

Quantitative Structure-Activity Relationship (QSAR) Study of Cyclooxygenase-2 (COX-2) Inhibitors

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A plethora of selective COX-2 inhibitors belonging to nine chemical classes (pyrrole, imidazole, cyclopentene, benzene, pyrazole, spiroheptene, spiroheptadiene, isoxazole, and thiophene) was subjected to quantitative structure-activity relationship (QSAR) analysis using semi-empirical (AM1)-computed quantum mechanical properties and electrotopological state (E-state) indices. These computed parameters were correlated with experimental inhibitory activities (pIC_{50}). Multilinear regression analyses produced three statistically acceptable models. Model 1 is based on quantum mechanical properties only, Model 2 is an all-E-state relationship, and Model 3 embraces both quantum mechanical and electrotopological parameters. All three models surpassed the commonly allowed minimum predictive squared correlation coefficient (q^2) of 0.60. These QSAR results and the probable pharmacophore features identified in this study offer important structural insight into designing novel anti-inflammatory drugs devoid of unwelcome side effects. Guided by the generated models, 18 chemical structures belonging to spiroalkene classes were designed with calculated pIC_{50} values higher than that of known potent COX-2 inhibitors.

Key Words: AM1, Electrotopological State, heterodiaryl, multilinear regression, quantum mechanical

INTRODUCTION

Cyclooxygenase-2 (COX-2) inhibition has been one of the most widely investigated areas of research in the last decade due to its crucial role in relieving pain and other inflammatory conditions. Nonsteroidal anti-inflammatory drugs (NSAIDs) are profoundly used in the treatment of wide variety of inflammatory conditions including osteoarthritis and rheumatoid arthritis (Vane et al. 1996). However, these drugs are associated with high risk of gastrointestinal and renal adverse effects (Allison 1992, DeWitt 1999, Deviere 2002). NSAIDs act by inhibition of cyclooxygenase (COX), the enzyme involved in the biosynthesis of prostaglandins, prostacyclins and thromboxanes from arachidonic acid (Dannhardt 2001; Bleumink 2003).

Cyclooxygenase exists in at least two isoforms, namely, the constitutive cyclooxygenase-1 (COX-1) and the inducible cyclooxygenase-2 (COX-2) (Fu et al. 1990). Inhibition of COX-1 is responsible for the adverse gastrointestinal and renal effects of NSAIDs while the inhibition of COX-2 accounts for NSAIDs' therapeutic effects (Dionne 2003). All classical NSAIDs, such as aspirin, ibuprofen, and indomethacin, can inhibit both COX-1 and COX-2, but bind more tightly to COX-1 (Laneville 1994). Selective COX-2 inhibitors have the same anti-inflammatory, anti-pyretic, and analgesic activities as do nonselective NSAIDs but without causing gastric ulceration, bleeding and perforation (Marnett 1998).

The importance of developing selective COX-2 inhibitors is manifested by the intense efforts devoted in this field that consequently resulted in the synthesis of hundreds

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of compounds, which displayed activity against the title enzyme. Chavatte and co-workers (Chavatte 2001) compiled the experimental data from various sources and performed 3D QSAR studies using comparative molecular analysis (CoMFA). Although the relationship of different molecular fields (i.e. steric, electrostatic, and lipophilic) and inhibitory activity were detailed in that study, no novel structures were presented as outcome of such modeling exercise. Moreover, the numerous QSAR studies in the recent past on COX-2 inhibitors involved only small data set of a particular class of compounds such as stilbenoid diaryls (Soltani 2010), pyrimidines (Shah 2009) and others (Manivannan 2009; Khoshneviszadeh 2008).

In this light, we are interested in designing a new set of COX-2-selective inhibitors based on simple but statistically sound QSAR models whose parameters can be easily obtained using commonly available and less costly computational programs. The generated models were the primary basis in designing new structures with potentially greater bioactivity. The alternative models obtained in our study proved to provide similar results to that indicated by CoMFA but with the advantage of simplicity, accessibility of parameters, and cost-effectiveness.

Herein, we report a quantitative structure-activity relationship (QSAR) study, which utilizes chemical properties obtained from quantum mechanical calculations (3-D parameters) and 2-D parameters known as Electrotopological states (or E-states) (Kier & Hall 1999) to derive predictive models from a wide series of 305 varied diarylheterocyclic derivatives studied by Chavatte (Chavatte 2001).

