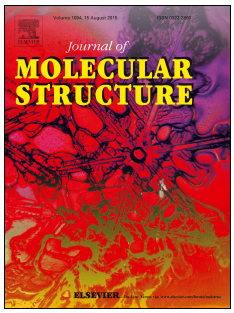

Synthesis, antimicrobial and antioxidant evaluation, and molecular docking study of 4,5-disubstituted 1,2,4-triazole-3-thiones

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Hamid Beyzaei: Supervision, Writing- Original Draft, Writing- Reviewing and Editing. **Maryam Ghanbari Kudeyani:** Methodology, Investigation. **Hojat Samareh Delarami:** Software, Data Curation. **Reza Aryan** Conceptualization, Formal Analysis.

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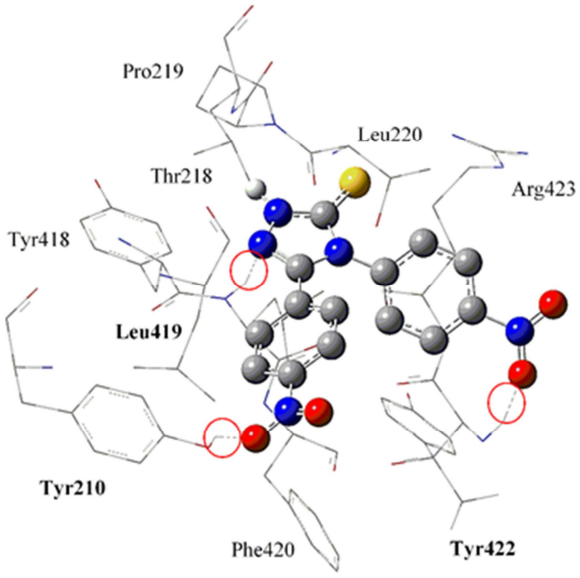
Department of Chemistry, Faculty of Science, University of Zabol, Zabol, Iran



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Hamid Beyzaei*, Maryam Ghanbari Kudeyani, Hojat Samareh Delarami, Reza Aryan

Department of Chemistry, Faculty of Science, University of Zabol, Zabol, Iran

*Corresponding author: Hamid Beyzaei; E-mail: hbeyzaei@yahoo.com and hbeyzaei@uoz.ac.ir; Tel.: +98 543 123 2186; Fax: +98 543 123 2180.

E-mail addresses: hbeyzaei@yahoo.com (H. Beyzaei), ghanbari.m.chemist@gmail.com (M. Ghanbari Kudeyani), hsd102012@gmail.com (H. Samareh Delarami), rezaaryanchemist@yahoo.com (R. Aryan).

Abstract

There is a growing need for new antibiotics and antifungal drugs to combat pathogenic bacteria and fungi. In order to develop potential antimicrobial agents, some 1,2,4-triazole-3-thiones were synthesized from the reaction of hydrazides with isothiocyanates under optimized conditions in deep eutectic solvent of potassium carbonate-glycerol (1:5 molar ratio). Blocking properties of all products were assessed on a variety of Gram-positive and Gram-negative bacterial as well as fungal pathogens. Good to excellent inhibitory effects especially against fungi were observed with all synthesized compounds. 5-(4-Hydroxyphenyl)-4-(4-nitrophenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (3b) showed significant antioxidant activity according to the results obtained from 2,2-diphenyl-1-picrylhydrazylf (DPPH) free radical scavenging experiments. The possible interaction mechanism of synthetic triazoles with 1IYL enzyme on *Aspergillus fumigatus* was investigated by molecular docking method. A complete agreement was found between experimental data and theoretical calculations. Hydrogen bond acceptor strength of N-1 in 1,2,4-triazole rings was the main cause of the observed differences.

Keywords 1,2,4-Triazole-3-thione, Deep eutectic solvent, Glycerol, 1IYL, Molecular docking, Antimicrobial and antioxidant evaluation.

Introduction

Triazoles are an important class of aromatic five-membered heterocyclic compounds with a wide variety of biological activities. They consist of two structural isomers: 12,3- and 1,2,4-triazole. Compounds based on 1,2,4-triazole scaffold show diverse biological and pharmacological activities [1-3]. 1,2,4-Triazole-3-thiones occupy a unique place among compounds containing 1,2,4-triazole ring. Lesinurad is a selective inhibitor of uric acid transporter 1 (URAT1) and organic anion transporter 4 (OAT4) indicated for the treatment of hyperuricemia [4]. Prothioconazole is a systemic fungicide that can be used alone or combined with other seed and foliar treatments [5]. The synthesized compounds having 1,2,4-triazole-3-thione skeleton have been reported to possess biological and pharmacological activities such as anticonvulsant, urease inhibition, antioxidant, analgesic. anti-parasitic, antiulcer, anticancer, anti-HIV, anti-tuberculosis, antiamoebic, antigiardial, antiepileptic, anti-inflammatory, antidepressant and anxiolytic [6-16]. This wide range of activities has encouraged chemists to design new synthetic methods. Cyclocondensation of hydrazinecarbothioamides or thiosemicarbazide with aldehydes, ethyl benzimidate or aroyl halides, reaction of methyl 3-acyldxithiocarbazates with amines, thermolysis of thiosemicarbazones, condensation of aroylthiosemicarbazides, reaction of 2-hydrazinoethanol with aroyl isothiocyanate, and reaction of hydrazides and isothiocyanates are a few examples [17-25].

