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## DRUG SYNTHESIS

# SYNTHESIS, ANTITUBERCULAR, ANTIFUNGAL AND ANTIBACTERIAL ACTIVITIES OF 6-SUBSTITUTED PHENYL-2-(3'-SUBSTITUTED PHENYL PYRIDAZIN-6'-YL)-2,3,4,5-TETRAHYDROPYRIDAZIN-3-ONE

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**Abstract:** A series of 6-substituted phenyl-2-(3'-substituted phenyl pyridazin-6'-yl)-2,3,4,5-tetrahydropyridazin-3-ones has been synthesized. An appropriate aromatic hydrocarbon reacts with succinic anhydride in presence of $AlCl_3$ to yield β-aroyl propionic acid. The corresponding acid was cyclized with hydrazine hydrate to give 6-(substituted aryl)-2,3,4,5-tetrahydro-3-pyridazinone, which was heated on steam bath with phosphorus(V) oxychloride to yield 3-chloro 6-substituted phenyl pyridazine. This intermediate after reaction with hydrazine hydrate was converted into 3-hydrazino-6-substituted phenyl pyridazine. The resulting product was converted into 6-substituted phenyl-2-(3'-substituted phenyl pyridazin-6'-yl)-2,3,4,5-tetrahydropyridazin-3-one by reacting with substituted aroyl propionic acid. Spectral data (IR, NMR, mass spectra) confirmed the structures of the synthesized compounds. The synthesized compounds were investigated for their *in vitro* antitubercular, antifungal and antibacterial activities. The results indicated that the synthesized compounds have mild to potent activities with reference to their appropriate reference standards.

**Keywords:** pyridazine derivatives, *in vitro* antitubercular, antifungal, antibacterial activity

Many human illnesses are caused by infections with microbes like viruses or bacteria or fungi. Amongst those various illnesses, certain tubercular, bacterial, viral and fungal infections are more common because of their tendency to develop new strains under any circumstances and developing resistance against the available drugs. This stimulated the scientists for development of novel molecules to combat these illnesses. Infectious microbial disease remain a pressing problem worldwide, because microbes have resisted prophylaxis or therapy longer than any other form of life.

Pyridazine and its derivatives are noteworthy for their physiological and biological importance. Medicinal chemists are working on pyridazines due to their wide range of biological activities like antibacterial (1-4), anti HIV (5), anticonvulsant (6, 7), antihypertensive (8-12), antiasthmatic (14-15), and anti-inflammatory (15, 16) etc. In view of above facts and inspired by the research going on pyridazinone and its derivatives, particularly in relation to microbial infections, 6-substituted phenyl-2-(3'-substituted phenyl pyridazin-6'-yl)-2,3,4,5-tetrahydropyridazin-3-ones have been synthesized. All the compounds were synthesized by reaction sequence outlined in Scheme 1. The Friedel-Crafts acylation of appropriate hydrocarbons with succinic anhydride, in presence of anhydrous $AlCl_3$ yielded β-aroyl propionic acid followed by cyclocondensation with hydrazine hydrate to get substituted pyridazinones, which reacted with phosphorus(V) oxychloride to give 3-chloro-6-substituted phenylpyridazines. These intermediates, after reaction with hydrazine hydrate, were converted into 3-hydrazino-6-substituted phenylpyridazines. These products were reacted with substituted aroyl propionic acids to yield 6-substituted phenyl-2-(3'-substituted phenylpyridazin-6'-yl)-2,3,4,5-tetrahydropyridazin-3-ones. All the final compounds were structurally confirmed on the basis of NMR, IR and mass spectral data, and the final synthesized compounds were evaluated for antitubercular, antifungal and antibacterial activities. The physiochemical data of the final synthesized compounds are presented in Table 1.

## EXPERIMENTAL

The melting points were determined on an X-4 microscope melting point apparatus and are uncorrected. 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-

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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. Solvent system used for TLC was toluene : ethyl formate : formic acid (5 : 4 : 1, v/v/v).

The synthesis of pyridazinone derivatives involves five steps and is exemplified by the synthesis of compound **1e.**

