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Synthesis and antibacterial activity evaluation of aminoguanidine or dihydrotriazine derivatives

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In the alarming context of rising bacterial antibiotic resistance, there is an urgent need to discover new antibiotics or increase and/or enlarge the activity of those currently in use. In this article, aminoguanidine and dihydrotriazine derivatives were designed, synthesized and evaluated in terms of their antibacterial and antifungal activities. Most of the synthesized compounds showed potent inhibitory activities against different bacteria and one fungus with minimum inhibitory concentrations (MICs) ranging from 1 to 64 μg/mL, which obviously better than the positives control drug. The compound 23a showed the best antibacterial activities, whose MIC value was 1 μg/mL against eight strains. The cytotoxic activity of the compound 4c, 8a and 23a were assessed in Human liver cancer cells. The preliminary docking results imply that compounds 21b and 23a possibly display their antibacterial activity through the interaction with DHFR protein by targeting residues of the active cavities of DHFR.

Keywords: Aminoguanidine, Antibacterial activities, Antifungal activities, Cytotoxicity, Dihydrotriazine

Numerous research groups throughout the world are currently involved in an urgent search for novel antibacterial and antifungal agents to overcome the emergence of new infectious diseases and the increasing number of multidrug-resistant microbial pathogens[1,2]. According to the World Health Organization (WHO), if nothing is done, the world will enter a post antibiotic era, in which current infections and minor wounds will be able to kill once again. Methicillin-resistant *S. aureus* (MRSA) is a major pathogen causing both hospital and community acquired infections. Among these organisms, MRSA alone accounts for nearly one-half of the deaths attributed to antibiotic-resistant infections[3]. Aminoguanidine derivatives have recently captured the attention of numerous researchers because of their diverse range of biological properties, including their antibacterial[4], antifungal[5], and anti-inflammatory activities[6]. 1,3,5-triazine derivatives have been reported to exhibit a wide range of interesting biological properties including anticancer, anti-HIV and antimicrobial activities[7-10]. The 1,2,3-triazoles are known for several biological activities such as antibacterial, antiviral and anti-inflammatory[11-13].

Recently, the concept of hybrid molecules has proved to be the most interesting topic in medicinal chemistry, where two or more pharmacophores are linked covalently resulting in one molecule[14-16]. Two units of the final molecule may act on different targets to exert dual drug action or one part can counter balance the side effects caused by another part. In our previous work, we reported the identification of aminoguanidine and dihydrotriazine derivatives, and demonstrated that all of the compounds belonging to this series showed outstanding bacteriostatic activity against Gram-positive bacteria, as exemplified by compounds A, B , and D (MIC = 0.5 or 32 μg/mL). Kumar Deepak *et al*. reported that compound C (Fig. 1) exhibited potent activity against Mycobacterium tuberculosis H37Rv strain with MIC_{99} values ranging from 0.3 μg/mL *in vitro*[17]. As a part of our ongoing studies toward the development of novel antibacterial agents, here we proposed to link aminoguanidine or dihydrotriazine and triazole entities together and synthesized novel derivatives. These two scaffolds were joined covalently into one

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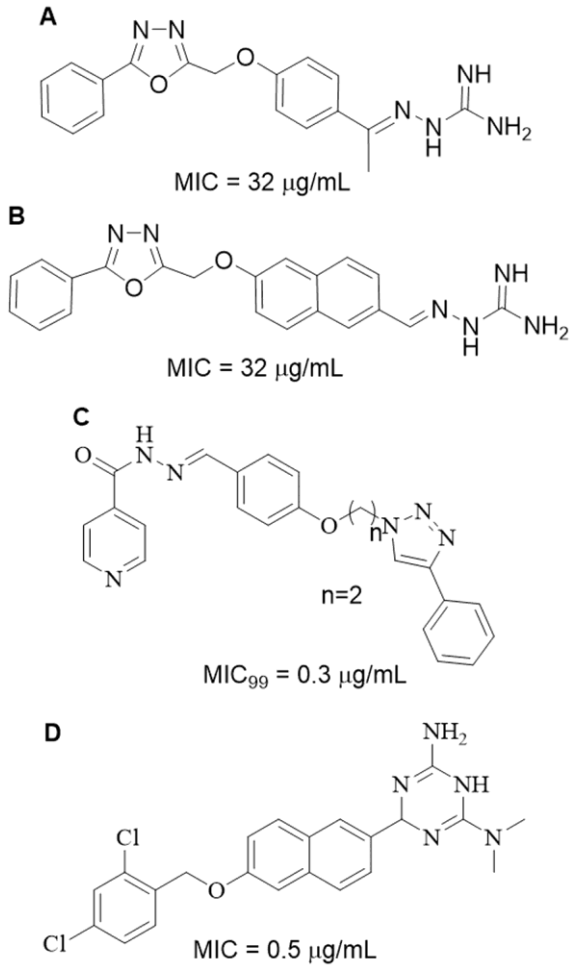


Fig. 1 — Previously reported antibacterial compounds

single derivative in anticipation that the hybrid may show potent antibacterial and antifungal activities.

Materials and Methods

Chemistry

Melting points were determined in open capillary tubes and are uncorrected. Reaction courses were monitored by TLC on silica gel-precoated F254 Merck plates. Developed plates were examined with UV lamps at wavelengths of 254 nm. ^{1}H-NMR and ^{13}C-NMR spectra were measured on a BURKER NMR spectrometer at 300 MHz and 126 MHz while using TMS as the internal standard. Mass Spectrometry was measured on anAXIMA-CFR plus (Shimadzu, Japan) MALDI TOF/TOF spectrometer. The other raw materials and solvents were purchased from their respective suppliers and underwent no further purification. Distilled water was self-prepared in our laboratory.

