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SYNTHESIS, ANTIMICROBIAL AND ANTIHUMAN LIVER CANCER ACTIVITIES OF NOVEL SULFONAMIDES INCORPORATING BENZOFURAN, PYRAZOLE, PYRIMIDINE, 1,4-DIAZEPINE AND PYRIDINE MOIETIES PREPARED FROM (*E*)-4-(3-(DIMETHYLAMINO)ACRYLOYL)-*N*-ETHYL-*N*-METHYLBENZ ENE-SULFONAMIDE

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Abstract – Reaction of compound **1** with dimethylformamide dimethylacetal gave enaminone **2**, which reacts with 1,4-benzoquinone and 4-amino-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one to afford **6** and **7**, respectively. Treatment of **2** with hydroxylamine hydrochloride gave cyanoacetyl derivative **14**. Enaminone **2** reacts with guanidine hydrochloride and phenylhydrazine to furnish 2-aminopyrimidine and pyrazole derivatives **15** and **16,** respectively. Enaminone **2** also reacts with ethylenediamine and acetylacetone to afford the corresponding derivatives of 1,4-diazepine **18** and pyridine **20**, respectively. The structures of the newly synthesized compounds were confirmed by elemental analysis, IR, ^{1}H NMR, ^{13}C NMR and MS spectral data. All the synthesized compounds were evaluated for antimicrobial activity. Compounds **6** and **20** were found to be highly active against the all microorganisms. In addition, all the compounds were tested *in-vitro* antihuman liver hepatocellular carcinoma cell line (HepG2). Compounds **14, 6, 18** and **16** with selectivity index (SI) values of 70.92, 55.56, 29.56 and 15.00, respectively, exhibited better activity than methotrexate (MTX) as a reference drug with SI value of 13.30. Virtual screening using molecular docking studies of the synthesized compounds was performed by

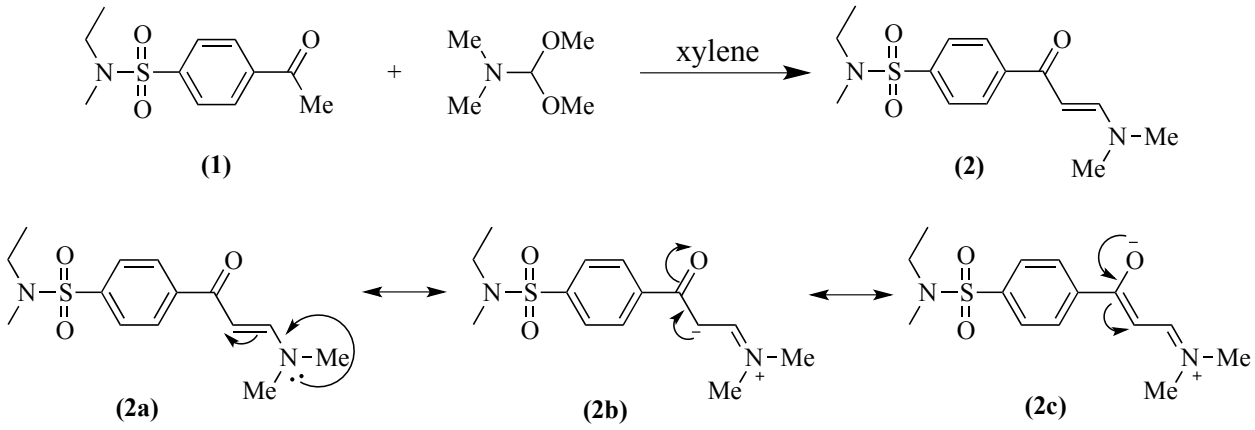
Molecular Operating Environment (MOE). The results indicated that some synthesized compounds suitable inhibitor against dihydrofolate reductase (DHFR) enzyme (PDB ID: 4DFR) with further modification.

INTRODUCTION

Enaminones constitute an interesting class of compounds that are versatile precursors for the synthesis of several heterocyclic compounds.[1,2] Sulfonamides have been demonstrated to possess antibacterial properties,[3,4] and some active sulfonamides as antibacterial agents are known for their antifungal,[6] insulin-releasing,[7,8] carbonic anhydrase inhibitory,[9-11] hypoglycemic,[12] anesthetic,[13] anti-inflammatory,[14,15] and anti-carcinogenic[16,17] activities. Liver cancer (hepatocellular carcinoma) remains one of the most important health problems in the world because it is the third foremost cause of cancer-related deaths worldwide.[18] In view of these reports and as a continuation of previous work[19-23] directed towards the synthesis of substituted heterocycles, incorporating with benzenesulfonamide with anticipated biological activities. Thus, in the present work, used enaminone **2** namely (*E*)-4-(3-(dimethylamino)acryloyl)-*N*-ethyl-*N*-methylbenzenesulfonamide as a starting material in the formation of a variety of heterocycles with substituted benzenesulfonamide moiety and investigated their antimicrobial and antihuman liver cancer activities.

RESULTS AND DISCUSSION

This article reports a new and convenient method for the synthesis of such ring systems that are required to medicinal chemistry utilizing. (*E*)-4-(3-(dimethylamino)acryloyl)-*N*-ethyl-*N*-methylbenzenesulfonamide **(2)**, as a starting material, which was prepared from the interaction of 4-acetyl-*N*-ethyl-*N*-methylbenzenesulfonamide **(1)**[24] with dimethylformamide dimethyl acetal (DMF-DMA) under reflux in dry xylene.

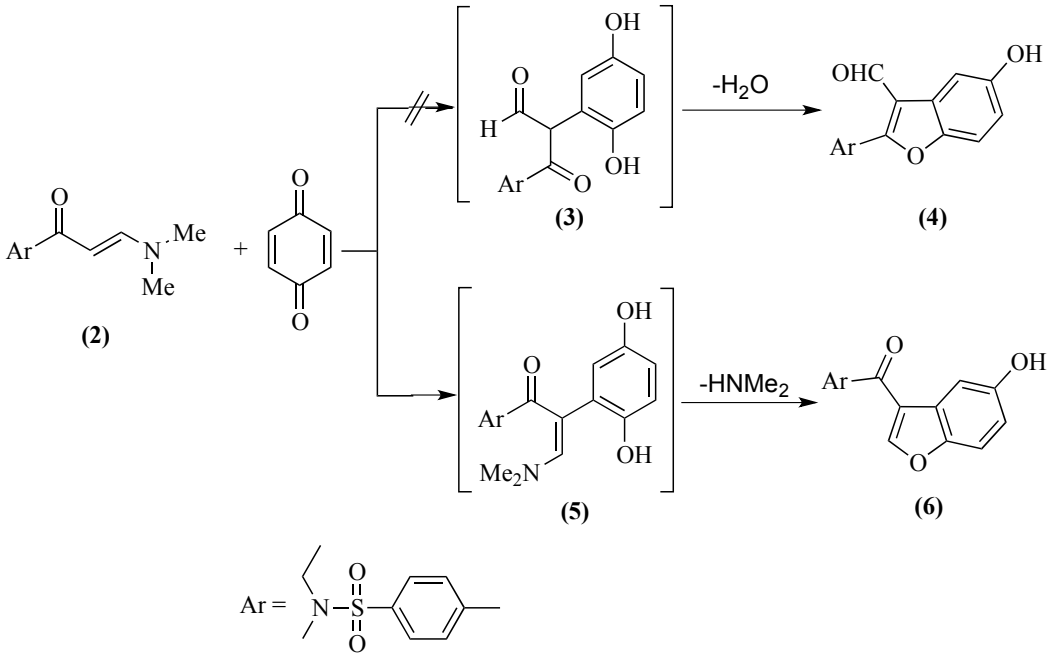


Scheme 1

Compound **2** can be represented by three isomeric structures (**2a-c**) (**Scheme 1**). Enaminone **2** was assigned an *E*-configuration based on its ^{1}H NMR spectrum which revealed that the coupling constant of

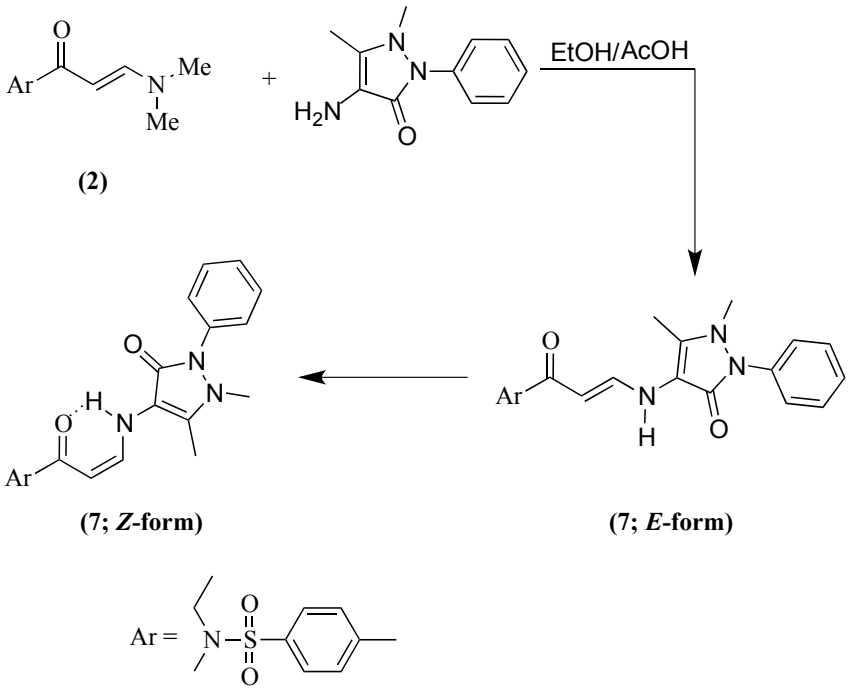
the doublet signals for olefinic protons equal to 12.8 *Hz* correlated to *E*-isomers.

