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Original Article

Synthesis and Anti-Tubercular Activity of 6-Substtitutedaryl-4-Arylidene-4, 5-Dihydropyridazin-3(*2H*)-One Derivatives against *Mycobacterium Tuberculosis*

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Abstract

Several 4-substituted-benzylidene-6-substituted-phenyl-4,5-dihydro-pyridazin-3(2*H*)-one derivatives (3a-q) were synthesized and evaluated for their antimicrobial activities with an aim to obtain promising antitubercular agents. In the first step, 6-aryl-2, 3, 4, 5-tetrahydro-pyridazin-3-ones (2) was prepared by reacting 4-aryl-4-oxobutanoic acids (1) with hydrazine hydrate. Then, aryl-aldehydes were reacted with compounds 2 to furnish pyridazinones (3a-q). Finally, the synthesized compounds were evaluated for their *in vitro* antitubercular activities against mycobacterium strains and compared to reference drugs streptomycin (MIC value of 6.25µg/mL) and pyrizinamide (MIC value of 3.125µg/mL). Compound 3m and 3n was found to have most significant action. Identity of these compounds was ascertained using IR, NMR and mass spectral data results.

Keywords: Pyridazine, antitubercular, mycobacterium, synthesis, spectral data.

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1. Introduction

Pyridazine is six membered nitrogen, containing heterocyclic ring and plays a vital role in the field of medicinal chemistry. Pyridazine ring is a part of the structures of a various drugs available in the market like hydralazine, minaprine, cefozopran, and pipofezine etc. Pyridazine derivatives have been reported to possess various pharmacological activities including antibacterial, antifungal, anti-tubercular, anticonvulsant, antihypertensive, analgesic and anti-inflammatory etc; they have been extensively investigated in the past years [1-8]. In view of the antimicrobial activities exhibited by nitrogen containing heterocyclic like pyridazines, it was thought worthwhile to

study their derivatives as potential antitubercular agents [9-11]. The increasing incidence of resistance to a large number of antibacterial agents is becoming a major concern. Due to the difficulty in managing tuberculosis because prolonged duration of the treatment, the emergence of drug resistance and co-infection with HIV. The effective treatment of tuberculosis requires new drugs that help in combating resistant forms of *Mycobacterium tuberculosis*, reduce the treatment duration and also effective against HIV [12-14]. These observations place new emphasis on the need of as well as search for alternative new and more effective antitubercular drugs. New research effort towards the development of novel antitubercular agents, discovering new classes of compounds, which are structurally different from known anti-tubercular drugs. Therefore, we thought to synthesize of pyridazine derivatives (3a-q) bearing different substituents on the phenyl rings in order to study the structure activity relationship by evaluating their antitubercular activities against *M. tuberculosis*.

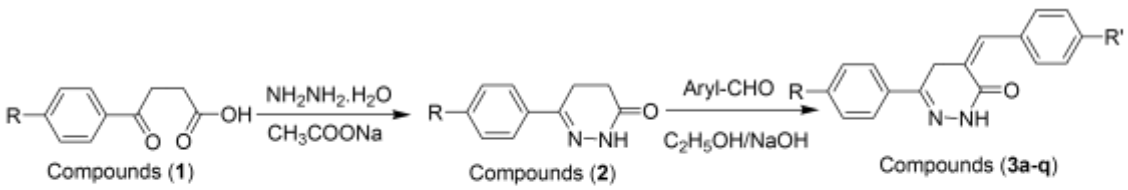
2. Materials and Methods

2.1. Experimental Section

Melting points (M.P) of the compounds 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. 1H-NMR spectra were recorded on Bruker 400 MHz instrument in $CDCl_3$/DMSO, using tetra methyl silane [$(CH_3)_4Si$] (TMS) as internal standard. Mass spectra were recorded on Jeol JMS-D300 instrument. The synthesis of intermediates, 6-aryl-2, 3, 4, 5-tetrahydropyridazin-3-ones (2) and target compounds 4-substituted-benzylidene-6-substituted-phenyl-4,5-dihydro-pyridazin-3(2*H*)-ones (3a-q) was achieved following Scheme 1.

