ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Synthesis, Anti-Breast Cancer and Molecular Docking of Some Heterocycles Incorporating N,N-Dibenzylbenzenesulfonamide

MAHMOUD SAYED BASHANDY

Chemistry Department, Faculty of Science (Boys), Al-Azhar University, 11884, Nasr City, Cairo, Egypt.
Chemistry Department, University College in Al-Jamoum, Umm Al-Qura University, 21955, Makkah, Saudi Arabia.

Manuscript Info

Manuscript History:

Received: 25 August 2014
Final Accepted: 29 September 2014
Published Online: October 2014

Key words:

Benzenesulfonamide, enaminone, anti-breast cancer activity, molecular docking

Corresponding Author

Mahmoud Sayed Bashandy

E-mail address:
bashandy_sci@yahoo.com

Abstract

Reaction of **1** with dimethylformamide-dimethylacetal (DMF-DMA) gave enaminone **2**, which reacts with *p*-toluidine and phenylhydrazine to afford **3** and **5**, respectively. Treatment of **2** with 1,4-benzoquinone gave 5-hydroxybenzofuran derivative **9**. Hydrazonyl bromide **10** reacts with **2** in refluxing xylene-TEA to give ethyl pyrazole-3-carboxylate derivative **15**, which on treatment with ethanolic hydrazine hydrate gave pyrazolo[3,4-d]pyridazine derivative **16**. Enaminone **2** reacts with heterocyclic amines to furnished new heterocyclic systems, pyrazolo[1,5-a]pyrimidine **22**, triazolo[4,3-a]pyrimidine **28** and pyrimido[1,6-a]pyrimidine **34**. Also, enaminone **2** reacts with guanidine.HCl, hydrazine hydrate, *p*-chlorobenzenediazonium chloride and hydroxylamine.HCl to afford the corresponding derivatives of 2-aminopyrimidine **35**, pyrazole **36**, 2-arylhydrazonopropanal **37** and cyanoacetyl **46**, respectively. Enaminone **2** reacts with phenylisothiocyanate in DMF-KOH through nucleophilic addition of enaminone C-2 to give **47**. Interaction of **2** with active methylene compounds afforded cyanopyridine **53** and imidazo[1,2-a]pyridine **56**, derivatives. On the other hand enaminone **2** reacts with ethylenediamine and alanine to afford derivatives of diazepine **58** and oxazepine **60**, respectively. Reaction of **2** with dimethyl acetylenedicarboxylate and acetyl acetone afforded derivatives of pyranone **64** and pyridine **66**, 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 newly synthesized compounds were tested *in-vitro* anti-breast cancer cell line (MCF7). Compounds **56, 53, 36, 15, 46, 37, 9, 16, 64, 28, 35** and **2** with IC_{50} values (170.3, 157.5, 153.1, 146.9, 143.2, 140.0, 127.9, 122.7, 121.3, 119.5, 117.3, 111.9, μM), respectively, exhibited better activity than methotrexate as a reference drug with IC_{50} value (74.6 μM). Virtual screening using molecular docking studies of the synthesized compounds was performed by (MOE), the molecular docking results indicate that, some synthesized compounds suitable inhibitor against dihydrofolate reductase (DHFR) enzyme (PDB ID: 4DFR) with further modification.

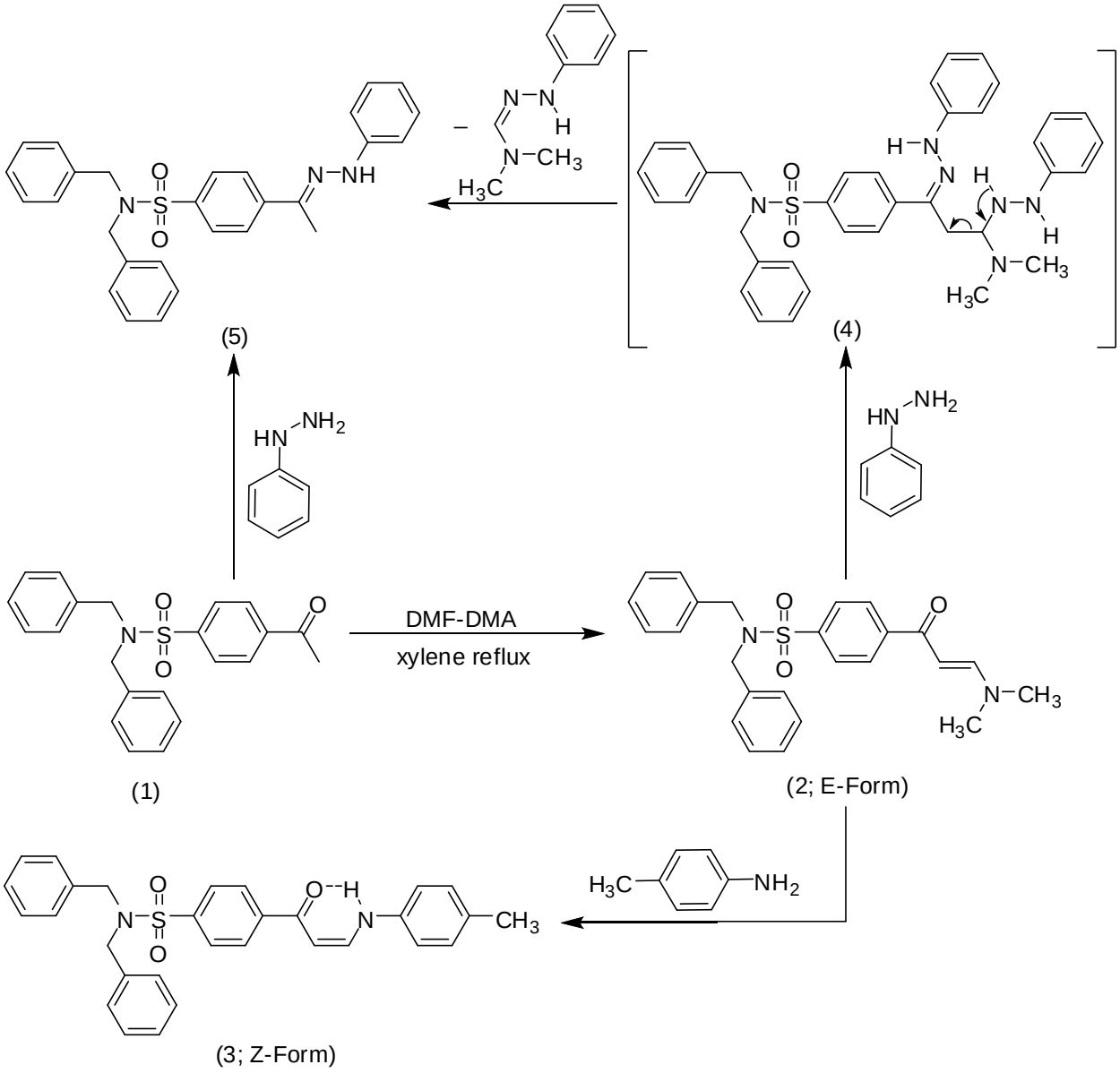
1. Introduction

Enaminones constitute an interesting class of compounds that are versatile precursors for the synthesis of several heterocyclic compounds. **[1,2]** Also Sulfonamides have been demonstrated to possess antibacterial, **[3,4]** antifungal, **[5]** insulin releasing, **[6,7]** carbonic anhydrase inhibitory, **[8-10]** hypoglycemic, **[11]** anesthetic, **[12]** anti-tumor, **[13,14]** anti-cancer and anti-inflammatory **[15-17]** activities. Some active sulfonamides as anti-bacterial are also known for their immune modifying effects **[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)-N,N-dibenzyl-4-(3-(dimethylamino)acryloyl)benzenesulfonamide as a starting material in the formation of a variety of heterocycles with substituted benzenesulfonamide moiety and investigated their anti-breast cancer activities.

2. Results and Discussion

2.1. Chemistry

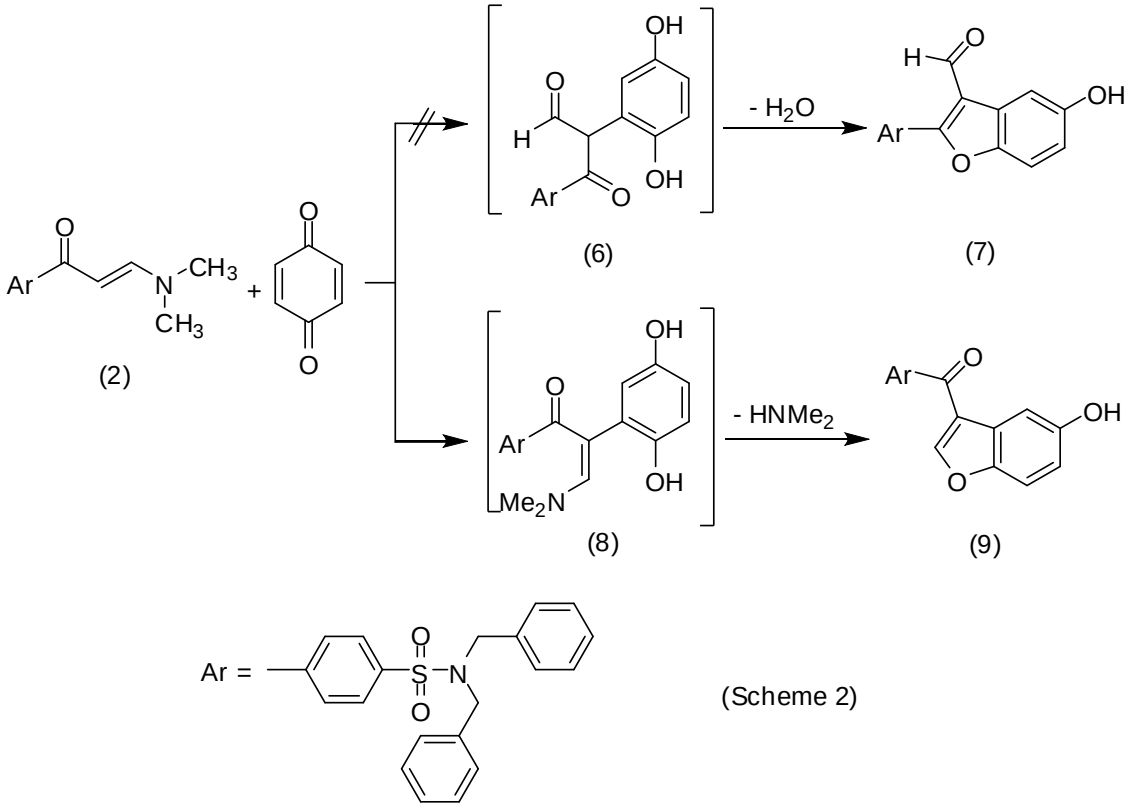
This article reports a new and convenient method for the synthesis of such ring systems that are required to medicinal chemistry utilizing. (E)-N,N-dibenzyl-4-(3-(dimethylamino)acryloyl)benzenesulfonamide **(2)**, as a starting material, which was prepared from interaction of 4-acetyl-N,N-dibenzylbenzenesulfonamide **(1)**[24] with dimethylformamide-dimethylacetal (DMF-DMA) under reflux in dry xylene. The enaminone **2** was assigned the E-configuration based on their ^{1}H-NMR spectrum which revealed that the coupling constant of the doublet signals for olefinic protons equal to 11.7 *Hz* correlated for E-isomers. Treatment of **2** with *p*-toluidine in a mixture of ethanol/acetic acid at reflux temperature afforded (Z)-N,N-dibenzyl-4-(3-(*p*-tolylamino)acryloyl)benzenesulfonamide **(3)**, ^{1}H-NMR spectrum of **3** 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. Also, where enaminone **2** was treated with phenylhydrazine in refluxing ethanol, to produce addition intermediate **4**, which undergoes elimination of N,N-dimethylformamide phenylhydrazone to afford N,N-dibenzyl-4-(1-(2-phenylhydrazono)ethyl)benzenesulfonamide **(5)**. Structure of compound **5** was established on the basis of its elemental analysis and spectral data. Thus IR spectrum indicated absorption bands at ν_{max}= 3301 for NH group and ν_{max}= 1338, 1151 cm^{-1} for SO_2 group. Compound **5** was obtained from condensation of **1** with phenylhydrazine, both products where identical in all aspects m.p., mixed m.p. and IR spectrum, **(Scheme 1).**



(1) (2; E-Form) (3; Z-Form) (4) (5)

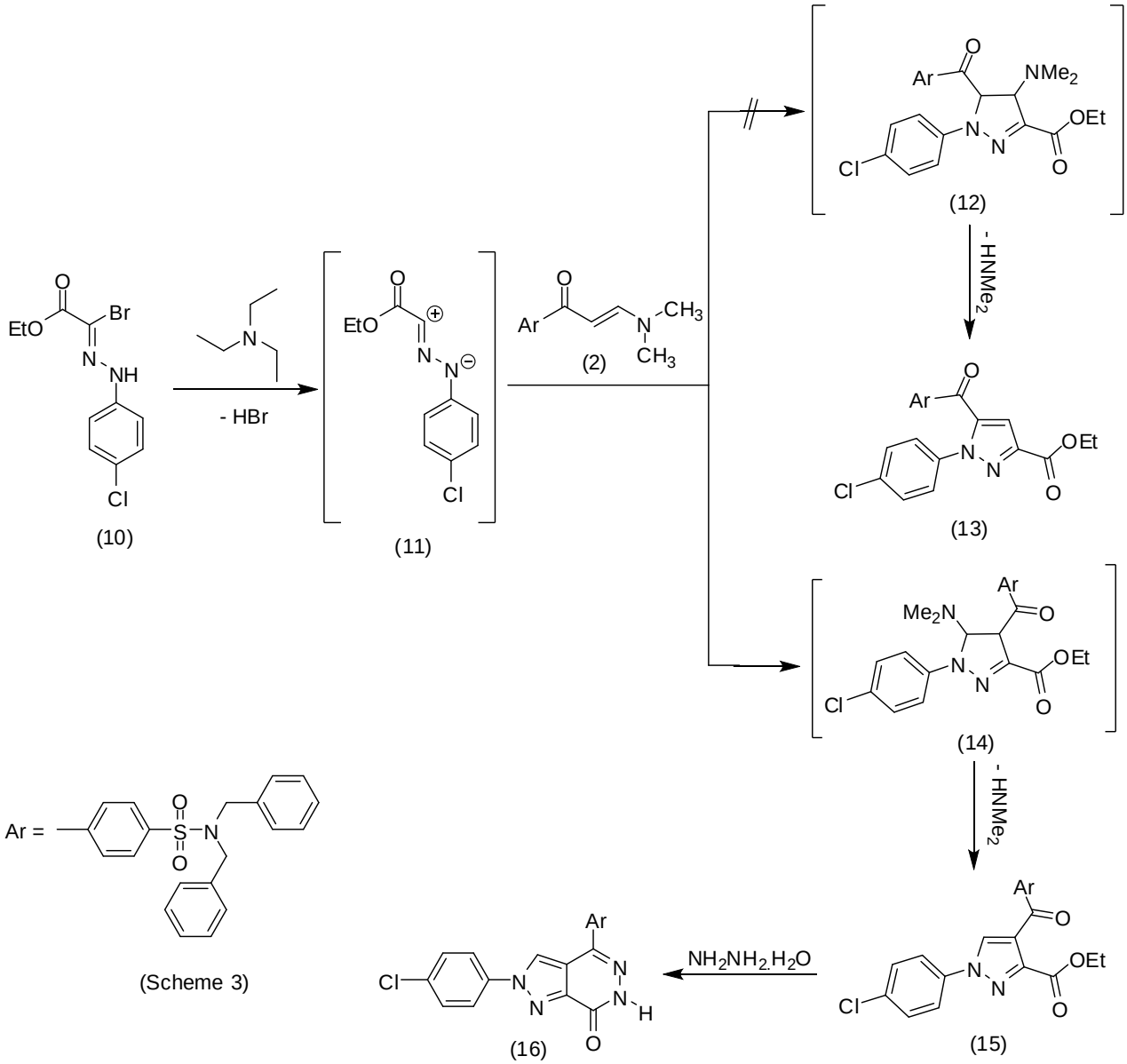
(Scheme 1)

Enaminone **2** reacted with 1,4-benzoquinone in glacial acetic acid at room temperature to yield a product which formulated as N,N-dibenzyl-4-(5-hydroxybenzofuran-3-carbonyl)benzenesulfonamide **(9)**. 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 **8** which then cyclized into **9** *via* dimethylamine elimination, **[25]** and not afforded N,N-dibenzyl-4-(3-formyl-5-hydroxybenzofuran-2-yl)benzenesulfonamide **(7)**. Elucidation of structure **9** and refusing of structure **7** was based on ^{1}H-NMR spectrum which indicates the disappearance of aldehydic signal and showed singlet signal at δ= 8.75 ppm. for benzo[b]furan H-2, **(Scheme 2).**



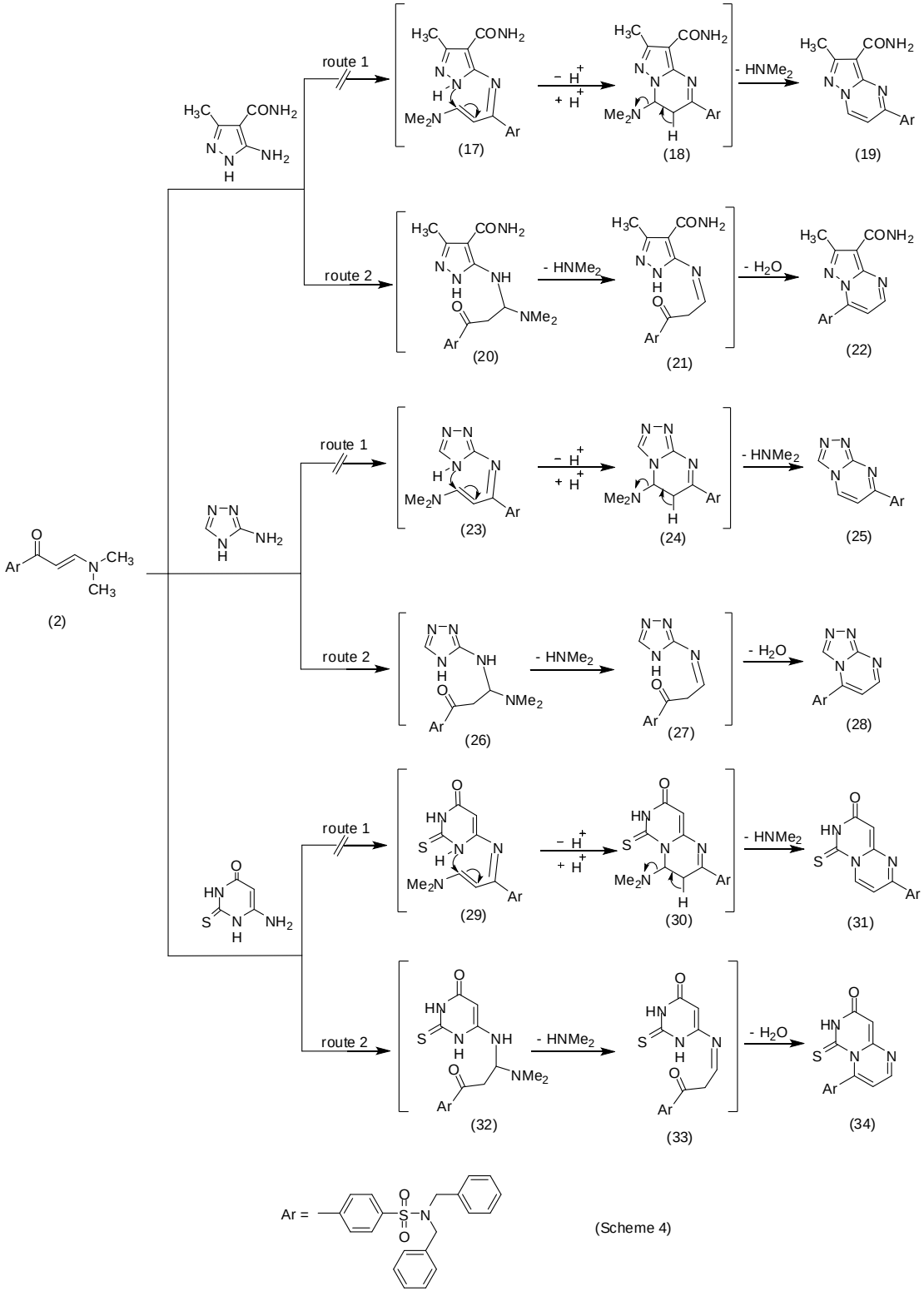
(Scheme 2)

