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Quantitative structure-activity relationship of some substituted phenothiazine as anti-inflammatory agents

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ABSTRACT

The invention of new anti-inflammatory agents peaks the first phase of an existing and fast paced effort to exploit a novel, target for nonsteroidal anti-inflammatory drugs (NSAIDs). A series of molecules has been reported as anti-inflammatory agents belonging to the class of phenothiazine nucleus. Various physicochemical and steric parameters were calculated. Quantitative structure-activity relationship models were generated employing sequential multiple regression method using VALSTAT. Statistically significant models with R-values 0.949 and 0.946 were obtained. Models were validated using leave one out and bootstrapping methods. The result shows that Boiling point, Stretch energy, Connolly molecular area and Partition coefficient are contributing to biological activity. Among these, Connolly molecular area and partition coefficient plays an important role as positive contribution is seen in the models. The obtained and validated models bring important structural insight to aid the design of novel anti-inflammatory activity prior to their synthesis.

KEYWORDS

Quantitative Structure-Activity Relationship;
Phenothiazine;
Anti-inflammatory Agents.

INTRODUCTION

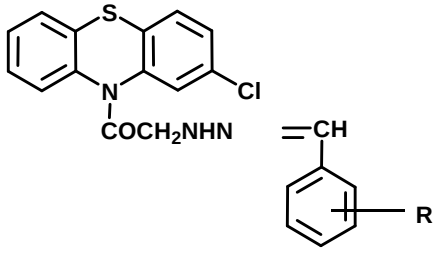
The anti-inflammatory agents represent an extremely interesting category of drugs as evident from the active research going on in numerous laboratories all over the world. There is continuous demand for new therapeutic agents for their therapeutic use, with high margin of safety and freedom for normally associated gastrointestinal side effects, notably dyspepsia, complications of peptic ulcers and renal toxicity of known anti-inflammatory drugs[1,2] it was thought to study QSAR analysis of some anti-inflammatory cyclic Phenothiazine derivatives reported by Saxena *et al*[3]. The above reported series of 20 compounds was subjected to quantitative structure-activity relationships (QSAR) analyses. As a part of our rational drug discovery programme on novel NSAIDs, the theoretical study is aimed for determining the quantitative relationship between various substitutions on phenothiazines and their anti-inflammatory activity.[4-6]

EXPERIMENTAL

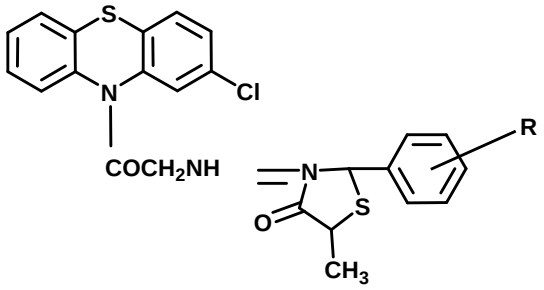
The activity data have been given as IC50 values, where IC50 refers to the experimentally determined molar concentration of the phenothiazine required to

inhibit carrageenan induced rat's paw oedema by 50%. The biological activity values [IC50 (μM)] reported in the literature were converted to molar units, and further to -log scale, and subsequently used as the response variable for QSAR analysis. Molecular modeling and quantum mechanical calculations were performed using CS Chem office software Version 6.0 (Cambridge Software)[7] running on P-IV processor.

The series of 20 compounds was generated by combination of four subsets of compounds of different nucleus (Figure 1) subset 1 Schiff's bases (compound **(1)** to **(5)**), subset 2-thiazolidinones (compound **(6)** to **(10)**), subset 3 ?[2]-triazolines (compound **(11)** to **(15)**) and subset 4-formazons (compound **(16)** to **(20)**) of 2-chlorophenothiazines subjected to QSAR analysis.



