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Chalcones and Flavonoids as Anti-Tuberculosis Agents

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Abstract—A series of flavonoids, chalcones and chalcone-like compounds were evaluated for inhibitory activity against *Mycobacterium tuberculosis H37Rv*. Among them, eight compounds exhibited >90% inhibition on the growth of the bacteria at a concentration of 12.5 μg/mL. Chalcones 1-(2-hydroxyphenyl)-3-(3-chlorophenyl)-2-propen-1-one (**22**) and 1-(2-hydroxyphenyl)-3-(3-iodophenyl)-2-propen-1-one (**37**) demonstrated 90 and 92% inhibition, respectively. Chalcone-like compounds (heterocyclic ring-substituted 2-propen-1-one) 1-(4-fluorophenyl)-3-(pyridin-3-yl)-2-propen-1-one (**48**), 1-(3-hydroxyphenyl)-3-(phenanthren-9-yl)-2-propen-1-one (**49**), 1-(pyridin-3-yl)-3-(phenanthen-9-yl)-2-propen-1-one (**50**) and 1-(furan-2-yl)-3-phenyl-2-propen-1-one (**51**) exhibited 98, 97, 96 and 96% inhibition, respectively. The actual minimum inhibitory concentrations (MIC), defined as the lowest concentration inhibiting 99% of the inoculum, for **22**, **37**, **48**, **49**, **50** and **51** were 20.3, 31.5, 48.3, >35.7, 6.8 and 19.2, respectively. A hydrophobic substituent on one aromatic ring, and a hydrogen-bonding group on the other aromatic ring resulted in increased anti-TB activity of the chalcones and chalcone-like compounds. Flavones and flavanones are more geometrically constrained than the corresponding chalcone analogues. The decreased activity of the flavones with respect to the chalcones may be due to the confinement of the terminal aromatic rings to the same plane.

Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (*Mtb*), a facultative intracellular bacillus, is the world's number one killer among infectious diseases and the leading cause of death among women of reproductive age.[1] Even though improved methods of prevention, detection, diagnosis and treatment have greatly reduced the number of people who contract the disease and die from it, the emergence of multidrug-resistant (MDR) strains and the global human immunodeficiency virus (HIV) pandemic have amplified the incidence of TB.[2–5]

Because of this, there is an urgent need for anti-TB drugs with improved properties such as enhanced activity against MDR strains, reduced toxicity, shortened duration of therapy, rapid mycobactericidal mechanism of action and the ability to penetrate host cells and exert anti-mycobacterial effects in the intracellular environment.

In our efforts to discover novel anti-tuberculosis agents, we screened a series of chalcones and flavonoids. In a previous paper,[6] we have reported the anti-tuberculosis (anti-TB) activities of biflavonoids. In this more recent study, we present the anti-TB screening results with a variety of chalcones, chalcone-like compounds, flavones and flavanones.

Results

Synthesis

Chalcones (1,3-diaryl-2-propen-1-ones) and chalcone-like compounds (1,3-heteroaromatic ring-substituted 2-propen-1-ones). Chalcones and chalcone-like compounds were prepared by base-catalyzed condensation of appropriately substituted ketones with substituted benzaldehydes or heterocylic aldehydes (Scheme 1). To a mixture of the substituted acetophenone and substituted benzaldehyde in alcohol was added a 60% solution of potassium hydroxide dropwise with stirring. The reaction mixture was kept at 0 °C for 2 days, then diluted with water and acidified with acetic acid. The precipitated chalcone was collected and recrystallized from alcohol to yield pure chalcone.[7]

Flavones. Flavones were synthesized by treating the corresponding chalcones with selenium dioxide in amyl

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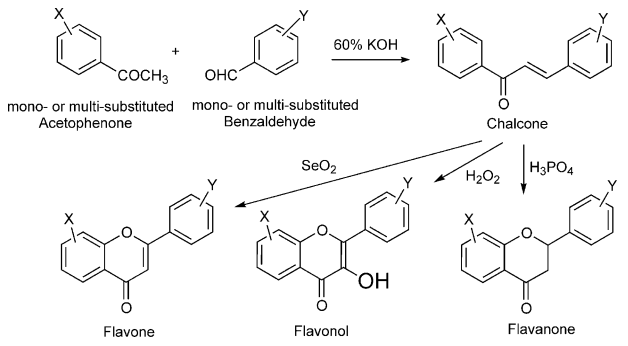


Scheme 1. Preparation of chalcones, chalcone-like compounds, flavones and flavanones.

alcohol (Scheme 1). Thus, 2′-, 3′- and 4′-monohalogenoflavones were prepared as usual by condensation of hydroxyacetophenone with *o*-, *m*- and *p*-halogenobenzaldehydes to provide the chalcones. This was followed by cyclization of the chalcones with selenium dioxide in amyl alcohol.[8–13] 6-Fluoro-, 6-chloro- and 6-bromoflavones and related compounds were prepared from 2-hydroxy-5-halogenoacetophenones.[13]