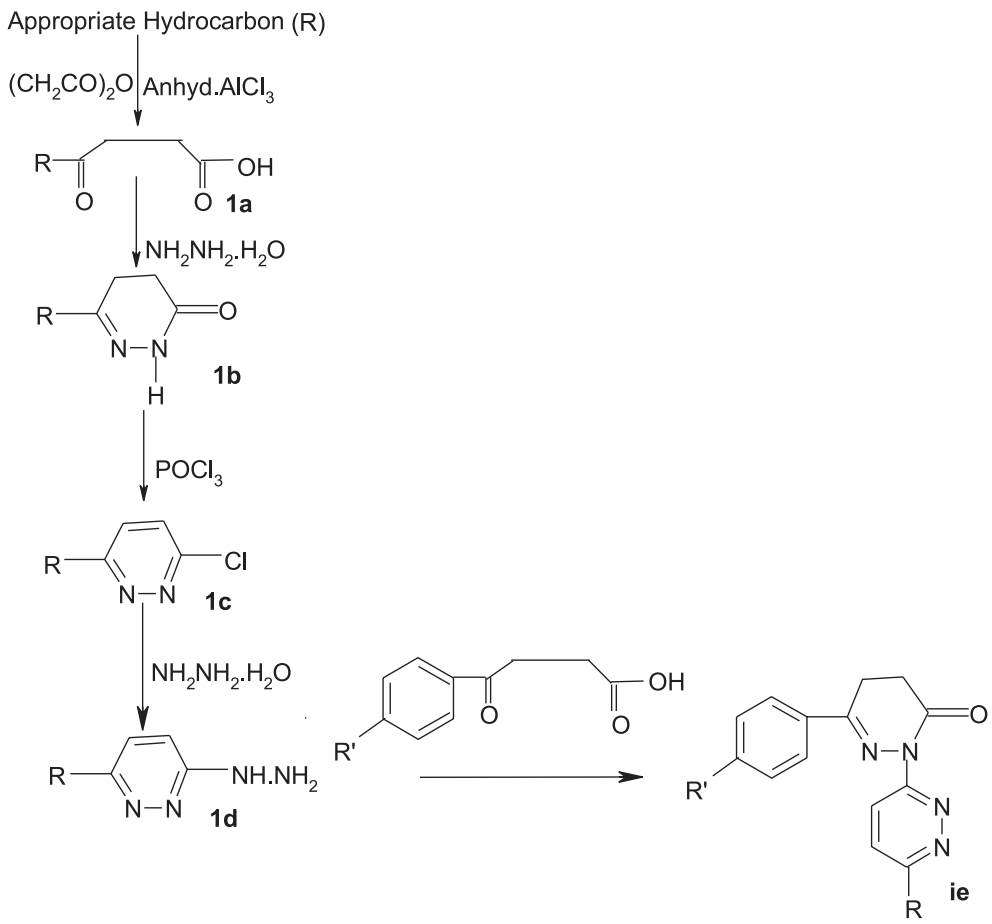
Synthesis of β-benzoyl propionic acid **(1a)**

After suspending anhydrous aluminum chloride (0.15 mol) in dry benzene (50 mL) under anhydrous conditions, the mixture 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 were continued for 6 h. The reaction mixture was left overnight at room temperature and then made acidic by addition of an ice cold solution of concentrated hydrochloric acid (2.5% v/v). The mixture was concentrated to a small volume by heating on a water bath. The separated precipitate 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. It was crystallized from aqueous ethanol to give a colorless compound, m.p. 125°C; $R_f$ 0.25; 73% yield; $^1$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




Scheme 1. Synthesis of 6-substituted phenyl-2-(3'-substituted phenylpyridazin-6'-yl)-2,3,4,5-tetrahydropyridazin-3-ones.

Table 1. Physicochemical data of 6-substituted phenyl-2-(3'-substituted phenylpyridazin-6'-yl)-2,3,4,5-tetrahydropyridazin-3-one.

| Compound no. | R | R' | m.p. [$^{o}$C] | Mol. formula | % yield |
|---|---|---|---|---|---|
| **1e** | $-C_6H_5$ | H | 198 | $C_{20}H_{16}N_4O$ | 78 |
| **1f** | $-C_6H_5$ | 3,5–$(CH_3)_2$ | 217 | $C_{22}H_{20}N_4O$ | 87 |
| **1g** | $-C_6H_5$ | 4–Cl– | 206 | $C_{20}H_{15}N_4OCl$ | 75 |
| **1h** | $-C_6H_5$ | 4–Br– | 238 | $C_{20}H_{16}N_4OBr$ | 89 |
| **1i** | $-C_6H_5$ | $-C_6H_5$ | 168 | $C_{26}H_{20}N_4O$ | 60 |
| **2a** | 4–$CH_3$–$C_6H_5$ | H | 169 | $C_{21}H_{18}N_4O$ | 72 |
| **2b** | 4–$CH_3$–$C_6H_5$ | 3,5–$(CH_3)_2$ | 172 | $C_{23}H_{22}N_4O$ | 78 |
| **2c** | 4–$CH_3$–$C_6H_5$ | $-C_6H_5$ | 225 | $C_{27}H_{22}N_4O$ | 82 |
| **2d** | 4–$CH_3$–$C_6H_5$ | 4–Br– | 219 | $C_{21}H_{17}N_4OBr$ | 86 |
| **3a** | 3,5–$(CH_3)_2$–$C_6H_5$ | H | 197 | $C_{22}H_{20}N_4O$ | 91 |
| **3b** | 3,5–$(CH_3)_2$–$C_6H_5$ | 4–Cl– | 228 | $C_{22}H_{19}N_4OCl$ | 58 |
| **3c** | 3,5–$(CH_3)_2$–$C_6H_5$ | $-C_6H_5$ | 217 | $C_{28}H_{24}N_4O$ | 62 |
| **4a** | $-C_{11}H_9O$ | H | 196 | $C_{25}H_{20}N_4O_2$ | 69 |
| **4b** | $-C_{11}H_9O$ | 3,5–$(CH_3)_2$ | 201 | $C_{27}H_{24}N_4O_2$ | 70 |
| **4c** | $-C_{11}H_9O$ | 4–Cl– | 193 | $C_{27}H_{23}N_4O_2Cl$ | 48 |
| **5a** | $-(C_6H_5)_2$ | H | 199 | $C_{26}H_{20}N_4O$ | 91 |
| **5b** | $-(C_6H_5)_2$ | p–$CH_3$– | 192 | $C_{27}H_{22}N_4O$ | 88 |
| **5c** | $-(C_6H_5)_2$ | $-C_5H_6O$ | 201 | $C_{37}H_{29}N_4O_2$ | 67 |
| **5d** | $-(C_6H_5)_2$ | $-C_6H_5$ | 206 | $C_{32}H_{24}N_4O$ | 73 |
| **5e** | $-(C_6H_5)_2$ | 4–Cl– | 213 | $C_{26}H_{19}N_4OCl$ | 80 |

