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2(3*H*)-FURANONES AND 2(3*H*)-PYRROLONES: SYNTHESIS AND ANTIMYCOBACTERIAL EVALUATION

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Abstract: A series of 3-arylidene-5-(substituted phenyl)-2(3*H*)-furanones (**3-10**) and their nitrogen analogues, 3-arylidene-5-(substituted phenyl)-1-benzyl-2(3*H*)-pyrrolone (**11-14**), and 3-arylidene-5-(substituted phenyl)-2(3*H*)-pyrrolones (**15-21**) were synthesized. All the compounds were screened for their antimycobacterial activity *in vitro* against *Mycobacterium tuberculosis* $H_{37}Rv$ (ATCC 27294) in BACTEC medium using the Microplate Alamar Blue Assay (MABA). Among the synthesized compounds, compound **19,** 3-(4-chlorobenzylidene)-5-(4-methylphenyl)-2(3*H*)-pyrrolone, emerged as a lead compound having the highest activity against *Mycobacterium tuberculosis*. The antimycobacterial activity was found to improve upon replacement of oxygen of furanone ring with nitrogen atom (pyrrolones), however, substitution with benzylamine moiety (1-benzylpyrrolones) markedly decreases the activity.

Keywords: furanones, pyrrolones, antimycobacterial, antitubercular

Tuberculosis (TB), one of the most common infectious diseases, continues to be major cause of morbidity and mortality all over the world (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). Resistance of *Mycobacterium tuberculosis* strains to antitubercular agents is an increasing problem worldwide. If the present trend continues, tuberculosis is likely to claim more than 30 million lives within the new decade. In this context, TB has become again an important public health problem worldwide due to two major factors, the AIDS epidemic and the advent of multidrug resistant strains (MDR).

The furanone ring system, also known as butyrolactone or butenolide, is a widely recognized component of natural products exhibiting a wide range of interesting biological activities. Different classes of synthetic furanones and pyrrolones possess an extensive spectrum of pharmacological activities. In particular, compounds bearing 2(3*H*)-furanone and 2(3*H*)-pyrrolone rings are known to exhibit important activities such as antibacterial, antifungal, antiviral, anticancer, antiinflammatory, vasodilating and anticonvulsant (3-10). The efficacy of several butyrolactone derivatives against mycobacteria suggests that these structures could serve as a new template for tuberculosis drug development (11). We had tested in these laboratories the antibacterial and antifungal activities of a number of furanones and pyrrolones and the results were encouraging (4, 5, 8). Our research effort towards the development of novel antitubercular agent is in the direction of discovering new classes of compounds, which are structurally different from known antitubercular drugs (12). The current work describes the synthesis of novel 2(3*H*)-furanones, a butyrolactone derivative, and its nitrogen analogues 2(3*H*)-pyrrolones with expected antimycobacterial activity.

EXPERIMENTAL

Chemicals were purchased from Merck and Sigma-Aldrich as 'synthesis grade' and used without further purification. Melting points (m.p.) were determined in open capillary tubes and are uncorrected. Microanalyses of the compounds were done on Perkin-Elmer model 240 analyzer and the values