General procedures for the synthesis of 4a-c, 8a-b, 12a and 17a

The hydroxybenzaldehyde, 4-formylbenzoic acid, 6-benzyloxynaphthalene, vanilline or hydroxyacetophenone was treated with propargyl bromide to get the compounds 2, 6, 10 and 15 , the cycloaddition of azide group and terminal alkyne *via* click reaction gave different triazole-based compounds 3, 7, 11 and 16 as depicted in (scheme 1)[17], Ketone derivatives or aldehyde derivatives were dissolved in absolute ethanol (10-20 mL), aminoguanidine bicarbonate (1 eq) and catalytic amounts of hydrochloric acid were added[18]. The reaction mixtures were heated at reflux for 24 h. The solvent was evaporated under reduced pressure and the crude product was purified by silica gel column chromatography (dichloromethane/methanol, 20:1) to afford the desired compounds as white solid. The yield, melting point and spectral data of each compound are given below.

(E)-2-(4-((1-(2,4-Dichlorobenzyl)-1H-1,2,3-triazol-4-yl) methoxy)benzylidene)hydrazine-1-carboximidamide (4a)

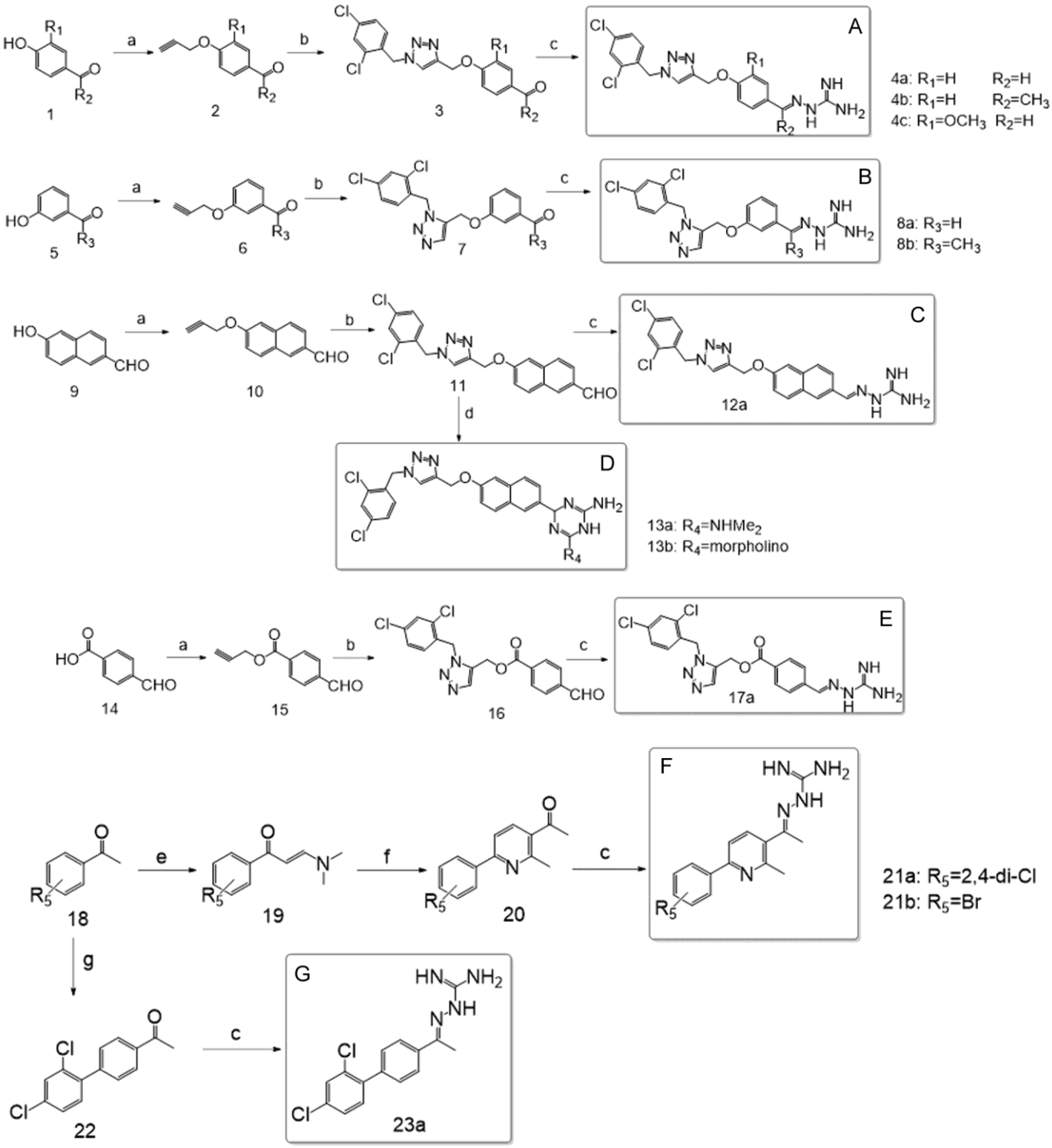
Yield 62%; m.p. 68-70°C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.86 (s, 1H, NH), 8.32 (s, 1H, CH=N), 8.11 (s, 1H, Ar-H), 7.77 (d, J = 25.3 Hz, 5H, NH and Ar-H), 7.49 (s, 2H, Ar-H), 7.29 (s, 1H, Ar-H), 7.11 (s, 2H, Ar-H), 5.73 (s, 2H, CH_2), 5.21 (s, 2H, CH_2). ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.31, 155.87, 146.93, 143.04, 134.49, 134.24, 132.83, 132.45, 129.67, 128.40, 126.85, 125.72, 115.43, 61.64, 50.57. MS (MALDI-TOF) m/z 418 (M+1).

