Section A-Research paper

Design, Synthesis, Characterization, Molecular Docking and Antimicrobial Properties of New 1,2,4-Triazole Derivatives

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ABSTRACT

In present study we report synthesis, characterization, antimicrobial activity and docking study of 1,2,4 – Triazole derivatives. 1-formyl-4-methyl-3-thiosemicarbazide used as starting material triazole Schiff bases (**4a-f**) were synthesized by reaction of hydrazide with various aromatic aldehydes in glacial acetic acid medium. Melting point, TLC, and spectral analyses, including IR and NMR, were used to confirm the structures of the numerous synthesized compounds. The newly synthesized compounds were tested for antibacterial and antifungal activity against bacterial pathogens *S.aureus, E.coli* and fungal species *C.albicans* and *A.niger.* The prepared derivatives underwent a docking study.The observations have demonstrated that when an aromatic ring is substituted, antibacterial activity decreases, whereas antifungal activity of *N*'-[(*E*)-(4-methoxyphenyl)methylidene]-2-[(4-methyl-4*H*-1,2,4-triazol-3-yl)sulfanyl]acetohydrazide (**4d**) against *C. albicans* and *A. niger* was at its peak. According to docking studies, compound 4d interacts with glucosamine-6-phosphate synthase through hydrogen bonds, arene-arene interactions, and hydrophobic interactions. The docking results for compound 4d were exactly in line with what the MIC found for that molecule.

Keywords - antibacterial and antifungal activity, computational study, active site.

INTRODUCTION

Electronic structures are typically used to categorise heterocyclic compounds. Due to their versatility in a variety of industries, including pharmaceuticals[1], research into the synthesis of nitrogen-containing heterocyclic derivatives has grown significantly over the past few decades. Triazolam, Alprazalam, Etizolam, Furacylin, Ribavirin, Hexaconazole, Triadimefon, Mycobutanil, Rizatriptan, Propicaconazole, and Fluotrimazol are a few examples of medications known to include the 1,2,4-triazole moiety[2]. These results support our ongoing work to create a variety of bioactive compounds with pharmaceutically active groups[3]. Here, we report on the synthesis, characterisation, antibacterial activity, and docking investigation of 1,2,4 - Triazole derivatives.

MATERIAL AND METHODS

Melting points were determined using microprocessor based melting point apparatus (Veego make) having liquid paraffin bath and are uncorrected. The IR spectra were recorded on a PERKIN-ELMER SPECTRUM 100 FTIR spectrophotometer using KBr pellets and the wave numbers were given in cm^{-1}. The ^{H1} NMR spectra were recorded in DMSO-d_6/$CDCl_3$ on a Bruker spectrophotometer (400 MHz). All chemical shifts are described in δ (ppm) using TMS as an internal standard. The progress of all reactions were monitored by TLC on 2cm x 5cm pre-coated silica gel 60 F_{254} (Merck) plates of thickness of 0.25 mm ($CHCl_3$:MeOH). The chromatograms were observed under UV (254 nm) and/or exposure to iodine vapours. All reagents used were of analytical grade (AR), obtained from S.D. Fine chemicals, Spectrochem, Qualigens and Sigma-Aldrich. Molecular modeling and pharmacophore modeling were carried out using Molecular Operating Enviorment (MOE) 2009 (Chemical Computing Group, Canada).

Here, focus was made mainly on the synthesis of 1,2,4-triazole-5-thione as the essential pharmacophore.