2.2. General Method

Compounds 6-aryl-2, 3, 4, 5-tetrahydropyridazin-3-one 2 were synthesized by reported method [20] with slight modifications. Their synthesis involved the reaction of 4-aryl-4-oxobutanoic acid (1) with hydrazine hydrate. Compounds 2 were used for the preparation of compounds; 4-



Scheme 1. The synthesis of intermediates, 6-aryl-2, 3, 4, 5-tetrahydropyridazin-3-ones (2) and target compounds 4-substituted-benzylidene-6-substituted-phenyl-4, 5-dihydro-pyridazin-3(2*H*)-ones (3a-q).

substituted-benzylidene-6-substituted-phenyl-4, 5-dihydro-pyridazin-3 (2*H*)-ones (3a-q).

2.3. General Procedure for Synthesis of 4-Substituted-Benzylidene-6-(aryl-phenyl)-4, 5-Dihydropyri-Dazin-3(2H)-one (3a-q)

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, sodium hydroxide solution 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.

2.3.1. (4E)-4-(2-Chlorobenzylidene)-6-Phenyl-4, 5-Dihydropyridazin-3(2H)-one (3a)

IR (cm^{-1}, KBr): 3284(NH), 1665(CO); ^1H NMR (δ, *ppm*): 2.94, t, 2H, CH_2; 7.38-7.56, m, 7H, H-3,4,5 & H-4',5',3',6'; 7.70, d, H-2,6; 7.92, 1H, arylidene; 10.90, bs,1H, CONH; Mass $[C_{17}H_{13}ClN_2O]$: *m/z* 297(M^+), 299(M^{+2}); R =H; R'=2-Cl; M.P (ºC)=201-203; Yield %= 73; R_f values =0.89.

2.3.2. (4E)-4-(3-Bromobenzylidene)-6-Phenyl-4, 5-Dihydropyridazin-3(2H)-one (3b)

IR (cm^{-1}, KBr): 3286(NH), 1672(CO); ^1H NMR (δ, *ppm*): 2.96, s, 2H; 7.38-7.54, m, 6H, H-3,4,5 & H-4',5', 2'; 7.71, d, 2H, H-2,6; 7.78, d, 1H, H-6'; 7.92, arylidene; 10.82, bs, 1H, CONH; Mass: $[C_{17}H_{13}BrN_2O]$ *m/z* 342(M^+), 344(M^{+2}); R=H R'=3-Bromo, M.P (ºC)= 191-193 Yield %=68; R_f values =0.78.

2.3.3. (4E)-4-(4-Bromobenzylidene)-6-Phenyl-4, 5-Dihydropyridazin-3(2H)-one (3c)

IR (cm^{-1}, KBr): 3292(NH), 1682(CO); 1H NMR (δ, *ppm*): 2.95, s, 2H;7.36-7.44, m, 3H, H-3,4,5; 7.72, d, 2H, H-2,6; 7.76, d, 2H, H-2',6'; 7.58, d, 2H, H-3',5'; 7.91, s, 1H, arylidene; 10.86, bs, 1H, CONH; Mass: $[C_{17}H_{13}BrN_2O]$ *m/z* 342(M^+), 344(M^{+2}); R =H; R'=4-Bromo; M.P (ºC)=178-180; Yield %= 62; R_f values =0.81.

2.3.4. (4E)-4-(4-Fluorobenzylidene)-6-Phenyl-4, 5-Dihydropyridazin-3(2H)-one (3d)

IR (cm^{-1}, KBr): 3296 (NH), 1688 (CO); ^1H NMR (δ, *ppm*): 2.94, s, 2H; 7.17-7.20, m, 2H, H-3',5'; 7.38-7.44, m, 3H, H-3,4,5; 7.70, d, 2H, H-2,6; 7.78, m, 2H, H-2',6'; 7.88, s, 1H, arylidene; 10.82, bs, 1H, CONH; Mass: $[C_{17}H_{13}FN_2O]$ *m/z* 281(M^+); R =H; R'=4-Fluoro; M.P (ºC)=198-200; Yield %= 58; R_f values =0.92.

