REPORT

Insight into the lipophilicity of selected monosubstituted chalcones

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Abstract: This study represents our attempt to understand how the antimicrobial activity of chalcones is modulated by their lipophilicity. To achieve this overall objective, a library of monosubstituted chalcones was targeted after careful consideration of the stereo electronic properties of the substituents appended in each of its constituent members. The lipophilicities of these derivatives were determined experimentally as well as by means of different validated computational programs. The theoretical determination was necessitated by the long-winded and time-consuming experimental protocols involved. It was gratifying to note the good correlation between these determinations which indicated the suitability of using such theoretical descriptors not only for assessing the lipophilicity of putative lead molecules but also for evaluating their biological activity. Standard disc diffusion technique employed against gram positive & negative bacteria as well as fungi revealed some preliminary information about the antimicrobial activity of these analogues.

Keywords: Lipophilicity, monosubstituted chalcones, partition coefficient, thin layer chromatography and antimicrobial activity.

INTRODUCTION

The pharmacokinetic and pharmacodynamic properties of a drug substance primarily depend on its lipophilicity. The importance of hydrophobicity (or lipophilicity) in tuning the biological activity of molecules has been clearly understood by the application of quantitative structure activity relationship (QSAR) methods to large compound libraries (Podunavac-Kuzmanović *et al.*, 2008). Through such an analysis, it is possible to mathematically estimate the inhibitory activity of drugs upon determining their lipophilicity values (Ghose *et al.*, 1988; Hopfinger and Battershell, 1976; Margabandu and Subramani, 2010). This property is usually characterized by the partition coefficient (log P), which, in turn, is determined from distribution studies of the compound between an immiscible polar and non-polar solvent pair. This quantitative descriptor of lipophilicity is one of the main determinants of the so-called absorption, distribution, metabolism and excretion (ADME) profile of a pharmaceutical entity (Hansch *et al.*, 1995; Lin and Lu, 1997; Van de Waterbeemd *et al.*, 2001). Among the practical approaches to measure log P - thin layer chromatography (TLC) - often used in case of rather hydrophobic compounds, represents an experimental alternative to shake-flask partition coefficient method.

A privileged group of compounds called chalcones act as biosynthetic precursors to the naturally abundant flavonoid family that is found in a variety of plant species. Structurally, the chalcone scaffold consists of two aromatic rings (conventionally referred to as ring-A and ring-B) which are linked by a three carbon α,β-unsaturated carbonyl system (1, fig. 1A). This linker fragment is regarded as the main pharmacophore since its partial or complete removal leads to a loss of bioactivity. Such an inherently simple chemical assembly coupled with diverse pharmacological profiles of these moieties have generated intensive scientific studies throughout the world (Dhar, 1981; Rahman, 2011). Along with their derivatives, chalcones are associated with different biological activities like anti-inflammatory (Balasubramanian and Vijayagopal, 2012), antioxidant (Iqbal *et al.*, 2014), anticancer (Prabhakar *et al.*, 2014) and antimicrobial (Awasthi *et al.*, 2009) activities to name a few.

The study described herein shall help to determine the best descriptor of log P leading to an optimized and most significant QSAR model, which could then be used to link the structure of these compounds with their inhibitory activity. In particular, the aim of the present study was to evaluate the activity of a mini series of monosubstituted chalcones against different microorganisms and to understand the role played by lipophilicity in antibacterial activity.

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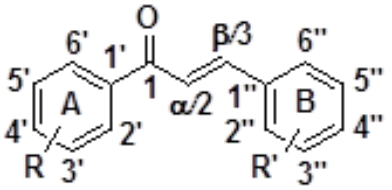
MATERIALS AND METHODS

Design, synthesis and characterization of target chalcones

Apart from the fully unsubstituted parent chalcone (2, fig. 1B) that was taken as the base compound, seven ring-A monosubstituted derivatives were designed based on fundamental medicinal chemistry principles. The task was to choose and append such substituents in ring-A that contained functional groups capable of withdrawing as well as releasing electrons by inductive and/or mesomeric effects (Balasubramanian and Vijayagopal, 2012). A focused library of this nature would, in principle, facilitate us to unambiguously implicate a specific substituent to pharmacological activity. The target compounds (fig. 1B) included 3'-nitrochalcone (3), 4'-cyanochalcone (4), 4'-trifluoromethylchalcone (5), 4'-methoxychalcone (6), 2'-methylchalcone (7), 4'-benzenesulphonamide chalcone (8) and 4'-phenylchalcone (9). The desired chalcones were synthesized via a single step Claisen-Schmidt condensation and characterized by ^{1}H as well as ^{13}C nuclear magnetic resonance (NMR) spectroscopy, Fourier transform infrared (FT-IR) spectroscopy and mass spectrometry (Jayapal *et al.*, 2010). The detailed synthetic protocols, physicochemical properties of the synthesized analogues and the spectral signals and/or bands have already been described elsewhere (Balasubramanian *et al.*, 2013).