Flavonols (3-hydroxyflavones). Flavonols were prepared by treating the corresponding chalcones with a 16% solution of aqueous sodium hydroxide and 15% hydrogen peroxide solution (v/v 1:1) (Scheme 1). The 6-halogenoflavonols were prepared in good yield by cyclization of the corresponding chalcones in cold alkaline hydrogen peroxide.[14,15]

Flavanones. Flavanones were prepared by refluxing the corresponding chalcones with phosphoric acid in alcohol for 2–3 days (Scheme 1). Generally, 2-hydroxy-5-halogenoacetophenones condensed smoothly with benzaldehyde or *p*-anisaldehyde in the presence of alcoholic alkali, giving chalcones, which were cyclized in phosphoric acid to obtain 6-halogenoflavanones.[16]

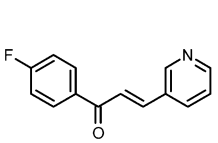
Anti-tuberculosis activity

Forty-seven chalcones (**1**–**47**), 21 chalcone-like compounds (**48**–**69**), 54 flavones (**70**–**124**) and 17 flavanones (**125**–**142**) were screened against *Mtb H37Rv* at a drug concentration of 12.5 μg/mL. Among the chalcones and chalcone-like compounds screened, two chalcones (**22** and **23**) and four chalcone-like compounds (**48**–**51**) demonstrated >90% inhibitory activity. Twenty-two compounds exhibited activity between 50 and 89% (four, 80–89%; five, 70–79%; eight, 60–69%; five, 50–59%) and 25 compounds displayed activity less than 50% (five, 40–49%; three, 30–39%; five, 20–29%; six, 10–19%; six, 1–9%). The remaining 16 compounds were inactive. Flavones, flavonols and flavanones were inactive or weakly to moderately active.

Chalcones (1,3-Diaryl-2-propen-1-ones). The results of anti-TB screening of chalcones are displayed in Table 1. Chalcones with a 2-hydroxyl group in the B ring and a 3-iodo- or 3-chloro-group in the A ring, **22** and **37**, respectively, demonstrated the strongest activity among this series of compounds, with 90 and 92% inhibition, respectively, against *Mtb* at a drug concentration of 12.5 μg/mL. Chalcones without halogen substitution in the molecule exhibited less activity compared to those with a halogen substitution.

The activity of 2′-hydroxy-chalcone **5** (61% inhibition) was enhanced by introducing a chloro- or a methoxy-group at the 4′-position of the B-ring, such as compounds **23** (89%) and **1** (78%). A bromo-, or an iodo-substituent at the 4′-position of ring B led to decreased activity [e.g., **32** (57%) and **42** (21%)]. The bromo-substituent at the 3′-position of the B-ring of 2′-hydroxy-chalcone (**5**) slightly increased the activity [**29** (79%) vs **5** (61%)]. The effect of various substituents at the 5′-position of the B-ring on anti-TB activity was as follows: phenyl (**3**, 68%) = Br (**31**, 68%) ~ Cl (**24**, 67%) > H (**5**, 61%) > I (**39**, 51%) > NH_2 (**12**, 6%). The anti-TB activity was also affected by a halogen-substituent on the B-ring of 2′-hydroxychalcone. A rank of the inhibitory activities of compounds with a halogen substituent on the B ring is outlined as follows: 4′-Cl (**23**, 89%) > 5′-Cl (**24**, 67%); 3′-Br (**29**, 79%) > 5′-Br (**31**, 68%) > 4′-Br (**32**, 57%); and 5′-I (**39**, 52%) > 4′-I (**42,** 21%) > 3′-I (**44**, 0%). Introducing an additional substituent on either the A- or B-ring of the above 2′-hydroxychalcones resulted in a significant decrease or loss of activity. This effect was shown in compounds **23** (4′-Cl–, 89%) versus **26** (4′-Cl-4-OCH_3–, 57%); **1** (4′-OCH_3–, 78%) versus **7** (4,4′,6-$(OCH_3)_3$–, 40%); **31** (5′-Br–, 68%) versus **34** (5′-Br-4-OCH_3–, 23%) and **36** (5′-Br-2-NH_2–, 8%); **24** (5′-Cl-, 67%) versus **27** (5′-Cl-4-OCH_3–, 20%); **32** (4-Br-, 57%) versus **33** (4-Br-4-OCH_3–, 25%) and **35** (4-Br-2-NH_2–, 12%), **37** (3-I–, 92%) versus **40** (3-I-4′-OCH_3, 47%) and **39** (5′-I–, 51%) versus **45** (5-I-4-OCH_3–, 0%).