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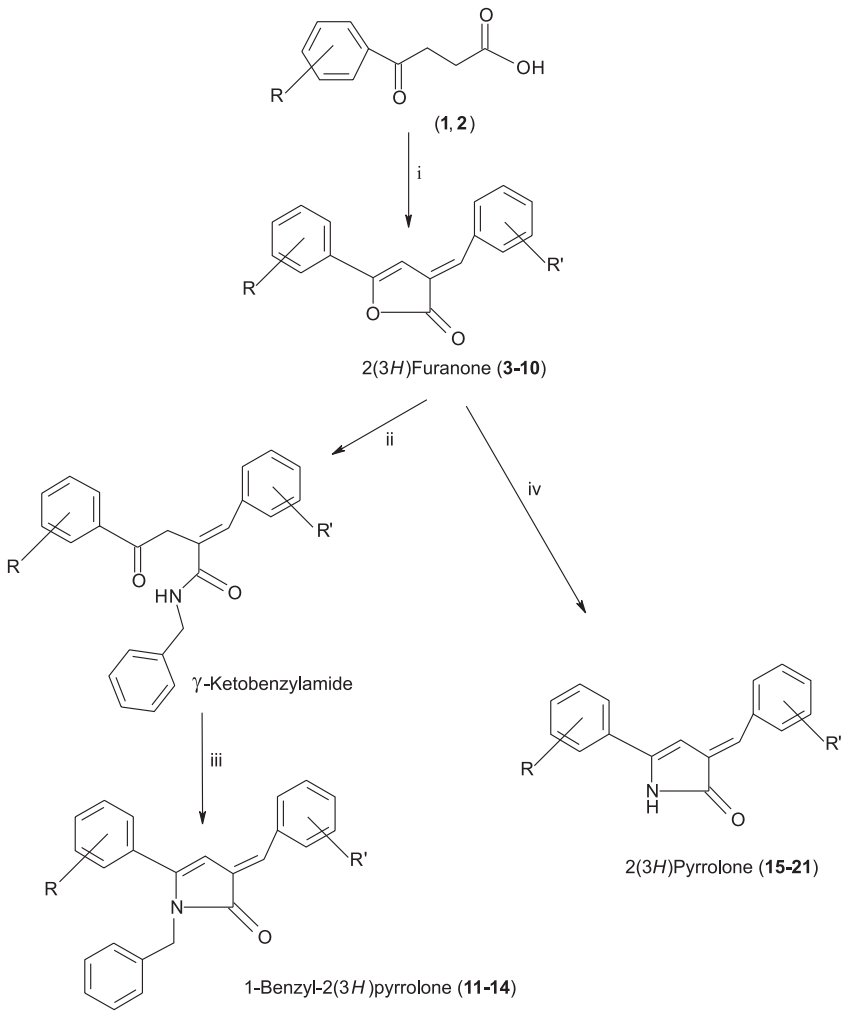
were found within ±0.4% of the theoretical values. The IR spectra were recorded in KBr pellets on Perkin-Elmer 1725X spectrophotometer. ^{1}H-NMR spectra were recorded on DPX-300 NMR spectrometer and BRUKER-400 Ultra Shield™ spectrometer. Chemical shifts (δ) are reported in parts per million (ppm); TMS was used as an internal standard. The splitting pattern abbreviations are as follows: s, singlet; d, doublet; dd, double doublet; t, triplet; q, quartet; m, multiplet. Mass spectroscopic analyses for compounds were performed on a Jeol JMS-D 300 instrument at 70 eV. Spectral data are consistent with the assigned structures. The molecular ion for compounds containing chloro substituent was calculated according to ^{35}Cl isotope. Thin-layer chromatography was carried out to monitor the reactions using silica gel and the solvent system – toluene : ethyl acetate : formic acid (5:4:1, v/v/v).

Preparation of 3-(4-bromo/methyl benzoyl)propionic acid (1, 2)

These compounds were obtained from dry bromobenzene/toluene by the method reported earlier (4, 5).

General procedure for the synthesis of 3-arylidene-5-(substituted phenyl)-2(3*H*)-furanone (3–10)

A solution of 3-(4-substituted benzoyl)-propionic acid **1** or **2** (3 mmol) and aromatic aldehyde (equimolar, 3 mmol) in acetic anhydride (15 mL) with triethylamine (3–4 drops) was refluxed for 4 h under anhydrous conditions. After completion of



Scheme 1. Protocol for synthesis of the title compounds (**3-21**). (i) Aryl aldehyde, Ac_2O, triethylamine, reflux; (ii) Dry benzene, Benzylamine; (iii) 6M HCl; (iv) Dry ammonia gas, Absolute ethanol.

reaction, the contents were poured onto crushed ice in small portions while stirring. A colored solid mass separated out, which was filtered, washed with water and crystallized from a mixture of methanol : chloroform (1:1, v/v).

3-(4-Chlorobenzylidene)-5-(4-bromophenyl)-2-(3*H*)-furanone (3)

Dark yellow needles, yield: 78%, m.p. 216°C. ^{1}H-NMR ($CDCl_3$, δ ppm) 6.89 (s, 1H, furanone ring), 7.34 (s, 1H, olefinic H), 7.41 and 7.53 (d, each, 2 × A_2B_2, arylidene ring), 7.45 and 7.63 (d, each, 2 × A_2B_2, *p*-substituted phenyl). MS (m/z): 361 (M^+), 184, 156, 105, 77. IR (KBr, cm^{-1}): 1763, 1603, 835.

3-(3-Nitrobenzylidene)-5-(4-bromophenyl)-2-(3*H*)-furanone (4)

Yellowish orange crystals, yield: 84%, m.p. 186-188°C. ^{1}H-NMR ($CDCl_3$, δ ppm): 6.83 (s, 1H, furanone ring), 7.48 (s, 1H, olefinic H), 7.53 and 7.65 (d, each, 2 × A_2B_2, *p*-substituted phenyl), 7.60 (m, 1H, H-5, arylidene ring), 7.84 (dd, 1H, H-6, arylidene ring) 8.22 (dd, 1H, H-4, arylidene ring), 8.30 (d, 1H, H-2, arylidene ring). MS (m/z): 372 (M^+), 184, 105, 77. IR (KBr, cm^{-1}): 1765, 1608, 834.

