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Synthesis, molecular modeling of novel 1,2,4-triazole derivatives with potential antimicrobial and antiviral activities

Sherin M. El-Feky[1], Laila A. Abou-zeid[1]*, Mohamed A. Massoud[1], Shady G. Shokralla[2] and Hassan M. Eisa[1]

[1]Departments of Pharmaceutical Organic Chemistry, [2]Departments of Microbiology, Faculty of Pharmacy, University of Mansoura, Mansoura 35516, Egypt.

Abstract

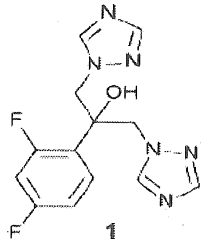
A new series of 3,5-disubstituted triazoles were synthesized and evaluated for in vitro antifungal and antibacterial activity. All compounds tested showed significant antifungal activity against micromycetes, compared to the commercial fungicide, clotrimazol. The binding modes of the docked compounds at the active site of 1EA1 enzyme were examined and the recognition modes of these new analogs with the catalytic triad amino acids were explored. Compound **8b** performed highest antifungal activity against *C. albicans* and antibacterial activity against *S. aureus*.

Keywords: Triazoles; Antifungal; Antibacterial; Molecular modeling; catalytic triad recognition; lipophilicity.

Introduction

Invasive fungal infections have increased in frequency and severity over the last two decades because of increasing the number of immuno-compromised hosts (Metcalf and Dockrell 2007, Ship et al. 2007, Cordonnier et al. 2008). Widespread uses of antifungal therapies for curative, pre-emptive or prophylactic purposes have been developed to overcome the threat of *Candida* colonization (Barker and Rogers 2006, Herbrecht 2006, Mai et al. 2007). Mounting the administration of antifungal drugs enhanced the development of resistance due to the genetic mutations towards the clinically used drugs, specifically fluconazole **1** (Xu et al. 2001, Redding et al. 2003, Varanasi et al. 2004, Cowen and Lindquist 2005). In the present work, authors spotlight to overcome the drug resistance by design and synthesis of new triazole analogs aiming to achieve novel lead with secure and broad antifungal scale.

1,2,4-Triazole derivatives possess wide spectrum actions including antibacterial and antifungal activities (Aufort et al. 2008, Liu et al. 2008, Sztanke et al. 2008,Yang et al. 2008). Fluorinated triazole analogs achieved enhanced antimicrobial activity relative to clotrimazole and ampicillin **2,3** (Tatsumi et al. 2001).



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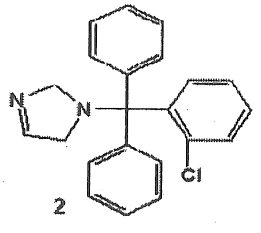

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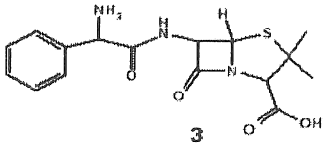

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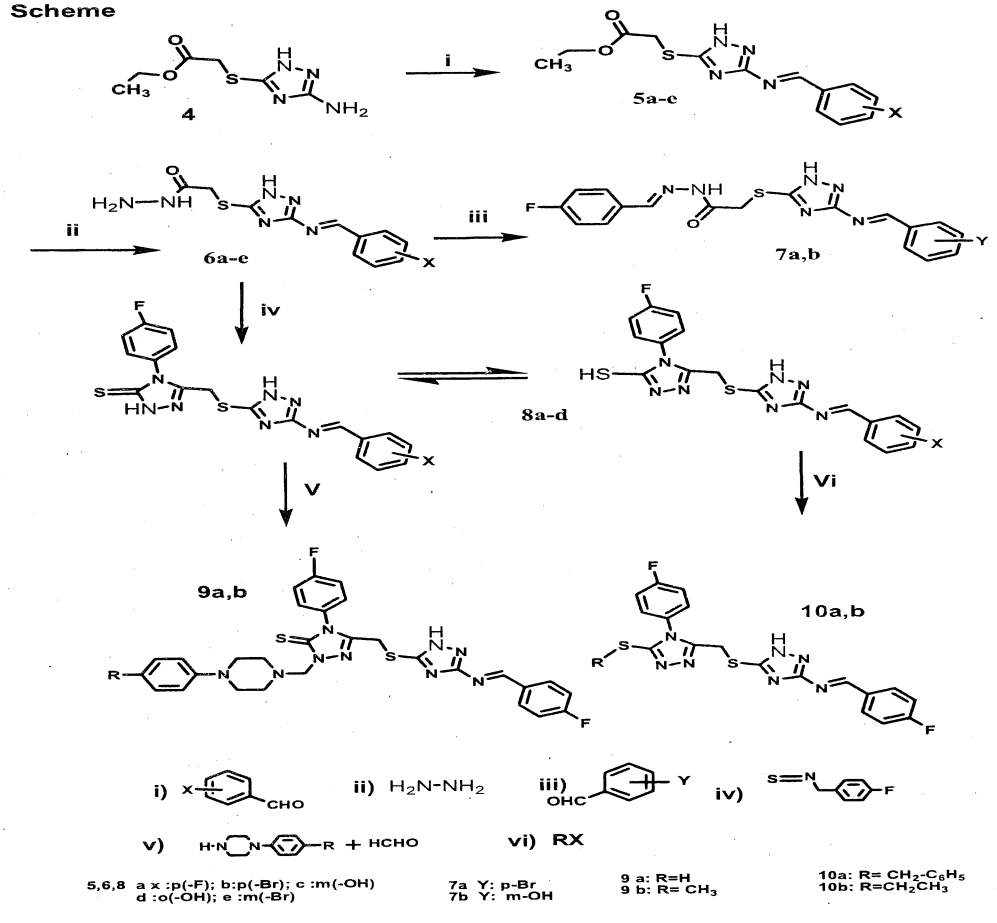
***Corresponding author:** labouzeid@yahoo.com

Chemistry

The synthetic pathways followed for the preparation of the title compounds were performed as shown in Scheme 1. Compound, ethyl 2- (3-amino-1*H* –[1,2,4]-triazol-5-ylthio) acetate **4**, was prepared according to the procedure described by Liu *etal* 2001. Stirring equimolar amount of ethyl chloroacetate, 3-amino-5-mercapto-1*H*-[1,2,4]-triazole and potassium carbonate in DMF solution. A new series of (*E*)-ethyl2-(3-(substituted-benzylideneamino)-1*H*-[1,2,4]-triazol-5-ylthio)acetate **5a-e** were prepared by treating compound **4** with equimolar amount of the appropriate benzaldehyde derivatives in presence of glacial acetic acid. Treatment of compounds **5a-e** with hydrazine hydrate under reflux to afford the corresponding (*E*)-2-(3-(Substituted-benzylideneamino)-1*H*-[1,2,4]-triazol-5-ylthio) acetohydrazide **6a-e** that were subjected for further reaction with 4-fluorophenyl isothiocyanate to give (E)-3-((3-(Substituted-benzylideneamino)-1H-[1,2,4]-triazol-5-ylthio)-methyl)-4-(4-fluorophenyl)-1H-[1,2,4]-triazole-5(4H)-thione **8a-d**.