Green chemistry is a branch of chemistry, which directs chemical processes to improve the quality of life of all living organisms. One of the main goals of green chemistry is the development of the use of environmentally friendly solvents rather than harmful solvents. Solvents such as water, glycerol, supercritical carbon dioxide and polyethylene glycol have been suggested for this purpose. Recently, the use of deep eutectic solvents (DESs) has been expanding due to their diverse applications [26]. Glycerol (glycerin)/potassium carbonate is a green catalytic media that its efficiency has been proven in the synthesis of different heterocyclic compounds [27, 28].

Molecular docking studies are efficient computational procedures which can predict how a ligand interacts with the protein's binding site. Some (2*R*,3*R*)-1-(1*H*-1,2,4-triazol-1-yl)-2-(2,4-difluorophenyl)-3-(*N*-substitutied)-2-butanole derivatives were designed and synthesized as antifungal agents by Wu *et al*., their inhibitory mechanism was determined with molecular docking [29]. A synthesized hybrid of ciprofloxacin and 1,2,4-triazole-3-thione derivative showed better inhibitory activities against *Candida albicans* versus itraconazole, it strongly binds to active site of target enzyme according to reasonable docking scores [30]. A novel synthetic series of 1*H*-1,2,4-triazole was evaluated for their potential antifungal activities, the interactions of the most effective compounds in binding site of protein were studied by molecular docking methods [31].

In this context, one-pot synthesis of 4,5-disubstituted 1,2,4-triazole-3-thione derivatives **3a-i** was reported. They were prepared *via* cyclocondensation of various aryl hydrazides **1a-e** with isothiocyanates **2a-e** in the presence of glycerol/K_2CO_3 as catalyst and reaction media. *In vitro* inhibitory potential of synthesized compounds was evaluated on pathogens including five Gram-negative and four Gram-positive bacteria as well as three fungi. In addition, their free radical scavenging capacity was assessed to determine antioxidant activity. The interaction of all derivatives in active site of 1IYL was explored to determine their possible inhibitory mechanism against *Aspergillus fumigatus* strain.

Material and Methods

Chemicals

All yields refer to isolated products. Melting points were recorded on a Kruss type KSP1N melting point apparatus and are uncorrected. The reaction progress was monitored by aluminum TLC plates pre-coated with silica gel 60 with fluorescent indicator F254 using *n*-hexane/ethyl acetate (8:2, v/v) as the desired mobile phase. The resulted TLC plates were visualized under UV radiation (254 nm). IR spectra of compounds were recorded on a Bruker Tensor-27 FT-IR spectrometer using KBr disks. ^{1}H and ^{13}C NMR spectra of products in DMSO-d_6 were recorded on a Bruker FT-NMR Ultra Shield-400 spectrometer (400 and 100 MHz, respectively). Elemental analyses for C, H, N and S atoms were performed on a Termo Finnigan Flash EA microanalyzer. UV/Vis spectroscopy was performed using a Jenway 6405 UV/Vis spectrophotometer.

General procedure for the synthesis of 1,2,4-triazole-3-thiones **3a-i**

A mixture of both 1 mmol of hydrazides **1a-i** and isothiocyanates **2a-i** were added to 1 g of prepared *in situ* glycerol/K_2CO_3 (5:1 molar ratio) DES. The contents were vigorously stirred at 100 °C until the end of the reaction was determined by TLC analysis. The reaction mixture was cooled downed to room temperature; 10 ml of ethanol was added with stirring to it. The precipitates were filtered off, washed respectively with ethanol (5 ml) and water (5 ml), dried in an oven. The products **3a-i** were obtained without need to further purification.

4-(4-Nitrophenyl)-5-phenyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**3a**)

Yield: 0.23 g, 78%; m.p.: 278-280 °C (Lit. [19]: 285 °C); reaction time: 10 h; IR (KBr) *v*: 3428 (NH), 1646 (C=N), 1591, 1511, 1328, 1242 (C=S), 1175, 1112, 753, 687 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.83 (s, 1H, NH),

8.26 (d, *J* = 8.1 Hz, 2H, H-3′,5′), 7.90 (s, 2H, H-2″,6″), 7.78 (d, *J* = 8.1 Hz, 2H, H-2′,6′), 7.57 (s, 3H, H-3″,4″,5″) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ: 117.3 (C-3′,5′), 125.9 (C-2″,6″), 126.2 (C-3″,5″), 129.2 (C-1″), 129.8 (C-2′,6′), 131.7 (C-4″), 141.5 (C-1′), 143.0 (C-4′), 145.3 (C-5), 159.6 (C-3); Anal. Calcd. for $C_{14}H_{10}N_4O_2S$: C 56.37, H 3.38, N 18.78, S 10.75. Found: C 56.41, H 3.39, N 18.75, S 10.73.

