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Original article

Design, synthesis, antimicrobial evaluation and molecular docking studies of some new thiophene, pyrazole and pyridone derivatives bearing sulfisoxazole moiety

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ABSTRACT

Development of new antimicrobial agents is a good solution to overcome drug-resistance problems. In this context, new functionalized thiophene, acrylamide, arylhydrazone, pyrazole and pyridone derivatives bearing sulfisoxazole moiety were designed, synthesized and evaluated for their *in vitro* antibacterial and antifungal activities. Among the synthesized compounds, thiophene **4d** and 6-thioglucosylpyridone **17** displayed significant antibacterial activities against *Escherichia coli* (MIC, 0.007 μg/mL *vs* gentamycin 1.95 μg/mL) and *Bacillis subtilis* (MIC, 0.007 μg/mL *vs* ampicillin 0.24 μg/mL), respectively. Whereas, the pyrazole **6** showed the highest antifungal activity against *Aspergillus fumigates* (MIC, 0.03 μg/mL *vs* amphotericin B 0.12 μg/mL). In general, most of the synthesized compounds exhibited better antimicrobial activities than sulfisoxazole; this might be attributed to the synergistic effect of the sulfonamide and attached heterocyclic moieties as well as the increased lipophilic characters of the synthesized compounds. Molecular docking studies indicated that the synthesized compounds could occupy both *p*-amino benzoic acid (PABA) and pterin binding pockets of the dihydropteroate synthase (DHPS), suggesting that the target compounds could act by the inhibition of microbial DHPS enzyme. The results provide important information for the future design of more potent antimicrobial agents.

1. Introduction

Many drug-resistant human pathogenic microbes have been observed in the past few decades [1]; the reasons are the misuse and widespread use of antimicrobial agents as well as inaccurate diagnosis [2]. Among these drug-resistant microbes are methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococci* and azole-resistant *Candida* species [3]. Treatment of these infections is a major impediment especially in immunocompromised patients [4]; to overcome this problem we need to search for new powerful antimicrobial agents [5]. The discovery of completely new antimicrobial pharmacophore and modifying the structure of a well known antimicrobial agent are the main two strategies to accomplish this [6]. In the second strategy; two or more different antimicrobial pharmacophores are often combined together in one molecule to get powerful synergistic effect [7].

Sulfonamides have diverse biological activities including antibacterial [8], carbonic anhydrase inhibitor [9], insulin release inducer [10], antiviral [11], antifungal [12], anticancer [13], and anti-inflammatory activities [14]. The antimicrobial sulfonamides act as competitive inhibitors to PABA substrate for the DHPS enzyme active site and thus inhibit the biosynthesis of dihydrofolic acid [15]. DHPS facilitate the biosynthesis of the folate intermediate, 7,8-dihydropteroic acid, from PABA and dihydropterin-6-hydroxymethyl pyrophosphate (DHPP). Despite the relative abundance of DHPS crystal structures in the Protein Data Bank (PDB), only two structures to-date were solved with a sulfa-related drug in the active site of the enzyme. Yun et al., reported the crystal structure of *Bacillus anthracis* dihydropteroate synthase (BaDHPS) bound to sulfathiazole-6-hydroxymethyl-7,8-dihydropterin-pyrophosphate (STZ-DHPP) adduct. They showed that STZ-DHPP adduct occupies both the PABA and pterin binding pockets of HDPS (Fig. I,

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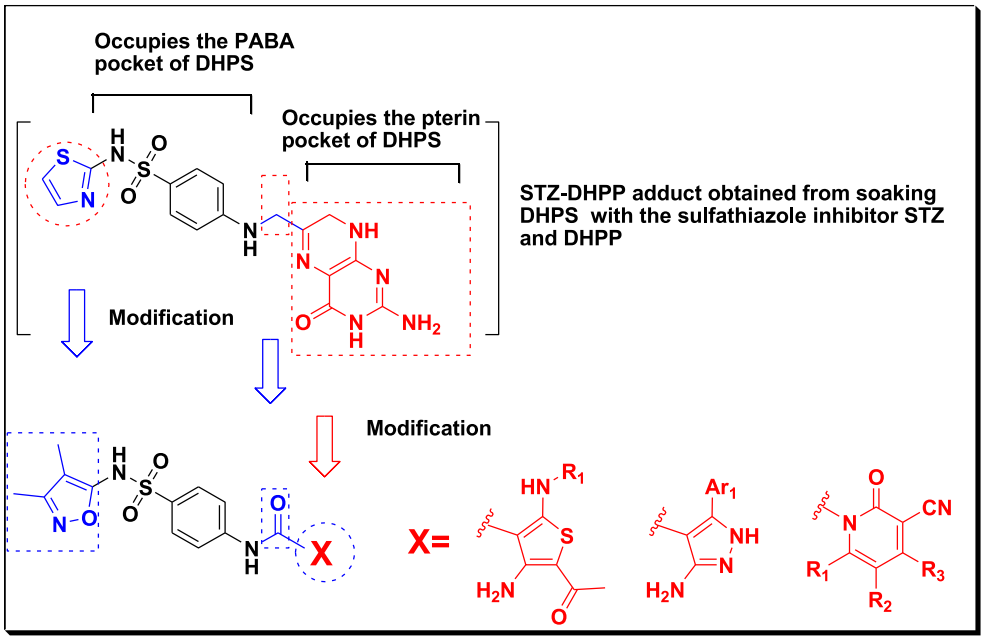


Fig. 1. Design of antimicrobial DHPS inhibitors.

supplementary data) [16]. The Co-administration of sulfonamides and the dihydrofolate reductase inhibitor, trimethoprim, increases the therapeutic efficacy [17].

Literature survey revealed that thiophene, acrylamide, arylhydrazone, pyrazole and 2-pyridone derivatives are important scaffolds in pharmacologically active compounds. Regarding thiophene ligands, their metal complexes possess antimicrobial activities [18]. For cyanothiophene scaffolds, they act as MurF ligase inhibitor which prevents the bacterial peptidoglycan biosynthesis [19]. Interestingly; several cyanoacrylamide [20], arylhydrazone [21], pyrazole [22], and 2-pyridone derivatives exhibit promising antimicrobial activities [23].

Sulfisoxazole is an available sulfa drug acting as PABA competitive inhibitor. Here we aimed to substitute its primary amino group by flexible antimicrobial pharmacophores to occupy both the PABA and pterin binding pockets for the DHPS enzyme (Fig. 1) to get new potent antimicrobial agents.

Based on the above considerations and as extension of our search for effective antimicrobial agents, we designed and synthesized new thiophene, acrylamide, arylhydrazone, pyrazole and pyridone compounds tagged with sulfisoxazole moiety. The synthesized compounds were evaluated *in vitro* for their antimicrobial activities against human pathogenic microbes. Molecular docking and lipophilicity studies were used to explain the obtained biological data.

2. Results and discussion

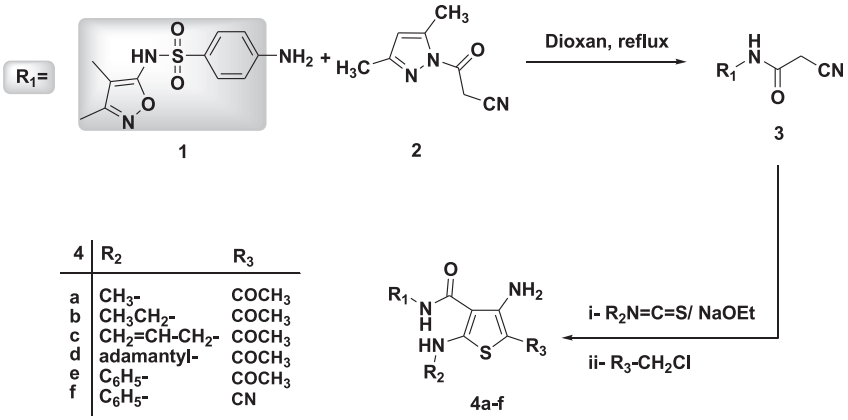
2.1. Chemistry

The synthetic strategies adopted for constructing the target molecules are illustrated in Schemes 1–3. The starting material, 2-cyano-*N*-(4-{[(3,4-dimethylisoxazole-5-yl)amino]sulfonyl}-phenyl)acetamide (**3**), was prepared in a quantitative yield by cyanoacetylation of sulfisoxazole **1** with 1-cyanoacetyl-3,5-dimethylpyrazole (**2**) in refluxing dry dioxan (Scheme 1). The reactivity of a CH_2 group of compound **3**, with various types of substituted isothiocyanates and each of chloroacetone and chloroacetonitrile, was evaluated. Thus, treatment of an ethanolic sodium ethoxide solution of compound **3** with a series of substituted isothiocyanates, namely, methyl-, ethyl-, allyl-, adamantly- and phenyl-isothiocyanates, at room temperature followed by addition of chloroacetone or chloroacetonitrile afforded, in each case, a single product, in moderate yields (55–65%). Elemental analyses and spectral data (IR, MS, ^{1}H and ^{13}C NMR spectra) confirmed the reaction products as 4-aminothiophene-3-carboxamides **4a**–**f**. The one-pot formation of the functionalized thiophenes **4a**–**f** starting from activated nitrile, isothiocyanates, and haloalkanes is in the line of the work reported earlier by Gewald et al. [24].

α,β-Unsaturated nitriles are versatile intermediates and were utilized for the synthesis of functionalized aminopyrazoles. In this context, the Knoevenagel condensation of compound **3** with aromatic and heteroaromatic aldehydes was investigated. Thus, treatment of **3** with various types of aldehydes in refluxing ethanol containing a catalytic amount of piperidine produced, in each case, a single stereoisomer, identified as (*E*)-2-cyano-3-aryl-acrylamides **5a**–**e** (Scheme 2). The *E*-configuration of compound **5a**, as representative example, was assigned based on its ^{1}H NMR spectrum which displayed a downfield singlet signal at δ 8.34 ppm due to the olefinic (CH=) proton that agrees with the chemical shift of the olefinic proton (8.36 ppm) of similar structure, (*E*)-*N*-(pyridin-2-yl)-2-cyano-3-phenylprop-2-enamide, confirmed by X-ray analysis [25].

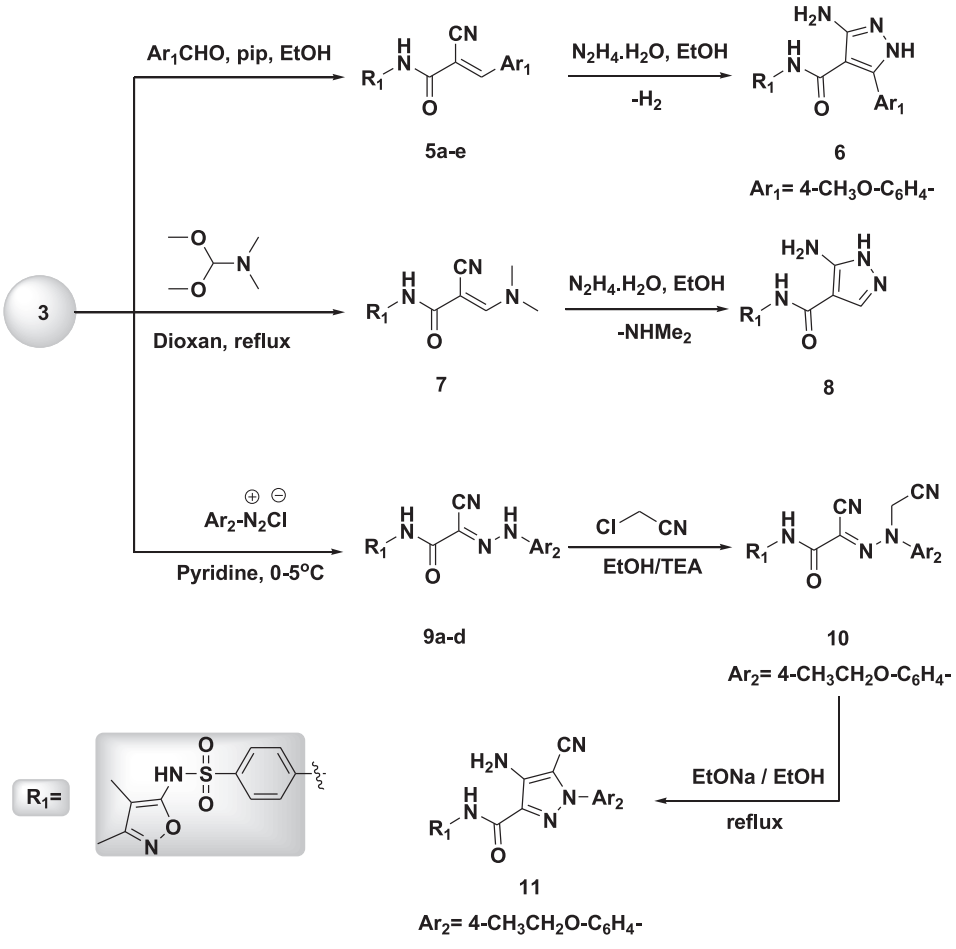
Treatment of (*E*)-2-cyano-3-(4-methoxyphenyl)acrylamide **5b** with hydrazine hydrate in ethanol, under reflux, furnished the functionalized pyrazole derivative **6**. Formation of pyrazole **6** is believed to proceed through the Michael addition of hydrazine hydrate to α,β-unsaturated nitrile **5b** and *in situ* intramolecular 1,5-dipolar cyclization *via* the addition of amino group to a cyano function to give the non-isolable dihydropyrazole which underwent auto-oxidation to give the target pyrazole. The pyrazole formation is in the line with our previous report [26].

Treatment of **3** with dimethylformamide-dimethylacetal (DMF-DMA), in dry dioxan, at reflux temperature, afforded a yellow product identified as (*E*)-2-cyano-3-(*N*,*N*-dimethylamino)



4	R_2	R_3
a	CH_3-	$COCH_3$
b	CH_3CH_2-	$COCH_3$
c	CH_2=CH-CH_2-	$COCH_3$
d	adamantyl-	$COCH_3$
e	C_6H_5-	$COCH_3$
f	C_6H_5-	CN

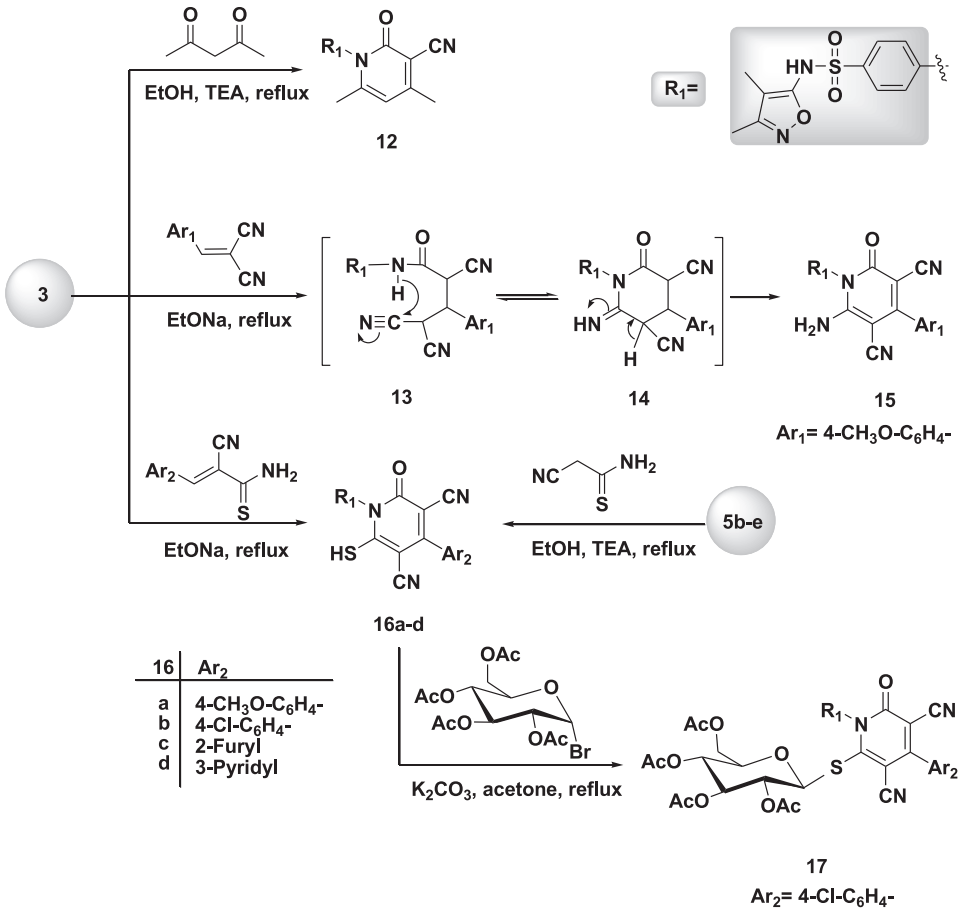
Scheme 1. Synthetic route to functionalized thiophenes tagged with sulfisoxazole.



