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QSAR Analysis of Substituted Benzylideneacetophenones as Lipid Peroxidation Inhibitors

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A quantitative structure activity relationship study was perfomred on a series of substituted benzylideneacetophenones with inhibitory effect on lipid peroxidation using combination of various thermodynamic, electronic and steric descriptors. Several statistical regression expressions were obtained using multiple linear regression analysis. The best QSAR model was further validated by leave one out cross validation method. Electronic parameter (total energy) and thermodynamic parameter [dipole (DPL_3) van der Waals' energy (E14)] were found to have significant correlationship.

Key Words: QSAR, Antiinflammatory, Antioxidant, Lipid peroxidation inhibitors, Substituted benzylideneacetophenones.

INTRODUCTION

Inflammation is a defense reaction caused by tissue damage or injury, characterized by redness, heat, swelling and pain. Inflammatory processes are associated with a large increase in oxygen-free radicals and other reactive species as prostaglandins, nitric oxide and lipid peroxidation products such as malondialdehyde and 4-hydroxy-2,3-trans-normal (HNE)[1]. These species injure cells and tissues directly *via* oxidative degradation of essential cellular components and indirectly by altering the protease/antiprotease balance[2]. These species initate and/or amplify inflammation *via* upregulation of several different genes involved in inflammation, such as those that code for proinflammatory cytokines and adhesion molecules.

Lipid peroxidation[3] is free radical initiated chain oxidation of unsaturated lipids that takes place in three steps: 1. initiation, 2. propagation and 3. termination.

$$LH + R^* \longrightarrow \underset{\text{Lipid radical}}{L^*} + RH$$

$$L^* + O_2 \longrightarrow \underset{\text{Lipid peroxyl radical}}{LOO^*}$$

$$LOO^{*} + LH \longrightarrow \underset{\text{Lipid hydroperoxide}}{LOOH} + L^{*}$$

$$LOOH \longrightarrow LO, LOO\text{-aldehydes}$$

Free redicals initiate the oxidation of polyunsaturated fatty acids (PUFA) by abstraction of hydrogen atom from methylene group of the fatty acid. The lipid radical formed tends to stabilize by molecular rearrangement to form conjugated diene. The conjugated diene readily combines with oxygen to give peroxy radical, the peroxy radicals are capable of abstraction of hydrogen from other lipid molecule propagating the chain reaction. The peroxy radical combines with hydrogen atom to give lipid hydroperoxide (lipid peroxide)[4,5].

The peroxy radicals are the carriers of the chain reactions, can oxidize further PUFA molecules and initiate new chains producing lipid hydroperoxides that can break down to yield radical species and wide range of compounds, mainly aldehyde. These aldehydes can diffuse from original site of attack and cause inflammation by activating the enzymes such as cycloxygenase and lipoxygenase[6,7].

Cycloxygenase[8] catalyzes incorporation of molecular oxygen to arachidonic acid leading to peroxidation at C-11 and C-15 followed by ring closure between C-8 and C-12. The products of reaction are cyclic peroxides PGG_2 and PGH_2 that are unstable and isomerized enzymatically or non-enzymatically into different products. The major products of endoperoxide metabolism are stablized PGD_2, PGF_2, $PGF_{2\alpha}$, TXA_2 and prostacyclins. On the other hand, lipoxygenase[9] catalyzes peroxidation of fatty acids giving rise to straight chain hydroperoxy acids (HPETEs), which are then converted into hydroxyl acids (HETEs), which are important proinflammatory mediators.

The peroxidation can be blocked by free radical scavengers as antiinflammatory agents. Most of the antiinflammatory agents, which inhibit the above mentioned enzymes, are associated with side effects such as ulceration and bleeding in gastro-intestinal tract; so the attention is focused on benzylideneacetophenones having antisinflammatory, antioxidant and gastric protectant activities by virtue of free radical scavengers[10]. The aim of the present study is to find QSAR models with good correlation between molecular structure and biological activity. Such an efforts would facilitate the design and development of lipid peroxidation inhibitors.