Equimolar amounts of **6b,c** and 4-substituted benzaldehyde were refluxed to yield (*E*)-2-(3-((*E*)-Substituted-benzylideneamino)-1*H*-[1,2,4]-triazol-5-ylthio)-N'-(4-fluorobenzylidene) acetohydrazide **7a,b** respectively. (*E*)-3-((3-(4-fluoro-benzylideneamino)-1*H*-[1,2,4]-triazol-5-ylthio)methyl)-4-(4-fluorophenyl)-1-((4 substituted -piperazin-1-yl)methyl)-1*H*-[1,2,4] -triazole-5(4*H*)-thione **9a,b** were synthesized by the reaction of **8a** with formaldehyde and derivatives of piprazine hydrochloride salts. Compound **8a** was alkylated with benzyl chloride or ethyl iodide to yield (*E*)-5-((5-(Alkyl/Arylthio)-4-(4-fluorophenyl)-4*H*-[1,2,4]-triazol-3-yl)methylthio)-N-(4-fluorobenzylidene)-1*H*-[1,2,4]-triazol-3-amine **10a,b** respectively.

Scheme



Material and Methods

Computational Molecular modeling methodology

All modeling experiments were conducted with Hyperchem 6.03 package from Hypercube, Moelgro molecular viewer (Hyperchem 1999, Moelgro molecular viewer MMV 2008) and MOE 2007.09, Chemical Computing Group Inc .

Enzyme structure

The coordinate of 1EA1 enzyme in tertiary complex with protoporphyrin ix containing Fe (**HEM**) and (**TPF**), codeID 1EA1, was obtained from the Protein Data Bank of Brookhaven National Laboratory (Figure 1).

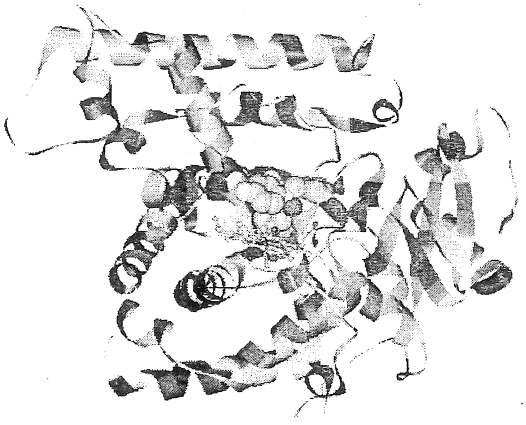


Figure 1. Flat-ribbon presentation of the crystallographic structure of ternary complex of TPF-HEME-1EA1, showing the stick models of TPF and the cofactor HEM

Molecular Structure of the synthesized triazoles:

The traizole analogs **5-10** were constructed from fragment libraries in the Hyperchem program followed by energy minimization using the "Amber force field". The partial atomic charges for each analogue were assigned with the semiemperical mechanical calculation method "AM1" implemented in Hyperchem 6.03. 1Conformational search was performed around all the rotatable bonds with an increment of 100 using conformational search module as implemented in HyperChem 6.03 All the conformers were minimized until the Root Mean Square (RMS) deviation was 0.01 Kcal/mol Å.

Docking and Molecular geometrical optimization:

Lowest energy conformer of each new analog "global-minima" was docked into the 1EA1 enzyme-binding domain. For each of the traizole analogs, energy minimizations were performed using 1000 steps of steepest descent, followed by conjugate gradient minimization to a RMS energy gradient of 0.01 Kcal/mol Å. Hydrogen bonds with a bond length up to 3.5 Å were considered. The active site of the enzyme was defined using a radius of 8.0 Å around TPF. The cofactor (**HEM**) as a part of the enzyme structure was not fixed during the geometrical optimization.

Antimicrobial Screening:

Disc Diffusion Assay:

Disc diffusion assay was performed according to the procedure of Xu et al. (2001) and Varanasi et al. (2004). Whatman No. 1 filter paper discs of 5 mm diameter were sterilized by autoclaving for 15 min at 121°C. The sterile discs were impregnated with the test compounds (500 µg/disc). Agar plates were prepared by pouring a suitable volume of melted nutrient agar into each 75 mm Petri plates. The volume of nutrient agar was enough to keep its depth at approximately 6 mm. The agar plates were surface inoculated with standard inoculums (10^5 cells/mL broth) of the test organisms (local strains) namely, *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. The impregnated discs were placed on the agar plate medium, suitably spaced apart and the plates were incubated at 5°C for one hr to permit diffusion and then transferred to an incubator at 37°C for 24 h for bacteria and at 28°C for 72 h for fungi. The inhibition zones caused by various compounds on the tested microorganisms were measured using a caliber, to the nearest 0.5 mm. Ampicillin

(10000 µg/mL) was used as a reference standard antibacterial, while clotrimazole (10000 µg/mL) and fluconazole (10000 µg/mL) were used as a reference standard antifungal drug.

Synthesis:

Ethyl 2-(3-amino-1H –[1,2,4]-triazol-5-ylthio) acetate(4)

Equimolar amounts of 3-amino-5-mercapto-1*H*-1,2,4-triazole (5g, 0.04 mole), anhydrous potassium carbonate (5.95 g, 0.04 mole) and ethyl chloroacetate (5.26g, 0.04 mole) in dry dimethyl formamide (30 mL) were stirred for 1hr at room temperature. The reaction mixture was filtered and concentrated under vacuum. The yellowish white precipitate was crystallized from absolute ethanol. M.P 123-125^{0}C, yield 88% (Liu et al. 2001, M.P 125–126 ^{0}**C**, yield 86 %).

