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RESEARCH ARTICLE

DESIGN AND *IN SILICO* ANALYSIS OF RING-A MONOSUBSTITUTED CHALCONES AS POTENTIAL ANTI-INFLAMMATORY AGENTS

Ranganathan Balasubramanian* and Rachana Vijaya Gopal

Department of Pharmaceutical Chemistry, Amrita School of Pharmacy, Kochi-682 041, Kerala, India

**E-mail*: ranga.balasubramanian@gmail.com
Tel.: +91-9995640710.

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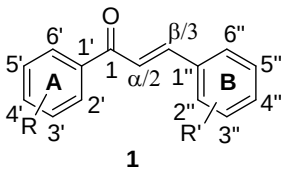
A 51-membered library of simple chalcones bearing a single substituent on ring-A was rationally designed. All the compounds therein were minimized using CORINA software and the target protein cyclooxygenase (COX) was normalized using CHIRON energy minimization server. Subsequently, each member of the library was docked onto an optimal, energy-minimized conformation of the human cyclooxygenase target using ArgusLab and AutoDock programs. About ten of these ligands had high docking scores, exhibited drug-likeliness and appeared to have enhanced binding to COX compared to the clinical anti-inflammatory agent - ibuprofen.

Key words: Chalcones, Rational design, Molecular docking, Anti-inflammatory agents.

INTRODUCTION

Chalcones are natural or synthetic *trans*-1,3-diaryl-2-propen-1-ones **(Figure 1)** belonging to the flavonoid family of natural products. Chemically, they contain an open-chain flavonoid skeleton in which two aromatic rings are linked by a three-carbon *α,β*-unsaturated carbonyl system (Rahman, 2011).



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R & R' are variable;
1,3-diaryl-prop-2-ene-1-ones

Fig. 1. General chemical structure of chalcones

They have been reported to possess many useful properties including anti-inflammatory (Kontogiorgis *et al* 2008; Nowakowska, 2007), anti-microbial (Cushnie and Lamb, 2005), anti-oxidant (Batovska and Todorova, 2010), and anti-cancer activities (Mojzis *et al* 2008) among others; therefore representing a class with enormous therapeutic potential. Anti-inflammatory activity of these compounds is manifested by their interaction with a number of targets (Kidd and Urban, 2001); some of which include inducible nitric oxide synthase (iNOS), nuclear factor-*κ*B (NF-*κ*B), heme oxygenase (HO) and cyclooxygenase (COX).

Among these, cyclooxygenase (COX), also known as prostaglandin H synthase (PGH synthase/PGHS/PHS) is a prominent and well-studied protein catalyzing the conversion of arachidonic acid to prostaglandin H_2 (PGH_2), the committed step in prostaglandin (PG) biosynthesis. There are two isoforms of this enzyme: COX-1 and COX-2; the former being a constitutive form that is expressed in most mammalian tissues for the maintenance of PG levels in organ homeostasis and the latter being an inducible form expressed only in certain tissues in response to inflammatory stimuli (Voet and Voet, 2004). The severe gastrointestinal (GI) side effects of the traditional non-steroidal anti-inflammatory drugs (NSAIDs) are attributed to their non-selective inhibition of COX. The resultant hunt for compounds with reduced GI toxicity culminated in the launch of newer selective COX-2 inhibitors (coxibs). Apparently, the utility of these agents is also questionable due to the statistically significant increase in cardiovascular

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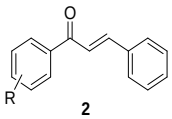
events associated with their intake prompting the withdrawal of blockbusters like rofecoxib and valdecoxib from the market (Flower, 2003). Hence, there is a great need to come up with safer anti-inflammatory agents.

Utilizing the inherent simplicity of chalcones, their potential for binding to COX and the clearly delineated structure of the target, we rationally designed a set of simple ring-A monosubstituted chalcones as putative anti-inflammatory agents. In continuation of drug design studies on compounds of biological interest (Bansal *et al* 2011; Sharma *et al* 2011), the present investigation reports preliminary findings by picking lead compounds exhibiting good binding to COX and having drug-like properties.