Hydrazonyl halides with 1,3-dipoles have been reported to be added to enaminones as dipolarophiles to yield a mixture of isomeric pyrazolines**[26-30]**. Thus, in the present work the reaction of enaminone **2** with nitrilimine **11** (liberated in situ by the action of triethylamine on the hydrazonyl bromide **10** in refluxing xylene) gave only one isolable product, as evidenced by TLC analysis. From which two proposed structures **13** or **15** seemed possible. ^{1}H-NMR spectrum provided a firm support for structure **15** and ruled out the other possible structure **13.** Thus, ^{1}H-NMR spectrum of **15** exhibits a singlet at δ= 8.78 ppm. this indicates the presence of pyrazole H-5 rather than H-4 **[31]**. Pyrazole derivative **15** was assumed to be formed *via* initial 1,3-dipolar cycloaddition of nitrilimine **11** to the activated double bond in compound **2** forming non isolable intermediate **14**, followed by the loss of dimethylamine. Interaction of pyrazole derivative **15** with hydrazine hydrate in refluxing ethanol afforded pyrazolo[3,4-d]pyridazine derivative **16**, **(Scheme 3).**



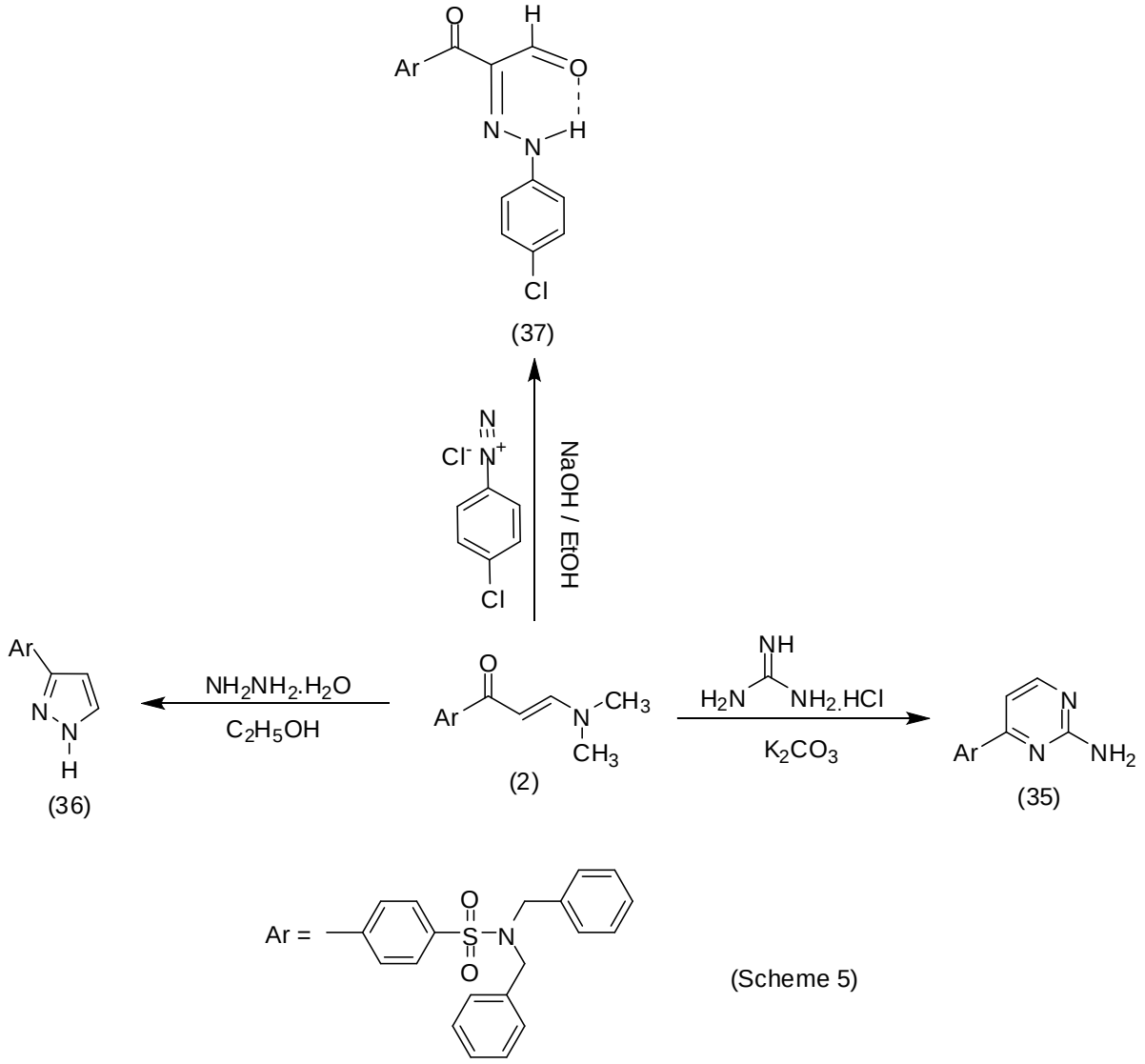
As previously reported enaminones can be used as potential precursors for fused heterocyclic systems when reacting with heterocyclic amines **[32]**. Thus, treatment of enaminone **2** with 5-amino-3-methyl-1H-pyrazole-4-carboxamide, 3-amino-1H-1,2,4-triazole and 6-amino-2-thioxo-2,3-dihydropyrimidin-4(1H)-one gave three new ring systems pyrazolo[1,5-a]pyrimidine **22**, triazolo[4,3-a]pyrimidine **28** and pyrimido[1,6-a]pyrimidine **34** respectively (**Scheme 4**). Condensation of enaminone **2** with 5-amino-3-methyl-1H-pyrazole-4-carboxamide can proceed in two possible ways, as shown in **Scheme 4**. In **route 1**, the exocyclic amino group of pyrazole attacks the carbonyl group to generate 5-substituted isomeric structure **19** *via* non isolable intermediates **17** and **18**. On the other hand, in **route 2**, the nucleophilic exocyclic amino group attacks the double bond *via* a Michael addition reaction, followed by elimination of the dimethylamino group. Cyclization of intermediate **20** with concurrent dehydration of **21** gave compound **22. Route 2** has been unambiguously substantiated through ^{1}H-NMR spectrum which showed

for pyrimidine ring protons two doublets at δ= 7.99 and 8.84 ppm., whose coupling constant; J=4.7 *Hz* has been described as characteristic for the pyrimidine H-5, H-6 sequence which congruent with the previous work.**[33](Scheme 4).**



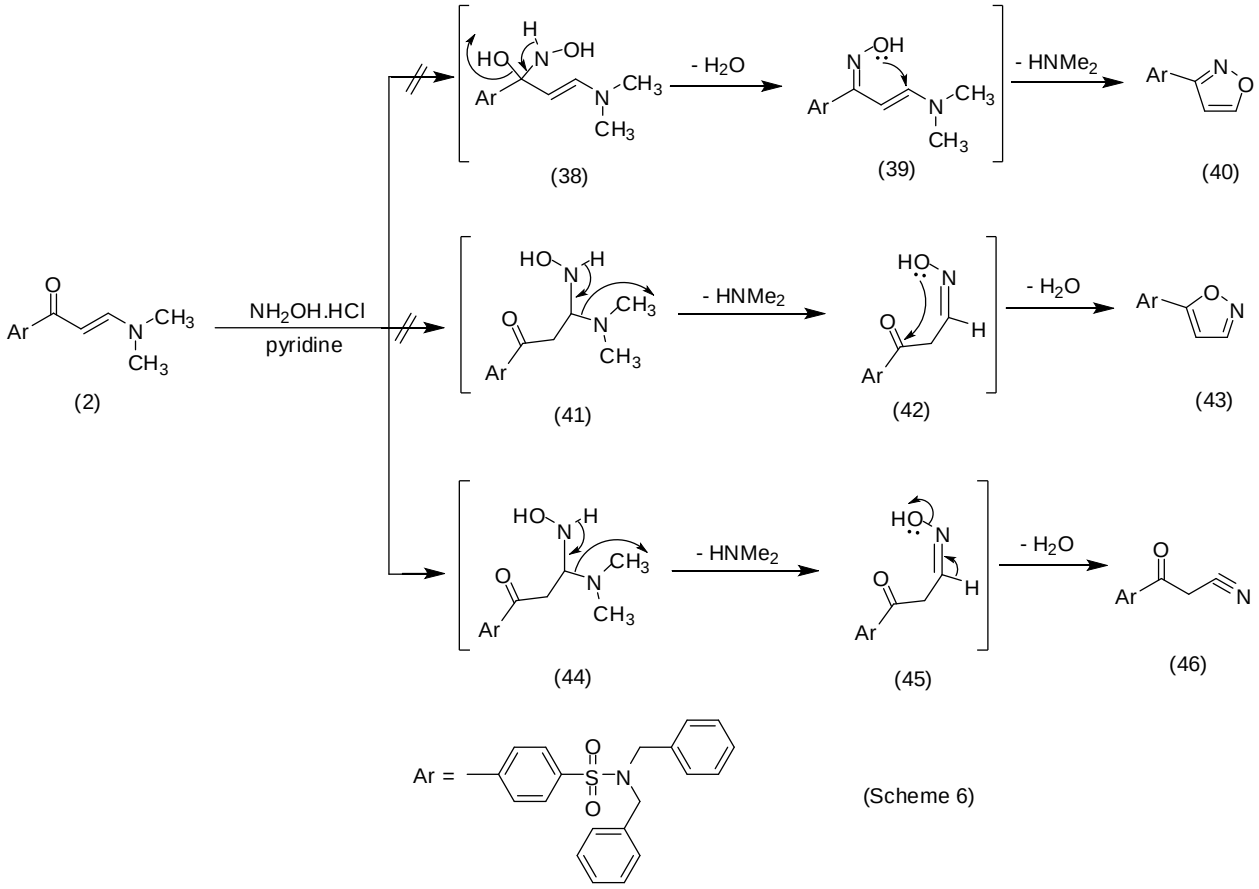
(Scheme 4)

Interaction of enaminone **2** with guanidine hydrochloride and hydrazine hydrate produced 2-aminopyrimidine **35** and pyrazole **36,** derivatives, respectively. The required 2-arylhydrazonopropanal **37** was prepared *via* coupling *p*-chlorobenzenediazonium chloride with the enaminones **2** in ethanolic sodium hydroxide solution, (**Scheme 5**).



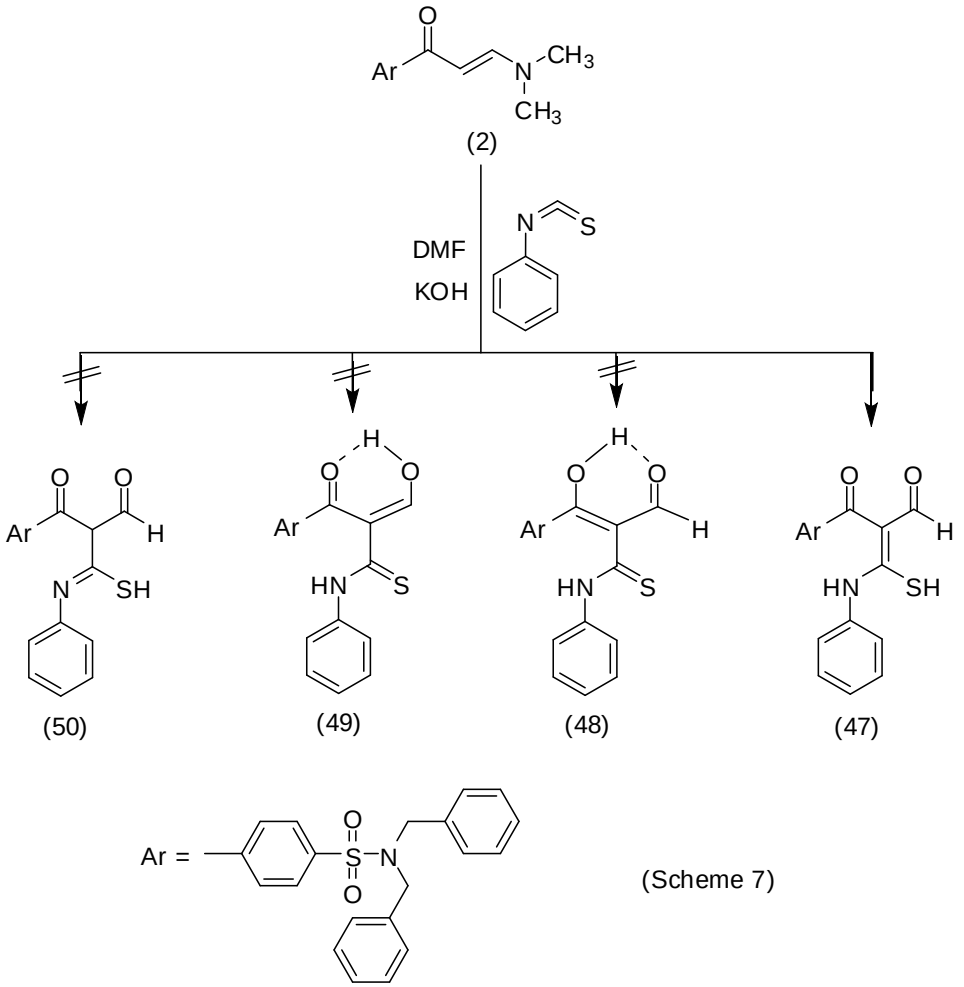
(Scheme 5)

Enaminone **2** reacts with hydroxylamine hydrochloride in refluxing pyridine to give one isolable product, namely, N,N-dibenzyl-4-(2-cyanoacetyl)benzenesulfonamide **(46)** rather than another isomeric forms **40** and **43**. Structure **46** 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=3.17 ppm. cyanoacetyl derivative **46** was assumed to be formed *via* initial addition of hydroxylamine to the activated double bond in enaminone **2** forming non isolable intermediate **44**, followed by the loss of dimethylamine to produce aldehyde oxime intermediate **45** then dehydration (**Scheme 6**).



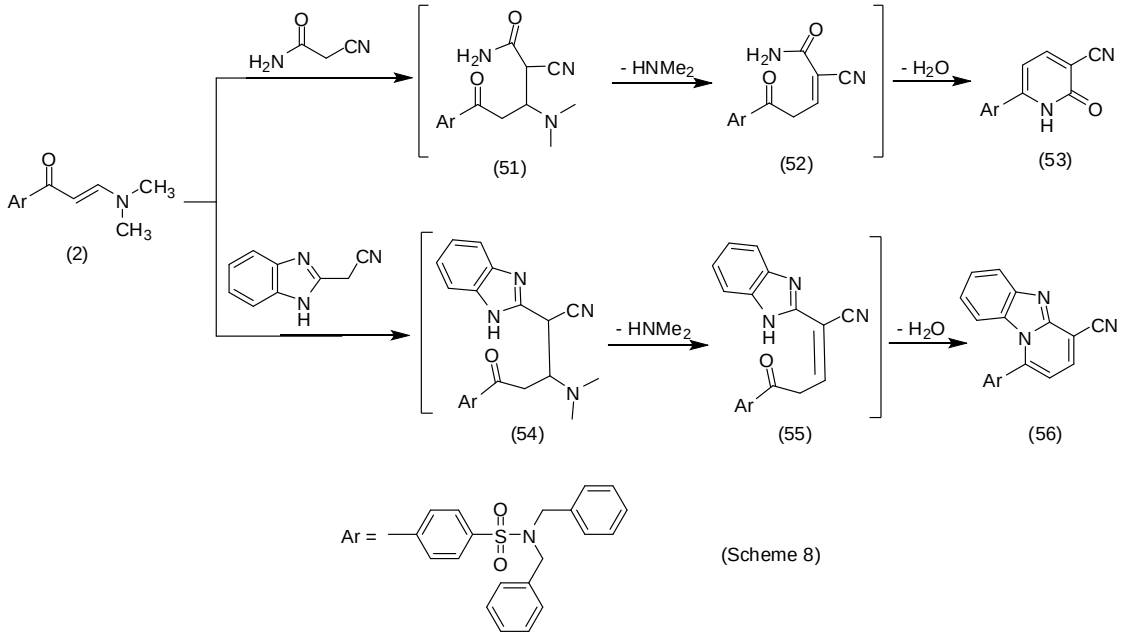
(Scheme 6)

It was found that the enaminone **2** reacted with phenylisothiocyanate in DMF in the presence of potassium hydroxide to afford after acidification with HCl molecular formula corresponding to structure **47** or its tautomeric forms **48-50** although Regitz *et al*. **[34]** have assigned hydroxy methylene structures **48** or **49** for diketothioamides, structure **47** better agreed with the obtained spectral data for these compounds thus structures **48** and **49** could be ruled out. structure **50** was also ruled out on the bases of IR spectrum due to appearance of band at ν_{max}= 3370 cm^{-1} for NH group, moreover ^{1}H-NMR spectrum indicated the presence of a low field aldehydic proton in the region of δ= 9.79 ppm. the existence of this proton at such value compared with that expected for aromatic aldehydes, is probably due to conjugated double bond, beside the appearance of singlet signal at δ= 12.13 ppm. for NH group which discharged with D_2O (**Scheme 7**).



