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Synthesis and antimicrobial activity of 4-substituted thiazol-2-yl hydrazine derivatives of 1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazole-4-carbaldehyde

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The manuscript reports synthesis and antimicrobial activity of several novel heterocyclic compounds in which 1,4-disubstituted 1,2,3-triazole synthesized *via* click chemistry approach and 4-aryl (**5a-h**) and 4-piperazinyl amide (**7a-e**) or 4-aryl amide (**8a-e**) substituted thiazole rings, are bridged through hydrazine linkage. Structures of all the synthesized compounds have been elucidated using ^{1}H and ^{13}C NMR and mass spectral analysis. *In vitro* antimicrobial screening of the target compounds has been carried out against six bacterial species *viz. E. coli*, *P. aeruginosa*, *B. subtilis*, *S. pyogenes*, *K. pneumoniae* and *S. aureus* and four fungal species *viz. C. albicans*, *T. viride*, *A. flavus* and *A. brasiliensis.* The activity study revealed that many of the compounds possess moderate to good activity against the tested microorganisms. The active compounds have been further studied to determine the minimum inhibitory concentration (MIC).

Keywords: 1,2,3-Triazole, thiazole, hydrazine, click chemistry, antibacterial activity, antifungal activity

The resistance observed for the existing drugs by newly emerging pathogenic bacteria has become one of the major concern for the medicinal scientists. Due to the resistive developments in the microorganisms, the existing drugs lack in the efficient treatment of microbial infections. Also, due to their slower effects, toxicity, and undesired side effects; these drugs become less efficient against the microorganisms. As a consequence, in past few decades the multi-drug resistant bacteria have drastically affected the human health. These facts have constantly underlined the need for more effective compounds.

The exhaustive research in the field of medicinal chemistry has proved that, a heterocyclic ring is the dominant part of biologically active compounds and most of the times is responsible for the activity of that compound.

Due to the wide range of activities, heterocyclic compounds have now become the most significant and interesting area of research. Among these bioactive heterocycles, 1,2,3-triazoles are found to have various biological activities such as antimicrobial[1-4], anti-phytopathogenic[5], antimycobacterial[6], anti-proliferative[7], anticancer[8-10], anti-Alzheimer's[11], anti-diabetic[12], antitubercular[13, 14], antiepileptic[15], antiviral[16].

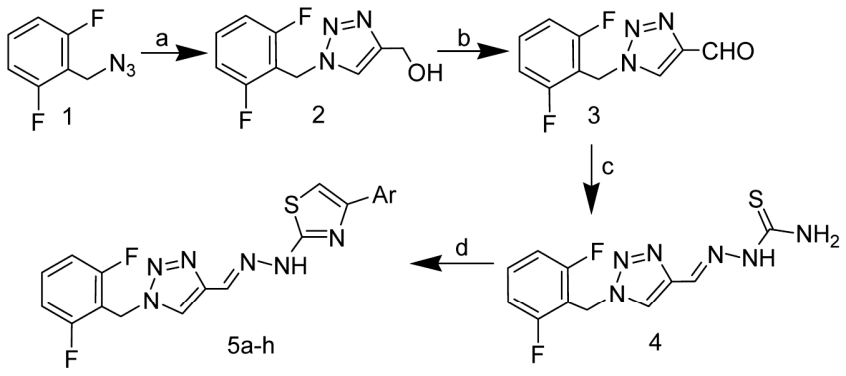
Several drugs like carboxyamidotriazole, cefatrizine, tazobactam bear 1,2,3-triazole in their structure. Moreover, Rufinamide, the amide of 1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazole-4-carboxylic acid is an antiepileptic drug, which is used in the treatment of partial seizures and drop attacks associated with the Lennox-Gastaut syndrome[17].

Similarly, thiazole ring has also marked its place in the field of medicinal chemistry. Alagebrium, amthamine, ceftibuten, clomethiazole**,** fentiazac are some drugs containing thiazole ring in their structure. Various activities possessed by different thiazole derivatives are reported in literature. Few of them are anti-inflammatory[18], anticancer[19,20], antiviral[21], antimicrobial[22-26], activities. Heterocyclic compounds containing thiazole along with hydrazine chain are also found to cover a broad spectrum of biological activities such as anti-parasitic[27], monoamine oxidase inhibitor[28], antimicrobial[29-31].

In view of these findings and in pursuing our continuous interest in designing and synthesizing different heterocyclic compounds as possible bioactive precursors[32-40], we herein report synthesis and antimicrobial evaluation of thiazolylhydrazine derivatives of 1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazole-4-carbaldehyde.

