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Structure–Activity Relationships of Antileishmanial and Antimalarial Chalcones

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Abstract—A series of oxygenated chalcones which have been evaluated earlier for antimalarial activity (*Plasmodium falciparum K1*) were tested for antileishmanial activity against *Leishmania donovani* amastigotes. A comparison of structure–activity relationships reveal that different physicochemical and structural requirements exist for these two activities. Antileishmanial activity is associated with less lipophilic chalcones, in particular those with 4′-hydroxyl-substituted B rings and hetero/polyaromatic A rings. In contrast, chalcones with good antimalarial activity have alkoxylated B rings and electron-deficient A rings. Visualization of the steric and electrostatic fields generated from comparative molecular field analysis (CoMFA) indicate that the ring A of chalcones make a more significant contribution to antileishmanial activity while both rings A and B are important for antimalarial activity. Despite different requirements, two alkoxylated chalcones (**8**, **19**) were identified which combined good antimalarial and antileishmanial activities.

Introduction

For a structurally simple group of compounds, chalcones have displayed an impressive array of pharmacological activities, among which anti-protozoal,[1–3] anti-inflammatory,[4] immunomodulatory,[5] nitric oxide inhibition,[6] anticancer[7] activities have been cited in the literature. Chalcones that combine one or more types of activities may be advantageous or otherwise. A combination of activities can enhance the likelihood of undesirable side effects during therapy. On the other hand, it may translate into a successful dual-acting drug, especially if closely related activities (for example, antiprotozoal) are involved. Whether the objective of drug design is exclusion or inclusion of more than one kind of activity, it would have to start with defining the structure–activity relationships for each pharmacophore.

The antileishmanial activity of several chalcones have been reported in the literature.[1,8–10] The most promising member to date is licochalcone A (Fig. 1), an oxygenated chalcone isolated from the roots of Chinese liquorice and presently thought to exert its action by inhibiting fumarate reductase, a selective target present in the parasite mitochondria.[10] The activity of licochalcone A has stimulated interest in the antileishmanial potential of other chalcones from natural and synthetic sources, and several members have been identified for lead development.[1,8,9,11] Antimalarial activity has also been found in licochalcone A[12] and other oxygenated chalcones.[2,3] However, reports on chalcones that combine activities against both protozoal infections are lacking, though the example of licochalcone A suggests that this is not an unreasonable expectation.

In this study, we have taken a group of oxygenated chalcones that have previously been evaluated for antimalarial activity and tested them for antileishmanial properties. Activities were analyzed by conventional

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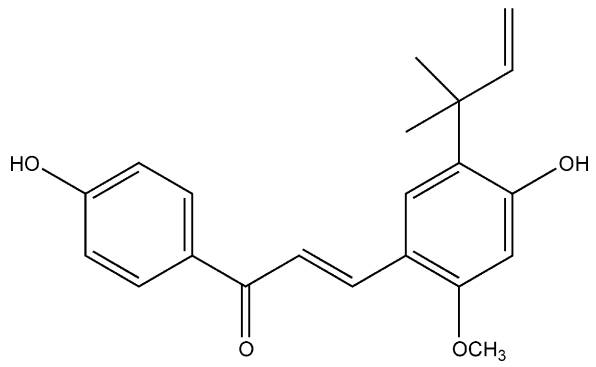


Figure 1. Structure of licochalcone A.

structure–activity relationship (SAR) approaches and by comparative molecular field analysis (CoMFA) with the aim of establishing the relevant SARs and to see if these are mutually exclusive or share some elements of similarity. Such information would be useful in either fine-tuning one activity to the exclusion of the other, or in developing dual-acting drugs against both infections.

Results and Discussion

Table 1 summarizes the antileishmanial and antimalarial activities of the chalcones expressed in terms of ED_{50} and IC_{50} values, respectively. The antimalarial activities have been reported previously (with the exception of the 2′-hydroxychalcones) but the antileishmanial activities are reported for the first time. It is noted that the ED_{50} values for antileishmanial activity span a narrow 30-fold range, in contrast to a wider 300-fold spread for antimalarial activity.

To facilitate discussion, the chalcones are classified into seven groups according to the substitution on the B ring, namely 2′,3′,4′-trimethoxy ($n=18$), 2′,4′-dimethoxy ($n=16$), 4′-methoxy ($n=14$), 4′-ethoxy ($n=13$), 2′,4′-dihydroxy ($n=12$), 4′-hydroxy ($n=16$) and 2′-hydroxy ($n=13$). In addition, activities are arbitrarily classified into 5 categories (A–E) for comparison. For antileishmanial activity, compounds with ED_{50} values $<5\,\mu M$ are classified as A (very good); $5–10\,\mu M = B$ (good); $10–20\,\mu M = C$ (fair); $20–30\,\mu M = D$ (poor) and $>30\,\mu M = E$ (very poor). IC_{50} values for antimalarial activities are similarly classified: $A<10\,\mu M$, B: $10–20\,\mu M$, C: $20–50\,\mu M$, D: $50–100\,\mu M$ and $E>100\,\mu M$.

The distribution of the chalcones according to this classification is shown in Figure 2. It can be seen that methoxylated chalcones (ring B substituted with trimethoxy, dimethoxy or methoxy substituents) are represented in every category (A–E) of antimalarial and antileishmanial activities. The ethoxychalcones are an exception, being notable in having poor antimalarial activity. Hydroxylated chalcones are better antileishmanial agents: they are not represented in category A antimalarial activity but many members are found to have category A antileishmanial activity.

Regression analyses show that antimalarial and antileishmanial activities are not correlated in any way ($r^2=0.05$ for linear regression). It is evident that chalcones have different structural requirements for antimalarial and antileishmanial activities. Based on the compounds with very good (A) antimalarial and antileishmanial activities, dual-acting chalcones are more likely to be found among methoxylated than hydroxylated chalcones. Indeed, the only two chalcones with combined category A activities are alkoxylated chalcones (2′, 4′-dimethoxy-4-ethylchalcone **8**, 4′-methoxy-4-hydroxychalcone **19**).

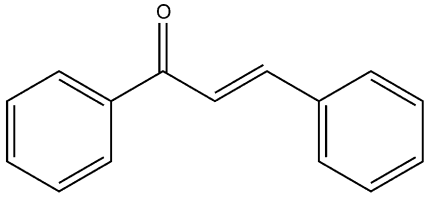
Previous structure–activity studies on the antileishmanial activity of chalcones have emphasized the importance of ring B for activity. In particular, *para*-substitution of ring B with oxygenated and non-bulky substituents have been reported to be desirable.[1,8] The good activity seen here with the 4′-hydroxychalcones is in keeping with these findings.