R & R' are variable
1,3-diaryl-prop-2-ene-1-ones (**1**)
A
R = R' = H (parent chalcone **2**)
R = 3'-NO_2, R' = H (derivative **3**)
R = 4'-CN, R' = H (derivative **4**)
R = 4'-CF_3, R' = H (derivative **5**)
R = 4'-OCH_3, R' = H (derivative **6**)
R = 2'-CH_3, R' = H (derivative **7**)
R= 4'-$C_6H_5SO_2NH$, R' = H (derivative **8**)
R = 4'-C_6H_5, R' = H (derivative **9**)
B

Fig. 1: Representation of the A. prototypical chalcone scaffold (1) and B. target chalcones (parent 2 and derivatives 3-9).

Determination of lipophilicities

Precoated silica gel plates (60 F_{254}, Merck-Germany) were employed for R_f value determination. An optimized binary mobile phase of dichloromethane (DCM)/hexane has been used for running the TLC's (Costanzo, 1997). Seven DCM/hexane mixtures ranging in concentrations from the more non-polar 25/75 mixture to the rather polar 60/40 ratio were prepared apart from the base composition of 50/50 that is moderately polar. All eight target chalcones were spotted in these eight different DCM/hexane mixtures and their R_f values were determined. The plates were developed and product spots visualized under an ultraviolet (UV) lamp and/or iodine chamber. The theoretical log P descriptor values (indicated in parentheses) were determined via different online software programs like ALOGPS & Chem Bio Draw Ultra 11.0 (Ac log P, A log P & C log P), Moriguchi log P model (M log P), Chem Axon log P Calculator (X log P2 & X log P3), Spartan (Ghose-Crippen log P, Villar log P & HINT log P) & ADMET predictor model (ADME log P). Linear regression analysis was carried out using Microsoft Excel and the best theoretical descriptor of log P was chosen based on correlation coefficient values obtained from this regression analysis (Kellogg *et al.*, 1992).

Evaluation of antimicrobial activity

Standard protocol for the Kirby-Bauer (KB) test, popularly known as disk diffusion method, was used for antimicrobial screening (Drew *et al.*, 1972). *Escherichia coli* and *Staphylococcus aureus* were the gram negative and gram positive organism respectively for antibacterial study while *Candida albicans* was the fungus of choice. The media used for the bacterial strains was Mueller Hinton agar while Potato dextrose agar was used for antifungal study. The stock solution of the test compounds was prepared in dimethyl sulfoxide. After allowing for incubation and drug diffusion, the zone diameter was measured 16-18 hours (for bacterial strains) and 36-40 hours (for fungal strains) post inoculation. These readings were then compared with the zone diameter exhibited by standard antibiotics i.e., gentamicin (10 µg/disc) in case of gram negative strain, ciprofloxacin (5 µg/disc) in case of gram positive strain and fluconazole (25 µg/disc) in case of fungal strain (Alvarez *et al.*, 2004; Batovska *et al.*, 2009; López *et al.*, 2001; Lunardi *et al.*, 2003).

RESULTS

The R_f values of each compound was determined in triplicate in eight different compositions of the optimized binary mobile phase and the average readings are indicated in table l. It may be noted that these average values were taken for calculating the R_M value using equation (a). The linear relationship between R_M values and the experimental lipophilicity of the compounds, indicated by R_{Mo}, is described as per equation (b) and the corresponding data shown in table l:

$$R_M = \log (1/R_f - 1) \quad (a)$$

$$R_M = R_{M0} + bC \quad (b)$$

Where b is the slope and C is the concentration of the polar component of the mobile phase. Thus, the R_{M0} value of each of the target chalcone was graphically determined by plotting R_M (y-axis) and the DCM concentration (x-axis). The intercepts of these graphs, which would then represent R_{M0} as per the equation of the straight line in (**b**), were obtained by extrapolating the line to zero concentration of DCM so that it intersects the y-axis.