3-(3,4-Dimethoxybenzylidene)-5-(4-bromophenyl)-2(3*H*)-furanone (5)

Dark yellow needles, yield: 76%, m.p. 110°C. ^{1}H-NMR ($CDCl_3$, δ ppm): 3.96 (s, 6H, 2 × OCH_3), 6.89 (s, 1H, furanone ring), 6.95 (d, 1H, H-5, arylidene ring), 7.12 (s, 1H, H-2, arylidene ring), 7.29 (d, 1H, H-6, arylidene ring), 7.39 (s, 1H, olefinic H), 7.43 and 7.74 (d, each, 2 × A_2B_2, *p*-substituted phenyl). MS (m/z): 387 (M^+), 184, 156. IR (KBr, cm^{-1}): 1771, 1598, 836.

3-(2,6-Dichlorobenzylidene)-5-(4-bromophenyl)-2(3*H*)-furanone (6)

Brown crystals, yield: 74%, m.p. 176-178°C. ^{1}H-NMR ($CDCl_3$, δ ppm): 6.31 (s, 1H, furanone ring), 7.28 and 7.40 (d, each, 2 × A_2B_2, *p*-substituted phenyl), 7.44 (s, 1H, olefinic H), 7.56 (m, 3H, H-3,4,5, arylidene ring). MS (m/z): 396 (M^+), 184, 156, 105, 77. IR (KBr, cm^{-1}): 1776, 1611, 832.

3-(4-Chlorobenzylidene)-5-(4-methylphenyl)-2-(3*H*)-furanone (7)

Yellow crystals, yield: 72%, m.p. 248-250°C. ^{1}H-NMR ($CDCl_3$, δ ppm): 2.41 (s, 3H, CH_3), 6.83 (s, 1H, furanone ring), 7.27 and 7.42 (d, each, 2 × A_2B_2, *p*-substituted phenyl), 7.34 (s, 1H, olefinic H), 7.55 and 7.65 (d, each, 2 × A_2B_2, arylidene ring). MS (m/z): 296 (M^+), 119, 91, 77. IR (KBr, cm^{-1}): 1756, 1601, 827.

3-(3-Nitrobenzylidene)-5-(4-methylphenyl)-2-(3*H*)-furanone (8)

Dark yellow needles, yield: 78%, m.p. 204°C. ^{1}H-NMR ($CDCl_3$, δ ppm): 2.42 (s, 3H, CH_3), 6.86 (s, 1H, furanone ring), 7.27 and 7.62 (d, each, 2 × A_2B_2, *p*-substituted phenyl), 7.37 (s, 1H, olefinic H), 7.62 (m, 1H, H-5, arylidene ring), 7.87 (dd, 1H, H-6, arylidene ring), 8.23 (dd, 1H, H-4, arylidene ring), 8.42 (d, 1H, H-2, arylidene). MS (m/z): 307 (M^+), 119, 91. IR (KBr, cm^{-1}): 1766, 1609, 828.

3-(3,4-Dimethoxybenzylidene)-5-(4-methylphenyl)-2(3*H*)-furanone (9)

Light yellow crystals, yield: 76%, m.p. 140-142°C. ^{1}H-NMR ($CDCl_3$, δ ppm): 2.40 (s, 3H, CH_3), 3.95 (s, 6H, 2 × OCH_3), 6.83 (s, 1H, furanone ring), 6.94 (d, 1H, H-5, arylidene ring), 7.11 (s, 1H, H-2, arylidene ring), 7.24 (m, 3H, merged, H-6 of arylidene ring + 2H, H-3,5, *p*-substituted phenyl), 7.63 (d, 2H, H-2,6, *p*-substituted phenyl), 7.35 (s, 1H, olefinic H). MS (m/z): 322 (M^+), 119, 91. IR (KBr, cm^{-1}): 1767, 1597, 836.

3-(2,6-Dichlorobenzylidene)-5-(4-methylphenyl)-2(3*H*)-furanone (10)

Red needles, Yield: 82%, m.p. 144°C. ^{1}H-NMR ($CDCl_3$, δ ppm): 2.37 (s, 3H, CH_3), 6.78 (s, 1H, furanone ring), 7.41 (s, 1H, olefinic H), 7.57 and 7.82 (d, each, 2 × A_2B_2, *p*-substituted phenyl), 7.62 (m, 3H, H-3,4,5, arylidene ring). MS (m/z): 331 (M^+), 119, 91, 77. IR (KBr, cm^{-1}): 1769, 1608, 829.

