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Synthesis, Docking, and Antibacterial Activity of Some Novel 4-Substituted s-triazino[1,2-*a*]benzimidazoles

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ABSTRACT

*Two novel series of 2-amino-3,4-dihydro[1,3,5]triazino[1,2-a]benzimidazoles, and 2-amino-4,4-disubstituted/-spiro[1,3,5]triazino[1,2-a]benzimidazoles were synthesized and evaluated for their in vitro antibacterial activity against Staphylococcus aureus and Escherichia coli. Molecular modeling and docking of the synthesized compounds into enoyl acyl carrier protein reductase (**FabI**) complexed with its bound inhibitor using Molsoft ICM 3.4-8C program was performed. Among the tested compounds, **2** and **16** were the most potent antibacterial (MIC = 25 ug/ml). Detailed synthesis, spectroscopic and biological data are reported.*

Keywords: 4-substituted s-triazino[1,2-*a*]benzimidazole, spiro-compounds, synthesis, docking, antibacterial activity.

INTRODUCTION

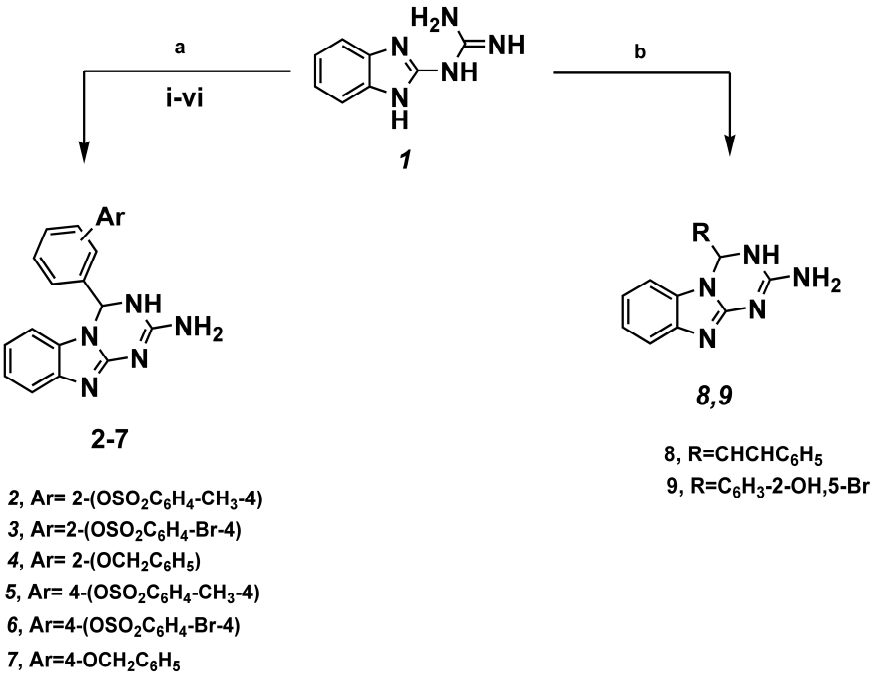
Benzimidazole is one of the most important heterocyclic rings possessing a wide variety of pharmacological activities [1]; these include antiepileptic, antidiabetic, [2] antifungal [3], antitumor [4] and antibacterial activities [5]. Developing new antibacterial agents, which operate with distinctly different mechanism of action other than the conventional ones offers hope of overcoming the increasing resistance of these pathogens [6]. A recent trend in developing new antibacterial agents is to identify and exploit new molecular targets in pathogenic strains. One of these new molecular targets is the enzyme, enoyl-acyl carrier protein reductase [6]. Bacterial Enoyl Acyl Carrier Protein Reductase Inhibitors (FabI) are considered a new antibacterial agents class that act by inhibiting the last step of fatty acid biosynthesis which is an essential process in bacterial growth supplying precursors for the assembly of important cell components such as phospholipids, lipoproteins, lipopolysaccharides, mycolic acids and cell envelope [7,8]. In addition fatty acids are synthesized by mammals (FASI), and bacteria (FASII), via different biosynthetic mechanisms thus enabling bacteria-specific drug targeting [9]. Triclosan, and isoniazid are among other antibacterial agents that inhibit efficiently ACP reductase in a number of pathogens [10].

s-Triazino[1,2-*a*]benzimidazoles attracted a great attention because of their antimicrobial effect; [11,12-15] and 2-amino-s-triazino[1,2-*a*]benzimidazoles displayed an efficient antibacterial activity. [11] Based on the above findings, our goal was to synthesize novel series of 2-amino-3,4- dihydro[1,3,5]triazino[1,2-*a*]benzimidazoles to investigate the effect of 4-substitution, and the formation of spiro-compounds on the *in vitro* antibacterial activity of these compounds against selected Gram-positive and Gram-negative FabI-containing organisms, also to study the SARs of the synthesized compounds after "in silico" screening via docking of the target compounds into the active site of **FabI**.

RESULTS AND DISCUSSION

2.1. Chemistry:

The target compounds, 2-amino-4-aryl-s-triazino[1,2-*a*]benzimidazoles (**2-7)** were synthesized according to schemes 1, where cyclocondensation of various aromatic aldehydes **i-vi** with 2-guanidinobenzimidazole (**1)** in ethanol / piperidine mixture was performed under reflux condition. Similarly; compounds **8** and **9** were prepared in high yield (100%), using stirring condition, (Scheme 1).