Among the different structural classes, the 4′-hydroxychalcones have yielded the largest number of category A antileishmanial compounds, with nearly half of the available members possessing ED_{50} values of less than $5\,\mu M$. Unfortunately, many of these compounds are also toxic against macrophages (Table 1). The toxicity of oxygenated chalcones against murine bone marrow-derived macrophages have been reported.[8] Further structural modifications to reduce toxicity while maintaining antileishmanial activity would be necessary before 4′-hydroxychalcones can be useful agents.

In chalcones, ring A and its substitution pattern are generally considered less important for antileishmanial activity compared to ring B.[1,8] However, this was not observed in our study where notwithstanding the nature of ring B, very good antileishmanial activity was observed when ring A is 1-naphthalenyl (**110**, **205**, **212**, **242**), 2-pyridinyl (**207**, **214**, **241**) and 4-quinolinyl (**28**, **30**, **34**, **215**). The position of attachment of ring A to the αβ unsaturated carbonyl linkage is also critical, as seen from the poor activities of the 3-quinolinyl (**213**, **29**) versus 4-quinolinyl (**215**, **30**), and 2-naphthalenyl (**216**, **243**) versus 1-naphthalenyl (**212**, **242**) derivatives. This is unlike antimalarial activity where better activities are associated with 3-quinolinyl (**27**, **29**, **31**, **33**) than 4-quinolinyl (**28**, **30**, **32**, **34**) derivatives.[3] This departure from earlier reports[1,8] may be due to the fact that Ring A in the present series of chalcones has not been restricted to substituted benzene rings (as in earlier studies) but includes heteroaromatic and polycyclic ring systems which were not previously investigated.

Correlation analysis show that the activities of chalcones with very good (A) antileishmanial properties are inversely correlated to lipophilicity (ClogP) (Pearson correlation -0.443, $p=0.034$, $n=23$). This differs from the general opinion that lipophilicity is an important determinant of antileishmanial activity.[8] However, a statistically significant correlation with ClogP could not be obtained by linear regression ($r^2=0.196$, $n=23$), possibly because of the narrow range of antileishmanial activity (30-fold) associated with our compounds.

Table 1. Antileishmanial, antimalarial activities and toxicity against mice macrophages of chalcones



Compd	Ring B	Ring A	ED_{50} (μM)[a]	IC_{50} (μM)[b]	Toxicity to macro-phages (μM)[c]	Compd	Ring B	Ring A	ED_{50} (μM)[a]	IC_{50} (μM)[b]	Toxicity to macro-phages (μM)[c]
3	2′,3′,4′-	2,4-Dichloro	13.24	5.4	30	**39**	4′-Ethoxy	4-Fluoro	>30	24.10	—
4	-Trimethoxy	4-Dimethylamino	7.81	18.0	10	**121**		2,4-Dichloro	>30	96.00	—
6		4-Trifluoromethyl	6.82	3.0	10	**122**		4-Trifluoromethyl	>30	24.00	30
11		2,4-Dimethoxy	13.15	16.5	30	**123**		2,4-Dimethoxy	24.17	30.00	—
12		4-Methyl	14.15	25.6	30	**124**		4-Methyl	>30	38.00	—
13		4-Ethyl	6.98	16.5	10	**125**		4-Nitro	25.23	39.00	—
27[d]		3-Quinolinyl	7.95	2.0	10	**126**		4-Dimethylamino	29.93	30.00	—
28[d]		4-Quinolinyl	4.38	60.0	10	**127**		4-Cyano	13.92	540.00	30
35		4-Methoxy	13.57	25.0	30	**136**		H	11.23	43.00	30
36		4-Fluoro	8.13	9.5	10	**41**[f]	4′-Butoxy	2,4-Dimethoxy	>30	108.00	30
40		4-Phenyl	21.52	26.2	30	**201**	2′,4′-	2,4-Dichloro	23.66	68.5	30
128		2,4-Difluoro	4.38	18.5	10	**202**[d]	-Dihydroxy	3-Quinolinyl	>30	16.1	—
129		4-Nitro	>30	22.5	—	**203**		2,4-Difluoro	>30	16.0	10
130		3,4-Dichloro	12.33	14.5	30	**204**		2,4-Dimethoxy	12.12	56.4	30
131		4-Chloro	4.89	14.5	10	**205**[d]		1-Naphthalenyl	4.03	24.8	10
132		2-Chloro	9.09	41.5	30	**206**		4-Trifluoromethyl	8.07	26.5	10
133		3-Chloro	4.39	24.4	10	**207**[d]		2-Pyridinyl	2.23	19.7	3
134		H	6.82	15.8	10	**208**[d]		2-Naphthalenyl	>30	20.0	—
1	2′,4′-	2,4-Dichloro	>30	18.75	—	**209**[d]		4-Pyridinyl	10.99	121.6	30
2	-Dimethoxy	4-Trifluoromethyl	>30	5.85	—	**210**[d]		4-Quinolinyl	ND	92.8	ND
5		2,4-Difluoro	13.16	6.23	30	**211**		4-Chloro	4.36	12.30	10
7		2,4-Dimethoxy	>30	2.10	—	**245**		4-Methyl	8.02	ND	10
8		4-Ethyl	4.38	2.42	10	**212**[d]	4′-Hydroxy	1-Naphthalenyl	1.38	39.9	3
29[d]		3-Quinolinyl	12.46	2.16	30	**213**[d]		3-Quinolinyl	>30	41.0	—
30[d]		4-Quinolinyl	1.5	27.00	3	**214**[d]		2-Pyridinyl	1.48	16.3	3
101		4-Methyl	6.82	93.75	10	**215**[d]		4-Quinolinyl	3.83	51.0	10
102		4-Methoxy	12.23	128.50	30	**216**[d]		2-Naphthalenyl	>30	27.5	—
104		4-Fluoro	>30	322.00	—	**217**		4-Chloro	6.98	38.0	10
105		4-Chloro	>30	342.00	—	**220**		4-Methoxy	5.77	32.2	10
106		4-Bromo	23.38	542.50	30	**221**		4-Methyl	4.4	25.4	10
107		2-Chloro-4-fluoro	11.89	600.00	30	**222**		3-Methyl	4.19	25.8	10
108		3,4-Dichloro	11.27	297.50	30	**224**		4-Trifluoromethyl	4.18	30.4	10
109		4-Nitro	>30	415.00	—	**225**		4-Nitro	>30	20.4	—
110[d]		1-Naphthalenyl	4.37	320.00	10	**226**		4-Fluoro	2.6	21.7	3
19	4′-	4-Hydroxy	4.18	7.00	10	**227**		3,4-Dichloro	>30	18.4	—
22	-Methoxy	2,4-Difluoro	12.51	26.75	30	**228**		4-Dimethylamino	8.13	17.70	10
23		4-Methoxy	13.13	21.70	30	**229**		2,4-Dichloro	4.39	24.50	10
31[d]		3-Quinolinyl	6.82	4.83	10	**230**		H	4.41	29.6	10
32[d]		4-Quinolinyl	8.13	43.00	10	**231**	2′-Hydroxy	2,4-Dichloro	11.74	35.45	30
38		4-Fluoro	ND	14.40	ND	**232**		4-Dimethylamino	>30	188.00	—
111		2,4-Dichloro	12.38	16.00	30	**233**[d]		3-Quinolinyl	13.16	28.00	30
112		4-Trifluoromethyl	>30	19.00	—	**234**		4-Chloro	6.82	12.85	10
113		2,4-Dimethoxy	23.38	6.40	30	**235**		4-Methyl	12.05	62.50	30
114		4-Methyl	ND	70.00	ND	**236**		4-Methoxy	13.16	61.50	30
115		4-Nitro	>30	100.00	—	**237**		2,4-Dimethoxy	10.85	25.50	30
116		4-Dimethylamino	>30	70.00	—	**238**		4-Trifluoromethyl	29.31	35.50	—
117		4-Cyano	6.91	94.50	10	**239**		4-Fluoro	29.31	47.00	30
135		H	12.2	55.50	30	**241**[d]		2-Pyridinyl	1.57	31.00	3
25	4′-Ethoxy	2,4-Difluoro	>30	28.10	—	**242**[d]		1-Naphthalenyl	4.07	32.50	10
26		4-Methoxy	29.37	33.00	—	**243**[d]		2-Naphthalenyl	11.73	29.50	30
33[d]		3-Quinolinyl	9.99	24.85	30	**244**[d]		4-Quinolinyl	12.63	ND	30
34[d]		4-Quinolinyl	4.39	100.00	10[e]						

