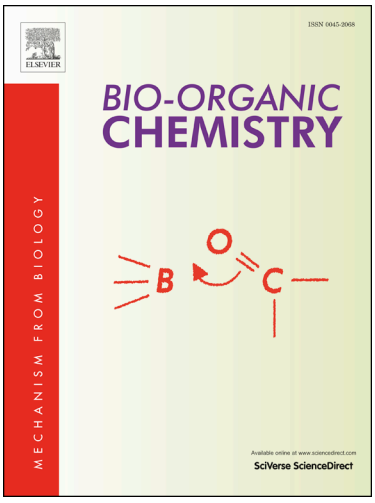

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Design, Synthesis and Molecular Modeling Study of certain 4-Methylbenzenesulfonamides with CDK2 inhibitory Activity as Anticancer and Radio-sensitizing agents

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Abstract

Two series of 2-aminopyridine derivatives **6-17** and tyrphostin **AG17** analogs **18-22** bearing 4-methylbenzenesulfonamide moiety were designed and synthesized as anticancer compounds. The synthesized compounds were biologically evaluated for their cytotoxic activity against human breast cancer cell line MCF-7. From 2-aminopyridine and tyrphostin **AG17** series, compounds **14**, **16** and **20** showed the best activities with IC_{50} values of 20.4, 18.3 and 26.3 µM, respectively compared to **E7070** IC_{50} 36.3 µM. Further biological evaluation of **14**, **16** and **20** against cyclin dependent kinase-2 (CDK2) revealed good inhibitory activity with IC_{50} of 2.53, 1.79 and 2.92 µM, respectively compared to roscovitine IC_{50} 0.43 µM. Additionally, capability of γ-radiation to augment the cytotoxic activity of **14**, **16** and **20** was studied and showed a dramatic increase in the cell killing effect at lower concentrations after irradiation. Docking was used to investigate the possible binding modes of compounds **14**, **16** and **20** inside the active site of CDK2 enzyme.

Keywords: 2-Aminopyridine, Tyrphostin **AG17** analogs, 4-methylbenzenesulfonamide, CDK2, cytotoxic, γ-radiation.

1. Introduction

In the last few decades, a great attention has been paid to cyclin-dependent kinases (CDKs). CDKs represent a class of serine/threonine kinases that play a pivotal role in cell cycle regulation [1, 2]. One of the main cell cycle deregulation causes is the abnormal CDK activity either due to CDKs overexpression or mutations of their encoded genes [3-5]. CDKs inhibition is still under intense investigation as one of the most important medicinal chemistry strategies in cancer management. Consequently, the design of a wide array of many CDKs ATP competitive inhibitors has been reported [5-9]. Remarkable growth inhibition of tumor cells transformed with several oncogenes has been obtained with selective CDK2 inhibitors [10]. Deans *et al* [11] has also demonstrated that CDK2 inhibitors constitute a potential target in the treatment of BRCA-deficient tumors. Recently, Galons *et al.* [12] and santo *et al.* [13] suggested CDK2 inhibitors as promising antitumor agents. On the other hand, it has been reported that the main reason for Trastuzumab resistance is the overexpression of cyclin E in HER2+ breast tumor patients, and treatment of such patients with CDK2 inhibitors may be a significant strategy to conquer this resistance [14]. Furthermore, Yang et al. [15] showed that CDK2 inhibitors are inspiring targets in ovarian carcinoma management.

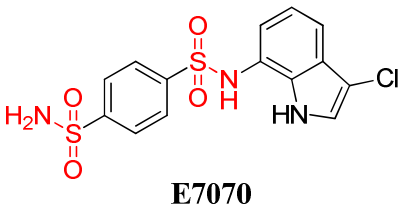
Sulfonamide derivatives have been greatly studied for various biological activities [16-18] including antitumor, antibacterial, antiparasitic and diuretic activity. Several mechanisms have been suggested to explain the antineoplastic activity of sulfonamides, such as cell cycle arrest in the G1 phase [19], binding to β-Tubulin, and disruption of microtubule assembly [20]. Furthermore, certain sulfonamides act as inhibitors for several enzymes as cyclin-dependent kinase (CDK) [21], carbonic anhydrase [22], methionine amino peptidases [23], matrix metalloproteinase [24] and nicotinamide adenine dinucleotide oxidase [25]. *N*-(3-Chloro-7-indolyl)-1,4-benzenesulfonamide **E7070** (**Indisulam**) (Fig. 1) is a disulfonamide anticancer agent against different cancer types which target the G1 phase of the cell cycle through CDK2 and cyclin E inhibition. Besides, **E7070** is indicated to be potent anti-breast cancer candidate against MCF-7 cell line by *in vitro* and *in vivo* findings [26 a-c]. Moreover, **SLC-0111**, is an urea derivative bearing one sulfonamide moiety (Fig. 1) currently in phase I clinical trials for the treatment of solid

tumors through the inhibition of carbonic anhydrase (CA, EC 4.2.2.1) isoform IX [27].

On the other hand, the antiproliferative activity of several aminopyridines has been examined against various cell lines and the observed cytotoxic activity of this class is mainly attributed to inhibition of cyclin dependent kinases (CDKs). The 2-aminopyridine moiety mimics the adenosine triphosphate (ATP) 6-aminopurine core and considered a good candidate for designing ATP-competitive kinase inhibitors. A series of aryl aminopyridine was evaluated for cytotoxic activity and the most active derivative was **sp24** (Fig. 1). The mechanism of action involves the disruption of signaling pathway and cell cycle through inhibition of CDK and several other tyrosine kinases. It is also indicated that the presence of a sulfonamide moiety with 2-aminopyridine contributed to selectivity and potency, for example, compound **I** (Fig. 1) inhibits CDK1 and CDK2 while not interacting with other kinases [28]. The corresponding *N*-[(4-hydroxyphenyl)amino]-3-pyridinyl]-4-methoxybenzenesulfonamide **E7010** (Fig. 1) is a sulfonamide derivative carrying 2-arylaminopyridine substituent, showed a cytotoxic activity against different cell lines by causing cell cycle arrest in the M phase due to inhibition of tubulin polymerization [29]. All the synthesized derivatives have a nitrile substituent attached whether to the aliphatic side chain or to the aminopyridine ring, as this group acts as a hydrogen bond acceptor and form nonspecific dipole interactions with protein side chain and thus promote penetration with sterically occluded protein kinases. Bosutinib (Fig. 1) is a known kinase inhibitor whose docking studies demonstrated a crucial hydrogen bond between threonine and nitrile nitrogen which is a common motif in these kinase inhibitors [30].

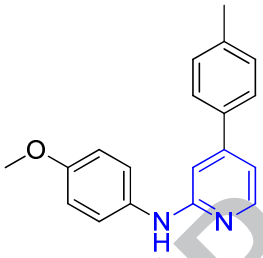
Tyrphostins are Low molecular weight compounds that act as general tyrosine kinases inhibitors. **AG17**, a benzylidene malononitrile derivative (Fig. 1) induces arrest at the G1 phase followed by apoptosis with a general reduction of the intracellular level of tyrosine phosphorylated proteins. Bcl-2 and cdk2 protein levels were not altered with **AG17**, whereas cdk2 kinase activity, as well as p21 and p16 protein levels, were markedly reduced. These findings suggest that the target of **AG17** is cdk2 inhibition [31]. The results offered by Burger *et al*. [32] reported that **AG17** is the most potent anti-breast cancer agent from tyrphostins series investigated. Recently, Alafeefy *et al.* [33] studied antitumor synergism from the coupling of certain quinazolines with tyrphostin **AG99** (Fig. 1) and discovered that ten of the synthesized candidates exhibited a significant antitumor activity against three carcinoma cell lines.

Based on these studies, two novel series of 2-aminopyridines **6-17** and tyrphostin **AG17** analogs **18-22** (Fig. 1) bearing 4-methylebenzenesulfonamide moiety were designed and synthesized. The *in vitro* cytotoxic activity of the synthesized compounds **4-22** was evaluated against breast carcinoma cell line MCF-7. In addition, the ability of γ-radiation to synergize the cytotoxic activity of the most active candidates from each series **14**, **16** and **20** was also studied. Furthermore, enzyme inhibition assay was carried out against CDK2 for the most active compounds **14**, **16** and **20**. Finally, molecular modeling was utilized to investigate the possible binding mode of the synthesized compounds in CDK2.



E7070

SLC-0111



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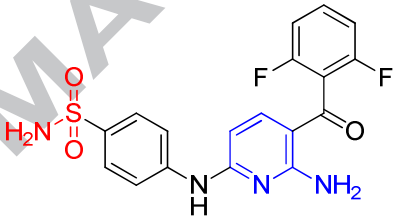
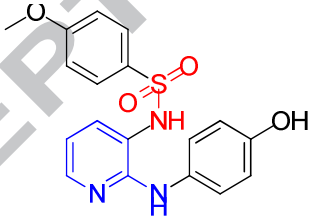
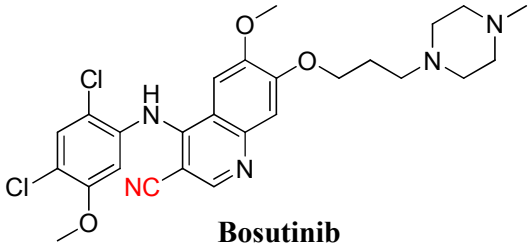