The behavior of enaminone **2** towards 1,4-benzoquinone was investigated. Thus, compound **2** reacted with 1,4-benzoquinone in glacial acetic acid at room temperature to yield a product which formulated as *N*-ethyl-4-(5-hydroxybenzofuran-3-carbonyl)-*N*-methylbenzenesulfonamide **(6)**. It is believed that electron rich (C-2) in the enaminone **2** initially adds to the activated double bond in the 1,4-benzoquinone yielding acyclic intermediate **5** which then cyclized into **6** *via* dimethylamine elimination,[25] and not afforded *N*-ethyl-4-(3-formyl-5-hydroxybenzofuran-2-yl)-*N*-methylbenzenesulfonamide **(4)**. Elucidation of structure **6** and refusing of structure **4** was based on ^{1}H NMR spectrum which indicated the disappearance of aldehydic signal and showed singlet signal at δ= 8.11 ppm. for benzo[*b*]furan H-2, **(Scheme 2)**.



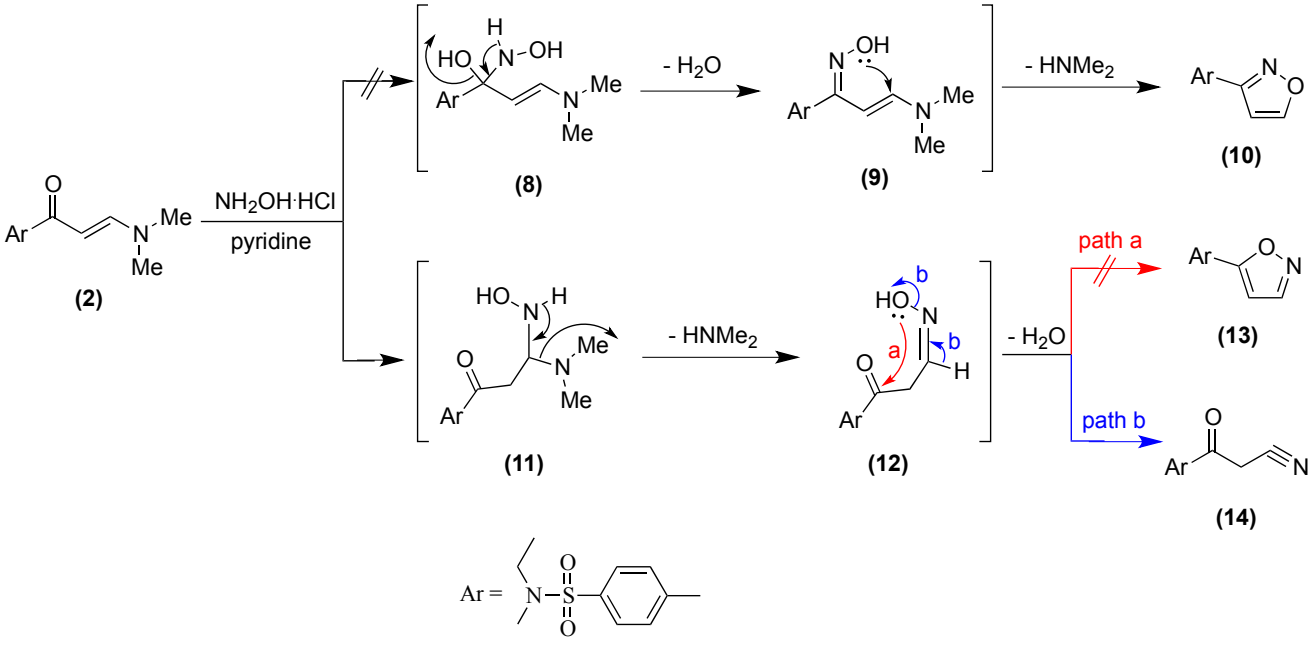
Scheme 2

Treatment of enaminone **2** with 4-amino-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one in a mixture of ethanol/acetic acid at reflux temperature afforded (*Z*)-4-(3-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-ylamino)acryloyl)-*N*-ethyl-*N*-methylbenzenesulfonamide **(7)**, ^{1}H NMR spectrum of compound **7** supports that this structure in (*Z*-form) not in (*E*-form), while the coupling constant of the doublet signals for olefinic protons equal to 8.5 *Hz*. *Z*-Form is stabilized by intramolecular hydrogen bonding (**Scheme 3**).



Scheme 3

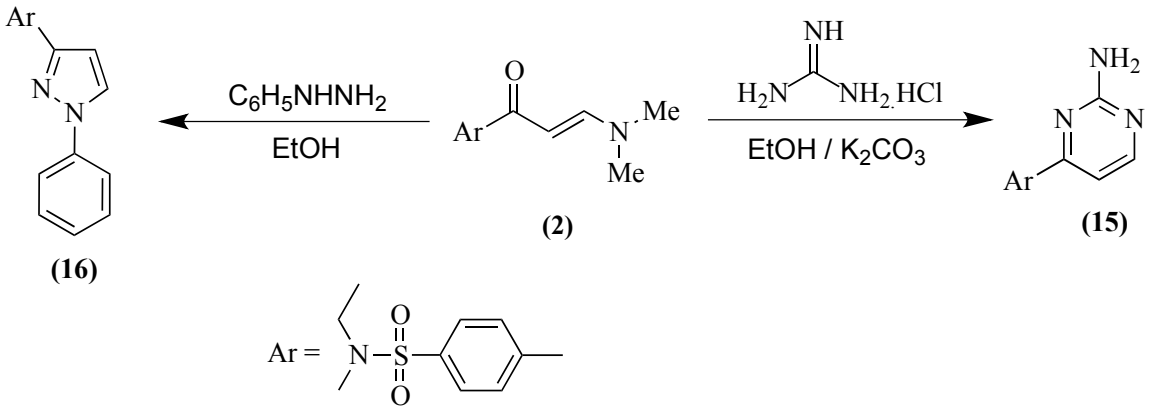
Enaminone **2** reacts with hydroxylamine hydrochloride in refluxing pyridine to give one isolable product, namely, 4-(2-cyanoacetyl)-*N*-ethyl-*N*-methylbenzenesulfonamide **(14)** rather than another isomeric forms of compounds **10** and **13**. Structure of compound **14** was assigned as the correct structure on the basis of its IR spectrum revealed absorption band at ν_{max}= 2220 cm^{-1} for cyano group, and ^{1}H NMR spectrum which showed singlet signal for methylene group at δ= 3.14 ppm.



Scheme 4

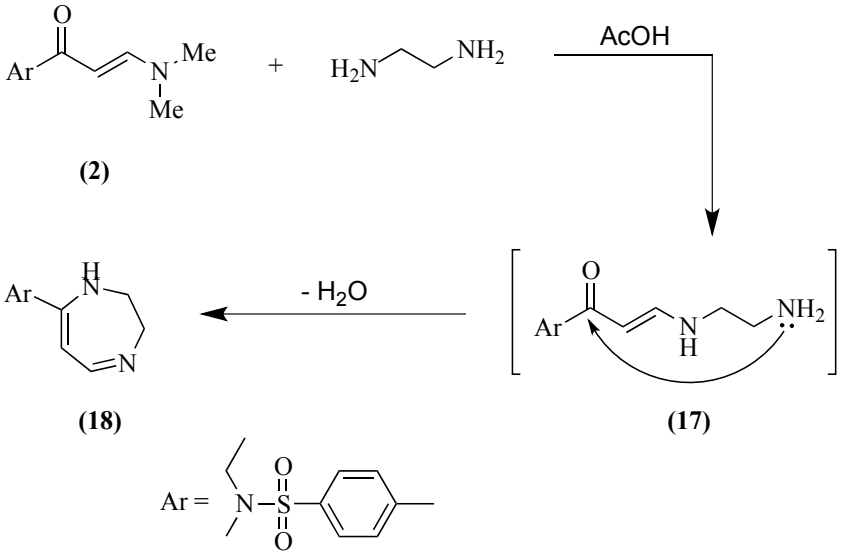
Cyanoacetyl derivative **14** was assumed to be formed *via* initial addition of hydroxylamine to the activated double bond in enaminone **2** forming non isolable intermediate **11**, followed by the loss of dimethylamine to produce aldehyde oxime intermediate **12** then dehydration (**Scheme 4**).

Interaction of enaminone **2** with guanidine hydrochloride and phenylhydrazine afforded 2-aminopyrimidine and pyrazole derivatives **15**,**16,** respectively (**Scheme 5**). The absorption band of carbonyl group disappeared in the IR spectra of compounds **15** and **16** in comparison with the IR spectrum of compound **2**. The IR spectrum of compound **15** showed two absorption bands at ν_{max}= 3448 and 3418 cm^{-1} for amino group. The ^1H NMR spectrum of compound **15** showed singlet signal at δ= 7.13 ppm for NH_2 group, which exchangeable with D_2O. The ^{13}C NMR spectrum of compound **16** revealed fourteen carbon types; for eighteen carbon atoms; the most important signals appeared at δ= 13.08 ppm for methyl group of $\underline{CH_3}$-CH_2, 26.87 ppm for CH_3-N and 34.01 ppm for methylene group of CH_3-$\underline{CH_2}$. The mass spectrum of **16** showed a molecular ion peak at *m/z* 341 as a base beak.