Although E-state parameters are incorporated in CoMFA (Kellog 1996), the use of less expensive software which is available in our group, and which equally allows calculation of these parameters is certainly instructive. Furthermore, while it is true that 3D QSAR permits use of different structural motifs, most QSAR studies are aimed at improving the potency of known candidate compounds through modification of substituents of a common core structure. Thus, while 3D QSAR may lead to another lead compound with markedly different scaffold, our present work provides next generation of candidate compounds with core structure similar to the parent compounds but with greater calculated activities.

Based on the models we created, eighteen chemical structures have been designed based on spiroalkene core with calculated pIC₅₀ greater than that of the potent known COX-2 inhibitors. The result of this work should facilitate further development of new selective COX-2 inhibitors.

METHODOLOGY

A data set of 305 cyclo-oxygenase 2 (COX-2) inhibitors composed of nine families with known structures and experimental activities was obtained from the literature (Chavatte 2001). These were used as the training set for developing the linear models. The biological activity was expressed as $-\log(\text{IC}_{50})$, where IC_{50} is the effective concentration of a compound to achieve 50% inhibition of cyclooxygenase-2 enzyme.

All computational work was performed on Pentium III workstation. All softwares used in this study were run on Windows 98 operating system. 3-dimensional models of the compounds were drawn using both HyperChem Pro (Hypercube, Inc.) and PC Spartan '04 V1.0.1 (Wavefunction, Inc). 2D Electrotopological state indices such as the atom related E-states, atom-type E-states and Hydrogen E-states (HE-states) were calculated utilizing the E-Calc program (Kier & Hall 1999). Management of the database and all statistical analyses were accomplished using SPSS V11.0 and Windows 97 Excel.

All structures were initially subjected to molecular mechanics geometry optimization using Molecular Mechanics Force Field (MMFF) (Clark et al. 1989). The resulting structures were then used as the input in the subsequent Austin-Method 1 (AM1) (Dewar et al. 1985) semi-empirical computations. Both the optimized geometry and quantum mechanics-based properties were computed at the AM1 level.

Linear models on the variation of biological activity as functions of computed structure-based properties were generated using ordinary least squares (also known as the classical multiple linear regression) procedure. The quality of the model was considered as statistically satisfactorily on the basis of squared correlation coefficient (r^2) standard deviation (s), F-statistics (F) and squared predictive correlation coefficient (q^2) when all the parameters in the model were significant at 95% confidence level ($p < 0.05$). The jackknife method was used to improve the overall quality of the regression model. The most satisfactory model was selected on the basis of maximum r^2 , collinearity diagnostics and analysis of variance. Model validation was accomplished using the Leave-One-Out (LOO) method described by Maw and Hall (Maw & Hall 2000; Maw & Hall 2001).

RESULTS AND DISCUSSION

The dataset composed of nine different families of compounds (Figure 1) were subjected to quantitative structure-activity relationship study. These include the derivatives of pyrrole (Family A), imidazole (Family B),