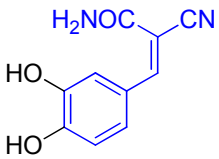
I



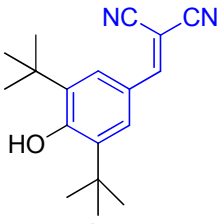
E7010



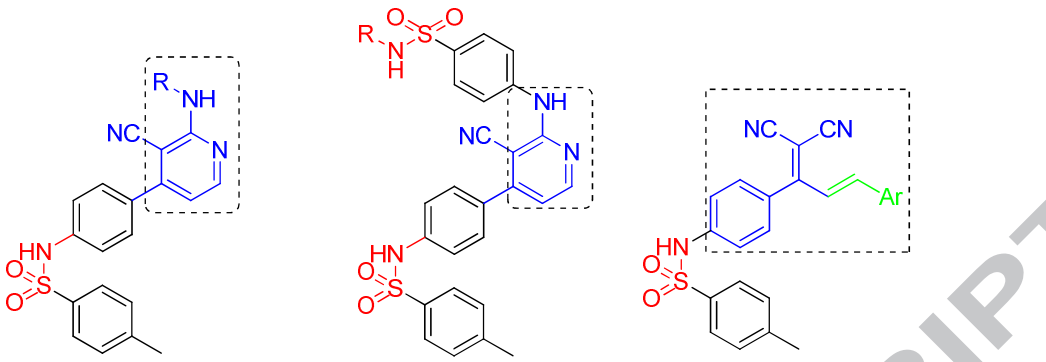
Bosutinib



AG99



AG17



2-Aminopyridines bearing one sulfonamide

2-Aminopyridines bearing two sulfonamides

Tyrphostin AG17 analogs targets

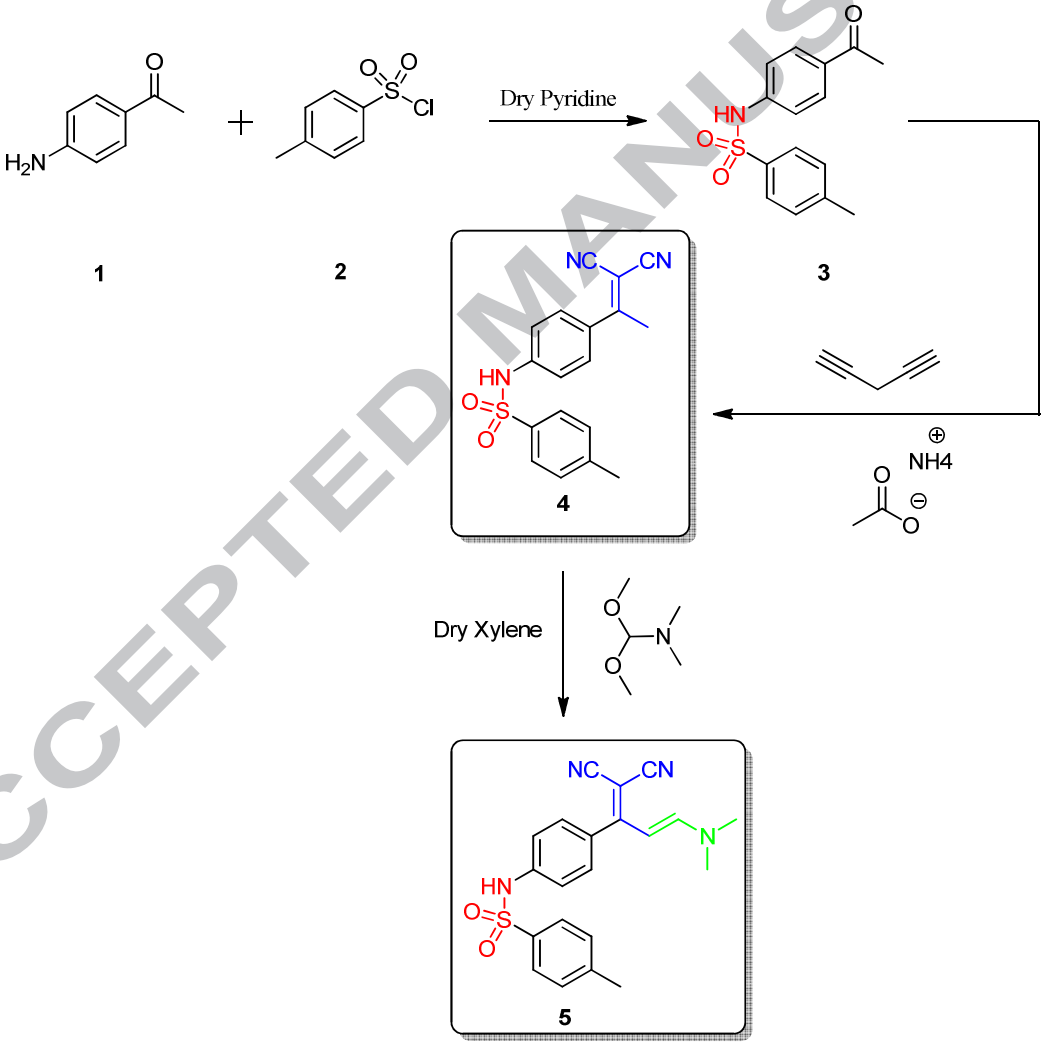
Fig. 1

2. Results and Discussion

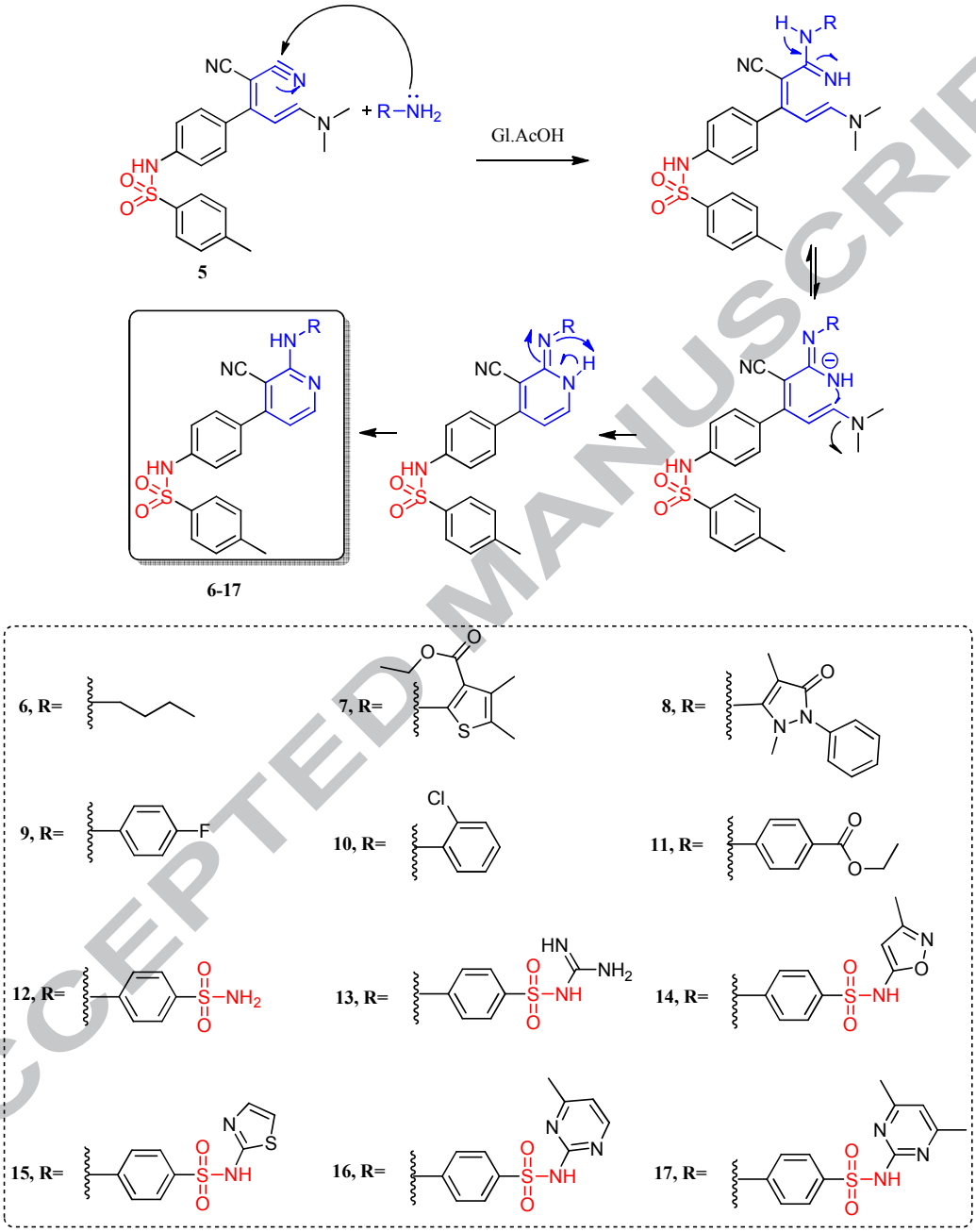
2.1. Chemistry

Schemes 1-3 show the synthetic routes adopted for the synthesis of target derivatives **4**-**22**. Ethylidenemalononitrile derivative **4,** was obtained via Knoevenagel condensation of the substituted acetophenone **3** [34] with malononitrile in presence of anhydrous ammonium acetate. IR spectrum of **4** revealed the absence of (C=O) band and the appearance of two strong bands at 2191 and 2160 cm^{-1} (2C≡N). ^{1}H-NMR displayed up-field two singlets at 2.32, 2.45 ppm corresponding to CH_3 (tolyl) and CH_3 (aliph.), respectively and a downfield singlet at 10.78 ppm (SO_2NH). ^{13}C-NMR exhibited a new signal at 86.3 ppm ($\underline{C}$=$(CN)_2$) and signal at 116.2 ppm (2C≡N). Reaction of **4** with dimethylformamide dimethylacetal (DMF-DMA) in dry xylene afforded the novel enaminonitrile derivative **5**. ^{1}H-NMR displayed the absence of a singlet at 2.45 ppm (CH_3) and presence of an up-field singlet at 3.32 ppm (N-$(CH_3)_2$) and two doublets at 7.05, 7.80 ppm (CH=CH). ^{13}C-NMR exhibited absence of a signal at 25.7 ppm (CH_3) and appearance of a new signal at 55.9 ppm (N-$(CH_3)_2$) (**Scheme 1**). Reaction of **5** with certain aliphatic and aromatic amines or 4-aminobenzenesulfonamides in glacial acetic acid yielded the required 2-aminopyridine derivatives **6-17**. ^{1}H-NMR spectra showed the disappearance of two doublets assigned to (CH=CH) and presence of another downfield singlets at the range of 10.60-12.25 ppm (2NH) and 2 doublets at 6.60-8.20 ppm (2CH pyridine) in addition to the characteristic peaks of each individual derivative (experimental section) (**Scheme 2**). Reaction of arylethylidenemalononitrile **4** with certain aromatic aldehydes in ethanol in presence of KOH afforded a new series of 1,1-dicyano-4-(substituted phenyl)buta-1,3-dien derivatives **18-22** (**Scheme**

