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ARTICLE

Design, Synthesis, *in vitro* and *in silico* Study of 5-(Methylthio)-4-(*H*)-1,2,4-triazole-2-amine and its Derivatives

Sushama Kadam✉

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ABSTRACT

In this work, design and synthesis of 5-(methylthio)-4-(*H*)-1,2,4-triazole-2-amine and its derivatives were carried out using *N*-cyano-carbonimidodithioic acid dimethyl ester and methane thiol hydrazine monohydrate to obtain an excellent yield of the desired core molecule. It was observed that free -NH_2 group available at 2nd position, which itself acts as a pharmacophore useful to connect with alkyl/aryl substituted isocyanate and form urea moiety as a bridge between 5-(methylthio)-4-(*H*)-1,2,4-triazole and alkyl/aryl substituent which exhibited the antimicrobial and docking activities of the synthesized molecules. The QSAR, toxicokinetics, docking studies with selected antimicrobial PDBs are useful *in silico* study of derivatives. In this study, it is emphasised that the results obtained *in vitro* and *in silico* correspond with each other and provide a better understanding of how and where medications exert their effects at the molecular level. This is based on the fact that the results were found using the same drug.

KEYWORDS

5-(Methylthio)-4-(*H*)-1,2,4-triazole-2-amine, MCRs, SBDD, Green synthetic approach, PDBs.

INTRODUCTION

Combinatorial chemistry is one of the new technologies developed by academics and researchers in the pharmaceutical & biotechnology industries to reduce time and cost associated with producing effective, marketable and competitive new molecule, which can be acts as a drug [1,2]. Instead of start to synthesizing new molecules high throughput screening of designed molecules offers systematic way to build a divers set of molecular entities. 3-Amino-5-mercapto-1,2,4-triazole as a core molecule and moreover, sulphur attached to it then it shows numerous biological activities such as antitumor, anti-cancer, anti-inflammatory, anticonvulsant, antimalarial, anti-viral, analgesics, antioxidant *etc*. [3,4]. By considering these, we get attracted to synthesize its derivatives, which may be enhance its medicinal values [5,6].

Toxicity testing of new compounds is essential for drug development process; various screening methods used for toxicity testing of the newly synthesized compounds [7-10]. In this study, we planned to synthesize of 5-(methylthio)-4-(*H*)-1,2,4-triazole-2-amine and its derivatives and investigate and compare the *vitro* and *in silico* biological screening of the synthesized compounds.

Author affiliations:

Department of Chemistry, DGM's Hon'ble B.J. Arts, Commerce & Science College (Affiliated to Savitribai Phule Pune University), Ale, Junnar-412411, India

✉To whom correspondence to be addressed:

E-mail: sushmakadam.24@gmail.com

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EXPERIMENTAL

All the solvents used were purified using the literature procedures and the synthesized compounds were characterized by comparison of their R_f values on TLC, IR and NMR spectra as well as melting point with the authentic samples. IR spectra were recorded on Shimadzu FTIR instrument and Perkin-Elmer instrument using KBr pallets. ^{1}H NMR was recorded by Bruker Instrument ($CDCl_3$, 200 MHz), (DMSO-d_6 MHz), ^{13}C NMR (50 MHz, DMSO-d_6), DEPT-135.

Step-I: Synthesis of 5-(methylthio)-4*H*-1,2,4-triazole-3-amine (TZB): In a round bottom flask, *N*-cyanocarbonimidodithioic acid dimethyl ester (1.74 mL) and methane thiol were added to acetonitrile (10 mL) solvent and refluxed for 2 h after cooling to room temperature, hydrazine monohydrate (2.56 mL) was added in the reaction mixture and it further refluxed for additional 5 h (**Scheme-I**). The solvent was evaporated under reduced pressure and the resulting product was recrystallized from ethyl acetate (m.p.: 152-156 ºC, yield: 90%).

Synthesis of 5-(methylthio)-4*H*-1,2,4-triazole-3-amines: In a round bottom flask, aryl/alkyl substituted isocyanate (0.714 mL, 6.54 mmol) was taken in ethanol (10 mL) at 0 ºC. To this cooled solution, 5-(methylthio)-4*H*-1,2,4-triazole-3-amine (0.5 g, 5.95 mmol) was added and then stirred at room temperature for 2 h (**Scheme-I**). The pro-gress of reaction was monitored by TLC analysis. Formation of new single spot and complete consumption of compound **1** was observed; the reaction mixture was concentrated on rotary *vacuo* and the residue was purified by silica-gel column chro-matography. The new spot was isolated and concentrated to dryness. White solid product obtained was characterized.