5	Ar_1
a	C_6H_5-
b	4-CH_3O-C_6H_4-
c	4-Cl-C_6H_4-
d	2-furyl
e	3-pyridyl

9	Ar_2
a	C_6H_5-
b	4-CH_3-C_6H_4-
c	4-CH_3O-C_6H_4-
d	4-CH_3CH_2O-C_6H_4-

Scheme 2. Synthetic route to functionalized pyrazoles bearing sulfisoxazole moiety.



Scheme 3. Synthesis of functionalized pyridones bearing sulfisoxazole moiety.

acrylamide **7**. The *E*-configuration of enaminonitrile **7** was confirmed according to the chemical shift of a methine proton of similar *E*-enaminonitriles reported earlier by Yogavel et al. [27].

In addition, the reactivity of enaminonitrile **7** with nitrogen binucleophile was investigated. Thus, when enaminonitrile **7** was treated with hydrazine hydrate in refluxing ethanol, it afforded a single product identified as 5-aminopyrazole **8**. The IR spectrum of compound **8** was free of absorption band corresponding to a nitrile function and showed bands corresponding to amino group, in addition to a strong absorption band corresponding to a conjugated carbonyl group. Its mass spectrum revealed a molecular ion peak at *m*/*z* 376 (M^+) corresponding to a molecular formula $C_{15}H_{16}N_6O_4S$. In addition, inspection of ^{1}H NMR spectrum enabled establishing structure **8** for this pyrazole derivative since the pyrazole H-3 appeared as a singlet at δ 8.23 ppm. We could not trace in the ^{1}H NMR spectrum any signals for the tautomeric 3-aminopyrazole as this could reveal pyrazole-H5 as a doublet signal. Formation of compound **8** is assumed to take place *via* a Michael type addition of the amino group of the hydrazine to the activated double bond in compound **7** to form the non-isolable intermediate which readily undergoes intramolecular cyclization followed by loss of dimethylamine molecule to form the target compound (Scheme 2).

The behavior of compound **3** towards diazotized aromatic amines as potential precursors for 4-aminopyrazoles was also investigated. Thus, coupling of compound **3** with a series of diazotized aromatic amines in pyridine furnished the arylhydrazones **9a**–**d** in excellent yields. The formation of arylhydrazones rather than arylazo derivatives (CH-azo) was confirmed by IR spectra. In addition, their ^{1}H NMR spectra were free of a singlet peak at δ 5.72 ppm characteristic for the CH-proton of the CH-azo compounds [28]. It is worth mentioning that the arylhydrazones **9a**–**d** can exist in, two geometric structures, *E*- and *Z*-configuration. The preference formation of *E*-configuration was elucidated from their ^{1}H NMR spectra which displayed the hydrazone proton (C═N–NH) signal at δ = 11.90–12.05 ppm while the chemical shift of the hydrazone proton of *Z*-configuration has been reported to resonate more downfield at δ = 13.83–13.92 ppm [29].

Alkylation of hydrazonyl nitrile **9d** with chloroacetonitrile in boiling ethanol containing a catalytic amount of triethylamine afforded a single product identified as 2-cyano-2-[(cyanomethyl)(4-ethoxyphenyl)hydrazono]-N-(4-{[(3,4-dimethylisoxazol-5-yl) am-ino]sulfonyl}phenyl)acetamide (**10**). The base catalyzed Thorpe-Ziegler cyclization of compound **10** led to the formation of 4-amino-3-cyano-1*H*-pyrazole-3-carboxamide derivative **11**.

It is well known that the reaction of *N*-hetaryl-2-cyanoacetamides with either 1,3-dicarbonyl compounds or α,β-unsaturated nitriles represent a facile and efficient synthetic route to attain functionalized pyridone derivatives [25,30]. In this context, we investigated the reaction of compound **3** with each of acetylacetone, arylidene malononitriles and arylidene cyanothioacetamides (Scheme 3). Thus, reaction of equimolar amount of the cyanoacetamide **3** and acetylacetone in boiling ethanol containing a catalytic amount of piperidine afforded 2-pyridone **12**. On the other hand, treatment of **3** with, Michael acceptor, 2-cyano-3-(4-methoxyphenyl)acrylonitrile, in ethanolic sodium ethoxide solution, under reflux, furnished the functionalized pyridone derivative **15**. Compound **15** was assumed to be formed *via* an initial Michael-

type adduct **13** followed by an intramolecular cyclization to the final product **15** (Scheme 3).

In a similar manner, compound **3** was reacted with a series of arylidene cyanothioacetamides under the same experimental condition to give the functionalized pyridonethiol derivatives **16a**–**d**. Elemental analysis and spectral data (IR, MS, ^{1}H, and ^{13}C NMR) confirmed the structure of the latter products. Additionally, the elucidation of structures **16a**–**d** was supported chemically through its alternative synthesis from the reaction of acrylamides **5a**–**d** with cyanothioacetamide in refluxing ethanol and in the presence of a catalytic amount of triethylamine.

Finally, coupling of the pyridonethiol **16b** with, activated sugar, 2′,3′,4′,6′-tetra-*O*-acetyl-α-*D*-glucopyranosyl bromide in refluxing acetone containing a catalytic amount of anhydrous potassium carbonate afforded the respective thioglycoside **17** in a moderate yield (66%). The structure of thioglycoside **17** was established on the basis of its elemental analysis and spectral data. The ^{1}H NMR spectrum of **17** showed the anomeric proton as a doublet at δ 6.10 ppm with a coupling constant ($J_{1',2'} = 9.8$ Hz) corresponding to a *trans* orientation of H-1′ and H-2′ protons indicating the β-configuration (Scheme 3).

2.2. Antimicrobial evaluation

The antimicrobial screening and minimal inhibitory concentrations of the tested compounds were carried out at the Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt. Twenty two of the newly synthesized target compounds were evaluated for their *in vitro* antimicrobial activities against the human pathogens *Streptococcus pneumoniae* (RCMB 010010), *Bacillis subtilis* (RCMB 010067) and *Staphylococcus epidermidis* (RCMB 010024) as examples of Gram-positive bacteria and *Escherichia coli* (RCMB 010052), *Proteous vulgaris* (RCMB 010085) and *Klebsiella pneumonia* (RCMB 010093) as examples of Gram-negative bacteria. They were also evaluated for their potential antifungal activities against the following fungal strains; *Aspergillus fumigatus* (RCMB 02568), *Syncephalastrum racemosum* (RCMB 05922) and *Geotricum candidum* (RCMB 05097). Agar-diffusion method was used for the preliminary screening of the antibacterial and antifungal activities. Ampicillin, gentamycin, amphotericin B and sulfisoxazole were used as reference drugs. The results were recorded for each tested compound as the average diameter of inhibition zones (IZ) of microbial growth around the disks in mm and were attributed to the original tested concentration (5 mg/mL) as a preliminary test. The minimum inhibitory concentration (MIC) measurements were determined for compounds showed significant growth inhibition zones (>10 mm) using twofold serial dilution method [31].

2.2.1. Antibacterial activity

Preliminary antibacterial screening was carried out for most of the newly synthesized compounds and the results were summarized in Table 1.

Some of the thiophene derivatives **4a**–**f** (Scheme 1) displayed significant antibacterial activities. It was observed that the 2-allylamino- and 2-adamantylamino-thiophenes **4c** and **4d** were the most potent among their series; they showed one to three folds the activity of sulfisoxazole against both Gram-positive and Gram-negative bacteria. Interestingly, the thiophene **4d** was more potent than gentamycin against *E. coli* (MIC, 0.007 μg/mL *vs* 1.95 μg/mL) and equipotent to ampicillin against *B. subtilis* (MIC, 0.24 μg/mL) (Table 1). Moreover, it was active against *P. vulgaris* taking advantage over sulfisoxazole. On the other hand, the thiophene **4c** was one fold more potent than ampicillin against *B. subtilis* (MIC, 0.12 μg/mL *vs* 0.24 μg/mL).

Regarding the antibacterial activities of acrylamides, the 4-chlorophenyl- and 3-pyridyl-derivatives **5c**, **5e** were the most active among this series **5a**–**e** (Scheme 2), their MIC values were (0.03–0.98 μg/mL) against Gram-positive bacteria and (0.06–3.9 μg/mL) against Gram-negative bacteria (Table 1). Acrylamides **5c** and **5e** were eight folds more potent than ampicillin

Table 1
Antibacterial inhibition zone in mm ± standard deviation and minimal inhibitory concentrations (MIC, μg/ml, between brackets) of some new synthesized compounds.

Compounds	Gram-positive bacteria			Gram-negative bacteria		
	S. pneumoniae	*B. subtilis*	*S. epidermidis*	*E. coli*	*P. vulgaris*	*K. pneumonia*
4a	16.6 ± 0.38 (16.63)	19.1 ± 0.41 (3.90)	18.7 ± 0.68 (7.81)	17.1 ± 0.37 (15.63)	18.2 ± 0.50 (3.90)	16.9 ± 0.31 (15.63)
4c	20.6 ± 0.34 (0.98)	23.7 ± 0.25 (0.12)	19.3 ± 0.63 (3.90)	18.3 ± 0.58 (7.81)	–[a]	18.9 ± 0.63 (3.90)
4d	21.6 ± 0.28 (0.98)	22.9 ± 0.24 (0.24)	20.9 ± 0.39 (0.98)	29.9 ± 0.51 (0.007)	17.4 ± 0.45 (15.6)	20.3 ± 0.34 (0.98)
4f	15.2 ± 0.44 (62.5)	17.4 ± 0.25 (15.63)	14.2 ± 0.63 (125)	11.2 ± 0.33 (500)	–	13.6 ± 0.63 (125)
5a	18.4 ± 0.29 (7.81)	20.3 ± 0.41 (1.95)	19.1 ± 0.56 (3.90)	20.6 ± 0.37 (1.95)	18.0 ± 0.48 (7.81)	21.9 ± 0.30 (0.49)
5c	20.3 ± 0.44 (0.98)	25.6 ± 0.63 (0.03)	22.3 ± 0.63 (0.49)	22.1 ± 0.25 (0.49)	19.2 ± 0.63 (3.90)	23.9 ± 0.61 (0.06)
5d	16.9 ± 0.44 (15.63)	17.4 ± 0.25 (15.63)	16.2 ± 0.53 (31.25)	14.9 ± 0.44 (0.12)	–	15.0 ± 0.58 (0.12)
5e	24.6 ± 0.43 (0.06)	26.2 ± 0.58 (0.03)	22.3 ± 0.53 (0.49)	23.7 ± 0.63 (0.12)	19.3 ± 0.58 (3.9)	24.2 ± 0.72 (0.12)
6	22.0 ± 0.33 (0.49)	20.4 ± 0.36 (1.95)	18.9 ± 0.46 (3.90)	17.8 ± 0.36 (7.81)	19.6 ± 0.50 (1.95)	17.1 ± 0.58 (15.63)
7	20.0 ± 0.43 (1.95)	21.4 ± 0.72 (0.98)	18.2 ± 0.58 (7.81)	16.3 ± 0.33 (31.25)	–	17.1 ± 0.58 (15.63)
8	17.8 ± 0.44 (7.81)	18.0 ± 0.67 (7.81)	16.1 ± 0.32 (31.25)	13.0 ± 0.46 (125)	11.0 ± 0.46 (500)	14.6 ± 0.46 (125)
9a	15.5 ± 0.37 (62.5)	18.4 ± 0.53 (7.81)	19.8 ± 0.58 (1.95)	13.5 ± 0.43 (125)	–	19.1 ± 0.44 (3.90)
9c	17.3 ± 0.63 (15.63)	21.2 ± 0.44 (0.98)	20.2 ± 0.44 (1.95)	15.9 ± 0.37 (31.25)	–	20.2 ± 0.58 (1.95)
9d	19.6 ± 0.63 (1.95)	20.0 ± 0.30 (1.95)	17.6 ± 0.53 (7.81)	15.9 ± 0.46 (31.25)	–	16.3 ± 0.58 (31.25)
11	20.0 ± 0.43 (1.95)	19.6 ± 0.53 (1.95)	18.9 ± 0.58 (3.90)	16.4 ± 0.58 (31.25)	18.2 ± 0.44 (7.81)	25.1 ± 0.44 (0.06)
12	23.8 ± 0.44 (0.12)	25.3 ± 0.58 (0.06)	21.4 ± 0.53 (0.98)	20.9 ± 0.72 (0.98)	19.5 ± 0.53 (1.85)	21.4 ± 0.63 (0.98)
15	20.3 ± 0.44 (0.98)	22.2 ± 0.67 (0.49)	18.1 ± 0.53 (7.81)	16.2 ± 0.46 (31.25)	17.2 ± 0.58 (15.63)	22.3 ± 0.53 (0.49)
16a	20.6 ± 0.63 (0.98)	22.4 ± 0.44 (0.98)	19.9 ± 0.53 (1.95)	20.6 ± 0.37 (0.98)	18.6 ± 0.44 (3.90)	21.4 ± 0.58 (0.98)
16b	22.3 ± 0.43 (0.49)	24.4 ± 0.53 (0.12)	20.2 ± 0.53 (0.49)	20.6 ± 0.25 (0.98)	19.3 ± 0.53 (3.90)	23.2 ± 0.44 (0.24)
16c	22.6 ± 0.63 (0.24)	23.6 ± 0.67 (0.12)	21.4 ± 0.32 (0.98)	21.6 ± 0.46 (0.98)	18.3 ± 0.46 (7.81)	22.6 ± 0.46 (0.24)
16d	21.3 ± 0.63 (0.98)	22.6 ± 0.58 (0.24)	20.0 ± 0.63 (1.95)	19.2 ± 0.58 (3.90)	17.6 ± 0.72 (7.81)	21.0 ± 0.58 (0.98)
17	25.1 ± 0.44 (0.06)	29.8 ± 0.58 (0.007)	24.2 ± 0.58 (0.12)	23.2 ± 0.19 (0.24)	21.2 ± 0.58 (0.98)	25.8 ± 0.72 (0.03)
Sulfisoxazole	19.6 ± 0.44 (1.95)	20.8 ± 0.63 (0.98)	17.5 ± 0.72 (7.81)	15.2 ± 0.25 (62.5)	–	18.2 ± 0.58 (7.81)
Ampicillin	23.8 ± 0.2 (0.12)	32.4 ± 0.3 (0.24)	25.4 ± 0.18 (0.06)	NT[b]	NT	NT
Gentamycin	NT	NT	NT	19.9 ± 0.3 (1.95)	23.4 ± 0.3 (0.24)	26.3 ± 0.15 (0.03)

[a] No activity.
[b] NT, Not tested.