General procedure for the synthesis of 3-arylidene-5-(4-bromophenyl)-1-benzyl-2(3*H*)-pyrrolones (11–14)

The synthesis of these compounds involved the following two steps:

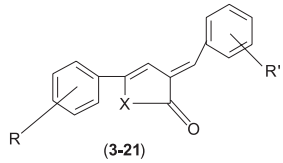
- Synthesis of γ-ketobenzylamide

2(3*H*)-Furanone (3 mmol) and benzylamine (4 mmol) were refluxed in dry benzene for 2 h. On completion of reaction, an excess of benzene was distilled off and a solid mass so obtained was washed with petroleum ether and dried. The compound obtained was used without crystallization.

- Cyclization of γ-ketobenzylamide

γ-Ketobenzylamide (3 mmol) was refluxed in 6 M hydrochloric acid (20 mL) for 1 h. The contents were then cooled and a solid mass so obtained was collected, washed with water and crystallized from methanol to give **11-14.**

Table 1: Antimycobacterial activity of the synthesized compounds (**3-21**)



(**3-21**)

Compd. no.	R	X	R'	IC_{90} (µg/mL)	C_{50} (µg/mL)
3	-Br-	-O-	4-Chloro	>100	>100
4	-Br-	-O-	3-Nitro	>100	>100
5	-Br-	-O-	3,4-Dimethoxy	>100	26.77
6	-Br-	-O-	2,6-Dichloro	>100	>100
7	$-CH_3-$	-O-	4-Chloro	>100	>100
8	$-CH_3-$	-O-	3-Nitro	>100	>100
9	$-CH_3-$	-O-	3,4-Dimethoxy	>100	>100
10	$-CH_3-$	-O-	2,6-Dichloro	>100	>100
11	-Br-	$-NCH_2C_6H_5-$	3-Nitro	>100	>100
12	-Br-	$-NCH_2C_6H_5-$	2,6-Dichloro	>100	>100
13	$-CH_3-$	$-NCH_2C_6H_5-$	3-Nitro	>100	>100
14	$-CH_3-$	$-NCH_2C_6H_5-$	3,4-Dimethoxy	>100	5.86
15	-Br-	-NH-	4-Chloro	>100	>100
16	-Br-	-NH-	3-Nitro	>100	63.40
17	-Br-	-NH-	3,4-Dimethoxy	>100	14.32
18	-Br-	-NH-	2,6-Dichloro	>100	>100
19	$-CH_3-$	-NH-	4-Chloro	**12.59**	11.34
20	$-CH_3-$	-NH-	3,4-Dimethoxy	>100	26.43
21	$-CH_3-$	-NH-	2,6-Dichloro	>100	41.47
Isoniazid	-	-	-	>100	-

3-(3-Nitrobenzylidene)-5-(4-bromophenyl)-1-benzyl-2(3*H*)-pyrrolone (**11**)

Dark brown crystals, yield: 42%, m.p. 154°C. ^{1}H-NMR ($CDCl_3$, δ ppm): 4.85 (s, 2H, CH_2), 6.21 (s, 1H, furanone ring), 6.96 and 7.32 (d, each, 2 × A_2B_2, *p*-substituted phenyl), 7.25 (m, 5H, H-2,3,4,5,6, benzyl), 7.49 (s, 1H, olefinic H), 7.57 (t, 1H, H-5, arylidene), 7.89 (d, 1H, H-6, arylidene), 8.18 (d, 1H, H-4, arylidene), 8.49 (s, 1H, H-2, arylidene). MS (m/z): 461 (M^+) 370, 183, 91, 77. IR (KBr, cm^{-1}):1738, 1615, 798.

3-(2,6-Dichlorobenzylidene)-5-(4-bromophenyl)-1-benzyl-2(3*H*)-pyrrolone (**12**)

Red crystals, yield: 46%, m.p. 200-202°C. ^{1}H-NMR ($CDCl_3$, δ ppm): 4.84 (s, 2H, CH_2), 6.24 (s, 1H, furanone ring), 7.10 (dd, 2H, H-2,6, benzyl), 7.18 and 7.40 (d, each, 2 × A_2B_2, *p*-substituted phenyl), 7.26 (m, 3H, H-3,4,5, benzyl), 7.47 (s, 1H, olefinic H), 7.52 (m, 3H, H-3,4,5, arylidene). MS (m/z): 485 (M^+) 314, 183, 91, 77. IR (KBr, cm^{-1}): 1748, 1598, 814.