General procedure for the synthesis of the (E)- Ethyl -2- (3-(substituted-benzylidene-amino)-1H-[1,2,4]-triazol-5-ylthio) acetate (5a-e):

Equimolar amounts of ethyl 2-(3-amino-1*H* –[1,2,4]-triazol-5-ylthio) acetate (**4**) (3.0g , 0.01 mol) and the appropriate benzaldehyde derivatives (0.01 mol) were mixed in (25 mL) of ethanol in presence of 0.5 mL of glacial acetic acid. The reaction mixtures were refluxed and the organic solvent was then evaporated. The separated solid was dried, and crystallized from the appropriate solvents.

(E)-Ethyl 2- (3- (4-flourobenzylideneamino)-1H-[1,2,4]-triazol-5-yl thio) acetate (5a)

The yellowish white crystalline precipitate with yield: 80%; M.P 125^{0}C. (Chohan et al. 2004: yield: 80%; 128 ^{0}C).

(E)-Ethyl 2- (3-(4-bromobenzylideneamino)-1H-[1,2,4]-triazol-5-yl thio) acetate (5b)

Yield: 85% (ethanol): M.P 180 ^{0}C; ^{1}H NMR ($CDCl_3$) δ in ppm: 1.50 (t, 3H, CH_3), 3.80 (s, 2H, CH_2), 4.00 (q, 2H, CH_2), 7.20-7.85 (m, 4H, Ar), 8.50 (s, 1H, ArHC=N-), 13.00 (*br*, 1H, NH). Anal. Calcd for $C_{13}H_{13}BrN_4O_2S$; C: 42.29; H: 3.55; N: 15.17. Found: C: 42.12; H: 3.76; N: 15.36.

(E)-Ethyl 2- (3- (3-hydroxybenzylideneamino)-1H-[1,2,4]-triazol-5-yl thio) acetate (5c)

Yield: 90% (aq. ethanol): M.P 130 ^{0}C; ^{1}H NMR ($CDCl_3$) δ in ppm: 1.34 (t, 3H, CH_3), 3.89 (s, 2H, CH_2), 4.33 (q, 2H, CH_2), 5.25 (s, 1H, OH), 6.90-7.45 (m, 4H, Ar), 9.25 (s, 1H, ArHC=N-), 12.53 (*br*, 1H, NH). Anal. Calcd for $C_{13}H_{14}N_4O_3S$; C: 50.97; H: 4.61; N: 18.29. Found: C: 50.91; H: 4.52; N: 18.30.

(E)-Ethyl 2- (3- (2-hydroxybenzylideneamino)-1H-[1,2,4]-triazol-5-yl thio) acetate (5d)

Yield: 90% (ethanol): M.P 160 ^{0}C; ^{1}H NMR ($CDCl_3$) δ in ppm: 1.27 (t, 3H, CH_3), 3.52 (s, 2H, CH_2), 4.11 (q, 2H, CH_2), 5.50 (s, 1H, OH), 7.00-7.45 (m, 4H, Ar), 9.15 (s, 1H, ArHC=N-), 13.20 (*br*, 1H, NH). Anal.: Calcd for $C_{13}H_{14}N_4O_3S$; C: 50.97; H: 4.61; N: 18.29. Found: C: 50.89; H: 4.60; N: 18.27.

(E)-Ethyl 2- (3- (3-bromobenzylideneamino)-1H-[1,2,4]-triazol-5-yl thio) acetate (5e)

Yield: 88% (ethanol): M.P 150 ^{0}C; ^{1}H NMR ($CDCl_3$) δ in ppm: 1.53 (t, 3H, CH_3), 3.83 (s, 2H, CH_2), 4.05 (q, 2H, CH_2), 7.32-7.86 (m, 4H, Ar), 8.52 (s, 1H, ArHC=N-), 12.95 (*br*, 1H, NH). Anal.: Calcd for $C_{13}H_{13}BrN_4O_2S$; C: 42.29; H: 3.55; N: 15.17. Found: C: 42.32; H: 3.49; N: 15.16.

General procedure for the Synthesis of (E)-2-(3-(Substituted-benzylideneamino) -1H-[1,2,4]-triazol-5-ylthio) acetohydrazide (6a-e):

Hydrazine hydrate (3.0 gm, 0.06 mol) and (0.006mol) ethyl 2-(3-(substituted benzylideneamino)-1*H*-[1,2,4]-triazol-5-ylthio) acetate (**5a-e**) in absolute ethanol (50 ml) were refluxed and the reaction mixtures were concentrated under vacuum. The obtained residue was crystallized from the appropriate solvents.

(E) -2- (3- (4-Flourobenzylideneamino)-1H-[1,2,4]-triazol-5-ylthio) acetohydrazide (6a):

Yield: 65% (aq. ethanol); M.P: 160 ^{0}C; ^{1}H NMR ($CDCl_3$) δ in ppm: 4.34 (s 2H, CH2), 6.20 (br, 2H, NH2), 6.82-7.94 (m, 4H, Ar), 8.25 (s, 1H, ArHC=N-), 9.64 (s,1H,NH), 11.89 (s, 1H, triazole-NH). Anal. Calcd for $C_{11}H_{11}FN_6OS$; C, 44.89; H, 3.77; N, 28.56. Found: C, 44.39; H, 4.16; N, 28.16.

(E)-2-(3- (4-Bromobenzylideneamino)-1H-[1,2,4]-triazol-5-ylthio) acetohydrazide (6b):

Yield: 65% (aq. ethanol); M.P165 ^{0}C; ^{1}H NMR ($CDCl_3$) δ in ppm: 4.25 (s 2H, CH2), 6.24 (br, 2H, NH2), 6.70-7.90 (m, 4H, Ar), 8.12 (s, 1H, ArHC=N-), 9.66 (s,1H,NH); 11.93 (s, 1H, triazole-NH). Anal. Calcd for $C_{11}H_{11}BrN_6OS$; C, 37.19; H, 3.12; N, 23.66. Found: C, 37.60; H, 2.87; N, 23.26.

(E)-2-(3-(3-Hydroxybenzylideneamino)-1H-[1,2,4]-triazol-5-ylthio)acetohydrazide (6c):

Yield: 80% (aq. ethanol); M.P240 ^{0}C; ^{1}H NMR ($CDCl_3$) δ in ppm: 4.25 (s 2H, CH2), 6.24 (br, 2H, NH2), 6.70-7.90 (m, 4H, Ar), 8.12 (s, 1H, ArHC=N-), 9.66 (s,1H,NH), 5.60 (s, 1H, OH); 11.93, (s, 1H, triazole-NH). Anal. Calcd for $C_{11}H_{12}N_6O_2S$; C, 45.20; H, 4.14; N, 28.75. Found: C, 45.79; H, 3.82; N, 28.55.

