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Synthesis, Biological Evaluation, and Molecular Docking of 1,4-Benzodioxan Derivatives as Potential Antibacterial Agents[1,2]

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Abstract—A series of novel 1,4-benzodioxan derivatives containing Schiff base are synthesized by the method of splicing active substructures. All compounds are assayed for antimicrobial activity. The preliminary results indicate that most of the products demonstrate higher antibacterial activity against Gram-negative bacteria strains than Gram-positive bacteria strains. Compounds **4d** and **4m** exhibit better antibacterial activity against *E. coli* (MIC = 0.78 and 0.17 μg/mL), respectively; compound **4g** displays better antibacterial activity against *P. aeruginosa* (MIC = 0.78 μg/mL). Eleven common antibacterial targets are selected for molecular docking of target compounds. The results demonstrate that all target compounds have the strongest binding energy to Tyrosine-tRNA synthetase (-CDOCKER_INTERACTION_ENERGY, kcal/mol: 47.1486 and 47.3776). Therefore, it is speculated that the target compounds can be used as novel tyrosine-tRNA synthetase inhibitors.

Keywords: 1,4-benzodioxan derivatives, Schiff base, antimicrobial activity, molecular docking
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INTRODUCTION

High bacteria resistance to the originally powerful antibiotics [1] initiates a proactive effort in developing novel antibacterial agents with excellent antimicrobial pharmacophores.

1,4-Benzodioxane is a type of structural moieties that contains multiple substitution sites and can promote the developed of a variety of derivatives. 1,4-Benzodioxane derivatives demonstrate a wide range of biological activities, such as anti-tumor, anti-bacterial, anti-inflammatory, and anti-adrenergic [2–4]. Owing to the broad spectrum of chemotherapeutic properties of 1,4-benzodioxane and Schiff bases [5–10], their combination in one molecule could result in creating potentially strong antimicrobial agents. This approach is promoted in the current study. Molecular docking allowed to explore the binding ability of the target compounds with the classical antibacterial active sites.

RESULTS AND DISCUSSION

The developed three steps synthetic approach to new target compounds **4a–4s** is presented in Scheme 1. In the first step 2,3-dihydrobenzo[*b*][1,4]dioxine-6-carboxylic acids 1 were refluxed with concentrated sulfuric acid in methanol to give methyl 2,3-dihydrobenzo[*b*][1,4]dioxine-6-carboxylate **2**. Concentrated sulfuric acid acted there as a catalyst. In the second step 2,3-dihydrobenzo[*b*][1,4]dioxine-6-carbohydrazides **3** were prepared by treatment of compound **2** with hydrazine hydrate (85%) in ethanol. Finally, the mixture of 2,3-dihydrobenzo[*b*][1,4]dioxine-6-carbohydrazides **3** with different substituents aldehydes in methanol was refluxed for 2 h. Upon removal of the solvent under reduced pressure the target compounds **4a–4s** were isolated as white powders with high yields.

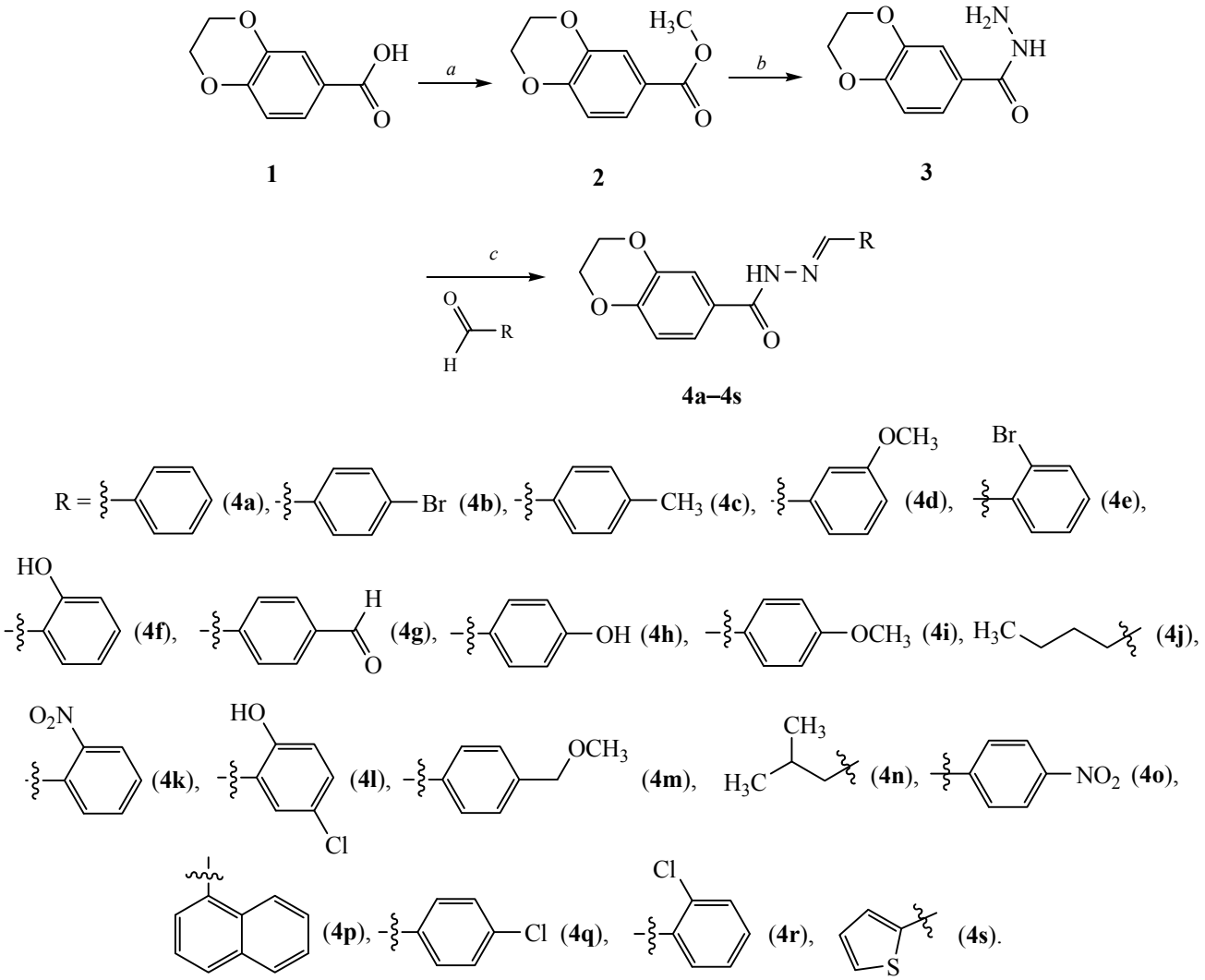
Crystal structure of compound 4b. Crystals of compound **4b** (CCDC: 1847855) were obtained from a methanol solution. The perspective view of the monomeric unit with the atomic numbering scheme is presented in Fig. 1. The crystal structure with inter- and intramolecular H-bonds is presented in Fig. 2. The crystallographic data and structure refinement parameters are listed in Table 1. Hydrogen bond lengths and angles are listed in Table 2.