MATERIALS AND METHODS

Design

The library was generated by designing ring-A monosubstituted chalcones with an unsubstituted ring-B. 17 substituents were chosen so as to cover the gamut of electronic and steric properties together with widely differing polarity. These substituents were appended to the 2'- (*o*-), 3'- (*m*-) and 4'- (*p*-) positions relative to the carbonyl group in ring-A leading to a 51-membered library **(Figure 2)**. The nonselective COX inhibitor ibuprofen, a clinically used anti-inflammatory agent was used as the reference standard in this study.



R = H, -OH, -NH_2, -COOH, SO_3H, -CHO, CF_3, -Cl, -Br, -OCH_3, -Ph, -NO_2, -$CONH_2$, -CH_3, -C_2H_5, -N_3, -CN, -SO_2NH_2

Fig. 2. Constituents of the ring-A mono-substituted chalcone library

General procedure for in silico analysis

The following step-wise protocol was used to assess the members of the library generated above:

Step 1: The chemical structures of the ligands were drawn using the ACD/ChemSketch freeware. *Step 2:* The generated structures were converted to 3D and minimized using CORINA- a fast, powerful 3D structure generator cum energy minimization software applied to small and medium-sized, typically drug-like molecules. *Step 3:* The target COX protein was normalized using CHIRON, a server that interactively minimizes the steric clashes in proteins through molecular dynamics simulations while causing minimal perturbation of the protein backbone. *Step 4:* The ligands were docked onto entry 2AW1 of Brookhaven protein databank (PDB), optimized as in *Step 3*, and representing a 1.46 Å high-resolution X-ray co-crystal structure of the protein with valdecoxib (Source: *Homo sapiens*). *Step 5:* Preliminary docking was performed using the robust ArgusLab molecular modeling program that was quick enough, yet reliable, to be used as a screening method. The docking score of the simplest unsubstituted chalcone (1,3-diphenyl-prop-2-en-1-one) was chosen as the threshold value and those ligands with a higher docking score were carried forward for more detailed analysis. This produced a focused library composed of ten members. *Step 6:* The ten prescreened ligands were validated using AutoDock version 4.0 which is a suite of automated docking tools that describe the target as a set of grids prior to the docking operation. Even though this second docking process was somewhat longer than the preliminary docking using ArgusLab, the limited number of ligands to be screened allowed efficient utility of the program. The docking scores obtained were more accurate and trustworthy. *Step 7:* This subset of compounds was further assessed for drug-likeness by calculating total polar surface area (TPSA) and the Lipinski parameters using the high-speed molecular properties calculator, a free module in the MolSoft software package.

RESULTS AND DISCUSSION

The scores obtained from the preliminary docking employing ArgusLab which served as a primary screen are listed in **Table 1**. The chalcone library was designed with an intention to include functional groups that vary widely with respect to their electronics, sterics as well as polarity. *-I* groups like trifluoromethyl and carboxamido; *+I* groups like alkyl (methyl, ethyl); *-M* groups like nitro and cyano; *+M* groups like alkoxy and halo; lipophilic, sterically demanding groups like phenyl as well as polar groups like carboxylic acid and sulfonamido, were utilized as substituents to get a fair sense of the interaction with the target.

