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ANTITUBERCULAR ACTIVITY OF SOME NEWER 6-PYRIDAZINONE DERIVATIVES

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ABSTRACT

*Two series of 6-pyridazinone derivatives (**17-30**) were synthesized and evaluated for antitubercular activities against Mycobacterium tuberculosis $H_{37}Rv$ strain. The results indicated that among the synthesized compounds, 5-(4-hydroxy-3-methoxybenzyl)-3-phenyl-1,6-dihydro-6-pyridazinone (**23**) showed good antitubercular activity. Three more compounds, (**18, 25** & **27**) were significant in their antitubercular action. The present study reveals the antitubercular potential of 6-pyridazinones.*

KEY WORDS: Pyridazinone, antitubercular, mycobacteria, furanone.

RESUMO

*Duas séries de derivados de 6-piridazona foram sintetizados e avaliados para a atividade antitubercular contra Mycobacterium tuberculosis da cepa $H_{37}Rv$. Os resultados experimentais indicaram que o composto 5-(4-hidroxi-3-metoxibenzil)-3-fenil-1,6-dihidro-6-piridazinona (**23**) apresentou boa atividade antitubercular. Outros três compostos (**18, 25 e 27**) mostraram atividade antitubercular significativa. O presente estudo revela o potencial antitubercular de 6-piridazinonas.*

PALAVRAS-CHAVE: Piridazinona, atividade contra tuberculose, micobactéria, furanona.

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INTRODUCTION

Resistance of *Mycobacterium tuberculosis* strains to available antitubercular drugs is an increasing problem worldwide. New potent antimycobacterial drugs with new mechanisms of action have not been developed in the last forty years[1]. TB is considered by the WHO to be the most important chronic communicable disease in the world. About 32% of the world's population is currently infected with TB. The emergence of AIDS, decline of socioeconomic standards and a reduced emphasis on tuberculosis control programs contribute to the disease's resurgence in industrialized countries[2]. If the present trend continues, tuberculosis is likely to claim more than 30 million lives within the new decade. A great deal of research is being directed towards the development of new antitubercular drug.

During recent years pyridazinones have been a subject of intensive research due to their wide spectrum of biological activities. Substituted pyridazinones have been found to have potent antibacterial, antifungal and antiviral including anti-HIV activities[3-6]. Various 3-(2*H*)-pyridazinone derivatives have shown anticancer[6], analgesic & anti-inflammatory[6-8], anticonvulsant[9], cardiotonic & hypotensive[10,11] and antiulcer activities[12]. Now research efforts are toward the search of new antimycobacterial agents (new classes of compounds), which are structurally different from known antimycobacterial drugs[13,14]. The present work describes the synthesis of newer 2(3*H*)-pyridazinones with encouraging antitubercular activity.

MATERIALS AND METHODS

Synthesis

Melting points were determined in open capillary tubes and are uncorrected. Thin-layer chromatography was carried out to monitor the reactions using silica gel G plates. The IR spectra were recorded in potassium bromide pellets using a Perkin-Elmer 1725X spectrophotometer. Elemental analyses were performed on a Perkin-Elmer 240 analyzer and the values were in range of ±0.4% theoretical value for the element analyzed (C, H, N). ^{1}H-NMR spectra were recorded on Bruker spectropsin DPX-300 MHz in $CDCl_3$; chemical shift (δ) values are reported in parts per million (*ppm*). The splitting pattern abbreviations are as follows: *s*, singlet; *d*, doublet; *m*, multiplet. Mass spectra under electron impact conditions (EI) were recorded at 70 eV ionizing voltage with a VG Prospec instrument and are presented as m/z. Spectral data are consistent with the assigned structures.

Preparation of 3-(4-Chloro/methyl benozyl)propionic acid (1,2). The compounds, **1** and **2**, were synthesized according to the reported method[14].

Preparation of 3-Arylidene-5-(4-chloro/methyl phenyl)-2(3H)-furanones (3-16). Compounds (**3-16**) were synthesized from 3-(4-chloro/methyl benozyl)propionic acid (**1,2**) following literature method[14].

