate hydrocarbons with succinic anhydride in the presence of anhydrous aluminium chloride yielded β-aroyl propionic acids **(1a)** followed by cyclocondensation with hydrazine hydrate to get substituted pyridazinones, (**1b**) which reacted with ethyl bromoacetate to give ethyl 3-oxo-6-(substituted aryl)-2,3,4,5-tetrahydropyridazinyl acetate **(1c)**. The ester was converted into 3-oxo-6-(substituted aryl)-2,3,4,5-tetrahydropyridazinyl acetohydrazide **(1d)** with slight excess of 99% hydrazine hydrate in absolute ethanol under reflux.These acetohydrazides act as starting material for the synthesis of various 2-substituted 1,3,4-oxadiazoles:

I) Equimolar quantities of acetohydrazide **(1d)** and cyanogen bromide in ethanol were refluxed and then neutralized with sodium bicarbonate to yield 5-[3'-oxo-6'-(substituted aryl) 2',3',4',5'-tetrahydropyridazin-2'-ylmethyl]-2-amino-1,3,4-oxadiazoles **(2a-e)**.

II) Equimolar quantities of acetohydrazide (**1d**) and carbon disulfide were refluxed in ethanol and then acidified with dil. HCl to give 5-[3'-oxo-6'-(substituted aryl) 2',3',4',5'-tetrahydropyridazin-2'-ylmethyl]-2-thione-1,3,4-oxadiazoles (**3a-e**).

III) The thiosemicarbazides **(1g)** were conveniently synthesized by refluxing acid hydrazide **(1d)** with aryl isothiocyanate in ethanol for 3 h.The thiosemicarbazides (**1g)** were oxidatively cyclized to 5-[3'-oxo-6'-(substituted aryl) 2',3',4',5'-tetrahydropyridazin-2'-ylmethyl]-N-(substituted aryl)-2-amino-1,3,4-oxadiazoles **(4a-e)** by elimination of H_2S using iodine and potassium iodide in ethanolic sodium hydroxide. All the final compounds of each series were structurally confirmed by elemental analysis and spectral data as shown in Table 1.

* Correspondence: e-mail: mojahidui@yahoo.co



Scheme 1. Synthesis of 5-[3'-oxo-6'-(substituted phenyl)-2',3',4',5'- tetrahydropyridazin-2'-yl]methyl-2-substituted 1,3,4-oxadiazoles.

Antibacterial activity

All the compounds are evaluated for their antibacterial activity against *E. coli, S. aureus, Micrococcus luteus* and *Klebsiella pneumoniae* by using cup plate (11) technique in the nutrient agar at 100 µg/mL concentration and the zone of inhibition (mm) of each compound is mentioned in Table 2. DMSO was used as a control. From the results, it was observed that most of the compounds were active against the microorganism having significant activity against these bacteria comparable to standard drugs, ampicillin and chloramphenicol.

Antitubercular activity

Antitubercular activity was determined using the BACTEC 460 system (12, 13). Stock solutions of test compounds were prepared in DMSO. MIC of rifampin was calculated by established procedures (12). All the synthesized compounds screened at 6.25 µg/mL showed the percentage inhibition ranging from 48 to 91%. Compound **(4a)** was highly active analogue in this series with 91% inhibition against *M. tuberculosis* H37 Rv comparable with that of standard rifampicin and isoniazid shown in Table 3.

Antifungal activity

All the final compounds were evaluated for antifungal activity against *C. albicans* and *C. neoformans* by using cup-plate method (14, 15) in the Sabouraud agar media (dextrose 4%, peptone 1%, agar 1.5%). The zone of inhibition (mm) of each compound was determined and compared with standard drug, fluconazole. The results are presented in Table 4.

Table 1. Physicochemical data of 5-[3'-oxo-6'-(substituted aryl)-2',3',4',5'-tetrahydropyridazin-2'-ylmethyl]-2-substituted 1,3,4-oxadiazoles.