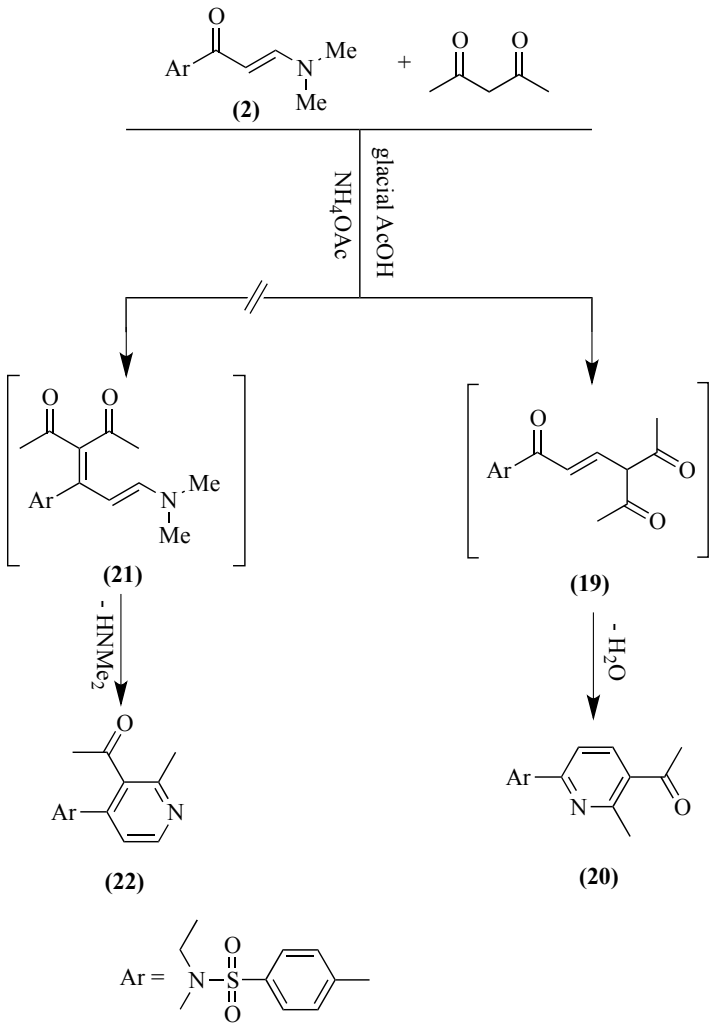


Scheme 5

Interaction of enaminone **2** with ethylenediamine in acetic acid afforded 1,4-diazepine derivative **18** *via* elimination of water from acyclic intermediate **17** (**Scheme 6**). The obtained product was established based on elemental analysis and spectral data. Thus, IR spectrum of compound **18** revealed absorption band at ν_{max}= 3230 cm^{-1} due to NH group. ^1H NMR spectrum showed multiplet signals at δ= 2.48-2.65 ppm due to two methylene protons of 1,4-diazepine and D_2O exchangeable signal at δ= 12.30 ppm due to NH proton. The other possible isomeric structure 4-(3-acetyl-2-methylpyridin-4-yl)-*N*-ethyl-*N*-methylbenzenesulfonamide **(22)** was discarded based on ^1H NMR data that revealed pyridyl hydrogens at C-4, C-5 as a pair of doublets at δ= 7.51, 7.72 ppm, respectively, with coupling constant (*J= 7.6 Hz)* assignable to 6-substituted pyridine **20**. The isomeric structure **22** should display pair of doublets corresponding to C-5, C-6 with a lower coupling constant (*J=2-3 Hz*)[26] (**Scheme 7**).



Scheme 6



Scheme 7

DOCKING AND MOLECULAR MODELING

Thymidylate synthase and dihydrofolate reductase are among the main targets involved in anticancer and antimicrobial activities.[27,28] Molecular modeling using Molecular Operating Environment (MOE)[29] module was performed in order to rationalize the observed anticancer activity of compounds **1**, **2**, **6**, **7**, **14, 15** and **16**. Molecular docking studies further help to understand the mode of action of the compounds through their various interactions with the active sites of DHFR.

Docking of MTX into DHFR: The active site revealed that hydrogen bond interactions beside hydrophobic interactions were considered responsible for the observed affinity as it acts as a hydrogen bond donor to the backbone Ile 5, Ile 94 residues, and the side chain Asp 27 residue. It also acts as a hydrogen bond accepter to Arg 52 and Arg 57 residues. This occurred along with many hydrophobic interactions with various amino acid residues: ILe 5, Ala 6, Ala7, Asp 27, Leu 28, Phe 31, Lys 32, Ser 49, Ile 50, Arg 52, Leu 54, Arg 57, Ile 94, Tyr 100 and Thr 113, as shown in **Figure 1.**

Docking simulation of the synthesized compounds 1, 2, 6, 7, 14, 15 and 16: MOE docking studies of the inhibitors were performed using DHFR co-crystallized with methotrexate (PDB ID: 4DFR) as a template.

Docking of compound 1 into DHFR: The active site revealed the presence of three hydrogen bond interactions because one oxygen atom of the SO_2 moiety acted as a hydrogen bond acceptor with the amino acid residue Ala 9 and Tyr 121 (2.64 Aº and 3.64 Aº, respectively) with a strength of 89.5% and 2.1%, respectively. In addition, a hydrogen bond interaction as the oxygen atom of carbonyl function acted as a hydrogen bond acceptor with the amino acid residue Ser 59 (3.27 Aº) with a strength of 3%. Hydrophobic interactions also occurred with the following amino acid residues: Ile 7, Val 8, Ala 9, Ile 16, Asp 21, Leu 22, Trp 24, Glu 30, Phe 34, Ser 59 and Tyr 121, as shown in **Figure 2**.

Docking of compound 2 into DHFR: The active site revealed that several molecular interactions were considered to be responsible for the observed affinity, because the two oxygen atoms of the SO_2 moiety acted as a hydrogen bond acceptor with the side chain residues; Thr 56 and Ser 59 (3.15 Aº and 2.43 Aº; respectively) with a strength of 56.5% and 57.9%, respectively. Furthermore, the oxygen atom of carbonyl function acted as a hydrogen bond acceptor with the side chain residues, Ala 9 and Tyr 121 (3.19 Aº and 3.16 Aº, respectively) with a strength of 13.8% and 3.6%, respectively. In addition, hydrophobic interactions occurred with the following amino acid residues: Val 8, Ala 9, Ile 16, Gly 17, Gly 20, Asp 21, Leu 22, Glu 30, Phe 34, Lys 55, Thr 56 , Ser 59, Tyr 121 and Thr 146, as shown in **Figure 3**.

Docking of compound 6 into DHFR: The active site illustrated the occurrence of several interactions of

one oxygen atom of the SO_2 moiety with different amino acid residues as it acted as a hydrogen bond acceptor with the side chain residues; Thr 56 and Tyr 121 (3.62 Aº and 3.79 Aº, respectively) with a strength of 2.7% and 2.1%, respectively. This occurred along with hydrophobic interactions among the other atoms of the compound and the following amino acid residues: Ile 7, Val 8, Ala 9, Phe 31, Phe 34, Gln 35, Thr 56, Ile 60, Pro 61, Asn 64, Leu 67, Val 115, Gly 116 and Tyr 121, as shown in **Figure 4**.

Docking of compound 7 into DHFR: The active site revealed the presence of hydrogen bond interactions between one oxygen atom of the SO_2 moiety because it acted as a hydrogen bond acceptor with the amino acid residue Thr 56 (3.27 Aº) with a strength of 7.5%. Moreover, the oxygen atom having a carbonyl function acted as a hydrogen bond acceptor with the side chain residue; Asn 64 (1.93 Aº) with a strength of 69.6%. In addition, hydrophobic interactions involved other atoms of the compound with the following amino acid residues: Ile 16, Asp 21, Leu 22, Phe 31, Phe 34, Thr 56, Ser 59, Ile 60, Pro 61, Asn 64, Leu 67, Val 115, Gly 116 and Gly 117, as shown in **Figure 5**.

Docking of compound 14 into DHFR: The active site revealed the presence of several molecular interactions in which one oxygen atom of the SO_2 moiety acted as a hydrogen bond acceptor with the amino acid residues Thr 56 and Ser 59 (2.28 Aº and 1.68 Aº, respectively) with a strength of 79% and 18.4%, respectively. Moreover, the nitrogen atom having a cyano function acted as a hydrogen bond acceptor with the amino acid residue Ala 9 (3.11 Aº) with a strength of 23.8%. In addition to hydrophobic interactions involved other atoms of the compound with the following amino acid residues: Val 8, Ala 9, Ile 16, Gly 17, Gly 20, Asp 21, Leu 22, Lys 55, Thr 56, Ser 59 and Thr 146, as shown in **Figure 6**.

Docking of compound 15 into DHFR: The active site revealed the presence of a hydrogen bond interaction between one oxygen atom of the SO_2 moiety because it acted as a hydrogen bond acceptor with the side chain residue; Thr 56 (2.62 Aº) with a strength of 88.8%. In addition, hydrophobic interactions occurred that involved the other atoms of the compound with the following amino acid residues: Ile 16, Phe 31, Thr 56, Ser 59, Ile 60, Pro 61, Asn 64, Leu 67, Val 115, Gly 116, Gly 117 and Tyr 121, as shown in **Figure 7**.

Docking of compound 16 into DHFR: The active site revealed the presence of a hydrogen bond interaction between one oxygen atom of the SO_2 moiety because it acted as a hydrogen bond acceptor with the side chain residues; Thr 56 (2.67Aº) with a strength of 98.4%. In addition, hydrophobic interactions occurred among other atoms of the compound with the following amino acid residues: Ile 16, Gly 17, Leu 22, Phe 31, Phe 34, Thr 56, Ser 59, Asn 64, Leu 67, Val 115, Gly 116 and Gly 117, as shown in **Figure 8**.

Conclusion of the docking simulation study: Docking was performed for the seven most active

anticancer compounds **1**, **2**, **6**, **7**, **14, 15** and **16** on DHFR in a trial to predict their mode of action as anticancer drugs. As revealed from the aforementioned data, the compounds showed several interactions with both enzymes but they exhibited strong interactions with DHFR, (mainly compounds **1, 2, 6** and **14)** leading to the conclusion that they might exert their action through inhibition of DHFR.

