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Exploring Selectivity Requirements for COX-2 versus COX-1 Binding of 3,4-Diaryloxazolones Using E-State Index

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Abstract—Considering the importance of developing selective COX-2 inhibitors, the present paper explores selectivity requirements for COX-2 versus COX-1 binding of 3,4-diaryloxazolones using electrotopological state (E-state) index. The study also shows the utility of E-state index in developing statistically acceptable model having direct physicochemical significance: electron density distribution of different atoms of the oxazolone ring and attached two phenyl rings are important for the selective binding with COX-2 over COX-1. Moreover, the use of indicator variable shows that presence of *ortho* R_1 substituent (except fluoro) on the N^3-phenyl ring decreases COX-2 selectivity. Further, an amino substituent at R_2 position (i.e., sulfonamide compound) is favorable for increasing COX-2 selectivity when the R_3 position is unsubstituted.

The use of non-steroidal anti-inflammatory drugs (NSAIDs) is common in alleviating pain, pyrexia and inflammation, and in patients with rheumatoid arthritis and osteoarthritis. However, these drugs are associated with high incidence of gastrointestinal adverse effects.[1–3] NSAIDs block prostaglandin biosynthesis by inhibiting the enzyme cyclooxygenase (COX), which exists in a constitutive (COX-1) and an inducible (COX-2) isoforms.[3] Nonselective NSAIDs suppress the activity of both isoforms of COX. Inhibition of COX-1 is primarily responsible for the adverse gastrointestinal effects of NSAIDs while the inhibition of COX-2 for their therapeutic effects.[4] Recognition of the two distinct COX isoforms prompted the development of drugs that selectively block the activity of COX-2, such as celecoxib, rofecoxib and valdecoxib. These selective inhibitors have the potential to reduce the risk of gastrointestinal bleeding associated with the use of nonselective blockers.[3,5] Moreover, as COX-2 is overexpressed in several human cancers, the potential utility of selective COX-2 inhibitors in the prevention and treatment of cancer is being presently explored.[5–8] COX-2 inhibitors may be recommended for average-risk individuals as chemopreventive agents.[9] Thus, exploring the requirements of COX inhibitors for selectively binding with COX-2 isoform over COX-1 is of current interest.

The present paper explores selectivity requirements for COX-2 versus COX-1 binding of 3,4-diaryloxazolones[10] using electrotopological state (E-state) index, developed by Kier and Hall.[11,12] Though the use of topological indices in QSAR has been criticized by some authors,[13] some misunderstandings on the role of such descriptors in Chemistry have been recently clarified by Estrada.[14] In another paper, Estrada has shown that topological descriptors may predict 3D structural parameters also.[15] In a recent paper, Rose and Hall[16] have commented that topological models directly give structural information to guide design of new molecules and the topological model developed by them in the paper was statistically better than a previous model[17] based on ab initio quantum mechanical calculations. The present group of authors also have used E-state index to explore QSAR of ligands acting on pharmacologically relevant targets of contemporary interest.[18,19] In continuation of such efforts, the present communication will show here the utility of E-state index in QSAR studies by exploring selectivity requirements of COX-2 versus COX-1 binding of 3,4-diaryloxazolones using E-state index.

The E-state index is an atom level descriptor[11,12] encoding both electronic character and topological

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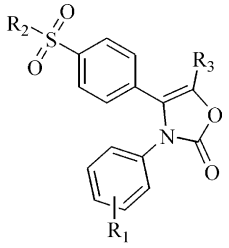
environment of each skeletal atom in a molecule. It is derived from graph theoretic approach and has two basic components: (a) intrinsic topological and electronic state of an atom; (b) effect of the environment influencing the atom (perturbation), considering differences in the intrinsic electrotopological states of different atoms and topological distance among them which determine the magnitude of the interactions. The E-state index has been projected as a useful tool in the context of QSAR studies and reported to have power to identify atoms or fragments in the molecules that are important for the biological activity.[20]