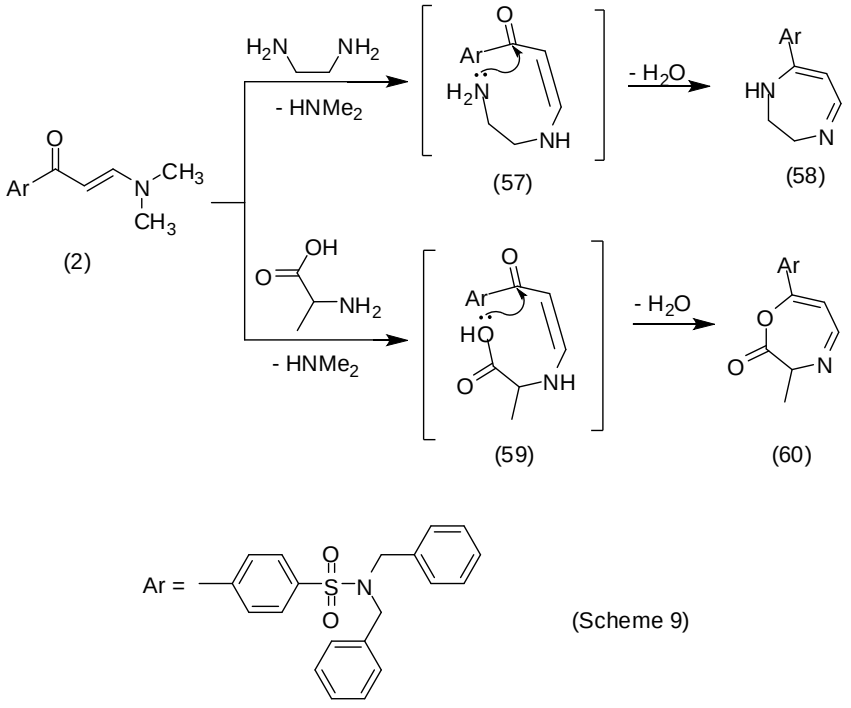
(Scheme 7)

Interaction of enaminone **2** with 2-cyanoacetamide afforded cyanopyridine derivative **53**, *via* elimination of dimethylamine and water from intermediates **51**, **52** respectively. In the same manner enaminone **2** reacted with 2-(1H-benzo[d]imidazol-2-yl)acetonitrile, in glacial acetic acid to give imidazo[1,2-a]pyridine derivative **56,** *via* elimination of dimethylamine and water from an intermediates **54, 55** respectively **(Scheme 8).**



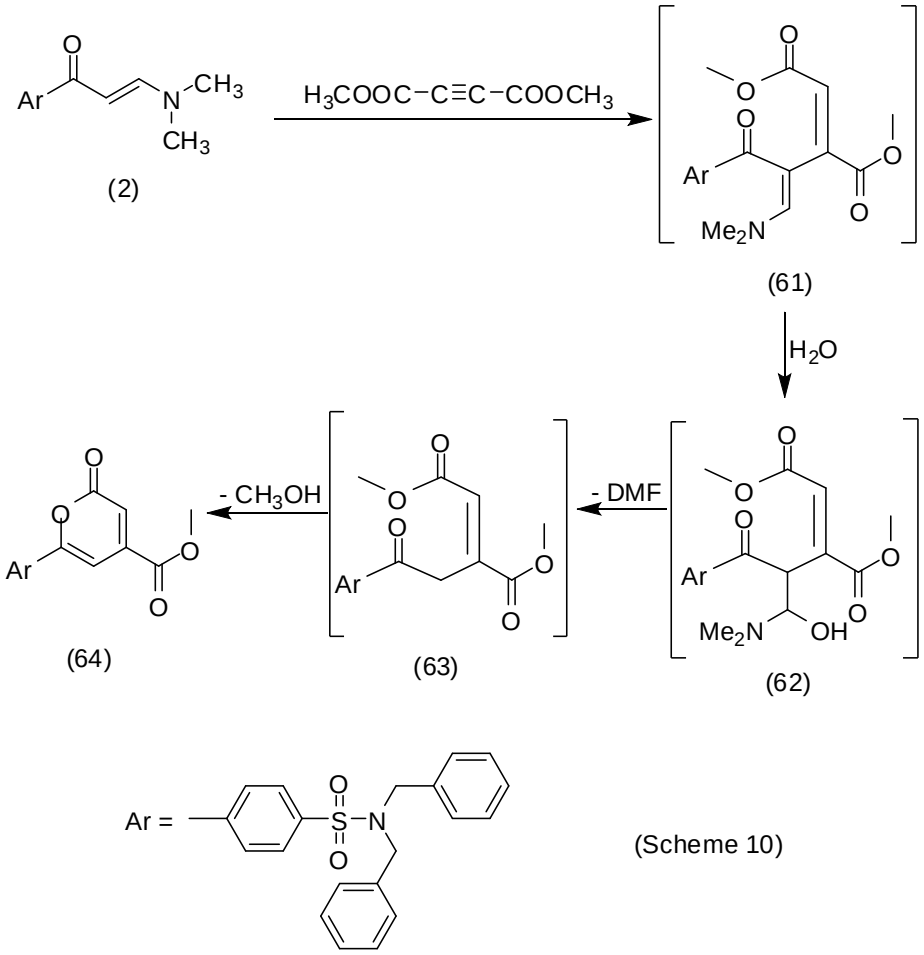
(Scheme 8)

On the other hand, interaction of enaminone **2** with ethylenediamine or alanine afforded derivatives of diazepine **58** and oxazepine **60** respectively *via* an elimination of water from intermediates **57** and **59,** respectively **(Scheme 9).**



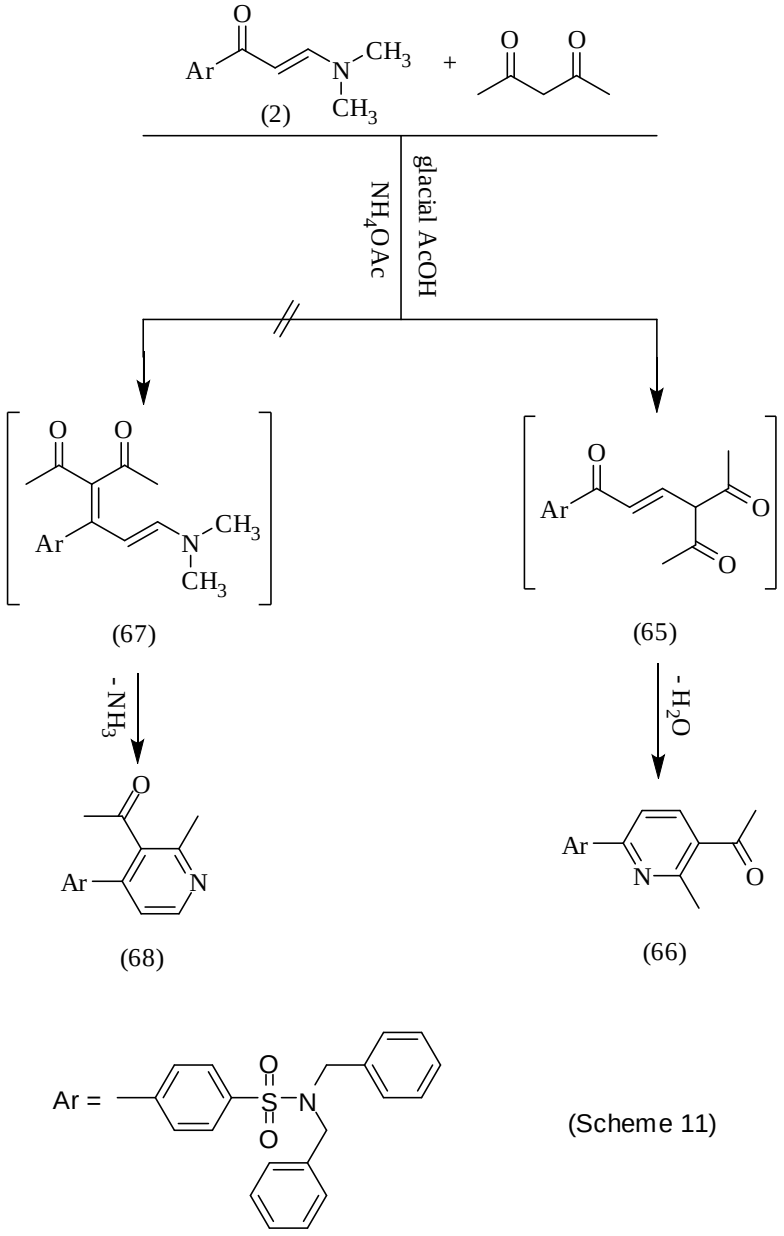
(Scheme 9)

When enaminone **2** was refluxed with dimethyl acetylenedicarboxylate, the pyranone **64** was formed. It is believed that **64** was formed *via* nucleophilic addition of enaminone C-2 to dimethyl acetylenedicarboxylate to yield adduct **61**, that then hydrolyze to **62** followed by elimination of dimethylformamide to produce **63**, finally afford **64** *via* cyclization with elimination of methanol under the reaction conditions, (**Scheme 10**).



(Scheme 10)

Reactions of enaminone **2** with *C*-nucleophiles such as acetyl acetone was carried out in glacial acetic acid in the presence of ammonium acetate gave 4-(5-acetyl-6-methylpyridin-2-yl)-N,N-dibenzylbenzenesulfonamide **(66)** *via* nucleophilic displacement of active methylene to the dimethylamino group followed by concurrent elimination of water molecule from non isolable intermediates **65.** The other possible isomeric structure 4-(3-acetyl-2-methylpyridin-4-yl)-N,N-dibenzylbenzenesulfonamide **(68)** was discarded based on ^{1}H-NMR data that revealed pyridyl hydrogens at C-4, C-5 as a pair of doublets at δ= 7.62, 7.83 ppm, respectively, with *J=8.1 Hz* assignable to 6-substituted pyridine **66**. The isomeric structure **68** should display pair of doublets corresponding to C-5, C-6 with a lower coupling constant (*J=2-3 Hz*)**[35]** (**Scheme 11**).



(Scheme 11)

2.2. Docking and molecular modeling

As revealed from the aforementioned introductory part, that thymidylate synthase and dihydrofolate reductase are among the main targets involved in anticancer and antimicrobial activity **[36,37]**. Molecular modeling study using Molecular Operating Environment (MOE)**[38]** module was performed in order to rationalize the observed anticancer activity of compounds **1**, **2**, **9**, **15**, **16, 28, 36, 46, 53, 56** and **64**. Molecular docking studies further help in understanding the mode of action of the compounds through their various interactions with the active sites of dihydrofolate reductase.

2.2.1. Docking of MTX into DHFR: active site revealed that hydrogen bond interactions beside hydrophobic interactions were considered to be responsible for the observed affinity as it acts as a hydrogen bond donor to the backbone Ile 5 and 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 beside 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 **Fig. (1).**

***2.2.2. Docking simulation study of the synthesized compounds* 1**, **2**, **9**, **15**, **16, 28, 36, 46, 53, 56** and **64:**

MOE docking studies of the inhibitors were performed using dihydrofolate reductase co-crystallized with methotrexate (PDB ID: 4DFR) as a template.

2.2.2.1. Docking of compound 1 into DHFR: active site revealed the presence of hydrogen bond interaction between two oxygen atoms of SO_2 moiety, as it acts as hydrogen bond acceptor with side chain Tyr 121 residue (3.02 Aº and 2.38 Aº) with a strength of 5.3 % and 86.8%; respectively. However, it revealed hydrophobic interactions between atoms of the compound and many amino acid residues: IIe 7, Val 8, Ala 9, IIe 16, Gly 20, Leu 22, Glu 30, Phe 31, Phe 34, Lys 55, Thr 56, Ser 59, Val 115 and Gly 116, as shown in **Fig. (2)**.

2.2.2.2. Docking of compound 2 into DHFR: active site revealed the presence of hydrogen bond interaction between one of the oxygen atom of SO_2 moiety, as it acts as hydrogen bond acceptor with side chain Ser 59 residue (2.66 Aº) with a strength of 93.6 %. Moreover, it showed a hydrogen bond interaction between oxygen atom of methanone moiety, as it acts as a hydrogen bond acceptor with side chain Asn 64 residue (2.55 Aº) with a strength of 52.8 %. However, it revealed presence of hydrophobic interactions between other atoms in the compound and many amino acid residues: IIe 7, Val 8, Asp 21, Leu 22, Phe 31, Phe 34, Thr 56, IIe 60, Pro 61, Leu 67, Arg 70, Val 115, and Tyr 121, as shown in **Fig. (3)**.

2.2.2.3. Docking of compound 9 into DHFR: active site revealed the presence of hydrogen bond interaction between the oxygen atom of C=O moiety, as it acts as hydrogen bond acceptor with side chain Asn 64 residue (2.53 Aº) with a strength of 23.5 %. In addition to other hydrogen bond interaction between the oxygen atom of hydroxyl group and the side chain Lys 68 residue (3.80 Aº) with a strength of 2.6 %. Also, it revealed presence of hydrogen bond interaction between the two oxygen atoms of SO_2 moiety, as it acts as hydrogen bond acceptor with side chain Ser 59 residue (3.28 Aº and 2.88 Aº) with a strength of 4.8 % and 32.8%; respectively. However, hydroxyl function act as hydrogen bond donor with side chain Asn 64 residue (3.89 Aº) with a strength of 4.8 %. Furthermore, it revealed hydrophobic interactions between other atoms in the compound and many amino acid residues: IIe 16, Leu 22, Phe 31, Phe 34, Gln 35, Thr 56, IIe 60, Pro 61, Asn 64, Leu 67, Arg 70 and Val 115, as shown in **Fig. (4)**.

2.2.2.4. Docking of compound 15 into DHFR: active site revealed that almost all atoms make hydrophobic interactions between many amino acid residues: Ala 9, IIe 16, Asp 21, Leu 22, Trp 24, Phe 31, Phe 34, Gln 35, Ser 59, IIe 60, Pro 61, Lys 63, Asn 64 and Leu 67, as shown in **Fig. (5)**.

2.2.2.5. Docking of compound 16 into DHFR: active site revealed the presence of hydrogen bond interaction between two oxygen atoms of SO_2 moiety, as it acts as hydrogen bond acceptor with side chain Ser 59 residue (1.83 Aº and 3.04 Aº) with a strength of 30.5 % and 3.9 %; respectively and Thr 56 residue (3.33 Aº) with a strength of 1.9 %. However, it revealed presence of hydrophobic interactions between other atoms in the compound and many amino acid residues: Val 8, Ala 9, IIe 16, Asp 21, Leu 22, Phe 31, Phe 34, Gln 35, Lys 55, IIe 60, Pro 61, Asn 64, Leu 67, Arg 70, Val 115 and Tyr 121, as shown in **Fig. (6)**.

2.2.2.6. Docking of compound 28 into DHFR: active site revealed the presence of hydrogen bond interaction between the two oxygen atoms of SO_2 moiety, as it acts as hydrogen bond acceptor with the side chain Ser 59 residue (2.86 Aº and 1.61 Aº) with a strength of 12.4 % and 83.1 %; respectively. In addition to hydrophobic interactions concerning carbon atoms of phenyl moieties, carbon and nitrogen atoms of triazolopyrimidine moiety

with many amino acid residues: IIe 16, Asp 21, Leu 22, Phe 31, Phe 34, Thr 56, IIe 60 and Pro 61, as shown in **Fig. (7)**.

2.2.2.7. Docking of compound 36 into DHFR: active site revealed the presence of hydrogen bond interaction between one of the oxygen atom of SO_2 moiety, as it acts as hydrogen bond acceptor with the side chain Ser 59 residue (2.36 Aº) with a strength of 79.1 %. In addition to hydrophobic interactions concerning carbon atoms of phenyl moieties, carbon and nitrogen atoms of pyrazole moiety with many amino acid residues: Val 8, Ala 9, Asp 21, Leu 22, Phe 31, Phe 34, Thr 56, IIe 60, Pro 61, Leu 67, Val 115 and Tyr 121, as shown in **Fig. (8)**.

2.2.2.8. Docking of compound 46 into DHFR: active site revealed the presence of hydrogen bond interaction between the two oxygen atoms of SO_2 moiety, as it acts as hydrogen bond acceptor with side chain Ser 59 residue (2.27 Aº and 3.06 Aº) with a strength of 98 % and 7 %; respectively. However, it revealed hydrophobic interactions between carbon, oxygen and nitrogen atoms of the compound and many amino acid residues: Val 8, Ala 9, Gly 20, Asp 21, Leu 22, Pro 26, Phe 31, Thr 56, Pro 61, Val 115 and Tyr 121, as shown in **Fig. (9)**.

2.2.2.9. Docking of compound 53 into DHFR: active site revealed the presence of hydrogen bond interaction between the oxygen atom on pyridinone ring, as it acts as hydrogen bond acceptor with side chain Phe 31 residue (2.61 Aº) with a strength of 19 %. However, it revealed presence of hydrophobic interactions between other atoms in the compound and many amino acid residues: Ala 9, IIe 16, Asp 21, Leu 22, Phe 34, Thr 56, Ser 59, Asn 64, Leu 67 and Val 115, as shown in **Fig. (10)**.

2.2.2.10. Docking of compound 56 into DHFR: active site revealed the presence of hydrogen bond interaction between one of the oxygen atom of SO_2 moiety, as it acts as hydrogen bond acceptor with side chain Asn 64 residue (1.94 Aº) with a strength of 65.7 %. However, it revealed hydrophobic interactions between atoms of the compound and many amino acid residues: IIe 16, Gly 17, Asp 21, Leu 22, Arg 28, Glu 30, Phe 31, Phe 34, Gln 35, Ser 59, IIe 60, Pro 61, Leu 67 and Arg 70, as shown in **Fig. (11)**.

2.2.2.11. Docking of compound 64 into DHFR: active site revealed the presence of hydrogen bond interaction between two oxygen atoms of SO_2 moiety, as it acts as hydrogen bond acceptor with the side chain Ser 59 residue (2.17 Aº and 3.29 Aº) with a strength of 98.8 % and 1.9%; respectively. Furthermore, it showed that the presence of hydrogen bond interaction between the oxygen atom of (C=O) at position two of pyranone moiety with the side chain Asn 64 residue (2.96 Aº with a strength of 11.6 %. In addition to hydrophobic interactions concerning carbon atoms of phenyl, pyranone moieties and aliphatic carbon atoms with many amino acid residues: Ala 9, IIe 16, Asp 21, Leu 22, Pro 26, Glu 30, Phe 31, Thr 56, Pro 61, Val 115, Gly 116, Gly 117 and Tyr 121, as shown in **Fig. (12)**.

2.2.2.12. Conclusion of docking simulation study:

Docking was performed for the eleven most active anticancer compounds **1**, **2**, **9**, **15**, **16, 28, 36, 46, 53, 56** and **64** on the dihydrofolate reductase in a trial to predict their mode of action as anticancer drugs. As revealed from the aforementioned data, the compounds show several interactions with both enzymes but they exhibit strong interactions with dihydrofolate reductase enzyme, mainly compounds **9, 16, 53, 56** and **64** giving rise to the conclusion that they might exert their action through inhibition of DHFR enzyme.

2.*3. In vitro* anticancer activity

The newly synthesized compounds were evaluated for their *in-vitro* cytotoxicity against human breast cancer cell line (MCF7) and some of the tested compounds were equipotent, while the others were more potent compared with Methotrexate as reference drug. From the obtained results (**Table 1**), we can observe that compound **56** having 4-cyanobenzo[4,5]imidazo[1,2-a]pyridine moiety with IC_{50} value (170.3 µM.), 5-cyano-6-oxo-1,6-dihydropyridin **53** with IC_{50} value (157.5 µM.), pyrazole **36** with IC_{50} value (153.1 µM.), ethyl pyrazole-3-carboxylate **15** with IC_{50} value (146.9 µM.), cyanoacetyl **46** with IC_{50} value (143.2 µM.), (4-chlorophenyl)hydrazono **37** with IC_{50} value (140.0 µM.), 5-hydroxybenzofuran **9** with IC_{50} value (127.9 µM.), compound **16** having pyrazolo[3,4-d]pyridazine moiety with IC_{50} value (122.7 µM.), compound **64** having methyl pyrane-4-carboxylate moiety with IC_{50} value (121.3 µM.), triazolo[4,3-a]pyrimidine **28** with IC_{50} value (119.5 µM.), 2-aminopyrimidine **35** with IC_{50} value (117.3 µM.), enaminone **2** with IC_{50} value (111.9 µM.), showed increased activity when compared to methotrexate with IC_{50} value (74.6 µM.), while compounds **34**, **47**, **1**, **58** and **60** with IC_{50} values (85.7, 80.1, 70.3, 63.1, 60.2 µM.), respectively, were found to be nearly as active as methotrexate. While the remaining compounds **22**, **3**, **5** and **66** with IC_{50} values (50.3, 44.7, 30.6, 15.7 µM.), 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 breast cancer cell line (MCF7). (**Table 1**) has showed that the cell killing potency follows the order **56 > 53 > 36 > 15 > 46 > 37 > 9 > 16 > 64 > 28 > 35 > 2 > 34 > 47 > methotrexate > 1 > 58 > 60 > 22 > 3 > 5 > 66**. 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 agent.

3. Experimental

3.1. Chemistry

Melting points (^{o}C, uncorrected) were determined in open capillaries on a GallenKemp melting point apparatus (Sanyo GallenKemp, 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.

3.1.1. (E)-N,N-dibenzyl-4-(3-(dimethylamino)acryloyl)benzenesulfonamide (2)

A mixture of 4-acetyl-N,N-dibenzylbenzenesulfonamide (**1;** 3.79 g, 0.01 mol) and DMF-DMA (1.43 g, 0.012 mol) in dry xylene (50 mL) was heated under reflux for 4 h, the separated solid was filtered off, washed with ethanol and recrystallized from benzene to give **2**. Yellow crystals, Yield, 71%; m.p. 151-152°C. IR (KBr, cm^{-1}): ν_{max}= 3050 (CH-aromatic), 2910 (CH-aliphatic), 1684 (CO), 1344, 1158 (SO_2). ^{1}H-NMR (DMSO-d_6): δ= 2.97, 3.18 (2s, 6H, $(CH_3)_2N$), 4.38 (s, 4H, $2CH_2$), 5.66, 8.33 (dd, 2H, *J= 11.7 Hz*, olefinic CH=CH), 7.25-8.20 (m, 14H, Ar-H). MS m/z (%) (**Chart 1**): 434 [M^+] (2.5), 287 (63.6), 196 (11.6), 194 (84.8), 174 (38.4), 130 (6.7), 105 (32.9), 104 (66.5), 91 (100), 76 (27.5). Anal. Calcd. for $C_{25}H_{26}N_2O_3S$ (434.55): C, 69.10; H, 6.03; N, 6.45; O, 11.05; S, 7.38. Found: C, 68.88; H, 5.98; N, 6.55; O, 10.92; S, 7.45%.