The substitution of a halogen group on the A-ring of 2′-hydroxychalcone led to an increase in anti-TB activity. Compounds with a halogen substituent at the 3-position demonstrated stronger anti-TB activity than those with a halogen substituent at the 2- or 4-position. Compounds with a 3-, 2- and 4-iodo substituent exhibited 92% (**37**, 3-I–), 41% (**41**, 2-I–) and 21% (**43**, 4-I–) inhibition against *Mtb*, respectively. Compound **28**, 2-bromo-2′-hydroxychalcone, demonstrated an inhibitory activity of 83% while compound **30** with a 4-bromo substituent exhibited an anti-TB activity of 70%. The activities of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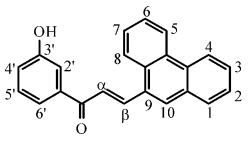
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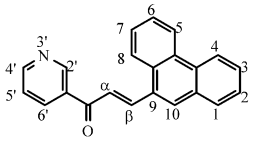
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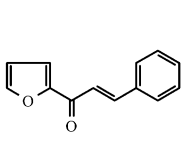
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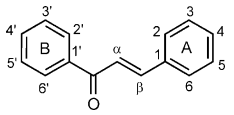
Table 1. Anti-tuberculosis activity of chalcones



Compd	B-ring					A-ring				Activity % inhibition at 12.5 mg/mL	MIC (μg/mL)
	2′–	3′–	4′–	5′–	6′–	2–	3–	4–	5–		
RMP											0.125–0.25
1	OH		OCH_3							78	—[a]
2	OH				OCH_3			OCH_3		75	—
3	OH			Phenyl						68	—
4			NO_2					OCH_3		62	—
5	OH									61	—
6		OH					OCH_2	O		53	—
7	OH		OCH_3		OCH_3			OCH_3		40	—
8	OH					OCH_3				39	—
9	OH		OCH_3					OCH_3		32	—
10	OH						OH			18	—
11	OH						NH_2			11	—
12	OH			NH_2						6	—
13			NH_2							5	—
14	OH		EtO		EtO					0	—
15	OH		OCH_3		OH		OCH_3	OCH_3	OCH_3	0	—
16	OH			COOH				OCH_3		0	—
17	OH						$NHCOCH_3$			0	—
18	F							OCH_3		82	—
19	OH				F			OCH_3		66	—
20	OH		F					OCH_3		63	—
21	OH	F						OCH_3		45	—
22	OH						Cl			90	20.3
23	OH		Cl							89	—
24	OH			Cl						67	—
25	OH							Cl		67	—
26	OH		Cl					OCH_3		57	—
27	OH			Cl				OCH_3		20	—
28	OH					Br				83	—
29	OH	Br								79	—
30	OH							Br		70	—
31	OH			Br						68	—
32	OH		Br							57	—
33	OH		Br					OCH_3		25	—
34	OH			Br				OCH_3		23	—
35	OH		Br			NH_2				12	—
36	OH			Br		NH_2				8	—
37	OH						I			92	31.5
38		NH_2				I				88	—
39	OH			I						51	—
40	OH		OCH_3				I			47	—
41	OH					I				41	—
42	OH		I							21	—
43	OH							I		21	—
44	OH	I								0	—
45	OH			I				OCH_3		0	—
46	OH	I						OCH_3		0	—
47	OH			COOH			I			0	—

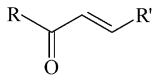
[a]12.5 and not determined.

compounds **22** (3-chloro-2′-hydroxychalcone) and **25** (4-chloro-2′-hydroxychalcone) are 90 and 67%, respectively. Since there was no example of fluoro compounds tested, it was not possible to predict which halogen would contribute most to the activity.

Introduction of an additional substituent, such as a methoxy, bromo or carboxyl group on the B-ring of 2′-hydroxy-3-iodochalcone (**37**) led to a dramatic decrease or a complete loss of activity. This was concluded by comparing the activities of compounds **40** (47%, 4′-methoxy-2-hydroxy-3-iodochalcone) and **47** (0%, 5′-carboxyl-2′-hydroxy-3-iodochalcone) to that of the parent compound **37** (92%, 2′-hydroxy-3-iodochalcone).

Chalcone-like compounds (1,3-heteroaromatic ring-substituted 2-propen-1-ones). Chlacone-like compounds demonstrated the most significant anti-TB activity among all the compounds evaluated. As presented in Table 2, compounds **48**, **49**, **50**, and **51** inhibited 98, 97,