Result and Discussion

As shown in Scheme I, the synthesis of target compounds **5a-h** was initiated using



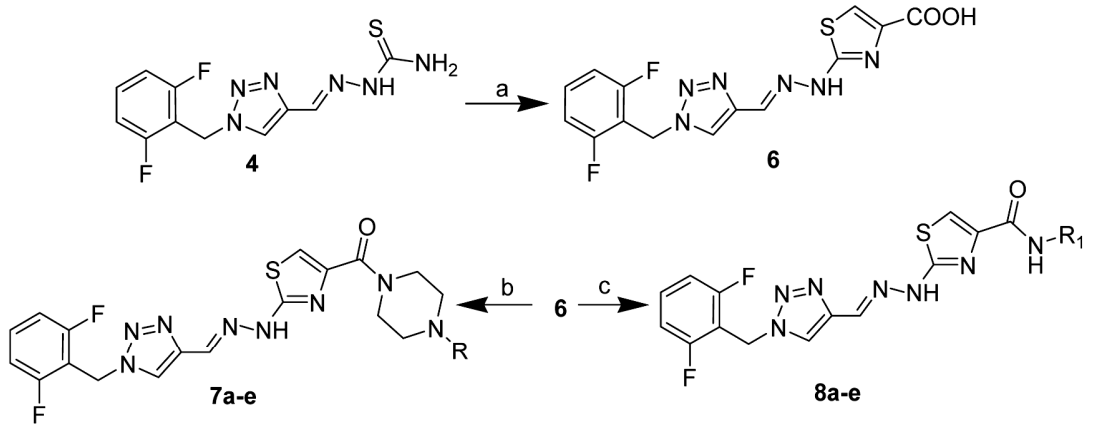
Compd	Ar	Compd	Ar
5a	Phenyl	**5e**	4-Methoxyphenyl
5b	4-Bromophenyl	**5f**	4-Methylphenyl
5c	4-Chlorophenyl	**5g**	2,4-Dichlorophenyl
5d	4-Fluorophenyl	**5h**	(3-Fluoro-4-methoxy)phenyl

Reagents and reaction conditions: (a) $CuSO_4.5H_2O$, Na-ascorbate, propargyl alcohol, $^tBuOH:H_2O$ (1:1), RT; (b) Jone's reagent, acetone, 0°C; (c) Thiosemicarbazide, ethanol, reflux; (d) Sub. phenacyl bromides, ethanol, reflux.

Scheme I — Synthetic scheme for the compounds **5a-h**

2,6-difluorobenzyl azide **1** which was prepared by literature reported procedure[37]. Compound **1** was cyclized with propargyl alcohol using click chemistry approach to obtain the corresponding (1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazol-4-yl)methanol (**2**). Structure of compound **2** was established by ^{1}H and ^{13}C NMR analysis. In ^{1}H NMR, two peaks were observed at δ 4.73 (-CH_2OH) and 5.60 (Ar-CH_2). In addition, a singlet at δ 7.57 (triazole-H) confirmed formation of expected 1,2,3-triazole ring. The ^{13}C NMR displayed two peaks at δ 41.4 and 56.3 for the two methylene carbons. Further, oxidation of the alcohol **2** to corresponding aldehyde **3** was achieved by using Jone's reagent at 0°C. A singlet for aldehydic proton was observed at δ 10.12 in the ^{1}H NMR spectrum of compound **3**. Refluxing aldehyde **3** with thiosemicarbazide gave the 1-((1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazol-4-yl) methylene)thiosemicarbazide **4**. Formation of compound **4** was ascertained by ^{1}H and ^{13}C NMR analysis. In the ^{1}H NMR, the disappearance of aldehydic proton and appearance of a new singlet at δ 8.43 for imine proton confirmed formation of the compound **4**. The thiosemicarbazide derivative **4** was reacted with different substituted phenacyl bromides in ethanol to afford the corresponding target compounds **5a-h**. Structures of all the synthesized target compounds **5a-h** were established by ^{1}H and ^{13}C NMR and LC-MS spectral analysis. For instance, the ^{1}H NMR spectrum of **5d** displayed a peak at δ 7.31 (thiazole ring proton) which confirmed the formation of thiazole ring. Further, the LC-MS spectrum showed $[M+H]^+$peak at 415.0944. (molecular formula $C_{19}H_{13}F_3N_6S$ and exact mass 414.0874).

Similarly, synthesis of the target compounds **7a-e** and **8a-e** was achieved as depicted in Scheme II. Compound **4** was treated with bromopyruvic acid in ethanol to afford the corresponding thiazole-4-carboxylic acid derivative **6**. Formation of thiazole ring was confirmed by ^{1}H NMR spectral analysis in which the thiazole proton appeared at δ 7.69.Coupling of acid **6** with different substituted piperazines and anilines provided the target compounds **7a-e** and **8a-e**, respectively. All the target compounds were characterized by ^{1}H NMR and LC-MS spectral analysis. ^{1}H NMR spectrum of compound **7d** displayed three peaks as broad singlet for piperazine protons at δ 2.92, 3.51 and 3.98, respectively while methyl group protons appeared as singlet at δ 2.15. LC-MS spectrum of compound **7d** having molecular formula$C_{25}H_{24}F_2N_8OS$ and exact mass 522.1762 showed $[M+H]^+$peak at 523.1828. ^{1}H NMR spectrum of compound **8c** revealed aromatic protons of 4-fluorophenyl rings as two multiplets at δ 7.07-7.18 and 7.87-7.91, respectively and two -NH protons at δ 10.25 and 12.55. LC-MS spectrum of compound **8c** showed $[M+H]^+$peak at 458.0999 against exact mass 457.0933. All the newly synthesized derivatives displayed suitable spectral data in accordance with the expected structures.



