

Article

# Synthesis of Some Novel Fused Pyrimido[4'',5'':5',6']-[1,2,4]triazino[3',4':3,4] [1,2,4]triazino[5,6-*b*]indoles with Expected Anticancer Activity

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**Abstract:** Our current goal is the synthesis of polyheterocyclic compounds starting from 3-amino-[1,2,4]triazino[5,6-*b*]indole **1** and studying their anticancer activity to determine whether increasing of the size of the molecules increases the anticancer activity or not. 1-Amino[1,2,4]triazino[3',4':3,4]-[1,2,4]triazino[5,6-*b*]indole-2-carbonitrile (**4**) was prepared by the diazotization of 3-amino[1,2,4]-triazino[5,6-*b*]indole **1** followed by coupling with malononitrile in basic medium then cyclization under reflux to get **4**. Also, new fused pyrimido[4'',5'':5',6']-[1,2,4]triazino-[3',4':3,4][1,2,4]triazino[5,6-*b*]indole derivative **6** was prepared and used to obtain polycyclic heterocyclic systems. Confirmation of the synthesized compounds' structures was carried out using elemental analyses and spectral data (IR, <sup>1</sup>H-NMR and <sup>13</sup>C-NMR and mass spectra). The anticancer activity of some of the synthesized compounds was tested against HepG2, HCT-116 and MCF-7 cell lines. The anticancer screening results showed that some derivatives display good activity which was more potent than that of the reference drug used. Molecular docking was used to predict the binding between some of the synthesized compounds and the prostate cancer 2q7k hormone and breast cancer 3hb5 receptors.

**Keywords:** triazino[5,6-*b*]indole; pyrimido[2',1':3,4]triazino[5,6-*b*]indole; anticancer activity; molecular docking

## 1. Introduction

Interestingly, we are now seeing the emergence of a new generation of drugs with hybrid molecular architectures combining the biological features of two or more small molecules. The expectation is that, in the long term, such molecular conjugates could become a dominant form of targeted infectious diseases. The idea of combining two or more potentially bioactive substructures to make an integrated new molecular framework with a higher anticipated therapeutic power is conceptually simple yet highly successful in the context of infectious diseases that require effective treatment. In previous works, we succeeded in constructing many small 1,2,4-triazine molecules that had a remarkable biological activity on cancer cells [1,2]. In addition, we were able to construct many fused 1,2,4-triazine systems which showed marvelous antimicrobial activities [3–10]. Herein, we aspire to construct huge fused triazine molecules of increased efficacy and decreased systemic toxicity which are a great challenge for medical science. One of the present goals in heterocyclic compound synthesis is the construction of macromolecules, in particular, by using of sequential reactions [11]. Such molecular explorations, yielding novel architectures, are of particular interest for the investigation of new bioactive agents with possible new modes of action, which could subsequently be elaborated in medicinal chemistry programs [12]. Approaches to the realization of these synthetic goals have often been explored in the context of complex multistep syntheses

of natural product targets [13]. Among the various clinical applications, heterocyclic compounds have a considerable active role as anti-viral [14], anti-bacterial [15,16], anti-inflammatory [17], anti-fungal [18], and anti-tumor drugs [19–21]. Numerous of biologically active macromolecules naturally occurring (e.g., with antimicrobial activity or anticancer) include homofascaplysin [22], fascaplysin (antimicrobial pigment) [23–26], staurosporine [27], iheyamines [28], and rebeccamycin [29] are shown in Figure 1.

The amazing biological activity of the polycyclic heterocyclic compounds encouraged us to continue our previous work on the synthesis of fused triazine[7,9,10] and their applications, by designing a polycyclic heterocyclic compounds containing five and/or six rings fused with each other in the hope to get a superior biological activity.

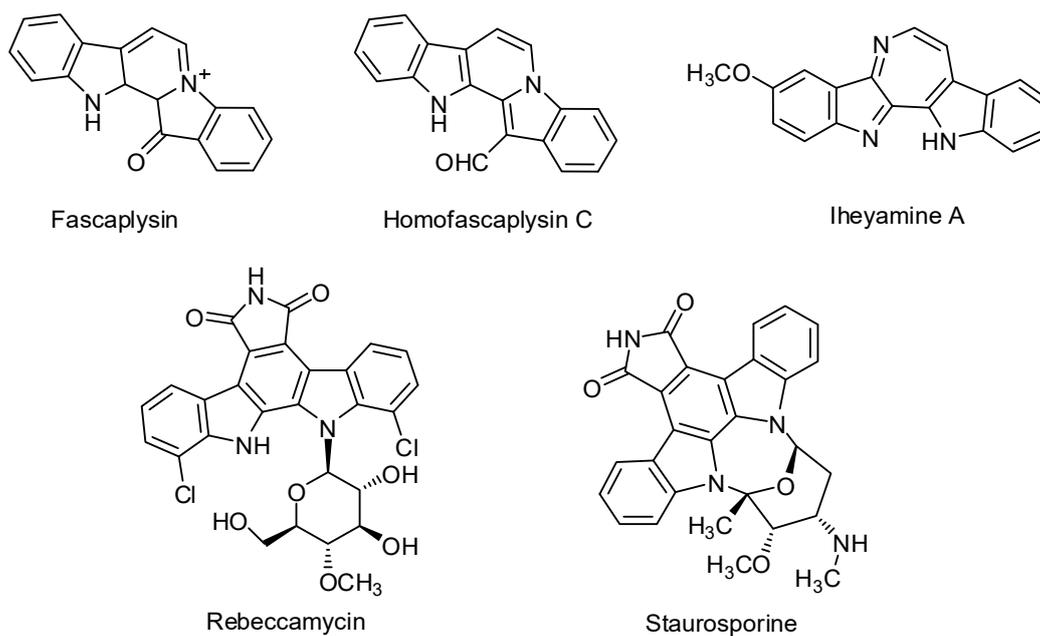
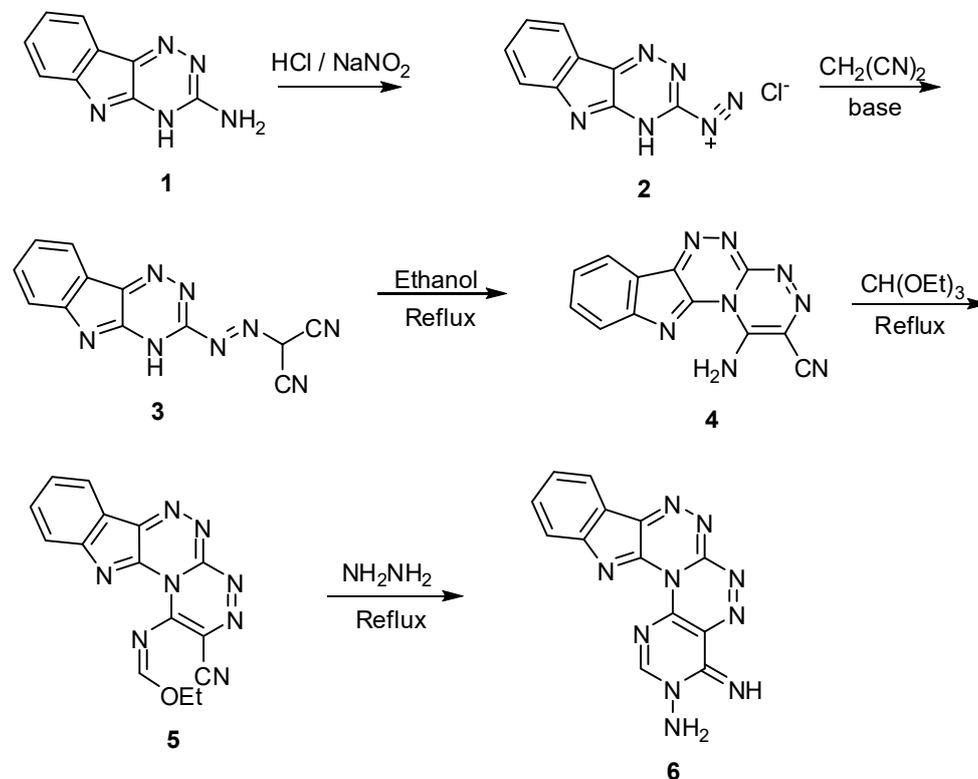


Figure 1. Some active poly cyclic natural compounds.

## 2. Results and Discussion

The previously prepared 3-amino-4*H*-[1,2,4]triazino[5,6-*b*]indole **1** [30] was used in this work as a starting unit on which the polycyclic compound will be built. Compound **1** was subjected to diazotization at 4 °C in conc. HCl with aqueous solution of NaNO<sub>2</sub> (Scheme 1). To the stirred solution an aqueous mixture of malononitrile and sodium acetate was added to obtain [4*H*-[1,2,4]triazino[5,6-*b*]indol-3-yl-diazenyl]malononitrile (**3**). The structure of **3** was elucidated from its IR spectrum, which showed two cyano groups signals at 2210, 2217 cm<sup>-1</sup> with disappearance of the band corresponding to the NH<sub>2</sub> group of compound **1**. Cyclization of compound **3** to 1-amino[1,2,4]triazino[3',4':3,4]-[1,2,4]triazino[5,6-*b*]indole-2-carbonitrile (**4**) was achieved by refluxing compound **3** in ethanol. The structure elucidation of compound **4** was supported from the disappearance of one cyano group peak in the IR, as only one CN group was detected at 2209 cm<sup>-1</sup>, in addition to the disappearance of the triazine NH peak at 3201 cm<sup>-1</sup> along with appearance of a NH<sub>2</sub> group in the 3351–3313 cm<sup>-1</sup> range. The <sup>1</sup>H-, <sup>13</sup>C-NMR data supported the same results, where the disappearance of the NH signal of compound **3** at δ = 11.42 ppm was compensated by the appearance of the NH<sub>2</sub> signal of compound **4** at δ = 6.90 ppm. The <sup>13</sup>C-NMR data, where one of the CN signals disappeared, also advocated the same results. Refluxing of compound **4** with triethyl orthoformate in ethanol resulted in the disappearance of the NH<sub>2</sub> signals in both the IR and <sup>1</sup>H-NMR spectra, respectively, due to the formation of the compound ethyl (2-cyano[1,2,4]triazino[3',4':3,4][1,2,4]triazino[5,6-*b*]indol-1-yl)imidofornate (**5**) with appearance

of ethyl group signals in the  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR, respectively. Compound **5** was subjected to reaction with hydrazine hydrate in boiling ethanol to get the first fifth cyclic compound 3-(4*H*)-amino-4-iminopyrimido[4'',5'':5',6'] [1,2,4]triazino[3',4':3,4][1,2,4]triazino[5,6-*b*]indole (**6**), which was considered as a second starting material for the formation of six-ring cyclic compounds (Scheme 1).

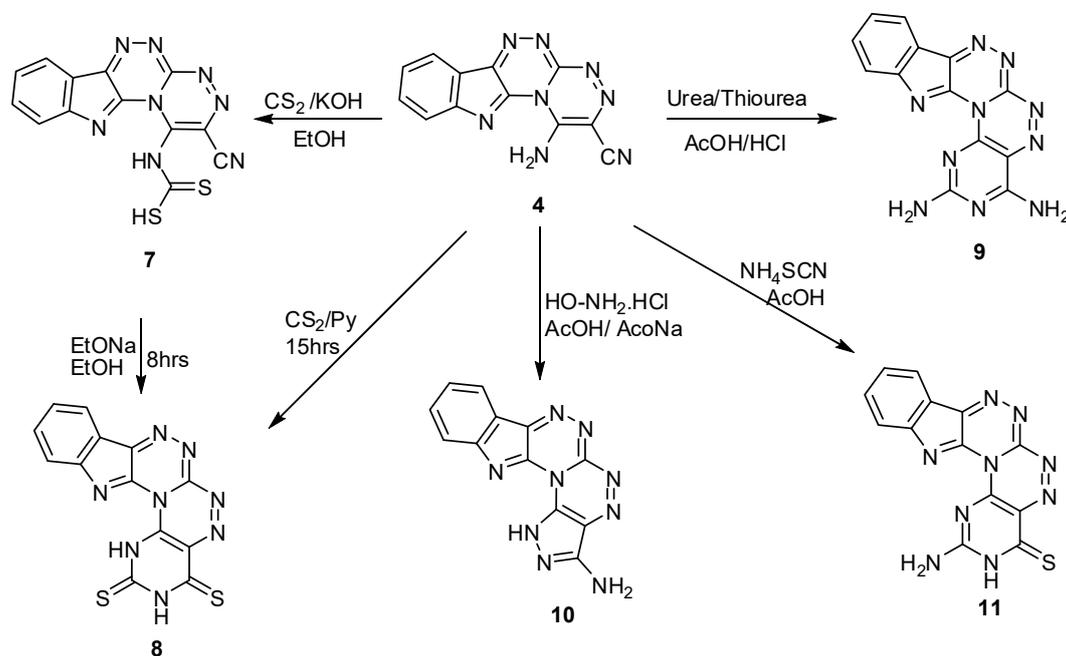


**Scheme 1.** Synthesis of 4-iminopyrimido[4'',5'':5',6'] [1,2,4]triazino[3',4':3,4][1,2,4]triazino-[5,6-*b*]indole-3(4*H*)-amine **6**.