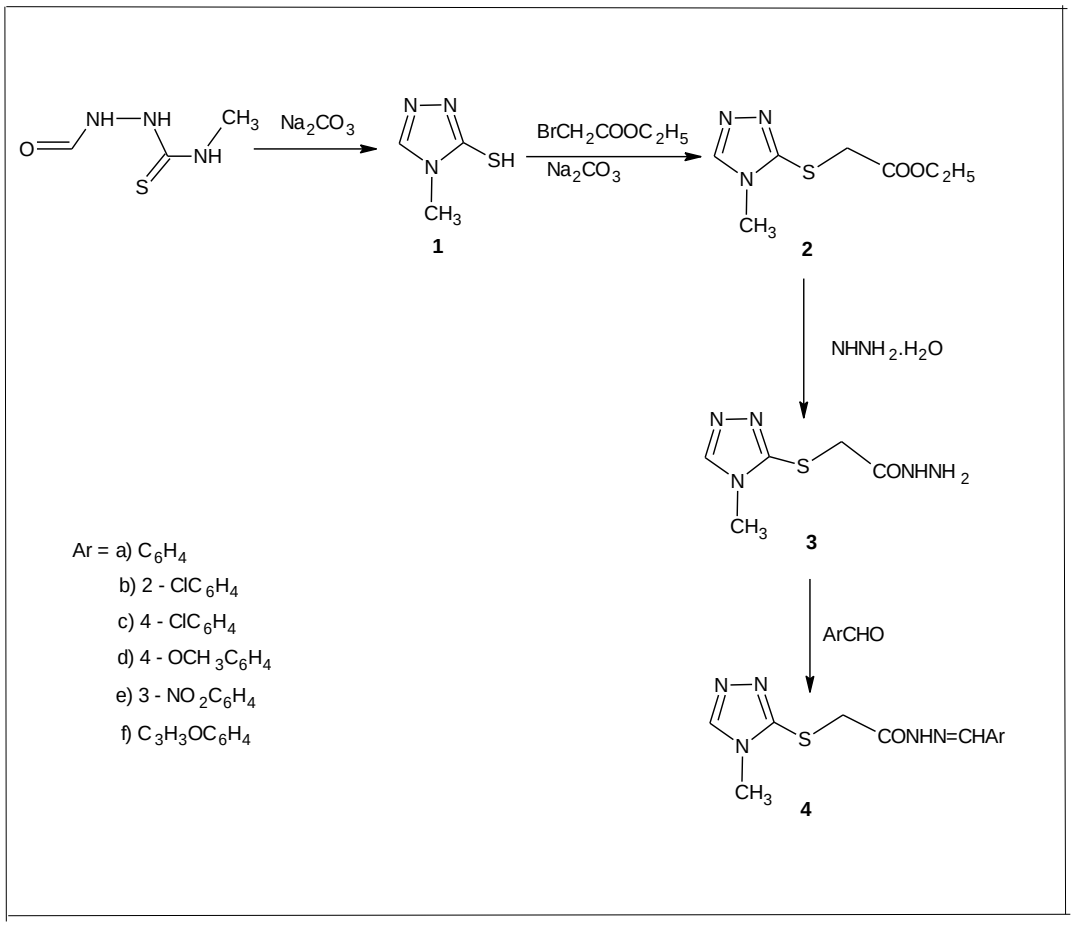
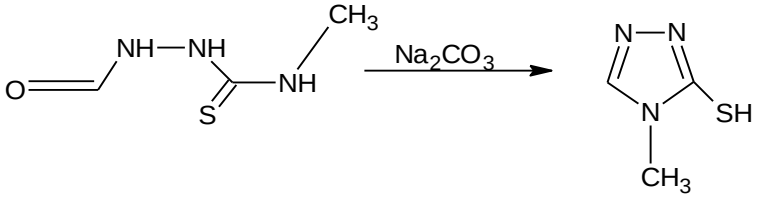


Figure 1 Scheme of work

Synthesis Of 4-Methyl-4h-1,2,4-Triazole-5-Thione (1)

In a round bottom flask with a 200 ml water solution of 1-formyl-4-methyl-3-thiosemicarbazide (0.5 mol) and sodium carbonate (1 mol), heat was applied for one hour using a steam bath. The solution was chilled in an ice bath for 30 minutes. The next step involved treating it with 100 ml of dilute hydrochloric acid. The 4-methyl-1,2,4-triazole-5-thione (1) that precipitated out was then collected by suction filtering after the reaction mixture had been cooled in an ice bath for two hours. 200 ml of boiling water were used to dissolve the thiol (1), and the resulting solution was filtered. The filter was chilled in an ice bath for an hour, and the compound that had recrystallized was then collected by suction filtration and left to air dry for the following day. There was a 58% yield. M.P. 162-164 °C [4].