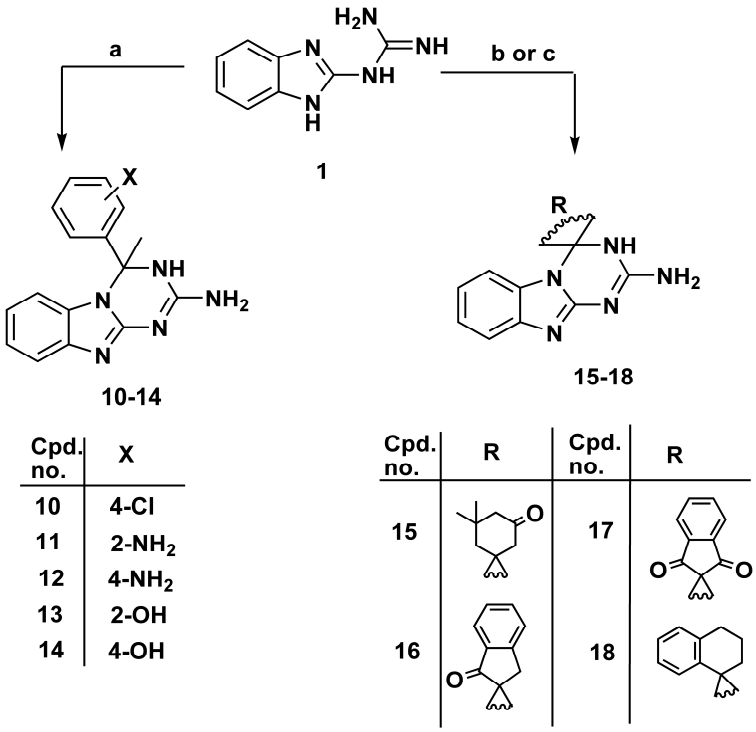


Scheme 1

Reagents and conditions: a; EtOH/pip, reflux 3-6h. **i** = OHC-C6H4-2-(OSO2C6H4-CH3-4), **ii** = OHC-C6H4-2-(OSO2C6H4-Br-4), **iii** = OHC-C6H4-4-(OSO2C6H4-CH3-4), **iv** = OHC-C6H4-4-(OSO2C6H4-Br-4), **v** = OHC-C6H4-2-(OCH2C6H5), **vi** = OHC-C6H4-4-(OCH2C6H5). **b**; EtOH/pip stirring 0.5 h, OHC:CH:CHC6H5, OHC-C6H3-2-OH,5-Br.

Moreover, reaction of starting material **1** with appropriate acetophenones in ethanol / piperidine [12] or in DMF / piperidine failed to give 2-amino-4,4-disubstituted-[1,3,5]triazino[1,2-*a*]benzimidazoles **11-14;** however **10** was produced successfully when the reaction was carried in DMF/piperidine. Using fusion condition, 2-amino-4,4-disubstituted-[1,3,5]triazino[1,2-*a*]benzimidazoles, **(10-14)** were produced.

Additionally, 2-amino-4-spiro[1,3,5]triazino[1,2-*a*]benzimidazoles (**15-18**) were synthesized by cyclocondensation with cyclic ketones namely, dimidone, indanedione, indanetrione, or tetralone in either ethanol / piperidine to afford **17** or in DMF / piperidine to give **15-18**, (Scheme 2).



scheme 2: reagent and condition:; **a**; acetophenons, DMF/pip, reflux or fusion. **b;** dimedone; indandione; tetralone , DMF/pip, reflux 1-16 h, **c**; ninhydrin, ethanol/pip, stirring 0.5 h.

Table 1: The (*in-vitro*) antibacterial activities of the tested compounds

Comound No.	*E.coli (MIC)*	*S.aureus (MIC)*
2	20±0.04 (25)	***19±0.05 (25)***
3	14±0.05 (50)	***18±0.02 (25)***
4	17±0.01 (25)	***14±0.02 (50)***
5	14±0.03 (50)	***11±0.01 (50)***
6	10±0.10 (100)	***13±0.04 (100)***
7	10±0.03 (200)	***13±0.05 (100)***
8	15±0.03 (50)	***12±0.03 (50)***
9	19±0.02 (50)	***21±0.03 (50)***
10	13±0.04 (100)	***16±0.1 (100)***
11	13±0.03 (50)	***12±0.03 (50)***
12	14±0.1 (50)	***16±0.05 (50)***
13	NA	***NA***
14	NA	***NA***
15	15±0.03 (50)	***17±0.04 (50)***
16	20±0.05 (25)	***23±0.03 (25)***
17	19±0.13 (50)	***16±0.01 (50)***
18	9±0.01 (200)	***13±0.01 (100)***
Gentamycin	24±0.01 (6.25)	***20±0.02 (12.5)***

MIC's are in µg/ml, NA: no activity; and data are expressed in the form of mean ±SD
Inhibition zone diameters are in mm.

2.2. Antimicrobial activity*:*
All the synthesized triazinobenzimidazoles, **2-18** were tested for their *in vitro* antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* (**Table 1**).

Among the tested compounds, **2** and **16** showed the highest activity against *S. aureus* and *E .coli* with MIC = 25 ug/ml. In studying the SARs of 2-amino-4-substitutedphenyl-s-triazino[1,2-*a*]benzimidazoles (**2-9)**, we can notice the effect of substitution as the 4-phenyl substitution on the antibacterial activity.

Compound **2** (MIC=25 ug/ml) showed higher activity against *E. coli* than compound **3** (MIC=50 ug/ml). This reveals that, the electron-releasing effect of 4-methylphenylsulfonate group imparts a higher activity, and is more favored for the antibacterial activity than 4-bromophenylsulfonate. Moreover; the position of the substitution influences the antibacterial activity, this is illustrated by the fact that compound **2** carrying 4-methylphenylsulfonate is more active than **5** (MIC=50 ug/ml), thus the 2-substituent on the 4-phenyl group enhances the activity more than 4-substituent. In the same manner, compound **4** bearing 2-benzyloxy moiety on the 4-phenyl group possesses a higher antibacterial activity than **7** which bears the same group on p-4; .this could be attributed further to the favored 2-position over 4-position.

Comparing the activity of 2-amino-4-methyl-4-substitutedphenyl-triazinobenzimidazoles **(11**&**12)** (MIC=50 ug/ml), as they are both carrying aniline group at p-4 of triazinobenzimidazole, showed that compound **12** bearing 4-NH_2 has a slightly better antibacterial (c.f. inhibition zones, Table 1) than **11** having 2-NH_2. Replacing aniline group by 4-chlorophenyl in **10** decreases the activity by half its value (MIC=100 ug/ml), while replacing it with phenol in **13**&**14** abolishes the activity.

Examining the antibacterial activities of the spiro-compounds, **15-18**, revealed that, compound **15** carrying dimidone moiety at p-4 of triazinobenzimidazole nucleus displayed good antibacterial activity (MIC=50 ug/ml). The analog **16** bearing 1'-oxo-indane was the most active antibacterial against both microorganisms (MIC=25 ug/ml), and by comparing the activity of **16** and its counterpart**, 17** (MIC=50 ug/ml) bearing 1',3'-dioxo-indane group at the same position, we can conclude that, the higher acidity of **17** may decrease the antibacterial activity. Moreover, compound **18** having 4-tetralene group lacks acidity and displayed lower activity (MIC=100, 200 ug/ml) than the other analogs (**15-17**).

2.3. Molecular modelling studies:
To understand the obtained biological data on a structural basis, the biologically tested compounds were evaluated through molecular modeling and docking techniques using "Internal coordinate Mechanics (Molsoft ICM 3.4-8C)". Molecular modeling docking studies are performed and ICM score values [16-18] combined with hydrogen bonds formed with the surrounding amino acid residues help to predict the correct binding geometry for each binder at the active site. **FabI** inhibition requires certain binding features including close proximity to the hydrophobic amino acids (Met206, Phe203), and hydrogen bonding interaction to the 2-ribose hydroxyl of NAD^+ and to the hydroxyl group of Tyr156 [6]. In our investigation, the 3D-coordinates in X-ray crystal structure of **FabI** in complex with 1,3,4,9-tetrahydro-2-(hydroxybenzoyl)-9-[(4-hydroxyphenyl)methyl]-6-methoxy-2H-pyrido[3,4-*b*]indole, **ligand** (PDB code 1i30) [6] were used as the receptor model in **FabI** docking simulation. The docked model of Ligand and the most active compounds; **2** and **16** with **FabI** are illustrated in **Fig. 1-3**.