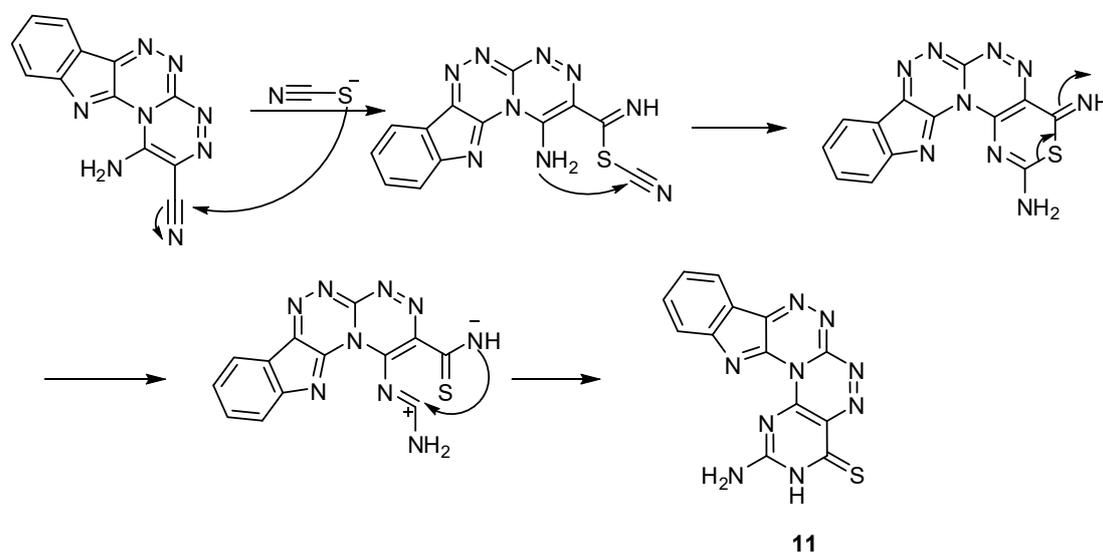
Confirmation of the structure of compound **6** was achieved from its  $^1\text{H}$ -NMR, where the signals corresponding to the ethyl group of compound **5** at  $\delta = 1.26$  and  $3.51$  ppm disappeared and new bands due to  $\text{NH}_2$  and  $\text{NH}$  at  $\delta = 5.83$  and  $10.13$  ppm appeared, while the  $^{13}\text{C}$ -NMR showed the disappearance of the  $\text{sp}^3$  carbons of the ethyl group of compound **5**. The first starting material, **4**, reacted with carbon disulphide in alcoholic KOH under reflux, to get the carbamodithioic acid derivative **7** which was cyclized to give pyrimido[4'',5'':5',6'] [1,2,4]triazino[3',4':3,4][1,2,4]triazino indole-2,4(1*H*,3*H*)-dithione (**8**) by refluxing compound **7** in hot ethanolic sodium ethoxide for 8 h. Compound **8** was also prepared by directly refluxing compound **4** with  $\text{CS}_2$  in boiling pyridine. The structures of compounds **7** and **8** were confirmed by their spectral analysis, where the  $^1\text{H}$ -NMR of compound **7** showed the appearance of a signal at  $\delta = 13.98$  ppm due to a SH group, which disappeared in compound **8** and 2 NH signals shown at  $\delta = 11.40$  and  $12.50$  ppm, respectively (Scheme 2).

Reactions of compound **4** with urea and/or thiourea in boiling glacial acetic acid in presence of a catalytic amount of HCl yielded 2,4-diaminopyrimido[4'',5'':5',6'] [1,2,4]triazino[3',4':3,4][1,2,4]triazino[5,6-*b*]indole (**9**), while 3-amino-1*H*-pyrazolo[3'',4'':5',6'] [1,2,4]triazino[3',4':3,4]-[1,2,4]triazino-[5,6-*b*]indole (**10**) and 2-aminopyrimido[4'',5'':5',6'] [1,2,4]triazino[3',4':3,4]-[1,2,4]triazino-indole-4(3*H*)-thione (**11**) were formed by the reactions of **4** with hydroxylamine hydrochloride in glacial acetic acid containing anhydrous sodium acetate as a catalyst and ammonium thiocyanate in glacial acetic acid. The structures of compounds **9**, **10** and **11** were confirmed from their spectral data, where the IR of all compounds showed the disappearance of the cyano group peak with appearance of peaks corresponding to  $\text{NH}_2$ ,  $\text{NH}$  in the  $3413$ – $3754$   $\text{cm}^{-1}$  range, and the IR of compound **11** showed

a C=S moiety at  $1349\text{ cm}^{-1}$ . The  $^1\text{H-NMR}$  of the three compounds show signals due to  $\text{NH}_2$  and in some NH signals in the  $\delta = 5.55\text{--}6.93$  and  $11.12\text{--}12.33$  ppm ranges, respectively (Scheme 2). The mechanism proposed for the formation of compound **11** is illustrated in Scheme 3.



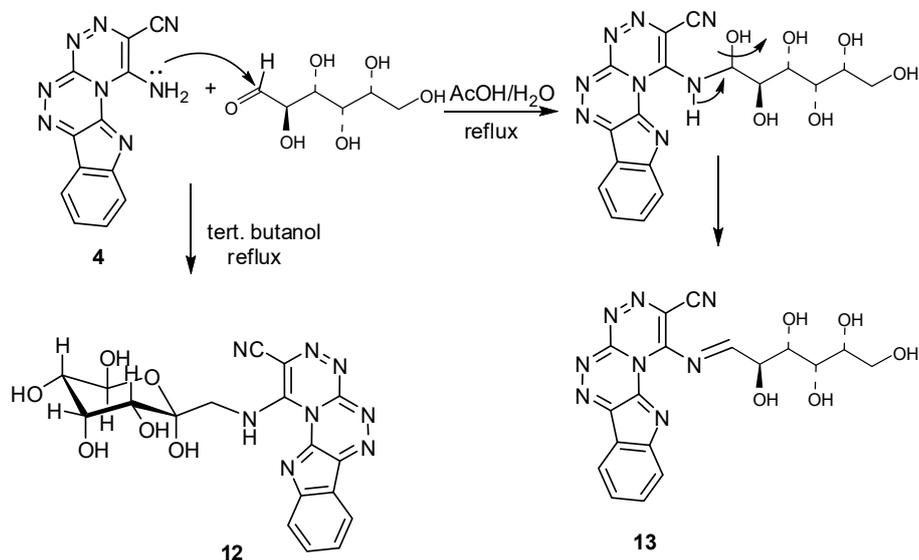
**Scheme 2.** Some reactions on 1-amino[1,2,4]triazino[3',4':3,4][1,2,4]triazino[5,6-*b*]indole-2-carbonitrile.



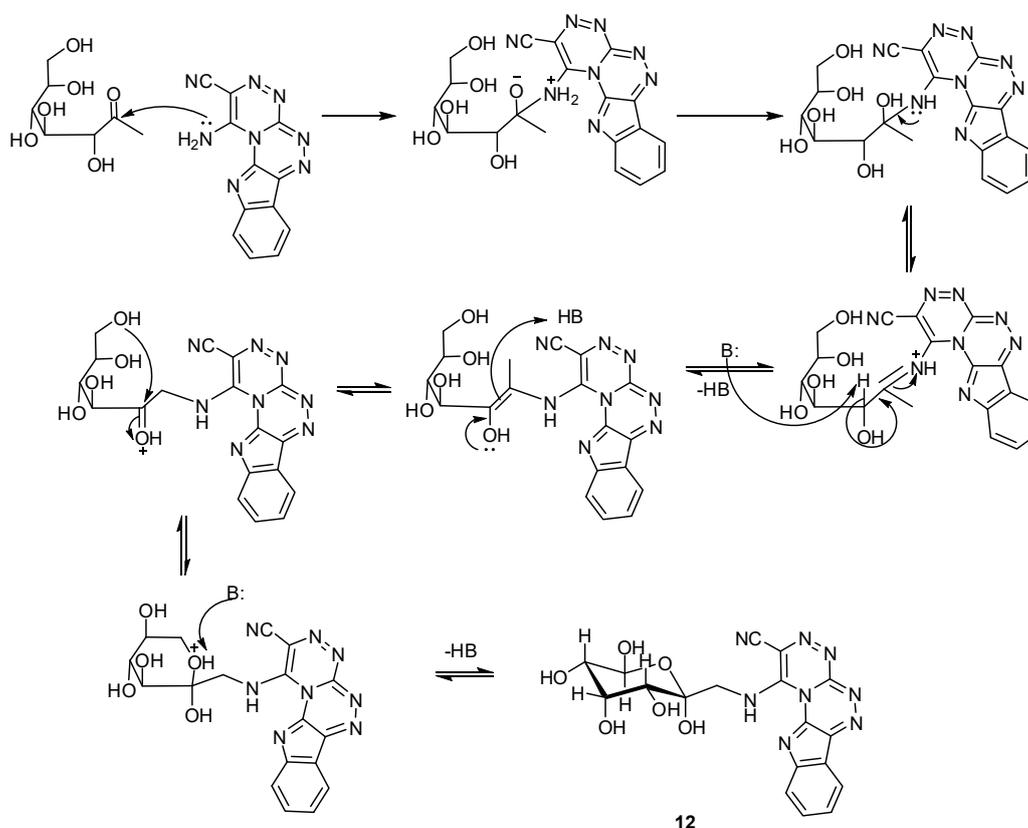
**Scheme 3.** Mechanism that explains the formation of compound **11**.

The product of the reaction of glucose with compound **4** varied depending on the solvent used in the reaction, whereby when the reaction carried out in *tert*-butanol the reaction followed the Maillard reaction pathway and the resulting compound was 1-( $\beta$ -D-glucopyranosyl-methylamino)-triazino[3',4':3,4][1,2,4]triazino[5,6-*b*]indole-2-carbonitrile (**12**), while, when the reaction was performed in hot acetic acid the main product is the Schiff base type product, 1-(2,3,4,5,6-pentahydroxyhexylideneamino)[1,2,4]triazino[3',4':3,4][1,2,4]triazino[5,6-*b*]indole-2-carbonitrile (**13**). The  $^1\text{H-NMR}$  spectra differentiate between the two compounds, whereby compound **12** showed two signals at  $\delta = 3.93$  and  $5.12$  ppm due to  $\text{CH}_2$  and  $\text{NH}$  groups, respectively, whereas the Schiff base

compound showed an olefinic proton absorption at  $\delta = 7.34$  ppm; the  $^{13}\text{C}$ -NMR also showed a signal at  $\delta = 163.2$  ppm in compound **13** corresponding to the  $\text{HC}=\text{N}$  carbon, while, in compound **12** the  $\delta = 52.66$  ppm signal is due to a  $\text{CH}_2$  group. The mechanisms illustrating the formation of compounds **12** and **13** are shown in Schemes 4 and 5.



**Scheme 4.** Reactions of 1-amino[1,2,4]triazino[3',4':3,4][1,2,4]triazino[5,6-*b*]indole-2-carbonitrile with glucose.

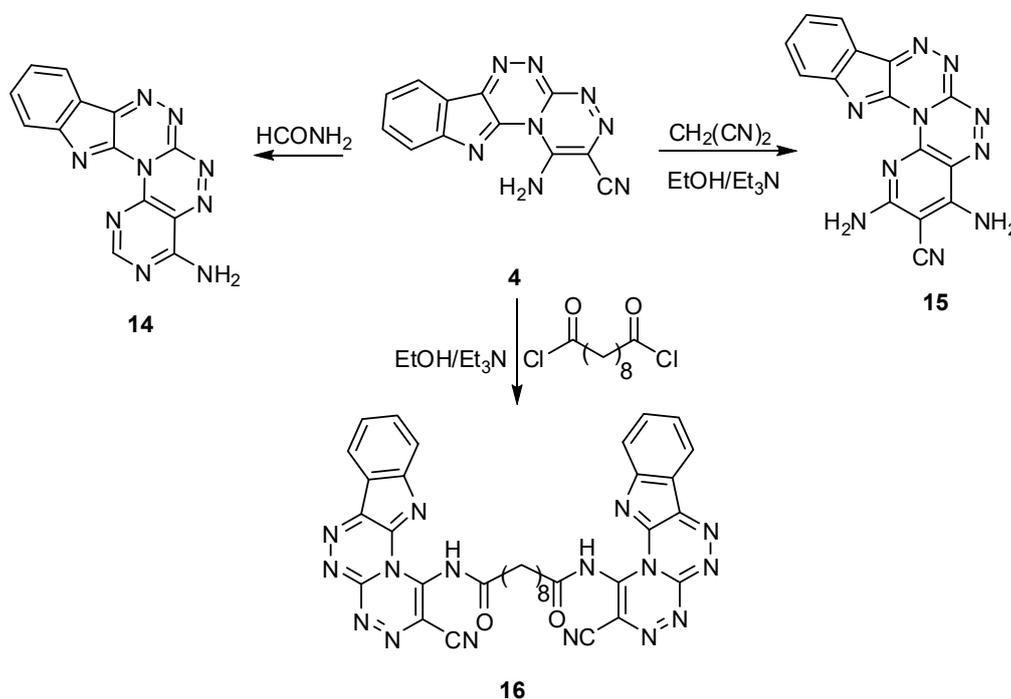


**Scheme 5.** Maillard-type mechanism for the reaction of compound **4** with glucose.

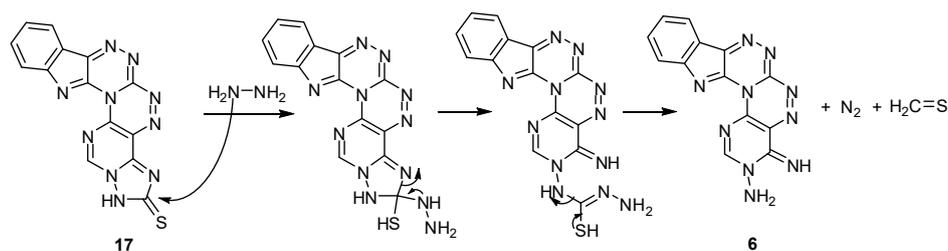
4-Aminopyrimido[4'',5'':5',6']-[1,2,4]triazino[3',4':3,4]-[1,2,4]triazino[5,6-*b*]indole (**14**) was synthesized by the reaction of compound **4** with formamide, while, the reaction of **4** with malononitrile in basic medium yielded the corresponding 2,4-diaminopyrimido[2'',3'':5',6']-[1,2,4]triazino[3',4':3,4]-triazino[5,6-*b*]indole-3-carbonitrile (**15**). On the other hand, the most amazing compound in this group of products, *N,N'*-bis(2-cyano[1,2,4]triazino[3',4':3,4]-[1,2,4]triazino[5,6-*b*]indol-1-yl)decanediamide (**16**) was formed from the reaction of **4** with sebacyl chloride in basic medium [Scheme 6]. The marvelous feature of compound **16** is the anticancer activity it shows against all types of cell lines, more than the standard drug used. The structures of compounds **14**, **15** and **16** are proved using their spectral data (see the Experimental section).