(E)-2-(3-(2-Hydroxybenzylideneamino)-1H-[1,2,4]-triazol-5-ylthio) acetohydrazide (6d):

Yield: 82% (aq. HAc); M.P 162 ^{0}C; ^{1}H NMR ($CDCl_3$) δ in ppm: 4.25 (s 2H, CH2), 6.24 (br, 2H, NH2), 6.70-7.90 (m, 4H, Ar), 8.12 (s, 1H, ArHC=N-), 9.66 (s,1H,NH), 5.60 (s, 1H, OH); 11.93, (s, 1H, triazole-NH). Anal. Calcd for $C_{11}H_{12}N_6O_2S$; C, 45.20; H, 4.14; N, 28.75. Found: C, 45.65; H, 4.67; N, 28.16.

(E)-2-(3- (3-Bromobenzylideneamino)-1H-[1,2,4]-triazol-5-ylthio) acetohydrazide (6e):

Yield: 70% (ethanol); M.P140 ^{0}C; ^{1}H NMR ($CDCl_3$) δ in ppm: 4.27 (s 2H, CH2), 6.22 (br, 2H, NH2), 7.36-8.19 (m, 4H, Ar), 8.70 (s, 1H, ArHC=N-), 9.60 (s,1H,NH); 11.90 (s, 1H, triazole-NH). Anal. Calcd for $C_{11}H_{11}BrN_6OS$; C, 37.19; H, 3.12; N, 23.66. Found: C, 36.86; H, 3.76; N, 23.23.

General procedure for the Synthesis of (E)-2-(3-((E)-Substituted-benzylideneamino)-1H-[1,2,4]-triazol-5-ylthio)-N'-(4-fluorobenzylidene) acetohydrazide(7a,b):

Equimolar amounts of (*E*)-2-(3-((*E*)-(substituted-benzylideneamino)-1*H*-[1,2,4]-triazol-5-ylthio) acetohydrazide (**6b,c**) (0.01 mol) and 4-flourobenzaldehyde (1.2gm, 0.01 mol) in absolute ethanol (25 ml) were refluxed to yield (**7a**) and (**7b**) respectively. The organic solvent was evaporated under vacuum. The obtained residues were crystallized from the ethanol.

5.3.4.1. (E)-2-(3-((E)-4-Bromobenzylideneamino)-1H-[1,2,4]-triazol-5-ylthio)-N'-(4fluoro-benzylidene) acetohydrazide (7a).

Yield: 80%; Mp: 135^{0}C; ^{1}H NMR ($CDCl_3$) δ in ppm: 3.85 (s 2H, CH2), 7.00-7.60 (m, 8H, Ar), 8.12 (s, 2H, ArHC=N-), 8.06 (s,1H,NH), 13.53, (s, 1H, triazole-NH). Anal. Calcd for $C_{18}H_{14}BrFN_6OS$; C, 46.86; H, 3.06; N, 18.22. Found: C, 46.43; H,3.66; N, 18.47.

(E)-2-(3-((E)-Hydroxybenzylideneamino)-1H-[1,2,4]-triazol-5-ylthio)-N'-(4-fluorobenzylidene) acetohydrazide (7b).

Yield: 85%; Mp: 180^{0}C; ^{1}H NMR ($CDCl_3$) δ in ppm: 3.85 (s 2H, CH2), 5.35 (s 1H, OH), 7.00-7.60 (m, 8H, Ar), 8.12 (s, 2H, ArHC=N-), 8.06 (s,1H,NH), 13.53, (s, 1H, triazole-NH). Anal. Calcd for $C_{18}H_{15}FN_6O2S$; C, 54.26; H, 3.79;N,21.09. Found: C, 53.88; H,3.25; N, 21.27.

General procedure for the Synthesis of (E)-3-((3-(Substitutedbenzylideneamino)-1H-1,2,4-triazol-5-ylthio) methyl)-4-(4-fluorophenyl)-1

(0.03 mol) of the appropriate 3-(substituted-benzylideneamino)-1*H*-[1,2,4]-triazol-5-ylthio) acetohydrazide (**6a-d**) and 4-fluorophenyl isothiocyanate in 5% ethanolic sodium hydroxide (20 ml) were refluxed overnight then the ethanolic solutions were evaporated under vacuum. The residues were crystallized from the appropriate solvents.

(E)-5-((3-(4-Flourobenzylideneamino)-1H-[1,2,4]-triazol-5-ylthio)methyl)-4-(4-fluorophenyl)-1H-1,2,4-triazole-5(4H)-thione (8a).

Yield: **70%** (Methanol); Mp: 85 ^{0}C; ^{1}H NMR ($CDCl_3$) δ in ppm: 3.85 (s 2H, CH2), 6.44-6.75 (m, 4H, ArH), 7.10-7.60 (m,4H, ArH), 8.10 (s, 1H, CH=N), 13.10 (br, 2H, NH triazole). Anal. Calcd for $C_{18}H_{13}F_2N_7S_2$; C, 50.34; H, 3.05; N, 22.83. Found: C, 50.30; H, 3.45; N, 23.14.

(E)-5-((3-(4-Bromobenzylideneamino)-1H-[1,2,4]-triazol-5-ylthio)methyl)-4-(4-fluorophenyl)-1H-1,2,4-triazole-5(4H)-thione (8b).

Yield: **75%** (Methanol); Mp: 90 ^{0}C; ^{1}H NMR ($CDCl_3$) δ in ppm: 3.79 (s, 2H, CH2), 6.44-6.75 (m, 4H, ArH), 7.40-7.60 (m, 4H, ArH), 8.15 (s, 1H, CH=N), 13.50 (br, 2H, NH triazole). Anal. Calcd for $C_{18}H_{13}BrFN_7S_2$; C, 44.09; H, 2.67; N, 19.99. Found: C, 44.77; H, 2.58; N, 21.32.

(E)-5-((3-(2-Hydroxybenzylideneamino)-1H-[1,2,4]-triazol-5-ylthio)methyl)-4-(4-fluoro-phenyl)-1H-1,2,4-triazole-5(4H)-thione (8c).

Yield: **70%** (Methanol); Mp: 195 ^{0}C; ^{1}H NMR ($CDCl_3$) δ in ppm: 3.80 (s, 2H, CH2), 5.00 (s, 1H, OH), 6.65-6.90 (m, 4H, ArH), 7.20-7.60 (m, 4H, ArH), 8.15 (s, 1H, CH=N), 13.10 (br, 2H, NH triazole). Anal. Calcd for $C_{18}H_{14}FN_7OS_2$; C, 50.57; H, 3.30; N, 22.94. Found: C, 52.15; H, 3.64; N, 22.55.