EXPERIMENTAL

The lipid peroxidation inhibitory data of substituted benzylideneacetophenones was taken from the reported work of Timmerman *et al.*[11] (Table-1 and Fig. 1). All the biological activity data (IC_{50} in μM) have been converted to negative logarithmic mole dose (pIC_{50}) for QSAR analysis. The correlations were sought between inhibitory activity and various substituent constants at position R_1 of molecule (Fig. 1) and indicator variable for presence of methoxy group at R_4 (I_{v1}) and presence of hydroxy group in the ring system at R_5 position (I_{v2}). The values of substituent constants like hydrophobic (π), steric (molar refractivity

or MR), hydrogen acceptor (HA), hydrogen donor (HD) and electronic (field effect or $\mathcal{F}$, resonance effect or $\mathcal{R}$ and Hammett's constant or σ) were taken from literature. The series was also subjected to molecular modeling *via* 3D-QSAR studies using CS Chem-Office Software version 6.0 (Cambridge Soft)[12] running on a P-III processor. Structures of all the compounds were sketched using builder module of the program. These structures were then subjected to energy minimization using force field molecular mechanics-2 (MM2) until the root mean square (RMS) gradient value became smaller than 0.1 kca/mol Å. Minimized molecules were subjected to reoptimization *via* Austin model-1 (AM1) method[13] until the RMS gradient attained a value smaller than 0.001 kcal/mol Å using MOPAC. The geometry optimization of the lowest energy structure was carried out using eigen vector following routine. The descriptor values for all the molecules were calculated using compute properties module of program.

TABLE-1
SUBSTITUTED BENZYLIDENEACETOPHENONES AND COMPARISON OF OBSERVED AND CALCULATED pIC_{50} VALUE OF COMPOUNDS USED IN EQN. 1

Compound	R_1	R_2	R_3	R_4	R_5	Observed pIC_{50}	Calculated pIC_{50}
1	CH_3O	H	H	H	H	–1.25	–1.202600
2	CH_3O	H	CH_3	H	H	–1.09	–1.096660
3	CH_3O	H	CH_3O	H	H	–1.26	–1.206380
4	CH_3O	H	Cl	H	H	–0.85	–1.068290
5	CH_3O	H	H	H	OH	–1.17	–1.202600
6	CH_3O	CH_3O	CH_3	H	H	–0.107	–0.413829
7	CH_3O	CH_3O	CH_3O	CH_3O	H	–0.704	–0.523548
8	CH_3O	H	OH	H	H	–1.35	–1.329340
9	CH_3O	H	F	H	H	–1.31	–1.176110
10	CH_3O	CH_3O	F	H	H	–0.62	–0.493281
11	*t*-Bu	*t*-Bu	F	CH_3O	H	–0.55	–0.307870
12	*t*-Bu	*t*-Bu	OH	H	H	–0.29	–0.461098
13	CH_3O	H	OH	H	H	–1.29	–1.329340
14	*i*-Pr	*i*-Pr	F	H	H	–0.70	–0.672596
15	*t*-Bu	*t*-Bu	CH_3	H	H	–0.55	–0.440287
16	*t*-Bu	*t*-Bu	C_2H_5O	H	H	–0.26	–0.262469
17	*t*-Bu	*t*-Bu	CH_3O	H	H	–0.29	–0.338137
18	*t*-Bu	*t*-Bu	OH	H	H	–0.22	–0.461099
19	*t*-Bu	*t*-Bu	Cl	H	H	–0.26	–0.200042

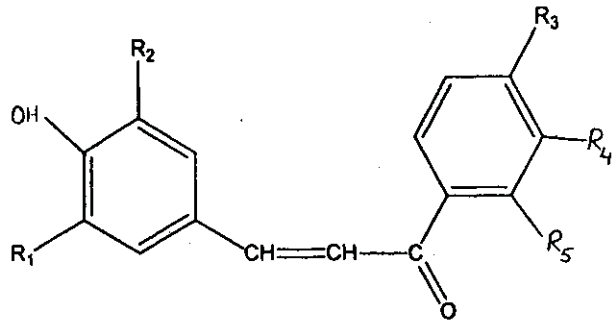

Fig. 1. Benzylideneacetophenones analogs used in the present study

Calculated thermodynamic descriptors[14] included critical temperature (T_c), ideal gas thermal capacity (C_p), critical pressure (P_c), boiling point (b.p.), Henry's law constant (H), bend energy (E_b), heat of formation (H_f), total energy (TE) and partition coefficient (PC).