Compd	R	Compd	R_1
7a	Boc	**8a**	Phenyl
7b	2-Methoxyphenyl	**8b**	4-Chlorophenyl
7c	2-(Trifluoromethyl)phenyl	**8c**	4-Fluorophenyl
7d	4-Methylphenyl	**8d**	4-Methoxyphenyl
7e	4-Chlorobenzyl	**8e**	Pyridin-4-yl

***Reagents and reaction conditions*:** (a) Bromopyruvic acid, ethanol, reflux; (b) HOBt, sub. piperazines, TEA, EDC.HCl, DMF, RT; (c) HOBt, sub. anilines, TEA, EDC.HCl, DMF, RT.

Scheme II — Synthetic scheme for the compounds **7a-e** and **8a-e**

Antimicrobial Activity

Antimicrobial screening of the target compounds **5a-h**, **7a-e** and **8a-e** was performed using agar disc diffusion assay method against six bacterial strains namely *E. coli*, *P. aeruginosa*, *B. subtilis*, *S. pyogenes*, *K. pneumonia*, *S. aureus* and four fungal strains, *C. albicans*, *T. viride*, *A. flavus* and *A. brasiliensis*. The zone of inhibition (in mm of diameter) and minimum inhibitory concentration values (MICs) in µg/mL were determined. Discs measuring 6mm diameter were prepared from sterile Whatmann paper. Test solution concentration and positive control concentration was kept 100 µg/mL. After loading the sample discs on the agar plate, the plates were inoculated at 37°C for 24 h and the zone of inhibition was measured thereafter using digital vernier caliper.

From the results obtained (Table I and Table II), it was evident that, many of the tested compounds exhibited moderate to good inhibition of the tested microorganisms. Aryl thiazole derivative **5c**with 4-chlorophenyl ring on thiazole, showed good antibacterial activity as compared to its 4-bromophenyl analogue **5b** and 4-fluorophenyl analogue **5d**. Compound **5h** bearing (3-fluoro-4-methoxy)phenyl substitution was inactive against all tested bacterial species but showed antifungal activity against three out of four tested species.

Compounds **7a-e**, **8d** and **8e** showed enhanced activity against bacteria *E. coli*. Piperazinyl amides **7a-e**were slightly more effective towards tested bacteria as compared to the N-aryl amide derivatives **8a-e**. In case of fungal strains, compound **8a** and **8c** exhibited noticeable activity against all tested fungal species. Most of the tested compounds exhibited promising activity against *A. brasiliensis*. N-aryl amides **8a-e**were found more active against the tested fungal strains as compared to piperazinyl amides **7a-e**.

Experimental Section

The reagents and solvents used were of AR grade, and used without any further purification. Thin Layer Chromatography was used to monitor reactions. ^{1}H and ^{13}C NMR spectra were recorded on Varian Mercury 300, Brucker Advance II 400 and 500 spectrometers. $CDCl_3$ or DMSO-d_6 were used as solvents. Chemical shifts are expressed in δ (ppm) unit downfield to internal standard TMS. The assignment of spectra is expressed using standard notations for chemical shifts (δ), splitting patterns (s, d, t, q, m), coupling constants (*J* in Hz). High resolution mass spectra were recorded on Waters Q-TOF Micromass analyzer or Brucker Mass analyzer.

Table I — Antimicrobial activity of the compounds **4**, **5a-h**, **6**, **7a-e** and **8a-e**; diameter of inhibition zone in mm

