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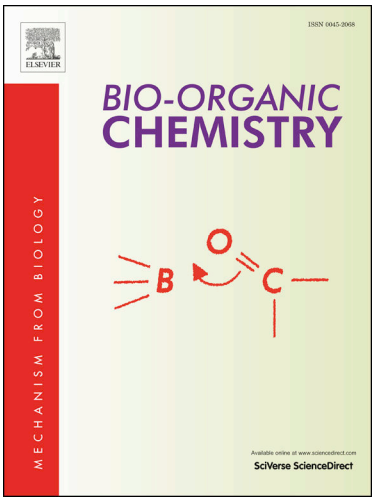

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The Rational Design, Synthesis, and Antimicrobial Investigation of 2-Amino-4-Methylthiazole Analogues Inhibitors of *GlcN-6-P Synthase*

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Abstract

A series of novel 2-Amino-4-Methylthiazole analogs were developed via three-step reaction encompassing hydrazine-1-carboximidamide motif to combat Gram-positive and Gram-negative bacterial and fungal infections. Noticeably, the thiazole-carboximidamide derivatives **4a-d** displayed excellent antimicrobial activity and the most efficacious analogue **4d** with MIC/MBC values of 0.5 and 4 μg/mL, compared to reference drugs with very low toxicity to mammalian cells, resulting in a prominent selectivity more than 100 folds. Microscopic investigation of **4d** biphenyl analogue showed cell wall lysis and promote rapid bactericidal activity though disrupting the bacterial membrane. In addition, an interesting *in vitro* investigation against *GlcN-6-P Synthase* Inhibition was done which showed potency in the nanomolar range. Meanwhile, this is the first study deploying a biomimicking strategy to design potent thiazole-carboximidamides that targeting *GlcN-6-P Synthase* as antimicrobial agents. Importantly, Molecular modeling simulation was done for the most active **4d** analogue to study the interaction of this analogue which showed good binding propensity to glucosamine binding site which support the *in vitro* data.

Keywords: Thiazole; aminoguanidine; antimicrobial activity, GlcN-6-P Synthase Inhibition.

1. Introduction

The need for novel antibacterial agents has intensified due to the continuing emergence of resistant bacteria limiting the utility of many drug therapies [1-3]. At the last few years, FDA have approved only seven new chemical entities as systemic antibacterials and, of these, only two drugs ,linezolid and daptomycin, work through novel modes-of-action compared to the previous antibiotics [4]. The marketplace includes many active antibacterial drugs against resistant strains because of either the increase in potency or decrease susceptibility to the metabolic degradations [5-7]. However, the resistance mechanisms are in increase and more expected in the future time. Nowadays, antibiotic-resistant bacteria accounts for 700,000 infections worldwide, and are responsible for over 25,000 deaths per year in the European Union only, which World Health Organization (WHO) identified as a major public health issue of the 21st century [8, 9]. Thiazole ring having a strong S-C-N fragment which is a member of the family of five-membered heterocyclic compounds; has been the subject of many different types of research to date. Thiazole ring is found to be a common structural component in many biological agents. On the other hand, natural compounds such as thiamine (known as vitamin B1), thiamine pyrophosphate (TPP), epothilone, bacitracin, penicillin antibiotics and a wide range of synthetic drug groups contain thiazole moiety [7]. Previous research has shown that it is an undeniable fact that thiazole based-compounds had effective different pharmacological importance [10-17]. Thiazole-based recent drugs have been extensively studied for their antibacterial and antifungal activities [18-21]. Glucosamine-6-phosphate (GlcN-6-P) synthase has been proposed as a potential target for design of antibacterial and antifungal agents. GlcN-6-P synthase catalyzes the first step in the biosynthesis of D-glucosamine-6-phosphate, which is a precursor of a number of amino sugar containing macromolecules, including chitin and mannoproteins in fungi, peptidoglycan and lipopolysaccharides in bacteria and glycoproteins in mammals [22, 23]. This enzyme was previously reported to be inhibited by a naturally occurring compound tetaine and peptide

A190095, and also by a number of chemically obtained compounds, especially electrophilic ones [24]. In fact, not all of these inhibitors are selective; consequently, they have ability to react with other enzymes in the fungal cell. The most effective and selective compounds, which are the strong inactivators of GlcN-6-P synthase, are the analogues of L-glutamine (the substrate of the enzyme) and one of these compounds is N^3-4-metoxyfumaroyl-(S)-2,3-diaminopropanoic acid (FMDP) [23]. Despite the effectiveness of the enzyme inhibition, the FMDP inhibitor shows none or very poor antimicrobial activity caused by the high polarity of the inhibitor molecule. Different trials were done for improving its antimicrobial and antifungal activity by increasing of the lipophilicity character through synthesis of ester and amide derivatives [25-27]. It was reported that polyguanidines have attracted considerable attention for their antibacterial and antifungal activities [28-30]. The supposed mechanism of action of guanidine compounds is based on the strong electrostatic interaction between their positive charges and the electronegative envelope of bacteria. This immediate binding to the components of the cytoplasmic membrane or the cell wall leads to the loss of bio-functions of phospholipids or to the disruption of the membrane and eventually the cell death [31, 32]. In our research work here, we proposed molecular hybridization of an interesting thiazole moiety linked to hydrophilic guanidine part and terminal lipophilic part mediated by imine hydrogen bonding linker, **Figure 1**. This molecular design is explained in the view of the fragments to the glucosamine substrate of the GlcN-6-P synthase and the capability to bind similarly in the enzyme pocket. In **Figure 1**, the amidine moiety looks like the phosphate group and thiazole to the tetrahydropyran that accommodation the same binding regions that might responsible to inhibitory effect of the scaffold derivatives.

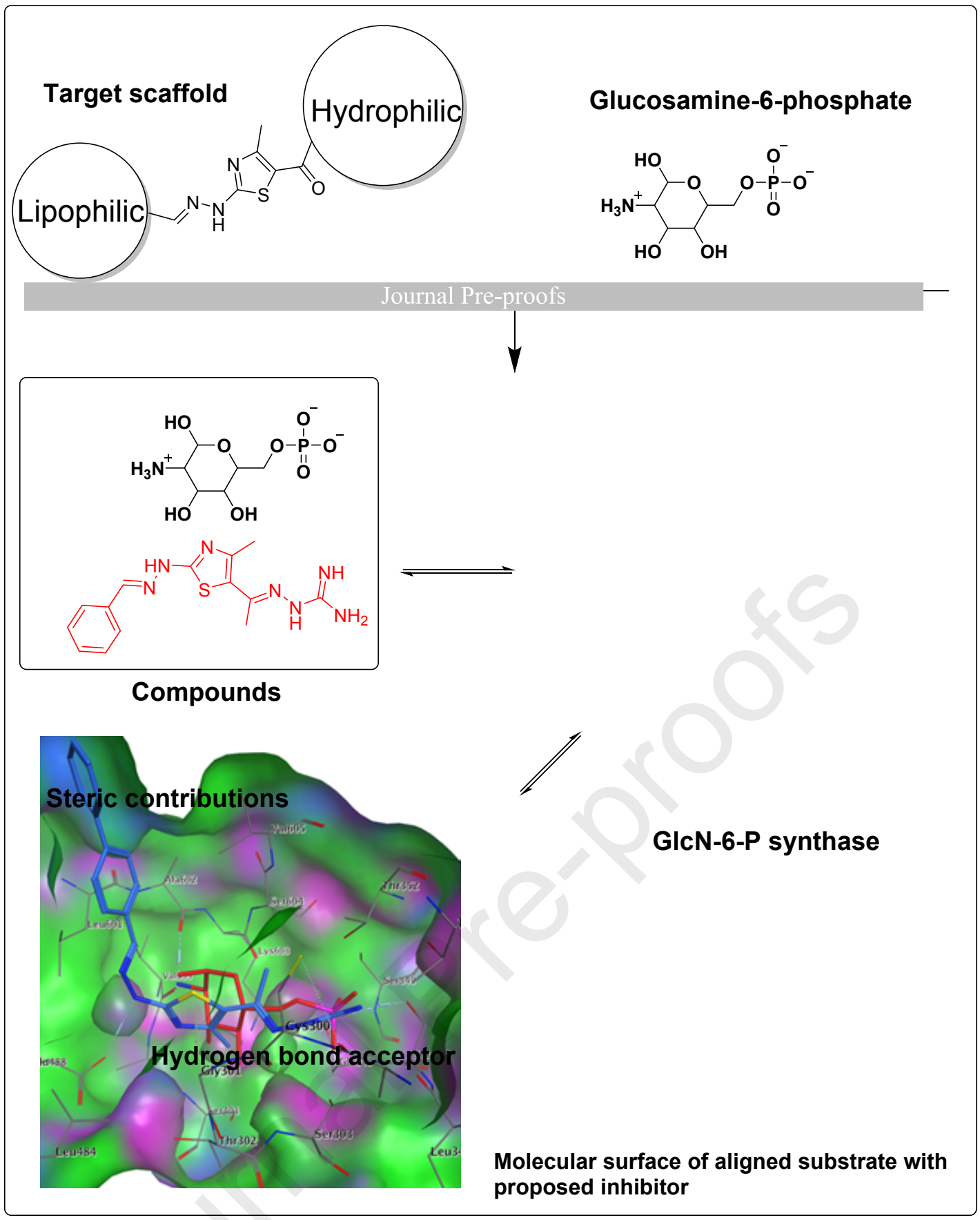


Figure 1. Rational discussion of study including the mapping of target scaffold inhibitor to that with the enzyme substrate and the binding site.