[a]In vitro sensitivity against *L. donovani* (L82) amastigotes determined in a mouse peritoneal macrophage model.[20] Compounds with ED_{50} values >30 μM are considered inactive. ED_{50} for pentosam® (sodium stilbogluconate) is 0.6 μM. Compounds were tested in triplicates.
[b]Inhibition of [^{3}H] hypoxanthine uptake into *P. falciparum* K1.[3] IC_{50} for chloroquine = 0.27 μM.
[c]Concentration at which toxicity to mouse macrophages was observed. (—) no toxicity observed at 30 μM. ND, not done.
[d]Ring A = heterocyclic or polyaromatic.
[e]Compound **34** is toxic at 10 μM, but parasites are present at this concentration.
[f]Compound **41** (2′,4′-dimethoxy-4′-butoxychalcone) has been reported to have antimalarial and antileishmanial activities.[9,12] Low levels of activities observed here may be due to different strains of parasites being used.

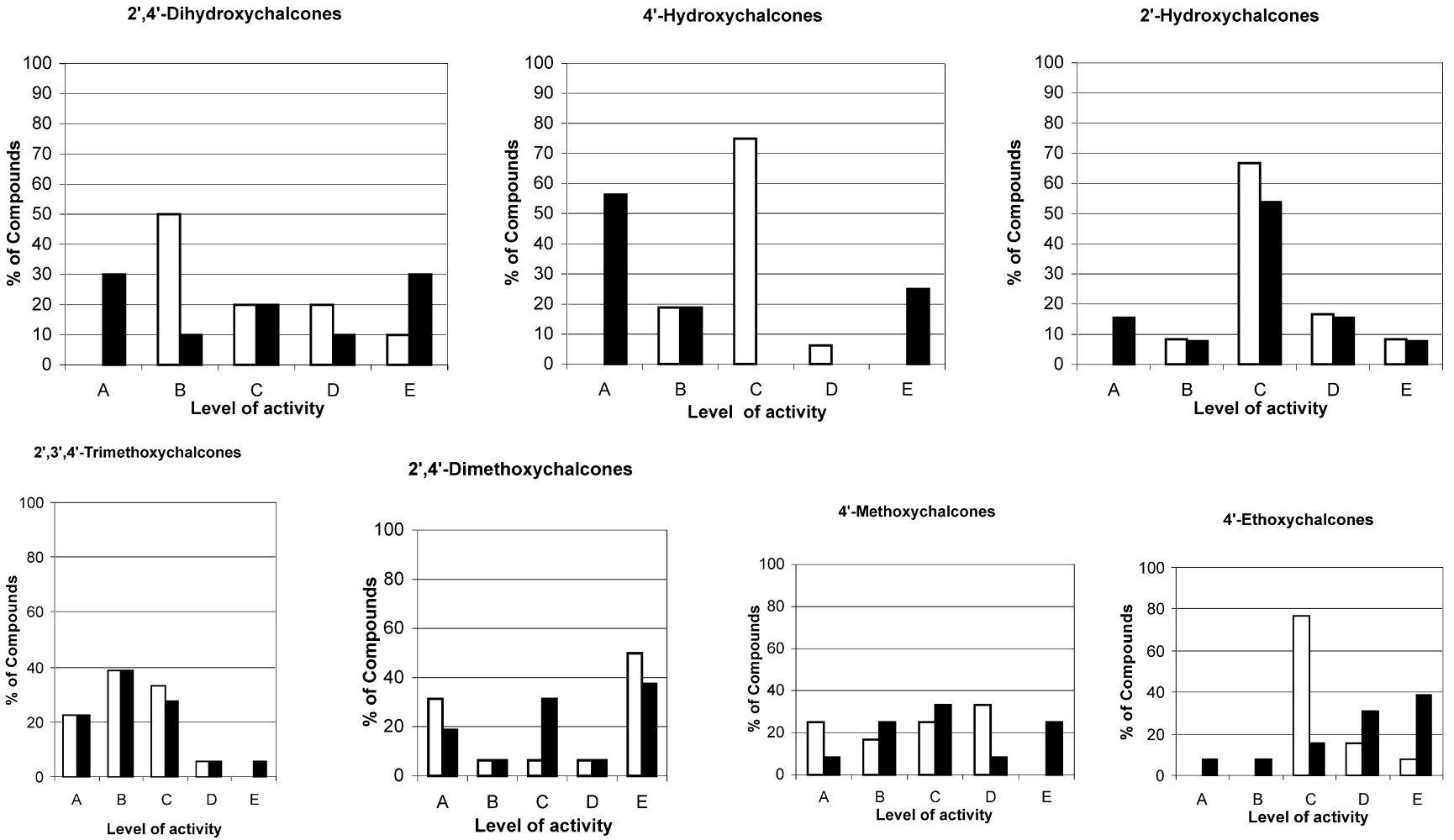


Figure 2. Distribution of antimalarial and antileishmanial activities of chalcones classified according to nature of Ring B. Antileishmanial activities ■ ED_{50} values: $<5\,\mu M$ (A), 5–10 μM (B), 10–20 μM (C), 20–30 μM (D), $>30\,\mu M$ (E). Antimalarial activities □ IC_{50} values: $<10\,\mu M$ (A), 10–20 μM (B), 20–50 μM (C), 50–100 μM (D), $>100\,\mu M$ (E).

The SAR for antimalarial activity has been reported earlier.[3] Using multivariate analysis, it was shown that a sterically larger ring B and a polar ring A had a positive influence on activity.

In the next stage of analysis, the three-dimensional structure–activity relationship study for antimalarial and antileishmanial activities were carried out using comparative molecular field analysis (CoMFA). Various CoMFA models were generated using all compounds, compounds classified according to B ring substitution pattern or according to activity levels (A–E). The best model ($r^2_{cv} = 0.547$, standard error of prediction, cross validated (SEP_{cv}) = 0.218, three components) for antimalarial activity was obtained using 'active' chalcones, namely alkoxylated chalcones with $IC_{50} < 10\,\mu M$, and hydroxylated chalcones with $IC_{50} < 20\,\mu M$. Coincidentally, these were the same compounds that had been successfully analyzed using multivariate analysis in an earlier study, with activity represented by the following equation:[3]

$$-\log\ IC_{50} = 6.4\ \log\ A - 10.6$$

$$n = 19,\quad r^2 = 0.690,\quad r^2 cv = 0.616$$

The equation shows a good correlation to Connolly surface area (log A) which is a measure of the solvent accessible surface area of the molecule. In view of this relationship, the CoMFA was re-done with the inclusion of log A. A statistically better model (r^2 cv = 0.655; $SEP_{cv} = 0.227$, three components) was obtained, with contributions by the steric, electrostatic and log A parameters at 33.6, 40.1 and 26.3%, respectively. In view of this improvement, the new model incorporating log A is adopted for subsequent discussion.

The best CoMFA model for antileishmanial activity was found for chalcones with hydroxylated B rings ($n = 40$) covering the entire range of antileishmanial activity (A–E). However, this model had low predictability ($r^2_{cv} = 0.358$, $SEP_{CV} = 0.383$, four components), with balanced steric (48.8%) and electrostatic (51.2%) contributions. In an attempt to improve the CoMFA model, ClogP was included as an additional parameter, since earlier results showed good antileishmanial activity to be inversely correlated to hydrophobicity. However, no significant improvement to the model was evident upon inclusion of the hydrophobic parameter ($r^2_{cv} = 0.352$, $SEP_{CV} = 0.373$). Thus, ClogP was not included in the CoMFA model for antileishmanial activity.

Visualization of the steric and electrostatic contours of the two CoMFA models was carried out using 1-(4′-hydroxy)-3-(2-pyridinyl)-2-propen-1-one (**214**), a