Compd. No.	R	M. p. ^{o}C	Mol. Formula	Yield %	IR (cm^{-1}); ^{1}H-NMR (δ, ppm)
2b	3,5-$(CH_3)_2$-C_6H_5	204-8	$C_{15}H_{18}N_5O$	98	3210 (NH), 3060 (CH),1682 (C=O), 1635 (C=N), 1032-1025 (C-O-); 2.31(s, 6H, 2×CH_3), 2.58 (s, 2H, CH_2), 2.92 (t, 2H, CH_2), 2.72 (s, 2H, NCH_2), 7.31 (s, 2H, NH_2), 7.9- 8.3 (m, 3H, Ar-H);
2c	4-CH_3-C_6H_5	185-7	$C_{14}H_{15}N_5O_2$	90	3205 (NH), 3051 (CH),1680 (C=O),1630 (C=N); 2.06 (s, 3H, CH_3), 2.46 (t, 2H, CH_2), 2.89 (t, 2H, CH_2), 3.09 (s, 2H, NCH_2), 7.40 (s, 2H, NH_2), 8.0-8.5 (m, 4H, Ar-H);
2d	4-(OC_6H_5)-C_6H_5	250-4	$C_{19}H_{17}N_5O_3$	98	3210 (NH), 3050 (CH), 1681 (C=O), 1630 (C=N), 1035 (C-O-C); 2.37 (t, 2H, CH_2), 2.68 (t, 2H, CH_2), 3.00 (s, 2H, NCH_2), 7.38 (s, 2H, NH_2), 8.01-8.43 (m, 9H, Ar-H);
2e	4-Cl-C_6H_5	186-8	$C_{13}H_{12}N_5O_2Cl$	90	3205 (NH), 3051 (CH), 1680 (C=O), 1630 (C=N), 710 (C-Cl); 2.46 (t, 2H, CH_2), 2.82 (t, 2H, CH_2), 3.1 (s, 2H, NCH_2), 7.40 (s, 2H, NH_2), 8.01-8.68 (m, 4H, Ar-H);
3b	3,5-$(CH_3)_2$-C_6H_5	209-12	$C_{15}H_{16}N_4O_2S$	90	3205 (NH), 3044 (CH), 1683 (C=O), 1602 (C=N), 1165 (C=S); 2.35 (s, 6H, 2×CH_3), 2.54 (s, 2H, CH_2), 2.86 (t, 2H, CH_2), 3.06 (s, 2H, CH_2), 7.19-7.50 (m, 3H, Ar-H),7.97 (s, 1H, 1CSNH);
3c	4-CH_3-C_6H_5	160-2	$C_{14}H_{14}N_4O_2S$	88	3120 (NH), 3043 (CH),1683 (C=O), 1590 (C=N), 1165 (C=S); 2.02 (s, 2H, CH_3), 2.30 (t, 2H, CH_2), 2.59 (t, 2H, CH_2), 3.01(s, 2H, CH_2), 7.18-7.52 (m, 4H, Ar-H), 8.05 (s, 1H, 1CSNH).
3d	4-(OC_6H_5)-C_6H_5	217-19	$C_{19}H_{16}N_4O_2S$	90	3110 (NH), 3060 (CH), 1680 (C=O), 1590 (C=N), 1168 (C=S); 2.30 (t, 6H, CH_2), 2.60 (s, 2H, CH_2), 3.0 (s, 2H, CH_2), 7.09-7.45 (m, 9H, Ar-H), 8.05 (s, 1H, 1NHCS).
3e	4-Cl-C_6H_5	179-82	$C_{13}H_{11}N_4O_2SCl$	89	3120 (NH), 3043 (CH), 1683 (C=O), 1590 (C=N), 1167 (C=S), 715 (C-Cl); 2.30 (t, 2H, CH_2), 2.60 (t, 2H, CH_2), 3.10 (s, 2H, CH_2), 7.17-7.50 (m, 4H, Ar-H), 7.94 (s, 1H, 1CSNH).
4b	3,5-$(CH_3)_2$-C_6H_5	199-204	$C_{15}H_{21}N_5O_2$	93	3145-3100 (NH),1680 (C=O), 1650-1600 (C=N), 1035-1021 (C-O-C); 2.3 (s, 9H, 3×CH_3), 2.59 (t, 2H, CH_2), 2.94 (t, 2H, CH_2), 3.7 (m, 2H, N-CH_2), 4.87 (s, 1H, NH), 7.03-7.39 (m, 7H, Ar-H).
4c	4-CH_3-C_6H_5	164-8	$C_{14}H_{19}N_5O_2$	76	3125 (NH), 1686 (C=O), 1676 (CN), 1026 (C-O-C); 2.02 (s, 3H, CH_3), 2.42 (t, 2H, CH_2), 2.84 (t, 2H, CH_2), 3.62 (m, 2H, NCH_2), 7.01-7.60 (m, 9H, Ar-H), 9.72 (s, 1H, Ar-NH).
4d	4-(OC_6H_5)-C_6H_5	203-08	$C_{19}H_{21}N_5O_3$	95	3135 (NH),1681 (C=O), 1672 (C=N), 1035 (C-O-C); 2.37 (t, 2H, CH_2), 2.68 (t, 2H, CH_2), 3.00 (s, 2H, N-CH_2),7.38 (s, 2H, NH_2), 8.01-8.43 (m, 14H, Ar-H).
4e	4-Cl-C_6H_5	185-8	$C_{13}H_{16}N_5O_2Cl$	99	3150 (NH), 1686 (C=O), 1670 (C=N), 1022 (C-O-C), 722 (C-Cl); 2.54 (t, 2H, CH_2), 2.89 (t, 2H, CH_2), 3.67 (m, 2H, NCH_2), 7.01-7.78 (m, 9H, Ar-H), 9.78 (s, 1H, Ar-NH).