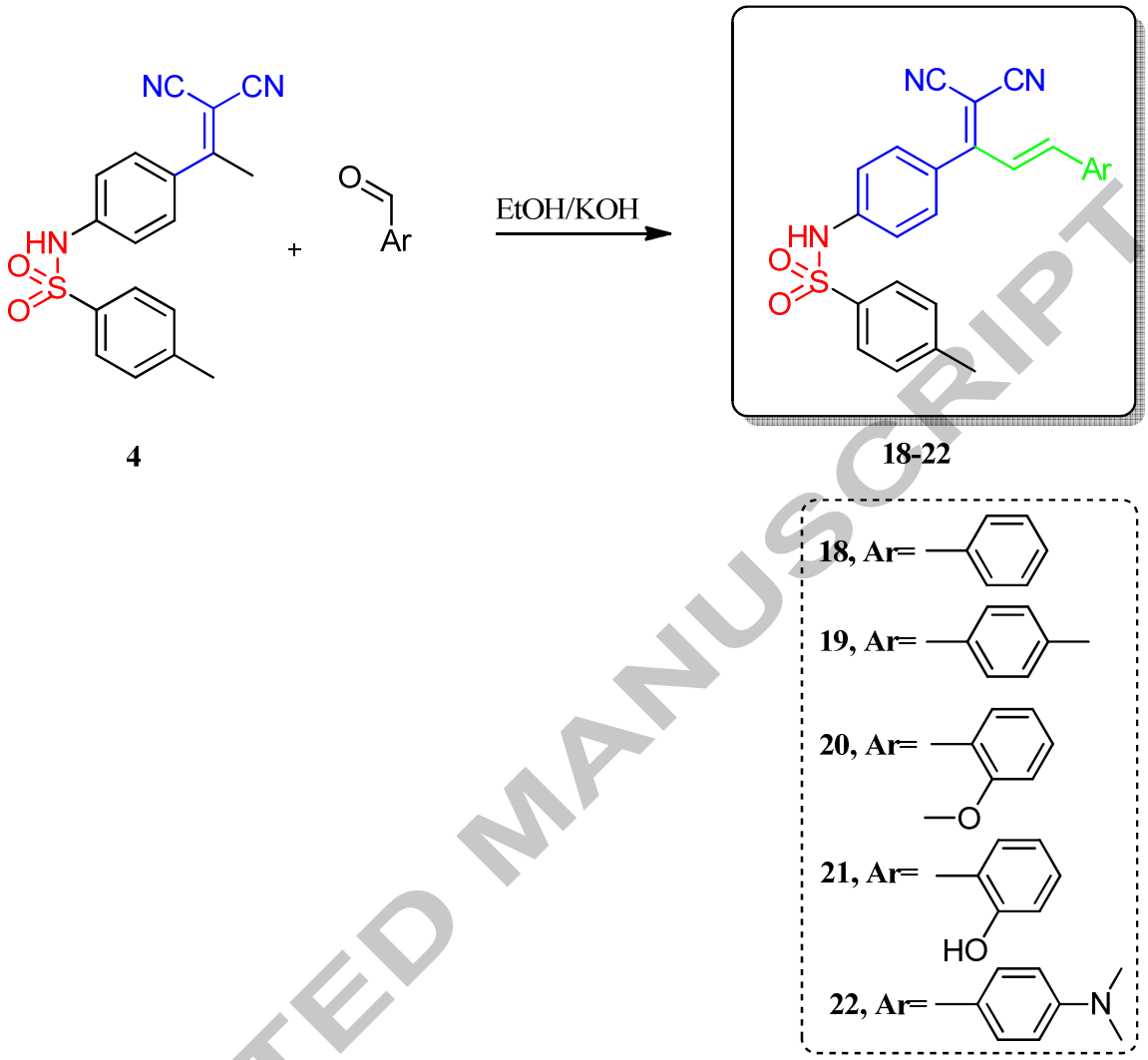
3). ^{1}H-NMR spectra showed the appearance of two doublets at a range of 6.54-7.93 ppm (CH=CH) and a characteristic signals for each individual compound. ^{13}C-NMR spectra showed two new down-field signals (CH=CH) (experimental section).



Scheme 1. Synthesis of compounds **4** and **5**



Scheme 2. Synthesis of 2-aminopyridine derivatives **6-17**.



Scheme 3. Synthesis of Tyrphostin analogs **18-22.**

2.2. Biological evaluation

*2.2.1. In-vitro anticancer evaluation against human tumor breast cancer (*MCF-7*)*

The results of the *in vitro* cytotoxic activity are shown in table 1. All the tested compounds have good cytotoxic activity with varies degrees. Thirteen compounds showed IC_{50} values lower than the reference compound, **E7070**. From the aminopyridine series **6-17**, compounds bearing one sulfonamide moiety **6-8**, **11** and all compounds with disulfonamide moieties **12-17** showed a remarkable cytotoxic activity with IC_{50} values between 18.3 and 35.7µM, compared to **E7070** (IC_{50}= 36.30 µM) [26c]. The substituents on the sulfonamide moiety attached to

the aminopyridine ring affected the cytotoxic activity, where the most active derivative was **16** with (4-methylpyrimidin-2-yl) group and IC_{50} value of 18.3 µM followed by **14** that has 5-methyl-2-isoxazolyl moiety with IC_{50} value of 20.4 µM then **17**, **13** and **15** derivatives bearing 4,6-dimethylpyrimidin-2-yl, diaminomethylene and thiazol-2-yl groups revealed a significant IC_{50} values of 21.7, 28.8 and 30.5 µM respectively compared to derivative **12** carrying free unsubstituted sulfonamide moiety attached to 2-aminopyridine ring, displayed the lowest activity in the disulfonamide series with IC_{50} 33.1 µM. The most active derivative with one sulfonamide moiety was **8** carrying pyrazolone ring which possessed IC_{50} value of 26.5 µM followed by the thiophene derivative **7** with IC_{50} value of 26.7 µM then butyl derivative **6** with IC_{50} value of 28.7 µM and ethylbenzoate derivative **11** with IC_{50} value of 34.3 µM. By contrast, the substituted phenyl derivatives **9, 10** revealed lower cytotoxic activities with IC_{50} values of 38.3 µM. Compound **12** showed comparable potency to **E7070**. Regarding to tyrphostin **AG17** analogs **18-22**, the most promising compound was the phenyl and 2-methoxy phenyl derivatives **18** and **20** with IC_{50} value of 35.7 and 26.3 µM. while the other derivatives revealed lower cytotoxic activity compared to **E7070** with IC_{50} values ranging from 37.5-39.8 µM.

Table 1: *In vitro* antitumor screening of the targeted compounds **4-22** against human breast carcinoma cell line (MCF-7).

Compound No.	IC_{50} (µM)[a]	Compound No.	IC_{50}
E7070	36.3 ± 3.60	**13**	28.8 ± 0.01
4	38.5 ± 0.03	**14**	20.4 ± 0.01
5	33.8 ± 0.01	**15**	30.5 ± 0.002
6	28.7 ± 0.01	**16**	18.3 ± 0.01
7	26.7 ± 0.02	**17**	21.7 ± 0.003
8	26.5 ± 0.02	**18**	35.7 ± 0.02
9	38.3 ± 0.02	**19**	37.5 ± 0.01
10	38.3 ± 0.02	**20**	26.3 ± 0.02
11	34.3 ± 0.02	**21**	39.8 ± 0.02
12	33.1 ± 0.01	**22**	38.8 ± 0.03

[a] Each value is the mean of three experiments ± standard error.

2.2.2. In vitro Cyclin dependent kinase 2 (CDK2) inhibitory assay

In order to investigate the CDK2 inhibitory activity of the synthesized compounds, the IC_{50} values of the most potent compounds from each series **14**, **16** and **20** were determined. From the results illustrated in Table 2, Compound **16** was the most potent with IC_{50} value of 1.79 µM, followed by compound **14** and **20** with IC_{50} value of 2.53 and 2.92 µM respectively, compared to Roscovitine which exhibited IC_{50} value of 0.43 µM. The CDK2 inhibitory activity matched the cytotoxic activities of compound **14** and **16** giving a good indication that the cytotoxic activity is due to inhibition of this enzyme.

Table 2: IC_{50} values for the most active compounds **14**, **16** and **20** against CDK2 enzyme.

Compound no.	CDK2 IC_{50} (µM)[a]
14	2.53±0.01
16	1.79±0.01
20	2.92±0.01
Roscovitine	0.43±0.01

[a] Each value is the mean of three experiments ± standard error.

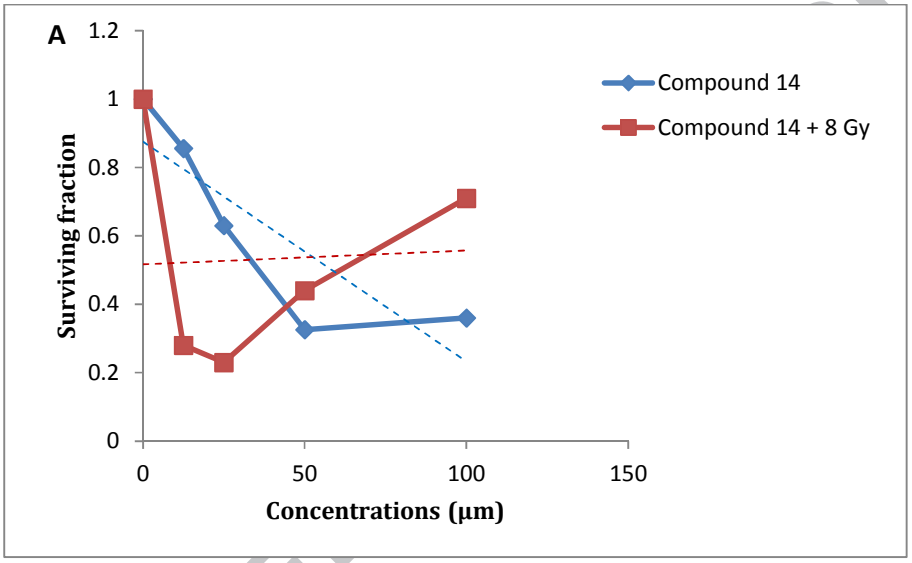
2.2.3. Radio-sensitizing evaluation

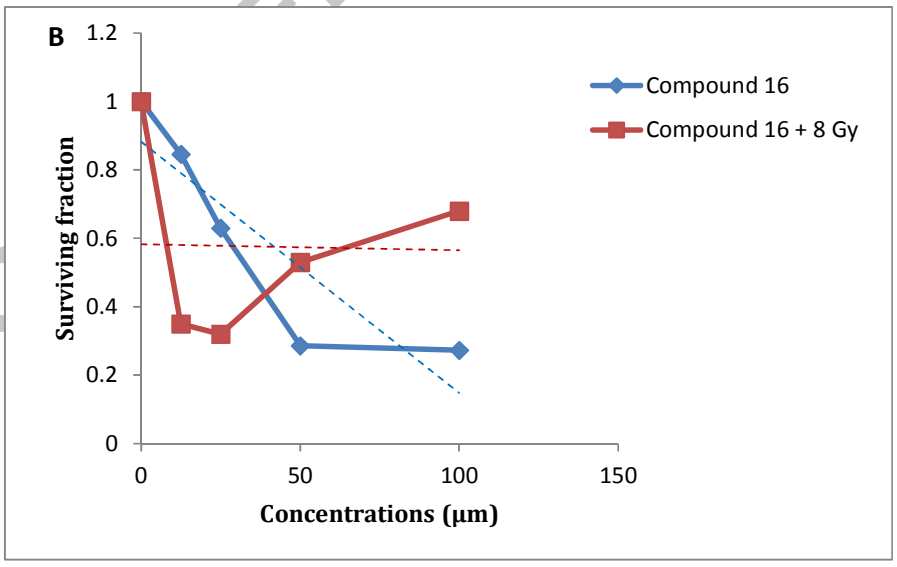
The capability of γ-irradiation to augment the cytotoxic activity of the tested compounds was also evaluated. The most active derivatives among the two series **14, 16** and **20** with IC_{50} 20.4, 18.3 and 26.3 µM respectively, were further evaluated against MCF-7 after exposure of the cells to a single dose of ɤ-irradiation. A dramatic cytotoxic activity enhancement was observed in potency for the tested compounds after irradiation. The IC_{50} values decreased to 11.8, 9.1 and 12.2 µM, respectively as shown in table 3. Thus γ-irradiation sensitize tumor cells to the cytotoxic effect of the tested compounds which allows decreasing the doses of the drug to avoid toxic effects. (**Fig**. **2**. **A**, **B** and **C**)

Table 3: *In vitro* antitumor screening of compounds **14** and **16** against human breast carcinoma cell line (MCF-7) in combination with γ-radiation (8Gy).