(E)-2-(1-(4-((1-(2,4-Dichlorobenzyl)-1H-1,2,3-triazol-4-yl) methoxy)phenyl)ethylidene)hydrazine-1-carboximidamide (4b)

Yield 55%; m.p. 155-156°C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.10 (s, 1H, NH), 8.30 (s, 1H, Ar-H), 7.93 (d, J = 8.8 Hz, 2H,Ar-H), 7.72 (d, J = 2.0 Hz, 3H, NH), 7.49 (dd, J = 8.3, 2.1 Hz, 1H, Ar-H), 7.28 (d, J = 8.4 Hz, 1H, Ar-H), 7.06 (d, J = 8.9 Hz, 2H, CH_2), 5.74 (d, J = 12.7 Hz, 3H, Ar-H), 5.21 (s, 2H, CH_2), 2.31 (s, 3H, CH_3). MS (MALDI-TOF) m/z 432 (M+1)].

(E)-2-(4-((1-(2,4-Dichlorobenzyl)-1H-1,2,3-triazol-4-yl) methoxy)-3-methoxybenzylidene)hydrazine-1-carboximidamide (4c)

Yield 58%; m.p. 196-197°C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.72 (s, 1H, NH), 8.30 (s, 1H, CH=N), 8.08 (s, 1H, Ar-H), 7.73 (d, J = 2.1 Hz, 3H, NH), 7.60-7.46 (m, 3H, Ar-H), 7.32-7.16 (m, 3H, Ar-H), 5.75 (d, J = 12.3 Hz, 2H, CH_2), 5.18 (s, 2H,CH_2), 3.80 (s, 3H, OCH_3). MS (MALDI-TOF) m/z 448 (M+1).



Scheme 1 — Synthetic route of compounds 4a-c, 8a-b, 12a, 13a-b 17a, 21a-b and 23a. Reagents and conditions: (A) Propargyl bromide, DMF, K_2CO_3, 35-40°C, 10 h; (B) 1-(Azidomethyl)-2,4-dichlorobenzene, Sodium ascorbate, $CuSO_4.5H_2O$, DMF:H_2O (1:1), RT, 20 h; (C) Aminoguanidine bicarbonate, EtOH, HCl, 78°C, 24 h; (D) Moroxydine hydrochloride or metformin hydrochloride, AcOH, 120°C, 4-8 h; (E) DMF-DMA, DMF, 80°C, 12 h; (F) Acetylacetone, AcOH, CH_3COONH_4, 80°C, 12 h; and (G) Orthoboric acid, Na_2CO_3, DME, H_2O, 85°C, 4 h

(E)-2-(3-((1-(2,4-Dichlorobenzyl)-1H-1,2,3-triazol-4-yl) methoxy)benzylidene)hydrazine-1-carboximidamide (8a)

Yield 65%; m.p. 108-110°C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.92 (s, 1H, NH), 8.34 (s, 1H, CH=N), 8.14 (d, J = 2.0 Hz, 1H, Ar-H), 7.96-7.57 (m, 5H, NH and Ar-H), 7.55-7.44 (m, 1H, Ar-H), 7.42-7.23 (m, 3H, Ar-H), 7.11 (d, J = 2.6 Hz, 1H, Ar-H), 5.73 (d, J = 2.1 Hz, 2H, CH_2), 5.20 (d, J = 2.0 Hz, 2H, CH_2). ^{13}C NMR (126 MHz, DMSO-d_6) δ 158.76, 155.96, 146.92, 143.16, 135.35, 134.50, 134.25, 132.85, 132.45, 130.29, 129.65, 128.39, 125.66, 121.62, 117.72, 112.94, 61.65, 50.57. MS (MALDI-TOF) m/z 418 (M+1).

(E)-2-(1-(3-((1-(2,4-Dichlorobenzyl)-1H-1,2,3-triazol-4-yl) methoxy)phenyl)ethylidene)hydrazine-1-carboximidamide (8b)

Yield 60%; m.p. 95-97°C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.19 (s, 1H, NH), 8.30 (s, 1H, Ar-H), 7.82 (s, 3H, NH), 7.72 (d, J = 1.9 Hz, 1H, Ar-H), 7.62 (s, 1H, Ar-H), 7.49 (dd, J = 5.7, 2.6 Hz, 2H, Ar-H), 7.39-7.26 (m, 2H, Ar-H), 7.11 (d, J = 8.0 Hz, 1H, Ar-H), 5.73 (s, 2H, CH_2), 5.22 (s, 2H, CH_2), 2.34 (s, 3H, CH_3). ^{13}C NMR (126 MHz, DMSO-d_6) δ 158.51, 156.58, 151.90, 143.30, 138.78, 134.50, 134.24, 132.85, 132.44, 129.86, 129.64, 128.39, 125.66, 120.10, 61.59, 50.56. MS (MALDI-TOF) m/z 432 (M+1).

(E)-2-((6-((1-(2,4-Dichlorobenzyl)-1H-1,2,3-triazol-4-yl) methoxy)naphthalen-2-yl)methylene)hydrazine-1-carboximidamide (12a)

Yield 68%; m.p. 190-192°C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.03 (s, 1H, NH), 8.36 (s, 1H, , CH=N), 8.29 (s, 1H, Ar-H), 8.16 (d, J = 10.0 Hz, 2H, Ar-H), 7.95-7.70 (m, 6H, NH and Ar-H), 7.57 (s, 1H, Ar-H), 7.47 (dd, J = 8.3, 2.1 Hz, 1H, Ar-H), 7.27 (dd, J = 14.2, 8.6 Hz, 2H, Ar-H), 5.75 (d, J = 10.1 Hz, 2H, CH_2), 5.29 (s, 2H, CH_2). ^{13}C NMR (126 MHz, DMSO-d_6) δ 157.53, 155.86, 147.50, 143.08, 135.82, 134.49, 134.25, 132.84, 132.46, 130.51, 129.69, 128.61, 128.39, 127.76, 125.79, 124.10, 119.80, 108.23, 61.67, 50.58. MS (MALDI-TOF) m/z 468 (M+1).