Table 2. *In vitro* antibacterial activity of the 5-[3'-oxo-6'-(substituted aryl)-2',3',4',5'-tetrahydropyridazin-2'-ylmethyl]-2-substituted 1,3,4-oxadiazoles.

Compound no.	Zone of inhibition (mm)			
	Gram positive bacteria		Gram negative bacteria	
	S. aureus	*M. luteus*	*E. coli*	*K. pneumoniae*
2a	10	11	9	8
2b	12	14	15	19
2c	15	18	17	14
2d	11	16	14	17
2e	20	18	21	18
3a	11	12	9	13
3b	12	14	16	15
3c	14	12	11	13
3d	11	13	12	14
3e	17	19	20	14
4a	7	10	11	9
4b	10	9	12	8
4c	14	16	18	13
4d	16	13	18	15
4e	19	21	21	18
Ampicillin	23	21	25	21
Chloramphenicol	22	25	23	20

EXPERIMENTAL

The melting points were determined on an X-4 microscope melting point apparatus and are uncorrected. Elemental analysis was carried out on elemental vario EL analyzer. The NMR spectra were recorded in $CDCl_3$ as a solvent (using TMS as an internal standard). The NMR and mass spectra were recorded on Jeol FX-100FT-NMR and Jeol BX 102/DA-6000 mass spectrometers, respectively. The infrared spectra in KBr were recorded on Buck Scientific M-500 Infrared Spectrophotometer. The reaction course was monitored by TLC [silica gel G, activation – 30 min at 110°C, solvent system: toluene : ethyl formate : formic acid (5 : 4 : 1, v/v/v)]. The oxadiazole derivatives were synthesized as shown in Scheme 1.