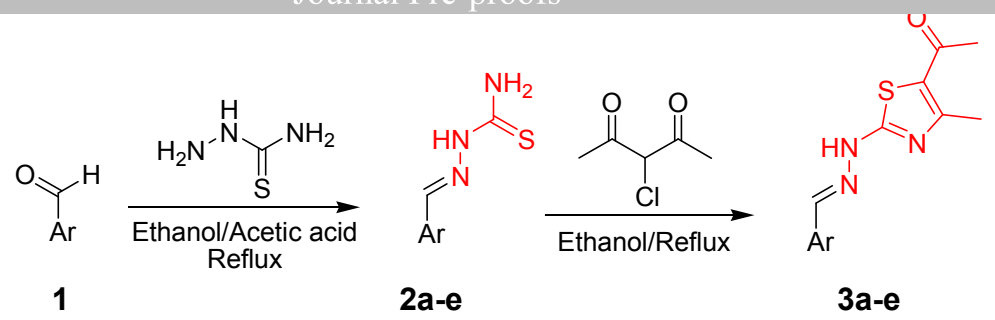
2. Results and discussions

2.1. Chemistry

2.1.1. Synthesis and Characterization

2-Benzylidenehydrazine-1-carbothioamides (**2a-e**) were synthesized by condensation of substituted aromatic aldehyde (1) with thiosemicarbazide (2) in ethanol (Scheme 1). The physical property, IR, ^{1}H NMR, ^{13}C NMR and MS in addition to the microanalysis (see Experimental section) are in support with the structures of the new compounds (**2a-e**) were confirmed by the analysis of IR, ^{1}H NMR and ^{13}C NMR spectroscopy in addition to the microanalysis (see Experimental section). ^{13}C NMR spectrum showed a requisite signal at δ = 177.71 ppm assignable to (C=S) group in support of the thione tautomeric form. The substituted 1-(2-(2-drevatives benzylidenehydrazineyl)-4-methylthiazol-5-yl)ethan-1-ones (**3a-e**) were prepared by heating a mixture of the substituted 2-benzylidenehydrazine-1-carbothioamides (**2a-e**) and 3-chloro-2,4-pentanedione in absolute ethanol, as illustrated in (Scheme 1). The structures of the new products (**3a-e**) were confirmed by the analysis of their IR, ^{1}H NMR and ^{13}C NMR spectroscopy in addition to the microanalysis (see Experimental section). The structures of the synthesized compounds (**3a-e**) were established on the basis of their analytical and

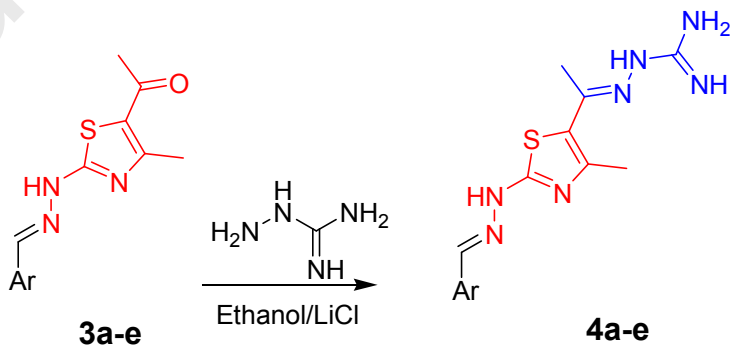
spectral data. The infrared spectra of compounds (**3a-e**) displayed sharp absorption band at 1666-1695 cm^{-1} which is a characteristic of the carbonyl group (C=O), The representative ^{1}H NMR spectrum of compounds (**3a-e**) (DMSO-d_6) showed two singlet signals at δ = 2.40 and 2.50 ppm, assigned to the two CH_3 protons. ^{13}C NMR spectrum showed a requisite signal at δ = 189.34 ppm assignable to (C=O) group.



Compound	Ar
a	4-Iodophenyl
b	4-Hydroxy, 3-Methoxyphenyl
c	3-Hydroxynaphthalene
d	Biphenyl-4-yl
e	3-Bromo-4,5-dimethoxy

Scheme 1. Synthesis of target compounds (**2a-e**) and (**3a-e**)

The hydrazinecarboximidamide derivatives (**4a-e**) (Scheme 2). In this procedure a suspension of compounds (**3a-e**) in ethanol and aminoguanidine hydrochloride in the presence of a catalytic amount of lithium chloride which was then refluxed for 24 hours. At the completion of the reaction the work-up gave compounds yeilds; **4a** (85%), **4b** (83%), **4c** (77%), **4d** (66%), and **4e** (87%). The structure of the compoundsd (**4a-e**) were confirmed by the analysis of their IR, ^{1}H NMR, ^{13}C NMR spectroscopy and LCMS in addition to microanalysis (see Experimental section).



Compound	Ar
a	4-Iodophenyl
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c	3-Hydroxynaphthalene
d	Biphenyl-4-yl
e	3-Bromo-4,5-dimethoxy

Scheme 2. Synthesis of hydrazinecarboximidamide derivatives (**4a-e**)

2.2. Biological screening

2.2.1. Antibacterial screening

All the newly synthesized compounds (**3a-e**) and (**4a-e**) and their precursors (**2a-e**) were screened for their antibacterial and antifungal activities via the agar diffusion well method [33] by recording the inhibition zones, and for the most effective derivatives, the minimum inhibitory concentrations (MIC)

and the minimum bactericidal concentrations (MBC) were determined by the serial dilution method [34]. The antimicrobial evaluation was done for all the three series of compounds varied from the benzylidenehydrazine-1-carbothioamide to the acetyl thiazole and passing to the final products thiazole-hydrazine-1-carboximidamide for clarifying the role of each reaction step in the activity and further showing the role of thiazole with and without the terminal carboximidamide. The activity of the synthesized compounds was tested against three Gram-positive bacteria (*Staphylococcus aureus* IFO 3060, *Bacillus subtilis* IFO 3007 and *Micrococcus luteus* IFO 3232), two Gram-negative bacteria (*Escherichia coli* IFO 3301 and *Pseudomonas aeuroginosa* IFO 3448), and three pathogenic fungi (*Candida albicans* IFO 0583, *Aspergillus oryzae* IFO 4177 and *Aspergillus niger* IFO 4414) with commercially available antibiotics from 3 different classes were used as positive controls; ampicillin, ciprofloxacin, and fluconazole, with GlcN-6-P synthase inhibitor FMDP1 for comparison [23]. The observed antimicrobial data of the target compounds and the reference drugs; the inhibition zone (IZ) and the minimum inhibitory and bactericidal concentrations (MIC, MBC) are given in **Tables 1** and **2**. The observed antimicrobial data *in vitro* indicated that a close to 90% of the tested compounds exhibited appreciable bacterial and fungal inhibition with inhibition zone range (10-36 mm) in comparison to the reference drugs.

In general, the synthesized compounds were more active against Gram-positive microorganism compared to the Negative ones. As shown in **Table1**, Compounds **2a-e** and **3a-e** showed moderate antibacterial activity against Gram-positive microorganism and weak activity against Gram-negative ones, with almost no activity against most of the tested fungal strains. On the other hand, the hydrazinecarboximidamide derivatives (**4a-e**) showed moderate to strong activity against Gram-positive tested microorganism with IZ from 12-36 mm. With respect to the activity against Gram negative microorganism compounds **4a-e** showed weak to moderated antimicrobial activity. Among this group Compound **4c** and **4d** were found to be the most active against all tested strains, except *P. aeruginosa*. Compound **4d** was the most active against *Staphylococcus aureus* with an inhibition zone (IZ = 34 mm) greater than that of the standard drug ampicillin (IZ = 28 mm) and equal to that of ciprofloxacin (IZ = 34 mm), this compound also showed high activity against *Bacillus subtilis* with an inhibition zone (IZ =36 mm) which is greater than the standard drug ampicillin (IZ = 30 mm) and almost equal to that of ciprofloxacin (IZ =38 mm) at the same tested concentration. In the case of the antibacterial activity against the Gram-positive bacteria, most of the novel final compounds were found to be comparable in activity to the reference drugs. Most of the *hydrazinecarboximidamide derivatives* described here revealed activity that was found to have good MIC and MBC values (0.5–16 µg/mL), as shown in **Table 2**. The best MIC and MBC value observed for **4d** with same value as the reference drug ciprofloxacin and even better values compared to that of Ampicillin followed by compound **4c.** A direct comparison could be made between highly active derivatives **4a-e** and less active ones **3a-e** a good correlation between activity and structural features could obtained. First and most profound effect was obtained after the addition of the hydrophilic group (guanidine) moiety significantly enhance the activity **4a-e** compared to **3a-e**. However, the **4a-e** derivatives showed difference antibacterial activity within the same group. The Lipophilicity is an important physicochemical parameter in the development of an antibacterial agent, and the aromatic ring structures produce additional affinity through hydrophobic interaction to the bacterial membrane and this could account for the high affinity for the gram positive compared to the gram-negative ones. From the structural point of view, it seems that the presence of the biphenyl ring at imine side chain of the thiazole ring produce derivative **4d** with the most promising antibacterial activity **(Scheme 2).** Also it revealed that the presence of polar group on hydrophobic side chain decrease the antibacterial activity as shown for **4c** compared to the most active one **4d.** Taken together, these data support the contention that both guanidine moiety and the terminal aryl moiety are required to get the high observed antibacterial activity**.**

2.2.2. Antifungal screening

Preliminary screening of the tested compound revealed good antifungal activity on the most with the final derivatives **4a-e,** with moderate activity was produce against *Aspergillus niger* and strong activity against both *Candida albicans* and Aspergillus *oryzae*. The best activity was observed for Compounds **4d** with IZ of 36 mm followed by 4c (IZ = 34 mm) then 4e (IZ = 26 mm), compared to the 21-mm IZ of the standard drug fluconazole against *Candida albicans,* same trend of activity was also observed against *Aspergillus oryzae* **(Table1).** The MIC and MBC values support the high

potency of the hydrazinecarboximidamide derivatives (**4a-e**) against the tested fungal species, compounds **4a-d** give better activity compared to the reference drug fluconazole (**Table 2**).

Table 1. Antimicrobial activity of the synthetic compounds (Inhibition Zone, IZ, diameter (mm)) (200 μg/8 mm disc).