Compd	Microorganisms*									
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
4	17.01	20.16	18.00	-†	19.94	18.97	20.04	-†	-†	-†
5a	19.06	20.14	-†	-†	19.97	-†	18.01	17.65	18.54	18.00
5b	19.00	16.54	16.62	15.58	16.37	17.68	18.00	-†	-†	18.87
5c	20.03	-†	18.97	17.52	-†	19.36	17.33	18.61	-†	16.44
5d	-†	19.00	-†	-†	-†	-†	-†	17.66	16.98	19.67
5e	18.87	19.23	-†	-†	18.88	-†	-†	-†	-†	-†
5f	-†	-†	18.97	17.25	-†	18.68	18.97	-†	-†	-†
5g	17.32	17.27	-†	-†	19.00	-†	18.00	-†	-†	-†
5h	-†	-†	-†	-†	-†	-†	-†	18.16	17.98	20.07
6	17.62	-†	19.11	20.00	18.67	19.03	17.24	-†	-†	-†
7a	18.61	18.94	-†	-†	19.98	-†	19.48	-†	20.08	19.88
7b	18.66	20.04	19.15	-†	-†	19.32	20.06	-†	-†	19.72
7c	19.32	19.23	19.66	-†	-†	20.11	19.76	-†	-†	19.53
7d	18.94	-†	-†	19.53	-†	-†	-†	-†	20.02	19.13
7e	18.92	-†	18.81	19.90	19.07	20.11	18.68	-†	-†	-†
8a	-†	-†	19.07	-†	-†	18.24	19.13	18.33	19.24	19.41
8b	-†	-†	-†	19.23	-†	-†	-†	-†	20.13	17.96
8c	-†	-†	19.28	-†	-†	19.37	20.19	19.12	20.14	19.53
8d	20.00	19.47	19.13	-†	-†	19.61	20.06	-†	-†	20.15
8e	19.32	18.64	-†	-†	18.88	-†	-†	-†	-†	-†
Chloram-phenicol	21.84	22.93	21.90	22.88	22.67	22.97	NA‡	NA‡	NA‡	NA‡
Nystatin	NA‡	NA‡	NA‡	NA‡	NA‡	NA‡	22.27	22.03	23.07	22.83

*(A) *E. coli*; (B) *P. aeruginosa*; (C) *B. subtilis*; (D) *S. pyogenes*; (E) *K. pneumonia*; (F) *S. aureus*; (G) *C. albicans*; (H)*T. viride*; (I) *A. flavus*; (J) *A. brasilliansis*; † (-) Inactive; ‡ (NA) Not Applicable

Table II — Antimicrobial activity of the compounds **4**, **5a-h**, **6**, **7a-e** and **8a-e**; minimum inhibitory concentrations (MIC) in µg/mL

Compd	Microorganisms*									
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
4	90	50	60	-†	60	60	50	-†	-†	-†
5a	70	50	-†	-†	60	-†	60	80	70	60
5b	70	80	80	90	80	80	60	-†	-†	60
5c	60	-†	60	80	-†	70	80	70	-†	80
5d	-†	60	-†	-†	-†	-†	-†	80	80	50
5e	80	60	-†	-†	70	-†	-†	-†	-†	-†
5f	-†	-†	60	80	-†	80	50	-†	-†	-†
5g	90	70	-†	-†	70	-†	60	-†	-†	-†
5h	-†	-†	-†	-†	-†	-†	-†	70	80	50
6	80	-†	60	60	80	70	90	-†	-†	-†
7a	70	60	-†	-†	70	-†	70	-†	60	70
7b	70	50	60	-†	-†	70	60	-†	-†	70
7c	60	60	60	-†	-†	60	70	-†	-†	70
7d	70	-†	-†	70	-†	-†	-†	-†	60	70
7e	70	-†	70	60	70	60	80	-†	-†	-†
8a	-†	-†	60	-†	-†	90	70	90	70	70
8b	-†	-†	-†	70	-†	-†	-†	-†	60	90
8c	-†	-†	60	-†	-†	70	60	80	60	70
8d	60	60	60	-†	-†	70	60	-†	-†	60
8e	70	70	-†	-†	80	-†	-†	-†	-†	-†
Chloram-phenicol	50	40	40	50	50	50	NA‡	NA‡	NA‡	NA‡
Nystatin	NA‡	NA‡	NA‡	NA‡	NA‡	NA‡	40	60	50	40

*(A) *E. coli*; (B) *P. aeruginosa*; (C) *B. subtilis*; (D) *S. pyogenes*; (E) *K. pneumonia*; (F) *S. aureus*; (G) *C. albicans*; (H)*T. viride*; (I) *A. flavus*; (J) *A. brasilliansis*; † (-) Inactive; (NA) Not Applicable

Synthesis of (1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazol-4-yl)methanol, 2

To a solution of sodium ascorbate (0.2 mol) in aqueous copper sulphate pentahydrate (0.2 mol), propargyl alcohol (1.0 mol) in *tert*-butanol was added slowly and the mixture was stirred for 15 min. Azide **2** (1.0 mol) was added thereafter, portion wise with stirring. After the complete addition, the reaction mixture was stirred further at RT. After completion of the reaction, the excess solvent was removed and the solid obtained was filtered, washed with water. The product was purified by column chromatography using hexane-ethyl acetate as eluent. Yield 88%. m.p.87-90°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.73 (s, 2H, CH_2-OH), 5.60 (s, 2H, -CH_2), 6.93-6.97 (m, 2H, Ar-H), 7.33-7.37 (m, 1H, Ar-H), 7.57 (s, 1H, triazole-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 41.4, 56.3, 110.7, 111.8, 112.0, 121.8, 131.5, 148.0, 160.1, 162.6.