(E)-5-((3-(3-Hydroxybenzylideneamino)-1H-[1,2,4]-triazol-5-ylthio)methyl)-4-(4-fluoro-phenyl)-1H-1,2,4-triazole 5(4H)-thione (8d).

Yield: **70%** (Methanol); Mp: 100 ^{0}C; ^{1}H NMR ($CDCl_3$) δ in ppm: 3.75 (s, 2H, CH2), 5.10 (s, 1H, OH), 6.45-6.70 (m, 4H, ArH), 6.90-7.40 (m, 4H, ArH), 8.00 (s, 1H, CH=N), 13.30 (br, 2H, NH triazole). Anal. Calcd for $C_{18}H_{14}FN_7OS_2$; C, 50.57; H, 3.30; N, 22.94. Found: C, 50.32; H, 4.12; N, 22.66.

General procedure for the Synthesis of (E)-3-((3-(4-Fluorobenzylideneamino)-1H-[1,2,4]-triazol-5-ylthio)-methyl)-4-(4-fluorophenyl) -1- ((4-substituted-piperazin-1-yl) methyl) -1H-[1,2,4]-triazole-5(4H)-thione (9a,b):

To an ethanolic solution of 5-((3-(4-fluorobenzylideneamino)-1*H*-[1,2,4]-triazol-5-yl-thio)-methyl)-4-(4-fluorophenyl)-4*H*-[1,2,4]-triazole-3-thiol (**8a**) (1gm, 0.002 mol), a mixture of formaldehyde solution and (0.002 mol) phenylpiprazine derivative were added. The reaction mixtures were refluxed. Reaction mixture was evaporated under vacuum to yield (**9a,b**) respectively.

(E)-3-((3-(4-Fluorobenzylideneamino)-1H-[1,2,4]-triazol-5-ylthio)methyl)-4-(4-fluoro-phenyl)-1-(4-phenylpiperazin-1-yl) methyl)-1H-[1,2,4]-triazole-5(4H)-thione (9a).

Yield: **70%** (ethanol); Mp: 125 ^{0}C; ^{1}H NMR ($CDCl_3$) δ in ppm: 2.65-3.50 (m, 8H, $4CH_2$- piperazine). 3.00 (s, 2H, CH2-S, *J*=4.15Hz), 4.50 (s, 2H, CH_2-piperazine, *J*=2.50Hz), 6.40-6.75 (m, 4H, ArH), 7.00-7.60 (m, 5H, ArH), 8.10 (s, 1H, CH=N), 13.50 (br, 1H, NH-triazole). Anal. Calcd for $C_{29}H_{27}F_2N_9S_2$; C, 57.69; H, 4.51; N, 20.88. Found: C, 58.13; H, 4.22; N, 20.53.

(E)-3-((3-(4-Fluorobenzylideneamino)-1H-[1,2,4]-triazol-5-ylthio)methyl)-4-(4-fluorophenyl)-1-(4-p-tolylpiperazin-1-yl)methyl)-1H-[1,2,4]-triazole-5(4H)-thione (9b).

Yield: **75%** (ethanol); Mp: 185 ^{0}C; ^{1}H NMR ($CDCl_3$) δ in ppm: 2.33 (s, 3H, CH_3), 2.55-3.45 (m, 8H, $4CH_2$-piperazine). 3.20 (s, 2H, CH2-S, *J*=4.35Hz), 4.55 (s, 2H, CH_2-piperazine, *J*=2.60Hz), 6.40-6.75 (m, 4H, ArH), 7.00-7.60 (m, 4H, ArH), 8.10 (s, 1H, CH=N), 13.50 (br, 1H, NH triazole). Anal. Calcd for $C_{30}H_{29}F_2N_9S_2$; C, 58.33; H, 4.73; N, 20.41. Found: C, 58.84; H, 4.52; N, 20.99.

General procedure for the Synthesis of (E)-5-((5-(Alkyl/ Arylthio)-4-(4-fluorophenyl)-4H-[1,2,4]-triazol-3-yl)methylthio)-N-(4-fluorobenzylidene)-1H-[1,2,4]-triazol-3-amine 10a,b:

5-((3-(4-fluoro-benzylideneamino)-1*H*-[1,2,4]-triazol-5-ylthio)methyl)-4-(4-fluorophenyl)-4*H*-[1,2,4]-triazole-3-thiol (**8a**) (1gm) and benzyl chloride (3gm) or ethyl iodide (0.31gm) in DMF were refluxed in water bath. Reaction mixture was evaporated under vacuum. The residues were crystallized to yield (**10a,b**) respectively.

(E)-5-((5-(Benzylthio)-4-(4-fluorophenyl)-4H-[1,2,4]-triazol-3-yl)--methylthio)-N-(4-fluoro-benzylidene)-1H-[1,2,4]-triazol-3-amine (10a).

Yield: **80%** (ethanol); Mp: 180 ^{0}C; ^{1}H NMR ($CDCl_3$) δ in ppm: 3.55 (s 2H, CH_2-S-triazole), 4.20 (s, 2H, CH_2-benzyl), 7.00-8.40 (m,12H, 3ArH), 8.00 (s, 1H, CH=N), 13.20 (br, 1H, NH triazole). Anal. Calcd for $C_{25}H_{19}F_2N_7S_2$; C, 57.79; H, 3.69; N, 18.87. Found: C, 58.22; H, 3.14; N, 19.24.

(E)-5-((5-(Ethylthio)-4-(4-fluorophenyl)-4H-[1,2,4]-triazol-3-yl)methylthio)-N-(4-fluoro-benzylidene)-1H-[1,2,4]-triazol-3-amine(10b).

Yield: **85%** (ethanol); Mp: 220 ^{0}C; ^{1}H NMR ($CDCl_3$) δ in ppm: 1.20 (t, 3H, CH_3, *J*=1.18Hz), 2.85 (q, 2H, CH_2, *J*= 1.18Hz), 3.55 (s 2H, CH_2-S-triazole), 7.00-7.80 (m,8H, 2ArH), 8.10 (s, 1H, CH=N), 13.10 (br, 1H, NH triazole). Anal. Calcd for $C_{20}H_{17}F_2N_7S_2$; C, 52.50; H, 3.75; N, 21.43. Found: C, 52.66; H, 3.58; N, 21.99.