General Procedure for the synthesis of 5-(substituted benzyl)-3-aryl-1,6-dihydro-6-pyridazinones (17-30). 2(3*H*)-Furanones (**3-16**) (0.005 mole) and hydrazine hydrate (1-2 mL) in *n*-propanol (5-6 mL) were refluxed for 3h. After refluxing reaction mixture was poured onto crushed ice, a precipitate was obtained, which was filtered, dried and recrystallized from methanol to give TLC pure 5-(substituted benzyl)-3-aryl-1,6-dihydro-6-pyridazinone derivatives (**17-30**).

5-Benzyl-3-phenyl-1,6-dihydro-6-pyridazinone (**17**): Yield: 58%; m.p. 168-170 °C; ^{1}H-NMR ($CDCl_3$, δ, ppm): 3.99 (s, 2H, CH_2), 7.26 (s, 1H, H-4, pyridazinone ring), 7.29-7.41 (m, 6H, 2xH-2,4,6, benzyl + phenyl), 7.58-7.65 (m, 4H, 2xH-3,5, benzyl + phenyl), 10.62 (s, 1H, NH); MS (*m/z*): 262(M^+); IR (cm^{-1}, KBr): 3186 (NH), 2949 (CH), 1683 (CO); Anal calcd. for $C_{17}H_{14}N_2O$ (CHN).

5-(2-Chlorobenzyl)-3-phenyl-1,6-dihydro-6-pyridazinone (**18**): Yield: 63%; m.p. 210 °C; ^{1}H-NMR ($CDCl_3$, δ, ppm): 4.42 (s, 2H, CH_2), 7.24 (s, 1H, H-4, pyridazinone ring), 7.26-7.64 (m, 9H, phenyl+ benzyl) 10.77 (s, 1H, NH); MS (*m/z*): 296/297 (M^+/M+1); IR (cm^{-1}, KBr): 3179 (NH), 2942 (CH), 1688 (CO), 726 (C-Cl); Anal calcd. for $C_{17}H_{13}ClN_2O$ (CHN).

5-(4-Chlorobenzyl)-3-phenyl-1,6-dihydro-6-pyridazinone (**19**): Yield: 68%; m.p. 188 °C; ^{1}H-NMR ($CDCl_3$, δ, ppm): 3.93 (s, 2H, CH_2), 7.24 (s, 1H, H-4, pyridazinone ring), 7.27 & 7.67 (d, each, $2xA_2B_2$, *p*-chlorophenyl), 7.30 (m, 1H, H-4, phenyl), 7.33 (m, 2H, H-2,6, phenyl), 7.46 (m, 2H, H-3,5, phenyl), 12.68 (s, 1H, NH); MS (*m/z*): 296/297(M^+/M+1); IR (cm^{-1}, KBr): 3173 (NH), 2936 (CH), 1672 (CO), 707 (C-Cl); Anal calcd. for $C_{17}H_{13}ClN_2O$ (CHN).

5-(3-Nitrobenzyl)-3-phenyl-1,6-dihydro-6-pyridazinone (**20**): Yield: 58%, m.p. 178-180 °C; ^{1}H-NMR ($CDCl_3$, δ, ppm): 3.89 (s, 2H, CH_2), 7.14 (s, 1H, H-4, pyridazinone ring), 7.26 (m, 1H, H-4, phenyl), 7.31 (m, 1H, H-4, phenyl), 7.44 (m, 1H, H-6, benzyl ring), 7.34 (m, 2H, H-2,6, phenyl), 7.50 (m, 2H, H-3,5, phenyl), 8.14 (m, 1H, H-5, benzyl ring), 8.16 (m, 1H, H-4, benzyl ring), 8.18 (m, 1H, H-2, benzyl ring), 10.98 (s, 1H, NH); MS (*m/z*): 307 (M^+); IR (cm^{-1}, KBr): 3178 (NH), 2942 (CH), 1686 (CO); Anal calcd. for $C_{17}H_{13}N_3O_3$ (CHN).