Synthesis of 1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazole-4-carbaldehyde, 3

To a solution of compound **2**(1.0 mol) in dry acetone at 0°C, freshly prepared Jone's reagent was added drop wise under cold conditions. After the addition was complete, the mixture was stirred further and reaction was monitored on TLC. After completion of the reaction, isopropyl alcohol was added slowly to consume the excess Jone's reagent. The mixture was then filtered through celite bed. The excess solvent was removed and the solid obtained was washed with water. Yield 84%. m.p.104-107°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.71 (s, 2H, -CH_2), 6.98-7.04 (m, 2H, Ar-H), 7.38-7.46 (m, 1H, Ar-H), 8.14 (s, 1H, triazole-H), 10.12 (s, 1H, -CHO); ^{13}C NMR (100 MHz, $CDCl_3$): δ 41.8, 109.7, 111.9, 112.1, 125.3, 132.0, 147.9, 160.0, 162.5, 185.1.

Synthesis of 1-((1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazol-4-yl)methylene)thiosemi-carbazide, 4

Mixture of aldehyde derivative **3** (1.0 mol) and thiosemicarbazide (1.0 mol) in ethanol was refluxed for 3 h. After the reaction was complete, the excess solvent was removed and the solid obtained was filtered, washed with cold ethanol. Yield 92%. m.p.154-156°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.67 (s, 2H, -CH_2), 7.04-7.08 (m, 2H, Ar-H), 7.42-7.49 (m, 1H, Ar-H), 7.77 (s, 1H, -NH), 8.02 (s, 1H, -SH), 8.15 (s, 1H, triazole-H), 8.43 (s, 1H, -CH=N), 11.5 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 41.0, 110.4, 111.5, 122.5, 126.1, 131.4, 134.1, 141.5, 143.9, 159.5, 162.0, 178.0, 178.9.

General procedure for the synthesis of 1-((1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazol-4-yl)methylene)-2-(4-arylthiazol-2-yl)hydrazine, 5a-h

Equimolar quantities of thiosemicarbazide derivative **4** and corresponding substituted phenacyl bromide were refluxed in ethanol for 2 h. The reaction mixture was then cooled to RT. The solid separated was filtered, washed with cold ethanol. All the synthesized compounds were purified by column chromatography using hexane-ethyl acetate as eluent.

1-((1-(2,6-Difluorobenzyl)-1*H*-1,2,3-triazol-4-yl) methylene)-2-(4-phenylthiazol-2-yl)hydrazine, 5a: Yield 82%. m.p.210-212°C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 5.71 (s, 2H, -CH_2), 6.97-7.06 (m, 3H, Ar-H, thiazole-H), 7.22-7.35 (m, 3H, Ar-H), 7.48-7.61 (m, 3H, Ar-H), 8.14 (s, 1H, triazole-H), 8.36 (s, 1H, -CH=N), 12.36 (s, 1H, -NH).

1-((1-(2,6-Difluorobenzyl)-1*H*-1,2,3-triazol-4-yl) methylene)-2-(4-(4-bromophenyl)thiazol-2-yl) hydrazine, 5b: Yield 86%. m.p.231-233°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.69 (s, 2H, -CH_2), 7.10-7.14 (m, 3H, Ar-H, thiazole-H), 7.46-7.53 (m, 1H, Ar-H), 7.73 (d, 2H, *J* = 7.8 Hz, Ar-H), 7.93 (d, 2H, *J* = 7.8 Hz, Ar-H), 8.17 (s, 1H, triazole-H), 8.82 (s, 1H, -CH=N); ^{13}C NMR (100 MHz, DMSO-d_6): δ 41.3, 110.5, 111.6, 111.7, 111.8, 122.3, 126.8, 128.2, 129.4, 129.6, 131.6, 131.8, 132.6, 138.0, 159.3, 159.5, 162.0.

1-((1-(2,6-Difluorobenzyl)-1*H*-1,2,3-triazol-4-yl) methylene)-2-(4-(4-chlorohenyl)thiazol-2-yl) hydrazine, 5c: Yield 88%. m.p.163-165°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.71 (s, 2H, -CH_2), 7.11-7.15 (m, 2H, Ar-H), 7.26 (s, 1H, thiazole-H), 7.40 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.47-7.52 (m, 1H, Ar-H), 7.84 (d, 2H, *J* = 8.1 Hz, Ar-H), 8.10 (s, 1H, triazole-H), 8.38 (s, 1H, -CH=N), 12.12 (s, 1H, -NH).

