Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research

ISSN No: 0975-7384
CODEN(USA): JCPRC5

J. Chem. Pharm. Res., 2011, 3(5):443-449

QSAR studies of Anthrax Lethal inhibitors through Quantum Chemical Indices

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ABSTRACT

*Bacillus Anthracis is a gram positive, rod shaped spore- forming bacterium that causes infectious disease Anthrax. Hydroxamate analogues are used as inhibitors of the anthrax lethal toxin. QSAR models by stepwise regression analysis is developed using quantum chemical descriptors. After considering training set and test set we have also designed seventeen compounds and predicted their activity. This work may be helpful in screening and synthesis of Anthrax inhibitor.***

Keywords: Anthrax lethal factor, descriptor, Hydrxamate analogue, QSAR.

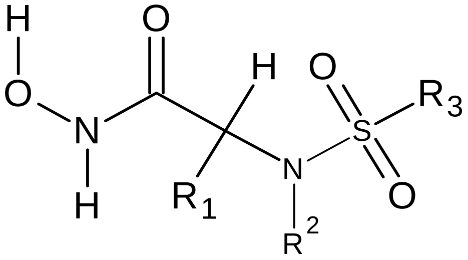
INTRODUCTION

Bacillus Anthracis is a gram positive, rod shaped spore- forming bacterium that causes infectious disease Anthrax [**1**]**.** The bacterium secreted three proteins protective antigen (PA), which is a pore forming sub unit; edema factor (EF), which is an adnylate cyclase that increases c AMP levels in cells and lethal factor (LF), which is a protease that cleaves mitogen activated protein kinase - kinase family [2-5]. These three proteins are individually non toxic but assemble to form two toxic complex, lethal toxin, and edema toxin [**6**]**.** Initially PA binds to host cell surface and cleaved by a cell associated furine type protease to form PA63 [7-10]. Then PA63 oligomerizes in to a heptameric pore- forming complex to which LF and EF bind [11]. The resulting pore-forming complex is internalized by receptor-mediated endocytosis and is trafficked in to a low pH endosome . Then complex undergoes a conformational change that allows the EF and LF to translocated in to the host cell cystol and exert their toxic action [12-14]. Inside the cystol, LF is then able to cleaves mitogen activated protein kinases kinases (MAPKK) and disrupting interaction with mitogen activated protein kinase, which in turn results in inhibition of the signaling path way [15-17]. Hydroxamate analogues are used as inhibitors of the anthrax lethal toxin [18].

In this paper, we developed six QSAR models by stepwise regression analysis with respect to their experimental value of lnIc50. The method is very useful in elucidating the mechanism of chemical and biological interaction in various biomolecules. The QSAR approach employs thermodynamically derived and computational based descriptors to correlate biological activity (lnIc50) in isolated receptors. Three standard quantum chemical descriptors routinely used in QSAR analysis are quantum, electronic, surface and steric. For screening of chemical database or virtual libraries before their synthesis, the use of QSAR models appears equally attractive to chemical manufacturers, pharmaceutical companies.

EXPERIMENTAL SECTION

Structural and biological data of hydroxamate analogue was collected from site Binding db (www.bindingdb.org) as shown in Table 1 [19-21].



Hydroxamate analogue

Table 1: Chemical structure of Hydroxamate analogue by substituting R1, R2, R3 of figure 1.