Subset 1 (Compound 1 to 5)



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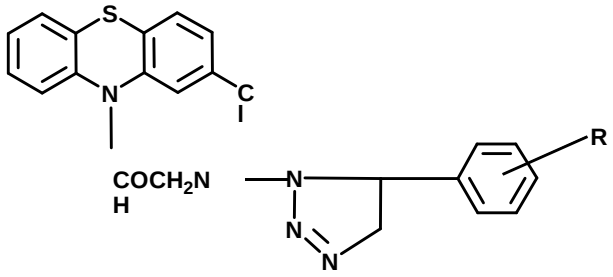
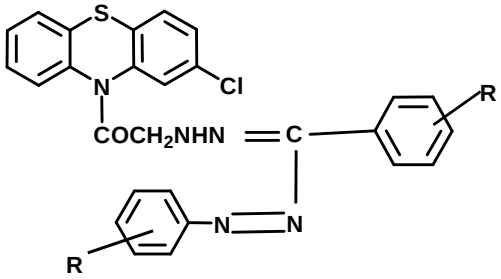


Subset 3 (Compound 11 to 15)



Subset 4 (Compound 16 to 20)

Figure 1 : General structure of phenothiazines

All the compounds **(1-20)** (TABLE 1) were sketched using CS Chem Draw Ultra module of CS Chem office. The sketched structures were imported to chem. 3D module in order to create its 3D model. Energy calculations were done using the Allinger's MM2 force field. Every structure was subjected to energy minimization process with root mean square gradient (RMS) 0.100 Kcal / mol A°. After the minimization process is over molecular dynamics uses Newtonian mechanics to stimulate motion of atoms; adding or subtracting kinetic energy, as the models temperature increases or decreases. Each and every compound reached to its most stable conformer and further, geometry optimization was done using semi-empirical AM1 (Austin model) Hamiltonian method, namely MOPAC (Version 6.0) module with the RMS value 0.001 Kcal / mol A°.

The optimized conformers were used for calculating physicochemical parameters by standard procedures given in QSAR plus modulus of CS Chem 3D. The

TABLE 1 : Substituents and anti-inflammatory data for phenothiazines

Compd. No.	R	IC_{50}	pIC_{50}
1	2-Cl	0.13	6.87
2	4-F	0.14	6.84
3	2,6-Cl_2	0.18	6.73
4	2-CH_3	0.09	7.01
5	4-CH_3	0.08	7.05
6	2-Cl	0.28	6.53
7	4-F	0.38	6.41
8	2,6-Cl_2	0.34	6.46
9	2-CH_3	0.27	6.56
10	4-CH_3	0.32	6.49
11	2-Cl	0.19	6.70
12	4-F	0.25	6.59
13	2,6-Cl_2	0.22	6.64
14	2-CH_3	0.19	6.71
15	4-CH_3	0.16	6.77
16	2-Cl	0.14	6.84
17	4-F	0.17	6.75
18	2,6-Cl_2	0.15	6.81
19	2-CH_3	0.12	6.91
20	4-CH_3	0.13	6.85

descriptors (TABLE 2) were calculated for QSAR[8,9] study (value of only those descriptors occurring in different equations are given in TABLE 3.