Steric descriptors[15] derived were Connolly accessible area (CAA), Connolly molecular area (CMA), Connolly solvent excluded volume (CSEV), exact mass (EM), molecular weight (MW), principal moment of inertia-X component (PMI-X), principal moment of inertia-Y component (PMI-Y) and principal moment of inertia-Z component (PMI-Z), molar refractivity (MR) and ovality (OVAL).

Electronic descriptors[16, 17] such as dipole (DPL), electronic energy (ElcE), highest occupied molecular orbital energy (HOMO), lowest unoccupied molecular orbital energy (LUMO), repulsion energy (NRE), VDW-1,4-energy (E14), Non-1,4-VDW energy (E_v) and total energy were calculated.

Stepwise multiple linear regression analysis method[18, 19] was used to perform QSAR analysis employing in-house VALSTAT program. The ± data within the parentheses are the standard deviation, associated with coefficient of descriptor in regression equation. The best model was selected on the basis of various statistical parameters such as correlation coefficient (r), standard error of estimation (SE), sequential Fischer test (F). Quality of each model was estimated from the cross validated squared correlation coefficient (Q^2), calculated root mean square error (S_{DEP}), chance statistics evaluated as the ratio of the equivalent regression equations to the total number of randomized sets; a chance value of 0.01 corresponds to 1% chance of fortuitous correlation and boot-strapping square correlation coefficient (r^2_{bs})[20] which confirm the robustness and applicability of QSAR equation on the structural analogs.

RESULTS AND DISCUSSION

When data set was subjected to stepwise multiple linear regression analysis, in order to develop 2D-QSAR between inhibitory activity as dependent variables and substituents constant as independent variables, several equations were obtained. The statistically significant equation [eqn. (1)] with coefficient of correlation (r = 0.94) was considered as model 1 (Tables 2, 3 and Fig. 2).

TABLE-2
DESCRIPTORS USED IN EQN. (1)

Compound No.	MR_1	$\mathcal{F}_2$	π_3
1	7.87	0.00	0.00
2	7.87	0.00	0.56
3	7.87	0.00	–0.02
4	7.87	0.00	0.71
5	7.87	0.00	0.00
6	7.87	0.26	0.56
7	7.87	0.26	–0.02
8	7.87	0.00	–0.67
9	7.87	0.00	0.14
10	7.87	–0.26	0.14
11	19.62	–0.07	0.14
12	19.62	–0.07	–0.67
13	7.87	0.00	–0.67
14	14.96	0.00	0.14
15	19.62	–0.05	0.56
16	19.62	–0.07	0.38
17	19.62	–0.07	–0.02
18	19.62	–0.07	–0.07
19	19.62	–0.07	0.71

TABLE-3
CORRELATION MATRIX FOR EQN. (1)

	MR_1	$\mathcal{F}_2$	π_3
MR_1	1.0000	—	—
$\mathcal{F}_2$	0.6090	1.0000	—
π_3	0.1630	0.2220	1.000000

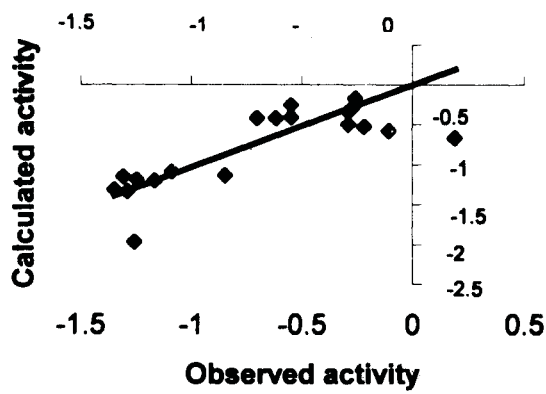

Fig. 2. A plot of observed pIC_{50} *vs*, calculated pIC_{50} for eqn. (1)