1-((1-(2,6-Difluorobenzyl)-1*H*-1,2,3-triazol-4-yl) methylene)-2-(4-(4-fluorohenyl)thiazol-2-yl) hydrazine, 5d: Yield 84%. m.p.223-225°C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 5.71 (s, 2H, -CH_2), 7.07-7.25 (m, 4H, Ar-H), 7.31 (s, 1H, thiazole-H), 7.48-7.57 (m, 1H, Ar-H), 7.81-7.90 (m, 2H, Ar-H), 8.12 (s, 1H, triazole-H), 8.50 (s, 1H, -CH=N), 12.18 (s, 1H, -NH); ^{13}C NMR (125 MHz, DMSO-d_6): δ 41.1, 111.1, 111.9, 115.4, 122.9, 126.8, 127.6, 129.9, 131.0, 131.8, 133.7, 143.1, 149.0, 159.8, 160.6, 161.8, 162.9, 167.9;LC-MS $[M+H]^+$: 415.0944.

1-((1-(2,6-Difluorobenzyl)-1*H*-1,2,3-triazol-4-yl) methylene)-2-(4-(4-methoxyphenyl)thiazol-2-yl) hydrazine, 5e: Yield 81%. m.p.227-230°C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 3.80 (s, 3H, -OCH_3), 5.68 (s, 2H, -CH_2), 6.97-7.02 (m, 4H, Ar-H), 7.14 (s, 1H, thiazole-H), 7.54-7.56 (m, 1H, Ar-H), 7.70-7.73 (m, 2H, Ar-H), 7.95 (s, 1H, triazole-H), 8.12 (s, 1H, -CH=N), 11.20 (s, 1H, -NH); ^{13}C NMR (125 MHz, DMSO-d_6): δ 41.5, 55.2, 101.8, 113.4, 113.5, 113.9, 114.3, 124.4, 126.3, 127.3, 129.8, 130.9, 144.9, 154.2, 159.4, 160.2.

1-((1-(2,6-Difluorobenzyl)-1*H*-1,2,3-triazol-4-yl) methylene)-2-(4-*p*-tolylthiazol-2-yl)hydrazine, 5f: Yield 89%. m.p.216-218°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.71 (s, 3H, -CH_3), 5.72 (s, 2H, -CH_2), 7.14-7.21 (m, 3H, Ar-H, thiazole-H), 7.49-7.53 (m, 3H, Ar-H), 7.78-7.82 (m, 2H, Ar-H), 8.13 (s, 1H, triazole-H), 8.36 (s, 1H, -CH=N), 12.09 (s, 1H, -NH).

1-((1-(2,6-Difluorobenzyl)-1*H*-1,2,3-triazol-4-yl) methylene)-2-(4-(2,4-dichloro--phenyl)thiazol-2-yl) hydrazine, 5g: Yield 83%. m.p.191-194°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.74 (s, 2H, -CH_2), 7.09-7.17 (m, 3H, Ar-H, thiazole-H), 7.39-7.53 (m, 3H, Ar-H), 7.95 (d, 1H, *J* = 8.5 Hz, Ar-H), 8.60 (s, 1H, triazole-H), 8.71 (s, 1H, -CH=N), 12.44 (s, 1H, -NH).

1-((1-(2,6-Difluorobenzyl)-1*H*-1,2,3-triazol-4-yl) methylene)-2-(4-(3-fluoro-4-methoxyphenyl)thiazol-2-yl)hydrazine, 5h: Yield 79%. m.p.219-220°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.91 (s, 3H, -OCH_3), 5.67 (s, 2H, -CH_2), 6.97-7.03 (m, 4H, Ar-H, thiazole-H), 7.47-7.51 (m, 3H, Ar-H), 7.95 (s, 1H, triazole-H), 7.97 (s, 1H, -CH=N).

Synthesis of 2-(((1-(2,6-difluorobenzyl)-1*H*-1,2, 3-triazol-4-yl)methylene)hydrazin-1-yl)thiazole-4-carboxylic acid, 6

Bromopyruvic acid (1.0 mol) was added to a solution of compound **4** (1.0 mol) in ethanol and the resulting mixture was refluxed for 3 h. After the reaction was complete, the excess solvent was removed. The solid obtained was filtered and washed with cold ethanol. Yield 82%. m.p.214-216°C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 5.71 (s, 2H, -CH_2), 7.17-7.20 (m, 2H, Ar-H), 7.49-7.54 (m, 1H, Ar-H), 7.69 (s, 1H, thiazole-H), 8.05 (s, 1H, triazole-H), 8.49 (s, 1H, -CH=N).

General procedure for the synthesis of (2-(((1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazol-4-yl)methylene) hydrazin-1-yl)thiazol-4-yl)(4-substitutedpiperazin-1-yl)methanone 7a-e or **N-(substitutedphenyl)-1-(2-(((1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazol-4-yl) methylene)hydrazin-1-yl)thiazole-4-carboxamide, 8a-e**

To a cooled solution of 2-(((1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazol-4-yl)methylene)hydrazin-1-yl) thiazole-4-carboxylic acid **6** (1.0 mol) in DMF, HOBt (1.0 mol) was added followed by corresponding piperazine (1.0 mol) or substituted aniline (1.0 mol). TEA (2.0 mol) and EDC.HCl (1.0 mol) were added thereafter and the reaction mixture was left overnight for stirring. The mixture was then poured on to crushed ice; the product was filtered and washed with water. The final products were purified by column chromatography using hexane-ethyl acetate as eluent.