mixture was refluxed for 6 h. After completion of the reaction, methanol was distilled off and the content was poured into cold water. The solid that separated out was filtered and crystallized from methanol, m.p. 250 $^{o}$C; $R_f$ 0.45; 72% yield; 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 3-chloro-6-phenylpyridazine (**1c**)

A mixture of 6-phenyl-2,3,4,5-tetrahydropyridazin-3-one (0.01 mole) and phosphorous(V) oxychloride ($POCl_3$) (20 mL), was heated on a steam bath for 6 h. After heating, the mixture was carefully poured on crushed ice and rendered alkaline by addition of sodium bicarbonate. Crude 3-chloropyridazine was collected by filtration. M.p. 135$^{o}$C, $R_f$ 0.70, 76% yield; IR ($cm^{-1}$): 1630 (C=N), 1590 (C=C), 940, 702 (C-Cl); $^{1}$H-NMR (δ, ppm): 7.42-7.51 ((m, 7H, Ar-H ).

Synthesis of 3-hydrazino-6-phenylpyridazine (**1d**)

To an ethanolic solution of 3-chloropyridazine (0.01 mole), hydrazine hydrate (99% -10 mL), was added and the resulting reaction mixture was refluxed on steam bath for 16 h. The mixture was concentrated, cooled and poured onto crushed ice. The resulting solid which separated out was filtered, washed with water, dried and recrystallized from ethanol. M.p. 168 $^{o}$C, $R_f$ 0.75, 79% yield; IR($cm^{-1}$): 3440 (NH), 961 (CH); 1638 (C=N), 1582 (C=C), 938, 680; $^{1}$H-NMR (δ, ppm): 2.5 (s, 2H, $NH_2$), 7.17-8.05 (m, 7H, Ar-H), 8.15 (m, 1H, Ar-NH);

Synthesis of 6-phenyl-2-(3'-phenylpyridazin-6'-yl)-2,3,4,5-tetrahydropyridazin-3-one (**1e**)

To an ethanolic solution of 3-hydrazino-6-phenylpyridazine (0.01 mole) benzoyl propionic acid (0.01 mole) and 300 mg of sodium acetate were added and the mixture was refluxed for 18 h. The content was concentrated, cooled and poured onto crushed ice. The separated solid was filtered, washed with water, dried and recrystallized from ethanol. M.p. 198$^{o}$C, $R_f$ 0.64, 78% yield. IR ($cm^{-1}$): 2919 (CH), 1682 (C=O), 1632 (C=N), 1590 (C=C), 1206, 752; $^{1}$H-NMR (δ, ppm) 2.56 (t, 2H, $CH_2$), 3.01 (t, 2H, $CH_2$), 7.10-7.89 (m, 12H, Ar-H). MS (m/z): 328 ($M^{+}$).

The remaining compounds were synthesized with analogous procedure and their melting points, yields and molecular formulas are presented in Table 1.

6-(2'',4''-Dimethyl)-2-(3'-phenylpyridazin-6'-yl)-2,3,4,5- tetrahydropyridazin-3-one (**1f**)

IR ($cm^{-1}$): 2919 (CH), 1671 (C=O), 1638 (C=N), 1591 (C=C), 757, 696; $^1$H-NMR (δ, ppm): 2.80 (s, 6H, 2×$CH_3$), 2.40 (m, 2H, $CH_2$), 2.80 (t, 2H, $CH_2$), 7.04-8.62 (m, 10H, Ar-H). MS (m/z): 356 ($M^+$), 309, 211.

6-(4''-Chlorophenyl-2-(3'-phenylpyridazin-6'-yl)-2,3,4,5- tetrahydropyridazin-3-one (**1g**)

IR ($cm^{-1}$): 1681 (C=O), 1630 (C=N), 1590 (C=C), 732 (C-Cl); $^1$H-NMR (δ, ppm): 2.45 (t, 2H, $CH_2$), 2.93 (t, 2H, $CH_2$), 7.31-8.31 (m, 11H, Ar-H).