TABLE 2 : Descriptors calculated for QSAR study

Sr. No.	Descriptor	Type
1	Heat of Formation (HF)	Thermodynamic
2	Boiling Point (BP)	Thermodynamic
3	Critical Pressure (CP)	Thermodynamic
4	Critical Temperature (CT)	Thermodynamic
5	Critical Volume (CV)	Thermodynamic
7	Henry's Law Constant (HLC)	Thermodynamic
8	Ideal Gas Thermal Capacity (IGTC)	Thermodynamic
9	Log P	Thermodynamic
10	Melting Point (MP)	Thermodynamic
11	Molar Refractivity (MR)	Thermodynamic
12	Standard Gibbs Free Energy (SGFE)	Thermodynamic
13	Connolly Accessible Area (CAA)	Steric
14	Connolly Molecular Area (CMA)	Steric
15	Connolly Solvent–Excluded Volume (CSEV)	Steric
16	Ovality (OVA)	Steric
17	Principal Moment of Inertia – X (PMI–X)	Steric
18	Principal Moment of Inertia – Y (PMI–Y)	Steric
19	Principal Moment of Inertia – Z (PMI–Z)	Steric
20	Dipole Moment (D)	Electronic
21	Dipole Moment –X Axis (DX)	Electronic
22	Dipole Moment –Y Axis (DY)	Electronic
23	Dipole Moment –Y Axis (DZ)	Electronic
24	Electronic Energy (EE)	Electronic
25	HOMO Energy (HOMO)	Electronic
26	LUMO Energy (LUMO)	Electronic
27	Repulsion Energy (RE)	Electronic
28	Bend Energy (E_b)	Thermodynamic
29	Charge–Charge Energy (CCE)	Thermodynamic
30	Charge–Dipole Energy (CDE)	Thermodynamic
31	Dipole–Dipole Energy (DDE)	Thermodynamic
32	Non–1, 4 VDW Energy (E_v)	Thermodynamic
33	Stretch Energy (SE)	Thermodynamic
34	Stretch–Bend Energy (SBE)	Thermodynamic
35	Torsion Energy (E_t)	Thermodynamic
36	Total Energy (E)	Thermodynamic
37	Van der Waals e 1,4 Energy (VDWE)	Thermodynamic
38	VDW 1,4 Energy (VDWE)	Thermodynamic
39	Partition coefficient	Thermodynamic

All the calculated descriptors were considered as independent variables and biological activity as dependent variable. The correlation was obtained by stepwise multiple regression analysis employing VALSTAT[10] software.

To generate QSAR equations, stepwise multiple regression analysis[11] method was used. The QSAR generated models were cross-validated using the leave one out and bootstrapping method. The best equation was judged by considering the following statistical measures.

TABLE 3 : Descriptors contributing to the anti-inflammatory activity of phenothiazines

Compd. No.	BP	CMA	SE	PC
1	817.612	358.791	11.898	4.700
2	796.242	349.819	12.169	4.730
3	836.305	371.536	12.170	4.853
4	810.515	353.843	7.8424	4.674
5	810.515	358.528	8.0754	4.974
6	939.901	413.392	14.185	6.442
7	918.533	395.947	14.426	5.872
8	958.595	419.139	14.788	7.155
9	932.805	399.799	10.346	6.178
10	932.805	404.014	9.422	6.228
11	869.679	384.189	12.117	5.906
12	848.311	378.673	17.167	5.336
13	888.373	393.112	15.777	6.619
14	862.583	387.447	15.732	5.642
15	862.583	393.144	16.675	5.692
16	940.207	448.017	12.518	8.437
17	918.839	440.937	17.497	7.867
18	958.902	458.297	15.398	9.150
19	933.112	457.467	12.884	8.223
20	933.112	437.803	12.701	8.223

n - Number of sample in regression
R - Correlation coefficient
R^2 - Squared correlation coefficient (coefficient of determination)
SD - Standard deviation
t-test - For statistical significance and correlation matrix to show mutual correlation among the parameters.
F-test - Ratio between the described parts and non-described part of the Y variance
r^2_{BS} - Bootstrapped strapped squared correlation coefficient
q^2 - Squared cross-correlation coefficient
S_{DEP} - Standard deviation of error of prediction
S_{PRESS} - Standard deviation of sum of square of difference between predicted and observed values

RESULTS AND DISCUSSION

All the calculated descriptors and log IC_{50} value of the 20 compounds were subjected to stepwise multiple regression analysis and several models were generated.

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Among these models obtained, model-I and model-II were considered based on the statistical criterion: correlation coefficient > 0.8 and chance correlation < 0.001.

MODEL-I

pIC_{50} = 10.539 (± 0.733) - 0.005(± 0.001)BP - 0.025(± 0.011)SE +0.185(± 0.041) PC

n=20, R= 0.946, R^2 = 0.894, F=45.456, variance=0.004, SD = 0.063.

MODEL-II

pIC_{50} = 9.035(± 0.524) - 0.006(± 0.001)BP + 0.009 (± 0.001)CMA - 0.030(± 0.0115) SE

n=20, R= 0.949, R^2 = 0.901, F=48.538, variance=0.003, SD = 0.061.

