HETEROCYCLES, Vol. 92, No. 3, 2016, pp. 431 - 469. © 2016 The Japan Institute of Heterocyclic Chemistry
Received, 1st December, 2015, Accepted, 15th January, 2016, Published online, 20th January, 2016
DOI: 10.3987/COM-15-13384

SYNTHESIS, MOLECULAR DOCKING AND ANTI-HUMAN BREAST CANCER ACTIVITIES OF NOVEL THIAZOLYLACETONITRILES AND THIAZOLYLACRYLONITRILES AND THEIR DERIVATIVES CONTAINING BENZENESULFONYLPYRROLIDINE MOIETY

Mahmoud S. Bashandy[1,2*] and Shimaa M. Abd El-Gilil[3]

[1]Chemistry Department, Faculty of Science (Boys), Al-Azhar University, 11884, Nasr City, Cairo, Egypt

[2]Chemistry Department, University College in Al-Jamoum, Umm Al-Qura University, 21955, Makkah, Saudi Arabia

[3]Department of Organic Chemistry, Faculty of Pharmacy-Girls, Al-Azhar University, Cairo, Egypt

*****Author to whom correspondence should be addressed; E-mail: bashandy_sci@yahoo.com

Abstract – This article describes the synthesis of some novel sulfonamides having the biologically active, thiazole **3**, **8**-**10**, **13**, **19**, **20**, **24**, **30**, **31**, **35**-**41**, pyrazolo[5,1-*c*][1,2,4]triazine **5**, 1*H*-1,2,4-triazole **6**, thiazolo[3,2-*a*]pyridine **14**, chromen-2-one **16**, benzo[*f*]chromen-3-imine **17**, benzo[*f*]chromen-3-one **18**, triazolo[4,3-*a*]pyrimidine **22**, pyrazolo[1,5-*a*]pyrimidine **23**, isoxazole **26**, 2,4-diaminopyrimidine **27**, benzo[4,5]imidazo[1,2-*a*]pyridine **28**, imidazolidine **32** and 1*H*-benzo[*d*]imidazolidene **33** moieties, starting with 2-(4-(4-(pyrrolidin-1-ylsulfonyl)phenyl)thiazol-2-yl)acetonitrile (**2**), which was prepared from cyclocondensation of phenacyl bromide derivative **1** with 2-cyanoethanethio-amide. 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 compounds were tested *in-vitro* antihuman breast cancer cell line (MCF7). Compounds **18**, **8**, **41** and **28** with IC_{50} values of 48.01, 49.11, 49.27 and 49.78 μM, respectively, exhibited better activity than doxorubicin (DOX) as a reference drug with IC_{50} value of 68.6 μM. Molecular Operating Environment (MOE) performed virtual screening using molecular docking studies of the synthesized compounds.

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

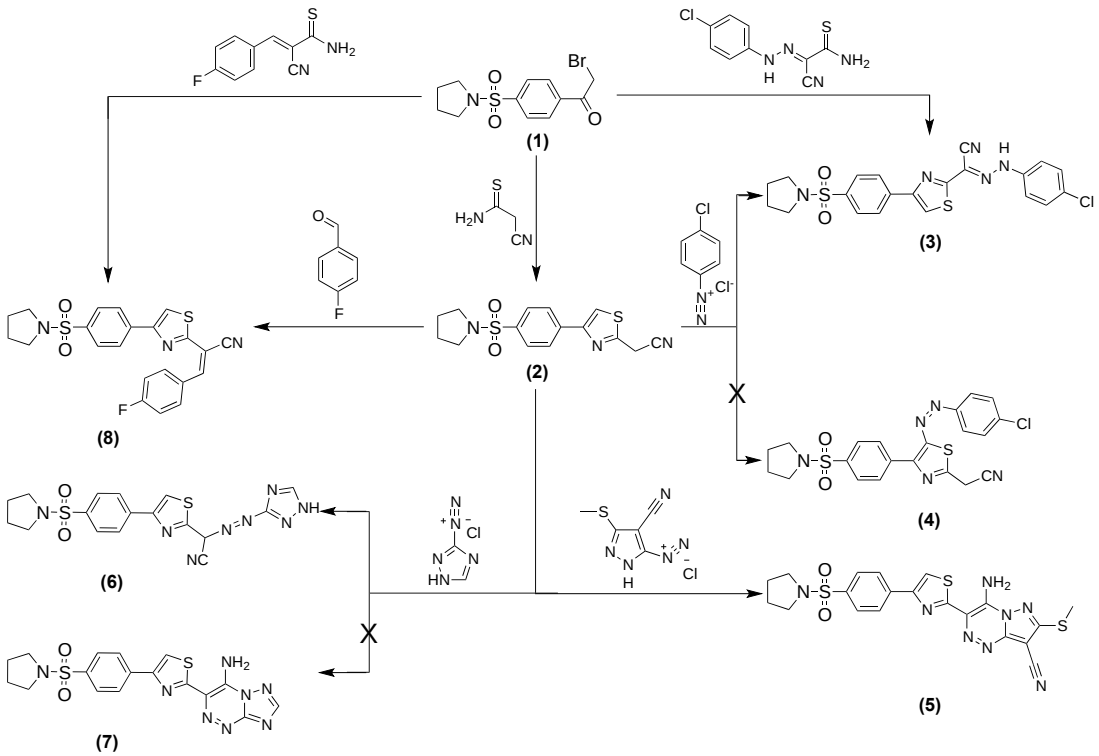
INTRODUCTION

Thiazole derivatives have attracted a great deal of interest owing to their antibacterial,[1] antifungal,[2] antiinflammatory,[3] and antiviral[4-6] activities. They are also useful as antiallergic,[7] anthelmintic[8] agents and as sedative hypnotics.[9] In addition to being used in the pharmaceutical industry, thiazoles also find a wide application in the dye[10] and photographic industry.[10] Sulfonamides also have been demonstrated to possess antibacterial,[11-13] antifungal,[14] insulin-releasing,[15,16] carbonic anhydrase inhibitory,[17-19] hypoglycemic,[20] anesthetic,[21] anti-inflammatory,[22,23] and anti-carcinogenic[24,25] activities. In view of these reports and as a continuation of previous work[26-30] directed towards the synthesis of substituted heterocycles, incorporating benzenesulfonamide with anticipated biological activities. Therefore, this article reports new and convenient methods for the synthesis of heterocyclic ring systems that are required to medicinal chemistry utilizing 2-(4-(4-(pyrrolidin-1-ylsulfonyl)phenyl)thiazol-2-yl)acetonitrile (**2**) as starting material and investigated their antihuman breast cancer activities.

RESULTS AND DISCUSSION

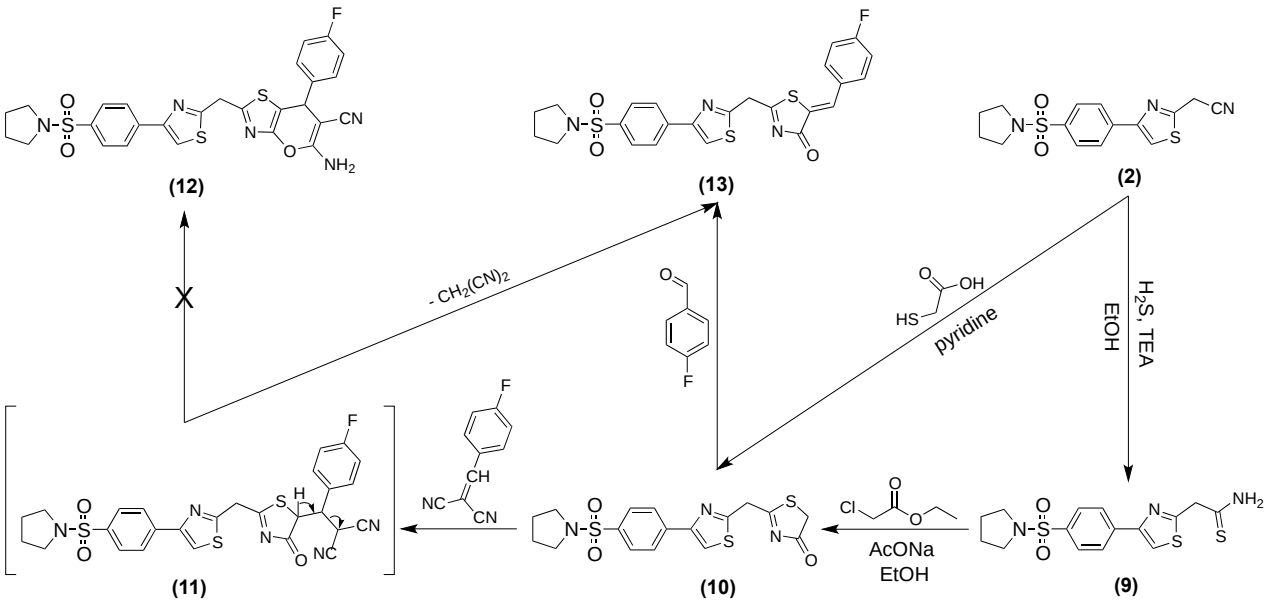
When 2-bromo-1-(4-(pyrrolidin-1-ylsulfonyl)phenyl)ethanone (**1**) was treated with 2-cyanoethanethio-amide in refluxing ethanol afforded a single product identified as 2-(4-(4-(pyrrolidin-1-ylsulfonyl)phenyl)thiazol-2-yl)acetonitrile (**2**) on the basis of elemental analysis and spectral data. Thus, IR spectrum of compound **2** revealed absorption band at ν 2220 cm^{-1} due to cyano group. ^1H NMR spectrum showed singlet signal at δ 4.01 ppm due to active methylene protons and singlet signal at δ 7.52 ppm for H_5 of thiazole. ^{13}C NMR spectrum showed signal at δ 18.9 ppm for aliphatic carbon atom of active methylene, two signals at δ 108.1 and 117.5 ppm corresponding to C_5 of thiazole and cyano group, respectively besides, the mass spectrum was compatible with the molecular formula $C_{15}H_{15}N_3O_2S_2$, confirmed the structure **2**. The methylene group in thiazolylacetonitrile derivative **2** proved to be highly reactive. Thus, compound **2** underwent coupling with an equimolar amount of 4-chlorobenzenediazonium chloride in ethanol solution containing sodium acetate, at 0-5 °C, to afford a yellow crystals product for which two isomeric structures **3** or **4** seemed possible. However the appearance of NH and cyano absorption bands at ν 3193 and 2222 cm^{-1}, respectively in the IR spectrum of the isolated product, the lack of the signal due to methylene protons, and the appearance of the signal due to the H_5 of thiazole at δ 7.40 ppm in the ^1H NMR spectrum provided a firm support for structure **3** and ruled out the other possible isomer **4**. The structure **3** was also obtained *via* direct reaction of phenacyl bromide derivative **1** with

2-amino-*N'*-(4-chlorophenyl)-2-thioxoacetohydrazonoyl cyanide. In the same manner, thiazolylacetonitrile **2** couples with 4-cyano-3-(methylthio)-1*H*-pyrazole-5-diazonium chloride to afford the pyrazolo[5,1-*c*][1,2,4]triazine derivative **5** in moderate yield. The latter structure was established on the basis of its elemental analysis and spectral data (**Scheme 1**). In contrast to their behavior, it has been found that a buffered solution of 1*H*-1,2,4-triazole-3-diazonium chloride couples smoothly, and in high yield, with compound **2** to afford a product for which two isomeric structures **6** or **7** seemed possible. However, the appearance of NH and cyano absorption bands at ν 3230 and 2231 cm^{-1}, respectively, in the IR spectrum of the isolated product and the absence of any signals corresponding to amino group provided a firm support for structure **6** and ruled out the other possible isomer **7** (**Scheme 1**). Formation of thiazolylacrylonitrile derivative **8** is assumed to take place *via* the condensation of the active methylene group in compound **2** with 4-fluorobenzaldehyde. The structure of compound **8** was established on the basis of their elemental analysis and spectral data. Thus, IR spectrum of compound **8** revealed absorption bands at ν 2220 and 1170 cm^{-1} due to C≡N group and C-F bond, respectively. ^{1}H NMR spectrum showed two singlet signals at δ 7.60 and 8.21 ppm due to H_5 of thiazole and proton of CH=C group. ^{13}C NMR spectrum showed two signals at δ 109.2 and 117.3 ppm for C_5 of thiazole and C≡N group, respectively. A final evidence for the proposed structure comes from synthesizing compound **8** *via* reaction of phenacyl bromide derivative **1** with 2-cyano-3-(4-fluorophenyl)prop-2-enethioamide to afford product identical in all aspects (mp, TLC and IR spectrum) with those obtained previously from the reaction of compound **2** with 4-fluorobenzaldehyde as described before (**Scheme 1**).



Scheme 1

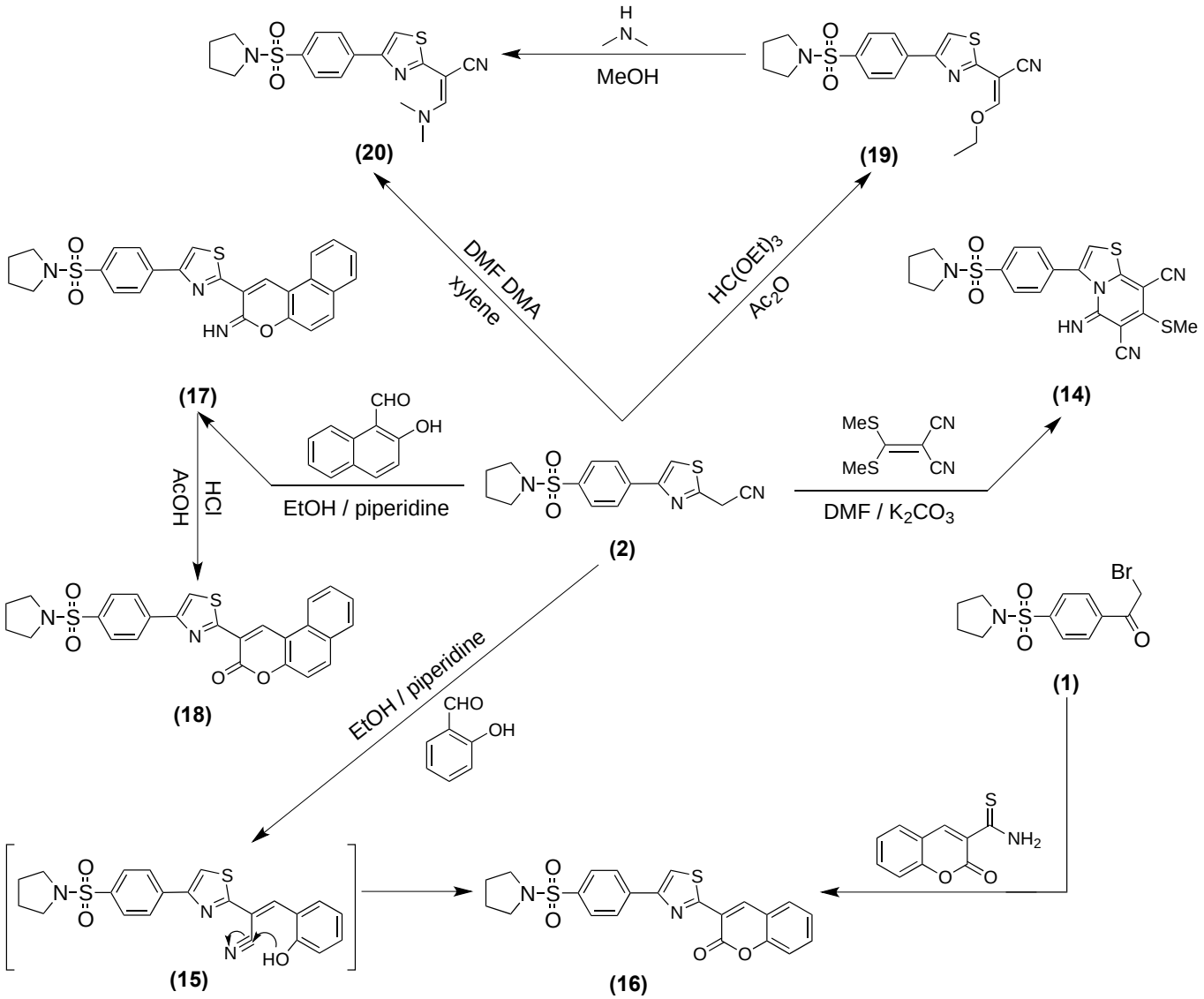
Compound **2** was treated with hydrogen sulfide gas in ethanol containing triethylamine as a catalyst at room temperature to afford 2-(4-(4-(pyrrolidin-1-ylsulfonyl)phenyl)thiazol-2-yl)ethanethioamide (**9**), which upon treatment with ethyl 2-chloroacetate in refluxing ethanol contains fused sodium acetate afforded 4-thiazolidinone derivative **10**. IR spectrum of compound **10** revealed absorption band at ν 1673 cm^{-1} due to C=O group. ^1H NMR spectrum showed two singlet signals at δ 3.18 and 4.25 ppm due to protons of acyclic and cyclic methylene groups, respectively and a singlet signal of one proton appeared in aromatic region at δ 7.40 ppm corresponding to H_5 of thiazole. ^{13}C NMR spectrum showed two signals at δ 35.3 and 41.6 ppm for carbons of acyclic and cyclic methylene groups, respectively and signal at δ 110.7 ppm for C_5 of thiazole. The latter compound was also obtained *via* direct reaction of thiazolylacetonitrile **2** with 2-mercaptoacetic acid in pyridine. Efforts to cyclize compound **10** with 2-(4-fluorobenzylidene)malononitrile to afford enaminonitrile **12** were not successful; instead the product was identified as 5-(4-fluorobenzylidene)-2-((4-(4-(pyrrolidin-1-ylsulfonyl)phenyl)thiazol-2-yl)methyl)-thiazol-4(5*H*)-one (**13**) on the basis of elemental analysis and spectral data. The structure of **13** was further confirmed by an independent synthesis of 4-thiazolidinone derivative **10** with 4-fluorobenzaldehyde in ethanolic piperidine solution. The formation of **13** from 4-thiazolidinone derivative **10** and 2-(4-fluorobenzylidene)malononitrile can be explained by the addition of an active methylene group of compound **10** at the olefinic bond of benzylidene malononitrile forming the intermediate **11**, which undergoes spontaneous elimination of malononitrile to give the final product **13** (**Scheme 2**).



