A theoretical model to study the interaction between boronic acids and insulin

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Abstract

Boronic acids are widely used in various applications in view of their ability to recognize and bind at specific sites of the biological molecules to mimic several processes. Therefore, this has attracted the researchers, academician and medical expertise to explore them. In the present work, the authors have designed a theoretical approach to study the interaction of boronic acid with insulin using computational tools. A library of boronic acids (114 compounds) are designed, optimized and interacted with insulin using computational tools i.e. iGEMDOCK. Further, their different biological activities and toxicity are determined. Results indicates the promising potential of the boronic acids on interaction with the insulin. Amongst, 114 molecules of boronic acids, 3-Benzyloxyphenylboronic acid (71) showed the best interaction with amino-acids of insulin and significant interaction was shown with the Glu21 and His5 residues. Further, these results were compared with the stabilizing agents and found to be more potent.

Keywords: Boronic acids, insulin, biological activity, toxicity.

1. Introduction

Boronic acids are Lewis acids and interact with Lewis bases. Their unique feature to form reversible covalent boronic esters with diols has been thoroughly explored in the area of sensor devices used in the sugars detection. The onlyproperty of boron is possibility to form reversible covalent bonds with nitrogen and oxygen atoms. The boronic acid or its ester equilibrium is sensitive towards pH, temperature, and competing hydroxyl compounds and is also influenced by the proximity of amines. Additionally, boronic acid functionalized materials have been deployed as enzyme inhibitors, as membrane transporter molecules, in boron neutron capture therapy, and in affinity column separation chromatography.[1-7]The reactivity of the boron centre is highly dependent upon their substituent as an alkyl group.[8-16]The most important feature of boronic acids is their interaction with diols, resulting in complexation and boronic ester formation. Consequently, it was widely advocated that a higher pH of the system would result in higher complexation between a diol and a boronic acid, and the lower the pKa enable them to make complexes with strong affinity. As a result boronic acid containing materials are generally thermoresponsive, pH-responsive and chemoresponsive. For example, many studies have been performed to design a boronic acid containing hydrogel that releases insulin in response to an increased blood glucose concentration.[1,3-5,7,17-25]

Insulin is a popular natural hormone produced by the pancreas of humans and being in use since last 100 years and commonly used therapeutic protein.[26-31] It has attracted the researchers from academics or industries. [26,32-37] the structure of the insulin has several issues like drastic reduction in stability and biological applications like drug delivery and it affects the potential to control the glucose level in the blood of the patients. Therefore, it is important to find the methods to avoid the aggregation of insulin to maintain its biological potency to control the sugar level. Boronic acids considers to have potential in different areas of chemical and life sciences and proposed to have ability to prevent the aggregation in the structure of insulin. In the present work, the authors have designed a library of the boronic acids to find their potential to avoid the aggregation of the structure of insulin using computational tool. This methodology is considered to be time saving, efficient and strategic. Further, Lipinski rule's of five was applied on the potential compounds was applied to designed compounds. Further, the biological potency and toxicity of top 15 compounds has been determined.

2. Experimental details

Computational tools were utilized as a part of planning, optimization and recognizable proof of physical, chemical and biological properties of boronic acid. The capability of computational devices is utilized to predict the interaction between boronic acid with insulin and the instruments utilized was iGEMDOCK. It has several parts to understand the interaction like preparation of the molecules and insulin, docking of the molecules and analysis after the docking.

2.1 Preparation of protein structure

It is very important to prepare the receptor i.e. the structure of the insulin after taking it from the RCSB. It is done by using the Chimera and considered to be mandatory to get the accurate results. This computational tools is used to for the removal of ligands present as well solvent present, addition of hydrogen as well the charges.

2.2 Preparation of compounds

A library of boronic acids has been created using the CS ChemDraw 12 and they were optimized using Gaussian 9.0 to get better results.

2.3 Molecular Docking

iGEMDock, a computational tool used to determine the interaction between the designed boronic acids and the insulin and gives binding energy, composed of the energy due to hydrogen bonding, van der Waals and electrostatic interactions. After giving a set of poses, iGEMDOCK calculates the binding energy of each molecule as in **Table 1**.

2.4 Scoring function

The calculation for the scoring function based on the mathematical methods gives binding energy for the formation of interaction between the small molecule and the receptor. This binding energy is obtained by the non-covalent interaction like hydrogen bonding, van der Waals and electrostatic interactions.