Antibacterial activity. The MIC (Minimum inhibitory concentration, μg/mL) of compounds **4a–4s** against the bacterial strains were tested by MTT

[1] The text was submitted by the authors in English.

[2] Supplementary materials are available from authors.

Scheme 1. Synthesis of the title compounds.



Reagents and conditions: (*a*) concentrated sulfuric acid, methanol, reflux; (*b*) hydrazinehydrate (85%), ethanol; (*c*) different substituents aldehydes, methanol, refluxing.

method (Table 3). Compounds **4a–4s** demonstrated significant selectivity and high antibacterial activity. Among those compounds **4d** and **4m** exhibited the highest antibacterial activity against *E. coli* (MIC = 0.78 and 0.17 μg/mL), and the compound **4g** was the most active against *P. aeruginosa* (MIC = 0.78 μg/mL).

Activities of the tested compounds could be correlated with their molecular structures. According to the accumulated data (Table 3), most of compounds demonstrated better selectivity and inhibitory activity against Gram-negative bacteria (*E. coli* and *P. aeruginosa*). The compounds containing the benzene ring exhibited higher inhibitory activity than those containing the naphthalene ring (**4p**) and the thiophene ring (**4s**). The compound **4c** (MIC =10.21 μg/mL for *E. coli*) with the methyl group located on the benzene ring was more active than the compounds **4j** (MIC = 16.25 μg/mL for *E. coli*) and **4n** (MIC > 20 μg/mL for *E. coli*) that contained butane or isobutane fragments. The compounds **4q** and **4r** with Cl on the benzene ring were more active than the compounds **4b** and **4e** with Br. The compounds containing Cl or Br atom in *p*-position demonstrated stronger inhibitory activity than their analogues with *o*-position of the same substituents.

Introduction of the aldehyde group in *p*-position of the benzene ring promoted the inhibitory activity (**4g**: MIC = 3.12 μg/mL for *E. coli* and MIC = 0.78 μg/mL

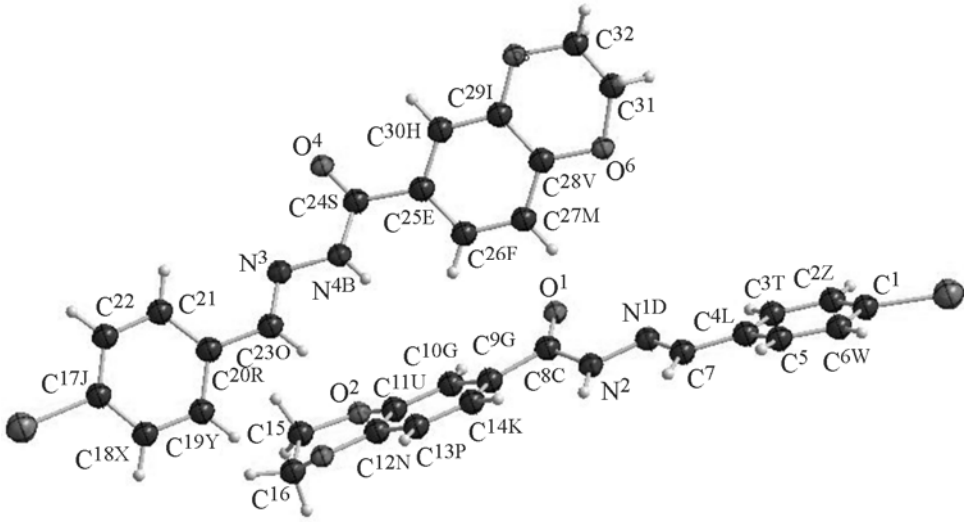


Fig. 1. Molecular structure of the compound **4b** with atomic numbering scheme.