Synthesis of β-benzoyl propionic acid (1a)

After suspending anhydrous aluminum chloride (0.15 mol) in dry benzene (50 mL) under anhydrous conditions, the content was refluxed on a water bath. Succinic anhydride (0.10 mol) was then added to the reaction mixture in small portions with continuous stirring. Stirring and heating was continued for 6 h. After leaving overnight at room temperature, ice cold solution of concentrated hydrochloric acid (2.5% v/v) was added to the reaction mixture which was then concentrated to a small volume by heating on a water bath. The solid compound which separated out, was filtered. It was purified by dissolving in 5% w/v sodium bicarbonate solution, followed by extraction with ether. The aqueous layer on acidification with dilute hydrochloric acid gave benzoyl propionic acid, crystallized from aqueous ethanol to give a colorless compound. M.p. 123-5°C; R_f 0.25; yield 73%; ^{1}H-NMR (δ, ppm): 2.59 (t, 2H, CH_2), 3.23 (t, 2H, CH_2), 7.53-7.62 (m, 3H, H-3'-H-5'), 7.97 (d, 2H, H-2', H-6'), 12.17 (s, 1H, COOH);

All the remaining acids were synthesized by analogous procedure with minor modification in temperature of reaction and use of nitrobenzene as a solvent.

Synthesis of 6-phenyl-2,3,4,5-tetrahydropyridazin-3-one (1b)

To a solution of β-benzoyl propionic acid **(1a)** (0.1 mol) in methanol (30 mL), hydrazine hydrate (1 mL) and sodium acetate (0.5 g) were added and the mixture was refluxed for 6 h. After completion of the reaction, methanol was distilled off and the residue was poured into cold water. The solid that separated out, was filtered and crystallized from methanol, m.p. 249-50°C; R_f 0.45; yield 72%; IR (cm^{-1}): 3306 (NH), 1678 (C=O); ^{1}H-NMR (δ, ppm):

2.45 (t, 2H, CH_2), 2.93 (t, 2H, CH_2), 7.41 (m, 3H, H-3'-H-5'), 7.74 (d, 2H, H-2', H-6'), 10.94 (s, 1H, CONH); MS (m/z): 174,159, 147, 130, 115, 109.

Synthesis of ethyl-3-oxo-6-phenyl 2,3,4,5-tetrahydropyridazinyl acetate (1c)

Compound (**1b)** was added to an ethanolic solution (50 mL) of sodium (0.46 g), the reaction mixture was refluxed for 30 min, and then cooled. Ethyl bromoacetate (3.34 g, 0.02 mole) was added and the mixture was refluxed for 24 h. The solvent was evaporated off and the resulting residue was triturated with diisopropyl ether. The solid formed was collected by filtration and dried. The compound was recrystalized from a mixture of ethanol – water (50:50); m.p. 186-9°C; R_f 0.69; yield 50%; IR (cm^{-1}): 1725 (C=O), 1680 (C=O), 1599 (C=C), 1345, 1207, 751; ^{1}H-NMR (δ, ppm): 1.2 (t, 3H, CH_3), 2.60 (s, 2H, N-CH_2CO), 2.64 (t, 2H, CH_2), 3.00 (t, 2H, CH_2), 4.2 (q, 2H, $COCH_2$) 7.41-7.72 (m, 5H, Ar-H); MS (m/z): 260 (M^+), 247, 186.8, 173.1.

Synthesis of 3-oxo-6-phenyl-2,3,4,5-tetrahydropyridazinyl acetohydrazide (1d)

To a solution of **(1c)** (0.01 mol) in methanol (30 mL), hydrazine hydrate (1 mL) was added and the mixture was refluxed for 8 h. Then is was concentrated, cooled and filtered to get the solid compound. It was recrystallized with ethanol to get TLC pure compound, m.p. 177-9°C; R_f 0.64; yield 50%; IR (cm^{-1}): 3150 ($NHNH_2$), 1701 (CONH), 1686 (CO), 1652 (C=C); ^{1}H-NMR (δ, ppm): 2.6 (t, 2H, CH_2), 2.8 (m, 2H, NH_2), 3.00 (t, 2H, CH_2), 3.2 (s, 2H, N-CH_2CO), 7.37-7.71 (m, 5H, Ar-H) 8.85 (s, 1H, CONH).