(1-(2,4-Dichlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl(E)-4-((2-carbamimidoylhydrazono)methyl)benzoate (17a)

Yield 55%; m.p. 246-247°C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.13 (s, 1H, NH), 8.33 (s, 1H, CH=N), 8.24 (s, 1H, Ar-H), 8.07-7.95 (m, 5H, NH and Ar-H), 7.88 (s, 2H, Ar-H), 7.72 (d, J = 2.0 Hz, 1H, Ar-H), 7.49 (dd, J = 8.3, 2.1 Hz, 1H, Ar-H), 7.31 (d, J = 8.3 Hz, 1H, Ar-H), 5.75 (d, J = 12.3 Hz, 2H, CH_2), 5.42 (s, 2H, CH_2). MS (MALDI-TOF) m/z 447 (M+1).

General procedures for the synthesis of 13a-b

Intermediate 11 and Metformin hydrochloride were refluxed in glacial acetic acid at 120°C for 4-6 h. The whole processes of the reactions were traced by TLC. The solvent was removed under reduced pressure. The crude products were purified by column chromatography (dichloromethane : methanol = 20 : 1)[19].

6-(6-((1-(2,4-Dichlorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy) naphthalen-2-yl)-N^2,N^2-dimethyl-3,6-dihydro-1,3,5-triazine-2,4-diamine (13a)

Yield 45%; m.p. 108-110°C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.86 (d, J = 14.1 Hz, 2H, NH_2), 8.35 (s, 1H, Ar-H), 7.90 (d, J = 8.4 Hz, 2H, Ar-H), 7.81 (s, 1H, Ar-H), 7.73 (d, J = 2.1 Hz, 1H, Ar-H), 7.59-7.45 (m, 3H, Ar-H), 7.28 (dd, J = 19.7, 8.7 Hz, 2H, Ar-H), 5.94 (s, 1H, NH), 5.75 (d, J = 10.3 Hz, 2H, CH_2), 5.27 (s, 2H, CH_2), 3.16 (d, J = 5.2 Hz, 3H, CH_3), 3.06 (s, 4H, CH and CH_3). ^{13}C NMR (126 MHz, DMSO-d_6) δ 157.82, 156.95, 156.32, 143.12, 136.26, 134.79, 134.50, 134.26, 132.85, 132.49, 130.18, 129.65, 128.38, 128.15, 125.76, 125.33, 124.86, 119.83, 107.76, 62.89, 61.62, 50.57, 37.37. MS (MALDI-TOF) m/z 523 (M+1).

4-(6-((1-(2,4-Dichlorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy) naphthalen-2-yl)-6-morpholino-1,4-dihydro-1,3,5-triazin-2-amine (13b)

Yield 50%; m.p. 100-102°C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.41 (s, 1H, NH_2), 9.14 (s, 1H, NH_2), 8.37 (s, 1H, CH), 7.89 (dd, J = 8.6, 5.6 Hz, 2H, Ar-H), 7.81 (s, 1H, Ar-H), 7.71 (d, J = 1.8 Hz, 1H, Ar-H), 7.51 (dt, J = 8.2, 5.2 Hz, 4H, Ar-H), 7.32 (d, J = 8.3 Hz, 1H, Ar-H), 7.24 (dd, J = 9.0, 2.1 Hz, 1H, Ar-H), 5.99 (s, 1H, NH), 5.75 (d, J = 10.3 Hz, 2H, CH_2), 5.27 (s, 2H, CH_2), 3.64 (s, 8H, CH_2). ^{13}C NMR (126 MHz, DMSO-d_6) δ 158.17, 156.99, 155.63, 143.11, 135.89, 134.83, 134.50, 134.27, 132.84, 132.50, 130.21, 129.65, 128.37, 128.18, 125.76, 125.40, 124.90, 119.85, 107.76, 66.07, 62.78, 61.62, 50.58, 44.96. MS (MALDI-TOF) m/z 562 (M+1).

General procedures for the synthesis of 21a-b

Intermediate 19 was synthesized according to the reported method[20]. To a solution of the enaminone 19 in glacial acetic acid, acetylacetone and ammonium acetate were added. The reaction mixture was heated under reflux for 3 h. After cooling and pouring into ice-water, the intermediate 20 obtained was filtered and washed with petroleum ether than with water and finally crystallized from ethanol[21].

(E)-2-(1-(6-(2,4-Dichlorophenyli-2-methylpyridin-3-yl) ethylidene)hydrazine-1-carboximidamide i21ai

Yield 60%; m.p. 228-230°C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.40 (s, 1H, NH), 7.91 (d, J = 8.0 Hz, 1H,

Ar-H), 7.82 (s, 3H, NH), 7.78-7.75 (m, 1H, Ar-H), 7.61-7.55 (m, 3H, Ar-H), 2.60 (s, 3H, CH_3), 2.38 (s, 3H, CH_3). ^{13}C NMR (126 MHz, DMSO-d_6) δ 156.69, 155.97, 154.51, 152.97, 137.92, 136.96, 134.36, 133.64, 133.36, 132.65, 129.83, 128.10, 122.14, 24.00, 19.21. MS (MALDI-TOF) m/z 336 (M+1).