ID	Diameter of Inhibition Zone (mm)							
	Gram positive			Gram negative		Fungi		
	S. aureus	*B. subtilis*	*M. luteus*	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. oryzae*	*A. niger*
Benzylidenehydrazine-1-carbothioamides								
2a	10	11	-	-	-	13	-	-
2b	24	22	12	12	10	25	-	-
2c	16	18	12	-	-	-	-	-
2d	18	20	14	14	12	-	-	-
2e	14	15	10	-	-	-	-	-
(4-Methylthiazol-5-yl)ethan-1-ones								
3a	12	14	-	-	-	-	-	-
3b	14	16	12	-	-	-	-	-
3c	14	16	12	-	-	-	-	-
3d	15	16	11	10	-	-	-	-
3e	16	18	12	16	12	14	12	-
((4-Methylthiazol-5-yl)ethylidene)hydrazinecarboximidamides								
4a	18	20	16	14	12	18	18	15
4b	20	24	12	16	-	14	10	-
4c	25	34	20	17	14	34	26	16
4d	34	36	18	18	10	36	25	18
4e	20	22	15	16	10	26	22	18
Reference drugs								
A	28	30	25	24	22	NT	NT	NT
C	34	38	32	38	36	NT	NT	NT
F	NT	NT	NT	NT	NT	21	22	24

- (-) Not active (<8 mm), Weak activity (8-12 mm), Moderate activity (12-16 mm), Strong activity (> 18 mm).
- Solvent: DMSO (8 mm). A; Ampicillin, C; Ciprofloxacin, F; Fluconazole.
- NT: Not tested.

Table 2. The minimum inhibitory and bactericidal concentrations (MIC, MBC in μg/mL) of the compounds initially screened against *S. aureus*, *B. subtilis*, *C. albicans*, and *A. oryzae* strains.

ID	MIC/MBC	*Gram positive bacteria*		*Fungi*	
		S. aureus	*B. subtilis*	*C. albicans*	*A. oryzae*
2b	MIC	2± 1.12	2± 0.14	8± 1.20	NT
	MBC	4± 0.55	4± 0.11	16± 1.41	NT
4a	MIC	8± 0.11	4± 0.11	4± 2.10	8± 0.47
	MBC	8± 0.10	8± 0.14	8± 1.10	16± 1.05
4b	MIC	4± 1.13	2± 0.10	8± 0.23	8± 1.32
	MBC	8± 0.14	4± 0.04	8± 0.54	8± 2.11
4c	MIC	2± 0.01	1± 0.09	4± 1.05	4± 0.35
	MBC	4± 1.11	2± 0.10	8± 2.25	8± 1.60
4d	MIC	1± 0.15	0.5± 0.15	4± 0.33	4± 0.33
	MBC	2± 0.13	1± 0.11	8± 0.51	8± 0.44
4e	MIC	4± 0.10	2± 0.10	8± 1.10	8± 1.48
	MBC	8± 0.52	4± 0.26	16± 0.27	16± 0.81
A	MIC	2± 0.11	1± 0.31	NT	NT
	MBC	4± 0.04	2± 0.20	NT	NT
C	MIC	1± 0.07	0.5± 0.11	NT	NT
	MBC	2± 0.12	1± 0.14	NT	NT
F	MIC	NT	NT	8± 0.32	4± 0.22
	MBC	NT	NT	16± 0.34	8± 0.36
FMDP[1]	MIC	64± 0.13	64± 0.51	64± 0.23	64± 0.25
	MBC	64± 0.10	64± 0.26	64± 0.41	64± 0.33

- Results are mean values from at least three experiments; **(NT)** Not Tested (not significant IZ).
- [1] FMDP "N3-(4-methoxyfumaroyl)-$_L$-2,3-diaminopropanoic acid" Data taken from reference [23]

- A; Ampicillin, C; Ciprofloxacin, F; Fluconazole.

2.2.2. Antibacterial Activity of selected compounds 4a-d against a panel of highly resistant strains

To further probe the activity of antimicrobial diamines **4a-d** as representative examples, these compounds were evaluated for the ability to kill human pathogenic strains; two Gram-positive bacteria including; methicillin-resistant Staphylococcus aureus (MRSA), Clostridium difficile (C.D), two Gram-negative bacteria including; two Escherichia coli isolates (E.C TM, E.C WT) *in vitro*. The data obtained are compared to control drugs (gentamicin, linezolid, vancomycin of the compounds and the reference drugs are given in Tables 3. The results of the *in vitro* antimicrobial screening revealed that, the series of **4a-d** exhibited promising Gram-positive antibacterial and low Gram-negative inhibitory effects. Table 2 showed that the compounds **4d** has best activity towards Gram-positive bacteria (E.C TM) with MIC 32 μg/mL. Whereas, the other derivatives **4a-c** showed weak inhibitory activity against both of bacteria, MIC 140 μg/mL. This performance is consistent with the results of antimicrobial assay on standard strains. SAR analysis proved that derivative with biphenyl lipophilic tail could inhibit the more resistant MRSA type.

Table 3. The minimum inhibitory concentration (MIC in μg/mL) of the compounds initially screened against *methicillin-resistant Staphylococcus aureus*, *Escherichia coli* and *Clostridium difficile* isolates.

Comp. ID	*S. aureus NRS 384 (MRSA USA 300)*	*E. coli JW55031 (TolC mutant strain)*	*E. coli BW525113 (wild type strain)*	*C. difficile (ATCCBAA1870)*
4a	128	130	145	150
4b	128	130	140	150
4c	128	130	130	150
4d	32	128	130	150
Linezolid	1	8	65	NT
Vancomycin	1	NT	NT	1
Gentamicin	NT	1	1	NT
Metronidazole	NT	NT	NT	0.25

[1]NT: Not tested

2.2.2. Cytotoxicity assay

The cytotoxicity of compound **4d** was also monitored in two mammalian cell lines, the HEK293T human kidney embryonic cell line and the A549 human alveolar basal epithelial adenocarcinoma cell line. The results of these studies are shown in **Table 4**. The selectivity index (SI) in each cell line was the ratio of the IC_{50} value of cytotoxicity against a given human cell line divided by the MIC values for given bacterial strains. Compound **4d** possessed a favorable SI between 0.5 and 100 against *S. aureus*, *B. subtilis*, *C. albicans*, and *MRSA* isolates. The results overall indicated that compound is selectively kill bacterial cells other than the human cells in most of cases. The SI against *MRSA* was lower (either 0.55 or 1.56), suggesting that additional structure optimization is required to identify a compound that kills *MRSA* strain with an acceptable SI.

Table 4. Cytotoxicity of Compound **4d** in immortalized human keratinocytes and normal human diploid fibroblasts

Organism type	MBC/MIC, μg/mL	Selectivity index[a]	
		HaCaT	**WI38**
Staphylococcus aureus IFO 3060	1	49.97	17.70
Bacillus subtilis IFO 3007	0.5	99.94	35.4
Candida albicans IFO 0583	4	12.49	4.42
MRSA USA 300	32	1.56	0.55

[a] Selectivity index = IC_{50}/MIC. The IC_{50} value of compound **4d** against normal HaCaT and WI38 cells were 49.97 and 17.70 μg/mL respectively. The IC_{50} value = the concentration that reduced HaCaT or WI38 cell viability by 50%.

2.2.3. Mechanism of Action of target compounds

The overall theory analysis of mechanism of action of these analogues are owing to their structure which bears variable lipophilic parts and terminal hydrophilic one. The lipophilic systems were in favor of assembly on the membrane due to the lipophilicity aiding in the embedding of the compound inside the cell. This emine leading to inhibition of cell wall. We hypothesized that the thicker, peptidoglycan-rich Gram-positive cell wall was prevented more than Gram-negative one and this was clear from the observed antimicrobial data. In addition, the mechanism of action of compound **4d** was investigated and fast killing activity was an indicative of the membrane-targeting activities, which could be mediated by an electrostatic interaction between the positively charged central nitrogens in **4d** and other analogues, and the negatively charged bacterial membrane. This hypothesis is consistent with the fact that positively charged antibacterial peptides can disrupt bacterial membrane integrity and depolarize bacterial membranes [32]. To test this hypothesis, Nikon Eclipse Ci, Japan microscopy was subsequently used to visualize bacterial cell morphology at high resolution and provide direct evidence of the membrane effects caused by **4d**. Untreated S. aureus (**Figure 2A**) and C. albicans (**Figure 2C**) possessed an intact cell envelope with both a well-defined peptidoglycan cell wall and intact membrane. Following treatment with **4d** at 2× MIC for 30–60 min, S. aureus formed numerous spherical, double layered mesosome-like structures (**Figure 2A**). Cellular debris of completely lysed cells was also observed after the treatment with 3 (**Figure 2B**). Similarly, C. albicans treated with 4d at 2× MIC (15 min) exhibited extensive membrane damage, as illustrated by the appearance of lysed cells and the release of cell contents (**Figure 2B and 2D**).