Table 2
Antifungal inhibition zone in mm ± standard deviation and minimal inhibitory concentrations (MIC, μg/ml, between brackets) of some new synthesized compounds.

Compounds	Fungi			Compounds	Fungi		
	A. fumigatus	*S. racemosum*	*G. candidum*		*A. fumigatus*	*S. racemosum*	*G. candidum*
4a	15.8 ± 0.33 (62.5)	14.1 ± 0.48 (62.5)	16.4 ± 0.26 (31.25)	**9a**	16.5 ± 0.36 (15.63)	17.3 ± 0.53 (15.63)	17.8 ± 0.38 (7.81)
4c	20.6 ± 0.63 (0.98)	21.1 ± 0.27 (0.98)	21.9 ± 0.35 (0.49)	**9c**	16.3 ± 0.44 (31.25)	18.6 ± 0.58 (3.90)	19.8 ± 0.25 (1.95)
4d	25.3 ± 0.28 (0.06)	26.9 ± 0.24 (0.007)	24.6 ± 0.39 (0.12)	**9d**	19.3 ± 0.44 (3.90)	18.6 ± 0.36 (3.90)	21.4 ± 0.58 (0.98)
4f	16.3 ± 0.25 (31.25)	15.2 ± 0.58 (62.5)	17.3 ± 0.17 (15.63)	**11**	23.0 ± 0.34 (0.24)	24.6 ± 0.52 (0.06)	21.9 ± 0.53 (0.49)
5a	17.1 ± 0.53 (15.63)	18.8 ± 0.42 (7.81)	20.9 ± 0.31 (0.98)	**12**	22.9 ± 0.44 (0.24)	20.3 ± 0.58 (1.95)	23.1 ± 0.37 (0.24)
5c	20.3 ± 0.39 (0.98)	21.6 ± 0.16 (0.98)	24.6 ± 0.58 (0.12)	**15**	20.6 ± 0.25 (0.98)	19.7 ± 0.34 (1.95)	20.9 ± 0.58 (0.98)
5d	13.9 ± 0.29 (125)	12.1 ± 0.18 (500)	14.6 ± 0.35 (62.5)	**16a**	20.4 ± 0.34 (0.98)	16.3 ± 0.52 (31.25)	22.4 ± 0.31 (0.49)
5e	23.4 ± 0.24 (0.49)	18.7 ± 0.62 (3.90)	25.6 ± 0.16 (0.03)	**16b**	21.6 ± 0.58 (0.98)	20.2 ± 0.25 (1.95)	22.8 ± 0.38 (0.24)
6	26.1 ± 0.29 (0.03)	24.4 ± 0.34 (0.12)	13.6 ± 0.46 (125)	**16c**	22.0 ± 0.36 (0.49)	20.6 ± 0.44 (0.98)	24.3 0.58 (0.12)
7	18.3 ± 0.34 (7.81)	21.1 ± 0.25 (0.98)	21.3 ± 0.38 (0.98)	**16d**	21.2 ± 0.58 (0.98)	19.2 ± 0.63 (3.90)	22.0 ± 0.37 (0.49)
8	18.6 ± 0.25 (7.81)	17.2 ± 0.34 (15.63)	18.9 ± 0.58 (7.81)	**17**	22.3 ± 0.25 (0.49)	20.3 ± 0.25 (3.90)	25.8 ± 0.58 (0.03)
Amphotericin B	23.7 ± 0.1 (0.12)	19.7 ± 0.2 (7.81)	28.7 ± 0.2 (0.007)	**Sulfisoxazole**	15.1 ± 0.39 (62.5)	13.2 ± 0.58 (125)	16.8 ± 0.58 (15.63)

against the growth of *B. subtilis* (MIC, 0.03 μg/mL *vs* 0.24 μg/mL). Moreover, **5e** displayed doubled potency of ampicillin against *S. pyogenes* (MIC, 0.06 μg/mL *vs* 0.12 μg/mL). Interestingly, the acrylamides **5c**–**5e** exhibited superior activities than gentamycin against *E. coli* (MIC, 0.49 μg/mL, 0.12 μg/mL and 0.12 μg/mL *vs* 1.95 μg/mL; respectively). While, acrylamide **5a** was equipotent to gentamycin against *E. coli*. Conversion of sulfisoxazole **1** to acrylamides **5a**, **5c** and **5e** enhanced the antibacterial activity against *P. vulgaris*. The *N,N*-dimethyl-aminoacrylamide **7** was almost equipotent to sulfisoxazole and less potent than ampicillin and gentamycin. In general, the acrylamides **5a**–**e** were more potent than the thiophenes **4a**–**f** against all tested bacteria.

In some cases, the arylhydrazone derivatives **9a**, **9c** and **9d** (Scheme 2) displayed better activities than sulfisoxazole and were less potent than ampicillin and gentamycin.

The pyrazole **6** (Scheme 2) displayed better antibacterial activities than sulfisoxasole against most of the tested bacteria. Similarly, the pyrazole **11** was more potent than its corresponding precursor **9d** against the *S. epidermidis*, *P. vulgaris* and *K. pneumonia*. On the other hand, transformation of the acrylamide **7** to pyrazole **8** reduced the antibacterial activities.

It was noticed that the 2-pyridones **12**–**17** (Scheme 3) were more potent than sulfisoxazole against all screened bacteria (Table 1). The 4,6-dimethylpyridone **12** was four folds stronger than ampicillin against *B. subtilis* (MIC, 0.06 μg/mL *vs* 0.24 μg/mL) and equipotent to ampicillin against *S. pneumoniae* (Table 1). Moreover, it showed double the potency of gentamycin against *E. coli*. Interestingly, the pyridonethiols **16b** and **16c** showed double potency of ampicillin in inhibiting the growth of *B. subtilis* (MIC, 0.12 μg/mL *vs* 0.24 μg/mL) while compound **16d** was equipotent to ampicillin against the same bacteria. On the other hand, the pyridonethiols **16a**–**c** displayed double activity of gentamycin against *E. coli*. The results displayed in Table 1 revealed that the pyridones **12** and **16a**–**d** had superior activity to the aminopyridone **15** against most screened bacteria; and only pyridonethiols **16a** and **16d** were less potent than the aminopyridone **15** against *K. pneumonia*. Transformation of pyridonethiols **16d** to thioglucoside **17** increased its antibacterial activities to a great extent. The thioglucoside **17** showed excellent activity against *B. subtilis* (MIC, 0.007 μg/mL *vs* 0.24 μg/mL for ampicillin) and double the potency of ampicillin against *S. pneumoniae* (MIC, 0.06 μg/mL *vs* 0.12 μg/mL). Additionally, the thioglucoside **17** showed eight folds the activity of gentamycin against *E. coli* (MIC, 0.24 μg/mL *vs* 1.95 μg/mL) and was equipotent to gentamycin against *K. pneumonia*.

2.2.2. Antifungal activity

Sulfisoxazole showed weaker antifungal activities than amphotericin B (Table 2) and some of the target compounds showed promising antifungal activities.

The 2-adamantylaminothiophene **4d** (Scheme 1) showed more potent antifungal activities than the 2-allylaminothiophene **4c**. The former was extremely stronger than amphotericin B against *S. racemosum* (MIC, 0.007 μg/mL *vs* 7.81 μg/mL) and displayed double the potency of amphotericin B against *A. fumigatus* (MIC, 0.06 μg/mL *vs* 0.12 μg/mL).

The acrylamides **5a**–**e** (Scheme 2) showed varied antifungal activities. Acrylamide **5c** was the most potent derivative; it had eight folds the activity of amphotericin B against *S. racemosum* (MIC, 0.98 μg/mL *vs* 7.81 μg/mL). While, the hetaryl acrylamides **5d** and **5e** showed moderate to weak antifungal activities. *N,N*-Dimethylaminoacrylamide **7** exhibited eight folds the activity of amphotericin B against *S. racemosum* (MIC, 0.98 μg/mL *vs* 7.81 μg/mL); However, it was less potent than amphotericin B against *A. fumigatus* and *G. candidum*.

The arylhydrazone derivatives **9a**, **9c** and **9d** showed good antifungal activities. 4-Ethoxyphenylhydrazones **9c** and **9d** were twofold more potent than amphotericin B against *S. racemosum* (MIC, 3.9 μg/mL *vs* 7.81 μg/mL).

The pyrazole **6** showed promising antifungal activities, it was much more active than amphotericin B against *S. racemosum* (MIC, 0.12 μg/mL *vs* 7.81 μg/mL) and four folds more active than amphotericin B against *A. fumigatus* (MIC, 0.03 μg/mL *vs* 0.12 μg/mL). Transformation of arylhydrazone **9d** to pyrazole **11** increased the antifungal activity. Pyrazole **11** exhibited much better antifungal activity than amphotericin B against *S. racemosum* (MIC, 0.06 μg/mL *vs* 7.81 μg/mL). On the other hand, the pyrazole **8** was less potent than its precursor acrylamide **7**.

The antifungal activities of the 2-pyridones **12**, **15** and **16b** were four folds more than amphotericin B against *S. racemosum* (MIC, 1.95 μg/mL *vs* 7.81 μg/mL). Moreover, the pyridonethiol **16c** showed eight folds the activity of amphotericin B against *S. racemosum* (MIC, 0.98 μg/mL *vs* 7.81 μg/mL). Compound **12** showed more antifungal activities than the pyridonethiols **16a**–**d** against *A. fumigatus*; it was equipotent to the pyridonethiol **16b** and twofold less potent than the pyridonethiol **16c** against *G. candidum*.

The thioglucoside **17** displayed double potency of amphotrecin B against *S. racemosum* (MIC, 3.9 μg/mL *vs* 7.81 μg/mL) and was more potent than its pyridonethiol precursor **16b** against *A. fumigatus* and *G. candidum*; moreover, it possessed better antifungal activities than the other 2-pyridones **12**, **15**, **16a**–**d** against *G. candidum*.

2.3. Molecular modeling

2.3.1. Validation of MOE docking methodology

The proposed docking algorithm was validated by self-docking in the BaDHPS crystal structure (PDB code 3TYE) [16], by removing the bound ligand, STZ-DHPP adduct, from the complex then docking it back into the binding site. The top ranked pose

exhibited heavy-atom root-mean-square deviation (RMSD) value of 0.62 Å from the experimental crystal structure (Fig. 2). This result indicates that Molecular Operating Environment (MOE) docking can reliably predict docking poses for the studied compounds to BaDHPS.

2.3.2. MOE docking results

In general, the top-ranked poses obtained from MOE docking show that the all studied compounds can interact with BaDHPS in a manner similar to that observed for the STZ-DHPP covalent adduct in the solved crystal structure. In addition, the obtained docking poses generally maintain most of the key interactions observed in the aforementioned complex. In the thiophene derivatives, for instance compound **4a**, the benzene-sulfonamide moiety occupies virtually the same position observed in the STZ-DHPP–BaDHPS complex; thereby establishing the characteristic hydrogen bond between its sulfonamide moiety and the backbone NH group of Ser221 while its phenyl ring packs against the side chains of Lys220 and Pro69 (Fig. 3). The predicted binding mode of the benzene-sulfonamide moiety, occupying the *p*-amino benzoic acid (PABA) pocket and preventing the key substrate from binding, is common for all sulfa drugs and is the basis of their inhibitory action on DHPS [32,33]. In addition to the PABA pocket, the substituted thiophene moiety of compound **4a** extends in to the pterin-binding pocket establishing hydrogen bonds between primary amino group and side chain of Lys220 and between the acetyl oxygen and the side chain of Asn120. Meanwhile, the thiophene ring itself forms a face-to-edge interaction with the aromatic side chain of Phe189 (Fig. 3).

Predicted binding modes for the acrylamide derivatives show essentially the same picture in the PABA-binding pocket, where the sulfonamide group makes the key hydrogen bond to Ser221. Additionally, the phenyl group in compound **5a** makes face-to-edge interaction with Phe189 (H-to-C distances ranging from 3.0 to 3.6 Å) and cation-π interaction with the positively-charged side chain of Lys220 (Fig. 3). In the acrylamide series, however, the terminal aryl substituent is mostly hydrophobic in nature. Therefore, the interactions in the pterin-binding pocket are dominated by van der Waals contacts to the hydrophobic side chains of residues such as Met145 and Ile122. In addition to the characteristic network of interactions at the PABA-pocket, the pyrazole ring in compounds **6** and **8** can form a hydrogen bond to the acidic side chain of Asp101 (Fig. 4). Similarly, compounds in the arylhydrazone series can form a hydrogen bond to one of the polar residues Asp101 or Asn120 in the pterin-binding pocket, e.g. compound **9a** (Fig. 4).

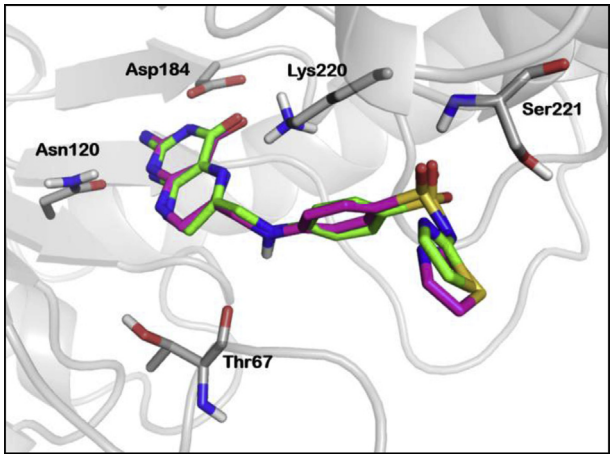


Fig. 2. Self-docking of STZ-DHPP adduct with BaDHPS active site. PDB code 3TYE, (STZ: sulfathiazole, DHPP: 6-hydroxymethyl-7,8-dihydropterin-pyrophosphate); magenta carbons: crystal structure pose, green carbons: top-ranked docking pose (red: oxygen, blue: nitrogen, yellow: sulfur, white: hydrogen). The protein is shown as light gray cartoons. Key residues in the binding site are shown as light gray sticks. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Docking studies for the 2-pyridone derivatives in Scheme 3 showed that, unlike the derivatives of the other classes, they do not have good poses with the BaDHPS enzyme. This could be attributed to the direct link between the pyridone ring and the sulfisoxazole phenyl ring, resulting in steric clash with binding site residues. Nevertheless, Yun et al. reported significant flexibility in the binding pocket of BaDHPS, as evidenced by the rearrangement of loop 1 and loop 2 to form the PABA binding pocket [16]. Therefore, bulky or rigid compounds that might not dock well into DHPS binding pocket could in principle bind to a slightly altered conformation of the enzyme, where pocket residues rearrange to accommodate the larger substituents. In the present work, docking studies were performed using a rigid representation of the enzyme rather than the methods that take receptor flexibility into account, e.g. induced-fit docking [34]. The reported DHPS binding pocket conformational changes involve some loop rearrangements [16], involving back-bone movements, which are more challenging to account for in docking simulations. Future DHPS enzymatic assay studies will be carried out to further confirm the target of the reported compounds.