(E)-2-(1-(6-(4-Bromophenyl)-2-methylpyridin-3-yl)ethylidene) hydrazine-1-carboximidamide (21b)

Yield 58%; m.p. 185-186°C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.32 (s, 1H, NH), 8.12 (d, J = 8.7 Hz, 4H, Ar-H), 7.90 (s, 3H, NH), 7.82 (s, 1H, Ar-H), 7.63 (s, 1H, Ar-H), 2.41 (s, 3H, CH_3), 2.30 (s, 3H, CH_3). MS (MALDI-TOF) m/z 346 (M+1).

General procedures for the synthesis of 23a

Bromoacetophenone, the corresponding boronic acid, tetrakis (triphenylphosphine) palladium (0) and sodium carbonate in 1:1 dimethoxyethane:water (6 mL) was heated at 85°C for 4 h, and the solvents were evaporated to dryness. The residue was sonicated for 15 min with 50 mL of EtOAc and filtered. Silica gel was added to the filtrate, and the solvents were evaporated to dryness. The residue was applied to a silica gel column and eluted using EtOAc-hexane as the mobile phase to give the intermediate **22**[22].

(E)-2-(1-(2',4'-Dichloro-[1,1'-biphenyl]-4-yl)ethylidene) hydrazine-1-carboximidamide(23a)

Yield 55%; m.p. 98-100°C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.16 (s, 1H, NH), 8.08 (d, J = 43.8 Hz, 3H, NH), 7.80 (t, J = 8.3 Hz, 2H, Ar-H), 7.59-7.40 (m, 4H, Ar-H), 7.28 (d, J = 2.1 Hz, 1H, Ar-H), 2.53 (s, 3H, CH_3). ^{13}C NMR (126 MHz, DMSO-d_6) δ 156.58, 151.59, 139.21, 138.64, 137.01, 133.63, 133.11, 132.79, 129.80, 129.64, 128.21, 127.17, 15.11. MS (MALDI-TOF) m/z 321 (M+1).

Evaluation of antibacterial activity *in vitro*

The micro-organisms used in the present study were *S. aureus* 4220, *E. Coli* 1942, *S. mutans* 3289 and *C. Albicans* 7535. The strains of multidrug-resistant clinical isolates were methicillin-resistant *S. aureus* (MRSA CCARM 3167 and 3506) and quinolone-resistant *S. aureus* (QRSA CCARM 3505 and 3519). Clinical isolates were collected from various patients hospitalized in several clinics. Test bacteria were grown to mid-log phase in Muellere Hinton broth (MHB) and diluted 1000-fold in the same medium. The bacteria of 10^5 CFU/mL were inoculated into MHB and dispensed at 0.2 mL/well in a 96-well microtiter plate. As positive controls, oxacillin and norfloxacin were used. Test compounds were prepared in DMSO, the final concentration of which did not exceed 0.05%. A two-fold serial dilution technique was used to obtain final concentrations of 64-0.25 µg/mL. The MIC was defined as the concentration of a test compound that completely inhibited bacteria growth during 24 h incubation at 37°C. Bacteria growth was determined by measuring the absorption at 650 nm using a microtiter enzyme-linked immunosorbent assay (ELISA) reader. All experiments were carried out three times[19].

Cytotoxicity on human cells

The cytotoxicity test of selected compounds was measured through the colorimetric MTT assay. Human liver cancer cells (BEL 7402) suspension in DMEM medium supplemented with 10% FBS and antimycotic was added in 96 well microplates at 1.8×10^4 cells/well. A variety of concentrations of the test compounds (200, 100, 50, 25, 12.5, 6.25, 3.125, 1.625 µM/L) dissolved by distilled 10% DMSO was added to each well. Incubation for 24 h at 37°C under 5% CO_2, 2.5 mg/mL of MTT solution was added to each well. Further, the plate was incubated for 4 h. Then, the medium was removed and the resulting formazan crystals were dissolved with 100 µL DMSO. After shaking 10 min, the optical density was measured at 570 nm using a microtiter ELISA reader. The selected compounds were used as a positive control, whereas untreated cells were used as negative controls. The IC_{50} values were defined as the concentrations inhibiting 50% of cell growth. All experiments were performed in triplicate[19].

Results and Discussion

***In vitro* antibacterial activity**

The *in vitro* antibacterial activities of the synthesized compounds were evaluated using a 96-well microtiter plate and a serial dilution method to obtain the minimum inhibitory concentrations (MICs) for different strains (including multidrug-resistant clinical isolates). Gatifloxacin, moxifloxacin, norfloxacin, and oxacillin were used as positive controls for antibacterial activity, and fluconazole and itraconazole were used as positive controls for antifungal activity, and the results are shown in (Table 1). Most of the newly synthesized compounds exhibited potent inhibitory activities against the different bacteria and single fungus tested in the current study with MICs in the range of 1-64 µg/mL. It is noteworthy that compound 23a exhibited the greatest activities of all of the compounds prepared in

Table 1 — Inhibitory activity (MIC, μg/mL) of compounds 4a-c, 8a-b, 12a, 13a-b, 17a, 21a-b and 23a against various bacteria

Compound	Gram-positive strains		Gram-negative strains	Fungus
	4220[a]	3289[b]	1924[c]	7535[d]
4a	4	8	8	4
4b	4	4	8	4
4c	8	16	8	8
8a	2	4	8	4
8b	4	4	4	4
12a	8	2	4	8
13a	4	8	4	4
13b	4	8	4	4
17a	32	64	32	16
21a	4	16	4	4
21b	16	16	16	16
23a	1	1	1	1
Gatifloxacin	0.25	0.25	2	0.5
Moxifloxacin	0.25	0.25	2	0.5
Fluconazole	n.d	n.d	n.d	1
Itraconazole	n.d	n.d	n.d	0.6