(2-(((1-(2,6-Difluorobenzyl)-1*H*-1,2,3-triazol-4-yl)methylene)hydrazin-1-yl)thiazol-4-yl)(4-boc-piperazin-1-yl)methanone, 7a: Yield 89%. m.p.175-177°C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.33 (s, 9H, -$C(CH_3)_3$), 2.91 (bs, 4H, piperazine-H), 3.55 (bs, 2H, piperazine-H), 4.01 (bs, 2H, piperazine-H), 5.89 (s, 2H, -CH_2), 7.10-7.14 (m, 2H, Ar-H), 7.35 (s, 1H, thiazole-H), 7.45-7.47 (m, 1H, Ar-H), 8.14 (s, 1H, triazole-H), 8.55 (s, 1H, -CH=N), 12.08 (bs, 1H, -NH); LC-MS $[M+H]^+$: 533.1886.

(2-(((1-(2,6-Difluorobenzyl)-1*H*-1,2,3-triazol-4-yl) methylene)hydrazin-1-yl)thiazol-4-yl)(4-(2-methoxy-phenyl)piperazin-1-yl)methanone, 7b: Yield 87%. m.p.180-182°C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.94 (bs, 4H, piperazine-H), 3.60 (bs, 2H, piperazine-H), 4.03 (bs, 2H, piperazine-H), 5.81 (s, 2H, -CH_2), 7.09-7.12 (m, 2H, Ar-H), 7.32 (s, 1H, thiazole-H), 7.49-7.51 (m, 1H, Ar-H), 8.17 (s, 1H, triazole-H), 8.56 (s, 1H, -CH=N), 12.10 (bs, 1H, -NH); LC-MS $[M+H]^+$: 539.1780.

(2-(((1-(2,6-Difluorobenzyl)-1*H*-1,2,3-triazol-4-yl) methylene)hydrazin-1-yl)thiazol-4-yl)(4-(2-(trifluoro-methyl)phenyl)piperazin-1-yl)methanone, 7c: Yield 84%. m.p.180-182°C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.89 (bs, 4H, piperazine-H), 3.58 (bs, 2H, piperazine-H), 4.02 (bs, 2H, piperazine-H), 5.77 (s, 2H, -CH_2), 7.06-7.10 (m, 2H, Ar-H), 7.38 (s, 1H, thiazole-H), 7.41-7.46 (m, 1H, Ar-H), 8.12 (s, 1H, triazole-H), 8.51 (s, 1H, -CH=N), 12.13 (bs, 1H, -NH); LC-MS $[M+H]^+$: 577.1555.

(2-(((1-(2,6-Difluorobenzyl)-1*H*-1,2,3-triazol-4-yl) methylene)hydrazin-1-yl)thiazol-4-yl)(4-(4-*p*-tolylpiperazin-1-yl)methanone, 7d: Yield 91%. m.p.150-151°C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.15 (s, 3H, -CH_3),2.92 (bs, 4H, piperazine-H), 3.51 (bs, 2H, piperazine-H), 3.98 (bs, 2H, piperazine-H), 5.71 (s, 2H, -CH_2), 6.84-6.86 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.02-7.06 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.10-7.15 (m, 2H, Ar-H), 7.35 (s, 1H, thiazole-H), 7.45-7.49 (m, 1H, Ar-H), 8.18 (s, 1H, triazole-H), 8.59 (s, 1H, -CH=N), 12.10 (bs, 1H, -NH); LC-MS $[M+H]^+$: 523.1828.

(2-(((1-(2,6-Difluorobenzyl)-1*H*-1,2,3-triazol-4-yl) methylene)hydrazin-1-yl)thiazol-4-yl)(4-(4-chlorobenzyl)piperazin-1-yl)methanone, 7e: Yield 82%. m.p.130-140°C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.92 (bs, 4H, piperazine-H), 3.51 (bs, 2H, piperazine-H), 3.98 (bs, 2H, piperazine-H), 4.30 (s, 2H, Ar-CH_2), 5.75 (s, 2H, -CH_2), 7.09-7.12 (m, 2H, Ar-H), 7.27-7.30 (m, 2H, Ar-H), 7.33 (s, 1H, thiazole-H), 7.46-7.52 (m, 3H, Ar-H), 8.13 (s, 1H, triazole-H), 8.48 (s, 1H, -CH=N), 12.15 (bs, 1H, -NH).