3.1.2. (Z)-N,N-dibenzyl-4-(3-(*p*-tolylamino)acryloyl)benzenesulfonamide (3)

A mixture of enaminone **2** (4.34 g, 0.01 mol) and *p*-toluidine (1.07 g, 0.01 mol) in ethanol/acetic acid (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 ethanol and recrystallized from ethanol to give **3**. White solid, Yield, 77%; m.p. 163-164°C. IR (KBr, cm^{-1}): ν_{max}= 3290 (NH), 3105 (CH-aromatic), 2920 (CH-aliphatic), 1681 (CO), 1340, 1156 (SO_2). ^{1}H-NMR (DMSO-d_6): δ= 2.30 (s, 3H, CH_3), 4.40 (s, 4H, 2 CH_2), 5.90, 8.11 (dd, 2H, *J= 8.5 Hz*, olefinic CH=CH), 6.99-7.93 (m, 18H, Ar-H), 11.98 (s, 1H, NH, Discharged with D_2O). MS m/z (%): 496 [M^+] (17.9), 467 (17.2), 427 (18.0), 289 (42.5), 229 (24.8), 158 (61.7), 141 (61.2), 101 (47.0), 85 (45.2), 58 (100). Anal. Calcd. for $C_{30}H_{28}N_2O_3S$ (496.62): C, 72.55; H, 5.68; N, 5.64; O, 9.66; S, 6.46. Found: C, 72.69; H, 5.47; N, 5.56; O, 9.39; S, 6.57%.

3.1.3. N,N-dibenzyl-4-(1-(2-phenylhydrazono)ethyl)benzenesulfonamide (5)

3.1.3.1. Procedure (A):

To a solution of the enaminone **2** (4.34 g, 0.01 mol) in ethanol (20 mL), phenylhydrazine (1.08 g, 0.01 mol) was added. The reaction mixture was refluxed for 4 h, and then cooled. The solid product so formed was filtered off, washed with ethanol, dried and recrystallized to give **5**.

3.1.3.2. Procedure (B):

To a solution of the **1** (3.79 g, 0.01 mol) in ethanol (50 mL), phenylhydrazine (1.08 g, 0.01 mol) was added. The reaction mixture was refluxed for 2 h, and then cooled. The obtained product was collected and recrystallized. m.p. and mixed m.p. determined with authentic sample gave no depression.

Golden yellow crystals, Yield, 63%; m.p. 251-252°C (ethanol). IR (KBr, cm^{-1}): ν_{max}= 3301 (NH), 3100 (CH-aromatic), 2931 (CH-aliphatic), 1338, 1151 (SO_2). ^{1}H-NMR (DMSO-d_6): δ= 2.95 (s, 3H, CH_3), 4.42 (s, 4H, 2 CH_2), 6.81-8.02 (m, 19H, Ar-H), 9.91 (s, 1H, NH, Discharged with D_2O). MS m/z (%): 469 [M^+] (**Chart 2**) (100), 453 (26.9), 438 (20.1), 424 (11.7), 410 (71.2), 391 (89.0), 377 (10.5), 362 (31.2), 346 (57.0), 340 (33.7), 323 (34.2), 314 (21.4). Anal. Calcd. for $C_{28}H_{27}N_3O_2S$ (469.60): C, 71.61; H, 5.80; N, 8.95; O, 6.81; S, 6.83. Found: C, 71.55; H, 5.73; N, 8.72; O, 6.73; S, 6.72%.

3.1.4. N,N-dibenzyl-4-(5-hydroxybenzofuran-3-carbonyl)benzenesulfonamide (9)

To a stirred solution of enaminone **2** (4.34 g, 0.01 mol) in glacial acetic acid (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 vacuo and the solid product was isolated by filtration and recrystallized from ethanol to give **9**. White solid, Yield, 69%; m.p. 210-211°C. IR (KBr, cm^{-1}): ν_{max}= 3200-3430 (OH), 3098 (CH-aromatic), 2909 (CH-aliphatic), 1673 (CO), 1345, 1163 (SO_2). ^{1}H-NMR (DMSO-d_6): δ= 4.40 (s, 4H, 2 CH_2), 7.20-8.70 (m, 17H, Ar-H), 8.75 (s, 1H, benzo[b]furan H-2), 9.30 (s, 1H, OH, Discharged with D_2O). MS m/z (%): 497 [M^+] (19.1), 363 (15.3), 302 (18.1), 288 (23.4), 166 (42.3), 152 (30.9), 105 (7.8), 91 (**Chart 2**) (100), 66 (80.2). Anal. Calcd. for $C_{29}H_{23}NO_5S$ (497.56): C, 70.00; H, 4.66; N, 2.82; O, 16.08; S, 6.44. Found: C, 69.86; H, 4.59; N, 2.93; O, 15.89; S, 6.68%.

3.1.5. ethyl 1-(4-chlorophenyl)-4-(4-(N,N-dibenzylsulfamoyl)benzoyl)-1H-pyrazole-3-carboxylate (15)

To a mixture of enaminone **2** (4.34 g, 0.01 mol) and ethyl 2-bromo-2-(2-(4-chlorophenyl)hydrazono)acetate (**10;** 3.03 g, 0.01 mol) in xylene (40 mL) an equivalent amount of triethylamine (1.01 g. 0.01 mol) was added. The reaction mixture was heated under reflux for 5 h. the solvent was distilled at reduced pressure and the residual viscous liquid was taken in ethanol then the resulting solid was collected by filtration, washed thoroughly with ethanol, dried and finally recrystallized from ethanol to give **15**. Buff crystals, Yield, 70%; m.p. 200-201°C. IR (KBr, cm^{-1}): ν_{max}= 3099 (CH-aromatic), 2930 (CH-aliphatic), 1715 (CO ester), 1685 (CO), 1343, 1158 (SO_2). ^{1}H-NMR (DMSO-d_6): δ= 1.30 (t, 3H, CH_3), 4.32 (q, 2H, CH_2), 4.45 (s, 4H, 2 CH_2), 7.10-8.30 (m, 19H, Ar-H), 8.78 (s, 1H, pyrazole H-5). MS m/z (%): 613 [M^+] (11.3), 412 (9.4), 379 (8.9), 308 (10.1), 288 (70.6), 196 (**Chart 2**) (100), 182 (30.1), 125 (10.4), 118 (60.5), 104 (55.3), 64 (40.2). Anal. Calcd. for $C_{33}H_{28}ClN_3O_5S$ (614.11): C, 64.54; H, 4.60; N, 6.84; O, 13.03; S, 5.22. Found: C, 64.73; H, 4.56; N, 6.71; O, 13.11; S, 5.10%.

3.1.6. N,N-dibenzyl-4-(2-(4-chlorophenyl)-7-oxo-6,7-dihydro-2H-pyrazolo[3,4-d]pyridazin-4-yl)benzene-sulfonamide (16)

A mixture of pyrazole derivative **15** (6.13 g, 0.01 mol) and hydrazine hydrate (0.50 g, 0.01 mol) in ethanol (50 ml) was heated under reflux for 4 h. the separated solid was filtered off, washed with ethanol and recrystallized from ethanol to give **16**. Yellowish white crystals, Yield, 88%; m.p. 260-261°C. IR (KBr, cm^{-1}): ν_{max}= 3280 (NH), 3133 (CH-aromatic), 2880 (CH-aliphatic), 1660 (CO), 1339, 1163 (SO_2). ^{1}H-NMR (DMSO-d_6): δ= 4.43 (s, 4H, 2 CH_2), 7.19-8.33 (m, 19H, Ar-H), 8.15 (s, 1H, NH, Discharged with D_2O). MS m/z (%): 581 [M^+] (40.7), 511 (20.9), 395 (22.6), 377 (26.5), 316 (49.5), 303 (70.3), 196 (**Chart 2**) (100), 135 (90.1), 93 (44.7). Anal. Calcd. for $C_{31}H_{24}ClN_5O_3S$ (582.07): C, 63.97; H, 4.16; N, 12.03; O, 8.25; S, 5.51. Found: C, 63.86; H, 4.09; N, 11.97; O, 8.09; S, 5.66%.

3.1.7. 7-(4-(N,N-dibenzylsulfamoyl)phenyl)-2-methylpyrazolo[1,5-a]pyrimidine-3-carboxamide (22)

A mixture of enaminone **2** (4.34 g, 0.01 mol) and 5-amino-3-methyl-1H-pyrazole-4-carboxamide (1.40 g, 0.01 mol) in ethanol/acetic acid (1:1), (60 mL) was heated under reflux for 5 h. during the reflux period, a crystalline solid was separated. The separated solid was filtered off, washed with ethanol and recrystallized from ethanol to give **22**. White crystals, Yield, 55%; m.p. 217-218°C. IR (KBr, cm^{-1}): ν_{max}= 3390, 3350 (NH_2), 3100 (CH-aromatic), 2950 (CH-aliphatic), 1653 (CO), 1340, 1155 (SO_2). ^{1}H-NMR (DMSO-d_6): δ= 2.38 (s, 3H, CH_3), 4.40 (s, 4H, 2 CH_2), 7.99 and 8.84 (dd, $J_{5,6}$= 4.7 *Hz*, 2H, pyrimidine-$H_{5,6}$), 7.22-7.82 (m, 14H, Ar-H), 8.20 (s, 2H, NH_2, Discharged with D_2O). MS m/z (%): 511 [M^+] (22.3), 409 (22.1), 377 (12.3), 362 (9.8), 196 (**Chart 2**) (100), 119 (43.7), 105 (20.6), 66 (30.1). Anal. Calcd. for $C_{28}H_{25}N_5O_3S$ (511.59): C, 65.74; H, 4.93; N, 13.69; O, 9.38; S, 6.27. Found: C, 65.66; H, 4.89; N, 13.78; O, 9.60; S, 6.05%.

3.1.8. 4-([1,2,4]triazolo[4,3-a]pyrimidin-5-yl)-N,N-dibenzylbenzenesulfonamide (28)

A mixture of enaminone **2** (4.34 g, 0.01 mol) and 3-amino-1H-1,2,4-triazole (0.84 g, 0.01mol) in acetic acid (30 mL) was refluxed for 5 h. during the reflux period, a crystalline solid was separated. The separated solid was filtered off, washed with ethanol and recrystallized from ethanol/benzene to give **28**. White solid, Yield, 65%; m.p. 300-301°C. IR (KBr, cm^{-1}): ν_{max}= 3030 (CH-aromatic), 2899 (CH-aliphatic), 1344, 1166 (SO_2). ^{1}H-NMR (DMSO-d_6): δ= 4.44 (s, 4H, 2 CH_2), 7.69 and 8.85 (dd, $J_{5,6}$= 4.6 *Hz*, 2H, pyrimidine-$H_{5,6}$), 6.98-7.55 (m, 15H, Ar-H). ^{13}C-NMR (DMSO-d_6): δ= 60.4 (2C), 125.8 (4C), 127.3 (4C), 123.2 (2C), 124.0 (2C), 127.7 (2C), 132.5 (2C), 170.3, 167.5, 161.1, 147.0, 143.6, 135.9, 110.1. MS m/z (%): 455 [M^+] (10.2), 428 (13.2), 311 (9.0), 252 (24.2), 226 (27.9), 194 (11.2), 177 (10.9), 152 (60.7), 139 (25.6), 119 (**Chart 2**) (100), 96 (14.3), 82 (17.1), 76 (88.7). Anal.

Calcd. for $C_{25}H_{21}N_5O_2S$ (455.53): C, 65.92; H, 4.65; N, 15.37; O, 7.02; S, 7.04. Found: C, 65.83; H, 4.59; N, 15.40; O, 6.89; S, 7.31%.

3.1.9. N,N-dibenzyl-4-(8-oxo-6-thioxo-7,8-dihydro-6H-pyrimido[1,6-a]pyrimidin-4-yl)benzenesulfonamide (34)

A mixture of enaminone **2** (4.34 g, 0.01 mol) and 6-amino-2-thioxo-2,3-dihydropyrimidin-4(1H)-one (1.43 g, 0.01 mol) in ethanol/acetic acid (1:1), (50 mL) was heated under reflux for 2 h. during the reflux period, a crystalline solid was separated. The separated solid was filtered off, washed with ethanol and recrystallized from ethanol to give **34**. Orange solid, Yield, 63%; m.p. 273-274°C. IR (KBr, cm^{-1}): ν_{max}= 3250 (NH), 3095 (CH-aromatic), 2905 (CH-aliphatic), 1650 (CO), 1344, 1160 (SO_2). ^{1}H-NMR (DMSO-d_6): δ= 4.42 (s, 4H, 2 CH_2), 8.01 and 8.90 (dd, $J_{5,6}$= 4.7 *Hz*, 2H, pyrimidine-$H_{5,6}$), 7.12-7.93 (m, 15H, Ar-H), 11.90 (s, 1H, NH, Discharged with D_2O). MS m/z (%): 514 [M^+] (10.3), 379 (5.2), 288 (14.4), 197 (13.3), 118 (12.9), 104 (16.5), 91 (**Chart 2**) (100). Anal. Calcd. for $C_{27}H_{22}N_4O_3S_2$ (514.62): C, 63.02; H, 4.31; N, 10.89; O, 9.33; S, 12.46. Found: C, 62.99; H, 4.52; N, 10.76; O, 9.56; S, 12.44%.

3.1.10. 4-(2-aminopyrimidin-4-yl)-N,N-dibenzylbenzenesulfonamide (35)

A mixture of enaminone **2** (4.34 g, 0.01 mol) and guanidine hydrochloride (0.95 g, 0.01 mol) in ethanol (30 mL), anhydrous potassium carbonate (2 g) 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 dioxane to give **35**. Pale yellow crystals, Yield, 71%; m.p. 290-291°C. IR (KBr, cm^{-1}): ν_{max}= 3460, 3410 (NH_2), 3090 (CH-aromatic), 2900 (CH-aliphatic), 1341, 1158 (SO_2). ^{1}H-NMR (DMSO-d_6): δ= 4.43 (s, 4H, 2 CH_2), 7.15 (s, 2H, NH_2, Discharged with D_2O), 7.25-8.02 (m, 16H, Ar-H). MS m/z (%): 430 [M^+] (13.8), 196 (26.6), 118 (11.3), 104 (10.2), 91 (**Chart 2**) (100). Anal. Calcd. for $C_{24}H_{22}N_4O_2S$ (430.52): C, 66.96; H, 5.15; N, 13.01; O, 7.43; S, 7.45. Found: C, 66.77; H, 5.27; N, 12.89; O, 7.59; S, 7.30%.

3.1.11. N,N-dibenzyl-4-(1H-pyrazol-3-yl)benzenesulfonamide (36)

To a solution of the enaminone **2** (0.45 g, 2 mmol) in ethanol (20 mL), hydrazine hydrate (0.50 g, 0.01 mol) was added. The reaction mixture was refluxed for 4 h, and then cooled. The solid product so formed was filtered off, washed with ethanol, dried and recrystallized to give **36**. White crystals, Yield, 66%; m.p. 222-224°C (ethanol/benzene). IR (KBr, cm^{-1}): ν_{max}= 3270 (NH), 3070 (CH-aromatic), 2907 (CH-aliphatic), 1340, 1158 (SO_2). ^{1}H-NMR (DMSO-d_6): δ= 3.88 (s, 4H, 2 CH_2), 7.11-8.30 (m, 16H, Ar-H), 11.93 (s, 1H, NH, Discharged with D_2O). ^{13}C-NMR (DMSO-d_6): δ= 50.3 (2C), 123.1 (2C), 128 (4C), 130.5 (4C), 124.5 (2C), 127.7 (2C), 140.3 (2C), 110.2, 133.5, 137.1, 142.4, 160.9%. MS m/z (%): 403 [M^+] (20.4), 376 (12.4), 356 (24.9), 335 (70.5), 296 (10.0), 271 (12.5), 247 (17.3), 220 (89.1), 196 (**Chart 2**) (100), 181 (10.3), 133 (11.7), 106 (91.2), 66 (11.8). Anal. Calcd. for $C_{23}H_{21}N_3O_2S$ (403.50): C, 68.46; H, 5.25; N, 10.41; O, 7.93; S, 7.95. Found: C, 68.27; H, 5.14; N, 10.32; O, 7.87; S, 7.89.

3.1.12. N,N-dibenzyl-4-(2-(2-(4-chlorophenyl)hydrazono)-3-oxopropanoyl)benzenesulfonamide (37)

A cold solution of *p*-chlorobenzenediazonium chloride (0.01 mol) (prepared by adding a cold solution of sodium nitrite (0.69 g, 0.01 mol) in water (5 mL) to a solution of *p*-chloroaniline (1.27 g, 0.01 mol) in conc. HCl (5 mL) at (0-5 ^oC) under stirring) was added to a cold solution of enaminone **2** (4.34 g, 0.01 mol), in ethanol (50 mL) containing NaOH (1.6 g). The mixture was then stirred at room temperature for 2 h, and the solid precipitated was collected and recrystallized from ethanol to give **37**. Deep orange solid, Yield, 45%; m.p. 209-210°C. IR (KBr, cm^{-1}): ν_{max}= 3283 (NH), 3070 (CH-aromatic), 2903 (CH-aliphatic), 1681, 1699 (2CO), 1346, 1150 (SO_2). ^{1}H-NMR (DMSO-d_6): δ= 4.41 (s, 4H, 2 CH_2), 7.08-8.35 (m, 18H, Ar-H), 10.03 (s, 1H, CHO), 12.77 (s, 1H, NH, Discharged with D_2O). MS m/z (%): 545 [M^+] (60.8), 495 (30.3), 480 (25.9), 393 (26.4), 311 (27.0), 286 (44.8), 236 (35.7), 196 (86.8), 149 (19.4), 118 (58.4), 91 (**Chart 2**) (100). Anal. Calcd. for $C_{29}H_{24}ClN_3O_4S$ (546.04): C, 63.79; H, 4.43; N, 7.70; O, 11.72; S, 5.87. Found: C, 63.62; H, 4.38; N, 7.65; O, 11.69; S, 5.76%.

3.1.13. N,N-dibenzyl-4-(2-cyanoacetyl)benzenesulfonamide (46)

A mixture of enaminone **2** (4.34 g, 0.01 mol) and hydroxylamine hydrochloride (0.69 g, 0.01 mol) in pyridine (30 mL) was refluxed for 8 h and then allowed to cool to 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 ethanol to give **46**. White solid, yield 78%; mp 200-201^oC. IR (KBr, cm^{-1}): ν_{max}= 3130 (CH-aromatic), 2900 (CH-aliphatic), 2220 (CN), 1672 (CO), 1345, 1157 (SO_2). ^{1}H-NMR (DMSO-d_6): δ= 3.17 (s, 2H, CH_2), 4.38 (s, 4H, 2 CH_2), 7.15-8.32 (m, 14H, Ar-H). ^{13}C-NMR (DMSO-d_6): δ= 33.6, 60.5 (2C), 120.1 (2C), 123.5 (4C), 125.1 (4C),

127.0 (2C), 130.5, 135.2 (2C), 137.1, 150.7, 190.9. MS m/z (%): 404 [M^+] (11.8), 375 (13.7), 341 (28.4), 302 (70.2), 276 (72.1), 264 (15.8), 250 (44.2), 196 (32.0), 177 (10.0), 146 (12.4), 117 (46.7), 91 (**Chart 2**) (100), 76 (33.9), 56 (29.0). Anal. Calcd. for $C_{23}H_{20}N_2O_3S$ (404.48): C, 68.30; H, 4.98; N, 6.93; O, 11.87; S, 7.93. Found: C, 68.19; H, 4.83; N, 6.98; O, 11.99; S, 7.84%.