Synthesis of 5-(3'-oxo-6'-phenyl-2',3',4',5'-tetrahydropyridazin-2'-ylmethyl)-2-amino-1,3,4-oxadiazole (2a)

Equimolar quantities of compound **(1d)** (0.001 mol) and cyanogen bromide (0.001 mol) in ethanol were refluxed for 14 h. The resulting solution was cooled and neutralized with sodium bicarbonate. The solid which separated out was filtered, washed with water, dried and recrystallized from methanol. M.p. 187-9°C; R_f 0.74; yield 80%; IR (cm^{-1}): 3210 (NH), 3050 (CH), 1680 (C=O), 1630 (C=N); ^{1}H-NMR (δ, ppm): 2.48 (t, 2H, CH_2), 2.84 (t, 2H, CH_2), 3.05 (s, 2H, NCH_2), 7.42 (s, 2H, NH_2), 8.02-8.68 (m, 5H, Ar-H).

Synthesis of 5-(3'-oxo-6'-phenyl-2',3',4',5'-tetrahydropyridazin-2'-ylmethyl)-2-mercapto-1,3,4-oxadiazole (3a)

An ethanolic solution of (**1d)** (0.001 mol), KOH (0.001 mol) and carbon disulfide (5 mL) was refluxed

Table 3. *In vitro* antitubercular activity of the 5-[3'-oxo-6'-(substituted aryl)-2',3',4',5'-tetrahydropyridazin-2'-ylmethyl]-2-substituted 1,3,4-oxadiazoles.

Compound no.	Concentration	%ge inhibition
2a	6.25	86
2b	6.25	52
2c	6.25	56
2d	6.25	48
2e	6.25	84
3a	6.25	88
3b	6.25	48
3c	6.25	51
3d	6.25	45
3e	6.25	87
4a	6.25	91
4b	6.25	52
4c	6.25	55
4d	6.25	49
4e	6.25	89
Rifampicin	0.25	98
Isoniazid	0.031	95
Tobramycin[1]	10.0	99
Clarithromycin[1]	26.0	99
Ethionamide[1]	1.17	99
PAS[1]	2.31	99
Ethambutol[1]	1.17	99
Gentamycin[1]	6.0	99
Doxycyclin[1]	12.0	99

[1] The concentration represents their MIC.

Table 4**.** *In vitro* antifungal activity of the 5-[3'-oxo-6'-(substituted aryl)- 2',3',4',5'-tetrahydropyridazin-2'-ylmethyl]-2-substituted 1,3,4-oxadiazoles.

Compound no.	Zone of inhibition(mm)	
	C. albicans	*C. neoformans*
2a	11	13
2b	21	20
2c	18	16
2d	11	14
2e	16	18
3a	12	13
3b	22	20
3c	19	18
3d	13	11
4a	10	12
4b	22	19
4c	20	18
4d	12	14
4e	17	19
Fluconazole	23	21

on water bath for 24 h.The solution was then concentrated, cooled and acidified with diluted HCl. The solid mass that separated out was filtered, washed with ethanol, dried and crystallized from ethanol. M.p. 167-9°C; R_f 0.74; yield 78%; IR (cm^{-1}): 3128 (NH), 3046 (CH), 1680 (C=O), 1593 (C=N), 1267 (C=S); ^{1}H-NMR (δ, ppm): 2.32 (t, 2H, CH_2), 2.61 (t, 2H, CH_2), 3.02 (s, 2H, CH_2), 8.04 (s, 1H, CSNH);