5-(4-Hydroxyphenyl)-4-(4-nitrophenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**3b**)

Yield: 0.24 g, 76%; m.p.: 180-182 °C; reaction time: 8 h; IR (KBr) *v*: 3454 (NH), 3338 (OH), 1657 (C=N), 1586, 1501, 1331, 1245 (C=S), 1178, 1112, 851, 746, 691 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ: 10.36 (brs, 1H, NH), 10.00 (s, 1H, OH), 8.17 (d, *J* = 7.7 Hz, 2H, H-3′,5′), 7.88 (d, *J* = 7.7 Hz, 2H, H-2′,6′), 7.80 (d, *J* = 7.2 Hz, 2H, H-2″,6″), 6.84 (d, *J* = 7.2 Hz, 2H, H-3″,5″) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ: 115.3 (C-3″,5″), 123.3 (C-1″), 124.0 (C-3′,5′), 125.2 (C-2′,6′), 130.4 (C-2″,6″), 141.5 (C-1′), 143.8 (C-4′), 146.2 (C-5), 161.2 (C-4″), 166.2 (C-3) ppm; Anal. Calcd. for $C_{14}H_{10}N_4O_3S$: C 53.50, H 3.21, N 17.83, S 10.20. Found: C 53.54, H 3.19, N 17.88, S 10.17.

4-(4-Nitrophenyl)-5-(4-(pyridin-4-yl)phenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**3c**)

Yield: 0.24 g, 80%; m.p. > 300 °C (Lit. [32]: 320-322 °C); reaction time: 9 h; IR (KBr) *v*: 3415 (NH), 3213, 2362, 1642 (C=N), 1605, 1556, 1500, 1462, 1327, 1244 (C=S), 1173, 1111, 846, 728, 696, 646, 502 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.97 (brs, 1H, NH), 8.77 (d, *J* = 3.9 Hz, 2H, H-3″,5″), 8.19 (d, *J* = 8.7 Hz, 2H, H-3′,5′), 7.81-7-89 (m, 4H, H-2′,6′,2″,6″) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ: 122,0 (C-2″,6″), 124.1 (C-3′,5′), 125.7 (C-2′,6′), 127.6 (C-1″), 139.8 (C-1′), 143.8 (C-4′), 146.0 (C-5), 150.7 (C-3″,5″), 164.9 (C-3) ppm; Anal. Calcd. for $C_{13}H_9N_5O_2S$: C 52.17, H 3.03, N 23.40, S 10.71. Found: C 52.21, H 3.05, N 23.41, S 10.66.

5-(4-(Furan-2-yl)phenyl)-4-(4-nitrophenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**3d**)

Yield: 0.22 g, 76%; m.p. 185-186 °C (Lit. [33]: 187-188 °C); reaction time: 8 h; IR (KBr) *v*: 3418 (NH), 2361, 1629 (C=N), 1551, 1510, 1408, 1334, 1267 (C=S), 1188, 1110, 844, 704 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.55 (brs, 1H, NH), 8.26 (d, *J* = 8.7 Hz, 2H, H-3′,5′), 7.99 (s, 1H, H-5″), 7.77 (d, *J* = 8.7 Hz, 2H, H-2′,6′), 7.17 (s, 1H, H-3″), 6.75 (s, 1H, H-4″) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ: 112.8 (C-4″), 113.4 (C-3″), 117.3 (C-3′,5′), 125.9 (C-2′,6′), 139.0 (C-5″), 140.7 (C-2″), 141.5 (C-1′), 144.3 (C-4′), 146.7 (C-5), 159.0 (C-3) ppm; Anal. Calcd. for $C_{12}H_8N_4O_3S$: C 50.00, H 2.80, N 19.44, S 11.12. Found: C 49.95, H 2.80, N 19.46, S 11.09.

4,5-Bis(4-nitrophenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**3e**)

Yield: 0.32 g, 93%; m.p. 249-250 °C; reaction time: 9 h; IR (KBr) *v*: 3479 (NH), 1627 (C=N), 1509, 1301, 1260 (C=S), 1176, 1107, 838, 624 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 14.35 (s, 1H, NH), 8.34 (d, *J* = 7.4 Hz, 2H, H-3″,5″), 8.31 (d, *J* = 7.0 Hz, 2H, H-3′,5′), 8.10 (d, *J* = 7.4 Hz, 2H, H-2″,6″), 7.75 (d, *J* = 7.0 Hz, 2H, H-2′,6′) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ: 117.1 (C-3′,5′), 123.0 (C-3″,5″), 127.7 (C-2″,6″), 130.4 (C-2′,6′), 131.2 (C-1′), 134.6 (C-1″), 145.3 (C-4′), 146.8 (C-5), 150.5 (C-4″), 159.1 (C-3) ppm; Anal. Calcd. for $C_{14}H_9N_5O_4S$: C 48.98, H 2.64, N 20.40, S 9.34. Found: C 48.95, H 2.63, N 20.44, S 9.32.