IN VITRO ANTIMICROBIAL ACTIVITY[30-32]

The results of the antimicrobial evaluation of the tested compounds are presented in (Table 1). All the newly synthesized compounds were evaluated for antibacterial activity against three strains of Gram-positive bacteria (*S. aureus* [RCMB 010027], *S. pneumonia* [RCMB 010010] and *B. subtilis* [RCMB 010067]), two strain Gram-negative bacteria (*K. pneumoniae* [RCMB 0010093] and *E. coli* [RCMB 010052]) and for the antifungal activity against *A. fumigatus* [RCMB 02568]. Compounds **6** and **20,** which contain 5-hydroxybenzofuran and 3-acetylpyridine moieties, respectively were found to be highly biologically active against all microorganisms. Compounds **1**, **14**, **15** and **18** contain 4-acetyl, 4-cyanoacetyl, 2-aminopyrimidine and 1,4-diazepine moieties, respectively, showed higher activity against *K. pneumonia* compared to gentamicine as the reference drug. Compounds **1** and **14** showed moderated activity against *S. pneumonia* compared to ampicillin as the reference drug. Compounds **1**, **14** and **15** revealed moderate activity against *B. subtilis* compared to ampicillin as the reference drug.

IN VITRO ANTICANCER ACTIVITY

The newly synthesized compounds were evaluated for their *in-vitro* cytotoxicity against a hepatocellular cancer cell line (HepG2) and some of the tested compounds were more potent compared with Methotrexate as the reference drug. From the obtained results (**Tables 2-4**), we can observe that compound **14** having cyanoacetyl moiety with SI value of 70.92, 5-hydroxybenzofuran derivative **6** with SI value of 55.56, [1,4]diazepine derivative **18** with SI value of 29.56, pyrazole derivative **16** with SI value of 15.00, showed increased activity when compared to methotrexate (MTX) with SI value of 13.30, while the remaining compounds **7**, **15**, **2**, **1** and **20** with SI values of 03.09, 02.57, 02.46, 02.44 and 02.06, respectively, showed decreased activity when compared to methotrexate. It is clear from the present data that the comparison of the cytotoxicity of the synthesized compounds against hepatocellular cancer cell line (HepG2). (**Chart 1**) has showed that, the cell killing potency follows the order **14 > 6 > 18 > 16 > MTX > 7 > 15 > 2 > 1 > 20**. These preliminary results of biological screening of the tested compounds could offer an encouraging framework in this field that may lead to the discovery of potent anticancer agents.

EXPERIMENTAL

Melting points (^{o}C, uncorrected) were determined in open capillaries on a Gallen Kemp melting point apparatus (Sanyo Gallen Kemp, Southborough, UK). IR spectra (KBr) were recorded on FT-IR 5300

spectrometer and Perkin Elmer spectrum RXIFT-IR system (ν, cm^{-1}). Pre-coated silica gel plates (silica gel 0.25 mm, 60 G F 254; Merck, Germany) were used for thin layer chromatography. The NMR spectra in (DMSO-d_6) were recorded at 300 *MHz* on a Varian Gemini NMR spectrometer (δ, ppm). Mass spectra were obtained on GC Ms-QP 1000 EX mass spectrometer at 70 ev. Elemental analyses were performed on Carlo Erba 1108 Elemental Analyzer (Heraeus, Hanau, Germany). All compounds were within ± 0.4 % of the theoretical values. Analyses were carried out by the Micro analytical Research Center, Faculty of Science, Cairo University and Al-Azhar University.

(*E*)-4-(3-(Dimethylamino)acryloyl)-*N*-ethyl-*N*-methylbenzenesulfonamide (2)

A mixture of 4-acetyl-*N*-ethyl-*N*-methylbenzenesulfonamide **1** (2.41 g; 0.01 mol) and DMF-DMA (1.43g; 0.012 mol) in dry xylene (50 mL) was heated under reflux for 4 h, the separated solid was filtered off, washed with EtOH and recrystallized from EtOH to give **2**. Yellow crystals, yield 63%; mp 170-171 °C. IR (KBr, cm^{-1}): ν_{max} = 1692 (C=O), 3031 (CH-aromatic), 2982 (CH-aliphatic), 1340, 1180 (SO_2). ^{1}H NMR (DMSO-d_6, ppm): δ= 1.43 (t, 3H, *J=7.2 Hz*, $\underline{CH_3}$-CH_2), 2.66 (s, 3H, SO_2NCH_3), 2.74, 2.77 (2s, 6H, $N(CH_3)_2$), 3.14 (q, 2H, *J=7.2 Hz*, CH_3-$\underline{CH_2}$), 5.30 (d, 1H, *J=12.8 Hz*, COCH=), 7.88, 8.11 (each d, each 2H, *J=8.5 Hz*, AB-system), 7.96 (d, 1H, *J=12.8 Hz*, =CHN). ^{13}C NMR (DMSO-d_6, ppm): δ = 13.18, 33.98, 37.97, 44.94 (2C), 77.23, 127.84 (2C), 129.37 (2C), 141.97, 143.03, 144.50, 196.86. MS *m/z* (%): 296 [M^+] (30.2), 252 (27.9), 238 (70.3), 198 (11.7), 174 (16.3), 155 (9.7), 122 (100.0), 58 (27.9), 44 (50.2). Anal. Calcd for $C_{14}H_{20}N_2O_3S$ (296.39): C, 56.73; H, 6.80; N, 9.45; S, 10.82. Found: C, 56.70; H, 6.90; N,9.50; S, 10.74.

***N*-Ethyl-4-(5-hydroxybenzofuran-3-carbonyl)-*N*-methylbenzenesulfonamide (6)**

To a stirred solution of enaminone **2** (2.96 g, 0.01 mol) in glacial AcOH 30 mL, 1,4-benzoquinone (1.08 g, 0.01 mol) was added, stirring was continued for 3 h, at room temperature. The reaction mixture was evaporated in vacuum and the solid product was isolated by filtration and recrystallized from EtOH to give **6**. Brown crystals, yield 73%; mp 253-254 °C. IR (KBr, cm^{-1}): ν_{max} = 3444-3250 (OH), 3050 (CH-aromatic), 2912 (CH-aliphatic), 1672 (C=O), 1340, 1160 (SO_2). ^{1}H NMR (DMSO-d_6, ppm): δ = 1.15 (t, 3H, *J=7.1 Hz*, $\underline{CH_3}$-CH_2), 2.66 (s, 3H, SO_2NCH_3), 3.16 (q, 2H, *J=7.1 Hz*, CH_3-$\underline{CH_2}$), 7.06-8.08 (m, 7H, Ar-H), 8.11 (s, 1H, CH of benzo[*b*]furan H-2), 8.43 (s, 1H, OH, Discharged with D_2O). ^{13}C NMR (DMSO-d_6, ppm): δ = 13.08, 27.87, 34.01, 77.03, 127.56, 127.64, 127.96, 128.86, 128.90 (2C), 130.50 (2C), 134.51, 137.78, 139.27, 141.96, 142.65, 193.18. MS *m/z* (%):359 [M^+] (77.3), 342 (10.4), 301 (11.8), 237 (17.8), 226 (90.4), 198 (43.9), 133 (77.4), 122 (100.0), 58 (36.7). Anal. Calcd for $C_{18}H_{17}NO_5S$ (359.40): C, 60.15; H, 4.77; N, 3.90; S, 8.92. Found: C, 60.20; H, 4.80; N, 4.10; S, 8.85.

(*Z*)-4-(3-((1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)amino)acryloyl)-*N*-ethyl-*N*-

methylbenzenesulfonamide (7)

A mixture of enaminone **2** (2.96 g, 0.01 mol) and 4-amino-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one (2.03 g, 0.01 mol) in EtOH/AcOH (1:1), 50 mL was heated under reflux for 3 h, during the reflux period, a crystalline solid was separated. The separated solid was filtered off, washed with EtOH and recrystallized from EtOH/benzene to give **7**. Yellowish white crystals, yield 66%; mp 290-291 °C. IR (KBr, cm^{-1}): ν_{max} = 3190 (NH), 1690 (C=O), 3100 (CH-aromatic), 2954 (CH-aliphatic), 1342, 1163 (SO_2). ^1H NMR (DMSO-d_6, ppm): δ = 1.14 (t, 3H, *J=7.1 Hz*, $\underline{CH_3}$-CH_2), 2.01 (s, 3H, CH_3 of pyrazole), 2.66 (s, 3H, SO_2NCH_3), 2.77 (s, 3H, CH_3-N of pyrazole), 3.13 (q, 2H, *J=7.1 Hz*, CH_3-$\underline{CH_2}$), 6.67, 8.11 (each d, each 2H, *J*=8.5 *Hz*, CH=CH), 7.87-7.89 (m, 5H, Ar-H), 8.07, 8.09 (each d, each 2H, *J=8.4 Hz*, AB-system), 10.74 (s, 1H, NH, Discharged with D_2O), MS *m/z* (%): 455 [M^++1] (25.1), 454 [M^+] (77.6), 396 (23.1), 377 (20.1), 332 (13.9), 256 (45.9), 228 (77.1), 196 (10.3), 91 (100.0). Anal. Calcd for $C_{23}H_{26}N_4O_4S$ (454.54): C, 60.77; H, 5.77; N, 12.33; S, 7.05. Found: C, 60.80; H, 5.90; N, 12.50; S, 6.91.