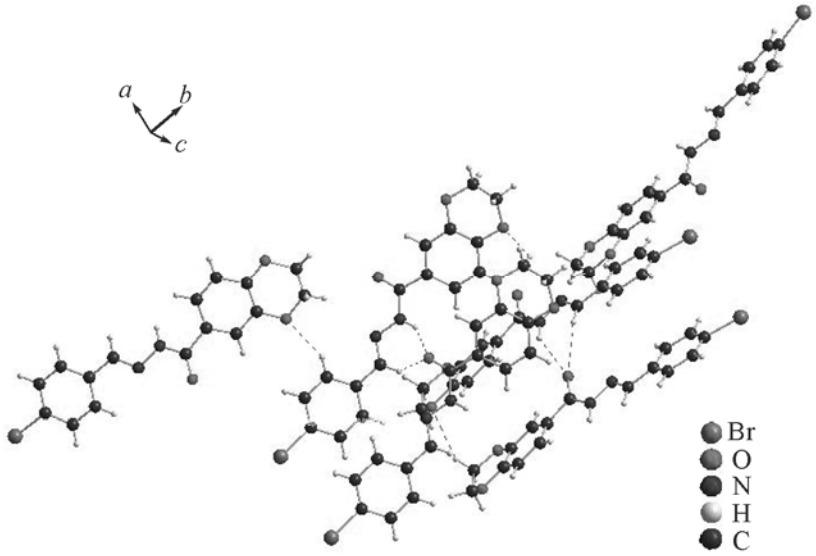


Fig. 2. Crystal packing and H-bonds in the compound **4b.**

for *P. aeruginosa*). The compound **4m** with the methoxy group in p-position of the benzene ring exhibited higher inhibitory activity (MIC = 0.17 μg/mL for *E. coli* and MIC = 1.22 μg/mL for *P. aeruginosa*) than the compound **4d**, and was significantly higher than the positive control penicillin.

Docking study. Molecular docking was performed for validating antibacterial target proteins interaction with the designed compounds. The molecular structures of the synthesized compounds were fitted into several antibacterial target protein, including 4ALM, 3S1Y, 3FY8, 2VVT, 1PFY, 4HEJ, 3G75, 1HNJ, 2VAM, 1JIJ, 1VBM. The results indicated that the designed compounds could be more potent against *E. coli* Tyrosyl-tRNA synthetase (PDB code: 1VBM).

The 2D and 3D simulations of compounds **4d** and **4m** with the highest binding ability to the target protein Tyrosine-tRNA synthetase were resolved using DS 4.5 software (Fig. 3). According to these data (Figs. 3c, 3d) the benzyl group of compound **4m** formed the carbon hydrogen bond with HIS98 and PHE236 of the target protein; the benzene ring formed Pi–Pi T-shaped with His 51, and Pi-alkyl forms with LEU227 and PRO54. The oxygen atom in 1,4-benzodioxan formed the conventional hydrogen bond with the THR76, and the hydrogen atom formed the

Table 1. Crystallographic data for compound **4b**

Parameter	Value
Formula	$C_{16}H_{13}N_2O_3Br$
Formula weight	361.18
Crystal system	Monoclinic
Space group	*P*21
a, Å	5.0735(6)
b, Å	17.9043(18)
c, Å	16.2456(18)
α, deg	90
β, deg	90
γ, deg	90
V, Å	1475.7(3)
Z	4
D_{calc}, g/cm^3	1.626
θ range, deg	2.3–28.3
F(000)	728
Reflections collected/unique	14170/6650
Date/restraints/parameters	2989/0.044/200
Absorption coefficient, mm^{-1}	0.71073
R_1/wR_2 [$I > 2\sigma(I)$]	0.0855/0.1428
R_1/wR_2 (all date)	0.0855/0.1428
GOOF	1.010

carbon hydrogen bond with the THR76. The compounds **4d** and **4m** could fully bind to the active pocket of the target protein, in which the benzene ring site was located inside the active pocket, and the 1,4-benzodioxane site was located in the opening of the active pocket.

EXPERIMENTAL

All chemicals and reagents used were of analytical grade. The reactions were monitored by TLC on Merck precoated silica gel GF254 plates. Melting points (uncorrected) were determined on a Digital Melting Point apparatus (Shenguang, Shanghai, China). ESI mass spectra were measured on a Mariner System 5304 mass spectrometer. ^{1}H NMR spectra were measured on a Bruker DPX300 spectrometer at room temperature using DMSO-d_6 as a solvent and TMS as an internal standard. Elemental analyses were performed on a CHN-O-Rapid instrument, and were within ±0.4% of the theoretical values.

(*E*)-*N*'-Benzylidene-2,3-dihydrobenzo[*b*][1,4]-dioxine-6-carbohydrazide (4a). mp 181–183°C. ^{1}H NMR spectrum, δ, ppm: 11.67 s (1H); 8.44 s (1H); 7.70 s (2H); 7.44–7.47 m (5H); 6.97–7.00 d (*J* = 5.58 Hz, 1H); 4.39–4.33 m (4H). MS: *m/z:* 282.1 [*M* + H]$^+$. Found, %: C 68.13; H 5.06; N 9.87. $C_{16}H_{14}N_2O_3$. Calculated, %: C 68.07; H 5.00; N 9.92.

(*E*)-*N*'-(4-Bromobenzylidene)-2,3-dihydrobenzo-[*b*][1,4]-6-carbohydrazide(4b). mp 244–246°C. ^{1}H NMR spectrum, δ, ppm: 11.75 s (1H); 8.41 s (1H); 7.66 s (4H); 7.43–7.47 m (2H); 6.97–7.00 d (*J* = 8.4 Hz, 1H); 4.29–4.33 m (4H). MS: *m/z:* 360.01 [*M* + H]$^+$. Found, %: C 53.27; H 3.59; Br, 22.17; N 7.71. $C_{16}H_{13}BrN_2O_3$. Calculated, %: C 53.21; H 3.63; Br, 22.12; N 7.76.