Compound No.	IC_{50} (μM)[a]
14	11.8 ± 3.6
16	9.10 ± 0.03
20	12.20 ± 0.04

[a] Each value is the mean of three experiments ± standard error.





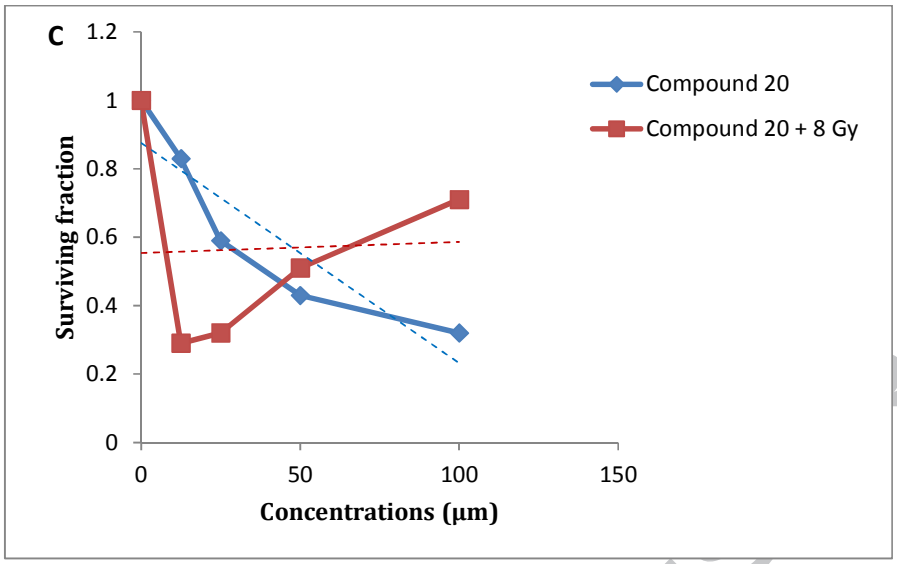


Fig. 2. Survival curve of MCF-7 for: (**A**) compound **14**, (**B**) compound **16** and (**C**) compound **20** alone or in combination with γ-irradiation (8 Gy).

2.3. Molecular modeling and docking study

The hypothetical binding modes of the most active compounds **14, 16** and **20** were calculated using GOLD docking in the active site of the CDK2-CA complex. Based on the structural similarity between the tested compounds and type II kinase inhibitors, a crystal structure of the inactive conformation of CDK2 (PDB: 2WXV) was chosen. This crystal structure was also selected because of the similar structural motifs in the tested compounds and the structure of the co-crystallized ligand, Pyrazolo[4,3-h]quinazoline-3-carboxamide (**Lead**). First, the docking algorithm was validated for its ability to reproduce the co-crystal binding mode of the reference compound. The reference compound was extracted from the complex and redocked in the active site using the same parameters that will be used for the tested compounds. As expected the GOLD algorithm could regenerate the co-crystal structure with RMSD of 0.5 Å giving high confidence in the calculated binding mode of compounds (**Fig. 3**).

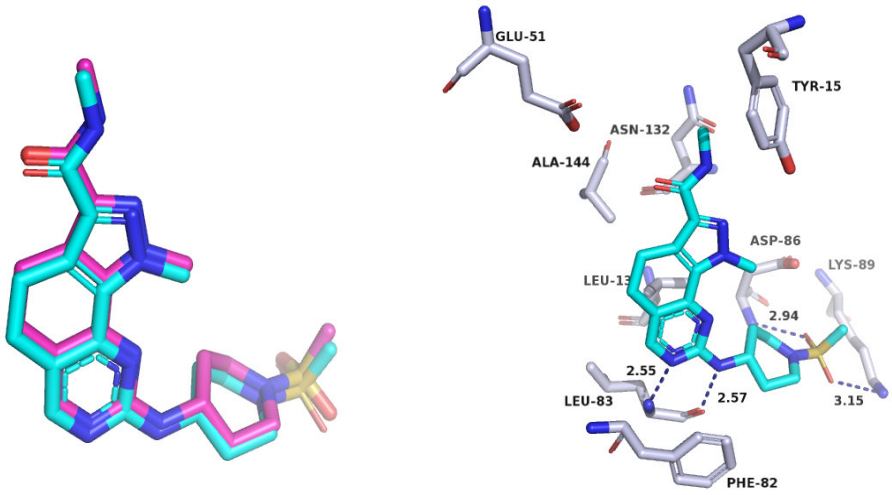


Fig. 3. RMSD, docking validation and binding mode of the **lead** compound (cyan) in CDK2 pocket.

The calculated binding modes of **14, 16** revealed very interesting results As shown in **Fig. 4-6**, the aminopyridine moiety in compound **16** showed strong binding to the hinge residue Leu83 with two hydrogen bonding. The nitrile group forced the sulfonamide side chain to the non-co-planar position giving the perfect orientation for hydrogen bonding of the sulfonamide group to two important residues Asp86 and Lys89. This pattern of binding was repeated in compound **14** because it has a similar structure. The side chains were directed to the solvent-exposed area which has some polar residues and solvent molecules. In compound **16**, the pyrimidine ring was directed to a solvent exposed area of the enzyme which was expected in the original design. **Fig. 5** and **6** demonstrated the binding mode of compound **16** and its superimposition with the **Lead** compound. The pyrimidine ring is very important as a solvent exposed group as the hydrophilic nature of this group will give the required stabilization of the correct binding mode of this compound. In compounds **14** the methyl isoxazole ring also was directed to the same area and showed very good binding interaction **Fig. 4**. Also, the most active molecule **20** in tyrphostin analogs **18-22,** was docked into CDK2 active site **Fig. 7**. Compound **20**, the nitrile group replaced the pyridine nitrogen as the hinge binder and showed good binding to the hinge residue Leu83 with one hydrogen bonding, while, the tolyl group was involved in the interaction with the tyrosyl residue. On the other hand, the methoxyphenyl group was directed to the solvent exposed area and the

polar amino acids in the binding site. The docking scores of **14**, **16** and **20** are listed in **table 4**.

Table 4: Docking scores of promising compounds **14**, **16** and **20** inside CDK2 active site.

Compound	GOLD Chemscore (Kcal/mol)	Amino acids	Interacting groups	Length (Å)
Lead	-43	Leu 83	N of quinazoline	2.55
		Leu 83	NH linked to quinazoline	2.57
		Lys 89	SO_2 of sulfonyl	3.15
		Asp 86	SO_2 of sulfonyl	2.94
14	-51	Leu 83	N of pyridine	2.83
		Leu 83	NH linked to pyridine	2.53
		Asp 86	SO_2 of sulfonamide	2.25
16	-52	Leu 83	N of pyridine	3.02
		Leu 83	NH linked to pyridine	2.61
		Lys 89	SO_2 of sulfonamide	3.09
		Asp 86	SO_2 of sulfonamide	2.89
		Tyr 15	SO_2 of tolyl sulfonamide	3.04
20	-45	Leu 83	CN of dinitrile	2.83

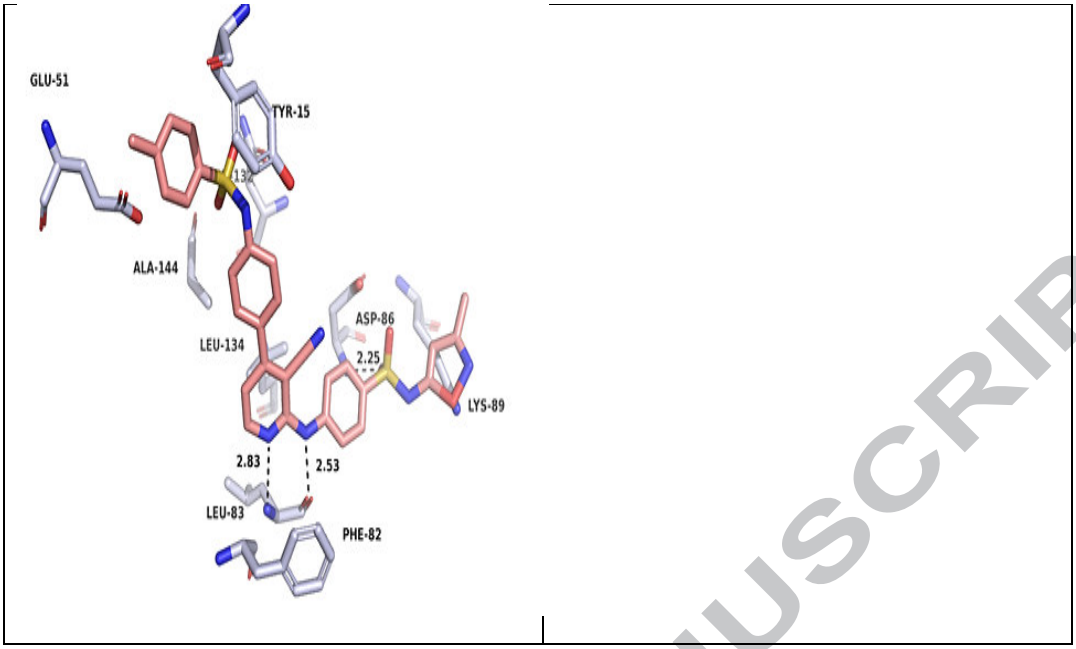
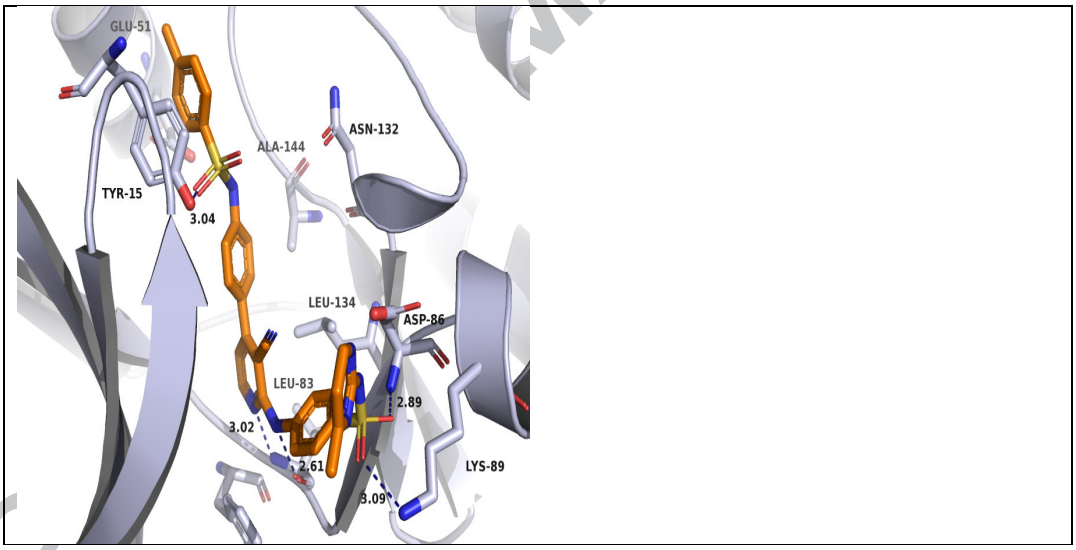


Fig. 4. Binding mode (stick view) of compound **14** (pink) in CDK2 pocket.