5-(4-Nitrophenyl)-4-phenyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**3f**)

Yield: 0.22 g, 74%; m.p.: 271-273 °C (Lit. [34]: 252-254 °C); reaction time: 6 h; IR (KBr) *v*: 3481 (NH), 1630 (C=N), 1591, 1480, 1301, 1252 (C=S), 1175, 1106, 842, 750, 624 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 14.27 (s, 1H, NH), 8.34 (d, *J* = 6.7 Hz, 2H, H-3″,5″), 8.11 (d, *J* = 6.7 Hz, 2H, H-2″,6″), 7.48 (m, 3H, H-3′,4′,5′), 7.38 (m, 2H, H-2′,6′) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ: 122.9 (C-3″,5″), 125.32 (C-4′), 129.1 (C-2′,6′), 129.6 (C-3′,5′), 129.8 (C-2″,6″), 134.6 (C-1′), 135.4 (C-1″), 147.7 (C-5), 150.7 (C-4″), 166.5 (C-3) ppm; Anal. Calcd. for $C_{14}H_{10}N_4O_2S$: C 56.37, H 3.38, N 18.78, S 10.75. Found: C 56.41, H 3.39, N 18.74, S 10.77.

4-(4-Fluorophenyl)-5-(4-nitrophenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**3g**)

Yield: 0.22 g, 64%; m.p.: 229-231 °C; reaction time: 5 h; IR (KBr) *v*: 3419 (NH), 1691 (C=N), 1600, 1500, 1459, 1417, 1281, 1237 (C=S), 1011, 966, 853, 773, 695, 607, 543 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 14.40 (s, 1H, NH), 8.37 (d, *J* = 7.2 Hz, 2H, H-3″,5″), 8.16 (d, *J* = 7.2 Hz, 2H, H-2″,6″), 7.45 (d, *J* = 7.8 Hz, 2H, H-2′,6′), 7.34 (d, *J* = 7.8 Hz, 2H, H-3′,5′) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ: 116.6 (C-3′,5′), 122.9 (C-3″,5″), 126.3 (C-2″,6″), 130,5 (C-1′), 131.4 (C-2′,6′), 134.4 (C-1″), 146.2 (C-5), 149.7 (C-4″), 160.6 (C-3), 164.5 (C-4′) ppm; Anal. Calcd. for $C_{14}H_9FN_4O_2S$: C 53.16, H 2.87, N 17.71, S 10.14. Found: C 53.21, H 2.88, N 17.74, S 10.10.

5-(4-Nitrophenyl)-4-(*p*-tolyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**3h**)

Yield: 0.27 g, 88%; m.p.: 169-170 °C; reaction time: 9 h; IR (KBr) *v*: 3414 (NH), 1695 (C=N), 1607, 1511, 1458, 1398, 1327, 1225 (C=S), 1097, 1011, 967, 842, 775, 723, 672, 624, 594 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.80 (s, 1H, NH), 8.33 (d, *J* = 6.9 Hz, 2H, H-3″,5″), 8.12 (d, *J* = 6.9 Hz, 2H, H-2″,6″), 7.25 (d, *J* = 5.9 Hz, 2H, H-2′,6′), 7.13 (d, *J* = 5.9 Hz, 2H, H-3′,5′), 2.32 (s, 3H, CH_3) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.2 (CH_3), 113.8 (C-3″,5″), 128.8 (C-3′,5′), 129.5 (C-2″,6″), 130.1 (C-2′,6′), 131.6 (C-1′), 132.8 (C-4′), 135.5 (C-1″), 150.4 (C-5), 151.7 (C-4″), 168.4 (C-3) ppm; Anal. Calcd. for $C_{15}H_{12}N_4O_2S$: C 57.68, H 3.87, N 17.94, S 10.26. Found: C 57.62, H 3.85, N 17.97, S 10.30.

4-Ethyl-5-(4-nitrophenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**3i**)

Yield: 0.20 g, 80%; m.p.: 174-176 °C; reaction time: 5 h; IR (KBr) *v*: 3427 (NH), 1697 (C=N), 1607, 1514, 1396, 1327, 1241 (C=S), 1020, 967, 819 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.69 (s, 1H, NH), 8.32 (d, *J* = 6.7 Hz, 2H, H-3″,5″), 8.13 (d, *J* = 6.7 Hz, 2H, H-2″,6″), 4.40 (q, *J* = 7.5 Hz, 2H, CH_2), 1.12 (t, *J* = 7.5 Hz, 3H, CH_3) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ: 13.8 (CH_3), 63.5 (CH_2), 113.9 (C-3″,5″), 129.7 (C-2″,6″), 130.1 (C-2′,6′), 136.4 (C-1″), 151.4 (C-5), 151.8 (C-4″), 166.7 (C-3) ppm; Anal. Calcd. for $C_{10}H_{10}N_4O_2S$: C 47.99, H 4.03, N 22.39, S 12.81. Found: C 48.02, H 4.05, N 22.38, S 12.77.

Biological assay

Gram-negative bacterial strains including *Salmonella enterica subsp. enterica* (PTCC 1709), *Pseudomonas aeruginosa* (PTCC 1310), *Shigella dysenteriae* (PTCC 1188), *Acinetobacter baumannii* (PTCC 1855), *Escherichia*

coli (PTCC 1399), Gram-positive bacterial strains including *Streptococcus pyogenes* (PTCC 1447), *Staphylococcus epidermidis* (PTCC 1435), *Listeria monocytogenes* (PTCC 1297), *Bacillus cereus* (PTCC 1665) and fungi including *Aspergillus fumigatus* (PTCC 5009), *Candida albicans* (PTCC 5027) and *Fusarium oxysporum* (PTCC 5115) were prepared from the Persian Type Culture Collection (PTCC), Karaj, Iran. Broth microdilution and streak plate methods were applied for antimicrobial testing according to the previously described procedures [27]. DPPH free radical scavenging method was also used to determine the antioxidant activity of synthetic heterocycles [27]. All biological tests were repeated at least three times. The results were reported as the mean of three independent experiments.