FabI is a member of the short chain alcohol dehydrogenase/ reductase family. This superfamily is characterized by a conserved triad of active site residues; the triad is comprised of Tyr146, Tyr156, and Lys163. The Tyr146F mutant was reported [10] to catalyze substrate reduction which suggests that the Tyr146 hydroxyl is directly involved in catalysis. Triclosan (antiseptic) is one of the marketed antibacterial agents that target FabI enzyme and its mode of action is well documented as antibacterial target. The diphenyl ether of triclosan adopted a conformation with a dihedral angle of about 90° between the two phenyl rings of the inhibitor [19].

Table 2: ICM Scores of the compounds, and hydrogen bonds formed with amino acid residues and their lengths.

Compounds	*ICM scores*	*No. of hydrogen bonds*	*Involved group of amino acid*	*Atom of ligand involved*	*Length of hydrogen bond(A)*
Ligand	-71.09	5	G93….hn	o3	***2.40***
			Y156…hh	o1	***1.90***
			I192…hn	o2	***1.39***
			G93……o	h23	***2.25***
			G190….o	h16	***1.43***

2	-109.43	5	Y156 …… hh	o2	***2.76***
			I92 …… o	h8	***2.01***
			K163 ….. hz2	n3	***2.50***
			I92 …… o	h7	***2.20***
			K163 …. hz1	n3	***1.90***
3	-104.73	6	Y156 ….. hh	o2	***2.47***
			I92 …….. o	h8	***2.46***
			K163... . hz1	n3	***2.78***
			K163 … hz2	n3	***2.48***
			I92…….. o	h7	***2.15***
			K163 … hz1	o2	***2.00***
4	-83.57	6	K163 … hz1	n1	***2.08***
			K163 … hz1	h8	***1.38***
			K163 … hz2	n3	***2.34***
			K163 … hz3	n3	***2.20***
			Y156…. oh	h5	***1.49***
			Y156 …. oh	n3	***0.97***
5	-99.32	3	K163 … hz1	o1	***2.10***
			K163… hz1	o2	***1.88***
			Y156 ….. hh	o2	***2.56***
6	-100.62	6	Y156….. hh	n1	***2.01***
			K163… hz1	n3	***1.43***
			I92…….. o	h7	***2.70***
			T194 … hg1	o3	***2.07***
			I92…….. o	h5	***2.57***
			K163… hz3	n3	***1.60***
7	-80.41	2	L144…. o	h8	***2.63***
			Y156… hh	o1	***2.35***
8	-75.93	9	Y146… .. hn	n3	***2.42***
			K163… hz1	n1	***1.71***
			K163… hz2	n1	***2.25***
			K163… hz3	n1	***2.75***
			S188 ….. og	h14	***1.51***
			S145…… o	h5	***1.29***
			S145 …… o	h15	***2.33***
			Y146…… o	h14	***2.68***
			S145 …. og	h15	***1.41***
9		7	Y156…. hh	n1	***2.05***
			K163.. hz1	n3	***1.59***
			S120 …. og	n3	***2.66***
			S91….. og	h8	***1.58***
			I92……. o	h5	***2.75***
			I92…… o	h7	***1.47***
			K163.. hz3	h12	***2.40***
10	-75.46	3	Y156… hh	n1	***2.09***
			G190…. o	h6	***2.48***
			A189…. o	h7	***2.58***

Table 2: Continued

11	-72.66	5	K163… hz1	n1	***1.98***
			S145…... o	h5	***2.72***
			A189…… o	h9	***1.36***
			Y156…. oh	h15	***2.45***
			S145…. o	h10	***0.98***
12	-72.26	4	Y156… hh	n1	***2.40***
			S145….. o	h15	***1.61***
			S91…… o	h10	***2.42***
			S91….. og	h15	***2.21***
13	-72.24	7	K163.. hz1	n1	***1.90***
			K163... hz1	n3	***0.84***
			Y156…. oh	n3	***2.11***
			K163... hz3	h9	***2.03***
			S145….. o	h15	***1.47***
			Y156… oh	h5	***1.47***

			K163.. hz2	n3	***2.10***
14	-66.21	7	Y156…. hh	n1	***2.05***
			K163.. hz1	n1	***2.53***
			I192…. hn	o1	***1.46***
			S145….. o	h15	***2.79***
			S145….. o	h10	***1.09***
			I192…. o	h9	***1.52***
			S145…. o	h5	***0.96***
15	-70.82	3	K163… hz1	n3	***1.81***
			Y156…. oh	h7	***1.66***
			Y156….. oh	h6	***2.56***
16	-64.48	3	Y156 … hh	n1	***1.95***
			K163 .. hz1	o1	***2.21***
			Y156 ... hh	n3	***2.11***
17	-72.26	8	Y156…. hh	o1	***2.80***
			K163 ... hz1	o1	***2.68***
			K163... hz1	n3	***2.59***
			K163 ... hz2	n3	***2.65***
			S145 …... o	h5	***2.66***
			L144….. o	h5	***2.58***
			L144 …. o	h7	***0.85***
			L144 …. o	h6	***1.12***
18	-65.56	3	Y156… hh	n3	***1.63***
			Y156… oh	h16	***1.19***
			K163.. hz1	n1	***1.76***