A group of six-ring compounds are synthesized using compound **6** as starting material. The reaction of compound **6** with carbon disulphide in alcoholic KOH under reflux for 9 h gave the cyclic derivative [1,2,4]triazolo[1''',5''':1'',6'']pyrimido[4'',5'':5',6']-[1,2,4]triazino[3',4':3,4]-triazino[5,6-*b*]indole-2(*1H*)-thione (**17**) directly and no evidence for the formation of the dithioic acid derivative, The <sup>1</sup>H-NMR of compound **17** showed no signal due to a SH group with the presence of a signal due to the NH of a triazolo ring at  $\delta = 11.30$  ppm. The reaction of compound **17** with hydrazine hydrate in boiling ethanol gave the starting material **6** in poor yield.

The mechanism proposed for this amazing reaction is illustrated in Scheme 7. Methylation of compound **17** with methyl iodide in sodium acetate yielded the *S*-methyl derivative **18**. The structure of compound **18** was confirmed by its IR, <sup>1</sup>H, and <sup>13</sup>C-NMR data. The IR showed the disappearance of the NH peak at  $3266\text{ cm}^{-1}$  and the C=S peak at  $1338\text{ cm}^{-1}$  in compound **17**. The <sup>1</sup>H-NMR of compound **18** showed a new signal due to the SCH<sub>3</sub> protons at  $\delta = 3.01$  ppm and no signal for a NH group. The <sup>13</sup>C-NMR spectrum also supported the methylation of the S atom by a signal appearing at  $\delta = 13.5$  ppm due to the SCH<sub>3</sub> carbon.

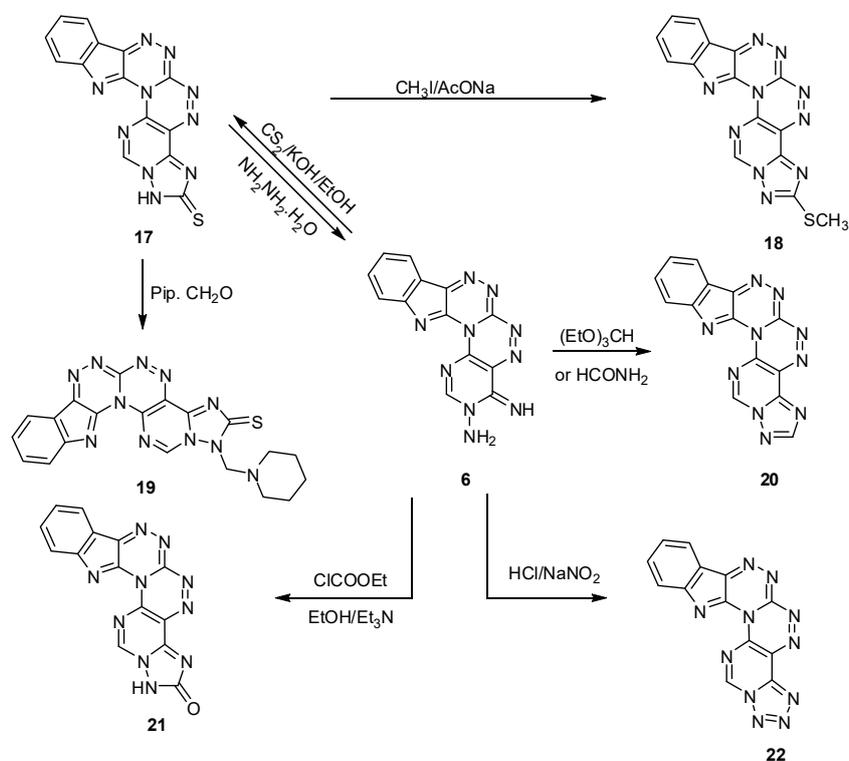


**Scheme 6.** Reactions of 1-amino[1,2,4]triazino[3',4':3,4]-[1,2,4]triazino[5,6-*b*]indole-2-carbonitrile with different reagents.



**Scheme 7.** The proposed mechanism for the conversion of compound **17** to its substrate by hydrazine hydrate.

Mannich reaction on compound **6** with formaldehyde and piperidine (Scheme 8) afforded 1-(piperidin-1-ylmethyl)[1,2,4]triazolo[1''',5''':1'',6'']pyrimido[4'',5'':5',6'] [1,2,4]triazino[3',4':3,4][1,2,4]-triazino[5,6-*b*]indole-2(1*H*)-thione (**19**). The structure of compound **19** was elucidated from its spectral analysis, where the <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra showed signals due to the piperidine ring hydrogen and carbons at  $\delta = 1.35$ – $2.35$  and  $24.03$ ,  $25.73$ ,  $50.23$ ,  $51.43$  ppm, respectively. Reaction of compound **6** with triethyl orthoformate in boiling DMF yielded [1,2,4]triazolo[1''',5''':1'',6'']pyrimido[4'',5'':5',6'] [1,2,4]triazino[3',4':3,4][1,2,4]-triazino-[5,6-*b*]-indole (**20**). The same product was obtained when compound **6** was reacted with formamide in boiling DMF (Scheme 8). The IR of compound **20** showed no peaks due to NH<sub>2</sub> or NH groups, and the same observation was obtained from the <sup>1</sup>H-NMR spectrum. Reaction of ethyl chloroformate with compound **6** in boiling ethanol using Et<sub>3</sub>N as a basic medium formed [1,2,4]triazolo[1''',5''':1'',6'']pyrimido[4'',5'':5',6'] [1,2,4]triazino[3',4':3,4][1,2,4]-triazino indol-2(1*H*)-one (**21**). On the other hand, diazotization of compound **6** yielded the tetrazolo derivative tetrazolo[1''',5''':1'',6'']pyrimido[4'',5'':5',6'] [1,2,4]triazino[3',4':3,4][1,2,4]triazino-[5,6-*b*]indole (**22**) (Scheme 8).

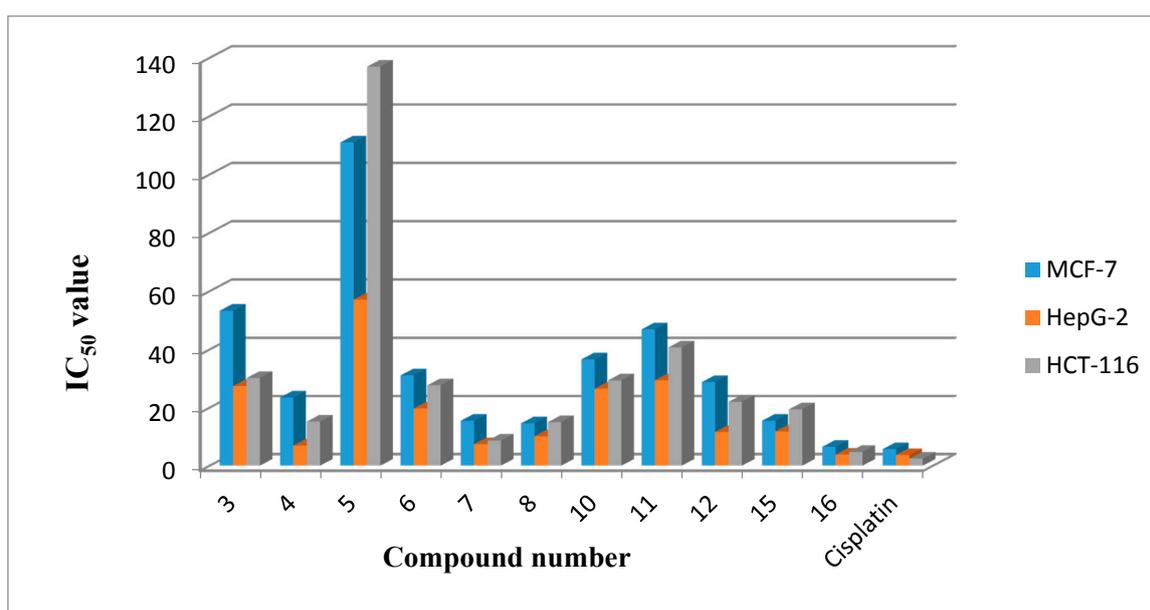


**Scheme 8.** Some reactions on 4-iminopyrimido[4'',5'':5',6'] [1,2,4]triazino[3',4':3,4][1,2,4]triazino [5,6-*b*]indol-3(4*H*)-amine.

The IR of compound **21** showed a peak due to a carbonyl group at  $1654\text{ cm}^{-1}$  with disappearance of the  $\text{NH}_2$  and  $\text{NH}$  peaks, while compound **22** showed no major functional groups in its IR except the  $\text{C}=\text{N}$  group.

### 3. Cytotoxic Activity

The *in vitro* growth inhibitory activity of the synthesized compounds was investigated in comparison with a well-known anticancer standard drug (cisplatin) under the same conditions using a colorimetric MTT assay. Data generated were used to plot a dose response curve from which the concentration of test compounds required to kill 50% of cell population ( $\text{IC}_{50}$ ) was determined (Figure 2). The results revealed that all the tested compounds showed inhibitory activity to the tested tumor cell lines in a concentration dependent manner. Cytotoxic activity was expressed as the mean  $\text{IC}_{50}$  of three independent experiments.



**Figure 2.** The antitumor activities of the tested compounds expressed as  $\text{IC}_{50}$  values and compared with reference standard drugs evaluated on breast, liver cancer and colon carcinoma cell lines.

Interestingly, the results, presented in Table 1 and Figure 2, show that compound **16** was the most active against the three tested carcinoma cell lines (HepG2, HCT-116 and MCF-7, respectively), giving  $\text{IC}_{50}$  values of  $3.82$ ,  $4.73$  and  $6.52\text{ }\mu\text{g/mL}$  compared with the cisplatin reference drug.

The order of activity against breast carcinoma cell line (MCF-7) was **16, 8, 15, 7, 4, 12, 6, 10, 11, 3** and **5**, with  $\text{IC}_{50}$  values of  $6.52 \pm 0.6$ ,  $14.6 \pm 1.2$ ,  $15.5 \pm 0.7$ ,  $15.5 \pm 0.8$ ,  $23.5 \pm 0.8$ ,  $28.8 \pm 0.8$ ,  $31.1 \pm 0.7$ ,  $36.6 \pm 1.8$ ,  $46.9 \pm 3.4$ ,  $53.3 \pm 1.9$ , and  $111 \pm 2.9\text{ }\mu\text{g/mL}$ , respectively.

The order of activity against the liver carcinoma cell line (HepG2) was **16, 4, 7, 8, 12, 15, 6, 10, 3, 11** and **5**, with  $\text{IC}_{50}$  values of  $3.82 \pm 0.3$ ,  $6.93 \pm 0.3$ ,  $7.4 \pm 0.5$ ,  $10.1 \pm 0.9$ ,  $11.6 \pm 0.8$ ,  $11.8 \pm 0.6$ ,  $19.7 \pm 0.9$ ,  $26.5 \pm 0.4$ ,  $27.4 \pm 0.8$ ,  $29.4 \pm 0.8$ , and  $57.1 \pm 1.4\text{ }\mu\text{g/mL}$ , respectively.