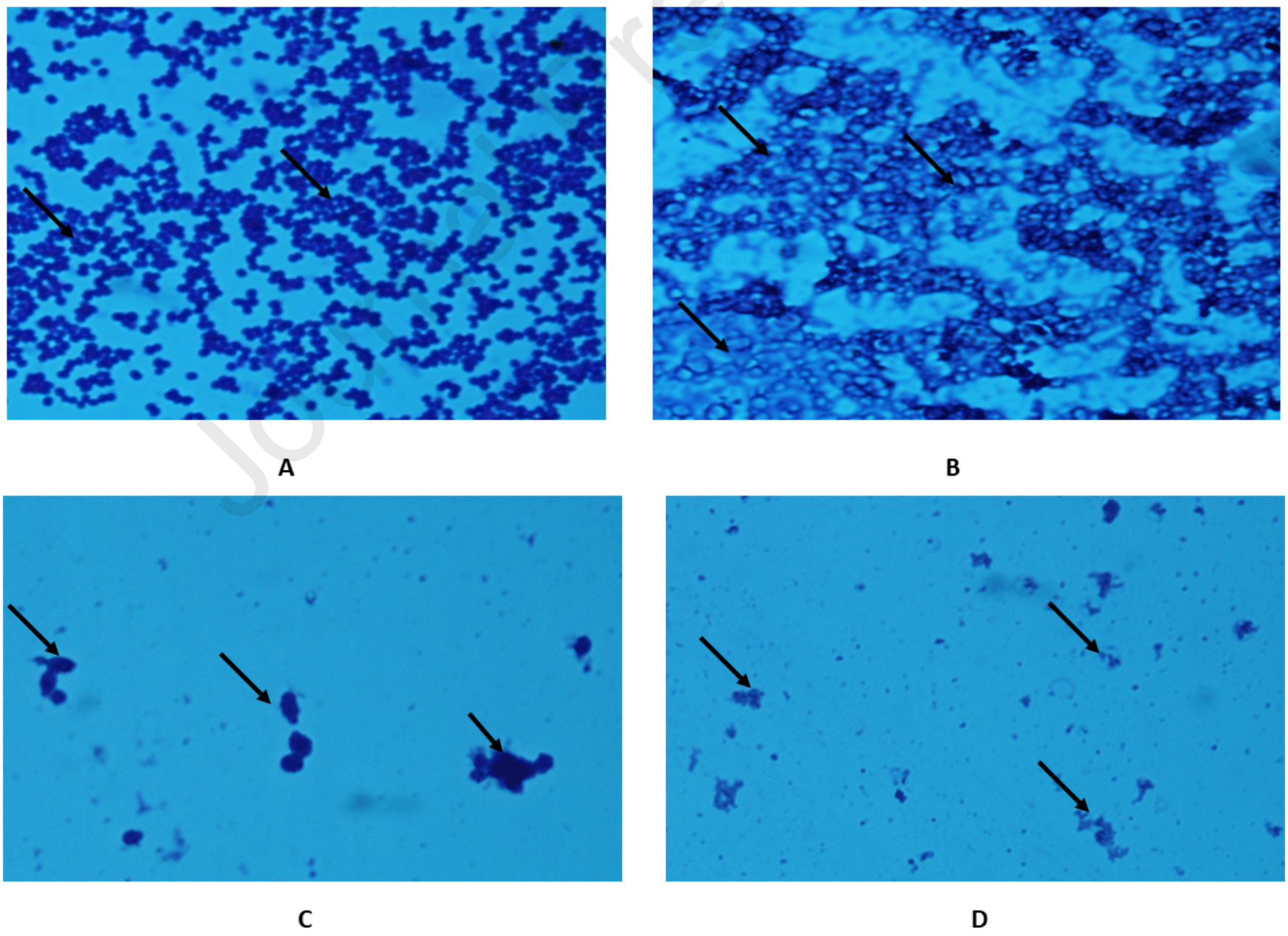


Figure 2. Confocal microscopy experiments performed on **A**) untreated S. aureus and **B**) Treated S. aureus ,**C**) Untreated C. albicans **D**) treated C. albicans, with 2× its' MIC **4d** analog and stained with Gram-stain using Nikon Eclipse Ci, Japan microscope. All images have been captured using a ×100 oil immersion objective. The areas of membrane damage and cell content release are marked with black arrows and clear from morphological analyses.

2.3. *In vitro GlcN-6-P Synthase* activity analysis

Glucosamine 6-P Synthase is considered an essential enzyme involved in the peptidoglycan N-acetyl-glucosamine (NAG) precursor. In this work, we introduced novel compounds bearing polar amidine motif similar to glucosamine and assumed to accommodate similar interactions as the substrate does with the enzyme. Hence, *in vitro* inhibitory analysis of the novel compounds was done, and the results are shown in **Table 5.** As shown in **Table 5**, the inhibitory activity of all synthesized compounds was evaluated against the GlcN-6-P Synthase, displaying IC50 in the micromolar range with high comparative results as that obtained for gentamicin. Highest inhibitory activity was obtained by **3e** which produce 50% inhibition of the enzyme at 1.25 μM for compound **3e**. Compounds **4a-e** consider the best activity when compared to both gentamicin as well as FMDP ''the selective in activator of GlcN-6-P Synthase''. This improvement in the inhibitory activity of the hydrazinecarboximidamide derivatives (**4a-e**) could be account due to the impact of the guanidine moiety which would interact with the enzyme on the same manner as the phosphate group do. Out of these final compounds, compound **4c** and **4d** give the best inhibitory activity which in turn very supportive to their antibacterial activity observed before as seen in **Table 1**, and **2** which also supported by their docking interaction **Figure 3**.

Table 5. *In vitro* inhibitory *GlcN-6-P Synthase* activity data for new compounds.

Compounds	IC_{50} (μM)
2a	8.63±0.21
2b	77.14±1.93
2c	15.26±0.38
2d	8.20±0.10
2e	7.18±0.10
3a	5.81±0.14
3b	4.66±0.12
3c	17.19±0.43
3d	3.72±0.093
3e	1.25±0.031
4a	4.06±0.10
4b	5.15±0.12
4c	3.87±0.097
4d	3.47±0.087
4e	4.93±0.12
Gentamicin	3.41±0.08
FMDP[1]	4.03 ± 0.26

- Data are presented as average IC_{50} ± SD (μM) values for at least three experiments.
- [1]FMDP data was reported [23].

2.3. Molecular properties and pharmacokinetic profiling

PK properties such as absorption, distribution, metabolism, excretion and toxicity (ADMET) profiling of compounds were determined using the pkCSM ADMET descriptors algorithm protocol [35-37]. Two important chemical descriptors correlate well with PK properties, the 2D polar surface area (PSA_2D, a primary determinant of fractional absorption) and the lipophilicity levels in the form of atom-based LogP. The absorption of drugs depends on factors including membrane permeability [indicated by colon cancer cell line (Caco-2)], intestinal absorption, skin permeability levels, P-glycoprotein substrate or inhibitor. The distribution of drugs depends on factors that include the blood–brain barrier (logBB), CNS permeability, and the volume of distribution (VDss). Metabolism is predicted based on the CYP models for substrate or inhibition (CYP2D6, CYP3A4, CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4). Excretion is predicted based on the total clearance model and renal OCT2 substrate. The toxicity of drugs is predicted based

on AMES toxicity, hERG inhibition, hepatotoxicity, and skin sensitization. These parameters were calculated for potent **4c**, **4d**, and **2b** and inactive **2e** analogs compared to marketed drugs ampicillin and fluconazole and checked for compliance with their standard ranges. After calculating the ADMET properties (**Table 6**), we can verify that the ligands **2b**, **4c**, **4d** and the reference ampicillin have a low percentage of human intestinal absorption (69.96%, 71.15%, 79.77%, 45.09% respectively), as they have very hydrophilic groups in their chemical structures, which would make it difficult to pass through the biological membranes [37]. Conversely, **2e** and fluconazole have human intestinal absorption values above 90%, demonstrating that these compounds may have good oral bioavailability when tested experimentally [37]. Analyzing the central nervous system (CNS) permeability, **2e**, **4c** and **4d** have good penetration in the CNS (Log PS> -2.0), while **2b**, ampicillin and fluconazole are unable to penetrate the CNS (Log PS <-3.0) [37]. These data may help that **2b**, ampicillin and fluconazole present lower side effects in the (CNS) compare **2e**, **4c** and **4d**, contributing to a better tolerability among patients. It was also possible to observe that only **4d** is able to inhibit the cytochrome P450, CYP3A4 isoform, the main enzyme responsible for drug metabolism. This possibly occurs because these ligands exhibit high lipophilicity and molecular size, thus favoring the inhibition of this enzyme and may lead to interactions with other drugs metabolized by this CYP and may cause serious adverse effects [37]. The excretion was evaluated as Total Clearance. This parameter is related to bioavailability and is important in determining dosage rates to achieve steady state concentrations. Our data demonstrated that **2e**, **4c**, ampicillin and fluconazole exhibit the higher Total Clearance values when compared to other ligands. Thus, these compounds, due to their high hydrophilicity, could be excreted rapidly by the kidneys, requiring shorter administration intervals to maintain desired therapeutic concentrations. The last parameter analyzed in our studies was hepatotoxicity. In **Table 6**, we can verify that no ligand showed hepatotoxic effects except **4c**. Therefore, these ligands of better interaction with TcTR may not have a physiological or pathological event that could be associated with hepatic injury, suggesting that these compounds may be well tolerated by the liver when they are tested in a biological environment especially the **4d** analogue.

Table 6. Predicted ADMET properties of selective target compounds.

Parameter	2e	2b	4c	4d	Ampicillin	Fluconazole
Molecular properties						
Molecular Weight	318.2	225.3	381.5	391.5	349.4	306.3
LogP	1.6	0.6	2.9	3.8	0.31	0.74
Rotatable Bonds	4	3	5	6	4	5
Acceptors	4	4	7	6	5	7
Donors	2	3	5	4	3	1
Surface Area	113	93	160	167	143	123
Absorption						
Water solubility	-3.196	-2.197	-3.036	-3.06	-2.43	-2.788
Caco2 permeability	0.696	0.665	-0.724	-0.531	0.398	1.213
Intestinal absorption (human)	91.068	69.968	71.157	79.771	45.097	95.275
Skin Permeability	-3.133	-3.407	-2.735	-2.735	-2.735	-2.756
P-glycoprotein substrate	No	No	Yes	Yes	No	No
P-glycoprotein I inhibitor	No	No	No	Yes	No	No
P-glycoprotein II inhibitor	No	No	Yes	Yes	No	No
Distribution						
VDss (human)	-0.276	-0.245	1.07	0.961	-1.114	-0.112
Fraction unbound (human)	0.416	0.519	0.009	0	0.759	0.269
BBB permeability	-0.591	-0.631	-1.532	-1.337	-0.861	-1.088
CNS permeability	-2.973	-3.004	-2.596	-2.383	-3.145	-3.235
Metabolism						
CYP2D6 substrate	No	No	Yes	No	Yes	No
CYP3A4 substrate	No	No	Yes	No	Yes	No
CYP1A2 inhibitor	Yes	No	Yes	Yes	No	Yes
CYP2C19 inhibitor	No	No	No	No	No	No
CYP2C9 inhibitor	No	No	No	No	No	No
CYP2D6 inhibitor	No	No	Yes	Yes	No	No
CYP3A4 inhibitor	No	No	No	Yes	No	No
Excretion						
Total Clearance	0.059	-0.03	0.138	-0.056	0.327	0.37
Renal OCT2 substrate	No	No	No	Yes	No	No
Toxicity						
AMES toxicity	No	No	No	Yes	No	No
Max. tolerated dose (human)	0.86	0.747	0.451	0.459	1.222	0.316
hERG I inhibitor	No	No	No	Yes	No	No

hERG II inhibitor	No	No	Yes	Yes	No	Yes
Oral Rat Acute Toxicity (LD_{50})	3.25	2.636	2.423	2.195	1.826	2.227
Oral Rat Chronic Toxicity (LOAEL)	1.971	1.6	1.779	1.725	1.594	1.079
Hepatotoxicity	No	No	No	Yes	Yes	Yes
Skin Sensitization	Yes	Yes	No	No	No	No
T. Pyriformis toxicity	1.251	0.2	0.289	0.293	0.285	0.298
Minnow toxicity	2.07	2.318	5.586	5.383	3.411	3.547