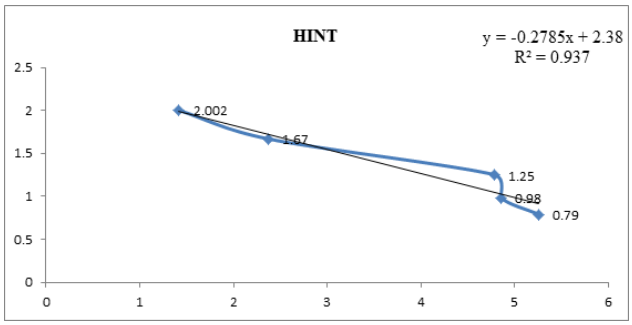


Fig. 2: R_{M0} (y-axis) v/s HINT log P (x-axis).

Ten different theoretical lipophilicity descriptors viz. Ac log P, A log P, M log P, X log P2, X log P3, Ghose-Crippen, Villar, HINT, ADME log P and C log P were selected based on their free availability and user-friendliness. Subsequently, log P values of all the target chalcones corresponding to each descriptor were computed and the results have been displayed in table 2.

The next aspect of the work represents our attempt to correlate, via linear regression analysis, the experimental lipophilicity (R_{M0}) values that have been determined chromatographically with the log P values theoretically computed through various descriptors as noted above. A graphical treatment in this regression analysis generated a family of straight lines, a representative member of which is illustrated in fig. 2.

The test chalcones were then subjected to standard KB test in which zone of inhibition is used as a barometer to evaluate the antimicrobial efficacy of a compound. As noted in table 3, the experiments were performed in triplicate and average values of the zone of inhibition (in mm) were calculated.

DISCUSSION

The R_{M0} values involve a rather simplistic approach whereas the inbuilt algorithms of the various online modules incorporate a rigorous analysis for the computation of lipophilicity. As a result, the experimental R_{M0} values as observed in table 1 are a gross underestimation of the values corresponding to the theoretical lop P descriptors (table 2) that reflect a more realistic estimate of a compound's hydrophobicity. It is worthwhile pointing out that the trends in lipophilicity values across the compound series derived from both these types of estimations is quite similar, a fact that makes the further analysis meaningful. The parent chalcone has a higher lop P value, on an average, compared to the nitro-, cyano- and the methoxy derivatives (3, 4 and 6 respectively). Moreover, the lipophilicity of these three derivatives is almost identical indicating that their relative polarities are similar. While the identical behavior of the nitro- and cyano derivatives is expected as both functional groups are electron withdrawing, the similar polarity of the electron releasing methoxy derivative presents an contrasting scenario. Likewise, log P values of chalcones 5, 7 and 8 can be considered similar enough for these derivatives to be grouped together on a polarity basis although the chalcone appended with the trifluoromethyl moiety (5) is slightly more lipophilic than the other two. Once again, the electronics of the substituent(s) does not seem to be a major factor in a compound's lipophilicity. However, there is consensus amongst all the ten theoretical log P descriptors in projecting the phenyl-substituted chalcone (9) as being the most lipophilic member of this library. This finding is along expected lines as steric bulk counts as a significant determinant of lipophilicity. In fact, all the descriptors other than M log P and X log P3 spit out values greater than the generally accepted log P maximum of 5 for orally available candidates. Interestingly, the benzenesulphonamide-based derivative 8 has the highest experimental R_{M0} value contrary to the consensus compound 9 as determined computationally.

Examining the extent of correlation between the lipophilicities determined by experimental versus computational techniques is an integral step towards achieving the overall objective of this study. Nine other graphs were obtained analogous to the one in fig. 2 (data not shown). In fact, all these linear relationships may be expressed in a rather concise manner by equations (c) to (l). This data would eventually help us better appreciate the role of lipophilicity in the antimicrobial activity of these chalcones.