Table 2. Anti-tuberculosis activity of chalcone-like compounds



Compd	R	R′	Activity % inhibition at 12.5 μg/mL	MIC (μ/mL)
RMP				0.125–0.25
48	4-Fuorophenyl-	Pyridin-3-yl-	98	48.3
49	3-Hydroxyphenyl-	Phenanthren-9-yl-	97	31.5
50	Pyridin-3-yl-	Phenanthren-9-yl-	96	6.8
51	Furan-2-yl-	3-Phenyl-	96	19.2
52	Phenanthren-2-yl-	2-Aminopyridino-3-yl-	74	—[a]
53	3-Fluorenyl-	2-Aminopyridino-3-yl-	53	—
54	Pyridin-2-yl-	Pyridin-2-yl-	42	—
55	Naphthalen-1-yl-	Phenyl-	37	—
56	Pyridin-2-yl-	4-Dimethylaminophenyl-	16	—
57	4-Bromo-2-hydroxyphenyl-	Furan-2-yl-	17	—
58	Pyridin-4-yl-	Indol-2-yl-	12	—
59	2-Hydroxy-4-methoxyphenyl-	Furan-2-yl-	3	—
60	4-Aminophenyl-	2-Aminopyridin-3-yl-	7	—
61	Pyridin-4-yl-	4-Dimethylaminophenyl-	1	—
62	2-Hydroxy-5-methoxyphenyl-	Furan-2-yl-	0	—
63	4-Methoxyphenyl-	Pyridin-4-yl-	0	—
64	4-Methoxyphenyl-	Pyridin-3-yl-	0	—
65	2-Hydroxy-5-chlorophenyl-	2-Aminopyridin-3-yl-	0	—
66	4-Aminophenyl-	2-Aminopyridin-3-yl-	0	—
67	3-Hydroxynaphthalen-2-yl-	2-Aminopyridin-3-yl-	0	—
68	Furan-2-yl-	Pyridin-4-yl-	0	—
69	Pyridin-2-yl-	4-Methoxyphenyl-	0	—

[a] > 12.5 and not determined.

96, and 96% growth, respectively, of *Mtb H37Rv* at a drug concentration of 12.5 μg/mL. The common structural feature is that all four compounds have a heteroaromatic ring or a phenyl ring with a hydrophilic group substituent on one side of the molecule, and an aromatic ring, such as phenyl or phenanthrenyl, with or without a hydrophobic substitution on the other side.

Additional hydrophilic substituents, such as methoxyl, hydroxyl and amino groups resulted in a dramatic decrease or complete loss of activity. From the above results, it was concluded that the active compounds may require a lipophilic group on one side and a hydrophilic group on the other side of 2-propen-1-one.

Flavones. The anti-TB activities of the flavones studied are presented in Table 3. All flavones tested, including carboxylated, halogenated, hydroxylated and methoxylated flavones were only moderately to weakly active or inactive, while halogenated flavones or halogenated flavonols (3-hydroxyflavones) demonstrated moderate activity. Flavones bearing an 8-bromo or 8-chloro substituent displayed 66% (**72**) and 62% (**73**) growth inhibition at a dose of 12.5 μg/mL against *Mtb*. Flavones with a halogen substitution at the 3-position (**120**), a 6-, 7- or 8-halogen substituent on ring A (**95**, **98**, **99**, **105**, **107**, **115**, **116**, and **117**) and 2′-, 3′- or 4′-halogen substituent on ring B (**77**, **90**, **92**, **93**, **94**, **104**, and **114**) were weakly active or inactive. Flavonol (3-hydroxyflavone) (**85**) exhibited a low potency of 38% inhibition. The presence of a 4′-methoxyl group on flavonol (**81**) led to a slight, 10% increase in inhibitory activity. Further modification, including a 6-chloro or 7-fluoro group on 4′-methoxyflavonol (**80** and **78**) did not affect the activity. However, the substitution of a 7- or 8-iodo group, a 6-fluoro or 6-bromo group or a 6-carboxy group on 4′-methoxyflavonol resulted in a significant decrease or a complete loss of activity.

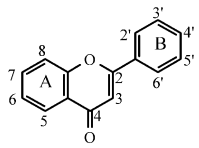
Flavanones. Eighteen flavanones were evaluated for anti-TB activity. The results are listed in Table 4. 5-Methoxy-8-bromoflavanone (**125**) demonstrated the most significant activity among these flavanones, with 87% inhibition against *Mtb*. The substitution of a bromo group on the B-ring demonstrated higher activity than that of a halogen-substituted A-ring [3-bromo-flavanone (**126**), 73%, vs 7-bromo-flavanone (**128**), 53%]. Due to the small sample size and the lack of diversity, it is difficult to provide a structure–activity relationship interpretation.

Discussion

SAR analysis and pharmacophore mapping analysis

Two chalcone compounds (**22** and **37**) and four chalcone-like compounds (**48**, **49**, **50**, and **51**) exhibited greater than 90% inhibition of the TB bacteria. The common structural feature of these six compounds is that they have two aromatic rings, one ring substituted with a heteroatom, and the other with or without hydrophobic substitutions. The presence of additional heteroatoms on the aromatic ring, such as methoxyl, hydroxyl and amino groups, results in decreased activity. With the exception of **48**, these compounds have a