(*E*)-*N*'-(4-Methylbenzylidene)-2,3-dihydrobenzo-[*b*][1,4]dioxine-6-carbohydrazide(4c). mp 205–207°C. ^{1}H NMR spectrum, δ, ppm: 11.61 s (1H); 8.40 s (1H); 7.46–7.62 m (2H); 7.45–7.44 m (2H); 7.25–7.28 m (2H); 6.96–6.99 d (*J* = 8.22 Hz, 1H); 4.29–4.32 m (4H); 2.34–2.49 d (*J* = 4.2 Hz, 3H). MS: *m/z*: 296.12 [*M* + H]$^+$. Found, %: C 68.96; H 5.40; N 9.49. $C_{17}H_{16}N_2O_3$. Calculated, %: C 68.91; H 5.44; N 9.45.

Table 2. Hydrogen bonds length and bond angles values for compound **4b**

D–H···A	*d*(D–H), Å	*d*(H···A), Å	*d*(D···A), Å	∠DHA, deg
$N^2\cdots H^2\cdots O^1$	0.86	2.33	2.961(9)	143
$N^{4B}\cdots H^{4B}\cdots O^4$	0.86	2.24	2.953(9)	141
$C^{3T}\cdots H^{3T}\cdots O^2$	0.93	2.48	3.305(10)	149
$C^7\cdots H^7\cdots O^1$	0.93	2.59	3.213(10)	125
$C^{21}\cdots H^{21}\cdots O^5$	0.93	2.45	3.291(11)	150
$C^{23O}\cdots H^{23O}\cdots O^4$	0.93	2.57	3.211(9)	127

Table 3. Antibacterial activity (MIC) of the synthesized compounds

Compound	MIC±SD, μg/mL			
	gram-negative bacteria		gram-positive bacteria	
	Escherichia coli	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Bacillus subtilis*
4a	12.52±1.235	15.00±1.996	>20	>20
4b	7.22±0.986	6.68±0.981	>20	>20
4c	10.21±1.669	13.79±1.967	>20	>20
4d	0.78±0.079	3.78±0.468	12.50±1.654	>20
4e	10.12±1.593	8.17±0.963	12.50±1.364	>20
4f	>20	18.03±	>20	>20
4g	3.12±0.997	0.78±0.083	>20	>20
4h	12.58±1.596	12.11±1.649	>20	>20
4i	15.32±1.779	>20	12.50±1.265	>20
4j	16.25±1.489	12.56±1.648	12.50±1.469	>20
4k	12.58±1.566	13.66±1.934	>20	>20
4l	>20	>20	>20	>20
4m	0.17±0.029	1.22±0.352	>20	>20
4n	>20	>20	>20	>20
4o	12.15±1.569	8.08±0.978	12.50±1.349	>20
4p	>20	18.71±1.679	>20	>20
4q	3.56±0.966	5.56±0.966	>20	>20
4r	5.18±0.998	8.12±0.987	12.50±1.497	12.50±1.675
4s	12.24±1.795	16.97±1.689	>20	>20
Penicillin	1.56±0.467	1.89±0.647	6.25±0.976	6.25±0.996

(*E*)-*N*'-(3-methoxybenzylidene)-2,3-dihydrobenzo[*b*][1,4]-6-carbohydrazide (4d). mp 179–181°C. ^{1}H NMR spectrum, δ, ppm: 11.69 s (1H); 8.41 s (1H); 7.40–7.47 m (2H); 7.35–7.3707 m (1H); 7.01–7.26 m (2H); 6.97–7.00 m (2H); 4.29–4.32 m (4H); 3.75–3.81 d (*J* = 16.44 Hz, 3H). MS: *m/z*: 312.11 $[M + H]^+$. Found, %: C 65.42; H 5.11; N 8.92. $C_{17}H_{16}N_2O_4$. Calculated, %: C 65.38; H 5.16; N 8.97.

(*E*)-*N*'-(2-Bromobenzylidene)-2,3-dihydrobenzo-[*b*][1,4]-6-carbohydrazide (4e). mp 232–235°C. ^{1}H NMR spectrum, δ, ppm: 11.94 s (1H); 8.80 s (1H); 7.98–8.00 d (*J* = 4.11 Hz, 1H); 7.67–7.71 m (1H); 7.44–7.50 m (3H); 7.33–7.39 m (1H); 6.98–7.01 d (*J* = 7.62 Hz, 1H); 4.29–4.34 m (4H). MS: *m/z*: 360.01 $[M + H]^+$. Found, %: C 53.27; H 3.60; Br, 22.16; N 7.70. $C_{16}H_{13}BrN_2O_3$. Calculated, %: C 53.21; H 3.63; Br, 22.12; N 7.76.

(*E*)-*N*'-(2-Hydroxybenzylidene)-2,3-dihydrobenzo[*b*][1,4]-6-carbohydrazide (4f). mp 176–177°C. ^{1}H NMR spectrum, δ, ppm: 11.95 s (1H), 11.34 s (1H); 8.61 s (1H); 7.30–7.53 m (3H); 7.28–7.27 m (1H); 7.00–7.02 m (1H); 6.90–6.95 m (2H); 4.29–4.34 m (4H). MS: *m/z*: 298.1 $[M + H]^+$. Found, %: C 64.47H, 4.69; N 9.34. $C_{16}H_{14}N_2O_4$. Calculated, %: C 64.42 H 4.73; N 9.39.