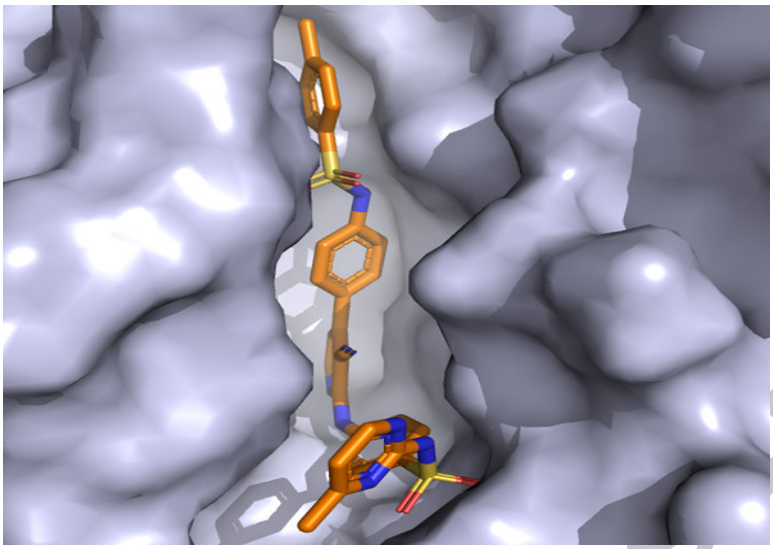


Fig. 5. Binding mode of compound **16** (orange) in CDK2 pocket and surface view displaying the hydrophobic interaction between the tyrosine residue and the p-tolyl group.

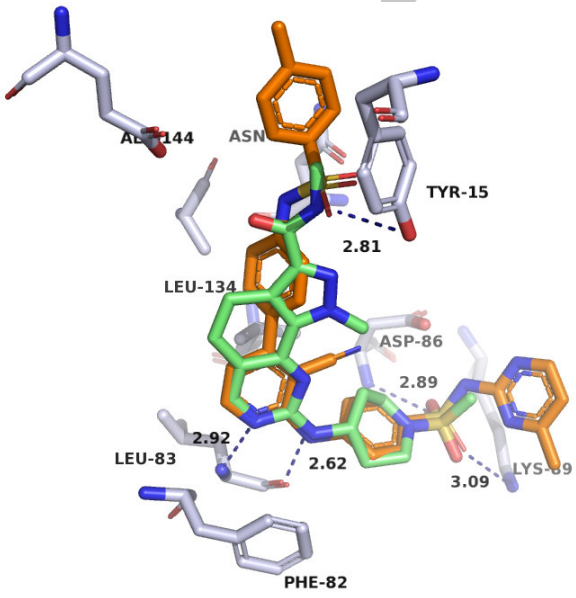


Fig. 6. Overlay of compound **16** (orange) with **lead** compound (green) in CDK2 pocket.

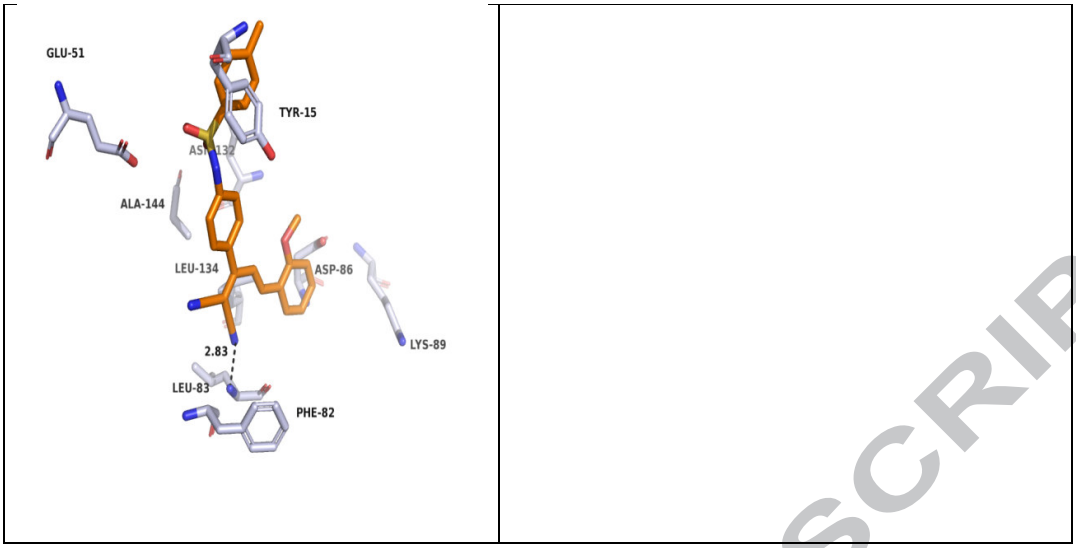


Fig. 7. Binding mode (stick view) of compound **20** in CDK2 pocket.

3. Conclusion

In Conclusion, two series of 2-aminopyridines **6-17** and tyrphostin **AG17** analogs **18-22** bearing 4-methylbenzenesulfonamide moiety have been designed and synthesized as prospective cytotoxic agents and CDK2 inhibitors. The candidates were tested for their cytotoxic activity against human breast carcinoma cell line (MCF-7). Compounds **6-8**, **11-18** and **20** were found to be more potent than **E7070**. From SAR, we may deduce that the presence of 4-methylbenzenesulfonamide scaffold bearing another sulfonamide moiety or tyrphostin **AG17** analog skeleton incorporating 2-methoxyphenyl moiety is associated with promising cytotoxic activity and gave the most active compounds **14, 16** and **20** (IC_{50}= 20.4, 18.3 and 26.3 µM), respectively. On the other hand, the enzyme inhibition assay of compounds **14**, **16** and **20** against CDK2 enzyme demonstrated good inhibitory activity with IC_{50} values of 2.53, 1.79 and 2.92 µM, respectively compared to Roscovitine (IC_{50} = 0.43 µM). Molecular modeling study showed that compound **16** has considerable docking score value (S= -52 Kcal mol^{-1}) in the CDK2 active site compared with the lead compound (S= -43 Kcal mol^{-1}). Finally, γ-irradiation sensitize tumor cells to the cytotoxic effect of the most active compounds **14**, **16** and **20** which allows to decrease the doses of drugs to avoid toxic effects.

4. Experimental

4.1. Chemistry

Within an open capillary tube on a melting point Stuart equipment (Stuart Scientific, Redhill, UK) melting points were obtained and were un rectified. Molecules IR spectra were listed on FT-IR Shimadzu spectrometer (Shimadzu, Tokyo, Japan). Mass spectra analyses were completed on MS-5988 HP Model (Hewlett Packard, Palo Alto, California, USA). ^{1}H-NMR and ^{13}C-NMR spectra were carried out using Bruker NMR spectrometers running at 300 and 400 MHz, respectively. Microanalyses were achieved on a Carlo Erba 1108 Elemental Analyzer (Heraeus, Hanau, Germany), and all readings were within ±0.4 % of the hypothetical values. Substances purity was confirmed through thin layer chromatography (TLC) with precoated Aluminum plates SiO_2 gel (HF_{254}, 200 mesh) and were visualized via UV lamp (Merck, Damstadt, Germany). Solvent system consumed was chloroform/ethylacetate (8:2) and the spots were shown in UV chamber. IR, ^{1}H-NMR, ^{13}C-NMR and elemental analyses were matched with the determined structures. IR was accomplished at NCRRT-EAEA (National Center for Radiation Research and Technology, Egyptian Atomic Energy Authority). While ^{1}H-NMR and ^{13}C-NMR was carried out at both of department of Chemical Warfare, Ministry of Defense and NCRRT, EAEA. Elemental analyses were done at the Laboratories Microanalytical chemistry of the Faculty of Science, Cairo University. P-toluenesulfonyl chloride and 4- aminoacetophenon were bought from Sigma-Aldrich.

*4.1.1.N-(4-(1,1-dicyanoprop-1-en-2-yl)phenyl)-4-methylbenzenesulfonamide (**4**).*

A mixture of *N*-(4-acetylphenyl)-4-methylbenzenesulfonamide **3** (3 g, 0.01 mol), malononitrile (0.66 g, 0.015 mole) and ammonium acetate (0.77 g, 0.01 mole) was fused together in an oil bath at 100 °C for 4 hrs. The resinous matter formed was dissolved in hot ethanol (15 ml) with continuous stirring. The mixture was left overnight in the refrigerator, and then poured onto ice water, the yellow solid obtained was dried and crystallized from dioxan to give **4**. Yield, 88 %; m.p. 120-122 °C. IR (KBr, cm^{-1}): 3246 (NH), 3043 (CH arom.), 2926, 2871 (CH aliph.), 2191, 2160 (2C≡N), 1310, 1121 (SO_2). ^{1}H-NMR (DMSO-d_6): 2.32 [s, 3H, CH_3 tolyl], 2.45 [s, 3H, CH_3], 7.21, 7.63, 7.72, 7.83 [4d, 8H, Ar-H AB system,

J= 8.7 Hz, *J*= 7.8 Hz], 10.78 [s, 1H, SO_2NH]. ^{13}C-NMR (DMSO-d_6): 22.1, 24.7, 86.3, 116.2 (2), 119.3 (2), 124.4, 128.1 (2), 129.1 (2), 129.8 (2), 135.2, 136.3, 137.1, 174.1. MS, *m/z* (%): 337 [M^+] (3.35), 274 (100). Anal. Calcd. For $C_{18}H_{15}N_3O_2S$ (337.40): C, 64.08; H, 4.48; N, 12.45. Found: C, 63.90; H, 4.68; N, 12.20.

*4.1.2.(E)-N-(4-(1,1-dicyano-4-(dimethylamino)buta-1,3-dien-2-yl)phenyl)-4-methylbenzenesulfonamide (**5**)*

A mixture of *N*-(4-(1,1-dicyanoprop-1-en-2-yl)phenyl)-4-methylbenzenesulfonamide **4** (3.3 g, 0.01 mol) and DMF/DMA (1.8 g, 0.015 mole) was refluxed for 24 hrs in dry xylene (20 ml). The mixture was concentrated and left in the refrigerator for 24 hrs. The obtained solid was crystallized from ethanol to give **5**. Yield, 45%; m.p. 90-92 °C. IR (KBr, cm^{-1}): 3267 (NH), 3031 (CH arom.), 2926, 2868 (CH aliph.), 2192 (2C≡N), 1319, 1115 (SO_2). ^{1}H-NMR (DMSO-d_6)δ: 2.32 [s, 3H, CH_3 tolyl], 3.32 [s, 6H, $N(CH_3)_2$], 7.05, 7.80 [2d, 2H, CH=CH], 7.17- 7.70 [m, 8H, Ar-H], 10.20 [s, 1H, SO_2NH]. ^{13}C-NMR (DMSO-d_6): 20.7, 55.9 (2), 67.3, 103.2, 113.2 (2), 119.4 (2), 122.0, 122.6 (2), 124.2, 126.2 (2), 127.2, 128.3, 134.8, 138.4, 155.5, 170.2. MS, *m/z* (%): 390 [M-2] (1.15), 91 (100). Anal. Calcd. For $C_{21}H_{20}N_4O_2S$ (392.47): C, 64.27; H, 5.14; N, 14.28. Found: C, 64.47; H, 5.54; N, 13.97.

