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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 17 (2007) 1695–1700

Synthesis, antimycobacterial activity evaluation, and QSAR studies of chalcone derivatives

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Received 12 July 2006; revised 7 December 2006; accepted 22 December 2006
Available online 12 January 2007

Abstract—In order to develop relatively small molecules as antimycobacterial agents, twenty-five chalcones were synthesized, their activity was evaluated, and quantitative structure–activity relationship (QSAR) was developed. The synthesis was based on the Claisen-Schimdt scheme and the resultant compounds were tested for antitubercular activity by luciferase reporter phage (LRP) assay. Compound $\mathbf{C_{24}}$ was found to be the most active (~99%) in this series based on the percentage reduction in Relative Light Units at both 50 and 100 μg/ml levels, followed by compound $\mathbf{C_{21}}$. Four compounds at the 50 μg/ml and eight compounds at the 100 μg/ml showed activity above 90% level. QSAR model was developed between activity and spatial, topological, and ADME descriptors for the 50 μg/ml data. The statistical measures such as r, r^2, q^2, and F values obtained for the training set were in acceptable range and hence this relationship was used for the test set. The predictive ability of the model is satisfactory ($q^2 = 0.56$) and it can be used for designing similar group of compounds.

Tuberculosis is a major and challenging health problem around the world and the current survey says that about two million deaths are caused annually due to the highly infective acid fast bacilli *Mycobacterium tuberculosis*.[1–3] Tuberculosis is mostly asymptomatic and is aggravated when impairment of immunity arises due to conditions like malnutrition, diabetes, malignancy, and AIDS. The last condition is susceptible to *Mycobacterium avium* complex (MAC).[4] Treatment of tuberculosis is a complex process due to several factors which include patient's inability to persist with combined treatment regimen, the spreading ability of the nontubercular mycobacteria (NTM) like *M. avium* complex (MAC), the ineffectiveness of the drugs on immunosuppressed patients, and MDR (Multi Drug Resistance).[5] Streptomycin was introduced in 1944 for the treatment of tuberculosis which was followed by first line tuberculosis drugs such as isoniazid, rifampicin, ethambutol, and pyrizinamide, and later by second line of drugs. Multidrug regimen was found to be effective for the treatment of tuberculosis (Isoniazid + Rifampicin + Pyrizinamide + Ethambutol). MDR is observed in all front line and second line of drugs. Resistance toward isoniazid has arisen due to mutation of *katG* and *inhA*, resistance toward rifampicin due to mutation of *rpoB*-gene, resistance toward pyrizinamide due to mutation of *pncA* gene, etc.[6] Over the past twenty years design of new drugs to address drug resistance is in progress, with few successes.

Use of small molecules in the current treatment regimen encouraged us to work on chalcone derivatives.[7] Moreover licochalcone, a natural product obtained from *Glycyrrhiza inflata*, showed low MICs (between 5 and 20 mg/L) against three of the species such as *M. tuberculosis*, *M. avium*, and *Mycobacterium bovis*.[8] Chalcones and flavanoids have also been found to be effective against *M. tuberculosis* H37Rv which proved that 2′-hydroxy substitution favors antimycobacterial activity.[9] Chalcones are known to posses antibacterial activity and, dimethylamino chalcones have been reported as iNOS inhibitor[10] (literature evidence points that NOS2 gene expression is involved in the production of NO in primary TB).[11] This has encouraged us to include methylthio and dimethyl amino substitutions at the R^3 position. In addition, effect of methoxy substitution at various positions in the A- and B-rings has also been studied in this paper (see Scheme 1).

The following synthetic procedure has been adopted for the preparation of twenty-five prop-2-en-1-one derivatives.[12]

Keywords: Chalcones; Antimycobacterial activity; QSAR.
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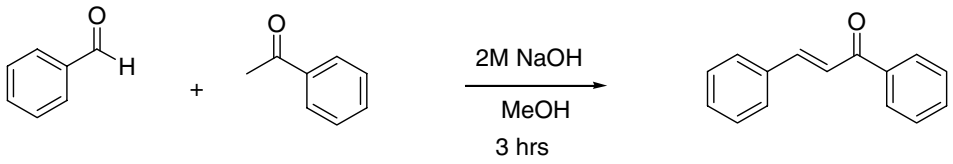
doi:10.1016/j.bmcl.2006.12.112



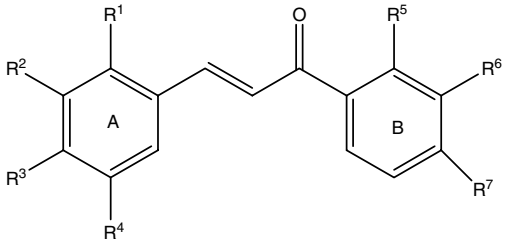
Scheme 1. Synthetic route for prop-2-en-1-one derivatives.