2.3.5. (4E)-4-(3, 4-Dimethoxybenzylidene)-6-Phenyl-4, 5-Dihydropyridazin-3(2H)-one (3e)

IR (cm^{-1}, KBr): 3294(NH), 1678(CO); ^1H NMR (δ, *ppm*): 2.94, CH2; 3.82, s, 3H, OCH_3; 3.84, s, 3H, OCH_3; 6.98, d, 1H, H-5'; 7.36-7.46, m, 4H, H-3,4,5, H-6'; 7.70, d, H-2,6; 7.64, s, 1H, H-2'; 7.90, 1H, arylidene; 10.82, CONH; Mass: $[C_{19}H_{18}N_2O_3]$ *m/z* 323(M^+); R =H; R'=3,4-Dimethoxy; M.P (ºC)=172-174; Yield %= 59; R_f values =0.88.

2.3.6. (4E)-4-[4-(Dimethylamino) Benzylidene]-6-Phenyl-4, 5-Dihydropyridazin-3(2H)-one (3f)

IR (cm^{-1}, KBr): 3290(NH), 1680 (CO); ^1H NMR (δ, *ppm*): 3.07, s, 3H; 3.09, s, 3H; 2.94, s, 2H; 6.78, d, 2H, H-3',5'; 7.38-7.46, m, 3H, H-3,4,5; 7.70-7.74, m, 4H, H-2,6 & H-2',6'; 7.90, s, 1H, arylidene; 10.86, bs, 1H, CONH; Mass: [$C_{19}H_{19}N_3O$] *m/z* 306(M^+); R =H; R'=4-*N*,*N*-Dimethyl; M.P (ºC)=185-187; Yield %= 68; R_f values =0.86.

2.3.7. (4E)-4-(3-Bromobenzylidene)-6-(4-Methylphenyl) -4, 5-Dihydropyridazin-3(2H)-one (3g)

IR (cm^{-1}, KBr): 3282 (NH), 1670 (CO); ^1H 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, H-2,6) 7.74 (d, 2H, 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}); R=4-CH_3, R′=3-Br; Yield: 52 %; M.P. (°C): 221-223; R_f : 0.87.

2.3.8. (4E)-4-(4-Bromobenzylidene)-6-(4-Methylphenyl)-4, 5-Dihydropyridazin-3(2H)-one (3h)

IR (cm^{-1}, KBr): 3295 (NH), 1683 (CO); ^1H NMR (δ, ppm): 2.38 (3H, CH_3), 2.96 (s, 2H, CH_2), 7.40 (d, 2H, H-3,5), 7.56 (d, 2H, H-3′,5′), 7.70 (d, 2H, H-2,6), 7.78 (d, 2H, 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}); R=4-CH_3, R′=4-Br; Yield: 48 %; M.P. (°C): 227-229; R_f: 0.86.

2.3.9. (4E)-4-(3, 4-Dimethoxybenzylidene)-6-(4-Methylphenyl)-4, 5-Dihydropyridazin-3 (2H)-one (3i)

IR (cm^{-1}, KBr): 3290 (NH), 1679 (CO); ^1H 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, H-5′),7.32 (d, 2H, H-3,5), 7.48 (s, 1H, H-2′), 7.58 (d, 1H, H-6′), 7.68 (d, 2H, 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^+); R=4-CH_3, R′=3,4-OCH_3; Yield: 61 %; M.p. (°C): 205-207; R_f: 0.83.

2.3.10. (4E)-4-[4-(Dimethylamino) Benzylidene]-6-(4-Methyl-Phenyl)-4, 5-Dihydropyridazin-3(2H)-One (3j)

IR (cm^{-1}, KBr): 3297(NH), 1683 (CO); ^1H 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, H-3′,5′), 7.34 (d, 2H, 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^+); R=4-CH_3, R′=4-$N(CH_3)_2$; Yield: 69%; M.P. (°C): 166-168; R_f: 0.82.

2.3.11. (4E)-4-(2-Chlorobenzylidene)-6-(4-Methylphenyl)-4, 5-Dihydropyridazin-3(2H)-one (4k)

IR (cm^{-1}, KBr): 3287 (NH), 1670 (CO); ^1H NMR (δ, *ppm*): 2.38, s, 3H, CH_3; 2.94, s, 2H, CH_2; 7.42-7.50, m, 5H, H-3,5 & H-3',4',5'; 7.60, d, 1H, H-6'; 7.66, d, 1H, H-2,6; 7.86, s, 1H, arylidene; 10.86, bs, 1H, CONH.; Mass: [$C_{18}H_{15}ClN_2O$] *m/z* 310 (M^+), 312(M^{+2}); R=CH_3; R'=2-Chloro; M.P. (ºC)=217-219; Yield %= 71; R_f values =0.75.