$$R_{M0} = - 0.896 \times \text{Ac log P} + 4.54 - \quad (c)$$
$$R_{M0} = - 0.796 \times \text{A log P} + 4.2927 - \quad (d)$$
$$R_{M0} = - 0.6068 \times \text{M log P} + 3.6029 - \quad (e)$$
$$R_{M0} = - 0.7039 \times \text{X log P2} + 4.2168 - \quad (f)$$
$$R_{M0} = - 0.806 \times \text{X log P3} + 3.8854 - \quad (g)$$
$$R_{M0} = - 0.6428 \times \text{Ghose-Crippen} + 3.8645 - \quad (h)$$
$$R_{M0} = - 0.1897 \times \text{Villar} + 0.5374 - \quad (i)$$
$$R_{M0} = - 0.2785 \times \text{HINT} + 2.38 - \quad (j)$$
$$R_{M0} = - 0.7701 \times \text{ADME} + 4.4849 - \quad (k)$$
$$R_{M0} = - 0.6774 \times \text{C log P} + 3.8937 - \quad (l)$$

It is evident from fig. 2 that five data points were considered while generating this graph. In this regard, we would like to clarify that from this point onwards, only five of the eight target chalcones were employed in the

Table I: R_f, R_M and R_{M0} values of target chalcones

% DCM	Average R_f values of compound							
	2	3	4	5	6	7	8	9
25	0.28	0.05	0.10	0.31	0.19	0.38	0.02	0.14
30	0.30	0.10	0.13	0.41	0.23	0.48	0.04	0.20
35	0.39	0.21	0.17	0.49	0.27	0.53	0.06	0.27
40	0.47	0.29	0.22	0.53	0.34	0.58	0.09	0.33
45	0.51	0.36	0.29	0.61	0.39	0.64	0.13	0.43
50	0.59	0.39	0.37	0.66	0.49	0.69	0.20	0.51
55	0.65	0.49	0.45	0.69	0.56	0.73	0.24	0.60
60	0.69	0.55	0.51	0.73	0.62	0.74	0.27	0.67
% DCM	Average R_M values of compound							
	2	3	4	5	6	7	8	9
25	0.41	1.27	0.95	0.34	0.62	0.21	1.69	0.78
30	0.36	0.95	0.82	0.15	0.52	0.03	1.38	0.60
35	0.19	0.57	0.68	0.01	0.43	-0.05	1.19	0.43
40	0.05	0.38	0.54	-0.05	0.28	-0.14	1.00	0.30
45	-0.01	0.24	0.38	-0.19	0.19	-0.24	0.82	0.12
50	-0.15	0.19	0.23	-0.28	0.01	-0.34	0.60	-0.01
55	-0.26	0.01	0.08	-0.34	-0.10	-0.43	0.50	-0.17
60	-0.34	-0.08	-0.01	-0.43	-0.21	-0.45	0.43	-0.30
	Experimental lipophilicities							
Compound	2	3	4	5	6	7	8	9
R_{M0}	0.99	2.00	1.67	0.80	1.26	0.62	2.49	1.52

Table 2: Theoretically determined log P values of the test compounds

Compound	Ac log P	A log P	M log P	X log P2	X log P3	Ghose-Crippen	Villar	HINT	ADME	C log P
2	3.51	3.70	3.70	3.85	3.08	3.76	4.00	4.86	3.95	3.62
3	3.38	3.60	3.60	3.74	2.91	3.79	4.55	1.42	3.93	3.53
4	3.32	3.58	3.32	3.57	2.80	3.79	4.03	2.37	3.71	3.23
5	4.27	4.64	4.64	4.77	3.96	4.68	4.56	5.26	4.84	4.64
6	3.40	3.69	3.40	3.76	3.05	3.63	3.97	4.79	4.00	3.84
7	3.82	4.19	4.01	4.28	4.05	4.25	4.24	5.4	4.53	4.12
8	4.12	4.66	3.63	4.54	4.00	#	#	#	4.30	4.42
9	5.19	5.22	4.95	5.79	4.71	5.43	5.83	6.73	5.93	5.51

[#] Right parameters not available to carry out the computation

study. The need for eliminating three of the compounds [2'-methylchalcone (7), 4'-benzenesulphonamide chalcone (8) and 4'-phenylchalcone (9)] from the test set arose due to reasons pertaining to the evaluation of their antimicrobial activity. While 2'-methylchalcone, being a viscous oil, could not be tested due to operational issues, the other two derivatives were completely inactive against all the microbes. Hence, the inclusion of these congeners would render the further analysis with respect to structure function correlation meaningless.