Aldehyde (10 mmol) and acetophenone (10 mmol) derivatives were added and stirred in methanol (10 ml) at room temperature. When 2 M NaOH was added under rapid stirring to the above mixture, in most of the cases an almost pale yellowish solid precipitated out. The solid was washed with 50% alcohol, followed by water and then dried. In majority of the cases the yield was more than 80%. Synthesized compounds were characterized by FT-IR, NMR, and mass spectrometry. Spectral data for compound 17 ($\mathbf{C_{17}}$) given in reference.[28]

Antimycobacterial activity of the synthesized compounds was evaluated by luciferase reporter phage assay method[13] against *M. tuberculosis H37Rv* at two concentrations (50 and 100 μg/ml) and the observed percentage inhibition is tabulated in Table 1. A compound is considered to be an antimycobacterial agent if fifty percent reduction in the Relative Light Units (RLU) is observed when compared to the control using a luminometer.

Reports indicate that substituted (either with electron-donating or electron-withdrawing groups) or unsubstituted A-ring did not affect the biological activity.[14] But A-ring with hydrophobic substitution showed increased anti-tubercular activity.[9] In the present study, introduction of substitutions like $-NMe_2$, –OMe, –SMe irrespective of their positions in the A-ring exhibited higher activity when compared to the parent compound $\mathbf{C_4}$ (without any substitution in the A-ring). Hydrophilic substituents such as $-NO_2$ or –OH in B-ring

Table 1. Structures and experimental antitubercular activity of chalcone derivatives



Compound	A-ring				B-ring			% Reduction in RLU		*C*log *P*
	R^1	R^2	R^3	R^4	R^5	R^6	R^7	50 μg/ml (data)	100 μg/ml (data)	
C_1			NMe_2					70.02	70.18	3.789
C_2			SMe					46.29	66.51	4.183
C_3			SMe				NO_2	30.95	48.06	4.088
C_4								41.93	50.64	3.624
C_5			SMe			OH		31.59	98.86	4.057
C_6			SMe				OMe	7.36	17.38	4.403
C_7			SMe		OH			68.41	90.52	4.517
C_8			SMe				Me	16.88	51.59	4.682
C_9			SMe				Cl	24.06	48.83	4.972
C_{10}			SMe			NO_2		0	0	4.088
C_{11}			OMe					58.75	65.08	3.543
C_{12}			SMe		Cl		Cl	0	0	5.451
C_{13}	OMe							66.07	72.98	3.543
C_{14}			SMe				Br	0	21.87	5.122
C_{15}		OMe	OMe	OMe				43.16	63.9	2.924
C_{16}			SMe				OH	29.25	55.16	4.057
C_{17}			OMe	OMe	Cl		Cl	0	12.17	4.550
C_{18}	OMe		OMe			NO_2		92.19	96.45	3.537
C_{19}			OMe	OMe		NO_2		64.61	89.27	3.187
C_{20}			NMe_2			NO_2		90.06	96.22	3.694
C_{21}	OMe						NO_2	94.30	98.15	3.448
C_{22}			NMe_2		Cl		Cl	31.55	33.8	5.057
C_{23}			OMe				Me	60.24	98.52	4.042
C_{24}			OMe			OH		98.90	99.04	3.417
C_{25}	OMe					NO_2		84.16	97.94	3.448