N-Phenyl-1-(2-(((1-(2,6-difluorobenzyl)-1*H*-1,2, 3-triazol-4-yl)methylene)hydrazin-1-yl)thiazole-4-carboxamide, 8a: Yield 79%; m,p: 143-145°C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.95 (bs, 4H, piperazine-H), 3.56 (bs, 2H, piperazine-H), 4.01 (bs, 2H, piperazine-H), 5.73 (s, 2H, -CH_2), 7.01-7.08 (m, 3H, Ar-H), 7.24-7.27 (m, 2H, Ar-H), 7.38 (s, 1H, thiazole-H), 7.49-7.54 (m, 1H, Ar-H), 7.79-7.84 (m, 2H, Ar-H), 8.09 (s, 1H, triazole-H), 8.51 (s, 1H, -CH=N), 10.27 (s, 1H, -NH), 12.09 (s, 1H, -NH); LC-MS $[M+H]^+$: 440.1098.

N-(4-Chlorophenyl)-1-(2-(((1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazol-4-yl)methylene) hydrazin-1-yl) thiazole-4-carboxamide, 8b: Yield 82%. m.p. 159-161°C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 5.85 (s, 2H, -CH_2), 7.07-7.11 (m, 2H, Ar-H), 7.36-7.39 (m, 3H, Ar-H, thiazole-H), 7.43-7.48 (m, 1H, Ar-H), 7.81 (d, 2H, *J* = 8.6 Hz, Ar-H), 8.05 (s, 1H, triazole-H), 8.59 (s, 1H, -CH=N), 10.25 (s, 1H, -NH), 12.51 (s, 1H, -NH).

N-(4-Fluorophenyl)-1-(2-(((1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazol-4-yl)methylene) hydrazin-1-yl) thiazole-4-carboxamide, 8c: Yield 77%. m.p.148-150°C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 5.82 (s, 2H, -CH_2), 7.07-7.18 (m, 4H, Ar-H), 7.39 (s, 1H, tiazole-H), 7.51-7.55 (m, 1H, Ar-H), 7.87-7.91 (m, 2H, Ar-H), 8.05 (s, 1H, triazole-H), 8.62 (s, 1H, -CH=N), 10.25 (s, 1H, -NH), 12.55 (s, 1H, -NH); LC-MS $[M+H]^+$: 458.0999.

N-(4-Methoxyphenyl)-1-(2-(((1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazol-4-yl)methylene)hydrazin-1-yl) thiazole-4-carboxamide, 8d: Yield 85%. m.p.105-107°C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 3.76 (s, 3H, -OCH_3), 5.82 (s, 2H, -CH_2),6.86 (d, 2H, *J* = 9.0 Hz, Ar-H), 7.10-7.14 (m, 2H, Ar-H), 7.34 (s, 1H, thiazole-H), 7.43-7.48 (m, 1H, Ar-H), 7.75-7.79 (d, 2H, *J* = 9.0 Hz, Ar-H), 8.07 (s, 1H, triazole-H), 8.53 (s, 1H, -CH=N), 10.21 (s, 1H, -NH), 12.53 (s, 1H, -NH); LC-MS $[M+H]^+$: 470.1191.

N-(Pyridin-4-yl)-1-(2-(((1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazol-4-yl)methylene)hydrazin-1-yl)thiazole-4-carboxamide, 8e: Yield 80%. m.p.165-167°C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 5.84 (s, 2H, -CH_2), 7.08-7.13 (m, 2H, Ar-H), 7.39 (s, 1H, thiazole-H), 7.45-7.49 (m, 1H, Ar-H), 7.90 (d, 2H, *J* = 8.4 Hz, Ar-H), 8.07 (s, 1H, triazole-H), 8.46 (d, 2H, *J* = 8.4 Hz), 8.53 (s, 1H, -CH=N), 10.21 (s, 1H, -NH), 12.53 (s, 1H, -NH); LC-MS $[M+H]^+$: 441.1047.

Conclusion

In conclusion, 1-((1-(2,6-difluorobenzyl)-1*H*-1, 2,3-triazol-4-yl)methylene)-2-(4-arylthiazol-2-yl) hydrazine derivatives **5a-h** and amide derivatives of 2-(((1-(2,6-difluorobenzyl)-1*H*-1,2,3-triazol-4-yl) methylene)hydrazin-1-yl)thiazole-4-carboxylic acid **7a-e** and **8a-e** were synthesized successfully. The antimicrobial evaluation of the synthesized compounds revealed that most of the compounds were active against the tested microorganisms.

Assessment of the antimicrobial activity of target compounds **5a-h**, **7a-e** and **8a-e** revealed that introduction of thiazole ring did not show any noticeable effect on the activity of thiosemicarbazide **4**. The results also showed that presence of amide linkage on thiazole ring was more effective as compared to the simple aryl substitution.

Supplementary Information

Supplementary information is available in the website http://nopr.niscair.res.in/handle/123456789/60.

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