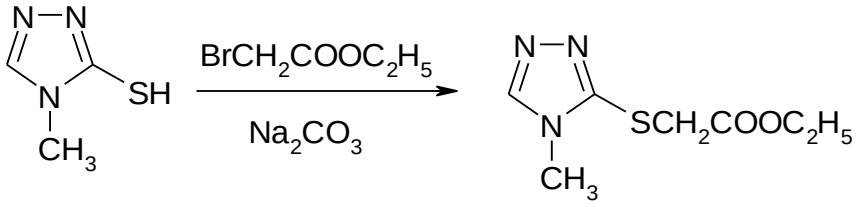


1

Synthesis Of Ethyl [(4-Methyl-4h-1,2,4-Triazol-3-Yl)Sulfanyl]Acetate (2)

Anhydrous sodium carbonate (0.0086mol) was added to a solution of 3-mercapto-4-methyl-4*H*-1,2,4-triazole (0.0086mol) in acetone (30ml). Ethyl bromoacetate (0.0086mol) was gradually added to the reaction mixture while being stirred at room temperature. Thin layer chromatography was used to track the reaction's

development while employing chloroform and ethanol (9:1) as the eluent. By using filtering, the sodium bromide by product was separated. The product's mother liquor was concentrated under vacuum to get rid of the acetone, and the resulting chemical was then purified using column chromatography. [5]



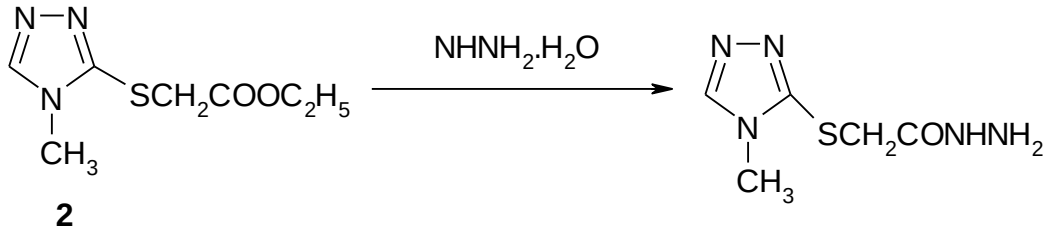
1 **2**

Analysis. Viscous orange colour liquid. TLC: Rf value (0.7)

IR (KBr): 1735, 1599, 1499 and 1182

Synthesis Of 2-[(4-Methyl-4h-1,2,4-Triazol-3-Yl)Sulfanyl]Acetohydrazide (3)

The above ester (**2**) (0.005mole***)*** was dissolved in methanol (30 ml). 99.9% hydrazine hydrate (0.01mol) was added to the clear solution and heated through reflux. Thin layer chromatography was used to track the reaction's development, with a 9:1 solution of chloroform and methanol serving as the eluent. To crystallise the reaction product, the reaction mixture was chilled to 0-5^{0}C. On filtration and washing with chilled methanol an acylated hydrazine derivative was produced. The yield was 65% and melts at 172^{0}C.[5]



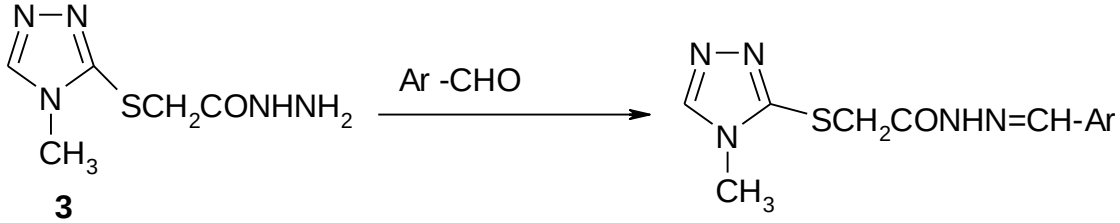
2 **3**

Analysis. White crystalline solid.TLC: Rf value (0.6)

IR (KBr): 1673, 1619, 1600, 3321 and 3241.

Synthesis of n'-[(e)-(substituted)methylidene]-2-[(4-methyl-4h-1,2,4-triazol-3-yl)sulfanyl] acetohydrazide 4(a-f)

Equimolar amounts of various aldehydes and hydrazide (3) were refluxed in alcohol for five hours while a few drops of glacial acetic acid were present. After the reaction was finished, the resulting mixture was poured over crushed ice. The precipitated product was dried and filtered. In ethanol, a crude solid chemical was recrystallized. There was a 60% yield. [6]



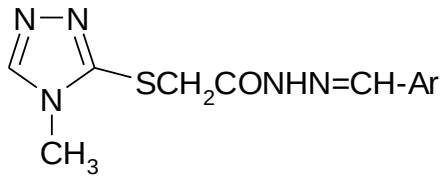
3 **4 (a-f)**

The newly synthesised compounds were tested for their antibacterial and antifungal effects against the bacterial species S. aureus and E. coli as well as the fungi C. albicans and A. niger. By using the broth micro dilution method, the antibacterial and antifungal standards Ciprofloxacin and Fluconazole were utilised, respectively.[7]