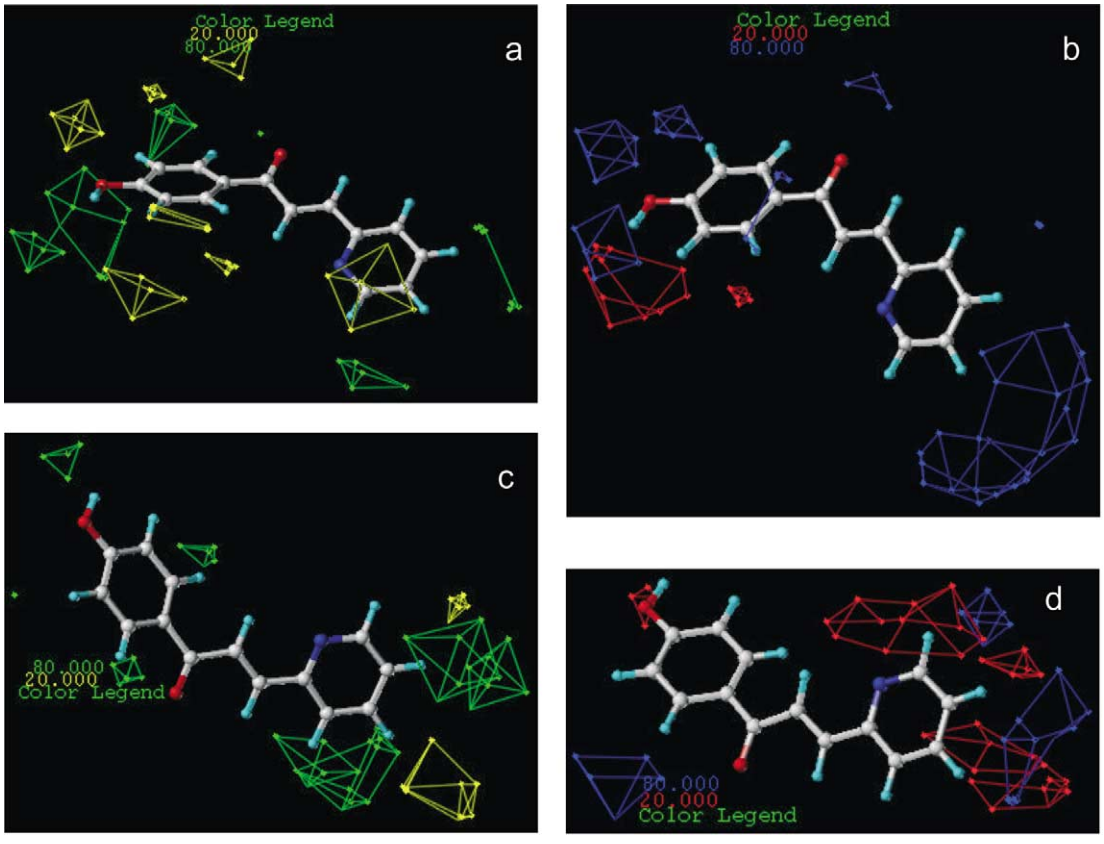


Figure 3. Steric and electrostatic contour maps of CoMFA models for antimalarial and antileishmanial activities, showing the orientation of 1-(4′-hydroxy)-3-(2-pyridinyl)-2-propen-1-one (**214**) in the antimalarial model (a,b) and antileishmanial model (c,d). In (a), the steric contours show that a large sized B ring (green) and a smaller sized A ring (yellow) is preferable. In (b), the blue contours indicate that an electron-deficient A ring is preferred. The steric (c) and electrostatic (d) contours in the antileishmanial model are concentrated around ring A, indicating the relative importance of this ring compared to ring B. Steric contours are colour-coded yellow (where bulk is undesirable) and green (where bulk is acceptable). The electrostatic contours are colour-coded blue (where positive charge is desirable) and red (where negative charge is preferred).

representative compound with an ED_{50} of 1.48 μM and IC_{50} of 16.25 μM for antileishmanial and antimalarial activities, respectively. Steric contours in the CoMFA model are colour-coded yellow (where bulk is undesirable) and green (where bulk is acceptable). The electrostatic contours are colour-coded blue (where positive charge is desirable) and red (where negative charge is preferred).

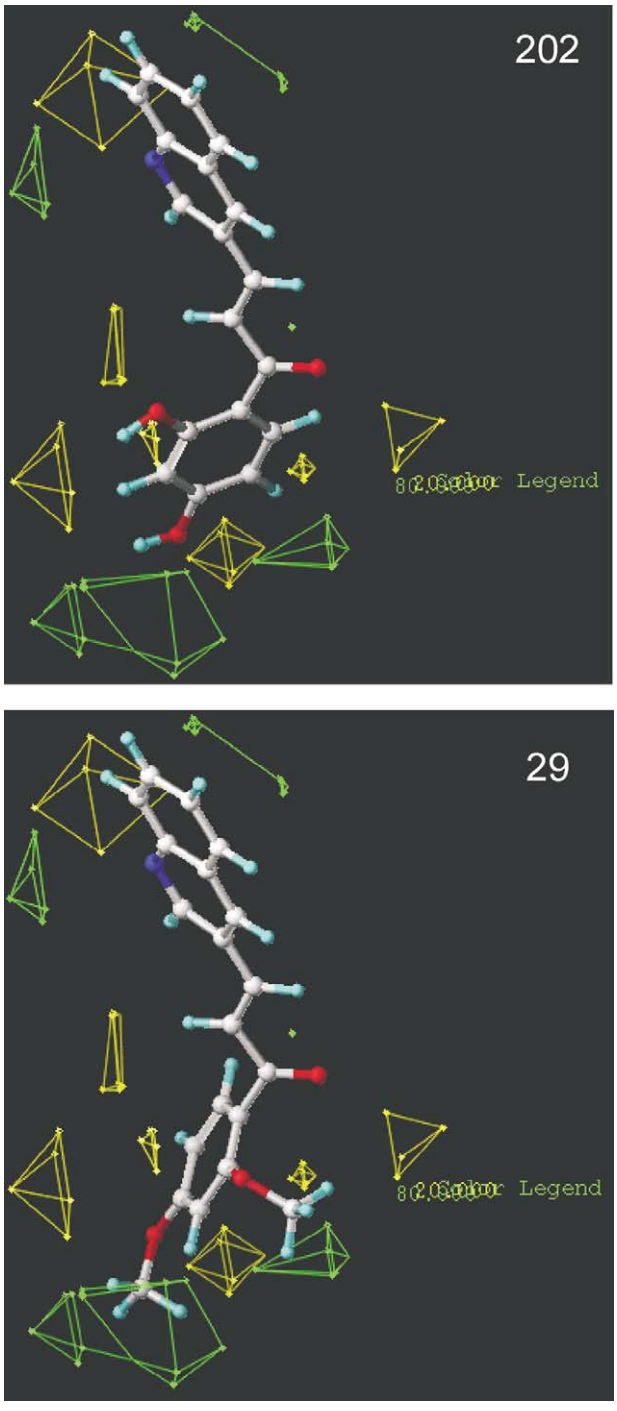


Figure 4. Orientation of 1-(2′,4′-dihydroxy)-3-(3-quinolinyl)-2-propen-1-one (**202**) and its dimethoxy analogue (**29**) in the steric contours of the CoMFA model for antimalarial acitivty. The methoxy groups on ring B of **29** are directed towards the green zone (where bulk is desirable), unlike the hydroxyl groups of **202**. This may account for the generally better antimalarial activities of alkoxylated chalcones.

The orientation of **214** in the steric (Fig. 3a) contours of the CoMFA model for antimalarial activity shows a concentration of green zones around ring B, indicating that more bulk is tolerated or even preferred in this region. Unfortunately, the solitary 4-hydroxyl substituent on ring B of **214** did not project into the green zones. An alkoxylated B ring would be more advantageously placed in the green zones and this was indeed observed when the steric contours around the 2′,4′ dihydroxy chalcone (**202**) and its 2′,4′-dimethoxy analogue (**29**) were compared (Fig. 4). The distribution of steric contours in this model would explain why alkoxylated chalcones are generally better antimalarials than their hydroxylated counterparts.

Unlike the preference for more bulk around ring B, the large yellow zone in the vicinity of ring A would suggest some degree of steric restriction in this region (Fig. 3a). The small-sized pyridine ring in **214** is still well-accommodated in this region, but so were the bigger sized quinoline and naphthalene rings.

The strong blue contours (preference for positive charged entities) around ring A suggests that the electronic characteristics of ring A would be important for activity. (Fig. 3b). Thus, the π deficient carbon atoms of the pyridine ring of **214** would be suitably placed in this region, as would the carbon atoms of the π deficient quinoline ring.