Table 3. Anti-tuberculosis activity of flavones

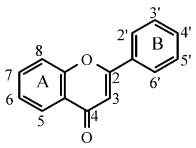


Compd	3	5	6	7	8	2′	3′	4′	Activity % inhibition at 12.5 μg/mL
70					Br				66
71					Cl				62
72	OH		I						64
73	OH						Br		60
74	OH			Cl					58
75	OH		Br						58
76	OH			Cl					52
77								I	51
78	OH			F				OCH_3	50
79	OAc			F				OCH_3	50
80	OH		Cl					OCH_3	48
81	OH							OCH_3	48
82	OCH_3		Br						44
83	OH				F				43
84	OH			I				OCH_3	43
85	OH								38
86								OCH_3	29
87	OH		F					OCH_3	29
88							NO_2	Cl	28
89	Br							OCH_3	28
90								Br	26
91	OH		Br					OCH_3	24
92						Br			23
93						I			22
94							I		22
95				I					20
96	Br			OCH_3				Cl	19
97								OH	18
98				F					15
99				Br					15
100			I					OCH_3	15
101	Br								13
102		OCH_3		OCH_3				OCH_3	12
103	Benzoyl			Benzoyl					12
104								Cl	7
105			F						7
106	OH				I			OCH_3	6
107					I				5
108			COOH					OCH_3	2
109						OH			1
110				OCH_3					0
111				F				OCH_3	0
112				OCH_3				OCH_3	0
113			F					OCH_3	0
114							Cl		0
115				Cl					0
116			Br						0
117			I						0
118		OCH_3		OCH_3	I			OCH_3	0
119		OCH_3		OCH_3			I	OCH_3	0
120	I								0
121				I				OCH_3	0
122	OH		COOH					OCH_3	0
123	Br	OCH_3		OCH_3				OCH_3	0
124		OCH_3		OCH_3				OH	0

hydrogen-bonding substituent on the B ring, while the A ring maintains its hydrophobicity. Although **48** has a heteroatom on the A ring with a hydrophobic substituent on the B ring, the A ring and B ring of **48** can be superimposed with the B ring and A ring, respectively, of the remaining compounds. An example of this superimposition model is shown in Figure 1 with compounds **48** and **51**.

Flavones and flavanones can be viewed as geometrically constrained chalcone analogues. These structural constraints may be the cause of the decreased activity with

Table 4. Anti-tuberculosis activity of flavanones



Compd	3	5	6	7	8	3′	4′	5′	Activity% inhibition at 12.5 μg/mL
125		OCH_3			Br				87
126						Br			73
127	OH					Cl			63
128				Br					53
129				OCH_3			Cl		48
130			Cl						30
131			I						27
132	Br_2	OCH_3		OCH_3		OCH_3	OCH_3	OCH_3	16
133				I					9
134			Br						8
135				F					7
136		OCH_3		OCH_3		OCH_3	OCH_3	OCH_3	0
137			COOH				OCH_3		0
138			COOH						0
139				OCH_3		NO_2			0
140	Br_2								0
141				I			OCH_3		0
142	Br_2								0

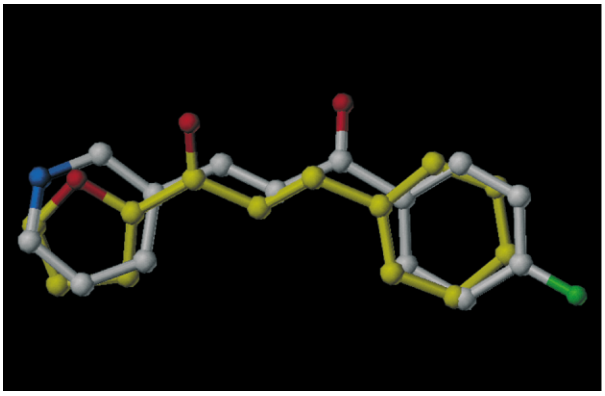


Figure 1. Superimposition of compounds **48** and **51**.

respect to their chalcone counterparts. Compared to flavanones, flavones are less active, and are structurally more constrained, with the two terminal aromatic rings in the same plane. The activity relationship of chalcones and their corresponding flavones is illustrated by the following examples: when chalcone **41** was converted to flavone **93**, the activity decreased from 41 to 22%. Similar activity relationships for chalcones and flavones were observed between other compounds including **41** (41%) to **93** (22%); **28** (83%) to **92** (23%); **30** (70%) to **90** (26%); **22** (90%) to **114** (0%) and **25** (67%) to **104** (7%). The exception to this trend is the activity relationship between chalcone **43** (21%) and flavone **77** (51%). However, there is no corresponding flavanone compound to determine the activity relationship with the active chalcone. It is worth mentioning that the important features in the active chalcone and the chalcone-like compounds described above do not exist in any of the flavone or flavanone compounds currently tested.

Conclusion

Two chalcones (**22** and **37**) and four chalcone-like compounds (**48**, **49**, **50**, and **51**) exhibited greater than 90% inhibitory activity against *Mtb*. The common structural feature for these six compounds is the two aromatic rings, one ring substituted with a heteroatom, and the other with or without hydrophobic substitutions. The presence of additional hydrophilic substituents, such as methoxyl, hydroxyl and amino groups, results in decreased activity. With the exception of **48**, all compounds have a hydrophobic A ring and a hydrogen-bonding substituent on the B ring. Structural superimposition analysis indicated that compound **48** could be inversely superimposed to the remaining five compounds.