2.4. Structure activity relationship (SAR)

It was observed that most of the synthesized *N*-substituted sulfisoxazole derivatives have superior antimicrobial activities than sulfisoxazole itself. The synthesized compounds have higher lipophilic characters than sulfisoxazole (Table 3) and hence they have more intracellular concentration due to their improved cell penetration. Consequently, the synergistic effect of two different pharmacophores (Fig. 5) and/or their ability to occupy both the PABA and pterin binding pockets of DHPS enzyme (Figs. 3 and 4) could account for the good results obtained.

Regarding the thiophene series **4a**–**d** (Scheme 1), the larger the *N*-alkyl substituents the better antimicrobial activities were noticed. This could be explained by the increased lipophilic characters of these compounds by increasing the size of the alkyl group (Table 3).

In case of the acrylamide **5c**–**e** and **7** (Scheme 2), the synergistic effect of both the sulfonamide and acrylamide moieties could account for the good antimicrobial activities observed. Moreover, the additional synergistic effect of the 3-pyridyl moiety in compound **5e** resulted in excellent antimicrobial activities. Additionally, the presence of the electron withdrawing chlorine atom in the acrylamide **5c** and the 2-pyridone **16b** increased the antimicrobial activities of these compounds. On the other hand, the presence of alkoxyphenyl substituent in pyrazoles **6** and **11** (Scheme 2) and the replacement of the amino group by the thiol moiety at position 6 in the 2-pyridone **16a** (Scheme 3) increased the Clog *P* values (Table 3) and hence their antimicrobial activities. Finally, the insertion of the thioglucosyl moiety at position 6 in the 2-pyridone **17** significantly increased the antimicrobial activities.

3. Conclusion

New series of thiophene, acrylamide, pyrazole and pyridone derivatives tagged with sulfisoxazole moieties were synthesized successfully and evaluated for their *in vitro* antimicrobial activities. Most of newly synthesized compounds were more potent than sulfisoxazole. Moreover, some of the targets exhibited better

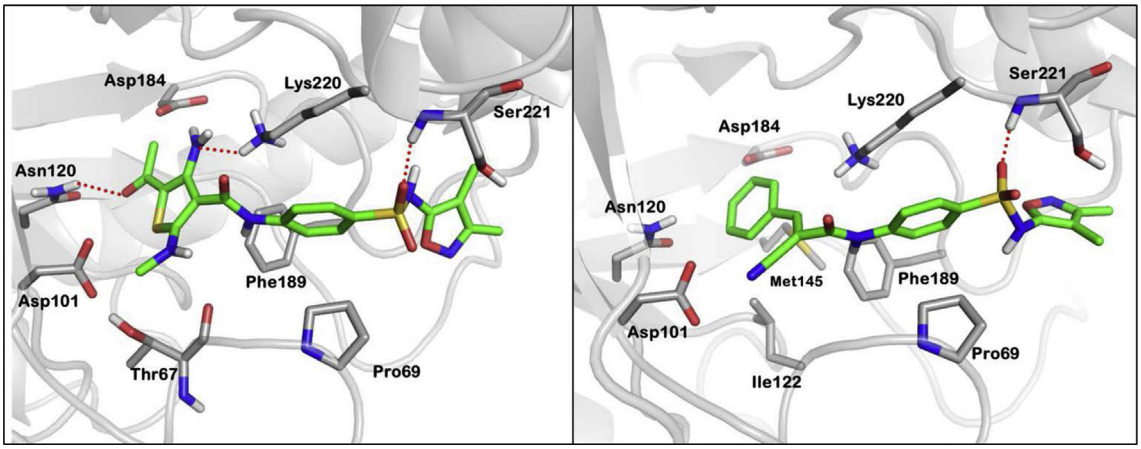


Fig. 3. Top-ranked docking poses of compound **4a** (left) and compound **5a** (right) showing key interactions with BaDHPS active site. Color codes as in Fig. 2. Hydrogen bonds are shown as red dotted lines. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

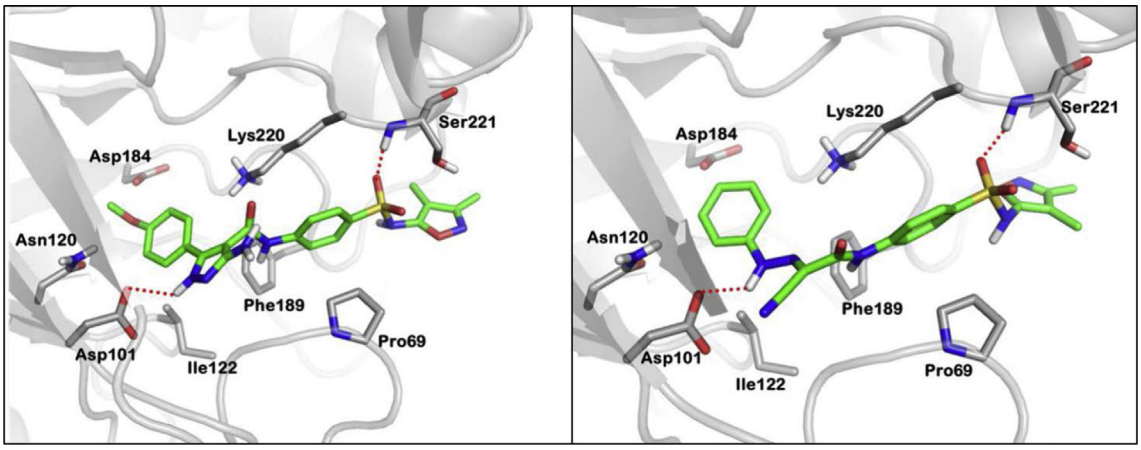


Fig. 4. Top-ranked docking poses of compound **6** (left) and compound **9a** (right) showing key interactions with BaDHPS active site. Color codes as in Fig. 2. Hydrogen bonds are shown as red dotted lines. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

antimicrobial activities than the reference drugs ampicillin, gentamycin and amphotericin B. Docking simulations showed that the studied compounds can be accommodated in the PABA pocket of DHPS thereby inhibiting the enzyme in the same manner reported for sulfa drugs. Additionally, the synergistic effect of combining sulfonamide and biologically active heterocyclic rings in one molecule could explain the targets' observed good results. It was observed that the biological activities of the target compounds increased as their CLog *P* values increased. Compounds **4d**, **5e**, **6** and **17** were the most active antimicrobial agents in this study.

Table 3
Calculated lipophilic characters of the target compounds.

Compounds	Clog *P*[a]	Compounds	Clog *P*[a]	Compounds	Clog *P*[a]
Sulfisoxazole	1.02	**5c**	3.59	**9d**	3.42
4a	1.66	**5d**	2.08	**11**	1.58
4b	2.10	**5e**	1.90	**12**	2.38
4c	2.27	**6**	2.17	**15**	1.64
4d	3.68	**7**	1.20	**16a**	3.41
4e	3.97	**8**	0.53	**16b**	4.12
4f	3.86	**9a**	3.09	**16c**	2.62
5a	2.98	**9b**	3.41	**16d**	2.43
5b	2.87	**9c**	2.99	**17**	4.03

[a] Calculated by online program OSIRIS Property Explorer.

4. Experimental

4.1. Chemistry

Melting points were determined on digital Gallen-Kamp MFB-595 instrument using open capillary tubes and are uncorrected. IR spectra were recorded on Schimadzu FTIR 440 spectrometer using KBr pellets. Mass spectra were performed on Shimadzu Qp-2010 plus mass spectrometer at 70 eV. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker model (500 MHz or 400 MHz) Ultra Shield NMR spectrometer in DMSO-d_6 using tetramethylsilane (TMS) as an internal standard; chemical shifts are reported as δ_{ppm} units. The elemental analyses were done at the Microanalytical Center, Cairo University, Cairo, Egypt. Sulfisoxazole **1** and (4-methoxybenzylidene) malononitrile were purchased from sigma Aldrich. 1-Cyanoacetyl-3,5-dimethylpyrazole **2** [35] and arylidene cyanothioacetamides [36] were synthesized according to the reported procedures.

4.1.1. 2-Cyano-N-(4-{[(3,4-dimethylisoxazole-5-yl)amino]sulfonyl} phenyl)acetamide (**3**)

To a hot solution of sulfisoxazole **1** (5.34 g, 0.02 mol) in dioxan (60 mL) was added 1-cyanoacetyl-3,5-dimethylpyrazole **2** (3.26 g, 0.02 mol) and the reaction mixture was refluxed for 6 h. It was

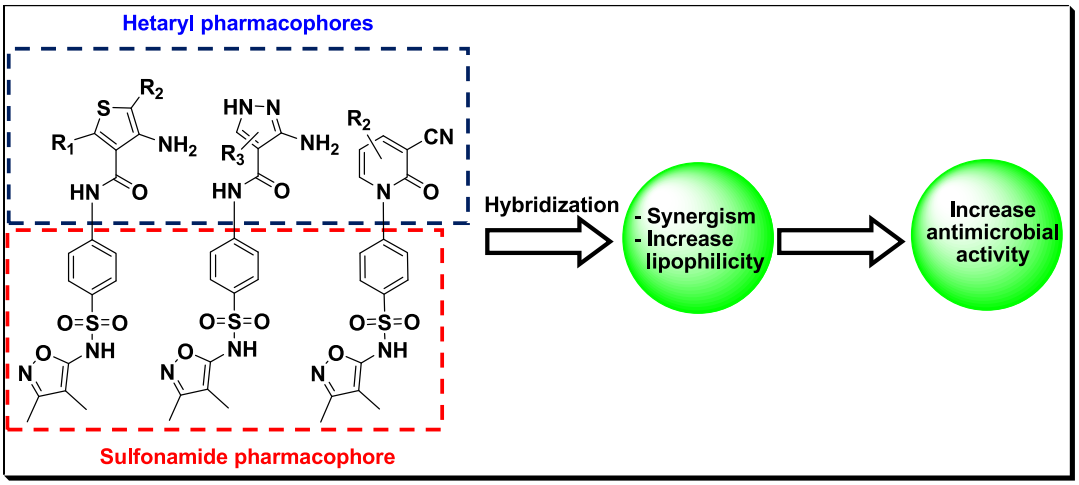


Fig. 5. Factors improving the antimicrobial activities.

allowed to cool down to room temperature and the obtained solid was filtered off, washed with dioxan and recrystallized from ethanol/DMF to afford the target compound.

Colorless crystals, yield (82%), mp 241–242 °C; IR (KBr) ν_{max}/cm^{-1}: 3263 (NH), 3183 (NH), 3073 (CH-Ar), 2964 (CH-sp^3), 2261 (CN), 1699 (CO); ^{1}H NMR (500 MHz, DMSO-d$_6$): δ_{ppm} = 1.63 (s, 3H, CH_3), 2.09 (s, 3H, CH_3), 3.98 (s, 2H, CH_2), 7.72–7.77 (m, 4H, CH_{Ar}), 10.73 (s, 1H, $NHSO_2$), 10.97 (s, 1H, NHCO); ^{13}C NMR (125 MHz, DMSO-d$_6$): δ_{ppm} = 5.8 (CH_3), 10.2 (CH_3), 27.0 (CH_2), 105.1 (CN), 115.5 (Phenyl-C$_4$), 119.0 (2C, Phenyl-C$_2$ and Phenyl-C$_6$), 128.0 (2C, Phenyl-C$_3$ and Phenyl-C$_5$), 134.3 (Phenyl-C$_1$), 142.5 (Isoxazole-C$_4$), 155.4 (Isoxazole-C$_3$), 161.3 (Isoxazole-C$_5$), 161.9 (CO); MS *m/z* (%): 335 ([M+H]$^+$, 22.8), 334 (M$^+$, 24.7), 223 (100), 175 (30.2), 159 (84.8), 111 (78.8), 96 (16.2), 83 (13.7), 68 (78.2); Anal. Calcd. for $C_{14}H_{14}N_4O_4S$ (334.35): C, 50.29; H, 4.22; N, 16.76%, Found: C, 50.33; H, 4.17; N, 16.81%.

*4.1.2. General procedure for the synthesis of functionalized thiophene derivatives **4a–f***

An ethanolic solution of sodium ethoxide [prepared from sodium metal (0.23 g, 0.01 mol) in 10 mL absolute ethanol] was added dropwise to a well stirred solution of compound **3** (3.34 g, 0.01 mol) in absolute ethanol (20 mL). After complete addition, the reaction mixture was stirred at room temperature for 2 h. Substituted isothiocyanate (0.01 mol) was added and the mixture was heated on a water-bath at 70 °C for 30 min then cooled to 25 °C. Chloroacetone (0.93 g, 0.01 mol) or chloroacetonitrile (0.76 g, 0.01 mol) was then added, and the temperature was raised to 60 °C for 30 min more, followed by addition of an ethanolic solution of sodium ethoxide [prepared from sodium (0.12 g, 0.005 mol) in 5 mL absolute ethanol] and heating was continued for additional 3 h. After cooling, water was added and the separated product was filtered, dried and recrystallized from ethanol to afford compounds **4a–f**.

*4.1.2.1. 5-Acetyl-4-amino-N-(4-{[(3,4-dimethylisoxazol-5-yl)amino] sulfonyl}phenyl)-2-(methylamino)thiophene-3-carboxamide (**4a**).* Brown powder, yield (58%), mp 156–157 °C; IR (KBr) ν_{max}/cm^{-1}: 3412–3354 (NH_2), 3314 (NH), 3247 (NH), 3173 (NH), 3047 (CH-Ar), 2987 (CH-sp^3), 1698 (CO), 1650 (CO); ^{1}H NMR (500 MHz, DMSO-d$_6$): δ_{ppm} = 1.63 (s, 3H, CH_3), 2.09 (s, 3H, CH_3), 2.30 (s, 3H, $COCH_3$), 3.56 (s, 3H, NCH_3), 7.70–7.85 (m, 6H, C_6H_4 and NH_2), 8.17 (s, 1H, NH), 9.98 (s, 1H, $NHSO_2$), 10.96 (s, 1H, CONH); MS *m/z* (%): 464 ([M+H]$^+$, 3.8), 463 (M$^+$, 24.8), 433 (30.1), 175 (6.2), 169 (16.2), 133 (100); Anal. Calcd. for $C_{19}H_{21}N_5O_5S_2$ (463.53): C, 49.23; H, 4.57; N, 15.11%, Found: C, 49.26; H, 4.54; N, 15.07%.