Table 1:- PHYSICOCHEMICAL DATA OF COMPOUND

Comp	**Ar**	**MF**	**MW**	**%Yield (%w/w)**	**MP (°C)**	**Analysis found(cacld.)%**		
						C	**H**	**N**
A	C_6H_4	$C_{12}H_{13}N_5OS$	275.33	65	78-82	52.40 (52.5)	4.78 (4.76)	25.0 (25.4)
B	2-Cl-C_6H_4	$C_{12}H_{12}ClN_5OS$	309.77	75	158-160	46.50 (46.5)	3.92 (3.90)	22.58 (22.6)
C	4-Cl-C_6H_4	$C_{12}H_{12}ClN_5OS$	309.77	78	222-226	46.55 (46.5)	3.88 (3.90)	22.60 (22.6)
D	4-OCH_3 C_6H_4	$C_{13}H_{15}N_5O_2S$	305.35	75	112-116	51.10 (51.1)	4.93 (4.95)	22.90 (22.9)
E	3-NO_2 C_6H_4	$C_{12}H_{12}N_6O_3S$	320.33	65	110-112	44.96 (44.9)	3.76 (3.78)	26.21 (26.2)
F	$C_3H_3OC_6H_4$	$C_{14}H_{15}N_5OS$	301.37	60	156-160	55.82 (55.7)	5.00 (5.02)	23.22 (23.2)

Table 2:- SPECTRAL DATA OF COMPOUND



4(a-f)

Comp.	**Ar**	**IR(KBr) cm^{-1}**	**^{H1} NMR (DMSO) δppm**
A	C_6H_4	3543(N-H), 1681(C=O) , 644(C-S) , 1319(C-N)	4.5 (s,2H CH_2), 7.1-7.7(m,5H-Ar), 8.1(m,H1 CH), 11.4 (s,H1 NH)
B	2-Cl-C_6H_4	3330(N - H), 1710(C=O), 630(C-S), 1320(C-N)	4.3(s,2H CH_2), 7.2-7.9(s, 4H-Ar), 8.1(m,H1 CH), 11.7 (s,H1 NH)
C	4-Cl-C_6H_4	3426(N-H) 1684(C=O), 547(C-S), 1306(C-N)	4.2(s,2H CH_2), 7.5-7.7(s, 4H-Ar), 8.3(m,H1 CH), 11.0 (s,H1 NH)
D	4-OCH_3 C_6H_4	3382(N-H) ,1674(C=O), 691(C-S) , 1249(C-N)	4.0(s,2H CH_2), 7.0-7.8(s, 4H-Ar), 8.5 (m,H1 CH), 11.07 (s,H1 NH)
E	3-NO_2 C_6H_4	3458(N-H) , 1667 (C=O), 678(C-S), 1227(C-N)	4.3(s,2H CH_2), 7.6-8.4(m, 4H-Ar), 8.4 (m,H1 CH), 11.8 (m,H1 NH)
F	$C_3H_3OC_6H_4$	3430(N-H) ,1672(C=O), 690(C-S) , 1207(C-N)	4.0(s,2H CH_2), 7.4-7.6(s, 4H-Ar), 7.5 (m,H1 CH), 10.58 (s,H1 NH)

Making use of the Molecular Operating Environment 2009, docking investigations were conducted.. The pdb 1moq subjected to energy and residue optimization by protonate 3D option in MOE programme. Substrates were docked within active site using the Monte Carlo docking procedure of MOE and repeated cycles of protein and substrate minimization. During the early stage of the docking procedure, side chains of the protein were fixed. The best ranking docking modes of the ligands were identified and energy minimized in the protein while allowing full side chain flexibility.[8]

Osiris and Molinspiration virtual screenings and molecular properties calculations

Mol inspiration calculations

MiLogP is determined using a method created by Molinspiration as the total of correction factors and fragment-based contributions. The sum of fragment contributions is used to determine the Total Polar Surface Area (TPSA). The approach created by Osiris is used to evaluate the toxicology hazards (mutagenicity,

tumorogenicity, irritation, reproduction) and physico-chemical characteristics (mi log P, solubility, drug similarity, and drug score) of compound 4a to f.