A cursory glance of the data indicates that the 2'- (*o*-) substituted chalcones have lower docking scores compared to their 3'- (*m*-) and 4'- (*p*-) substituted counterparts. Therefore, in general, substitution at the 2'-position in ring-A is not conducive for binding to the target. However, the 2'-hydroxychalcone (docking score = -8.14 kcal/mol) is a known anti-inflammatory compound through induction of HO at μM

Table 1: ArgusLab docking scores for the designed chalcone library

Ligand	Docking score (kcal/mol)	Ligand	Docking score (kcal/mol)	Ligand	Docking score (kcal/mol)
2'-OH	-8.14	3'-OH	-7.58	4'-OH	-7.80
2'-CHO	-8.01	3'-CHO	-8.20	4'-CHO	-7.90
2'-NH_2	-7.20	3'-NH_2	-7.42	4'-NH_2	-7.73
2'-SO_3H	-7.70	3'-SO_3H	-7.68	4'-SO_3H	-7.75
2'-CF_3	-7.33	3'-CF_3	-7.67	4'-CF_3	-7.18
2'-Cl	-7.89	3'-Cl	-7.75	4'-Cl	-7.14
2'-Br	-7.20	3'-Br	-7.83	4'-Br	-7.82
2'-OCH_3	-7.38	3'-OCH_3	-7.43	4'-OCH_3	-8.01
2'-Ph	-7.16	3'-Ph	-8.26	4'-Ph	-8.02
2'-CH_3	-7.49	3'-CH_3	-7.77	4'-CH_3	-7.87
2'-NO_2	-7.68	3'-NO_2	-7.56	4'-NO_2	-7.78
2'-C_2H_5	-7.92	3'-C_2H_5	-7.89	4'-C_2H_5	-7.96
2'-COOH	-7.56	3'-COOH	-7.59	4'-COOH	-7.74
2'-$CONH_2$	-7.68	3'-$CONH_2$	-7.57	4'-$CONH_2$	-7.41
2'-N_3	-8.15	3'-N_3	-7.55	4'-N_3	-7.51
2'-SO_2NH_2	-7.50	3'-SO_2NH_2	-7.60	4'-SO_2NH_2	-8.31
2'-CN	-7.66	3'-CN	-7.93	4'-CN	-7.88
Ibuprofen reference		-6.84	Unsubstituted chalcone		-7.94