2.3.12. (4E)-4-(4-Fluorobenzylidene)-6(4-Methylphenyl)-4, 5-Dihydropyridazin-3(2H)-one (3l)

IR (cm-1, KBr): 3298 (NH), 1686 (CO); ^{1}H NMR (δ, *ppm*): 2.38, s, 3H, CH3; 2.96, s, 2H, CH_2; 7.20, t, 2H, H-3',5'; 7.36, d, 2H, H-3,5; 7.64, d, 2H, H-2,6; 7.74, m, 2H, H-2',6'; 7.88, s, 1H, arylidene; 10.86, bs, 1H, CONH; Mass: [$C_{18}H_{15}FN_2O$] *m/z* 294 (M^+); R=CH_3; R'=4-Fluoro; M.P. (ºC)=203-205; Yield %=55; R_f values=0.90.

2.3.13. (4E)-4-(3-Bromobenzylidene)-6-(4-Chlorophenyl)-4, 5-Dihydropyridazin-3(2H)-one (3m)

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, H-6′), 7.70 (d, 2H, H-2′,6′), 7.92 (s, 1H, arylidene), 10.84 (bs, 1H, CONH). Mass [$C_{17}H_{12}$Br ClN_2O]: *m/z* 377 (M^+), 379 (M^{++2}); R=4-Cl, R′= 3-Br; Yield: 56 %; M.p. (°C): 203-205; R_f: 0.82.

2.3.14. (4E)-4-(4-Bromobenzylidene)-6-(4-Chlorophenyl)-4, 5-Dihydropyridazin-3(2H)-one (3n)

IR (cm-1, KBr): 3297 (NH), 1684 (CO); 1H NMR (δ, ppm): 2.96 (s, 2H, CH2), 7.52-7.56 (m, 4H, H-3,5 and H-3′,5′), 7.68 (d, 2H, H-2′, 6′), 7.72 (d, 2H, 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}); R = 4-Cl, R′ = 4-Br; Yield: 47 %; M.p. (°C): 197-199; R_f : 0.81.

2.3.15. (4E)-4-(3, 4-Dimethoxybenzylidene)-6-(4-Chlorophenyl)-4,5-Dihydropyridazin-3(2H)-one (3o)

IR (cm^{-1}, KBr): 3289 (NH), 1672 (CO); 1H 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, H-5′), 7.50-7.54 (m, 3H, H-3,5 and H-2′), 7.60 (d, 1H, H-6′), 7.68 (d,2H, 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}); R=4-Cl, R′=3,4-OCH_3; Yield: 61 %; M.p. (°C): 223-225; R_f : 0.82.

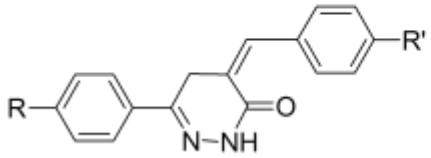
2.3.16. (4E)-4-[4-(Dimethylamino) Benzylidene]-6-(4-Chlorophenyl)-4, 5-Dihydropyridazin-3(2H)-one (3p)

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, 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; mass [$C_{19}H_{18}ClN_2O$] *m/z* 339 (M^+), 341 (M^{+2}); R=4-Cl, R'=4-Dimethyl amino; M.P. (ºC) 186-188; Yield % 69; R_f values 0.89.

2.4. Antitubercular Activity Using Microplate Alamar Blue Dye Assay Method

The Antitubercular activity of title compounds (3a-j) was assessed against *Mycobacterium tuberculosis* H37Rv strain by using Microplate Alamar Blue Assay (MABA) method. This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method [16]. In this method, 200µl of sterile de-ionized water was added to

Table 1. Structure and Antitubercular activity of (4*E*)-4-substituted benzylidene-6-substituted-phenyl-4,5-dihydropyridazin-3(2*H*)-one (3a-q).