*4.1.3.N-(4-(3-cyano-2-((4-(N-(substituted)sulfamoyl)phenyl)amino)pyridin-4-yl)phenyl)-4-methylbenzenesulfonamides(**6-17**)*

A mixture of **5** (0.5 g, 0.0013 mol) and substituted amines (0.0013 mole) namely, butan-1-amine, ethyl 2-amino-4,5-dimethylthiophene-3-carboxylate, 4-amino-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one, 4-fluoroaniline, 2-chloroaniline, ethyl-4-aminobenzoate, sulfanilamide, sulfaguanidine, sulfathiazole, sulfamethoxazole, sulfamerazine, sulfamethazine, was refluxed for 20 hrs in glacial acetic acid (10 ml). The mixture was cooled then poured onto ice water. The solid obtained was crystallized from ethanol to give **6-17**, respectively.

*4.1.4.N-(4-(2-(butylamino)-3-cyanopyridin-4-yl)phenyl)-4-methylbenzenesulfonamide (**6**)*

Yield, 53.11%; m.p. 126-128 °C. IR (KBr, cm^{-1}): 3248, 3199 (2NH), 3043 (CH arom.), 2922, 2872 (CH aliph.), 2189 (C≡N), 1331, 1149 (SO_2). ^{1}H-NMR (DMSO-d_6)δ: 0.88 [t, 3H, CH_3], 1.34 [m, 2H, $\underline{CH_2}CH_3$], 1.56 [m, 2H, $\underline{CH_2}CH_2CH_3$], 2.32 [s, 3H, CH_3 tolyl], 3.03 [t, 2H, NH-$\underline{CH_2}$], 7.00-7.65 [m, 8H, Ar-H], 7.72, 7.83 [2d, 2H, 2CH pyridine, J = 7.2 Hz], 10.20, 10.78 [2s, 2H, SO_2NH, NH]. ^{13}C-NMR (DMSO-d_6): 15.2, 20.9, 22.0, 26.1, 40.3, 84.2, 113.2, 115.8, 119.5 (2), 122.6 (2), 124.1 (2), 126.2, 127.2 (2), 128.2, 134.8, 138.2, 152.2, 155.5, 165.1. Anal. Calcd. For $C_{23}H_{24}N_4O_2S$ (420.53): C, 65.69; H, 5.75; N, 13.32. Found: C, 65.41; H, 5.52; N, 13.56.

*4.1.5.Ethyl 2-((3-cyano-4-(4-(4-methylphenylsulfonamido)phenyl)pyridin-2-yl)amino)-4,5-dimethylthiophene-3-carboxylate (**7**)*

Yield, 54.9%; m.p. 108-110 °C. IR (KBr, cm^{-1}): 3359, 3295 (2NH), 3044 (CH arom.), 2974, 2870 (CH aliph.), 2208 (C≡N), 1709.01 (C=O), 1360, 1156 (SO_2). ^{1}H-NMR (DMSO-d_6)δ: 1.23 [t, 3H, $CH_2\underline{CH_3}$], 2.06 [s, 3H, CH_3 tolyl], 2.32, 2.45 [2s, 6H, $2CH_3$ thiophene], 4.16 [q, 2H, $\underline{CH_2}CH_3$], 6.91-7.54 [m, 8H, Ar-H], 7.74, 7.86 [2d, 2H, 2CH pyridine, J = 8.7 Hz], 10.34, 10.79 [2s, 2H, SO_2NH, NH]. ^{13}C-NMR (DMSO-d_6): 9.5, 10.5, 21.1, 22.0, 67.0, 84.2, 113.2, 115.8, 118, 119.4 (2), 122.6 (2), 124.1 (2), 126.2, 127.2 (2), 128.2, 134.6, 138.4, 140.1, 145.1, 150.0, 152.2, 155.6, 165.1, 168.1. Anal. Calcd. For $C_{28}H_{26}N_4O_4S_2$ (546.66): C, 61.52; H, 4.79; N, 10.25. Found: C, 61.30; H, 4.51; N, 10.56.

*4.1.6.N-(4-(3-cyano-2-((1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)amino)pyridin-4-yl)phenyl)-4-methylbenzenesulfonamide (**8**)*

Yield, 54.3%; m.p. 128-130°C. IR (KBr, cm^{-1}): 3316, 3198 (2NH), 3047 (CH arom.), 2918, 2870 (CH aliph.), 2201 (C≡N), 1663 (C=O), 1334, 1157 (SO_2). ^{1}H-NMR (DMSO-d_6)δ: 2.32 [s, 3H, CH_3 pyrazol], 2.36 [s, 3H, CH_3 tolyl], 2.55 [s, 3H, N-CH_3], 7.05-7.66 [m, 13H, Ar-H], 7.83, 7.89 [2d, 2H, 2CH pyridine, J = 8.1 Hz], 10.13, 10.80 [2s, 2H, SO_2NH, NH]. ^{13}C-NMR (DMSO-d_6): 12.4, 22.4, 34.2, 84.2, 113.4, 115.8, 116.4, 119.5 (2), 122.6 (2), 124.1 (2), 126.2, 127.2 (2), 128.2, 130.1 (2), 131.0 (2) 132.0, 134.8, 136.0, 137.0, 138.2, 152.2, 155.6, 165.2, 170.2. Anal. Calcd. For $C_{30}H_{26}N_6O_3S$ (550.63): C, 65.44; H, 4.76; N, 15.26. Found: C, 65.66; H, 4.54; N, 15.57.

*4.1.7.N-(4-(3-cyano-2-((4-fluorophenyl)amino)pyridin-4-yl)phenyl)-4-methylbenzenesulfonamide (**9**)*

Yield, 50 %; m.p. 126-128 °C. IR (KBr, cm^{-1}): 3328, 3260 (2NH), 3043 (CH arom.), 2927, 2870 (CH aliph.), 2191 (C≡N), 1352, 1186 (SO_2). ^{1}H-NMR (DMSO-d_6)δ: 2.37 [s, 3H, CH_3 tolyl], 7.08-7.92 [m, 12H, Ar-H], 7.83, 7.92 [2d, 2H, 2CH pyridine, *J* = 8.7 Hz], 10.32, 10.80 [2s, 2H, SO_2NH, NH]. ^{13}C-NMR (DMSO-d_6): 20.9, 84.2, 113.2, 115.8, 116.2 (2), 119.5 (2), 120.2 (2), 122.6 (2), 124.1 (2), 126.2, 127.2 (2), 128.2, 134.8, 136.0, 138.2, 150.0, 152.2, 155.6, 165.2. Anal. Calcd. For $C_{25}H_{19}FN_4O_2S$ (458.51): C, 65.49; H, 4.18; N, 12.22. Found: C, 65.79; H, 4.30; N, 12.56.

*4.1.8.N-(4-(2-((2-chlorophenyl)amino)-3-cyanopyridin-4-yl)phenyl)-4-methylbenzenesulfonamide (**10**)*

Yield, 40 %; m.p. 136-138 °C. IR (KBr, cm^{-1}): 3328, 3248 (2NH), 3046 (CH arom.), 2955, 2925 (CH aliph.), 2190 (C≡N), 1391, 1129 (SO_2), 652 (C-Cl). ^{1}H-NMR (DMSO-d_6)δ: 2.32 [s, 3H, CH_3], 6.78-7.42 [m, 12H, Ar-H,], 7.80, 7.89 [2d, 2H, 2CH pyridine, *J* = 8.7 Hz], 10.49, 10.79 [2s, 2H, SO_2NH, NH]. ^{13}C-NMR (DMSO-d_6): 20.9, 88.2, 113.2, 115.8, 116.2 (2), 119.5 (2), 120.2 (2), 122.6 (2), 124.1 (2), 126.2, 127.2 (2), 128.2, 134.8, 136.1, 138.2, 152.2, 155.6, 157.5 165.2, Anal. Calcd. For $C_{25}H_{19}ClN_4O_2S$ (474.96): C, 63.22; H, 4.03; N, 11.80. Found: C, 63.35; H, 4.28; N, 11.53.

*4.1.9.Ethyl-4-((3-cyano-4-(4-(4-methylphenylsulfonamido)phenyl)pyridin-2-yl)amino)benzoate (**11**)*

Yield, 56.6 %; m.p. 144-146 °C. IR (KBr, cm^{-1}): 3315, 3246 (2NH), 3049 (CH arom.), 2973, 2942 (CH aliph.), 2204 (C≡N), 1731 (C=O), 1363, 1142 (SO_2). ^{1}H-NMR (DMSO-d_6)δ: 1.65 [t, 3H, $CH_2\underline{CH_3}$], 2.33 [s, 3H, CH_3 tolyl], 4.16 [q, 2H, $\underline{CH_2}CH_3$], 7.03-7.73 [m, 12H, Ar-H,], 7.80, 7.89 [2d, 2H, 2CH pyridine, *J* = 8.1 Hz], 10.54, 10.93 [2s, 2H, SO_2NH, NH]. ^{13}C-NMR (DMSO-d_6): 14.4, 20.9, 60.8, 84.2, 113.2, 115.8, 116.2 (2), 119.5 (2), 120.2 (2), 122.6 (2), 124.1 (2), 126.2, 127.2 (2), 128.2, 134.8, 136.3, 138.2, 150.4, 152.2, 155.6, 165.2, 172.2. Anal. Calcd. For $C_{28}H_{24}N_4O_4S$ (512.58): C, 65.61; H, 4.72; N, 10.93. Found: C, 65.36; H, 4.99; N, 11.23.

*4.1.10.N-(4-(3-cyano-2-((4-sulfamoylphenyl)amino)pyridin-4-yl)phenyl)-4-methylbenzenesulfonamide (**12**)*

Yield, 48 %; m.p. 138-140 °C. IR (KBr, cm^{-1}): 3480, 3356, 3249, (NH_2, NH), 3039 (CH arom.), 2922, 2855 (CH aliph.), 2193 (C≡N), 1333, 1181 ($2SO_2$). ^{1}H-NMR (DMSO-d_6)δ: 2.43 [s, 3H, CH_3], 6.60, 7.80 [2d, 2H, 2CH pyridine, *J*= 8.1 Hz], 7.22-7.82 [m, 12H, Ar-H], 8.10 [s, 2H, SO_2NH_2] 10.20, 10.70 [2s, 2H, SO_2NH, NH]. ^{13}C-NMR (DMSO-d_6):

20.9, 84.2, 113.2, 115.8, 116.2 (2), 119.5 (2), 120.2 (2), 122.6 (2), 124.1 (2), 126.2, 127.2 (2), 128.2, 134.8, 136.4, 138.2, 150.3, 152.2, 155.6, 165.2. Anal. Calcd. For $C_{25}H_{21}N_5O_4S_2$ (519.60): C, 57.79; H, 4.07; N, 13.48. Found: C, 57.99; H, 4.37; N, 13.18.