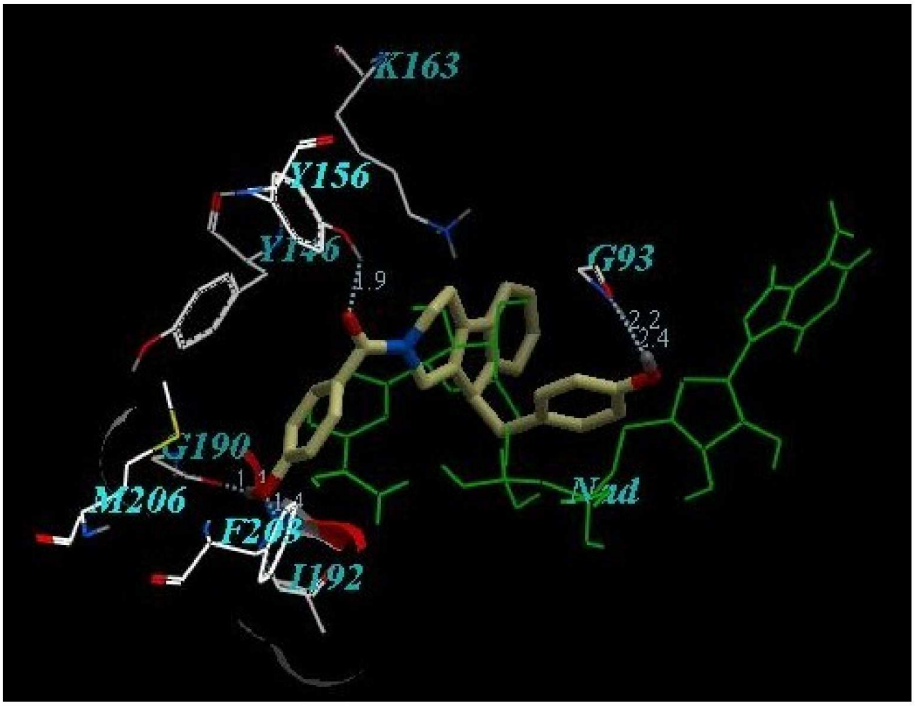


Figure 1: Ligand in the active site of FabI with NAD

As shown in **Table 2**, most of new compounds showed ΔG= -(72.2-109.4) lower than that of ligand (ΔG = -71.1), which indicates a higher stability and affinity of these compounds to the active site of **FabI** compared to the ligand. In modeling process, the 3D structure of all compounds revealed that, 4-aryl group rotated 90° of the plane and became non coplanar with the triazinobenzimidazoles scaffold. Also, the ligand reveals ICM score of -71.1 and

forms five H bonds with: phenolic group of Tyr156 (strong H bond), with Gly93 (2 H bonds), Ile192 (strong H bond), and Gly190 (strong H bond), (Fig.1).

Tolylsulfonate moiety In compound **2** resembles p-hydroxybenzamide moiety of the ligand; tolyl group faces hydrophobic pocket of Met206 and Phe203 (hydrophobic contacts), SO_3 binds by hydrogen bond with Tyr156, N of s-triazine binds with Lys163 via two hydrogen bonds and NH_2 group binds to Ile92 with another two hydrogen bonds. Compound **5** showed moderate activity, this could be attributed to the lack of binding to important amino acids such as Ile192 and Gly93, (Fig.2).

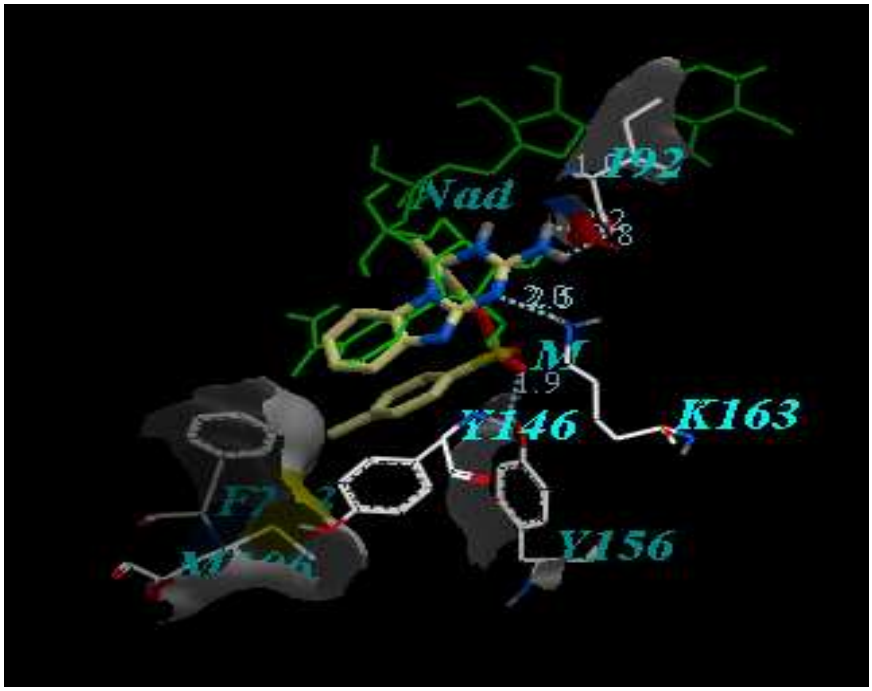


Figure 2: Compound 2 in the active site of FabI with NAD

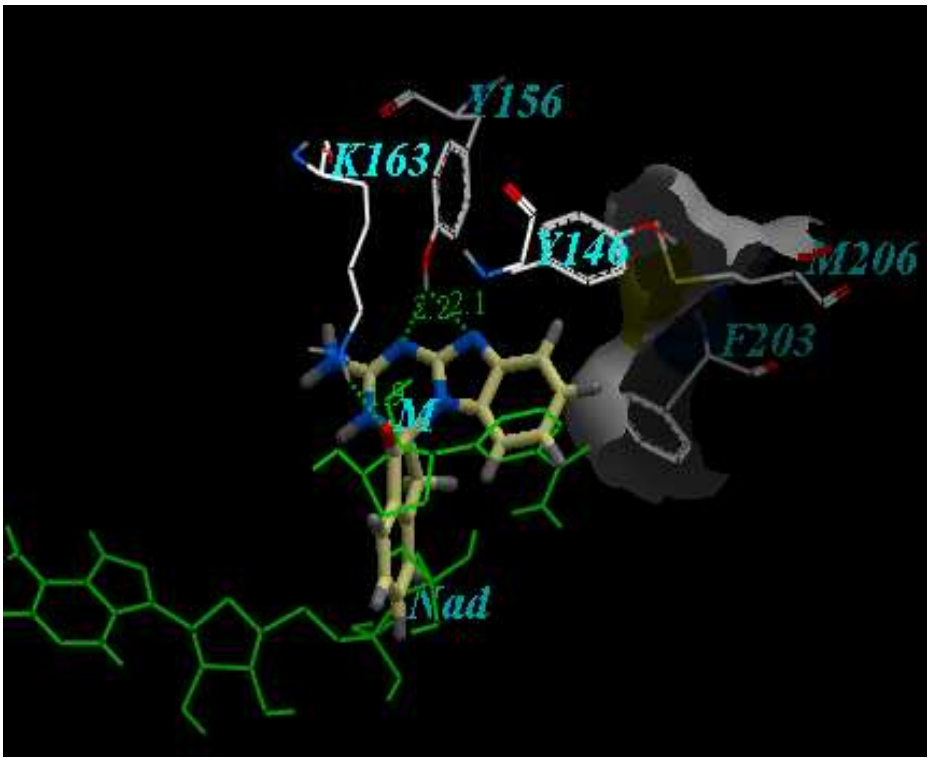


Figure 3: Compound 16 in the active site of FabI with NAD

Compounds **6** and **10** displayed low activity (MIC = 100 ug/ml), as they were not fitted in the correct binding site like the ligand, N1 of benzimidazole binds to Tyr156 instead of O_2 of SO_3**,** as well as, both compounds **13** and **14;** possessed ΔG of -72.2, -66.2 respectively; they bind to Tyr156 with N1 of benzimidazole, and lack antibacterial activity. Compound **7** binds with only one of the important amino acid in the active site of FabI.