[a]*Staphylococcus aureus RN* 4220.
[b]*Streptococcus mutans* 3289.
[c]*Escherichia coli KCTC* 1924.
[d]*Candida albicans* 7535; n.d.: Not determined

Table 2 — Inhibitory activity (MIC, μg/mL) of compounds 4a-c, 8a-b, 12a, 13a-b, 17a, 21a-b and 23a against clinical isolates of multidrug-resistant Gram-positive strains

Compound	Multidrug-resistant Gram-positive strains			
	MRSA		QRSA	
	3167[a]	3506[b]	3505[c]	3519[d]
4a	8	4	8	8
4b	8	4	8	8
4c	8	8	8	16
8a	4	2	4	4
8b	4	4	4	8
12a	4	4	8	8
13a	4	2	4	4
13b	4	4	4	4
17a	32	32	32	32
21a	8	4	8	8
21b	16	16	16	16
23a	1	1	1	1
Gatifloxacin	2	2	8	4
Moxifloxacin	1	1	4	4
Norfloxacin	8	4	>64	>64
Oxacillin	>64	>64	1	1

[a]*Methicillin-resistant S. aureus* CCARM 3167.
[b]*Methicillin-resistant S. aureus* CCARM 3506.
[c]*Quinolone-resistant S. aureus* CCARM 3505.
[d]*Quinolone-resistant S. aureus* CCARM 3519.

Table 3 — Antibacterial activity and cytotoxicity for 4c, 8a , and 23a

	Test organisms	4c	8a	23a
MIC (μg/mL)	*S. aureus* 4220	8	2	1
	MRSA 3519	16	4	1
IC_{50}[a] (μmol/L)	BEL7402[b]	6.13	11.02	6.95

[a]IC_{50} is the concentration required to inhibit cell growth by 50%. Data represent the average of three independent experiments running in triplicate. Variation was generally between 5–10%.
[b]Human liver cancer cells.

the current study with MIC value of 1 μg/mL against eight strains, making its potency comparable to those gatifloxacin and moxifloxacin. In terms of its activity towards the fungus *C. albicans* 7535, compound 23a displayed the strongest activity of all of the compounds synthesized in the current study with an MIC value of 1 μg/mL, making it equipotent to fluconazole. Furthermore, compound 23a was determined to be 4-fold more potent than moxifloxacin (MIC = 4 μg/mL) and 8-fold more potent than Gatifloxacin (MIC = 8 μg/mL) towards QRSA CCARM 3505. Against MRSA CCARM 3506 (Table 2), compounds 8a and 13a were equipotent to the positive controls gatifloxacin (MIC = 2 μg/mL).

Analysis of these results revealed several structure activity relationships. A comparison of the potency of compounds 13a and 13b revealed that isosteric replacement with a morpholine or dimethylamine group at the *N*-position of the dihydro-1,3,5-triazine ring generally did not affect the antimicrobial activity. It is also noteworthy that compound 21a, bearing a 2,4-dichloro-substituted phenyl ring, showed excellent antimicrobial activities. These results therefore, provide further evidence that the inclusion of a 2,4-dichloro-substituted benzene ring is critical to the activity of these compounds, which is consistent with the results obtained for a previously reported series of aminoguanidine and dihydrotriazine derivatives [19,23]. A comparison of the results for compounds 21a, 21b and 23a revealed that the inclusion of a benzene ring led to an increase in antimicrobial activity. Taken together, these results suggest that compound 23a could be used as hits for the development of a suitable lead compound for further investigation, especially for novel antifungal agents.

Cytotoxicity study

To determine whether the compounds synthesized in the current study were selectively toxic towards bacteria, we evaluated the cytotoxicities of several representative compounds (4c, 8a, 23a) using a standard technique. As shown in the (Table 3),