4-(2-Cyanoacetyl)-*N*-ethyl-*N*-methylbenzenesulfonamide (14)

A mixture of enaminone **2** (2.96 g, 0.01 mol) and hydroxylamine hydrochloride (0.69 g, 0.01 mol) in 30 mL pyridine was refluxed for 8 h, and then allowed to cool at room temperature and diluted with ice-cold water (20 mL). The solid product so formed was collected by filtration, washed with water, dried, and recrystallized from dioxane to give **14**. White crystals, yield 66%; mp 200-202 °C. IR (KBr, cm^{-1}): ν_{max} = 2220 (CN), 1698 (C=O), 3099 (CH-aromatic), 2991 (CH-aliphatic), 1336, 1158 (SO_2). ^1H NMR (DMSO-d_6, ppm): δ = 1.14 (t, 3H, *J=7.3 Hz*, $\underline{CH_3}$-CH_2), 2.32 (s, 3H, SO_2NCH_3), 2.75 (q, 2H, *J=7.3 Hz*, CH_3-$\underline{CH_2}$), 3.14 (s, 2H, $COCH_2CN$), 7.76, 8.51 (each d, each 2H, *J=8.4 Hz*, AB-system). ^{13}C NMR (DMSO-d_6, ppm): δ = 12.04, 33.96, 37.92, 44.91, 126.57 (2C), 127.91 (2C), 135.87, 138.19, 140.70, 154.83. MS *m/z* (%): 266 [M^+] (100.0), 240 (34.8), 208 (77.8), 198 (13.5), 144 (66.3), 122 (76.2), 91 (32.1), 64 (30.0), 58 (10.1). Anal. Calcd for $C_{12}H_{14}N_2O_3S$ (266.32): C, 54.12; H, 5.30; N, 10.52; S, 12.04. Found: C, 54.20; H, 5.40; N, 10.60; S, 12.13.

4-(2-Aminopyrimidin-4-yl)-*N*-ethyl-*N*-methylbenzenesulfonamide (15)

A mixture of enaminone **2** (2.96 g, 0.01 mol) and guanidine hydrochloride (0.95 g, 0.01 mol) in EtOH 30 mL, anhydrous potassium carbonate (2.0 g; 0.01 mol) was added the resulting mixture was refluxed for 4 h, and then allowed to cool at room temperature and diluted with water (20 mL). The solid product so formed was collected by filtration, washed with water and recrystallized from EtOH to give **15**. Faint yellow crystals; yield 65%; mp 235-236 °C. IR (KBr, cm^{-1}): ν_{max} = 3448, 3418 (NH_2), 3051 (CH-aromatic), 2914 (CH-aliphatic), 1340, 1162 (SO_2). ^1H NMR (DMSO-d_6, ppm): δ = 1.14 (t, 3H, *J=7.2 Hz*, $\underline{CH_3}$-CH_2), 2.66 (s, 3H, SO_2NCH_3), 3.14 (q, 2H, *J=7.2 Hz*, CH_3-$\underline{CH_2}$), 7.13 (s, 2H, NH_2,

Discharged with D_2O), 7.27-8.11 (m, 6H, Ar-H + $H_{5,6}$ of pyrimidine). ^{13}C NMR (DMSO-d_6, ppm): δ = 13.08, 27.68, 33.94, 77.24, 127.56 (2C), 128.86 (2C), 139.63, 140.05, 141.96, 196.84, 196.85. MS *m/z* (%): 292 [M^+] (30.9), 234 (70.2), 198 (100.0), 170 (35.2), 122 (78.2), 91 (66.9), 64 (13.2), 58 (11.4). Anal. Calcd for $C_{13}H_{16}N_4O_2S$ (292.36): C, 53.41; H, 5.52; N, 19.16; S, 10.97. Found: C, 53.50; H, 5.60; N, 19.30; S, 10.83.

_N_-Ethyl-_N_-methyl-4-(1-phenyl-1_H_-pyrazol-3-yl)benzenesulfonamide (16)

A mixture of enaminone **2** (2.96 g, 0.01 mol) and phenylhydrazine (1.08 g, 0.01 mol) in EtOH (30 mL) was refluxed for 3 h, after cooling the solid which formed was collected and recrystallized from AcOH to give **16**. Brown crystals, yield 70%; mp 250-252 °C. IR (KBr, cm^{-1}): ν_{max} = 3111 (CH-aromatic), 2917 (CH-aliphatic), 1342, 1161 (SO_2). ^1H NMR (DMSO-d_6, ppm): δ = 1.44 (t, 3H, *J=7.1 Hz*, $\underline{CH_3}$-CH_2), 2.66 (s, 3H, SO_2NCH_3), 3.13 (q, 2H, *J=7.1 Hz*, CH_3-$\underline{CH_2}$), 6.92 (d, 1H, H_4 of pyrazole), 7.76-8.11 (m, 10H, Ar-H + H_5 of pyrazole). ^{13}C NMR (DMSO-d_6, ppm): δ = 13.08, 26.87, 34.01, 103.03, 127.56 (2C), 127.64, 128.86 (2C), 130.50 (2C), 134.51 (2C), 137.78, 139.27, 141.96, 142.65, 193.18. MS *m/z* (%): 341 [M^+] (100.0), 283 (30.7), 264 (80.2), 219 (90.2), 198 (19.3), 143 (66.0), 122 (100), 64 (32.7), 58 (84.9). Anal. Calcd for $C_{18}H_{19}N_3O_2S$ (341.43): C, 63.32; H, 5.61; N, 12.31; S, 9.39. Found: C, 63.40; H, 5.60; N, 12.40; S, 9.12.

4-(2,3-Dihydro-1_H_-1,4-diazepin-7-yl)-_N_-ethyl-_N_-methylbenzenesulfonamide (18)

A mixture of enaminone **2** (2.96 g, 0.01 mol) and ethylenediamine (0.56 g, 0.01 mol) in AcOH (20 mL) was refluxed for 6 h. the obtained solid after cooling was recrystallized from EtOH to give **18**. Brown crystals, yield 80%; mp 299-300 °C. IR (KBr, cm^{-1}): ν_{max} = 3230 (NH), 3101 (CH-aromatic), 2930 (CH-aliphatic), 1314, 1156 (SO_2). ^1H NMR (DMSO-d_6, ppm): δ = 1.02 (t, 3H, *J=7.3 Hz*, $\underline{CH_3}$-CH_2), 2.48-2.65 (m, 4H, CH_2-CH_2), 2.68 (s, 3H, SO_2NCH_3), 3.03 (q, 2H, *J=7.3 Hz*, CH_3-$\underline{CH_2}$), 3.34 (m, 1H, CH), 7.88, 8.13 (each d, each 2H, *J=8.6 Hz*, AB-system), 8.00 (d, 1H, N=CH), 12.30 (s, 1H, NH, Discharged with D_2O). ^{13}C NMR (DMSO-d_6, ppm): δ = 12.76, 27.03, 33.84, 39.60, 56.00, 80.64, 126.69 (2C), 127.15 (2C), 137.62, 139.66, 164.15, 197.35. MS *m/z* (%): 294 [M^++1] (16.6), 293 [M^+] (55.2), 277 (14.0), 243 (67.3), 198 (10.5), 171 (67.2), 122 (17.9), 95 (38.2), 91 (100.0), 64 (33.9), 58 (34.9), 41 (16.3). Anal. Calcd for $C_{14}H_{19}N_3O_2S$ (293.38): C, 57.31; H, 6.53; N, 14.32; S, 10.93. Found: C, 57.40; H, 6.60; N, 14.50; S, 10.99.

4-(5-Acetyl-6-methylpyridin-2-yl)-_N_-ethyl-_N_-methylbenzenesulfonamide (20)

To a mixture of enaminone **2** (2.96 g, 0.01 mol) and acetylacetone (1.00 g, 0.01 mol), ammonium acetate (1.54 g, 0.02 mol) in glacial AcOH (50 mL) was refluxed for 4 h, and then allowed to cool at room

temperature and diluted with ice-cold water. The solid product so formed was collected by filtration, washed with water, dried, and recrystallized from AcOH to give **20**. Yellow crystals, yield 77%; mp 298-299 °C. IR (KBr, cm^{-1}): ν_{max} = 1670 (C=O), 3086 (CH-aromatic), 2970 (CH-aliphatic), 1340, 1160 (SO_2). ^1H NMR (DMSO-d_6, ppm): δ = 1.02 (t, 3H, *J=7.0 Hz*, $\underline{CH_3}$-CH_2), 2.49 (s, 3H, CH_3CO), 2.92 (s, 3H, SO_2NCH_3), 3.34 (q, 2H, *J=7.0 Hz*, CH_3-$\underline{CH_2}$), 7.51 (d, 1H, *J=7.6 Hz*, H_4 of pyridine), 7.72 (d, 1H, *J=7.6 Hz*, H_5 of pyridine), 7.81, 8.42 (each d, each 2H, *J=8.5 Hz*, AB-system). ^{13}C NMR (DMSO-d_6, ppm): δ = 12.77, 24.61, 29.57, 33.89, 44.49, 118.31, 127.01 (2C), 128.05 (2C), 130.15, 136.92, 139.66, 140.95, 141.06, 154.96, 197.36. MS *m/z* (%): 332 [M^+] (70.3), 289 (76.3), 257 (56.2), 212 (78.3), 167 (11.8), 134 (34.9), 122 (34.6), 91 (100.0), 76 (15.9). Anal. Calcd for $C_{17}H_{20}N_2O_3S$ (332.42): C, 61.42; H, 6.06; N, 8.43; S, 9.65. Found: C, 61.50; H, 6.20; N, 8.40; S, 9.83.

DOCKING AND MOLECULAR MODELING CALCULATIONS

Materials: All the molecular studies were carried out on an Intel Pentium 1.6 GHz processor, 512 MB memory with the windows XP operating system using Molecular Operating Environment (MOE 2005.06; Chemical Computing Group, Montreal, Canada) as the computational software. All minimizations were performed with MOE until achieving an RMSD gradient of 0.05 K Cal/mol A° with an MMFF94X force field. The partial charges were automatically calculated.