Compd No.	R1	R2	R3
1	-CH(CH$_3$)$_2$	-H	4-C$_6$H$_4$-OCH$_3$
2	-CH(CH$_3$)$_2$	4-CH$_2$-Pyrdinyl	4-C$_6$H$_4$-OCH$_3$
3	-CH(CH$_3$)$_2$	3-CH$_2$CH$_2$Thiophenyl	4-C$_6$H$_4$-OCH$_3$
4	-CH(CH$_3$)$_2$	1-CH$_2$-CH$_2$-Imidazoly	4-C$_6$H$_4$-OCH$_3$
5	-CH(CH$_3$)$_2$	-H	C$_6$H$_5$
6	-CH(CH$_3$)$_2$	-H	2-F-C$_6$H$_5$
7	-CH(CH$_3$)$_2$	-H	3-Cl-C$_6$H$_5$
8	-CH(CH$_3$)$_2$	-H	4-F-C$_6$H$_5$
9	-CH(CH$_3$)$_2$	-H	4-CH$_3$-C$_6$H$_5$
10	-CH(CH$_3$)$_2$	-H	3-CH$_3$-C$_6$H$_5$
11	-CH(CH$_3$)$_2$	-H	4-F-3-CH$_3$-C$_6$H$_5$
12	-CH(CH$_3$)$_2$	-H	3-F-4-CH$_3$-C$_6$H$_5$
13	-CH(CH$_3$)$_2$	-H	-CH$_2$-CH$_2$-C$_6$H$_5$
14	-CH(CH$_3$)$_2$	-H	3-Chloro- benzyl
15	-CH(CH$_3$)$_2$	-H	4-Hydroxy-3-methyl- C$_6$H$_5$
16	-CH(CH$_3$)$_2$	-H	4-Fluoro-3-hydroxymethyl- C$_6$H$_5$
17	-CH(CH$_3$)$_2$	-H	4-Fluoro-2-hydroxymethyl- C$_6$H$_5$
18	-CH(CH$_3$)$_2$	-H	2-Chloro-5-Thiophenyl
19	-CH(CH$_3$)$_2$	-H	2-{5-(1,2,3-Thiadiazolyl)-thiophenyl
20	-CH$_3$	-H	4-Fluro -3-methyl - C$_6$H$_5$
21	-CH$_2$CH$_3$	-H	4-Fluro -3-methyl - C$_6$H$_5$
22	-(CH$_2$)$_3$CH$_3$	-H	4-Fluro -3-methyl - C$_6$H$_5$
23	-C(CH$_3$)$_3$	-H	4-Fluro -3-methyl - C$_6$H$_5$
24	Cyclopropyl	-H	4-Fluro -3-methyl - C$_6$H$_5$
25	Cyclobutyl	-H	4-Fluro -3-methyl - C$_6$H$_5$

26	Cyclopentyl	-H	4-Fluro -3-methyl - C_6H_5
27	Cyclohexyl	-H	4-Fluro -3-methyl - C_6H_5
28	Phenyl	-H	4-Fluro -3-methyl - C_6H_5
29	Cyclohexyl	-H	4-Fluro -3-methyl - C_6H_5
30	3-Tetrahydrofuranyl	-H	4-Fluro -3-methyl - C_6H_5
31	$-CH_2(CH_3)CH_2CH_3$	-H	4-Fluro -3-methyl - C_6H_5
32	$-CH_2(CH_3)CH_2CH_3$	-H	4-Fluro -3-methyl - C_6H_5
33	2-(Cyclopropyl)Methyl	-H	4-Fluro -3-methyl - C_6H_5
34	2-(1Cyclopropyl)Ethyl	-H	4-Fluro -3-methyl - C_6H_5

In this work natural logarithm of experimental Ic50 was computed and taken as response variable for QSAR and is listed in Table 2.

Table 2: Values of quantum chemical indices and experimental value of ln IC_{50} of training set

Compd	WSA	MR	SA	POL	logP	HOMO	LUMO	DM	$\ln IC_{50}$
1	6.0866	4.2868	6.24	3.3996	0.961	-0.2462	-0.0339	6.7728	6.8024
2	6.3491	4.6061	6.3343	3.6623	1.315	-0.2135	-0.0433	6.4262	8.0392
3	6.1647	4.6454	6.1191	3.7402	2.598	-0.1933	-0.0414	4.2424	7.6497
4	6.3631	4.6009	6.4264	3.6855	0.888	-0.2045	-0.0367	9.1421	9.8574
5	5.9753	4.1938	6.1289	3.2883	0.904	-0.2298	-0.0448	7.7259	7.0031
6	5.796	4.1975	6.1255	3.2846	1.02	-0.2438	-0.0542	7.6089	8.7948
7	5.7643	4.1975	5.8296	3.2915	1.044	-0.1461	-0.039	8.3645	7.9374
8	5.8181	4.1975	5.9459	3.2918	1.068	-0.2156	-0.0582	8.4473	5.6699
9	6.0542	4.2671	6.2168	3.3706	1.353	-0.2406	-0.0408	5.1837	8.0064
10	6.053	4.2671	6.2184	3.3639	1.329	-0.2204	-0.0462	7.4782	5.9915
11	6.0678	4.2701	6.2316	3.363	1.444	-0.2451	-0.0482	7.3547	4.8675
12	5.8508	4.2701	5.9275	3.3734	1.444	-0.2175	-0.0488	5.5005	8.5526
13	5.902	4.3257	5.9437	3.433	1.175	-0.2247	-0.0119	6.2454	9.3826
19	6.0945	4.4011	6.2802	3.4391	1.487	-0.2492	-0.0761	4.8421	6.697
20	5.9221	4.1357	6.1201	3.2122	0.665	-0.2543	-0.0376	1.5427	4.8675
21	5.7737	4.2056	5.8657	3.2995	1.201	-0.245	-0.0398	1.4663	4.6052
22	6.1395	4.3342	6.3489	3.4447	2.265	-0.2482	-0.0377	3.2164	4.2485
23	5.9504	4.3298	6.0264	3.3986	2.025	-0.2035	-0.0571	8.362	5.7038
24	6.0125	4.2446	6.1579	3.3285	1.192	-0.2037	-0.0532	7.8314	4.7875
25	6.0789	4.3085	6.2031	3.4004	1.366	-0.1316	-0.0342	3.3367	4.8675
19	6.0945	4.4011	6.2802	3.4391	1.487	-0.2492	-0.0761	4.8421	6.697
20	5.9221	4.1357	6.1201	3.2122	0.665	-0.2543	-0.0376	1.5427	4.8675
21	5.7737	4.2056	5.8657	3.2995	1.201	-0.245	-0.0398	1.4663	4.6052
22	6.1395	4.3342	6.3489	3.4447	2.265	-0.2482	-0.0377	3.2164	4.2485
23	5.9504	4.3298	6.0264	3.3986	2.025	-0.2035	-0.0571	8.362	5.7038
24	6.0125	4.2446	6.1579	3.3285	1.192	-0.2037	-0.0532	7.8314	4.7875
25	6.0789	4.3085	6.2031	3.4004	1.366	-0.1316	-0.0342	3.3367	4.8675