Synthesis of 5-(3'-oxo-6'-phenyl 2',3',4',5'-tetrahydropyridazin-2'-ylmethyl)-2-phenylamino-1,3,4-oxadiazole (4a)

An ethanolic solution of (**1d)** (0.001 mol) and phenyl isothiocyanate (0.001 mol) was refluxed for 3 h.The contents was concentrated and poured onto crushed ice, filtered and dried to get crude thiosemicarbazide (**1g**).To the crude thiosemicarbazide (**1g**) (0.003 mol) in ethanol (60 mL), aq. 6 M NaOH (5 mL) was added. 10% solution of I_2 in KI was then added dropwise and the reaction mixture was kept at 10°C. The addition of I_2 was continued till the color of I_2 persisted and the reaction mixture was then refluxed for 4 h. On cooling, the separated solid was washed thoroughly with water and recrystallised from ethanol; m.p. 155-8°C, R_f 0.84; yield 80%; IR (cm^{-1}):1030-1020 (C-O-C),1680-1600 (CN), 1678 (C=O), 3140-3100 (NH); ^{1}H-NMR (δ, ppm:) 2.59 (t, 2H, CH_2), 2.94 (t, 2H, CH_2), 3.7 (m, 2H, NCH_2), 7.01-7.68 (m, 10H, Ar-H), 9.86 (s, 1H, Ar-NH);

The remaining compounds were synthesized with analogous procedures and their m.p.s, yields and molecular formulas are presented in Table 1.

BIOLOGICAL EVALUATION

Antibacterial activity (11)

The antibacterial activity of the synthesized compounds was performed by adopting cup plate method. Freshly prepared liquid agar medium (35 mL/Petri dish) was poured into the Petri dishes (8 Petri dishes/sample) and kept for solidification. Then 200 µL of 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 on each plate.The synthetic compounds were diluted with dimethyl sulfoxide (DMSO).The concentrations (100 µg/mL) of the sample solutions was added to each well separately. The Petri dishes were kept aseptically for 4 to 5 h for diffusion of the sample. After the completion of diffusion period, all Petri dishes were kept for incubation at 37°C for 24 h. After 24 h the activity of sample i.e. zone of inhibition was observed for each compound against four (2 Gram positive and 2 Gram negative) microorganisms, namely *Staphylococcus aureus, Micrococcus luteus* , *Escherichia coli and Klebsiella pneumoniae*.

Antitubercular activity (Alamar blue susceptibility test (MABA) (12, 13)

The antitubercular activity of the synthesized compounds was performed by adopting Alamar blue susceptibility test (MABA). Antimicrobial susceptibility testing was performed in black, clear-bottomed, 96-well microplates (black view plates; Packard Instrument Company, Meriden, Conn.) in order to minimize background fluorescence. Outer perimeter wells were filled with sterile water to prevent dehydration in experimental wells. Initial drug dilutions were prepared in either dimethyl sulfoxide or distilled deionized water, and subsequent twofold dilutions were performed in 0.1 mL of 7H9GC (no Tween 80) in the microplates. BACTEC 12B-passaged inocula were initially diluted 1 : 2 in 7H9GC, and 0.1 mL was added to wells. Subsequent determination of bacterial titers yielded 1 X106 CFU/mL in plate wells for *M. tuberculosis* H37Rv. Frozen inocula were initially diluted 1 : 20 in BACTEC 12B medium followed by a 1 : 50 dilution in 7H9GC. Addition of 1/10 mL to wells resulted in final bacterial titers of 2.0 X105 CFU/mL for *M. tuberculosis* H37Rv. Wells containing drug only were used to detect autofluorescence of compounds. Additional control wells consisted of bacteria only (B) and medium only (M). Plates were incubated at 37°C. Starting at day 4 of incubation, 20 µL of 10X Alamar Blue solution (Alamar Biosciences/ Accumed, Westlake, Ohio) and 12.5 mL of 20% Tween 80 were added to one B well and one M well, and plates were reincubated at 37°C. Wells were observed at 12 and 24 h for a color change from blue to pink and for a reading of 50,000 fluorescence units (FU). Fluorescence was measured in a Cytofluor II microplate fluorometer (PerSeptive Biosystems, Framingham, Mass.) in bottom-reading mode with excitation at 530 nm and emission at 590 nm. If the B wells became pink by 24 h, reagent was added to the entire plate. If the well remained blue or 50,000 FU was measured, additional M and B wells were tested daily until a color change occurred, at which time reagents were added to all remaining wells. Plates were then incubated at 37°C, and results were recorded at 24 h after reagent addition. Visual minimum inhibitory concentration (MIC) was defined as the lowest concentration of drug that prevented a color change. For fluorometric MICs, a background subtraction was performed on all wells with a mean of triplicate M wells. Percent inhibition was defined

