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Molecular docking studies of some novel 2 & 3-(4-aminobenzamido) benzoic acid derivatives as DHFR inhibitors for treatment of tuberculosis

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Abstract : A Novel series 2 & 3-(4-aminobenzamido) benzoic acid derivatives were designed virtually considering the basic pharmacophore N-(3,5-bis (trifluoromethyl) phenyl)-5-chloro-2-hydroxybenzamide.The energy minimized conformers of each molecule was generated and docked with *M. tuberculosis* DHFR enzyme with PDB id: 1DF7 using Autodock 4.2.5.1. Most of the molecules have shown significant binding interaction with the receptor. Among the test compounds, **DX-35, DY-24, DX-18, DX-31 & DY-23** have shown highest free energy of binding -9.51 to -8.92 kcal/mol and also the very good estimated inhibitory constant in a range of 0.11 to 0.29 Ki μM, which is comparable to that of the reference standard methotrexate and the standard Anti-Tb drug Ciprofloxacin.
Keywords : Docking, Methotrexate, Ciprofloxacin, Autodock, Benzamide & Benzoic acid.

Introduction

In current years as per WHO TB is the tenth primary cause of death worldwide and the leading cause from a single infectious agent, ranking above HIV/AIDS. In 2017, there were an estimated 1.3 million TB (Tuberculosis) deaths among HIV-negative people and an additional 300 000 deaths among HIV-positive people. An estimated 10 million people fell ill with TB in 2017. Around 558 000 new cases of resistance was found in 2017.[1]

Dihydrofolate reductase (DHFR) is an important enzyme in the folate metabolic pathway which is necessary for the biosynthesis of essential amino acids required for DNA, RNA, and protein synthesis in eukaryotic and prokaryotic cells. Inhibition of the enzyme leads to the arrest of DNA synthesis causing cell death. Dihydrofolate reductase is one of the well-established targets, where around 100 or more 3D-structures

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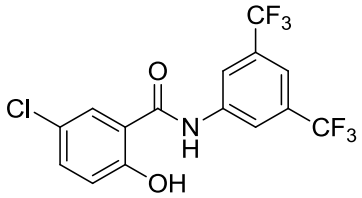
submitted to Protein Data bank. DHFR is the sole source of tetrahydrofolate Thus, the enzyme has already attracted a lot of researcher for the anticancer (methotrexate)[2-3], antibacterial (trimethoprim)[4], antimalarial (pyrimethamine)[5] drugs and anti*coxiela burnetti*[6]. Moreover, specific inhibitors of *Mycobacterium tuberculosis* DHFR (*mt*DHFR) which are active against live *M. tuberculosis* cells have been developed, suggesting that such inhibitors may be useful for treating tuberculosis. [7-15]

In the Protein (PDB: 1DF7) a sequence alignment comparison between human and *M. tuberculosis* DHFRs indicates only 26 % sequence identity and, hence, key differences in the active sites leading to quite specific suggestions for design of new selective inhibitors of M. tuberculosis DHFR.

In recent years amino benzamide derivative have gain lot of importance for their potential as highly effective in combating *Mycobacterium tuberculosis* Infection.[16-17] we also found N-(3,5-bis (trifluoromethyl) phenyl)-5-chloro-2-hydroxybenzamide (IMD-0354) as a lead compound. IMD-0354 is a potent inhibitor of 1ƙB kinase β, and was reached till Phase I/II clinical trials for both atopic dermatitis and chronic obstructive pulmonary disease.[18]

By considering the above facts and also that this compounds are already in human trials, we wish to report the docking studies of some novel 35 newly designed (Table No.1) 2 & 3-(4-aminobenzamido) benzoic acid derivatives with *mt*DHFR.



N-(3,5-bis(trifluoromethyl)phenyl)-5-chloro-2-hydroxybenzamide

Fig 1. Drugs under clinical trials

Fig 2. The structure of *mt*DHFR (1DF7).