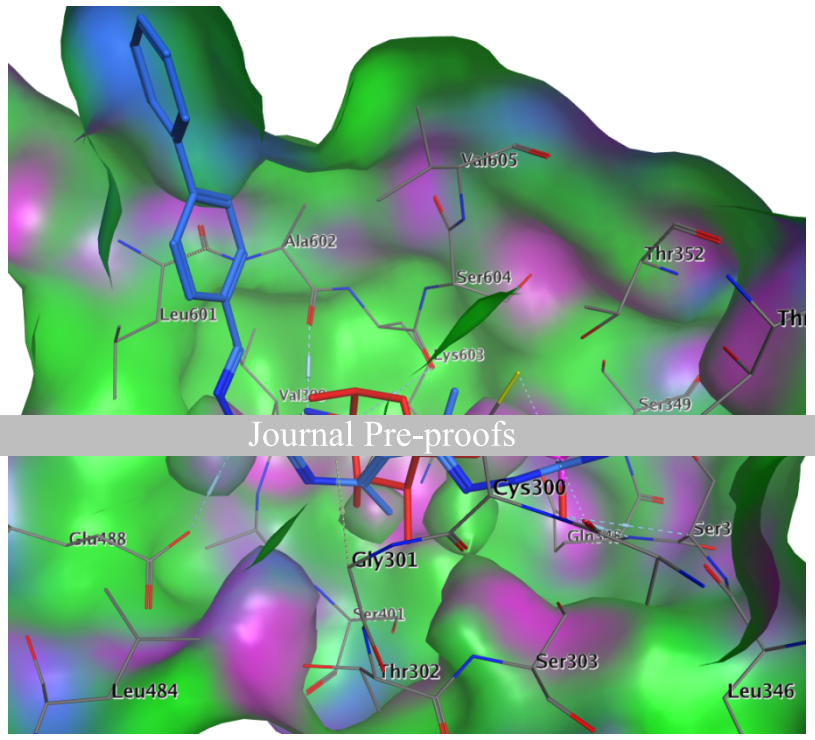
- http://biosig.unimelb.edu.au/pkcsm/prediction

2.4. Molecular docking studies

For the docking calcule-6-phosphate synthase in complex with glucosamine-6- phosphate were utilized by the Autodock 4.2.3 package [38] as a receptor model for interaction analysis. Ligand was built by means of MOE2012 package [39], the geometry was optimized with the CHARMm forcefield and then prepared for docking calculations with the python scripts available in the Autodock package. For the compound **4d**, 50 runs were performed, and the resulting poses were clustered with 1.8 Å tolerance. Lamarckian GA was used for the conformational space search with initial population set to 150, and fitness function evaluations set to 25000000. The most abundant low energy clusters were selected for analysis. As shown in **Figure 3**, the docking simulations suggested that the compound **4d** interact with the active site in a fashion similar to the glucosamine bound substrate observed in the reported crystal structure of the target enzyme, **Figure 3**. Firstly, the placement of substrate in the complex, **Figure 3A**, consists of a long, narrow tunnel, leading to a cavity that contains the phosphate terminal polar part surrounded by set of hydrophilic amino acid residues showing group of hydrogen bonding interactions.

The group of thiazole-based analogues as GlcN-6-P synthase inhibitors mimic the transition state of the reaction taking place in the enzyme's C-terminal sugar isomerizing domain which consider to be transition state inhibitor which is known to be more effective type of inhibitors. The interaction map of target compound **4d** exhibited pivotal hydrogen bonding of amidine motif to the corresponding amino acid residues; Ser347 and Gln348 with consistent distance 3.01 Å compared to glucosamine-6-phosphate in the polar site, **Figure 3B**. In addition, 2-amino thiazole fragment as antimicrobial scaffold shared the interaction with its chelation part through Sulphur and amino atoms with Glu488 that stabilize the compound within the binding pocket. Moreover, terminal lipophilic part of biphenyl accommodates the hydrophobic pocket formed of Leu601, Val605, and Val399 that correctly oriented within the substrate sugar, **Figure 3C**. Overall, the docking simulation of potent analog **4d** could explain the *in vitro* activity of the thiazole design involving two essential part; lipophilic aromatic and terminal polar amidine parts which contributes strongly in the substrate pocket.

A **B**



C

Figure 3. **Docking modes of active compounds in the binding pocket of GlcN-6-P synthase**. Interactions between the protein (PDB ID: 2VF5), the interactions of compound **4d** and the target substrate are shown as dotted lines; the residues as colored codes as presented in the software; and the ligands as black line models. **(A)** *2D* Predicted binding mode of substrate with GlcN-6-P synthase. **(B)** *2D* Predicted binding mode of **4d** inhibitor with GlcN-6-P synthase. **(C)** *3D* aligned map of **4d** (blue sticks) and glucosamine-6-phosphate (red sticks) inside the pocket.

3. Conclusions

In this study, we designed and synthesized a series of thiazole derivatives as potential GlcN-6-P synthase inhibitors. Most of the derivatives including acetylthiazole **3a-e** and thiazole-carboximidamide **4a-e** target the enzyme and inhibit it through competing with the glucosamine substrate and the IC_{50} values were determined to be in the micromolar range. One of these compounds **4d** exhibit significant antimicrobial activity with MIC/MBC 1-8 μg/mL with prominent pharmacokinetic profile compared to reference marketed drugs. Interestingly, the mechanism of such derivatives on cell wall was imaged and linked to the disruption of peptidoglycan formation. Computational docking studies were done for binding interaction profile for the potent **4d** compound as a representative example of this class and confirming the role of each fragment in target activity. Moreover, the cytotoxicity profiling reported that none of the compounds have normotoxicity activity against human normal cells. Molecular docking. Finally, the compounds identified here can be considered as initial leads for the development of improved antibacterial agents.

4. General Chemistry

4.1. Chemistry

The starting reagents and solvents were purchased from the Aldrich chemical company and were used as received. The melting points were determined with a Mel-Temp apparatus and are uncorrected. All the new compounds were characterized by the ^{1}H-NMR and ^{13}C-NMR, using a ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra, and were measured in DMSO at room temperature. The chemical shifts (δ) were reported in ppm to a scale calibrated for tetramethylsilane (TMS), which is used as an internal standard. The IR spectra were recorded on a KBr disc on a Schimadzu 8201 PC, FTIR spectrophotometer (ν max in cm^{-1}). Melting points (Mp) were determined in open capillary tubes using Electrothermal apparatus (Stuart, UK) and are uncorrected. HPLC-Mass Spectrometry were performed on Agilent 1100 / ZQ MSD including C18 column and diod-array UV detector. The mobile phase (containing 0.01 M ammonium acetate) was gradient starting from 20% acetonitrile/80% water to 80% acetonitrile/20% water. Purities are reported according to percentage of Peak Areas at wavelength 254 nm. The follow up of the reactions and the check of the purity of the compounds were made by the TLC on silica gel-protected aluminum sheets (Type 60 GF254, Merck), and the spots were detected by exposure to a UV-lamp at λ 254 nm for a few seconds.

4.1.1. General procedure for the synthesis of substituted 2-benzylidenehydrazine-1-carbothioamides (2a-e)

The appropriate aldehyde (1.1 mmol) and thiosemicarbazide (1.0 mmol) was dissolved in ethanol (10 ml) with addition of glacial acetic acid (0.2 mmol). The reaction mixture was refluxed for 5-6 hours and then cooled to room temperature. The resulting precipitate was filtered, washed by ether and recrystalized from ethanol to obtain the corresponding thio-semicarbazones, **(2a-e)**. All the compounds were characterized by spectral techniques and recrystallized from absolute ethanol to give the pure products (**2a-e**).

2-(4-Iodobenzylidene)hydrazinecarbothioamide (2a)

Yellow crystals from EtOH (83%), m.p. 145-146 °C; IR (KBr) cm^{-1}: υ_{max} 3401, 3180 (N-H, NH_2), 1602 (C=N); ^{1}H-NMR (DMSO-d_6 400 MHz) δ (ppm): 7.59-8.23 (4H, m, Ar-H + 2H, NH_2 +1H, C-H=), 11.48 (1H, s, NH, D_2O exch.); ^{13}C-NMR (DMSO-d_6 100 MHz) δ (ppm): 96.86, 129.53, 129.50, 134.10, 137.92, 137.95, 141.71, 178.26 (C=S) ppm; HRMS (m/z) 306.0556 [M+H]+, calcd 305.1386; Anal. Calcd for $C_8H_8IN_3S$: C, 31.49; H, 2.64; N, 13.77. Found: C, 31.48; H, 2.64; N, 13.76%.