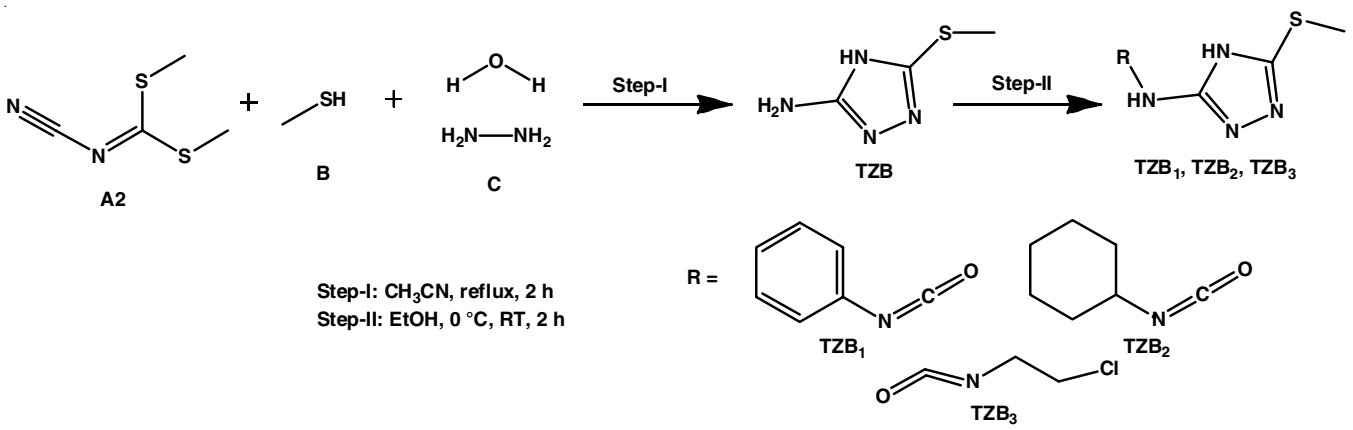
1-(5-(Methylthio)-4*H*-1,2,4-triazol-3-yl)-3-phenylurea (TZB_1): Yield: 42%, White solid, *m.f.*: $C_{10}H_{11}ON_5S$, *m.w.*: 249, LC-MS: 250.0 (M+1), R_t = 1.072 min. ^{1}H NMR (200 MHz, DMSO-d_6) δ ppm: 2.55 (s, 3H), 7.13 (t, *J* = 7.6 Hz, 1H), 7.31-7.46 (m, 4H), 7.63-6.67 (m, 2H), 9.87 (s, 1H). ^{13}C NMR (50 MHz, DMSO-d_6) δ ppm: 13.3 (q), 121.2 (d, 2C), 128.7 (d, 2C), 128.8 (d), 137.1 (s), 148.5 (s), 157.2 (s), 160.1 (s). IR (KBr, ν_{max}, cm^{-1}): 3353 (NH *str.*), 1644 (N-H bend), 1443 for CH_3 (C-H bend), 1527, 1436 (Ar-C=C *str.*), 1707.66 (-C=O), 1343-1254 (C=N *str.*), 839.38 (=C-H *str.*) 743, 686, 603, 556 (=C-H -Ar- *str.*).

1-Cyclohexyl-3-(5-(methylthio)-4*H*-1,2,4-triazol-3-yl) urea (TZB_2): Yield: 41%, White solid, *m.f.*: $C_{10}H_{17}ON_5S$, *m.w.*: 255, LC-MS: 256.1 (M+1), R_t = 1.816 min. ^{1}H NMR (200 MHz, DMSO-d_6) δ ppm: 1.11-1.44 (m, 5H), 1.56-1.79 (m, 5H), 2.50 (s, 3H), 3.54-3.58 (m, 1H), 7.31 (br s, 2H), 7.70 (d, *J* = 7.9 Hz, 1H). ^{13}C NMR (50 MHz, DMSO-d_6) δ ppm: 13.3 (q), 24.9 (t, 2C), 25.0 (t), 32.1 (t, 2C), 49.1 (d), 149.7 (d), 157.0 (s), 159.5 (s). IR (KBr, ν_{max}, cm^{-1}): 3385.42 (NH *str.*), 1483.96 (C-H bend), 1493, 1405, 1358 (cyclohexyl C-H *str.*), 1702.84 (-C=O), 1650 (N-H bend), 1269 (C=N *str.*), 988-749 (=C-H *str.*) 539, 503 for (C-H *str.*).