The order of activity against colon carcinoma cell line (HCT-116) was **16, 7, 4, 8, 15, 12, 6, 10, 3, 11** and **5**, with  $\text{IC}_{50}$  values of  $4.73 \pm 0.7$ ,  $8.64 \pm 0.8$ ,  $15.2 \pm 0.4$ ,  $15 \pm 0.6$ ,  $19.4 \pm 1.8$ ,  $22 \pm 0.7$ ,  $27.7 \pm 0.9$ ,  $29.4 \pm 0.7$ ,  $30.2 \pm 0.8$ ,  $40.7 \pm 2.3$ , and  $137 \pm 6.4\text{ }\mu\text{g/mL}$ , respectively.

**Table 1.** The antitumor activities of the tested compounds expressed as IC<sub>50</sub> values and compared with reference standard drugs evaluated on breast, liver cancer and colon carcinoma cell lines

Compound Number	MCF-7	HepG-2	HCT-116
3	53.3 ± 1.9	27.4 ± 0.8	30.2 ± 0.7
4	23.5 ± 0.8	6.93 ± 0.3	15.2 ± 0.4
5	111 ± 2.9	57.1 ± 1.4	137 ± 6.4
6	31.1 ± 0.7	19.7 ± 0.9	27.7 ± 0.9
7	15.5 ± 0.8	7.4 ± 0.5	8.64 ± 0.8
8	14.6 ± 1.2	10.1 ± 0.9	15 ± 0.6
10	36.6 ± 1.8	26.5 ± 0.4	29.4 ± 0.7
11	46.9 ± 3.4	29.4 ± 0.8	40.7 ± 2.3
12	28.8 ± 0.8	11.6 ± 0.8	22 ± 0.7
15	15.5 ± 0.7	11.8 ± 0.6	19.4 ± 1.8
16	6.52 ± 0.6	3.82 ± 0.3	4.73 ± 0.7
Cisplatin	5.71 ± 0.4	3.67 ± 0.2	2.43 ± 0.2

#### 4. Molecular Docking Studies

Molecular docking is a key tool in computer drug design [31]. The focus of molecular docking is to simulate the molecular recognition process. Molecular docking aims to achieve an optimized conformation for both protein and drug with relative orientation between them such that the free energy of the overall system is minimized. The results showed a possible arrangement between free compound as a drugs and receptors (2q7k) and (3hb5). The docking study showed a favorable interaction between the compounds and the receptor, and the calculated energy is presented in Tables 2 and 3. According to our results (Figures 3 and 4), HB plots indicate that the drug compounds bind to the 2q7k and 3hb5 proteins with hydrogen bond interactions and there are decomposed interaction energies of synthesized compounds with 2q7k and 3hb5 (Tables 4 and 5). The calculated efficiency is favorable. K<sub>i</sub> values (estimated using AutoDock) were compared with experimental K<sub>i</sub> values, when available, and the Gibbs free energy is negative. Also, based on these data, we can propose that an interaction between the 2q7k and 3hb5 receptors and our compounds is possible. The 2D plots of interaction between the compound 3 and compound 4 and prostate cancer receptor (2q7k) and receptor breast cancer enzymes are shown in Figures 5 and 6. From an analysis of the values, it is evident that the binding energy of our compounds decreases. Binding energies are the most widely used mode of measuring the binding affinity of a compound. Thus, a decrease in binding energy due to mutation will increase the binding affinity of the compounds towards the receptor. The characteristic feature of the compounds is represented by the presence of several active sites available for hydrogen bonding. This feature gives them the ability to be good binding inhibitors to the protein and will help to produce augmented inhibitory compounds. The results confirm that the fused triazines as a drug compounds are efficient, preventing prostate cancer 2q7k and breast cancer 3hb5 hormone effectively, which inhibits assembly. This interaction could activate apoptosis in cancer cells for interactions with synthesized compounds. Binding energies are most widely used as a mode of measuring the binding affinity of compounds. Thus, a decrease in binding energy due to mutation will increase the binding affinity of the compounds towards the receptor [25–29]. The characteristic feature of compounds was the presence of several active sites available for hydrogen bonding. This software includes code developed by the Theoretical and Computational Biophysics Group in the Beckman Institute for Advanced Science and Technology at the University of Illinois at Urbana-Champaign [31–34].

**Table 2.** Energy values obtained in docking calculations of some synthesized compounds with receptor prostate cancer (2q7k).

Compd.	Est. Free Energy of Binding kcal/mol	Est. Inhibition Constant, K <sub>i</sub> uM	vdW + Hbond + desolv Energy kcal/mol	Electrostatic Energy kcal/mol	Total Intermolec. Energy kcal/mol	Interact Surface
3	-7.04	6.90	-8.22	+0.04	-8.17	509.119
4	-7.51	3.12	-8.09	-0.02	-8.11	465.35
5	-8.31	8.14	-9.44	-0.04	-9.48	523.761
6	-7.98	1.41	-8.03	-0.25	-8.28	508.448
7	-8.13	1.09	-9.18	-0.03	-9.21	519.896
8	-6.35	22.23	-6.41	+0.06	-6.35	522.705
12	-0.96	1.97	-1.87	+0.61	-1.26	583.589
13	-0.26	6.47	-7.64	+0.01	-7.63	576.654
16	-7.95	1.49	-8.16	-0.40	-8.56	540.988

**Table 3.** Energy values obtained in docking calculations of some synthesized compounds with receptor breast cancer (3hb5).

Compd.	Est. Free Energy of Binding kcal/mol	Est. Inhibition Constant, K <sub>i</sub> uM	vdW + Hbond + desolv Energy kcal/mol	Electrostatic Energy kcal/mol	Total Intermolec. Energy kcal/mol	Interact. Surface
3	-7.55	2.90	-8.28	-0.18	-8.47	700.877
4	-7.66	2.43	-8.03	-0.06	-8.09	639.72
5	-7.68	2.34	-8.94	+0.03	-8.91	740.419
6	-7.93	1.53	-8.22	-0.01	-8.23	719.146
9	-9.31	14.94	-11.12	-0.85	-11.97	1288.56
12	-9.15	19.78	-9.76	+0.31	-9.44	851.928
13	-8.01	1.35	-10.31	-0.08	-10.39	911.407
14	-8.87	31.24	-9.49	-0.59	-10.08	908.153
15	-8.74	39.33	-9.08	+0.34	-8.74	846.707

**Table 4.** Decomposed energy of some synthesized compounds with prostate cancer receptor (2q7k).

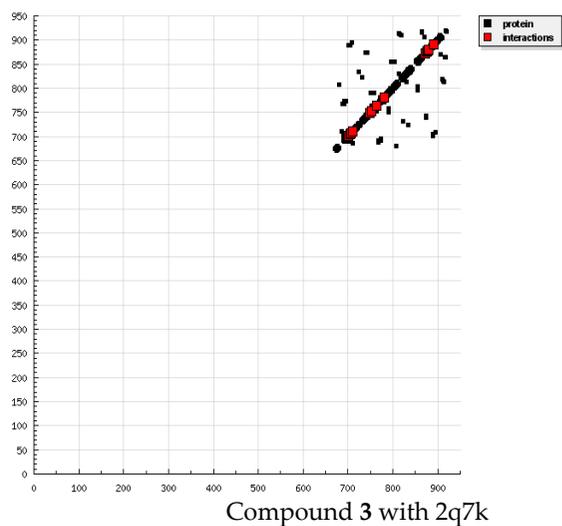
Compd.	Hydrogen Bonds	Polar	cation-pi	Hydrophobic	Other
3	LEU704 (-0.9588) ASN705 (-0.2861) THR877 (0.0967) LEU873 (0.2094)			PHE764 (-0.9244)	GLN711 (-0.3009) ARG752 (-0.2282) PHE891 (-0.1823)
				LEU707 (-0.5332)	
				PHE876 (-0.4595)	
				MET780 (-0.3859)	
				MET749 (-0.3551)	
				LEU701 (-0.1735)	
LEU880 (0.031)					
4	ASN705 (-0.6684) THR877 (-0.4461)			PHE764 (-1.321)	LEU704 (-1.2982)
				LEU873 (-0.6886)	PHE876 (-0.5103)
				LEU707 (-0.4798)	MET742 (-0.4068)
				LEU701 (-0.2013)	PHE891 (-0.3538)
				LEU880 (0.2352)	
5	PHE764 (-1.0213) GLN711 (-0.5111)	ARG752 (-0.2593)		LEU704 (-1.3263)	MET749 (-0.5969) ASN705 (-0.5916) MET787 (-0.2392)
				LEU873 (-0.8151)	
				LEU707 (-0.6963)	
				MET745 (-0.6501)	
				MET742 (-0.5522)	
				PHE876 (-0.4912)	
				VAL746 (-0.4163)	
				TRP741 (-0.3903)	
				LEU701 (-0.2362)	
MET780 (-0.0943)					
6	MET742 (0.5327) ASN705 (1.1422)	THR877 (-12.988)	PHE891 (0.4955) PHE876 (1.6611)	PHE764 (-1.6911)	GLN711 (-0.3671)
				MET749 (-0.4918)	VAL746 (-0.3662)
				LEU707 (-0.1047)	ARG752 (-0.132)
				LEU704 (0.1685)	LEU880 (0.7122)
				LEU873 (1.6514)	LEU701 (1.3032)

Table 4. Cont.

Compd.	Hydrogen Bonds	Polar	cation-pi	Hydrophobic	Other
7	GLN711 (−0.2307) MET787 (2.5309)	ARG752 (−0.3853)		LEU873 (−9.3441) PHE764 (−0.6167) MET749 (−0.5857) LEU704 (−0.5848) LEU701 (−0.1264) PHE876 (0.1242) MET780 (0.6784)	MET745 (−0.7229) LEU707 (−0.3795) ASN705 (−0.255) TRP741 (−0.2472) VAL746 (−0.1409) THR877 (0.2176)
8	LEU704 (−2.7674) ASN705 (21.4001)	THR877 (−1.5585)	PHE876 (−0.703)	PHE764 (−2.7458) LEU701 (−2.0955) LEU873 (−1.5548) MET780 (−0.6025) MET749 (−0.457) VAL746 (1.5949) MET787 (3.0657)	PHE891 (−1.637) LEU880 (−0.6967)
16	GLN711 (−0.4793) MET745 (0.1156)	ASN705 (−1.6327) ARG752 (0.3987)		LEU701 (−2.1158) LEU880 (−0.6528) PHE764 (−0.5839) MET749 (−0.3359) PHE876 (1.1185)	LEU873 (−0.4761) LEU707 (−0.4553) MET787 (−0.2292) MET895 (−0.0748) THR877 (1.3407)

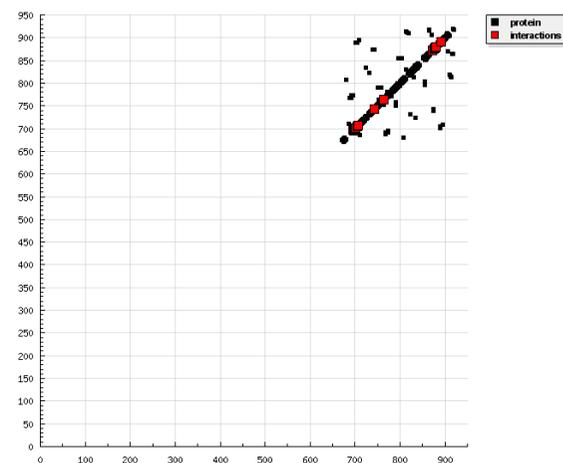
Table 5. Decomposed energy of some synthesized compounds with breast cancer receptor (3hb5).