5-(4-Methoxybenzyl)-3-phenyl-1,6-dihydro-6-pyridazinone (**21**): Yield: 52%; m.p. 186 °C; ^{1}H-NMR ($CDCl_3$, δ, ppm): 3.82 (s, 3H, OCH_3), 3.93 (s, 2H, CH_2), 6.82 (s, 1H, H-4, pyridazinone ring), 6.83 & 7.41 (d, each, $2xA_2B_2$, *p*-methoxy benzyl ring), 7.21 (m, 1H, H-4, phenyl ring), 7.27 (m, 2H, H-2,6, phenyl ring), 7.65 (m, 2H, H-3,5, phenyl ring), 10.87 (s, 1H, NH); MS (*m/z*): 292 (M^+); IR (cm^{-1}, KBr): 3173 (NH), 2936 (CH), 1684 (CO); Anal calcd. for $C_{18}H_{16}N_2O_2$ (CHN).

5-(3,4-Dimethoxybenzyl)-3-phenyl-1,6-dihydro-6-pyridazinone (**22**): Yield: 56%; m.p. 198-200 °C; ^{1}H-NMR ($CDCl_3$, δ, ppm): 3.63 (s, 2H, $2xOCH_3$), 3.86 (s, 2H, CH_2), 7.26 (s, 1H, H-4, pyridazinone ring), 7.28 (m, 1H, H-4, phenyl ring), 7.38 (m, 1H, H-6, benzyl), 7.61 (m, 2H, H-3,5, phenyl ring), 7.64 (m, 1H, H-5, benzyl), 7.66 (m, 1H, H-2, benzyl), 11.31 (s, 1H, NH); MS (*m/z*): 322 (M^+); IR (cm^{-1}, KBr): 3167 (NH), 3002 (CH), 1673 (CO); Anal calcd. for $C_{19}H_{18}N_2O_3$ (CHN).

5-(4-Hydroxy-3-methoxybenzyl)-3-phenyl-1,6-dihydro-6-pyridazinone (**23**): Yield: 62%; m.p. 191-193 °C; ^{1}H-NMR ($CDCl_3$, δ, ppm): 3.48 (s, 2H, CH_2), 3.64 (s, 3H, OCH_3), 6.53 (s, 1H, H-4, pyridazinone ring), 7.07-7.29 (m, 5H, phenyl), 7.39-7.72 (3H, H-2,5,6, benzyl), 10.82 (s, 1H, NH); MS (*m/z*): 308 (M^+); IR (cm^{-1}, KBr): 3185 (NH), 2955 (CH), 1686 (CO); Anal calcd. for $C_{18}H_{16}N_2O_3$ (CHN).

5-(4-Fluorobenzyl)-3-phenyl-1,6-dihydro-6-pyridazinone (**24**): Yield: 57%; m.p. 201 °C; ^{1}H-NMR ($CDCl_3$, δ, ppm): 3.57 (s, 2H, CH_2), 7.09 (s, 1H, H-4, pyridazinone ring), 7.28 (m, 1H, H-4, phenyl), 7.29 (d, H-2,6, *p*-fluorobenzyl), 7.37 (m, 2H, H-2,6, phenyl), 7.53 (m, 2H, H-3,5, *p*-fluorobenzyl), 7.57 (m, 2H, H-3,5, phenyl), 11.73 (s, 1H, NH); MS (*m/z*): 280 (M^+); IR (cm^{-1}, KBr): 3182 (NH), 2949 (CH), 1673 (CO); Anal calcd. for $C_{17}H_{13}FN_2O$: (CHN).