as 1 – (test well FU/mean FU of triplicate B wells) X100. The lowest drug concentration effecting an inhibition of > 90% was considered the MIC.

Antifungal activity (14, 15)

The antifungal activity of the synthesized compounds was performed by adopting cup plate method. The Sabouraud agar medium (dextrose 4%, peptone 1%, agar 1.5%) was used for antifungal activity. The medium was prepared and sterilized in an autoclave at 15 Psi for 15 min. Then, it was poured in sterilized Petri plates, aseptically. The fungal strains C*andida albicans* and C*ryptococcus neoformans* were inoculated on the surface of Petri plates separately after 2 h of pouring the agar media, when the media sets on Petri plates the cups (diameter 6 mm) were made in the Sabouraud agar medium using sterilized cup borer under aseptic conditions. 0.1 mL of each standard and test (10 mg/mL) prepared by dissolving it in DMSO was added into cups. The Petri plates were incubated at 28 ± 2^{o}C for 48 h growth and zone of inhibition (in mm) was recorded.

RESULTS AND DISCUSSION

The synthesized compounds were evaluated for their antibacterial activity against *E. coli, S. aureus, Micrococcus luteus* and *Klebsiella pneumoniae* by using cup plate technique in the nutrient agar at 100 µg/mL concentration and the zone of inhibition (mm) of each compound is shown in Table 2. Compound **(2e)** and **(4e)** are the most active derivatives as compared to that of standard drugs – ampicillin and chloramphenicol.

All the final synthesized compounds were evaluated for antitubercular activity. Stock solutions of test compounds were prepared in DMSO. MIC of rifampin was calculated by established procedures. All the synthesized compounds were tested for antitubercular activity at 6.25 µg/mL concentration and showed percentage of inhibition ranging from 45 to 90%. Compound **4a** emerged as highly active analogue of the series with 91% inhibition against *M. tuberculosis* H37 Rv.The order of activity was found to be H > Cl > *o*-toluidine > *m*-xylyl > diphenyl ether. The results are presented in Table 3.

All the synthesized final compounds were evaluated for antifungal activity against *C. albicans* and *C. neoformans* by using cup-plate method and the Sabouraud agar medium (dextrose 4%, peptone 1%, & agar 1.5%).The zone of inhibition (mm) of each compound was determined and compared with standard drug fluconazole and the zons of inhibition (in mm) were recorded. Compounds **2b, 3b**, and **4b** were found to be highly active as compared with the standard drug, fluconazole.The results are shown in Table 4.

CONCLUSION

From the above results, it was concluded that compounds **2a** and **4a** are active against Gram positive and Gram negative bacteria. Both the compounds are unsubstituted derivatives of the series and most potent against the *S. aureus* and *M. luteus*. However, compound **4a** is active against *M. tuberculosis* H37 Rv. For antitubercular activity, unsubstituted compounds were more active than after the substitution of chlorine at the *para* position in the phenyl ring. Compounds **2b**, **3b** and **4b** are potent antifungal agents against the *C. albicans*.

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