Experimental

General

Melting points were determined in open glass capillary tubes and are uncorrected. ^1H NMR and ^{13}C NMR spectra were recorded on a Varian XL300 NMR spectrometer in $CDCl_3$, DMSO-d_6 or acetone-d_6 as specified, using TMS as an internal standard. Chemical shifts are expressed in parts per million (δ, ppm). IR spectra were recorded using a Perkin-Elmer 1000 FT-IR spectrometer. Mass spectral data were recorded using a Finnegan LCQ mass spectrometer. Analytical thin-layer chromatography (TLC) was carried out on precoated plates (Silica gel F_{254} from EM Science). Column chromatography was performed with silica gel 60 (70–230 mesh from EM Science). The structure and purity of compounds were confirmed by TLC profiles as well as IR, NMR and MS spectra.

3-Chloro-2′-hydroxychalcone (22). To a solution of 2-hydroxyacetophenone (1.5 g, 11 m mol) and 3-chlorobenzaldehyde (1.32 g, 9 mmol) in EtOH (10 mL) was added a 60% of KOH (10 g) solution dropwise at 0 °C. The reaction was stirred at 0 °C for 1 day. Cool water was added and the reaction mixture was neutralized with cold acetic acid. The yellow precipitate was collected, washed with water and recrystallized from EtOH to yield yellow needles (1.8 g, yield 76%), mp 108–108.5 °C, APCIMS m/z 257.3 $[M-H]^+$ (relative intensity 100%), 259.2 $[M-H+2]^+$ (isotope) (58%); IR (KBr) cm^{-1}: 3091, 3060, 3017 (aromatic CH, =C–H), 3010–2800 (br, –O–H), 1647 (chalcone C=O), 1582, 1492 (arom); ^1H NMR ($CDCl_3$) δ 12.710 (1H, s, OH-2′), 7.923 (1H, dd, $J=8.1$ Hz, 1.5 Hz, H-6′), 7.846 (1H, d, $J=15.6$ Hz, H-β), 7.657 (1H, d, $J=1.5$ Hz, H-2), 7.651 (1H, d, $J=15.6$ Hz, H-α), 7.552–7.749 (2H, m, H-5′,6), 7.45–7.35 (2H, m, H-4,5), 7.043 (1H, dd, $J=8.1$ Hz, 1.2 Hz, H-3′), and 6.967 (1H, ddd, $J=8.1$ Hz, 6.9 Hz, 1.2 Hz, H-4′); ^{13}C NMR ($CDCl_3$) δ 193.598 (>C=O), 163,792 (=C<), 143.783 (=CH–), 136.756 (=CH–), 136.521 (=C<), 135.165 (=C<), 130.784 (=CH–), 130.382 (=CH–), 129.760 (=CH–), 128.106 (=CH–), 127.119 (=CH–), 121.521 (=CH–), 119.956 (=CH–), 119.030 (=C<), and 118.780 (=CH–).

3-Iodo-2′-hydroxychalcone (37). Compound **37** was prepared from 2-hydroxyacetophenone and 3-iodobenzaldehyde by the procedure described above, mp 161–163 °C; APCIMS m/z 349.1 $[M-H]^+$; IR (KBr) cm^{-1}: 3010–2800 (br, –O–H), 1638 (chalcone C=O), 1564, 1485 (arom); ^1H NMR ($CDCl_3$) δ 12.712 (1H, s, OH-2′), 8.019 (1H, d, $J=1.5$ Hz, H-2), 7.923 (1H, dd, $J=8.1$, 1.5 Hz, H-6), 7.898 (1H, d, $J=15.6$ Hz, H-β), 7.756 (1H, dd, $J=7.5$, 1.5 Hz, H-6), 7.625 (1H, d, $J=15.6$ Hz, H-α), 7.603 (1H, dd, $J=7.8$, 1.2 Hz, H-6′), 7.519 (1H, t,d, $J=$ 7.8, 1.2 Hz, H5), 7.179 (1H, t, $J=8.4$ Hz, H-5′), 7.040 (1H, dd, $J=7.2$, 1.2 Hz, H-3′), 6.966 (1H, ddd, $J=8.2$, 7.2 Hz, 1.2 Hz, H-4′); ^{13}C NMR ($CDCl_3$) δ 193.578 (>C=O), 163.818 (>C=), 143.649 (=CH–), 139.665 (=CH–), 137.070 (=CH–), 136.903 (>C=), 136.774 (=CH–), 130.764 (=CH–), 129.816 (=CH–), 128.184 (=CH–), 121.431 (=CH–), 119.982 (>C=), 119.056 (=CH–), 118.805 (=CH–), 94.880 (>C=).