(*E*)-*N*'-(4-Formylbenzylidene)-2,3-dihydrobenzo-[*b*][1,4]-6-carbohydrazide (4g). mp 330–337°C. ^{1}H NMR spectrum, δ, ppm: 11.77 s (1H); 8.46 s (1H);

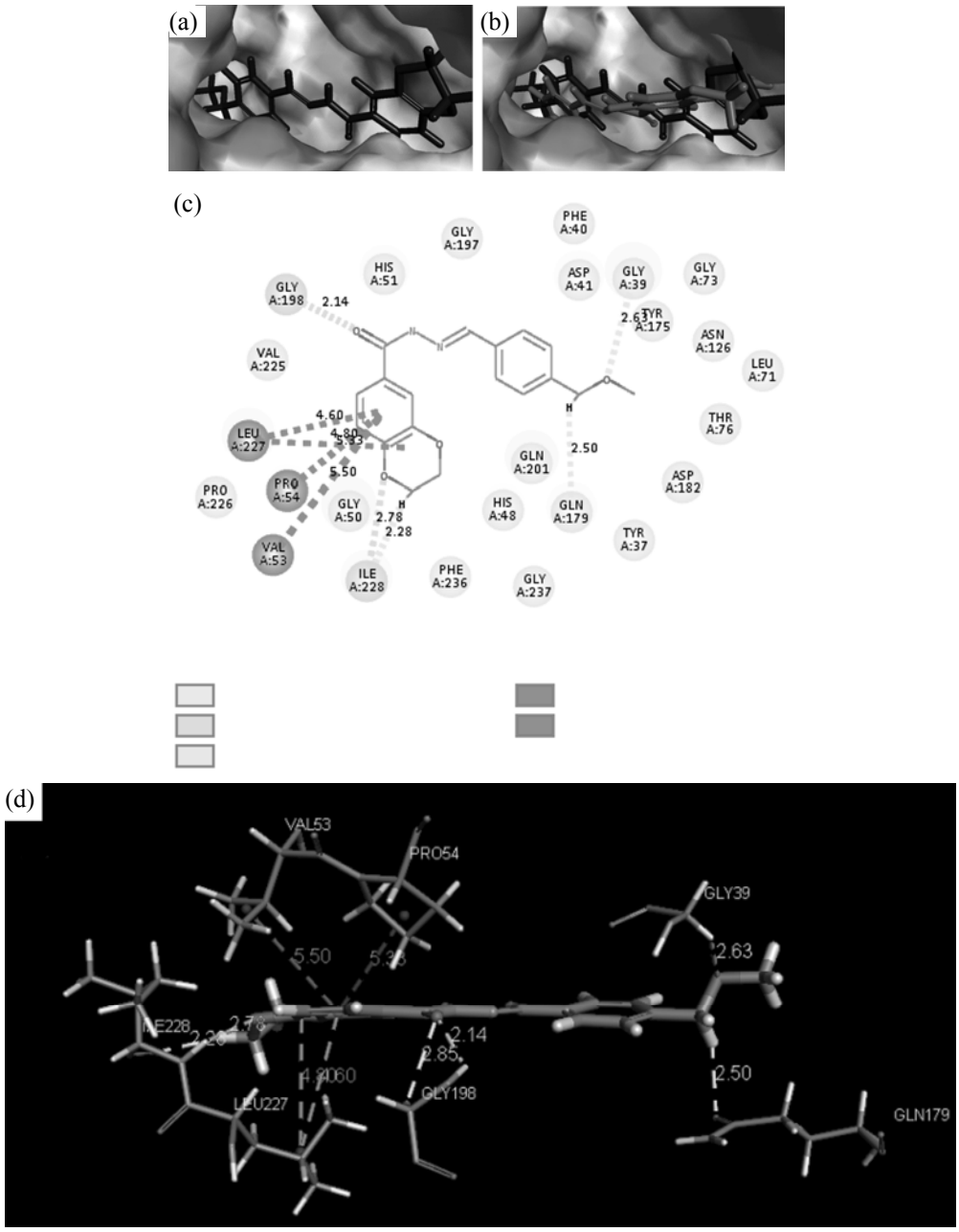


Fig. 3. (a) The best pose of **4m** (blue) obtained from the docking study in the active site of *E. coil* Tyrosyl-tRNA synthetase (1VBM), (b) **4d** (red) And **4m** (blue) in 1VBM, (c) 2D schematic presentation of compound **4m**, and (d) important amino acid residues associated with compound **4m**. Green is the conventional hydrogen bond, cyan is the carbon hydrogen bond, and purple is the Alkyl and *Pi*-Alkyl.

7.74–8.00 m (1H); 7.71–7.79 m (2H); 7.45–7.49 m (4H); 6.98–7.01 d (J = 8.58 Hz, 1H); 4.31 s (4H); 3.17 s (1H). MS: *m/z*: 310.1 $[M + H]^+$. Found, %: C 65.85 H 4.51; N 9.34. $C_{17}H_{14}N_2O_4$. Calculated, %: C 65.80 H 4.55; N 9.30.

(*E*)-*N*'-(4-Hydroxybenzylidene)-2,3-dihydrobenzo[*b*][1,4]-6-carbohydrazide (4h). mp 247–250°C. ^{1}H NMR spectrum, δ, ppm: 11.47 s (1H); 9.89 s (1H); 8.33 s (1H); 7.53–7.55 d (J = 8.43 Hz, 2H); 7.42–7.45 m (2H); 6.96–6.98 d (J = 8.04 Hz, 1H); 6.82–6.85 d (J = 8.58 Hz, 2H); 4.30 s (4H). MS: *m/z*: 298.1 $[M + H]^+$. Found, %: C 64.46; H 4.73; N 9.35. $C_{16}H_{14}N_2O_4$. Calculated, %: C 64.42; H 4.78; N 9.39.