Scheme 2

Reaction of compound **2** with 2-(bis(methylthio)methylene)malononitrile[31,32] in *N*,*N*-dimethylformamide

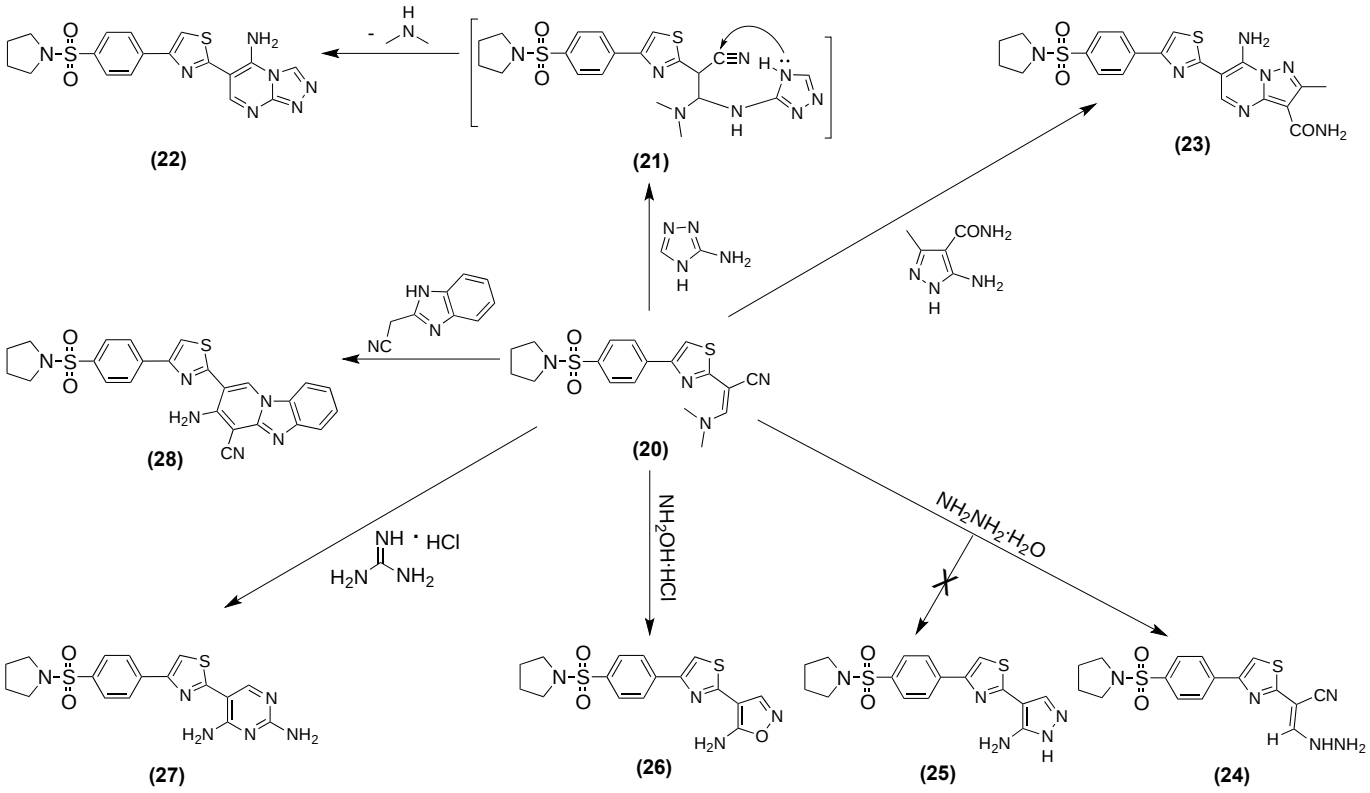
(DMF) in the presence of anhydrous potassium carbonate led to 5-imino-7-(methylthio)-3-(4-(pyrrolidin-1-ylsulfonyl)phenyl)-5*H*-thiazolo[3,2-*a*]pyridine-6,8-dicarbonitrile (**14**) (**Scheme 3**). The elemental analysis and spectral data of the latter structure were in agreement with its assigned structure. Thus, IR spectrum of compound **14** revealed absorption bands at ν 3235, 2220 and 2218 cm^{-1} due to NH and two cyano groups, respectively. The ^{1}H NMR spectrum showed two singlet signals at δ 2.83 and 7.52 ppm due to SCH_3 and H_5 of thiazole, and (D_2O exchangeable) singlet signal at δ 8.52 ppm due to NH proton. ^{13}C NMR spectrum showed two signals at δ 16.2 and 118.2 ppm for SCH_3 and C_5 of thiazole, respectively. Besides, the mass spectrum was compatible with the molecular formula $C_{20}H_{17}N_5O_2S_3$, *m/z* 455 confirmed structure **14**. Our investigation was also extended to study the reaction of compound **2** with *o*-phenolic aldehydes,[33] namely 2-hydroxybenzaldehyde and 2-hydroxy-1-naphthaldehyde. Thus, cyclocondensation of compound **2** with an equimolar amount of 2-hydroxybenzaldehyde in boiling ethanol solution containing piperidine as a catalyst gave the corresponding 3-(4-(4-(pyrrolidin-1-ylsulfonyl)phenyl)thiazol-2-yl)-2*H*-chromen-2-one (**16**). The formation of compound **16** was assumed to occur *via* the intermediacy of the Knovenagel condensed intermediate **15**, intramolecular cyclization *via* an anticipated Michael-type addition[34] of the acidic hydroxyl group to the cyano function, and spontaneous hydrolysis of the imino function into a carbonyl function under the experimental reaction conditions employed. Similar hydrolysis phenomena have been previously reported[35,36] (**Scheme 3**). The latter structure established based on its elemental analysis and spectral data. A final evidence for the proposed structure comes by boiling 2-oxo-2*H*-chromene-3-carbothioamide with phenacyl bromide derivative **1** in ethanol. However, when compound **2** was treated with 2-hydroxy-1-naphthaldehyde at the same reaction condition, the reaction afforded the isolable benzo[*f*]chromen-3-imine derivative **17**, which when heated with glacial acetic acid in the presence of few drops of hydrogen chloride afforded benzo[*f*]chromen-3-one derivative **18** (**Scheme 3**). On the other hand, when compound **2** was allowed to react with triethoxymethane at reflux temperature, afforded 3-ethoxy-2-(4-(4-(pyrrolidin-1-ylsulfonyl)-phenyl)thiazol-2-yl)acrylonitrile (**19**), which on treatment with dimethylamine in refluxing methanol afforded 3-(dimethylamino)-2-(4-(4-(pyrrolidin-1-ylsulfonyl)phenyl)thiazol-2-yl)acrylonitrile (**20**) which confirmed by elemental analysis and spectral data. Thus, IR spectrum revealed an absorption band at *v* 2218 cm^{-1} corresponding to cyano group. ^{1}H NMR spectrum showed three singlet signals at δ 2.99, 7.42 and 7.65 ppm corresponding to $(CH_3)_2N$, CH=C and H_5 of thiazole, respectively. ^{13}C NMR spectrum showed signal at δ 41.6 ppm for two carbon atoms of $(CH_3)_2N$ moiety and two signals at δ = 108.3 and 118.8 ppm corresponding to C_5 of thiazole and cyano group, respectively. The latter compound was also obtained *via* direct reaction of thiazolylacetonitrile **2** with dimethylformamide dimethyl acetal (DMF-DMA) in refluxing xylene (**Scheme 3**).



Scheme 3

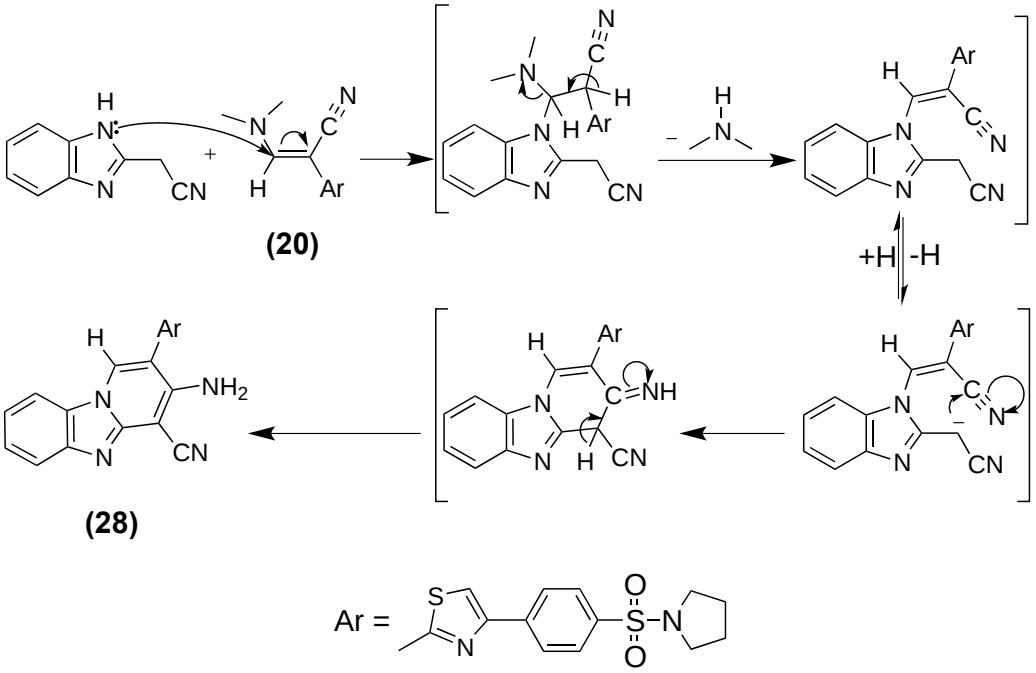
The behavior of 3-dimethylamino thiazolylacrylonitrile derivative **20** towards some heterocyclic amines are investigated, thus compound **20** reacted with 4*H*-1,2,4-triazol-3-amine in DMF[37] under reflux to afford triazolo[4,3-*a*]pyrimidine derivative **22**. The formation of **22** is assumed to proceed through the addition of exocyclic amino group of aminotriazole to α,β-unsaturated moiety of **20** to yield acyclic intermediate **21**, which undergoes intramolecular cyclization with elimination of dimethylamine to afford the final product triazolo[4,3-*a*]pyrimidine **22** (**Scheme 4**). In the same manner, compound **20** reacted with 5-amino-3-methyl-1*H*-pyrazole-4-carboxamide[38] in refluxing DMF to give pyrazolo[1,5-*a*]-pyrimidine derivative **23**. The reactivity of compound **20** towards some nitrogen nucleophiles was also investigated. Thus, when compound **20** was treated with hydrazine hydrate in refluxing ethanol, the reaction afforded acyclic product (3-hydrazinothiazolyl)acrylonitrile derivative **24** and ruled out the amino pyrazole derivative **25**, due to the fact that the IR spectrum showed an absorption band at ν 2218

cm^{-1} assignable to cyano group. Its ^{1}H NMR spectrum revealed two singlet signals at δ 6.75 and 7.45 ppm corresponding to CH=C moiety and H_5 of thiazole, respectively, and (D_2O exchangeable) two singlet signals at δ 5.08 and 10.22 ppm, corresponding to NH_2 and NH protons, respectively. The mass spectrum of **24** showed a molecular ion peak at *m/z* 375. Similarly, compound **20** reacts with hydroxylamine hydrochloride and guanidine hydrochloride in refluxing ethanol containing sodium acetate to afford isoxazole derivative **26** and 2,4-diaminopyrimidine derivative **27**, respectively (**Scheme 4**). Moreover compound **20** reacts smoothly with 2-(1*H*-benzo[*d*]imidazol-2-yl)acetonitrile in refluxing dioxane to afford only one isolable product, identified as benzo[4,5]imidazo[1,2-*a*]pyridine derivative **28**. The IR spectrum of **28** showed, two bi-forked characteristic absorption bands at ν 3416 and 3295 cm^{-1} assignable to amino group and another absorption band at ν 2220 cm^{-1} for cyano group. ^{1}H NMR spectrum showed (D_2O exchangeable) singlet signal at δ 6.81 ppm, corresponding to amino protons, and its mass spectrum was compatible with molecular formula $C_{25}H_{20}N_6O_2S_2$, (M^+: 500) (**Scheme 4**).



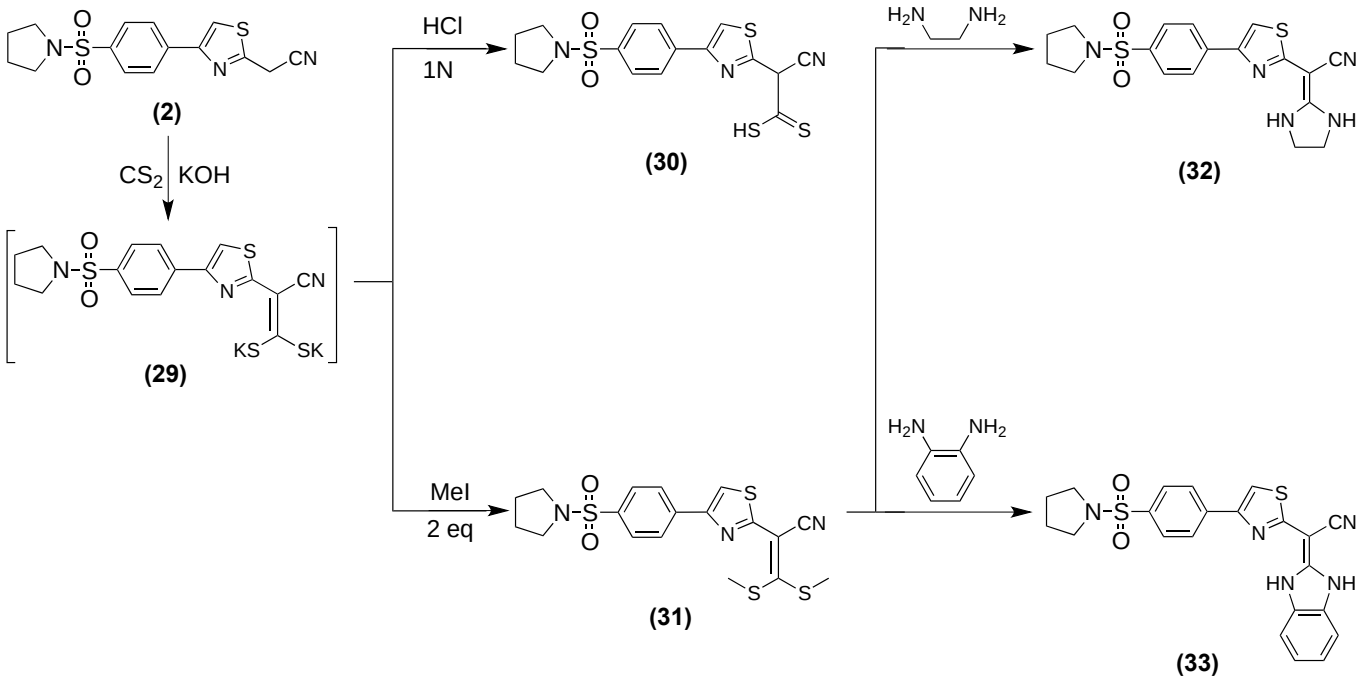
Scheme 4

Formation of compound **28** is assumed to proceed *via* Michael addition of 2-(1*H*-benzo[*d*]imidazol-2-yl)-acetonitrile to ylidenic bond in 3-dimethylamino thiazolylacrylonitrile derivative **20** forming an acyclic intermediate, which cyclized by elimination of dimethylamine then nucleophilic attack of the active methylene on the cyano group followed by tautomerization to the final product **28** (**Scheme 5**).



Scheme 5

The base-promoted nucleophilic addition of the thiazolylacetonitrile **2** to an equimolar amount of carbon disulfide in dry DMF in the presence of KOH at room temperature afforded the non-isolable potassium dithiolate salt **29** and converted into (2-cyanothiazolyl)ethanedithioic acid derivative **30** by treatment with 1N hydrogen chloride. However, when compound **29** was treated with two moles of iodomethane afforded [3,3-bis(methylthio)thiazolyl]acrylonitrile derivative **31** (**Scheme 6**). The IR spectrum of

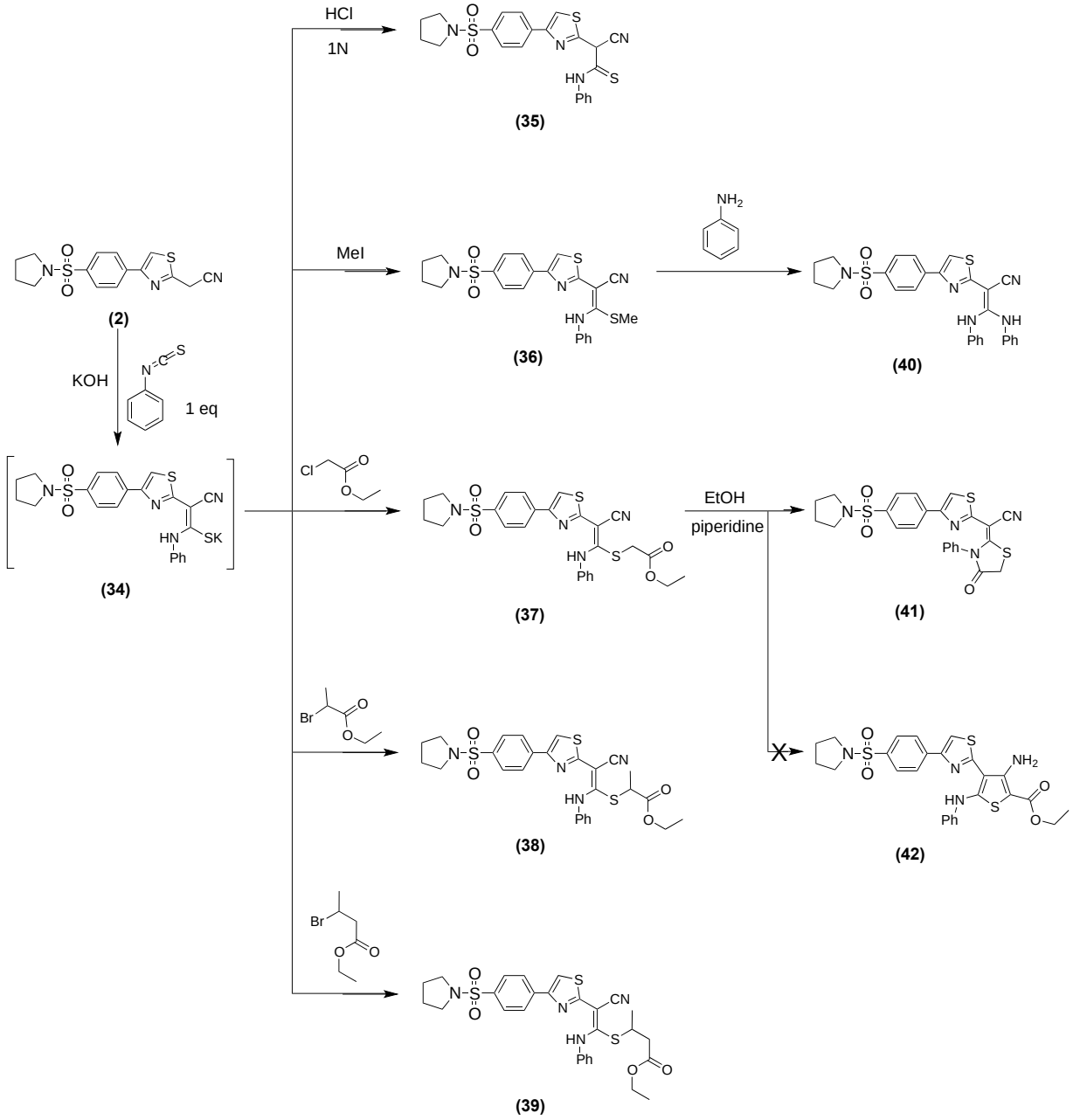


Scheme 6

compound **31** revealed an absorption band at *v* 2218 cm^{-1} corresponding to cyano group, and two bands at *v* 1189 and 1150 cm^{-1} for two (C-S) bonds. ^1H NMR spectrum revealed two singlet signals at δ 2.85 and 7.31 ppm for $2SCH_3$ and H_5 of thiazole, respectively. ^{13}C NMR spectrum showed signal at δ 16.9 ppm for two carbon atoms of $2SCH_3$ moieties and two signals at δ 109.6 and 119.1 ppm corresponding to C_5 of thiazole and cyano group, respectively, and its mass spectrum was compatible with molecular formula $C_{18}H_{19}N_3O_2S_4$, (M^+: 437). Cyclization of compound **31** with 1,2-diamine derivatives namely; ethane-1,2-diamine and benzene-1,2-diamine afforded derivatives of imidazolidine **32** and 1*H*-benzo[*d*]imidazolidine **33**, respectively (**Scheme 6**).

Additionally, when thiazolylacetonitrile **2** was reacted with isothiocyanatobenzene in dry DMF in the presence of KOH at room temperature afforded the non-isolable potassium salt **34**, which acidified with 1N hydrogen chloride to yield the (2-cyano-*N*-phenylthiazolyl)ethanethioamide derivative **35**. Interaction of intermediate **34** with halogenated compounds, namely; iodomethane, ethyl 2-chloroacetate, ethyl 2-bromopropanoate and ethyl 3-bromobutanoate gave acyclic compounds **36-39**, respectively (**Scheme 7**). The IR spectrum of compound **36** revealed an absorption bands at *v* 3230, 2222 and 1160 cm^{-1} corresponding to NH, C≡N and C-S groups, respectively. ^1H NMR spectrum revealed two singlet signals at δ 2.72 and 7.32 ppm for protons of SCH_3 and H_5 of thiazole, respectively, in addition to, D_2O exchangeable singlet signal at δ 9.35 ppm due to NH proton. ^{13}C NMR spectrum showed signal at δ 16.3 ppm for aliphatic carbon atom of SCH_3 moiety and two signals at δ 111.3 and 115.9 ppm corresponding to C_5 of thiazole and cyano group, respectively, and its mass spectrum was compatible with molecular formula $C_{23}H_{22}N_4O_2S_3$, (M^+: 482). IR spectrum of compound **37** revealed an absorption bands at *v* 3220, 2220 and 1697 cm^{-1} corresponding to NH, C≡N and C=O groups, respectively. ^1H NMR spectrum revealed a triplet at δ 1.31 ppm for CH_3 of ethyl group, a quartet at δ 4.34 ppm due to CH_2 of ethyl group, two singlet signals at δ 3.79 and 7.31 ppm for protons of CH_2 and H_5 of thiazole, respectively, in addition to, D_2O exchangeable singlet signal at δ 10.99 ppm due to NH proton. ^{13}C NMR spectrum showed two signals at δ 18.3 and 59.3 ppm for CH_3 and CH_2 of ethyl group, respectively and three signals at δ 40.2, 110.2 and 121.0 ppm corresponding to CH_2, C_5 of thiazole and cyano group, respectively, and its mass spectrum was compatible with molecular formula $C_{26}H_{26}N_4O_4S_3$, (M^+: 554). Interaction of compound **36** with aniline in refluxing ethanol afforded [3,3-bis(phenylamino)thiazolyl]acrylonitrile derivative **40**, which was established on the basis of elemental analysis and spectral data. However, when compound **37** heated in ethanol in the presence of piperidine as a catalyst, afforded only one isolable product from two proposed structures 4-thiazolidinone **41** and thiophene **42**. IR spectrum indicated the disappearance of amino group bands and showed absorption band for cyano group at *v* 2220 cm^{-1}. ^1H NMR spectrum showed the lack of the signal due to protons of ester group and the appearance of a signal due to the methylene group at δ 4.01 ppm. ^{13}C NMR spectrum showed signals at δ 41.4, 108.8, 120.3 and 173.8 ppm

corresponding to the methylene, C_5 of thiazole, cyano and carbonyl groups, respectively. These spectral data provided a firm support for structure **41** and ruled out the other possible structure **42** (**Scheme 7**).