1-(4-Fluorophenyl)-3-(pyridin-3-yl)-2-propen-1-one (48).[17] Compound **48** was prepared from 4-fluoroacetophenone and 3-pyridinecarboxyaldehyde by the procedure described above, mp 126–127 °C; APCIMS m/z 228.2 $[M+H]^+$; ^1H NMR ($CDCl_3$) δ 8.866 (1H, d, $J=1.8$ Hz, H-2), 8.638 (1H, dd, $J=4.8$ Hz, 1.5 Hz, H-4), 8.0 (2H, ddd, $J=9$, 5.4 Hz, 2.1 Hz, H-2′,6′), 7.958 (1H, dt, $J=8.1$, 2.1 Hz, H-6), 7.805 (1H, d, $J=15.9$ Hz, H-β), 7.580 (1H, d, $J=15.9$ Hz, H-α), 7.380 (1H, dd, $J=7.8$, 4.8 Hz, H-5), 7.203 (ddd, H = 9.0 Hz, 8.1 Hz, 2.1 Hz, H-3′,5′); ^{13}C NMR ($CDCl_3$) δ 188.321 (=C<), 167.628 (=C<), 164.236 (=C<), 151.314 (=CH–), 150.061 (=CH–), 141.252 (=CH–), 134.726 (=CH–), 134.180 (=C<), 131.319 (=CH–), 131.198 (=CH–), 130.638 (=C<), 123.867 (=CH–), 123.435 (=CH–), 116.105 (=CH–), 115.817 (=CH–).

1-(3-Hydroxyphenyl)-3-phenanthren-9-yl-2-propen-1-one (49). The compound was synthesized from 3-hydroxyacetophenone and phenanthrene-9-carboxaldehyde by the same method described above, mp 212–213 °C; APCIMS m/z 325.1 $[M+H]^+$; ^1H NMR (acetone-d_6) δ 8.926 (1H, m, H-8), 8.86 (1H, d, $J=8.1$ Hz, H-5), 8.781 (bs, OH), 8.643 (1H, d, $J=15.6$ Hz, H-β), 8.478 (1H, s, H-10), 8.360 (1H, m, H-4), 8.09 (1H, dd, $J=7.8$, 1.8 Hz, H-1), 7.964 (1H, d, $J=15.5$ Hz, H-α), 7.818–7.643 (6H, m, H-2,3,6,7,2′,6′), 7.442 (1H, t, $J=7.8$ Hz, H-5′), 7.167 (1H, dt, $J=7.2$ Hz, H-4′); ^{13}C NMR (acetone-d_6) δ 190.016 (>C=O), 158.956 (=C<), 140.729 (=C<), 141.905 (=CH–), 132.465 (=C<), 132.276 (=C<), 132.207 (=C<), 131.562 (=C<), 131.373 (=C<), 130.910 (=CH–), 130.432 (=CH–), 128.944 (=CH–), 128.383 (=CH–), 128.163 (=CH–), 128.231 (=CH–), 127.768 (=CH–), 126.425 (=CH–), 125.226 (=CH=), 124.467 (=CH–), 123.799 (=CH–), 121.143 (=CH–), 121.022 (=CH–), 115.892 (=CH–).

1-(Furan-2-yl)-3-phenyl-2-propen-1-one (51).[18] Compound **51** was prepared from 2-acetylfuran and benzaldehyde as colorless crystals, mp 94–95 °C. APCIMS m/z 199.1 $[M+H]^+$. ^1H NMR ($CDCl_3$) δ 7.890 (1H, d, $J=$ 15.9 Hz, H-β), 7.661 (1H, dd, $J=1.5$, 0.9 Hz, H-5′). 7.659 (2H, m, H-2,6), 7.46 (1H, d, $J=15.6$ Hz, H-α), 7.421 (3H, m, H-3,4,5), 7.341 (1H, dd, $J=3.3$, 0.9 Hz, H-3′), 6.603 (1H, dd, $J=3.6$ Hz, 1.5 Hz, H-4′); ^{13}C NMR ($CDCl_3$) δ 178.195 (>C=O), 153.822 (=C<, C-2′), 146.636 (=CH–, C-5′), 144.101 (C-β), 134.814 (=C<, C-1), 130.686 (=CH–, C-4), 129.016 (=CH–, C-3,5), 128.607 (=CH–, C-2,6), 121.216 (=CH–, C-4′), 117.566 (=CH–, C-α), 112.596 (=CH–, C-3′).