Quantum chemical descriptors namely HOMO, LUMO, dipole moment (DM) were calculated using GAMESS [22]. Vandewalls surface area (WSA) , molar refractivity (MR), molecular surface area (SA), polarizability (Pol), Partition coefficient (logP) were calculated using Mervin logP calculator. The 2D geometry of compounds was drawn in ChemSketch and converted into 3D model in 3D viewer. Energy minimization was done under MOPAC module according to AM1 (Austin Model 1) method using RHF (restricted HartreeeFock: closed shell) wave function.

RESULTS AND DISCUSSION

A total of thirty four compounds were selected as potent anthrax inhibitors and were divided into in to training set and test set. A multivariate regression was performed using eight (8) different indices by stepwise addition method [23]. Since HOMO and LUMO plays an important role in transition as well as reaction so we consider these two indexes as our initial step. Using these two quantum chemical parameters Model 1 (Table 3) was constructed and shows a lower F value and correlation coefficient.

Table 3: Regression equations and Fischer F-value using different indices

Model No.	Regression equations	r	r^2	F
Model 1	$\ln Ic_{50} = 9.728269 + (7.6253)HOMO + (27.6314)LUMO$	0.298	0.089	3.57
Model 2	$\ln Ic_{50} = 9.054719 + (8.1413)HOMO + (7.6253)LUMO + (0.1561)DM$	0.379	0.144	7.76
Model 3	$\ln IC_{50} = 9.304326 + (-0.1809)\log \mathrm{P} + (8.2142)\mathrm{HOMO} + (29.2211)\mathrm{LUMO} + 0.1523)\mathrm{DM}$	0.383	0.147	10.32
Model 4	$\ln IC_{50} = (-10.881087) + (5.9332)\mathrm{Pol} + (-0.7994)\mathrm{logP} + (5.1477)\mathrm{HOMO} + (21.2275)\mathrm{LUMO} + (0.1146)\mathrm{DM}$	0.547	0.299	19.91
Model 5	$\ln IC_{50} = 0.29 + (-17.8827)\mathrm{MS} + (8.1232)\mathrm{MV} + (19.6404)\mathrm{Pol} + (-1.4596)\mathrm{logP} + (2.4232)\mathrm{HOMO} + (31.0984)\mathrm{LUMO} + (0.1962)\mathrm{DM}$	0.757	0.573	29.11
Model 6	$\ln IC_{50} = (-0.9232) + (-18.1715)\mathrm{MV} + (2.3851)\mathrm{MR} + (8.1614)\mathrm{MS} + (17.4222)\mathrm{Pol} + (-1.4521)\mathrm{logP} + (2.1142)\mathrm{HOMO} + (32.8694)\mathrm{LUMO} + (0.1934)\mathrm{DM}$	0.758	0.575	31.29

$\ln IC_{50}$- natural logarithm of Ic50, HOMO- value of HOMO energy, LUMO- value of LUMO energy, DM- dipole moment, SA- molecular surface area, WSA-Vandewalls surface area. MR- molar refractivity, Pol- polarisibility, LogP – octanol/water partition coefficient.