6-(4''-Bromophenyl)-2-(3'-phenylpyridazin-6'-yl)-2,3,4,5- tetrahydropyridazin-3-one (**1h**)

IR ($cm^{-1}$): 2927 (CH), 1685 (C=O), 1630 (C=N), 1588 (C=C), 1340, 1210, 750; $^1$H-NMR (δ, ppm): 2.49 (t, 2H, $CH_2$), 3.04 (t, 2H, $CH_2$), 7.40-7.98 (m, 11H, Ar-H).

6-(4''-Biphenyl)-2-(3'-phenylpyridazin-6'-yl)-2,3,4,5- tetrahydropyridazin-3-one (**1i**)

IR ($cm^{-1}$): 2930 (CH), 1681 (C=O), 1632 (C=N), 1586 (C=C), 1340, 1210; $^1$H-NMR (δ, ppm): 2.49 (t, 2H, $CH_2$), 3.04 (t, 2H, $CH_2$), 7.41-8.21 (m, 16H, Ar-H).

6-Phenyl-2-[3'-(4''-tolyl)pyridazin-6'-yl]-2,3,4,5-tetrahydropyridazin-3-one (**2a**)

IR ($cm^{-1}$): 2970 (CH), 1682 (C=O), 1630 (C=N), 1590 (C=C), 762; $^1$H-NMR (δ, ppm): 2.40 (s, 3H, $CH_3$), 2.74 (t, 2H, $CH_2$), 3.25 (t, 2H, $CH_2$), 7.25-7.93 (m, 11H, Ar-H).

6-(2''',4'''-Dimethylphenyl)-2-[3'-(4''-tolyl)pyridazin-6'-yl]-2,3,4,5- tetrahydropyridazin-3-one (**2b**)

IR ($cm^{-1}$): 2968 (CH), 1682 (C=O), 1637 (C=N), 1609 (C=C); $^1$H-NMR (δ, ppm): 2.46 ( s, 9H, 3×$CH_3$), 2.7 (t, 2H, $CH_2$), 3.19 (t, 2H, $CH_2$), 7.03-7.90 (m, 9H, Ar-H). MS (m/z): 368 (M-2), 357, 332, 298, 105, 83.

6-(4'''-Biphenyl)-2-[3'-(4''-tolyl)pyridazin-6'-yl)-2,3,4,5- tetrahydropyridazin-3-one (**2c**)

IR ($cm^{-1}$): 2980 (CH), 1682 (C=O), 1615 (C=N), 1580 (C=C); $^1$H-NMR (δ, ppm): 2.5 ( s, 3H, $CH_3$), 2.82 ( t, 2H, $CH_2$), 3.49 (t, 2H, $CH_2$), 7.43-7.98 (m, 15H, Ar-H).

6-(4'''-Bromo phenyl)-2-[3'-(4''-tolyl)pyridazin-6'-yl]-2,3,4,5- tetrahydropyridazin-3-one (**2d**)

IR ($cm^{-1}$): 2914 (CH), 1680 (C=O), 1612 (C=N), 1588 (C=C); $^1$H-NMR (δ, ppm): 2.42 ( s, 3H, $CH_3$), 2.80 (t, 2H, $CH_2$), 3.29 (t, 2H, $CH_2$), 7.41-8.01 (m, 10H, Ar-H).

Table 2. *In vitro* antitubercular activity of 6-substituted phenyl-2-(3'-substituted phenyl pyridazin-6'-yl)-2,3,4,5-tetrahydropyridazin-3-ones and standard drugs.

| Compound | Concentration (μg/mL) | Inhibition % |
|---|---|---|
| **1e** | 6.25 | 48 |
| **1f** | 6.25 | 89 |
| **1g** | 6.25 | 60 |
| **1h** | 6.25 | 62 |
| **1i** | 6.25 | 51 |
| **2a** | 6.25 | 70 |
| **2b** | 6.25 | 92 |
| **2c** | 6.25 | 62 |
| **2d** | 6.25 | 64 |
| **3a** | 6.25 | 89 |
| **3b** | 6.25 | 95 |
| **3c** | 6.25 | 72 |
| **4a** | 6.25 | 47 |
| **4b** | 6.25 | 54 |
| **4c** | 6.25 | 32 |
| **5a** | 6.25 | 38 |
| **5b** | 6.25 | 41 |
| **5c** | 6.25 | 34 |
| **5d** | 6.25 | 39 |
| **5e** | 6.25 | 40 |
| 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 |
| Gentamicin[1] | 6.0 | 99 |
| Doxycyclin[1] | 12.0 | 99 |

[1] The concentration represents their MIC's.