$$pIC_{50} = 0.090(\pm 0.018)^{*}MR_1 + 2.626(\pm 0.896)^{*}\mathcal{F}_2 + 0.189(\pm 0.176)^{*}\pi_3 - 1.907$$

$$n = 19, r = 0.944, r^2 = 0.891, SE = 0.159, F = 40.829 \quad (1)$$

Eqn. (1) explains for 89.1% of the variance in activity with low standard error of estimation. The model showed overall internal statistical significance level better than 99.9% as it exceeded the tabulated $F_{(3, 15\alpha 0.001)} = 10.8$. The study revealed that steric effect, *i.e.*, molar refractivity, electronic parameter, *i.e.*, field effect and hydrophobic nature contributed positively to the R_1, R_2 and R_3 positions respectively. The analysis is also usggested that A ring of the parent system (Fig. 1) is more prone to modification for the activity as compare to the B ring.

The series was also subjected to molecular modeling using 3D-QSAR; all the descriptor values (Table-4) for the molecules calculated from the program were considered as independent variables and inhibitory concentration data (pIC_{50}) were taken as dependent variables. Regression gave various multivariant equations with significant correlation coefficients. The statistically significant equations with coefficient of correlation (r) = 0.875 were considered. The equations showed overall internal statistical significance level better than 99.9% as it exceeded the tabulated $F_{(3, 15\alpha 0.001)} = 10.8$.

TABLE-4
DESCRIPTORS USED IN EQNS. (2) AND (3)

Compd. No.	DPL_3	TE	E_{14}	CP	PMIX
1	-0.7724	-3200.88	10.9958	28.9051	410.400
2	0.0177	-3356.62	11.5177	25.7411	432.890
3	-1.7187	-3676.76	13.6988	25.3027	540.085
4	-0.0293	-3560.85	11.4017	27.3542	462.780
5	0.1111	-3521.35	9.58329	34.1586	514.281
6	-0.1890	-3832.59	14.1468	22.6973	811.884
7	1.7986	-4152.60	15.9159	22.3341	958.052
8	-1.3785	-3997.16	11.5543	29.5690	516.358
9	-0.7218	-3672.09	10.3445	27.1267	467.074
10	-0.0856	-3672.15	10.5547	27.1267	429.107
11	0.1274	-4441.80	17.8407	15.3546	1447.330
12	0.9355	-4766.79	17.3305	16.3778	1533.420
13	-0.3674	-3521.50	9.78172	34.1586	473.340
14	-0.4344	-4131.14	15.0042	17.6245	1112.330
15	-0.9029	-4126.47	18.6209	14.7588	1457.600
16	-0.1812	-4601.58	17.4433	13.5563	1635.700
17	-1.5562	-4446.48	20.4279	14.5679	1534.820
18	-0.0662	-4290.96	15.9752	18.2161	1447.230
19	-0.2121	-4330.60	18.5439	15.4513	1480.740

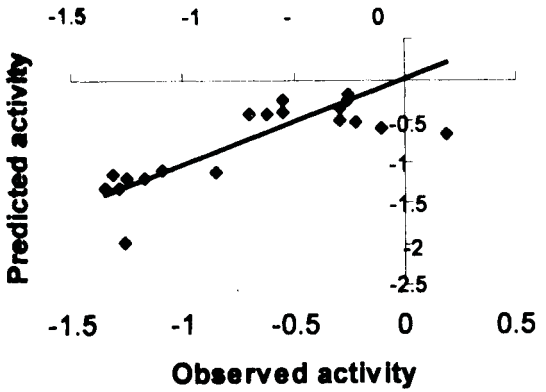

Fig. 3. A plot of observed pIC_{50} *vs.* predicted (leave one out) pIC_{50} for eqn. (2)

$$pIC_{50} = 0.234(\pm 0.188)DPL_3 - 7.594e\text{-}005(\pm 8.080e\text{-}005)TE + 0.088(\pm 0.036)E14 - 2.210$$