General methodology: The coordinates of the X-ray crystal structure of MTX bound to DHFR (PDB ID: 4DFR) were obtained from Protein Data Bank (PDB ID: 1BID). Enzyme structures were checked for missing atoms, bonds and contacts. Hydrogen atoms were added to the enzyme structure. Water molecules and bound ligands were manually deleted. The ligand molecules were constructed using the builder molecule and were energy minimized. The active site was generated using the MOE-Alpha site finder. Dummy atoms were created from the obtained alpha spheres. Ligands were docked within the dihydrofolate reductase active sites using the MOE-Dock with simulated annealing used as the search protocol and the MMFF94X molecular mechanics force field for 8000 interactions. The lowest energy conformation was selected and subjected to an energy minimization using the MMFF94X force field.

Docking on the active site of dihydrofolate reductase (DHFR): The recent determination of the three dimensional co-crystal structure of dihydrofolate reductase complexes with the potent inhibitor, methotrexate (MTX) (PDB ID: 4DFR) has led to the development of a model for the topography of the binding site of dihydrofolate reductase.

IN VITRO ANTIMICROBIAL SCREENING

Antibacterial and antifungal activities of all newly synthesized compounds tested by measuring the

inhibitory effects of such compounds against Gram-positive, Gram-negative bacteria and fungi using an agar diffusion technique.

Materials: Gram-positive bacteria (*Staphylococcus aureus* [RCMB 010027], *Streptococcus pneumonia* [RCMB 010010], *Bacillus subtilis* [RCMB 010067]), Gram-negative bacteria (*Klebsiella pneumoniae* [RCMB 0010093], *Escherichia coli* [RCMB 010052]) and fungi (*Asperigillus fumigatus* [RCMB 02568]), were used against all newly synthesized compounds. The standard controls for the microorganisms were ampicillin for the Gram-positive bacteria, gentamicine for the Gram-negative bacteria and *Amphotericin B* for the Fungi.

Agar diffusion test: Nutrient agar was melted and poured each into empty sterile petri dishes and left for 24 h. A specific culture of each organism was spread with a dry sterile swab on the surface of previously prepared plates. Sterile discs (6-9 mm diameter) were impregnated with solutions of tested compounds, left to dry and were then placed on the surface of the inoculated plates. Discs of antimicrobial standards were placed in the agar plate culture and inoculated at 37 ^{o}C for 24 h. After inoculation, the plates were examined visually and the zone of inhibition was measured.

IN VITRO ANTICANCER SCREENING

Cytotoxicity activity was measured *in vitro* for the newly synthesized compounds using the Sulfo-Rhodamine-B stain (SRB) assay.[33] Cells were plated in 96-multiwell micro titer plates (10^4 cells/well) for 24 h. before treatment with the compound(s) to allow attachment of cells to the wall of the plate. Test compounds were dissolved in DMSO and diluted with saline to the appropriate volume. Different concentrations of the compound under test (50, 25, 12.5, 6.25 and 3.125 μg/mL) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compound(s) for 48 h at 37 ^{o}C in an atmosphere of 5% CO_2. After 48 h cells were fixed, washed, and stained for 30 min with 0.4% (wt/vol) with SRB dissolved in 1% acetic acid. Excess unbound dye was removed by four washes with 1% acetic acid and attached stain was recovered with Tris-EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fraction and drug concentration was plotted to obtain the survival curve for breast tumor cell after the specified time.[33] The molar concentration required for 50% inhibition of cell viability (IC_{50}) was calculated and the results are presented (**Tables 2-4**).

Table 1. *In-vitro* Antimicrobial activity of the newly synthesized compounds

Comp. No.	Gram-positive Bacteria			Gram-Negative Bacteria		Fungi
	S. aureus	*S. pneumoniae*	*B. subtilis*	*K. pneumoniae*	*E. Coli*	*A. fumigatus*
1	19.3±0.44	20.6±0.63	22.4±0.44	19.3±0.44	16.9±0.58	17.8±0.63
2	13.2±0.44	14.6±0.43	16.2±0.53	14.6±0.58	13.7±0.25	12.6±0.58

6	29.2±0.63	27.6±0.44	27.4±0.67	22.4±0.56	28.6±0.46	24.9±0.25
7	16.0±0.44	18.3±0.63	19.8±0.46	15.3±0.58	13.0±0.46	10.6±0.25
14	17.3±0.63	20.2±0.63	22.3±0.25	18.9±0.45	15.9±0.37	16.3±0.44
15	18.6±0.58	19.6±0.63	20.0±0.32	20.3±0.58	15.9±0.46	18.6±0.63
16	15.4±0.58	16.9±0.44	19.3±0.25	17.3±0.44	14.9±0.44	15.6±0.44
18	16.2±0.15	19.8±0.58	20.1±0.58	18.3±0.58	15.3±0.53	15.7±0.33
20	30.3±0.43	26.4±0.58	28.4±0.44	19.6±0.44	27.9±0.25	25.6±0.58
St. Control*	28.9±0.14	25.3±0.58	26.3±0.34	17.3±0.12	27.3±0.44	23.7±0.10

*St. Control: *Ampicillin* for the Gram-positive bacteria, *Gentamicine* for the Gram-negative bacteria and *Amphotericin B* for the Fungi.

Table 2. *In-vitro* anticancer screening of the synthesized compounds against human liver hepatocellular carcinoma cell line (HepG2)

Comp. No.	Validity for sample Conc. (μg/mL)					IC_{50}
	50	25	12.5	6.25	3.125	
1	18.56±0.21	29.81±0.12	60.62±0.20	72.83±0.13	85.72±0.23	16.80
2	15.93±0.32	24.56±0.19	62.81±0.23	83.34±0.18	89.08±0.43	16.70
6	17.84±0.34	29.08±0.61	71.36±0.48	86.43±0.32	94.82±0.62	01.80
7	10.48±0.58	23.86±0.52	37.34±0.29	78.16±0.51	89.42±0.32	10.60
14	04.50±0.25	07.30±0.23	14.20±0.33	24.28±0.34	36.30±0.19	01.41
15	18.32±0.22	27.89±0.37	64.20±0.48	83.12±0.64	91.54±0.36	17.40
16	04.48±0.14	07.22±0.41	14.35±0.45	24.25±0.34	36.20±0.38	01.38
18	35.48±0.22	49.52±0.47	73.14±0.60	82.63±0.25	94.06±0.19	02.70
20	37.16±0.29	68.42±0.54	80.94±0.39	89.58±0.22	94.46±0.59	39.70
MTX	06.82±0.31	08.89±0.11	14.83±0.21	16.17±0.13	22.28±0.15	03.21

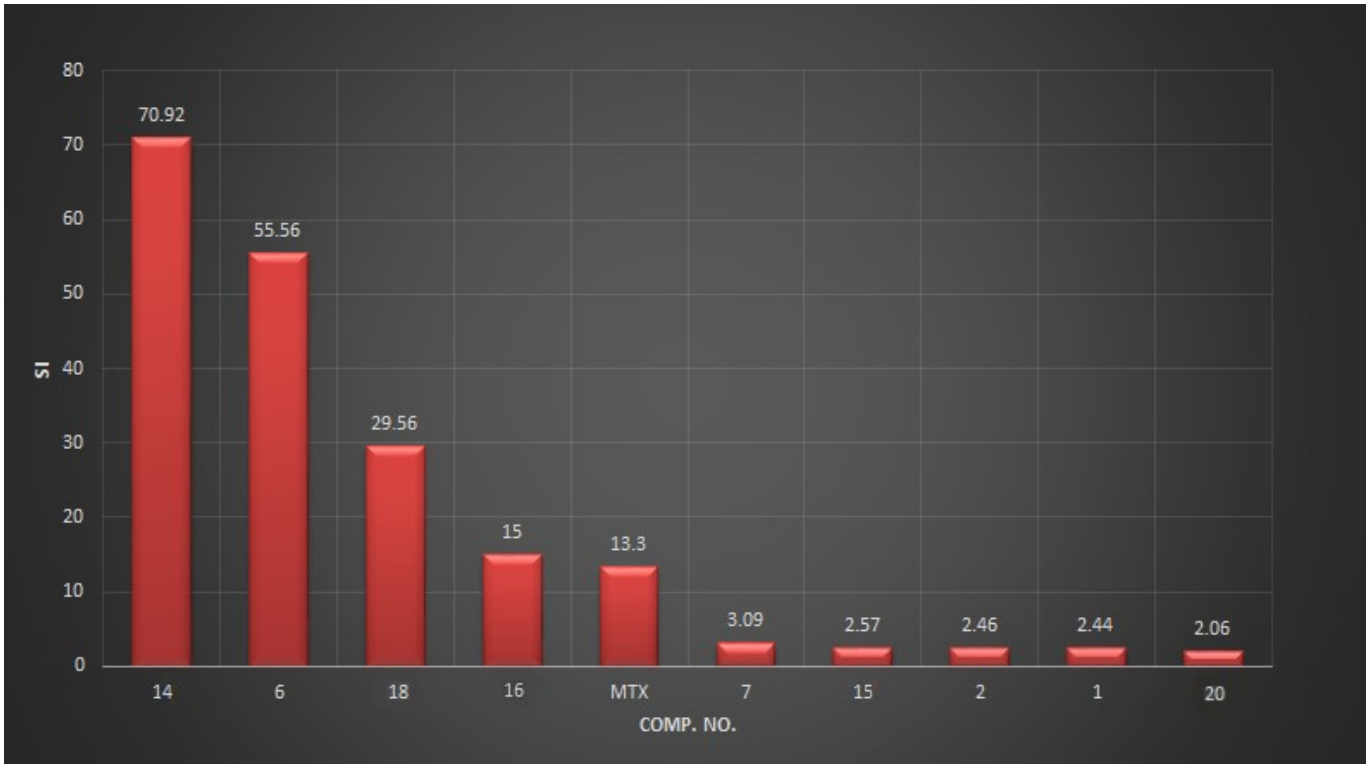
Table 3. *In-vitro* cytotoxicity screening of the synthesized compounds against mammalian cells of African green monkey kidney cell line (VERO)