Computational details

Geometrical optimization of 1,2,4-triazole compounds was performed using the Gaussian 09 program package [35] at the B3LYP level of theory [36]. Vibrational frequency calculations were also performed at the same level to ensure that the structures are local minima.

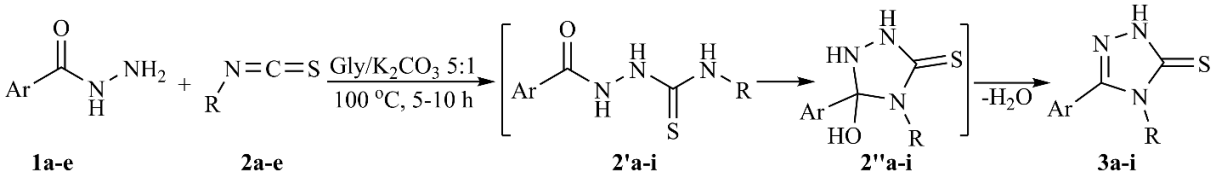
For molecular docking of 1,2,4-triazole compounds, we selected the X-ray structure of *N*-myristoyltransferase from *Candida albicans* (CaNMT, PDB ID: 1IYL) from the Protein Data Bank as fungal target. The molecular docking was performed using MGL tools 1.5.6 including the AutoDock4.2 scoring function to predict the interactions between ligand and receptor [37]. Then, the ligand and receptor was considered flexible and rigid, respectively. The best-docked conformation of the ligand-receptor complex is according to the lowest binding energy.

A blind docking was performed to recognize the binding sites of 1IYL receptor. For 1IYL grid size was set at 126×126×126 Å in the x, y, and z-axes, respectively, with 0.375 Å grids spacing to cover the whole receptor. The interaction energies between each amino acid with the best pose of docked 1,2,4-triazoles into 1IYL binding site were calculated by Molegro Molecular Viewer 2.5 (MMV) (http://www.molegro.com/mmv-product.php).

Results and Discussion

Chemistry

1,2,4-Triazole-3-thiones **3a-i** were synthesized through reaction of aryl hydrazides **1a-e** with isothiocyanates **2a-e**, as shown in Scheme 1 and Fig. 1. Reactions were progressed in a fixed and optimized 5:1 molar ratio of glycerol/K_2CO_3 at 100 °C for 5-10 h.



Ar = C_6H_5, 4-HO-C_6H_4, 4-O_2N-C_6H_4, 4-pyridine, 2-furan; R = C_2H_5, C_6H_5, 4-F-C_6H_4, 4-H_3C-C_6H_4, 4-O_2N-C_6H_4