RESULTS AND DISCUSSION

The infrared spectrum of hydrazide (**3**) showed strong absorption band at 1673 cm^{-1} characteristic of C=O, and two bands in the range of 3190-3320 cm^{-1} are the stretching modes of NH_2 and C=N. The ^{H1} NMR spectra of hydrazide showed a singlet at 9.15 ppm (H1, HNC=O) and broad singlet at 4.26 ppm (2H) assigned to the hydrazide NH_2. The absence of SH proton confirmed that the ester was converted into hydrazide.

The formation of Schiff bases was indicated by the presence of the CH=N stretching band near to the 1600 cm^{-1}, combined with the disappearnce of the NH_2 streching band. Because the aromatic ring was generated from the aldehyde moiety at the aromatic region, the 1H NMR spectra of compounds 4a–f showed extra signals,While the signal from the hydrazide structure's -NH2 group was absent. Two sets of signals, one from the -SCH2 group and the other from the -N=CH group, were seen in the H1 NMR spectra of compounds 4a through 4f between 4.00 and 8.50 ppm.

In addition, compound **4b** and **4c** shows absorption band at 1091 and 1065 cm^{-1} respectively, characteristic of C-Cl. $–OCH_3$ group of compound **4d** resonated at 3.83 ppm integrating three protons as a singlet in the ^{H1} NMR spectrum. Moreover, the absoption band from NO_2 group in compound **4e** and the signals derived from CH=CH group in compound **4f** were recorded at 5.2 and 7.03 ppm as doublet in the ^{H1} NMR spectrum.

Rotation is impeded by the N=CH double bond, leading to the production of *E* and *Z* isomers. The compounds having arylidene- hydrazide structure may exit as *E/Z* isomers about –C=N double bond and as cis/trans amide conformers (Figure 2).