Results and discussion

Molecular modeling study

As a reference to our modeling and docking studies, the tertiary complex of 1EA1 enzyme, coupled with HEM and TPF as inhibitor, was used as a template (Podust et al. 2001). Studying the triazole ring hydrogen bonding interaction of TPF with the 1EA1 active site revealed that the N_4 of the triazole ring and the

carbonyl oxygen contributed preferable hydrogen bonds with the key pocket residue Cys394 and with the cofactor HEM. The N_1, N_2, N_4 atoms of the second triazole ring of TPF conferred trifurcated H-bonds with the `catalytic triad' residues of 1EA1 pocket Phe255, Ala256, and His259 respectively (Figure 1).

Molecular modeling studies of **5a-e** analogs indicated that the triazole-N_1 was able to form hydrogen bond with **Cys394** and to chelate with the Iron-Fe atom of the cofactor, HEM in a proper manner as TPF. The ester carbonyl oxygen performed bifurcated Hydrogen bonds with **Met254** and **His258**. Due to the presence of aromatic *meta*-hydroxy group in compound **5c**, the conformational orientation of the phenyl ring was sandwiched within the catalytic triad and was directed to allow the recognition by additional hydrogen bond with the residue **Met79** at the binding active site. While *ortho*-hydroxy group of **5d** is oriented away from the sandwich pocket and that may explain its lower affinity and less antibacterial activity relative to **5c** (Figure 2).

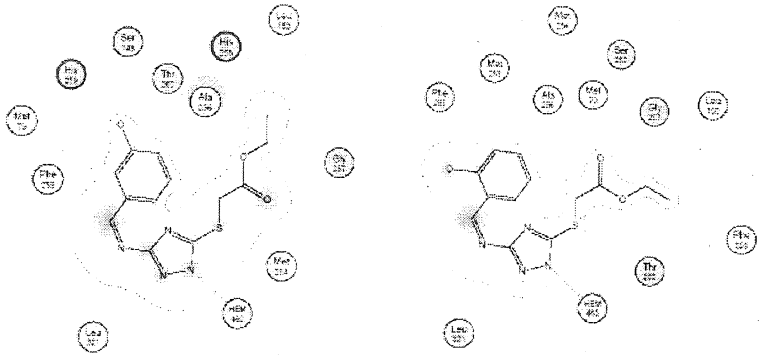


Figure 2. Binding mode for compounds 5c, 5d docked and minimized in the 1EA1 binding pocket, showing residues involved in its recognition

Compounds **6a-e,** carrying elongated semicarbazide side chain, affect the conformational configuration leading to extending the ligands away from the conserved residues at the binding active site. As a result, **6a-e** changed their overall conformation to avoid clashes with the protein backbone and to accommodate by the *van der Waals* interactions. The phenyl ring of **6a,b,d** is freely rotated around the torsional angle of the carbon-nitrogen double bond providing proper hydrophobic interaction with the surrounding pocket residues. These ligands performed better lipophylic recognition within the binding pocket due to the embedding of halo-substituted phenyl ring within hydrophobic aromatic rings of the surrounding residues namely, **Tyr76**, **Phe78**. However, the hydrogen bond interactions of these analogs were performed with only one of the conserved amino acid residues, **Ala256** in addition to **Ser261** (Figure 3).

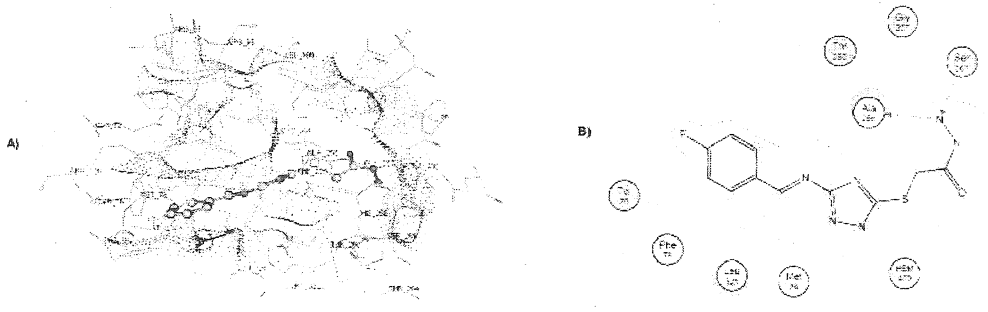


Figure 3. A) Binding mode of compound 6a docked and minimized in the 1EA1 binding pocket, showing residues involved in its recognition: 6a showed as ball and sticks, amino acid residues showed as thin sticks, electrostatic surface showed as doted surface. B) Binding mode of compound (6a) docked and minimized in the 1EA1 binding pocket, showing the hydrophilic & lipophilic characterization of the pocket residues surrounding the ligand 6a.

Meta-hydroxy group of **6d** showed single HB with the amino acid **Ile323** and the hydrazone moiety stabilized the binding recognition by HB with one of the triad catalytic residues, Ala256. The conformationaly stable form of **6c** leads to losing the hydrogen bonding interaction of *ortho*-hydroxy group with the active site, however the hydrazone moiety stabilized the binding recognition by bifurcated HB with **Ser261** and **Ala 256** (Figure 4).

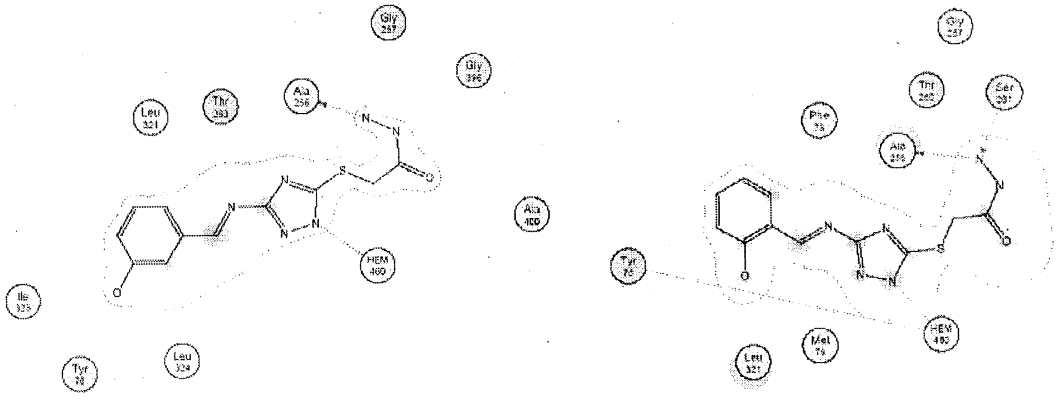


Figure 4. Binding mode of compounds 6c, 6d docked and minimized in the 1EA1 binding pocket, showing residues involved in its recognition

Compounds **7a,b** have hydrophilic bridge linked between the triazole and *para*-flourophenyl ring that showed extensive conformational changes because of the bulkiness of the two terminal phenyl groups that led to the conformational bending of ligands and both phenyl rings were laid between hydrophobic aromatic amino acid residues namely, **Tyr76**, **Phe78**, **Phe255**, **Tyr169** and **His258**. The turn over U shaped conformation of ligand **7a** allowed the exposure of the N_1 of triazole ring and the carbonyl oxygen that were stabilized by hydrogen bonding with amino acids **Thr260** and **Ser261** respectively. The later hydrogen bond was weak and cracked during the geometrical optimization of the tertiary **7a**-1EA1 complex (Figure 5).