Compd.	Hydrogen Bonds	Polar	cation-pi	Hydrophobic	Other
3	SER12 (−0.443) THR190 (0.8132)			PHE192 (−1.7843) VAL188 (−1.341) PHE226 (−0.4523)	ALA191 (−0.3955) TYR155 (−0.2826) SER142 (−0.0999)
4	ASN90 (−1.0823) SER12 (−0.2674) GLY9 (−0.009) THR190 (0.5483)			ILE14 (−0.9193) CYS185 (1.4843)	TYR155 (−1.2233) PHE192 (−1.1514) THR140 (−0.9162) SER142 (4.0984)
5	ASN90 (−0.9996) SER12 (−0.3889) GLY9 (−0.145) THR190 (1.0804)			PHE192 (−1.0039) ILE14 (−0.9349) CYS185 (−0.1611)	TYR155 (−0.5008) LYS159 (−0.2892) SER142 (−0.2522)
6	VAL188 (−1.233) GLY186 (−0.7452) TYR155 (−0.6104) SER142 (−0.2699) THR190 (0.5399)			PHE192 (−1.5739) ILE14 (−1.0434)	ASN90 (−0.9337) PHE226 (−0.238) THR140 (−0.2162) PRO187 (1.0923)
8	VAL188 (−1.1105) ASN90 (−0.4933) THR190 (0.7801)	TYR155 (−0.4568)		PHE192 (−1.5267) ILE14 (−1.0426)	THR140 (−0.3635) LYS159 (−0.2711) SER142 (−0.0517)
9	GLY94 (−0.9894) GLY92 (−0.811) SER11 (−0.6928) SER12 (9.0552)	LYS195 (−1.1394) TYR155 (−1.0113)	PHE192 (5.8101)	LEU93 (−1.8663) ILE14 (−1.7045) VAL196 (−1.0286) ALA106 (−0.3699) LEU95 (1.3807)	ASN90 (−2.3374) ALA91 (−1.5627) ARG37 (−0.5212) SER142 (−0.3955) CYS185 (−0.3436)
14	ASN90 (−0.8782) GLY92 (−0.822) SER12 (−0.5469)	ARG37 (−1.3972) SER11 (−0.4518) LYS159 (−0.3494)		PHE192 (−1.2886) ALA91 (−0.6477) VAL113 (−0.4438) VAL66 (−0.273)	LEU93 (−0.5816) THR140 (−0.3649) TYR155 (−0.3056)
15	GLY92 (−0.5283) GLY9 (−0.4326)	ARG37 (−1.8678) SER12 (−0.5819) THR190 (−0.2891)	PHE192 (−1.547)	VAL113 (−0.8616) ALA91 (−0.383) VAL66 (−0.2579)	LEU93 (−0.6114) ARG67 (−0.4835) ALA191 (−0.1625) ASP65 (−0.0007)



Interactions

- 701: LEU
- 704: LEU
- 705: ASN
- 707: LEU
- 711: GLN
- 749: MET
- 752: ARG
- 764: PHE
- 780: MET
- 873: LEU
- 876: PHE
- 877: THR
- 880: LEU
- 891: PHE

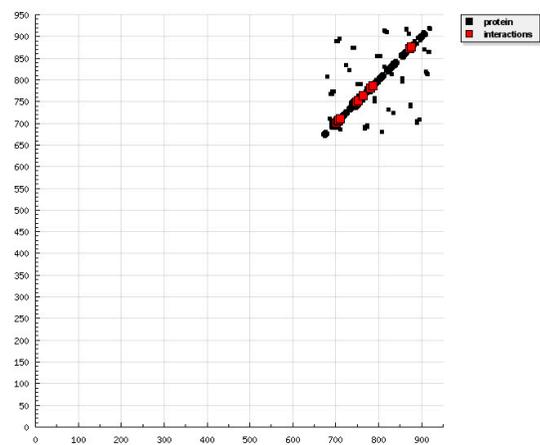


Interactions

- 701: LEU
- 704: LEU
- 705: ASN
- 707: LEU
- 742: MET
- 764: PHE
- 873: LEU
- 876: PHE
- 877: THR
- 880: LEU
- 891: PHE

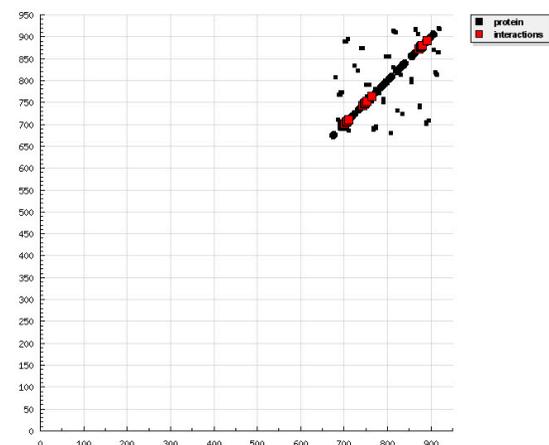
Compound 3 with 2q7k

Compound 4 with 2q7k



Interactions

- 701: LEU
- 704: LEU
- 705: ASN
- 707: LEU
- 711: GLN
- 741: TRP
- 742: MET
- 745: MET
- 746: VAL
- 749: MET
- 752: ARG
- 764: PHE
- 780: MET
- 787: MET
- 873: LEU
- 876: PHE



Interactions

- 701: LEU
- 704: LEU
- 705: ASN
- 707: LEU
- 711: GLN
- 742: MET
- 746: VAL
- 749: MET
- 752: ARG
- 764: PHE
- 873: LEU
- 876: PHE
- 877: THR
- 880: LEU
- 891: PHE

Compound 5 with 2q7k

Compound 6 with 2q7k

Figure 3. Cont.

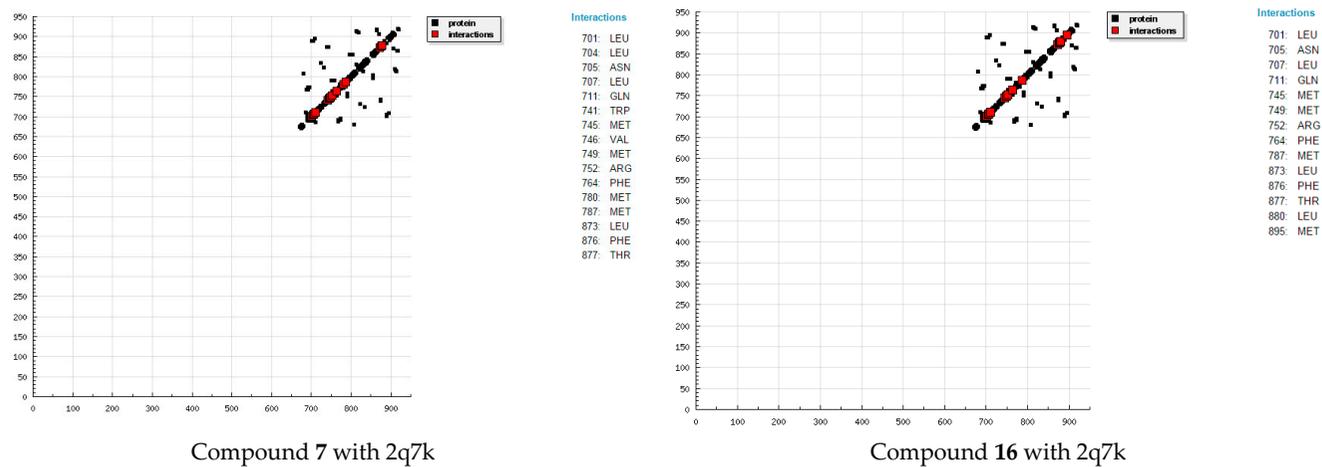


Figure 3. HB plots of interaction between some synthesized compounds and prostate cancer 2q7k hormone.

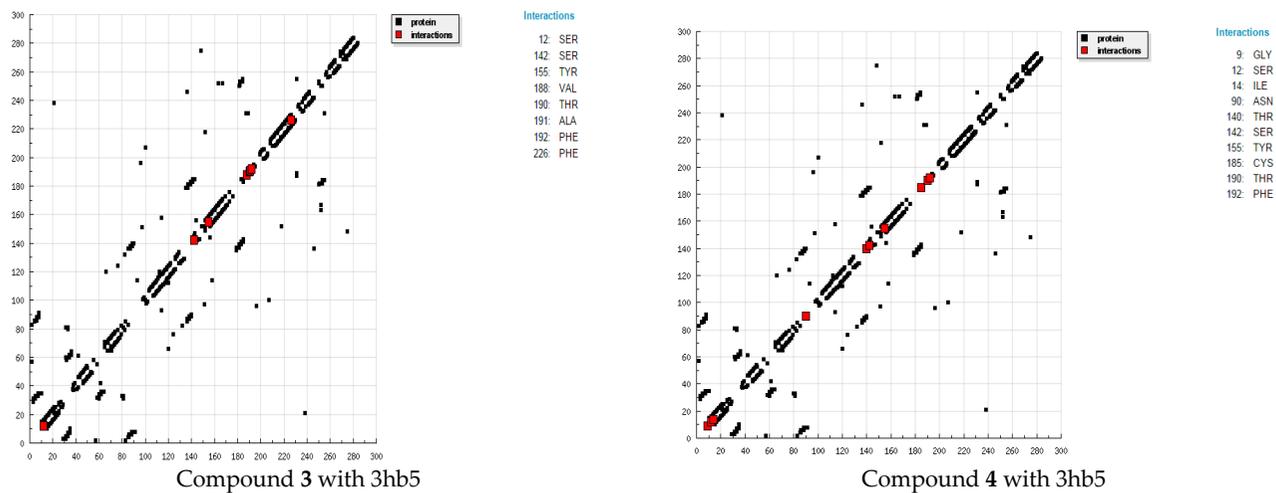


Figure 4. Cont.

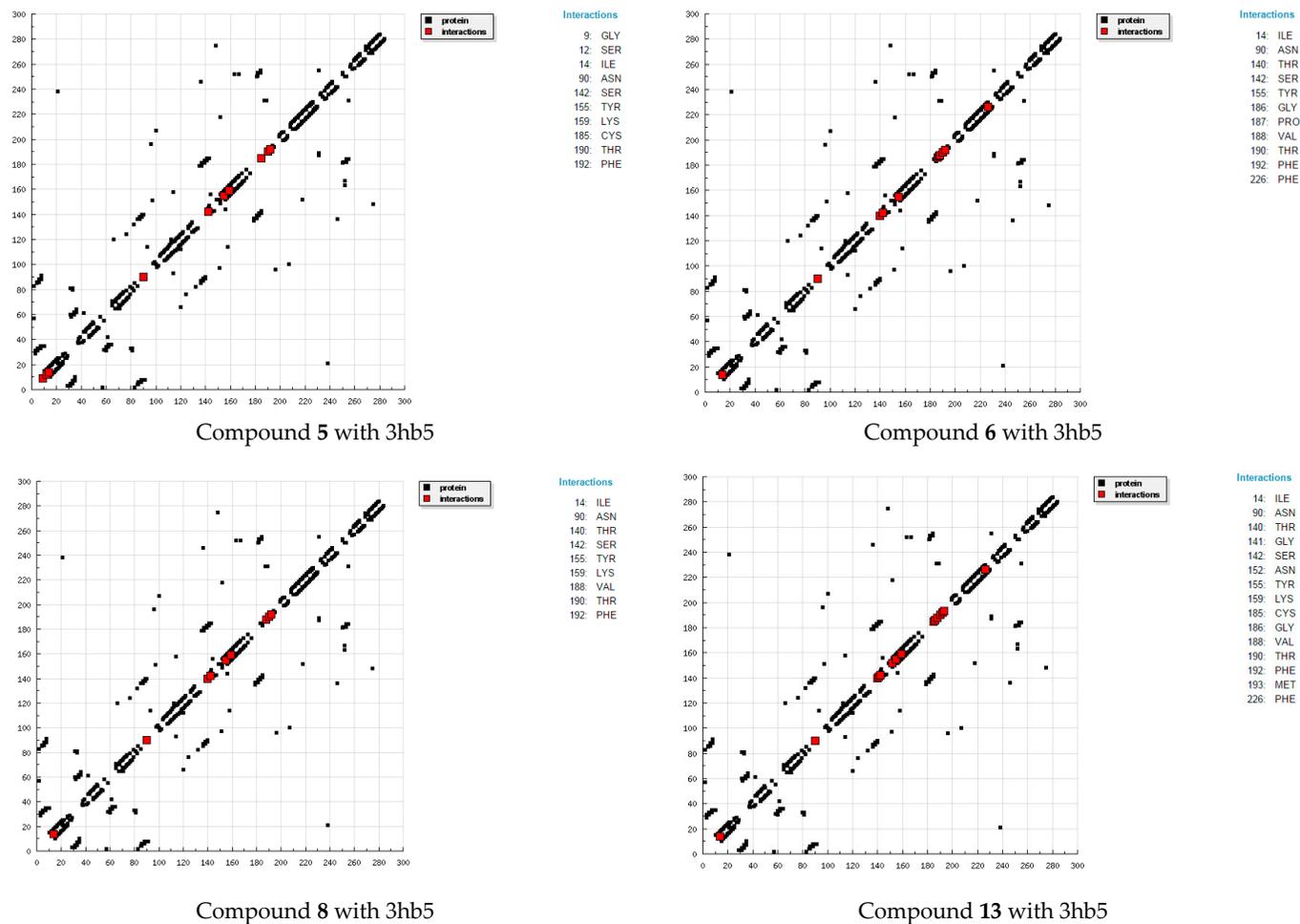
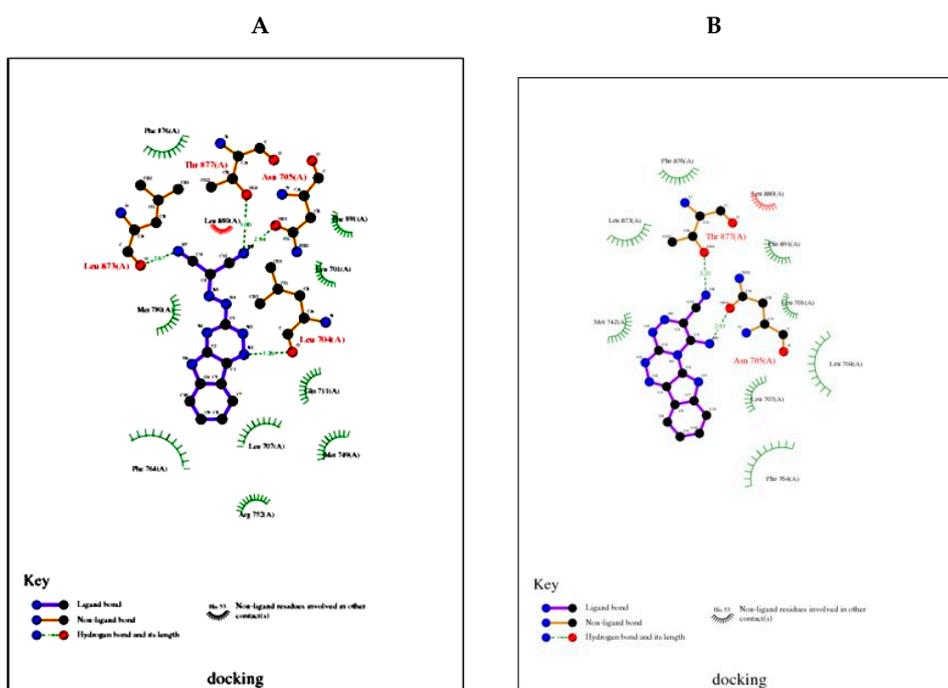
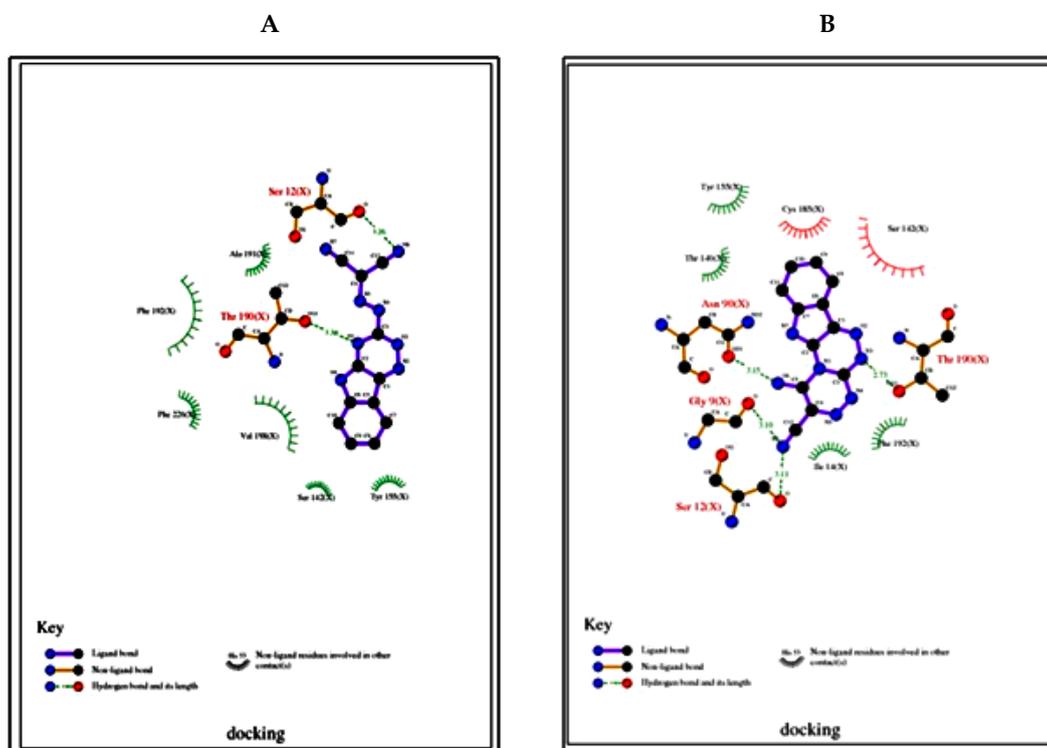