(*E*)-*N*'-(4-Methoxybenzylidene)-2,3-dihydrobenzo[*b*][1,4]-6-carbohydrazide (4i). mp 185–189°C. ^{1}H NMR spectrum, δ, ppm: 11.5496 s (1H). 8.74 s

Table 4. Molecular docking values for compounds **4a–4s**

Comp. no.	-CDOCKER_INTERACTION_ENERGY, kcal/mol					
	1VBM	1KZE	1PFY	2VVT	3FY8	3SLY
4a	39.3609	19.0786	26.0215	31.9964	34.9746	35.6504
4b	42.9604	19.9851	31.4072	30.9021	36.069	37.957
4c	43.1962	20.818	33.2328	30.8796	39.0212	36.1243
4d	47.1486	24.2729	35.7799	35.5791	39.5175	37.6954
4e	42.4443	20.6997	25.46	35.6387	38.6448	34.221
4f	40.1377	21.5187	29.1245	33.5681	36.7413	35.0902
4g	44.8369	20.3402	29.394	32.1584	32.5522	35.4947
4h	43.1038	22.6902	36.752	32.8772	34.9377	34.7404
4i	42.6011	20.228	38.8938	34.7772	31.6437	39.2301
4j	37.896	20.2057	29.3292	32.3345	33.3973	34.4477
4k	43.8237	23.9406	39.5657	36.1728	42.296	37.6678
4l	41.4125	26.8925	35.8983	38.2088	42.4505	38.5191
4m	47.3776	19.3733	29.2344	33.0106	36.2841	41.0415
4n	40.2734	22.626	27.0826	33.2413	37.7435	31.2961
4o	45.8463	22.2428	27.9109	30.8815	41.2824	38.8345
4p	43.0021	21.4705	30.7128	40.087	43.1369	38.4059
4q	39.9589	19.774	32.4531	31.2672	37.5451	32.7445
4r	43.0277	21.1262	32.856	35.4361	35.2899	34.5612
4s	37.6805	21.7116	30.4133	30.6372	32.7496	28.605
Comp. no.	4ALM	2VAM	4HEJ	3G75	1HNJ	1JIJ
4a	20.3747	31.9594	32.5264	37.6327	28.1742	37.1694
4b	28.8793	36.0918	35.8462	37.2929	34.1572	38.5207
4c	32.9551	30.658	35.1048	36.8286	31.7989	37.6191
4d	30.0192	29.9581	35.9255	39.7354	34.8809	41.89
4e	29.7274	33.9133	34.9509	39.8847	34.2174	40.1967
4f	24.2476	34.4743	36.8656	40.1922	31.2649	39.5337
4g	26.9871	35.1196	37.2149	35.5737	32.8235	37.6679
4h	37.3166	29.9219	35.7176	37.5847	33.7495	38.8959
4i	36.4327	32.105	34.1819	38.6838	37.7495	38.3272
4j	31.5603	31.9174	34.251	37.8953	31.2935	31.804
4k	33.3979	34.6051	36.685	40.6257	36.1148	38.9517
4l	31.9442	37.5439	39.2061	40.5534	35.89	40.554
4m	37.0251	34.8603	36.1478	44.7868	37.5197	43.2117
4n	25.6653	34.6221	31	38.5031	30.8043	34.7123
4o	28.0438	27.3347	38.1462	37.364	35.5181	37.3882
4p	35.0272	32.3559	39.5794	38.9022	32.4042	41.6013
4q	22.5643	28.0774	35.1469	36.9761	31.8476	37.4253
4r	28.4941	34.2285	34.3075	38.8953	32.8022	39.1652
4s	25.026	27.408	32.5096	34.0752	29.8731	21.7116

(1H); 7.64–7.67 d (J = 11.4 Hz, 2H); 7.43–7.46 m (2H); 6.96–7.03 m (3H); 3.81 s (3H); 4.31 s (4H). MS: *m/z*: 312.11 $[M + H]^+$. Found, %: C 65.43; H 5.12; N 8.93. $C_{17}H_{16}N_2O_4$. Calculated, %: C 65.38; H 5.16; N 8.97.

(*E*)-2,3-Dihydro-*N*'-pentylidenebenzo[*b*][1,4]-dioxine-6-carbohydrazide (4j). mp 147–149°C. ^{1}H NMR spectrum, δ, ppm: 7.71 s (1H); 7.36–7.39 m (2H); 6.93–6.95 m (1H); 4.27–4.31 m (4H); 2.21–2.28 m (2H); 1.40–1.52 m (2H); 1.29–1.38 m (2H); 0.88–0.93 m (3H). MS: *m/z*: 262.13 $[M + H]^+$. Found, %: C 64.15; H 6.88; N 10.63. $C_{14}H_{18}N_2O_3$. Calculated, %: C 64.10; H 6.92; N 10.68.

(*E*)-*N*'-(2-Nitrobenzylidene)-2,3-dihydrobenzo[*b*]-[1,4]-6-carbohydrazide (4k). mp 221–223°C. ^{1}H NMR spectrum, δ, ppm: 12.03 s (1H); 8.85 s (1H); 8.06–8.13 m (2H); 7.70–7.70 m (1H); 7.65–7.67 m (1H); 7.47–7.50 d (J = 8.4 Hz, 2H); 6.98–7.01 d (J = 8.22 Hz, 1H); 4.31–4.32 m (4H). MS: *m/z*: 327.09 $[M + H]^+$. Found, %: C 58.76; H 3.97; N 12.81. $C_{16}H_{13}N_3O_5$. Calculated, %: C 58.77; H 4.04; N 12.80.