The structural features of the compounds and their COX-binding affinities are given in Table 1. In the present study, we have tried to incorporate hydrophobicity parameter (partition coefficient, log P), steric factor (molar refractivity, MR) and some indicator or integer variables along with E-state parameters to improve the quality of final relations. As we see from previous papers,[16,21] that use of molecular connectivity along with E-state index helps to develop better models, we have tried to include first order molecular connectivity ($^1\chi^v$) also in the relations. The physicochemical parameter values (log P and MR) were calculated by Chem

Table 1. Structural features, observed, calculated and predicted cyclooxygenase binding affinity of 3,4-diaryloxazolones



1-31

Sl. no.	Substituents			Biological activity [pIC_{50} (mM)][a]						Selectivity log [IC_{50} (COX-1)/IC_{50} (COX-2)]		
				COX-2			COX-1					
	R_1	R_2	R_3	Obs.	Calc.[b]	Pred.[b]	Obs.	Calc.[c]	Pred.[c]	Obs.	Calc.[d]	Pred.[d]
1	H	Me	H	2.796	2.727	2.679	1.474	1.640	1.659	1.322	1.464	1.485
2	2-F	Me	H	3.180	2.996	2.977	1.873	1.574	1.542	1.301	1.295	1.295
3	4-F	Me	H	3.292	3.103	3.078	2.292	2.066	2.047	1.000	1.199	1.233
4	4-Cl	Me	H	3.495	3.273	3.232	1.866	2.092	2.113	1.623	1.344	1.316
5	4-CF_3	Me	H	2.699	2.957	2.973	—	—	—	—	—	—
6	4-Me	Me	H	3.319	3.365	3.377	1.936	1.962	1.968	1.380	1.422	1.427
7	4-Et	Me	H	3.161	3.451	3.529	1.373	1.646	1.678	1.785	1.437	1.392
8	2,4-di-F	Me	H	2.870	2.832	2.828	2.159	2.000	1.987	0.699	1.029	1.212
9	H	NH_2	H	2.620	2.517	2.457	1.613	1.626	1.628	1.000	1.037	1.043
10	2-F	NH_2	H	2.658	2.786	2.803	1.554	1.560	1.561	1.114	0.868	0.845
11	4-F	NH_2	H	2.824	2.893	2.899	1.879	2.052	2.066	0.954	0.772	0.745
12	2-Cl	NH_2	H	2.187	2.021	1.852	1.451	1.607	1.623	0.699	0.556	0.412
13	3-Cl	NH_2	H	2.824	3.068	3.079	1.967	2.075	2.084	0.845	0.993	1.011
14	4-Cl	NH_2	H	2.921	3.063	3.070	2.155	2.078	2.072	0.778	0.917	0.929
15	4-CF_3	NH_2	H	2.699	2.747	2.758	—	—	—	—	—	—
16	2-Me	NH_2	H	2.013	2.179	2.348	1.538	1.632	1.642	0.477	0.620	0.765
17	3-Me	NH_2	H	3.292	3.186	3.180	2.180	1.950	1.900	1.114	1.050	1.039
18	4-Me	NH_2	H	3.102	3.155	3.158	1.963	1.949	1.946	1.146	0.995	0.977
19	4-Et	NH_2	H	2.807	3.241	3.271	1.983	1.632	1.593	0.845	1.010	1.031
20	4-OMe	NH_2	H	3.678	3.153	3.126	3.046[e]	—	—	0.602[e]	—	—
21	2,4-di-F	NH_2	H	2.638	2.622	2.617	1.815	1.986	1.999	0.845	0.602	0.487
22	3,4-di-Cl	NH_2	H	3.398	3.074	3.057	2.824	2.527	2.419	0.602	0.871	0.896
23	3-F-4-OMe	NH_2	H	2.947	2.946	2.946	2.292	2.353	2.370	0.699	0.786	0.797
24	3-Cl-4-OMe	NH_2	H	3.108	3.164	3.168	2.237	2.388	2.431	0.845	0.889	0.893
25	Cyclohexyl[f]	NH_2	H	1.724	1.839	1.996	—	—	—	—	—	—
26	1-Naphthyl[f]	NH_2	H	2.328	2.212	2.055	1.431[e]	—	—	0.903[e]	—	—
27	H	NH_2	Me	2.745	2.917	3.060	1.307	1.247	1.217	1.431	1.459	1.463
28	4-F	NH_2	Me	3.292	3.293	3.293	1.339[e]	—	—	1.954[e]	—	—
29	3-Me	NH_2	Me	3.032	2.925	2.818	1.772	1.571	1.481	1.255	1.435	1.457
30	4-Me	NH_2	Me	2.770	2.876	2.983	1.362	1.568	1.663	1.415	1.417	1.417
31	3,4-di-Cl	NH_2	Me	3.638	3.474	3.393	2.060	2.144	2.215	1.580	1.292	1.261