In summary, the CoMFA model for antimalarial activity indicates a preference for a sterically large (alkoxylated) ring B and an electron deficient Ring A. These conclusions compare favourably with those derived from multivariate analysis (large size ring B and polar ring A).[3]

Examination of the steric and electronic contours around **214** in the CoMFA model for antileishmanial activity shows that both contours are concentrated around ring A (Fig. 3c and d). Ring A may thus have a greater influence than ring B in the activity of hydroxylated chalcones. The interspersing of red and blue zones around ring A suggest that the ring can be electron-deficient or electron-rich.

Smaller electronic contours are found around Ring B and a careful examination shows that the good activity of 4′-hydroxylated chalcones may be traced to the favourable orientation of the 4′-hydroxyl group away from a blue zone (preference for positive charge) and towards a negative charge-seeking red zone, as seen in Figure 3d for **214**. When the B ring carries 2′,4′-dihydroxyl substituents, the 2′-hydroxyl group impinges into the blue zone (undesirable) although some members (**205**, **207**) have their B rings twisted so as to avoid this interaction. Similarly, some 2′-hydroxychalcones (**241**, **242**) orient their hydroxyl groups away from this blue zone. These members manifest better antileishmanial activity than those that do not adopt a twisted B ring conformation (**235**, **238**, **239**). This is shown in Figure 5, using **207**, **241** and **238** as examples. The reason as to why some hydroxylated chalcones adopt a twisted

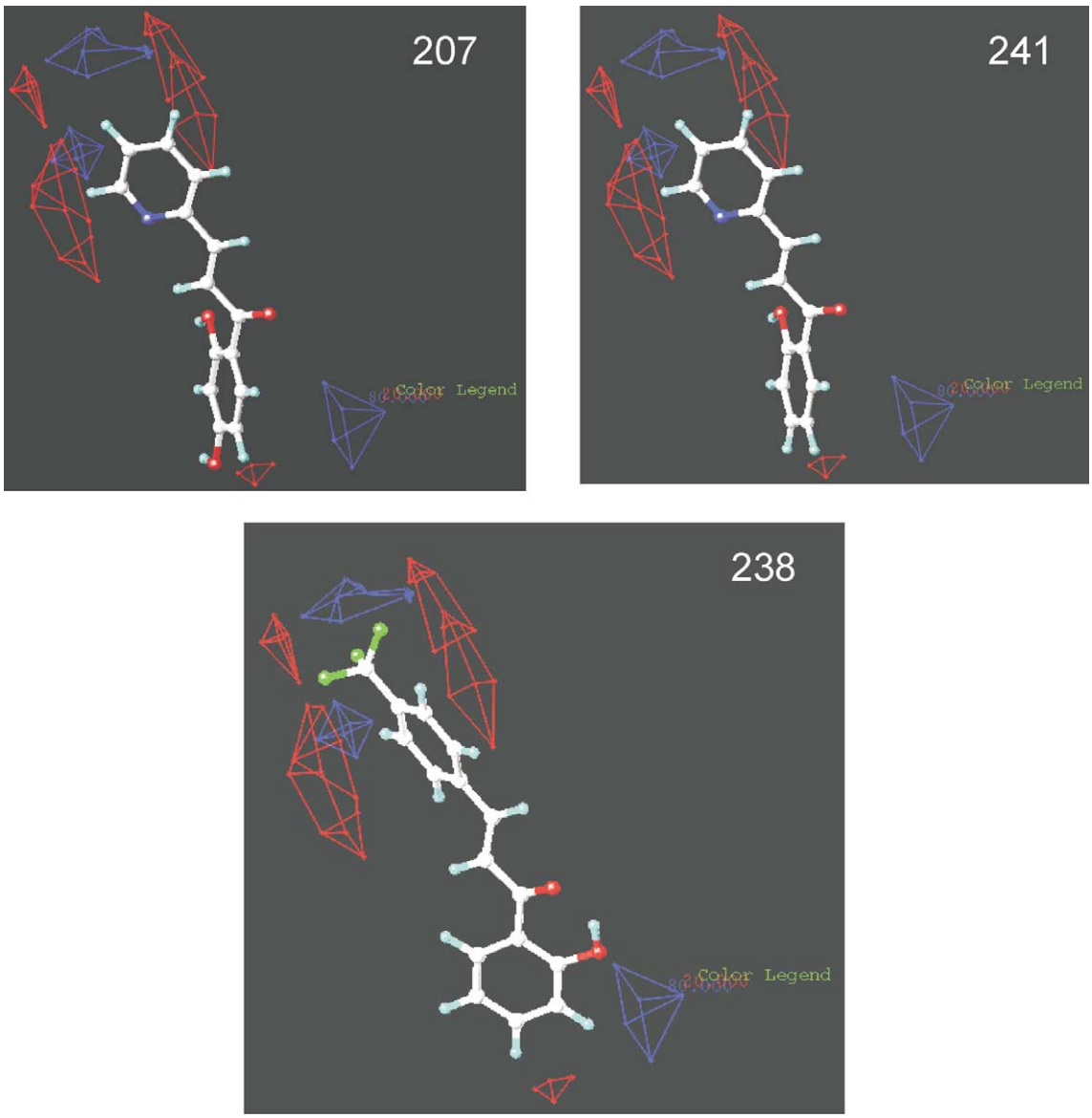


Figure 5. Orientation of 1-(2′,4′-dihydroxy)-3-(2-pyridinyl)-2-propen-1-one (**207**), 1-(2′-hydroxy)-3-(2-pyridinyl)-2-propen-1-one (**241**) and 2′hydroxy-4-trifluoromethylchalcone (**238**) in the electrostatic contours of the CoMFA model for antileishmanial activity. The B rings of **207** and **241** are 'twisted', and their 2′-hydroxyl groups (electron rich oxygen) do not point towards the blue zone. In **238**, the B ring is not twisted and the 2′-hydroxyl group points towards the blue zone which is undesirable. ED_{50} of **238** is 29.3 μM compared to 2.23 and 1.57 μM for **207** and **241**, respectively.

versus non-twisted B ring conformation is not known, but these differences do exist.