Table 4: Values of experimental ln IC_{50} and predicted ln IC_{50} of training set using model 6

Compd No.	$\ln IC_{50}$	$\ln IC_{50}$ (Model 6)
1	6.8024	7.128667
2	8.0392	7.647253
3	7.6497	8.522042
4	9.8574	9.905629
5	7.0031	6.022826
6	8.7948	8.668462
7	7.9374	7.763879
8	5.6699	6.943415
9	8.0064	5.891324
10	5.9915	6.143184
11	4.8675	5.665155
12	8.5526	6.995867
13	9.3826	9.097739
19	6.697	5.751855
20	4.8675	4.816607
21	4.6052	6.279258
22	4.2485	5.260135
23	5.7038	6.02852
24	4.7875	5.785872
25	4.8675	6.028028

Table 5: Values of quantum chemical indices, experimental and predicted (using Model 6) value of ln IC_{50} of test set

Compd No.	WSA	MR	SA	POL	logP	HOMO	LUMO	DM	$\ln IC_{50}$	$\ln IC_{50}$ (Model 6)
26	6.1422	4.3686	6.2372	3.4634	2.105	-0.2146	-0.0449	5.8735	4.1589	5.276187
27	6.0387	4.4252	6.0776	3.5092	2.611	-0.2021	-0.0567	8.7496	3.6376	6.234704
28	6.1003	4.4116	6.2636	3.4624	1.917	-0.1599	-0.0541	9.5845	3.912	7.125803
29	6.1722	4.3954	6.2541	3.4881	1.08	-0.2164	-0.0418	4.8182	3.9889	6.750255
30	5.8979	4.3357	5.9139	3.4241	0.809	-0.1224	-0.0219	3.5729	4.7875	8.711773
31	5.8965	4.3325	5.9143	3.4135	1.947	-0.2207	-0.0366	3.8311	4.2047	6.253476
32	6.1393	4.3325	6.2605	3.4206	1.947	-0.2151	-0.0514	8.1483	4.2047	5.131939
33	6.0846	4.3095	6.2236	3.3995	1.463	-0.2386	-0.0405	1.2967	3.9318	5.118746
34	5.9663	4.3679	6.0579	3.4706	1.939	-0.2074	-0.0535	7.2957	4.1744	7.375374

In the next step together with HOMO, LUMO we introduce dipole moment (DM). As a result it shows a higher F (7.76) and r (0.379) value than previous one. To modify the results we introduce logP (Model 3) in Model 2. It shows a higher r^2 and F value. Polarisibility, molecular surface area (SA), Vandewalls surface area (WSA) are added stepwise in Model 4 and Model 5. Model 5 shows a larger r (0.757) and F (29.11) value than Model 4. From this step it is clear that molecular surface and Vandewalls surface area plays an active role in activity. Finally with Model 5 molar refractivity is introduced and constructed Model 6. Model 6 exhibits slightly greater r value (0.758) but modified F (31.29) value. The model 6 having good correlation coefficient value (0.758) explains 57.5% variance in the anthrax lethal toxin inhibitory activity. The higher F value indicate the model is statistically more significant than the other model but model 6 and model 5 both r , r^2, F values are very closes to each other which indicate that little contribution of molar refractivity on regression equation. Therefore steric effect does not improve the activity of hydoxamate inhibitor. Experimental activities and predicted activities of training set represented in Table 4. It was observed that the predicted biological activities of the compounds in the test set (Table 5) using model 6 are in good agreement with their corresponding experimental activities.

Hydroxamate analogue (1) exhibit high activity against anthrax lethal toxin. When hydrogen atom of amino group replaced by methyl pyridinyl, ethyl thophenyl, ethyl imidazolyl group of hydroxamate inhibitor (1) of table 1, exhibited high value of inhibition activity, which is evident from the hydroxamate derivatives (2, 3, 4). When methoxy group is replased by fluorine atom (8), the inhibition activity was reduced. But fluorine atom present at ortho and meta position of phenyl ring (6, 7) displayed high activity. Introduction of methyl group at the para position of phenyl ring (9) would improve the inhibition activity. But methyl group present at the meta position of phenyl ring (10), the activity decreases. Replacing benzene ring with ethyl phenyl group (13) and Chloro benzyl group (14) displayed outstanding activity. When isopropyl group substituted by ethyl, butyl, t-butyl, cyclopropyl, cyclobutyl and similar groups (20-34) are present inhibition activity towards anthrax lethal toxin reduces. On the basis of these observations we have designed 17 inhibitors and calculated their activity using Regression model 6 exhibits in Table 6. It is found that designed compounds are significant value of inhibition activity (Table 7).