1-(2-Chloroethyl)-3-(5-(methylthio)-4*H*-1,2,4-triazol-3-yl)urea (TZB_3): Yield: 83%, white solid, *m.f.*: $C_5H_{10}ON_5SCl$, *m.w.*: 235, LC-MS: 236.0 (M+1), R_t = 1.452 min. ^{1}H NMR (200 MHz, DMSO-d_6) δ ppm: 2.50 (s, 3H), 3.53 (t, *J* = 6.9 Hz, 2H), 3.73 (t, *J* = 6.9 Hz, 2H), 7.34 (bs, 2H), 8.23 (t, *J* = 6.9). ^{13}C NMR (50 MHz, DMSO-d_6) δ ppm: 13.2 (q), 41.3 (t), 42.9 (t), 150.6 (s), 156.9 (s), 159.9 (s). IR (KBr, ν_{max}, cm^{-1}): 3324.68 (NH *str.*), 1500 (C-H bend), 1695 (-C=O), 1627 (N-H bend), 1507, 1405, 1321, 1192 (C=N *str.*), 750.17 (C-Cl *str.*).

Docking studies: In order to predict the antibacterial and antifungal activity of the synthesized compounds, six different drug targets based on their biological importance and wide range of reporting in the literature were selected [11]. The DHFR (PDB ID: 3Q1H); DNA Gyrase (PDB ID: 2XCT); topoisomerase IV (PDB ID: 3FV5); S-adenosyl homocysteine nucleosidase (PDB ID: 4YML) for antibacterial activity and penicillin binding protein (PDB ID: 1VQQ) and lanosterol 14 α-demethylase (PDB ID: 4LXJ) for antifungal activity [12-15]. Results from the docking studies predicted the IC_{50} value range of 5.24 µm to 1.41 µm levels with binding energy in a range of -4.00 to -8.00 Kcal/mol for selected drug target, respectively.

Antimicrobial activity: The antimicrobial study of the synthesized molecules were done by *in vitro* Kirby-Bauer Method. The NCIM provides Gram-positive bacterial strains *Staphallococcus aureus* (Sa-2178), *Bacillus subtilis* (Bs-2239), Gram-negative bacterial strains *Eschereschia coli* (Ec-25744), *Klibesiella aerogenus* (Ka-2249); antifungal strains *Aspergillus niger* (An-504) and *Penicillium chrysogenum* (Pc-709). The antimicrobial effects of the substances were tested quantitatively in respective broth media by means of double micro dilution and the minimal inhibition concentration (MIC) values (l µg/mL) were determined.