Comp. No.	Validity for sample Conc. (μg/mL)					CC_{50}
	50	25	12.5	6.25	3.125	
1	39.74±0.44	68.16±0.26	82.83±0.42	91.57±0.56	98.94±0.63	41.00
2	41.56±0.47	65.38±0.37	81.47±0.48	93.61±0.45	100.0±0.55	41.10
6	79.83±0.18	92.58±0.23	98.24±0.12	100.0±0.34	100.0±0.13	>100
7	31.73±0.68	58.30±0.11	83.14±0.31	94.26±0.56	97.61±0.28	32.80
14	69.72±0.58	82.94±0.12	91.53± 0.34	97.48±0.23	99.16±0.49	>100
15	43.28±0.52	75.34±0.18	89.12±0.61	94.56±0.42	98.08±0.37	44.80
16	19.78±0.34	38.14±0.19	72.36±0.49	83.54±0.45	92.62±0.28	20.70
18	73.36±0.21	87.82±0.56	91.16±0.23	94.35±0.23	98.54±0.62	79.80
20	73.24±0.66	89.34±0.29	96.34±0.56	99.18±0.54	100.0±0.45	81.90
MTX	42.33±0.59	77.54±0.76	83.05 ± 0.33	90.75±0.44	100.0±0.49	42.70

Table 4. Cytotoxicity of human liver hepatocellular carcinoma cell line (HepG2) and mammalian cells of African green monkey kidney cell line (VERO)

Comp. No.	CC_{50} (μg /mL)	CC_{50} (μM)	IC_{50} (μg /mL)	IC_{50} (μM)	SI
1	41.00	170.12	16.80	69.71	02.44
2	41.10	138.85	16.70	56.42	02.46
6	>100	278.55	01.80	05.01	55.56
7	32.80	72.25	10.60	23.35	03.09
14	>100	375.94	01.41	05.30	70.92
15	44.80	153.42	17.40	59.59	02.57
16	20.70	60.70	01.38	04.05	15.00
18	79.80	272.35	02.70	09.22	29.56
20	81.90	246.69	39.70	119.58	02.06
MTX	42.70	94.05	03.21	07.07	13.30

Chart 1. Comparison of the Selective index (SI) for the synthesized compounds against human liver hepatocellular carcinoma cell line (HepG2)