*4.1.2.2. 5-Acetyl-4-amino-N-(4-{[(3,4-dimethylisoxazol-5-yl)amino] sulfonyl}phenyl)-2-(ethylamino)thiophene-3-carboxamide (**4b**).* Yellow powder, yield (51%), mp 140–141 °C; IR (KBr) ν_{max}/cm^{-1}: 3422–3362 (NH_2), 3310 (NH), 3244 (NH), 3170 (NH), 3049 (CH-Ar), 2978 (CH-sp^3), 1699 (CO), 1646 (CO); ^{1}H NMR (500 MHz, DMSO-d$_6$): δ_{ppm} = 1.23 (t, *J* = 7.0 Hz, 3H, CH_3), 1.70 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 2.11 (s, 3H, $COCH_3$), 3.24 (q, *J* = 7.0 Hz, 2H, CH_2N), 7.65 (broad s, 2H, NH_2), 7.71 (d, *J* = 9.0 Hz, 2H, Phenyl-H$_2$, Phenyl-H$_6$), 7.81 (d, *J* = 9.0 Hz, 2H, Phenyl-H$_3$, Phenyl-H$_5$), 8.10 (s, 1H, NH), 9.90 (s, 1H, $NHSO_2$), 10.94 (s, 1H, CONH); ^{13}C NMR (125 MHz, DMSO-d$_6$): δ_{ppm} = 5.9 (CH_3), 10.3 (CH_3), 13.9 (CH_3), 27.9 ($\underline{CH_3}CO$), 41.5 (CH_2N), 99.5, 104.9, 119.1, 119.9, 125.7, 127.4, 133.4 (9C, C_6H_4, Thiophene-C$_3$, Thiophene-C$_4$, Thiophene-C$_5$), 143.6 (Isoxazole-C$_4$), 155.5 (Isoxazole-C$_3$), 161.4 (Isoxazole-C$_5$), 163.3 (CONH), 164.2 (Thiophene-C$_2$), 185.4 (CO); MS *m/z* (%): 478 ([M+H]$^+$, 45.0), 477 (M$^+$, 45.0), 226 (32.5), 211 (32.5), 183 (2.5), 175 (19.7), 57 (100); Anal. Calcd. for $C_{20}H_{23}N_5O_5S_2$ (477.56): C, 50.30; H, 4.85; N, 14.66%, Found: C, 50.28; H, 4.89; N, 14.67%.

*4.1.2.3. 5-Acetyl-2-(allylamino)-4-amino-N-(4-{[(3,4-dimethylisoxazol-5-yl)amino] sulfonyl}phenyl)thiophene-3-carboxamide (**4c**).* Brown powder, yield (49%), mp 155–156 °C; IR (KBr) ν_{max}/cm^{-1}: 3417–3361 (NH_2), 3314 (NH), 3280 (NH), 3193 (NH), 3096 (CH-Ar), 2983 (CH-sp^3), 1697 (CO), 1650 (CO); ^{1}H NMR (500 MHz, DMSO-d$_6$): δ_{ppm} = 1.71 (s, 3H, CH_3), 2.09 (s, 3H, CH_3), 2.52 (s, 3H, CH_3CO), 3.87 (m, 2H, CH_2N), 5.21 (dd, *J* = 5.0 Hz, 12.0 Hz, 1H, CH_2=), 5.28 (dd, *J* = 5.0 Hz, 12.0 Hz, 1H, CH_2=), 5.88 (m, 1H, CH=), 7.62 (broad s, 2H, NH_2), 7.71 (d, *J* = 9.0 Hz, 2H, Phenyl-H$_2$ and Phenyl-H$_6$), 7.81 (d, *J* = 9.0 Hz, 2H, Phenyl-H$_3$ and Phenyl-H$_5$), 8.28 (s, 1H, NH), 9.93 (s, 1H, $NHSO_2$), 10.94 (s, 1H, CONH); ^{13}C NMR (125 MHz, DMSO-d$_6$): δ_{ppm} = 5.9 (CH_3), 10.3 (CH_3), 27.9 (CH_3CO), 48.7 (CH_2N), 100.5, 104.9, 105.0, 117.0, 119.9, 127.4, 133.3, 133.4, 141.5 (11C, C_6H_4, –CH=CH_2, Thiophene-C$_3$, Thiophene-C$_4$, Thiophene-C$_5$), 143.6 (Isoxazole-C$_4$), 155.6 (Isoxazole-C$_3$), 161.4 (Isoxazole-C$_5$), 163.3 (CONH), 164.3 (Thiophene-C$_2$), 185.6 (CO); MS *m/z* (%): 490 ([M+H]$^+$, 9.9), 489 (M$^+$, 7.9), 448 (5.9), 446 (3.6), 433 (6.3), 378 (4.4), 238 (5.7), 223 (8.3), 175 (2.3), 167 (100); Anal. Calcd. for $C_{21}H_{23}N_5O_5S_2$ (489.57): C, 51.52; H, 4.74; N, 14.31%, Found: C, 51.55; H, 4.78; N, 14.35%.

*4.1.2.4. 5-Acetyl-2-(adamantylamino)-4-amino-N-(4-{[(3,4-dimethylisoxazol-5-yl)amino] sulfonyl}phenyl) thiophene-3-carboxamide (**4d**).* Yellow powder, yield (61%), mp 262–263 °C; IR (KBr) ν_{max}/cm^{-1}: 3417–3370 (NH_2), 3282 (NH), 3238 (NH), 3165 (NH), 3079 (CH-Ar), 2910 (CH-sp^3), 1696 (CO), 1643 (CO); ^1H NMR (500 MHz, DMSO-d_6): δ_{ppm} = 1.61–1.73 (m, 9H, 9 × Adamantyl-H), 1.92–2.17 (m, 6H, CH_3, 3 × Adamantyl-H), 2.25 (s, 3H, 3 × Adamantyl-H), 2.42 (s, 3H, CH_3), 2.50 (s, 3H, CH_3CO), 7.61 (broad s, 2H, NH_2),7.68 (d, J = 9.0 Hz, 2H, Phenyl-H_2 and Phenyl-H_6), 7.78 (d, J = 9.0 Hz, 2H, Phenyl-H_3 and Phenyl-H_5), 8.07 (s, 1H, NH), 9.49 (s, 1H, $NHSO_2$), 10.83 (s, 1H, CONH); MS m/z (%): 583 (M^+, 72.5), 582 (50.4), 568 (47.8), 408 (51.3), 332 (71.7), 294 (60.2), 231 (100), 175 (16.8), 150 (16.8), 135 (7.1), 96 (21.2); Anal. Calcd. for $C_{28}H_{33}N_5O_5S_2$ (583.72): C, 57.61; H, 5.70; N, 12.00%, Found: C, 57.64; H, 5.75; N, 12.04%.

*4.1.2.5. 5-Acetyl-4-amino-N-(4-{[(3,4-dimethylisoxazol-5-yl)amino] sulfonyl}phenyl)-2-(phenylamino)thiophene-3-carboxamide (**4e**).* Yellow powder, yield (58%), mp 188–189 °C; IR (KBr) ν_{max}/cm^{-1}: 3418–3362 (NH_2), 3287 (NH), 3241 (NH), 3166 (NH), 3062 (CH-Ar), 2928 (CH-sp^3), 1697 (CO), 1647 (CO); ^1H NMR (400 MHz, DMSO-d_6): δ_{ppm} = 1.74 (s, 3H, CH_3), 2.17 (s, 3H, CH_3), 2.57 (s, 3H, $COCH_3$), 7.40–7.50 (m, 5H, Ar-H), 7.56 (s, 2H, NH_2), 7.75 (d, J = 9.0 Hz, 2H, Phenyl-H_2, Phenyl-H_6), 7.90 (d, J = 9.0 Hz, 2H, Phenyl-H_3, Phenyl-H_5), 9.86 (s, 1H, NH), 10.30 (s, 1H, $NHSO_2$), 10.99 (s, 1H, CONH); ^{13}C NMR (100 MHz, DMSO-d_6): δ_{ppm} = 6.4 (CH_3), 10.8 (CH_3), 28.5 (CH_3), 105.5 (Thiophene-C_3), 119.2, 120.4, 121.0, 124.6, 128.0, 129.6, 129.8, 134.2, 138.5, 141.2 (14C, C_6H_4, C_6H_5, Thiophene-C_4, Thiophene-C_5), 143.9 (Isoxazole-C_4), 156.0 (Isoxazole-C_3), 158.6 (Isoxazole-C_5), 161.8 (CONH), 162.5 (Thiophene-C_2), 187.0 (CO); Anal. Calcd. for $C_{24}H_{23}N_5O_5S_2$ (525.60): C, 54.84; H, 4.41; N, 13.32%, Found: C, 54.80; H, 4.44; N, 13.35%.

*4.1.2.6. 4-Amino-5-cyano-N-(4-{[(3,4-dimethylisoxazol-5-yl)amino] sulfonyl}phenyl)-2-(phenylamino)thiophene-3-carboxamide (**4f**).* Yellowish green powder, yield (52%), mp 141–142 °C; IR (KBr) ν_{max}/cm^{-1}: 3405–3348 (NH_2), 3306 (NH), 3243 (NH), 3168 (NH), 3052 (CH-Ar), 2976 (CH-sp^3), 2183 (CN), 1652 (CO); ^1H NMR (400 MHz, DMSO-d_6): δ_{ppm} = 1.75 (s, 3H, CH_3), 2.16 (s, 3H, CH_3), 7.35–7.55 (m, 5H, Ar-H), 7.56 (s, 2H, NH_2), 7.77 (d, J = 9.0 Hz, 2H, Phenyl-H_2, Phenyl-H_6), 7.93 (d, J = 9.0 Hz, 2H, Phenyl-H_3, Phenyl-H_5), 9.83 (s, 1H, NH), 10.41 (s, 1H, $NHSO_2$), 11.15 (s, 1H, CONH); ^{13}C NMR (100 MHz, DMSO-d_6): δ_{ppm} = 6.8 (CH_3), 10.9 (CH_3), 98.1 (Thiophene-C_5), 103.5 (Thiophene-C_3), 111.5 (CN), 116.3, 118.4, 119.9, 120.5, 121.6, 129.1, 129.8, 130.0, 130.9 (13C, C_6H_4, C_6H_5, Thiophene-C_4), 143.4 (Isoxazole-C_4), 156.6 (Isoxazole-C_3), 159.5 (Isoxazole-C_5), 161.8, 162.5 (2C, CONH, Thiophene-C_2); MS m/z (%): 509 ($[M+H]^+$, 2.6), 508 (M^+, 1.5), 493 (2.0), 294 (1.5), 257 (4.5), 111 (10.1), 80 (100); Anal. Calcd. for $C_{23}H_{20}N_6O_4S_2$ (508.57): C, 54.32; H, 3.96; N, 16.52%, Found: C, 54.35; H, 4.01; N, 16.48%.

*4.1.3. General procedure for the synthesis of compounds **5a–d***

To a solution of compound **3** (3.34 g, 0.01 mol) and piperidine (5 drops) in absolute ethanol (30 mL), aryl/hetaryl aldehyde (0.01 mol) was added. The reaction mixture was refluxed for 6 h, then cooled to room temperature and poured onto (100 mL) ice/water. The obtained solid was filtered off, washed with water and recrystallized from ethanol/DMF to afford the target compounds.

*4.1.3.1. 2-Cyano-N-(4-{[(3,4-dimethylisoxazol-5-yl)amino]sulfonyl} phenyl)-3-phenyl-acrylamide (**5a**).* Yellow powder, yield (88%), mp 220–221 °C; IR (KBr) ν_{max}/cm^{-1}: 3329 (NH), 3216 (NH), 3056 (CH-Ar), 2987 (CH-sp^3), 2219 (CN), 1683 (CO); ^1H NMR (500 MHz, DMSO-d_6): δ_{ppm} = 1.66 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 7.60–7.65 (m, 3H, Phenyl-$H_{3'}$, Phenyl-$H_{4'}$, Phenyl-$H_{5'}$), 7.80 (d, J = 9.0 Hz, 2H, Phenyl-$H_{2'}$, Phenyl-$H_{6'}$), 7.91 (d, J = 9.0 Hz, 2H, Phenyl-H_2, Phenyl-H_6), 8.01 (d, J = 9.0 Hz, 2H, Phenyl-H_3, Phenyl-H_5), 8.34 (s, 1H, acrylamide-H_3), 10.82 (s, 1H, $NHSO_2$), 11.00 (s, 1H, CONH); ^{13}C NMR (125 MHz, DMSO-d_6): δ_{ppm} = 5.9 (CH_3), 10.3 (CH_3), 105.1, 106.9, 115.9, 120.3, 127.8, 129.3, 130.1, 131.7, 132.6, 134.8, 142.6 (15C, Acrylamide-C_2, CN, C_6H_4, $C_{6'}H_{5'}$, Isoxazole-C_4), 151.5 (Acrylamide-C_3), 155.5 (Isoxazole-C_3), 161.1 (Isoxazole-C_5), 161.4 (CO); MS m/z (%): 423 ($[M+H]^+$, 0.14), 422 (M^+, 0.24), 326 (1.2), 311 (2.4), 247 (3.4), 171 (1.3), 156 (100), 128 (67.4), 111 (2.1), 90 (5.4), 77 (47.4); Anal. Calcd. for $C_{21}H_{18}N_4O_4S$ (422.46): C, 59.70; H, 4.29; N, 13.26%, Found: C, 59.72; H, 4.33; N, 13.31%.

*4.1.3.2. 2-Cyano-N-(4-{[(3,4-dimethylisoxazol-5-yl)amino]sulfonyl} phenyl)-3-(4-methoxylphenyl)-acrylamide (**5b**).* Yellow powder, yield (91%), mp 175–176 °C; IR (KBr) ν_{max}/cm^{-1}: 3317 (NH), 3205 (NH), 3043 (CH-Ar), 2977 (CH-sp^3), 2211 (CN), 1671 (CO); ^1H NMR (500 MHz, DMSO-d_6): δ_{ppm} = 1.63 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 3.88 (s, 3H, OCH_3), 7.18 (d, J = 9.0 Hz, 2H, Phenyl-$H_{3'}$, Phenyl-$H_{5'}$), 7.74 (d, J = 9.0 Hz, 2H, Phenyl-$H_{2'}$, Phenyl-$H_{6'}$), 7.81 (d, J = 9.0 Hz, 2H, Phenyl-H_2, Phenyl-H_6), 8.04 (d, J = 9.0 Hz, 2H, Phenyl-H_3, Phenyl-H_5), 8.25 (s, 1H, Acrylamide-H_3), 10.60 (s, 1H, $NHSO_2$), 10.90 (s, 1H, CONH); ^{13}C NMR (100 MHz, DMSO-d_6): δ_{ppm} = 6.6 (CH_3), 10.8 (CH_3), 56.1 (OCH_3), 99.8, 103.7, 115.2, 117.1, 120.5, 124.7, 127.8, 133.2, 138.5, 141.9 (14C, Acrylamide-C_2, CN, C_6H_4, $C_{6'}H_{4'}$), 151.3 (Acrylamide-C_3), 160.4 (Isoxazole-C_4), 161.0 (Isoxazole-C_3), 161.8 (Isoxazole-C_5), 163.3 (CO); MS m/z (%): 453 ($[M+H]^+$, 0.3), 452 (M^+, 0.2), 356 (0.8), 294 (0.5), 277 (2.4), 201 (0.9), 186 (58.9), 175 (0.3), 158 (10.9), 111 (0.7), 107 (0.8), 84 (100); Anal. Calcd. for $C_{22}H_{20}N_4O_5S$ (452.48): C, 58.40; H, 4.46; N, 12.38%, Found: C, 58.42; H, 4.43; N, 12.35%.