[a]Observed values are taken from ref 10. Obs. = Observed, Calc. = Calculated, Pred. = Predicted.
[b]Calculated/Predicted from eq. 2.
[c]Calculated/Predicted from eq. 3.
[d]Calculated/Predicted from eq. 5.
[e]Compounds **20**, **26** and **28** were not used in COX-1 and selectivity data modeling.
[f]Cyclohexyl and 1-naphthyl groups replace the respective phenyl groups in **25** and **26**.

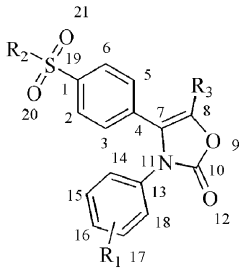


Figure 1. General structure of 3,4-diaryloxazolones: the common atoms have been numbered 1–21.

Draw Ultra 5.0 software[22] using Crippen's fragmentation method.[23] All compounds considered in the present study contain 21 common atoms (excluding hydrogens). The atoms of the molecules were numbered keeping serial numbers of the common atoms same in all the compounds (as shown in Fig. 1). The E-state index and $^1\chi^v$ values were calculated using ELECTRO1 and 1CHIV programs,[24] respectively. The program AUTOREG[24] was used to relate biological activity with E-state index of different atoms and also to different combinations of them along with appropriate physicochemical variables, connectivity parameter and indicator variables in order to find out the best multivariate relations. For this purpose, all possible combinations of predictor variables were tried with a restriction that predictor variables used in a equation are not much intercorrelated ($|r| < 0.5$). Using the program RRR98,[24] regression coefficients with corresponding standard errors and various statistical parameters reflecting quality[25] (like explained variance R_a^2, correlation coefficient R, standard error of estimate s, variance ratio F and average of absolute values of the residuals $AVRES$) of the equations were found out. A compound was considered as an outlier for a particular equation when the residual value exceeded twice the standard error of estimate of the equation. Leave-one-out cross-validation[26] was done using the programs KRPRES1 and KRPRES2,[24] which generate predicted variance (Q^2), predicted residual sum of squares (PRESS), standard deviation of error of prediction (SDEP) and average of absolute values of predicted residuals ($Pres_{av}$). While deriving the final relations, log P, MR and connectivity values could not find place in the best equations. Though many relations were generated, only the final ones will be reported here for brevity. The regression coefficients and variance ratios of all reported equations are significant at 95 and 99% levels, respectively. For convenience, definitions of different variables appearing in the reported equations are given in Table 2.

In case of COX-2 binding activity, the best relation involving all 31 compounds was the following:

$$\begin{aligned} pC_2 &= 2.101(\pm1.41)S_3 + 1.318(\pm0.90)S_{12} \\ &- 0.495(\pm0.30)I_{R_1\text{-H}} - 1.075(\pm0.34)I_{R_1\text{-o}} \\ &- 1.537(\pm0.39)I_{Ph} - 0.624(\pm0.39)I_{R_3\text{-Me}} \\ &- 15.973(\pm11.012) \\ n &= 31,\ Q^2 = 0.71,\ R_a^2 = 0.76,\ R^2 = 0.81,\ R = 0.90, \\ s &= 0.22,\ F = 16.8(\text{df}6, 24),\ \text{AVRES} = 0.15, \\ \text{PRESS} &= 1.73,\ \text{SDEP} = 0.24,\ \text{Pres}_{av} = 0.20 \end{aligned} \quad (1)$$