Model -I shows good correlation (R= 0.946) between descriptors (BP, SE, PC) and biological activity. The thermodynamic descriptor boiling point (BP) and stretch energy (SE) shows negative contribution while partition coefficient (PC) shows positive contribution with anti-inflammatory activity. BP is the temperature at which the kinetic energy of molecule is sufficient to overcome attractive forces. Negative contribution of BP indicates decrease in intermolecular attractive forces and is conducive for activity. Stretch energy, a thermodynamic parameter, deals with the stretching or the conformational flexibility of the molecule. The descriptor in first model bears a negative coefficient, indicating, substituents that decrease the flexibility of phenothiazines and will enhance the anti-inflammatory activity. Partition coefficient calculated using atom based approach and represents the hydrophobicity of the molecules[12]. Partition coefficient is positively correlated with the biological activity. This property assumes significance in the present case because of the fact that the molecules under study contain lipophilic groups. Thus, model-I suggests that partition coefficient is of significance having high value of t-test indicating statistical significance of calculated regression coefficient.

To confirm these results, the value of pIC_{50} was estimated using leave one-out and correlated with observed value pIC_{50}. The value of bootstrapping r^2, chance and q^2 in randomized biological activity indicates the statistical significance of the model as given below.

r^2_{BS} = 0.901, chance = <0.001, q^2 = 0.836, S_{PRESS} = 0.078, S_{DEP} = 0.070.

The predicted activity data of model-I is shown in TABLE 4. A plot of observed activity versus predicted activity of the compounds is shown in Figure 2.

TABLE 4 : Predicted activity data for model-I

Compd. No.	Log BA	Calculated Activity	Predicted Activity
1	6.87	6.831	6.825
2	6.84	6.875	6.884
3	6.73	6.822	6.832
4	7.01	6.953	6.931
5	7.05	6.989	6.965
6	6.53	6.495	6.489
7	6.41	6.463	6.475
8	6.46	6.412	6.398
9	6.56	6.532	6.523
10	6.49	6.598	6.639
11	6.72	6.730	6.732
12	6.59	6.661	6.683
13	6.64	6.584	6.576
14	6.71	6.695	6.692
15	6.77	6.718	6.707
16	6.84	6.856	6.860
17	6.75	6.774	6.782
18	6.81	6.745	6.728
19	6.91	6.974	7.009
20	6.85	6.802	6.794

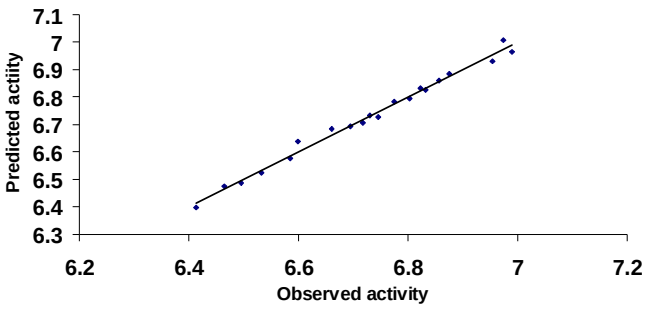

Figure 2 : Graph between observed activity and predicted activity of model-I.

Model-II shows good correlation (r=0.949) between descriptors (BP, CMA, SE) and biological activity. BP, SE negatively contributed to the biological activity. BP and SE are thermodynamic parameters. It plays an important role in governing molecular reactivity and properties. The model suggests that BP is of significance indicating that lowering the BP will favor

the activity. Stretch energy, a thermodynamic parameter, deals with the stretching or the conformational flexibility of the molecule. The descriptor in the model bears a negative coefficient, indicating, substituents that decrease the flexibility of phenothiazines and will enhance the anti-inflammatory activity. Connolly's solvent molecular area (CMA), a steric descriptor, represents the surface area that is in contact with the solvent. The descriptor bears positive coefficient in the model, suggesting decrease in the bulkiness of the substituents and molecular solvent molecular surface area is not conducive to the activity.