Scheme-I: Synthesis of TZB and their derivatives

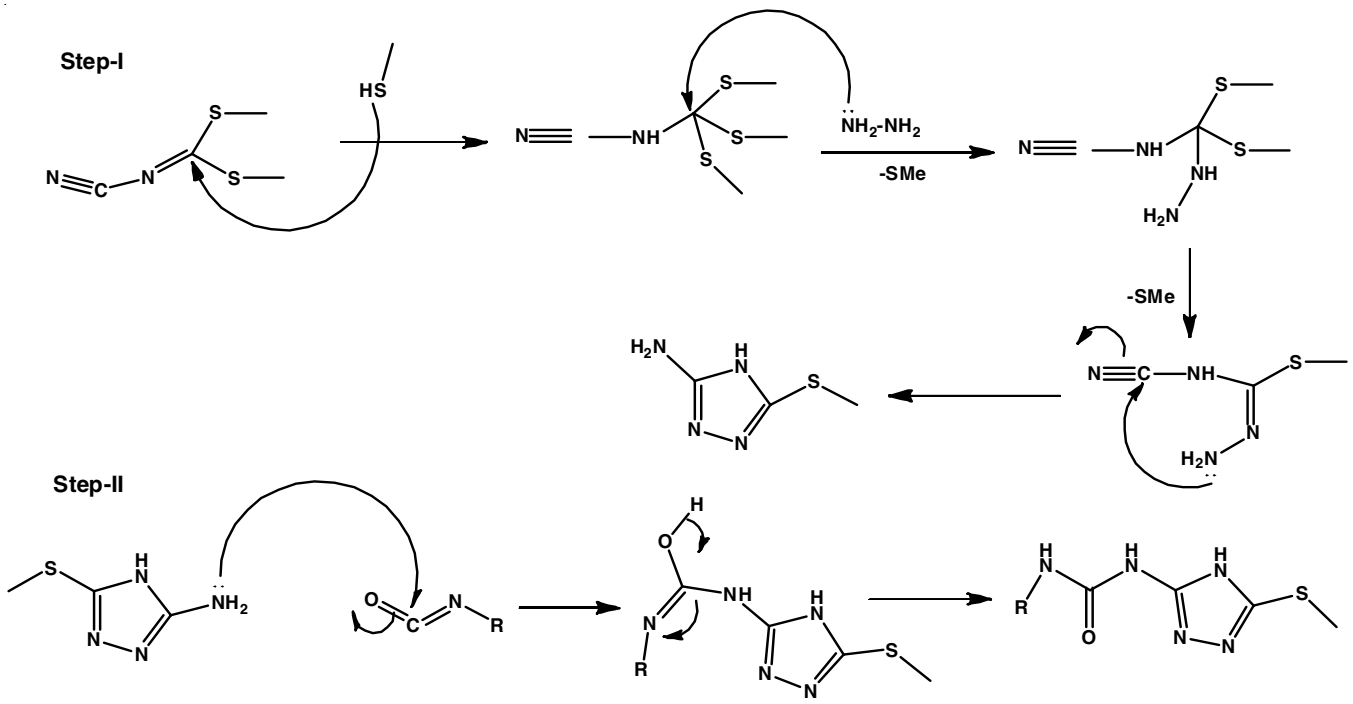
RESULTS AND DISCUSSION

The synthesis of 5-(methylthio)-4-(*H*)-1,2,4-triazole-2-amine (TZB) and its derivatives (TZB_1, TZB_2 and TZB_3) were carried out using *N*-cyano-carbonimidodithioic acid dimethyl ester and methane thiol hydrazine monohydrate to obtain an excellent yield of the desired core molecule. In next step, aryl/alkyl substituted isocyanate in ethanol was stirred with TZB for 2 h at room temperature and then the desired product was obtained in 99% yield. Free -NH_2 group available at 2 position, which itself acts as a pharmacophore is useful to connect with alkyl/aryl substituted isocyanate and form urea moiety as a bridge between 5-(methylthio)-4-(*H*)-1,2,4-triazole and alkyl/aryl substituent (**Scheme-II**), which enhances the antimicrobial and docking activities of molecules.

Based on Table-1, the antibacterial and antifungal activity (*in vitro*) ciprofloxacin (10 µg) and fluconazole (5 µg) were used as standard antibacterial and antifungal drugs, respectively. Dimethyl sulfoxide with dilution of 1:10 was used as solvent control. 5-(Methylthio)-4*H*-1,2,4-triazole-3-amine (TZB), which is the core structure of a molecule with preferable bioactive properties but it is inactive against Gram-positive bacteria and *E. coli* but 42% active against *K. aerogenus*. Derivatives TZB_2 and TZB_3 were inactive against *B. subtilis*, whereas TZB_1 TZB_2 and TZB_3 showed good antimicrobial activity against the selected microorganism. Toxins may be evaluated qualitatively or quantitatively.

Toxicology profiles & ADMET parameters are depicted in Table-2. The toxicity predictions of the present studied synthesized derivatives using Osiris Property Explorer were based on the functional group similarity for the query molecule with *in vitro* and *in vivo* validated compounds in the database. The toxicity study should be carried out with a minimum of three doses *viz.* low, medium and high doses in the experimental animals and the toxic effect compared with data from a control group of animals. Alkyl substituent increases hydrophobic interactions and increase in log P values has support the *in vitro* evaluation virtual screening of these molecules has displayed comparative same result [16].