*4.1.11.N-(4-(3-cyano-2-((4-(N-(diaminomethylene)sulfamoyl)phenyl)amino)pyridin-4-yl)phenyl)-4-methylbenzenesulfonamide (**13**)*

Yield, 38.4 %; m.p. 148-50 °C. IR (KBr, cm^{-1}): 3345, 3319, 3278 (NH_2, NH), 3044 (CH arom.), 2920, 2894 (CH aliph.), 2203 (C≡N), 1331, 1158 ($2SO_2$). ^{1}H-NMR (DMSO-d_6)δ: 2.31 [s, 3H, CH_3], 2.62 [s, 1H, NH guanido], 6.05 [s, 2H, NH_2], 6.85, 8.21 [2d, 2H, 2CH pyridine, *J*= 8.4 Hz], 7.20-7.90 [m, 12H, Ar-H], 10.21, 10.62, 10.93 [3s, 3H, $2SO_2NH$, NH]. ^{13}C-NMR (DMSO-d_6): 21.4, 84.2, 113.2, 115.8, 116.2 (2), 119.5 (2), 120.2 (2), 122.6 (2), 124.1 (2), 126.2, 127.2 (2), 128.2, 134.8, 136.2, 138.2, 150.3, 152.2, 155.6, 158.2, 160.4. Anal. Calcd. For $C_{26}H_{23}N_7O_4S_2$ (561.64): C, 59.03; H, 4.10; N, 11.87; Found: C, 59.28; H, 4.43; N, 12.05.

*4.1.12.N-(4-(3-cyano-2-((4-(N-(5-methylisoxazol-3-yl)sulfamoyl)phenyl)amino)pyridin-4-yl)phenyl)-4-methylbenzenesulfonamide (**14**)*

Yield, 44.9 %; m.p. 135-137 °C. IR (KBr, cm^{-1}): 3319, 3217 (3NH), 3016 (CH arom.), 2972, 2873 (CH aliph.), 2202 (C≡N), 1324, 1116 ($2SO_2$). ^{1}H-NMR (DMSO-d_6)δ: 2.28 [s, 3H, CH_3 tolyl], 2.32 [s, 3H, CH_3 isoxazol], 6.07 [s, H, CH isoxazol], 6.56-7.47 [m, 12H, Ar-H], 7.72, 7.83 [2d, 2H, CH pyridine, *J*= 8.4 Hz], 10.89 [s, 3H, $2SO_2NH$+NH]. ^{13}C-NMR (DMSO-d_6): 37.8, 45.1, 88.1, 95.2, 112.5, 113.1, 115.8, 117.9 (2), 124.2 (2), 126.6 (2), 128.7, 129.6 (2), 129.8, 132 (2), 136.5, 137.1, 138.9, 142.2, 148.5, 151.9, 152.8, 153.2, 157.2, 169.8. Anal. Calcd. For $C_{29}H_{24}N_6O_5S_2$ (600.12): C, 57.99; H, 4.03; N, 13.99. Found: C, 58.19; H, 4.20; N, 14.30.

*4.1.13.N-(4-(3-cyano-2-((4-(N-(thiazol-2-yl)sulfamoyl)phenyl)amino)pyridin-4-yl)phenyl)-4-methylbenzenesulfonamide (**15**)*

Yield, 31 %; m.p. 122-124 °C. IR (KBr, cm^{-1}): 3270, 3216 (3NH), 3107 (CH arom.), 2967, 2926 (CH aliph.), 2191 (C≡N), 1335, 1157 ($2SO_2$). ^{1}H-NMR (DMSO-d_6)δ: 2.34 [s, 3H, CH_3], 5.40, 6.55 [2d, 2H, CH thiazole, *J*= 8.1 Hz], 7.10-7.80 [m, 12H, Ar-H], 7.85, 7.95 [2d, 2H, CH pyridine, *J*= 8.2 Hz], 10.60, 10.80 [2s, 3H, $2SO_2NH$, NH]. ^{13}C-NMR

(DMSO-d_6): 22.4, 84.2, 110.2, 113.2, 115.8, 116.2 (2), 119.5 (2), 120.2 (2), 122.6 (2), 124.1 (2), 126.2, 127.2 (2), 128.2, 130.3, 134.8, 136, 138.2, 150.3, 152.2, 155.6, 165.2, 172.1. Anal. Calcd. For $C_{28}H_{22}N_6O_4S_3$ (602.71): C, 55.80; H, 3.68; N, 13.94. Found: C, 55.46; H, 3.88; N, 14.24.

4.1.14.N-(4-(3-cyano-2-((4-(N-(4-methylpyrimidin-2-yl)sulfamoyl)phenyl)amino)pyridin-4-yl)phenyl)-4-methylbenzenesulfonamide ***(16)****.*

Yield, 35 %; m.p. 132-135 °C. IR (KBr, cm^{-1}): 3316, 3260 (2NH), 3032 (CH arom.), 2919, 2873 (CH aliph.), 2195 (C≡N), 1335, 1150 ($2SO_2$). ^{1}H-NMR (DMSO-d_6)δ: 2.33 [s, 3H, CH_3 tolyl], 2.41 [s, 3H, CH3], 6.95, 7.84 [2d, 2H, 2CH pyrimidine, *J*= 6.9 Hz], 7.20-7.67 [m, 12H, Ar-H], 7.68, 7.73 [2d, 2H, CH pyridine, *J*= 10.2 Hz] 10.50, 10.90 [2s, 3H, $2SO_2NH$, NH]. ^{13}C-NMR (DMSO-d_6): 20.4, 25.3, 85.6, 110.5, 113.6, 113.9 (2), 115.2, 117.2 (2), 128.1 (2), 128.4 (2), 129.1, 129.4 (2), 129.8, 130.2 (2), 136.6, 137.6, 138.9, 144.2, 152.2, 152.8, 154.5, 155.4, 168.8, 170.1 Anal. Calcd. For $C_{30}H_{25}N_7O_4S_2$ (611.69): C, 58.91; H, 4.12; N, 16.03. Found: C, 58.63; H, 4.42; N, 15.88.

*4.1.15.N-(4-(3-cyano-2-((4-(N-(4,6-dimethylpyrimidin-2-yl)sulfamoyl)phenyl)amino)pyridin-4-yl)phenyl)-4-methylbenzenesulfonamide (****17****)*

Yield, 43 %; m.p. 108-110 °C. IR (KBr, cm^{-1}): 3363, 3236 (2NH), 3022 (CH arom.), 2924, 2844 (CH aliph.), 2204 (C≡N), 1311, 1129 ($2SO_2$). ^{1}H-NMR (DMSO-d_6)δ: 2.24 [s, 6H, $2CH_3$ pyrimidine], 2.32 [s, 3H, CH_3 tolyl], 6.53-7.64 [m, 12H, Ar-H], 6.74 [s, H, CH pyrimidine], 7.69, 7.83 [2d, 2H, CH pyridine, *J*= 8.7 Hz], 10.53, 10.78, 12.25 [3s, 3H, $2SO_2NH$, NH]. ^{13}C-NMR (DMSO-d_6): 20.3, 24.1, 96.1, 110.5, 111.7, 113.9 (2), 115.2, 117.2 (2), 126.7 (2), 128.7 (2), 129.1, 129.4 (2), 129.8, 130.2 (2), 136.6, 137.6, 138.9, 144.2, 152.2, 152.8, 154.5, 155.4, 166.2 (2), 168.1. Anal. Calcd. For $C_{31}H_{27}N_7O_4S_2$ (625.72): C, 59.50; H, 4.35; N, 15.67. Found: C, 59.22; H, 4.55; N, 15.82.

*(E)-N-(4-(1,1-dicyano-4-(substitutedphenyl)buta-1,3-dien-2-yl)phenyl)-4-methylbenzenesulfonamides (****18-22****)*

General procedure

A mixture of **4** (0.5 g, 0.0015 mol), aromatic aldehydes (0.0015 mole) namely, benzaldehyde, 4-methylbenzaldehyde, 2-methoxybenzaldehyde,

2-hydroxybenzaldehyde and 4-dimethylaminobenzaldehyde in ethanolic KOH (1.2 g, 20 ml) was stirred overnight while a precipitate was formed. The obtained product was separated and crystallized from dioxane to give **18-22**, respectively.

*4.1.16.(E)-N-(4-(1,1-dicyano-4-phenylbuta-1,3-dien-2-yl)phenyl)-4-methylbenzenesulfonamide (**18**)*

Yield, 40 %; m.p. 140-142°C. IR (KBr, cm^{-1}): 3203 (NH), 3083 (CH arom.), 2993, 2920 (CH aliph.), 2203 (2C≡N), 1374, 1183 (SO_2). ^{1}H-NMR (DMSO-d_6)δ: 2.31 [s, 3H, CH_3], 6.96-7.80 [m, 13H, Ar-H], 7.82, 7.92 [2d, 2H, CH=CH, *J*= 7.8 Hz], 9.34 [s, 1H, SO_2NH]. ^{13}C-NMR (DMSO-d_6): 20.8, 88.2, 114.2 (2), 118.6 (2), 122.2, 126.8, 127.8, 128.3 (2), 128.5 (2), 128.8 (2), 129.3 (2), 130.2 (2), 132.2, 135.2, 136.4, 136.9, 137.4, 162.1. Anal. Calcd. For $C_{25}H_{19}N_3O_2S$ (425.5): C, 70.57; H, 4.50; N, 9.88. Found: C, 70.77; H, 4.81; N, 9.68.

*4.1.17.(E)-N-(4-(1,1-dicyano-4-(p-tolyl)buta-1,3-dien-2-yl)phenyl)-4-methylbenzenesulfonamide (**19**)*

Yield, 36.4 %; m.p. 240-242°C. IR (KBr, cm^{-1}): 3218 (NH), 3033 (CH arom.), 2916, 2871 (CH aliph.), 2217 (2C≡N), 1338, 1146 (SO_2). ^{1}H-NMR (DMSO-d_6)δ: 2.26 [s, 6H, $2CH_3$], 6.54, 6.61 [2d, 2H, CH=CH, *J*= 8.7 Hz], 6.76-7.57 [m, 12H, Ar-H], 12.82 [s, 1H, SO_2NH]. ^{13}C-NMR (DMSO-d_6): 20.7 (2), 55.9, 115.6 (2), 116.8 (2), 125.8, 126.4, 127.6, 128.3 (2), 128.6 (2), 128.8 (2), 129.4 (2), 130.2 (2), 132.2, 135.2, 136.4, 136.9, 137.4, 155.4. MS, *m/z* (%): 440.5 [M^+] (5.58), 62.28 (100). Anal. Calcd. For $C_{26}H_{21}N_3O_2S$ (439.53): C, 71.05; H, 4.82; N, 9.56. Found: C, 71.34; H, 4.78; N, 9.71.