enhanced the antimycobacterial activity. Eight compounds namely **C_5**, **C_7**, **C_{18}**, **C_{20}**, **C_{21}**, **C_{23}**, **C_{24}**, and **C_{25}** exhibited greater than 90% inhibition activity at 100 μg/ml level. In this group of compounds except for **C_7**, **C_{21}**, and **C_{23}**, other five compounds have substitution at the R^6 position. Compound **C_{19}** with 89.3% activity with $-NO_2$ at the R^6 position just falls outside the above group. Nine compounds show inhibition activity between 50% and 90%, and hence can also be considered active. In this group, except for compounds **C_8**, **C_{16}**, and **C_{19}** other six compounds do not have any substitution in the B-ring. Halogen substitution at the R^7 position inactivates the chalcone derivatives (**C_9 and C_{14}**), and introduction of an additional halogen in the B-ring increases the hydrophobicity of the B-ring reducing the activity further (**C_{12}**, **C_{17}**, **and C_{22}**). Moreover, nitro and methoxy substitution in the B ring at R^7 position yields inactive compounds (**C_3** and **C_6**) at the 100 μg/ml level. In brief at 50 μg/ml level, 11 compounds are active in nature, out of which four compounds (**C_{18}**, **C_{20}**, **C_{21}**, and **C_{24}**) have more than 90% activity and the rest of the 14 compounds are inactive (less than 50% inhibition).

It is reported that there is a relationship between % inhibition and *C*log *P*.[7,15] Percentage inhibition increased with increase in *C*log *P*. Lipinski's rule states that compounds with *C*log *P* greater than 5 will not be active, which is also observed in our studies (**C_{12}**, **C_{14}**, and **C_{22}** are inactive). *C*log *P* is an indication of the lipophilicity of molecules, and more lipophilic molecules can easily enter the lipid-enriched mycobacterial cell wall.[16] Cluster analysis for antimycobacterial activity at 50 μg/ml is shown in Figure 1. The figure shows four clusters based on their activity at 50 μg/ml. Clusters 1 and 2 contain moderate and highly active compounds. Cluster 2 has compounds with activity in the range of 80–100% reduction in RLU due to their hydrophilic substituents at the B-ring. Fifty percent of compounds in Cluster 1 does not have any substitution in B-ring. Clusters 3 and 4 have inactive compounds, which is due to the introduction of hydrophobic substituents in the B-ring. Cluster 4 has compounds whose activity is in the range of 0–10%. From these observations it is evident that antimycobacterial activity totally depends on the substitution in the B-ring and substitution in the A-ring has little impact.

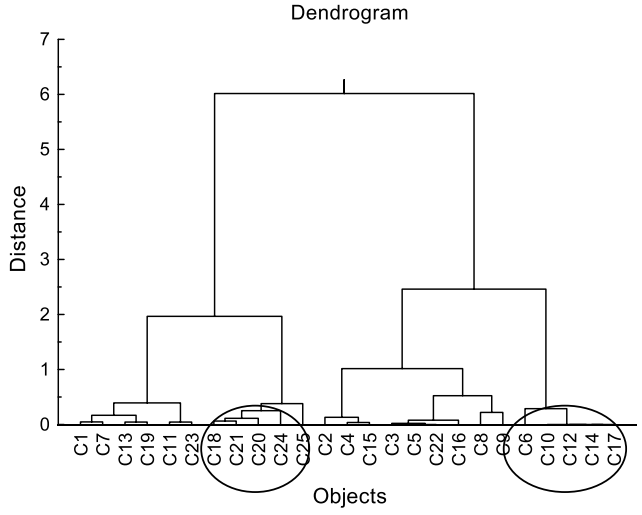


Figure 1. Cluster analysis of compounds at 50 μg/ml concentration level.

The structure of the various molecules as listed in Table 1 was drawn and the minimum energy conformation was determined using Cerius^2 software® using Universal force field (Acceryls Inc., USA). Two hundred and forty nine descriptors that included topological, charge, geometrical, aromaticity indices, constitutive properties, quantum mechanics, and thermodynamics were evaluated for each compound. Several literature reports give a very detailed description of descriptors.[17–19] Equations were developed between the observed activity and the descriptors. The set of descriptors that would give the statistically best models (r, r^2, $q^2 > 0.5$) were selected from the large pool using a Genetic function approach. While r^2 is an indication of the model data fit, q^2 is an indication of the predictive capability of the model.