Docking results of compounds targeting (Table-3) DHFR (PDB ID: 3Q1H), DNA gyrase (PDB ID: 2XCT) topoisomerase IV (PDB ID: 3FV5) S-adenosyl homocysteine nucleosidase (PDB ID: 4YML) for antibacterial activity shows excellent result almost -6 to -10 Kcal/mol binding energy and IC_{50} value 2 to 4 µm.



Scheme-II: Proposed plausible mechanism of TZB and their derivatives

TABLE-1
ANTIMICROBIAL SCREENING OF THE SYNTHESIZED TZB AND THEIR DERIVATIVES

Compd.	Antibacterial activity								Antifungal activity			
	Gram-positive				Gram-negative				*Aspergillus niger*		*Penicillium chrysogenum*	
	S. aureus		*B. subtilis*		*E. coli*		*K. aurogenus*					
	MZI (mm)	Activity (%)	MZI (mm)	Activity (%)	MZI (mm)	Activity (%)	MZI (mm)	Activity (%)	MZI (mm)	Activity (%)	MZI (mm)	Activity (%)
TZB	–	–	–	–	–	–	10.5	42.0	–	–	–	–
TZB_1	8.50	34.0	9.5	38	9.2	36.8	6.8	27.2	–	–	–	–
TZB_2	9.40	37.6	–	–	8.8	35.2	7.0	28.0	–	–	7.4	26.42
TZB_3	11.25	45.0	–	–	7.2	28.8	6.8	27.2	–	–	9.2	32.85

TABLE-2
ADMET PARAMETERS AND TOXICOLOGY PROFILE OF THE SYNTHESIZED TZB AND THEIR DERIVATIVES

Compd.	ADMET parameters					Toxicity			
	Log P	H-bond donors	H-Bond acceptors	Rotatable bonds	TPSA	Mutagenic	Tumerogenic	Effect on reproductive system	Eye irritant
TZB	-0.1	2	4	1	92.8	None	None	None	None
TZB_1	1.6	3	6	3	108	None	None	None	None
TZB_2	1.5	3	6	3	108	None	None	None	None
TZB_3	0.5	3	6	4	108	High	High	High	None

TABLE-3
DOCKING RESULTS OF SYNTHESIZED TZB AND THEIR DERIVATIVES

Compd.	**Antibacterial activity**							
	DHFR (PDB ID: 3Q1H		DNA Gyrase (PDB ID: 2XCT)		Topoisomerase IV (PDB ID: 3FV5)		S-Adenosyl homocysteine nucleosidase (PDB ID: 4YML)	
	Binding energy (Kcal/mol)	Predicted IC_{50}	Binding energy (Kcal/mol)	Predicted IC_{50}	Binding energy (Kcal/mol)	Predicted IC_{50}	Binding energy (Kcal/mol)	Predicted IC_{50}
TZB	-6.3	3.75 µM	-6.4	162.39 nM	-6.4	1.97 µM	-6.0	7.84 µM
TZB_1	-7.8	1.89 µM	-8.3	36.14 nM	-6.6	1.89 µM	-6.7	6.92 µM
TZB_2	-7.4	2.83 µM	-8.2	31.14 nM	-6.6	1.89 µM	-6.4	7.38 µM
TZB_3	-7.2	2.98 µM	-7.8	54.62 nM	-7.3	1.38 µM	-7.1	6.43 µM

Compd.	**Antifungal activity**			
	Penicillin binding protein (PDB ID: 1VQQ)		Lanosterol 14 α-demethylase (PDB ID: 4LXJ)	
	Binding energy (Kcal/mol)	Predicted IC_{50}	Binding energy (Kcal/mol)	Predicted IC_{50}
TZB	-6.5	4.28 µM	-8.0	486.3 nM
TZB_1	-7.6	2.98 µM	-9.3	286.2 nM
TZB_2	-8.0	2.69 µM	-10.0	137.5 nM
TZB_3	-8.1	2.58 µM	-9.7	168.3 nM

Conclusion

All the synthesized derivatives from 5-(methylthio)-4*H*-1,2,4-triazole-3-amine exhibited the versatile biological activities. The antimicrobial evaluation by *in vitro* and based on computational and bioinformatics studies these synthesized derivatives will be useful as some anti-infective agents similarly cytotoxicity studies, which will help in future to make it more medicinally useful to the society.

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