Compound **16** was the most potent in the spiro-compounds (**15-18**), its benzene ring of benzimidazole nucleus binds by hydrophobic contacts with Met206 and Phe203**,** the carbonyl oxygen binds with hydrogen bond to Lys163, N-3 of triazine binds with hydrogen bond to Tyr156 and N-1 of benzimidazole binds to phenolic-OH of Tyr156 with another hydrogen bond, (Fig.3).

CONCLUSION

All the target compounds were evaluated for their *in vitro* antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*; compounds, **2** and **16** exhibited the greatest antibacterial activity. From the correlation of the structure to the activity, we can conclude that, for 2-amino-4-monosubstituted-3,4-dihydro[1,3,5]triazino[1,2-*a*]benzimidazoles **2-9**, the 2-substituent is favored than corresponding 4-substituent and electron-releasing group is favored than electron-withdrawing one. In 2-amino-4-methyl-4-substitutedphenyl[1,3,5]triazino[1,2-*a*]benzimidazoles, **10-14**, aniline group is important for activity than 4-chlorophenyl group; however the phenol group abolished the activity. Moderate acidity is required for antibacterial activity of 2-amino-4-spiro [1,3,5]triazino[1,2-*a*]benzimidazoles, **15-18**.

4. Experimental protocols

4.1. Chemistry

Melting points were measured in open capillary tubes using Griffin apparatus and were uncorrected. Elemental microanalyses were carried out at Micro analytical Unit, Cairo University. The infrared (IR) spectra were recorded using potassium bromide disc technique on Schimadzu 435 IR Spectrophotometer at Micro analytical Unit, Cairo University. The proton nuclear magnetic resonance (^{1}HNMR) spectra were performed on Varian Gemini 300 MHZ Spectrophotometer using tetramethylsilane (TMS) as internal standard. Chemical shift values (δ) are given using parts per million scales (ppm.) at Faculty of scince, Cairo University. Mass spectra were recorded on DI-50 unit of Shimadzu GC/ MS-QP 5050A at the Regional Centre for Mycology and Biotechnology at AL-Azhar University and Hewlett Packard 5988 Spectrometer at Micro analytical Unit, Cairo University. All reactions were monitored by TLC using precoated Aluminium sheets silica gel Merck 60 F 254 and were visualized by UV lamp.
The used aldehydes: 2-formylphenyl 4-methylbenzenesulfonate (**i)**, 2-formylphenyl-4-bromo benzenesulfonate (**ii)**, 2-(benzyloxy)benzaldehyde **(iii)**, 4 formylphenyl-4-methylbenzenesulfonate (**iv)**, 4-formylphenyl-4-methylbenzenesulfonate (**v)**, and 4-(benzyloxy)benzaldehyde **(vi)** were prepared according to the reported method [20, 21].

*General procedure for synthesis of compounds **2-7***
A mixture of 2-guanidinobenzimidazole **1** (0.01 mol), appropriate aromatic aldehyde (0.01 mol) and 0.5 ml piperidine in ethanol (30 ml) was heated under reflux for 3-6 h. After cooling the product was filtered, washed with hot ethanol and dried.

4.1.1. 2-(2-Amino-3,4-dihydro[1,3,5]triazino[1,2-a]benzimidazol-4-yl)phenyl-4-methylbenzenesulfonate (2) :
Yield: 31%; m.p. 220-222 ^{o}C; **IR (KBr, cm^{-1}):** 1608 (C=N), 3226 (NH_2), 3472 (NH); **^{1}H NMR (300 MHz, DMSO-d_6):** δ 2.45 (s, 3H, CH_3); 6.30 (d, 1H, H4); 6.43 (s, 2H, NH_2- D_2O exchangeable); 6.72-6.86 (m, 4H, 4Ar-H); 6.92 (t, 1H, H7), 7.24 (m, 2H, H6, H9), 7.37 (t, 1H, H8), 7.53 (d, 2H, H3, H5, *J*=7.8 Hz); 7.76 (s, 1H, NH- D_2O exchangeable), 7.95 (d, 2H, H2, H6, *J*=7.8 Hz); **MS (m/z, %)**: 433 (M^+, 7.3);. Anal.Calcd. for $C_{22}H_{19}N_5O_3S$: C 60.96, H 4.42, N 16.16; Found: C 61.15, H 4.68, N 16.37.

4.1.2. 2-(2-Amino-3,4-dihydro[1,3,5]triazino[1,2-a]benzimidazol-4-yl)phenyl-4-bromobenzenesulfonate (3)
Yield: 56%; m.p. 240-242^{o}C; **IR (KBr, cm^{-1}):** 1650 (C=N), 3280, 3310 (NH_2) 3380 (NH); **^{1}H NMR (300 MHz, DMSO-d_6):** δ 6.29 (d, 1H, H_4); 6.40 (br.s, 2H, NH_2- D_2O exchangeable); 6.66-7.00 (m, 4H, 4-Ar-H); 7.20-7.39 (m, 3H, H6,7,9), 7.40 (t, 1H, H8), 7.78 (s, 1H, NH- D_2O exchangeable), 7.96 (m, 4-H, BrPh-H); **MS (m/z, %)**: 498 (M^+, 0.7). .Anal.Calcd. for $C_{21}H_{16}BrN_5O_3S$: C 50.61, H 3.24, N14.05; Found: C 50.91, H 3.29, N 14.22.

4.1.3. 4-[2-(Benzyloxy) phenyl]-3, 4-dihydro [1 ,3 ,5]triazino[1,2-a]benzimidazol-2-amine (4)
Yield: 41%; m.p. 240 -242^{o}C; **IR (KBr, cm^{-1}):** 1624 (C=N), 3234, 3314 (NH_2); 3426 (NH); **^{1}H NMR (300 MHz, DMSO-d_6):** δ 5.26 (s, 2H, CH_2); 6.28 (s, 2H, NH_2- D_2O exchangeable); 6.65 (s, 1H, H4); 6.82-7.00 (m, 7H, H 7,8 + 5 benzyloxy-H); 7.19-7.39 (m, 5H, 4 Ar-H + H6), 7.50 (d, 1H, H9), 7.66 (s, 1H, NH- D_2O exchangeable). **MS (m/z, %)**: 369 (M^+, 1.18); Anal.Calcd. for $C_{22}H_{19}N_5O$: C 71.53, H 5.18, N18.96; Found: C 71.75, H 5.19, N 19.04.