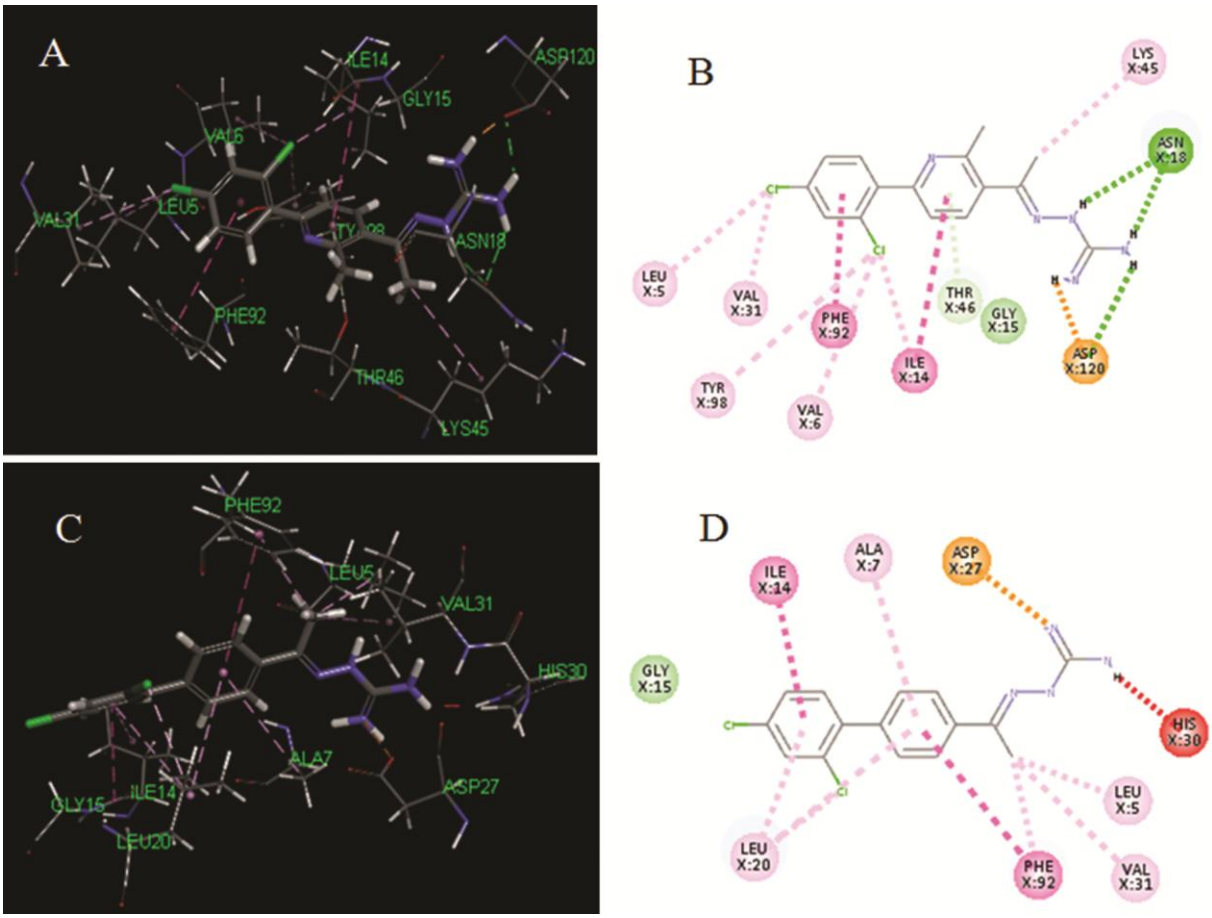


Fig. 2 — Docking result of compound 21b and 23a. (A) Key residues in binding site surrounding 21b; (B) 2D molecular docking modeling of compound 21b with 3fra; (C) Key residues in binding site surrounding 23a; and (D) 2D molecular docking modeling of compound 23a with 3fra.

compound 4c exhibited weaker activity than 23a against the different bacteria, in spite of its slight greater cytotoxicity than 23a, comparably indicating that the promising antibacterial activity of these compounds may not be due to their cytotoxicity, but some unknown mechanism of action.

***In silico* docking study**

To rationalize the observed antibacterial activity and understand the possible mechanism of action of these compounds, a docking investigation was undertaken. The crystal structure data (*S. aureus* DHFR) were obtained from the protein data bank (PDB ID: 3fra)[24,25]. Enzyme structures were checked for missing atoms, bonds and contacts. Hydrogen atoms were added to the enzyme structure. Water molecules and bound ligands were manually deleted[26]. Preferred coordination modes of 21b and 23a with dihydrofolate reductase (DHFR) protein are presented in (Fig. 2). Fragment A of 21b is bound into the active site, in which the benzene ring shows Amide-Pi stacked interaction with Phe 92. The chloro atom of 21b interacted with Leu 5 *via* a pi-alkyl bond. Fragment B of 23a is bound into the active site where the aminoguanidine group shows attractive charge interaction with Asp 27 and the methyl group of 23a formed alkyl bond with Leu 5 and Phe 92, the benzene ring of 23a formed a pi-alkyl bond with Leu 20, comparably indicated its more potent activity than that of 21b. The preliminary docking results imply that compounds 23a and 21b possibly display their antibacterial activity through the interaction with DHFR protein by targeting residues of the active cavities of DHFR.

Conclusion

In summary, we have synthesized aminoguanidine or dihydrotriazin derivatives and evaluated their antibacterial and antifungal activities. Compound 23a showed the highest levels of activity against eight strains with a MIC value of 1 μg/mL. Preliminary docking study showed that these compounds have a

good interaction with the active cavities of DHFR, possibly exhibit their potency *via* inhibiting DHFR.