3.1.14. N,N-dibenzyl-4-(2-formyl-3-mercapto-3-(phenylamino)acryloyl)benzenesulfonamide (47)

To a stirred solution of potassium hydroxide (0.56 g, 0.01 mol) in dimethylformamide (20 mL) was added enaminone **2** (4.34 g, 0.01 mol). After stirring for 2 h, phenylisothiocyanate (1.35 g, 0.01 mol) was added to the resulting mixture. Stirring was continued for 6 h, at room temperature; the mixture was poured onto ice-water (100 g), and acidified with dilute HCl. The solid product formed collected by filtration, washed with water and dried then recrystallized from ethanol/benzene to give **47**. Orange crystals, Yield, 41%; m.p. 198-200°C. IR (KBr, cm^{-1}): ν_{max}= 3370 (NH), 3060 (CH-aromatic), 2950 (CH-aliphatic), 2591 (SH), 1660, 1654 (2CO), 1350, 1155 (SO_2). ^{1}H-NMR (DMSO-d_6): δ= 2.53 (s, 1H, SH, Discharged with D_2O), 4.44 (s, 4H, 2 CH_2), 6.67-7.90 (m, 19H, Ar-H), 9.79 (s, 1H, CHO), 12.13 (s, 1H, NH, Discharged with D_2O). MS m/z (%): 544 [M^++2] (37.0), 442 [M^+] (17.3), 518 (20.3), 433 (30.2), 418 (45.2), 401 (68.2), 343 (35.9), 298 (30.0), 266 (55.1), 248 (88.3), 189 (28.4), 153 (68.2), 132 (11.0), 114 (12.4), 105 (13.7), 66 (100). Anal. Calcd. for $C_{30}H_{26}N_2O_4S_2$ (542.67): C, 66.40; H, 4.83; N, 5.16; O, 11.79; S, 11.82. Found: C, 66.29; H, 4.71; N, 5.09; O, 11.64; S, 11.74%.

3.1.15. N,N-dibenzyl-4-(5-cyano-6-oxo-1,6-dihydropyridin-2-yl)benzenesulfonamide (53)

A mixture of enaminone **2** (4.34 g, 0.01 mol) and 2-cyanoacetamide (0.84 g, 0.01 mol) in glacial acetic acid (50 mL) was heated under reflux for 2 h. during the reflux period, a crystalline solid was separated. The separated solid was filtered off, washed with ethanol and recrystallized from acetic acid to give **53**. White crystals, Yield, 87%; m.p. >360°C. IR (KBr, cm^{-1}): ν_{max}= 3300 (NH), 3100 (CH-aromatic), 2890 (CH-aliphatic), 2210 (CN), 1656 (CO), 1331, 1160 (SO_2). ^{1}H-NMR (DMSO-d_6): δ= 4.42 (s, 4H, 2 CH_2), 7.23-7.99 (m, 16H, Ar-H), 8.78 (s, 1H, NH, Discharged with D_2O). MS m/z (%): 455 [M^+] (10.1), 379 (12.1), 207 (12.5), 196 (**Chart 2**) (100), 184 (19.1), 119 (59.6), 104 (53.6), 76 (46.1). Anal. Calcd. for $C_{26}H_{21}N_3O_3S$ (455.53): C, 68.55; H, 4.65; N, 9.22; O, 10.54; S, 7.04. Found: C, 68.50; H, 4.63; N, 9.48; O, 10.39; S, 7.21%.

3.1.16. N,N-Dibenzyl-4-(4-cyanobenzo[4,5]imidazo[1,2-a]pyridin-1-yl)benzenesulfonamide (56)

A mixture of enaminone **2** (4.34 g, 0.01 mol) and 2-(1H-benzo[d]imidazol-2-yl)acetonitrile (1.57 g, 0.01 mol) in glacial acetic acid (30 mL) was refluxed for 2 h. The solid product which obtained after cooling was collected by filtration and recrystallized from DMF to give **56**. White solid, Yield, 62%; m.p. 311-313°C. IR (KBr, cm^{-1}): ν_{max}= 3101 (CH-aromatic), 2915 (CH-aliphatic), 2222 (CN), 1335, 1153 (SO_2). ^{1}H-NMR (DMSO-d_6): δ= 4.29 (s, 4H, 2 CH_2), 7.23-8.70 (m, 20H, Ar-H). ^{13}C-NMR (DMSO-d_6): δ= 58.3 (2C), 107.7, 113.7 (2C), 119.9, 122.7 (2C), 123.6, 125.8 (2C), 127.1 (2C), 129.2 (4C), 132.5 (2C), 135.4 (4C), 136.7, 138.0 (2C), 140.7, 144.2, 150.0, 152.3, 152.7, 180.2. MS m/z (%): 528 [M^+] (70.9), 505 (32.9), 467 (49.1), 427 (22.9), 385 (34.1), 362 (67.2), 327 (39.6), 295 (34.0), 288 (39.1), 241 (88.2), 221 (37.2), 206 (10.3), 118 (9.8), 91 (**Chart 2**) (100), 76 (19.3). Anal. Calcd. for $C_{32}H_{24}N_4O_2S$ (528.62): C, 72.71; H, 4.58; N, 10.60; O, 6.05; S, 6.07. Found: C, 72.64; H, 4.67; N, 10.73; O, 6.01; S, 6.18%.

3.1.17. N,N-dibenzyl-4-(2,3-dihydro-1H-1,4-diazepin-7-yl)benzenesulfonamide (58)

A mixture of enaminone **2** (4.34 g, 0.01 mol) and ethylenediamine (0.60 g, 0.01 mol) in ethanol (20 ml) was refluxed for 6 h. the obtained solid after cooling was recrystallized from ethanol/benzene to give **58**. White crystals, Yield 33%; m.p. 170-172°C. IR (KBr, cm^{-1}): ν_{max}= 3373 (NH), 3082 (CH-aromatic), 2898 (CH-aliphatic), 1342, 1157 (SO_2). ^{1}H-NMR (DMSO-d_6): δ= 1.61-2.82 (m, 4H, CH_2-CH_2), 3.87 (d, 1H, *J=7.7 Hz*, CH), 4.42 (s, 4H, 2 CH_2), 6.57-8.03 (m, 14H, Ar-H), 8.43 (d, 1H, *J=8.5 Hz*, N=CH), 11.14 (s, 1H, NH, Discharged with D_2O). MS m/z (%): 431 [M^+] (40.9), 392 (33.2), 380 (50.9), 302 (54.2), 272 (77.2), 223 (31.8), 212 (86.3), 198 (96.2), 184 (10.4), 166 (16.2), 152 (86.3), 140 (65.9), 105 (68.4), 66 (100). Anal. Calcd. for $C_{25}H_{25}N_3O_2S$ (431.55): C, 69.58; H, 5.84; N, 9.74; O, 7.41; S, 7.43. Found: C, 69.47; H, 5.73; N, 9.62; O, 7.30; S, 7.33%.

3.1.18. N,N-dibenzyl-4-(3-methyl-2-oxo-2,3-dihydro-1,4-oxazepin-7-yl)benzenesulfonamide (60)

A mixture of enaminone **2** (4.34 g, 0.01 mol) and alanine (0.89 g, 0.01 mol) in ethanol/acetic acid (1:1) (50 mL) containing triethylamine (0.5 mL) as a catalyst, was refluxed for 12 h. the obtained solid was collected and recrystallized from dioxane to give **60**. White solid, Yield, 31%; m.p. 181-182°C. IR (KBr, cm^{-1}): ν_{max}= 3101 (CH-aromatic), 2911 (CH-aliphatic), 1710 (CO), 1344, 1166 (SO_2). ^{1}H-NMR (DMSO-d_6): δ= 1.72 (d, 3H, CH_3), 2.43 (m,

1H, CH-N), 3.77 (d, 1H, *J=7.8 Hz*, CH oxazepine), 4.19 (s, 4H, 2 CH_2), 7.00-7.97 (m, 14H, Ar-H), 8.78 (d, 1H, *J=7.5 Hz*, N=CH oxazepine). MS m/z (%): 460 [M^+] (35.9), 442 (35.7), 391 (73.9), 365 (46.2), 330 (12.4), 301 (94.6), 278 (84.0), 261 (43.6), 227 (42.1), 219 (11.0), 196 (100), 166 (56.3), 151 (9.3), 118 (11.9), 78 (17.8). Anal. Calcd. for $C_{26}H_{24}N_2O_4S$ (460.54): C, 67.81; H, 5.25; N, 6.08; O, 13.90; S, 6.96. Found: C, 67.76; H, 5.14; N, 5.89; O, 13.87; S, 6.83%.

3.1.19. methyl 6-(4-(N,N-dibenzylsulfamoyl)phenyl)-2-oxo-2H-pyran-4-carboxylate (64)

A mixture of enaminone **2** (4.34 g, 0.01 mol), and dimethyl acetylenedicarboxylate (1.42 g, 0.01 mol) in acetic acid (20 mL) was refluxed for 12 h. The reaction mixture then cooled and poured onto ice water. The residue, so formed, was collected then recrystallized from ethanol to give **64**. Yellow crystals, Yield, 50%; m.p. 188-190°C. IR (KBr, cm^{-1}): ν_{max}= 3090 (CH-aromatic), 2960 (CH-aliphatic), 1700 (CO ester), 1697 (CO), 1340, 1158 (SO_2). ^{1}H-NMR (DMSO-d_6): δ= 3.70 (s, 3H, CH_3), 4.46 (s, 4H, 2 CH_2), 6.50-7.89 (m, 16H, Ar-H). MS m/z (%): 489 [M^+] (35.2), 464 (10.6), 409 (71.3), 365 (31.6), 335 (42.6), 270 (33.7), 208 (28.0), 197 (10.0), 183 (21.5), 152 (90.2), 120 (100), 105 (77.4), 66 (18.9). Anal. Calcd. for $C_{27}H_{23}NO_6S$ (489.54): C, 66.24; H, 4.74; N, 2.86; O, 19.61; S, 6.55. Found: C, 66.12; H, 4.90; N, 2.63; O, 19.53; S, 6.75 %.

3.1.20. 4-(5-acetyl-6-methylpyridin-2-yl)-N,N-dibenzylbenzenesulfonamide (66)

To a solution of enaminone **2** (4.34 g, 0.01 mol), in glacial acetic acid, in the presence of ammonium acetate (0.5 g), was added acetylacetone (1.00 g, 0.01 mol). The reaction mixture was heated under reflux for 7 h. The solvent was evaporated under reduced pressure and the oil residue was triturated with ethanol then recrystallized from acetic acid to give **66**. Yellow crystals, Yield, 72%; m.p. >360°C. IR (KBr, cm^{-1}): ν_{max}= 3077 (CH-aromatic), 2870 (CH-aliphatic), 1666 (CO), 1344, 1162 (SO_2). ^{1}H-NMR (DMSO-d_6): δ= 1.98 (s, 3H, CH_3CO), 2.17 (s, 3H, CH_3), 4.13 (s, 4H, 2 CH_2), 7.23-7.36 (m, 10H, Ar-H), 7.62, 7.83 (dd, *J= 8.1 Hz*, 2H, pyridine-$H_{4,5}$), 7.95, 8.58 (dd, *J=6.7 Hz*, 4H, AB-system). MS m/z (%): 470 [M^+] (12.1), 427 (15.6), 410 (25.8), 323 (70.1), 274 (87.4), 261 (33.6), 252 (58.2), 237 (85.1), 261 (90.1), 252 (73.9), 215 (33.8), 190 (17.9), 169 (94.2), 152 (**Chart 2**) (100), 135 (10.0), 111 (18.3), 85 (54.2). Anal. Calcd. for $C_{28}H_{26}N_2O_3S$ (470.58): C, 71.46; H, 5.57; N, 5.95; O, 10.20; S, 6.81. Found: C, 71.38; H, 5.47; N, 5.83; O, 10.18; S, 6.74%.

3.2. Docking and molecular modeling calculations

Docking and molecular modeling calculations were carried out in the department of pharmaceutical chemistry, Faculty of pharmacy, Alexandria University.

3.2.1. Materials

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

3.2.2. General methodology

The coordinates of the X-ray crystal structure of methotrexate (MTX) bound to dihydrofolate reductase (DHFR) enzyme (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 MMFF94X molecular mechanics force field for 8000 interactions. The lowest energy conformation was selected and subjected to an energy minimization using MMFF94X force field.

3.2.3. Docking on the active site of dihydrofolate reductase (DHFR):

The recent determination of the three dimensional co-crystal structure of dihydrofolate reductase complexed 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.

3.*3. In vitro* anticancer screening

The cytotoxicity activity was measured *in vitro* for the newly synthesized compounds using the Sulfo-Rhodamine-B stain (SRB) assay. **[39]** The *in vitro* anticancer screening was done by the pharmacology unit at the National Cancer Institute, Cairo University. Cells were plated in 96-multiwell micro titer plate (10^4 cells/well) for 24h. before treatment with the compound(s) to allow attachment of cell 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 (0, 1, 2.5, 5, and 10 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 48h. at 37 ^{o}C and in atmosphere of 5% CO_2 after 48h., 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 is plotted to get the survival curve for breast tumor cell after the specified time. **[39]** The molar concentration required for 50% inhibition of cell viability (IC_{50}) was calculated and the results are given in (**Table 1**).

Table 1: Anticancer screening of the newly synthesized compounds against human breast cell line (MCF7)

Compd. No.	Cytotoxicity (IC_{50})	
	IC_{50}[a] (µg/mL)	IC_{50}[b] (µM.)
1	26.6	70.3
2	48.5	111.9
3	22.2	44.7
5	14.3	30.6
9	63.5	127.9
15	90.2	146.9
16	71.4	122.7
22	25.7	50.3
28	54.4	119.5
34	44.0	85.7
35	50.4	117.3
36	61.7	153.1
37	76.4	140.0
46	57.8	143.2
47	43.4	80.1
53	71.7	157.5
56	89.9	170.3
58	27.2	63.1
60	27.7	60.2
64	59.3	121.3
66	7.4	15.7
Methotrexate	33.9	74.6

[a] Mean of three results obtained from three experiments.
[b] IC_{50} value: Concentration causing 50% inhibition of cell viability.

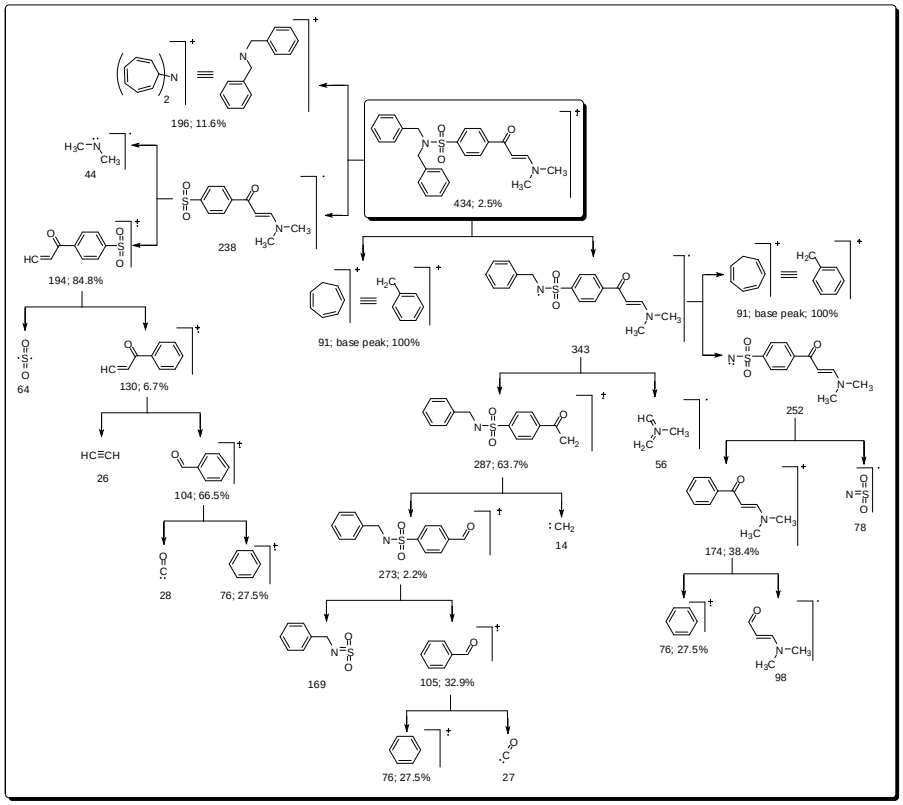


Chart (1): Fragmentation Pattern for Enaminone **2**

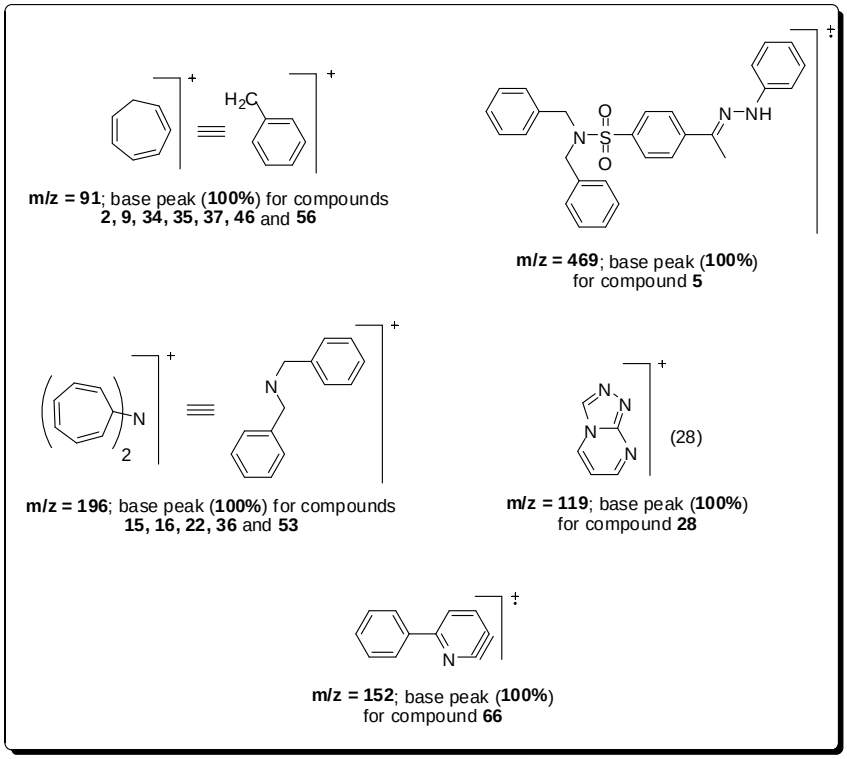


Chart (2): Base Peaks for Compounds **2, 5, 9, 15, 16, 22, 28, 34-37, 46, 53, 56** and **66**