Figure 4. HB plots of interaction between some synthesized compounds and breast cancer (3hb5) hormone.



**Figure 5.** (A). 2D plot of interaction between the compound 3 receptor prostate cancer (2q7k); (B). 2D plot of interaction between the compound 4 and receptor prostate cancer (2q7k).



**Figure 6.** (A). 2D plot of interaction between the compound 3 and receptor breast cancer (3hb5); (B). 2D plot of interaction between the compound 4 and receptor breast cancer (3hb5).

## 5. Materials and Methods

### 5.1. General Information

All chemicals were purchased from Sigma-Aldrich (Taufkirchen, Germany). The melting points were measured by a digital Electrothermal IA 9100 Series apparatus Cole-Parmer, Beacon Road, Stone, Staffordshire, ST15 OSA, UK) and were uncorrected. IR spectra were recorded on an ATRAlpha FTIR spectrophotometer (Billerica, MA, USA) from 400 to 4000  $\text{cm}^{-1}$ .  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  spectra were recorded on an AC-850 Hz instrument (Bruker, Billerica, Massachusetts). Chemical shifts were expressed as (ppm) relative to TMS as an internal standard, and DMSO- $d_6$  was used as the solvent. Mass spectra were recorded on a GC-MS-QP 1000 EX spectrometer (Shimadzu, Kyoto, Japan). The pharmacological study was carried out at Al-Azhar University, The Regional Center for Mycology & Biotechnology, Elemental analyses were performed at the Micro-analytical Center of Cairo University.

*[4H-[1,2,4]Triazino[5,6-b]indol-3-yl]diazanylmalononitrile (3)*. 3-Amino-4H-[1,2,4]triazino[5,6-b]indole (1.85 g, 0.01 mol) was dissolved in conc. HCl (6 mL) and cooled to 4 °C, then  $\text{NaNO}_2$  (0.7 g, 0.01 mol) dissolved in  $\text{H}_2\text{O}$  (5 mL) was added to the previous solution, and the prepared mixture was added to a stirred cold solution of malononitrile (0.66 g, 0.01 mol) and sodium acetate (2 g) in  $\text{H}_2\text{O}$  (15 mL). After stirring 15 min a yellow precipitate was formed, filtered off and washed with water, dried and washed with ethanol, yield, 85%, m.p.: 278–280 °C. IR: 3201  $\text{cm}^{-1}$  (NH), 2210, 2217  $\text{cm}^{-1}$  (2CN) and 1618  $\text{cm}^{-1}$  (C=N).  $^1\text{H-NMR}$  (DMSO- $d_6$ ):  $\delta$  = 6.93 (s, H,  $\text{CH}(\text{CN})_2$ ), 7.01 (dd, 1H,  $\text{C}_8\text{H}$ ), 7.92 (d, 1H,  $J$  = 15.6 Hz,  $\text{C}_9\text{H}$ ), 7.32 (dd, 1H,  $\text{C}_7\text{H}$ ), 7.70 (d, 1H,  $J$  = 15.0 Hz,  $\text{C}_6\text{H}$ ), 11.42 (s, 1H, NH).  $^{13}\text{C-NMR}$  (DMSO- $d_6$ ):  $\delta$  = 73.51 (CH), 110.6 (CN), 119.4, 120.7, 121.6, 122.3, 126.5, 132.0, 137.0, 142.9, 156.0, and 161.7 (Ar-C, C=C and C=N). Anal. Calcd. for  $\text{C}_{12}\text{H}_6\text{N}_8$  (262.23): C, 54.96; H, 2.31; N, 42.73; Found: C, 54.79; H, 2.12; N, 42.65. LCMS (ESI)  $m/z$  (int. %) (263 (9.1)) ( $\text{M} + \text{H}^+$ ); calcd. for ( $\text{C}_{12}\text{H}_6\text{N}_8$ ) (262.23): 262 (82.8), 195 (78.9), 168 (100), 140 (47.4), 113 (44.6), 71 (54.6), 69 (48.9), 57 (100), 55 (77.9).

*1-Amino[1,2,4]triazino[3',4':3,4][1,2,4]triazino[5,6-b]indole-2-carbonitrile (4)*. Compound 3 (2.62 g, 0.01 mol) was refluxed in ethanol (20 mL) for 3 h during which the yellow precipitate was converted into red crystals, which were filtered off and washed with ethanol. Yield, 94%, m.p.: 310–312 °C. IR: 3351–3313  $\text{cm}^{-1}$  ( $\text{NH}_2$ ), 2209  $\text{cm}^{-1}$  (CN) and 1623  $\text{cm}^{-1}$  (C=N).  $^1\text{H-NMR}$  (DMSO- $d_6$ ):  $\delta$  = 6.90 (s, 2H,  $\text{NH}_2$ ), 7.09 (dd, 1H,  $\text{C}_8\text{H}$ ), 7.52 (dd, 1H,  $\text{C}_7\text{H}$ ), 7.57 (d, 1H,  $J$  = 12.4 Hz,  $\text{C}_9\text{H}$ ), 7.84 (d, 1H,  $J$  = 15.6 Hz,  $\text{C}_6\text{H}$ ).  $^{13}\text{C-NMR}$  (DMSO- $d_6$ ):  $\delta$  = 111.5 (CN), 111.6, 113.0, 118.6, 121.2, 122.9, 125.8, 137.8, 146.5, 150.5, 152.1, and 163.7 (Ar-C, C=C and C=N). Anal. Calcd. for  $\text{C}_{12}\text{H}_6\text{N}_8$  (262.23): C, 54.96; H, 2.31; N, 42.73; Found: C, 54.81; H, 2.21; N, 42.69. LCMS (ESI)  $m/z$  (int. %) (263 (2.9)) ( $\text{M} + \text{H}^+$ ); calcd. for [( $\text{C}_{12}\text{H}_6\text{N}_8$ ) (262.23)], 262 (82.8), 195 (100), 169 (49.4), 168 (66.4), 167 (41.6), 140 (61.6), 115 (20.5), 113 (43.3), 87 (25.5), 62 (23.1), 51 (10.9), 50 (13.3).

*Ethyl (2-cyano[1,2,4]triazino[3',4':3,4][1,2,4]triazino[5,6-b]indol-1-yl)imidofornate (5)*. A solution of compound 4 (2.62 g, 0.01 mol) in triethyl orthoformate (10 mL) was refluxed for 1 h, and the yellow-orange powder that was formed was filtered, dried, and crystallized from methanol. Yield, 62%, m.p.: 217–219 °C. IR: 3001–2890  $\text{cm}^{-1}$  (CH), 2211  $\text{cm}^{-1}$  (CN) and 1620  $\text{cm}^{-1}$  (C=N).  $^1\text{H-NMR}$  (DMSO- $d_6$ ):  $\delta$  = 1.26 (t, 3H,  $J$  = 7.1 Hz,  $\text{CH}_3$ ), 3.51 (q, 2H,  $J$  = 12.4 Hz,  $\text{CH}_2$ ), 7.11 (dd, 1H,  $\text{C}_8\text{H}$ ), 7.54 (dd, 1H,  $\text{C}_7\text{H}$ ), 7.58 (d, 1H,  $J$  = 12.8 Hz,  $\text{C}_9\text{H}$ ), 7.85 (d, 1H,  $J$  = 15.4 Hz,  $\text{C}_6\text{H}$ ).  $^{13}\text{C-NMR}$  (DMSO- $d_6$ ):  $\delta$  = 13.77 ( $\text{CH}_3$ ), 63.02 ( $\text{CH}_2$ ), 119.3 (CN), 120.2, 120.8, 121.1, 128.6, 132.6, 132.8, 140.6, 149.3, 151.9, 159.8, 160.2 and 162.5 (Ar-C, C=C and C=N). Anal. Calcd. for  $\text{C}_{15}\text{H}_{10}\text{N}_8\text{O}$  (318.29): C, 56.60; H, 3.17; N, 35.20; Found: C, 56.40; H, 3.02; N, 35.13. LCMS (ESI)  $m/z$  (int. %) (318 (52.1)) ( $\text{M} + \text{H}^+$ ); calcd. for [( $\text{C}_{15}\text{H}_{10}\text{N}_8\text{O}$ ) (318.29)], 203 (30.7), 175 (92.5), 161 (16.9), 133 (23.4), 132 (24.9), 119 (17.9), 105 (25.6), 104 (100), 103 (22.0), 90 (17.4), 78 (21.0), 77 (37.7), 76 (28.3), 65 (12.4), 64 (9.8), 63 (12.4), 58 (14.7), 51 (20.6), 50 (15.6).

*3(4H)-Amino-4-iminopyrimido[4'',5'':5',6']][1,2,4]triazino[3',4':3,4][1,2,4]triazino[5,6-b]indole (6)*. A mixture of compound 5 (3.18 g, 0.01 mol) and hydrazine hydrate (3 mL (excess), 80%) in DMF (10 mL) was

refluxed for 4 h. A yellow precipitate that formed after cooling which filtered off, dried, and crystallized from DMF. Yield, 54%, m.p.: 298–300 °C. IR: 3381–3174  $\text{cm}^{-1}$  ( $\text{NH}_2$ , H), and 1621  $\text{cm}^{-1}$  ( $\text{C}=\text{N}$ ).  $^1\text{H-NMR}$  ( $\text{DMSO-}d_6$ ):  $\delta$  = 5.83 (s, 2H,  $\text{NH}_2$ ), 7.09 (dd, 1H,  $\text{C}_8\text{H}$ ), 7.52 (dd, 1H,  $\text{C}_7\text{H}$ ), 7.57 (d, 1H,  $J$  = 12.8 Hz,  $\text{C}_9\text{H}$ ), 7.83 (d, 1H,  $J$  = 15.4 Hz,  $\text{C}_6\text{H}$ ), 10.13 (s, 1H, NH).  $^{13}\text{C-NMR}$  ( $\text{DMSO-}d_6$ ):  $\delta$  = 120.2, 120.8, 121.1, 128.6, 132.6, 132.8, 140.6, 145.3, 149.3, 151.9, 159.8, 160.2 and 162.5 (Ar-C, C=C and C=N). Anal. Calcd. for  $\text{C}_{13}\text{H}_8\text{N}_{10}$  (304.27): C, 51.32; H, 2.65; N, 46.03; Found: C, 51.13; H, 2.49; N, 46.13. LCMS (ESI)  $m/z$  (int. %): (304 (0.9)) ( $\text{M} + \text{H}^+$ ); calcd. for [ $\text{C}_{13}\text{H}_8\text{N}_{10}$ ] (304.27), 252 (4.4), 224 (1.8), 196 (2.3), 149 (3.1), 141 (1.9), 127 (2.5), 113 (4.1), 111 (3.0), 99 (8.2), 97 (8.1), 96 (2.3), 85 (27.9), 83 (12.5), 82 (4.3), 71 (51.5), 69 (19.2), 57 (100), 56 (17.3), 55 (36.5).