6-Phenyl 2-[3'-(2'',4''-dimethylphenyl)pyridazin-6'-yl]-2,3,4,5- tetrahydropyridazin-3-one (**3a**)

IR ($cm^{-1}$): 2948 (CH), 1688 (C=O), 1622 (C=N), 1594 (C=C); $^1$H-NMR (δ, ppm): 2.50 ( s, 6H, 2×$CH_3$), 2.58 (t, 2H, $CH_2$), 3.25 (t, 2H, $CH_2$), 7.53-8.0 (m, 10H, Ar-H). MS (m/z): 356(M+), 331, 233, 161, 141.

6-(4'''-Chlorophenyl)-2-[3'-(2'',4''-dimethylphenyl)pyridazin-6'-yl]-2,3,4,5-tetrahydropyridazin-3-one (**3b**)

Table 3. *In vitro* antibacterial activity of 6-substituted phenyl-2-(3'-substituted phenyl pyridazin-6'-yl)-2,3,4,5-tetrahydropyridazin-3-ones.

| Compound | Concentration (µg/mL) | Zone of inhibition (in mm) | | | |
|---|---|---|---|---|---|
| | | Gram positive | | Gram negative | |
| | | *Staph. aureus* | *M. luteus* | *E. coli* | *K. pneumoniae* |
| **1e** | 50 | – | – | – | – |
| | 100 | – | – | 8 | – |
| | 200 | 10 | 12 | 13 | – |
| **1f** | 50 | – | 8 | – | – |
| | 100 | 12 | 14 | 12 | 13 |
| | 200 | 18 | 20 | 19 | 18 |
| **1g** | 50 | – | – | – | – |
| | 100 | 6 | – | 8 | 11 |
| | 200 | 13 | – | 16 | 18 |
| **1h** | 50 | – | – | – | – |
| | 100 | – | 9 | – | – |
| | 200 | 12 | 10 | 11 | 14 |
| **1i** | 50 | – | – | – | – |
| | 100 | – | 6 | 4 | – |
| | 200 | – | 13 | 10 | 9 |
| **2a** | 50 | – | – | – | – |
| | 100 | 7 | 17 | 9 | 17 |
| | 200 | 17 | 19 | 18 | 17 |
| **2b** | 50 | – | – | – | 6 |
| | 100 | 8 | 6 | 9 | 11 |
| | 200 | 21 | 20 | 22 | 21 |
| **2c** | 50 | – | – | – | – |
| | 100 | – | – | – | – |
| | 200 | 13 | 12 | 11 | 10 |
| **2d** | 50 | – | – | – | – |
| | 100 | 8 | 7 | 8 | – |
| | 200 | 16 | 14 | 12 | 13 |
| **3a** | 50 | – | – | – | 4 |
| | 100 | 8 | 9 | 6 | 7 |
| | 200 | 18 | 20 | 19 | 18 |
| **3b** | 50 | – | – | – | – |
| | 100 | – | 15 | – | – |
| | 200 | 17 | 18 | 16 | 13 |
| **3c** | 50 | – | – | – | 6 |
| | 100 | 10 | 11 | 9 | 12 |
| | 200 | 18 | 17 | 17 | 17 |
| **4a** | 50 | – | – | – | – |
| | 100 | – | – | – | – |
| | 200 | 10 | 9 | 8 | 7 |
| **4b** | 50 | – | – | – | – |
| | 100 | – | – | – | – |
| | 200 | 12 | – | 9 | – |
| **4c** | 50 | – | – | – | – |
| | 100 | – | 12 | – | 10 |
| | 200 | 14 | 12 | 13 | 16 |
| **5a** | 50 | – | – | – | – |
| | 100 | – | – | 8 | – |
| | 200 | 13 | 18 | 17 | 12 |

Table 3. cont.

| Compound | Concentration (μg/mL) | Zone of inhibition (in mm) | | | |
|---|---|---|---|---|---|
| | | Gram positive | | Gram negative | |
| | | *Staph. aureus* | *M. luteus* | *E. coli* | *K. pneumoniae* |
| **5b** | 50 | – | – | – | – |
| | 100 | 5 | – | – | – |
| | 200 | 12 | 10 | – | – |
| **5c** | 50 | – | – | – | – |
| | 100 | – | – | – | – |
| | 200 | 8 | 7 | – | 3 |
| **5d** | 50 | – | – | – | – |
| | 100 | – | – | – | – |
| | 200 | – | 11 | 12 | – |
| **5e** | 50 | – | – | – | – |
| | 100 | 7 | 9 | 5 | 11 |
| | 200 | 13 | 18 | 16 | 12 |
| Ampicillin | 50 | 23 | 21 | 25 | 21 |
| Chloramphenicol | 50 | 22 | 25 | 23 | 20 |

– shows no antibacterial activity.

IR ($cm^{-1}$): 2976 (CH), 1683 (C=O), 1619 (C=N), 1585 (C=C); $^1$H-NMR (δ, ppm): 2.38 (s, 6H, 2×$CH_3$), 2.76 (t, 2H, $CH_2$), 3.12 (t, 2H, $CH_2$), 7.58-8.21 (m, 9H, Ar-H).