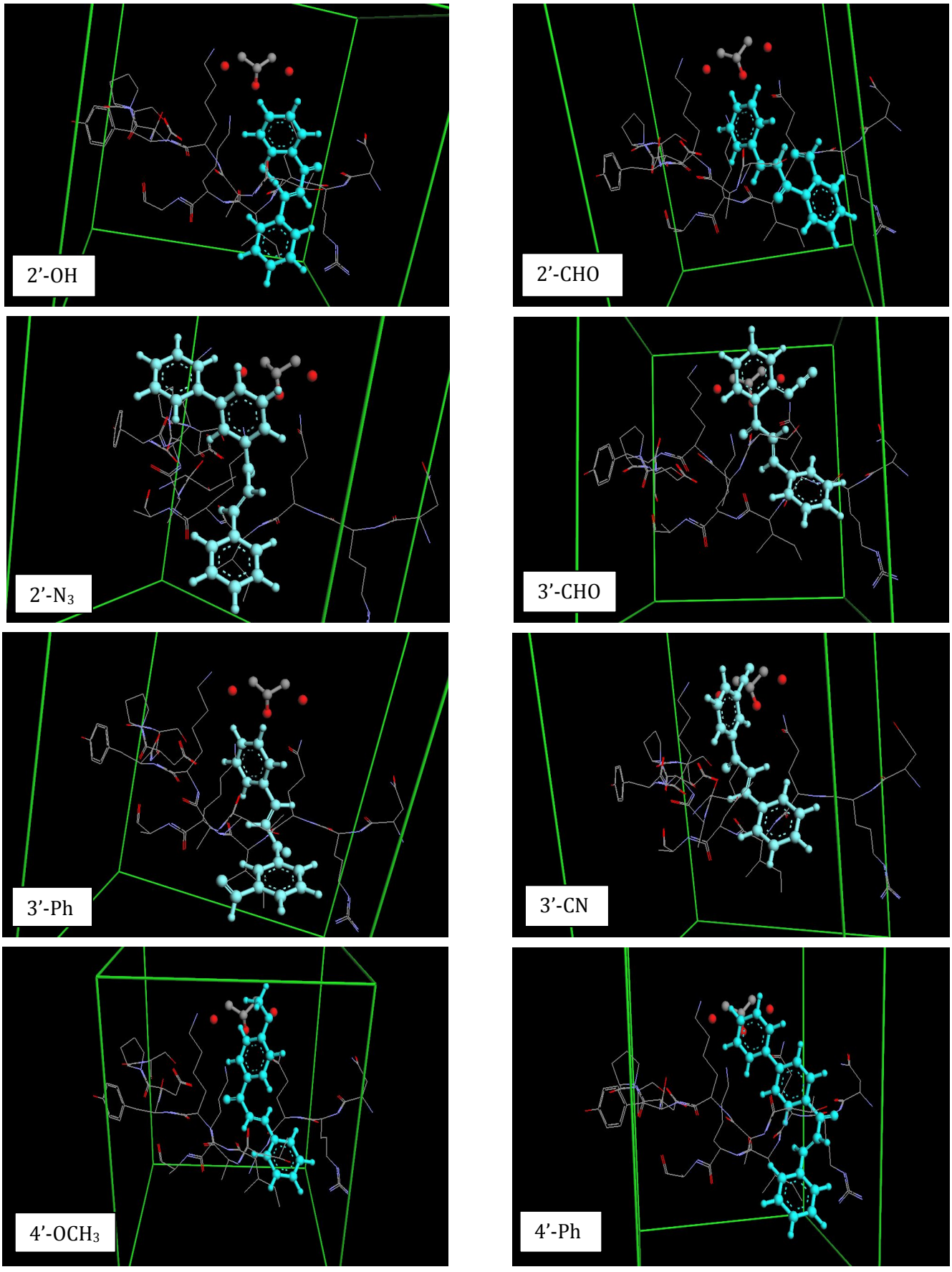
concentrations (Sawle *et al* 2008) as well as *via* reduction of tumour necrosis factor-α (TNF-α)-induced NF-κB levels (Nowakowska, 2007). Apparently, this analogue also has good affinity to COX as observed from our study. Moreover, the free phenolic –OH group seems to be a requirement for binding as the corresponding 2'-methoxychalcone has a much lower docking score (-7.38 kcal/mol). Besides, the linear, dipolar azide moiety, an established COX pharmacophore (Zarghi *et al* 2006) was also incorporated in the library. This yielded a compound 2'-azidochalcone with good binding along expected lines (docking score = -8.15 kcal/mol).

Among the 3'- (*m*-) substituted derivatives, the docking scores point to a preference for electron withdrawing groups (EWGs) in that the cyano and the aldehyde substituents are amongst the compounds that show good binding. In fact, the aldehyde seems to be a key contributor to binding with both the *o*- and *m*- congeners exhibiting good score and the *p*- analogue just falling below the threshold value.

The 4'- (*p*-) position is the most tolerant to substitution and different kinds of substituents show decent to good binding. The ethyl analogue is a borderline case showing similar binding as that of the unsubstituted chalcone (docking score = -7.96 kcal/mol). The methoxy derivative has decent binding to COX. This can be attributed to the fact that the methoxy group is a proven moiety with the mono-, di- and trimethoxylated ring-A as well as ring-B chalcones showing good anti-inflammatory activity (Rojas *et al* 2003). It is interesting to note that 4'-methoxychalcone has a higher docking score (-8.01 kcal/mol) than the corresponding 4'-hydroxylated derivative (-7.80 kcal/mol); precluding the requirement of a free phenolic –OH group at the 4' position for binding to COX as opposed to the 2'- and 3'-positions. The highly polar and slightly acidic sulfonamido group is a popular substituent in anti-inflammatory agents such as in the non-selective COX inhibitor piroxicam and the COX-2 selective inhibitor nimesulide. Accordingly, a ring-A *p*-sulfonamide moiety produced the best binder in the entire library (docking score = -8.31 kcal/mol). Furthermore, the 3'-(*m*-) and 4'-(*p*-) positions can accommodate bulky, lipophilic groups indicated by the good binding potential of the corresponding phenyl analogues (docking scores = -8.26 and -8.02 kcal/mol respectively).