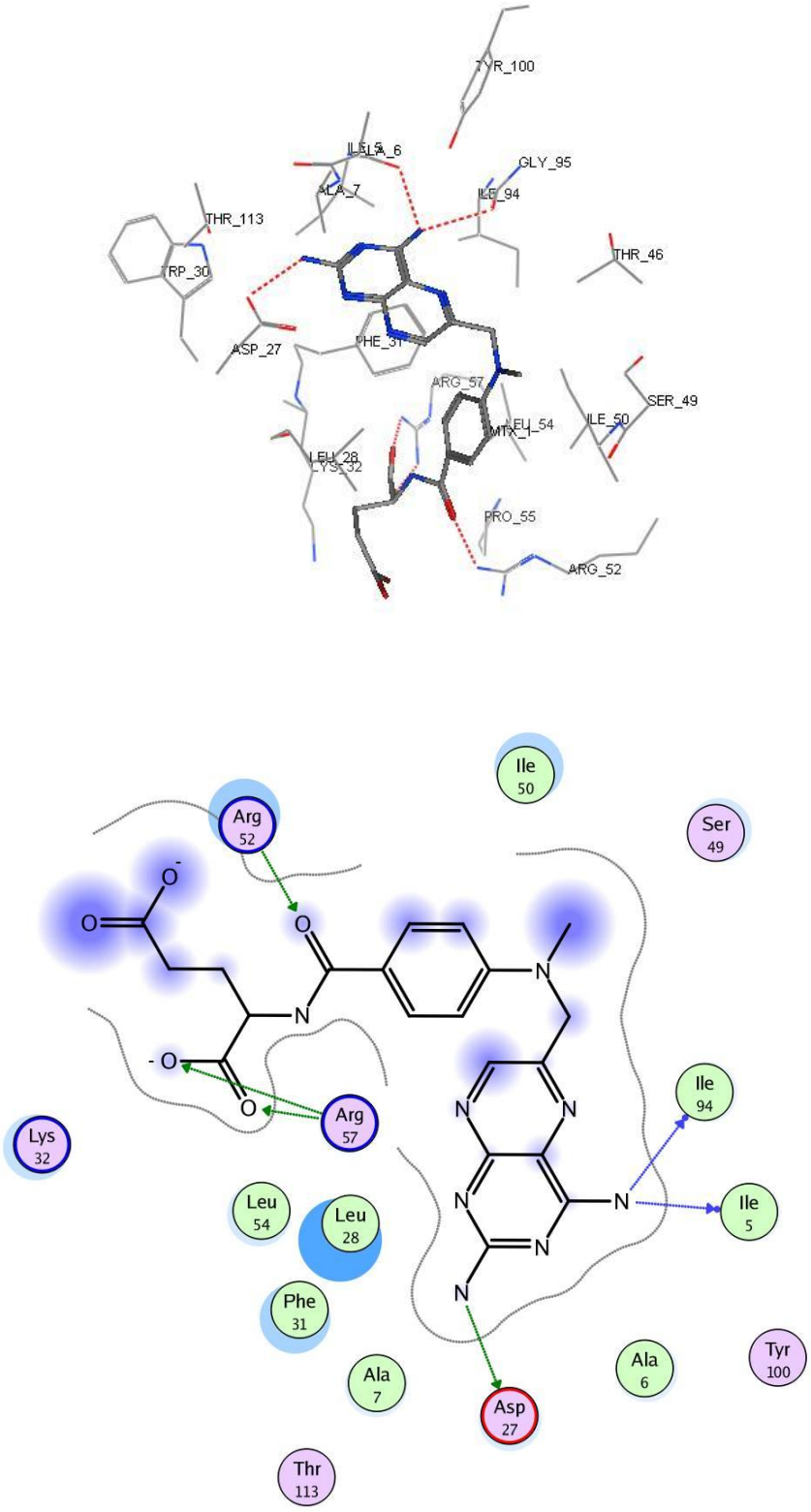


Fig (1) Docking of MTX in the active site of DHFR

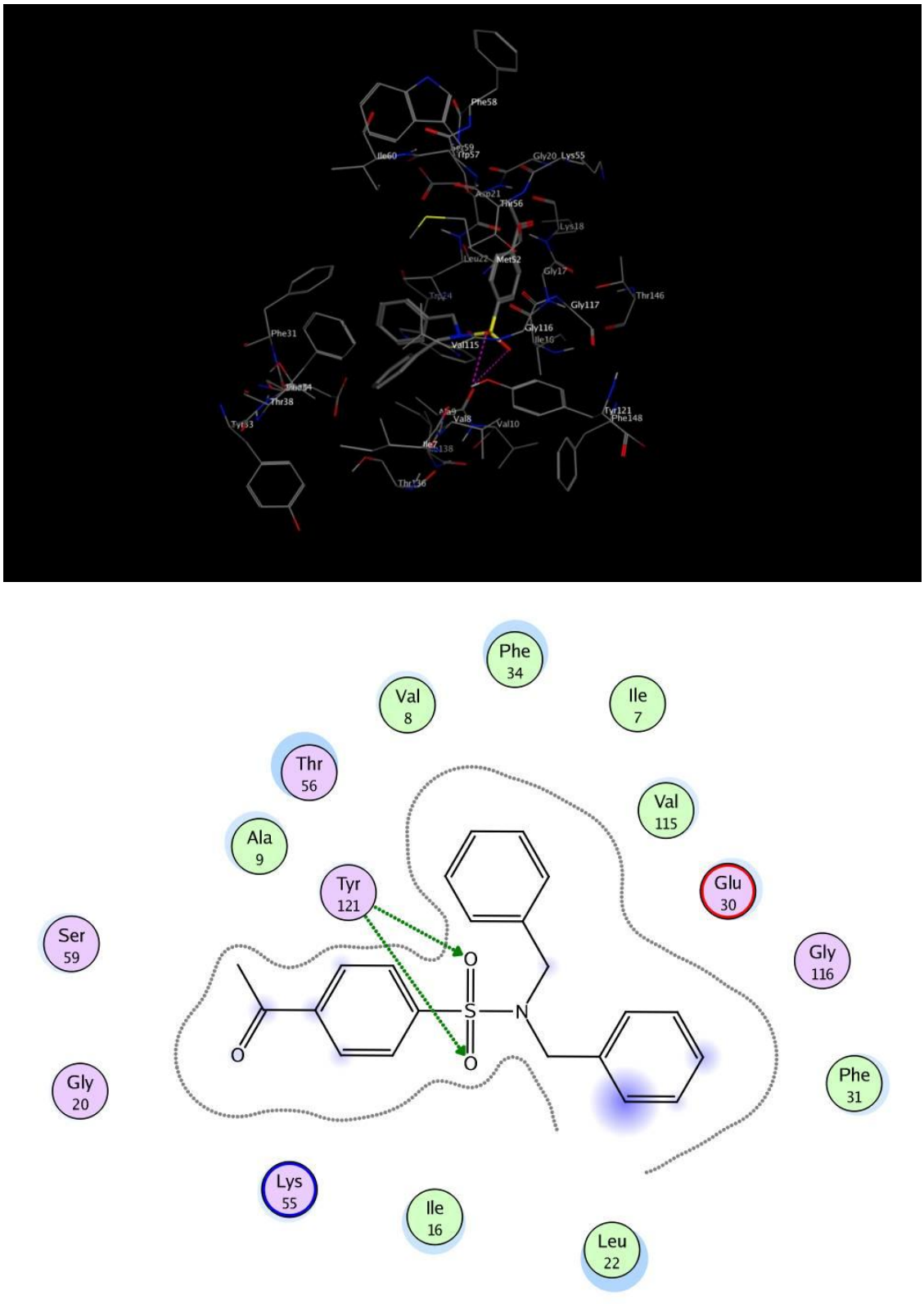


Fig. (2) Docking of compound 1 into DHFR

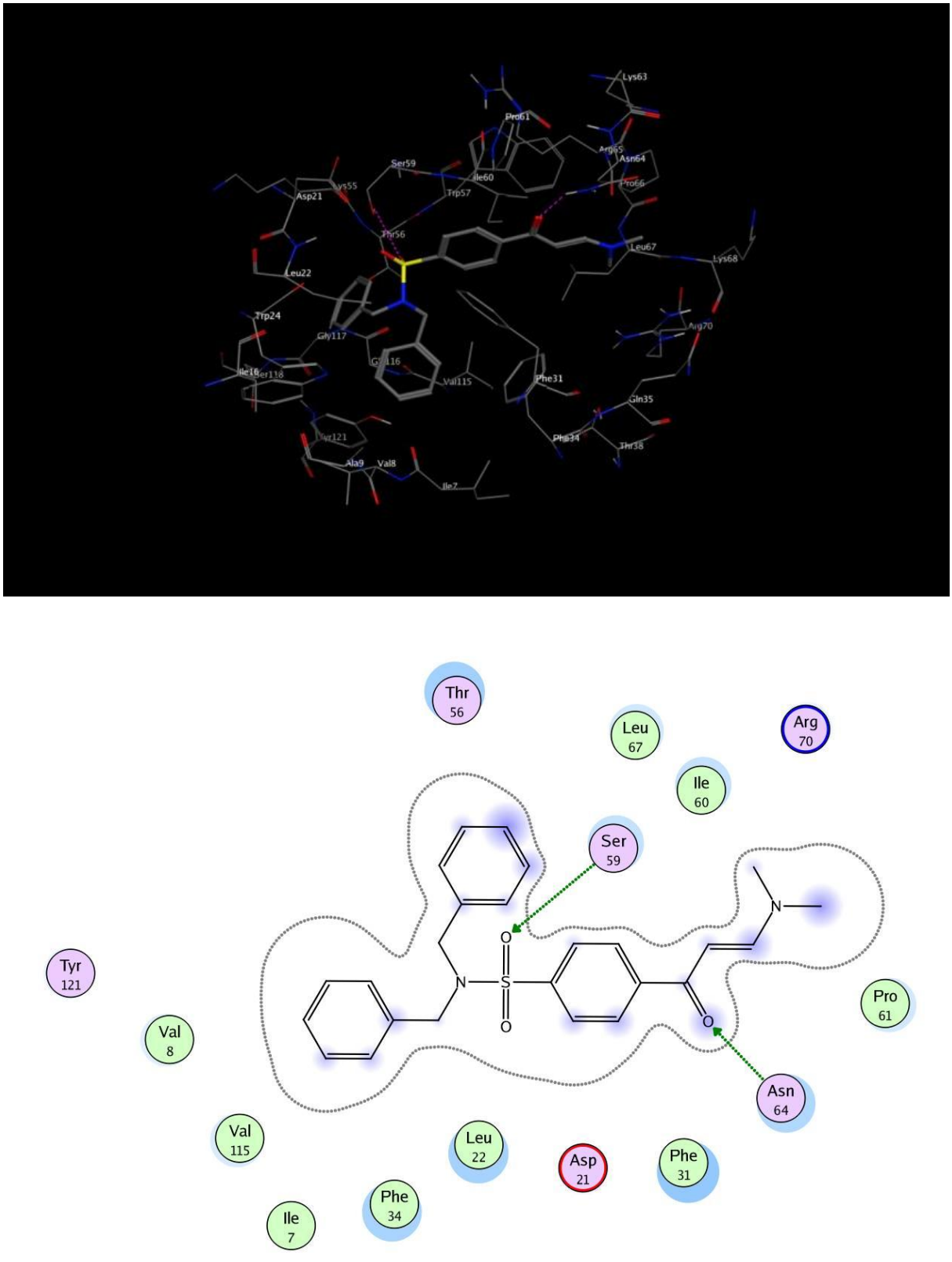
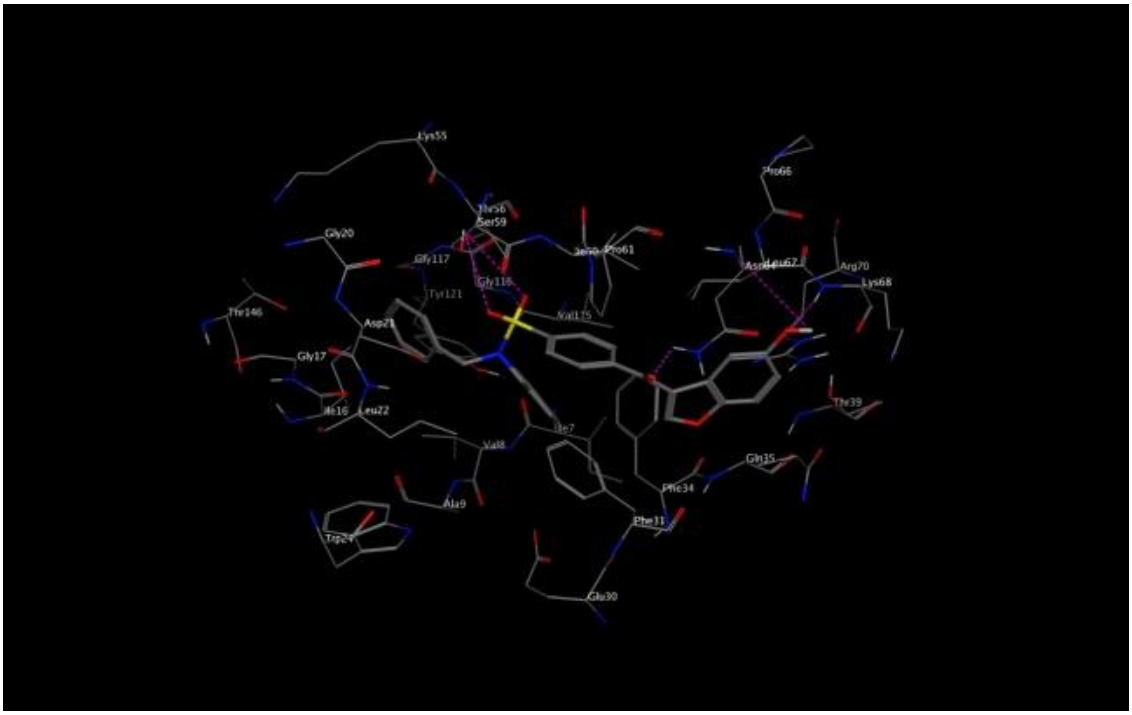


Fig. (3) Docking of compound 2 into DHFR



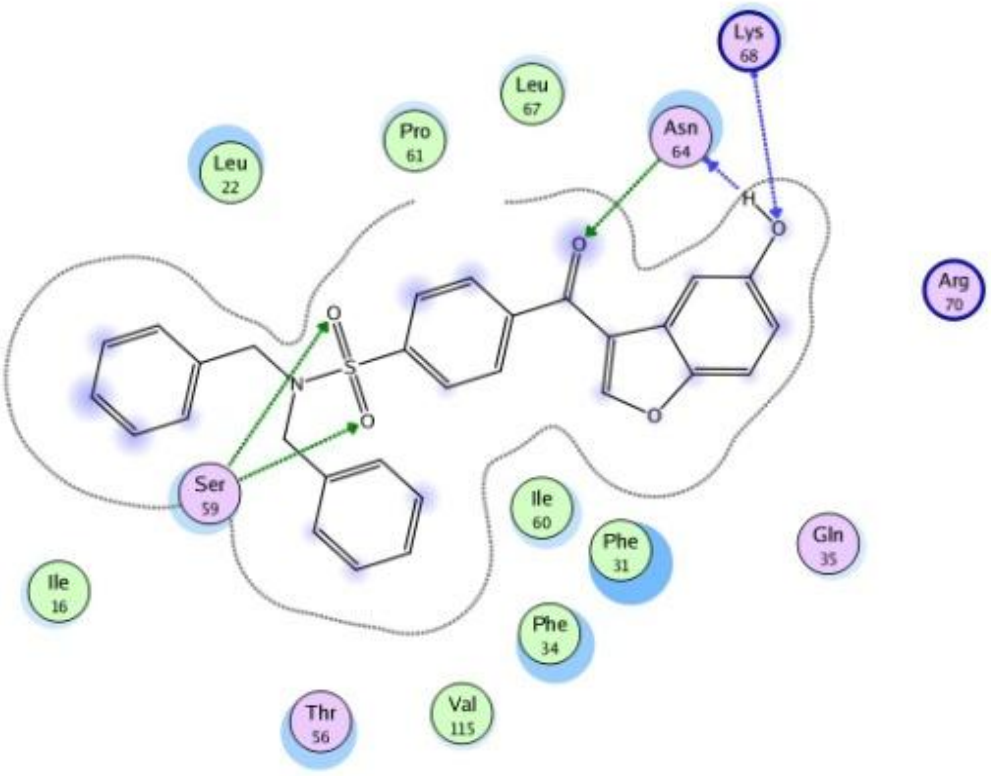
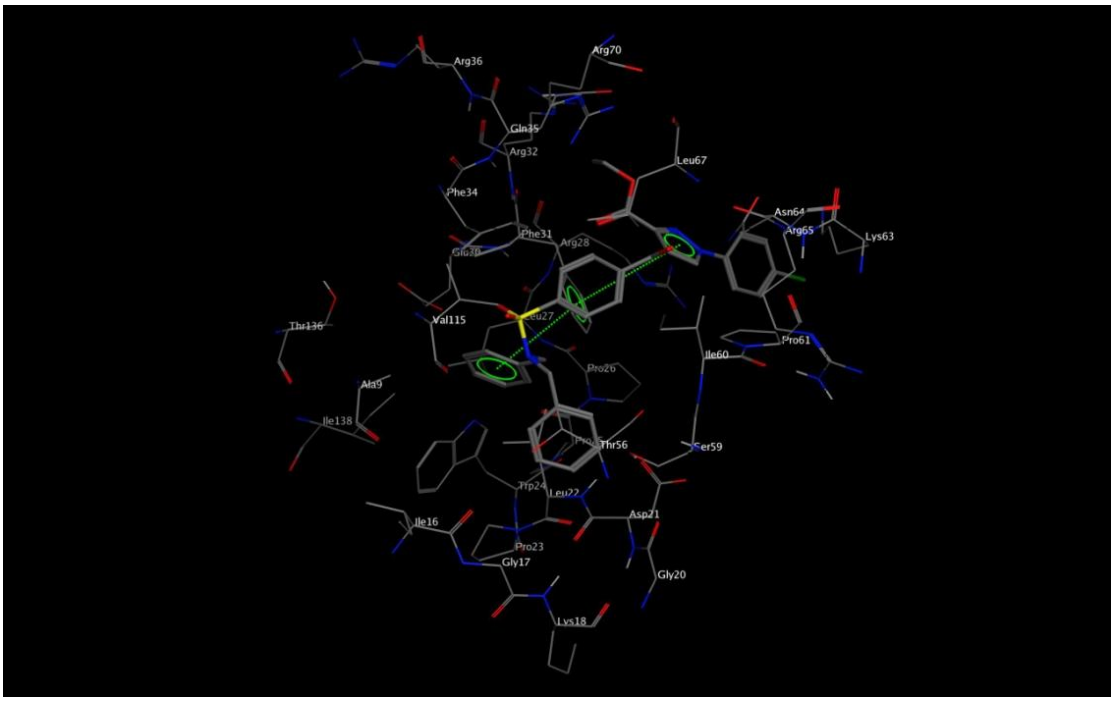


Fig. (4) Docking of compound 9 into DHFR



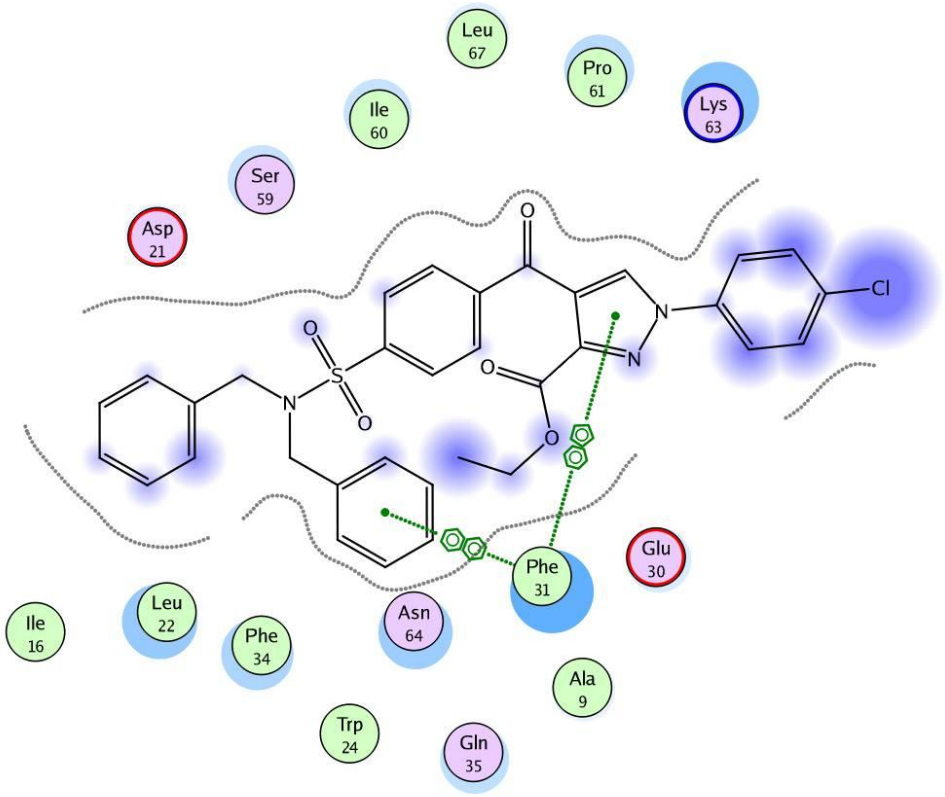
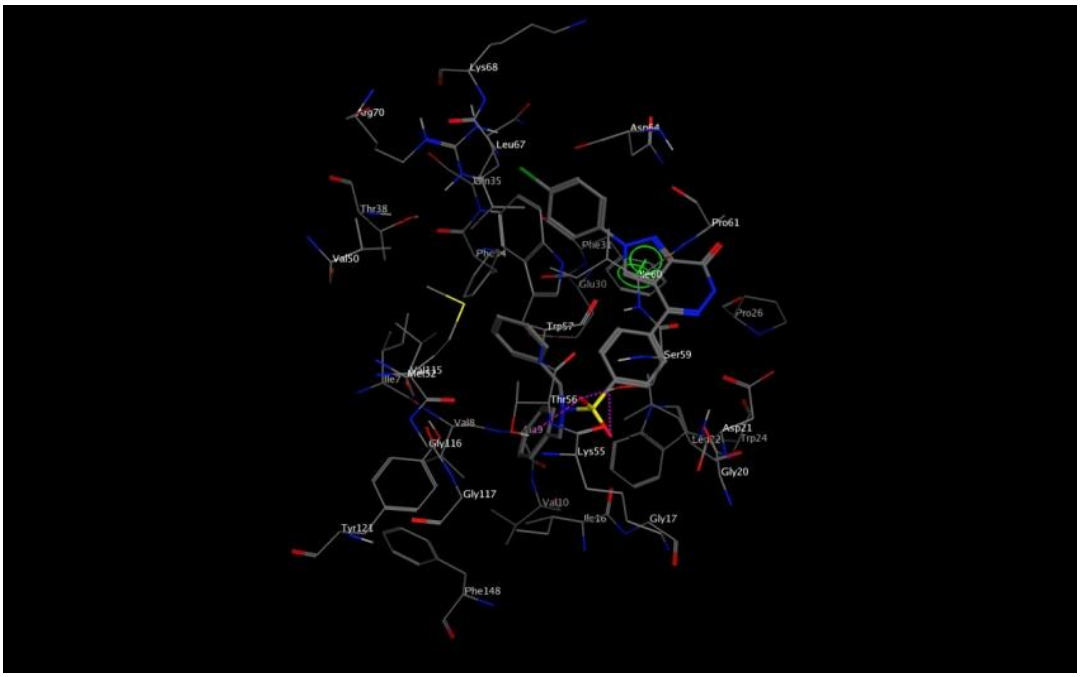