2-(4-Hydroxy-3-methoxybenzylidene)hydrazinecarbothioamide (2b)

Red crystals from EtOH (78%), m.p. 150-152 °C; IR (KBr) cm^{-1}: υ_{max} 3480 (OH), 3250, 3180 (N-H, NH_2), 1605 (C=N); ^{1}H-NMR (DMSO-d_6 400 MHz) δ (ppm): 3.82 (3H, s, OCH_3), 7.59-8.23 (3H, m, Ar-H + 2H, NH_2, 1H, C-H=), 9.56 (1H, bs, OH), 11.26 (1H, s, NH, D_2O exch.); ^{13}C-NMR (DMSO-d_6 100 MHz) δ (ppm): 56.17, 109.62, 115.63, 122.85, 126.01, 143.46, 148.51, 149.22. 177.71 (C=S) ppm; HRMS (m/z) 225.0566 [M+H]+, calcd 225.0566; Anal. Calcd for $C_9H_{11}N_3O_2S$: C, 47.99; H, 4.92; N, 18.65. Found: C, 47.98; H, 4.95; N, 18.65%.

2-[(2-Hydroxynaphthalen-1-yl)methylidene]hydrazinecarbothioamide (2c)

Orange crystals from EtOH (92%), m.p. 136-137 °C; IR (KBr) cm^{-1}: υ_{max} 3375 (OH), 3250, 3154 (N-H, NH_2), 1602 (C=N); ^{1}H-NMR (DMSO-d_6 400 MHz) δ (ppm): 7.19-9.03 (6H, m, Ar-H + 2H, NH_2, 1H, C-H=), 10.56 (bs, 1H, OH, D_2O exch.), 11.41 (1H, s, NH, D_2O exch.); ^{13}C-NMR (DMSO-d_6 100 MHz) δ (ppm): 110.25, 118.76, 123.46, 123.98, 128.44, 128.53, 129.18, 131.93, 133.06, 143.52, 157.12, 177.63 (C=S) ppm; HRMS (m/z) 246.0695 $[M+H]^+$, calcd 245.0617; Anal. Calcd for $C_{12}H_{11}N_3OS$: C, 58.76; H, 4.52, N, 17.13. Found: C, 58.75; H, 4.55; N, 17.15 %.

2-(Biphenyl-4-ylmethylidene)hydrazinecarbothioamide (2d)

Orange crystals from EtOH (75%), m.p. 133-134 °C; IR (KBr) cm^{-1}: υ_{max} 3320, 3154 (N-H, NH_2), 1610 (C=N); ^{1}H-NMR (DMSO-d_6 400 MHz) δ (ppm): 7.44-8.28 (9H, m, Ar-H + 2H, NH_2, 1H, C-H=), 11.53 (1H, s, NH, D_2O exch.); ^{13}C-NMR (DMSO-d_6 100 MHz) δ (ppm): 126.00, 126.10, 126.21, 127.20, 127.26, 127.29, 128.38, 128.53, 132.60, 132.62, 138.67, 140.65, 141.27, 177.18 (C=S) ppm; HRMS (m/z) 256.0913 [M+H]+, calcd 255.0824; Anal. Calcd for $C_{14}H_{13}N_3S$: C, 65.85, H, 5.13, N, 16.46. Found: C, 65.82; H, 5.15; N, 16.65%.

2-(3-Bromo-4,5-dimethoxybenzylidene)hydrazinecarbothioamide (2e)

Orange crystals from EtOH (92%), m.p. 136-137 °C; IR (KBr) cm^{-1}: υ_{max} 3150, 3105 (N-H, NH_2), 1607 (C=N); ^{1}H-NMR (DMSO-d_6 400 MHz) δ (ppm): 3.80 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 7.15-8.36 (2H, m, Ar-H + 2H, NH_2, 1H, C-H=), 11.52 (1H, s, NH, D_2O exch.); ^{13}C-NMR (DMSO-d_6 100 MHz) δ (ppm): 56.47, 58.21, 109.60, 115.65, 125.38, 141.77, 149.06, 151.51, 178.14 (C=S) ppm; HRMS (m/z) 318.1979 [M+H]+, calcd 318.1902; Anal. Calcd for $C_{10}H_{12}BrN_3O_2S$: C, 37.75, H, 3.80, N. 13.21. Found: C, 37.51; H, 3.30; N, 13.18%.

4.1.2. General procedure for the synthesis of substituted 1-(2-(2-drevatives benzylidenehydrazineyl)-4-methylthiazol-5-yl)ethan-1-ones (3a-e)

The thiosemicarbazone derivatives (**2a-e**) (1.0 mmol) and α-chloropentanedione (1.1 mmol) were dissolved in ethanol (10 ml). The reaction mixture was refluxed for 1-3 hours and then was cooled to room temperature. The resulting precipitate was filtered, washed with cold ethanol and recrystallized from ethanol to obtain the corresponding 1-(2-(2-drevatives benzylidenehydrazineyl)-4-methylthiazol-5-yl)ethan-1-ones (**3a-e**) in analytically pure form. All the compounds were characterized by spectral techniques and the data confirm the proposed structures.

a
1-(2-(2-(4-Iodobenzylidene)hydrazinyl)-4-methylthiazol-5-yl)ethanone (3a)

Antique white solid from EtOH (85%). m.p. 189-191°C; IR (KBr) cm^{-1}: υ_{max} 3401, 3180 (N-H), 1665 (C=O), 1602 (C=N); ^{1}H-NMR (DMSO-d_6 400 MHz) δ (ppm): 2.41 (s, 3H, CH_3), 2.51 (s, 3H, C=O-CH_3), 7.48 (d, *J*- 8.08 Hz, 2H), 7.82 (d, *J*- 8.08 Hz, 2H), 8.05 (s, 1H, CH=), 12.63 (bs, 1H, N-H, D_2O exch.); ^{13}C NMR (DMSO-d_6 100 MHz) δ (ppm): 17.54, 29.98, 97.04, 105.12, 128.99, 129.06, 133.97, 138.18, 138.20, 143.32, 156.74, 170.65, 189.60; HRMS (m/z) 386.9704 [M+H]+, calcd 385.2233; Anal. Calcd for $C_{13}H_{12}IN_3OS$: C, 40.53, H, 3.14, N, 10.91. Found: C, 40.51; H, 3.20; N, 10.90%.

1-(2-(2-(4-Hydroxy-3-methoxybenzylidene)hydrazinyl)-4-methylthiazol-5-yl)ethanone (3b)

Light yellow from EtOH (88% yield). m.p. 226-229 °C; IR (KBr) cm^{-1}: υ_{max} 3480 (OH), 3250, 3180 (NH), 1666 (C=O), 1612 (C=N); ^{1}H-NMR (DMSO-d_6 400 MHz) δ (ppm): 1.96 (s, 3H, CH_3), 2.40 (s, 3H, C=O-CH_3), 3.82 (s, 3H), 8.10 (s, 1H, CH=), 7.97 (s, 1H), 7.47 (d, *J*-8.0, 1H), 6.85 (d, *J*-8.0, 1H), 10.87 (s, 1H, OH, D_2O exch.), 11.88 (s, 1H, NH, D_2O exch.); ^{13}C NMR (DMSO-d_6 100 MHz) δ (ppm): 17.24, 29.63, 66.80, 109.97, 118.83, 122.91, 124.05, 128.31, 131.70, 133.18, 147.71, 158.10, 167.50, 189.20; HRMS (m/z) 306.0868 [M+H]+, calcd 305.3522; Anal. Calcd for $C_{14}H_{15}N_3O_3S$: C, 55.07, H, 4.95, N, 13.76. Found: C, 55.10; H, 4.91; N, 13.75%.

1-(2-(2-((2-Hydroxynaphthalen-1-yl)methylene)hydrazinyl)-4-methylthiazol-5-yl)ethanone (3c)

Light yellow from EtOH (78% yield). m.p. 226-229 °C; IR (KBr) cm^{-1}: υ_{max} 3380 (OH), 3250, 3180 (NH), 1695 (C=O), 1607 (C=N); ^{1}H-NMR (DMSO-d_6 400 MHz) δ (ppm): 2.47 (s, 3H, CH_3), 2.38 (s, 3H, C=O-CH_3), 8.10 (s, 1H, CH=), 7.24-8.63 (m, 6H, Ar), 10.87 (bs, 1H, OH, D_2O exch), 11.70 (s, 1H, NH, D_2O exch.); ^{13}C NMR (DMSO-d_6 100 MHz) δ (ppm): 17.30, 28.97, 110.04, 113.54, 116.92, 123.91, 125.05, 128.31, 130.70, 132.88, 140.22, 145.71, 156.51, 159.24, 168.50, 189.34; HRMS (m/z) 326.0919 [M+H]+, calcd 325.3849; Anal. Calcd for $C_{17}H_{15}N_3O_2S$: C, 62.75, H, 4.65, N, 12.91. Found: C, 62.71; H, 4.63; N, 12.95%.

1-(2-(2-(Biphenyl-4-ylmethylene)hydrazinyl)-4-methylthiazol-5-yl)ethanone (3d)

Light green solid from EtOH (80% yield). m.p. 242-245 °C; IR (KBr) cm^{-1}: υ_{max} 3320, 3154 (NH), 1695 (C=O), 1607 (C=N); ^{1}H-NMR (DMSO-d_6 400 MHz) δ (ppm): 2.51 (s, 3H,), 2.46 (s, 3H, C=O-CH_3), 8.15 (s, 1H, CH=), 7.78-7.39 (m, 9H, Ar), 12.58 (s, 1H, NH D_2O exch.); ^{13}C NMR (DMSO-d_6 100 MHz) δ (ppm): 16.93, 27.80, 125.98, 126.82, 127.31, 128.54, 129.22, 129.51, 130.70, 131.05, 132.6, 140.45, 144.12, 145.71, 156.51, 159.24, 168.50, 189.34; HRMS (m/z) 335.1092 [M+H]+, calcd 335.4228; Calcd for $C_{19}H_{17}N_3OS$: C, 68.03, H, 5.11, N, 12.53. Found: C, 68..03; H, 5.09; N, 12.49%.