QSAR was developed between the observed activity (Table 2) and various molecular descriptors for the 50 μg/ml data set. Twenty-one compounds from this data set were divided into training and test sets, the former set consisting of 16 randomly chosen compounds and the remaining in the latter set. Four compounds with RLU equal to zero were not included for developing the QSAR. The model developed using the training set was used to predict the activity of the compounds in the test set. The multi-linear regression model with 16 compounds gave very good fit. The best model was selected based on the r, r^2, F-ratio, and q^2. The predictive capability of the equation is determined using leave-one-out cross-validation method. The relation for q^2 is as shown below,

$$q^2 = 1 - \mathrm{PRESS}/\mathrm{TOTAL}$$

$\sum(Y_{\mathrm{predicted}} - Y_{\mathrm{observed}})^2$ is the predictive error sum of squares (=PRESS). $\sum(Y_{\mathrm{observed}} - Y_{\mathrm{mean}})^2$ is the total sum of squares (=TOTAL), where $Y_{\mathrm{predicted}}$, Y_{observed}, and Y_{mean} are the predicted, observed, and mean values of activity, respectively. The activity is defined as $\log(p/100 - p)$, where p is the percent reduction in RLU at the 50 μg/ml.

The best QSAR is given in Table 3 with corresponding r^2, q^2, F ratio, and standard error between the model predictions and data. Figure 2 shows a comparison of the experimental and predicted activity for the 16 compounds taken in the training set at 50 μg/ml concentration level. Table 2 lists the values of the selected descriptors and the model-predicted activity of the training set (16 compounds). Cross-correlation between these selected descriptors is given in Table 4. A low cross-correlation indicates that the least correlated descriptors are chosen for developing the QSAR, which is generally desired. Figure 3 shows a comparison of the experimental and predicted activity for the five compounds selected in the test set with the 95% confidence regions. Here

Table 2. Values of the selected descriptors and the predicted activity of the training set (16 compounds)

Compound	ADME_solubility_level	CHI-V-1	Shadow-Zlength	Observed activity = log(p/100 − p)	Predicted activity	Residual
C_2	2	6.93398	5.24383	−0.065	−0.283	0.218
C_3	2	7.43342	5.3824	−0.349	−0.536	0.187
C_4	2	5.18617	4.02785	−0.141	0.292	−0.433
C_5	2	7.06827	5.3608	−0.336	−0.317	−0.019
C_7	2	7.07425	7.6019	0.336	0.63	−0.294
C_8	2	7.34466	5.20032	−0.692	−0.558	−0.134
C_{11}	2	5.70923	4.26311	0.154	0.065	0.089
C_{16}	2	7.06827	6.10955	−0.384	0	−0.384
C_{18}	2	6.74369	7.41913	1.072	0.759	0.313
C_{19}	2	6.74369	7.31911	0.261	0.716	−0.455
C_{20}	2	6.72031	6.47091	0.957	0.371	0.586
C_{21}	2	6.21465	7.47266	1.219	1.112	0.107
C_{22}	2	7.17614	6.10791	−0.336	−0.068	−0.268
C_{23}	2	6.11991	5.10662	0.18	0.167	0.013
C_{24}	3	5.84352	5.11937	1.954	1.954	0
C_{25}	2	6.21465	5.44659	0.725	0.252	0.473

Table 3. QSAR of 50 μg/ml data

	n	r^2	r^2_{adj}	q^2	F	Standard error
−1.398 + 1.609 * ADME_solubility_level − 0.624 * CHI-V-1 + 0.424 * Shadow-Zlength	16	0.81	0.76	0.56	17.08	0.325

n, number of molecules in the data set; r^2, squared correlation coefficient; q^2, cross-validated squared correlation coefficient.

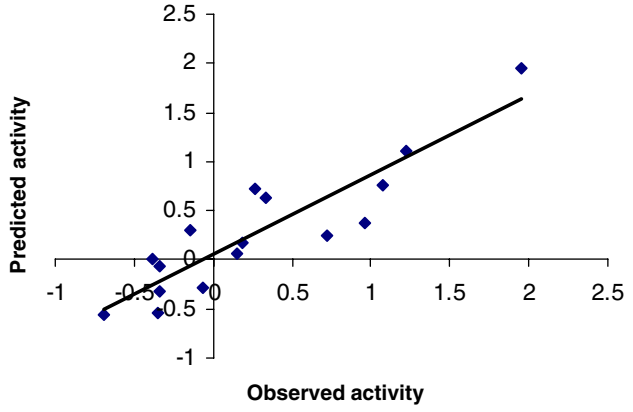


Figure 2. Comparison of observed and predicted activities of the compounds in the training set.