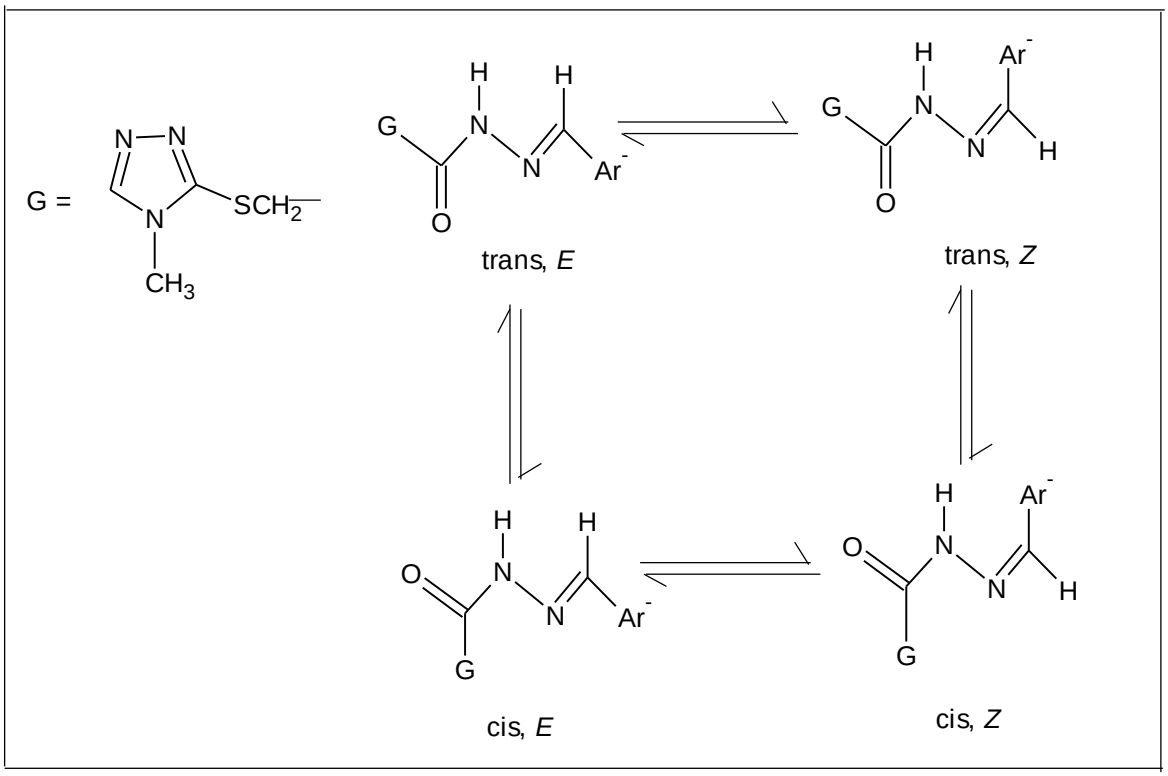


Figure 2 isomers

Antimicrobial activity

The antibacterial and antifungal properties of the synthesized compounds 4a–4f were assessed. Every synthetic compound that was studied exhibited some antimicrobial action. With a MIC of 200 g/ml, compound 4a and 4d demonstrated the best antibacterial activity when compared to the other derivatives against all bacterial species. With a MIC of 400 g/ml, compounds 4b and 4e exhibited the least antibacterial activity against all bacterial species. Among all the synthesized compounds, molecules having an unsubstituted aromatic ring demonstrated the best antibacterial activity, according to the antibacterial activity data. When compared to the standard, ciprofloxacin, the compounds 4c and 4f showed slightly less action, whilst compounds 4b and 4e showed poor activity.

It is clear from the discussion above that compounds 4a and 4d, which have MICs of 200 g/ml, are the most effective antibacterial triazoles.

The results of the antifungal activity demonstrated an increase in activity with substitution of a chloro group at position two on the aromatic ring and a methoxy group at position four on the aromatic ring. The activity is decreased when a 3-nitro or 4-chloro group is substituted. When compared to fluconazole, the compounds 4a and 4c showed slightly less action, while compounds 4e and 4f showed low activity. The most efficient antifungal drug against C. albicans was thus compound 4b and 4d.

computational research

According to Lipinski's rule, the computed milogP values for each compound ranged between -1.3 and 2.13(5), which is the upper limit for the medications' ability to pass through biomembranes. Therefore, it is anticipated that these molecules (4a–f) will have high bioavailability.

Compound 4c in the current series displayed a PSA value of 118.004, which is well in line with Rule of Five. All chemicals, according to Table 3, fall within this range. Compounds 4a- f's similarity is listed in Table 4. The four criteria of known successful pharmacological activity in the areas of GPCR ligand activity, nuclear receptor ligand activity, ion channel modulation, and kinase inhibition activity were carefully applied to the activity of all six compounds and the reference medication.

Table 5's results are presented for all compounds using numerical tagging. Similarly, each compound consistently has negative values across all categories. It is therefore easy to see that all the compounds are anticipated to have very similar activity to standard drug.

The tables in Osiris are the results of the calculations. According to the data analysed in Table 5, all structures should be non-mutagenic, non-irritating, and have no impacts on reproduction when put through the same mutagenicity assessment system as commonly used medications. All of the compounds 4a–f have log P values that meet the required threshold. In addition, compounds 4a, which have had positive results from antibacterial screening, have low log P values when compared to the other compounds in the series.

Table 3 showing molinspiration properties calculations for synthesized compounds.

CALCULATION OF MOLECULAR PROPERTIES									
COMP NO.	MW (g/mol)	miLogP	TPSA	NO of atom	NO. of ON	NO. of OH-NH	n violation	n rotb	Volume
4a	275.337	1.452	72.18	19.00	6	1	0	5	238.644
4b	309.782	2.082	72.18	20.00	6	1	0	5	252.18
4c	309.782	2.13	72.18	20.00	6	1	0	5	252.18
4d	305.363	1.508	81.414	21.00	7	1	0	6	264.19
4e	320.334	1.387	118.004	22.00	6	1	0	6	261.978
4f	301.375	1.675	72.18	21.00	6	1	0	6	266.061
Cipro	331.347	-0.701	74.569	24.0	6	2	0	3	285.460
Fluco	306.276	-0.118	81.664	22.0	7	1	0	5	248.957

Table 4 showing bioactive scores for synthesized compounds.

DRUGLIKENESS						
COMP	GPCR	ICM	KI	NRL	PI	EI
4a	-1.24	-1.65	-1.24	-1.71	-1.36	-0.75
4b	-1.20	-1.62	-1.17	-1.67	-1.34	-0.75
4c	-1.14	-1.57	-1.16	-1.60	-1.30	-0.74
4d	-1.10	-1.58	-1.09	-1.48	-1.22	-0.72
4e	-1.17	-1.50	-1.14	-1.49	-1.24	-0.79
4f	0.93	-1.53	-1.25	-1.46	-1.08	-0.60
Cipro	0.12	-0.04	-0.07	-0.19	-0.21	0.28
Fluco	0.04	0.01	-0.09	-0.23	-0.09	0.03

Table 5 showing Toxicity Risk for synthesized compounds **4a-f** by Osiris Calculations.