1-(2-(2-(3-Bromo-4,5-dimethoxybenzylidene)hydrazinyl)-4-methylthiazol-5-yl)ethanone (3e)

Light yellow solid from EtOH (88% yield). m.p. 177-180 °C; ; IR (KBr) cm^{-1}: υ_{max} 3150, 3105 (NH); ^{1}H-NMR (DMSO-d_6 400 MHz) δ (ppm): 2.40 (s, 3H), 2.50 (s, 3H, CH_3), 3.80 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 8.34 (s, 1H,C-H=), 7.39-7.24 (m, 2H, Ar), 11.52 (1H, s, NH D_2O exch.); ^{13}C NMR (DMSO-d_6 100 MHz) δ (ppm): 17.20, 28.98, 62.58, 110.25, 115.83, 122.91, 124.05, 143.3, 145.31, 153.51, 156.10, 168.34, 189.68; HRMS (m/z) 398..9939 [M+H]+, calcd 398.2748; Anal. Calcd for $C_{15}H_{16}BrN_3O_3S$: C, 45.24, H, 4.05, N, 10.55. Found: C, 45.52; H, 3.99; N, 10.63%.

4.1.3. General procedure for the synthesis of substituted 2-(1-(2-(-2-(4-benzylidene)hydrazinyl)-4-methylthiazol-5-yl)ethylidene)hydrazinecarboximidamides (4a-e)

The thiazole derivatives (**3a-e**) (0.954 mmol) was dissolved in absolute ethanol (50 mL), and aminoguanidine hydrochloride (1.14 mmol) and a catalytic amount of LiCl (15 mg) were added. The reaction mixture was heated at reflux for 24 hours. The solvent was evaporated under reduced pressure. The crude product was purified by crystallization from 70% methanol and then recrystallized from methanol to afford the desired compound.

2-(1-(2-((*E*)-2-(4-Iodobenzylidene)hydrazinyl)-4-methylthiazol-5 yl)ethylidene)hydrazinecarboximidamide (4a)

Light yellow solid (65% yield): mp 273-274 °C (MeOH). IR (KBr) cm^{-1}: υ_{max} 3401, 3180 (NH, NH_2), 1602 (C=N); ^{1}H-NMR (DMSO-d_6 400 MHz) δ (ppm): 2.38 (s, 3H, CH_3), 2.48 (s, 3H, CH_3), 7.65-8.25 (m, 4H, Ar, 2H, NH_2, 1H, NH), 8.84 (s, 1H, CH=), 11.36 (bs, 1H, NH, D_2O exch.), 12.20 (bs, 1H, NH, D_2O exch.); ^{13}C NMR (DMSO-d_6 100 MHz) δ (ppm): 17.20, 19.53, 97.31, 130.65, 130.87, 132.43, 132.64, 137.74, 137.75, 143.53, 155.63,

156.54, 158.51, 170.58; HRMS (m/z) 442.0266 [M+H]+, calcd 441.2932; Anal. Calcd. For $C_{14}H_{16}IN_7S$: C, 38.10; H, 3.65; N, 22.22; Found: C, 38.14; H, 3.62; N, 22.25.

2-(1-(2-((*E*)-2-(4-Hydroxy-3-methoxybenzylidene)hydrazinyl)-4-methylthiazol-5-yl)ethylidene)hydrazinecarboximidamide (4b)

Light yellow solid (66% yield): mp 252-254 °C (MeOH). IR (KBr) cm^{-1}: υ_{max} 3451 (OH), 3401, 3180 (NH, NH_2), 1608 (C=N); ^{1}H-NMR (DMSO-d_6 400 MHz) δ (ppm): 1.96 (s, 3H, CH_3), 2.49 (s, 3H, CH_3), 3.81 (s, 3H, OCH_3), 6.84-7.59 (m, 3H, Ar, 2H, NH_2, 1H, NH), 8.10 (s, 1H, CH=), 9.55 (bs, 1H, OH, D_2O exch.), 10.87 (bs, 1H, NH, D_2O exch.), 11.88 (bs, 1H, NH, D_2O exch.); ^{13}C NMR (DMSO-d_6 100 MHz) δ (ppm): 17.22, 19.43, 56.10, 112.15, 117.21, 123.21, 131.10, 132.45, 143.41, 149.35, 151.10, 155.66, 156.51, 158.52, 170.45; HRMS (m/z) 362.1354 [M+H]+, calcd 361.4221; Anal. Calcd. For $C_{15}H_{19}N_7O_2S$: C, 49.85; H, 5.30; N, 27.13; Found: C, 49.81; H, 5.25; N, 27.15.

2-(1-(2-((*E*)-2-((2-Hydroxynaphthalen-1-yl)methylene)hydrazinyl)-4-methylthiazol-5-yl)ethylidene)hydrazinecarboximidamide (4c)

Light yellow solid (35% yield): m.p. 262-263 °C (MeOH). IR (KBr) cm^{-1}: υ_{max} 3351 (OH), 3201, 3180 (NH, NH_2), 1605 (C=N); ^{1}H-NMR (DMSO-d_6 400 MHz) δ (ppm): 2.34 (s, 3H, CH_3), 2.49 (s, 3H, CH_3), 7.35-8.21 (m, 6H, Ar, 2H, NH_2, 1H, NH), 8.31 (s, 1H, CH=), 10.25 (bs, 1H, OH, D_2O exch.), 10.93 (bs, 1H, NH, D_2O exch.), 11.93 (bs, 1H, NH, D_2O exch.); ^{13}C NMR (DMSO-d_6 100 MHz) δ (ppm): 17.12, 19.52, 106.94, 107.26, 111.61, 115.82, 123.51, 125.40, 126.756.10, 112.15, 117.21, 123.21, 131.10, 132.45, 143.41, 149.35, 151.10, 155.66, 156.51, 158.52, 170.45; HRMS (m/z) 382.1405 [M+H]+, calcd 381.1372; Anal. Calcd. For $C_{15}H_{19}N_7O_2S$: C, 49.85; H, 5.30; N, 27.13; Found: C, 49.81; H, 5.25; N, 27.15.

2-(Biphenyl-4-ylmethylene)hydrazinyl)-4-methylthiazol-5-yl)ethylidene)hydrazinecarboximidamide (4d)

Light yellow solid (66% yield): mp 262-264 °C (MeOH). IR (KBr) cm^{-1}: υ_{max} 3401, 3180 (NH, NH_2), 1604 (C=N); ^{1}H-NMR (DMSO-d_6 400 MHz) δ (ppm): 1.96 (s, 3H, CH_3), 2.49 (s, 3H, CH_3), 6.84-7.59 (m, 9H, Ar, 2H, NH_2, 1H, NH), 8.10 (s, 1H, CH=), 10.87 (bs, 1H, NH, D_2O exch.), 11.88 (bs, 1H, NH, D_2O exch.); ^{13}C NMR (DMSO-d_6 100 MHz) δ (ppm): 17.22, 19.43, 112.15, 117.21, 123.21, 127.26, 127.62, 128.93, 129.43, 131.10, 132.45, 143.41, 149.35, 151.10, 155.66, 156.51, 158.52, 170.45; HRMS (m/z) 392.1613 [M+H]+, calcd 391.4926; Anal. Calcd. For $C_{20}H_{21}N_7S$: C, 61.36; H, 5.41; N, 25.04; Found: C, 61.33; H, 5.39; N, 25.01.

2-(3-bromo-4,5-dimethoxybenzylidene)hydrazinyl)-4-methylthiazol-5-yl)ethylidene)hydrazinecarboximidamide (4e)

Orange solid (58% yield): mp 242-244 °C (MeOH). IR (KBr) cm^{-1}: υ_{max} 3401, 3180 (NH, NH_2), 1602 (C=N); ^{1}H-NMR (DMSO-d_6 400 MHz) δ (ppm): 1.96 (s, 3H, CH_3), 2.49 (s, 3H, CH_3), 3.85 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 6.84-7.59 (m, 2H, Ar, 2H, NH_2, 1H, NH), 8.10 (s, 1H, CH=), 10.87 (bs, 1H, NH, D_2O exch.), 11.88 (bs, 1H, NH, D_2O exch.); ^{13}C NMR (DMSO-d_6 100 MHz) δ (ppm): 17.22, 19.43, 56.10, 112.15, 117.21, 123.21, 131.10, 132.45, 143.41, 149.35, 151.10, 155.66, 156.51, 158.52, 170.45; HRMS (m/z) 454.5421 [M+H]+, calcd 454.3447; Anal. Calcd. For $C_{16}H_{20}BrN_7O_2S$: C, 42.30; H, 4.44; N, 21.58; Found: C, 42.35; H, 4.39; N, 21.61.

4.2. Biological assays procedures

4.2.1. Antimicrobial screening

The disc diffusion method was utilized for antimicrobial evaluation of the tested compounds. The microorganism inoculums were uniformly spread, using sterile cotton swabs on a sterile Petri dish with malt extract agar (for fungi) and nutrient agar (for bacteria). One hundred cubic millimeters of each sample was added to each well (10-mm-diameter holes were cut in the agar gel, 20 mm apart from one another). The systems were incubated for 24-48 hr. at 37 ^oC (for bacteria) and at 28 ^oC (for fungi) recommended by Clinical and Laboratory Standards Institute (CLSI) [40] with the positive control of clinically antimicrobial drugs ampicillin, ciprofloxacin, fluconazole, and GlcN-6-P synthase inhibitor FMDP1 [23]. After incubation, the microorganism's growth was observed. The inhibition zones of the bacterial and fungal growth were measured in millimeters. The tests were performed in triplicate experiments [35,36]. Minimal inhibitory concentration (MIC, μg/mL) referred to the lowest concentration of new compounds that required to completely arrest the growth of microbes. Minimal bactericidal concentration (MBC, μg/mL) referred to the lowest concentration of new compounds that required to completely kill microbes [34]. All results are mean values from at least three experiments.

4.2.1.1. Antibacterial assays

The prepared compounds were evaluated for their *in vitro* antibacterial activities against three Gram--positive bacteria (*Staphylococcus aureus* IFO 3060, *Bacillus subtilis* IFO 3007 and *Micrococcus luteus* IFO 3232), two Gram--negative bacteria (*Escherichia coli* IFO 3301 and *Pseudomonas aeuroginosa* IFO 3448). The bacterial suspension was adjusted with sterile saline to a concentration of 1×10^5 CFU (Colony Forming Unit). Initially the compounds were dissolved in DMSO to prepare the stock solutions, then the tested compounds and reference drugs were prepared in Mueller-Hinton broth [41] to obtain the required (two-fold serial dilutions) eight wanted concentrations 0.5 to 64 μg/mL. These dilutions were inoculated and incubated at 37 ºC for 24 h.