5-(4-Hydroxy-3-ethoxybenzyl)-3-phenyl-1,6-dihydro-6-pyridazinone (**25**): Yield: 65%; m.p. 180 °C; ^{1}H-NMR ($CDCl_3$, δ, ppm): 1.46 (t, 3H, $OCH_2\underline{CH_3}$), 3.48 (s, 2H, CH_2), 4.07 (m, 2H, $O\underline{CH_2}CH_3$), 6.53 (s, 1H, H-4, pyridazinone ring), 7.07-7.29 (m, 5H, phenyl), 7.39-7.72 (3H, H-2,5,6, benzyl), 10.82 (s, 1H, NH); MS (*m/z*): 322 (M^+); IR (cm^{-1}, KBr): 3184 (NH), 2966 (CH), 1678 (CO); Anal calcd. for $C_{19}H_{18}N_2O_3$ (CHN).

5-(2-Chlorobenzyl-3-(4-chlorophenyl)-1,6-dihydro-6-pyridazinone (**26**): Yield: 63%; m.p. 186-188 °C; ^{1}H-NMR ($CDCl_3$, δ, ppm): 3.93 (s, 2H, CH_2), 7.21 (s, 1H, H-4, pyridazinone ring), 7.28 (m, 2H, H-4,6, phenyl), 7.38 (m, 1H, H-5, phenyl ring),7.40 & 7.59 (d, each, $2xA_2B_2$, *p*-chlorophenyl ring), 7.44 (m, 1H, H-3, phenyl), 11.52 (s, 1H, NH); MS (*m/z*): 330/331/333 (M^+/M+1/M+3); IR (cm^{-1}, KBr): 3185 (NH), 2952 (CH), 1676 (CO), 714 (C-Cl); Anal calcd. for $C_{17}H_{12}Cl_2N_2O$ (CHN).

5-(2-Hydroxybenzyl)-3-(4-chlorophenyl)-1,6-dihydro-6-pyridazinone (**27**): Yield: 58%; m.p. 182-184 °C; ^{1}H-NMR ($CDCl_3$, δ, ppm): 3.61 (s, 2H, CH_2), 7.26 (s, 1H, H-4, pyridazinone ring), 7.34 (m, 1H, H-4, phenyl ring), 7.41 & 7.62 (d, each, $2xA_2B_2$, *p*-chlorophenyl), 7.64 (m, 2H, H-2,5, phenyl), 8.37 (m, 1H, H-3, phenyl), 8.57 (s, 1H, OH), 9.33 (s, 1H, NH); MS (*m/z*): 312/313 (M^+/M+1); IR (cm^{-1}, KBr): 3174 (NH), 2939 (CH), 1683 (CO), 718 (C-Cl); Anal calcd. for $C_{17}H_{13}ClN_2O_2$ (CHN).

5-(3-Hydroxybenzyl)-3-(4-chlorophenyl)-1,6-dihydro-6-pyridazinone (**28**): Yield: 54%; m.p. 189 °C; ^{1}H-NMR ($CDCl_3$, δ, ppm): 3.64 (s, 2H, CH_2), 7.19 (s, 1H, H-4, pyridazinone ring), 7.42 & 7.55 (d, each, $2xA_2B_2$, *p*-chlorophenyl), 7.57 (m, 1H, H-6, benzyl), 7.98 (m, 1H, H-5, benzyl), 8.07 (m, 1H, H-4, benzyl), 8.17 (m, 1H, H-2, benzyl), 9.35 (s, 1H, NH); MS (*m/z*): 312/313 (M^+/M+1); IR (cm^{-1}, KBr): 3168 (NH), 2944 (CH), 1681 (CO), 722 (C-Cl); Anal calcd. for $C_{17}H_{13}ClN_2O_2$: (CHN).