6-(4'''-Biphenyl)-2-[3'-(2'',4''-dimethylphenyl) pyridazin-6'-yl]-2,3,4,5-tetrahydropyridazin-3-one (**3c**)

IR ($cm^{-1}$): 2928 (CH), 1681 (C=O), 1630 (C=N), 1590 (C=C); $^1$H-NMR (δ, ppm) 2.24 (s, 6H, 2×$CH_3$), 2.51 (t, 2H, $CH_2$), 3.02 (t, 2H, $CH_2$), 7.42-8.76 (m, 14H, Ar-H).

6-Phenyl-2-[3'-(6''-methoxynaphthyl)pyridazin-6'-yl]-2,3,4,5- tetrahydropyridazin-3-one (**4a**)

IR ($cm^{-1}$): 2998 (CH), 1681 (C=O), 1620 (C=N), 1595 (C=C); $^1$H-NMR (δ, ppm): 2.48 (s, 3H, $OCH_3$), 2.78 (t, 2H, $CH_2$), 3.04 (t, 2H, $CH_2$), 7.38-8.21 (m, 13H, Ar-H).

6-(2'',4'''-Dimethyl)-2-[3'-(6''-methoxynaphthyl)pyridazin-6'-yl]-2,3,4,5-tetrahydropyridazin-3-one (**4b**)

IR ($cm^{-1}$): 2992 (CH), 1680 (C=O), 1619 (C=N), 1592 (C=C); $^1$H-NMR (δ, ppm): 2.2 (s, 6H, 2×$CH_3$), 2.44 (s, 3H, $OCH_3$), 2.67 (t, 2H, $CH_2$), 3.18 (t, 2H, $CH_2$), 7.18-7.97 (m, 11H, Ar-H).

6-(4'''-Chlorophenyl)-2-[3'-(6''-methoxynaphthyl)pyridazine-6'-yl]-2,3,4,5-tetrahydropyridazin-3-one (**4c**)

IR ($cm^{-1}$): 2994 (CH), 1682 (C=O), 1617 (C=N), 1588 (C=C), 710 (C-Cl); $^1$H-NMR (δ, ppm): 2.41 (s, 3H, $OCH_3$), 2.62 (s, 2H, $CH_2$), 3.08 (t, 2H, $CH_2$), 7.16-8.03 (m, 12H, Ar-H).

6-Phenyl-2-[3'-(4''-biphenyl)pyridazin-6'-yl]-2,3,4,5-tetrahydropyridazin-3-one (**5a**)

IR ($cm^{-1}$): 2926 (CH), 1685 (C=O), 1343, 1203; $^1$H-NMR (δ, ppm): 2.6 (t, 2H, $CH_2$), 2.9 (t, 2H, $CH_2$), 7.26-8.11 (m, 16H, Ar-H). MS (m/z): 404($M^+$), 375, 309, 211.

6-(4'''-Tolyl)-2-[3'-(4''-biphenyl)pyridazin-6'-yl]-2,3,4,5-tetrahydropyridazin-3-one (**5b**)

IR ($cm^{-1}$): 2920 (CH), 1681 (C=O), 1610 (C=C), 1250, 965; $^1$H-NMR (δ, ppm): 2.4 (s, 3H, $CH_3$), 2.8 (t, 2H, $CH_2$), 3.18 (t, 2H, $CH_2$), 7.03-7.94 (m, 15H, Ar-H).

6-(5'''-Methoxynaphthyl)-2-[3'-(4''-biphenyl)pyridazin-6'-yl]-2,3,4,5- tetrahydropyridazin-3-one (**5c**)

IR ($cm^{-1}$): 2986 (CH), 1689 (C=O), 1624 (C=N), 1604 (C=C); $^1$H-NMR (δ, ppm): 2.38 (s, 3H, $OCH_3$), 2.72 (t, 2H, $CH_2$), 3.09 (t, 2H, $CH_2$), 7.19-8.07 (m, 17H, Ar-H).

6-(4'''-Biphenyl)-2-[3'-(4''-biphenyl)pyridazin-6'-yl]-2,3,4,5- tetrahydropyridazin-3-one (**5d)**

IR ($cm^{-1}$): 2980 (CH), 1682 (C=O), 1618 (C=N), 1598 (C=C); $^1$H-NMR (δ, ppm): 2.5 (t, 2H, $CH_2$), 2.98 (t, 2H, $CH_2$), 7.41-8.05 (m, 20H, Ar-H).