The 95% confidence intervals of the regression coefficients are shown within parentheses. eq 1 could predict 71.2% and explain 76.0% of the variance of COX-2 binding affinity. As E-state values of atom numbers 2, 3, 5 and 6 are highly intercorrelated ($|r| > 0.8$), a new variable S_{2356} was defined as sum of E-state values of the atoms. Similarly, another variable S_{912} was defined from E-state values of atoms 9 and 12. The statistical quality of the resultant equation was comparable to that of eq 1.

$$\begin{aligned} pC_2 &= 0.426(\pm0.35)S_{2356} + 0.790(\pm0.48)S_{912} \\ &- 0.540(\pm0.29)I_{R_1\text{-H}} - 1.054(\pm0.33)I_{R_1\text{-o}} \\ &- 1.478(\pm0.38)I_{Ph} - 0.679(\pm0.41)I_{R_3\text{-Me}} \\ &- 12.803(\pm8.44) \\ n &= 31,\ Q^2 = 0.70,\ R_a^2 = 0.76,\ R^2 = 0.81,\ R = 0.90, \\ s &= 0.22,\ F = 17.1(\text{df}6, 24),\ \text{AVRES} = 0.15, \\ \text{PRESS} &= 1.81,\ \text{SDEP} = 0.24,\ \text{Pres}_{av} = 0.20 \end{aligned} \quad (2)$$

The predictor variables used in eqs 1 and 2 are not much intercorrelated. The variable S_{2356} in eq 2 implies the importance of electron density distribution of the 4-phenyl ring which is in turn dependent on the R_2 substituent (*p*-methylsuphone or sulfonamide compound). Again, the variable S_{912} indicates the impor-

Table 2. Definitions of variables

Variable	Definition
S_3	E-state value of atom no. 3
S_{12}	E-state value of atom no. 12
S_{2356}	Sum of E-state values of atom nos. 2, 3, 5 and 6
S_{912}	Sum of E-state values of atom nos. 9 and 12
$I_{R_1\text{-H}}$	Indicator variable having value 1 when $R_1 = H$, value 0 otherwise
$I_{R_1\text{-o}}$	Indicator variable having value 1 when R_1 is an *ortho* substituent (other than fluoro), value 0 otherwise
I_{Ph}	Indicator variable having value 1 when the N^3-phenyl ring is replaced by other ring system, value 0 otherwise
$I_{R_3\text{-Me}}$	Indicator variable having value 1 when $R_3 = CH_3$ and $R_1 = CH_3$, value 0 otherwise
S_8	E-state value of atom no. 8
$I_{R_1\text{-Me}}$	Indicator variable having value 1 when $R_1 = CH_3$ (*meta* or *para*), value 0 otherwise
N_X	Integer variable denoting number of *meta* and/or *para* halogen substituents (R_1 position) on the N^3-phenyl ring
S_{15}	E-state value of atom no. 15
S_C	Sum of E-state values of atom nos. 4, 7, 10, 11, 13, 14, 15 and 16
$I_{R_2-NH_2}$	Indicator variable having value 1 when $R_2 = NH_2$ and $R_3 = H$, value 0 otherwise

tance of two oxygen atoms of the oxazolone ring. The negative coefficient of $I_{R_1\text{-H}}$ indicates that substitutions on N^3-phenyl ring increase COX-2 binding affinity. However, an *ortho* R_1 substituent (except fluoro) decreases the affinity. The negative coefficient of I_{Ph} indicates that replacement of the N^3-phenyl ring with other ring systems decreases affinity. Further, the variable $I_{R_3\text{-Me}}$ suggests that a methyl substituent present at R_3 position decreases binding affinity if it is simultaneously present with another methyl substituent at R_1 position. The calculated and predicted COX-2 binding affinity values according to eq 2 are given in Table 1.