*4.1.4.4-(2-Amino-3,4-dihydro[1,3,5]triazino[1,2-a]benzimidazol-4-yl)phenyl-4-methylbenzenesulfonate **(5)***
Yield: 8%; m.p. 200-202 °C; **IR (KBr, cm^{-1}):** 1610 (C=N), 3108 (NH_2); 3342 (NH); **^{1}H NMR (300 MHz, DMSO-d_6):** δ 2.40 (s, 3H, CH_3); 6.36 (s, 2H, NH_2- D_2O exchangeable); 6.62 (d, 2H, Ar-H, H3, H5); 6.72 (s, 1H, H4); 6.79 (t, 1H, H7); 6.93 (t, 1H, H8); 7.06 (d, 2H, tolyl-H, H3, H5); 7.24 (d, 2H, Ar-H, H2, H6); 7.37 (d, 1H, H6); 7.41 (d, 1H, H9); 7.68 (d, 2H, tolyl-H, H2, H6), 7.97 (s, 1H, NH-D_2O exchangeable), **MS (m/z,%)**: 432 (M^{-1}, 0.4); Anal.Calcd. for $C_{22}H_{19}N_5O_3S$: C 60.96, H 4.42, N16.16; Found: C 60.69, H 4.54, N 15.90.

*4.1.5. 4-(2-Amino-3,4-dihydro[1,3,5]triazino[1,2-a]benzimidazol-4-yl) phenyl 4-bromobenzenesulfonate **(6)***
Yield: 9%; m.p. 180 -182°C; **IR (KBr, cm^{-1}):** 1614 (C=N), 3180, 3200 (NH_2); 3300 (NH); **^{1}H NMR (300 MHz, DMSO-d_6):** δ 6.37 (s, 2H, NH_2- D_2O exchangeable); 6.62 (d, 2H, Ar-H3,5); 6.73 (s, 1H, H4); 6.77 (t, 1H, H7); 6.91 (t, 1H, H8); 7.10 (d, 2H, bromophenyl-H3,H5); 7.21 (d, 2H, Ar-H2,H6); 7.37 (d, 2H, bromophenyl-H2,H6); 7.74 (d, 1H, H6); 7.82 (d, 2H, H9); 7.96 (s, 1H, NH- D_2O exchangeable); Anal.Calcd. for $C_{21}H_{16}BrN_5O_3S$: C 50.61, H 3.24, N 14.05; Found: C 50.72, H 3.28, N 14.14.

*4.1.6. 4-[4-(Benzyloxy)phenyl]-3,4-dihydro[1,3,5]triazino[1,2-a]benzimidazol-2-amine **(7)***
Yield: 19%; m.p. 250 -252°C; **IR (KBr, cm^{-1}):** 1646 (C=N), 3150, 3200 (NH_2); 3420 (NH); **^{1}H NMR (300 MHz, DMSO-d_6):** δ 5.08 (s, 2H, CH_2); 6.31 (s, 2H, NH_2- D_2O exchangeable); 6.66 (s, 1H, H_4); 6.69 (d, 2H, Ar-H2,6), 6.77 (t, 1H, H7), 6.91 (t, 1H, H8), 7.01 (d, 2H, benzyloxy-H), 7.20 (d, 2H, Ar-H3,H5), 7.25-7.42 (m, 5H, H6,H9 + 3 benzyloxy-H); 7.86 (s, 1H, NH-D_2O exchangeable). **MS (m/z, %)**: 368 (M^{-1}, 0.4); Anal.Calcd. for $C_{22}H_{19}N_5O$: C 71.53, H 5.18, N18.96; Found: C 71.59, H 5.20, N 18.57.

*General procedure for synthesis of compounds **8** and **9***
A mixture of 2-guanidinobenzimidazole 1 (0.01 mol), appropriate aldehyde (0.01 mol) and 0.5 ml piperidine in ethanol (30 ml) was stirred for 30'. The product was filtered, washed with hot ethanol and dried.

*4.1.7. 4-[(E)-2-phenylethenyl]-3,4-dihydro[1,3,5]triazino[1,2-a]benzimidazol-2-amine **(8)***
Yield: 100%; m.p. 258 -260°C; **IR (KBr, cm^{-1}):** 1650 (C=N), 3122 (br-NH_2); 3342 (NH); **^{1}H NMR (300 MHz, DMSO-d_6):** δ 6.30 (s, 1H, H_4); 6.36 (s, 2H, NH_2- D_2O exchangeable); 6.85-6.99 (m, 2H, H7 + CH=CH-ph); 7.1 (d, 1H, CH=CH-ph), 7.24-7.35 (m, 6H, H8+ 5 styryl-H), 7.47 (d, 2H, H6, H9), 7.97 (s, 1H, NH-D_2O exchangeable). **MS (m/z, %)**: 289 (M$^+$, 1.71); Anal.Calcd. for $C_{17}H_{15}N_5$: C 70.57, H 5.23, N 24.21; Found: C 70.34, H 5.12, N 24.08.

*4.1.8. 2-(2-Amino-3,4-dihydro[1,3,5]triazino[1,2-a]benzimidazol-4-yl)-3-bromophenol **(9)***
Yield: 100%; m.p. 290 -292°C; **IR (KBr, cm^{-1}):** 1632 (C=N), 3374 (NH_2); 3470 (NH, OH) cm^{-1}; **^{1}H NMR (300 MHz, DMSO-d_6):** δ 6.36 (s, 2H, NH_2- D_2O exchangeable); 6.82-6.99 (m, 6H, H4, 3 Ar-H, H7, H8); 7.23 (d, 1H, H6), 7.30 (d, 1H, H9), 7.80 (s, 1H, NH-D_2O exchangeable); 10.20 (s, 1H, OH-D_2O exchangeable)). **MS (m/z, %)**: 356 (M^{-2}, 0.1). Anal. Calcd. for $C_{15}H_{12}BrN_5O$: C 50.30, H 3.38, N 19.55; Found: C 50.61, H 3.48, N 19.19.

*General procedure for synthesis of compounds **10-14***
Equimolar amounts of ***1*** (0.01 mol), appropriate acetophenone (0.01 mol) and few drops of piperidine were reacted under fusion condition. The residue was digested in boiling ethanol, filtered and dried.