Conclusion

The present study shows that different physicochemical and structural requirements exist for the antimalarial and antileishmanial activities of chalcones. Conventional structure activity relationships show that antileishmanial activity is favoured by chalcones with more hydrophilic character, with the most active members found among 4′-hydroxychalcones. In contrast, good antimalarial activity is found among alkoxylated chalcones with polar A rings, in particular those substituted with electron withdrawing groups or replaced by quinoline rings. The relative contributions of rings A and B were assessed by CoMFA models for antimalarial and antileishmanial activities. For antileishmanial activity, the steric and electronic characteristics of ring A appear to be more important than that of ring B. Ring A could be electron-rich or electron poor. The good antileishmanial activities of the naphthalenyl (**110**, **205**, **212**, **242**) and pyridinyl (**207**, **214**, **241**) derivatives suggest that considerable tolerance for the size of ring A exists. For antimalarial activity, the size characteristics of ring B (large, alkoxylated) and the electronic characteristics of ring A (electron deficient) are important. However, one shortcoming of these CoMFA models is that they have been derived from different subsets of compounds. The antimalarial model was obtained using alkoxylated and hydroxylated chalcones with category A activities, while the antileishmanial model was based only on hydroxylated chalcones exhibiting the full range (A–E) of activity. This may create

some biase in the interpretation of the results. Despite this limitation, CoMFA confirms the differing requirements for antimalarial and antileishmanial activities. Notwithstanding these differences, two chalcones (**8**, **19**) have been identified which have good in vitro antimalarial and antileishmania activities ($<10\,\mu M$) and moderate toxicities (observed at $10\,\mu M$) against mice macrophages. **8** is of particular interest as it has been shown to increase the survivability of *P. berghei* ANKA infected mice at a dose of 100 mg/kg.[3]

Experimental

Synthesis

The chalcones were synthesized by a base-catalyzed Claisen–Schmidt condensation of an aromatic aldehyde with the appropriate ketone. The syntheses of the chalcones, except the 2-hydroxychalcones, have been reported earlier.[3] The 2′-hydroxyl chalcones (**231**–**239**, **241**–**244**) were synthesized without protection of the 2-hydroxy group in 2′-hydroxyacetophenone, following the method of Nielsen et al.[1] Briefly, the method involved adding 2′-hydroxyacetophenone (10 mmol, 10 mL methanol) dropwise to a stirred mixture of the aldehyde (10 mmol) and NaOH (3% w/v) in methanol (20 mL) and stirring at room temperature (28 °C) for 4–18 h. Stirring in an ice bath was necessary if heat was generated during addition. The solid formed was removed by filtration, washed with cold methanol and recrystallized. When no precipitate was obtained, the solution was diluted with water, neutralized with HCl, and extracted with ethyl acetate. The organic layer was dried with anhydrous Na_2SO_4 and removed by evaporation under reduced pressure to give either a solid or liquid residue. The solid residue was treated as above. The liquid residue was passed through a column of silica gel (230–400 mesh ASTM) and eluted with chloroform or chloroform–hexane. Recrystallization was done twice and purity checked by TLC before characterization by ^{1}H NMR, IR, accurate mass determination and elemental analyses (Table 2). The 2′-hydroxyl chalcones (**231**–**239**, **241**–**244**) have been reported in literature[13–19] and a comparison of the physical and spectroscopic data of the synthesized compounds with reported values (where available) showed good agreement.

Antimalarial and antileishmanial activity

The in vitro sensitivity of *Leishmania donovani* (L82) amastigotes to the test compounds were determined in a mouse peritoneal model.[20] Briefly, peritoneal macrophages were isolated from CD1 mice and maintained in culture for 24 h before being infected with *L. donovani* amastigotes isolated from the spleen of an infected hamster. The test compounds were then added to the infected macrophages 24 h later, with each compound tested in quadruplicate at $30\,\mu M$. After 5 days of incubation, parasite growth is assessed microscopically after staining the cells with 10% Giemsa solution. The percentage of infected cells was determined and expressed as% reduction in parasite burden compared to the control. Damage to macrophages was concurrently assessed in order to determine toxicity. Where antileishmanial activity was detected at $30\,\mu M$, the compound was retested at an appropriate range of concentrations to obtain its ED_{50}, which was calculated by linear regression with P_{95} confidence limits.

The in vitro antimalarial activities of the chalcones were evaluated by determining the concentration required to inhibit the uptake of [^{3}H] hypoxanthine into a chloroquine-resistant strain of *P. faciparum* (K1) as reported earlier.[3]

Correlation and regression analyses

The following parameters were determined from the force-field minimized geometries of the chalcones using the SYBYL 6.6 force field MMFF94 (Tripos Associates, St Louis, MO, USA), with calculations continued until the root mean square (rms) gradient was less than 0.001 kcal mol^{-1} Å: ClogP (Version 3), molecular refractivity, total dipole moment, Connolly surfaces (volume and surface area, calculated from MOLCAD in SYBYL), negative charge on carbonyl oxygen calculated using the Gasteiger–Huckel method, HOMO and LUMO (MOPAC, QCPE program 455, version 6.0, interfaced with SYBYL). Multiple regression and Pearson correlation analyses of these properties were carried out using SPSS 10 (SPSS Inc., Chicago, IL, USA). The regression equations were assessed by the following statistical parameters: r^2 (explained variance), standard error (SE), cross validated r^2 and cross validated SE (determined using the QSAR module of SYBYL).

Comparative molecular field analysis (COMFA)

To obtain the CoMFA for antimalarial chalcones, the energy-minimized conformations of the chalcones were aligned using the geometry of the αβ unsaturated carbonyl linkage of the most potent member for antimalarial activity (**27**). In the case of antileishmanial activity, the process was repeated using the most active antileishmanial chalcone (**212**). Both compounds have a *trans* orientation of rings A and B, and comparable torsion angles (9–11°) for the carbon–carbon single bond in the –C(O)–CH=CH– linkage.

CoMFA analysis was carried out using the QSAR module of SYBYL 6.6 with the following settings: 2 Å grid spacing, 4 Å extension of the region beyond the van der Waals volume of the molecules, sp^3 carbon probe atom with +1 charge and a distance dependent $(1/r^2)$ dielectric constant. Computation of atomic charge was carried out using the Gasteiger–Huckel method. The steric and electrostatic CoMFA fields were scaled using default settings which gives identical weight to CoMFA fields and additional variables. Column filtering was set at 2.0 kcal and steric/electrostatic cutoff values at 30 kcal/mol. The CoMFA QSAR equations were derived using Partial Least Squares (PLS) 'leave-one-out' cross validation procedure and the models were evaluated on the basis of cross validated r^2, optimum number of components and cross validated standard error of prediction (SEP_{cv}).