To confirm these results, the value of pIC_{50} was estimated using leave one-out and correlated with observed value pIC_{50}. The value of bootstrapping r^2, chance and q^2 in randomized biological activity indicates the statistical significance of the model as follows.

r^2_{BS} = 0.911, chance = <0.001, q^2 = 0.835, S_{PRESS} = 0.079, S_{DEP} = 0.070.

The predicted activity data of model-II is shown in TABLE 5. A plot of observed activity versus predicted activity of the compounds is shown in Figure 3.

TABLE 5 : Predicted activity data for model-II

Compd. No.	Log BA	Calculated Activity	Predicted Activity
1	6.87	6.824	6.817
2	6.84	6.935	6.961
3	6.73	6.747	6.750
4	7.01	6.959	6.939
5	7.05	7.009	6.992
6	6.53	6.449	6.429
7	6.41	6.449	6.459
8	6.46	6.468	6.470
9	6.56	6.534	6.525
10	6.49	6.567	6.599
11	6.70	6.770	6.774
12	6.59	6.648	6.667
13	6.64	6.712	6.720
14	6.71	6.666	6.660
15	6.77	6.652	6.626
16	6.84	6.860	6.866
17	6.75	6.740	6.738
18	6.81	6.822	6.827
19	6.91	6.848	6.834
20	6.85	6.853	6.853

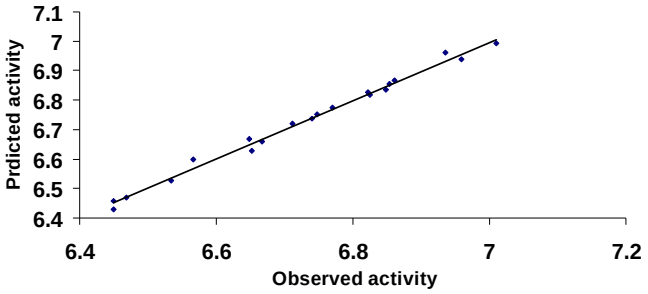

Figure 3 : Graph between observed activity and predicted activity of model-II.

Comparison of model-I and model-II reveals that model-II shows better correlation (r=0.949) between descriptors and biological activity than model-I (r=0.946). The bootstrapping r^2 (r^2_{BS} = 0.911) reflects the significance of model-II when compared to model-I. But the correlation matrix for model-I and model-II in TABLE 6 and TABLE 7, respectively, reveals that descriptors in the model-II are not significantly intercorelated indicating that their contribution is independent to the biological activity. By evaluating both the models, it was concluded that thermodynamic (BP, SE, CMA and PC) descriptors play an important role for the activity. From the above analysis, it was inferred that model-II could be used as theoretical prediction of biological activity for design of new molecules.

TABLE 6 : Correlation matrix of model-I

Parameters	BP	SE	PC
BP	1.000	-	-
SE	0.300	1.000	-
PC	0.842	0.345	1.000

TABLE 7 : Correlation matrix of model-II

Parameters	BP	CMA	SE
BP	1.000	-	-
CMA	0.887	1.000	-
SE	0.300	0.384	1.000

CONCLUSION

QSAR analysis was performed on a series of anti-inflammatory phenothiazines using molecular modeling program Chemoffice 2001. QSAR models were proposed for anti-inflammatory activity of the phenothiazines using ChemSAR descriptors employing sequential multiple regression analysis method. The selected models

were checked for multicolinearity. The predictive power of each model was estimated with bootstrapping r^2 method and leaves one-out cross validation method. It was observed from the selected models that biological activity of phenothiazine derivatives is governed by thermodynamic and steric properties of the molecules. The models also provide valuable insight into the mechanism of action of these compounds. The result of the study suggests involvement of partition coefficient in the mechanism of anti-inflammatory action of phenothiazine and bulky substituents are favorable for activity. The study will be helpful in the design of better anti-inflammatory analogs of phenothiazine derivatives for anti-inflammatory activity.

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ABBREVIATIONS

NSAIDs - Non-Steroidal Anti-inflammatory Agents; QSAR - Quantitative Structural-Activity Relationship.

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