From the correlation coefficient and standard error of mean (SEM) for equations (c) to (l) listed in table 4, it is obvious that HINT possesses a significantly higher correlation coefficient than any of the other descriptors and hence can be conveniently used instead of R_{M0}. In other words, HINT leads to the best fit among all the descriptors and this linear relationship has already been depicted in fig. 2. However, it is very important to appreciate the contextual nature of this correlation and realize that HINT log P is not a universally superior descriptor. An interesting aspect of the correlation that has been arrived at in this scenario is the fact that both the best and the worst (Villar) theoretical descriptors are modules of the same software Spartan.

Based on the data in table 3, no zone of inhibition was evident against *E. coli*. To corroborate this observation, the compounds were also tested against *Pseudomonas aeruginosa*. The lack of activity against both these organisms point to the fact that the target chalcones are perhaps inactive against gram negative microbes.

Table 3: Zone of inhibition of target chalcones on various strains

Organism & strain	Exp. No.	Compound coding (100 µg/well or 100 µL or 1 mg/mL solution)					
		2	3	4	5	6	Standard
E. coli (NCIM 2574)	1	*	*	*	*	*	27
	2	*	*	*	*	*	25
	3	*	*	*	*	*	28
	Average	*	*	*	*	*	26.66
P. aeruginosa (NCIM 2036)	1	*	*	*	*	*	21
	2	*	*	*	*	*	22
	3	*	*	*	*	*	21
	Average	*	*	*	*	*	21.33
C. albicans (NCIM 2079)	1	15	13	12	13	13	40
	2	18	10	13	12	12	26
	3	16	11	13	10	12	24
	Average	16.33	11.33	12.66	11.66	12.33	30
S. aureus (NCIM 3471)	1	15	13	15	12	17	28
	2	16	11	13	10	14	26
	3	15	10	14	11	15	28
	Average	15.33	11.33	14	11	15.33	27.33

* Zone of inhibition not detected

Moderate antimicrobial activity was observed against the gram positive and fungal strains. Interestingly, the unsubstituted parent chalcone 2 exhibited the best antimicrobial activity among the congeners screened.

Lipophilicity of the five chalcones was then correlated with their zones of inhibition measured against *S. aureus* and *C. albicans*. Such a correlation was not carried out for the gram negative strains owing to the absence of a detectable zone of inhibition in this case. Both the experimental log P (R_{M0}) as well as the best theoretical log P (HINT log P) were independently and separately correlated with the compounds' zones of inhibition by linear regression analysis that yielded equations (m) to (p).

Inhibition for *S .aureus* = - 0.7389 × Experimental log P + 14.385 - (m)

Inhibition for *S. aureus* = - 0.3796 × HINT log P + 11.98 - (n)

Inhibition for *C. albicans* = - 1.6514 × Experimental log P + 15.068 - (o)

Inhibition for *C. albicans* = - 0.4469 × HINT log P + 11.191 - (p)

The study was completed by carrying out statistical analysis involving the calculation of correlation coefficient and SEM (table 5) for each of the four linear relationships estimated. In general, the data suggests poor correlation between the properties under investigation. A major reason for such a result could be due to our underlying assumption that antimicrobial activity is solely determined by lipophilicity which may not be appropriate in all contexts. Furthermore, even if the activity was a function of only the lipophilicity, the relationship may not necessarily be linear in nature which constitutes our second layer of assumption.

CONCLUSION

There exists a good correlation between the experimental and theoretical lipophilicites. HINT log P emerged as the best descriptor for this group of compounds and can therefore be reliably used for quantitating structure function relationships pertaining to other biological activities. All the compounds were found to be inactive against gram negative strains while exhibiting moderate activity against gram positive bacteria and fungi. Moreover, chalcone 2 was superior in this regard compared to the other substituted derivatives and a general trend of enhancement in antimicrobial activity with an increase in lipophilicity was observed. It is likely that a single substituent on ring-A is not sufficient to impart antimicrobial activity and that additional substituent (s) in one or both of the phenyl rings may be necessary. It is expected that this preliminary information will come in handy during further design considerations involving this scaffold.

ACKNOWLEDGEMENTS

The authors deeply appreciate the insightful comments provided by Dr. Pietro Cozzini affiliated to the University of Parma, Italy during the course of the work. We are thankful to Ms. Aparna Banerji, Ms. Shahna V. and Ms. Hiba Iqbal for their assistance in performing the various experiments described herein.

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