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References

1 Zafer AK, Gulhan TZ, Ahmet O & Gilbert R, New triazole and triazolothiadiazine derivatives as possible antimicrobial agents. *Eur J Med Chem*, 43 (2008) 155.
2 Arya S, Kumar N, Roy P & Sondhi SM, Synthesis of amidine and bis amidine derivatives and their evaluation for anti-inflammatory and anticancer activity. *Eur J Med Chem*, 59 (2013) 7.
3 Fischbach MA & Walsh CT, Antibiotics for emerging pathogens. *Science*, 325 (2009) 1089.
4 Eissa IH, Mohammad H, Qassem OA, Younis W, Abdelghany TM, Elshafeey A, Seleem MN & Mayhoub AS, Diphenylurea derivatives for combating methicillin- and vancomycin-resistant *Staphylococcus aureus*. *Eur J Med Chem*, 130 (2017) 73.
5 Shrestha SK, Kril LM, Green KD, Kwiatkowski S Sviripa VM, Nickell JR, Dwoskin LP, Watt DS & Garneau-Tsodikova S, Bis(*N*-amidinohydrazones) and *N*-(amidino)-*N*′-aryl-bishydrazones: New classes of antibacterial/antifungal agents. *Bioorg Med Chem*, 25 (2017) 58.
6 Li YR, Li C, Liu JC, Guo M, Zhang TY, Sun LP, Zheng CJ & Piao HR, Synthesis and biological evaluation of 1,3-diaryl pyrazole derivatives as potential antibacterial and anti-inflammatory agents. *Bioorg Med Chem Lett*, 25 (2015) 5052.
7 Giacomelli G, Porcheddu A & DeLuca L, [1,3,5]-Triazine: a versatile heterocycle in current applications of organic chemistry. *Curr Org Chem*, 8 (2004) 1497.
8 Naicker KP, Jiang S, Lu H, Ni J, Boyer-Chatenet L, Wang LX & Debnath AK, Synthesis and anti-HIV-1 activity of 4-[4-(4,6-bisphenylamino-triazin-2-ylamino)-5-methoxy-2-methylphenylazo]-5-hydroxynaphthalene-2,7-disulfonic acid and its derivatives. *Bioorg Med Chem*, 12 (2004) 1215.
9 Lebreton S, Newcombe N & Bradley M, Antibacterial single-bead screening. *Tetrahedron*, 59 (2003) 10213.
10 Yang Y, Wedajo W, Yamada Y, Dahlroth SL, Dick T & Chui WK, 1,3,5-triazaspiro[5.5]undeca-2,4-dienes as selective Mycobacterium tuberculosis dihydrofolate reductase inhibitors with potent whole cell activity. *Eur J Med Chem*, 144 (2018) 262.
11 Biagi G, Dell'Omodarme G, Ferretti M, Giorgi I, Livi O & Scartoni V, Studies on 1,2,3-triazole derivatives as *in vitro* inhibitors of prostaglandin synthesis. *Farmaco*, 45 (1990) 1181.
12 Dabak K, Sezer O, Akar A & Anac O, Synthesis and investigation of tuberculosis inhibition activities of some 1,2,3-triazole derivatives. *Eur J Med Chem*, 38 (2003) 215.
13 Banfi E, Scialino G, Zampieri D, Mamolo MG, Vio L, Ferrone M, Fermeglia M, Paneni MS & Pricl S, Antifungal and antimycobacterial activity of new imidazole and triazole derivatives. A combined experimental and computational approach. *J Antimicrob Chemother*, 58 (2006) 76.
14 Meunier B. Hybrid molecules with a dual mode of action: dream or reality? *Acc Chem Res*, 41 (2008) 69.
15 Pokrovskaya V & Baasov T, Dual-acting hybrid antibiotics: a promising strategy to combat bacterial resistance. *Expert Opin Drug Discov*, 5 (2010) 883.
16 Tietze LF, Bell HP & Chandrasekhar S, Natural product hybrids as new leads for drug discovery. *Angew Chem Int Ed*, 42 (2003) 3996.
17 Kumar D, Beena, Khare G, Kidwai S, Tyaqi AK, Singh R & Rawat DS, Synthesis of novel 1,2,3-triazole derivatives of isoniazid and their *in vitro* and *in vivo* antimycobacterial activity evaluation. *Eur J Med Chem*, 81 (2014) 301.
18 Zhang TY, Li C, Li YR, Li XZ, Sun LP, Zheng CJ & Piao HR, Synthesis and Antimicrobial Evaluation of Aminoguanidine and 3-amino-1,2,4-triazole Derivatives as Potential Antibacterial Agents. *Lett Drug Des Discov*, 13 (2016) 1063.
19 Zhang TY, Yu ZK, Jin XJ, Li MY, Sun LP, Zheng CJ & Piao HR, Synthesis and evaluation of the antibacterial activities of aryl substituted dihydrotriazine derivatives. *Bioorg Med Chem Lett*, 28 (2018) 1657.
20 Mohamed H, Abdelrahman S M, Ghafoor A, Soofi M, Alajlouni RA, Cushman M & Seleem MN, Discovery and Characterization of Potent Thiazoles versus Methicillin- and Vancomycin-Resistant *Staphylococcus aureus*. *J Med Chem*, 57 (2014) 1609.
21 Eldehna WM, Altoukhy A, Mahrous H & Abdel-Aziz HA, Design, synthesis and QSAR study of certain isatin-pyridine hybrids as potential anti-proliferative agents. *Eur J Med Chem*, 90 (2015) 684.
22 Li B, Pai R, Di M, Aiello D, Barnes MH, Butler MM, Tashjian TF, Peet NP, Bowlin TL & Moir DT, Coumarin-Based Inhibitors of *Bacillus anthracis* and *Staphylococcus aureus* Replicative DNA Helicase: Chemical Optimization, Biological Evaluation, and Antibacterial Activities. *J Med Chem*, 55 (2012) 10896.
23 Wei ZY, Chi KQ, Yu ZK, Liu HY, Sun LP, Zheng CJ & Piao HR, Synthesis and biological evaluation of chalcone derivatives containing aminoguanidine or acylhydrazone moieties. *Bioorg Med Chem Lett*, 26 (2016) 5920.
24 Oefner C, Bandera M, Haldimann A, Laue H, Schulz H, Mukhija S, Parisi S, Weiss L, Lociuro S & Dale GE, Increased hydrophobic interactions of iclaprim with Staphylococcus aureus dihydrofolate reductase are responsible for the increase in affinity and antibacterial activity. *J Antimicrob Chemother*, 63 (2009) 687.
25 Lam T, Hilgers MT, Cunningham ML, Kwan BP, Nelson KJ, Brown-Driver V, Trzoss M, Hough G, Shaw KJ & Finn J, Structure-Based Design of New Dihydrofolate Reductase Antibacterial Agents: 7-(Benzimidazol-1-yl)-2,4-diaminoquinazolines. *J Med Chem*, 57 (2014) 651.
26 Jasmine R, Ganesh KA & Rajaram R, Probing the mechanism of the anti-diabetic potential of a terpenoid from *Elephantopus scaber* L., an Indian ethnomedicinal plant in STZ diabetic rats- *In vivo* and *in silico* analysis. *Indian J Biochem Biophys*, 55 (2018) 384.