Fig. (5) Docking of compound 15 into DHFR



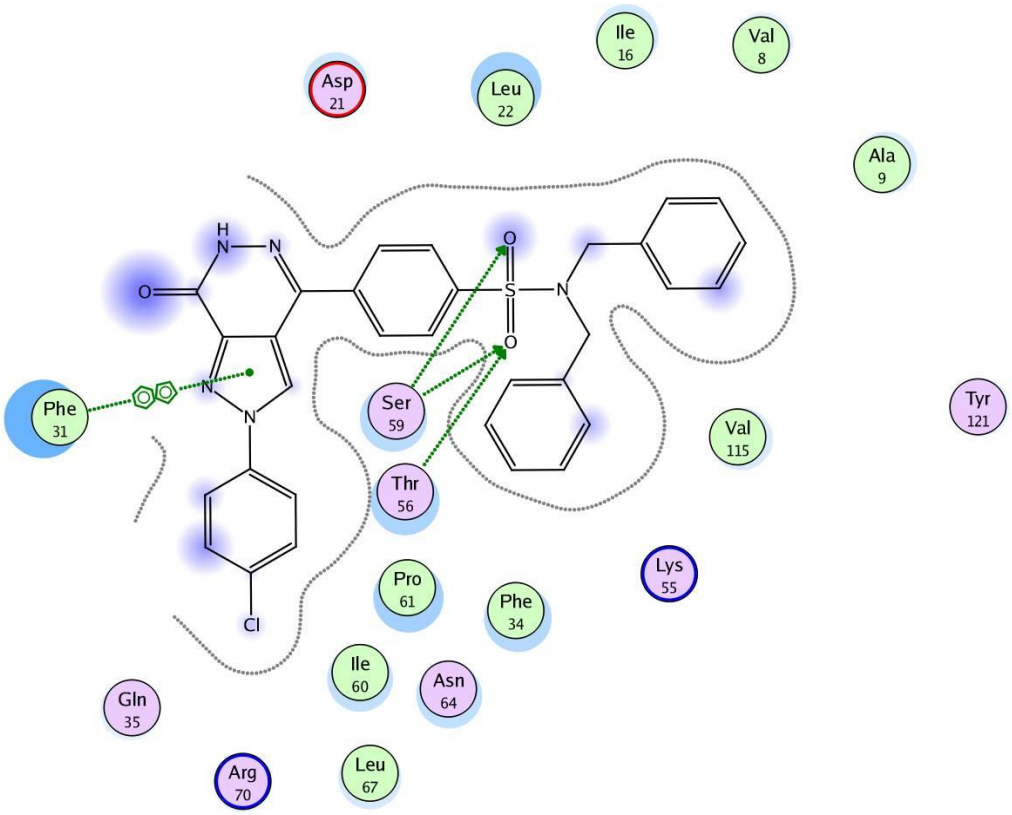
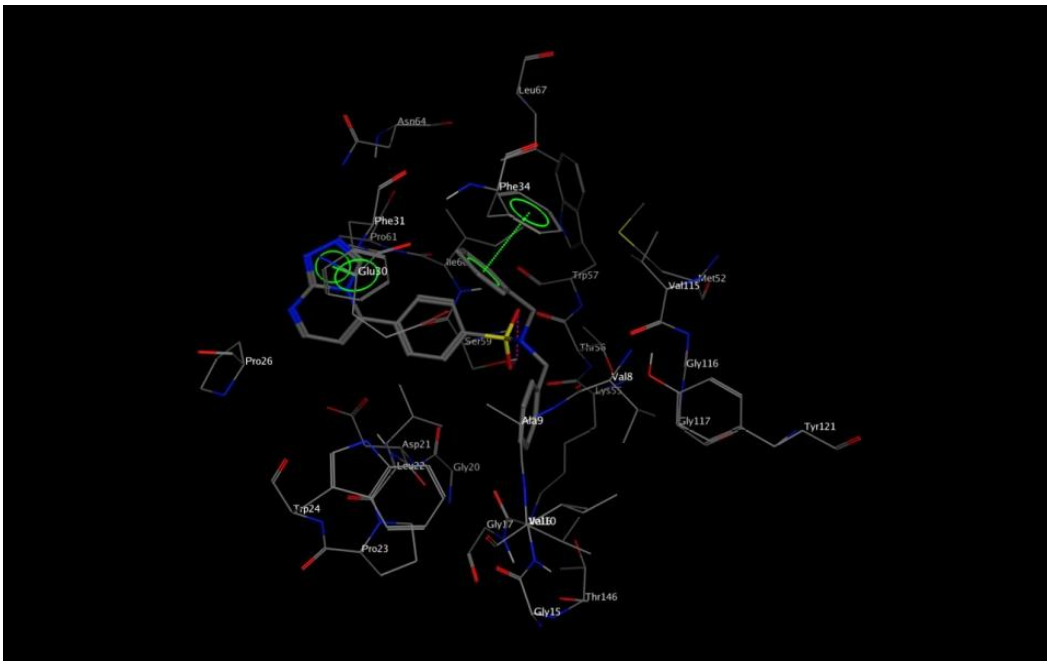


Fig. (6) Docking of compound 16 into DHFR



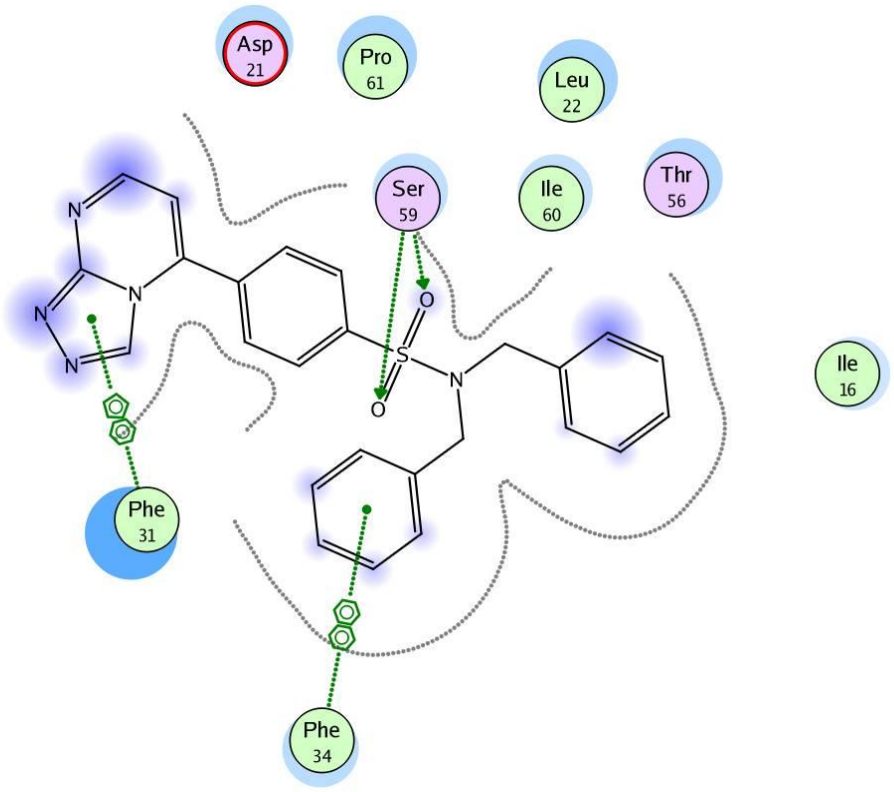
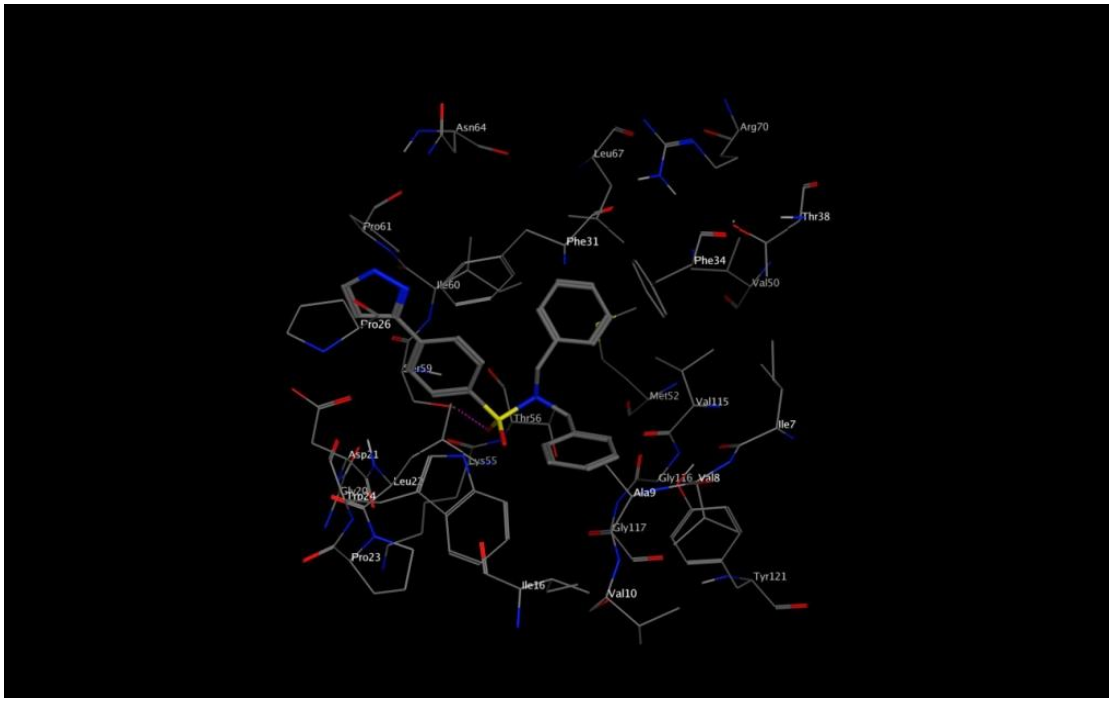


Fig. (7) Docking of compound 28 into DHFR



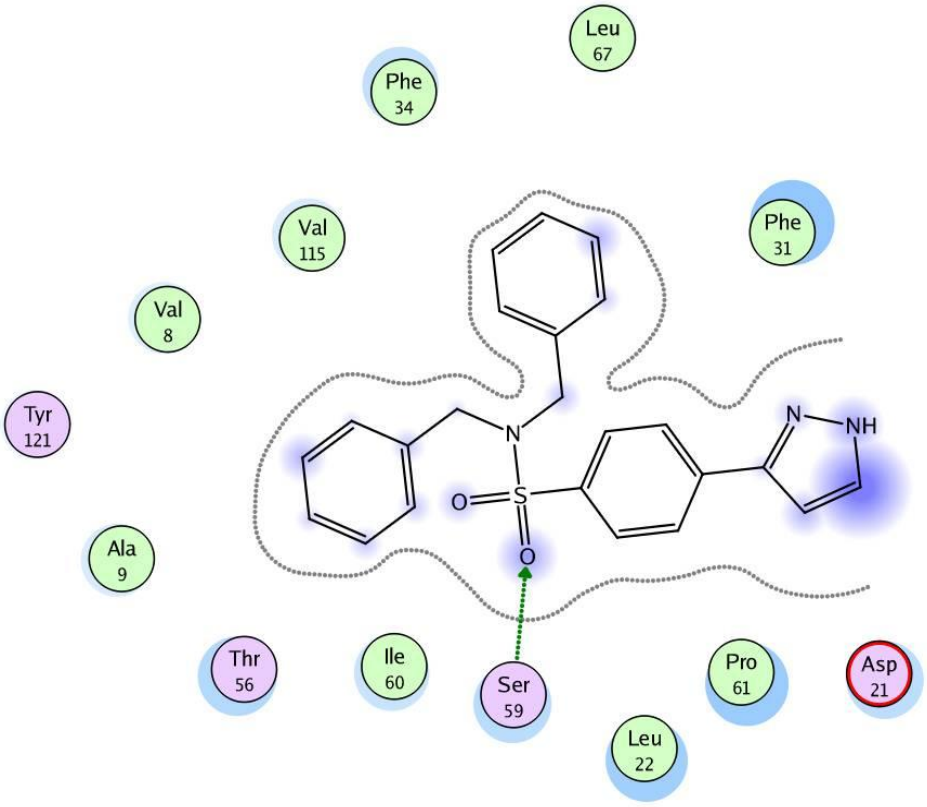
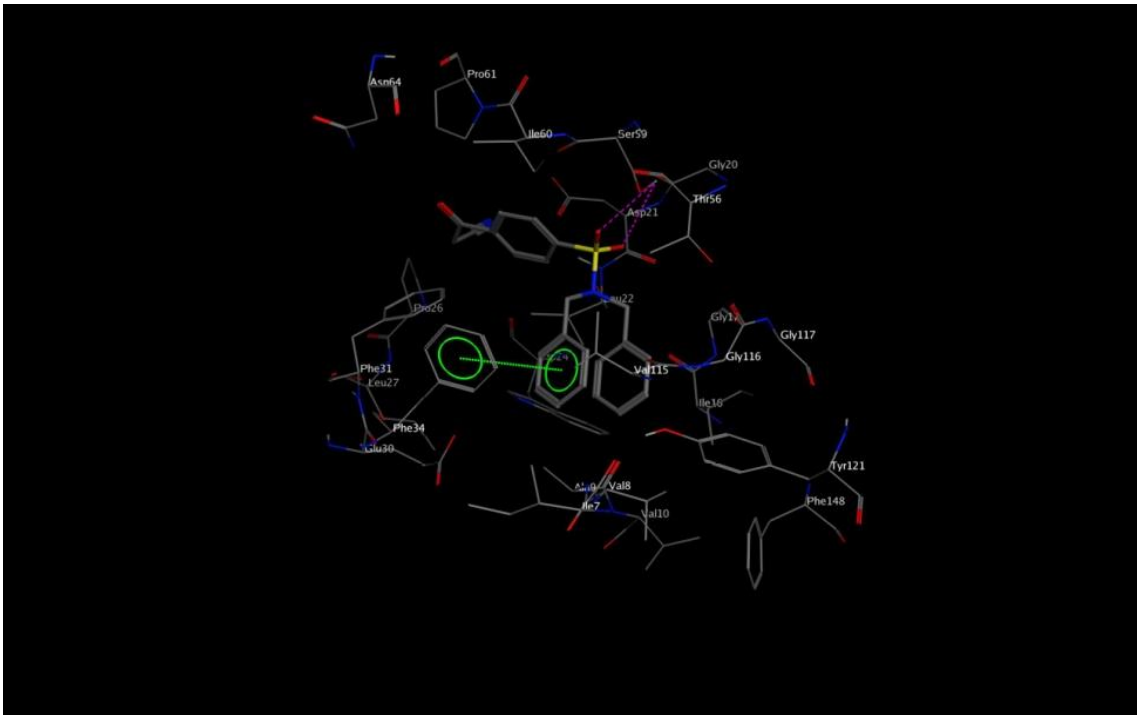


Fig. (8) Docking of compound 36 into DHFR



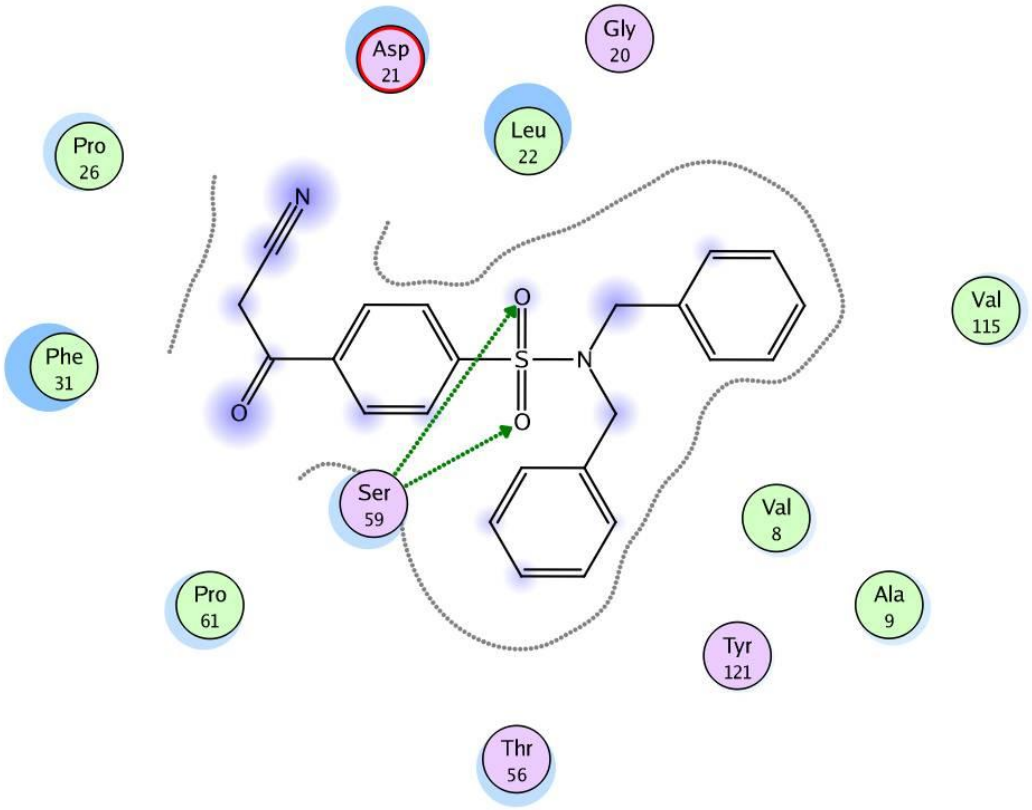
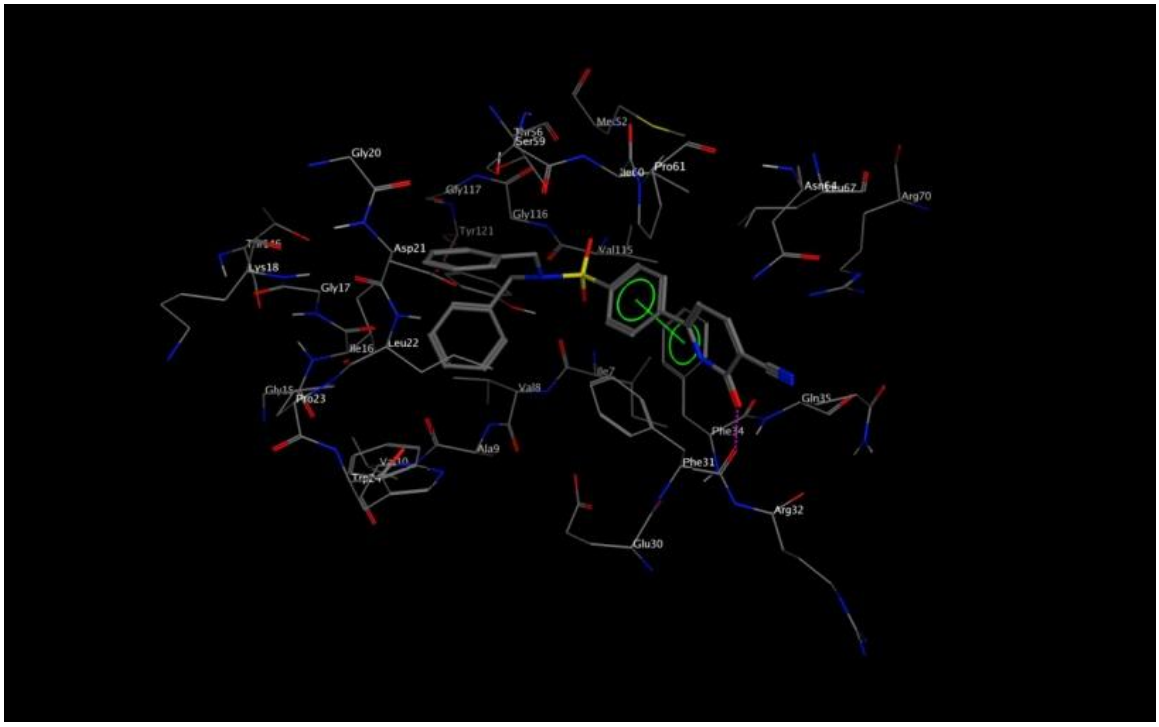