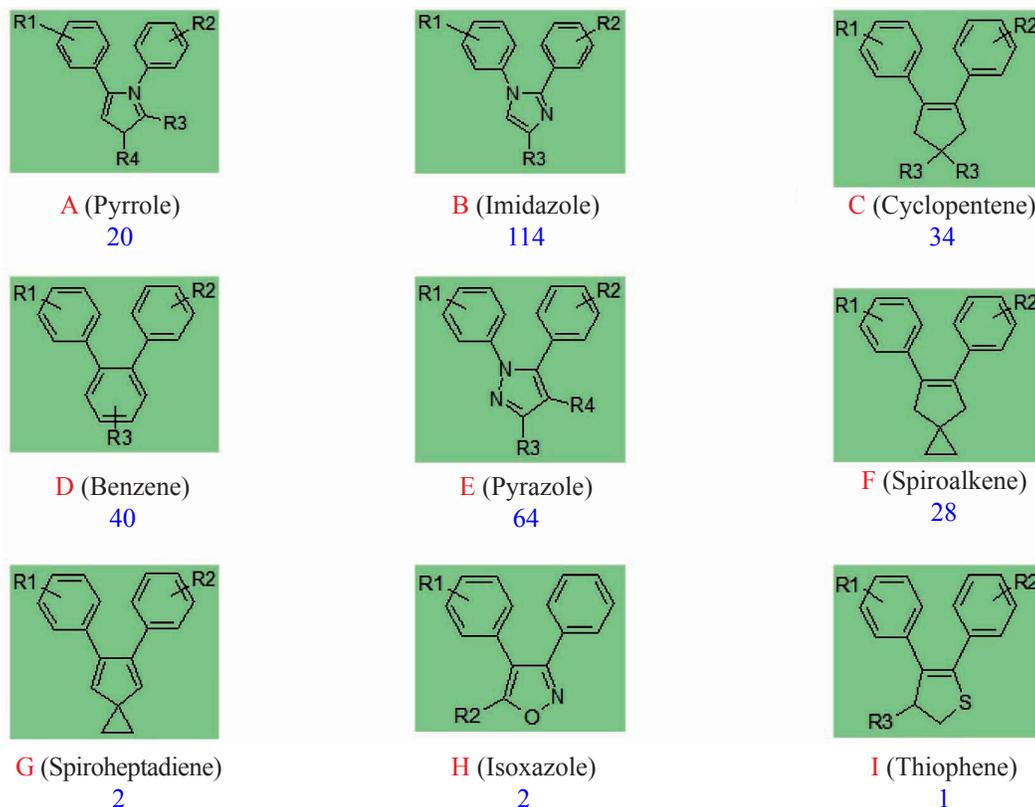


Figure 1. Nine families of 305 diarylheterocyclic COX-2 inhibitors (Chavatte et al. 2001). The number of compounds in each family is indicated below each group.

cyclopentene (Family C), benzene (Family D), pyrazole (Family E), spiroheptene (Family F), spiroheptadiene (Family G), isoxazole (Family H), and thiophene (Family I). One hundred two (102) parameters, 3D (e.g. surface area), whole molecule (e.g. mass, dipole moment), electronic (e.g. partial charge, energy), hydrophobic (e.g. log P) and steric (e.g. volume) properties, and topological (2D) indices (e.g. SCsat, SHCunsat, etc.) of the COX-2 inhibitors were successfully computed and encoded.

A principal component analysis (PCA) was first conducted in order to know the number of variables that significantly account for the observed variation in biological activity and thus the number of predictors the model equations should contain (Harman 1976). The results of PCA indicated that a statistically sound model should contain three predictors.

The generated model (Model 1) based on quantum properties can be written as:

$$\begin{aligned}
 -\log IC_{50} = & -13.19 C1_{pc} (\pm 0.85) + 15.48 C4_{pc} (\pm 1.35) \\
 & - 9.10 C8_{pc} (\pm 0.90) + 5.46 (\pm 0.14) \quad (\text{Model 1}) \\
 (n = 150 \quad r = 0.933 \quad r^2 = 0.870, \quad s = 0.363 \\
 F = 325.06 \quad q = 0.930 \quad q^2 = 0.865)
 \end{aligned}$$

This model indicates that among the QSAR parameters derived from quantum mechanical calculations, the charges on three carbon atoms significantly account for the variability in the observed inhibitory activity against COX-2. The structure of a representative diaryl compound and the atoms in the structure common to all compounds were numbered as shown below (Figure 2). We will refer the phenyl ring containing sulfonamide substituent as ring A and the other with variable substituents R2 as ring B. In COX-2, highly polar ring A interacts with polar residues such as ARG499 whereas aromatic ring B with variable substituents positions itself in the vicinity of aromatic residues namely TYR334, TRP373, and PHE504 (Figure 3).

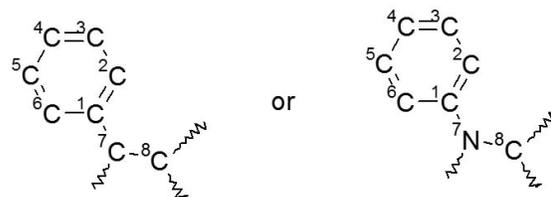


Figure 2. Common skeletal framework of diarylheterocyclic COX-2 inhibitors. The partial charges of carbons 1, 4, and 8 are crucial for COX-2 inhibitory activity as indicated by model 1.