Compounds (**3a-q**)

Compd	R	R'	MIC values (µg/mL)	Compd	R	R'	MIC values (µg/mL)
3a	H	2-Cl	12.5	3i	4-CH_3	R′=3,4-OCH_3	25
3b	H	3-Br	12.5	3j	4-CH_3	4-$N(CH_3)_2$	25
3c	H	4-Br	12.5	3k	CH_3	2-Cl	12.5
3d	H	4-F	12.5	3l	CH_3	4-F	12.5
3e	H	3,4-OCH_3	25	3m	4-Cl	3-Br	6.25
3f	H	4-$N(CH_3)_2$	25	3n	4-Cl	4-Br	6.25
3g	4-CH_3	R′=3-Br	12.5	3o	4-Cl	3,4-OCH_3	12.5
3h	4-CH_3	R′=4-Br	12.5	3p	4-Cl	4-$N(CH_3)_2$	12.5
Pyrazinamide			3.125µg/ml	Streptomycin			6.25 µg/ml

all outer perimeter wells of sterile 96 wells plate to minimize evaporation of medium in the test wells during incubation. The 96 wells plate received 100 µl of the Middle brook 7H9 broth and serial dilutions of the compounds were made directly on plate. The final drug concentrations tested were 100 to 0.2 µg/ml. Plates were covered, sealed with parafilm and incubated at 37ºC for five days. After this, 25µl of freshly prepared 1:1 mixture of Almar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. Blue color in the well was interpreted as no bacterial growth, pink color scored as growth [15]. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink. The MIC of the standard drug streptomycin and pyrizinamide were 6.25µg/mL and 3.125µg/mL. The results of the pharmacological evaluation have been listed in Table 1.

3. Results and Discussion

3.1. Chemistry

The reaction of 4-aryl-4-oxobutanoic acids (1a-c) with hydrazine hydrate gave 6-aryl-2, 3, 4, 5-tetrahydropyridazin-3-one (2a-c). Aryl-aldehydes were reacted with ethanolic solution of 2a-c to obtain (4*E*)-4-arylidene-6-aryl-4, 5-dihydro-pyridazin-3(2*H*)-ones (3a-p) [17]. The structures of the compounds were established on the basis of modern analytical techniques; IR, ^{1}H-NMR, Mass and elemental analysis data results. The spectral and analytical data are in full agreement with the proposed structures.

3.2. Antitubercular Evaluation

The Antitubercular screening was carried out against *M. tuberculosis* H37Rv (Table 1). The results illustrated that compound (3m) and (3n) had best anti-TB activity among all the synthesized compounds with MIC value

6.25µg/mL. Ten compounds (3a-3d), (3h), (4k), (3l), (3o) and (3p) also had antitubercular action with MIC value of 12.5µg/mL. The rest of compounds; (4e), (4f), (3i) and (3j) were exhibited MIC values of 25µg/mL. The result indicated that compounds bearing electron withdrawing group exhibited better antitubercular activity than the compounds bearing electron releasing groups. Further, compound bearing electron withdrawing group on both phenyl ring (3m and 3n) was found to be the most active antitubercular agent. Pyridazinone nucleus can be exploited for development of various synthetic compounds and their substituted pyridazinone derivatives to improved pharmacological activities [16-20].

4. Conclusion

In conclusion, compounds, 6-aryl-4-arylidene-4, 5-dihydropyridazin-3(2H)-one (3a-j) were successfully synthesized through multistep synthesis. So we synthesized compounds (3a-q) were evaluated against *M. tuberculosis* H37Rv strain for their antitubercular activity. The results illustrated that among all the synthesized compounds, only 3m and 3n exhibited the highest antitubercular activity, with MIC value of 6.25µg/ml, equivalent to reference drug, streptomycin. Antitubercular screening of these compounds shows the antitubercular potential of the pyridazine derivatives. From the antitubercular data, it could be concluded that compound bearing electron withdrawing group appeared most active against bacteria, whereas compound bearing electron releasing group exhibited less potent activity. In future, novel pyridazinone compounds will develop for new and effective antitubercular agent with more effective particularly against resistant strains of tuberculosis.

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