*4.1.3.3. 2-Cyano-N-(4-{[(3,4-dimethylisoxazol-5-yl)amino]sulfonyl} phenyl)-3-(4-chlorophenyl)acrylamide (**5c**).* Yellow powder, yield (86%), mp 125–126 °C; IR (KBr) ν_{max}/cm^{-1}: 3335 (NH), 3229 (NH), 3067 (CH-Ar), 2993 (CH-sp^3), 2227 (CN), 1686 (CO); ^1H NMR (400 MHz, DMSO-d_6): δ_{ppm} = 1.70 (s, 3H, CH_3), 2.14 (s, 3H, CH_3), 7.60–8.00 (m, 8H, 2 × C_6H_4), 8.65 (s, 1H, Acrylamide-H_3), 10.85 (s, 1H, $NHSO_2$), 10.05 (s, 1H, CONH); ^{13}C NMR (100 MHz, DMSO-d_6): δ_{ppm} = 6.4 (CH_3), 10.8 (CH_3), 105.0, 119.5, 120.0, 120.3, 128.3, 128.6, 130.8, 134.0, 135.6, 142.7, 148.2 (15C, Acrylamide-C_2, CN, C_6H_4, $C_{6'}H_{4'}$, Isoxazole-C_4), 153.9 (Acrylamide-C_3), 156.4 (Isoxazole-C_3), 160.9 (Isoxazole-C_5), 161.8 (CO); MS m/z (%): 456 (M^+, 0.1), 345 (1.8), 281 (3.9), 266 (2.2), 190 (1.2), 173 (100), 124 (2.1), 111 (7.3), 96 (4.9); Anal. Calcd. for $C_{21}H_{17}ClN_4O_4S$ (456.90): C, 55.20; H, 3.75; N, 12.26%, Found: C, 55.23; H, 3.78; N, 12.27%.

*4.1.3.4. 2-Cyano-N-(4-{[(3,4-dimethylisoxazol-5-yl)amino]sulfonyl} phenyl)-3-(2-furyl)-acrylamide (**5d**).* Brown powder, yield (71%), mp 231-231 °C; IR (KBr) ν_{max}/cm^{-1}: 3326 (NH), 3216 (NH), 3037 (CH-Ar), 2956 (CH-sp^3), 2214 (CN), 1681 (CO); ^1H NMR (500 MHz, DMSO-d_6): δ_{ppm} = 1.63 (s, 3H, CH_3), 2.02 (s, 3H, CH_3), 7.37 (t, J = 4.0 Hz, 1H, Furan-H_4), 7.75 (d, J = 9.0 Hz, 2H, Phenyl-H_2, Phenyl-H_6), 7.80 (d, J = 9.0 Hz, 2H, Phenyl-H_3, Phenyl-H_5), 7.96 (d, J = 4.0 Hz, 1H, Furan-H_5), 8.17 (d, J = 4.0 Hz, 1H, Furan-H_3), 8.56 (s, 1H, acrylamide-H_3), 10.57 (s, 1H, $NHSO_2$), 10.90 (s, 1H, CONH); ^{13}C NMR (125 MHz, DMSO-d_6): δ_{ppm} = 6.1 (CH_3), 10.3 (CH_3), 102.3 (Acrylamide-C_2), 116.1, 119.5, 120.0, 127.3, 128.7, 135.6, 135.7, 137.9, 138.2 (11C, CN, C_6H_4, Furan-C_2, Furan-C_3, Furan-C_4, Furan-C_5), 141.2 (Isoxazole-C_4), 144.3 (Acrylamide-C_3), 156.3 (Isoxazole-C_3), 160.4 (Isoxazole-C_5), 160.7 (CO); MS m/z (%): 412 (M^+, 2.8), 345 (0.3), 301 (19.5), 237 (14.9), 146 (100), 118 (12.5), 111 (6.9), 96 (2.3), 80 (3.5), 67 (1.9); Anal. Calcd. for $C_{19}H_{16}N_4O_5S$ (412.42): C, 55.33; H, 3.91; N, 13.58%, Found: C, 55.37; H, 3.93; N, 13.55%.

*4.1.3.5. 2-Cyano-N-(4-{[(3,4-dimethylisoxazol-5-yl)amino]sulfonyl} phenyl)-3-(3-pyridyl)acrylamide (**5e**).* Yellow powder, yield (69%), mp 158–159 °C; IR (KBr) ν_{max}/cm^{-1}: 3372 (NH), 3236 (NH), 3045 (CH-Ar), 2963 (CH-sp^3), 2195 (CN), 1677 (CO); ^{1}H NMR (500 MHz, DMSO-d_6): δ_{ppm} = 1.66 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 7.74 (t, J = 5.0 Hz, 1H, Pyridine-H_5), 7.79 (d, J = 9.0 Hz, 2H, Phenyl-H_2, Phenyl-H_6), 7.94 (d, J = 9.0 Hz, 2H, Phenyl-H_3, Phenyl-H_5), 8.51 (s, 1H, acrylamide-H_3), 8.55 (m, 1H, Pyridine-H_6), 8.81 (d, J = 5.0 Hz, 1H, Pyridine-H_4), 9.09 (s, 1H, Pyridine-H_2), 10.90 (s, 1H, $NHSO_2$), 11.05 (s, 1H, CONH); ^{13}C NMR (125 MHz, DMSO-d_6): δ_{ppm} = 5.8 (CH_3), 10.3 (CH_3), 105.2 (Acrylamide-C_2), 109.5, 115.4, 120.3, 124.6, 127.8, 128.0, 134.9, 137.0, 142.5, 148.2, 150.3, 151.6 (14C, CN, C_6H_4, C_5H_4N, Isoxazole-C_4, Acrylamide-C_3), 155.4 (Isoxazole-C_3), 160.6 (Isoxazole-C_5), 161.4 (CO); MS m/z (%): 423 (M^+, 0.9), 345 (0.3), 312 (4.9), 248 (7.6), 175 (3.7), 175 (3.7), 172 (5.6), 157 (100), 129 (21.5), 111 (23.0), 96 (9.4), 91 (23.3), 78 (53.1); Anal. Calcd. for $C_{20}H_{17}N_5O_4S$ (423.45): C, 56.73; H, 4.05; N, 16.54%, Found: C, 56.70; H, 4.01; N, 16.49%.

*4.1.4. Synthesis of 5-amino-N-(4-{[(3,4-dimethylisoxazol-5-yl) amino]sulfonyl}phenyl)-3-(4-methoxyphenyl)-1H-pyrazole-4-carboxamide (**6**)*

A solution of compound **5b** (0.452 g, 0.001 mol) and hydrazine hydrate (1 mL) in ethanol (30 mL) was refluxed for 2 h then cooled to room temperature and poured onto (100 mL) ice/water. The obtained solid was filtered off, washed with water and recrystallized from ethanol to afford the target compound.

Yellowish white powder, yield (67%), mp 161–162 °C; IR (KBr) ν_{max}/cm^{-1}: 3481 (NH), 3399–3312 (NH_2), 3286 (NH), 3208 (NH), 3046 (CH-Ar), 2983 (CH-sp^3), 1657 (CO); ^{1}H NMR (500 MHz, DMSO-d_6): δ_{ppm} = 1.65 (s, 3H, CH_3), 2.1 (s, 3H, CH_3), 3.78 (s, 3H, OCH_3), 6.10 (s, 2H, NH_2), 7.23 (d, J = 9.0 Hz, 2H, Phenyl-$H_{3'}$, Phenyl-$H_{5'}$), 7.75 (d, J = 9.0 Hz, 2H, Phenyl-$H_{2'}$, Phenyl-$H_{6'}$), 7.86 (d, J = 9.0 Hz, 2H, Phenyl-H_2, Phenyl-H_6), 7.93 (d, J = 9.0 Hz, 2H, Phenyl-H_3, Phenyl-H_5), 8.95 (s, 1H, NH), 9.72 (s, 1H, $NHSO_2$), 10.93 (s, 1H, CONH); MS m/z (%): 482 (M^+, 1.9), 451 (2.3), 371 (2.2), 294 (1.9), 231 (2.2), 175 (0.9), 127 (100), 107 (11.2), 96 (0.9), 77 (74.7); Anal. Calcd. for $C_{22}H_{22}N_6O_5S$ (482.51): C, 54.76; H, 4.60; N, 17.42%, Found: C, 54.73; H, 4.62; N, 17.46%.

*4.1.5. Synthesis of 2-cyano-3-(N,N-dimethylamino)-N-(4-{[(3,4-dimethylisoxazol-5-yl) amino]sulfonyl}phenyl)acrylamide (**7**)*

To a solution of compound **3** (0.6 g, 1.8 mmol) in dry dioxan (30 mL) was added dimethylformamide-dimethylacetal (0.28 mL, 2.2 mmol) and the mixture was refluxed for 6 h. Then, the solvent was distilled off under reduced pressure and the residual viscous liquid was taken in ether and the resulting solid was collected by filtration, washed with ether, air dried, and finally recrystallized from ethanol to afford compound **7**.

Yellow powder, yield (71%), mp 158–159 °C; IR (KBr) ν_{max}/cm^{-1}: 3307 (NH), 3178 (NH), 3032 (CH-Ar), 2930 (CH-sp^3), 2191 (CN), 1675 (CO); ^{1}H NMR (500 MHz, DMSO-d_6): δ_{ppm} = 1.80 (s, 3H, CH_3), 2.17 (s, 3H, CH_3), 3.25 (s, 3H, NCH_3), 3.31 (s, 3H, NCH_3), 7.64 (d, J = 9.0 Hz, 2H, Phenyl-H_2, Phenyl-H_6), 7.86 (d, J = 9.0 Hz, 2H, Phenyl-H_3, Phenyl-H_5), 7.88 (s, 1H, acrylamide-H_3), 9.65 (s, 1H, $NHSO_2$), 10.90 (s, 1H, CONH); ^{13}C NMR (100 MHz, DMSO-d_6): δ_{ppm} = 6.56 (CH_3), 10.4 (CH_3), 36.7 (NCH_3), 38.2 (NCH_3), 108.6 (Acrylamide-C_2), 118.8 (CN), 119.5, 119.7, 128.4, 129.3, 144.6, 156.9, 158.4 (9C, C_6H_4, Acrylamide-C_3, Isoxazole-C_4, Isoxazole-C_3), 161.7 (Isoxazole-C_5), 164.4 (CO); MS m/z (%): 389 (M^+, 1.7), 294 (1.5), 278 (16.8), 214 (12.2), 138 (2.9), 123 (100), 111 (2.1), 96 (7.8), 95 (9.5), 57 (2.4); Anal. Calcd. for $C_{17}H_{19}N_5O_4S$ (389.43): C, 52.43; H, 4.92; N, 17.98%, Found: C, 52.48; H, 4.96; N, 17.95%.

*4.1.6. Synthesis of 5-amino-N-(4-{[(3,4-dimethylisoxazol-5-yl) amino]sulfonyl}phenyl)-1H-pyrazole-4-carboxamide (**8**)*

A solution of compound **7** (0.389 g, 1 mmol) and hydrazine hydrate (1 mL) in ethanol (30 mL) was refluxed for 4 h then cooled to room temperature and poured onto (50 mL) ice/water. The obtained solid was filtered off, washed with water and recrystallized from ethanol to afford the target compound.

Yellow white powder, yield (56%), mp 173–174 °C; IR (KBr) ν_{max}/cm^{-1}: 3477 (NH), 3405–3319 (NH_2), 3286 (NH), 3201 (NH), 3053 (CH-Ar), 2985 (CH-sp^3), 1664 (CO); ^{1}H NMR (500 MHz, DMSO-d_6): δ_{ppm} = 1.68 (s, 3H, CH_3), 2.20 (s, 3H, CH_3), 6.08 (s, 2H, NH_2), 7.88 (d, J = 9.0 Hz, 2H, Phenyl-H_2, Phenyl-H_6), 7.96 (d, J = 9.0 Hz, 2H, Phenyl-H_3, Phenyl-H_5), 8.23 (s, 1H, Pyrazole-H_3), 8.75 (s, 1H, NH), 9.83 (s, 1H, $NHSO_2$), 10.98 (s, 1H, CONH); MS m/z (%): 376 (M^+, 2.4), 294 (3.3), 266 (7.5), 175 (100), 111 (12.3), 110 (1.8), 96 (17.0); Anal. Calcd. for $C_{15}H_{16}N_6O_4S$ (376.39): C, 47.87; H, 4.28; N, 22.33%, Found: C, 47.91; H, 4.32; N, 22.36%.

*4.1.7. General procedure for the synthesis of compounds **9a–d***

To a cold solution of compound **3** (1 g, 3 mmol) in pyridine (20 mL) was added the appropriate arene diazonium salts [prepared by treating a cold solution of aromatic amines (3 mmol) in 6 M HCl (4.5 mL) with sodium nitrite solution (0.21 g, 3 mmol) in cold water (9 mL)]. The addition was carried out portionwise with stirring at 0–5 °C over a period of 30 min. After the complete addition, the reaction mixture was stirred for further 4 h, then kept in ice-chest for 12 h, and finally diluted with water. The obtained solid was filtered off, washed with water, air dried, and recrystallized from ethanol/DMF (5:1) to afford the corresponding arylhydrazones **9a–d**.

*4.1.7.1. 2-Cyano-N-(4-{[(3,4-dimethylisoxazol-5-yl)amino]sulfonyl} phenyl)-2-(phenyl-hydrazono)acetamide (**9a**).* Brown powder, yield (92%), mp 235–236 °C; IR (KBr) ν_{max}/cm^{-1}: 3421 (NH), 3376 (NH), 3247 (NH), 3067 (CH-Ar), 2977 (CH-sp^3), 2215 (CN), 1676 (CO); ^{1}H NMR (400 MHz, DMSO-d_6): δ_{ppm} = 1.65 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 7.16 (m, 1H, Ar-H′), 7.40–7.44 (m, 2H, 2 × Ar-H′), 7.74–7.78 (m, 5H, NH, Phenyl-H_2, Phenyl-H_6, 2 × Ar-H′), 7.98 (d, J = 9.0 Hz, 2H, Phenyl-H_3, Phenyl-H_5), 10.29 (s, 1H, NH), 12.1 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ_{ppm} = 6.6 (CH_3), 10.7 (CH_3), 106.0 (C=N), 108.0 (CN), 112.5, 116.9, 121.0, 125.0, 128.1, 129.6, 135.0, 142.0 (12C, C_6H_4, $C_{6'}H_{5'}$), 143.0 (Isoxazole-C_4), 156.0 (Isoxazole-C_3), 161.0 (Isoxazole-C_5), 163.3 (CO); MS m/z (%): 438 (M^+, 41.7), 412 (5.7), 361 (3.7), 346 (3.9), 327 (43.2), 263 (44.3), 251 (2.5), 187 (3.8), 172 (58.4), 111 (16.8), 96 (10.8), 92 (49.2), 80 (100); Anal. Calcd. for $C_{20}H_{18}N_6O_4S$ (438.46): C, 54.79; H, 4.14; N, 19.17%, Found: C, 54.76; H, 4.16; N, 17.14%.