*4.1.18.(E)-N-(4-(1,1-dicyano-4-(2-methoxyphenyl)buta-1,3-dien-2-yl)phenyl)-4-methylbenzenesulfonamide (**20**)*

Yield, 41 %; m.p. 236-238°C. IR (KBr, cm^{-1}): 3360 (NH), 3089 (CH arom.), 2973, 2868 (CH aliph.), 2188, 2160 (2C≡N), 1376, 1124 (SO_2). ^{1}H-NMR (DMSO-d_6)δ: 2.26 [s, 3H, CH_3], 3.91 [s, 3H, OCH_3], 6.62, 6.64 [2d, 2H, CH=CH, *J*= 8.7 Hz], 7.05-7.57 [m, 12H, Ar-H], 10.36 [s, 1H, SO_2NH]. ^{13}C-NMR (DMSO-d_6): 20.7, 55.9, 68.1, 115.8 (2), 116.4 (2), 118.8, 120.6, 125.7, 125.9, 127.2, 128.4 (2), 128.8, 129.2 (2), 130.2 (2), 131.1, 135.2, 136.4, 136.8, 137.6, 159.2, 191.3. Anal. Calcd. For $C_{26}H_{21}N_3O_3S$ (455.53): C, 68.55; H, 4.65; N, 9.22. Found: C, 68.77; H, 4.88; N, 9.55.

*4.1.19.(E)-N-(4-(1,1-dicyano-4-(2-hydroxyphenyl)buta-1,3-dien-2-yl)phenyl)-4-methylbenzenesulfonamide (**21**)*

Yield, 52.85 %; m.p. 218-220°C. IR (KBr, cm^{-1}): 3585 (OH), 3388 (NH), 3032 (CH arom.), 2970, 2862 (CH aliph.), 2289, 2229 (2C≡N), 1384, 1164 (SO_2). ^{1}H-NMR (DMSO-d_6)δ: 2.25 [s, 3H, CH_3], 5.65 [s, H, OH], 6.56, 6.60 [2d, 2H, CH=CH, *J*= 8.7 Hz], 6.82-7.56 [m, 12H, Ar-H], 8.55 [s, 1H, SO_2NH]. ^{13}C-NMR (DMSO-d_6): 20.7, 55.8, 114.8 (2), 117.4 (2), 119.8, 121.2, 122.4, 125.8, 126.6, 128.2 (2), 128.6, 129.1, 129.4 (2), 130.2 (2), 131.4, 136.2, 136.8, 137.4, 157.2, 166.4. Anal. Calcd. For $C_{25}H_{19}N_3O_3S$ (441.5): C, 68.01; H, 4.34; N, 9.52. Found: C, 68.24; H, 4.59; N, 9.19.

*4.1.20.(E)-N-(4-(1,1-dicyano-4-(4-(dimethylamino)phenyl)buta-1,3-dien-2-yl)phenyl)-4-methylbenzenesulfonamide (**22**)*

Yield, 53 %; m.p. 116-118°C. IR (KBr, cm^{-1}): 3393 (NH), 3042 (CH arom.), 2966, 2865 (CH aliph.), 2189, 2155 (2C≡N), 1376, 1122 (SO_2). ^{1}H-NMR (DMSO-d_6)δ: 2.32 [s, 3H, CH_3], 3.04 [s, 6H, $N(CH_3)_2$], 6.74, 7.83 [2d, 2H, CH=CH, *J*= 8.7 Hz], 6.77-8.00 [m, 12H, Ar-H], 10.79 [s, 1H, SO_2NH]. ^{13}C-NMR (DMSO-d_6): 20.8, 40.3 (2), 64.2, 110.6 (2), 115.6 (2), 119.4 (2), 124.2, 125.4, 126.8, 128.2 (2), 129.4 (2), 129.8 (2), 130.4 (2), 131.4, 136.4, 136.8, 143.2, 143.6, 163.2. Anal. Calcd. For $C_{27}H_{24}N_4O_2S$ (468.5): C, 69.21; H, 5.16; N, 11.96. Found: C, 68.96; H, 5.38; N, 11.68.

4.2. Biological evaluation

4.2.1. In-vitro cytotoxic evaluation against human tumor breast cancer cell line (MCF-7)

The *in-vitro* cytotoxic activity was evaluated for nineteen novel compounds on human breast cancer cell line (MCF-7) via the Sulfo-Rhodamine-B stain (SRB) assay in the pharmacology unit at the National Cancer Institute, Cairo University. SRB assay stays one of the most broadly used techniques for *in-vitro* cytotoxic screening which was extended in 1990 [35]. The assay based on the capability of SRB to join to protein constituents of cellular materials that have been linked to tissue-culture sheets by trichloroacetic acid (TCA). SRB is a luminous-

pink aminoxanthene pigment with double sulfonic groups that attach to basic amino-acid residues under moderate acidic conditions, and detach under basic conditions. The quantity of pigment extracted from stained cellular materials is directly comparative to the cellular material mass [36]. Cellular materials were plated in 96-multiwell plate (104 cells/ well) for 24 hrs before conduction with the molecules to permit binding of the Cellular materials to plate wall. Test molecules were disoluted in dimethylsulfoxide (DMSO) and diluted with saline to the appropriate volume. Four different concentrations of the compounds under test (12.5, 25, 50 and 100 μM) were inserted to the cell monolayer. For each individual dose, Triplicate wells were prepared. Monolayer cells were incubated with the compounds for 48 hrs at 37°C and in atmosphere of 5% CO_2. After 48 hrs, cells were fixed, washed and stained for 30 min. with 0.4% (wt/ vol) SRB dissolved in 1% acetic acid. Acetic acid 1% conc. was used to remove unbounded pigment via four washes, and bounded stain was recovered with Tris-EDTA buffer. The intensity of colour was estimated through an enzyme linked immunosorbent assay (ELISA) reader. The relation between drug concentration and surviving fraction is plotted to get the survival curve of each carcinoma cell line after the definite time. The concentration demanded for 50% mortality of viable cell (IC_{50}) was calculated and compared with **E7070** which is presented to be effective anti-breast cancer agent by *in vitro* and *in vivo* findings [26 b,c] and the results are displayed in table 1.

4.2.2. *In vitro Cyclin dependent kinase 2 (CDK2) inhibitory assay*

In vitro enzymatic inhibitory assay was completed in the Egyptian Company for Production of Vaccines, Sera and Drugs (VACSERA), Dokki, Egyptian. Sandwich enzyme immunoassay is a kit used for *in vitro* quantitative evaluation of CDK2 in human tissue homogenates and other biological fluids (Clound-Clone.Corp.). The microtiter plate has been pre-coated with an antibody specific to CDK2. Standards or samples are further added to the proper microtiter plate wells with a biotin-conjugated antibody specific to CDK2. Then, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each individual microplate well and incubated. After addition of TMB substrate solution, only those well that include CDK2, enzyme conjugated Avidin and biotin conjugated antibody will exhibit alteration in color. The enzyme-substrate reaction is finished by the sulphuric acid solution addition and the variation in color is determined spectrophotometrically at 450 nm

$\pm$ 10 nm wavelength. CDK2 concentration in the samples is further determined by comparing the optical density (O.D.) of the samples to the standard curve. Prepare all reagents, samples and standards; add 100 µL standard or sample to each well. Incubate 2 hrs at 37 ºC; aspirate and add 100 µL prepared Detection Reagent A. Incubate 1 hr at 37 ºC. Aspirate and wash 3 times; add 100 µL prepared Detection Reagent B. Incubate 30 minutes at 37 ºC. Aspirate and wash 5 times; add 90 µL substrate solution. Incubate 15-25 minutes at 37 ºC; add 50 µL Stop Solution. Read at 450 nm instantly. The results are expressed as IC_{50} and presented in Table 2.

4.2.3. Radio-sensitizing evaluation

Irradiation was completed in the National Center for Radiation Research and Technology (NCRRT), Egyptian Atomic Energy Authority (EAEA), using Gamma cell-40 (^{137}Cs) source. The impressive derivatives **14**, **16** and **20** were further elected to be re-evaluated for their *in-vitro* cytotoxic activity after combination with γ-irradiation. Cells were plated in 96-multiwell plate (10^4cells/well) for one day before γ-irradiation with one single dose of 8 Gy. Cells were incubated for two days at 37 ˚C in atmosphere of 5% CO_2. After two days, cells were fixed, washed and pigmented with 0.4% (wt/vol) SRB dissolved in 1% acetic acid, for 30 minutes. Extra unbound dye was separated via four washes with 1% acetic acid and attached stain was recovered with Tris–EDTA buffer. The intensity of color was measured in an ELISA reader at 570 nm wavelength. In another multiwell plate, cells were incubated with the compounds **14**, **16 and 20** in molar concentrations of 12.5, 25, 50 and 100 µM. After 2 h., cells were subjected to a single dose of γ-radiation at a dose level of 8 Gy with a dose rate of 0.758 rad/sec for 17.73 min, and then the cytotoxicity was measured two days after irradiation. The surviving fractions were measured using the above stated techniques by ELISA reader. The surviving fractions were expressed as mean values ± standard error. The results were analyzed using 1-way ANOVA test and given in Table 3.

4.4. Molecular modeling and docking

For docking calculations the x-ray crystal structure of CDK2-CA protein was downloaded from the protein data bank website (code: 2WXV, res 2.6 Å) [37]. The protein structure was organized for docking via Accelry's Discovery Studio 2.5.5 according to the following criteria; the unwanted monomers were deleted leaving only one kinase and cyclin complex, the amino acid residues were corrected for errors and the missing ones were added via loop builder, the pH for ionization was adjusted at 7.4 and dielectric constant of 10 and protein was protonated accordingly, the hydrogen atoms were minimized using CHARMm force fields to relieve bumping and orient hydrogen atoms in the right direction, finally, all of the water molecules were deleted and only the water molecules involved in bridging the protein structure or those present in the binding site of ligand, were retained for accuracy. The ligands for docking were constructed using the drawing tools in Accelry's discovery studio and then they were prepared for docking by adding hydrogens and minimization using smart minimizer for 1000 steps. The docking calculations were performed using genetic optimization of ligand docking method in GOLD 4.1 software. The chemscore_kinase template was loaded and the protein and ligands structures were saved by GOLD. The binding site atoms were defined as all the atoms within 8 Å around the co-crystalized ligand. The genetic algorithm parameters were used without change except that the number of iterations was increased to 300000 to obtain the maximum accuracy for the binding poses. The early termination option was disabled and the top 10 poses were saved for each ligand. Chemscore scoring function was the primary scoring function used by the chemscore-kinase template and the ligand poses were rescored using GOLDscore. At last, all the figures were produced using Pymol 0.99.

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Graphical Abstract