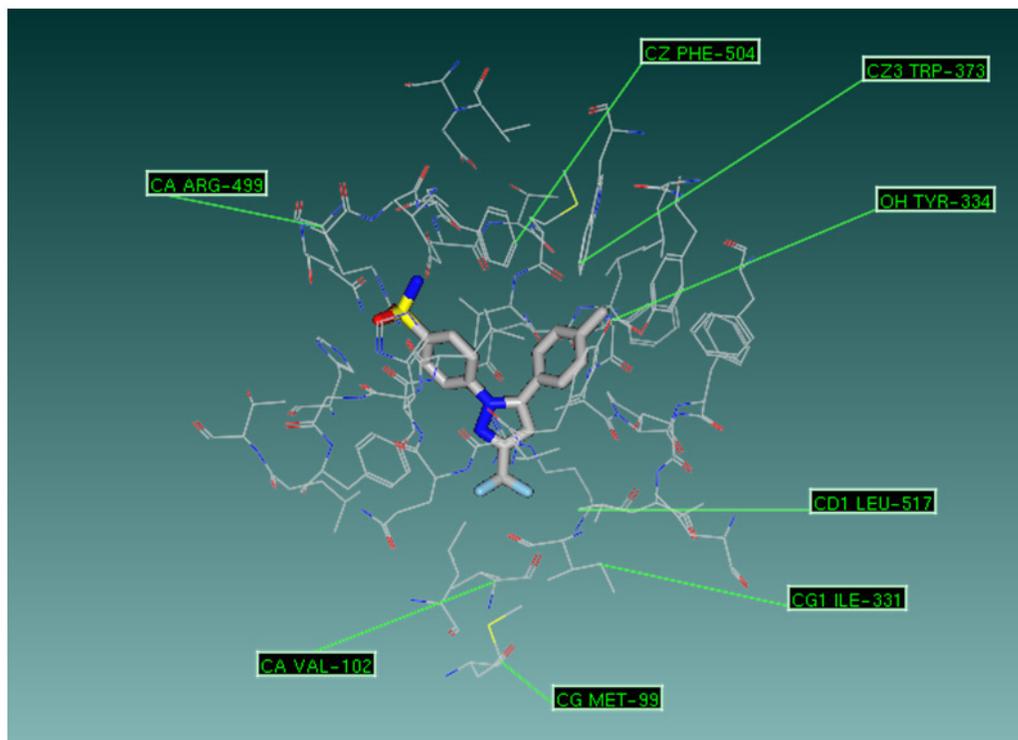


Figure 3. COX-2 with bound Celecoxib (compound 260). The PDB file 3LN1.pdb was modeled using MarvinSpace 5.3.8 viewer.

In Model 1, the partial charge on C1 accounts for 68% in the variability of observed activity. Additionally, the charges on C4 and C8 each explains approximately 10% of the variation of observed pIC_{50} . The model shows that a small partial electronic charge on carbons 1 and 8 and a large partial charge on carbon 4 favor the inhibition of COX-2. For example, substitution of F and Cl in R2 of nonsulfonamide-containing ring (ring B) with NH_2 and Br substituents enriches the C8 position (*vide infra*) and would enhance the activity. These substitutions are consistent with CoMFA models, which predicts that bulkier and less electron-withdrawing groups in this ring are associated with increased activity (Chavatte 2001). Although our substitution is dictated by charge requirement at C8, the same substituents satisfy the CoMFA derived models, albeit different characteristics of the substituents are operative.

A model based on Electrotological State (E-State) parameters was also derived. The E-state, S_i , of atom or group of atoms, i , is expressed as the intrinsic state of atom or group, I_i , plus the sum of all perturbations $\sum_j \Delta I_{ij}$ (i.e. $S_i = I_i + \sum_j \Delta I_{ij}$). The I value encodes the electronegativity and topological environment, and thus can be related to charge and lipophilicity indices. The ΔI_{ij} values are computed as a function of the separations of every pair of atoms using the number of atoms in the path of the separation as the distance (Kier and Hall 1999). The E-State values encode a unification of both

electronic and topological attributes of a molecule.

The E-state model equation (model 2) can be written as:

$$-\log IC_{50} = 0.17 G_{\min} (\pm 0.02) - 0.23 SH_{\text{other}} (\pm 0.02) + 0.52 SHC_{\text{sat}} (\pm 0.90) + 5.46 (\pm 0.14) \quad (\text{Model 2})$$

(n = 150 r = 0.893 r² = 0.797, s = 0.444
F = 190.73 q = 0.887 q² = 0.787)

In model 2, G_{\min} accounts for 55% in the variability of COX-2 inhibitors and SH_{other} and SHC_{sat} contribute additional 18% and 9%, respectively. G_{\min} is the minimum E-state value in the molecule. It must be the index for the most electron poor atom or moiety in the molecule. SH_{other} is the sum of Hydrogen atom-type E-state indices for all nonpolar Hydrogen atoms while SHC_{sat} is the sum of all hydrogen E-states of saturated carbons ($-CH_3$, $-CH_2-$, and $>CH-$). The model suggests higher G_{\min} and SHC_{sat} values and lower SH_{other} value for improved COX-2 inhibitory activity.

In general, the E-state value is a measure of the electron richness of an atom or a particular group of atoms. Thus, the model indicates that the most electron deficient part of the molecule (G_{\min}) must be improved, that is, the electrons must become more accessible in that region for enhanced activity.