*4.1.7.2. 2-Cyano-N-(4-{[(3,4-dimethylisoxazol-5-yl)amino]sulfonyl} phenyl)-2-[(4-methyl phenyl)hydrazono]acetamide (**9b**).* Yellow powder, yield (83%), mp 248–249 °C; IR (KBr) ν_{max}/cm^{-1}: 3416 (NH), 3369 (NH), 3241 (NH), 3063 (CH-Ar), 2921 (CH-sp^3), 2215 (CN), 1668 (CO); ^{1}H NMR (400 MHz, DMSO-d_6): δ_{ppm} = 1.62 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 2.31 (s, 3H, CH_3), 7.20 (d, J = 9.0 Hz, 2H, Phenyl-$H_{3'}$, phenyl-$H_{5'}$), 7.59 (d, J = 9.0 Hz, 2H, Phenyl-$H_{2'}$, phenyl-$H_{6'}$), 7.71 (d, J = 9.0 Hz, 2H, Phenyl-H_2, Phenyl-H_6), 7.94 (d, J = 9.0 Hz, 2H, Phenyl-H_3, Phenyl-H_5), 10.24 (s, 1H, NH), 11.15 (s, 1H, NH), 11.95 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ_{ppm} = 6.3 (CH_3), 10.7 (CH_3), 20.9 (CH_3), 105.6 (C=N), 107.0 (CN), 112.0, 116.9, 121.0, 128.1, 130.0, 134.3, 134.7, 140.0 (12C, 2 × C_6H_4), 143.0 (Isoxazole-C_4), 156.0 (Isoxazole-C_3), 160.7 (Isoxazole-C_5), 161.8 (CO); MS m/z (%): 452 (M^+, 4.1), 437 (6.3), 341 (8.5), 332 (1.0), 186 (1.5), 158 (7.5), 112 (100); Anal. Calcd. for $C_{21}H_{20}N_6O_4S$ (452.49): C, 55.74; H, 4.46; N, 18.57%, Found: C, 55.71; H, 4.42; N, 18.58%.

*4.1.7.3. 2-Cyano-N-(4-{[(3,4-dimethylisoxazol-5-yl)amino]sulfonyl} phenyl)-2-[(4-methoxyphenyl)hydrazono]acetamide (**9c**).* Yellow powder, yield (88%), mp 244–245 °C; IR (KBr) ν_{max}/cm^{-1}: 3419 (NH), 3364 (NH), 3231 (NH), 3065 (CH-Ar), 2929 (CH-sp^3), 2218 (CN), 1667 (CO); ^1H NMR (400 MHz, DMSO-d_6): $\delta_{ppm} = 1.65$ (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 3.78 (s, 3H, OCH_3), 6.99 (d, $J = 9.0$ Hz, 2H, Phenyl-$H_{3'}$, phenyl-$H_{5'}$), 7.69 (d, $J = 9.0$ Hz, 2H, Phenyl-$H_{2'}$, phenyl-$H_{6'}$), 7.74 (d, $J = 9.0$ Hz, 2H, Phenyl-H_2, Phenyl-H_6), 7.97 (m, 3H, NH, Phenyl-H_3, Phenyl-H_5), 10.21 (s, 1H, NH), 12.05 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ_{ppm} = 6.3 (CH_3), 10.7 (CH_3), 55.8 (OCH_3), 105.0 (C=N), 106.0 (CN), 111.5, 114.8, 118.4, 120.9, 128.1, 136.0, 137.0, 143.0, 155.0 (13C, C_6H_4, $C_{6'}H_{4'}$, Isoxazole-C_4), 156.0 (Isoxazole-C_3), 161.8 (Isoxazole-C_5), 162.0 (CO); MS *m/z* (%): 468 (M^+, 0.8), 437 (3.5), 357 (8.6), 346 (3.6), 293 (1.0), 266 (2.1), 217 (0.7), 174 (0.7), 112 (100), 111 (7.5), 96 (21.0); Anal. Calcd. for $C_{21}H_{20}N_6O_5S$ (468.49): C, 53.84; H, 4.30; N, 17.94%, Found: C, 53.80; H, 4.32; N, 17.99%.

*4.1.7.4. 2-Cyano-N-(4-{[(3,4-dimethylisoxazol-5-yl)amino]sulfonyl} phenyl)-2-[(4-ethoxy-phenyl)hydrazono]acetamide (**9d**).* Orange powder, yield (83%), mp 215–216 °C; IR (KBr) ν_{max}/cm^{-1}: 3418 (NH), 3368 (NH), 3233 (NH), 3059 (CH-Ar), 2955 (CH-sp^3), 2219 (CN), 1657 (CO); ^1H NMR (400 MHz, DMSO-d_6): $\delta_{ppm} = 1.36$ (t, $J = 6.8$ Hz, 3H, CH_3), 1.69 (s, 3H, CH_3), 2.14 (s, 3H, CH_3), 4.08 (q, $J = 6.8$ Hz, 2H, OCH_2), 6.98 (d, $J = 8.8$ Hz, 2H, Phenyl-$H_{3'}$, phenyl-$H_{5'}$), 7.73 (d, $J = 8.8$ Hz, 2H, Phenyl-$H_{2'}$, phenyl-$H_{6'}$), 7.80 (d, $J = 8.8$ Hz, 2H, Phenyl-H_2, Phenyl-H_6), 8.01 (d, $J = 8.8$ Hz, 2H, Phenyl-H_3, Phenyl-H_5), 10.26 (s, 1H, NH), 10.90 (s, 1H, NH), 11.90 (s, 1H, NH),; ^{13}C NMR (100 MHz, DMSO-d_6): δ_{ppm} = 6.3 (CH_3), 10.8 (CH_3), 15.1 (CH_3), 63.7 (OCH_2), 105.0 (C=N), 106.0 (CN), 112.5, 115.3, 115.5, 118.4, 120.8, 128.1, 133.5, 135.8 (12C, C_6H_4, $C_{6'}H_{4'}$), 143.0 (Isoxazole-C_4), 156.4 (Isoxazole-C_3), 161.0 (Isoxazole-C_5), 162.0 (CO); MS *m/z* (%): 482 (M^+, 0.7), 307 (4.0), 251 (1.5), 231 (2.1), 216 (1.0), 188 (6.7), 175 (4.8), 150 (1.6), 136 (26.8), 121 (26.7), 111 (5.5), 108 (100), 96 (2.8); Anal. Calcd. for $C_{22}H_{22}N_6O_5S$ (482.51): C, 54.76; H, 4.60; N, 17.42%, Found: C, 54.80; H, 4.62; N, 17.39%.

*4.1.8. Synthesis of 2-cyano-2-[(cyanomethyl)4-(ethylphenyl) hydrazono]-N-(4-{[(3,4-dimethyl-isoxazol-5-yl)amino]sulfonyl} phenyl)acetamide (**10**)*

To a solution of compound **9d** (1 g, 2.07 mmol) and chloroacetonitrile (0.13 mL, 2.07 mmol) in ethanol (30 mL), triethylamine (0.5 mL) was added. The reaction mixture was refluxed for 6 h then cooled to room temperature and poured onto (100 mL) ice/water and neutralized by HCl. The obtained solid was filtered off, washed with water and recrystallized from ethanol to afford the target compound.

Brown powder, yield (68%), mp 194–195 °C; IR (KBr) ν_{max}/cm^{-1}: 3379 (NH), 3235 (NH), 3025 (CH-Ar), 2980 (CH-sp^3), 2259 (CN), 2211 (CN), 1661 (CO); ^1H NMR (400 MHz, DMSO-d_6): δ_{ppm} = 1.34 (t, $J = 6.8$ Hz, 3H, CH_3), 1.67 (s, 3H, CH_3), 2.05 (s, 3H, CH_3), 4.01 (q, $J = 6.8$ Hz, 2H, OCH_2), 4.65 (s, 2H, CH_2CN), 6.93 (d, $J = 8.8$ Hz, 2H, Phenyl-$H_{3'}$, phenyl-$H_{5'}$), 7.73 (d, $J = 8.8$ Hz, 2H, Phenyl-$H_{2'}$, phenyl-$H_{6'}$), 7.77 (d, $J = 8.8$ Hz, 2H, Phenyl-H_2, Phenyl-H_6), 7.91 (d, $J = 8.8$ Hz, 2H, Phenyl-H_3, Phenyl-H_5), 10.38 (s, 1H, $NHSO_2$), 11.51 (s, 1H, NHCO); MS *m/z* (%): 521 (M^+, 8.3), 481 (2.0), 294 (3.0), 266 (1.0), 189 (1.0), 175 (1.5), 121 (26.0), 108 (100); Anal. Calcd. for $C_{24}H_{23}N_7O_5S$ (521.55): C, 55.27; H, 4.44; N, 18.80%, Found: C, 55.31; H, 4.47; N, 18.78%.

*4.1.9. Synthesis of 4-amino-5-cyano-N-(4-{[(3,4-dimethylisoxazol-5-yl)amino]sulfonyl} phenyl)-1-(4-ethoxyphenyl)-1H-pyrazole-3-carboxamide (**11**)*

To a solution of compound **10** (1 g, 1.91 mmol) in absolute ethanol (30 mL), sodium ethoxide [prepared by dissolving sodium metal (44 mg, 1.91 mmol) in absolute ethanol (10 mL)] was added. The reaction mixture was cooled to room temperature and poured onto (100 mL) ice/water and neutralized by HCl. The obtained solid was filtered off, washed with water and recrystallized from ethanol to afford the target compound.

Brown powder, yield (72%), mp 163–164 °C; IR (KBr) ν_{max}/cm^{-1}: 3387–3326 (NH_2), 3278 (NH), 3204 (NH), 3034 (CH-Ar), 2987 (CH-sp^3), 2219 (CN), 1671 (CO); ^1H NMR (400 MHz, DMSO-d_6): $\delta_{ppm} = 1.35$ (t, $J = 6.8$ Hz, 3H, CH_3), 1.68 (s, 3H, CH_3), 2.15 (s, 3H, CH_3), 4.11 (q, $J = 6.8$ Hz, 2H, OCH_2), 6.20 (s, 2H, NH_2), 6.88 (d, $J = 8.8$ Hz, 2H, Phenyl-$H_{3'}$, phenyl-$H_{5'}$), 7.54 (d, $J = 8.8$ Hz, 2H, Phenyl-$H_{2'}$, phenyl-$H_{6'}$), 7.78 (d, $J = 8.8$ Hz, 2H, Phenyl-H_2, Phenyl-H_6), 7.93 (d, $J = 8.8$ Hz, 2H, Phenyl-H_3, Phenyl-H_5), 8.32 (s, 1H, $NHSO_2$), 10.92 (s, 1H, NHCO); MS *m/z* (%): 522 ($[M+H]^+$, 3.3), 521 (M^+, 8.0), 505 (3.0), 294 (1.0), 270 (3.0), 251 (2.5), 175 (2.0), 121 (74), 80 (100); Anal. Calcd. for $C_{24}H_{23}N_7O_5S$ (521.55): C, 55.27; H, 4.44; N, 18.80%, Found: C, 55.25; H, 4.39; N, 18.76%.

*4.1.10. Synthesis of 4-(3-cyano-4,6-dimethyl-2-oxopyridin-1(2H)-yl)-N-(3,4-dimethyl-isoxazol-5-yl)benzenesulfonamide (**12**)*

To a mixture of compound **3** (1.67 g, 0.005 mol) and acetylacetone (0.51 mL, 0.005 mol) in absolute ethanol (30 mL) was added triethylamine (0.5 mL). The reaction mixture was refluxed for 9 h then cooled down to room temperature, poured onto (100 mL) ice/water and the medium was neutralized by dilute HCl. The obtained solid was filtered off, washed with water and recrystallized from ethanol to afford the target compound.

White powder, yield (77%), mp 182–183 °C; IR (KBr) ν_{max}/cm^{-1}: 3200 (NH), 3068 (CH-Ar), 2985 (CH-sp^3), 2218 (CN), 1655 (CO); ^1H NMR (400 MHz, DMSO-d_6): δ_{ppm} = 1.60 (s, 3H, CH_3), 1.97 (s, 3H, CH_3), 1.99 (s, 3H, CH_3), 2.33 (s, 3H, CH_3), 6.47 (s, 1H, Pyridone-H_5), 7.44 (d, $J = 8.8$ Hz, 2H, Phenyl-H_2, Phenyl-H_6), 7.89 (d, $J = 8.8$ Hz, 2H, Phenyl-H_3, Phenyl-H_5), 8.90 (s, 1H, $NHSO_2$); ^{13}C NMR (100 MHz, DMSO-d_6): $\delta_{ppm} = 6.7$ (CH_3), 10.9 (CH_3), 21.1 (CH_3), 21.8 (CH_3), 97.2 (Pyridone-C_5), 100.4 (Pyridone-C_3), 109.5 (CN), 116.2 (Phenyl-C_4), 128.0 (2C, Phenyl-C_2, Phenyl-C_6), 128.8 (2C, Phenyl-C_3, Phenyl-C_5), 139.8 (Phenyl-C_1), 145.9 (Isoxazole-C_4), 152.2, 160.3, 160.6, 160.8 (4C, Pyridone-C4, Pyridone-C_6, Isoxazole-C_3, Isoxazole-C_3), 162.2 (CO); MS *m/z* (%): 399 ($[M+H]^+$, 1.2), 398 (M^+, 3.3), 383 (2.0), 302 (100), 287 (0.98), 251 (1.4), 223 (21.8), 175 (0.4), 147 (0.3), 111 (8.0); Anal. Calcd. for $C_{19}H_{18}N_4O_4S$ (398.44): C, 57.27; H, 4.55; N, 14.06%, Found: C, 57.31; H, 4.56; N, 14.09%.

*4.1.11. Synthesis of 4-[6-amino-3,5-dicyano-4-(4-methoxyphenyl)-2-oxopyridin-1(2H)-yl]-N-(3,4-dimethylisoxazol-5-yl) benzenesulfonamide (**15**)*

To a solution of compound **3** (1.67 g, 0.005 mol) and (4-methoxybenzylidene)malononitrile (0.92 g, 0.005 mol) in absolute ethanol (30 mL), sodium ethoxide [prepared by dissolving sodium metal (115 mg, 0.005 mol) in absolute ethanol (5 mL)] was added. The reaction mixture was refluxed for 5 h then allowed to cool to room temperature. Then, it was poured onto (100 mL) ice/water and the medium was neutralized by dilute HCl. The obtained solid was filtered off, washed with water and recrystallized from ethanol to afford the target compound.