(*E*)-*N*'-(5-Chloro-2-hydroxybenzylidene)-2,3-dihydrobenzo[*b*][1,4]-6-carbohydrazide (4l). mp 194–196°C. ^{1}H NMR spectrum, δ, ppm: 12.04 s (1H). 8.59 s (1H); 7.64 s (1H); 7.47–7.50 d (J = 8.43 Hz, 2H); 7.28–7.32 m (1H); 6.93–7.01 m (2H); 4.31–4.31 m (5H). MS: *m/z*: 332.06 $[M + H]^+$. Found, %: C 57.79; H 3.90; Cl 10.61; N 8.48. $C_{16}H_{13}ClN_2O_4$. Calculated, %: C 57.75; H 3.94; Cl 10.65; N 8.42.

(*E*)-*N*'-(4-(Methoxymethyl)benzylidene)-2,3-dihydrobenzo[*b*][1,4]-6-carbohydrazide (4m). mp 201–204°C. ^{1}H NMR spectrum, δ, ppm: 11.55 s (1H), 8.38 s (1H); 7.64–7.67 d (J = 8.86 Hz, 2H); 7.43–7.48 m (2H); 7.08–7.12 d (J = 10.41 Hz, 2H); 6.96–6.99 d (J = 8.43 Hz, 1H); 4.31 s (4H); 3.32 s (3H). MS: *m/z*: 326.13 $[M + H]^+$. Found, %: C 66.31; H 5.52; N 8.63. $C_{18}H_{18}N_2O_4$. Calculated, %: C 66.25; H 5.58; N 8.58.

(*E*)-2,3-Dihydro-*N*'-(-3-methylbutylidene)benzo-[*b*][1,4]dioxine-6-carbohydrazide (4n). mp 148–151°C. ^{1}H NMR spectrum, δ, ppm: 11.19 s (1H). 7.65–7.66 d (J = 5.13 Hz, 1H); 7.36–7.39 m (2H); 6.92–6.95 d (J = 8.07 Hz, 1H); 4.26–4.31 m (4H); 3.32 s (1H); 2.49–2.51 m (2H); 1.01–1.07 m (6H). MS: *m/z*: 262.13 $[M + H]^+$. Found, %: C 64.16; H 6.88; N 10.63. $C_{14}H_{18}N_2O_3$. Calculated, %: C 64.10; H 6.92; N 10.68.

(*E*)-*N*'-(4-Nitrobenzylidene)-2,3-dihydrobenzo[*b*]-[1,4]-6-carbohydrazide (4o). mp 264–269°C. ^{1}H NMR spectrum, δ, ppm: 11.98 s (1H), 8.53 s (1H); 8.29–8.32 d (J = 8.79 Hz, 2H); 7.96–7.99 d (J = 8.58 Hz, 2H); 7.46–7.49 m (2H); 6.99–7.02 d (J = 8.96 Hz, 1H); 4.30–4.34 m (4H). MS: *m/z*: 327.09 $[M + H]^+$. Found, %: C 58.76; H 3.93; N 12.80. $C_{16}H_{13}N_3O_5$. Calculated, %: C 58.72; H 4.00; N 12.84.

(*E*)-2,3-Dihydro-*N*'-[(naphthalen-1-yl)methlene]-benzo[*b*][1,4]dioxine-6-carbohydrazide (4p). mp 262–265°C. ^{1}H NMR spectrum, δ, ppm: 11.77 s (1H); 9.09 s (1H); 8.84–8.87 d (J = 11.4 Hz, 1H); 8.01–8.04 d (J = 8.04 Hz, 2H); 7.91–7.93 d (J = 6.93 Hz, 1H); 7.59–7.67 m (3H); 7.50–7.53 d (J = 11.4 Hz, 2H); 7.01–7.03 d (J = 8.04 Hz, 1H); 4.33 s (4H). MS: *m/z*: 332.12 $[M + H]^+$. Found, %: C 72.33; H 4.81; N 8.46. $C_{20}H_{16}N_2O_3$. Calculated, %: C 72.28; H 4.85; N 8.43.

(*E*)-*N*'-(4-Chlorobenzylidene)-2,3-dihydrobenzo-[*b*][1,4]-6-carbohydrazide (4q). mp 234–236°C. ^{1}H NMR spectrum, δ, ppm: 11.69 s (1H); 8.43 s (1H); 7.75–7.79 m (2H); 7.43–7.47 m (2H); 7.27–7.33 m (2H); 6.97–7.00 d (J = 8.4 Hz, 1H); 4.31–4.33 m (4H). MS: *m/z*: 316.06 $[M + H]^+$. Found, %: C 60.74; H 4.08; Cl 11.14; N 8.80. $C_{16}H_{13}ClN_2O_3$. Calculated, %: C 60.67; H 4.14; Cl 11.19; N 8.84.

(*E*)-*N*'-(2-Chlorobenzylidene)-2,3-dihydrobenzo-[*b*][1,4]-6-carbohydrazide (4r). mp 185–186°C. ^{1}H NMR spectrum, δ, ppm: 11.91 s (1H); 8.85 s (1H); 8.01 s (1H); 7.43–7.55 m (5H); 6.98–7.01 d (J = 8.22 Hz, 1H); 4.31–4.33 m (4H). MS: *m/z*: 316.06 $[M + H]^+$. Found, %: C 60.72; H 4.09; Cl 11.15; N 8.87. $C_{16}H_{13}ClN_2O_3$. Calculated, %: C 60.67; H 4.14; Cl 11.19; N 8.84.