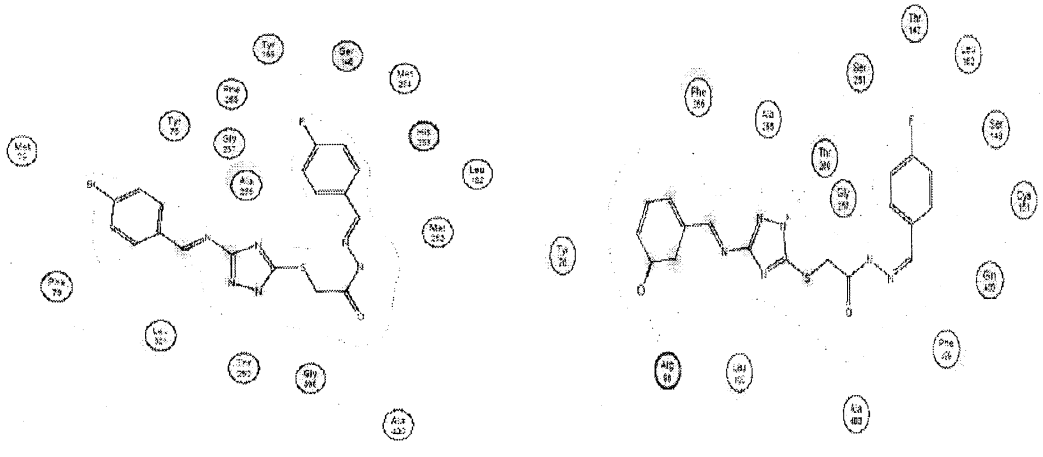


Figure 5. Binding mode for compounds 7a,b docked and minimized in the 1EA1 binding pocket, showing residues involved in its recognition

Compounds **8a-d** showed restricted flexibility due to the bulkiness of the adjacent phenyltriazolethione substituent. That rotated around the flexible thio-methylene spacer and performed proper hydrophobic recognition with hydrophobic patch of the neighbor amino acids at the binding pocket, namely, **Tyr76**, **Phe78**. Geometrically optimized ligand **8b** arranged in the *trans*-conformation that showed proper and stable

complex with high lipophilic interaction where the two halo-phenyl rings were embedded within the hydrophobic active site residues. The ligands exposed to the conserved residues and showed proper chelation with the iron atom of the HEM cofactor. The *trans*-alignment enhanced electrostatic hydrogen bonding with the polar residues and also facilitated the hydrophobic interaction with the greasy amino acid residues at the active site. The hydroxy related analogs **8c** showed a fruitful recognition with the conserved residues of the active site including polar and non polar amino acids that stabilized the *E*-**8c**-1EA1 complex with hydrogen bonding and hydrophobic interactions. In comparison between **8c** and **8d** where *meta*-hydroxy group changed the accommodation within the binding pocket led to loss of recognition with the conserved residue, **His259** that reflected improvement in the biological activity of compound **8c** relative to **8d** (Figure 6).

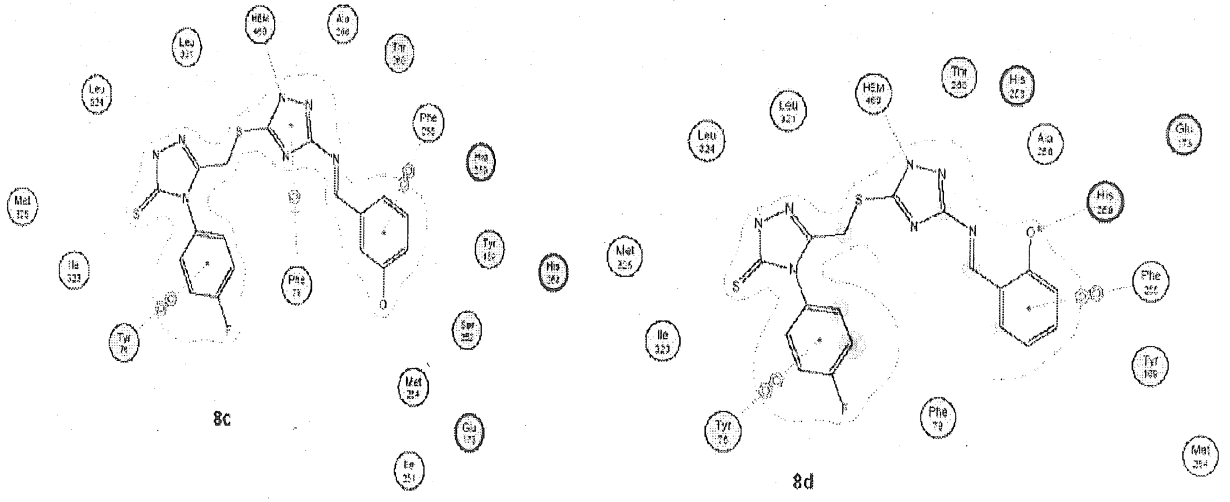


Figure 6. Binding mode for compounds 8c, d docked and minimized in the 1EA1 binding pocket, showing residues involved in its recognition

Compounds **9a-b** showed *trans* orientation that stabilized the structural conformation, however both of the phenylpiperazine and phenyltriazole moieties showed proper hydrophobic accommodation with the catalytic triad **Ala256**, **Phe255** and **His259**. Hydrogen bonding interactions was performed between the N_4 of the triazole ring and the **Ala256** residue. Besides, **Tyr76** showed proper hydrogen bonding interaction with N_1 of the other triazole ring. Piperazine substitution with tolyl group expressed steric hindrance due to the bulkiness (Figure 7).