(2-Cyano[1,2,4]triazino[3',4':3,4][1,2,4]triazino[5,6-*b*]indol-1-yl)carbamodithioic acid (7). A mixture of compound 4 (2.62 g, 0.01 mol), carbon disulfide (1.0 mL) and potassium hydroxide (0.06 g, 0.01 mol) in ethanol (20 mL) was heated under reflux for 3 h. After cooling, the mixture was poured onto water and then acidified with dilute HCl. The red solid obtained was filtered off, dried, and then crystallized from ethanol to give red crystals. Yield, 85%, m.p.: 307–309 °C. IR: 3287  $\text{cm}^{-1}$  (NH), 2589  $\text{cm}^{-1}$  (SH), 2209  $\text{cm}^{-1}$  (CN), 1618  $\text{cm}^{-1}$  ( $\text{C}=\text{N}$ ), and 1340  $\text{cm}^{-1}$  ( $\text{C}=\text{S}$ ).  $^1\text{H-NMR}$  ( $\text{DMSO-}d_6$ ):  $\delta$  = 7.12 (dd, 1H,  $\text{C}_8\text{H}$ ), 7.57 (dd, 1H,  $\text{C}_7\text{H}$ ), 7.59 (d, 1H,  $J$  = 12.8 Hz,  $\text{C}_9\text{H}$ ), 7.82 (d, 1H,  $J$  = 15.4 Hz,  $\text{C}_6\text{H}$ ), 10.86 (s, 1H, NH), 13.98 (s, 1H, SH).  $^{13}\text{C-NMR}$  ( $\text{DMSO-}d_6$ ):  $\delta$  = 117.3 (CN), 119.5, 120.8, 121.3, 125.4, 127.1, 132.0, 136.8, 152.6, 156.8, 159.0, 161.3 and 174.4 (Ar-C, C=C, C=N and C=S). Anal. Calcd. for  $\text{C}_{13}\text{H}_6\text{N}_8\text{S}_2$  (338.37): C, 46.14; H, 1.79; N, 33.12; S, 18.95; Found: C, 45.95; H, 1.64; N, 33.01; S, 18.77. LCMS (ESI)  $m/z$  (int. %): (338 (9.1)) ( $\text{M} + \text{H}^+$ ); calcd for [ $\text{C}_{13}\text{H}_6\text{N}_8\text{S}_2$ ] (338.37), 262 (4.0), 192 (2.3), 157 (11.5), 129 (9.14), 104 (21.8), 103 (32.9), 96 (11.9), 90 (14.8), 88 (13.5), 76 (67.5), 63 (100), 59 (37.9).

Pyrimido[4'',5'':5',6'] [1,2,4]triazino[3',4':3,4][1,2,4]triazino[5,6-*b*]indol-2,4(1H,3H)-dithione (8). Method A: A mixture of compound 7 (0.34 g, 0.001 mol) in ethanolic sodium ethoxide (20 mL, 0.07 g, 0.001 mol) was refluxed for 8 h. After cooling, the mixture was poured onto water and acidified with HCl. The orange yellow solid formed was collected by filtration and then crystallized from dimethylformamide to give compound 8. Yield, 74%, m.p.: over 300 °C. Method B: Compound 4 (0.26 g, 0.001 mol) and carbon disulfide (2 mL) in dry pyridine (15 mL) was refluxed for 15 h. After cooling, the mixture was poured onto water and then acidified with HCl. The obtained precipitate was collected by filtration and then crystallized from dimethylformamide to yield 8. Yield 67%, m.p. over 300 °C. IR: 3392–3347  $\text{cm}^{-1}$  (2NH), 1623  $\text{cm}^{-1}$  ( $\text{C}=\text{N}$ ), and 1351–1250  $\text{cm}^{-1}$  (2C=S).  $^1\text{H-NMR}$  ( $\text{DMSO-}d_6$ ): 6.93 (d, 1H,  $J$  = 15.0 Hz,  $\text{C}_9\text{H}$ ), 7.01 (dd, 1H,  $\text{C}_8\text{H}$ ), 7.31 (dd, 1H,  $\text{C}_7\text{H}$ ), 7.69 (d, 1H,  $J$  = 14.4 Hz,  $\text{C}_6\text{H}$ ), 11.40 (s, 1H, NH), 12.50 (s, 1H, NH).  $^{13}\text{C-NMR}$  ( $\text{DMSO-}d_6$ ):  $\delta$  = 110.9, 119.5, 120.8, 121.7, 122.4, 126.6, 132.0, 133.3, 137.0, 142.9, 156.0, 161.8, and 164.0 (Ar-C, C=C, C=N and C=S). Anal. Calcd. for  $\text{C}_{13}\text{H}_6\text{N}_8\text{S}_2$  (338.37): C, 46.14; H, 1.79; N, 33.12; S, 18.95; Found: C, 46.01; H, 1.69; N, 32.94; S, 18.78.

LCMS (ESI)  $m/z$  (int. %) (338 (26.5)) ( $\text{M} + \text{H}^+$ ); calcd. for ( $\text{C}_{13}\text{H}_6\text{N}_8\text{S}_2$ ) (338.37), 203 (25.5), 175 (82.3), 161 (14.8), 133 (21.3), 132 (20.5), 119 (16.2), 118 (12.2), 105 (24.8), 104 (100), 90 (17.4), 78 (23.0), 77 (42.4), 76 (32.4), 75 (12.5), 64 (11.8), 63 (14.8), 58 (16.8), 57 (11.9), 51 (26.1), 50 (19.2).

2,4-Diaminopyrimido[4'',5'':5',6'] [1,2,4]triazino[3',4':3,4][1,2,4]triazino[5,6-*b*]indole (9). A mixture of 4 (0.26 g, 0.001 mol) and urea and/or thiourea (0.001 mol) was refluxed in glacial AcOH (20 mL) containing HCl (1 mL) for 3 h. After cooling the reaction mixture was poured onto cold water and then neutralized with ammonia solution. The precipitate formed was filtered off and then crystallized from methanol to give orange-red crystals. Yield, 57% from urea and 72% from thiourea, m.p.: over 300 °C. IR: 3413–3761  $\text{cm}^{-1}$  (2 $\text{NH}_2$ ), and 1620  $\text{cm}^{-1}$  ( $\text{C}=\text{N}$ ).  $^1\text{H-NMR}$  ( $\text{DMSO-}d_6$ ):  $\delta$  = 5.55 (s, 2H,  $\text{NH}_2$ ), 5.67 (s, 2H,  $\text{NH}_2$ ), 6.94 (d, 1H,  $J$  = 15.6 Hz,  $\text{C}_9\text{H}$ ), 7.02 (dd, 1H,  $\text{C}_8\text{H}$ ), 7.28 (dd, 1H,  $\text{C}_7\text{H}$ ), 7.70 (d, 1H,  $J$  = 14.4 Hz,  $\text{C}_6\text{H}$ ).  $^{13}\text{C-NMR}$  ( $\text{DMSO-}d_6$ ):  $\delta$  = 110.9, 111.2, 119.5, 120.6, 121.7, 122.4, 132.1, 137.0, 142.9, 156.0, 157.3, 160.8, and 161.8 (Ar-C, C=C, and C=N). Anal. Calcd. for  $\text{C}_{13}\text{H}_8\text{N}_{10}$  (304.27): C, 51.32; H, 2.65; N, 46.03; Found: C, 51.18; H, 2.56; N, 45.89. LCMS (ESI)  $m/z$  (int. %) (304 (76.5)) ( $\text{M} + \text{H}^+$ ); calcd.

for (C<sub>13</sub>H<sub>8</sub>N<sub>10</sub>) (304.27), 203 (16.8), 175 (42.3), 157 (6.95), 146 (3.48), 133 (11.9), 132 (12.6), 119 (10.44), 104 (57.9), 103 (20.7), 90 (11.6), 77 (29.5), 76 (100), 63 (11.2), 59 (17.9), 51 (16.6).

**3-Amino-1H-pyrazolo[3'',4'':5',6']][1,2,4]triazino[3',4':3,4][1,2,4]triazino[5,6-b]indole (10).** Compound 4 (0.26 g, 0.001 mol) was refluxed for 2 h with hydroxylamine hydrochloride (0.007 g, 0.001 mol) in glacial acetic acid (20 mL) containing anhydrous sodium acetate (0.08 g, 0.001 mol) as a catalyst. After cooling the reaction mixture was poured onto cold water to give an orange-yellow precipitate which filtered, dried and crystallized from ethanol. Yield, 81%, m.p.: 297–299 °C. IR: 3392–3754 cm<sup>-1</sup> (NH<sub>2</sub>), and 1621 cm<sup>-1</sup> (C=N). <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>): δ = 6.10 (s, 2H, NH<sub>2</sub>), 6.92 (d, 1H, *J* = 15.0 Hz, C<sub>9</sub>H), 7.02 (dd, 1H, C<sub>8</sub>H), 7.30 (dd, 1H, C<sub>7</sub>H), 7.68 (d, 1H, *J* = 14.4 Hz, C<sub>6</sub>H), 12.33 (s, 1H, NH). <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>): δ = 117.9, 121.4, 121.7, 128.3, 132.8, 135.2, 139.4, 139.7, 140.5, 149.3, 159.1 and 159.9 (Ar-C, C=C, and C=N). Anal. Calcd. for C<sub>12</sub>H<sub>7</sub>N<sub>9</sub> (277.24): C, 51.99; H, 2.54; N, 45.47; Found: C, 52.12; H, 2.50; N, 45.32. LCMS (ESI) *m/z* (int. %) (277 (53.5)) (M + H)<sup>+</sup>; calcd. for (C<sub>12</sub>H<sub>7</sub>N<sub>9</sub>) (277.24), 203 (30.4), 176 (10.3), 175 (100), 161 (21.1), 133 (18.7), 132 (20.5), 119 (12.2), 105 (16.8), 104 (63.7), 103 (12.7), 90 (11.2), 78 (13.3), 77 (23.7), 76 (16.4), 51 (12.9).

**2-Aminopyrimidol[4'',5'':5',6']][1,2,4]triazino[3',4':3,4][1,2,4]triazino[5,6-b]indol-4(3H)-thione (11).** A mixture of compound 4 (0.26 g, 0.001 mol) and ammonium thiocyanate (0.23 g, 0.003 mol) in acetic acid (20 mL) was refluxed for 9 h. After cooling, the mixture was poured onto cold water; the solid thus separated was filtered off, dried, and crystallized from ethanol. Yield, 74%, m.p.: over 286–288 °C. IR: 3401–3811 cm<sup>-1</sup> (NH<sub>2</sub>), 1621 cm<sup>-1</sup> (C=N) and 1349 cm<sup>-1</sup> (C=S). <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>): δ = 6.93 (s, 2H, NH<sub>2</sub>), 6.91 (d, 1H, *J* = 15.6 Hz, C<sub>9</sub>H), 7.02 (dd, 1H, C<sub>8</sub>H), 7.32 (dd, 1H, C<sub>7</sub>H), 7.68 (d, 1H, *J* = 14.4 Hz, C<sub>6</sub>H), 11.12 (s, 1H, NH). <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>): δ = 117.9, 121.4, 121.7, 128.3, 132.8, 135.2, 139.4, 139.7, 140.5, 149.3, 159.1 and 159.9 (Ar-C, C=C, and C=N). Anal. Calcd. for C<sub>13</sub>H<sub>7</sub>N<sub>9</sub>S (321.32): C, 48.59; H, 2.20; N, 39.23; S, 9.98; Found: C, 48.42; H, 2.07; N, 39.11; S, 9.78. LCMS (ESI) *m/z* (int. %) (321 (56.4)) (M + H)<sup>+</sup>; calcd for (C<sub>13</sub>H<sub>7</sub>N<sub>9</sub>S) (321.32), 203 (22.7), 175 (66.5), 161 (11.7), 133 (17.7), 132 (17.1), 119 (13.8), 118 (11.3), 104 (100), 103 (25.4), 91 (10.0), 90 (20.4), 77 (51.2), 76 (42.2), 63 (18.8), 59 (22.8), 58 (20.6), 50 (25.9).