$$n = 19, r = 0.857, r^2 = 0.734, SE = 0.248, F = 13.775 \quad (2)$$

$$ICWP < 0.570, r^2_{bs} = 0.728, chance < 0.01, Q^2 = 0.417, S_{PRESS} = 0.367, S_{DEP} = 0.327$$

$$pIC_{50} = 0.116(\pm 0.158)DPL_3 - 0.039(\pm 0.031)C_p + 1.986e\text{-}004(\pm 4.062e\text{-}004)PMIX - 0.003$$

$$n = 19, r = 0.852, r^2 = 0.725, SE = 0.252, F = 13.215 \quad (3)$$

$$ICWP < 0.795, r^2_{bs} = 0.736, chance < 0.01, Q^2 = 0.628, S_{PRESS} = 0.294, S_{DEP} = 0.261$$

Eqns. (2) and (3) having nearly same variance, *i.e.*, 72.5% but eqn. (2) having intercorrelation among the physiochemical descriptors is less than eqn. (3) which is desired for robustness of the regression expression (Table-5). Therefore, eqn. 2 is considered as the model. To acertain the predictivity of the model, internal validation using leave one out method of cross-validation process (Table-6), bootstrapping techniques and randomization test was performed. The equation was further subjected to cross-validation method to confirm the internal consistency; the cross-validated squared correlation coefficient ($Q^2 = 0.417$), standard deviation of error of prediction (S_{press}) = 0.367, standard deviation of error of prediction (S_{DEP}) = 0.327 suggested good predictive ability of the activity. The robustness and wide pragmatism of the equation was further supported by $r^2_{bs} = 0.728$ and chance < 0.01. At par value of bootstrapping squared correlation coefficient (r^2_{bs}) with conventional squared correlation coefficent (r^2), suggested that the model is a proper representative of analogs.

TABLE-5
CORRELATION MATRIX FOR EQNS. (2) AND (3)

	DPL_3	TE	E14	CP	PMIX
DPL_3	1.000	—	—	—	—
TE	0.567	1.000	—	—	—
E14	0.068	0.094	1.000	—	—
CP	0.231	0.317	0.124	1.000	—
PMIX	0.451	0.458	0.415	0.791	1.000

TABLE-6
COMPARISON OF OBSERVED AND LEAVE ONE OUT PREDICTED
pIC_{50} VALUE OF COMPOUNDS USED IN EQUNS. (2) AND (3)

Compd. No.	Observed activity	Predicted activity of model 2	Predicted activity of model 3
1	–1.250	–1.167390	–1.122990
2	–1.090	–0.913637	–0.895784
3	–1.260	–1.062480	–1.026770
4	–0.850	–0.947379	–0.995169
5	–1.170	–1.044860	–1.239540
6	–0.107	–0.749158	–0.787126
7	–0.704	0.136186	–0.311158
8	–1.350	–1.173010	–1.177270
9	–1.310	–1.165690	–1.024130
10	–0.620	–1.077560	–1.027460
11	–0.550	–0.210952	–0.259955
12	–0.290	0.182152	–0.207131
13	–1.290	–1.140870	–1.279320
14	–0.700	–0.668566	–0.763627
15	–0.550	–0.439953	–0.358924
16	–0.260	–0.372239	–0.219527
17	–0.290	–0.524295	–0.518314
18	–0.220	–0.511789	–0.466217
19	–0.260	–0.295008	–0.347576

The above studies reveal that the DPL_3 and E14 descriptors are contributed positively whereas E contributed negatively to biological activity. Dipole moments on Z-component play a significant role in scavenging the free radical. Van der Waals' 1,4-energy explains the depth of the attraction potential and how easy

it is to scavenge the free radical. Therefore, substitution of groups, which favour DPL_3 and E14, may enhance the activity. The overall QSAR analysis suggested that at R_1 position bulkiness is favourable for the activity while at R_2 position the groups which enhance the electronic properties favour the activity by stabilizing the free radical.

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