Scheme 1 Synthesis of 1,2,4-triazole-3-thione derivatives 3a-i

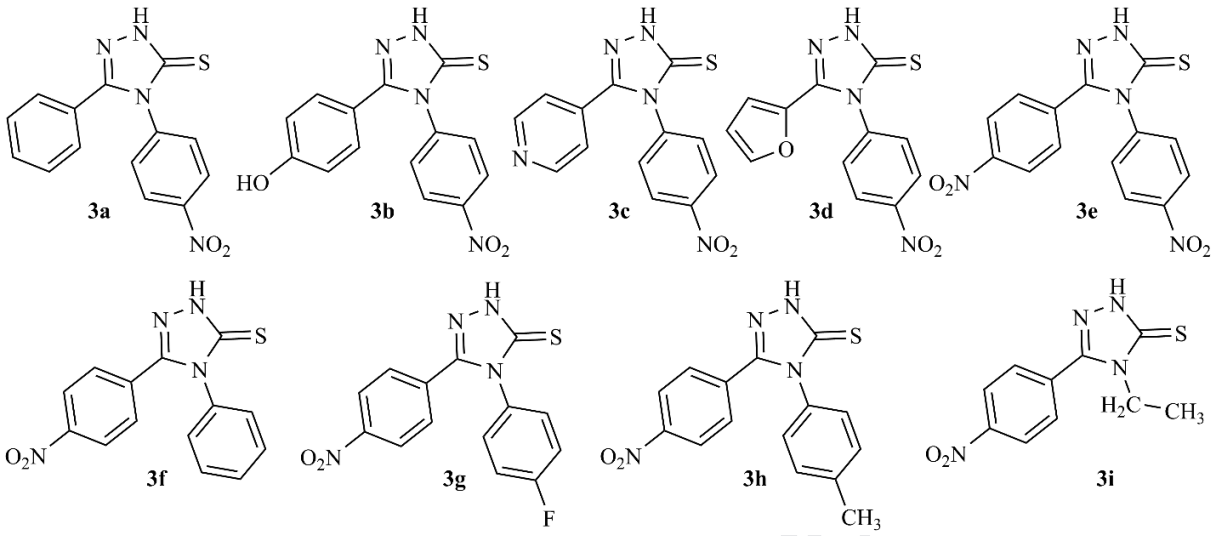


Fig. 1 Chemical structure of the synthesized 1,2,4-triazole-3-thiones

The reaction conditions were optimized in terms of solvent and temperature (Table 1). Reaction of 1 mmol of both benzhydrazide and 4-nitrophenyl isothiocyanate in 2 g of solvent was considered as a model. Condensation of intermediate carbothioamides to 1,2,4-triazole-3-thione derivatives occurred at a minimum temperature of 100 °C as well as by-products were formed at higher temperatures; therefore, it was kept constant in all processes. No condensation was observed in glycerol media (Entry 1). Traces of 1,2,4-triazoles were afforded in DESs containing choline chloride (ChCl) as hydrogen bond acceptor and urea, glucose, citric acid and tartaric acid as hydrogen bond receptors (Entries 2-5). Reaction yield was improved in choline chloride/glycerol (Entry 6). Reaction progress was studied in different molar ratios of K_2CO_3/glycerol (Entries 7-12). The highest yield and shortest time of reaction was achieved in K_2CO_3/glycerol (1:5 Molar, Entry 10).

Table 1 Optimization of the model reaction conditions for the preparation of 1,2,4-triazole-3-thione 3a

Entry	Solvent	Time (h)	Yield (%)
1	Glycerol	2	0
2	ChCl/urea 1:2	4	Trace
3	ChCl/Glucose 2:1	9	Trace
4	ChCl/Citric acid 1:1	10	Trace
5	ChCl/Tartaric acid 1:1	7	Trace
6	ChCl/glycerol 1:2	10	66
7	K_2CO_3/glycerol 1:2	7	32
8	K_2CO_3/glycerol 1:3	8	59
9	K_2CO_3/glycerol 1:4	4	43
10	K_2CO_3/glycerol 1:5	5	78
11	K_2CO_3/glycerol 1:6	9	54
12	K_2CO_3/glycerol 1:7	8	Trace

The chemical structure of all synthesized triazoles was characterized by FT-IR, ^{1}H- and ^{13}C-NMR spectroscopies and elemental analysis. The presence of thiocarbonyl groups in triazole rings as predominant tautomer was proved

by their absorption bands in the region of 1225-1267 cm^{-1} and carbon-13 chemical shifts appeared at 159.0-168.4 ppm [38].

Among the various methods proposed for the preparation of 1,2,4-triazole-3-thiones, reaction of hydrazides and isothiocyanates have been well developed. The reaction occurs in solvents such as benzene, diethyl ether, DMSO, 1,4-dioxane, dichloromethane and *N*,*N*-dimethyl acetamide [18, 25, 39-42]. Intermediate 1-acylthiosemicarbazides produced during this reaction are usually separated, purified and used in the next step [32, 33, 43]. They are converted to 1,2,4-triazole and 1,3,4-thiadiazole in the presence of basic or acidic catalysts, respectively [18, 25]. Caustic soda and potash are mainly applied under basic conditions. 1,2,4-Tiazole-3-thione derivatives have been synthesized *via* a one-step reaction in short times under microwave irradiation [44].

It is predicted that the reaction proceeds in three steps. The first step is the formation of 1-acylthiosemicarbazides **2'a-i** *via* the nucleophilic addition of hydrazides **1a-e** to isothiocyanates **2a-e**. In the next step, the intramolecular attachment of NH group belonging to the isothiocyanate component to carbonyl group can produce 4,5-disubstituted 5-hydroxy-1,2,4-triazolidine-3-thiones **2''a-i**. Finally, 1,2,4-triazole-3-thiones **3a-i** are generated by the elimination of water. The reaction times of isothiocyanates **2a-d** are approximately 1.5-2 times longer than those of isothiocyanates **2f-i**. If the first step was the rate-determining step, the total reaction times should be reduced in the presence of isothiocyanates containing strong electron withdrawing NO_2 groups. As a result, the second stage is predicted as the rate-determining step. In this step, the nucleophilic attack of NH bond to carbonyl group becomes faster as the electron-attracting ability of substituents on isothiocyanates decreases; in fact, the nucleophilicity of NH groups diminishes in the presence of adjacent electron withdrawing substituents.

Biological study

In vitro inhibitory potentials of 1,2,4-triazoles **3a-i** were studied against a variety of pathogenic Gram-positive and Gram-negative bacteria as well as fungi. The results were compared with those from gentamicin and terbinafine, and presented as MIC (the Minimum Inhibitory Concentration), MBC (the Minimum Bactericidal Concentration) and MFC (the Minimum Fungicidal Concentration) values in Tables 2 and 3.