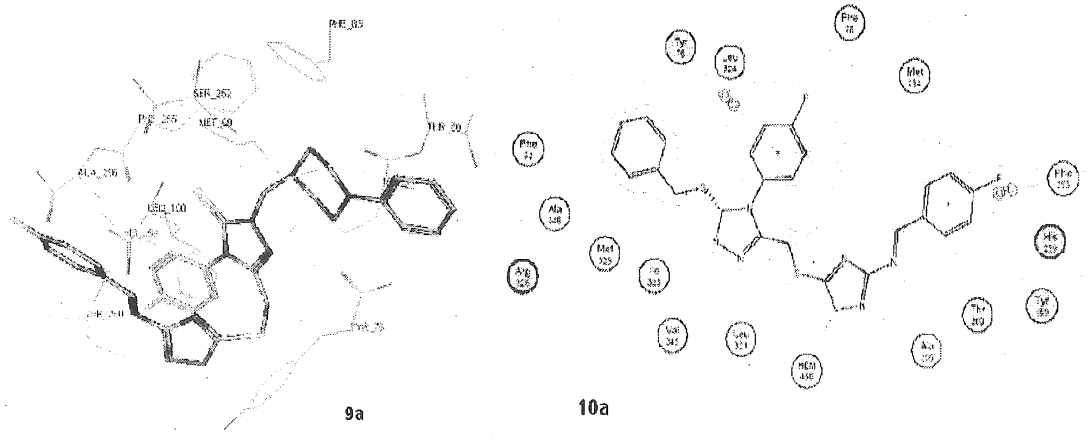


Figure 7. Binding mode for compound 9a and 10a docked and minimized in the 1EA1 binding pocket, showing residues involved in its recognition

Comparative studies of the conformational accommodation of the s-benzyl analog, **10a** and the s-ethyl analog, **10b** indicated that the introducing of the bulky benzyl group improve the hydrophobic interaction with the increase of the logp characters and without interfering with the electrostatic recognition with the catalytic triad residues. However, the *trans* conformational structure showed the proper and stable complex performance. Computational analysis of structurally conserved core indicated that the docked ligand **10a** showed that the root mean squared distance (RMSD) of the backbone atoms (C, N, S) in the conserved residues of the active site experience high degree of divergence to remain the proper ligand recognition and that may explain the variation between the high degree of docking complementarity and the expressed biological activities (Figure 7).

In conclusion recognition with "catalytic triad" three key amino acids, **Phe255, Ala256** and **His259**, is essential for binding and that reflected improvement in the biological activity. The amino acid **Tyr76** is not one of the key residues for recognition of the parent ligand TPF but it is playing a crucial rule in the recognition of the tested ligands and that was designated from the elucidation of the biological data. The overall outcome of this molecular docking study revealed that: 1) Triazole or its fused triazolo pyrimidine rings are an essential for CYP51 inhibition through their N_1, N_4 or both as recognition features with the Iron Fe atom of HEM cofactor, key amino acids residues at the enzyme pocket. 2) Ethyl carboxylate functional side chain that's freely rotated about torsion angles and that's not extended to other side chains is important for allowing carbonyl oxygen to bond with the conserved residues at the binding site. 3) Flexible thio-methylene spacer was necessary to allow hydrophobic recognition of phenyl rings with hydrophobic patch of amino acids at the enzyme pocket. 4) *Trans* conformation at carbon nitrogen double bond stabilized ligand-enzyme complex and enhanced electrostatic hydrogen bonds and hydrophobic interaction at the enzyme binding site. 5) Halo-substituted phenyl rings allowed lipophilic recognition within the binding pocket.

Lipophilicity studies

The attempt to correlate antibacterial and antifungal activities of the synthesized compounds with lipophilicity was unsuccessful. It is obvious from the obtained results (Table 1) that lipophilicity does not affect so much the antibacterial/ antifungal activities of synthesized compounds. Probably the structural characteristics of the synthesized molecules are more important for this kind of activity.

Antimicrobial Activity:

All the newly synthesized compounds were screening for their *in vitro* antimicrobial activities.

Activity against Escherichia coli:

Compounds **6ab** the hydrazide analogs were found to be the most reactive. Compound **5d** a Schiff's base derivative was found to be highly active. Compounds **5c**, **6d**, **9a** were found to be moderate in their activity. Compounds **8b,c** are bitriazoles that exhibited slight activity (Table 1).

Activity against Staphylococcus aureus.

Three of the tested 33 compounds were found to be highly active; **5d**, **8b** and **9a.** Compounds **8c** and **9b** were moderately active and compounds **5c**, **6a** and **8a** were slightly active. That reflected that bitriazoles and hydrazides have an effect on the activity. Piprazine moiety also contributed to the activity (Table 1).

Activity against fungi.

Compounds **5b,c** ; **8b,c,d** and **9a** were highly reactive against *Candida albicans*. Compounds **5d and 9b** were found to be active (Table 1).

Table 1. LogP and the antimicrobial activity of compounds (5-10)

		Microorganisms		
		Gram -ve bacteria	Gram +ve bacteria	*Fungi*
Comp. No.		*E.coli*	*S. aureus*	*C. albicans*
5b	**1.65**	-	-	28
5c	**1.65**	18	12	34
5d	**2.73**	23	21	20
5e	**0.83**	-	-	-
6[a]	**1.48**	29	11.5	-
6b	**0.40**	26	21	14
6c	**0.40**	-	-	-
6d	**1.48**	18.2	-	-
6e	**2.96**	-	-	-
7a	**3.61**	-	-	-
7b	**2.53**	-	-	-
8[a]	**2.53**	-	16	-
8b	**3.51**	13	21	38
8c	**4.40**	13.7	16	33
8d	**3.66**	-	-	27
9a	**3.73**	15.4	20	35
9b	**2.28**	-	13	22
10a	1.21	-	-	19
10b	**3.36**	-	-	-
Ampicillin	**1.7**	31	23	-
Clotrimazol	**3.50**	-	-	40
Fluconazole	**2.84**	-	-	38

*Degree of activity is measured by the zone of inhibition:, (-): No inhibition (resistant, not sensitive), (10-15 mm, slightly active), (15-20), mm, moderately active), (20-25 mm, highly active), > 25 mm, very active.

Conclusion

The most potent compounds exhibited bitriazole in their structures which may indicate structural similarity to the known antifungal fluconazole. Further molecular modeling work was important to confirm that. The screening results were found to be in coherence with the physicochemical parameters determined for the compounds. **5d**, **6a**,**b**,**e** showed proper degree of lipophilicity in agreement with their potential activity as antimicrobial agents related to that of ampicillin, clotrimazole. Also, that could be attributed to the rotation of phenyl triazole around flexible thio methyl spacer allowing hydrophobic recognition with neighboring amino acids at the CYP51 enzyme binding pocket.

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