*4.1.9.4-(4-Chlorophenyl)-4-methyl-3,4-dihydro[1,3,5]triazino[1,2-a]benzimidazol-2-amine **(10)***
*Another procedure for preparing compound **10***
Equimolar amounts of ***1*** (0.01 mol) and p-chloroacetophenone (0.01 mol) were reacted under reflux condition for 3 in DMF with few drops of piperidine. The product was filtered, washed with hot ethanol and dried.
Yield: 53%; m.p. 305-307 °C; **IR (KBr, cm^{-1}):** 3324 (br NH_2, NH) cm^{-1}; **^{1}H NMR (300 MHz, DMSO-d_6):** δ 2.04 (s, 3H, CH_3); 6.56 (s, 2H, NH_2), 7.06 (d, 2H, H2, H6); 7.47 (d, 2H, H3, H5), 7.60 (m, 2H, H7, H8); 8.22 (d, 2H, H6, H9); 8.71 (s, 1H, NH).; Anal.Calcd. for $C_{16}H_{14}ClN_5$: C 61.64, H 4.53, N 22.46; Found: C 61.85, H 4.41, N 22.61.

*4.1.10. 4-(2-Aminophenyl)-4-methyl-3[H] [1,3,5]triazino[1,2-a]benzimidazol-2-amine **(11)***
Yield: 18%; m.p. >300 °C; **IR (KBr, cm^{-1}):** 3286 (2NH_2, NH); **^{1}H NMR (300 MHz, DMSO-d_6):** δ 2.4 (s, 3H, CH_3); 6.52 (t,1H, H5 aniline-H); 6.72 (d, 1H, H3 aniline-H); 7.14 (s, 2H, NH_2); 7.46 (m, 2H, H4, H6 aniline-H); 7.88 (d, 2H, H6, H9); 8.08 (m, 4H, H7, H8 + 2H-NH_2); 11.45 (s, 1H, NH). **MS (m/z, %)**: 292 (M$^+$); Anal. Calcd. for $C_{16}H_{16}N_6$: C 65.74, H 5.52, N 28.75; Found: C 66.04, H 5.39, N 28.60.

*4.1.11. 4-(4-Aminophenyl)-4-methyl-3[H][1,3,5]triazino[1,2-a]benzimidazol-2-amine **(12)***
Yield: 12%; m.p. >300 °C; **IR (KBr, cm^{-1}):** 1650 (C=N), 3136 (NH2); 3324 (NH2,NH) cm^{-1}; **^{1}H NMR (300 MHz, DMSO-d_6):** δ 2.37 (s, 3H, CH_3); 6.54 (d, 2H, H3, H5 aniline-H); 7.14-8.35 (m, 6H, 4 benzimidazole-H + H2, H6

aniline-H); 6.67 (s, 4H, $2NH_2$-D_2O exchangeable); 10.57 (s,1H, NH-D_2O exchangeable). **MS (m/z, %)**: 292 (M^+, 0.07); Anal. Calcd. for $C_{16}H_{16}N_6$: C 65.74, H 5.52, N 28.75; Found: C 65.94, H 5.35, N 28.72.

*4.1.12. 2-(2-Amino-4-methyl-3,4-dihydro[1,3,5]triazino[1,2-a]benzimidazol-4-yl)phenol (**13**)*
Yield: 15%; m.p. >300 ^oC; **IR (KBr, cm^{-1}):** 1658 (C=N), 3122 (br-NH_2); 3320 (NH, OH) cm^{-1}; **^1H NMR (300 MHz, DMSO-d_6):** δ 2.49 (s, 3H, CH_3); 6.81 (s, 2H, NH_2-D_2O exchangeable); 7.07 (m, 2H, H3, H5 phenol-H); 7.26 (t, 2H, H7, H8); 7.42 (m, 2H, H4, H6 phenol-H); 7.82 (s, 2H, NH, OH- D_2O exchangeable); 8.06 (d, 2H, H6, H9). **MS (m/z, %)**: 293 (M^+, 4.39); Anal.Calcd. for $C_{16}H_{15}N_5O$: C 65.51, H 5.15, N 23.88; Found: C 65.88, H 5.43, N 23.58.

*4.1.13. 4-(2-Amino-4-methyl-3,4-dihydro[1,3,5]triazino[1,2-a]benzimidazol-4-yl)phenol (**14**)*
Yield: 12%; m.p. >300^oC; **IR (KBr, cm^{-1}):** 3112 (br-NH_2); 3322 (NH, OH); **^1H NMR (300 MHz, DMSO-d_6):** δ 2.48 (s, 3H, CH_3); 6.91-7.49 (m, 6H, 4 phenol-H +H7, H8); 7.80 (s, 2H, NH_2-D_2O exchangeable); 7.82 (s, 2H, NH, OH-D_2O exchangeable); 8.06 (d, 2H, H6, H9); **MS (m/z, %)**: 293 (M^+, 0.09). Anal.Calcd. for $C_{16}H_{15}N_5O$: C 65.51, H 5.15, N 23.88; Found: C 65.78, H 5.45, N 23.94.

*General procedure for synthesis of compounds **15-18***
Equimolar amounts (0.01mol) of ***1***, and appropriate cyclic ketones: dimidone, indan-1-one, ninhydrin or tetralone (0.01mol) in DMF (15 ml) containing few drops of piperidine were refluxed for 1-16 h. The separated solid was filtered, washed with hot ethanol and dried.

*4.1.14. 2-Amino-5,5-dimethyl spiro[5.5] -3-oxo-cyclohexyl[1,3,5]triazino[1,2-a]benzimidazole (**15**)*
Yield: 36%; m.p. >300 ^oC; **IR (KBr, cm^{-1}):** 1640 (C=N), 1680 (CO); 3308 (NH_2, NH) cm^{-1}; **^1H NMR (300 MHz, DMSO-d_6):** δ 1.07 (s, 6H, $2CH_3$); 2.50 (s, 2H, CH_2 cyclohexanone-H); 3.00 (s, 2H, CH_2 cyclohexanone-H); 3.28 (s, 2H, CH_2 cyclohexanone-H); 7.09 (m, 2H, H7, H8); 6.48 (d, 2H, H6, H9) ;11.90 (s, 3H, NH_2, NH-D_2O exchangeable); .Anal. Calcd. for $C_{16}H_{19}N_5O$: C 64.63, H 6.44, N 23.55; Found: C 64.90, H 6.19, N 23.23

*4.1.15. 3-Amino-spiro[1'-oxo-indane-2',1] [1,3,5]triazino[1,2-a]benzimidazole **16**)*
Yield: 29%; m.p. >300 ^oC; **IR (KBr, cm^{-1}):** 1610 (C=N), 1708 (CO); 3250, 3300 (NH_2), 3404 (NH) cm^{-1}; **^1H NMR (300 MHz, DMSO-d_6):** δ 3.28 (s, 2H, CH2 indanyl-H); 7.17-7.49 (m, 6H, H7, H8+ 4-indanyl-H), 7.62 (m, 2H, H6, H9); 7.76 (br s, 3H, NH_2, NH-D_2O exchangeable); **MS (m/z, %)**: 303 (M^+, 4.84); Anal.Calcd. for $C_{17}H_{13}N_5O$: C 67.32, H 4.32, N 23.09; Found: C 67.51, H 4.59, N 23.33.