The good binders were identified as those having a docking score greater than or equal to that of 1,3-diphenyl-2-propen-1-one. This simplest unsubstituted chalcone had a score of -7.94 kcal/mol that was arbitrarily chosen as the

threshold value, resulting in a focused library of ten compounds having the following substituents: 2'-OH, 2'-CHO, 2'-N_3, 3'-CHO, 3'-Ph, 3'-CN, 4'-OCH_3, 4'-Ph, 4'-C_2H_5 and 4'-SO_2NH_2. **Figure 3** shows these derivatives assuming a specific pose in complex with the COX target.



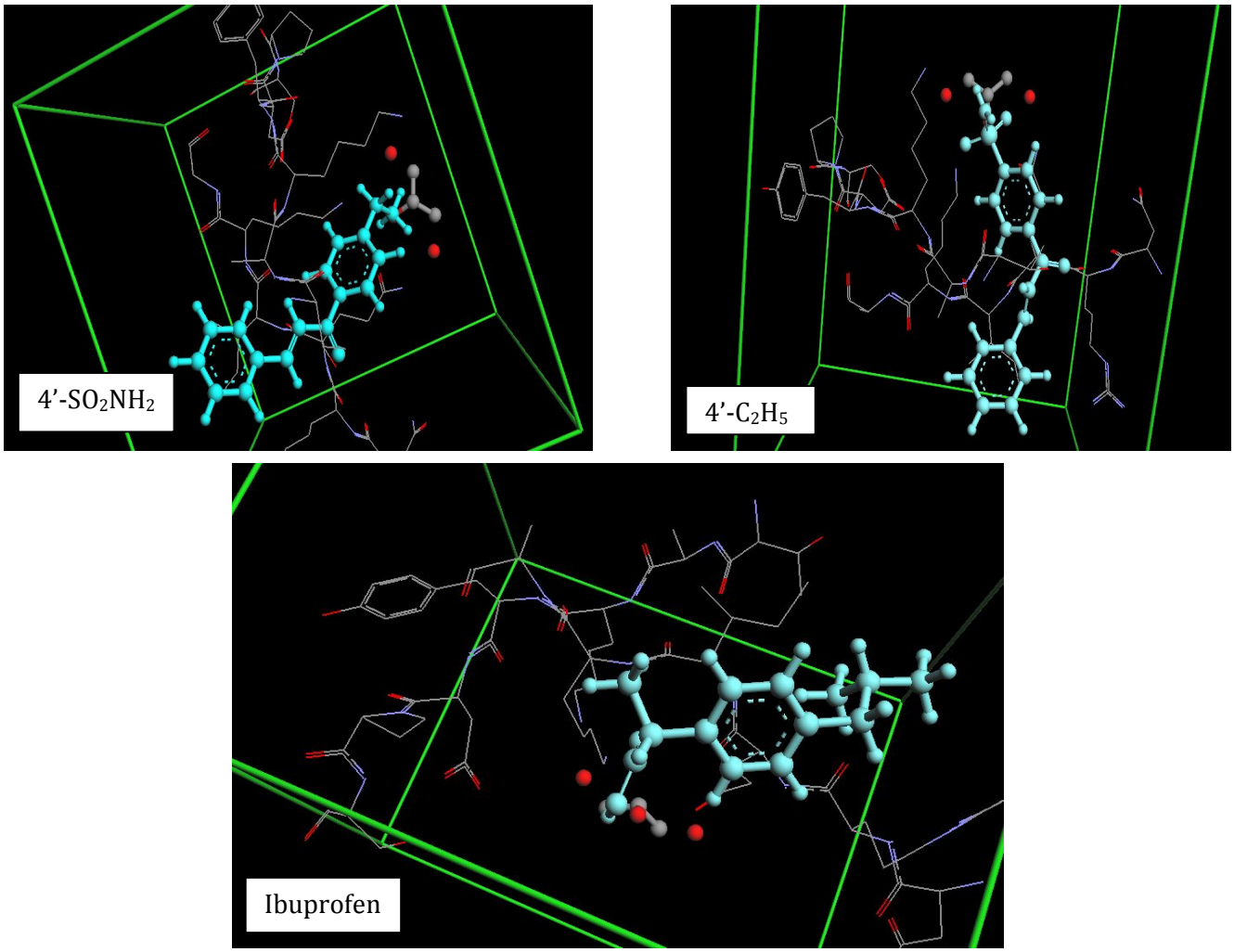
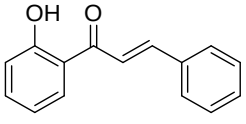
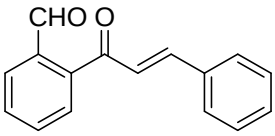
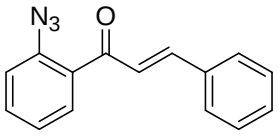
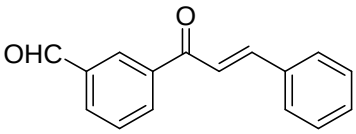
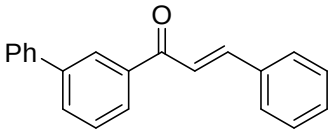
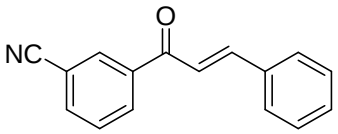
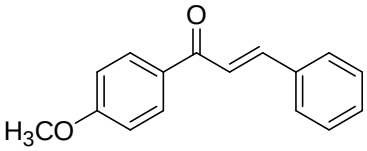
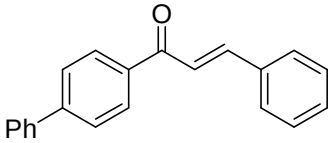
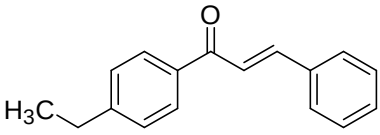
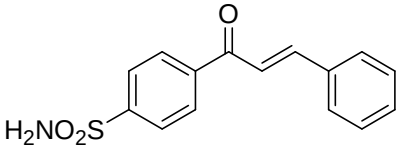
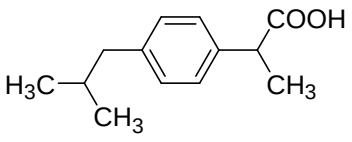