(*E*)-2,3-Dihydro-*N*'-[(thiophen-2-yl)methylene]-benzo[*b*][1,4]dioxine-6-carbohydrazide (4s). mp 183–187°C. ^{1}H NMR spectrum, δ, ppm: 11.63 s (1H); 8.65 s (1H); 7.65–7.67 d (J = 5.02 Hz, 1H); 7.41–7.45 m (3H); 7.12–7.15 m (1H); 6.96–6.99 d (J = 8.04 Hz, 1H); 4.30 s (4H). MS: *m/z*: 288.06 $[M + H]^+$. Found (%): C 58.38; H 4.14; N 9.76. $C_{14}H_{12}N_2O_3S$. Calculated, %: C 58.32; H 4.20; N 9.72.

X-Ray crystallography. Single crystal X-ray diffraction data were collected on a Bruker D-8 venture diffractometer at 293 K. The X-ray generator was operated at 50 KV and 35 mA using MoK_α radiation (λ = 0.71,073 Å). The data were collected using SMART software package and reduced by SAINT-PLUS. The packages SADABS and XPREP were used for empirical absorption correction and determination of the space group. The crystal structure was resolved by direct methods using SIR92 and refined by full-matrix least-squares method using SHELXL97 [11, 12]. All non-hydrogen atoms were refined

anisotropically and hydrogen atoms have been refined in the riding mode on their carrier atoms wherever applicable.

Antibacterial assay. Antibacterial activity of the synthesized compounds was tested against *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa,* and *Staphylococcus aureus* using LB medium (Luria-Bertani medium: yeast extract 5.0 g, peptone 10.0 g, sodium chloride 5.0 g, distilled water 1000 mL). Minimum inhibitory concentration of the tested compounds was determined by the colorimetric method using the dye MTT. Activity of the reference compound penicillin was determined under identical conditions. A stock solution of synthesized compounds (1000 μmol/mL) in DMSO were prepared, and graded quantities of the test compounds were incorporated in the specified quantity of sterilized liquid LB medium. Drug stocks were formulated in DMSO and then the compounds were diluted in media to final working concentrations of 25, 12.5, 6.25, 3.125, 1.5625 μg/mL. A specified amount of the medium containing the compound was added to a 96-well plate. Then, a bacterial suspension containing the concentration of ca 10^5 cfu/mL was added to a 96-well plate and incubated at 37°C for 24 h. Afterwards, PBS (10 μL) containing MTT (4 mg/mL) was added to each well. Incubation was continued at 37°C for 4 h. The content of each well was removed, and 150 μL of DMSO was added to extract the dye. The optical density was measured with an ELISA plate reader at 492 nm.

Molecular docking study. Automated docking studies were carried out using Discovery Studio (version 2.5) as implemented through the graphical user interface DS-CDocker protocal [13]. Three-dimensional structures of the studied compounds were constructed using Chem. 3D ultra 11.0 software [Chemical Structure Drawing Standard; Cambridge Soft corporation, USA (2009)]. The crystal structure complexes of FabI (PDB code: 4ALM), β-Lactamase (PDB code: 3SIY), Dihydrofolate reductas (PDB code: 3FY8), Glutamate recemase (MurI) (PDB code: 2VVT), Methionyl-tRNA synthetase (1PFY), Bacterial thymidylate kinase (PDB code: 4HEJ), Bacterial DNA gyrase (PDB code: 3G75), the ATP binding site of FabH (PDB code:1HNJ), FtsZ (PDB code: 2VAM), S. aureus Tyrosyl-tRNA synthetase (PDB code: 1JIJ), and E.coil Tyrosyl-tRNA synthetase (PDB code: 1VBM) were retrieved from the RCSB Protein Data Bank (http://www.rcsb.org/pdb/home/home.do). The bound water and ligands were eliminated from the protein, and the polar hydrogens and the Kollman-united charges were added to the proteins.

The molecular docking procedure was performed by using CDocker protocol for receptor–ligand interactions section of Discovery Studio 4.5 (Accelrys Software Inc, San Diego, CA). Initially both a ligand and a receptor were pretreated. For ligand preparation, the 3D structures of all synthesized compounds were generated with ChemBioOffice 2010 and optimized with MMFF94 method. For enzyme preparation, hydrogen atoms were added, pH of the protein was in the range of 7.25–7.65.

The binding poses of active compounds were selected through CDocker interaction energy. Docking algorithm utilized: CDocker algorithm; all protein SDB Radius: 10; scoring function: CDOC-KER interaction energy; rigid receptor: PDB code 4ALM, 3SIY, 3FY8, 2VVT, 1PFY, 4HEJ, 3G75, 1HNJ, 2VAM, 1JIJ, 1VBM; flexible ligand docking: YES; cluster analysis of docking poses: ten optimal poses were retained.

CONCLUSIONS

New 1,4-benzodioxane derivatives containing Schiff base are synthesized and tested for their antimicrobial activity. The preliminary results indicate that most of the compounds demonstrate higher antibacterial activity against gram-negative than gram-positive bacteria strains. Molecular docking with eleven common antibacterial activity targets and the sites of action of the target compounds are screened. These indicate that the target compounds have the strongest binding ability to Tyrosine-tRNA synthetase. The binding energy of compounds **4d** and compound **4m** to *E. coli* Tyrosine-tRNA synthetase is the highest. According to the docking simulation, the target compounds can be completely embedded in the active site with the phenyl ring located inside the active pocket, and 1,4-benzodioxane is located at the opening of the active pocket.

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CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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