3-(3-Nitrobenzylidene)-5-(4-methylphenyl)-1-benzyl-2(3*H*)-pyrrolone (**13**)

Brown crystals, yield: 44%, m.p. 170°C. ^{1}H-NMR ($CDCl_3$, δ ppm): 2.38 (s, 3H, CH_3), 4.85 (s, 2H, CH_2), 6.22 (s, 1H, furanone ring), 6.97 and 7.28 (d, each, 2 × A_2B_2, *p*-substituted phenyl), 7.05 (m, 2H, H-2,6, benzyl), 7.25 (m, 3H, H-3,4,5, benzyl), 7.47 (s, 1H, olefinic H), 7.62 (t, 1H, H-5, arylidene), 7.94 (d, 1H, H-4, arylidene), 8.21 (dd, 1H, H-6, arylidene), 8.55 (t, 1H, H-2, arylidene). MS (m/z): 396 (M^+) 396, 118, 91, 77. IR (KBr, cm^{-1}): 1742, 1606, 808.

Table 2: Physical constants of the title compounds (**3-21**)

Compd. no.	Mol. formula	R_f-value	M. p. (°C)	Mol. weight	Elemental analyses (%)					
					Calculated			Obtained		
					C	H	N	C	H	N
3	$C_{17}H_{10}BrClO_2$	0.80	216	361.61	56.46	2.79	-	56.54	2.80	-
4	$C_{17}H_{10}BrNO_4$	0.78	186-188	372.16	54.86	2.71	3.76	54.70	2.72	3.73
5	$C_{19}H_{15}BrO_4$	0.71	110	387.22	58.93	3.90	-	58.82	3.91	-
6	$C_{17}H_9Cl_2BrO_2$	0.72	176-178	396.06	51.55	2.29	-	51.39	2.28	-
7	$C_{18}H_{13}ClO_2$	0.78	248-250	296.74	72.85	4.42	-	72.71	4.41	-
8	$C_{18}H_{13}NO_4$	0.76	204	307.3	70.35	4.26	4.56	70.23	4.22	4.58
9	$C_{20}H_{18}O_4$	0.78	140-142	322.35	74.52	5.63	-	74.38	5.65	-
10	$C_{18}H_{12}Cl_2O_2$	0.74	144	331.19	65.28	3.65	-	65.12	3.64	-
11	$C_{24}H_{17}BrN_2O_3$	0.72	154	461.31	62.49	3.71	6.07	62.62	3.74	6.05
12	$C_{24}H_{16}BrCl_2NO$	0.73	200-202	485.19	59.41	3.32	2.89	59.58	3.33	2.90
13	$C_{25}H_{20}N_2O_3$	0.74	170	396.44	75.74	5.08	7.07	75.86	5.05	7.08
14	$C_{27}H_{25}NO_3$	0.70	142	411.49	78.81	6.12	3.40	78.94	6.10	3.38
15	$C_{17}H_{11}BrClNO$	0.74	230-232	360.63	56.62	3.07	3.88	56.48	3.04	3.91
16	$C_{17}H_{11}BrN_2O_3$	0.69	210	371.18	55.01	2.99	7.55	55.18	2.98	7.57
17	$C_{19}H_{16}BrNO_3$	0.78	194	386.23	59.08	4.18	3.63	59.16	4.20	3.62
18	$C_{17}H_{10}BrCl_2NO$	0.74	248-250	395.07	51.68	2.55	3.55	51.54	2.51	3.58
19	$C_{18}H_{14}ClNO$	0.69	218	295.76	73.10	4.77	4.74	72.94	4.66	4.75
20	$C_{20}H_{19}NO_3$	0.72	186-188	321.37	74.75	5.96	4.36	74.58	5.92	4.40
21	$C_{18}H_{13}Cl_2NO$	0.68	218-220	330.21	65.47	3.97	4.24	65.62	3.94	4.26

3-(3,4-Dimethoxybenzylidene)-5-(4-methylphenyl)-1-benzyl-2(3*H*)-pyrrolone (14)

Dark yellow flakes, yield: 52%, m.p. 142°C. ^{1}H-NMR ($CDCl_3$, δ ppm): 2.37 (s, 3H, CH_3), 3.91 (s, 6H, 2 × OCH_3), 4.84 (s, 2H, CH_2), 6.18 (s, 1H, furanone ring), 6.90 (d, 1H, H-5, arylidene), 7.07 (d, 1H, H-6, arylidene), 7.09 (s, 1H, H-2, arylidene), 7.24 (m, merged, *p*-substituted phenyl), 7.24 (m, merged, benzyl), 7.44 (s, 1H, olefinic H). MS (m/z): 411 (M^+) 320, 118, 91, 77. IR (KBr, cm^{-1}): 1746, 1612, 810.