Yellow powder, yield (71%), mp 131–132 °C; IR (KBr) ν_{max}/cm^{-1}: 3401–3345 (NH_2), 3217 (NH), 3057 (CH-Ar), 2985 (CH-sp^3), 2217 (CN, broad), 1650 (CO); ^1H NMR (400 MHz, DMSO-d_6): δ_{ppm} = 1.63 (s, 3H, CH_3), 2.08 (s, 3H, CH_3), 3.74 (s, 3H, OCH_3), 6.61 (s, 2H, NH_2), 6.91 (d, $J = 8.8$ Hz, 2H, Phenyl-$H_{3'}$, phenyl-$H_{5'}$), 7.14 (d, $J = 8.8$ Hz, 2H, Phenyl-$H_{2'}$, phenyl-$H_{6'}$), 7.51 (d, $J = 8.8$ Hz, 2H, Phenyl-H_2, Phenyl-H_6), 7.75 (d, $J = 8.8$ Hz, 2H, Phenyl-H_3, Phenyl-H_5), 10.51 (s, 1H, $NHSO_2$); ^{13}C NMR (100 MHz, DMSO-d_6): δ_{ppm} = 6.5 (CH_3), 10.7 (CH_3), 55.8 (OCH_3), 105.0, 113.1, 114.5, 114.6 (4C, Pyridone-C_3, Pyridone-C_2, 2 × CN), 122.3, 125.1, 127.6, 128.0, 128.5, 129.1, 130.6, 131.7 (12C, 2 × C_6H_4), 154.0, 157.5 (2C, Isoxazole-C_4, Pyridone-C_6), 161.0, 161.5 (2C, Isoxazole-C_3, Isoxazole-C_5), 162.0 (CO), 168.0 (Pyridone-

C_4); MS *m/z* (%): 517 ([M+H]$^+$, 1.9), 516 (M$^+$, 1.9), 490 (2.5), 405 (3.0), 265 (6.7), 251 (4.1), 111 (1.4), 96 (4.3), 80 (100); Anal. Calcd. for $C_{25}H_{20}N_6O_5S$ (516.53): C, 58.13; H, 3.90; N, 16.27%, Found: C, 58.17; H, 3.93; N, 16.30%.

4.1.12. General procedure for the synthesis of pyridone derivatives ***16a–d***

4.1.12.1. Method (A). To a solution of compound **3** (1.0 g, 2.99 mmol) and arylidene cyanothioacetamides (2.99 mmol) in absolute ethanol (30 mL), sodium ethoxide [prepared by dissolving sodium metal (69 mg, 2.99 mmol) in absolute ethanol (5 mL)] was added. The reaction mixture was refluxed for 4 h then allowed to cool down to room temperature. Then, poured onto (100 mL) ice/water and the medium was neutralized by dilute HCl. The obtained solid was filtered off, washed with water and recrystallized from ethanol to afford the target compounds.

4.1.12.2. Method (B). To a solution of cyanothioacetamide (1 g, 0.01 mol) and the corresponding arylidene derivatives **5b–e** (0.01 mol) in absolute ethanol (30 mL), triethylamine (0.5 mL) was added. The reaction mixture was refluxed for 9 h then allowed to cool to room temperature. The reaction mixture was poured onto (100 mL) ice/water and the medium was neutralized by HCl. The obtained solid was filtered off, washed with water and recrystallized from ethanol to afford the target compound.

*4.1.12.3. Synthesis of 4-[3,5-dicyano-6-mercapto-4-(4-methoxyphenyl)-2-oxopyridin-1(2H)-yl]-N-(3,4-dimethylisoxazol-5-yl)benzenesulfonamide (**16a**).* Yellow powder, yield (77%), mp 120–121 °C; IR (KBr) ν_{max}/cm^{-1}: 3217 (NH), 3052 (CH-Ar), 2971 (CH-sp^3), 2212 (CN), 1651 (CO); ^{1}H NMR (500 MHz, DMSO-d$_6$): $\delta_{ppm} = 1.62$ (s, 3H, CH_3), 2.08 (s, 3H, CH_3), 3.82 (s, 3H, OCH_3), 6.91 (d, $J = 9.0$ Hz, 2H, Phenyl-H$_{3'}$, Phenyl-H$_{5'}$), 7.04 (d, $J = 9.0$ Hz, 2H, Phenyl-H$_{2'}$, Phenyl-H$_{6'}$), 7.71–7.72 (m, 4H, C_6H_4), 10.50 (s, 1H, $NHSO_2$), 11.91 (s, 1H, SH); ^{13}C NMR (125 MHz, DMSO-d$_6$): $\delta_{ppm} = 5.7$ (CH_3), 10.2 (CH_3), 55.0 (OCH_3), 104.2, 109.5, 110.5, 112.6, 113.5, 113.9, 124.5, 128.0, 128.6, 129.5, 129.9, 139.5, 141.0 (17C, 2 × CN, Pyridone-C$_3$, Pyridone-C$_5$, Pyridone-C$_6$, 2 × C_6H_4), 153.2, 156.2, 160.5 (3C, Isoxazole-C$_3$, Isoxazole-C$_4$, Isoxazole-C$_5$), 161.1 (CO), 167.0 (Pyridone-C$_4$); MS *m/z* (%): 533 (M$^+$, 6.3), 507 (0.9), 426 (5.6), 422 (1.3), 282 (0.7), 251 (2.4), 111 (24.7), 96 (29.3), 57 (100); Anal. Calcd. for $C_{25}H_{19}N_5O_5S_2$ (533.58): C, 56.27; H, 3.59; N, 13.13%, Found: C, 56.31; H, 3.62; N, 13.15%.

*4.1.12.4. Synthesis of 4-[3,5-dicyano-6-mercapto-4-(4-chlorophenyl)-2-oxopyridin-1(2H)-yl]-N-(3,4-dimethylisoxazol-5-yl) benzenesulfonamide (**16b**).* Yellow powder, yield (67%), mp 180–181 °C; IR (KBr) ν_{max}/cm^{-1}: 3217 (NH), 3050 (CH-Ar), 2974 (CH– sp^3), 2206 (CN), 1656 (CO); ^{1}H NMR (400 MHz, DMSO-d$_6$): $\delta_{ppm} = 1.63$ (s, 3H, CH_3), 2.08 (s, 3H, CH_3), 7.44–7.48 (m, 4H, $C_{6'}H_{4'}$), 7.56 (d, $J = 9.0$ Hz, 2H, Phenyl-H$_2$, Phenyl-H$_6$), 7.82 (d, $J = 9.0$ Hz, 2H, Phenyl-H$_3$, Phenyl-H$_5$), 10.49 (s, 1H, $NHSO_2$), 11.85 (s, 1H, SH); MS *m/z* (%): 540 ([M+2]$^+$, 1.3), 538 (M$^+$, 4.1), 440 (3.7), 426 (7.4), 422 (1.3), 251 (2.4), 111 (25.6), 96 (2.2), 80 (100); Anal. Calcd. for $C_{24}H_{16}ClN_5O_4S_2$ (538.04): C, 53.58; H, 3.00; N, 13.02%, Found: C, 53.61; H, 3.02; N, 13.05%.

*4.1.12.5. Synthesis of 4-[3,5-dicyano-4-(2-furyl)-6-mercapto-2-oxopyridin-1(2H)-yl]-N-(3,4-dimethyl-isoxazol-5-yl)benzenesulfonamide (**16c**).* Yellow powder, yield (72%), mp 132–133 °C; IR (KBr) ν_{max}/cm^{-1}: 3207 (NH), 3059 (CH-Ar), 2973 (CH– sp^3), 2211 (CN), 1654 (CO); ^{1}H NMR (400 MHz, DMSO-d$_6$): $\delta_{ppm} = 1.62$ (s, 3H, CH_3), 2.08 (s, 3H, CH_3), 6.70 (t, $J = 5.8$ Hz, 1H, Furan-H$_4$), 7.20 (d, $J = 5.8$ Hz, 1H, Furan-H$_3$), 7.70–7.85 (m, 5H, C_6H_4 and Furan-H$_5$), 10.59 (s, 1H, $NHSO_2$), 11.90 (s, 1H, SH); MS *m/z* (%): 494 ([M+H]$^+$, 0.3), 493 (M$^+$, 0.3), 478 (0.2), 467 (0.3), 460 (0.3), 381 (0.4), 251 (1.9), 175 (1.6), 111 (1.9), 96 (48.9), 57 (100); Anal. Calcd. for $C_{22}H_{15}N_5O_5S_2$ (493.52): C, 53.54; H, 3.06; N, 14.19%, Found: C, 53.59; H, 3.02; N, 14.23%.

*4.1.12.6. Synthesis of 4-[3,5-dicyano-4-(3-pyridyl)-6-mercapto-2-oxopyridin-1(2H)-yl]-N-(3,4-dimethylisoxazol-5-yl)benzenesulfonamide (**16d**).* Yellow powder, yield (57%), mp 146–147 °C; IR (KBr) ν_{max}/cm^{-1}: 3216 (NH), 3068 (CH-Ar), 2971 (CH– sp^3), 2219 (CN), 1655 (CO); ^{1}H NMR (400 MHz, DMSO-d$_6$): $\delta_{ppm} = 1.62$ (s, 3H, CH_3), 2.08 (s, 3H, CH_3), 7.45–8.10 (m, 6H, C_6H_4, Pyridine-H$_5$ and Pyridine-H$_6$), 8.50–8.80 (m, 2H, Pyridine-H$_2$, Pyridine-H$_4$), 10.55 (s, 1H, $NHSO_2$), 11.98 (s, 1H, SH); MS *m/z* (%): 505 ([M+H]$^+$, 8.8), 504 (M$^+$, 6.4), 408 (8.0), 251 (11.1), 175 (1.1), 80 (100), 78 (3.4); Anal. Calcd. for $C_{23}H_{16}N_6O_4S_2$ (504.54): C, 54.75H, 3.20; N, 16.66%, Found: C, 54.78; H, 3.22; N, 16.63%.

*4.1.13. Synthesis of 4-[3,5-dicyano-6-(2′,3′,4′,6′-tetra-O-acetyl-β-D-glucopyranosylthio)-4-(4-chlorophenyl)-2-oxopyridin-1(2H)-yl]-N-(3,4-dimethylisoxazol-5-yl)benzenesulfonamide (**17**)*

A solution of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (0.41 g, 0.001 mol) in dry acetone (10 mL) was added to a mixture of compound **16b** (0.54 g, 0.001 mol) and potassium carbonate (0.3 g) in dry acetone (20 mL). The reaction mixture was refluxed for 18 h, cooled down to room temperature and filtered. The filtrate was evaporated under vacuum. The obtained residue was triturated with distilled water (10 mL), filtered, air dried and purified by column chromatography using 60 N silica gel and hexane/ethylacetate (10:1).

Yellow powder, yield (66%), mp 130–131 °C; IR (KBr) ν_{max}/cm^{-1}: 3203 (NH), 3069 (CH-Ar), 2939 (CH-sp^3), 2207 (CN), 1752 (CO), 1650 (CO); ^{1}H NMR (400 MHz, DMSO-d$_6$): $\delta_{ppm} = 1.95$ (s, 3H, CH_3), 1.99–2.01 (4s, 12H, 4 × CH_3CO), 2.09 (s, 3H, CH_3), 3.90–4.30 (m, 3H, Glucose-H$_{6a'}$, Glucose-H$_{6b'}$,Glucose-H$_{5'}$), 4.90–5.20 (m, 2H, Glucose-H$_{4'}$, Glucose-H$_{3'}$), 5.40 (m, 1H, Glucose-H$_{2'}$), 6.10 (d, $J = 9.80$ Hz, 1H, Glucose-H$_{1'}$), 7.40–7.70 (m, 8H, 2 × C_6H_4), 10.30 (s, 1H, $NHSO_2$); MS *m/z* (%): 870 ([M+2]$^+$, 10.7), 869 ([M+H]$^+$, 21.8), 832 (13.3), 824 (10.3), 808 (11.2), 251 (6.5), 175 (7.1), 153 (100); Anal. Calcd. for $C_{38}H_{34}ClN_5O_{13}S_2$ (868): C, 52.56H, 3.95; N, 8.07%, Found: C, 52.61; H, 3.99; N, 8.03%.

4.2. Antimicrobial evaluation

4.2.1. Antimicrobial screening

The disks of Whatman filter paper were prepared with standard size (6.0 mm diameter) and kept into 1.0 Oz screw capped wide mouthed containers for sterilization. These bottles are kept into hot air oven at a temperature of 150 °C. Then, the standard sterilized filter paper disks impregnated with a solution of the test compound in DMF (100 μL, 5 mg/mL) were placed on nutrient agar plate seeded with the appropriate test organism in triplicates. Standard concentrations of 10^6 CFU/mL (Colony Forming Units/mL) and 10^4 CFU/mL were used for antibacterial and antifungal assay, respectively. Pyrex glass Petri dishes (9 cm in diameter) were used and two disks of filter paper were inoculated in each plate. The utilized test organisms were *S. pneumoniae*, *B. Subtilis* and *S. epidermidis* as examples of Gram-positive bacteria and *E. coli*, *P. vulgaris* and *K. pneumonia* as examples of Gram-negative bacteria. They were also evaluated for their in *vitro* antifungal potential against *A. fumigatus*, *S. racemosum* and *G. candidum* fungal strains. Ampicillin and gentamycin were used as standard antibacterial agents; while amphotericin B was used as standard antifungal agent. The starting antimicrobial agent sulfisoxazole was also used as reference drug. DMF alone was used as control at the same above-mentioned concentration and due this there was no visible change in bacterial growth. The plates were incubated at 37 °C for 24 h for bacteria and for 48 h at 25 °C for fungi. The mean zone of inhibition measured in mm ± standard deviation

beyond well diameter (6 mm) produced on a range of environmental and clinically pathogenic microorganisms. Compounds that showed growth inhibition zones (>10 mm) using the twofold serial dilution technique, were further evaluated for their minimal inhibitory concentrations (MICs).

4.2.2. Minimal inhibitory concentration (MIC) measurement

The microdilution susceptibility test in Müller–Hinton Broth (Oxoid) and Subouraud Liquid Medium (Oxoid) was used for the determination of antibacterial and antifungal activity, respectively. Stock solutions of the tested compounds, ampicillin, gentamycin, amphotericin B and sulfisoxazole were prepared in DMF at concentrations 1000 μg/mL. Each stock solution was diluted with standard method broth (Difco) to prepare serial twofold dilutions in the range of 500–0.007 μg/mL. 10 mL of the broth containing about 10^6 CFU/mL of test bacteria or 10^4 CFU/mL of the test fungus was added to each well of 96-well microtiter plate. The sealed microplates were incubated at 37 °C for 24 h for antibacterial activity and at 25 °C for 48 h for antifungal activity in a humid chamber. At the end of the incubation period, the minimal inhibitory concentrations (MIC) values were recorded as the lowest concentrations of the substance that had no visible turbidity. Control experiments with DMF and uninoculated media were run parallel to the test compounds under the same conditions.

4.3. Molecular modeling

Docking simulations were performed using the crystal structure of DHPS from *B. anthracis* (BaDHPS, PDB code: 3TYE) bound to the covalent adduct of STZ-DHPP [16]. The PDB file was retrieved from the Protein Data Bank and chain B was deleted. Structure of chain A was processed using the Structure Preparation application in MOE [37]. Subsequently, the Protonate 3D application of MOE was used to add the missing hydrogens and properly assign the ionization states [38]. The resultant model was further refined by energy minimization to a gradient of 0.01 kcal mol A^{-2} keeping atoms tethered within 0.5 Å from their crystal structure positions. The default procedure in the MOE Dock application was used to find the favorable binding configurations of the studied ligands. Initial placement poses generated by the Alpha Triangle matcher were rescored and filtered using the London dG Scoring method to pick those exhibiting maximal hydrophobic, ionic, and hydrogen-bond contacts to the protein. This was followed by a refinement stage. The generated poses were energy minimized using the MMFF94x force field. Finally, the optimized poses were ranked using the GBVI/WSA ΔG free-energy estimates [39]. Docking poses were visually inspected and interactions with binding pocket residues were analyzed.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.07.052.

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