5-(3-Nitrobenzyl)-3-(4-chlorophenyl)-1,6-dihydro-6-pyridazinone (**29**): Yield: 56%; m.p. 198 °C; ^{1}H-NMR ($CDCl_3$, δ, ppm): 4.05 (s, 2H, CH_2), 7.15 (s, 1H, H-4, pyridazinone ring), 7.24 & 7.53 (d, each, $2xA_2B_2$, *p*-chlorophenyl), 7.55 (m, 1H, H-6, benzyl), 8.21 (m, 1H, H-5, benzyl), 8.31 (m, 1H, H-4, benzyl), 8.49 (m, 1H, H-2, benzyl), 9.23 (s, 1H, NH); MS (*m/z*): 341/342 (M^+/M+1); IR (cm^{-1}, KBr): 3180 (NH), 2934 (CH), 1687 (CO), 717 (C-Cl); Anal calcd. for $C_{17}H_{12}ClN_3O_3$ (CHN).

5-(3,4-Dimethoxybenzyl)-3-(4-chlorophenyl)-1,6-dihydro-6-pyridazinone (**30**): Yield: 59%; m.p. 186-188 °C; ^{1}H-NMR ($CDCl_3$, δ, ppm): 3.87 (s, 6H, 2x-OCH_3), 3.71 (s, 2H, CH_2), 6.81 (s, 1H, H-4, pyridazinone ring), 6.83 (m, 2H, H-2,6, benzyl), 7.26 (m, 1H, H-5, benzyl), 7.39 & 7.61 (d, each, $2xA_2B_2$, *p*-chlorophenyl), 11.54 (s, 1H, NH); MS (*m/z*): 356/357 (M^+/M+1); IR (cm^{-1}, KBr): 3176 (NH), 2959 (CH), 1681 (CO), 725 (C-Cl); Anal calcd. for $C_{19}H_{17}ClN_2O_3$ (CHN).

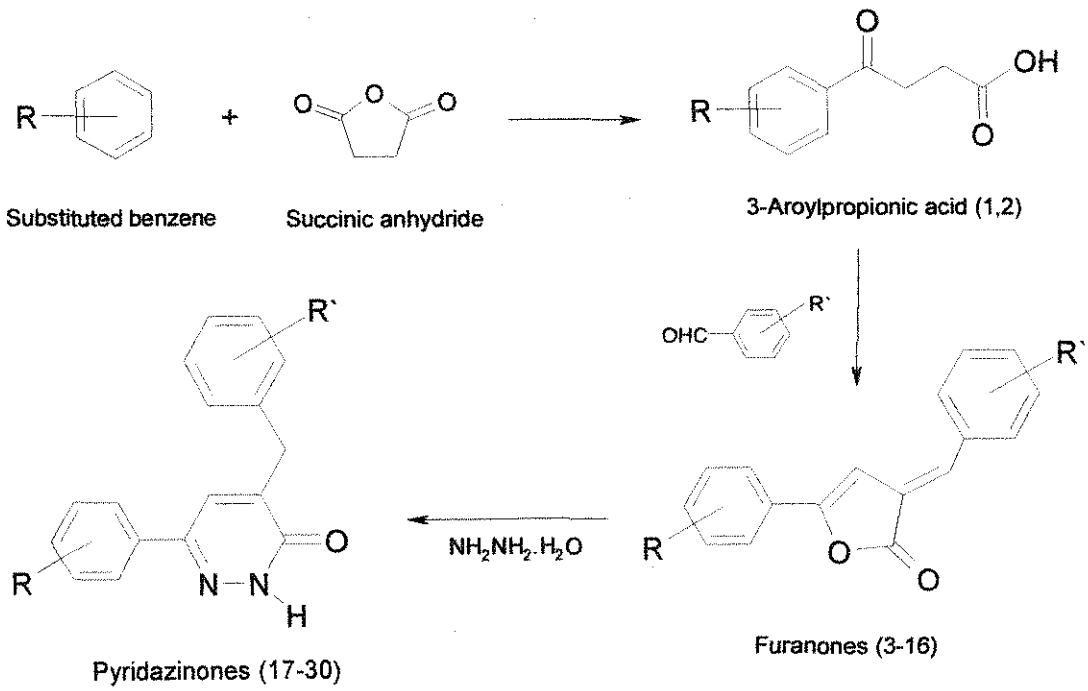
Antitubercular activity[15,16]