Scheme 7

DOCKING AND MOLECULAR MODELING

Thymidylate synthase and dihydrofolate reductase are among the main targets involved in anticancer and antimicrobial activity.[39,40] Molecular modeling study using Molecular Operating Environment (MOE)[41] module was performed in order to rationalize the observed anticancer activity of the newly synthesized compounds. 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.

DOCKING OF DOXORUBICIN INTO DHFR:

The active site revealed that several molecular interactions were considered responsible for the observed affinity, as the hydroxyl group of tetracene acted as a hydrogen bond donor with the side chain residue Asp 21 (2.65 Å) with a strength of 1.1%. While, the hydroxyl group of acetyl pyran acted as a hydrogen bond acceptor with the side chain residue Ser 59 (3.02 Å) with a strength of 16.2%. However, the amino group acted as a hydrogen bond donor with the side chain residue Glu 30 (3.31 Å) with a strength of 5%. Moreover, there is arene-arene interaction between the phenyl ring of tetracene and the side chain residue Phe 31. In addition to, hydrophobic interactions involving other atoms of the compound and the following amino acid residues: Val 8, Gly 20, Asp 21, Leu 22, Phe 31, Phe 34, Thr 56, Ile 60, Pro 61, Asn 64, Leu 67, Val 115 and Tyr 121, as shown in **Figure 1**.

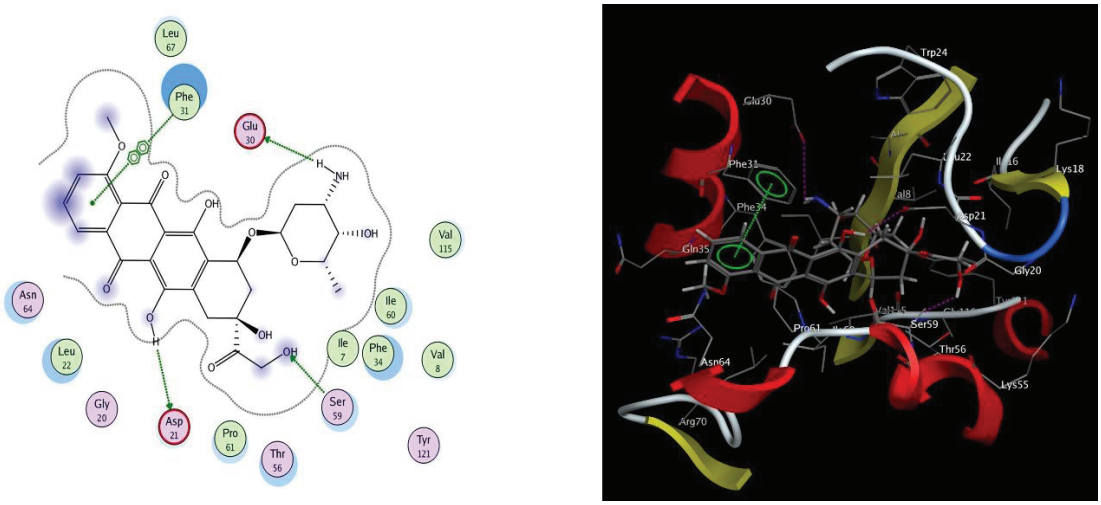


Figure 1. Docking of DOX into DHFR

DOCKING SIMULATION STUDY OF THE SYNTHESIZED COMPOUNDS 1, 2, 8, 10, 16, 18, 22, 27, 28, 30 AND 41:

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

DOCKING OF COMPOUND 1 INTO DHFR:

The active site revealed that one oxygen atom of SO_2 moiety acted as a hydrogen bond acceptor with the side chain residues; Thr 56 and Ser 59 (3.63 Å and 3.16 Å, respectively) with a strength of 2.4% and 10.9%, respectively. Besides to, hydrophobic interactions involving the bromine atom, oxygen atom of carbonyl function and other carbons as well the second oxygen atom of SO_2 moiety and the following amino acid residues: Ile 16, Leu 22, Phe 31, Ile 60, Pro 61, Val 115 and Tyr 121, as shown in **Figure 2**.

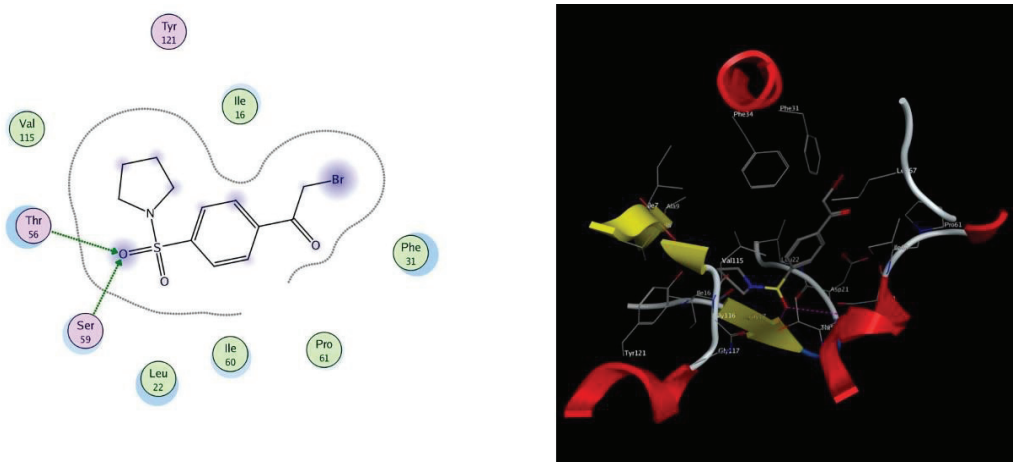


Figure 2. Docking of Compound **1** into DHFR

DOCKING OF COMPOUND 2 INTO DHFR:

The active site revealed the presence of three hydrogen bond interactions as one oxygen atom of SO_2 moiety acted as a hydrogen bond acceptor with the amino acid residues Ser 59 (2.68 Å) with a strength of (84.2%). Moreover, the nitrogen atom in cyano function acted as a hydrogen bond acceptor with the amino acid residues Val 8 and Thr 136 (3.79 Å and 2.80 Å; respectively) with a strength of 1.4% and 56.6%, respectively. In addition to, hydrophobic interactions with the following amino acid residues: Ile 7, Val 8, Ala 9, Ile 16, Gly 17, Asp 21, Leu 22, Glu 30, Phe 34, Thr 56, Phe 134 and Thr 136, as shown in **Figure 3**.

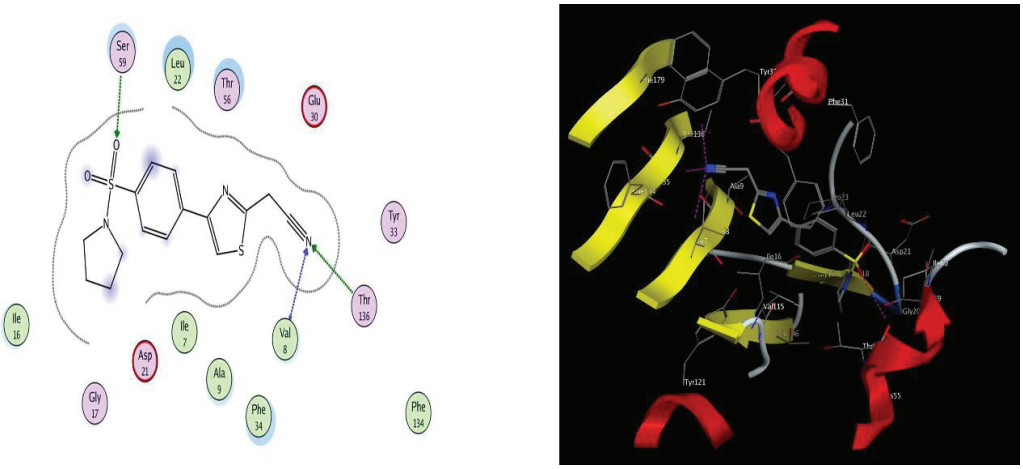


Figure 3. Docking of Compound **2** into DHFR

DOCKING OF COMPOUND 8 INTO DHFR:

The active site revealed that the two oxygen atoms of SO_2 moiety acted as a hydrogen bond acceptor with the amino acid residues Asn 64 (2.88 Å and 3.43 Å, respectively) with a strength of 32.6% and 3.1%, respectively. Furthermore, the nitrogen atom in cyano function acted as a hydrogen bond acceptor with the amino acid residue Thr 56 (3.21 Å) with a strength of 15.3%. In addition to, hydrophobic interactions

with the following amino acid residues: Val 8, Ala 9, Ile 16, Leu 22, Glu 30, Phe 31, Phe 34, Thr 56, Ser 59, Ile 60, Pro 61, Asn 64, Val 115 and Tyr 121, as shown in **Figure 4**.

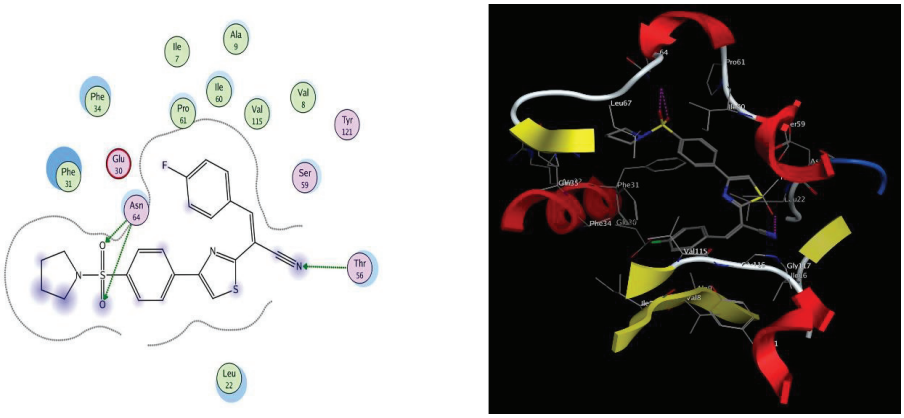


Figure 4. Docking of Compound **8** into DHFR

DOCKING OF COMPOUND 10 INTO DHFR:

The active site revealed the presence of hydrogen bond interactions between one oxygen atom of SO_2 moiety as it acted as a hydrogen bond acceptor with the side chain residues Thr 56 and Ser 59 (3.77 Å and 2.81 Å, respectively) with a strength of (1.4% and 59.1%, respectively). Moreover, the oxygen atom of carbonyl function acted as a hydrogen bond acceptor with the amino acid residues Lys 68 and Arg 70 (3.52 Å and 3.2 Å, respectively) with a strength of 3.3% for both. In addition to, hydrophobic interactions among other atoms of the compound with the following amino acid residues: Leu 22, Phe 31, Phe 34, Gln 35, Thr 56, Ser 59, Pro 61, Asn 64, Leu 67, Lys 68, Arg 70, Val 115 and Tyr 121, as shown in **Figure 5**.

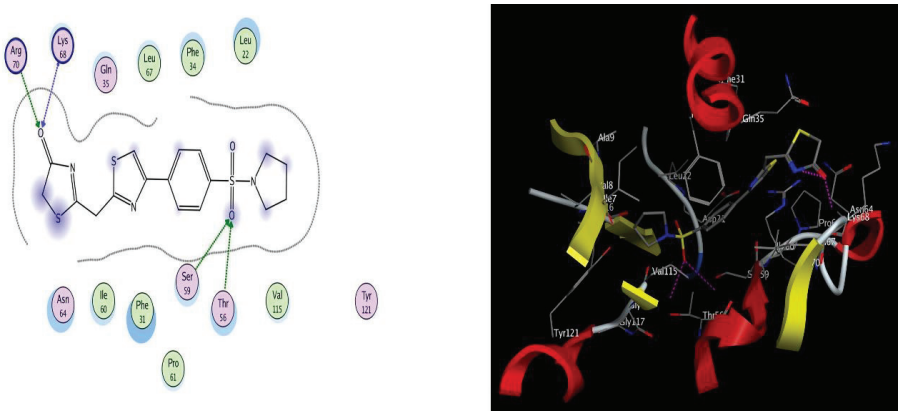


Figure 5. Docking of Compound **10** into DHFR

DOCKING OF COMPOUND 16 INTO DHFR:

The active site illustrated that the one oxygen atom of SO_2 moiety acted as a hydrogen bond acceptor with the side chain residue Arg 70 (3.35 Å) with a strength of 6%. In addition to, hydrophobic interactions among other atoms of the compound with the following amino acid residues: Ile 16, Asp 21, Leu 22, Phe

31, Phe 34, Gln 35, Thr 56, Ser 59, Ile 60, Pro 61, Asn 64, Leu 67, Lys 68 and Arg 70, as shown in **Figure 6**.

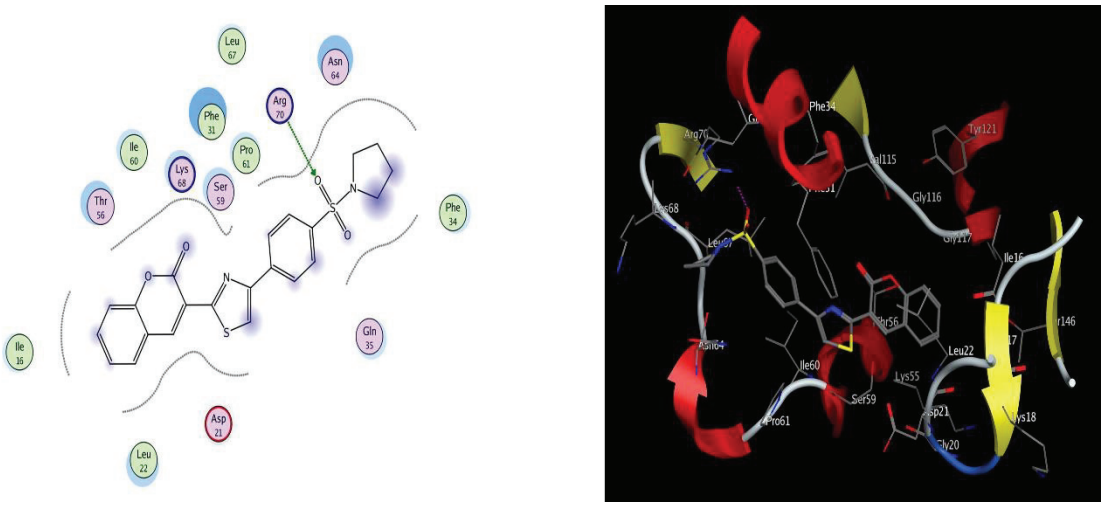


Figure 6. Docking of Compound **16** into DHFR

DOCKING OF COMPOUND 18 INTO DHFR:

The active site illustrated that the one oxygen atom of SO_2 moiety acted as a hydrogen bond acceptor with the side chain residue Arg 70 (3.56 Å) with a strength of 3.9%. In addition to, hydrophobic interactions among other atoms of the compound with the following amino acid residues: Ile 16, Gly 20, Asp 21, Leu 22, Phe 31, Phe 34, Gln 35, Thr 56, Ser 59, Ile 60, Pro 61, Asn 64, Leu 67, Lys 68, Arg 70 and Thr 146, as shown in **Figure 7**.

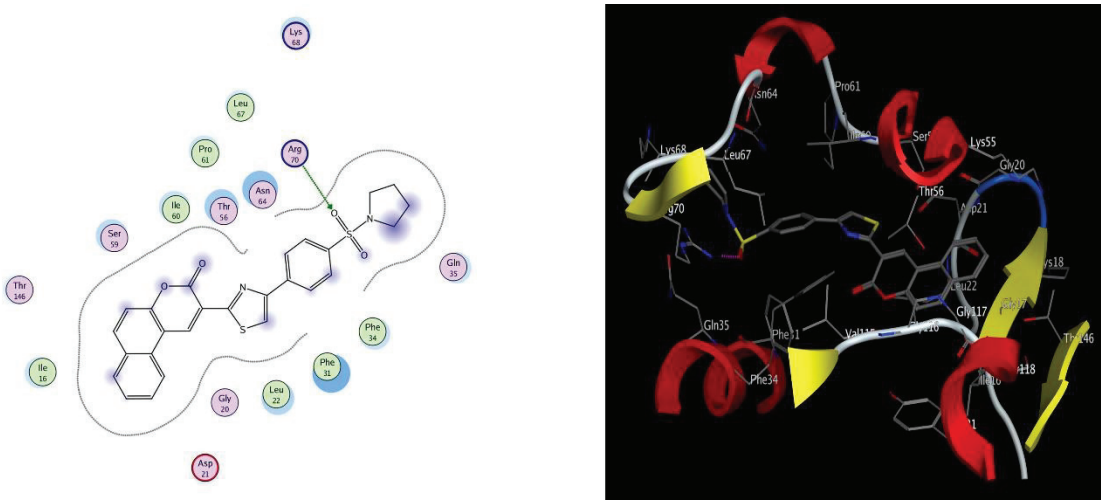


Figure 7. Docking of Compound **18** into DHFR

DOCKING OF COMPOUND 22 INTO DHFR:

The active site revealed the presence of several molecular interactions in which two oxygen atoms of SO_2 moiety acted as a hydrogen bond acceptor with the amino acid residues Thr 56 and Ser 59 (2.81 Å and 3.58 Å, respectively) with a strength of 43.6% and 2.3%, respectively. In addition to, hydrophobic

interactions 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 and Tyr 121, as shown in **Figure 8**.

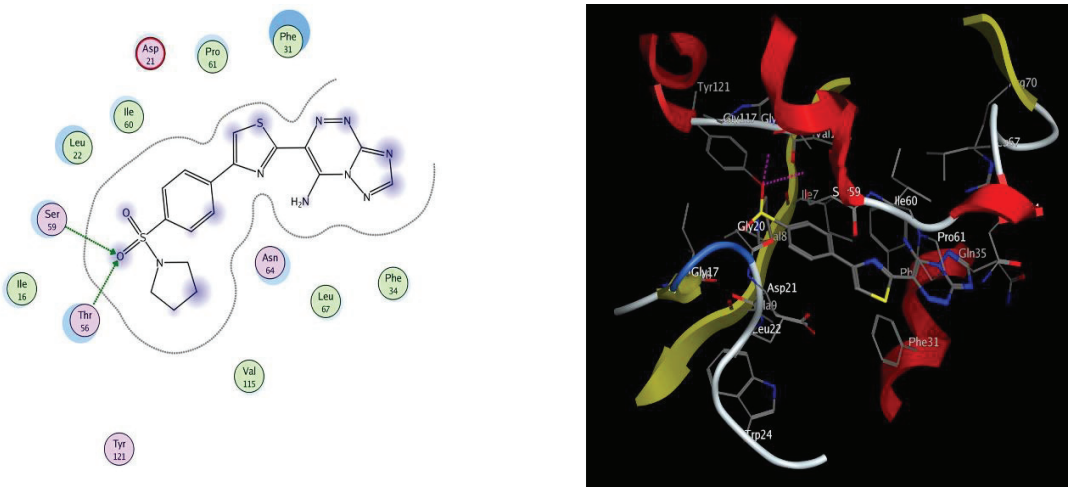


Figure 8. Docking of Compound **22** into DHFR

DOCKING OF COMPOUND 27 INTO DHFR:

The active site revealed the presence of hydrogen bond interactions as the one oxygen atom of SO_2 moiety acted as a hydrogen bond acceptor with the side chain residue Thr 56 (2.86 Å) with a strength of 26%. Furthermore, the nitrogen atom of pyrimidine moiety acted as a hydrogen bond acceptor with the amino acid residue Asn 64 (3.25 Å) with a strength of (1.5%). In addition to, hydrophobic interactions with the following amino acid residues: Ile 16, Asp 21, Leu 22, Phe 31, Thr 56, Ser 59, Ile 60, Pro 61, Asn 64, Leu 67, Val 115 and Tyr 121, as shown in **Figure 9**.

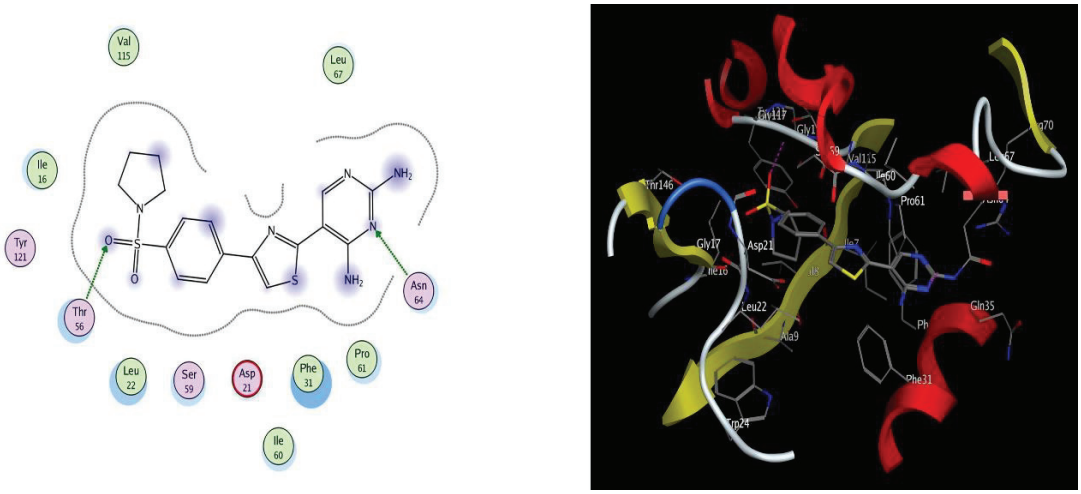


Figure 9. Docking of Compound **27** into DHFR

DOCKING OF COMPOUND 28 INTO DHFR:

The active site revealed the presence of one hydrogen bond interaction as one oxygen atom of SO_2 moiety

acted as a hydrogen bond acceptor with the side chain residue; Asn 64 (3.60 Å) with a strength of 1.7%. Besides, hydrogen atoms of amino function acted as hydrogen bond donor with the amino acid residues Gly 20 and Asp 21 (2.94 Å and 2.48 Å, respectively) with a strength of 2.8% and 6.6%, respectively. Moreover, there is arene-arene interaction between phenyl ring and amino acid residue Phe 31. In addition to, hydrophobic interactions involving the other atoms of the compound with the following amino acid residues: Ile 7, Val 8, Asp 21, Leu 22, Phe 31, Phe 34, Gln 35, Ser 59, Pro 61, Asn 64, Lys 68, Arg 70, Val 115 and Tyr 121, as shown in **Figure 10**.

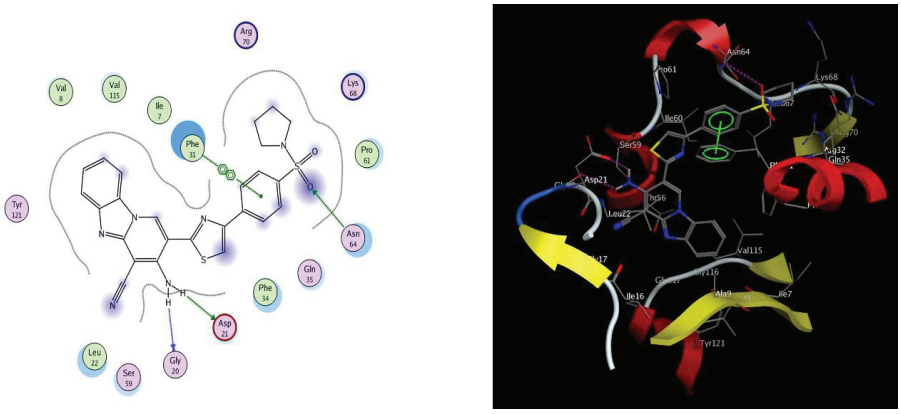


Figure 10. Docking of Compound **28** into DHFR

DOCKING OF COMPOUND 30 INTO DHFR:

The active site revealed the presence of a hydrogen bond interaction between the nitrogen atom of cyano function as it acted as a hydrogen bond acceptor with the side chain residue Arg 70 (3.21 Å) with a strength of 23.8%. Moreover, there is arene-arene interaction between phenyl ring and amino acid residue Phe 31. In addition to, hydrophobic interactions among other atoms of the compound with the following amino acid residues: Ile 16, Phe 31, Phe 34, Gln 35, Thr 56, Ile 60, Asn 64, Leu 67, Arg 70, Val 115 and Tyr 121, as shown in **Figure 11**.

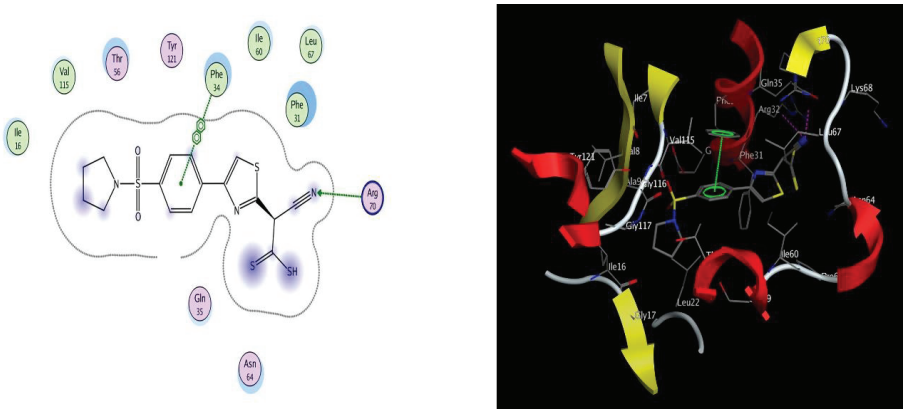


Figure 11. Docking of Compound **30** into DHFR

DOCKING OF COMPOUND 41 INTO DHFR:

The active site revealed the presence of four hydrogen bond interactions as one oxygen atom of SO_2 moiety acted as a hydrogen bond acceptor with the side chain residue; Asn 64 (2.70 Å) with a strength of 31.1%. Moreover, the oxygen atom of carbonyl function acted as hydrogen bond acceptor with the side chain residue; Ala 9 (3.05 Å) with a strength of 7.4%. Besides, the nitrogen atom of cyano function acted as hydrogen bond acceptor with the amino acid residues Thr 56 and Ser 59 (3.50 Å and 3.18 Å; respectively) with a strength of 6.3% and 15.1%, respectively. In addition to, hydrophobic interactions with the following amino acid residues: Val 8, Ala 9, Ile 16, Asp 21, Leu 22, Glu 30, Phe 31, Phe 34, Gln 35, Thr 56, Ser 59, Ile 60, Pro 61, Asn 64, Leu 67, Val 115 and Tyr 121, as shown in **Figure 12**.

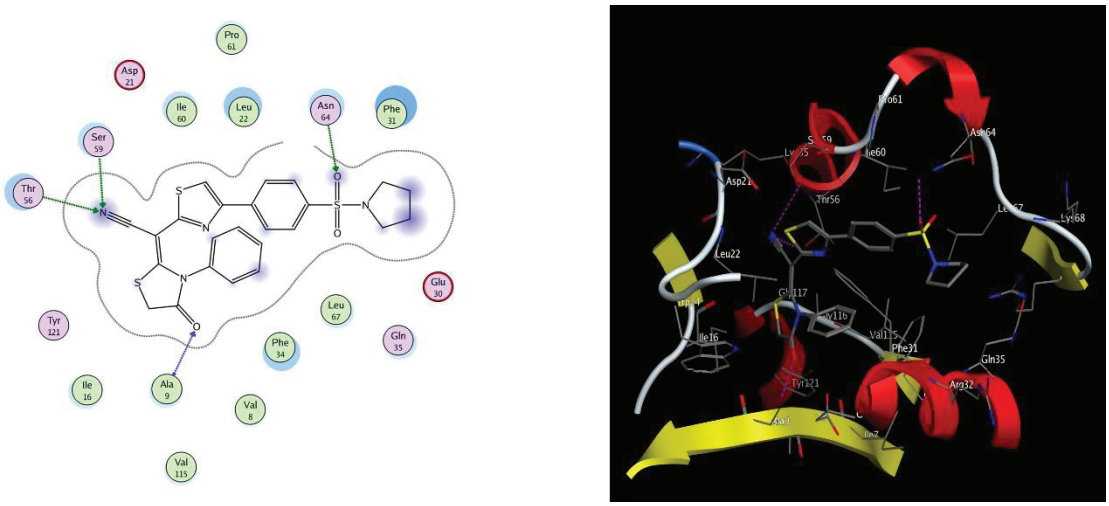


Figure 12. Docking of Compound **41** into DHFR

DOCKING AND MOLECULAR MODELING

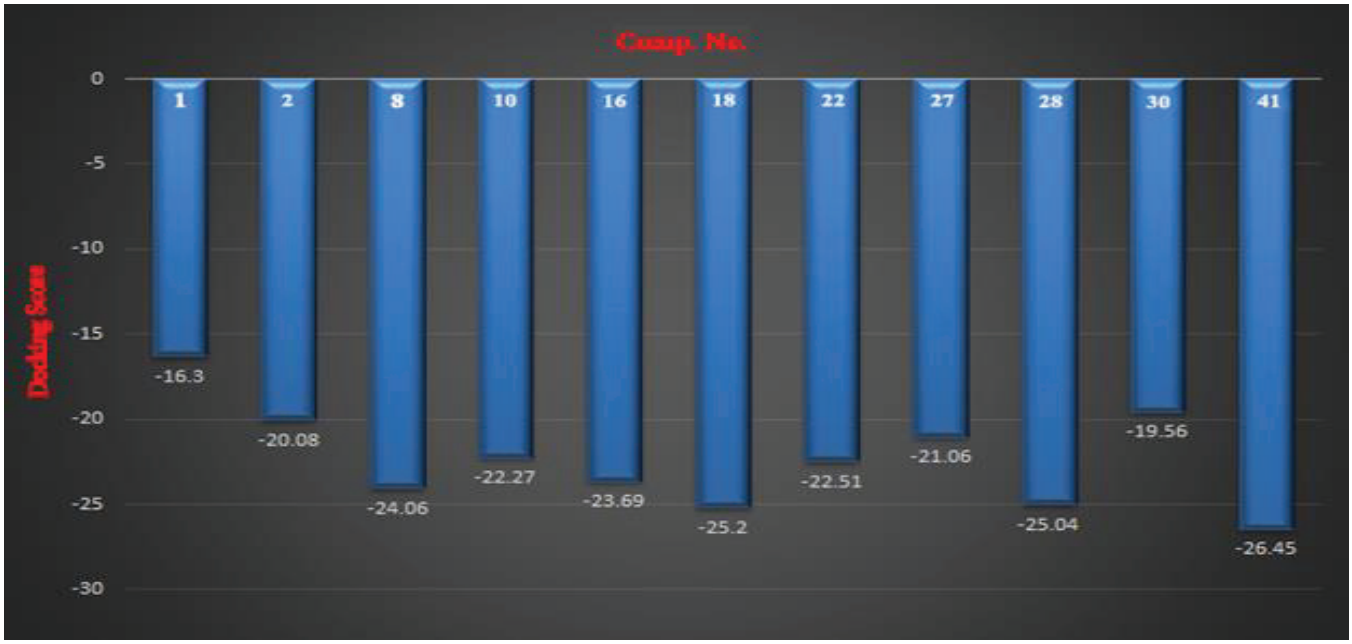
Docking was performed for the compounds **1, 2, 8, 10, 16, 18, 22, 27, 28, 30 and 41** on the dihydrofolate reductase in a trial to predict their mode of action as anticancer drugs. The compounds show several interactions with dihydrofolate reductase enzyme. Particularly noteworthy are the compounds **41**, **18**, **28**, **8**, **16** and **10**, which suggest that they might exert their action through inhibition of the DHFR enzyme (**Table 1**). It is clear from the present data that the comparison of the docking score energy for tested compounds that the compounds follows the order **41** > **18** > **28** > **8** > **16** > **10** > **22** > **27** > **2** > **30** > **1** (**Chart 1**).

Table 1. Docking score energy of the selective newly synthesized compounds

Comp. No.	Score	E-conf	E-place	E-score 1	E-score 2	E-refine
1	-16.30	-0.8400	-57.580	-8.810	-16.30	-15.73
2	-20.08	-9.0900	-69.280	-9.470	-20.08	15.74
8	-24.06	12.920	-94.590	-10.59	-24.06	-21.41
10	-22.27	-12.100	-114.23	-10.05	-22.27	-21.74
16	-23.69	30.260	-110.00	-10.38	-23.69	-23.20
18	-25.20	52.400	-117.45	-10.13	-25.20	-28.47
22	-22.51	76.680	-104.09	-10.29	-22.51	-12.51
27	-21.06	-127.01	-84.420	-10.57	-21.06	-14.41
28	-25.04	40.870	-83.700	-10.42	-25.04	-7.280
30	-19.56	-15.580	-102.91	-10.97	-19.56	-17.83
41	-26.45	41.260	-115.86	-10.17	-26.45	-16.25

Score: For all scoring functions, lower scores indicate more poses that are favorable. The unit for all scoring functions is kcal/mol. **E-conf:** the energy of the conformer. If there is a refinement stage, this is the energy calculated at the end of the refinement. **E-place:** Score from the placement stage (*Placement*. A collection of poses is generated from the pool of ligand conformations using one of the placement methods). **E-score 1:** Score from the first rescoring stage. **E-score 2:** Score from the second rescoring stage. **E-refine:** Score from the refinement stage (Refinement: Energy minimization of the system is carried out using the conventional molecular mechanics setup).

Chart 1. Comparison of the docking score energy of the selective newly synthesized compounds



IN VITRO ANTICANCER ACTIVITY

The newly synthesized compounds were evaluated for their *in-vitro* cytotoxicity against human breast cancer cell line (MCF7). Some of the tested compounds were more potent compared with doxorubicin as the reference drug. From the obtained results in **Table 2** and **Chart 2**, observe that compound **18** having

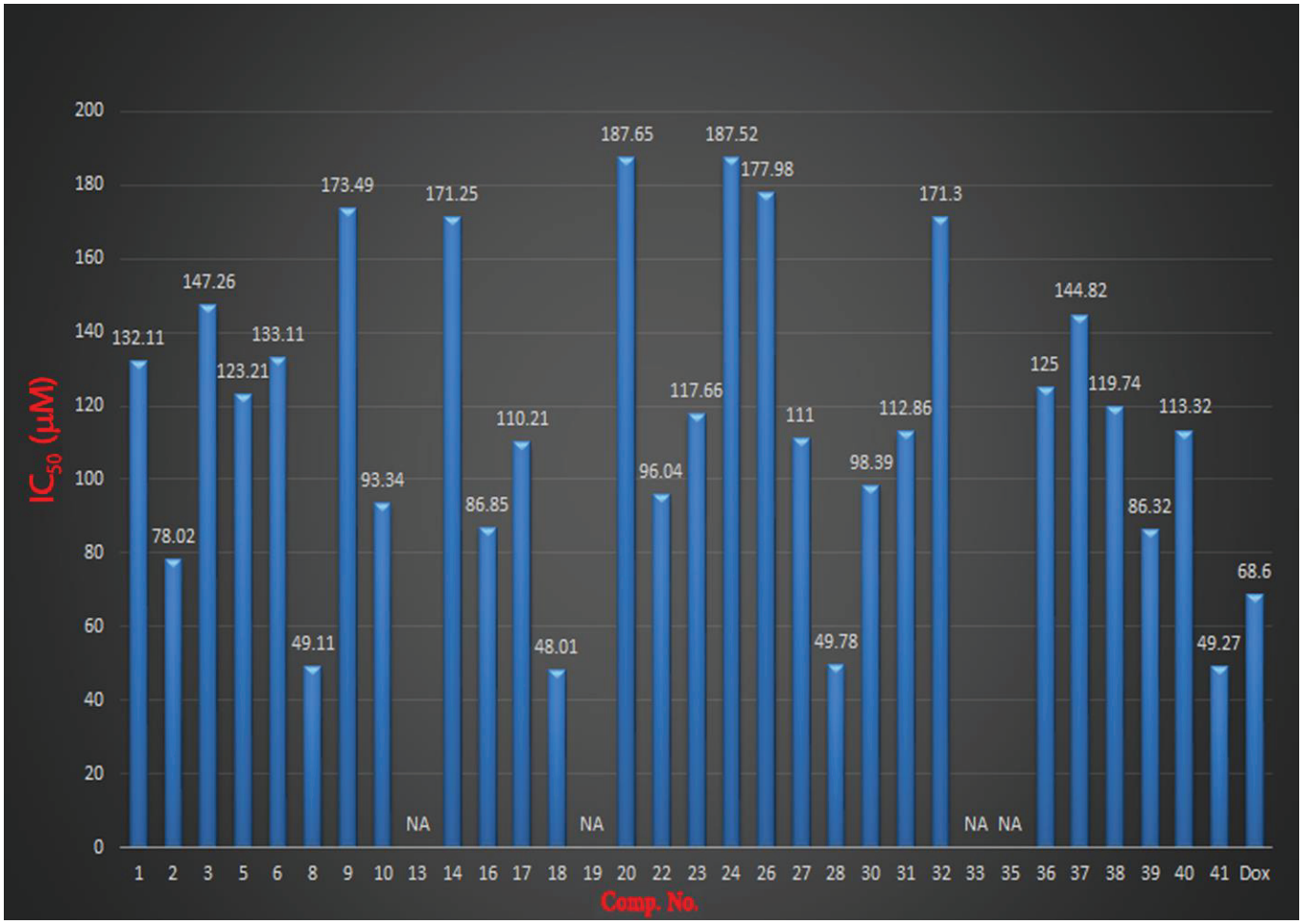
benzo[*f*]chromen-3-one moiety with IC_{50} value 48.01 μM, (thiazolyl)acrylonitrile **8** with IC_{50} value 49.11 μM, *N*-pheny-4-thiazolidinone **41** with IC_{50} value 49.27 μM and benzo[4,5]imidazo[1,2-*a*]pyridine-4-carbonitrile **28** with IC_{50} value 49.78 μM, showed increased activity when compared to doxorubicin with IC_{50} value 68.6 μM, while compounds **2**, **39**, **16**, **10**, **22** and **30** with IC_{50} values 78.02, 86.32, 86.85, 93.34, 96.04 and 98.39 μM, respectively, were found to be nearly as active as doxorubicin. While the compounds **17**, **27**, **31**, **40**, **23**, **38**, **5**, **36**, **1**, **6**, **37**, **3**, **14**, **32**, **9**, **26**, **24** and **20** with IC_{50} values 110.21, 111, 112.86, 113.32, 117.66, 119.74, 123.21, 125, 132.11, 133.11, 144.82, 147.26, 171.25, 171.3, 173.49, 177.98, 187.52 and 187.65 μM, respectively were less active than doxorubicin. While the remaining compounds **13**, **19, 33** and **35** were non-active (NA). It is clear from the present data that the comparison of the IC_{50} for the synthesized compounds against human breast cancer cell line (MCF7). **Chart 2** has showed that, the cell killing potency follows the order **18** > **8** > **41** > **28** > **Dox** > **2** > **39** > **16** > **10** > **22** > **30** > **17** > **27** > **31** > **40** > **23** > **38** > **5** > **36** > **1** > **6** > **37** > **3** > **14** > **32** > **9** > **26** > **24** > **20** > **13, 19, 33** and **35**. 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.

Table 2. Cytotoxicity of the newly synthesized compounds against human breast cancer cell line (MCF7)[a]

Comp. No.	IC_{50}[b] (μg /mL)	IC_{50}[b] (μM)	Comp. No.	IC_{50}[b] (μg /mL)	IC_{50}[b] (μM)
1	43.86	132.11	**23**	56.83	117.66
2	25.98	78.02	**24**	70.32	187.52
3	69.36	147.26	**26**	66.92	177.98
5	61.36	123.21	**27**	44.62	111
6	56.97	133.11	**28**	24.89	49.78
8	21.56	49.11	**30**	40.24	98.39
9	63.67	173.49	**31**	49.32	112.86
10	37.99	93.34	**32**	68.69	171.3
13	NA	NA	**33**	NA	NA
14	77.92	171.25	**35**	NA	NA
16	38.04	86.85	**36**	60.25	125
17	53.67	110.21	**37**	80.23	144.82
18	23.43	48.01	**38**	68.01	119.74
19	NA	NA	**39**	50.24	86.32
20	72.81	187.65	**40**	59.72	113.32
22	41.01	96.04	**41**	25.03	49.27
Dox	37.25	68.6	**Dox**	37.25	68.6

[a] Mean of three results obtained from three experiments.
[b] IC_{50} value: Concentration causing 50% inhibition of MCF7 cell viability.

Chart 2. Comparison of the IC_{50} values for the synthesized compounds against human breast cancer cell line (MCF7)



CONCLUSION

This article proved that compounds having benzenesulfonylpyrrolidine moiety attached to different heterocyclic moieties such as benzo[*f*]chromen-3-one **18**, thiazolylacrylonitrile **8**, *N*-pheny-4-thiazolidinone **41** and benzo[4,5]imidazo[1,2-*a*]pyridine-4-carbonitrile **28**, showed a significant cytotoxic activity against human breast cancer cell line (MCF7) compared to the reference drug doxorubicin.

EXPERIMENTAL

Melting points (°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 600 *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.

2-(4-(4-(Pyrrolidin-1-ylsulfonyl)phenyl)thiazol-2-yl)acetonitrile (2)

A mixture of phenacyl bromide derivative **1** (3.32 g, 0.01 mol) and 2-cyanoethanethioamide (1.00 g, 0.01 mol) in EtOH (50 mL) was heated under reflux for 1 h. The solvent was concentrated; after cooling, the solid product that formed was collected and recrystallized from EtOH to give **2**. White crystals, Yield, 74%; mp 130-131 °C. IR (KBr, cm^{-1}): ν_{max} 3074 (CH aromatic), 2960, 2908 (CH aliphatic), 2220 (C≡N), 1594 (C=N), 1560 (C=C), 1336, 1160 (SO_2). ^{1}H NMR (DMSO-d_6): δ 1.93 (m, 4H, CH_2-CH_2 of pyrrolidine), 3.30 (t, 4H, *J*=9.8 *Hz*, CH_2-N-CH_2 of pyrrolidine), 4.01 (s, 2H, CH_2), 7.52 (s, 1H, H_5 of thiazole), 7.80, 8.05 (dd, 4H, "each d, each 2H, *J*=8.6 *Hz*", AB system). ^{13}C NMR (DMSO-d_6): δ 18.9 (CH_2), 22.7 (2C, CH_2-CH_2 of pyrrolidine), 60.1 (2C, CH_2-N-CH_2 of pyrrolidine), 108.1 (C_5 of thiazole), 117.5 (C≡N), 125.9 (2C), 128.6 (2C), 137.4, 140.5, 160.0, 169.2. MS *m/z* (%): 334.06 [M^++1] (33.19), 333.03 [M^+] (53.04), 332.02 (15.47), 262.97 (14.88), 216.04 (14.16), 200.05 (29.81), 199.02 (45.92), 158.96 (13.55), 133.01 (11.85), 89.04 (35.62), 70.06 (100.00), 69.04 (5.23), 63.02 (9.19), 43.06 (11.04), 42.02 (44.71), 41.02 (12.00). Anal. Calcd for $C_{15}H_{15}N_3O_2S_2$ (333.43): C, 54.03; H, 4.53; N, 12.60; S, 19.23. Found: C, 54.12; H, 4.67; N, 12.74; S, 19.05%.

***N′*-(4-Chlorophenyl)-4-(4-(pyrrolidin-1-ylsulfonyl)phenyl)thiazole-2-carbohydrazonoyl cyanide (3)**

Procedure A: 4-Chlorobenzenediazonium chloride (prepared by adding sodium nitrite (0.69 g, 0.01 mol) to 4-chloroaniline (1.27 g, 0.01 mol) in conc. hydrogen chloride (6 mL) at 0-5 ºC under stirring) was added drop wise to a cold solution of thiazolylacetonitrile derivative **2** (3.33 g, 0.01 mol) in EtOH (20 mL) containing sodium acetate (6.56 g, 0.08 mol), the obtained product was collected and recrystallized from EtOH to give **3** (yield 91%).

Procedure B: A mixture of phenacyl bromide derivative **1** (3.32 g, 0.01 mol) and 2-amino-*N′*-(4-chlorophenyl)-2-thioxoacetohydrazonoyl cyanide (2.38 g, 0.01 mol) in EtOH (50 mL) was heated under reflux for 1 h. The resulting solid was collected to give the compound **3**, mp and mixed mp determined with authentic sample, which was obtained in procedure **A** gave no depression. Yellow crystals, Yield, 87%; mp 150-152 °C. IR (KBr, cm^{-1}): ν_{max} 3193 (NH), 3017 (CH aromatic), 2983, 2914, 2895 (CH aliphatic), 2222 (C≡N), 1610 (C=N), 1582 (C=C), 1345, 1151 (SO_2), 740 (C-Cl). ^{1}H NMR (DMSO-d_6): δ 1.96 (m, 4H, CH_2-CH_2 of pyrrolidine), 3.27 (t, 4H, *J*=10.1 *Hz*, CH_2-N-CH_2 of pyrrolidine), 7.23, 7.31 (dd, 4H, "each d, each 2H, *J*=8.6 *Hz*", AB system of chlorophenyl), 7.40 (s, 1H, H_5 of thiazole), 7.91, 8.06 (dd, 4H, "each d, each 2H, *J*=8.6 *Hz*", AB system of benzenesulfonyl), 12.76 (s, 1H, NH, Discharged with D_2O). ^{13}C NMR (DMSO-d_6): δ 23.0 (2C, CH_2-CH_2 of pyrrolidine), 63.4 (2C,

CH_2-N-CH_2 of pyrrolidine), 111.2 (C_5 of thiazole), 115.9 (2C), 121.3 (C≡N), 126.7 (2C), 128.1, 130.5 (2C), 133.2 (2C), 135.8, 140.7, 142.5, 145.2, 155.9, 166.0. MS *m/z* (%): 471.17 [M^+] (62.41), 232.06 (15.13), 194.10 (27.25), 193.11 (35.52), 192.06 (7.75), 143.98 (12.08), 131.05 (15.92), 129.08 (19.70), 127.05 (100.00), 125.04 (23.92), 111.04 (10.32), 92.10 (18.65), 88.04 (50.90), 65.07 (8.83), 60.06 (10.42). Anal. Calcd for $C_{21}H_{18}ClN_5O_2S_2$ (471.98): C, 53.44; H, 3.84; N, 14.84; S, 13.59. Found: C, 53.58; H, 3.72; N, 14.65; S, 13.66%.

General Procedure for Preparation of (5, 6)

To a stirred solution of compound **2** (3.33 g, 0.01 mol) in EtOH (50 mL) containing sodium acetate (3 g) 4-cyano-3-(methylthio)-1*H*-pyrazole-5-diazonium chloride, and/or 1*H*-1,2,4-triazole-3-diazonium chloride (prepared by adding sodium nitrite (0.69 g, 0.01 mol) to heterocyclic amines (0.01 mol) in conc. hydrogen chloride (6 mL) at 0-5 °C under stirring) was added drop wise while cooling to 0-5 °C and stirring. The reaction mixture was then left at room temperature for 2 h, and the solid product formed was collected by filtration and recrystallized from the appropriate solvent to give **5** and **6**, respectively.

4-Amino-7-(methylthio)-3-(4-(4-(pyrrolidin-1-ylsulfonyl)phenyl)thiazol-2-yl)pyrazolo[5,1-*c*][1,2,4]-triazine-8-carbonitrile (5)

White crystals, Yield, 53%; mp 202-204 °C (EtOH/benzene). IR (KBr, cm^{-1}): ν_{max} 3440, 3317 (NH_2), 3019 (CH aromatic), 2971, 2923 (CH aliphatic), 2217 (C≡N), 1583 (C=N), 1561 (C=C), 1408 (N=N), 1337, 1155 (SO_2), 1179 (C-S). ^{1}H NMR (DMSO-d_6): δ 1.99 (m, 4H, CH_2-CH_2 of pyrrolidine), 3.29 (t, 4H, *J*=9.9 *Hz*, CH_2-N-CH_2 of pyrrolidine), 2.55 (s, 3H, CH_3), 7.32 (s, 1H, H_5 of thiazole), 7.72, 8.30 (dd, 4H, "each d, each 2H, *J*=8.6 *Hz*", AB system), 8.51 (s, 2H, NH_2, Discharged with D_2O). MS *m/z* (%): 498.53 [M^+] (80.51), 309.10 (25.47), 245.11 (25.14), 201.09 (40.52), 192.08 (24.64), 191.06 (16.54), 176.06 (71.75), 175.06 (71.69), 174.07 (16.30), 148.06 (19.49), 146.05 (13.45), 122.08 (11.38), 121.05 (15.87), 109.07 (100.00), 104.08 (17.94), 89.06 (36.83), 82.00 (34.88), 80.00 (38.47), 77.06 (51.86), 69.05 (16.35), 43.07 (16.17), 41.07 (21.39). Anal. Calcd for $C_{20}H_{18}N_8O_2S_3$ (498.60): C, 48.18; H, 3.64; N, 22.47; S, 19.29. Found: C, 48.30; H, 3.59; N, 22.28; S, 19.37%.

2-((1*H*-1,2,4-Triazol-3-yl)diazenyl)-2-(4-(4-(pyrrolidin-1-ylsulfonyl)phenyl)thiazol-2-yl)acetonitrile (6)

Yellow crystals, Yield, 87%; mp 156-158 °C (EtOH). IR (KBr, cm^{-1}): ν_{max} 3230 (NH), 3090, 3085 (CH aromatic), 2973, 2908 (CH aliphatic), 2231 (C≡N), 1613 (C=N), 1592 (C=C), 1453 (N=N), 1341, 1159 (SO_2). ^{1}H NMR (DMSO-d_6): δ 1.92 (m, 4H, CH_2-CH_2 of pyrrolidine), 3.27 (t, 4H, *J*=9.8 *Hz*, CH_2-N-CH_2 of pyrrolidine), 4.99 (s, 1H, CH), 7.73 (s, 1H, H_5 of thiazole), 7.90, 8.07 (dd, 4H, "each d, each 2H, *J*=8.6 *Hz*", AB system), 8.33 (s, 1H, H_5 of triazole), 10.98 (s, 1H, NH, Discharged with D_2O). MS *m/z* (%):

428.06 [M^+] (50.81), 376.03 (23.13), 289.00 (47.17), 287.99 (51.35), 242.00 (27.83), 222.02 (23.93), 198.04 (20.01), 153.03 (41.96), 146.02 (24.78), 144.02 (19.33), 133.01 (33.87), 120.98 (22.64), 119.05 (28.60), 115.03 (36.90), 113.11 (25.85), 105.04 (18.00), 92.99 (27.92), 78.06 (19.58), 77.04 (100.00), 63.02 (68.67), 62.02 (23.33), 55.05 (25.64), 51.02 (42.62), 47.00 (42.76), 44.01 (25.92), 43.06 (81.12). Anal. Calcd for $C_{17}H_{16}N_8O_2S_2$ (428.49): C, 47.65; H, 3.76; N, 26.15; S, 14.97. Found: C, 47.38; H, 3.58; N, 26.33; S, 15.00%.

3-(4-Fluorophenyl)-2-(4-(4-(pyrrolidin-1-ylsulfonyl)phenyl)thiazol-2-yl)acrylonitrile (8)

Procedure A: A mixture of compound **2** (3.33 g, 0.01 mol) and 4-fluorobenzaldehyde (1.24 g, 0.01 mol) in EtOH (40 mL) containing few drops of piperidine was refluxed for 2 h. The obtained product that formed was collected by filtration and recrystallized from EtOH to give **8** (yield 72%).

Procedure B: A mixture of phenacyl bromide derivative **1** (3.32 g, 0.01 mol) and 2-cyano-3-(4-fluorophenyl)prop-2-enethioamide (2.06 g, 0.01 mol) in EtOH (40 mL) was refluxed for 2 h. The product obtained was collected and recrystallized. mp and mixed mp determined with authentic sample gave no depression. White crystals, Yield, 88%; mp 162-163 °C. IR (KBr, cm^{-1}): ν_{max} 3101 (CH aromatic), 2931 (CH aliphatic), 2220 (C≡N), 1630 (C=N), 1594 (C=C), 1364, 1181 (SO_2), 1170 (C-F). ^{1}H NMR (DMSO-d_6): δ 1.88 (m, 4H, CH_2-CH_2 of pyrrolidine), 3.28 (t, 4H, J=10.5 *Hz*, CH_2-N-CH_2 of pyrrolidine), 6.97-7.44 (m, 4H, Ar-H), 7.55, 8.01 (dd, 4H, "each d, each 2H, J=8.5 *Hz*", AB system of benzenesulfonyl), 7.60 (s, 1H, H_5 of thiazole), 8.21 (s, 1H, CH=C). ^{13}C NMR (DMSO-d_6): δ 24.2 (2C, CH_2-CH_2 of pyrrolidine), 64.5 (2C, CH_2-N-CH_2 of pyrrolidine), 100.4, 109.2 (C_5 of thiazole), 113.1 (2C), 117.3 (C≡N), 124.7 (2C), 129.9 (2C), 133.0 (2C), 135.2, 139.4, 144.3, 154.7, 159.2, 163.4, 169.9. MS *m/z* (%): 440.10 [M^++1] (21.76), 439.09 [M^+] (46.31), 438.07 (12.57), 305.03 (22.40), 134.02 (9.01), 133.02 (9.66), 89.04 (64.99), 70.06 (100.00), 63.02 (19.44), 43.06 (21.32), 42.04 (83.04), 41.04 (24.43). Anal. Calcd for $C_{22}H_{18}FN_3O_2S_2$ (439.53): C, 60.12; H, 4.13; N, 9.56; S, 14.59. Found: C, 60.09; H, 4.11; N, 9.51; S, 14.71%.

2-(4-(4-(Pyrrolidin-1-ylsulfonyl)phenyl)thiazol-2-yl)ethanethioamide (9)

To a solution of compound **2** (3.33 g, 0.01 mol) in EtOH (30 mL) triethylamine (1.01 g, 0.01 mol) was added, and the mixture was saturated with H_2S for 3 h and then left overnight. The precipitated solid was separated by filtration and purified by recrystallization from EtOH to give **9**. Yellow crystals, Yield, 54%; mp 146-148 °C. IR (KBr, cm^{-1}): ν_{max} 3401, 3299 (NH_2), 3019 (CH aromatic), 2995, 2957, 2910 (CH aliphatic), 1593 (C=N), 1554 (C=C), 1544, 1276, 1144, 1056 (N-C=S), 1358, 1170 (SO_2), 1340 (C=S). ^{1}H NMR (DMSO-d_6): δ 1.94 (m, 4H, CH_2-CH_2 of pyrrolidine), 3.33 (t, 4H, J=10.6 *Hz*, CH_2-N-CH_2 of pyrrolidine), 3.51 (s, 2H, CH_2), 7.31 (s, 1H, H_5 of thiazole), 7.57, 8.11 (dd, 4H, "each d, each 2H, J=8.5

Hz", AB system), 8.61 (s, 2H, NH_2, Discharged with D_2O). ^{13}C NMR (DMSO-d_6): δ 22.6 (2C, CH_2-CH_2 of pyrrolidine), 48.4 (CH_2), 61.8 (2C, CH_2-N-CH_2 of pyrrolidine), 113.5 (C_5 of thiazole), 126.6 (2C), 128.8 (2C), 137.0, 141.4, 156.5, 171.7, 203.7 (C=S). MS *m/z* (%): 367.45 [M^+] (90.13), 333.05 (14.20), 309.08 (25.31), 269.08 (18.15), 268.09 (19.48), 263.01 (19.65), 241.09 (32.91), 200.05 (30.76), 199.04 (43.35), 191.07 (15.84), 187.14 (18.21), 176.09 (19.94), 175.10 (56.20), 174.07 (17.12), 168.97 (18.68), 159.95 (16.53), 134.06 (33.23), 133.07 (17.71), 127.89 (18.73), 89.06 (41.73), 81.96 (22.21), 79.97 (16.80), 69.07 (100.00), 64.01 (17.16), 49.03 (28.48). Anal. Calcd for $C_{15}H_{17}N_3O_2S_3$ (367.51): C, 49.02; H, 4.66; N, 11.43; S, 26.17. Found: C, 48.89; H, 4.54; N, 11.40; S, 26.32%.

2-((4-(4-(Pyrrolidin-1-ylsulfonyl)phenyl)thiazol-2-yl)methyl)thiazol-4(5*H*)-one (10)

Procedure A: A mixture of compound **9** (3.67 g, 0.01 mol) and ethyl 2-chloroacetate (1.22 g, 0.01 mol) in EtOH (20 mL) containing 1 g of sodium acetate was refluxed for 2 h. The obtained product that formed was collected and recrystallized from EtOH to give **10** (yield 46%).

Procedure B: A mixture of compound **2** (3.33 g, 0.01 mol) and 2-mercaptoacetic acid (0.92 g, 0.01 mol) in pyridine (10 mL) was refluxed for 45 min. The solid obtained was filtered off and recrystallized; mp and mixed mp determined with authentic sample gave no depression. White crystals, Yield, 61%; mp 203-204 °C. IR (KBr, cm^{-1}): ν_{max} 3102, 3056 (CH aromatic), 2977, 2945, 2911 (CH aliphatic), 1673 (C=O), 1611 (C=N), 1586 (C=C), 1371, 1181 (SO_2). ^1H NMR (DMSO-d_6): δ 1.92 (m, 4H, CH_2-CH_2 of pyrrolidine), 3.31 (t, 4H, *J*=10.2 *Hz*, CH_2-N-CH_2 of pyrrolidine), 3.18 (s, 2H, CH_2), 4.25 (s, 2H, CH_2 of thiazolidinone), 7.40 (s, 1H, H_5 of thiazole), 7.83, 8.26 (dd, 4H, "each d, each 2H, *J*=8.4 *Hz*", AB system). ^{13}C NMR (DMSO-d_6): δ 22.1 (2C, CH_2-CH_2 of pyrrolidine), 35.3 (CH_2), 41.6 (CH_2 of thiazolidinone), 63.4 (2C, CH_2-N-CH_2 of pyrrolidine), 110.7 (C_5 of thiazole), 125.0 (2C), 129.2 (2C), 138.1, 142.5, 156.9, 167.9, 170.3, 179.2. MS *m/z* (%): 407.06 [M^+] (71.25), 388.15 (18.57), 255.12 (11.19), 254.14 (40.61), 253.11 (75.20), 252.09 (13.91), 239.10 (13.16), 238.07 (10.54), 212.09 (14.90), 211.08 (17.71), 152.09 (18.05), 123.07 (12.19), 120.09 (37.16), 119.11 (49.97), 105.08 (11.00), 72.13 (100.00). Anal. Calcd for $C_{17}H_{17}N_3O_3S_3$ (407.53): C, 50.10; H, 4.20; N, 10.31; S, 23.60. Found: C, 50.33; H, 4.13; N, 10.22; S, 23.51%.

5-(4-Fluorobenzylidene)-2-((4-(4-(pyrrolidin-1-ylsulfonyl)phenyl)thiazol-2-yl)methyl)thiazol-4(5*H*)-one (13)

Procedure A: A mixture of compound **10** (4.07 g, 0.01 mol) and 2-(4-fluorobenzylidene)malononitrile (1.72 g, 0.01 mol) in EtOH (50 mL) was refluxed for 2 h. The solid obtained was filtered off and recrystallized from AcOH to give **13** (yield 66%).

Procedure B: A mixture of compound **10** (4.07 g, 0.01 mol) and 4-fluorobenzaldehyde (1.24 g, 0.01 mol)

in EtOH (20 mL) and few drops of piperidine was refluxed for 3 h. The mixture was cooled and the separated solid was filtered off and recrystallized, mp and mixed mp determined with authentic sample gave no depression. White crystals, Yield, 83%; mp 213-215 °C. IR (KBr, cm^{-1}): ν_{max} 3090 (CH aromatic), 2910, 2899 (CH aliphatic), 1655 (C=O), 1580 (C=N), 1551 (C=C), 1345, 1155 (SO_2), 1172 (C-F). ^{1}H NMR (DMSO-d_6): δ 1.87 (m, 4H, CH_2-CH_2 of pyrrolidine), 3.32 (t, 4H, *J*=11.1 *Hz*, CH_2-N-CH_2 of pyrrolidine), 3.15 (s, 2H, CH_2), 7.69 (s, 1H, H_5 of thiazole), 7.80 (s, 1H, CH=C), 7.10-7.54 (m, 4H, Ar-H), 7.96, 8.52 (dd, 4H, "each d, each 2H, *J*=8.5 *Hz*", AB system of benzenesulfonyl). MS *m/z* (%): 513.88 [M^+] (57.28), 513.12 (42.87), 354.16 (19.89), 351.07 (6.02), 315.11 (6.95), 311.19 (9.84), 310.16 (100.00), 298.14 (16.56), 282.12 (18.52), 254.11 (14.16), 238.10 (30.62), 224.10 (23.21), 216.08 (5.44), 200.08 (29.68), 199.09 (11.64), 193.08 (16.61), 189.11 (10.88), 155.10 (11.41), 97.14 (41.79), 79.09 (31.20), 60.04 (79.33), 56.11 (8.64), 46.07 (16.68), 40.17 (8.84). Anal. Calcd for $C_{24}H_{20}FN_3O_3S_3$ (513.63): C, 56.12; H, 3.92; N, 8.18; S, 18.73. Found: C, 56.35; H, 3.79; N, 8.09; S, 18.56%.

5-Imino-7-(methylthio)-3-(4-(pyrrolidin-1-ylsulfonyl)phenyl)-5*H*-thiazolo[3,2-*a*]pyridine-6,8-dicarbonitrile (14)

A mixture of compound **2** (3.33 g, 0.01 mol), anhydrous potassium carbonate (1.66 g, 0.012 mol) and 2-(bis(methylthio)methylene)malononitrile (1.70 g, 0.01 mol) in *N*,*N*-dimethylformamide (30 mL) was heated at 50-60 ºC with stirring until odor of the methane thiol is ceased. After cooling, the reaction mixture poured into crushed ice (100 g) then acidified with 1N hydrogen chloride. The solid product that formed was collected and recrystallized from dioxane to give **14**. White crystals, Yield, 62%; mp 203-205 °C. IR (KBr, cm^{-1}): ν_{max} 3235 (NH), 3070 (CH aromatic), 2990 (CH aliphatic), 2220, 2218 (2C≡N), 1644 (C=N), 1568 (C=C), 1337, 1159 (SO_2), 1180 (C-S). ^{1}H NMR (DMSO-d_6): δ 1.92 (m, 4H, CH_2-CH_2 of pyrrolidine), 2.83 (s, 3H, SCH_3), 3.30 (t, 4H, *J*=10.6 *Hz*, CH_2-N-CH_2 of pyrrolidine), 7.52 (s, 1H, H_5 of thiazole), 7.67, 8.10 (dd, 4H, "each d, each 2H, *J*=8.8 *Hz*", AB system), 8.52 (s, 1H, NH, Discharged with D_2O). ^{13}C NMR (DMSO-d_6): δ 16.2 (CH_3), 23.6 (2C, CH_2-CH_2 of pyrrolidine), 64.7 (2C, CH_2-N-CH_2 of pyrrolidine), 77.2, 85.6, 114.0 (2C, 2C≡N), 118.2 (C_5 of thiazole), 126.7 (2C), 132.0 (2C), 135.7, 136.9, 141.3, 155.8, 160.0, 166.3. MS *m/z* (%): 455.43 [M^+] (75.92), 401.20 (14.46), 308.15 (21.86), 267.13 (6.25), 266.11 (4.76), 206.14 (5.95), 205.12 (4.81), 139.10 (6.16), 131.11 (8.08), 123.12 (7.56), 120.10 (4.16), 118.11 (14.45), 117.10 (10.07), 112.13 (6.69), 91.09 (75.75), 84.11 (100.00), 78.10 (5.70), 77.08 (9.85), 65.08 (10.22). Anal. Calcd for $C_{20}H_{17}N_5O_2S_3$ (455.58): C, 52.73; H, 3.76; N, 15.37; S, 21.12. Found: C, 52.64; H, 3.56; N, 15.10; S, 21.40%.

3-(4-(4-(Pyrrolidin-1-ylsulfonyl)phenyl)thiazol-2-yl)-2*H*-chromen-2-one (16)

Procedure A: To a mixture of compound **2** (3.33 g, 0.01 mol) and 2-hydroxybenzaldehyde (1.22 g, 0.01

mol) in EtOH (50 mL), a few drops of piperidine was added as a catalyst. The reaction mixture was refluxed for 3 h, and the solid product was collected by filtration and recrystallized from EtOH/benzene to give **16** (yield 90%).

Procedure B: A mixture of compound **1** (3.32 g, 0.01 mol) and 2-oxo-2*H*-chromene-3-carbothioamide (2.05 g, 0.01 mol) in EtOH (50 mL) was refluxed for 2 h. The solid obtained was filtered off and recrystallized; mp and mixed mp determined with authentic sample gave no depression. Yellowish white crystals, Yield, 85%; mp 245-246 °C. IR (KBr, cm^{-1}): ν_{max} 3100 (CH aromatic), 2953 (CH aliphatic), 1708 (C=O), 1628 (C=N), 1589 (C=C), 1364, 1180 (SO_2), 1276, 1037 (C-O-C). ^{1}H NMR (DMSO-d_6): δ 1.93 (m, 4H, CH_2-CH_2 of pyrrolidine), 3.27 (t, 4H, *J*=9.8 *Hz*, CH_2-N-CH_2 of pyrrolidine), 7.00 (s, 1H, H_5 of thiazole), 7.17-7.79 (m, 4H, Ar-H), 7.85, 8.17 (dd, 4H, "each d, each 2H, *J*=8.6 *Hz*", AB system), 9.11 (s, 1H, H_4 of coumarin). MS *m/z* (%): 439.18 [M^++1] (25.27), 438.24 [M^+] (59.50), 364.59 (39.76), 364.08 (30.74), 358.16 (17.76), 344.40 (19.43), 335.09 (32.38), 330.47 (27.88), 329.12 (19.68), 325.47 (33.45), 315.12 (30.68), 304.21 (59.19), 283.55 (37.76), 269.16 (21.25), 250.13 (19.41), 240.09 (17.59), 233.44 (58.81), 232.14 (33.39), 212.14 (38.16), 204.14 (57.33), 159.11 (51.86), 157.13 (100.00), 145.12 (35.16), 97.12 (81.54), 49.08 (18.58). Anal. Calcd for $C_{22}H_{18}N_2O_4S_2$ (438.52): C, 60.26; H, 4.14; N, 6.39; S, 14.62. Found: C, 60.01; H, 4.32; N, 6.45; S, 14.70%.

2-(4-(4-(Pyrrolidin-1-ylsulfonyl)phenyl)thiazol-2-yl)-3*H*-benzo[*f*]chromen-3-imine (17)

To a mixture of compound **2** (3.33 g, 0.01 mol) and 2-hydroxy-1-naphthaldehyde (1.72 g, 0.01 mol) in EtOH (50 mL), a few drops of piperidine was added as catalyst. The reaction mixture was refluxed for 5 h. The isolated product was collected and recrystallized from EtOH to give **17**. White solids, Yield, 72%; mp 199-201 °C. IR (KBr, cm^{-1}): ν_{max} 3199 (NH), 3019 (CH aromatic), 2993 (CH aliphatic), 1583 (C=N), 1554 (C=C), 1349, 1170 (SO_2), 1247, 1056 (C-O-C). ^{1}H NMR (DMSO-d_6): δ 1.93 (m, 4H, CH_2-CH_2 of pyrrolidine), 3.30 (t, 4H, *J*=10.0 *Hz*, CH_2-N-CH_2 of pyrrolidine), 7.16 (s, 1H, H_5 of thiazole), 7.22-8.97 (m, 10H, Ar-H), 9.17 (s, 1H, H_1 of benzocoumarin), 9.78 (br, 1H, NH, Discharged with D_2O). MS *m/z* (%): 487.04 [M^+] (36.42), 436.36 (65.48), 372.01 (58.90), 222.03 (14.83), 213.84 (37.09), 176.36 (33.76), 158.29 (42.91), 99.26 (16.53), 89.25 (11.52), 81.23 (27.35), 77.63 (72.59), 70.42 (100.00), 44.73 (10.94), 41.83 (9.37). Anal. Calcd for $C_{26}H_{21}N_3O_3S_2$ (487.59): C, 64.04; H, 4.34; N, 8.62; S, 13.15. Found: C, 63.89; H, 4.12; N, 8.53; S, 13.09%.

2-(4-(4-(Pyrrolidin-1-ylsulfonyl)phenyl)thiazol-2-yl)-3*H*-benzo[*f*]chromen-3-one (18)

To a mixture of 30 mL AcOH and 3 mL of hydrogen chloride, (4.87 g, 0.01 mol) of compound **17** was added and refluxed for 2 h. The precipitate obtained after cooling was filtered off and recrystallized from EtOH/benzene to give **18**. Yellow crystals, Yield, 91%; mp 203-205 °C. IR (KBr, cm^{-1}): ν_{max} 3105, 3065

(CH aromatic), 2973, 2910 (CH aliphatic), 1703 (C=O), 1630 (C=N), 1582 (C=C), 1333, 1179 (SO_2), 1208, 1042 (C-O-C). ^{1}H NMR (DMSO-d_6): δ 1.87 (m, 4H, CH_2-CH_2 of pyrrolidine), 3.29 (t, 4H, *J*=10.0 *Hz*, CH_2-N-CH_2 of pyrrolidine), 7.19 (s, 1H, H_5 of thiazole), 7.22-8.86 (m, 10H, Ar-H), 9.19 (s, 1H, H_1 of benzocoumarin). MS *m/z* (%): 489.08 [M^++1] (32.09), 488.38 [M^+] (59.06), 487.76 (12.68), 486.99 (14.32), 471.10 (16.35), 470.04 (42.94), 336.03 (22.59), 193.05 (39.88), 176.95 (23.46), 176.22 (31.85), 165.06 (17.47), 164.03 (16.15), 134.03 (27.78), 133.01 (18.73), 102.06 (13.38), 101.04 (26.18), 91.06 (17.77), 90.07 (18.35), 89.04 (100.00), 75.04 (12.24), 70.07 (69.38), 63.04 (34.79), 43.07 (34.77), 42.04 (92.64), 41.06 (35.88). Anal. Calcd for $C_{26}H_{20}N_2O_4S_2$ (488.58): C, 63.92; H, 4.13; N, 5.73; S, 13.13. Found: C, 63.76; H, 4.09; N, 5.54; S, 13.07%.

3-Ethoxy-2-(4-(4-(pyrrolidin-1-ylsulfonyl)phenyl)thiazol-2-yl)acrylonitrile (19)

A mixture of compound **2** (3.33 g, 0.01 mol) and triethoxymethane (1.48 g, 0.01 mol) in acetic anhydride (1.02 g, 0.01 mol) was refluxed for 3 h. The reaction mixture was concentrated to 10 mL. After cooling, the solid product was collected by filtration and washed with EtOH then recrystallized from benzene to give **19**. White crystals, Yield, 61%; mp 174-176 °C. IR (KBr, cm^{-1}): ν_{max} 3107, 3064 (CH aromatic), 2944, 2921, 2904 (CH aliphatic), 2220 (C≡N), 1611 (C=N), 1592 (C=C), 1347, 1155 (SO_2), 1254, 1035 (C-O-C). ^{1}H NMR (DMSO-d_6): δ 1.26 (t, 3H, *J*=7.2 *Hz*, $\underline{CH_3}$-CH_2), 1.85 (m, 4H, CH_2-CH_2 of pyrrolidine), 3.32 (t, 4H, *J*=10.5 *Hz*, CH_2-N-CH_2 of pyrrolidine), 4.52 (q, 2H, *J*=7.2 *Hz*, CH_3-$\underline{CH_2}$), 7.40 (s, 1H, CH=C), 7.73 (s, 1H, H_5 of thiazole), 7.96, 8.55 (dd, 4H, "each d, each 2H, *J*=8.6 *Hz*", AB system). MS *m/z* (%): 389.03 [M^+] (42.02), 333.03 (6.20), 262.97 (5.30), 216.02 (7.67), 200.04 (17.22), 199.03 (24.45), 159.00 (7.30), 134.06 (9.25), 133.02 (9.22), 89.03 (35.71), 70.06 (100.00), 63.02 (8.19), 43.05 (16.98), 42.03 (46.51), 41.05 (14.96). Anal. Calcd for $C_{18}H_{19}N_3O_3S_2$ (389.49): C, 55.51; H, 4.92; N, 10.79; S, 16.47. Found: C, 55.34; H, 4.75; N, 10.88; S, 16.31%.

3-(Dimethylamino)-2-(4-(4-(pyrrolidin-1-ylsulfonyl)phenyl)thiazol-2-yl)acrylonitrile (20)

Procedure A: A mixture of compound **19** (3.89 g, 0.01 mol) and dimethylamine (0.45 g, 0.01 mol) in MeOH (40 mL) was refluxed for 3 h. The mixture was then cooled, and the separated solid was collected by filtration and recrystallized from benzene to give **20** (yield 71%).

Procedure B: A mixture of compound **2** (3.33 g, 0.01 mol) and DMF-DMA (1.19 g, 0.01 mol) in dry xylene (30 mL) was refluxed for 3 h. Then the cooled precipitated product was filtered off, washed with light petroleum ether, and recrystallized, mp and mixed mp determined with authentic sample gave no depression. White crystals, Yield, 85%; mp 189-190 °C. IR (KBr, cm^{-1}): ν_{max} 3090, 3038 (CH aromatic), 2991, 2963, 2905 (CH aliphatic), 2218 (C≡N), 1575 (C=N), 1557 (C=C), 1355, 1161 (SO_2). ^{1}H NMR (DMSO-d_6): δ 1.95 (m, 4H, CH_2-CH_2 of pyrrolidine), 2.99 (s, 6H, $(CH_3)_2$N), 3.40 (t, 4H, *J*=11.0 *Hz*,

CH_2-N-CH_2 of pyrrolidine), 7.42 (s, 1H, CH=C), 7.65 (s, 1H, H_5 of thiazole), 7.97, 8.63 (dd, 4H, "each d, each 2H, *J*=8.7 *Hz*", AB system). ^{13}C NMR (DMSO-*d*$_6$): δ 22.7 (2C, CH_2-CH_2 of pyrrolidine), 41.6 (2C, $(CH_3)_2N$), 62.8 (2C, CH_2-N-CH_2 of pyrrolidine), 83.2, 108.3 (C_5 of thiazole), 118.8 (C≡N), 123.3 (2C), 128.0 (2C), 138.4, 143.5, 156.4, 159.9, 166.4. MS *m/z* (%): 389.15 [M^++1] (26.83), 388.12 [M^+] (98.13), 387.10 (7.80), 255.11 (16.63), 254.12 (47.16), 253.11 (100.00), 252.11 (18.22), 239.09 (14.46), 238.09 (12.67), 134.05 (11.64), 119.09 (15.71), 89.03 (22.86), 72.10 (17.98), 42.06 (13.32). Anal. Calcd for $C_{18}H_{20}N_4O_2S_2$ (388.51): C, 55.65; H, 5.19; N, 14.42; S, 16.51. Found: C, 55.44; H, 5.26; N, 14.62; S, 16.46%.

6-(4-(4-(Pyrrolidin-1-ylsulfonyl)phenyl)thiazol-2-yl)-[1,2,4]triazolo[4,3-*a*]pyrimidin-5-amine (22)

A mixture of compound **20** (3.88 g, 0.01 mol) and 4*H*-1,2,4-triazol-3-amine (0.84 g, 0.01 mol) in *N*,*N*-dimethylformamide (30 mL) was refluxed for 3 h. After cooling, the product was collected by filtration then washed with EtOH and recrystallized from dioxane to give **22**. White crystals, Yield, 83%; mp 310-312 °C. IR (KBr, cm^{-1}): ν_{max} 3417, 3310 (NH_2), 3074 (CH aromatic), 2934, 2901 (CH aliphatic), 1583 (C=N), 1558 (C=C), 1343, 1155 (SO_2). ^{1}H NMR (DMSO-*d*$_6$): δ 1.80 (m, 4H, CH_2-CH_2 of pyrrolidine), 3.27 (t, 4H, *J*=11.1 *Hz*, CH_2-N-CH_2 of pyrrolidine), 7.61 (br, 2H, NH_2, Discharged with D_2O), 7.72-8.96 (m, 7H, Ar-H + H_5 of thiazole). ^{13}C NMR (DMSO-d$_6$): δ 22.2 (2C, CH_2-CH_2 of pyrrolidine), 65.4 (2C, CH_2-N-CH_2 of pyrrolidine), 109.1 (C_5 of thiazole), 113.6, 126.3 (2C), 130.9 (2C), 134.9, 138.2, 143.7, 156.8, 157.4, 160.3, 163.4, 170.2. MS *m/z* (%): 428.82 [M^++1] (30.65), 427.02 [M^+] (72.76), 305.09 (21.09), 270.22 (17.64), 218.15 (18.72), 188.13 (18.54), 169.07 (25.29), 168.10 (16.04), 135.09 (18.55), 112.13 (21.29), 110.12 (38.84), 100.08 (18.90), 98.11 (27.42), 95.09 (33.95), 86.10 (43.97), 84.08 (100.00), 83.09 (17.05), 77.08 (44.30), 66.08 (14.28), 65.07 (25.35), 59.08 (29.66), 56.09 (74.85), 55.08 (45.89), 54.08 (79.34), 53.08 (26.00), 47.03 (15.46). Anal. Calcd for $C_{18}H_{17}N_7O_2S_2$ (427.50): C, 50.57; H, 4.01; N, 22.93; S, 15.00. Found: C, 50.46; H, 4.28; N, 22.78; S, 15.19%.

7-Amino-2-methyl-6-(4-(4-(pyrrolidin-1-ylsulfonyl)phenyl)thiazol-2-yl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide (23)

The same experimental procedure described above for the synthesis of compound **22** has been followed except for using 5-amino-3-methyl-1*H*-pyrazole-4-carboxamide (1.40 g, 0.01 mol), instead of 4*H*-1,2,4-triazol-3-amine. White crystals, Yield, 77%; mp 270-271 °C (dioxane). IR (KBr, cm^{-1}): ν_{max} 3418, 3400, 3371, 3218 ($2NH_2$), 3072 (CH aromatic), 2961, 2942 (CH aliphatic), 1651 (C=O), 1613 (C=N), 1571 (C=C), 1352, 1185 (SO_2). ^{1}H NMR (DMSO-*d*$_6$): δ 1.99 (m, 4H, CH_2-CH_2 of pyrrolidine), 2.52 (s, 3H, CH_3), 3.33 (t, 4H, *J*=10.4 *Hz*, CH_2-N-CH_2 of pyrrolidine), 7.31 (s, 1H, H_5 of thiazole), 7.55 (s, 2H, $CONH_2$, Discharged with D_2O), 7.71 (s, 2H, NH_2, Discharged with D_2O), 7.97, 8.63 (dd, 4H,

"each d, each 2H, *J*=8.8 *Hz*", AB system), 8.12 (s, 1H, H_5 of pyrazolopyrimidine). MS *m/z* (%): 483.21 [M^+] (53.48), 465.82 (14.92), 389.17 (24.27), 387.12 (15.64), 374.31 (21.33), 322.43 (12.87), 309.11 (12.77), 308.11 (16.15), 296.11 (23.31), 291.27 (25.65), 264.09 (14.32), 262.25 (28.13), 261.05 (25.47), 258.25 (22.85), 234.11 (14.02), 233.13 (22.46), 216.04 (100.00), 175.07 (21.65), 142.10 (39.31), 109.07 (12.19), 60.01 (42.41), 52.03 (16.12), 47.98 (66.00), 40.16 (65.02). Anal. Calcd for $C_{21}H_{21}N_7O_3S_2$ (483.57): C, 52.16; H, 4.38; N, 20.28; S, 13.26. Found: C, 52.23; H, 4.16; N, 20.01; S, 13.34%.

3-Hydrazinyl-2-(4-(4-(pyrrolidin-1-ylsulfonyl)phenyl)thiazol-2-yl)acrylonitrile (24)

To a solution of the compound **20** (3.88 g, 0.01 mol) in EtOH (20 mL) hydrazine hydrate (99%, 0.50 g, 0.01 mol) was added. The reaction mixture was refluxed for 2 h, then cooled. The solid product formed was filtered off, washed with EtOH, and dried, then recrystallized from EtOH to give **24**. White solids, Yield, 56%; mp 212-214 °C. IR (KBr, cm^{-1}): ν_{max} 3440, 3418 (NH_2), 3190 (NH), 3013 (CH aromatic), 2972, 2943 (CH aliphatic), 2218 (C≡N), 1630 (C=N), 1595 (C=C), 1336, 1159 (SO_2). ^{1}H NMR (DMSO-d_6): δ 1.83 (m, 4H, CH_2-CH_2 of pyrrolidine), 3.40 (t, 4H, *J*=9.9 *Hz*, CH_2-N-CH_2 of pyrrolidine), 5.08 (s, 2H, NH_2, Discharged with D_2O), 6.75 (s, 1H, CH=C), 7.45 (s, 1H, H_5 of thiazole), 7.86, 8.56 (dd, 4H, "each d, each 2H, *J*=8.4 *Hz*", AB system), 10.22 (s, 1H, NH, Discharged with D_2O). MS *m/z* (%): 375.40 [M^+] (70.54), 331.42 (51.72), 302.56 (17.43), 263.43 (11.92), 221.82 (22.73), 183.49 (18.43), 128.34 (11.54), 101.69 (47.83), 91.37 (66.90), 80.83 (15.32), 71.34 (100.00), 60.53 (12.43), 51.93 (15.76), 44.73 (17.53), 41.69 (18.34). Anal. Calcd for $C_{16}H_{17}N_5O_2S_2$ (375.47): C, 51.18; H, 4.56; N, 18.65; S, 17.08. Found: C, 51.09; H, 4.78; N, 18.70; S, 17.31%.

4-(4-(4-(Pyrrolidin-1-ylsulfonyl)phenyl)thiazol-2-yl)isoxazol-5-amine (26)

To a mixture of compound **20** (3.88 g, 0.01 mol) and hydroxylamine hydrochloride (1.04 g, 0.015 mol) in EtOH (30 mL), sodium acetate (2.05 g, 0.025 mol) was added. The resulting mixture was refluxed for 3 h and then allowed to cool to room temperature and diluted with water (20 mL). The solid product so formed was collected by filtration, washed with water, and dried, then recrystallized from EtOH/benzene to give **26**. White crystals, Yield, 75%; mp 256-258 °C. IR (KBr, cm^{-1}): ν_{max} 3411, 3316 (NH_2), 3015 (CH aromatic), 2962 (CH aliphatic), 1635 (C=N), 1580 (C=C), 1349, 1156 (SO_2). ^{1}H NMR (DMSO-d_6): δ 1.95 (m, 4H, CH_2-CH_2 of pyrrolidine), 3.32 (t, 4H, *J*=9.8 *Hz*, CH_2-N-CH_2 of pyrrolidine), 6.73 (br, 2H, NH_2, Discharged with D_2O), 7.21 (s, 1H, H_5 of thiazole), 7.88, 8.71 (dd, 4H, "each d, each 2H, *J*=8.4 *Hz*", AB system), 8.23 (s, 1H, H_3 of isoxazole). ^{13}C NMR (DMSO-d_6): δ 22.5 (2C, CH_2-CH_2 of pyrrolidine), 64.9 (2C, CH_2-N-CH_2 of pyrrolidine), 98.3, 112.0 (C_5 of thiazole), 125.5 (2C), 129.6 (2C), 138.3, 142.8, 147.3, 150.7, 157.3, 161.7. MS *m/z* (%): 376.07 [M^+] (70.91), 375.07 (30.31), 374.09 (28.46), 333.05 (5.59), 253.07 (8.82), 239.05 (8.14), 216.04 (10.99), 199.04 (44.19), 172.04 (9.88), 159.02 (16.30),

134.04 (30.03), 133.03 (20.59), 127.01 (16.75), 119.08 (14.62), 89.04 (95.58), 70.07 (100.00), 63.03 (30.54), 44.03 (14.40), 43.06 (35.82), 42.05 (84.74), 41.05 (45.09). Anal. Calcd for $C_{16}H_{16}N_4O_3S_2$ (376.45): C, 51.05; H, 4.28; N, 14.88; S, 17.04. Found: C, 50.89; H, 4.12; N, 14.76; S, 17.23%.

5-(4-(4-(Pyrrolidin-1-ylsulfonyl)phenyl)thiazol-2-yl)pyrimidine-2,4-diamine (27)

The same experimental procedure described above for the synthesis of compound **26** has been followed except for using guanidine hydrochloride (0.95 g, 0.01 mol) instead of hydroxylamine hydrochloride. Brown crystals, Yield, 79%; mp 301-303 °C (AcOH). IR (KBr, cm^{-1}): ν_{max} 3400, 3366, 3258, 3187 ($2NH_2$), 3030 (CH aromatic), 2983 (CH aliphatic), 1611 (C=N), 1584 (C=C), 1359, 1161 (SO_2). ^{1}H NMR (DMSO-d_6): δ 1.81 (m, 4H, CH_2-CH_2 of pyrrolidine), 3.28 (t, 4H, *J*=9.8 *Hz*, CH_2-N-CH_2 of pyrrolidine), 7.06, 7.37 (2s, 4H, $2NH_2$, Discharged with D_2O), 7.40 (s, 1H, H_5 of thiazole), 7.79, 8.83 (dd, 4H, "each d, each 2H, *J*=8.6 *Hz*", AB system), 8.35 (s, 1H, H_6 of pyrimidine). ^{13}C NMR (DMSO-d_6): δ 25.0 (2C, CH_2-CH_2 of pyrrolidine), 60.5 (2C, CH_2-N-CH_2 of pyrrolidine), 99.7, 110.7 (C_5 of thiazole), 123.1 (2C), 130.1 (2C), 135.3, 141.3, 146.4, 149.8, 153.5, 159.4, 169.2. MS *m/z* (%): 402.17 [M^+] (64.21), 360.21 (100.00), 291.17 (19.66), 256.16 (25.83), 241.14 (38.00), 232.12 (21.19), 229.12 (23.06), 216.09 (38.49), 190.09 (24.68), 188.11 (17.26), 181.11 (23.24), 180.12 (32.87), 168.09 (20.48), 155.09 (23.18), 154.09 (21.21), 131.09 (30.79), 127.08 (27.93), 118.08 (20.43), 111.08 (33.46), 101.08 (33.06), 94.10 (34.52), 78.08 (24.21), 73.07 (25.82), 71.10 (47.88), 57.09 (34.58). Anal. Calcd for $C_{17}H_{18}N_6O_2S_2$ (402.49): C, 50.73; H, 4.51; N, 20.88; S, 15.93. Found: C, 50.65; H, 4.62; N, 20.74; S, 15.81%.

3-Amino-2-(4-(4-(pyrrolidin-1-ylsulfonyl)phenyl)thiazol-2-yl)benzo[4,5]imidazo[1,2-*a*]pyridine-4-carbonitrile (28)

A mixture of compound **20** (3.88 g, 0.01 mol) and 2-(1*H*-benzo[*d*]imidazol-2-yl)acetonitrile (1.57 g, 0.01 mol) in dioxane (50 mL) was refluxed for 5 h. The solid product that was obtained after cooling was collected by filtration and recrystallized from DMF to give **28**. Brown crystals, Yield, 81%; mp 306-307 °C. IR (KBr, cm^{-1}): ν_{max} 3416, 3295 (NH_2), 3027 (CH aromatic), 2908 (CH aliphatic), 2220 (C≡N), 1630 (C=N), 1597 (C=C), 1345, 1155 (SO_2). ^{1}H NMR (DMSO-d_6): δ 1.85 (m, 4H, CH_2-CH_2 of pyrrolidine), 3.31 (t, 4H, *J*=10.3 *Hz*, CH_2-N-CH_2 of pyrrolidine), 6.81 (s, 2H, NH_2, Discharged with D_2O), 7.44-8.78 (m, 10H, Ar-H + H_5 of thiazole). MS *m/z* (%): 500.12 [M^+] (70.29), 385.08 (15.09), 370.12 (11.52), 338.02 (13.57), 333.06 (19.05), 310.13 (9.12), 263.04 (20.74), 126.20 (22.97), 91.07 (92.84), 65.06 (36.93), 60.04 (12.42), 57.08 (48.03), 51.04 (21.34), 50.03 (21.35), 45.02 (34.30), 44.08 (100.00), 43.09 (57.32). Anal. Calcd for $C_{25}H_{20}N_6O_2S_2$ (500.60): C, 59.98; H, 4.03; N, 16.79; S, 12.81. Found: C, 59.73; H, 4.12; N, 16.67; S, 12.59%.

2-Cyano-2-(4-(4-(pyrrolidin-1-ylsulfonyl)phenyl)thiazol-2-yl)ethanedithioic acid (30)

Carbon disulfide (0.76 g, 0.01 mol) was added gradually to a cold solution of thiazolylacetonitrile **2** (3.33 g, 0.01 mol) in *N,N*-dimethylformamide (20 mL) containing finely ground potassium hydroxide (1.12 g, 0.02 mol). The reaction mixture left at room temperature for an additional 24 h. The reaction mixture was then triturated with cold water (50 mL) and neutralized with 1N hydrogen chloride. The resulting precipitated solid was collected by filtration, washed with water dried and recrystallized from EtOH to give **30**. White solids, Yield, 90%; mp 142-144 °C. IR (KBr, cm^{-1}): ν_{max} 3074 (CH aromatic), 2915 (CH aliphatic), 2590 (SH), 2222 (C≡N), 1613 (C=N), 1588 (C=C), 1360, 1172 (SO_2), 1344 (C=S). ^{1}H NMR (DMSO-d_6): δ 1.61 (s, 1H, SH), 1.80 (m, 4H, CH_2-CH_2 of pyrrolidine), 3.27 (t, 4H, *J*=10.4 *Hz*, CH_2-N-CH_2 of pyrrolidine), 5.25 (s, 1H, CH), 7.60 (s, 1H, H_5 of thiazole), 7.77, 8.86 (dd, 4H, "each d, each 2H, *J*=8.6 *Hz*", AB system). MS *m/z* (%): 409.04 [M^+] (81.72), 200.08 (7.13), 199.06 (9.89), 159.05 (5.03), 134.06 (9.64), 133.05 (7.90), 89.06 (40.13), 76.02 (11.00), 70.09 (100.00), 63.06 (12.97), 43.10 (22.54), 42.08 (63.34), 41.09 (22.69). Anal. Calcd for $C_{16}H_{15}N_3O_2S_4$ (409.57): C, 46.92; H, 3.69; N, 10.26; S, 31.32. Found: C, 46.81; H, 3.47; N, 10.10; S, 31.14%.

3,3-Bis(methylthio)-2-(4-(4-(pyrrolidin-1-ylsulfonyl)phenyl)thiazol-2-yl)acrylonitrile (31)

The same experimental procedure described above for the synthesis of compound **30** has been followed except iodomethane was added (2.84 g, 0.02 mol) before the reaction mixture was left at room temperature. Orange crystals, Yield, 89%; mp 177-179 °C (EtOH). IR (KBr, cm^{-1}): ν_{max} 3105 (CH aromatic), 2990 (CH aliphatic), 2218 (C≡N), 1575 (C=N), 1560 (C=C), 1333, 1153 (SO_2), 1189, 1150 (2C-S). ^{1}H NMR (DMSO-d_6): δ 1.89 (m, 4H, CH_2-CH_2 of pyrrolidine), 2.85 (s, 6H, 2SCH_3), 3.32 (t, 4H, *J*=10.4 *Hz*, CH_2-N-CH_2 of pyrrolidine), 7.31 (s, 1H, H_5 of thiazole), 7.76, 8.56 (dd, 4H, "each d, each 2H, *J*=8.7 *Hz*", AB system). ^{13}C NMR (DMSO-d_6): δ 16.9 (2C, 2SCH_3), 25.0 (2C, CH_2-CH_2 of pyrrolidine), 63.7 (2C, CH_2-N-CH_2 of pyrrolidine), 84.5, 109.6 (C_5 of thiazole), 119.1 (C≡N), 124.9 (2C), 128.9 (2C), 133.6, 139.5, 148.4, 156.4, 164.3. MS *m/z* (%): 437.33 [M^+] (61.41), 420.60 (16.69), 339.11 (19.21), 324.15 (12.02), 312.14 (7.56), 309.13 (15.93), 279.14 (17.71), 252.12 (14.32), 244.15 (14.52), 238.15 (100.00), 237.14 (12.17), 226.11 (49.14), 184.10 (9.63), 183.08 (81.36), 153.30 (31.22), 150.09 (21.77), 125.14 (16.95), 123.10 (13.31), 98.13 (21.59), 85.13 (23.36), 84.12 (31.33), 82.11 (35.01), 71.13 (36.77), 69.11 (44.20). Anal. Calcd for $C_{18}H_{19}N_3O_2S_4$ (437.62): C, 49.40; H, 4.38; N, 9.60; S, 29.31. Found: C, 49.28; H, 4.17; N, 9.55; S, 29.53%.

General Procedure for Preparation of 32 and 33

A mixture of ketene dithioacetal **31** (4.38 g, 0.01 mol) and the appropriate 1,2-diamines namely (ethane-1,2-diamine or benzene-1,2-diamine) (0.01 mol) in EtOH (50 mL) was refluxed for 3 h. The obtained product was collected by filtration and recrystallized from an appropriate solvent to give **32** and

33, respectively.

2-(Imidazolidin-2-ylidene)-2-(4-(4-(pyrrolidin-1-ylsulfonyl)phenyl)thiazol-2-yl)acetonitrile (32)

White crystals, Yield, 86%; mp 196-198 °C (EtOH/benzene). IR (KBr, cm^{-1}): ν_{max} 3215, 3199 (2NH), 3010 (CH aromatic), 2962 (CH aliphatic), 2219 (C≡N), 1630 (C=N), 1579 (C=C), 1337, 1159 (SO_2). ^{1}H NMR (DMSO-d_6): δ 1.90 (m, 4H, CH_2-CH_2 of pyrrolidine), 3.02 (s, 4H, CH_2-CH_2 of imidazoline), 3.30 (t, 4H, *J*=10.0 *Hz*, CH_2-N-CH_2 of pyrrolidine), 7.30, 7.43 (2br, 2H, 2NH, Discharged with D_2O), 7.60 (s, 1H, H_5 of thiazole), 7.80, 8.55 (dd, 4H, "each d, each 2H, *J*=8.3 *Hz*", AB system). ^{13}C NMR (DMSO-d_6): δ 24.0 (2C, CH_2-CH_2 of pyrrolidine), 43.2 (2C, CH_2-CH_2 of imidazoline), 57.9, 66.0 (2C, CH_2-N-CH_2 of pyrrolidine), 109.9 (C_5 of thiazole), 117.6 (C≡N), 124.8 (2C), 129.4 (2C), 132.7, 137.9, 155.4, 163.2, 169.7. MS *m/z* (%): 402.12 [M^++1] (21.73), 401.08 [M^+] (55.91), 268.06 (93.22), 238.05 (33.28), 174.08 (62.85), 163.02 (100.00), 150.03 (80.59), 122.03 (24.12), 119.07 (43.32), 118.07 (30.38), 106.07 (20.63), 105.06 (27.95), 104.05 (49.57), 91.07 (23.05), 90.05 (23.03), 77.06 (37.98), 76.04 (59.92), 75.02 (23.57), 70.07 (97.52), 65.04 (28.20), 63.04 (23.22), 50.03 (18.47), 43.08 (36.99), 42.06 (72.85), 41.05 (38.04). Anal. Calcd for $C_{18}H_{19}N_5O_2S_2$ (401.51): C, 53.85; H, 4.77; N, 17.44; S, 15.97. Found: C, 53.73; H, 4.58; N, 17.38; S, 15.73%.

2-(1*H*-Benzo[*d*]imidazol-2(3*H*)-ylidene)-2-(4-(4-(pyrrolidin-1-ylsulfonyl)phenyl)thiazol-2-yl)acetonitrile (33)

Brown crystals, Yield, 82%; mp >360 °C (dioxane). IR (KBr, cm^{-1}): ν_{max} 3190, 3200 (2NH), 3018 (CH aromatic), 2986, 2915 (CH aliphatic), 2218 (C≡N), 1583 (C=N), 1555 (C=C), 1349, 1182 (SO_2). ^{1}H NMR (DMSO-d_6): δ 1.92 (m, 4H, CH_2-CH_2 of pyrrolidine), 3.39 (t, 4H, *J*=11.1 *Hz*, CH_2-N-CH_2 of pyrrolidine), 6.65-8.01 (m, 8H, Ar-H), 7.55 (s, 1H, H_5 of thiazole), 9.58, 11.01 (2br, 2H, 2NH, Discharged with D_2O). MS *m/z* (%): 449.43 [M^+] (80.75), 402.90 (38.86), 304.02 (11.29), 301.99 (21.50), 217.02 (12.37), 211.02 (16.09), 187.90 (22.28), 185.04 (5.04), 173.04 (9.58), 168.01 (10.70), 151.48 (100.00), 144.04 (5.61), 142.00 (42.90), 138.04 (13.20), 127.06 (10.12), 122.02 (5.18), 120.95 (84.98), 105.05 (19.15), 85.10 (6.96), 75.04 (9.03), 73.11 (27.60), 60.03 (18.77), 44.05 (23.54), 43.06 (87.76), 40.21 (41.74). Anal. Calcd for $C_{22}H_{19}N_5O_2S_2$ (449.55): C, 58.78; H, 4.26; N, 15.58; S, 14.27. Found: C, 58.65; H, 4.08; N, 15.46; S, 14.13%.

2-Cyano-*N*-phenyl-2-(4-(4-(pyrrolidin-1-ylsulfonyl)phenyl)thiazol-2-yl)ethanethioamide (35)

To a cooled suspension of finely grounded potassium hydroxide (0.56 g, 0.01 mol) in dry *N*,*N*-dimethylformamide (40 mL), thiazolylacetonitrile **2** (3.33 g, 0.01 mol) and subsequently isothiocyanatobenzene (1.35 g, 0.01 mol) were added. The reaction mixture was stirred overnight at room temperature, and left at room temperature for an additional 24 h. The reaction mixture was then triturated

with cold water (50 mL) and neutralized with 1N hydrogen chloride. The resulting precipitated solid was collected by filtration, washed with water, dried and recrystallized from EtOH to give **35**. White crystals, Yield, 86%; mp 256-258 °C. IR (KBr, cm^{-1}): ν_{max} 3190 (NH), 3074 (CH aromatic), 2961, 2957 (CH aliphatic), 2217 (C≡N), 1581 (C=N), 1550 (C=C), 1333, 1161 (SO_2), 1343 (C=S). ^{1}H NMR (DMSO-d_6): δ 1.87 (m, 4H, CH_2-CH_2 of pyrrolidine), 3.30 (t, 4H, *J*=9.9 *Hz*, CH_2-N-CH_2 of pyrrolidine), 5.10 (s, 1H, CH), 7.11-7.90 (m, 10H, Ar-H + H_5 of thiazole) 10.26 (s, 1H, NH, Discharged with D_2O). MS *m/z* (%): 468.07 [M^+] (70.11), 216.02 (12.35), 200.03 (23.79), 199.02 (29.16), 135.00 (49.09), 134.03 (7.25), 133.01 (7.61), 94.06 (8.08), 93.04 (95.53), 92.06 (10.74), 91.05 (6.75), 89.03 (38.27), 78.03 (10.40), 77.03 (59.05), 75.95 (80.27), 70.05 (100.00), 66.04 (7.55), 65.02 (8.79), 63.02 (12.21), 51.02 (18.12), 50.01 (7.31), 44.02 (6.61), 43.06 (8.44), 42.03 (20.29), 41.04 (6.65). Anal. Calcd for $C_{22}H_{20}N_4O_2S_3$ (468.61): C, 56.39; H, 4.30; N, 11.96; S, 20.53. Found: C, 56.24; H, 4.18; N, 11.75; S, 20.48%.

General Procedure for Preparation of (36-39)

To a cooled suspension of finely grounded potassium hydroxide (0.56 g, 0.01 mol) in dry *N*,*N*-dimethylformamide (40 mL), thiazolylacetonitrile **2** (3.33 g, 0.01 mol) and subsequently isothiocyanatobenzene (1.35 g, 0.01 mol) were added. The reaction mixture was stirred overnight at room temperature, then treated with the appropriate halo compounds namely (iodomethane, ethyl 2-chloroacetate, ethyl 2-bromopropanoate and ethyl 3-bromobutanoate) (0.01 mol), and left at room temperature for an additional 24 h. The reaction mixture was then triturated with cold water (50 mL) and neutralized with 1N hydrogen chloride. The resulting precipitated solid was collected by filtration, washed with water, dried and recrystallized from an appropriate solvent to give compounds **36**-**39**, respectively.

3-(Methylthio)-3-(phenylamino)-2-(4-(4-(pyrrolidin-1-ylsulfonyl)phenyl)thiazol-2-yl)acrylonitrile (36)

Yellow crystals, Yield, 88%; mp 269-270 °C (EtOH). IR (KBr, cm^{-1}): ν_{max} 3230 (NH), 3099 (CH aromatic), 2908 (CH aliphatic), 2222 (C≡N), 1611 (C=N), 1576 (C=C), 1335, 1177 (SO_2), 1160 (C-S). ^{1}H NMR (DMSO-d_6): δ 1.87 (m, 4H, CH_2-CH_2 of pyrrolidine), 2.72 (s, 3H, SCH_3), 3.29 (t, 4H, *J*=10.3 *Hz*, CH_2-N-CH_2 of pyrrolidine), 6.85-7.11 (m, 5H, Ar-H), 7.32 (s, 1H, H_5 of thiazole), 7.83, 8.10 (dd, 4H, "each d, each 2H, *J*=8.7 *Hz*", AB system), 9.35 (s, 1H, NH, Discharged with D_2O). ^{13}C NMR (DMSO-d_6): δ 16.3 (SCH_3), 22.8 (2C, CH_2-CH_2 of pyrrolidine), 60.6 (2C, CH_2-N-CH_2 of pyrrolidine), 82.5, 111.3 (C_5 of thiazole), 115.9 (C≡N), 120.8, 123.2 (2C), 126.2 (2C), 131.6 (2C), 135.8 (2C), 137.3, 140.2, 144.7, 156.4, 166.9, 171.2. MS *m/z* (%): 482.13 [M^+] (72.36), 435.13 (13.59), 301.09 (19.52), 198.04 (15.25), 197.02 (11.27), 134.04 (38.84), 133.02 (14.34), 105.04 (24.23), 102.05 (25.43), 91.05

(12.94), 90.07 (15.07), 89.04 (57.38), 77.03 (100.00), 76.03 (11.76), 70.07 (87.14), 69.03 (18.67), 63.03 (21.60), 51.02 (40.51), 48.00 (14.68), 47.00 (17.01), 44.99 (24.58), 44.02 (23.76), 43.06 (41.68), 42.04 (74.95), 41.06 (44.45). Anal. Calcd for $C_{23}H_{22}N_4O_2S_3$ (482.64): C, 57.24; H, 4.59; N, 11.61; S, 19.93. Found: C, 57.18; H, 4.48 N, 11.52; S, 20.04%.

Ethyl 2-((2-cyano-1-(phenylamino)-2-(4-(4-(pyrrolidin-1-ylsulfonyl)phenyl)thiazol-2-yl)vinyl)thio)-acetate (37)

White solids, Yield, 79%; mp 257-258 °C (EtOH/benzene). IR (KBr, cm^{-1}): ν_{max} 3220 (NH), 3085 (CH aromatic), 2985, 2964 (CH aliphatic), 2220 (C≡N), 1697 (C=O), 1630 (C=N), 1593 (C=C), 1354, 1161 (SO_2), 1288, 1055 (C-O-C), 1182 (C-S). ^{1}H NMR (DMSO-d_6): δ 1.31 (t, 3H, *J*=7.3 *Hz*, $\underline{CH_3}$-CH_2), 1.91 (m, 4H, CH_2-CH_2 of pyrrolidine), 3.28 (t, 4H, *J*=10.7 *Hz*, CH_2-N-CH_2 of pyrrolidine), 3.79 (s, 2H, CH_2), 4.35 (q, 2H, *J*=7.3 *Hz*, CH_3-$\underline{CH_2}$), 6.40-7.22 (m, 5H, Ar-H), 7.31 (s, 1H, H_5 of thiazole), 7.80, 8.42 (dd, 4H, "each d, each 2H, *J*=8.6 *Hz*", AB system), 10.99 (s, 1H, NH, Discharged with D_2O). ^{13}C NMR (DMSO-d_6): δ 18.3 ($\underline{CH_3}$-CH_2), 23.6 (2C, CH_2-CH_2 of pyrrolidine), 40.2 (CH_2), 59.3 (CH_3-$\underline{CH_2}$), 64.5 (2C, CH_2-N-CH_2 of pyrrolidine), 81.4, 110.2 (C_5 of thiazole), 121.0 (C≡N), 123.6, 125.9 (2C), 127.4 (2C), 130.7 (2C), 135.8 (2C), 137.9, 140.8, 143.6, 156.9, 167.3, 169.0, 173.2 (C=O). MS *m/z* (%): 554.16 [M^+] (62.08), 487.11 (20.54), 470.11 (42.31), 193.06 (23.08), 134.03 (30.80), 133.04 (19.00), 102.06 (14.45), 101.07 (14.39), 90.08 (14.50), 89.05 (77.46), 84.07 (25.66), 82.06 (13.60), 77.05 (40.77), 70.07 (90.14), 69.06 (24.43), 68.07 (21.26), 63.04 (23.05), 56.06 (18.79), 55.06 (30.75), 54.05 (17.73), 53.07 (14.17), 51.04 (22.39), 44.02 (63.52), 43.06 (49.48), 42.06 (100.00), 41.06 (70.56). Anal. Calcd for $C_{26}H_{26}N_4O_4S_3$ (554.70): C, 56.30; H, 4.72; N, 10.10; S, 17.34. Found: C, 56.27; H, 4.69; N, 10.31; S, 17.10%.

Ethyl 2-((2-cyano-1-(phenylamino)-2-(4-(4-(pyrrolidin-1-ylsulfonyl)phenyl)thiazol-2-yl)vinyl)thio)-propanoate (38)

White crystals, Yield, 73%; mp 244-246 °C (EtOH/benzene). IR (KBr, cm^{-1}): ν_{max} 3190 (NH), 3074 (CH aromatic), 2983, 2953 (CH aliphatic), 2217 (C≡N), 1710 (C=O), 1633 (C=N), 1588 (C=C), 1340, 1155 (SO_2), 1206, 1021 (C-O-C), 1178 (C-S). ^{1}H NMR (DMSO-d_6): δ 1.33 (t, 3H, *J*=7.2 *Hz*, $\underline{CH_3}$-CH_2), 1.72 (d, 3H, *J*=7.9 *Hz*, $\underline{CH_3}$-CH), 1.94 (m, 4H, CH_2-CH_2 of pyrrolidine), 3.31 (t, 4H, *J*=10.1 *Hz*, CH_2-N-CH_2 of pyrrolidine), 3.70 (q, 2H, *J*=7.2 *Hz*, CH_3-$\underline{CH_2}$), 4.36 (q, 1H, CH), 6.75-7.31 (m, 5H, Ar-H), 7.45 (s, 1H, H_5 of thiazole), 7.84, 8.11 (dd, 4H, "each d, each 2H, *J*=8.5 *Hz*", AB system), 11.62 (s, 1H, NH, Discharged with D_2O). MS *m/z* (%): 567.99 [M^+] (81.33), 389.22 (5.73), 365.18 (8.23), 358.37 (6.74), 346.10 (8.72), 341.16 (5.59), 332.39 (6.12), 317.50 (10.41), 305.11 (7.93), 291.56 (9.30), 271.11 (14.20), 269.14 (6.57), 249.13 (9.34), 246.11 (6.96), 244.51 (7.34), 309.24 (100.00), 229.11 (24.15), 224.12 (6.58), 192.11 (11.39), 168.11 (12.54), 162.10 (12.44), 136.09 (7.98), 123.11 (14.38), 94.10 (14.56), 75.06

(18.76), 64.03 (8.80). Anal. Calcd for $C_{27}H_{28}N_4O_4S_3$ (568.73): C, 57.02; H, 4.96; N, 9.85; S, 16.91. Found: C, 56.89; H, 4.80; N, 9.72; S, 16.78%.

Ethyl 3-((2-cyano-1-(phenylamino)-2-(4-(4-(pyrrolidin-1-ylsulfonyl)phenyl)thiazol-2-yl)vinyl)thio)-butanoate (39)

White solids, Yield, 62%; mp 235-237 °C (EtOH/benzene). IR (KBr, cm^{-1}): ν_{max} 3220 (NH), 3072 (CH aromatic), 2961, 2945 (CH aliphatic), 2220 (C≡N), 1707 (C=O), 1627 (C=N), 1581 (C=C), 1358, 1170 (SO_2), 1287, 1053 (C-O-C), 1199 (C-S). ^{1}H NMR (DMSO-d_6): δ 1.27-1.38 (m, 6H, $2CH_3$), 1.99 (m, 4H, CH_2-CH_2 of pyrrolidine), 2.64-2.99 (m, 2H, CH_2CO), 3.27 (t, 4H, *J*=11.7 *Hz*, CH_2-N-CH_2 of pyrrolidine), 3.52-3.83 (m, 1H, CH), 4.61 (q, 2H, *J*=8.8 *Hz*, CH_2), 6.87-7.27 (m, 5H, Ar-H), 7.69 (s, 1H, H_5 of thiazole), 7.85, 8.11 (dd, 4H, "each d, each 2H, *J*=8.5 *Hz*", AB system), 10.32 (s, 1H, NH, Discharged with D_2O). MS *m/z* (%): 581.61 [M^+] (41.99), 537.39 (16.76), 534.90 (12.13), 503.27 (10.36), 475.34 (12.59), 465.26 (18.18), 436.37 (28.08), 402.46 (66.80), 369.09 (34.48), 359.28 (17.04), 354.38 (25.55), 346.27 (16.26), 330.30 (100.00), 296.49 (25.68), 282.13 (28.31), 279.23 (14.65), 200.05 (15.28), 175.06 (12.65), 174.07 (11.57), 85.07 (21.90), 81.02 (10.89), 79.98 (25.53). Anal. Calcd for $C_{28}H_{30}N_4O_4S_3$ (582.76): C, 57.71; H, 5.19; N, 9.61; S, 16.51. Found: C, 57.65; H, 5.05; N, 9.47; S, 16.36%.

3,3-Bis(phenylamino)-2-(4-(4-(pyrrolidin-1-ylsulfonyl)phenyl)thiazol-2-yl)acrylonitrile (40)

A mixture of compound **36** (4.82 g, 0.01 mol) and aniline (0.93 g, 0.01 mol) in EtOH (50 mL) was refluxed for 3 h, after cooling. The solid product which formed was collected by filtration and recrystallized from EtOH/benzene to give **40**. White crystals, Yield, 83%; mp 292-294 °C. IR (KBr, cm^{-1}): ν_{max} 3215, 3201 (2NH), 3039 (CH aromatic), 2972 (CH aliphatic), 2222 (C≡N), 1575 (C=N), 1553 (C=C), 1335, 1165 (SO_2). ^{1}H NMR (DMSO-d_6): δ 1.88 (m, 4H, CH_2-CH_2 of pyrrolidine), 3.31 (t, 4H, *J*=10.4 *Hz*, CH_2-N-CH_2 of pyrrolidine), 6.42-7.26 (m, 10H, Ar-H), 7.52 (s, 1H, H_5 of thiazole), 7.82, 8.15 (dd, 4H, "each d, each 2H, *J*=8.7 *Hz*", AB system), 10.36, 11.27 (2br, 2H, 2NH, Discharged with D_2O). ^{13}C NMR (DMSO-d_6): δ 22.3 (2C, CH_2-CH_2 of pyrrolidine), 46.5, 60.9 (2C, CH_2-N-CH_2 of pyrrolidine), 108.7 (C_5 of thiazole), 117.8 (C≡N), 119.5 (2C), 121.4 (2C), 123.7 (2C), 125.3 (2C), 129.2 (2C), 132.6 (2C), 136.7 (2C), 140.3, 144.8, 150.0 (2C), 157.4, 168.9, 172.1. MS *m/z* (%): 527.29 [M^+] (75.09), 392.18 (20.34), 288.34 (34.01), 189.17 (100.00), 164.13 (45.32), 147.11 (38.24), 112.14 (42.31), 93.06 (55.26), 83.08 (61.06), 71.08 (42.37). Anal. Calcd for $C_{28}H_{25}N_5O_2S_2$ (527.66): C, 63.73; H, 4.78; N, 13.27; S, 12.15. Found: C, 63.54; H, 4.65; N, 13.10; S, 12.03%.

2-(4-Oxo-3-phenylthiazolidin-2-ylidene)-2-(4-(4-(pyrrolidin-1-ylsulfonyl)phenyl)thiazol-2-yl)aceto-nitrile (41)

To a mixture of EtOH (30 mL) and 1 mL of piperidine, 5.54 g (0.01 mol) of compound **37** was added and

refluxed for 3 h. The precipitate obtained after cooling was filtered off and recrystallized from dioxane to give **41**. White crystals, Yield, 72%; mp 303-305 °C. IR (KBr, cm^{-1}): ν_{max} 3103, 3085 (CH aromatic), 2990, 2937, 2879 (CH aliphatic), 2220 (C≡N), 1669 (C=O), 1635 (C=N), 1598 (C=C), 1339, 1159 (SO_2). ^{1}H NMR (DMSO-d_6): δ 1.92 (m, 4H, CH_2-CH_2 of pyrrolidine), 3.33 (t, 4H, *J*=11.9 *Hz*, CH_2-N-CH_2 of pyrrolidine), 4.01 (s, 2H, CH_2 of thiazolidinone), 6.99-7.33 (m, 5H, Ar-H), 7.56 (s, 1H, H_5 of thiazole), 7.88, 8.52 (dd, 4H, "each d, each 2H, *J*=8.3 *Hz*", AB system). ^{13}C NMR (DMSO-d_6): δ 22.9 (2C, CH_2-CH_2 of pyrrolidine), 41.4 (CH_2 of thiazolidinone), 62.9 (2C, CH_2-N-CH_2 of pyrrolidine), 82.5, 108.8 (C_5 of thiazole), 120.3 (C≡N), 124.5 (2C), 129.0 (2C), 133.2, 135.9 (2C), 139.2 (2C), 140.0, 142.5, 147.3, 156.9, 167.2, 169.5, 173.8 (C=O). MS *m/z* (%): 509.14 [M^++1] (31.70), 508.12 [M^+] (71.80), 347.12 (17.88), 268.11 (32.93), 174.11 (21.63), 163.08 (42.80), 134.06 (38.28), 119.10 (36.98), 105.09 (19.76), 104.08 (29.84), 91.10 (22.77), 90.10 (17.54), 89.08 (37.69), 77.09 (65.64), 76.08 (31.72), 71.11 (14.43), 70.10 (100.00), 69.10 (24.08), 63.08 (15.03), 55.09 (20.13), 51.07 (21.91), 45.07 (18.09), 44.07 (59.63), 43.11 (48.89), 42.09 (64.15), 41.08 (51.81). Anal. Calcd for $C_{24}H_{20}N_4O_3S_3$ (508.64): C, 56.67; H, 3.96; N, 11.02; S, 18.91. Found: C, 56.54; H, 3.83; N, 11.14; S, 19.07%.

Docking and molecular modeling calculations

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

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 Å with MMFF94X force field and the partial charges were automatically calculated.

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.

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.

In vitro anticancer screening

The cytotoxicity activity was measured *in vitro* for the newly synthesized compounds using the Sulfo-Rhodamine-B stain (SRB) assay.[42] The *in vitro* anticancer screening was done by The Regional Center for Mycology and Biotechnology RCMB, Al-Azhar University, Cairo, Egypt. Cells were plated in 96-multiwell micro titer plate (10^4 cells/well) for 24 h. 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 48 h at 37 ºC and in 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 is plotted to get the survival curve for breast tumor cell after the specified time.[42] The molar concentration required for 50% inhibition of cell viability (IC_{50}) was calculated and the results are given in **Table 1**.

REFERENCES

1. L. M. T. Frija, A. J. L. Pombeiro, and M. N. Kopylovich, *Coord. Chem. Rev.*, 2016, **308**, 32.
2. C. D. Monte, S. Carradori, B. Bizzarri, A. Bolasco, F. Caprara, A. Mollica, D. Rivanera, E. Mari, A. Zicari, A. Akdemir, and D. Secci, *Eur. J. Med. Chem.*, 2016, **107**, 82.
3. K. R. A. Abdellatif, M. A. Abdelgawad, H. A. H. Elshemy, and S. S. R. Alsayed, *Bioorg. Chem.*, 2016, **64**, 1.
4. K. M. Dawood, T. M. A. Eldebss, H. S. A. El-Zahabi, and M. H. Yousef, *Eur. J. Med. Chem.*, 2015, **102**, 266.
5. F. Y. Li, X. F. Guo, Z. J. Fan, Y. Q. Zhang, G. N. Zong, X. L. Qian, L. Y. Ma, L. Chen, Y. J. Zhu, K. Tatiana, Y. Y. Morzherin, and N. P. Belskaya, *Chinese Chem. Lett.*, 2015, **26**, 1315.
6. A. Ayati, S. Emami, A. Asadipour, A. Shafiee, and A. Foroumadi, *Eur. J. Med. Chem.*, 2015, **97**,

699.

7. A. Rouf and C. Tanyeli, *Eur. J. Med. Chem.*, 2015, **97**, 911.
8. N. D. Amnerkar, B. A. Bhongade, and K. P. Bhusari, *Arabian J. Chem.*, 2015, **8**, 545.
9. C. B. Mishra, S. Kumari, and M. Tiwari, *Eur. J. Med. Chem.*, 2015, **92**, 1.
10. R. S. Keri, M. R. Patil, S. A. Patil, and S. Budagumpi, *Eur. J. Med. Chem.*, 2015, **89**, 207.
11. S. Konda, S. Raparthi, K. Bhaskar, R. K. Munaganti, V. Guguloth, L. Nagarapu, and D. M. Akkewar, *Bioorg. Med. Chem. Lett.*, 2015, **25**, 1643.
12. M. M. Gamal El-Din, M. I. El-Gamal, M. S. Abdel-Maksoud, K. H. Yoo, and C. H. Oh, *Eur. J. Med. Chem.*, 2015, **90**, 45.
13. S. P. Sadarangani, L. L. Estes, and J. M. Steckelberg, *Mayo Clinic Proceedings*, 2015, **90**, 109.
14. M. Farahi, B. Karami, and H. M. Tanuraghaj, *Tetrahedron Lett.*, 2015, **56**, 1833.
15. F. M. Awadallah, T. A. El-Waei, M. M. Hanna, S. E. Abbas, M. Ceruso, B. E. Oz, O. O. Guler, and C. T. Supuran, *Eur. J. Med. Chem.*, 2015, **96**, 425.
16. M. Bozdag, F. Carta, D. Vullo, A. Akdemir, S. Isik, C. Lanzi, A. Scozzafava, E. Masini, and C. T. Supuran, *Bioorg. Med. Chem.*, 2015, **23**, 2368.
17. A. Grandane, M. Tanc, R. Zalubovskis, and C. T. Supuran, *Bioorg. Med. Chem.*, 2015, **23**, 1430.
18. Z. Y. Yu, D. Q. Yin, and H. P. Deng, *Ecotoxicol. Environ. Saf.*, 2015, **111**, 66.
19. M. Karakaya, Y. Sert, S. Sreenivasa, P. A. Suchetan, and C. Cirak, *Spectrochim. Acta Mol. Biomol. Spectrosc.*, 2015, **142**, 169.
20. S. A. Booker, N. Pires, S. Cobb, P. S. Silva, and I. Vida, *Neuropharmacology*, 2015, **93**, 103.
21. N. D. Reddy, M. H. Shoja, B. S. Jayashree, P. G. Nayak, N. Kumar, V. G. Prasad, K. S. R. Pai, and C. M. Rao, *Chem. Bio. Interact*, 2015, **233**, 81.
22. M. Angel, A. Nieto, T. Apan, and G. Delgado, *Eur. J. Pharm.*, 2015, **752**, 40.
23. Z. Chen, Z. C. Wang, X. Q. Yan, P. F. Wang, X. Y. Lu, L. W. Chen, H. L. Zhu, and H. W. Zhang, *Bioorg. Med. Chem. Lett.*, 2015, **25**, 1947.
24. M. T. Tavares, K. F. M. Pasqualoto, J. Streek, A. K. Ferreira, R. A. Azevedo, M. C. Damiao, C. P. Rodrigues, P. L.Junior, J. A. M. Barbuto, R. Filho, and F. F. Ferreira, *J. Mol. Struct.*, 2015, **1088**, 138.
25. S. Medici, M. Peana, V. M. Nurchi, J. I. Lachowicz, G. Crisponi, and M. A. Zoroddu, *Coord. Chem. Rev.*, 2015, **284**, 329.
26. M. S. Bashandy, M. S. Alsaid, R. K. Arafa, and M. M. Ghorab, *J. Enzym. Inhib. Med. Chem.*, 2014, **29**, 619.
27. M. S. Bashandy, M. S. Al-Said, S. I. Al-qasoum, and M. M. Ghorab, *Arzneimittel-Forschung Drug Res.*, 2011, **61**, 521.

28. M. S. Al-Said, M. S. Bashandy, and M. M. Ghorab, *Arzneimittel-Forschung Drug Res.*, 2011, **61**, 527.
29. M. S. Bashandy, S. M. Hassan, O. A. Fathalla, A. F. Eweas, and A. H. Khalel, *Egyptian J. Chem.*, 2012, **55**, 659.
30. M. S. Al-Said, M. S. Bashandy, S. I. Al-qasoumi, and M. M. Ghorab, *Eur. J. Med. Chem.*, 2011, **46**, 137.
31. K. A. Jensen and L. Henriksen, *Acta Chem. Scand.*, 1968, **22**, 1107.
32. R. Gompper and W. Toepfl, *Chem. Ber.*, 1962, 2861.
33. M. A. A. Elneariry, T. M. Abdel-Rahman, and A. M. Hammad, *J. Chem. Res. (S)*, 1998, 684.
34. S. M. Sayed, *J. Chinese Chem. Soc.*, 2003, **50**, 1061.
35. K. Sato and T. Amakasu, *J. Org. Chem.*, 1968, **33**, 2446.
36. O. Methcohn and B. Tarnowski, *Synthesis*, 1978, 56.
37. R. Rohrkasten, P. Raatz, R. P. Kreher, and M. Balaszkewicz, *Z. Natureforsch.*, 1997, **52b**, 1526.
38. Y. Tominaga, Y. Honkawa, M. Hara, and A. Hosomi, *J. Heterocycl. Chem.*, 1990, **27**, 775.
39. Q. R. Du, D. D. Li, Y. Z. Pi, J. R. Li, J. Sun, F. Fang, W. Q. Zhong, H. B. Gong, and H. L. Zhu, *Bioorg. Med. Chem.*, 2013, **21**, 2286.
40. K. N. Rao and S. R. Venkatachalam, *Bioorg. Med. Chem.*, 1999, **7**, 1105.
41. S. Vilar, G. Cozza, and S. Moro, *Curr. Top. Med. Chem.*, 2008, **8**, 1555.
42. P. Skehan, R. Storegng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney, and M. R. Boyd, *J. Natl. Cancer Inst.*, 1990, **82**, 1107.