Alternatively, since the E-State indices are less familiar chemical parameters, their implications on the observed property of the molecule can be better understood by relating them to more familiar chemical properties. For example, the Gmin parameter in Model 2 is represented by the partial charge on Carbon 8 ($C8_{pc}$) in models 1 and 3. The models indicate that C8 must be made more negative in order to enhance the activity. This nicely corroborates model 2 in which Gmin must increase (i.e. more electron-rich) to achieve superior activity.

When all parameters were included in the MLR analysis, we obtained the combined model (model 3), which can be written as:

$$-\log IC_{50} = 10.66 C_{pc} 8 (\pm 0.99) + 7.56 (\pm 0.01) \times (10^{-2} LUMO - 0.16 SH_{other} (\pm 0.02) + 10.93 (\pm 0.31)) \quad (\text{Model 3})$$

($n = 181$ $r = 0.901$ $r^2 = 0.811$, $s = 0.450$
 $F = 253.08$ $q = 0.901$ $q^2 = 0.812$)

In model 3, the partial charge on C8 explains 62% of the variation in observed COX-2 activity. The energy of LUMO and SH_{other} each contributes additional 11% and 9%, respectively. Again, a decrease in partial charge on C8 favors the inhibition of COX-2. In other words, substituents that increase the electron density on C8 will tend to increase the activity of the compound. This result is in accord with that observed in Model 1. Additionally, in Model 3 the E_{LUMO} becomes the second most important predictor for the set of 181 COX-2 inhibitors. The model indicates that the LUMO energy must increase, that is, it must become more destabilized to improve the inhibitory activity of the compound.

Detailed examination of the three models revealed that they are equivalent to each other. The $C8_{pc}$ and E_{LUMO} in model 3 are highly correlated with G_{min} and SHC_{sat} in model 2. Likewise, the E_{LUMO} and SH_{other} in model 3 represent the $C1_{pc}$ and $C4_{pc}$ in model 1. Delightfully, the LUMO isosurface of a representative inhibitor (compound 294) encompasses the A ring consisting of C1 and C4 and to a lesser extent the B ring centered on C8 (Figure 4). This simply indicates that a change in electron density around these relevant centers influences the energy of the LUMO. Being an important predictor in model 3 and correlated with the other predictors in models 1 and 2, LUMO remarkably dictates the biological activity of diaryl inhibitors. Its ubiquity in the models also implies that these variants of diaryl heterocycles serve as electron acceptors in their interaction with cyclooxygenase-2 enzyme.

An acceptable model is one in which the squared predictive correlation coefficient, q^2 is greater than 0.60

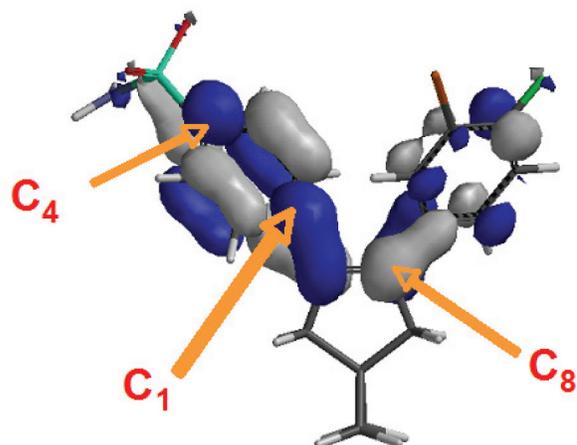


Figure 4. Lowest unoccupied molecular orbital (LUMO) isosurface of compound 294. (Generated using Spartan 04)

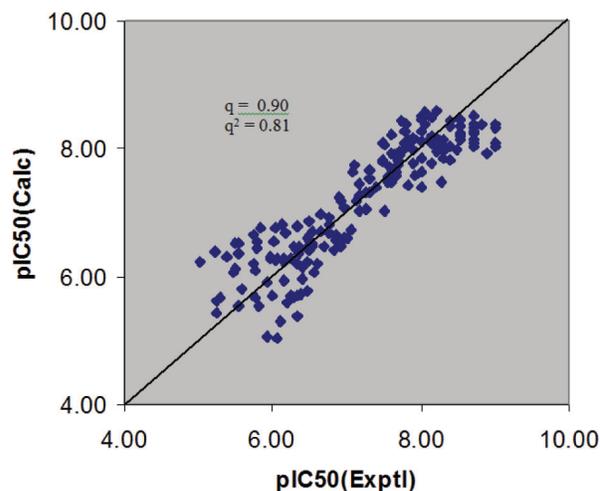


Figure 5. Predicted values versus experimental values for the validation test of Model 3 using leave-one-out (LOO) method.