Table 4. *In vitro* antifungal activity of 6-substituted phenyl-2-(3'-substituted phenylpyridazin-6'-yl) 2,3,4,5-tetrahydropyridazin-3-one.

| Compound | Concentration (μg/mL) | Zone of inhibition (in mm) | |
|---|---|---|---|
| | | *C. albicans.* | *C. neoformans* |
| **1e** | 100 | – | – |
| | 250 | 6 | 8 |
| | 500 | 12 | 13 |
| **1f** | 100 | – | – |
| | 250 | 7 | 5 |
| | 500 | 20 | 19 |
| **1g** | 100 | – | – |
| | 250 | | |
| | 500 | 18 | 17 |
| **1h** | 100 | 4 | – |
| | 250 | 8 | 14 |
| | 500 | 17 | 16 |
| **1i** | 100 | – | – |
| | 250 | 5 | 6 |
| | 500 | 13 | 14 |
| **2a** | 100 | 3 | – |
| | 250 | 8 | 4 |
| | 500 | 19 | 17 |
| **2b** | 100 | – | – |
| | 250 | 11 | 11 |
| | 500 | 21 | 19 |
| **2c** | 100 | 3 | – |
| | 250 | 8 | 11 |
| | 500 | 15 | 13 |
| **2d** | 100 | – | – |
| | 250 | 12 | 13 |
| | 500 | 19 | 18 |
| **3a** | 100 | – | – |
| | 250 | 8 | 6 |
| | 500 | 19 | 19 |
| **3b** | 100 | 5 | 4 |
| | 250 | 11 | 9 |
| | 500 | 22 | 20 |
| **3c** | 100 | – | – |
| | 250 | 13 | 14 |
| | 500 | 17 | 15 |
| **4a** | 100 | – | 3 |
| | 250 | 6 | 8 |
| | 500 | 14 | 12 |
| **4b** | 100 | – | – |
| | 250 | 5 | 7 |
| | 500 | 14 | 19 |
| **4c** | 100 | – | 2 |
| | 250 | 8 | 5 |
| | 500 | 17 | 18 |
| **5a** | 100 | – | – |
| | 250 | 11 | 8 |
| | 500 | 14 | 12 |

Table 4. cont.

| Compound | Concentration (µg/mL) | Zone of inhibition (in mm) | |
|---|---|---|---|
| | | *C. albicans.* | *C. neoformans* |
| **5b** | 100 | – | – |
| | 250 | 11 | 12 |
| | 500 | 17 | 18 |
| **5c** | 100 | – | – |
| | 250 | 6 | 6 |
| | 500 | 14 | 12 |
| **5d** | 100 | – | – |
| | 250 | 5 | 6 |
| | 500 | 13 | 14 |
| **5e** | 100 | – | – |
| | 250 | 7 | 9 |
| | 500 | 16 | 18 |
| Fluconazole | 100 | 24 | 28 |
| Griseofulvin | 100 | 23 | 26 |

– shows no antifungal activity.

6-(4'''-Chlorophenyl)-2-[3'-(4''-biphenyl)pyridazin-6'-yl]-2,3,4,5- tetrahydropyridazin-3-one (**5e)**

IR ($cm^{-1}$): 2985 (CH), 1683 (C=O), 1620 (C=N), 1594 (C=C); $^1$H-NMR (δ, ppm): 2.32 (t, 2H, $CH_2$), 2.87 (t, 2H, $CH_2$), 7.38-7.97 (m, 15H, Ar-H).

## BIOLOGICAL EVALUATION

Antitubercular activity (Alamar blue susceptibility test (MABA) (18, 19)

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 $X10^6$ 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 the wells resulted in final bacterial titers of 2.0 $X10^5$ CFU/mL for *M. tuberculosis* H37Rv. The 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 the plates were reincubated at 37°C. The wells were observed at 12 and 24 hours 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, the 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 the results were recorded at 24 h post-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) ×100. The lowest drug concentration effecting an inhibition of > 90% was considered the MIC. The percentage of inhibition of bacterial growth at 6.25 µg/mL of all the selected synthesized compounds from all the three series are presented in Table 2 along with the MIC's of standard drugs. The compounds which shown 90 and above of percentage of

inhibition has been taken up for second phase of screening to determine their actual MIC's .

Antibacterial activity (20)

The antibacterial activity of the synthesized compounds from all three series have been performed by adopting cup plate method. This method depends on the diffusion of a sample solution from a vertical cylinder or a cavity through the solidified agar layer of a Petri dish, such 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.

Freshly prepared liquid agar medium (35 mL/ Petri dish) was poured in to the Petri dishes (8 Petri dishes/sample) and kept for solidification. Then the 200 µL-standardized culture (99 mL Nutrient broth media + 1 mL culture) of organism was spread on each Petri dishes by L-shaped spreader. With the help of the borer (5 mm), three bores were made on each plate. The synthesized compounds were diluted with dimethyl sulfoxide (DMSO). Different concentrations (50 µg, 100 µg, and 200 µg/mL) of the sample solutions were 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$^{o}$C for 24 h. Then the activity of sample (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*. The antibacterial activity of the synthesized compounds from all the series is presented in Table 3.

Antifungal activity (21)

The sabouraud agar medium (dextrose 4%, peptone 1%, agar 1.5%) was used for determination of antifungal activity. The medium was prepared and sterilized in an autoclave at 15 Psi for 15 min. Then it was poured on sterilized Petri plates, aseptically. The fungal strains *Candida albicans* and C*ryptococcus neoformans* were inoculated on the surface of Petri plates separately. After 2 h of pouring the agar medium on Petri plates, the cups (diameter 6 mm) were made in the sabouraud agar medium using sterilized cup borer under aseptic conditions. The 0.1 mL of each standard and tested compound (10 mg/mL) prepared by dissolving it in DMSO was added to the cups. The Petri plates were incubated at 28 ± 2$^{o}$C for 48 h growth and zone of inhibition (in mm) was recorded. The antifungal activity of the synthesized compounds from all the series is presented in Table 4.