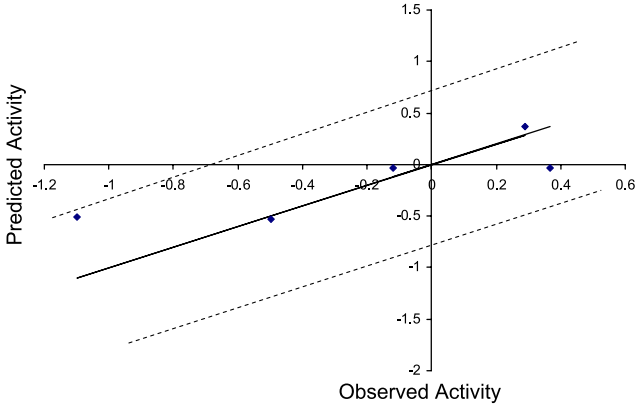


Figure 3. Comparison of observed and predicted activities of the compounds in the test set (dotted lines indicate 95% confidence limits).

Table 4. Matrix of correlation between the selected descriptors

	ADME_solubility_level	CHI-V-1	Shadow-Zlength	Activity
ADME_solubility_level	1			
CHI-V-1	−0.311042	1		
Shadow-Zlength	−0.173082	0.430808	1	
Activity	0.616005	−0.447219	0.326398	1

the activity was predicted using the QSAR developed with the training set. The r^2 (squared correlation coefficient between this observed and the predicted activity) was found to be 0.70.

ADME_solubility_level, CHI-V-1, and shadow-Zlength are the three descriptors that appear in the QSAR at 50 μg/ml concentration level. Contributions of ADME properties and connectivity indices toward TB activity have been observed by us for chalcones, chalcone-like compounds, flavones, and flavonones.[20] ADME_solubility_level indicates the aqueous solubility of the molecule. It is reported that aqueous solubility of organic compounds can be predicted from molecular descriptors such as number of H-bond donor and acceptors, dipole moment, number of rotatable bonds, and surface area.[21] Aqueous solubility plays an important role in drug absorption. Palm et al. explained that the dynamic polar

molecular surface area (PSA_d) is also an ideal surface descriptor for the differentiation of drugs which in turn considers three-dimensional shape and flexibility of the molecules[22]. Tangallapally et al. observed that hydrophilic substitutions improved the solubility of cyclic secondary amine-substituted phenyl and benzyl nitrofuranyl amides. The improved solubility improved the antimycobacterial activity.[23]

CHI-V-1 is the valence modified connectivity index, which is an indication of the number of bonds in the molecule. Importance of connectivity indices in virtual screening of new active agents against *M.avium* complex is explained by Garcia et al.[24] Shadow indices are a set of geometric descriptors that characterizes the shape of the molecule and Shadow-*Z*length is the length of the molecule in the *Z* dimension. This shows positive contribution in the QSAR equation, which means that increasing the length of the molecule (also related to hydrophobicity) increases the antimycobacterial activity of the compound.

The above short-listed descriptors describe the molecular size, degree of branching, flexibility, overall shape, and aqueous solubility and it is known that they in turn are related to the hydrophlicity–hydrophobicity ratio of the molecule (log*p*). Our studies corroborate the previous research that hydrophobicity[25–27] and solubility of the compound play an important role in the antitubercular ctivity.

Acknowledgment

Authors thank Dr. Moni (SAIF, IIT) for providing spectral analysis.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.12.112.

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28. selected spectral data for $\mathbf{C}_{17}$: IR (KBr) 1659 cm^{-1} (Carbonyl), ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.92 (s, 6 H), δ 7.26–7.48 (m, 3H), δ 6.88 (d, 1H), δ 6.96 (d, 1H) $J = 16$ Hz, δ 7.08 (s, 1H), δ 7.14 (d, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 192.71, 151.89, 149.31, 146.98, 137.67, 136.61, 132.24, 130.23, 130.11, 127.21, 127.17, 123.98, 123.58, 111.09, 109.98, 56.01, and 55.92. ESI MS: $[M]^+$ 337.20.