4.2.1.2 Antifungal assays

The newly synthesized compounds were evaluated for their *in vitro* antifungal activities against three pathogenic fungi (*Candida albicans* IFO 0583, *Aspergillus oryzae* IFO 4177 and *Aspergillus niger* IFO 4414). A spore suspension in sterile distilled water was prepared from one day old culture of the fungi of the fungi growing on Sabouraud agar (SA) media. The final spore concentration was 1-5 × 1033 spore mL^{-1}. From the stock solutions of the tested compounds and reference antifungal drug Fluconazole, dilutions in sterile RPMI 1640 medium were made resulting in eight wanted concentrations (0.5 to 64 μg/mL) of each tested compound. These dilutions were inoculated and incubated at 28 ºC for 48 h.

4.2.1.3. Antibacterial assay against resistant strains

The minimum inhibitory concentrations (MICs) of the tested compounds and control drugs; linezolid, vancomycin, gentamicin (antibiotics) and fluconazole and 5-fluorocytosine (5-FC) (antifungal drugs) were determined using the broth microdilution method, according to guidelines outlined by the Clinical and Laboratory Standards Institute [42] against clinically-relevant bacterial (methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli, Clostridium difficile* and *Neisseria gonorrhea* strains) and fungal (*Candida albicans*) strain. *S. aureus* and *E.coli* were grown aerobically overnight on tryptone soy agar plates at 37° C. *difficile* was grown anaerobically on brain heart infusion supplemented agar at 37° C for 48 hours. *N. gonorrhea* was grown on Brucella broth supplemented with yeast extract, neopeptone, hematin, pyridoxal and NAD at 37° C for 24 hours in presence of 5% CO_2. *C. albicans* was grown aerobically overnight on yeast peptone dextrose (YPD) agar plate at 35° C. Afterwards, a bacterial solution equivalent to 0.5 McFarland standard was prepared and diluted in cation-adjusted Mueller-Hinton broth (CAMHB) (for *S. aureus* and *E. coli*) to achieve a bacterial concentration of about 5×10^5 CFU/mL. *C. difficile* was diluted in brain heart infusion supplemented broth, supplemented with yeast extract, hemin and vitamin K to achieve a bacterial concentration of about 5×10^5 CFU/mL. *N. gonorrhea* was diluted in Brucella broth supplemented with yeast extract, neopeptone, hematin, pyridoxal and NAD to achieve a bacterial concentration of about 1×10^6 CFU/mL. *C. albicans* was diluted in Roswell Park Memorial Institute (RPMI 1640) medium with glutamine and without bicarbonate (GIBCO by Life Technologies, Green Island, NY, USA) which was buffered to pH 7.0 with 0.165 M of [3-(N-morpholino) propanesulfonic acid] (MOPS) (dot scientific inc., Burton, MI, USa) to achieve a fungal concentration of about 1.5×10^3 CFU/mL. Compounds and control drugs were added in the first row of the 96-well plates and serially diluted with the corresponding media containing bacteria/fungi. Plates were then, incubated aerobically at 37° C for 18-20 hours (for *S. aureus* and *E. coli*). *C.albicans* was incubated aerobically at 37° C for 24 hours. MICs defined as the minimum concentration of the compounds and control drugs that could completely inhibit the visual growth of bacteria/fungi [43].

4.2.1.4. GlcN-6-P synthase activity determination

A standard incubation mixture consisted of 10 mM Fru-6-P, 10 mM L-Gln, 1 mM EDTA, 1 mM DTT and appropriately diluted enzyme preparation in buffer D (final protein concentration 0.5–1.0 μg/mL). The reaction started by adding the enzyme, the mixture was incubated at 37 °C for 30 min, and the reaction was terminated by boiling for 1 min. The concentration of GlcN-6-P formed was determined using a modified Elson-Morgan procedure [44]. One unit of the specific activity (U) was defined as an amount of the enzyme that catalyzed formation of 1 μmol GlcN-6-P min−1 mg protein−1.

For the determination of kinetic constants, the enzyme activity was determined at variable (0–10 mM) initial concentrations of appropriate substrates. Data were plotted as Lineweaver-Burk plots. For the studies on Inhibition of GlcN-6-P synthase and its muteins activity by UDP-GlcNAc, the incubation mixtures contained constant amounts of substrates (10 mM Fru-6-P, 10 mM L-Gln), variable concentrations of UDP-GlcNAc (0–5 mM) and Glc-6-P, 10 mM, when indicated [45].

4.3. Molecular modeling

The newly synthesized 2VF5) that used as receptor model. The AutoDock 3.0 [38] and the MOE software [39] were used for all the docking calculations. The AutoDock Tools package was employed to generate the docking input files and to analyze the docking results. A grid box size of 90×90×90 points with a spacing of 0.375 Å between the grid points was generated that covered almost the entire protein surface. Ligands were built by means of the MOE builder interface, their geometries were optimized with the CHARMm forcefield [46] and then prepared for docking calculations with the python scripts available in the Autodock package. For each ligand 50 runs were performed, and the resulting poses were clustered with 1.8 Å tolerance. Lamarckian GA was used for the conformational space search with initial population set to 150, and fitness function evaluations set to 25000000. The most abundant low energy clusters were selected for analysis. The protein-ligand interaction plots were generated, using MOE 2012.10 [39]. The quantum mechanical calculations and the surface molecular orbitals were generated by the simulation module in the MOE software.

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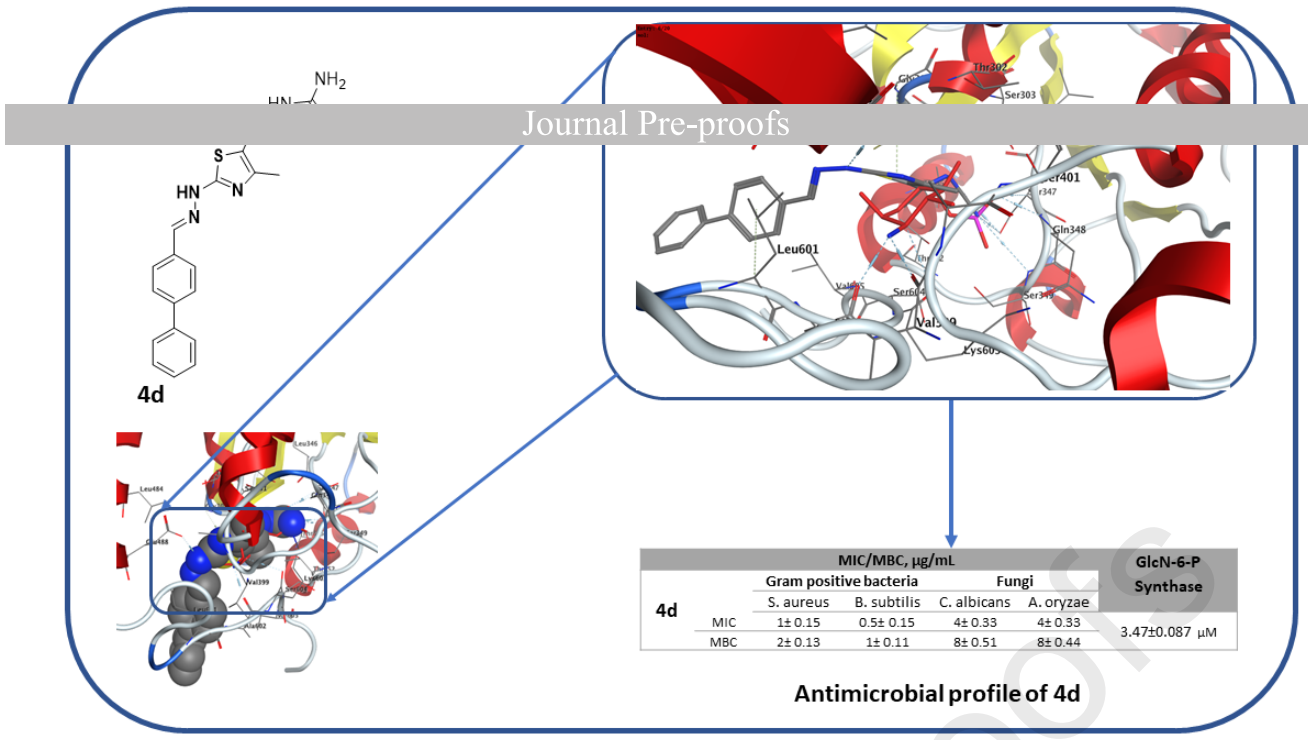
Graphical abstract

The Rational Design, Synthesis, and Antimicrobial Investigation of 2-Amino-4-Methylthiazole Analogues Inhibitors of *GlcN-6-P Synthase*



A series of novel 2-Amino-4-Methylthiazole analogs were developed via three-step reaction encompassing hydrazine-1-carboximidamide motif to combat Gram-positive and Gram-negative bacterial and fungal infections. Noticeably, the thiazole-carboximidamide derivatives **4a-d** displayed excellent antimicrobial activity and the most efficacious analogue **4d** with MIC/MBC values of 0.5 and 4 μg/mL, compared to reference drugs with very low toxicity to mammalian cells, resulting in a prominent selectivity more than 100 folds. Microscopic investigation of **4d** biphenyl analogue showed cell wall lysis and promote rapid bactericidal activity though disrupting the bacterial membrane.

1. A series of novel thiazole-based derivatives were synthesized and evaluated for their antimicrobial inhibiting activity.
2. Antimicrobial activity, GlcN-6-P Synthase inhibitory activity were tested
3. Molecular docking analyses was done for SAR interpretation

Conflict of Interest and Authorship Conformation Form

Please check the following as appropriate:

- All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.

- This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue.

- The following authors have affiliations with organizations with direct financial interest in the subject matter discussed in the manuscript:

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