Toxicity Risk				
Compound	MU	TU	IR	REP
4a E				
4b E				
4c E				
4d E				
4e E				

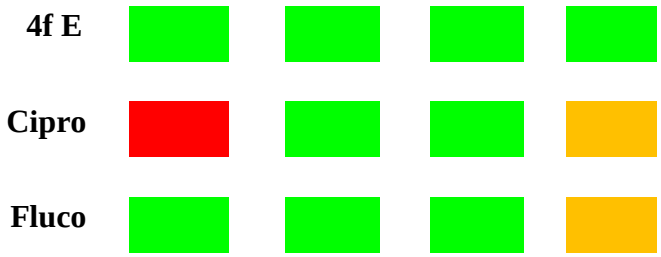


Table 6 showing physico-chemical properties for synthesized compounds 4a-f by Osiris Calculations

Osiris Calculations					
Compound	MW	CLogP	S	DL	D-S
4a(E-isomer)	275	1.38	-2.65	4.94	0.91
4b(E-isomer)	275	1.38	-2.64	4.94	0.91
4c(E-isomer)	309	2	-3.38	5.36	0.85
4d(E-isomer)	309	2	-3.38	5.78	0.85
4e(E-isomer)	305	1.28	-2.66	5.08	0.9
4f(E-isomer)	301	1.75	-2.94	3.81	0.88
Cipro	331.0	0.13	-3.32	2.07	0.39
Fluco	306.0	-0.21	-2.17	-1.13	0.46

The aqueous solubility

The estimated log S value is the base-10 stripped logarithm of the solubility, expressed in mol/liter, of a substance. Log S values for compounds 4a through 4f are less than -4. As indicated in Table 7, we determined the total drug score (DS) for compounds 4a through f and compared it to standard drug. The drug score is DS. In comparison to standard drug used , the described compounds 4a–f demonstrated a moderate to good drug score.

Studies on molecular docking

The outcomes of the biological screening motivate us to conduct receptor-based virtual screening of the most powerful antifungal chemical 4d against glucosamine-6-phosphate, which may be used as a target for future antifungal medication. [9.10]

Through hydrogen bonds, arene-arene interactions, and hydrophobic contacts, chemical 4d interacts with and inhibits glucosamine-6-phosphate synthase, according to docking research. In Figure 03 With an interatomic distance of 3.09 A0 and a proportion of hydrogen bonding of 11%, the terminal 4-methoxy phenyl substituent interacts with tyr 387, while the terminal phenyl ring substituent interacts with tyr 387 once more via arene-arene contact. The mercapto triazole and the next carbonyl oxygen both display hydrogen bonding with tyr476;

the percentage of hydrogen bonding was reported to be 13% with an interatomic distance of 2.06 A0. Additionally, one of the triazole's nitrogen atoms forms a hydrogen connection with tyr476 at an interatomic distance of 3.33A0 and a hydrogen bonding percentage of 13%. There is also evidence of arene-arene interaction with tyr497 in the triazole ring.

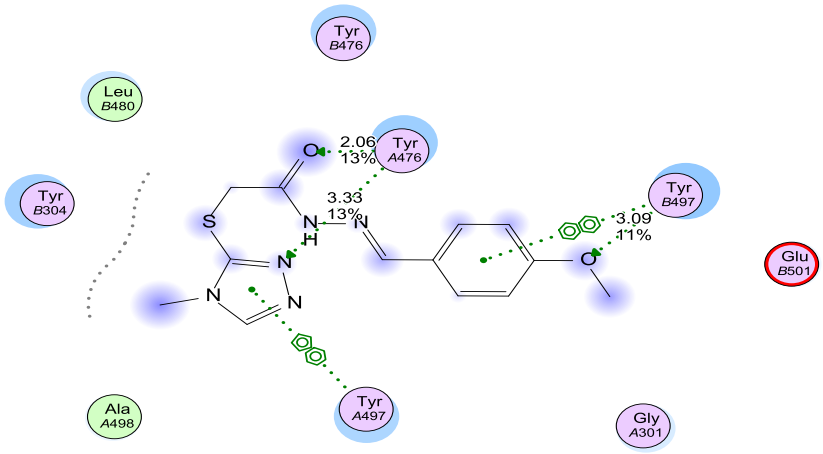


Figure 3 showing hydrogen bonding interaction of compound 4d and glucosamine-6-phosphate enzyme.