Experimental

Materials and Methods

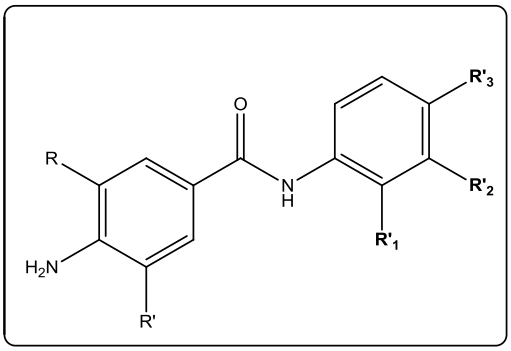
All computational studies were carried out using Autodock 4.2.5.1 (version: 1.5.6) installed in a single machine running on a 2.10 GHz Intel core2 duo processor with 2GB RAM and 500 GB hard disk with windows 7 ultimate as an operating system.

In the present study, the X-ray crystal structure of the antimicrobial agent methotrexate bound to *mt*DHFR was obtained from the Protein Data Bank (http://www.rscb.org/pdb/) (Fig. 2). (PDB ID: 1DF7). The protein consists of 159 amino acid and resolution of 1.7 to 2.0 Å was processed by removing water and methotrexate and energy minimization of this clean protein was carried out in the software Tripos's Sybyl-X 2.0 using the Tripose force field force field and combination of 10,000 steepest descent and conjugate gradient steps with 0.02 Å step size. 2D and 3D structures of 2 & 3-(4-aminobenzamido) benzoic acid derivatives were drawn and Geometry optimization was also performed using Sybyl-X 2.0. The energy minimized protein and geometry optimized structures of compounds were processed in MGLtools1.5.4. Docking simulation was carried out in Autodock 4.2.[19-20]

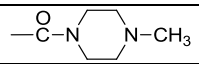
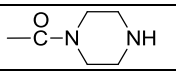
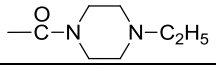
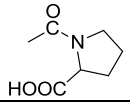
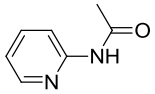
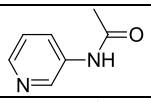
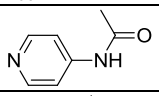
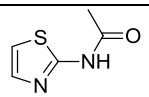
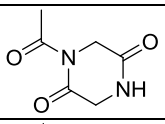
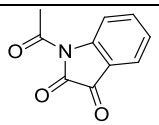
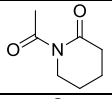
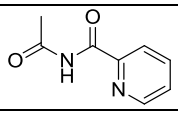
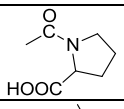
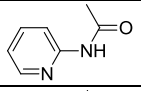
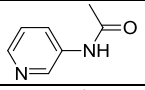
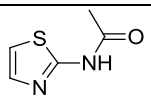
Autodock 4.2.5.1 was used to explore the binding conformation of methotrexate and active test molecules. The Autodock Tools package version 1.5.4 was employed to generate the docking input files and to analyze the docking results. Nonpolar hydrogens present in the protein were merged along with removal of water molecules. For the docking, a grid spacing of 0.375 Å and 60 x 60 x 60 number of points was used. The grid was centered on the ligand coordinates i.e. methotrexate. Autodock generated 25 possible binding conformations, i.e. 25 runs for each docking by using Genetic Algorithm (GA-LS) searches. A default protocol was applied, with an initial population of 150 randomly placed individuals, a maximum number of 2.5 x 105 energy evaluations, and a maximum number of 2.7 x 104 generations. A mutation rate of 0.02 and a crossover rate of 0.8 were used.

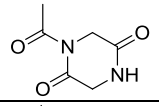
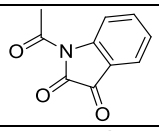
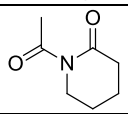
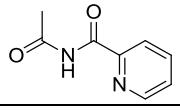
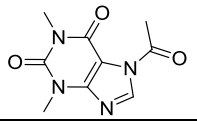
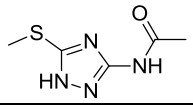
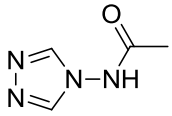
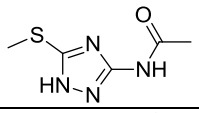
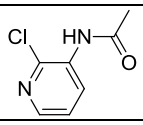
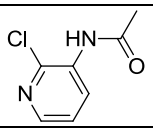
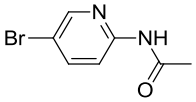
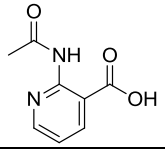
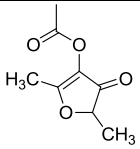
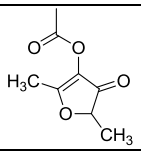
The analysis of binding free energy and interactions of ligands with residues at active site was carried out by using Pymol, Openbabel and Discovery studio 3.5. To validate the use of the Autodock program, redocking was performed on the reference compound methotrexate. Autodock successfully reproduced the experimental binding conformations of the reference drug methotrexate with acceptable root-mean-square deviation (RMSD) of 0.69 Å.