2.5 Drug likeness calculation on the basis of Lipinski rule:

Herein, based on the docking score, the drug likeness score of best fifteen compounds was determined. Different properties like milogP (partition coefficient), molecular weight, and number of heavy atom, number of hydrogen donor, number of hydrogen acceptor and number of violation etc are calculated as in **Table 2**.[38-40]

2.6 Bioactivity score:

Further, the bioactive score of the best fifteen molecules was determined. Herein, various activity scores for the GPCR ligand, ion channel modulator, nuclear receptor ligand, kinase inhibitor, protease inhibitor, enzyme inhibitor is determined. The properties were determined by using an online server i.e. www.molinspiration.com.[38] The milogP of best fifteen compounds was found to be less than five and considered to have good permeability across the cell membrane. Further, with o or 1 violation in Lipinski rule of five means a molecule bind with the receptor effectively.

2.7 Toxicity Prediction:

Herein, the toxicity of the best 15 compounds based on **Table 1** was predicted by using online web server (https://lazar.in-silico.de/predict) and various chemical properties of best 15 compounds as in **Table 3**. It gives a range of dose or concentration and based on it, indicates lethal dose, the organs affected and the time to onset, duration and severity of effects.[41,42]

3. Result & discussion

Molecular docking is a promising approach to understand the interaction between small molecule and the receptor and screening is done based on the physical data in the form of binding energy. Authors examined this interaction by using computational tool, iGEMDOCK via drug screening.[43-47] Herein, the molecule finds the best site in the receptor and binds having lowest energy for the formation of complex. The purpose of this work is to stabilize the most important hormone i.e. insulin by studying its interaction with the boronic acid and its derivatives using computational tool. The total energy contributed by the interaction decides the stability of the insulin. The association between the protein i.e. insulin and the boronic acid was considered in beam of mainly Vander Waal's, hydrogen bonding as well as electrostatic interactions.[48-57] In this work, the key part is played by the total energy contributed is Vander Waal's interaction between the boronic acid and insulin (80-90%) yet other components also have also important like energy contributed by hydrogen bonding is additionally imperative up to some degree to choose the most potential competitor in adjustment of insulin and it is about (10-20%) while the electrostatic interaction can be ignored.

Effect of boronic acids binding on the conformational stability of insulin

The docking result of insulin (5MAM) protein with designed boronic acids (114 ligands)is mentioned in **Table 1** with their total binding energy. It is observed that 3-benzyloxyphenyl boronic acid, 71 gave the most stable conformation to the insulin as mentioned in **Table 1**. From the docking result it is found that 3-benzyloxyphenyl boronic acid (**71**) showed highest binding affinity with minimum total energy –78.4613kcal/mol**(Figure 1)**. The docked poses of the ligands are represented in (**Figure 2**) shows (a) Docked view, (b) electro-, (c) hydrophobic and (d) pose obtained by the interaction of insulin with 3-Benzyloxyphenylboronic acid (71). The ligand 3-benzyloxyphenyl boronic acid showed lowest binding energy against insulin (PDB ID: 5MAM) and these ligand show interactions with Glu21 and His5 residue of insulin as in **Graph 1**.The Drug likeness score and bioactivity score of this ligand and other top 15 ligands were calculated in table 2. Thus, milogP values of derivatives of boronic acid compounds were found to be in the range of below 5 so it as obey the Lipinski rules. In general, higher the bioactive scope or value, more attention will be paid to the molecule, The compounds taken are potentially biologically active and participates in various physiological actions. Therefore, bioactivity score for GPCRs is found to be -0.13 to -1.04 for top 15 compounds and these 5 compounds (C. No. 74, 91, 53, 55 & 102) are not in the GPCRs range -0.50 to 0.00 and remaining 10 compounds obey in this range.

4. Conclusion

In the present work, authors have developed a theoretical model to investigate the interaction between the active site of the insulin with boronic acids using computational tool i.e. iGemDock. A very few stabilizing agents are reported and it was observed that 3-benzyloxyphenyl boronic acid, 71 gave the most stable conformation to the insulin. It showed better stability of insulin in comparison of other reagents. A novel compound derivative of boronic acid is found to be excellent insulin stabilizer and drug candidate based on the molecular docking studies, bioactivity score, Lipinski score and its ADME properties.

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Table 1 Energy obtained due to interaction of boronic acids (C. No.1-114) with insulin using a computational tool iGEMDOCK[45-49] as well logs and log K values

S.No.	`C. No.	Ligand	E_{total}	logS	Solubility (mg/ml)	logKp (cm/s)
1.	71	3-Benzyloxyphenylboronic acid	-78.4613	-2.95	0.253	-6.07
2.	74	3-Carboxy-5-nitrophenylboronic acid	-77.7919	-1.36	9.22	-7.46
3.	97	4-Benzyloxyphenylboronic acid	-77.4836	-2.95	0.253	-6.07
4.	26	2-Anthraceneboronic acid	-75.835	-3.86	0.0308	-5.29
5.	95	4,4'-Biphenyldiboronic acid	-75.1476	-2.49	0.780	-6.81
6.	72	3-Biphenylboronic Acid	-74.7128	-3.07	0.168	-5.77
7.	99	4'-Bromo-4-biphenylboronic acid	-73.883	-3.36	0.0305	-5.76
8.	40	2-Fluoro-4-biphenylboronic acid	-72.1959	-3.21	0.134	-5.81
9.	91	4-(Ethoxycarbonyl)phenylboronic acid	-70.587	-1.76	3.39	-6.74
10.	27	2-Biphenylboronic Acid	-70.3698	-3.07	0.168	-5.77
11.	12	2,4-Bis(trifluoromethyl)phenylboronic acid	-70.242	-3.13	0.189	-6.03
12.	53	2-Nitrophenylboronic acid	-70.2281	-1.53	4.94	-6.85
13.	63	3,5-Bis(trifluoromethyl)-phenylboronic Acid	-70.0491	-3.13	0.189	-6.03
14.	55	3-(Bromomethyl)phenylboronic acid	-69.9194	-2.31	1.04	-6.63
15.	102	4-Carbamoylphenylboronic acid	-69.1293	-0.91	20.2	-7.52
16.	10	2,3-Dimethoxyphenylboronic acid	-69.0658	-1.60	4.60	-6.86
17.	56	3-(Ethoxycarbonyl)phenylboronic acid	-68.2942	-1.76	3.39	-6.74
18.	4	2-(Ethoxycarbonyl)phenylboronic acid	-68.0229	-1.76	3.39	-6.74
19.	92	4-(Hydroxymethyl)phenylboronic acid	-67.4829	-1.02	14.6	-7.27
20.	6	2-(Methoxycarbonyl)phenylboronic acid	-67.2027	-1.53	5.34	-6.91
21.	68	3-Acetamidophenylboronic	-67.108	-1.09	14.4	-7.39
22.	93	4-(Methoxycarbonyl)phenylboronic acid	-67.0982	-1.53	5.34	-6.91
23.	58	3-(Methoxycarbonyl)phenylboronic acid	-66.3049	-1.53	5.34	-6.91
24.	98	4-Biphenylboronic Acid	-66.2792	-3.07	0.168	-5.77
25.	79	3-Cyanophenylboronic acid	-65.936	-1.43	5.47	-6.81
26.	88	3-Nitrophenylboronic acid	-65.9255	-1.52	5.01	-6.86
27.	65	3,5-Difluorophenylboronic acid	-65.8186	-1.81	2.47	-6.53
28.	66	3,5-Dimethoxyphenylboronic acid	-65.7448	-1.60	4.60	-6.86
29.	19	2,5-Dimethoxyphenylboronic acid	-65.3143	-1.60	4.60	-6.86
30.	24	2,6-Dimethoxyphenylboronic acid	-65.3054	-1.60	4.60	-6.86
31.	35	2-Chloropyridine-5-boronic acid	-65.1689	-1.65	3.55	-6.75
32.	108	4-Cyano-3-fluorophenylboronic acid	-64.9647	-1.58	4.38	-6.84
33.	49	2-Methoxypyridine-3-boronic acid	-64.9643	-1.10	12.2	-7.19
34.	62	3,4-Dimethoxyphenylboronic acid	-64.7995	-1.60	4.60	-6.86
35.	109	4-Cyanophenylboronic acid	-64.4827	-1.43	5.47	-6.81
36.	104	4-Chloro-2-(trifluoromethyl)-phenylboronic Acid	-64.1636	-2.89	0.289	-6.01
37.	15	2,4-Dimethoxyphenylboronic acid	-64.119	-1.60	4.60	-6.86

38.	34	2-Chloropyridine-4-boronic acid	-63.9093	-1.65	3.55	-6.75
39.	36	2-Cyanophenylboronic acid	-63.8188	-1.43	5.47	-6.81
40.	30	2-Carboxyphenylboronic acid	-63.4666	-1.33	7.81	-7.06
41.	38	2-Ethylphenylboronic acid	-63.3185	-2.39	0.669	-5.77
42.	112	4-Fluoro-3-(trifluoromethyl)-phenylboronic acid	-63.1665	-1.94	1.75	-6.32
43.	75	3-Carboxyphenylboronic acid	-63.1531	-1.33	7.81	-7.06
44.	37	2-Ethoxyphenylboronic acid	-63.1486	-1.77	2.81	-6.49
45.	22	2,6-Difluoro-3-pyridineboronic acid	-62.9184	-1.55	4.44	-6.83
46.	105	4-Chloro-3-(trifluoromethyl)-phenylboronic Acid	-62.8762	-2.89	0.289	-6.01
47.	57	3-(Hydroxymethyl)phenylboronic acid	-62.3348	-1.02	14.6	-7.27
48.	1	1,4-Phenylenediboronic acid	-62.3117	-0.94	19.2	-7.50
49.	42	2-Fluoro-5-(trifluoromethyl)-phenylboronic Acid	-61.9802	-2.45	0.730	-6.28
50.	80	3-Ethoxyphenylboronic acid	-61.9268	-1.77	2.81	-6.49
51.	96	4-Acetylphenylboronic acid	-61.6325	-1.42	6.29	-6.94
52.	69	3-Acetylphenylboronic acid	-61.4461	-1.42	6.29	-6.94
53.	2	1-Naphthaleneboronic acid	-61.2231	-2.72	0.328	-5.87
54.	50	2-Methoxypyridine-5-boronic acid	-61.1585	-1.10	12.2	-7.19
55.	31	2-Chloro-4-methoxyphenylboronic acid	-60.9687	-2.13	1.37	-6.42
56.	60	3,4-(Methylenedioxy)phenylboronic acid	-60.5811	-1.58	4.40	-6.86
57.	48	2-Methoxyphenylboronic acid	-60.4785	-1.56	4.21	-6.66
58.	70	3-Aminophenylboronic Acid	-60.4639	-1.16	9.44	-7.03
59.	101	4-Butylphenylboronic acid	-60.2212	-2.73	0.333	-5.46
60.	83	3-Formylphenylboronic acid	-60.1662	-1.22	8.95	-7.01
61.	5	2-(Hydroxymethyl)phenylboronic acid	-60.0975	-1.04	14.6	-7.27
62.	44	2-Fluoropyridine-3-boronic acid	-59.6923	-1.21	8.66	-7.02
63.	103	4-Carboxyphenylboronic acid	-59.6311	-1.33	7.81	-7.06
64.	52	2-Naphthaleneboronic acid	-59.4001	-2.72	0.328	-5.87
65.	110	4-Ethoxyphenylboronic acid	-59.1857	-1.77	2.81	-6.49
66.	33	2-Chloropyridine-3-boronic acid	-59.0891	-1.65	3.55	-6.75
67.	29	2-Bromopyridine-5-boronic acid	-58.9003	-1.96	2.21	-6.98
68.	14	2,4-Difluorophenylboronic acid	-58.0484	-1.81	2.47	-6.53
69.	41	2-Fluoro-4-methylphenylboronic acid	-57.7531	-1.94	1.75	-6.32
70.	85	3-Hydroxyphenylboronic acid	-57.5718	-1.37	5.89	-6.81
71.	16	2,4-Dimethylphenylboronic acid	-57.5072	-2.09	1.22	-6.11
72.	94	4-(Methylthio)phenylboronic acid	-57.1428	-2.00	1.69	-6.37
73.	89	3-Pyridylboronic Acid	-57.0923	-0.88	16.3	-7.22
74.	113	4-Fluoro-3-methylphenylboronic acid	-57.0877	-2.45	0.730	-6.28
75.	111	4-Ethylphenylboronic acid	-56.9452	-2.06	1.30	-6.06
76.	39	2-Fluoro-4-(trifluoromethyl)-phenylboronic Acid	-56.6364	-2.45	0.730	-6.28
77.	106	4-Chloro-2-methylphenylboronic	-56.6316	-2.38	0.709	-6.05

78.	77	3-Chloro-4-methylphenylboronic acid	-56.6198	-2.38	0.709	-6.05
79.	114	4-Fluoro-methylphenylboronic acid	-56.064	-1.94	1.75	-6.32
80.	64	3,5-Dichlorophenylboronic acid	-55.9604	-2.67	0.407	-5.99
81.	47	2-Hydroxyphenylboronic acid	-55.9475	-1.72	2.65	-6.42
82.	67	3,5-Dimethylphenylboronic acid	-55.9026	-2.09	1.22	-6.11
83.	11	2,3-Dimethylphenylboronic acid	-55.8925	-2.09	1.22	-6.11
84.	23	2,6-Difluorophenylboronic acid	-55.8472	-1.81	2.47	-6.53
85.	86	3-Methoxyphenylboronic acid	-55.8192	-1.56	4.21	-6.66
86.	45	2-Formylphenylboronic acid	-55.6917	-1.22	8.95	-7.01
87.	13	2,4-Dichlorophenylboronic acid	-55.5268	-2.67	0.407	-5.99
88.	3	2-(Bromomethyl)phenylboronic acid	-55.4612	-2.31	1.04	-6.63
89.	18	2,5-Difluorophenylboronic acid	-55.2963	-1.81	2.47	-6.53
90.	81	3-Fluoro-4-methylphenylboronic acid	-55.2656	-1.94	1.75	-6.32
91.	7	2-(Methylthio)phenylboronic acid	-55.2383	-2.00	1.69	-6.37
92.	25	2,6-Dimethylphenylboronic acid	-55.2258	-2.09	1.22	-6.11
93.	59	3-(Methylthio)phenylboronic acid	-55.0253	-2.00	1.69	-6.37
94.	61	3,4-Dichlorophenylboronic acid	-54.8509	-2.67	0.407	-5.99
95.	21	2,6-Dichlorophenylboronic acid	-54.7706	-2.67	0.407	-5.99
96.	20	2,5-Dimethylphenylboronic acid	-54.7279	-2.09	1.22	-6.11
97.	73	3-Bromophenylboronic acid	-54.5926	-2.42	0.762	-6.45
98.	17	2,5-Dichlorophenylboronic acid	-54.5644	-2.67	0.407	-5.99
99.	9	2,3-Difluorophenylboronic acid	-54.396	-1.81	2.47	-6.53
100.	8	2,3-Dichlorophenylboronic acid	-53.8515	-2.67	0.407	-5.99
101.	76	3-Chloro-4-fluorophenylboronic acid	-53.8003	-2.24	1.01	-6.26
102.	100	4-Bromophenylboronic acid	-53.0016	-2.42	0.762	-6.45
103.	78	3-Chlorophenylboronic acid	-52.977	-2.10	1.24	-6.22
104.	82	3-Fluorophenylboronic acid	-52.6656	-1.67	2.98	-6.49
105.	107	4-Chlorophenylboronic acid	-52.4022	-2.10	1.24	-6.22
106.	43	2-Fluorophenylboronic acid	-52.2772	-1.67	2.98	-6.49
107.	87	3-Methylphenylboronic Acid	-52.2568	-1.81	2.10	-6.28
108.	51	2-Methylphenylboronic Acid	-52.2056	-1.81	2.10	-6.28
109.	32	2-Chlorophenylboronic acid	-51.7109	-2.10	1.24	-6.22
110.	28	2-Bromophenyllboronic Acidcid	-51.7042	-2.42	0.762	-6.45
111.	46	2-Furylboronic Acid	-50.207	-0.89	14.5	-7.03
112.	54	2-Thiopheneboronic Acid	-48.3435	-1.37	5.46	-6.70
113.	90	3-Thiopheneboronic Acid	-47.5265	-1.35	5.70	-6.72
114.	84	3-Furylboronic Acid	-47.4753	-0.86	15.4	-7.06

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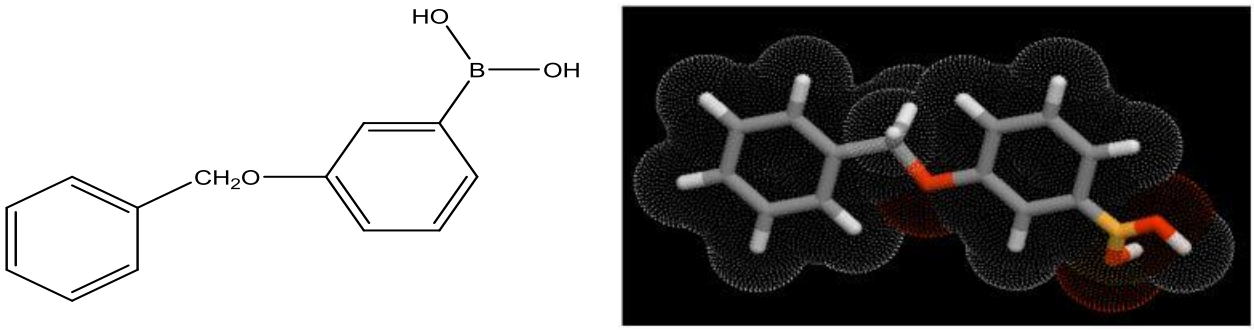
Table 2 Bioactivity and drug likeness score of top 15 compounds as in **Table 1**

C. No.	milogP	No. of violations	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor	Lipinski.
71	2.61	0	-0.24	-0.02	-0.07	0.74	0.59	1.17	Yes
74	0.78	0	-0.71	-0.21	-0.54	0.57	0.08	1.12	Yes
97	2.63	0	-0.24	-0.02	-0.07	0.74	0.59	1.17	Yes
26	3.33	0	-0.24	0.02	0.11	0.63	0.58	1.19	Yes
95	1.82	0	-0.13	0.06	0.13	0.75	0.60	1.18	Yes
72	2.75	0	-0.36	-0.00	-0.07	0.65	0.50	1.28	Yes
99	1.79	0	-0.43	-0.09	-0.07	0.51	0.35	1.13	Yes
40	2.87	0	-0.20	0.16	0.13	0.77	0.57	1.31	Yes
91	1.53	0	-0.83	-0.30	-0.64	0.47	0.15	1.00	Yes
27	2.73	0	-0.36	-0.00	-0.07	0.51	0.39	1.11	Yes
12	2.70	0	-0.16	0.11	0.04	0.74	0.48	1.00	Yes
53	0.89	0	-1.04	-0.33	-0.74	0.04	-0.07	0.90	Yes
63	2.70	0	-0.16	0.12	0.05	0.74	0.47	1.08	Yes
55	1.67	0	-0.84	-0.59	-0.92	0.14	0.46	1.24	Yes
102	-0.20	0	-0.76	-0.41	-0.29	0.18	0.50	1.39	Yes

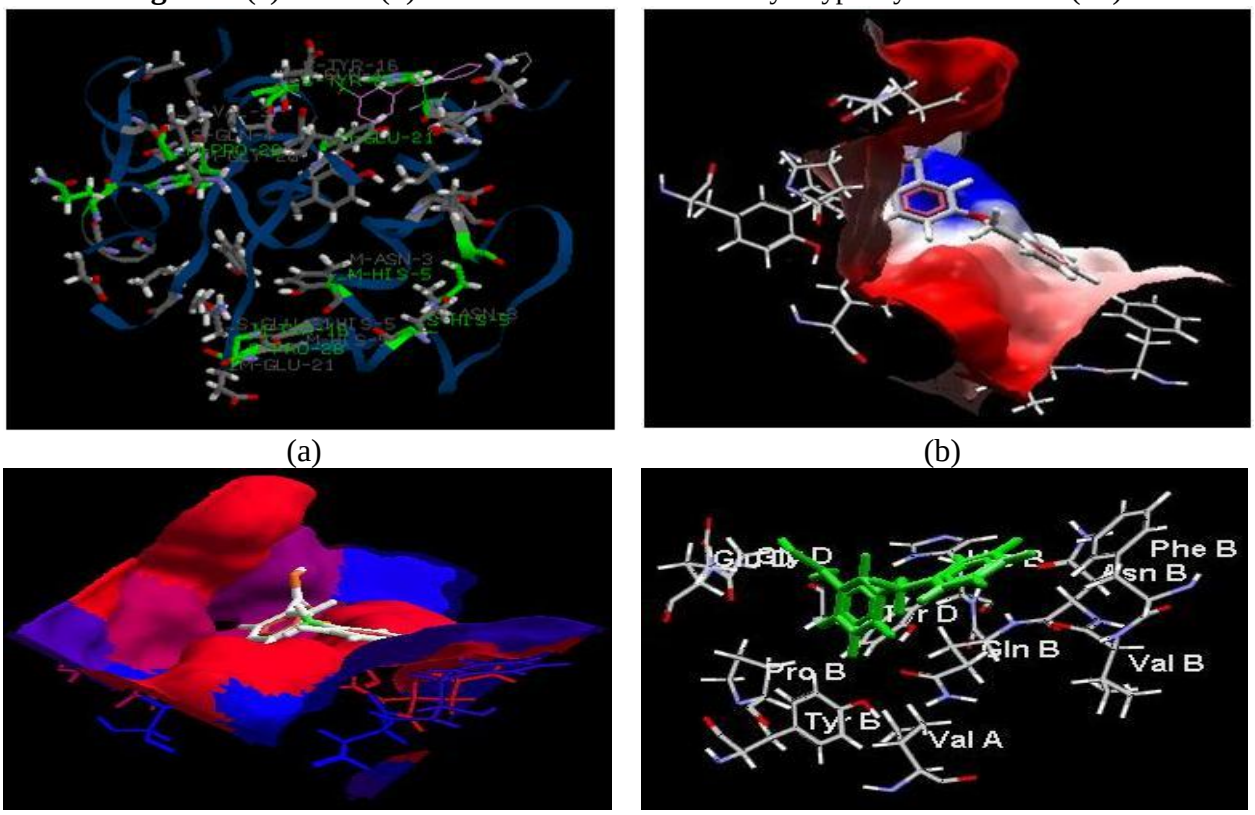
Table 3 Toxicity and other parameters of the top 15 molecules as in **Table 1**

C. No.	Acute toxicity (Fathead minnow) (mg/mL)	Acute toxicity (Daphnia magna) (mg/mL)	Blood Brain barrier Penetration (Human)	Carcinogenicity (Rodent multiple species/ sites)	Carcinogenicity (Mouse)	Mutagenicity (Salmonella typhimurium)
71	21.1	35.1	Penetrating	Non-carcinogenic	Non-carcinogenic	Mutagenic
74	14.8	21.7	Penetrating	Non-carcinogenic	Non-carcinogenic	Mutagenic
97	25.4	31.1	Penetrating	Non-carcinogenic	Non-carcinogenic	Mutagenic
26	9.58	0.247	Penetrating	Carcinogenic	Carcinogenic	Mutagenic
95	15.9	0.359	Penetrating	Carcinogenic	Carcinogenic	Mutagenic
72	8.29	2.85	Penetrating	Carcinogenic	Carcinogenic	Mutagenic
99	35.1	3.84	Penetrating	Carcinogenic	Non-carcinogenic	Mutagenic
40	8.37	2.91	Non-penetrating	Non-carcinogenic	Non-carcinogenic	Mutagenic
91	29.6	46.6	Cannot create prediction	Non-carcinogenic	Non-carcinogenic	Non-mutagenic
27	8.09	2.71	Penetrating	Carcinogenic	Carcinogenic	Mutagenic
12	9.93	1.17	Penetrating	Carcinogenic	Non-carcinogenic	Non-mutagenic
53	11.7	16.4	Penetrating	Carcinogenic	Non-carcinogenic	Mutagenic
63	9.93	**Highly toxic**	Penetrating	Non-carcinogenic	Non-carcinogenic	Non-mutagenic
55	**Highly toxic**	**Highly toxic**	Penetrating	Carcinogenic	Non-carcinogenic	Mutagenic
102	162.0	37.8	Penetrating	Non-carcinogenic	Non-carcinogenic	Non-mutagenic



(a) (b)

Figure 1 (a) 2D- & (b) 3D- structure of the 3-Benzyloxyphenylboronic acid (71)



(a) (b)

(c) (d)

Figure 2 (a) Docked view, (b) electro-; (c) hydrophobic and (d) pose obtained by the interaction of insulin with 3-benzyloxyphenylboronic acid (71)

Graph 1 Interaction of amino-acids of insulin showed interaction with compound no. 71

	H-M-HIS-5	H-M-GLU-21	V-M-ASN-3	V-S-ASN-3	V-M-GLN-4	V-S-GLN-4	V-M-HIS-5	V-S-HIS-5	V-S-TYR-16
E due to various aa	-3.5	-3.20961	-4.64505	-4.06509	-10.3269	-8.0283	-7.26829	-8.65503	-7.20764

Interacted amino-acids of insulin

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