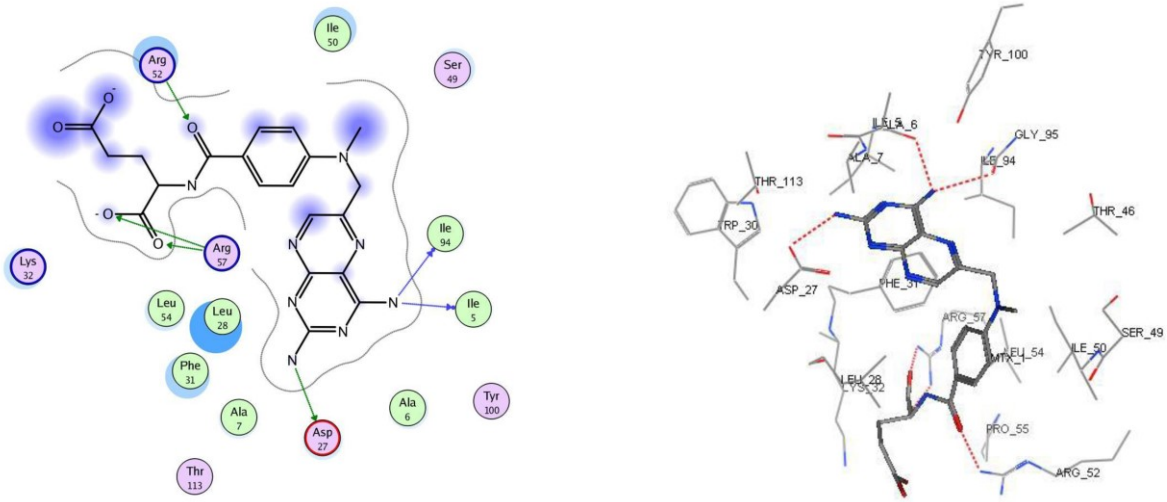


Figure 1. Docking of MTX into DHFR

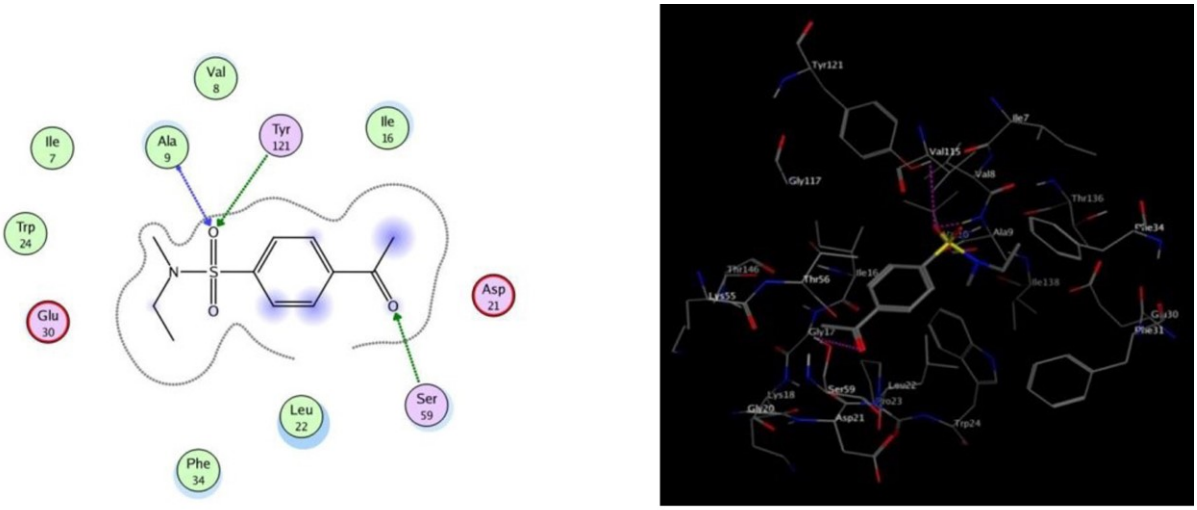


Figure 2. Docking of compound 1 into DHFR

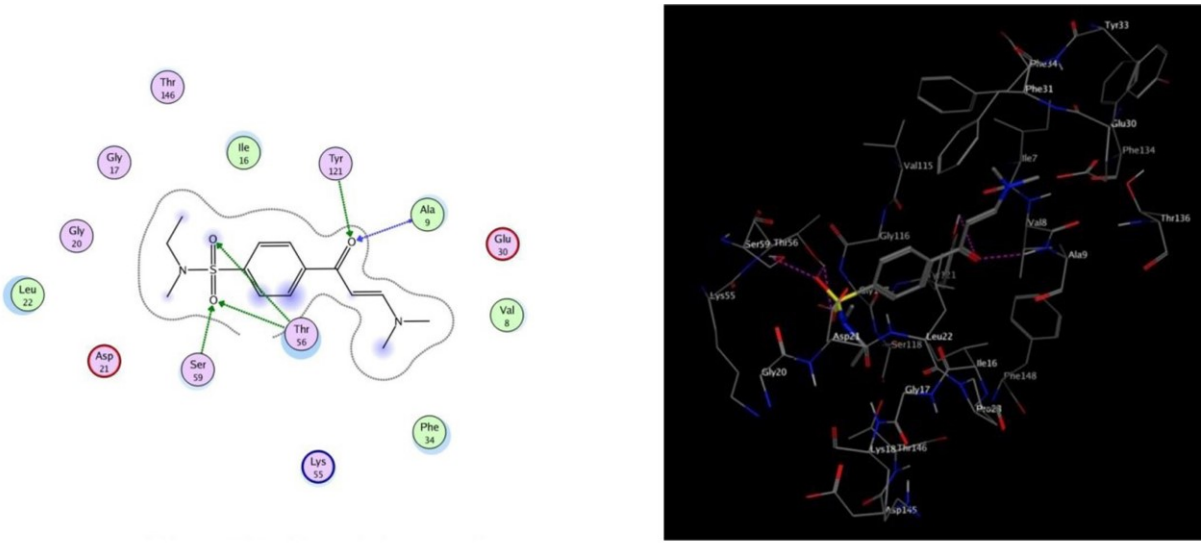


Figure 3. Docking of compound 2 into DHFR

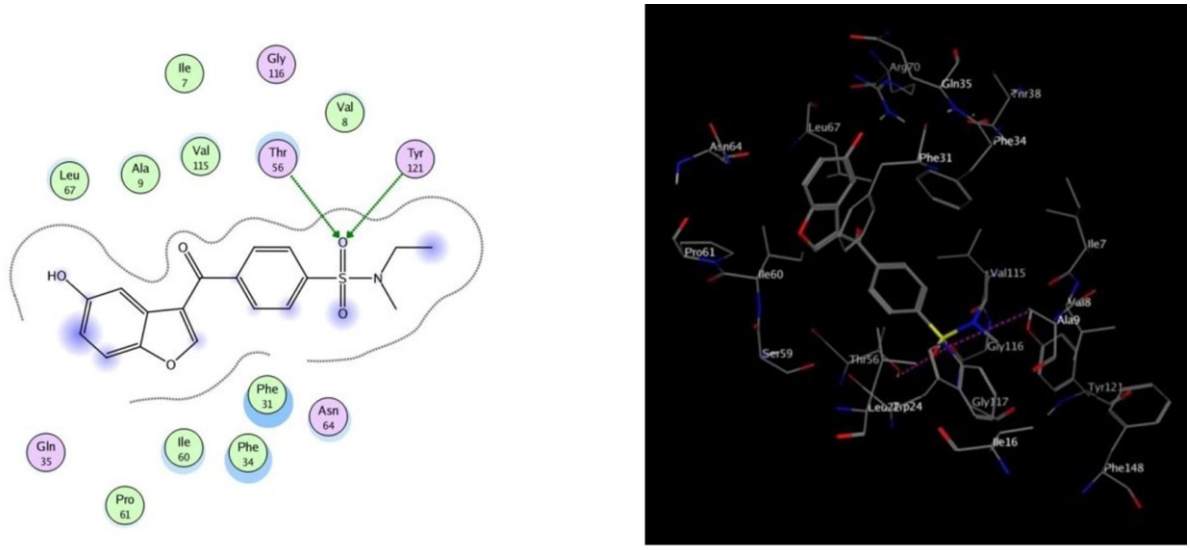


Figure 4. Docking of compound 6 into DHFR

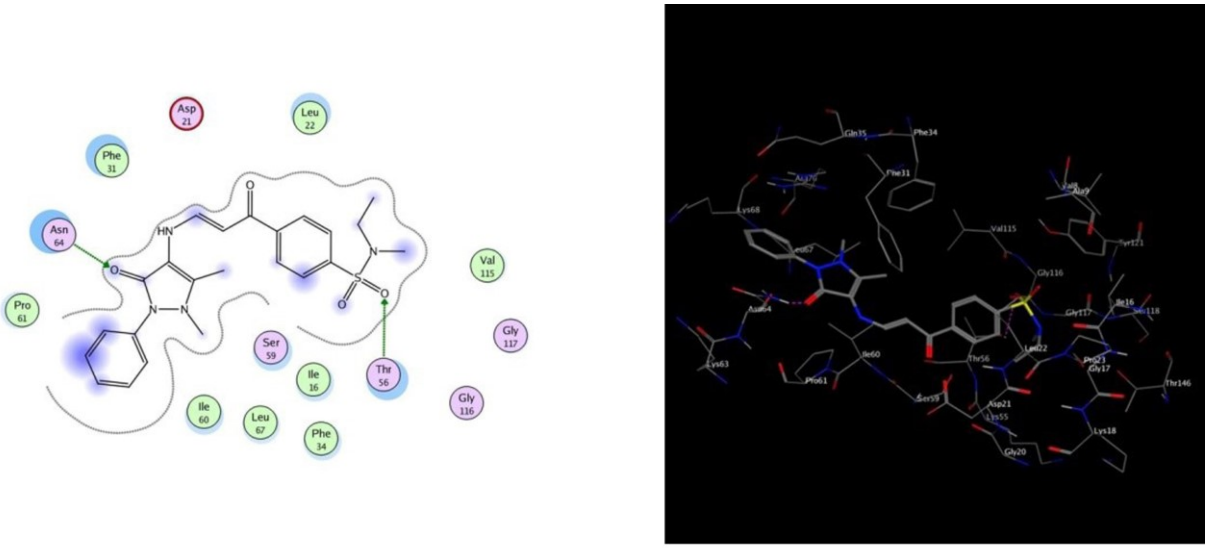


Figure 5. Docking of compound 7 into DHFR

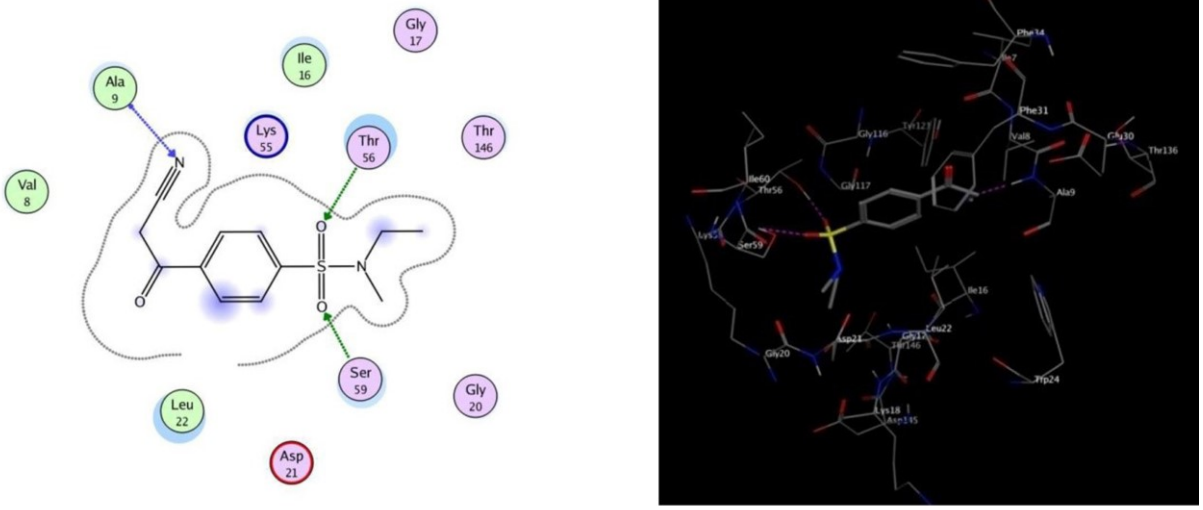


Figure 6. Docking of compound 14 into DHFR

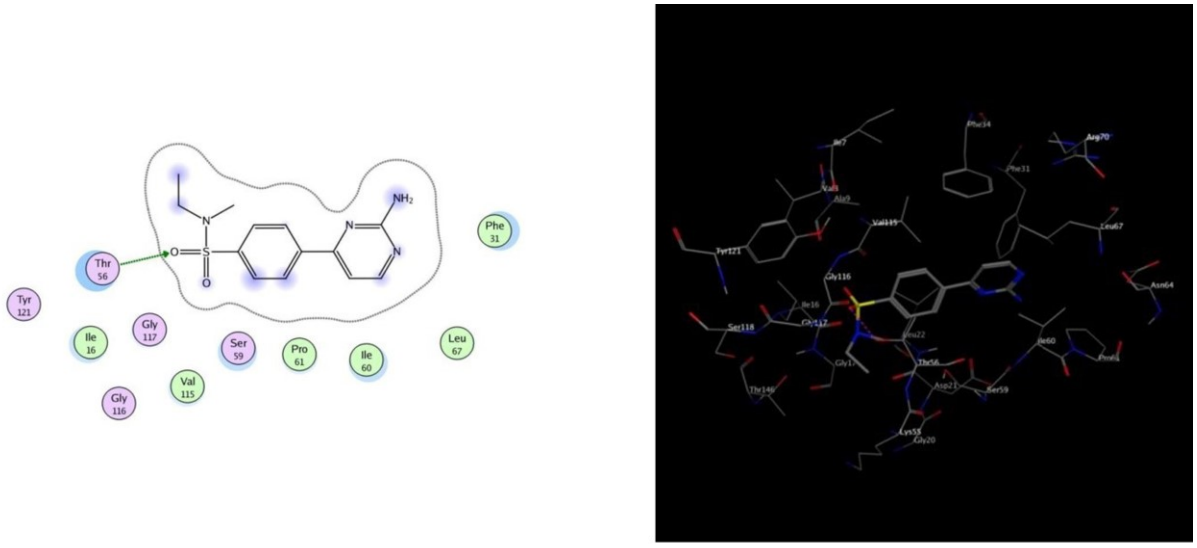


Figure 7. Docking of compound 15 into DHFR

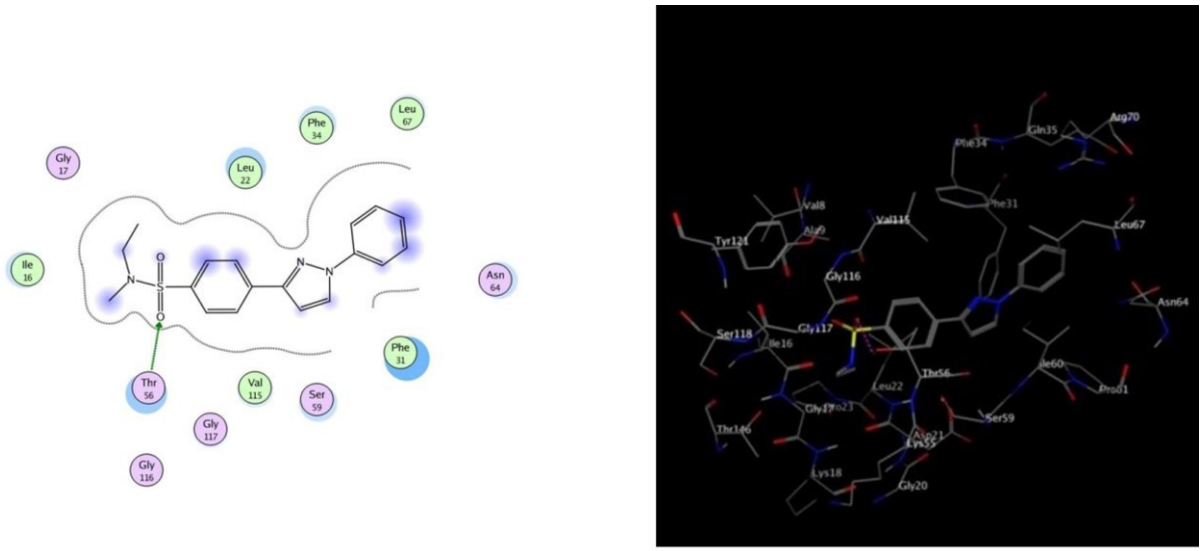


Figure 8. Docking of compound 16 into DHFR

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