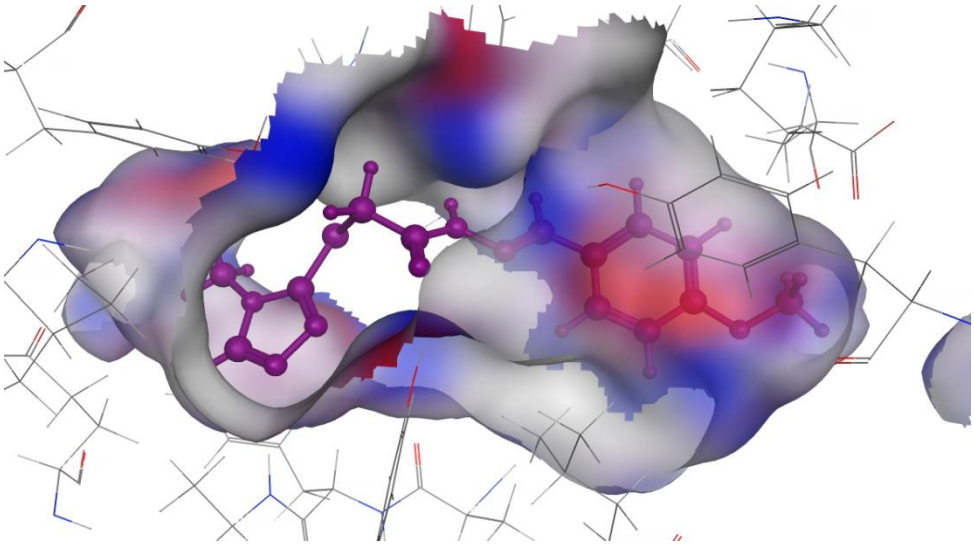


Figure 04 is the Connolly surface representations of the active site of glucosamine-6-phosphate synthase with the bound compound 4d, shown in Ball and Stick model. Connolly surface of the active site of the is coloured according to a charge spectrum: Blue colour for H-bonding region, white colour indicates lipophilic area while red colour is used to reveal mild polar area.

According to surface analysis, chemical 4d had a significant match with drug receptors, supporting the biological findings. The terminal phenyl ring is positioned over the hydrophobic surface of the receptor, indicating that phenyl ring replacement is advantageous and may cause hydrophobic contact with the receptor. Additional acetohydrazide substituents are positioned over the receptor's hydrogen bonding surface, indicating the possibility that these substituents may form a hydrogen bond with the receptor.

CONCLUSION

A number of triazole derivatives have been successfully synthesised with a appreciable yield, and their antimicrobial activity has been tested. According to the results of the activity investigations, the antibacterial activity of all triazole derivatives decreases when an aromatic ring is substituted, however compound 4d exhibited the highest overall activity against C.albicans and A. niger. Through hydrogen bonds, arene-arene interactions, and hydrophobic interactions, chemical 4d interacts with glucosamine-6-phosphate synthase, according to docking research. The docking results for compound 4d were exactly in line with what the MIC found for that molecule. Glucosamine-6-phosphate synthase is a good and prospective target for new antifungal medications, according to our investigations. A thorough understanding of its catalysis and inhibition mechanisms may lead to the creation of an entirely new family of its inhibitors as potential antifungal medications. A methyl group substitution at position 4 was found to be advantageous for activity, according to investigations on the structure-activity connection of this molecule utilising MOE. It is predicted that future structural modifications will not change the substitution of the methyl group. The presence of the acetohydrazide chain is advantageous for activity and it is possible that it will interact with drug receptors, keeping the structure stable for future structural modifications.

1. The presence of the terminal phenyl ring is advantageous for activity since it gives a significant amount of hydrophobicity and is engaged in the interaction with the drug receptor. It will therefore remain constant during future structure optimization.
2. The triazole nucleus works as the compound's lead component by interacting with receptors via hydrogen bonds and arene-arene interactions, remaining constant during further structural optimization.
3. Substitution of any hydrogen bond acceptor or donor, such as NH2, may improve drug receptor interation and increase the effectiveness of an antifungal medication. The activity and toxicity of the synthesised compounds were assessed using online property calculations offered by Molinspiration and Osiris. Molinspiration calculations revealed that all synthesised molecules met the Lipinski Rule of Five, displayed a favourable bioavailability profile, and had activity that was comparable to that of conventional medications. The results of the Osiris computation showed that all the compounds had log P values that met the required standards, showing that the compounds were all synthesised without any ADME issues.

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