*4.1.16. 3-Amino-spiro[1',3'-di-oxo-indane-2',1] [1,3,5]triazino[1,2-a]benzimidazole (**17**)***:**
A mixture of 2-guanidinobenzimidazole 1 (0.01 mol) and ninhydrin (0.01 mol) and 0.5 ml piperidine in ethanol (30 ml) was stirred for 30'. The product was filtered, washed with hot ethanol and dried.Yield: 67%; m.p. 250 252 ^oC; **IR (KBr, cm^{-1}):** 1652 (C=N), 1722 (2CO); 3272 (br NH_2), 3350 (NH) cm^{-1}; **^1H NMR (300 MHz, DMSO-d_6):** δ 6.93 (m, 2H, H7, H8); 7.60 (m, 2H, H2, H3 indandione-H); 7.77 (d, 2H, H6, H9); 7.91 (d, 2H, H1, H4 indandione-H) ;11.28 (s, 3H, NH2, NH-D_2O exchangeable); **MS (m/z, %)**: 317 (M^+, 0.53); Anal.Calcd. for $C_{17}H_{11}N_5O_2$: C 64.35, H 3.49, N 22.07; Found: C 64.55, H 3.3.20, N 22.18

*4.1.17. 2-Amino-spirotetralene[1',1][1,3,5]triazino[1,2-a]benzimidazole (**18**)***:**
Yield: 21%; m.p. 280 -282 ^oC; **IR (KBr, cm^{-1}):** 1645 (C=N), 3300, 3340 (NH_2, NH) cm^{-1}; **^1H NMR (300 MHz, DMSO-d_6):** δ 2.48 (m, 2H, CH_2 tetralene-H); 2.88 (d, 2H, CH_2 tetralene-H); 2.93 (d, 2H, CH_2 tetralene-H); 7.05 (m, 2H, H6, H7 tetralene-H); 7.35 (d, 2H, H5, H8 tetralene-H); 7.43 (m, 2H, H7, H8); 8.27 (d, 2H, H6, H9); 11.75 (s, 3H, NH_2, NH- D_2O exchangeable). **MS (m/z, %)**: 305 (M^+, 1.85); Anal.Calcd. for C_{18} H_{17} N_5: C 71.27, H 5.65, N 23.09; Found: C 71.44, H 5.49, N 23.26.

4.2. Antimicrobial activity:
Agar diffusion method: The microorganism's inoculums were uniformly spread using sterile cotton swab on a sterile Petri dish nutrient agar. 100uL of each sample was added to each well (10 mm diameter holes cut in the agar gel, 20 mm apart from one another). The systems were incubated for 24 h at 37°C. After incubation, microorganism's growth was observed. Inhibition of the bacterial growth were measured in mm. Tests were performed in triplicate [22].

Minimum Inhibitory Concentration (MIC) measurement [23]:
The test organisms were grown in suitable broth for 24 hours for bacteria at 37°C. Two-Fold serial dilutions of the test compound solutions were prepared using the suitable broth to obtain different concentrations. The tubes were then inoculated with the test organisms and were incubated at 37°C for 48 hours. The tubes were then observed for

the presence or absence of microbial growth. The lowest concentration showing no growth was taken as the minimum inhibitory concentration (MIC), (Table 1).

4.3. Docking studies:

All docking studies were performed using "Internal coordinate Mechanics (Molsoft ICM 3.5-0a)"

4.3.1. ***Generation of ligand and enzyme structures***

The crystal structure of Enoyl Acyl Carrier Protien reductase (1I30) was retrieved from the Protein Data Bank http://www.pdb.org/pdb/explore/explore.do?structureId=1I30 All bound waters ligands and cofactors were removed from the protein. The amino acids of the binding site where defined using data in pdbsum http://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/pdbsum/GetPage.pl

4.3.2. ***Preparation of small molecule***

A set of 1, 3, 5 triazino[1,2-a]benzimidazoles, was designed to bind with Enoyl Acyl Carrier Protien reductase (**FabI**), ChemDraw 3D structures were constructed using ChemDraw 3D ultra 9.0 software [Cambridge Soft Corporation, USA (2004)], and then they were energetically minimized by using MOPAC (semi-empirical quantum mechanics) with MM2, Jop Type with 100 iterations and minimum RMS gradient of 0.01, and saved as MDL MolFile (*.mol).

4.3.3. ***Docking using Molsoft ICM 3.4-8C program*** [24, 25]

4.3.3.1. *Convert our PDB file 1I30 into an ICM object*:

This conversion involves addition of hydrogen bonds, assignment of atoms types, and charges from the residue templates. Click on MolMechanics/Convert/Protein, and then delete water molecules.

4.3.3.2. *To perform ICM small molecule docking*:

a) Setup docking project:

i) Set Project Name: Click on Docking/Set project name, press OK.

ii) Setup the receptor: Click on Docking/Receptor Setup, enter the receptor molecule in the receptor molecule data entry box (a_*) will do, then click on identify the binding sites button to identify the potential ligand binding pockets, press OK. After the receptor setup is complete, the program normally displays the receptor with selected binding site residues highlighted in yellow xstick presentation.

iii) Review and adjust binding site: ICM makes a box around the ligand binding site based on the information entered in the receptor setup section. The position of the box encompasses the residues expected to be involved in ligand binding. Click on the menu Docking/Review/Adjust ligand/Box.

vi) Make receptor maps: The step now is to construct energy maps of the environment within the docking box. Click on menu Docking/Make Receptor Maps, select the resolution of the map by entering a value into the grid cell size data entry box which is 0.5, this step takes few minutes.

b) Start docking simulation: Use interactive docking to dock one ligand at a time. Click on menu Docking/Interactive docking/Mol Table Ligand, use the drop down arrow to find the table of ligand and/or Compounds we wish to dock, and then enter the thoroughness which represent the length of simulation. Generally 1 is reasonable value, select Calc ICM Score, then select Display run which display the ligand sampling the energy in the ligand binding project.

4.3.3.3. *Display the result. Click Docking/Browse/Stack Conformations*.

ICM stochastic global optimization algorithm attempts to find the global minimum of the energy function that include five grid potentials describing interaction of the flexible ligand with the receptor and internal conformational energy of the ligand, during this process a stack of alternative low energy conformations is saved. The mode of interaction of ligand within 1I30 was used as a standard docked model as well as for RMSD (results differing by less than 1.0 Å in positional root-mean-square deviation) calculation. All inhibitors were compared according to the best binding free energy (minimum) obtained among all the run.

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