5-Methoxy-8-bromo-flavanone (125). 5′-Methoxy-8′-bromo-chalcone was prepared from 5-methoxy-8-bromo-acetophenone and benzaldehyde by the usual method. A solution of the chalcone (0.5 g) and phosphoric acid (10 g) in EtOH (150 mL) was refluxed for 10 days. Concentration of the solution and dilution with water gave colorless needles (0.12 g), mp 134–165 °C, APCIMS m/z 333.1 $[M]^+$ (relative intensity 100%), 335.0 $[M+2]^+$ (94%). ^1H NMR ($CDCl_3$) δ 7.656 (1H, d, $J=8.7$ Hz, H-6), 7.511 (2H, dt, $J=7.5$, 1.8 Hz, H-2′,6′), 7.526–7.360 (5H, m, B-ring protons), 6.495 (1H, d, $J=9.0$ Hz, H-7), 5.572 (1H, m, H-2), 3.030 (2H, m, H-3α and H-3β); ^{13}C NMR ($CDCl_3$) δ 190.132 (>C=O), 160.211 (=C<), 158.959 (=C<), 138.964 (=CH–), 138.296 (=C<), 128.887 (=CH–), 128.652 (=CH–), 125.844 (=CH–), 112.474 (=C<), 105.326 (=CH–), 102.534 (=C<), 78.034 (>CH–, C-2), 58.353 (–CH_3, 5-OCH_3), and 45.297 (>CH_2, C-3).

In vitro evaluation of anti-tuberculosis activity.[19] The screening was conducted on a drug concentration of 12.5 μg/mL against *Mtb H38Rv* in BACTEC 12B medium using the BACTEC 460 radiometric system. The assay procedure was carried out according to the method described previously.[23] Compounds were solubilized in dimethylsulfoxide at 1 mg/mL and sterilized by passage through 0.22 μm PFTE filters. Fifty microliters was added to 4 mL BACTEC 12B medium (Becton Dickinson) to achieve a final concentration of 12.5 μg/mL. Approximately 4×10^5 colony forming units of *M. tuberculosis* H37Rv ATCC 27294 were added and

the cultures were incubated at 37 °C. Starting on the second day of incubation, the Growth Index (GI, 1 GI unit = 0.0025 dpm $^{14}CO_2$) was determined daily until the controls (drug-free) achieved a GI of 999. The percent inhibition was calculated as 1− (test sample GI÷control GI)×100.

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References and Notes

1. Lopez, A. In *Disease Control Priorties in Developing Countries*; Jamison, D. T., Mosely, W. H., Eds.; Oxford: New York, 1993; p 35.
2. Nivin, B.; Nicholas, P.; Gayer, M.; Frieden, T. R.; Fujiwara, P. I. *Clin. Infect. Dis.* **1998**, *26*, 303.
3. Pablos-Mendez, A.; Raviglione, M. C.; Laszlo, A.; Binkin, N.; Rieder, H. L.; Bustreo, F.; Cohn, D. L.; Lambregts-van Weezenbeek, C. S. B.; Kim, S. J.; Chaulet, P.; Nunn, P. *N. Eng. J. Med.* **1998**, *338*, 1641.
4. Murray, C. J. L.; Styblo, K.; Rouillon, A. In *Disease Control Priorities in Developing Countries*; Jamison, D. T., Mosely, W. H., Eds.; Oxford: New York, 1993; p 233.
5. Styblo, K. *Bull. Int. Union Tuberc.* **1990**, *65*, 24.
6. Lin, Y.-M.; Flavin, M. T.; Cassidy, C. S.; Mar, A.; Chen, F.-C. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2101.
7. Ariyan, Z. S.; Suschitzky, H. *J. Chem. Soc.* **1961**, 2242.
8. Chen, F. C.; Yang, C. H.; Hsu, K. K. *J. Formosan Sci.* **1953**, *7*, 51.
9. Chen, F. C.; Hsu, K. K. *J. Formosan Sci.* **1953**, *7*, 54.
10. Chen, F. C.; Lai, P. C.; Hsieh, H. C. *J. Formosan Sci.* **1953**, *7*, 57.
11. Chen, F. C.; Lin, C.; Lai, S. C. *J. Formosan Sci.* **1953**, *7*, 63.
12. Chen, F. C.; Lin, C.; Tu, T. T. *J. Formosan Sci.* **1954**, *8*, 71.
13. Chang, C. T.; Chen, F. C.; Chen, T. S.; Hsu, K. K.; Ueng, T.; Hung, M. *J. Chem. Soc.* **1961**, 3414.
14. Chen, F. C.; Chang, C. T. *J. Formosan Sci.* **1954**, *8*, 74.
15. Chen, F. C.; Hsu, K. K. *J. Taiwan Pharm. Assoc.* **1953**, *5*, 49.
16. Chen, F. C.; Chang, C. T. *J. Chem. Soc.* **1958**, 146.
17. Chen, F. C.; Chen, Y. H.; Chen, C. Y. *J. Formosan Sci.* **1972**, *26*, 52.
18. Chen, F. C.; Chen, Y. H.; Chen, L. S. *J. Formosan Sci.* **1972**, *26*, 50.
19. Collins, L. S.; Franzblau, S. G. *Antimicrob. Agents Chemother.* **1997**, *41*, 1004.