In case of COX-1 binding activity, quantitative data were not available for compounds **5**, **15** and **25**. Among the rest of the compounds, **26** is the only compound without having N^3-phenyl moiety; thus, it was not included in the study during modeling of COX-1 binding affinity. Moreover, two more compounds, **20** and **28** were deleted for their significant outlier behavior. The best relation involving 25 compounds was the following:

$$pC_1 = 0.431(\pm0.26)S_8 + 0.320(\pm0.19)I_{R_1-Me} + 0.464(\pm0.13)N_X + 1.052(\pm0.33)$$
$$n = 25,\ Q^2 = 0.63,\ R_a^2 = 0.71,\ R^2 = 0.74,\ R = 0.86,$$
$$s = 0.20,\ F = 20.3(\text{df}3, 21),\ \text{AVRES} = 0.15,$$
$$\text{PRESS} = 1.17,\ \text{SDEP} = 0.22,\ \text{Pres}_{av} = 0.18 \quad (3)$$

eq 3 could predict 63.3% and explain 70.7% of the variance of COX-1 binding affinity. The predictor variables of eq 3 are not much intercorrelated. The positive coefficient of the variable S_8 in eq 3 indicates that increase of E-state value of atom 8 increases COX-1 binding affinity. This, in turn, indicates the importance of the R_3 substituent. The positive coefficients of variables $I_{R_1\text{-Me}}$ and N_X indicates that presence of *meta*- or *para*-methyl and halogen substituents at R_1 position increases COX-1 binding affinity. The calculated and predicted COX-1 binding affinity binding affinity values according to eq 3 are given in Table 1.

While exploring selectivity relations involving the same 25 compounds as used in the case of COX-1 modeling, the following best relation was obtained:

$$\log(C_1/C_2) = 0.473(\pm0.31)S_{15} - 0.397(\pm0.32)I_{R_1-o} - 0.411(\pm0.17)I_{R_2-NH_2} + 0.551(\pm0.52)$$
$$n = 25,\ Q^2 = 0.55,\ R_a^2 = 0.67,\ R^2 = 0.71,\ R = 0.84,$$
$$s = 0.20,\ F = 17.3(\text{df}3, 21),\ \text{AVRES} = 0.15,$$
$$\text{PRESS} = 1.30,\ \text{SDEP} = 0.23,\ \text{Pres}_{av} = 0.19 \quad (4)$$

As E-state values of atom numbers 4, 7, 10, 11, 13, 14, 15 and 16 are highly intercorrelated ($|r| > 0.8$), a new variable S_C was defined as sum of E-state values of the atoms.

$$\log(C_1/C_2) = 0.068(\pm0.047)S_C - 0.430(\pm0.33)I_{R_1-o} - 0.412(\pm0.18)I_{R_2-NH_2} + 0.893(\pm0.33)$$
$$n = 25,\ Q^2 = 0.53,\ R_a^2 = 0.66,\ R^2 = 0.70,\ R = 0.84,$$
$$s = 0.20,\ F = 16.5(\text{df}3, 21),\ \text{AVRES} = 0.16,$$
$$\text{PRESS} = 1.37,\ \text{SDEP} = 0.23,\ \text{Pres}_{av} = 0.19 \quad (5)$$

The predictor variables of eqs 4 and 5 are not much intercorrelated. eq 5 could predict 52.5% and explain 66.0% of the variance of selectivity for binding with COX-2 over COX-1. The variable S_C in eq 5 indicates that electron density distributions of different atoms of the oxazolone ring and attached two phenyl rings are important for the selective binding with COX-2 over COX-1. Moreover, the variable I_{R_1-o} indicates that presence of *ortho* R_1 substituent (except fluoro) on the N^3-phenyl ring decreases COX-2 selectivity. Further, the variable $I_{R_2\text{-}NH_2}$ implies that an amino substituent at R_2 position (i.e., sulfonamide compound) is favorable for increasing COX-2 selectivity when the R_3 position is unsubstituted.

The present QSAR study could through some light on the selectivity requirements of 3,4-diaryloxazolones for binding with COX-2 over COX-1. The study also shows the utility of E-state index in developing statistically acceptable model having direct physicochemical significance. Based on the importance of different positions of the basic skeletal structure of the compounds and contribution pattern of different substituents, selective COX-2 inhibitors may be designed. However, more data points covering wider features of substitution pattern need be considered to reach a conclusion.

Acknowledgements

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