Table 2 Antibacterial effects of 1,2,4-triazoles 3a-i

Bacterial species		Products									Antibiotic
		3a	3b	3c	3d	3e	3f	3g	3h	3i	Gentamicin
1310	MIC	1024	128	256	1024	2048	2048	-	-	-	0.063
	MBC	2048	256	512	2048	2048	4096	-	-	-	0.063
1709	MIC	1024	256	128	-	1024	2048	-	-	2048	8
	MBC	1024	256	256	-	1024	2048	-	-	2048	8
1399	MIC	512	2048	1024	-	-	-	-	-	-	8
	MBC	512	4096	2048	-	-	-	-	-	-	8
1188	MIC	512	2048	512	-	-	512	-	2048	2048	0.031
	MBC	1024	2048	512	-	-	512	-	4096	4096	0.063
1855	MIC	-	32	256	-	2048	1024	2048	-	2048	16

	MBC	-	64	256	-	4096	2048	4096	-	4096	32
1665	MIC	256	-	2048	512	2048	1024	1024	-	2048	0.25
	MBC	512	-	2048	1024	4096	1024	2048	-	2048	4
1435	MIC	-	32	512	1024	512	512	-	-	-	1
	MBC	-	64	1024	1024	512	512	-	-	-	2
1297	MIC	1024	-	512	-	2048	-	-	-	-	2
	MBC	1024	-	512	-	4096	-	-	-	-	2
1447	MIC	4096	256	1024	512	512	512	-	2048	512	2
	MBC	2048	256	1024	512	512	1024	-	4096	1024	2

– No noticeable antibacterial effect at concentration of 2048 μg ml^{-1}, MIC (μg ml^{-1}), MBC (μg ml^{-1}).

Table 3 Antifungal effects of 1,2,4-triazoles 3a-i

Fungal species		Products									Antifungal
		3a	3b	3c	3d	3e	3f	3g	3h	3i	Terbinafine
5009	MIC	2048	64	64	512	2	128	128	2048	256	32
	MFC	4096	128	64	512	4	256	256	4096	512	32
5115	MIC	4	4	4	2	8	8	4	2	4	32
	MFC	4	8	8	4	8	8	8	4	8	64
5027	MIC	-	64	64	1024	1	2	128	32	-	32
	MFC	-	128	64	2048	2	4	128	64	-	64

– No noticeable antifungal effect at concentration of 2048 μg ml^{-1}, MIC (μg ml^{-1}), MFC (μg ml^{-1}).

At least a 4-nitrophenyl substituent is present at 4- or 5-positions of the 1,2,4-triazole ring due to antimicrobial potential of aromatic nitro compounds [45]. They were incorporated *via* 4-nitrophenyl isothiocyanate and/or 4-nitro benzohydrazide. Efficacy of 1,2,4-triazole derivative **3c** was proven on all tested bacteria, even though the best results was belonged to heterocycle **3b**. Generally, antibacterial activity of triazoles **3a-d** was more significant than those of derivatives **3f-i**. Derivatives **3a-c** were the only blockers of *Escherichia coli*. No remarkable antibacterial properties were observed with 1,2,4-triazole **3e** having two 4-nitrophenyl groups. As expected, the synthesized derivatives could effectively inhibit the growth of pathogenic fungi [46]. *Aspergillus fumigatus* and *Fusarium oxysporum* were inhibited with all of them. The best antifungal effects were observed with triazole **3e**. There was no notable difference between derivatives **3a-d** and **3f-i**. Antioxidant property of all products was assessed against DPPH radical. Acceptable effect was recorded only for triazole **3b** containing 4-hydroxyphenyl substituent with IC_{50} of 9.19 μg ml^{-1}. It is comparable to antioxidant activity of ascorbic acid (IC_{50} = 3.94 μg ml^{-1}).

Molecular docking analyses

Molecular docking is a technique which helps to prove binding and interaction poses of ligands within the binding pocket of macromolecules. Analogue of voriconazole containing 1,2,4-triazole ring showed excellent blocking properties against ten fungal pathogens especially *Aspergillus fumigatus* strain, it can be strongly attached to the enzyme lanosterol 14α-demethylase (CYP51) *via* its morpholine side chain [29]. A hybrid of fluoroquinolone and 1,2,4-triazole-3-thione with comparable antifungal activity to itraconazole can attach to Gly465, Cys470, Val311 and Leu312 amino acid residues *via* hydrophobic and hydrogen bonding interactions [30]. A synthetic 1,2,4-triazole

containing diflurophenyl and ethyl phenyl carbonate side chains could effectively interact with the active site of CACYP51 using hydrophobic and Van der Waals forces according to the docking results [31].

In this study, molecular docking procedure was applied to determine interactions of 1,2,4-triazoles **3a-i** in active site of *Aspergillus fumigatus N*-myristoyltransferase (NMT). The NMT is an essential enzyme in antifungal agents [47]. It facilities the attachment of the fatty acid myristate from myristoyl-CoA to the *N*-terminal glycine residue in living organisms such as *Aspergillus fumigatus* and *Candida albicans*. *Aspergillus fumigatus* was selected as target fungus because it was the only pathogen with relatively different MIC and MFC values. It should also be noted that cytochrome P450 14 alpha-sterol demethylase (CYP51) (PDB IDs: 5V5Z and 1EA1) is also selected for the molecular docking study, but the obtained results are not in agreement with the experimental data.