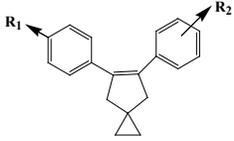
(Wold 1991). It can be seen that the validation results for all three models were very satisfactory. The high q and q^2 values for all models were simply remarkable. A representative plot of calculated versus experimental pIC_{50} (for Model 3) is shown in Figure 5.

The plots of calculated versus experimental biological activity clearly demonstrate the high predictive ability of the three models. The typical number of predictors in an acceptable model is 15-20% of the number of compounds. Since this study involved over a hundred inhibitors, with only three predictors in each model, it is overwhelming to have such quality of results. The scatter plots (not shown) also revealed that the error of predicted values was distributed randomly around zero indicating no established bias in the calculated activity.

The important end of QSAR studies is to be able to design better and more potent drugs. QSAR allows *a priori* determination of the biological activity of a novel analogue. Using the derived model equations and the information gathered, a number of novel inhibitors are proposed. Listed in Tables 1, 2 and 3 are the novel inhibitors and their corresponding structures and theoretical activities derived using the combined model (Model 3).

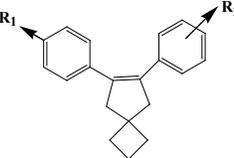
Our models do not indicate that the size (*i.e.* steric effect) of the substituents in ring B is relevant for COX-2 inhibition. Thus, we focused on the electronic effects of substituents because our models clearly reveal their significance in fine-tuning the inhibitory activity of diarylheterocyclic compounds against COX-2. The main basis of our molecular structure design was our observation that COX-2 inhibition was favored if

Table 1. Structures and predicted COX-2 inhibitory activities (using Model 3) of designed spiroheptene derivatives.



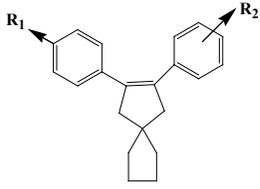
Compound	R ₁	R ₂	pIC ₅₀ (predicted)
A	SO ₂ NH ₂	4,5-diBr-2-NH ₂	9.72
B	SO ₂ CH ₃	4,5-diCl-2-NH ₂	9.63
C	SO ₂ CH ₃	3,5-diBr-2-NH ₂	9.64
D	SO ₂ CH ₃	3,5-diBr-2-CN	9.68
E	SO ₂ CH ₃	4,5-diBr-2-CN	9.68
F	4-SO ₂ NH ₂	3,5-diCl-2-CN	9.72
G	4-SO ₂ NH ₂	3,5-diCl-4-NH ₂	9.79
H	4-SO ₂ NH ₂	3,5-diBr-4-NH ₂	9.80
I	4-SO ₂ NH ₂	3,5-diBr-4-CN	9.75

Table 2. Structures and predicted COX-2 inhibitory activities of designed spirooctene derivatives.



Compound	R ₁	R ₂	pIC ₅₀ (predicted)
J	4-SO ₂ NH ₂	3,5-diBr-4-CN	9.65
K	4-SO ₂ NH ₂	3,5-diBr-4-NH ₂	9.71
L	4-SO ₂ NH ₂	3,5-diCl-4-CN	9.64
M	4-SO ₂ NH ₂	3,5-diCl-4-NH ₂	9.70

Table 3. Structures and predicted COX-2 inhibitory activities of designed spirononene derivatives.



Compound	R ₁	R ₂	pIC ₅₀ (calculated)
N	4-SO ₂ NH ₂	3,5-diBr-4-CN	9.55
O	4-SO ₂ NH ₂	3,5-diBr-4-NH ₂	9.61
P	4-SO ₂ NH ₂	3,5-diF-4-CN	9.48
Q	4-SO ₂ NH ₂	3,5-diF-4-NH ₂	9.52
R	4-SO ₂ NH ₂	3,5-diCl-4-CN	9.54

the partial charges on C1 and C8 were decreased or electron density was increased at these positions. C1 and C8 positions also contributed predominantly in the formation of their lowest unoccupied molecular orbital (LUMO), which also played an important role in their binding with COX-2.

With this in mind, we considered several structures whose R₂ substituents have strongly donating or moderately electron withdrawing ability. NH₂ for example is a known strong electron-donating group and Br is a weak electron withdrawing substituent due to its smaller electronegativity value (Solomons 1990). Since cyano (CN) is a moderately deactivating group it is expected to decrease the electron density at the *meta* position with respect to the carbon to which it is attached. This will in effect enrich the carbon adjacent to it – the C8. NH₂ on the other hand, being a strong activator, would directly enrich the *ortho* carbon (C8). It is therefore likely, that substitution of F and Cl at R₂ with NH₂ and Br, and substitution of cyano at the *ortho* position would improve the activity.

The newly designed inhibitors maintain the pharmacophoric diarylheterocyclic motif, which is common in the dataset of COX-2 inhibitors. Examination of Table 1 in SI revealed that among the nine families considered here, spiroalkenes are the most potent inhibitors. Considering also that most spiroalkenes formed a sizable part of the training set (*i.e.* they were retained after jackknife elimination), we focus our attention to these classes of compounds in designing novel COX-2 inhibitors. In fact, the four most potent inhibitors in the data set are all spiroheptenes with experimental pIC₅₀ of 9.00. Table 1 and Table 2 (SI) shows that the designed spiroheptenes with Br, NH₂, or CN substituent at R₂ have pIC₅₀ values greater than 9.00. Thus, the designed compounds containing diarylheterocyclic core with model-guided functionalization at key positions are expected to inhibit more strongly the COX-2 enzyme than any other

compound known to date.

We also hypothesized that attachment of a large cycloalkane moiety to the heterocyclic ring at the center would also enhance the electron accessibility at C1 and C8. In addition, a bigger and more nonpolar central ring is perfectly compatible with the hydrophobic residues such as MET99, VAL102, ILE331, and LEU 517 surrounding this portion of the molecule (Figure 3). Indeed, the calculated pIC_{50} values surged remarkably as the size of the cycloalkane ring increases (Tables 2 and 3). Among the spirononenes (Table 3), the relatively low pIC_{50} values of P and Q are due to the inductive effect of F substituents at 3 and 5 positions of ring B. The superior predicted potency of the designed inhibitors compared to known diarylcyclic inhibitors is clearly manifested in Figure 6, these proposed compounds having outperformed even the commercial drugs known as coxibs (encircled). These fascinating results should encourage the synthetic chemists to prepare them and evaluate their activities against cyclooxygenase-2 enzyme.

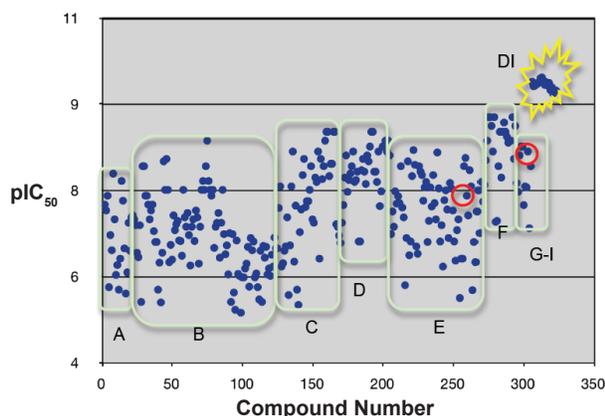


Figure 6. Biological activity (pIC_{50}) of diarylheterocyclic comprising nine families (A to I) of COX-2 inhibitors and predicted pIC_{50} of designed inhibitors (DI). The two data encircled data in red are for commercial anti-inflammatory drugs known as “coxibs”.

SUMMARY AND CONCLUSION

The usefulness of 3D and 2D parameters in predicting the biological activity of a group of compounds is demonstrated in this study. The parameters either all-quantum-mechanical, all- E-state, or combined give statistically acceptable models.

The results indicate that the electron density on carbon 1 and carbon 8 must be increased in order to improve the biological activity. The models also indicate that the destabilization of the LUMO enhances the potency of these diaryl heterocyclic compounds as COX-2 inhibitors.

It is very satisfying to find that simple models utilizing 2D-structure parameters (i.e. E-state indices) provided satisfactory results and gave essentially similar description of the system as provided by 3D QSAR studies.

As a consequence, 18 novel inhibitors based on spiroalkene family were designed. The calculated activity of these compounds exceeds the activity of the most potent known inhibitors to date. These fascinating results should prompt the synthesis of these compounds and the evaluation of their COX-2 inhibitory activity.

RECOMMENDATION

A follow up study on synthesis of the proposed inhibitors and evaluation of their activity is highly encouraged.