General procedure for the synthesis of 3-arylidene-5-(substituted phenyl)-2(3*H*)-pyrrolone (15–21)

Dry ammonia gas was passed over the anhydrous ethanolic solution of 2(3*H*)-furanones (3 mmol) for 1 h at room temperature, ethanol was distilled off under reduced pressure and the solid mass so obtained was crystallized from methanol/acetone to give **15**–**21**.

3-(4-Chlorobenzylidene)-5-(4-bromophenyl)-2-(3*H*)-pyrrolone (15)

Brown crystals, yield: 72%, m.p. 230-232°C. ^{1}H-NMR ($CDCl_3$, δ ppm): 6.18 (s, 1H, pyrrolone ring), 7.19 (s, 1H, olefinic H), 7.38 and 7.46 (d, each, 2 × A_2B_2, arylidene ring), 7.48 and 7.67 (d, each, 2 × A_2B_2, *p*-substituted phenyl), 9.72 (s, 1H, NH). MS (m/z): 360 (M^+), 183, 156, 77. IR (KBr, cm^{-1}): 3384, 1706, 1616, 822.

3-(3-Nitrobenzylidene)-5-(4-bromophenyl)-2-(3*H*)-pyrrolone (16)

Orange needles, yield: 42%, m.p. 210şC ^{1}H-NMR ($CDCl_3$, δ ppm): 6.12 (s, 1H, pyrrolone ring), 7.25 (s, 1H, olefinic H), 7.62 and 7.81 (d, each, 2 × A_2B_2, *p*-substituted phenyl), 7.56 (m, 2H, H-5,6, arylidene ring), 7.81 (m, 1H, H-2, arylidene ring), 8.14 (dd, 1H, H-4, arylidene ring), 8.91 (s, 1H, NH). MS (m/z): 371 (M^+), 183, 156. IR (KBr, cm^{-1}): 3378, 1718, 1606, 824.

3-(3,4-Dimethoxybenzylidene)-5-(4-bromophenyl)-2(3*H*)-pyrrolone (17)

Yellowish orange needles, yield: 68%, m.p. 194°C. ^{1}H-NMR ($CDCl_3$, δ ppm): 3.91 (s, 6H, 2 × OCH_3), 6.16 (s, 1H, pyrrolone ring), 6.91 (d, 1H, H-5, arylidene ring), 7.10 (s, 1H, H-2, arylidene ring), 7.21 (d, 1H, H-6, arylidene ring), 7.31 (s, 1H, olefinic H), 7.48 and 7.79 (d, each, 2 × A_2B_2, *p*-substituted phenyl), 9.41 (s, 1H, NH). MS (m/z): 386 (M^+), 183, 156. IR (KBr, cm^{-1}): 3392, 1715, 1611, 828.

3-(2,6-Dichlorobenzylidene)-5-(4-bromophenyl)-2(3*H*)-pyrrolone (18)

Brown crystals, Yield: 64%, m.p. 248-250°C. ^{1}H-NMR ($CDCl_3$, δ ppm): 6.10 (s, 1H, pyrrolone ring), 7.09 (s, 1H, olefinic H), 7.43 (m, 3H, H-3,4,5, arylidene ring), 7.49 and 7.61 (d, each, 2 × A_2B_2, *p*-substituted phenyl), 10.03 (s, 1H, NH). MS (m/z): 395 (M^+), 183, 156. IR (KBr, cm^{-1}): 3454, 1676, 1596, 809.

3-(4-Chlorobenzylidene)-5-(4-methylphenyl)-2(3*H*)-pyrrolone (19)

Red crystals, Yield: 68%, m.p. 218°C. ^{1}H-NMR ($CDCl_3$, δ ppm): 2.38 (s, 3H, CH_3), 6.52 (s, 1H, pyrrolone ring), 6.92 and 7.32 (d, each, 2 × A_2B_2, *p*-substituted phenyl), 7.18 and 7.45 (d, each, 2 × A_2B_2, arylidene ring), 7.25 (s, 1H, olefinic H), 8.96 (s, 1H, NH). MS (m/z): 295 (M^+), 118, 91, 77. IR (KBr, cm^{-1}): 3454, 1686, 1596, 812.