Fig. 3. Complex of the ligands with the binding site of COX. The ligands are indicated in the respective panels. The following colour codes have been employed: Aqua (in foreground) denotes the ligand; red and light blue represent oxygen and nitrogen atoms respectively; ashen gray (in background) forms the peptide carbon backbone of the COX protein.

The chalcone analogues listed above were subjected to a more rigorous second docking procedure using the AutoDock program that typically yields lower yet more realistic scores compared to ArgusLab. The docking scores of these good binders along with that of the reference ibuprofen are shown in **Table 2**. All these prescreened ligands had docking scores greater than that of ibuprofen (-5.84 kcal/mol) indicating that they bind the target better.

Table 2. AutoDock docking scores for the prescreened ligands

Ligand	Structure	Docking score (kcal/mol)
2'-OH		-6.30
2'-CHO		-6.91

2'-N_3		-7.34
3'-CHO		-6.71
3'-Ph		-7.49
3'-CN		-6.38
4'-OCH_3		-6.02
4'-Ph		-6.81
4'-C_2H_5		-6.43
4'-SO_2NH_2		-7.01
Ibuprofen		-5.84

Two key observations can be made from the data presented in **Table 2**. First, a higher ArgusLab docking score for a ligand over another doesn't necessarily imply the same result in AutoDock. This can be seen by comparing the corresponding docking scores of 4'-sulfonamido and 2'-azido analogues in the two programs: 4'-$SO_2NH_{2ArgusLab}$ (-8.31 kcal/mol) > 2'-$N_{3ArgusLab}$ (-8.15 kcal/mol) whereas 4'-$SO_2NH_{2AutoDock}$ (-7.01 kcal/mol) < 2'-$N_{3AutoDock}$ (-7.34 kcal/mol). Secondly, there is no correlation amongst the magnitude of the difference between the docking scores of two ligands in ArgusLab and AutoDock. The approximately 1 kcal/mol energy difference in the docking scores of the 2'-hydroxychalcone and 2'-azidochalcone in the AutoDock program doesn't compare well with their almost identical scores in ArgusLab. The ten good binders obtained after the secondary docking procedure were then evaluated for their drug-likeness. The original set of empirical rules called the Lipinski's Rule of Five (Lipinski *et al* 2001) has spawned many extensions to include parameters like TPSA (Ghose *et al* 1999).

Table 3. Assessment of drug-likeness of the prescreened ligand

Ligands	Molecular formula	Molecular weight (g/mol)	Number of hydrogen bond acceptor(s)	Number of hydrogen bond donor(s) BD	MLogP	TPSA(Å^2)
2'-OH	$C_{15}H_{12}O_2$	224.08	2	1	4.09	37.30
2'-CHO	$C_{16}H_{12}O_2$	236.08	2	0	3.57	34.14
2'-N_3	$C_{15}H_{11}N_3O$	249.09	3	0	4.10	66.82
3'-CHO	$C_{16}H_{12}O_2$	236.08	2	0	3.57	34.14
3'-Ph	$C_{21}H_{16}O$	284.12	1	0	5.86	17.07
3'-CN	$C_{16}H_{11}NO$	233.08	2	0	3.76	40.86
4'-OCH_3	$C_{16}H_{14}O_2$	238.1	2	0	4.00	26.30
4'-Ph	$C_{21}H_{16}O$	284.12	1	0	5.93	17.07
4'-C_2H_5	$C_{17}H_{16}O$	236.12	1	0	4.84	26.30
4'-SO_2NH_2	$C_{15}H_{13}NO_3S$	287.06	4	2	2.67	77.23

The values calculated for the monosubstituted chalcones which have been presented in **Table 3** indicated that most of the derivatives would likely be orally active drugs in humans. In particular, all the analogues have a molecular weight of less than 500 daltons (optimal range 160-500 daltons) and possess an average of about 33 atoms including H-bond donors and H-bond acceptors (optimal range 20-70). Moreover, none of the compounds exhibited a TPSA greater than 140 Å^2. 3'- and 4'-phenyl chalcones were the outliers in this dataset as reflected in their partition coefficient values (5.86 and 5.93 respectively) that are outside the acceptable limit of 5 as stipulated in the original set of rules and also fell outside the optimal range of -0.4 to +5.6 as suggested in later extensions. However, it is important to realize that this is only a rule of thumb which allows one violation per compound and is characterized by a number of exceptions to the rule.

CONCLUSION

Even though a number of natural and synthetic chalcones have been studied in a variety of different pharmaceutical contexts, there is no systematic structure activity relationship (SAR) data available in the literature. Considering its prominent role in inflammation and also taking into account the paucity of safe anti-inflammatory agents in the clinic, we decided to explore the chalcone scaffold and probe its SAR. As a first step toward that goal, we have developed a protocol for identifying viable compounds to be synthesized from a large collection of rationally designed chalcones. This robust and handy protocol, when taken together with the final filter of assessing drug-likeliness and oral absorption, is a simple yet powerful strategy to shift the subsequent medicinal chemistry SAR efforts towards the region that favors oral activity. Although such computational methods give only probabilistic values, they will help us make choices with regard to the synthesis or purchase of ligands when used in such an early discovery setting.

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