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REFERENCES

- ALLISON MC, HOWATSON AG, TORRANCE CJ, LEE FD, RUSSELL RI. 1992. Gastrointestinal damage associated with the use of nonsteroidal antiinflammatory drugs. *N Engl J Med* 327: 749-754.
- BLEUMINK GS, FEENSTRA J, STURKENBOOM MC, STRICKER BH. 2003. Non-steroidal anti-inflammatory drugs and heart failure. *Drugs* 63: 525-534.
- CLARK M, CRAMER III RD, VAN OPDENSCH NJ. 1989. Validation of the general purpose tripos 5.2 force field *J Comput Chem* 10: 982-1012.
- CHAVATTE P, YOUS S, MAROT C, BAURIN N, LESIEUR DJ. 2001. Three-Dimensional Quantitative Structure-Activity Relationships of Cyclo-oxygenase-2 (COX-2) Inhibitors: A Comparative Molecular Field Analysis. *J Med Chem* 44: 3223-3230.
- DEVIERE J. 2002. Do selective cyclo-oxygenase inhibitors eliminate the adverse events associated with nonsteroidal anti-inflammatory drug therapy? *Eur J Gastroenterol Hepatol* 14(Suppl. 1): S29-33.
- DEWAR MJ, ZOEBSCH EG, HEALY EF, STEWART JJP. 1985. AM1: a new general purpose quantum mechanical molecular model. *J Am Chem Soc* 107: 3902-3909.

- DEWITT DL. 1999. COX-2-selective inhibitors: the new super aspirins. *Mol Pharmacol* 55: 625-631.
- DANNHARDT G, KIEFER W. 2001. Cyclooxygenase inhibitors--current status and future prospects. *Eur J Med Chem* 36: 109-126.
- DIONNE R. 2003. Relative efficacy of selective COX-2 inhibitors compared with over-the-counter ibuprofen. *Int J Clin Pract Suppl* 135: 18-22.
- FU JY, MASFERRER JL, SIEBERT K, RAZ A, NEEDLEMAN PJ. 1990. The induction of prostaglandin-H₂ synthase (cyclooxygenase) in human monocytes. *J Biol Chem* 265: 16737-16747.
- HARMAN HH. 1976. *Modern Factor Analysis* (3rd Ed). Chicago: University of Chicago Press. 322p.
- KELLOG GE, KIER LB, GAILLARD P, HALL LH. 1996. E-state fields: applications to 3D QSAR. *J Comput Aided Mol Des* 10(6): 513-520.
- KHOSHNEVISZADEH M, EDRAKI N, MIRI R, HEMMATEENEJAD B. 2008. Exploring QSAR for substituted 2-sulfonyl-phenyl-indol derivatives as potent and selective COX-2 inhibitors using different chemometrics tools. *Chem Biol Drug Des* 72(6): 564-574.
- KIER LB, HALL LH. 1999. *Molecular Structure Description: The Electrotopological State*; Lemont B. (ed). New York: Academic Press. ____ p.
- LANEUVILLE O, BREUER DK, DEWITT DL, HLA T, FUNK CD, SMITH WL. 1994. Differential inhibition of human prostaglandin endoperoxide H synthases-1 and -2 by nonsteroidal anti-inflammatory drugs. *J Pharmacol Exp Ther* 271: 927-934.
- MANIVANNAN E, CHATURVEDI SC. 2009. QSAR investigations on benzylideneamino and phenyliminomethyl scaffolds for selective COX-2 inhibitor: a Hansch approach. *Med Chem* 5(5): 440-445.
- MARNETT LJ, KALGUTKAR AS. 1998. Design of selective inhibitors of cyclooxygenase-2 as nonulcerogenic anti-inflammatory agents. *Curr Opin Chem Biol* 2: 482-490.
- MAW HH, HALL LH. 2000. E-State modeling of modeling of dopamine transporter binding: validation of model for small data set. *J Chem Inf Comput Sci* 40: 1270-1275.
- MAW HH, HALL LH. 2001. E-State modeling of corticosteroids binding affinity: validation of model for small data set. *J Chem Inf Comput Sci* 41: 1248-1254.
- SOLOMONS TWG. 1990. *Fundamentals of Organic Chemistry* (3rd Ed.) New York: John Wiley & Sons. p494.
- SHAH UA, DEOKAR HS, KADAM SS, KULKARNI VM. 2009. Pharmacophore generation and atom-based 3D-QSAR of novel 2-(4-methylsulfonylphenyl) pyrimidines as COX-2 inhibitors. *Mol Divers.* ____ p.
- SOLTANI S, ABOLHASANI H, ZARGHIA, JOUYBAN A. 2010. QSAR analysis of diaryl COX-2 inhibitors: comparison of feature selection and train-test data selection methods. *Eur J Med Chem* 2010 45(7): 2753-2760.
- VANE JR, BOTTING RM. 1996. Mechanism of action of anti-inflammatory drugs. *Scand J Rheumatol Suppl* 102: 9-21.
- WOLD S. 1991. Validation of QSAR's. *Quant Struct Act Relat* 10: 191-193.