Fig. (9) Docking of compound 46 into DHFR



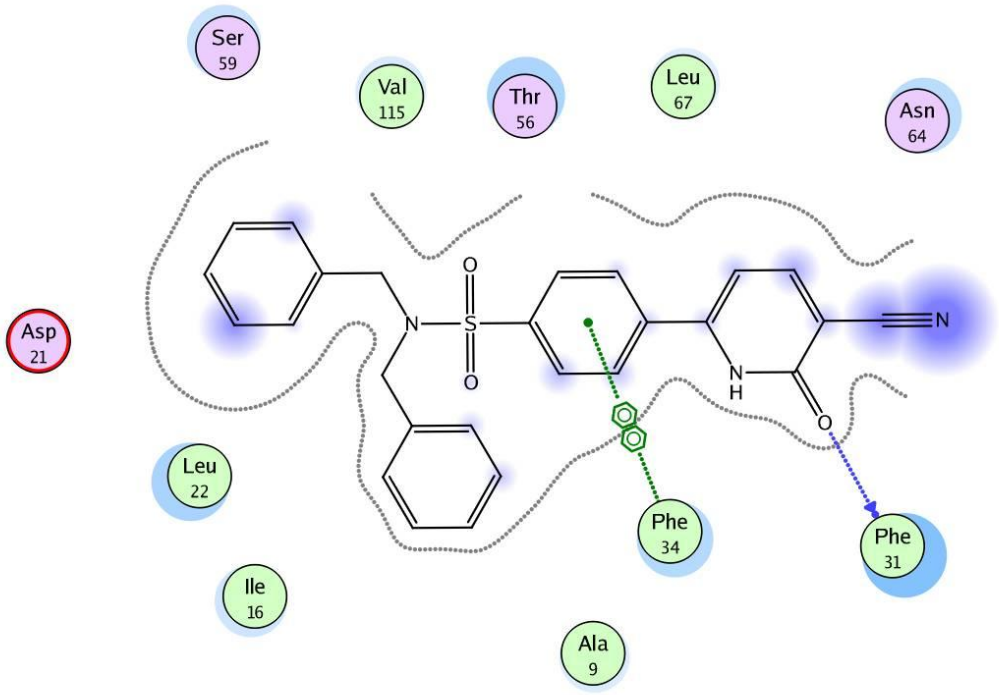
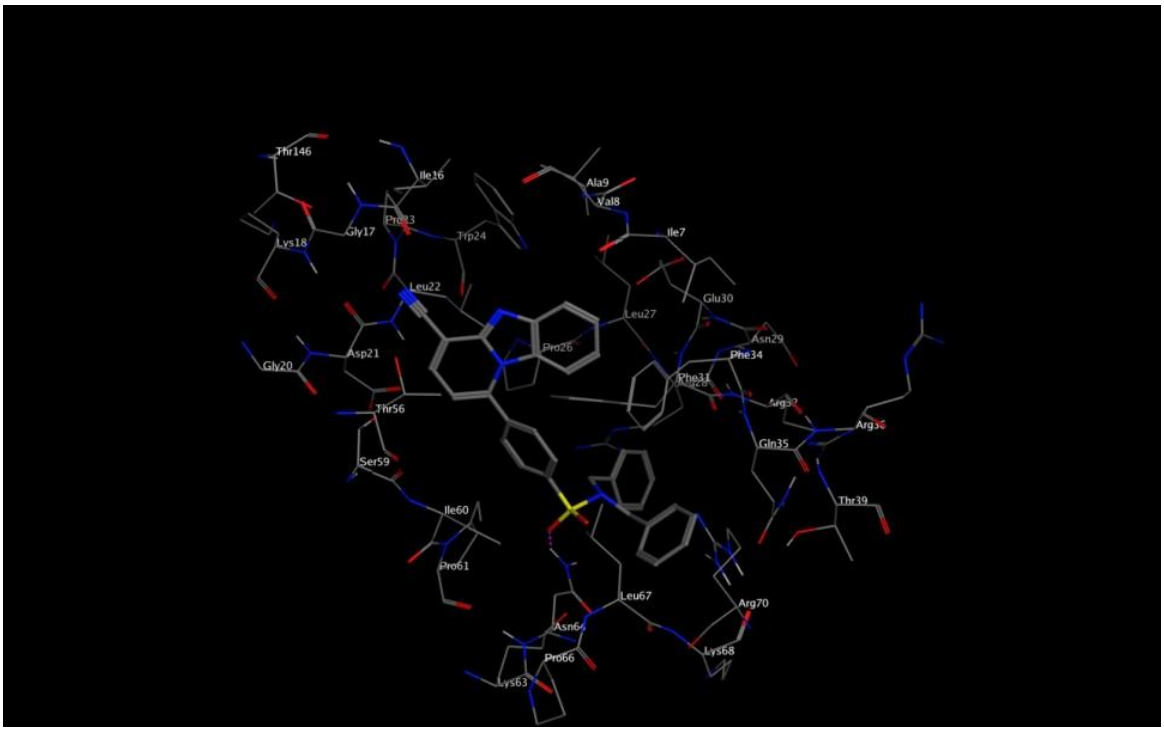


Fig. (10) Docking of compound 53 into DHFR



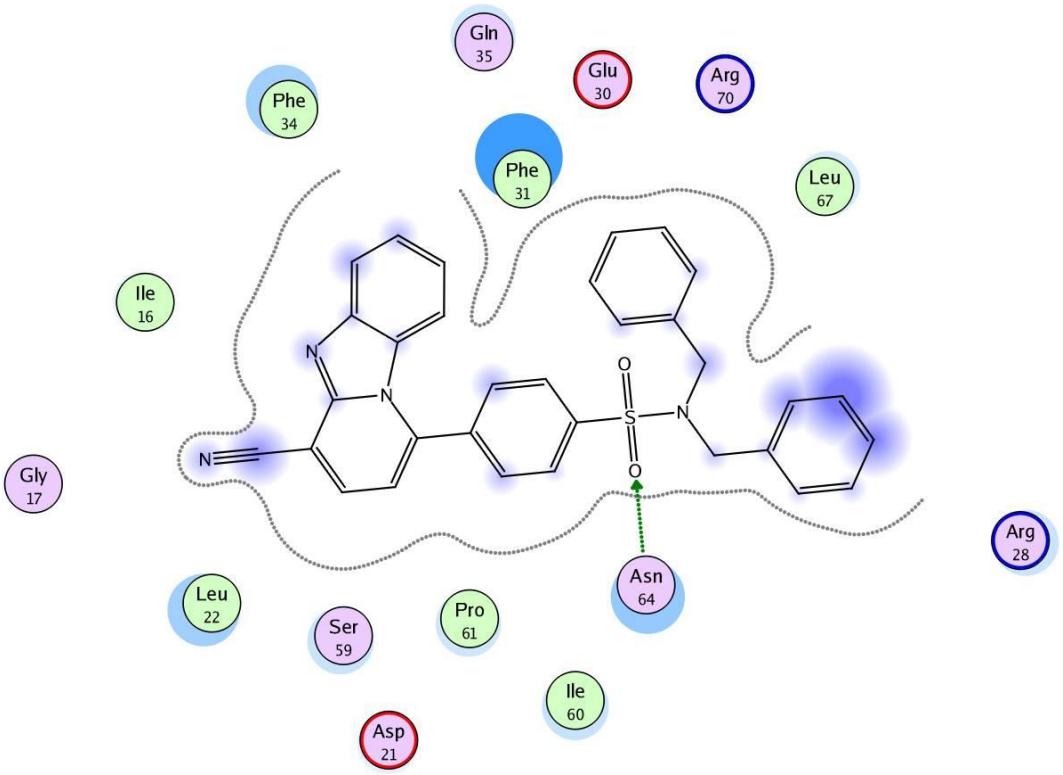
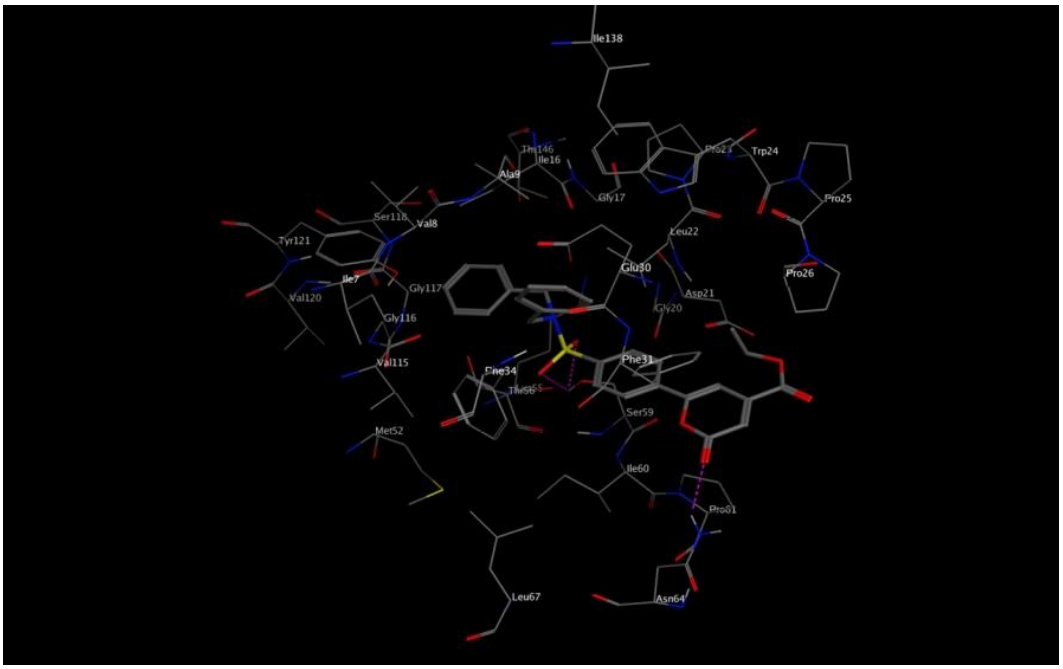


Fig. (11) Docking of compound 56 into DHFR



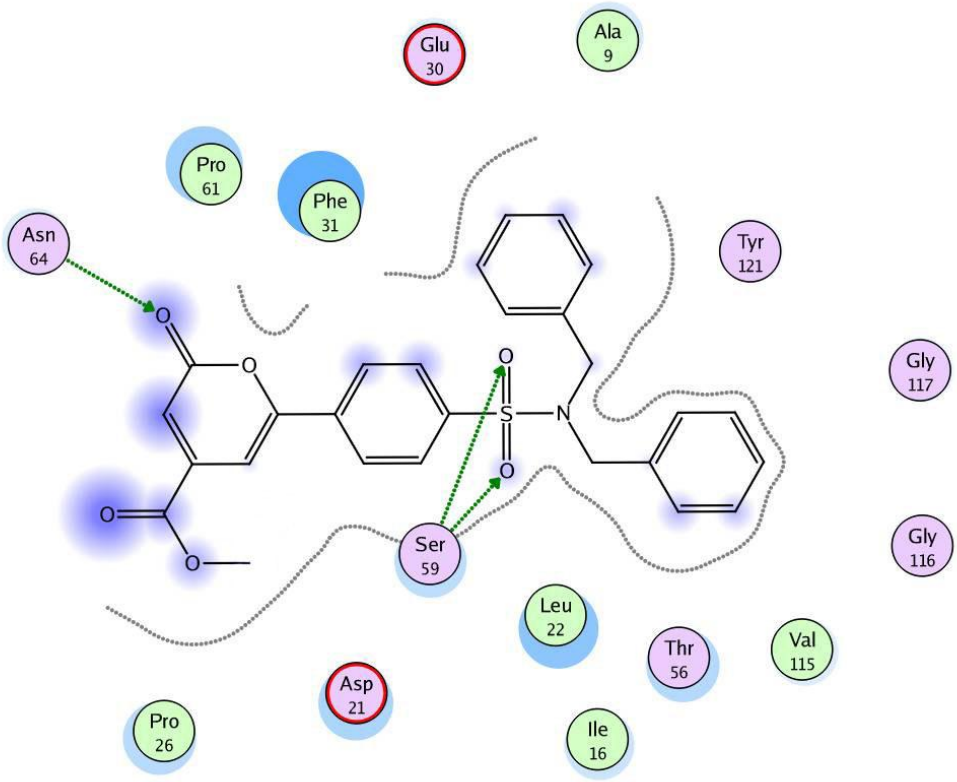


Fig. (12) Docking of compound 64 into DHFR

References

1. A. A. Fadda, M. S. Amine, M. M. H. Arief, E. K. Farahat, *Pharmacologia*; **5**, 1 (**2014**)
2. Y. Liu, B. Huang, X. Cao, D. Wu, J. Wan, ***RSC Adv.***, **4**, 37733 (**2014**)
3. M. A. Abbasi, N. Raza, A. Rehman, S. Rasool, K. M. Khan, M. Ashraf, U. Alam, R. Nasar, World J. Pharm. Sci., **2**, 161 (**2014**)
4. K. Zessel, S. Mohring, G. Hamscher, M. Kietzmann, J. *Stahl*, *Chemosphere*, **100**, 167 (**2014**)
5. E. C. Tavares, M. M. M. Rubinger, C. H. C. Zacchi, S. A. Silva, M. R. L. Oliveira, S. Guilardi, A. F. C. Alcantara, D. Pilo-Veloso, L. Zambolim, J. *Mol. Struc.*, **1067**, 43 (**2014**)
6. M. Kumar, B. Narasimhan, P. Kumar, K. Ramasamy, V. Mani, R. K. Mishra, A. Abdul Majeed, *Arabian J. Chem.*, **7**, 436 (**2014**)
7. R. A. Siegel, J. *Controlled Release*, **190**, 337 (**2014**)
8. A. M. Alafeefy, M. Ceruso, A. S. Al-Tamimi, S. D. Prete, C. Capasso, C. T. Supuran, *Bioorg. Med. Chem.*, **22**, 5133 (**2014**)
9. M. Ceruso, S. D. Prete, Z. AlOthman, S. M. Osman, A. Scozzafava, C. Capasso, C. T. Supuran, *Bioorg. Med. Chem. Lett.*, **24**, 4006 (**2014**)
10. G. Nasr, A. Cristian, M. Barboiu, D. Vullo, J. Winum, C. T. Supuran, *Bioorg. Med. Chem.*, **22**, 2867 (**2014**)
11. D. Nematollahi, S. S. H. Davarani, P. Mirahmadpour, F. Varmaghani, *Chinese Chem. Lett.*, **25**, 593 (**2014**)
12. A. Thakare, K. Bhate, R. Kathariya, *Acta Anaesthesiologica Taiwanica*, **52**, 59 (**2014**)
13. V. Akurathi, L. Dubois, S. Celen, N. G. Lieuwes, S. K. Chitneni, B. J. Cleynhens, A. Innocenti, C. T. Supuran, A. M. Verbruggen, P. Lambin, G. M. Bormans, *Eur. J. Med. Chem.*, **71**, 374 (**2014**)
14. M. Chen, K. Wen, X. Tao, J. Xie, L. Wang, Y. Li, S. Ding, H. Jiang, *Protein Exp. Purification*, **100**, 19 (**2014**)
15. D. Nematollahi, S. S. H. Davarani, P. Mirahmadpour, F. Varmaghani, *Chinese Chem. Lett.*, **25**, 593 (**2014**)
16. M. F. Zayed, M. H. Hassan, *Saudi Pharma. J.*, **22**, 157 (**2014**)
17. M. Mojzych, A. Bielawska, K. Bielawski, M. Ceruso, C. T. Supuran, *Bioorg. Med. Chem.*, **22**, 2643 (**2014**)
18. T. Liu, B. Huang, P. Zhan, E. D. Clercq, X. Liu, *Eur. J. Med. Chem.*, **86**, 481 (**2014**)
19. M. S. Bashandy, M. S. Alsaid, R. K. Arafa, M. M. Ghorab, *J. Enzyme Inhib. Med. Chem.*, **29**, 619 (**2014**)
20. M. S. Bashandy, M. S. Al-Said, S. I. Al-qasoum, M. M. Ghorab, *Arzneimittel-Forschung Drug Res.*, **61**, 521 (**2011**)
21. M. S. Al-Said, M. S. Bashandy, M. M. Ghorab, *Arzneimittel-Forschung Drug Res.*, **61**, 527 (**2011**)
22. M. S. Bashandy, S. M. Hassan, O. A. Fathalla, A. F. Eweas, A. H. Khalel, *J. Egy. Chem.*, **55**, 6 (**2012**)
23. M. S. Al-Said, M. S. Bashandy, S. I. Al-qasoumi, M. M. Ghorab, *Eur. J. med. Chem.*, **46**, 137 (**2011**)
24. M. S. Bashandy, *Asian J. Chem.*, **23**, 3191 (**2011**)
25. F. M. El-Taweel, M. H. Elnagdi, *J. Heterocyclic Chem.*, **38**, 981 (**2001**)
26. N. F. Eweiss, A. O. Abdelhamide, *J. Heterocyclic. Chem.*, **17**, 1713 (**1980**)
27. M. Nagakura, T. Ota, N. Shimadzu, K. Kawamura, Y. Eto, Y. Wada, *J. Med. Chem.*, **22**, 48 (**1979**)
28. A. S. Shawali, A. O. Abdelhamide, *Bull. Soc. Japan*, **49**, 321 (**1976**)
29. K. M. Al-Zaydi, *Molecules*, **8**, 541 (**2003**).
30. H. Biere, I. Bottcher, J. Kapp, *Arch. Pharm.*, **316**, 608 (**1983**).
31. F. M. A. El-Taweel, M. H. Elnagdi, *J. Heterocycl. Chem.*, **38**, 981 (**2001**)
32. K. M. Dawood, *J. Heterocycl. Chem.*, **42**, 221 (**2005**)
33. C. Mustazza, M. Del Giudice, A. Borioni, F. Gatta, *J. Heterocyclic Chem.*, **38**, 1119 (**2001**).
34. M. Regitz, A. Liedhegener, Liebigs Ann. Chem., 118 (**1967**)
35. B. Al-Saleh, M. M. Abdel-Khalik, A. M. Eltoukhy, M. H. Elnagdi, *J. Heterocycl. Chem.*, **39**, 1035 (**2002**)
36. Q. R. Du, D. D. Li, Y. Z. Pi, J. R. Li, J. Sun, F. Fang, W. Q. Zhong, H. B. Gong, H. L. Zhu, *Bioorg. Med. Chem.*, **21**, 2286 (**2013**)
37. K. N. Rao, S. R. Venkatachalam, *Bioorg. Med. Chem.*, **7**, 1105 (**1999**)
38. S. Vilar, G. Cozza, S. Moro, *Current topics med. Chem.*, **8**, 1555 (**2008**)
39. P. Srehan, R. Storegng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney, M. R. Boyd, *J. Natl. Cancer Inst.*, **82**, 1107 (**1990**)