The antitubercular screening was carried out against *Mycobacterium tuberculosis* $H_{37}Rv$ (ATCC 27294) in Middle brook 7H11 agar medium with OADC (oleic acid albumin dextrose catalase) growth supplement. 10 fold serial dilutions of each test compound/drug (in DMSO/Water mixture) were incorporated into the agar medium. Inoculum of *M. tuberculosis* $H_{37}Rv$ were prepared from fresh Middlebrook 7H11 agar slants with OADC growth supplement adjusted to 1 mg/mL (wet weight) in Tween 80 (0.05%) saline diluted to 10^{-2} to give a concentration of approximately 10^7 cfu/mL. A 5 µL amount of bacterial suspension was spotted into 7H11 agar tubes containing 10-fold serial dilutions of drugs per mL. The tubes were incubated at 37 °C, and final readings were recorded after 30 days. The minimum inhibitory concentration (MIC) is defined as the minimum concentration of compound required to give complete inhibition of bacterial growth. The MIC of the standard drug streptomycin was 10 µg/mL. The results are presented in **Table 1**.

RESULTS AND DISCUSSION

Synthesis

The starting material, 3-(4-chlorobenzoyl/benzoyl)propionic acid (**1,2**) was synthesized from dry benzene or chlorobenzene following Friedal Craft's acylation reaction conditions[14]. 2(3*H*)-Furanones (**3-16**) were prepared using 3-aroylopionic acid (**1,2**) following the previously



Scheme 1: Protocol for synthesis of title compounds

Table 1: Antitubercular activity of the 6-pyridazinone derivatives **17-30.**

Compound	R	R`	*MIC values*
17	H	H	50
18	H	2-Cl	25
19	H	4-Cl	50
20	H	3- NO_2	50
21	H	4-OCH_3	50
22	H	3,4-$(OCH_3)_2$	50
23	H	4-OH; 3-OCH_3	12.5
24	H	4-F	50
25	H	4-OH; 3-OC_2H_5	25
26	4-Cl	2-Cl	50
27	4-Cl	2-OH	25
28	4-Cl	3-OH	50
29	4-Cl	3- NO_2	50
30	4-Cl	3,4-$(OCH_3)_2$	50

benzyl)-3-aryl-1,6-dihydro-6-pyridazinone derivatives (**17-30**) (Scheme-1). Spectral data and microanalysis data were in agreement with the proposed structures.

Antitubercular activity

The antitubercular screening was carried out against *Mycobacterium tuberculosis* $H_{37}Rv$ (ATCC 27294) (**Table 1**). The results indicated that 5-(4-hydroxy-3-methoxybenzyl)-3-phenyl-1,6-dihydro-6-pyridazinone (**23**) showed best antitubercular activity among the synthesized compounds with *MIC*-12.5 μg/mL. Three compounds, 5-(2-chlorobenzyl)-3-phenyl-1,6-dihydro-6-pyridazinone (**18**), 5-(4-hydroxy-3-ethoxybenzyl)-3-phenyl-1,6-dihydro-6-pyridazinone (**25**) and 5-(2-hydroxybenzyl)-3-(4-chlorophenyl)-1,6-dihydro-6-pyridazinone (**27**) were also significant in their antitubercular action with *MIC*-25 μg/mL. Rests of the compounds showed *MIC*-values of 50 μg/mL. Disubstituted phenyl rings having hydroxyl group (**23** & **25**) at 5th position of pyridazinone ring showed good antitubercular activity than unsubstituted or mono-substituted phenyl rings. Among the mono-substituted phenyl rings at 5th position of pyridazinone ring, presence of 2-chloro or 2-hydroxyl group (**18** & **27**) showed significant antitubercular activity.

Conclusions

To sum up, among the synthesized 14 newer pyridazinones, compound **23**, 5-(4-hydroxy-3-methoxybenzyl)-3-phenyl-1,6-dihydro-6-pyridazinone emerged as lead compound with good antitubercular activity. The study showed the antitubercular potential of 6-pyridazinone derivatives.

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