Table 6: Chemical structure of designed compounds

CompdNo.	R_1	R_2	R_3
d1	$-CH(CH_3)_2$	-H	4-Nitro-C_6H_5
d2	$-CH(CH_3)_2$	-H	4-$N(CH_3)_2$-C_6H_5
d3	$-CH(CH_3)_2$	4(Pyridnyl)-CH_2-CH_2	4- OCH_3-C_6H_4
d4	$-CH(CH_3)_2$	3-CH_2-Thiophenyl	4- OCH_3-C_6H_4
d5	$-CH(CH_3)_2$	-H	2CH_3-C_6H_5
d6	$-CH(CH_3)_2$	-H	4-CCl_3-C_6H_5
d7	$-CH(CH_3)_2$	-H	2-(2F-C_6H_5) Ethyl
d8	$-CH(CH_3)_2$	-H	4-SH-3-CH_3-C_6H_5
d9	$-CH(CH_3)_2$	-H	4-F-3-(CH_2-SH)-C_6H_5
d10	$-CH(CH_3)_2$	-H	4-OCF_3-C_6H_5
d11	2(2-Oxo pyrimidinyl)	-H	4-F-3-CH_3-C_6H_5
d12	$-CH(CH_3)_2$	-CH_2-(2-Oxopyridinyl)	4- OCH_3-C_6H_4
d13	$-CH(CH_3)_2$	-CH_2-(4NO2-C_6H_5)	4- OCH_3-C_6H_4
d14	$-CH(CH_3)_2$	4- Methoxy- benzyl	4- OCH_3-C_6H_4
d15	$-CH(CH_3)_2$	-H	4(NH_2-CO-NH)C_6H_5
d16	$-CH(CH_3)_2$	-H	4(NH_2-CS-NH)C_6H_5
d17	$-CH(CH_3)_2$	-H	4(NH_2-CO-NH-NH)C_6H_5

Table 7: Designed compounds and their predicted $\ln IC_{50}$ using Model 6

Compd No.	WSA	MR	SA	POL	logP	HOMO	LUMO	DM	$\ln IC_{50}$
d1	6.0766	4.2986	6.1874	3.3629	0.863	-0.2698	-0.1244	2.0264	2.474801
d2	6.1779	4.3908	6.2804	3.4637	1.006	-0.1244	-0.0324	11.7828	8.379723
d3	6.403	4.6525	6.4824	3.7243	1.721	-0.1024	-0.0307	7.5891	9.355408
d4	6.3271	4.5987	6.3447	3.6736	2.192	-0.2015	-0.0475	7.9294	7.219331
d5	6.054	4.2671	6.2052	3.3596	1.305	-0.233	-0.0264	7.0286	6.522726
d6	5.8884	4.463	5.9371	3.5683	2.723	-0.1991	-0.0876	6.7121	7.386881
d7	5.9017	4.3285	5.9257	3.4362	1.291	-0.2393	-0.0117	5.7886	8.740487
d8	6.1042	4.3735	6.2703	3.4638	1.51	-0.2388	-0.0504	0.4293	5.835871
d9	5.8844	4.3737	5.9317	3.465	1.287	-0.1256	-0.0397	3.7106	8.637351
d10	6.1344	4.2391	6.2477	3.4006	1.874	-0.201	-0.0389	6.0376	4.69501
d11	6.0601	4.3939	6.1824	3.4247	-0.601	-0.1203	-0.0404	5.4162	9.896373
d12	6.3268	4.6096	6.334	3.6861	0.282	-0.0789	-0.0368	4.9994	10.19898
d13	6.4303	4.6966	6.4724	3.7443	2.563	-0.2288	-0.0785	14.2525	7.458973
d14	6.4424	4.6887	6.5033	3.7598	2.661	-0.1998	-0.0411	8.2498	7.7299
d15	6.1249	4.3797	6.2087	3.4527	0.038	-0.1051	-0.0224	4.954	8.993963
d16	6.1355	4.4751	6.2037	3.5119	-0.059	-0.1956	-0.0528	12.4105	10.41185
d17	6.1726	4.4193	6.2776	3.4792	-0.203	-0.111	-0.0276	5.4492	9.507978

CONCLUSION

QSAR analysis was performed on a series of 38 inhibitors of anthrax lethal factor and validated through six QSAR model. Descriptors are employing sequential multiple regression analysis method. A satisfactory QSAR model (model 6) was obtained with LOO cross- validation values of 0.56. The model 6 possesses predictive ability as differentiate by the testing on the external test set and also useful to explain the relationship between compound structure and biological activities and make easy to design of more potent hydroxamate inhibitors.

Acknowledgements
The authors are thankful to Uttam Kr. Mondal, Department of Chemistry, Raiganj College (University College), for his valuable suggestion during preparation of the manuscript.

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