## RESULTS AND DISCUSSION

All the final synthesized compounds were evaluated for antitubercular activity. Stock solutions of test compounds were prepared in DMSO. The MIC value of rifampicin was calculated by established procedures. All the synthesized compounds screened at 6.25 µg/mL show the percentage of inhibition ranging from 32 to 94%. Compound **3b** emerged as highly active analogue in this series with 95% inhibition against *M. tuberculosis* H37 Rv comparable with those of standard rifampicin and isoniazid shown in Table 2. The results indicate that the pyridazine with chloro group and dimethyl group **(3b)** showed comparable activity with the reference drugs. The final synthesized compounds were evaluated for their antibacterial activity against *E. coli, S. aureus, Micrococcus luteus* and *Klebsiella pneumonia* by using cup plate technique in the nutrient agar at 100 µg/mL concentration (Table 3). DMSO was used as a control, The results of antibacterial evaluation show that all compounds have comparable activity against the bacterial strains. Compounds **1f, 2b** and **3a** are the most active derivatives, which show significant activity against the bacteria comparable to standard drug, ampicillin and chloramphenicol. All the final compounds were evaluated for antifungal activity against *C. albicans* and *C. neoformans* by using cup-plate method in the sabouraud agar medium. The zone of inhibition (mm) of each compound was determined and compared with standard drug – fluconazole. The compounds **2b** and **3b** were found to be active derivatives of this series against the microorganisms used**.**

## CONCLUSION

From the above results, it is concluded that compounds **1f**, **2b** and **3a** are active against Gram positive and Gram negative bacteria, whereas compound **3b** is active against *M. tuberculosis* H37 Rv. Compounds **2b** and **3b** exhibited potent antifungal activity.

## REFERENCES

1. Junge L.N., Boyd D.B., Alborn W.E.: J. Med. Chem. 34, 172 (1991).
2. Patel H.K., Fernandes P.S.: Ind. J. Chem. 88, 733 (1989).

3. Katrusiak A., Katrusiak A., Bałoniak S.: Tetrahedron. 50, 12933 (1994).
4. Kraiso V.G., Goal A., Haider N., Matyus P.: J. Mol. Struct. (Theochem) 3, 528 (2000).
5. Livermone D.G.H., Bethell R.C., Cammack N.: J. Med. Chem. 36, 3784 (1993).
6. Siddiqui A.A., Shehroz M.M., Amir M.: Oriental J. Chem. 20, 303 (2004).
7. Rubat C., Coudert P., Refouvelet, B., Tronche, P., Bashde P., Bastide J.: Chem. Pharm. Bull. 38, 3009 (1990).
8. Seki T., Nakao T., Masuda T., Hasumi K., Gotanda K., Yasuda K.: Chem. Pharm. Bull. 44, 2061 (1996).
9. Cignarella G., Barlocco D., Pinna G.A.: J. Med. Chem. 32, 2277 (1989).
10. Vega A.M., Aldana I., Font M.: J. Pharm. Sci. 71, 1406 (1989).
11. Longo J.G., Verde I., Castro M.E.: J. Pharm. Sci. 82, 286 (1993).
12. Siddiqui A.A., Wani S.M., Ind. J. Chem. 43B, 1574 (2004).
13. Nagai H., Suda H., Iwama T., Daikoku M., Yanagihara Y., Khoda A.: Int. Arch. Allergy Immunol. 98, 57 (1992).
14. Suda H., Nagai H., Iwama T., Khoda A.: Int. Arch. Allergy Immunol. 97, 187 (1992).
15. Iwama T., Nagai H., Khoda A.: J. Pharm. Pharmacol. 45, 335 (2001).
16. Bebot M., Coudert P., Rubat C.: Chem. Pharm. Bull. 45, 659 (1997).
17. Pieretti S., Dal V., Malucci R., Giovannoni M. P., Galli A.: Life Sci. 65, 1381 (1999).
18. Inderland C.B., Nash K.A., Lorian V., Antibiotics in Laboratory Medicine, 4th ed., p.127, Williams and Wilkins, Baltimore 1996.
19. Inderland C.B., Salfinger M., Murray P.R., Baron E., Pfaller M.A., Tenover F.C., Yoken R.H.: Manual of Clinical Microbiology, 6th ed., p. 1385, ASM Press, Washington D.C.1995.
20. Greenwood D., Slack R.C.B., Peutherer J. F., Microbiology, 14th ed., p.1, Churchill and Livingstone, Spencerswood 1992.
21. Indian Pharmacopoeia., 3rd ed, p. 90, Govt. of India, New Delhi 1985.

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