3-(3,4-Dimethoxybenzylidene)-5-(4-methylphenyl)-2(3*H*)-pyrrolone (20)

Yellowish orange crystals, yield: 72%, m.p. 186-188°C. ^{1}H-NMR ($CDCl_3$, δ ppm): 2.49 (s, 3H, CH_3), 3.96 (s, 6H, 2 × OCH_3), 6.48 (s, 1H, pyrrolone ring), 6.51 (d, 1H, H-5, arylidene ring), 6.92 and 7.53 (d, each, 2 × A_2B_2, *p*-substituted phenyl), 7.11 (s, 1H, H-2, arylidene ring), 7.28 (d, 1H, H-6, arylidene ring), 7.37 (s, 1H, olefinic H), 8.78 (s, 1H, NH). MS (m/z): 321 (M^+), 118, 91. IR (KBr, cm^{-1}): 3394, 1691, 1586, 817.

3-(2,6-Dichlorobenzylidene)-5-(4-methylphenyl)-2(3*H*)-pyrrolone (21)

Dark red crystals, Yield: 69%, m.p. 218-220°C. ^{1}H-NMR ($CDCl_3$, δ ppm): 2.41 (s, 3H, CH_3), 6.52 (s, 1H, pyrrolone ring), 7.17 and 7.48 (d, each, 2 × A_2B_2, *p*-substituted phenyl), 7.47 (m, 3H, H-3,4,5, arylidene ring), 7.41 (s, 1H, olefinic H), 9.11 (s, 1H, NH). MS (m/z): 330 (M^+), 118, 91, 77. IR (KBr, cm^{-1}): 3424, 1686, 1601, 808.

Antimycobacterial evaluation

The *in vitro* antimycobacterial activity was assayed by Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF), antitubercular drug discovery program. The initial screen is conducted against *Mycobacterium tuberculosis* H37Rv (ATCC 27294) in BACTEC 12B medium using the Microplate Alamar Blue Assay (MABA) (13). Compounds are tested in ten 2-fold dilutions, typically from 100 µg/mL to 0.19 µg/mL. Isoniazid was used as a reference drug.

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 1×10^6, 2.5×10^6, and 3.25×10^5 CFU/mL in plate wells for $H_{37}Rv$, $H_{37}Ra$, and *M. avium*, respectively. 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 2.0×10^5 and 5×10^4 CFU/mL for $H_{37}Rv$ and $H_{37}Ra$, respectively. 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 10× alamar Blue solution (Alamar Biosciences/Accumed, Westlake, Ohio) and 12.5 µL of 20% Tween 80 were added to one B well and one M well, and plates were re-incubated 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 MICs were 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 calculated using the following formula:

Percent inhibition = 1-(test well FU/mean FU of triplicate B wells) × 100.

The IC_{90} is defined as the concentration effecting a reduction in fluorescence of 90% relative to controls. This value is determined from the dose-response curve using a curve-fitting program.

RESULTS AND DISCUSSION

Chemistry

Overall nineteen compounds **3-21** were synthesized as outlined in Scheme 1. Synthesis of various 2(3*H*)-furanones **3-10** was brought about by single step reaction using modified Perkin reaction conditions. Biologically active 3-arylidene-5-(4-bromophenyl)-2(3*H*)-furanones **3-6** or 3-arylidene-5-(4-methylphenyl)-2(3*H*)-furanones **7-10** were prepared by condensing different aromatic aldehydes with 3-(4-bromobenzoyl)propionic acid **1** or 3-(4-methylbenzoyl)propionic acid **2** in the presence of triethylamine and acetic anhydride under anhydrous conditions.

Some of the synthesized 2(3*H*)-furanones (**3-10**) were further converted to nitrogen derivatives i.e. 1-benzylpyrrolones (**11-14**). The 3-arylidene-5-(4-bromophenyl)-1-benzyl-2(3*H*)-pyrrolones (**11-12**) and 3-arylidene-5-(4-methylphenyl)-1-benzyl-2(3*H*)-pyrrolones (**13-14**) were synthesized by reacting appropriate 2(3*H*)-furanone with benzylamine in dry benzene to give γ-ketobenzylamide, which was then cyclized in 6 M HCl to give the corresponding 1-benzylpyrrolone.

The 3-arylidene-5-(4-bromophenyl)-2(3*H*)-furanones (**3-6**) as well as 3-arylidene-5-(4-methylphenyl)-2(3*H*)-furanones (**7**, **9**, **10**) were also converted to 3-arylidene-5-(4-bromophenyl)-2(3*H*)-pyrrolones (**15-18**) and 3-arylidene-5-(4-methylphenyl)-2(3*H*)-pyrrolones (**19-21**), by reacting them with ammonia in absolute ethanol. Two configurations (*E* and *Z*) are possible for the proposed structure of furanones and pyrrolones. Calculations of δ-values using incremental parameters for the hydrogen (semicyclic double bond) suggest (*E*)-configuration. This is in accordance with the previous reported results (4, 8).