Table 2. Physical and analytical data of 2′-hydroxychalcones

Compd	Melting point (°C)[a]	Yield (%)	Elemental analysis	Accurate mass (M + 1)	^{1}H NMR (δ ppm)
			C, H,	292.0067 ($C_{15}H_{10}O_2Cl_2$ = 292.0058)	In DMSO, 12.65 (s, OH) 8.26 (s, J = 15.44 Hz, βH) 7.62 (s, J = 15.44 Hz, αH) 7.88–6.95 (m, 2′H, 3′H, 5′H, 6′H, 3H, 5H, 6H)
231	176–178	10.0	Cl: calcd 23.95, found 24.33		
232	69–71	25.0	C, H,	267.1251 ($C_{17}H_{17}O_2N$ = 267.1259)	In DMSO, 12.72 (s, OH) 7.95 (s, J = 15.07 Hz, βH) 7.59 (s, J = 15.07 Hz, αH) 7.95–6.68 (m, 2′H, 3′H, 5′H, 6′H, 2H, 3H, 5H, 6H) 3.03 (s, –N–$(CH_3)_2$)
			N: calcd 5.24, found 5.39		
233	196–198	32.6	C, H,	275.0938 ($C_{18}H_{13}O_2N$ = 275.0946)	In DMSO, 12.42 (s, OH), 9.20 (d, 2H) 8.26 (s, J = 15.83 Hz, βH), 7.53 (s, J = 15.82 Hz, αH), 8.30–6.82 (m, 4H, 5H, 6H, 7H, 8H, 2′H, 3′H, 5′H, 6′H)
			N: calcd 5.09, found 5.18		
			C: calcd 69.76, found 70.04	258.0443 ($C_{15}H_{11}O_2Cl$ = 258.0447)	In DMSO, 12.77 (s, OH), 7.84 (m, J = 15.83 Hz, βH), 7.61 (m, J = 15.45 Hz, αH), 7.89–6.94 (m, 2H, 3H, 5H, 6H, 2′H, 3′H, 5′H, 6′H)
234	149–150	48.1	H: calcd 4.30, found 4.75		
			Cl: calcd 13.55, found 13.28		
235	114–115 120–121[b]	44.6	C, H	238.0986 ($C_{16}H_{14}O_2$ = 238.0994)	In DMSO, 12.88 (s, OH), 7.91 (s, J = 15.45 Hz, βH), 7.62 (d, J = 15.45 Hz, αH), 7.92–6.94 (m, 2H, 3H, 5H, 6H, 2′H, 3′H, 5′H, 6′H), 2.36 (s, CH_3)
236	89–91 91–92[b]	35.4	C, H	254.0946 ($C_{16}H_{14}O_3$ = 254.0943)	In DMSO, 12.95 (s, OH), 7.90 (s, J = 15.45 Hz, βH), 7.54 (s, J = 15.45 Hz, αH), 7.92–6.95 (m, 2H, 3H, 5H, 6H, 2′H, 3′H, 5′H, 6′H), 3.82 (s, OCH_3)
			C,	284.1049 ($C_{17}H_{16}O_4$ = 284.1049)	In DMSO, 12.90 (s, OH), 7.96 (d, J = 15.8 Hz, βH), 7.65 (d, J = 15 Hz, αH), 7.98–6.57 (m, 2H, 3H, 5H, 6H, 2′H, 3′H, 5′H, 6′H), 3.90 (s, OCH_3), 3.82 (s, OCH_3)
237	111–113	25.0	H: calcd 5.68, found 5.47		
			C: calcd 65.74, found 64.95	292.0704 ($C_{16}H_{11}O_2F_3$ = 292.0711)	In DMSO, 12.69 (s, OH), 8.03 (m, J = 15.82 Hz, βH), 7.70 (m, J = 15.45 Hz, αH), 8.11–6.90 (m, 2H, 3H, 5H, 6H, 2′H, 3′H, 5′H, 6′H)
238	< 50	46.4	H: calcd 3.80, found 3.78		
			F: calcd 19.51, found 19.71		
			C,	242.0732 ($C_{15}H_{11}O_2F$ = 242.0743)	In DMSO, 12.37 (s, OH), 7.88 (m, J = 15.45 Hz, βH), 7.64 (d, J = 15.45 Hz, αH), 8.08–6.88 (m, 2H, 3H, 5H, 6H, 2′H, 3′H, 5′H, 6′H)
239	< 50	43.5	H: calcd 4.58, found 4.50		
			F: calcd 7.85, found 7.62		
			C,	225.0785 ($C_{14}H_{11}O_2N$ = 225.0790)	In DMSO, 12.73 (s, OH), 8.43–8.38 (d, J = 15.45 Hz, βH), 7.77–7.72 (d, J = 15.45 Hz, αH), 8.82–6.93 (m, 3H, 4H, 5H, 6H, 2′H, 3′H, 5′H, 6′H)
241	89–91	25.3	H: calcd 4.93, found 5.03		
			N: calcd 6.22, found 6.38		
242	106–108	41.1	C, H	274.0993 ($C_{19}H_{14}O_2$ = 274.0994)	In DMSO, 12.42 (s, OH), 8.69 (d, J = 15.45 Hz, βH), 7.73 (d, J = 15.44 Hz, αH) 8.50–6.70 (m, 2H, 3H, 4H, 5H, 6H, 7H, 8H, 2′H, 3′H, 5′H, 6′H)
			C,	274.1003 ($C_{19}H_{14}O_2$ = 274.0994)	In DMSO, 12.16 (s, OH), 8.07–8.02 (d, J = 15.44 Hz, βH), 7.76–7.71 (d, J = 15.45 Hz, αH) 8.40–6.83 (m, 1H, 3H, 4H, 5H, 6H, 7H, 8H, 2′H, 3′H, 5′H, 6′H)
243	146–148	39.6	H: calcd 5.15, found 5.10		
			C,	275.0960 ($C_{18}H_{13}O_2N$ = 275.0946)	In DMSO, 12.39 (s, OH), 8.48–8.43(d, J = 15.45 Hz, βH), 8.37–8.31 (d, J = 15.45 Hz, αH), 9.27–6.93 (m, 2H, 3H, 5H, 6H, 7H, 8H, 2′H, 3′H, 5′H, 6′H)
244	151–153	11.2	H: calcd 4.76, found 4.89		
			N: calcd 5.09, found 5.21		

[a]Recrystallized from methanol.
[b]Cited in product catalog (2000/2001) of Indofine Chemical Company, Inc. (Somerville, NJ, USA).

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