The results of the docking for the prepared 1,2,4-triazoles with 1IYL have been presented in Tables 4 and 5, as well as Fig. 2 (A and B). As seen, the order of binding energies for the interaction of triazoles to 1IYL is **3e** > **3c** > **3b** > **3f** > **3g** > **3i** > **3d** > **3a** > **3h**, which is in agreement with the order obtained from experimental MIC and MFC values. These results show that the lowest binding energy of compounds to 1IYL corresponds to **3e** by -8.68 kcal mol^{-1}. As shown in Fig. 2A, there are three conventional H-bonds between N-1, and nitro groups of 4-nitrophenyl substituents at positions 4 and 5 of the 1,2,4-triazole ring **3e** and Leu419, Tyr422 and Tyr210 amino acid residues, while such interactions are not observed between the compounds **3h** and **3i** and the 1IYL receptor. Furthermore, Tyr418, Pro219, Thr218, Arg423, Phe420 and Leu220 residues are placed close to the complex, allowing for possible Van der Waals interactions.

Table 4 Molecular docking results for interaction between the triazole 3a-i and 1IYL enzyme

1,2,4-Triazoles	BE (kcal.mol^{-1})	k_i (μM)	vdW+Hbond+ desolv
3a	-7.35	4.10	-8.46
3b	-8.08	1.20	-9.1
3c	-8.15	1.07	-9.20
3d	-7.79	1.95	-8.64
3e	-8.68	0.43	-9.55
3f	-7.82	1.86	-9.39
3g	-7.77	2.00	-8.56
3h	-7.59	2.73	-8.37
3i	-6.83	-	-

Table 5 Hydrogen bonding interaction energies between the triazole 3a-i and responsive amino acid residues in molecular docking

1,2,4-Triazoles	Amino acid	Energy (kcal mol^{-1})	Bond length (Å)
3a	Tyr335	-0.87	3.43
3b	Leu419	-2.50	2.76
3c	Leu177	-1.56	3.16

	Thr211	-1.00	3.40
3d	Tyr107	-2.50	2.70
3e	Leu419	-2.5	2.78
	Tyr210	-0.87	3.43
	Tyr422	-0.15	3.34
3f	Leu419	-2.50	2.71
3g	Thr211	-2.5	2.95
3h	-	-	-
3i	-	-	-

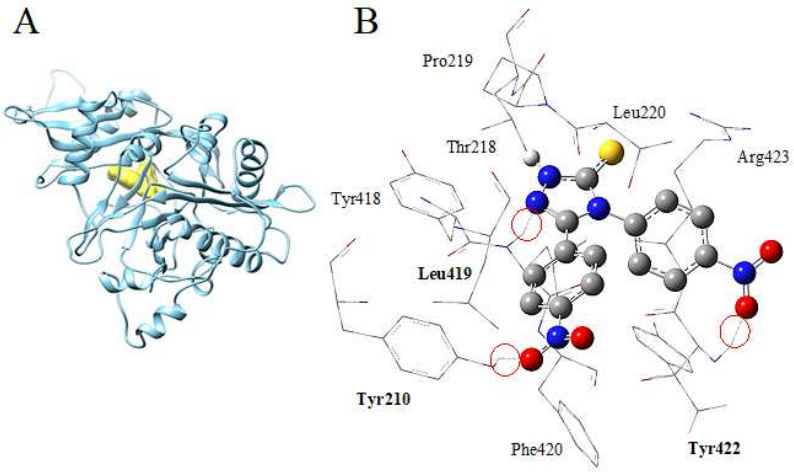


Fig. 2 (A) 3D depiction of the 1,2,4-triazole 3e bound to active site on 1IYL. (B) The interaction between the 1,2,4-triazole 3e and amino acid residues of 1IYL.

Conclusions

A series of 1,2,4-triazole-3-thiones were generated in glycerol/potassium carbonate as deep eutectic solvent. All products were screened for their antifungal and antibacterial potentials. Significant inhibitory effects were observed with them especially against fungal pathogens. Molecular docking calculations explored to identify ligand-protein interactions. When 1IYL was used as target enzyme, computational results were strongly correlated with the data obtained from the biological screening on *Aspergillus fumigatus*, which revealed compound 3e containing 4-nitrophenyl substituents has the highest binding affinity towards the receptor. Hydrogen bonds between N-1 of triazole rings and some amino acid residues on target enzyme are predominant interactions. These studies may be helpful in the development of antifungal drug discovery.

Conflict of Interest

The authors declare that they have no conflict of interest.

CRediT Author Statement

Hamid Beyzaei: Supervision, Writing- Original Draft, Writing- Reviewing and Editing. **Maryam Ghanbari Kudeyani:** Methodology, Investigation. **Hojat Samareh Delarami:** Software, Data Curation. **Reza Aryan** Conceptualization, Formal Analysis.

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Highlights

- Nine 4,5-disubstituted 1,2,4-triazole-3-thione derivatives were synthesized *via* a new and efficient process.
- The *in vitro* inhibitory properties of all synthesized products were evaluated against four Gram-positive and six Gram-negative pathogenic bacteria as well as three fungal pathogens.
- The inhibitory activities of derivatives as MIC, MBC and MFC values were determined by broth microdilution and streak plate methods.
- DPPH free radical scavenging experiments were applied to determine antioxidant activity of synthetic heterocycles.
- Molecular docking method was applied to find interaction between synthetic compounds and target enzyme (1IYL) on *Aspergillus fumigatus*.
- Hydrogen bond acceptor strength of N-1 in 1,2,4-triazole rings was the main factor that caused the observed differences.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: