Dapson in Heterocyclic Chemistry, Part V: Synthesis, Molecular Docking and Anticancer Activity of Some Novel Sulfonylbiscompounds Carrying Biologically Active Dihydropyridine, Dihydroisoquinoline, 1,3-Dithiolan, 1,3-Dithian, Acrylamide, Pyrazole, Pyrazolopyrimidine and Benzochromenemoieties

Mostafa Mohammed Ghorab,*[,a] Mansour Sulaiman Al-Said,[a] and Yassin Mohammed Nissan[b]

[a] *Medicinal, Aromatic and Poisonous Plants Research Center (MAPPRC), College of Pharmacy, King Saud University; P.O. Box 2457, Riyadh 11451, Saudi Arabia: and* [b] *Pharmaceutical Chemistry Department, Faculty of Pharmacy, Cairo University; Cairo 11562, Egypt.* Received April 2, 2012; accepted May 1, 2012

***N*,*N*′-(4,4′-Sulfonylbis(4,1-phenylene))bis(2-cyanoacetamid) 2 was utilized as a key intermediate for the synthesis of novel dihydropyridines 3, 4, 8, dihydroisoquinolines 5–7, dithiolan 10, dithian 11, acrylamide 12, benzochromenes 17 and 18 and chromenopyridones 19 and 20. Compound 2 was the starting material in the synthesis of the acrylamide derivative 14, the pyrazole derivative 15 and the pyrazolopyrimidine derivative 16. All the synthesized compounds were evaluated for their *in vitro* anticancer activity against human breast cancer cell line (MCF7). Compound 19 showed the best cytotoxic activity with IC_{50} value 19.36 μM. In addition, molecular docking study of the synthesized compounds on the active sites of farnesyltransferase and arginine methyltransferase was performed in order to give a suggestion about the mechanism of action of their cytotoxic activity.**

Key words sulfone; pyridine; pyrazolopyrimidine; benzochromene; anticancer activity

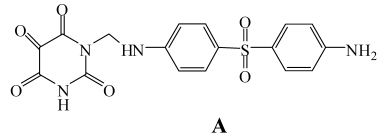
A large number of sulfone derivatives have been found to exhibit a wide variety of pharmacological activities.[1–8] Also, diphenylsulfones and bisheterocyclic compounds are reported to have a broad spectrum of biological activities. Some are endowed with antitumor,[9] or antifungal properties.[10] On the other hand, some pyridine and isoquinoline derivatives have various biological properties such as antimicrobial,[11] anticancer activities.[12–15]

Recent studies have proved the remarkable effect of Dapson **1** on inhibiting cell growth in glioblastoma by acting as antivascular endothelial growth factor (VEGF) and anti-angiogenic agent *via* depriving glioblastoma of neutrophil-mediated growth promoting effects.[16] Allantodapson **A**, a dapson derivative showed high activity as anticancer through inhibition of arginine methyltranseferase (PRMT1) an enzyme which plays an important role in hormone dependant cancers. A series of acylated diarylsulfone derivatives were evaluated for the same activity and compound **B** exihibited good activity as (PRMT1) inhibitor.[17] Some diarylsulfone derivatives bearing imidazole ring were evaluated for their anticancer activity through their action on inhibition of farnesyl-protein transferase (FTase), a zinc metalloenzyme which catalyzes the lipidation of a 3,4 cysteine in the C-terminal tetrapeptide sequence. Compound **C** showed the lowest IC_{50} as FTase inhibitor.[18] Some other styryl heterocyclic sulfone derivatives **D** were interesting as anticancer agents as they block the mitogen activated protein kinase (MAPK) cascade which phosphorylates a variety of proteins including several transcription factors, which translocate into the nucleus and activate gene transcription. Negative regulation of this pathway could arrest the cascade of these events and will inhibit the proliferation of cancer cells.[19,20]

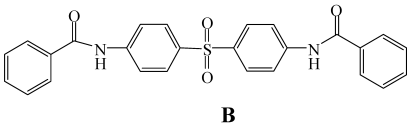
On the preceding and as a part of our studies on new heterocyclic compounds derived from Dapson as new potential anticancer agents,[21–24] we have synthesized a novel series of dihydropyridines, dihydroisoquinolines, 1,3-dithiolan, 1,3-dithian, acrylamide, pyrazole, pyrazolopyrimidine and benzochromene derivatives to evaluate their anticancer activity.

Results and Discussion

Chemistry The solvent-free reaction of arylamines with ethyl cyanoacetate is well known to constitute one of the most widely used synthetic methods. Thermal fusion of 4,4′-sulfonyldianiline (Dapson) **1** above its melting point with ethyl-2-cyanoacetate yielded the corresponding



A



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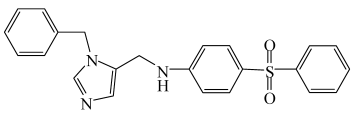

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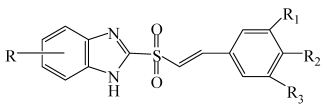
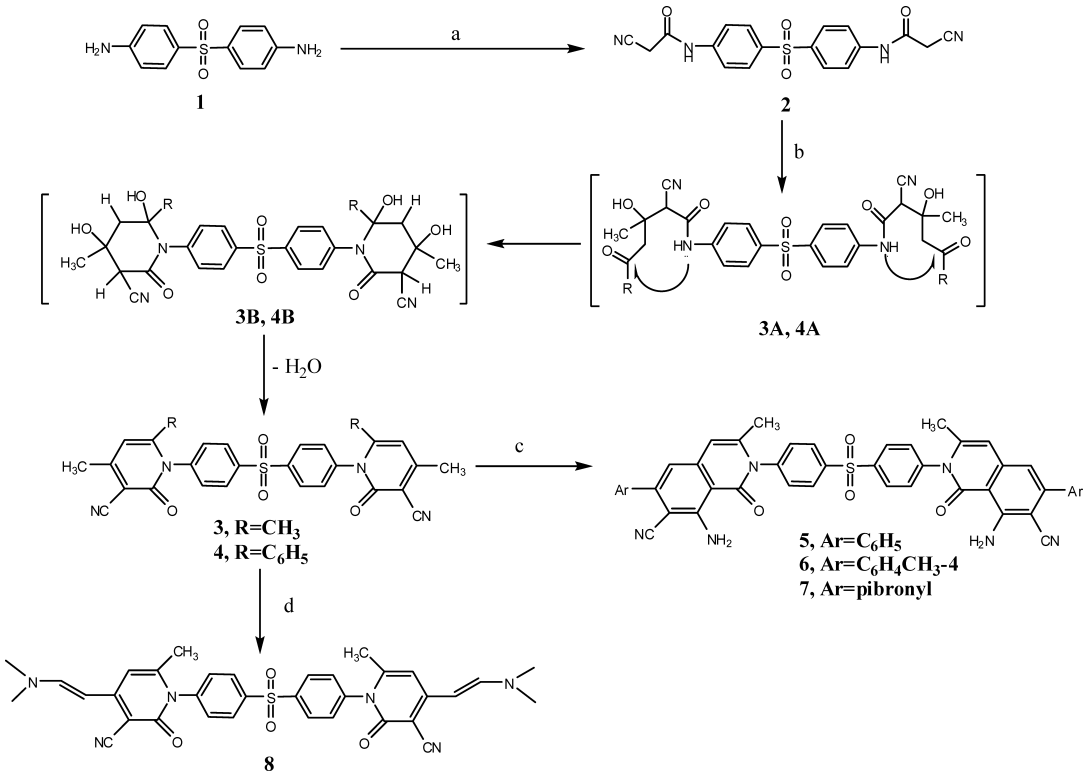


D

The authors declare no conflict of interest.

* To whom correspondence should be addressed. e-mail: mmsghorab@yahoo.com



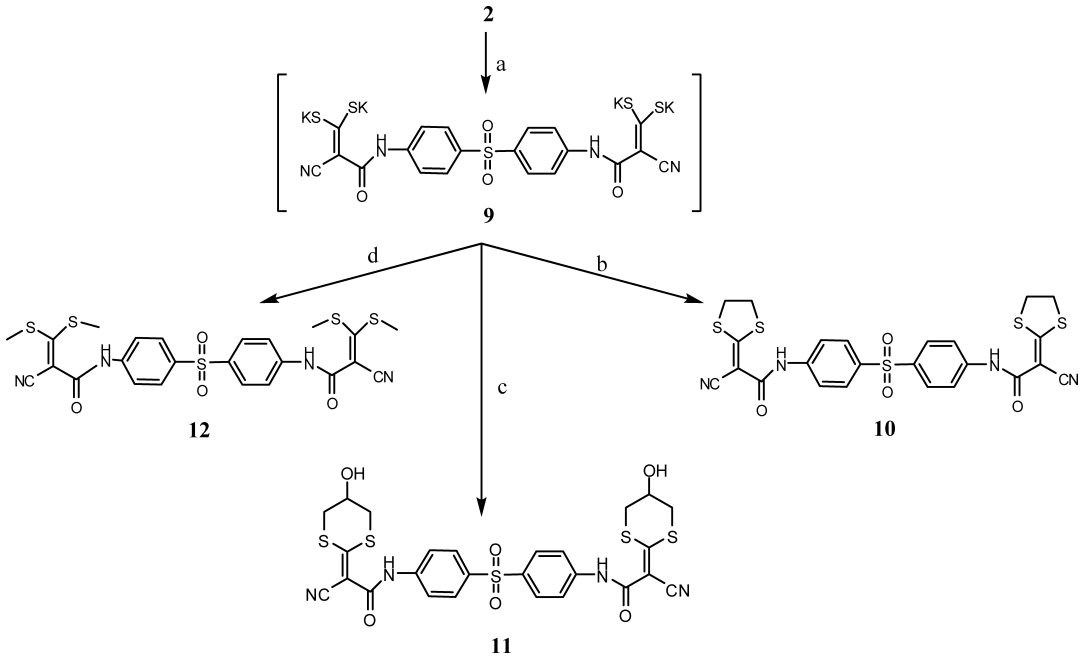
Reagents (a) ethylcyanoacetate, (b) acetylacetone or benzoylacetone, (c) benzylidenemalononitriles, (d) DMF/DMA.
Chart 1

N,*N*′-(4,4′-sulfonylbis(4,1-phenylene))bis(2-cyanoacetamide) **2**. IR spectrum of **2** revealed bands at 3448, 3363 cm^{-1} (2NH), 2256 cm^{-1} (2C≡N), 1701 cm^{-1} (2C=O) and 1342, 1180 cm^{-1} (SO_2). ^{1}H-NMR spectrum in (DMSO-d_6) of **2** exhibited signals at 4.0 ppm due to CH_2 group and 7.4–7.9 ppm corresponding to aromatic protons. It is well known that many pyridine-2-one derivatives exhibited diverse biological activity such as anticancer activity.[25] The present study was continued to report the reactivity of cyanoacetamide **2** towards certain nucleophilic reagents. Thus, when compound **2** was refluxed with acetylacetone or benzoylacetone in ethanol containing a catalytic amount of pipredine cyclocondensation reaction occurred and the corresponding 1,2-dihydropyridines **3** and **4** were smoothly afforded, respectively. It can be postulated that the reaction initially proceeds *via* a nucleophilic attack to form aldol adducts **3A** and **4A** which in turn cyclized to adducts **3B** and **4B** then lost four molecules of water and afforded the pyridine derivatives **3** and **4** (Chart 1). Compounds **3** and **4** were proved on the basis of elemental analysis, IR, ^{1}H- and ^{13}C-NMR. IR spectrum of **3** showed bands at 2218 cm^{-1} (2C≡N), 1695 cm^{-1} (2C=O) and 1330, 1161 cm^{-1} (SO_2). Its ^{1}H-NMR spectrum in (DMSO-d_6) displayed signals at 1.9 ppm for CH_3 groups, 6.2 ppm due to CH of pyridine and 7.4–8.0 ppm corresponding to aromatic protons. IR spectrum of **4** showed bands at 2218 cm^{-1} (2C≡N), 1655 cm^{-1} (2C=O) and 1365, 1145 cm^{-1} (SO_2). Its ^{1}H-NMR spectrum in (DMSO-d_6) displayed signals at 1.6 ppm corresponding to CH_3 groups.

Application of Michael addition of **3** to benzylidenemalononitriles in the presence of piperidine furnished the corresponding 1,2-dihydroisoquinoline derivatives **5**–**7**, respectively. The structures of compounds **5**–**7** were verified on the basis of analytical and spectral data. IR spectrum of **5** showed bands at 3390, 3367 cm^{-1} ($2NH_2$) and 2160 cm^{-1} (2C≡N). Its ^{1}H-NMR spectrum in (DMSO-d_6) displayed signals at 6.2 ppm for NH_2 group and 6.4 ppm for CH of pyridone. IR spectrum of **6** showed bands at 3425, 3260 cm^{-1} ($2NH_2$), 2187 cm^{-1} (2C≡N) and 1373, 1153 cm^{-1} (SO_2) . Its ^{1}H-NMR spectrum in (DMSO-d_6) displayed signals at 1.6 ppm for CH_3 group of pyridone and 2.4 ppm corresponding to tolyl CH_3. IR spectrum of **7** showed bands at 3464, 3350 cm^{-1} ($2NH_2$) and 2218 cm^{-1} (2C≡N). Its ^{1}H-NMR spectrum in (DMSO-d_6) displayed signals at 1.9 ppm due to CH_3 groups and 6.5 ppm due to CH_2 of piperonyl group.

Treatment of compound **3** with dimethylformamide-dimethylacetal (DMF-DMA) in xylene produced the enaminone derivative **8**. IR spectrum of **8** showed bands at 2950 cm^{-1} (CH, aliph.), 2198 cm^{-1} (2C≡N) and 1377, 1161 cm^{-1} (SO_2). ^{1}H-NMR spectrum in (DMSO-d_6) of **8** revealed signals at 2.4 ppm for $N(CH_3)_2$, doublet at 5.00, 5.10 ppm due to CH=CH group and 6.3 ppm corresponding to CH of pyridone.

Upon stirring the cyanoacetamide **2** with carbon disulfide in the presence of potassium hydroxide in dimethylformamide followed by cycloalkylation with 1,2-dibromoethane afforded the corresponding 1,3-dithiolan derivative **10**. Also, stirring of compound **2** under the same reaction conditions with 1,3-dichloropropan-2-ol yielded the dithiolan derivative **11**. Furthermore, reaction of compound **2** with carbon disulfide in the presence of KOH and dimethylsulfate while stirring and cooling afforded *N*,*N*′-(4,4′-sulfonylbis(4,1-phenylene))bis(2-cyano-3,3-bis(methylthio)acrylamide) **12** smoothly (Chart 2). The IR spectrum of compound **12** revealed bands at 3367 cm^{-1} (NH) and 2179 cm^{-1} (C≡N). ^{1}H-NMR (DMSO-d_6) spectrum of **12** revealed signals at 2.4 ppm for SCH_3 group and 7.0–7.9 ppm due to aromatic protons.

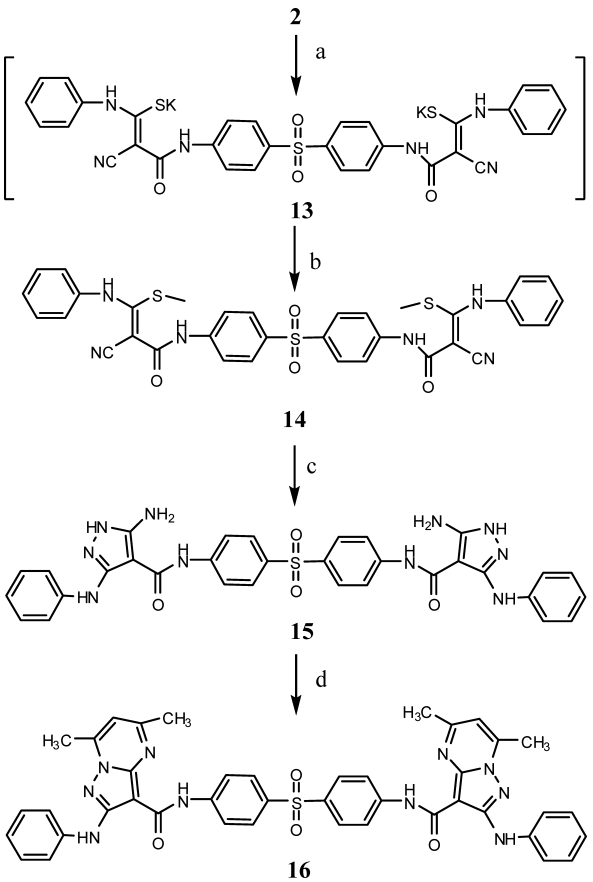


Reagents (a) KOH/CS_2, (b) 1,2-dibromoethane, (c) 1,3-dichloropropan-2-ol, (d) dimethyl sulphate.

Chart 2

When compound **2** was left to react with phenyl isothiocyanate in the presence of dimethylsulfate in alkaline medium, the corresponding methylated derivative **14** was afforded through the formation of intermediate **13**. The IR spectrum of compound **14** revealed bands at 2191 cm^{-1} (C≡N) and 1380, 1149 cm^{-1} (SO_2). ^{1}H-NMR spectrum in (DMSO-d_6) of **14** revealed signals at 2.3 ppm for SCH_3 group, 8.8 ppm for NH aniline and 10.1 ppm for NHCO group. Cyclocondensation of the acrylamide derivative **14** with hydrazine hydrate in refluxing ethanol gave the pyrazole derivative **15**. Presumably, formation of the aminopyrazole derivative **15** is assumed to proceed *via* Michael addition of the hydrazinoamino group to the ethylenic bond side chain in **14** with elimination of SCH_3 group followed by intramolecular cyclization at the cyano group. Its IR spectrum revealed the presence of (NH, NH_2) bands at 3490, 3344, 3260 $cm^{-1}$and a band of (C=N) at 1589 cm^{-1}. ^{1}H-NMR spectrum in (DMSO-d_6) of **15** revealed signals at 6.00 ppm corresponding to NH_2 group and 11.20 ppm due to NH of pyrazole. When compound **15** was heated under reflux with acetylacetone in glacial acetic acid, the corresponding pyrazolopyrimidine derivative **16** was obtained. ^{1}H-NMR spectrum in (DMSO-d_6) of **16** displayed signals at 2.3, 2.4 ppm corresponding to CH_3 groups, 8.9 ppm for CH of pyrimidines, 10.0 ppm for NH anilino and 10.3 ppm for NHCO groups (Chart 3).

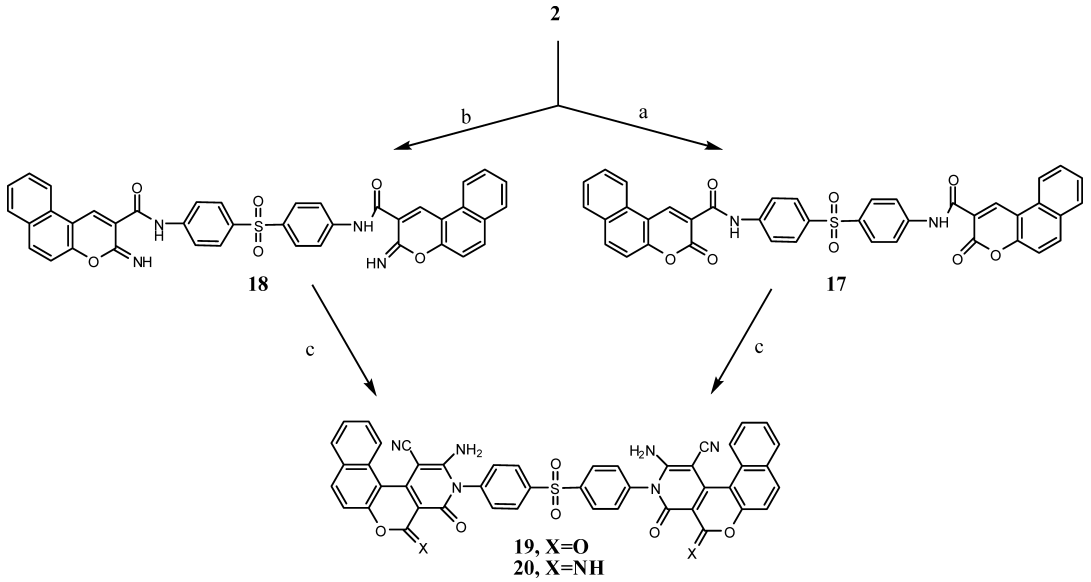
Furthermore, Perkin reaction was carried out by reacting compound **2** with 2-hydroxyl-1-naphthaldehyde in acetic anhydride in presence of sodium acetate to give the corresponding chromene-2-one derivative **17**, while reaction of **2** with the same reagent in ammonium acetate yielded the chromen-2-imino derivative **18**. Compounds **17** and **18** were supported in the basis of analytical and spectral data. Thus, the IR spectra of compounds **17** and **18** showed the absence of (C≡N) bands. The chromene derivatives **17** and **18** were further reacted with malononitrile in ammonium acetate furnished the corresponding chromenopyridones **19** and **20**, respectively. The IR spectra of compounds **19** and **20** exhibited the presence of (C≡N) bands (Chart 4).



Reagents (a) phenyl isothiocyanate/KOH/DMF, (b) dimethylsulfate, (c) hydrazine hydrate, (d) acetylacetone.

Chart 3

Molecular Docking The development of inhibitors of



Reagents (a) 2-hydroxy-1-naphthaldehyde/acetic anhydride/sodium acetate, (b) 2-hydroxy-1-naphathaldehyde/ammonium acetate/ethanol, (c) ammonium acetate/malononitrile.

Chart 4

farnesyltransferase (FTIs) has been pursued with the initial aim of targeting aberrant rat sarcoma (RAS) function in cancer.[26,27] As a critical step in the processing of RAS, inhibition of farnesylation alone may be sufficient to abrogate the cell signaling and transforming function of RAS in cancer. Various tumors, including those of colon, bladder, lung, prostate and pancreas showed dose-dependent growth inhibition and prophylactic administration of farnesyltransferase inhibitors daily delayed both tumor onset and growth.[28] Blocking by FTIs of farnesylation and RAS processing in transformed cells prevents many of the morphological changes associated with neoplastic growth.[29] Likewise, signaling pathways such as the proto-oncogene serine/threonine protein kinase (RAF)/MEKK/MAPK cascade, which are activated in H-RAS-transformed fibroblasts, were down regulated after exposure to FTI. Thus, there was cytoplasmic accumulation of soluble and inactive unprocessed proto-oncogene serine serine/therionine protein kinare (RAS)/RAF complexes.[30] Although FTIs were originally conceived as targeting mutant or aberrant RAS function in cancer, the observation that a broad range of human cancer cells are susceptible to FTI therapy independently of RAS mutation status led to the search for other critical prenylated proteins that may be targets for FTI therapy.[31]

On the other hand, epigenetic modifications, such as changes in DNA methylation patterns and histone alterations, play important roles in cancer.[32,33] Given the prevalence of PRMT1 substrates in the cell, it is highly probable that PRMT1 and arginine methylation is linked to many diseases.[34] PRMT1 is thought to contribute to as much as 85% of all cellular PRMT activity.[35] The PRMT1 mRNA is found in all embryonic and adult tissues examined demonstrating the widespread importance of this enzyme in cellular functions.[36] The activity of PRMT1 can be regulated in several fashions. PRMT1 is present in both the cytoplasm and the nucleus, and is highly mobile between these compartments.[37]

Thus, the present investigation is concerned with the synthesis of novel anticancer agents and trying to understand their mechanism of action. In order to perform the aim of the present investigations the authors have performed molecular docking of the synthesized compounds on the active sites of both farnesyltransferase and PRMT1 which may lead to an understanding of their effect as antitumor agents. These two enzymes were selected for docking based on the activity of several diaryl sulfone derivatives as PRMT1 inhibitors[17] and as FTIs.[18]

Molecular Docking on the Active Site of Farnesyltransferase The protein data bank file (PDB ID: 3E30) was selected for this purpose. The file contains farnesyltransferase enzyme co-crystallized with a sulfone ligand. All docking procedures were achieved by MOE (Molecular Operating Environment) software 10.2008 provided by chemical computing group, Canada. Docking on the active site of farnesyltransferase enzyme was performed for all synthesized compounds **2**–**8**, **10**–**12**, **14**–**20**.

Docking protocol was verified by redocking of the co-crystallized ligand in the vicinity of the active site of the enzyme with energy score (S)=−25.6345 kcal/mol and root mean standard deviation (RMSD)=2.8268 (Fig. 1). The sulfone ligand interacts with the active site of farnesyltransferase by four interactions: Try B361 with a hydrogen bond of 2.95 Å and arene-arene interaction, Trp B102 with a hydrogen bond of 2.83 Å and Zn with the lone pair of imidazole nitrogen. All synthesized compounds were fit to the active site of farnesyltransferase enzyme with good energy scores (S) suggesting activity as farnesyltransferase inhibitors. Energy scores (S) and amino acid interactions for all synthesized compounds were listed in Table 1. Compound **11** showed the best energy score (S)=−39.3306 kcal/mol and interacted with Lys B356 with a hydrogen bond of 2.95 Å, Lys A164 with a hydrogen bond of 2.82 Å and with Zn through its OH of the 1,3-dthiolan ring (Fig. 2). 3D interaction of compound **19** with the amino acid of active site of the enzyme as well as Zn^{+2} is illustrated in Fig. 3.

Molecular Docking on the Active Site of Arginine Methyltransferase (PRMT1) The protein data bank file (PDB ID: 3Q7E) was selected for this purpose. The file contains

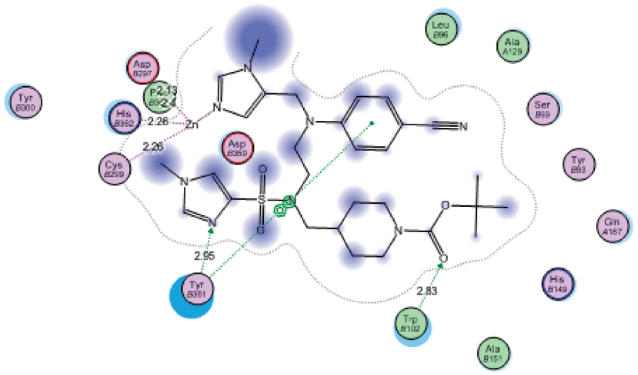


Fig. 1. Co-crystallized Sulfone Ligand on the Active Site of Farnesyltransferase

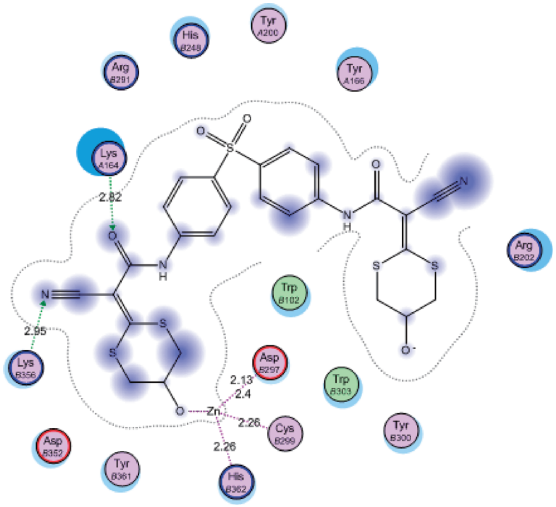


Fig. 2. Compound **11** on the Active Site of Farnesyltransferase

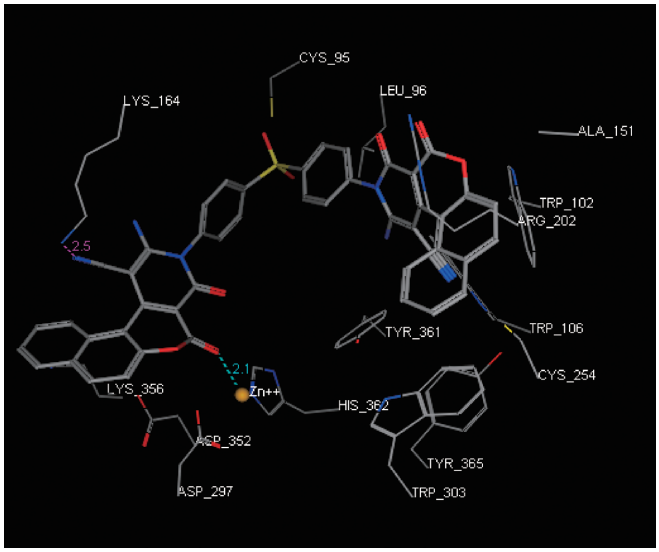


Fig. 3. Compound **19** on the Active Site of Farnesyltransferase

arginine methyltransferase co-crystallized with its ligand (*S*-adenosyl methionine). All docking procedures were achieved by MOE (Molecular Operating Environment) software 10.2008 provided by chemical computing group, Canada. Docking on the active site of arginine methyltransferase enzyme was performed for all synthesized compounds **2**–**8**, **10**–**12**, **14**–**20**.

Docking protocol was verified by redocking of the co-crystallized ligand in the vicinity of the active site of the enzyme with energy score (S)=−18.5932 kcal/mol and root mean standard deviation (RMSD)=0.3523 The ligand interacts with the active site of arginine methyltransferase by five interactions: Val 128 with a hydrogen bond of 3.00 Å, Arg 54 with a hydrogen bond of 2.64 Å, Gly 78 with a hydrogen bond of 1.81 Å and Glu 100 with two hydrogen bonds of 181, 186 Å (Fig. 4). All synthesized compounds were fit to the active site of arginine methyltransferase enzyme with good energy scores (S) suggesting activity as arginine methyltransferase inhibitors for most of the synthesized compounds. Energy scores (S) and amino acid interactions for all synthesized compounds were listed in Table 2. Compound **16** showed the best energy score (S)=−27.9591 kcal/mol and interacted with Glu 100 with a hydrogen bond of 1.97 Å, Lys 127 and Arg 327 with arene cation interactions (Fig. 5). 3D interaction of compound **19** with the amino acid of active site of the enzyme (Fig. 6).

***In Vitro* Antitumor Activity** The newly synthesized compounds were evaluated for their *in vitro* cytotoxic activity against human breast cancer cell line (MCF7). Doxorubicin which is one of the most effective anticancer agents was used as the reference drug in this study. The relationship between surviving fraction and drug concentration was plotted to obtain the survival curve of breast cancer cell line (MCF7). The response parameter calculated was the IC_{50} value, which corresponds to the concentration required for 50% inhibition of cell viability. Table 3 shows the *in vitro* cytotoxic activity of the synthesized compounds where all compounds exhibited significant activity compared to the reference drug.

All the synthesized compounds showed better cytotoxic activity than Doxorubicin especially the benzochromene derivatives **17**–**20** which showed IC_{50} values 34.95, 34.53, 19.36 and 35.45 μM, respectively. Compounds **2**–**7** showed cytotoxic activity with IC_{50} values in the range of 37.04 to 57.69 μM. On the other hand, compound **8** showed better cytotoxic activity than compounds **2**–**7** with IC_{50} value 30.70 μM. Finally compounds **10**–**16** showed cytotoxic activity with IC_{50} values in the range of 38.42 to 51.95 μM. The promising results of cytotoxic activity of the synthesized compounds especially compound **19** which was the most active compound with IC_{50} value 19.36 μM urge more investigations for their mechanism of action. The trial in the present investigation to predict an assumption on the mechanism of action of the synthesized compounds were conducted through molecular docking on the active site of two enzymes based on the similarities between the synthesized compounds and the enzyme inhibitors of these enzymes.

The results of molecular docking and biological activity may suggest better activity of the synthesized compounds as farnesyltransferasse (Table 1) inhibitor than as arginine methyltransferase inhibitors (Table 2) as good energy scores and amino acid interactions were better in the case of farnesyltransferase also good interaction with Zn^{+2} was observed with most of the synthesized compounds. However, we could not define a relationship between the cytotoxic activity and the docking results which may suggest other mechanisms of action for the synthesized compounds. On the other hand,

Table 1. Binding Scores and Amino Acid Interactions of the Docked Compounds on the Active Site of Farnesyltransferase (FT)

Compound No.	S (kcal/mol)	Amino acid interactions	H bond length (Å)	Interaction with Zn
2	−22.2685	Leu B295, Lys B294	3.37, 2.76	No interaction
3	−25.1744	Trp B102, Arg B202, Tyr A166, Tyr B251	3.16, 2.89, 2.87, 2.97	CN
4	−30.8654	Gln A187	3.00	CN
5	−29.8040	Arg B202	2.73	SO_2
6	−25.7866	Lys A164, Ser B357	2.62, 2.72	No interaction
7	−30.7866	Arg B281, Lys B284	3.21, 2.87	No interaction
8	−23.3447	Arg B202, Lys B294	3.10, 2.60	No interaction
10	−23.3689	Arg B202, Arg B291, Lys B294	2.57, 2.93, 2.68	No interaction
11	−39.3306	Lys A164, Lys B356	2.82, 2.95	OH
12	−29.2271	His B361	3.17	CN
14	−27.9248	Arg B281, Lys B294	2.75, 2.95	No interaction
15	−27.2643	Leu B295, Lys B363, Asp B352	1.87–2.12, 2.53, 1.65	No interaction
16	−36.9713	Arg B202, Arg B352	2.55, 1.29	NHCO
17	−26.5360	Lys A164, Lys B294, Asp B352	2.44, 2.72, 1.45	NHCO
18	−20.2098	Arg B281, Lys B294	2.6, 3.3	No interaction
19	−23.8669	Lys A164	2.54	C=O
20	−35.0407	Lys A164, Arg B202	3.25, 2.47–2.7	C=NH

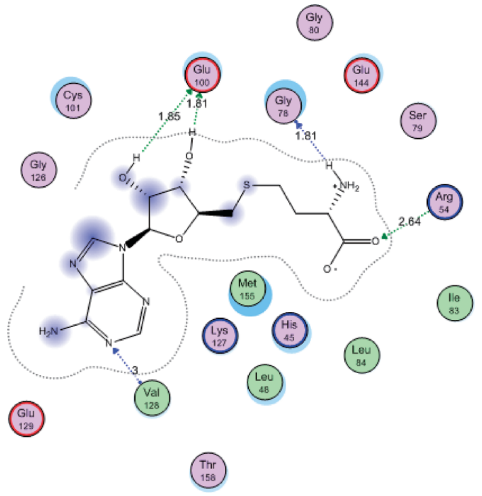


Fig. 4. Co-crystallized *S*-Adenosyl Methionine Ligand on the Active Site of Arginine Methyltransferase (PRMT1)

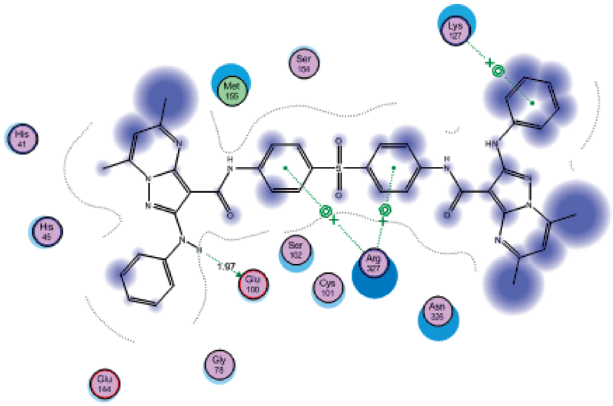


Fig. 5. : Compound **16** on the Active Site of Arginine Methyltransferase (PRMT1)

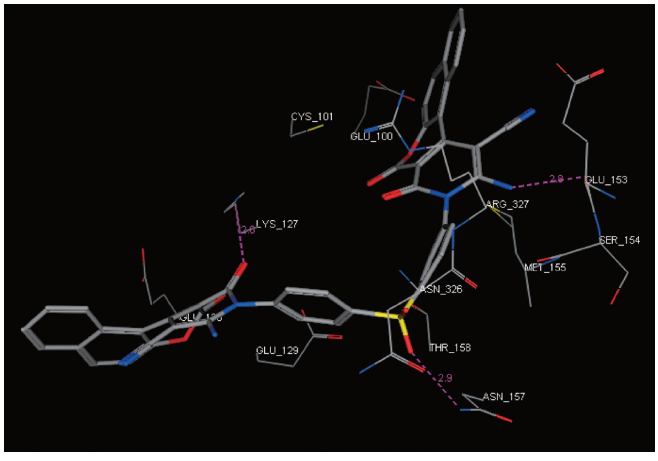


Fig. 6. Compound **19** on the Active Site of Arginine Methyltransferase (PRMT1)

the promising cytotoxic results of the synthesized compounds make them a good trial to discover new anticancer agents and urge the researchers to seek for more derivatives of diarylsulfone derivatives.

Experimental

Chemistry Melting points (°C, uncorrected) were determined in open capillaries on a Gallenkemp melting point apparatus (Sanyo Gallenkemp, Southborough, U.K.) and were uncorrected. Precoated silica gel plates (silica gel 0.25 mm, 60 G F254; Merck, Germany) were used for thin layer chromatography, dichloromethane–methanol (9.5 : 0.5) mixture was used as a developing solvent system and the spots were visualized by UV light and/or iodine. Infra-red spectra were recorded in KBr discs using IR-470 Shimadzu spectrometer (Shimadzu, Tokyo, Japan). NMR spectra (in DMSO-d_6) were recorded on Bruker AC-300 Ultra Shield NMR spectrometer (Bruker, Flawil, Switzerland, δ ppm) at 300 MHz using tetramethylsilane (TMS) as internal standard and peak multiplicities are designed as follows: s, singlet; d, doublet; t, triplet; m, multiplet. Elemental analyses were performed on Carlo Erba 1108 Elemental Analyzer (Heraeus, Hanau, Germany).

Table 2. Binding Scores and Amino Acid Interactions of the Docked Compounds on the Active Site of Arginine Methyltransferase (PRMT1)

Compound No.	S (kcal/mol)	Amino acid interactions	H bond length (Å)
2	−20.0584	Lys 127, His 293	2.65, 2.81
3	−17.4225	Lys 127	2.67
4	−23.2276	Lys 127, His 293	3.02, 3.10
5	−18.4486	His 45	3.02
6	−14.9319	Asn 326, Arg 327	3.06, 2.88–2.92
7	−23.3465	Arg 327, Asn 157, Lys 127	2.55–2.57, 2.88, 3.03
8	−18.1201	Val 128	3.19
10	−22.1917	Lys 127	2.94
11	−11.6688	Thr 158, His 127	2.85, 2.44
12	−17.2712	Lys 127, Arg 3.27	2.45, 2.95
14	−16.3269	Arg 127, Glu 136	2.40–3.11, 1.60
15	−18.4808	Glu 100, Glu 130, Lys 127	1.38–1.74, 3.20, 2.55
16	−27.9591	Glu 100	1.97
17	−11.4933	Lys 127, Arg 327	2.40, 2.57
18	−18.6764	Arg 327, Glu 129	2.41, 1.72
19	−24.5515	Asn 157, Glu 153, Lys 127	2.88, 1.89, 2.58
20	−19.2200	Lys 127, His 45	2.49, 3.03

***N*,*N*′-(4,4′-Sulfonylbis(4,1-phenylene))bis(2-cyanoacetamid) (2)** A mixture of Dapson **1** (2.48 g, 0.01 mol) and excess ethyl cyanoacetate was fused at 220°C in an oil bath for 2 h. Excess ethyl cyanoacetate was evaporated under vacuum. The solid product remained was triturated with diethylether (100 mL) then filtered. The solid obtained was crystallized from ethanol to give **2**. Yield 92%, mp 137.5°C. IR: ν_{max}/cm^{-1} 3448, 3363 (2NH), 3062 (CH arom.), 2960, 2931 (CH aliph.), 2256 (C≡N), 1701 (2C=O), 1342, 1180 (SO_2). ^{1}H-NMR (DMSO-d_6, D_2O) δ: 4.0 (s, 4H, 2CH_2), 7.4–7.9 (m, 8H, Ar-H), 10.7 (s, 2H, 2NH exchangeable). ^{13}C-NMR (DMSO-d_6): 24.4(2), 115.6(2), 119.2(2), 119.3(2), 128.1(2), 129.2(2), 137.8(2), 142.7(2), 162.2(2). *Anal.* Calcd for $C_{18}H_{14}N_4O_4S$ (382.39): C, 56.54; H, 3.69; N, 14.65. Found: C, 56.81; H, 3.84; N, 14.29.

General Procedure for Synthesis of Compounds 3 and 4 Equimolar amounts of compound **2** (3.82 g, 0.01 mol) and acetylacetone (2.00 g, 0.02 mol) or benzoylacetone (3.24 g, 0.02 mol) were refluxed in ethanol (50 mL) containing piperidine (0.5 mL) for 5 h. After trituration with ethanol, the solid obtained was filtered and crystallized from dioxane to give **3** and **4**, respectively.

1,1′-(4,4′-Sulfonylbis(4,1-phenylene))bis(4,6-dimethyl-2-oxo-1,2-dihydropyridine-3-carbonitrile) (**3**): Yield 89%, mp 358.1°C. IR: ν_{max}/cm^{-1} 3074 (CH arom.), 2940, 2843 (CH aliph.), 2218 (2C≡N), 1655 (2C=O), 1330, 1161 (SO_2) . ^{1}H-NMR (DMSO-d_6, D_2O) δ: 1.9 (s, 12H, 4CH_3), 6.2 (s, 2H, 2CH of pyridines), 7.4–8.0 (m, 8H, Ar-H). ^{13}C-NMR (DMSO-d_6): 20.6(2), 21.4(2), 109.1(2), 113.0(2), 115.6(2), 124.3(4), 129.7(4), 140.6(2), 142.9(2), 143.9(2), 153.9(2), 106.2(2). *Anal.* Calcd for $C_{28}H_{22}N_4O_4S$ (510.56): C, 65.87; H, 4.34; N, 10.97. Found: C, 66.13; H, 4.21; N, 11.26

1,1′-(4,4′-Sulfonylbis(4,1-phenylene))bis(4-methyl-2-oxo-6-phenyl-1,2-dihydropyridine-3-carbonitrile) (**4**): Yield 79%, mp 238.2°C. IR: ν_{max}/cm^{-1} 3055 (CH arom.), 2935, 2858 (CH aliph.), 2218 (2C≡N), 1655 (2C=O), 1365, 1145 (SO_2) . ^{1}H-NMR (DMSO-d_6, D_2O) δ: 1.6 (s, 6H, 2CH_3), 6.2 (s, 2H, 2CH of pyridones), 6.5–8.0 (m, 18H, Ar-H). ^{13}C-NMR (DMSO-d_6): 15.6(2), 91.2(2), 115.4(2), 116.2(2), 124.3(2), 126.9(4), 127.9(2), 128.7(4), 129.3(4), 130.6(2), 135.5(2), 140.7(2), 144.0(2), 159.2(2), 160.8(2). *Anal.* Calcd for $C_{38}H_{26}N_4O_4S$ (634.70): C, 71.91; H, 4.13; N, 8.83. Found: C, 72.20; H, 4.41; N, 8.66.

General Procedure for Synthesis of Compounds 5–7 A mixture of compound **3** (5.1 g, 0.01 mol) and benzylidenemalononitrile (0.02 mol) in ethanol (50 mL) containing piperidine (0.5 mL) was refluxed for 6 h. The reaction mixture was cooled

Table 3. *In Vitro* Anticancer Screening of the Synthesized Compounds against Human Breastcell Line (MCF7)

Compound	Compound concentration (μM)				IC_{50} (μM)
	10 μM	25 μM	50 μM	100 μM	
	Surviving fraction (mean±S.E.)[a]				
Doxorubicin	0.721±0.020	0.546±0.020	0.461±0.010	0.494±0.030	71.80
2	0.727±0.134	0.427±0.055	0.307±0.029	0.317±0.021	46.58
3	0.59±0.089	0.499±0.140	0.400±0.078	0.380±0.107	50.65
4	0.773±0.188	0.547±0.031	0.387±0.016	0.380±0.044	57.69
5	0.817±0.023	0.567±0.058	0.333±0.012	0.307±0.025	52.95
6	0.797±0.021	0.470±0.101	0.213±0.059	0.340±0.053	47.30
7	0.547±0.129	0.327±0.076	0.298±0.067	0.323±0.087	37.04
8	0.531±0.053	0.237±0.009	0.283±0.019	0.281±0.026	30.70
10	0.722±0.040	0.533±0.075	0.329±0.051	0.297±0.022	48.82
11	0.791±0.050	0.537±0.025	0.336±0.020	0.307±0.013	51.95
12	0.827±0.050	0.615±0.012	0.359±0.051	0.190±0.041	49.82
14	0.822±0.006	0.496±0.090	0.245±0.059	0.297±0.033	47.47
15	0.748±0.160	0.427±0.085	0.224±0.004	0.268±0.036	41.79
16	0.574±0.077	0.274±0.045	0.371±0.047	0.309±0.076	38.42
17	0.676±0.095	0.318±0.069	0.189±0.011	0.249±0.022	34.95
18	0.563±0.079	0.238±0.140	0.401±0.056	0.239±0.070	34.52
19	0.370±0.037	0.237±0.057	0.209±0.005	0.240±0.034	19.36
20	0.540±0.092	0.375±0.066	0.242±0.015	0.305±0.013	35.45

a) Each value is the mean of three values±standard error.

and poured on crushed ice and acidified by dil HCl. The solid product was filtered off and crystallized from dioxane to give compounds **5**–**7**, respectively.

2,2′-(4,4′-Sulfonylbis(4,1-phenylene))bis(8-amino-3-methyl-1-oxo-6-phenyl-1,2-dihydroisoquinoline-7-carbonitrile) (**5**): Yield 68%, mp 174.5°C. IR: ν_{max}/cm^{-1} 3390, 3367 (2NH$_2$), 3060 (CH arom.), 2939, 2858 (CH aliph.), 2160 (2C≡N), 1654 (2C=O), 1360, 1153 (SO$_2$). ^{1}H-NMR (DMSO-d_6, D$_2$O) δ: 1.9 (s, 6H, 2CH$_3$), 6.2 (s, 2H, 2NH$_2$, exchangeable), 6.4 (s, 2H, 2CH of pyridones), 7.3–7.9 (m, 20H, Ar-H). ^{13}C-NMR (DMSO-d_6, D$_2$O): 20.7(2), 92.9(2), 114.6(2), 115.7(2), 119.6(2), 122.8(4), 127.9(2), 128.3(4), 129.1(4), 130.1(4), 133.6(2), 134.8(2), 137.1(2), 138.6(2), 140.9(2), 143.8(2), 151.8(2), 153.9(2). *Anal.* Calcd for $C_{46}H_{32}N_6O_4S$ (764.85): C, 72.24; H, 4.22; N, 10.99. Found: C, 72.11; H, 4.50; N, 10.68.

2,2′-(4,4′-Sulfonylbis(4,1-phenylene))bis(8-amino-3-methyl-1-oxo-6-p-tolyl-1,2-dihydroisoquinoline-7-carbonitrile) (**6**): Yield 81%, mp 227.3°C. IR: ν_{max}/cm^{-1} 3425, 3260 (2NH$_2$), 2931, 2870 (CH aliph.), 2187 (2C≡N), 1655 (2C=O), 1373, 1153 (SO$_2$) . ^{1}H-NMR (DMSO-d_6, D$_2$O) δ: 1.6 (s, 6H, 2CH$_3$ pyridones), 2.4 (s, 6H, 2CH$_3$ tolyls), 6.1 (s, 4H, 2NH$_2$, exchangeable), 6.4 (s, 2H, 2CH pyridones), 7.2–7.9 (m, 18H, Ar-H). ^{13}C-NMR (DMSO-d_6): 21.6(2), 23.1(2), 94.2(2), 99.1(2), 115.9(2), 118.2(2), 119.1(2), 125.1(4), 127.1(4), 128.6(4), 129.9(4), 132.9(2), 137.6(2), 138.1(2), 139.8(2), 140.6(2), 142.3(2), 143.8(2), 148.3(2), 154.8(2). *Anal.* Calcd for $C_{48}H_{36}N_6O_4S$ (792.90): C, 72.71; H, 4.58; N, 10.60. Found: C, 72.99; H, 4.38; N, 10.29.

2,2′-(4,4′-Sulfonylbis(4,1-phenylene))bis(8-amino-6-(benzo[*d*][1,3]dioxol-5-yl)-3-methyl-1-oxo-1,2-dihydroisoquinoline-7-carbonitrile) (**7**): Yield 66%, mp >350°C. IR: ν_{max}/cm^{-1} 3464, 3350 (2NH$_2$), 3074 (CH arom.), 2218 (2C≡N), 1655 (2C=O), 1330, 1161 (SO$_2$) . ^{1}H-NMR (DMSO-d_6, D$_2$O) δ: 1.9 (s, 6H, 2CH$_3$), 6.2 (s, 4H, 2NH$_2$, exchangeable), 6.4 (s, 2H, 2CH pyridones), 6.5 (s, 4H, 2CH$_2$ pipernonyl), 6.6–8.0 (m, 16H, Ar-H). ^{13}C-NMR (DMSO-d_6): 21.4(2), 93.8(2), 98.8(2), 102.7(2), 113.0(2), 115.1(2), 115.9(2), 116.6(2), 117.3(2), 120.7(2), 122.1(4), 129.3(4), 129.9(2), 137.7(2), 138.6(2), 139.2(2), 142.8(2), 145.2(2), 149.1(2), 150.8(2), 152.6(2), 153.9(2). *Anal.* Calcd for $C_{48}H_{32}N_6O_8S$ (852.87): C, 67.60; H, 3.78; N, 9.85. Found: C, 67.98; H, 3.43; N, 9.64.

(*E*)-1,1′-(4,4′-Sulfonylbis(4,1-phenylene))bis(4-((*E*)-2-(dimethylamino)vinyl)-6-methyl-2-oxo-1,2-dihydropyridine-3-carbonitrile) (8) To a solution of compound **3** (5.1 g, 0.01 mol) in xylene (50 mL), DMF-DMA (2.30 g, 0.02 mol) was added. The reaction mixture was refluxed for 6 h, cooled and the solid obtained was crystallized from ethanol to give **8**. Yield 65%, mp >350°C. IR: ν_{max}/cm^{-1} 3100 (CH arom.), 2920, 2870 (CH aliph.), 2198 (2C≡N), 1654 (2C=O), 1377, 1161 (SO$_2$). ^{1}H-NMR (DMSO-d_6, D$_2$O) δ: 1.8 (s, 6H, 2CH$_3$ pyridones), 2.4 (s, 12H, 4N(CH$_3$)$_2$), 5.0, 5.1 (2d, 4H, 2CH=CH, *J*=7.3, 7.6 Hz), 6.3 (s, 2H, 2CH pyridones), 7.5–7.9 (m, 8H, Ar-H). ^{13}C-NMR (DMSO-d_6): 21.6(2), 44.8(4), 99.9(2), 108.2(2), 109.1(2), 116.7(2), 119.9(4), 128.6(4), 130.9(2), 131.8(2), 132.9(2), 148.2(2), 155.4(2), 177.1(2). *Anal.* Calcd for $C_{34}H_{32}N_6O_4S$ (620.72): C, 65.79; H, 5.20; N, 13.54. Found: C, 65.90; H, 5.11; N, 13.20.

General Procedure for Synthesis of Compounds 10–12 To a stirred suspension of finely powdered potassium hydroxide (1.12 g, 0.02 mol) in dry dimethylformamide (20 mL), cyanoacetamide **2** (3.82 g, 0.01 mol) was added. The resulted mixture was cooled at 0°C in an ice bath, then carbon disulfide (1.52 g, 0.02 mol) was added slowly over the course of 10 min. After complete addition, stirring of the reaction mixture was continued for additional 3 h. dibromoethane (1.88 g, 0.02 mol) or 1,3-dichloropropan-2-ol (2.58 g, 0.02 mol) or dimethylsulfate (2.52 g, 0.02 mol) was added to the reaction mixture while cooling (−15°C) and stirring for 2 h. then poured into crushed ice, the resulting precipitate was filtered off and crystallized from ethanol to give compounds **10**–**12**, respectively.

N,*N*′-(4,4′-Sulfonylbis(4,1-phenylene))bis(2-cyano-2-(1,3-dithiolan-2-ylidene)acetamide) (**10**): Yield 74%, mp 218.1°C. IR: ν_{max}/cm^{-1} 3448 (2NH), 2203 (2C≡N), 1652 (2C=O), 1315, 1184 (SO$_2$). ^{1}H-NMR (DMSO-d_6, D$_2$O) δ: 3.5–3.6 (m, 8H, 4CH$_2$), 7.5–8.0 (m, 8H, Ar-H) 10.2 (s, 2H, 2NH, exchangeable). ^{13}C-NMR (DMSO-d_6): 38.9(4), 89.4(2), 116.3(2), 123.5(4), 129.1(4), 135.9(2), 142.1(2), 162.9(2), 183.8(2). *Anal.* Calcd for $C_{24}H_{18}N_4O_4S_5$ (586.75): C, 49.13; H, 3.09; N, 9.55. Found: C, 49.40; H, 3.32; N, 9.31.

N,*N*′-(4,4′-Sulfonylbis(4,1-phenylene))bis(2-cyano-2-(5-hydroxy-1,3-dithian-2-ylidene)acetamide) (**11**): Yield 78%, mp 213.6°C. IR: ν_{max}/cm^{-1} 3433 (2OH), 3385 (2NH), 2202 (2C≡N),1675 (2C=O), 1350, 1184 (SO$_2$). ^{1}H-NMR (DMSO-d_6, D$_2$O) δ: 3.44, 3.45 (2d, 8H, 4CH$_2$, *J*=7.6, 7.4 Hz), 4.2 (m, 4H, 2CH+2OH) 7.5–7.9 (m, 8H, Ar-H) 10.5 (s, 2H, 2NH, exchangeable). ^{13}C-NMR (DMSO-d_6): 37.6(4), 78.0(2), 78.9(2), 115.5(2), 123.6(4), 129.1(4), 136.9(2), 143.6(2), 162.2(2), 183.8(2). *Anal.* Calcd for $C_{26}H_{22}N_4O_6S_5$ (646.80): C, 48.28; H, 3.43; N, 8.66 Found: C, 48.61; H, 3.18; N, 8.42.

N,*N*′-(4,4′-Sulfonylbis(4,1-phenylene))bis(2-cyano-3,3-bis(methylthio)acrylamide) (**12**): Yield 69%, mp 113.6°C. IR: ν_{max}/cm^{-1} 3367 (2NH), 3065 (CH arom.), 2920, 2870 (CH aliph.), 2179 (2C≡N), 1655 (2C=O), 1390, 1149 (SO$_2$). ^{1}H-NMR (DMSO-d_6, D$_2$O) δ: 2.4 (s, 12H, 4SCH$_3$), 7.0–7.9 (m, 8H, Ar-H) 13.5 (s, 2H, 2NH, exchangeable). ^{13}C-NMR (DMSO-d_6, D$_2$O): 17.80(4), 17.75(4), 95.0(2), 115.7(2), 123.3(4), 129.2(4), 138.1(2), 144.1(2), 164.0(2), 178.9(2). *Anal.* Calcd for $C_{24}H_{22}N_4O_4S_5$ (590.78): C, 48.79; H, 3.75; N9.48. Found: C, 48.92; H, 3.44; N 9.20

(2*Z*,2′*Z*)-*N*,*N*′-(4,4′-Sulfonylbis(4,1-phenylene))bis(2-cyano-3-(methylthio)-3-(phenylamino)acrylamide) (14) To a suspension of potassium hydroxide (1.12 g, 0.02 mol) in dry dimethylformamide (20 mL) cyanoacetamide derivative **2** (3.82 g, 0.01 mol) was added during stirring, phenylisothiocyanate (2.70 g, 0.02 mol) was dropped slowly to the reaction mixture. After complete addition, stirring of the reaction mixture was continued for 7 h, and dimethylsulfate (2.52 g, 0.02 mol) was added. The reaction mixture was stirred for 2 h. then poured into crushed ice water. The resulting precipitate was filtered off, dried and crystallized from methanol to give **14**. Yield 76%, mp 103.5°C. IR: ν_{max}/cm^{-1} 3332, 3290 (4NH), 3060 (CH arom.), 2940, 2860 (CH aliph.), 2191 (2C≡N),1654 (2C=O), 1380, 1149 (SO$_2$). ^{1}H-NMR (DMSO-d_6, D$_2$O) δ: 2.3 (s, 6H, 2SCH$_3$), 6.7–7.9 (m, 18H, Ar-H), 8.8 (s, 2H, 2NH anilino, exchangeable). ^{13}C-NMR (DMSO-d_6): 16.4(2), 79.2(2), 117.9(2), 118.4(4), 120.4(2), 124.4(4), 128.7(4), 129.8(4), 139.4(2), 143.0(2), 144.2(2), 167.8(2), 179.6(2). *Anal.* Calcd for $C_{34}H_{28}N_6O_4S_3$ (680.82): C, 59.98; H, 4.15; N, 12.34. Found: C, 60.11; H, 4.41; N, 12.01

***N*,*N*′-(4,4′-Sulfonylbis(4,1-phenylene))bis(5-amino-3-(phenylamino)-1*H*-pyrazole-4-carboxamide) (15)** A mix-

ture of compound **14** (6.80 g, 0.01 mol) and hydrazine hydrate (1.00 g, 0.02 mol) in ethanol (50 mL) was refluxed for 5 h, and allowed to cool. The solid product obtained was filtered and crystallized from dioxane. Yield 71%, mp 245°C. IR: ν_{max}/cm^{-1} 3490, 3344, 3260 (NH, NH_2), 1654 (2C=O), 1589 (2C=N), 1340, 1145 (SO_2). ^{1}H-NMR (DMSO-d_6, D_2O) δ: 6.0 (s, 4H, $2NH_2$, exchangeable), 7.2–7.9 (m, 18H, Ar-H), 8.5 (s, 2H, 2NH anilino, exchangeable) 9.1 (s, 2H, 2NHCO, exchangeable). ^{13}C-NMR (DMSO-d_6, D_2O): 87.9(2), 117.9(4), 118.1(2), 119.5(4), 128.1(4), 128.8(4), 136.6(2), 142.9(2), 143.1(2), 150.6(2), 152.8(2), 163.1(2). *Anal.* Calcd for $C_{32}H_{28}N_{10}O_4S$ (648.69): C, 59.25; H, 4.35; N, 21.59. Found: C, 59.00; H, 4.62; N, 21.31.

***N*,*N*′-(4,4′-Sulfonylbis(4,1-phenylene))bis(5,7-dimethyl-2-(phenylamino)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide) (16)** A mixture of compound **15** (6.48 g, 0.01 mol) and acetylacetone (2.00 g, 0.02 mol) in glacial acetic acid (30 mL) was refluxed for 5 h. then left to cool. The obtained solid was filtered and crystalized from acetic acid. Yield 69%, mp 286.5–287°C. IR: ν_{max}/cm^{-1} 3448, 3313, (4NH), 3055 (CH arom.), 2924, 2860 (CH aliph.), 1670 (2C=O), 1385, 1149 (SO_2). ^{1}H-NMR (DMSO-d_6, D_2O) δ: 2.3, 2.4 (2s, 12H, $4CH_3$), 6.9–7.9 (m, 18H, Ar-H), 8.9 (s, 2H, 2CH pyrimidines),10.0 (s, 2H, 2NH anilino, exchangeable), 10.3 (s, 2H, 2NHCO, exchangeable). ^{13}C-NMR (DMSO-d_6): 24.1(2), 86.0(2), 109.3(2), 117.1(4), 118.9(2), 121.0(4), 128.5(4), 128.8(4), 135.0(2), 139.7(2), 142.7(2), 143.6(2), 146.4(2), 162.1(2), 169.1(2), 171.9(2). *Anal.* Calcd for $C_{42}H_{36}N_{10}O_4S$ (776.86): C, 64.93; H, 4.67; N, 18.03. Found: C, 65.06; H, 4.49; N, 17.94.

***N*,*N*′-(4,4′-Sulfonylbis(4,1-phenylene))bis(3-oxo-3*H*-benzo[*f*]chromene-2-carboxamide) (17)** To a solution of **2** (3.82 g, 0.01 mol) in acetic anhydride (30 mL), 2-hydroxyl-1-nphathaldehyde (3.44 g, 0.02 mol) and fused sodium acetate (1.60 g, 0.02 mol) were added. The reaction mixture was refluxed for 2 h, cooled and the solid obtained was crystallized from ethanol. Yield 59%, mp 126.3°C. IR: ν_{max}/cm^{-1} 3425 (2NH), 3093 (CH arom.), 1766, 1720 (4C=O), 1369, 1190 (SO_2). ^{1}H-NMR (DMSO-d_6, D_2O) δ: 7.2–8.0 (m, 20H, Ar-H), 8.4 (s, 2H, 2CH chromene), 10.3 (s, 2H, 2NH, exchangeable). ^{13}C-NMR (DMSO-d_6): 118.2(2), 119.6(2), 120.8(2), 122.1(2), 125.1(4), 125.9(2), 127.3(2), 128.6(4), 128.7(2), 128.8(2), 130.5(2), 130.7(2), 131.6(2), 140.5(2), 144.4(2), 146.9(2), 168.4(2), 169.0(2). *Anal.* Calcd for $C_{40}H_{24}N_2O_8S$ (692.69): C, 69.36; H, 3.49; N, 4.04. Found: C, 69.78; H, 3.33; N, 3.91.

***N*,*N*′-(4,4′-Sulfonylbis(4,1-phenylene))bis(3-imino-3*H*-benzo[*f*]chromene-2-carboxamide) (18)** A mixture of **2** (3.82 g, 0.01 mol), 2-hydroxyl-1-nphathaldehyde (3.44 g, 0.02 mol) and anhydrous ammonium acetate (2.30 g, 0.02 mol) in ethanol (50 mL) was refluxed for 3 h. The solid product was filtered and crystallized from dioxane. Yield 71%, mp 276.9°C. IR: ν_{max}/cm^{-1}3383, 3294 (4NH), 3072 (CH arom.), 1685 (2C=O), 1620 (2C=N), 1395, 1149 (SO_2). ^{1}H-NMR (DMSO-d_6, D_2O) δ: 6.1 (s, 2H, 2CH chromene), 6.6–8.1 (m, 20H, Ar-H), 9.2 (s, 2H, 2NHCO exchangeable), 13.1 (s, 2H, 2NH imino, exchangeable). ^{13}C-NMR (DMSO-d_6): 115.6(2), 118.6(2), 119.5(2), 121.7(4), 125.8(2), 127.9(4), 128.7(4), 128.8(2),129.2(2), 129.6(2), 134.8(2), 137.3(2), 137.8(2), 141.7(2), 153.4(2), 155.7(2), 160.3(2), 161.0(2). *Anal.* Calcd for $C_{40}H_{26}N_4O_6S$ (690.72): C, 69.55; H, 3.79; N, 8.11. Found: C, 69.84; H, 3.66; N, 8.07.

General Procedure for Synthesis of Compounds 19 and 20 Equimolar amounts of compound **17** or **18**, malononitrile (1.32 g, 0.02 mol) and anhydrous ammonium acetate (2.20 g, 0.02 mol) in ethanol (50 mL) were refluxed for 5 h. The solid obtained was separated by filteration and crystallized from ethanol to give **19** and **20**, respectively.

3,3′-(4,4′-Sulfonylbis(4,1-phenylene))bis(2-amino-4,5-dioxo-4,5-dihydro-3*H*-benzo[*f*]chromeno[3,4-*c*]pyridine-1-carbonitrile) (**19**): Yield 75%, mp 348°C. IR: ν_{max}/cm^{-1} 3460, 3336, 3232 ($2NH_2$), 2199 (2C≡N), 1690, 1645 (4C=O), 1356, 1180 (SO_2). ^{1}H-NMR (DMSO-d_6, D_2O) δ: 6.3 (s, 4H, $2NH_2$, exchangeable), 7.3–7.9 (m, 20H, Ar-H). ^{13}C-NMR (DMSO-d_6): 75.1(2), 116.4(2), 116.6(2), 117.1(2), 117.3(2), 118.8(2), 123.0(4), 124.6(2), 126.5(2), 128.4(4), 128.5(2), 129.9(2), 130.7(2), 131.5(2), 135.6(2), 142.9(2), 153.2(2), 157.8(2), 159.4(2), 162.3(2), 169.0(2). *Anal.* Calcd for $C_{46}H_{24}N_6O_8S$ (820.78): C, 67.31; H, 2.95; N, 10.24. Found: C, 67.02; H, 3.08; N, 10.11.

3,3′-(4,4′-Sulfonylbis(4,1-phenylene))bis(2-amino-5-imino-4-oxo-4,5-dihydro-3*H*-benzo[*f*]chromeno[3,4-*c*]pyridine-1-carbonitrile) (**20**): Yield 78%, mp 246.4°C. IR: ν_{max}/cm^{-1} 3448, 3348, 3217 (NH, NH_2), 2214 (2C≡N), 1716 (2C=O), 1334, 1145 (SO_2). ^{1}H-NMR (DMSO-d_6, D_2O) δ: 6.6 (s, 4H, $2NH_2$, exchangeable), 7.2–8.2 (m, 20H, Ar-H), 9.3 (s, 2H, 2NH, exchangeable). ^{13}C-NMR (DMSO-d_6): 64.4(2), 113.2(2), 116.3(2), 117.0(2), 119.6(2), 122.4(2), 123.1(4), 124.6(2), 126.6(2), 128.8(4), 128.9(2), 129.8(2), 130.4(2), 131.1(2), 135.6(2), 138.0(2), 152.6(2), 155.8(2), 156.8(2), 160.2(2), 166.4(2) *Anal.* Calcd for $C_{46}H_{26}N_8O_6S$ (818.81): C, 67.47; H, 3.20; N, 13.68. Found: C, 67.76; H, 3.35; N, 13.30.

Molecular Docking All the molecular modeling 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, 10.2008) software. All the minimizations were performed with MOE until a RMSD gradient of 0.05 kcal mol^{-1}Å^{-1} with MMFF94X forcefield and the partial charges were automatically calculated. The X-ray crystallographic structure of franesyltransferase and arginine methyltransferase (PRMT1) complexes with their ligands (PDB ID: 3E30, 3Q7E) were obtained from the protein data bank. The enzymes were prepared for docking studies where: (i) Ligand molecule was removed from the enzyme active site. (ii) Hydrogen atoms were added to the structure with their standard geometry. (iii) MOE Alpha Site Finder was used for the active sites search in the enzyme structure and dummy atoms were created from the obtained alpha spheres. (iv) The obtained model was then used in predicting the ligand enzymes interactions at the active site.

Biological Screening. *In Vitro* Antitumor Activity Human tumor breast cell line (MCF7) was used in this study. The cytotoxic activity was measured *in vitro* for the newly synthesized compounds using the Sulfo-Rhodamine-B stain (SRB) assay using the method of Skehan *et al.*[38] The *in vitro* anticancer screening was done by the pharmacology unit at the National Cancer Institute, Cairo University.

Cells were plated in 96-multiwell plate (104 cells/well) for 24 h before treatment with the compound(s) to allow attachment of cell to the wall of the plate. The tested compounds were dissolved in dimethylsulfoxide. Different concentrations of the compound under test (10, 25, 50, 100 μM) were added to the cell monolayer. Triplicate wells were prepared for each individual concentration. Monolayer cells were incubated with the compound(s) for 48 h at 37°C and in atmosphere of 5% CO_2. After 48 h, cells were fixed, washed and stained for 30 min with 0.4% (w/v) 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 ethylenediaminetetraacetic acid (EDTA) buffer. Color intensity was measured in an enzyme-linked immunosorbent assay (ELISA) reader. The relation between surviving fraction and drug concentration is plotted to get the survival curve for breast tumor cell line after the specified time. The molar concentration required for 50% inhibition of cell viability (IC_{50}) was calculated and compared to the reference drug doxorubicin (CAS, 25316-40-9). The surviving fractions were expressed as means±standard error and the results are given in Table 3.

Calculation of IC_{50} The surviving fractions of cells for each tested compound and the reference drug in concentrations (10, 25, 50, 100 μM) were the average of three tests. Surviving fraction of cells in control test (0 μM) was considered as 100% viable cells. Concentrations of the tested compounds and the reference drug were plotted against surviving fractions of the cells using Microsoft Excel (2007). A trend line was drawn for each curve and a corresponding equation was obtained. Each equation was solved considering the surviving fraction of cells as 50% and the concentration obtained in μM representing IC_{50}.

Statistical Analysis of Data Data obtained from animal experiments were expressed as mean±standard error (± S.E.M.). Statistical differences between the treatments and the control were tested by one-way analysis of variance (ANOVA) followed by *post hoc* test using SPSS 11.0 software. A value of $p<0.05$ was considered to be significant.

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