Table No.1: Novel 35 newly designed 2 & 3-(4-aminobenzamido) benzoic acid derivatives



General structure of 2 & 3-(4-aminobenzamido) benzoic acid derivatives

Sr. No.	Comp. Code	R	R'	R'1	R'2	R'3
1.	D-5	H	H	$-COOC_2H_5$	H	H
2.	D-6	H	H	$-COOC_3H_7$	H	H
3.	D-9	H	H	$-COOC_4H_9$	H	H
4.	D-8	H	H		H	H
5.	D-10	H	H	H		H
6.	D-16	H	H	H	Cl	F
7.	D-22	H	H		H	H
8.	D-23	H	H		H	H
9.	DX-1	H	H		H	H
10.	DX-3	H	H		H	H
11.	DX-5	H	H		H	H
12.	DX-7	H	H		H	H
13.	DX-9	H	H		H	H
14.	DX-12	H	H		H	H
15.	DX-14	H	H		H	H
16.	DX-18	H	H		H	H
17.	DX-20	H	H		H	H
18.	DX-26	H	H	H		H
19.	DX-27	H	H	H		H
20.	DX-28	H	H	H		H
21.	DX-29	H	H	H		H

22.	DX-30	H	H	H		H
23.	DX-31	H	H	H		H
24.	DX-33	H	H	H		H
25.	DX-34	H	H	H		H
26.	DX-35	H	H	H		H
27.	DY-2	H	H		H	H
28.	DY-6	H	H		H	H
29.	DY-9	H	H	H		H
30.	DY-11	H	H	H		H
31.	DY-12	H	H		H	H
32.	DY-18	H	H		H	H
33.	DY-15	H	H	H		H
34.	DY-23	H	H		H	H
35.	DY-24	H	H	H		H

Results and Discussion

Molecular Docking and Binding Mode Analysis

An *in-silico* method has been used to generate the candidate model of *mt*DHFR derivatives using AUTODOCK-4 software. The 3D & 2D structures of the ligand molecule were built considering the pharmacophoric feature of the compound methotrexate. The ADT package was employed here to generate the docking input files of the ligand.

The highest ranking model was used further for ligand docking studies. Docking study also shows that model can be used for virtual screening of Anti-TB activity. The docking studies were carried out by using above mentioned validated docking protocol. The free energy of binding reflects the interaction energy between the ligand-protein complex and which has the lowest energy showed more stable interactions. Docking simulation results can be seen in Table 2.

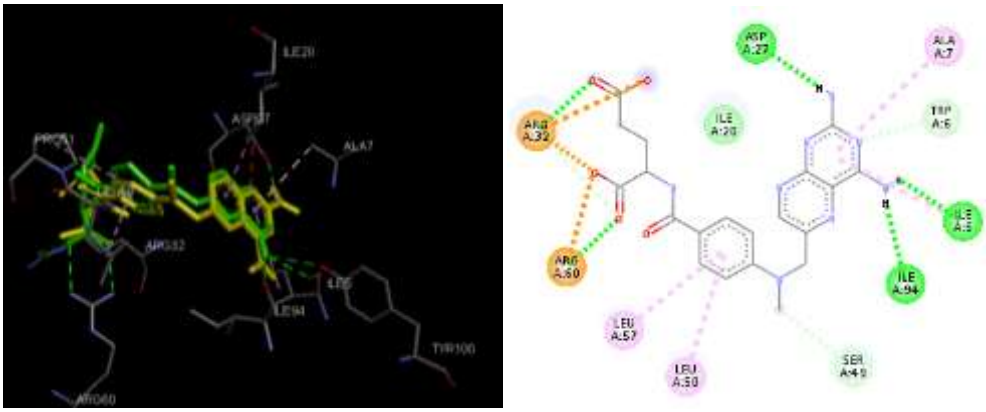
Table 2 Results of docking studies

Sr. No.	Comp. Code	Free energy of binding (kcal/mole)	Estimated inhibitory constant, Ki (µM)	Receptor-Ligand Interactions
1.	DX-35	-9.51	0.11	ALA7, THR46
2.	DY-24	-9.13	0.20	ALA7,THR46,TYR100,TRP6,GLY96
3.	DX-18	-8.99	0.26	ALA7, TRP22
4.	DX-31	-8.96	0.27	ALA7, THR46
5.	DY-23	-8.92	0.29	ALA7,TYR100, ILE94, ILE20
6.	DY-9	-8.82	0.34	ALA7, ILE94, ARG32
7.	DY-2	-8.7	0.42	ALA7, THR46, SER49, ASP19, GLY18, GLY96
8.	DX-1	-8.66	0.45	ILE94, ARG32, ARG60, GLN28
9.	DX-14	-8.64	0.46	ALA7, LEU24
10.	DY-12	-8.63	0.47	ALA7, TRP6, ILE5, TRP22
11.	DX-27	-8.59	0.50	TYR100, TRP22, ILE94, ASP27
12.	D-10	-8.57	0.52	ALA7, TRP22
13.	DX-28	-8.57	0.52	ALA7, ASP27, TRP22, ILE94
14.	DX-33	-8.5	0.58	ALA7
15.	DX-9	-8.42	0.68	ALA7, TRP22, SER49
16.	DX-12	-8.4	0.70	ALA7, TRP22, ILE14
17.	DX-20	-8.12	1.12	ALA7, TRP22
18.	DY-11	-8.1	1.15	ALA7, TYR100, ASP27, ASP19, SER49
19.	D-23	-8.07	1.21	TRP22
20.	DX-29	-8.05	1.25	ALA7, TYR100, ASP27
21.	DY-18	-8.05	1.26	ALA7, ILE5, TRP22
22.	DX-30	-8.04	1.27	ALA7,ILE20, ASP27,THR113

23.	DY-6	-8.04	1.28	ALA7, ASP27, ILE94, SER49, GLY96
24.	DX-7	-8.01	1.35	TYR100, ASP27, SER49
25.	DX-3	-7.93	1.55	TYR100, ASP27, ILE20
26.	DX-34	-7.78	1.97	ALA7, TYR100, ASP19, SER49
27.	DY-15	-7.75	2.07	ALA7, ILE94, ARG32
28.	DX-5	-7.72	2.18	TYR100, ASP27
29.	D-8	-7.64	2.52	ALA7, TRP22
30.	DX-26	-7.54	2.96	ALA7, ILE94, ASP27, SER49
31.	D-22	-7.33	4.21	TRP22, ASP27
32.	D-9	-7.17	5.58	ALA7, ILE94
33.	D-6	-7.12	6.08	TRP6, GLN28
34.	D-16	-6.83	9.78	TYR100, ASP27
35.	D-5	-6.77	10.85	ILE94, ASP27
Ciprofloxacin		-7.58	2.79	GLN28, TRP6
Methotrexate (Reference standard)		-10.21	32.59	ALA7,TRP6, ARG32, ARG60,ASP27, ILE5, ILE94

It was found that compound DX-35, DY-24, DX-18, DX-31 & DY-23 have a significant favorable free energy of binding in a range of -9.51 to -8.92 kcal/mol and also the very good estimated inhibitory constant in a range of 0.11 to 0.29 Ki μM which seems to be much close to that of the reference standard methotrexate for the energy minimized *mt*DHFR protein.

The docking studies were also compared with the standard Anti Tb drug Ciprofloxacin. The residues ALA7, TYR100, ASP27 & ILE94 are important in making important hydrogen interactions. The best conformer generated in docking showed same interactions as shown in Figure 3. The 2D diagram of important interactions between active site residues and all the compounds is shown in figure 4& 5.



(A) **(B)**

Figure 3: A) Docked conformer of Methotrexate (docked conformer shown in yellow and original pose of Methotrexate shown in green fluorescent color) B) 2D Docking Interactions of Methotrexate in with mtDHFR

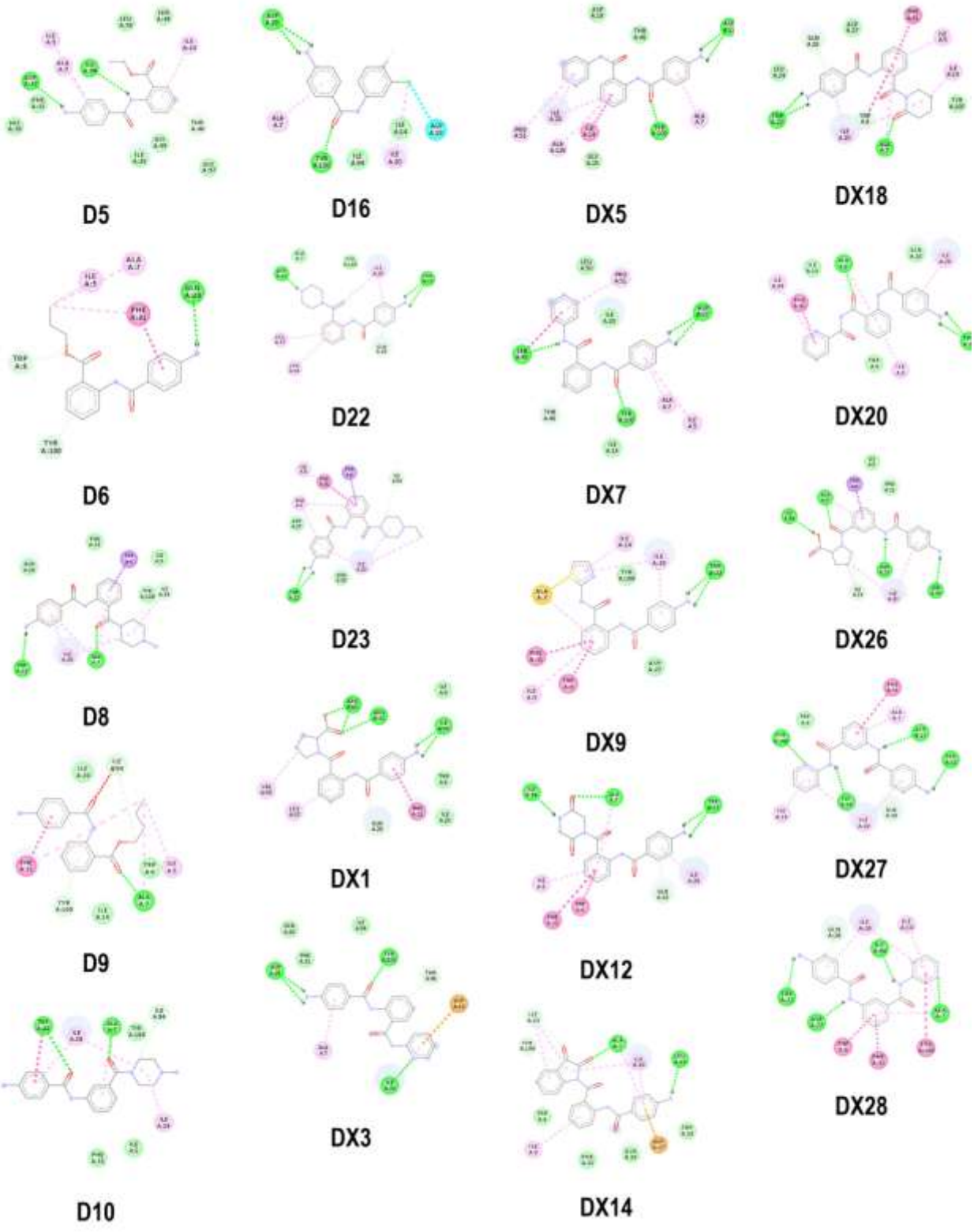


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Figure 4: 2D Interaction diagram for 2 & 3-(4-aminobenzamido) benzoic acid derivatives

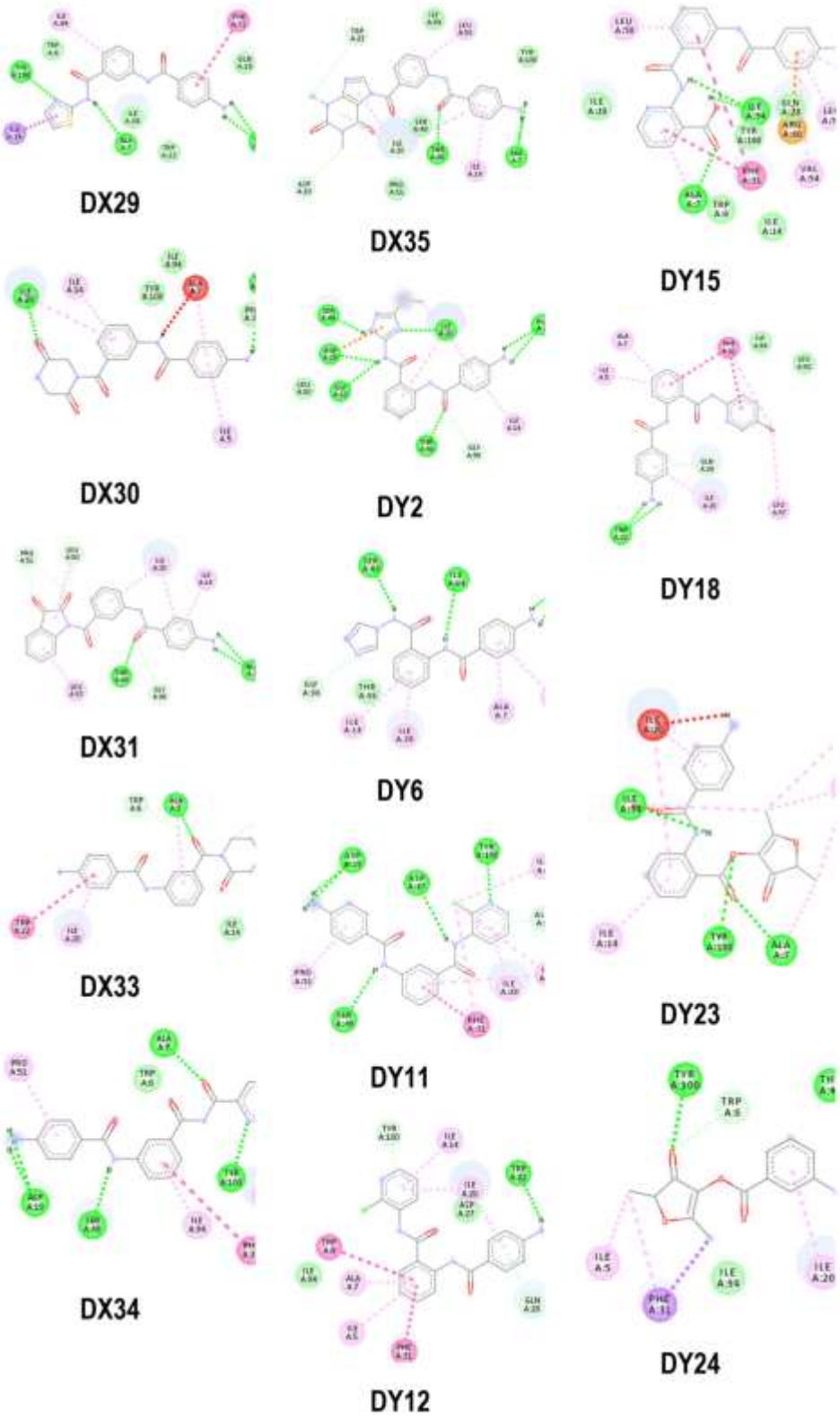

Figure 5: 2D Interaction diagram for 2 & 3-(4-aminobenzamido) benzoic acid derivatives

Conclusion

The molecular docking studies illustrate that the active analogs DX-35, DY-24, DX-18, DX-31 & DY-23 adopt an acceptable conformation within the active site of *mt*DHFR protein. Along with the significant binding interactions have well been pointed out from the above figures. The estimated inhibitory constant of all compounds are in a good range. On the basis of the above findings, these compounds can be further subjected to the QSAR Prediction model and help in rational design of novel and potent *mt*DHFR inhibitors for the treatment of Tuberculosis.

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