In the ^{1}H-NMR spectral data all the compounds showed two singlets of one proton each around δ 6.5 and δ 7.4 which could be assigned to the ring β-H and the olefinic hydrogen of the arylidene substituent. The mass spectral data of 3-arylidene-5-(4-substituted-phenyl)-2(3*H*)-furanones showed M^+ peak in reasonable intensities. The fragmentation pattern indicates major fragment to be $Br/CH_3–C_6H_4–C=O^+$ arising from the heterocyclic oxygen and γ-carbon with its substituent. Subsequently, it loses CO to give $Br/CH_3–C_6H_4^+$. There appeared a peak at m/z 77 that corresponds to $C_6H_5^+$. Occasionally the aryl ring of the arylidene moiety also appeared as Ar^+. In the case of pyrrolones, the major fragmentation is through $Br/CH_3–C_6H_4–C=N^+H$, which is followed by loss of HCN to give $Br/CH_3–C_6H_4^+$. In case of aryl groups having chloro substituent(s), the molecular ion peak or their fragments having halogen(s) appeared as cluster of peaks. In case of 1-benzylpyrrolones, a loss of 91 mass units corresponding to benzyl moiety from the molecular ion is observed along with peaks at m/z 91, 77. Other pathway is via $R\text{-}C=N^+H$ arising from C-2 and its substituent, which appears to be novel. This also loses HCN to give $R\text{-}C_6H_4^+$. In case of aryl group having chlorine as a substituent(s), the molecular ion or other related ions produced the appropriate isotopic abundances. The analytical data are mentioned in Table 2.

Antimycobacterial activity

The synthesized compounds (**3-21**) were tested for their antimycobacterial activity by determining their IC_{90} and IC_{50} value against *Mycobacterium tuberculosis* $H_{37}Rv$ (ATCC 27294) in BACTEC 12B medium using Microplate Alamar Blue Assay (13). Among the nineteen synthesized compounds one compound, 3-(4-chlorobenzylidene)-5-(4-methylphenyl)-2(3*H*)-pyrrolone (**19**) was found to be the most promising with IC_{90} and IC_{50} values 12.59 and 11.34, respectively (Table 1). In addition, 3-(3,4-dimethoxybenzylidene)-5-(4-bromophenyl)-2(3*H*)-furanone (**5**), 3-(3,4-dimethoxybenzylidene)-5-(4-methylphenyl)-1-benzyl-2(3*H*)-pyrrolone (**14**), 3-(3-nitrobenzylidene)-5-(4-bromophenyl)-2(3*H*)-pyrrolone (**16**), 3-(3,4-dimethoxybenzylidene)-5-(4-bromophenyl)-2(3*H*)-pyrrolone (**17**), 3-(3,4-dimethoxybenzylidene)-5-(4-methylphenyl)-2(3*H*)-pyrrolone (**20**) and 3-(2,6-dichlorobenzylidene)-5-(4-methylphenyl)-2(3*H*)-pyrrolone (**21**) were found to be active with IC_{50} values 26.77, 5.86, 63.40, 14.32, 26.43 and 41.47, respectively. Among them, compounds with dimethoxy substitution at 3rd and 4th position of arylidene moiety were found to have better antimycobacterial activity as compared to those having nitro, chloro, and dichloro functions. How-

ever, activity increases on replacement of oxygen of furanone ring with nitrogen atom (pyrrolones) while substitution with benzylamine moiety (1-benzylpyrrolones) markedly decreases activity.

Among the synthesized derivatives, compound (**19**) showed a promising activity *in vitro*. It is conceivable that the derivatives showing antimycobacterial activity can be further modified to exhibit better potency than the standard drugs.

CONCLUSION

The present study reveals the antimycobacterial potential of 2(3*H*)-furanones and their nitrogen analogues i.e. 2(3*H*)-pyrrolones. Among the new derivatives, 3-(4-chlorobenzylidene)-5-(4-methylphenyl)-2(3*H*)-pyrrolone (compound **19**) emerged as a lead compound. The results showed that introduction of nitrogen in place of oxygen atom (pyrrolones) in the furanone ring resulted in enhanced antimycobacterial activity. Further studies to acquire more information about quantitative structure-activity relationship (QSAR) are in progress in our laboratory. Some of the synthesized furanones and pyrrolones have shown higher degree of activity and obviously may have future commitment.

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