**1-(β-D-Glucopyranosymethylamino)[1,2,4]triazino[3',4':3,4][1,2,4]triazino[5,6-b]indol-2-carbonitrile (12).** A mixture of compound 4 (0.26 g, 0.001 mol) and glucose (0.18 g, 0.001 mol) in *tert*-butanol (15 mL) was refluxed for 5 h. After cooling, the formed yellow-orange crystals were filtered off, dried, and crystallized from ethanol. Yield, 71%, m.p.: 281–283 °C. IR: 3439 cm<sup>-1</sup> (OH), 3210 m<sup>-1</sup> (NH), 3011–2859 cm<sup>-1</sup> (CH, aromatic and aliphatic), 2210 cm<sup>-1</sup> (CN), 1621 cm<sup>-1</sup> (C=N). <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>): δ = 3.32–4.99 (m, 4H, 4CH), 3.93 (s, 2H, CH<sub>2</sub>), 4.01 (s, 1H, NH), 4.62–4.78 (m, 5H, 5OH), 6.95 (d, 1H, *J* = 16.2 Hz, C<sub>9</sub>H), 7.03 (dd, 1H, C<sub>8</sub>H), 7.33 (dd, 1H, C<sub>7</sub>H), 7.70 (d, 1H, *J* = 14.4 Hz, C<sub>6</sub>H), 5.12 (s, 1H, NH). <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>): δ = 52.66 (CH<sub>2</sub>), 71.61, 72.00, 75.90, 93.55, 101.2 (sp<sup>3</sup> carbons, CHOH), 111.0, 119.32 (CN), 120.7, 121.8, 125.3, 127.3, 132.9, 136.2, 145.2, 152.3, 156.2, and 161.1 (Ar-C, C=C, and C=N). Anal. Calcd. for C<sub>18</sub>H<sub>16</sub>N<sub>8</sub>O<sub>6</sub> (440.37): C, 49.09; H, 3.66; N, 25.45; Found: C, 48.91; H, 3.51; N, 25.40.

**1-(2,3,4,5,6-Pentahydroxyhexylideneamino)[1,2,4]triazino[3',4':3,4][1,2,4]triazino[5,6-b]indol-2-carbonitrile (13).** A mixture of 4 (0.26 g, 0.001 mol) and glucose (0.18 g, 0.001 mol) in acetic acid/water (15/5 mL) was refluxed for 8 h. After cooling, the resulting solution was poured onto cold water. The red solution was extracted with CH<sub>2</sub>Cl<sub>2</sub> (30 mL). After evaporation of the CH<sub>2</sub>Cl<sub>2</sub> a pale red solid resulted which was filtered off, dried, and crystallized from ethanol. Yield, 52%, m.p.: 268–270 °C. IR: 3432 cm<sup>-1</sup> (OH), 2992–2860 cm<sup>-1</sup> (CH, aromatic and aliphatic), 2208 cm<sup>-1</sup> (CN), 1618 cm<sup>-1</sup> (C=N). <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>): δ = 3.44–5.11 (m, 4H, 4CH), 4.73–4.93 (m, 2H, CH<sub>2</sub>), 4.30–4.81 (m, 5H, 5OH), 6.94 (d, 1H, *J* = 15.0 Hz, C<sub>9</sub>H), 7.03 (dd, 1H, C<sub>8</sub>H), 7.31 (dd, 1H, C<sub>7</sub>H), 7.34 (d, 1H, CH=N), 7.70 (d, 1H, *J* = 14.4 Hz, C<sub>6</sub>H). <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>): δ = 63.51, 69.21, 69.58, 71.17, 72.63 (sp<sup>3</sup> carbons, CHOH, CH<sub>2</sub>), 122.6 (CN), 122.7, 1123.8, 125.4, 127.2, 133.3, 135.2, 140.5, 155.0, 155.6, 160.2, 163.2 and 164.3 (Ar-C, C=C, and C=N). Anal. Calcd. for C<sub>18</sub>H<sub>16</sub>N<sub>8</sub>O<sub>5</sub> (424.37): C, 50.94; H, 3.80; N, 26.40; Found: C, 50.81; H, 3.67; N, 26.33.

4-Aminopyrimido[4''5'':5',6'] [1,2,4]triazino[3',4':3,4] [1,2,4]triazino[5,6-b]indole (**14**). Compound **4** (0.26 g, 0.001 mol) was refluxed for 3 h in formamide (10 mL). After cooling, a reddish brown precipitate was formed. The precipitate was collected by filtration, dried, and crystallized from ethanol. Yield, 41%, m.p.: over 300 °C. IR: 3392–3352 cm<sup>-1</sup> (NH<sub>2</sub>), 1619 cm<sup>-1</sup> (C=N). <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>): δ = 6.81 (s, 2H, NH<sub>2</sub>), 6.91 (d, 1H, *J* = 15.6 Hz, C<sub>9</sub>H), 7.02 (dd, 1H, C<sub>8</sub>H), 7.32 (dd, 1H, C<sub>7</sub>H), 7.68 (d, 1H, *J* = 14.4 Hz, C<sub>6</sub>H). <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>): δ = 117.2, 120.6, 121.3, 127.01, 129.6, 132.8, 139.6, 146.2, 156.4, 156.7, 159.8, 160.3 and 162.7 (Ar-C, C=C, and C=N). Anal. Calcd. for C<sub>13</sub>H<sub>7</sub>N<sub>9</sub> (289.25): C, 53.98; H, 2.44; N, 43.58; Found: C, 53.72; H, 2.34; N, 43.41.

2,4-Diaminopyrimido[2'',3'':5',6'] [1,2,4]triazino[3',4':3,4] [1,2,4]triazino[5,6-b]indol-3-carbonitrile (**15**). A suspension of compound **4** (0.26 g, 0.001 mol) in ethanol (20 mL) was treated with malononitrile (0.06 g, 0.001 mol) with triethylamine as a catalyst. The mixture was refluxed for 10 h. After cooling, an orange precipitate formed which was filtered off and dried. Crystallization from dioxane resulted compound **15**. Yield, 81%, m.p.: over 300 °C. IR: 3395–3355 cm<sup>-1</sup> (2NH<sub>2</sub>), 2203 cm<sup>-1</sup> (CN), 1622 cm<sup>-1</sup> (C=N). <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>): δ = 6.10 (s, 2H, NH<sub>2</sub>), 6.59 (s, 2H, NH<sub>2</sub>), 6.93 (d, 1H, *J* = 15.6 Hz, C<sub>9</sub>H), 7.03 (dd, 1H, C<sub>8</sub>H), 7.31 (dd, 1H, C<sub>7</sub>H), 7.70 (d, 1H, *J* = 14.4 Hz, C<sub>6</sub>H). <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>): δ = 75.23, 115.6 (CN), 117.6, 120.3, 121.8, 127.5, 127.9, 132.5, 139.3, 145.1, 156.1, 159.4, 162.5, 165.2 and 165.9 (Ar-C, C=C, and C=N). Anal. Calcd. for C<sub>15</sub>H<sub>8</sub>N<sub>10</sub> (328.29): C, 54.88; H, 2.46; N, 42.67; Found: C, 54.77; H, 2.31; N, 42.52. LCMS (ESI) *m/z* (int. %) (328 (67.2)) (M + H)<sup>+</sup>; calcd. for (C<sub>15</sub>H<sub>8</sub>N<sub>10</sub>) (328.29), 309 (43.6), 308 (26.9), 294 (16.4), 111 (10.5), 104 (10.1), 97 (22.5), 96 (11.0), 95 (16.6), 87 (10.0), 85 (26.5), 83 (33.6), 81 (22.1), 76 (22.1), 73 (27.5), 67 (23.6), 60 (25.0), 59 (21.7), 57 (100), 56 (24.9), 55 (78.7), 51 (13.1), 50 (16.4).

*N,N'*-bis(2-Cyano[1,2,4]triazino[3',4':3,4] [1,2,4]triazino[5,6-b]indol-1-yl)decanediamide (**16**). A mixture of **4** (0.26 g, 0.001 mol), sebacoyl chloride (0.24 g, 0.21 mL, 1 mmol) and a catalytic amount of TEA in DMF (20 mL) was refluxed for 5 h. The reaction was cooled and poured onto ice water. The solid was filtered off and crystallized from ethanol to result in **16** as orange-yellow crystals. Yield 67%, m.p. 284–286 °C. IR: 3271–3284 cm<sup>-1</sup> (2NH), 2203–2210 cm<sup>-1</sup> (2CN), 1674–1661 cm<sup>-1</sup> (2C=O), 1624 cm<sup>-1</sup> (C=N). <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>): δ = 1.01 (m, 4H, 2CH<sub>2</sub>), 1.13 (m, 4H, 2CH<sub>2</sub>), 2.20 (m, 4H, 2CH<sub>2</sub>), 3.39 (t, 4H, 2COCH<sub>2</sub>), 6.94 (d, 2H, *J* = 15.6 Hz, 2C<sub>9</sub>H), 7.07 (dd, 2H, 2C<sub>8</sub>H), 7.34 (dd, 2H, 2C<sub>7</sub>H), 7.83 (d, 2H, *J* = 15.6 Hz, 2C<sub>6</sub>H), 11.37 (s, 2H, 2NH). <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>): δ = 25.36, 26.89, 28.40, 35.27 (4CH<sub>2</sub>), 106.7 (CN), 111.7, 119.5, 121.7, 122.8, 125.7, 132.1, 137.8, 146.9, 150.6, 156.0, 161.9 and 163.6 (Ar-C, C=C, and C=N). Anal. Calcd. for C<sub>34</sub>H<sub>26</sub>N<sub>16</sub>O<sub>2</sub> (690.68): C, 59.13; H, 3.79; N, 32.45; Found: C, 58.93; H, 3.65; N, 32.32.

[1,2,4]Triazolol[1''',5''':1'',6'']pyrimido[4''5'':5',6'] [1,2,4]triazino[3',4':3,4] [1,2,4]triazino[5,6-b]indol-2(1H)-thione (**17**). To a stirred solution of compound **6** (0.30 g, 0.001 mol) in ethanol (20 mL), potassium hydroxide (0.06 g, 0.001 mol) and CS<sub>2</sub> (1.5 mL) were added. The mixture was then refluxed for 9 h and then the solvent evaporated. After that the residue obtained was dissolved in water and neutralized with dil. HCl. The solid thus separated filtered off and crystallized from ethanol to give red crystals. Yield, (86%), m.p.: 297–299 °C. IR: 3266 cm<sup>-1</sup> (NH), 1624 cm<sup>-1</sup> (C=N), 1338 cm<sup>-1</sup> (C=S). <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>): δ = 6.91 (d, 1H, *J* = 15.6 Hz, C<sub>9</sub>H), 7.02 (dd, 1H, C<sub>8</sub>H), 7.32 (dd, 1H, C<sub>7</sub>H), 7.68 (d, 1H, *J* = 14.4 Hz, C<sub>6</sub>H), 9.69 (s, 1H, CH<sub>pyrimidine</sub>), 11.30 (s, 1H, NH). <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>): δ = 120.1, 121.8, 122.0, 128.2, 128.5, 140.3, 141.5, 144.9, 147.2, 149.0, 151.3, 160.2, 161.5 and 172.3 (Ar-C, C=C, C=N and C=S). Anal. Calcd. for C<sub>14</sub>H<sub>6</sub>N<sub>10</sub>S (346.33): C, 48.55; H, 1.75; N, 40.44; S, 9.26; Found: C, 48.43; H, 1.66; N, 40.31; S, 9.03.

2-(Methylthio)[1,2,4]triazolol[1''',5''':1'',6'']pyrimido[4''5'':5',6'] [1,2,4]triazino[3',4':3,4] [1,2,4]triazinoindole (**18**). To a mixture of compound **17** (0.35 g, 0.001 mol) and sodium acetate (1.63 g, 0.02 mol) in ethanol (20 mL) was added the methyl iodide (0.14 g, 0.062 mL, 0.001 mol), then the reaction mixture was refluxed for 5 h. After cooling the solid thus formed was filtered off, washed with water, and crystallized from the ethanol to give yellowish powder of **18**. Yield, (86%), m.p.: 283–285 °C. IR: 3001–2871 cm<sup>-1</sup> (CH aromatic and aliphatic), 1618 cm<sup>-1</sup> (C=N). <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>): δ = 3.01 (s, 3H,

SCH<sub>3</sub>), 6.93 (d, 1H, *J* = 15.6 Hz, C<sub>9</sub>H), 7.04 (dd, 1H, C<sub>8</sub>H), 7.33 (dd, 1H, C<sub>7</sub>H), 7.71 (d, 1H, *J* = 14.4 Hz, C<sub>6</sub>H), 9.62 (s, 1H, CH<sub>pyrimidine</sub>). <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>): δ = 13.5 (SCH<sub>3</sub>), 120.6, 121.7, 122.2, 128.1, 128.9, 132.8, 137.8, 140.4, 141.1, 149.4, 151.8, 160.0, 161.6 and 162.0 (Ar-C, C=C, C=N and C-S). Anal. Calcd. for C<sub>15</sub>H<sub>8</sub>N<sub>10</sub>S (360.36): C, 50.00; H, 2.24; N, 38.87; S, 8.90; Found: C, 49.86; H, 2.16; N, 38.77; S, 8.62.

1-(Piperidin-1-ylmethyl)[1,2,4]triazolo[1''',5''':1'',6'']pyrimido[4'',5'':5',6']][1,2,4]triazino-[3',4':3,4][1,2,4]-triazino[5,6-*b*]indol-2(1H)-thione (**19**). A mixture of **17** (0.35 g, 0.001 mol), aqueous formaldehyde (36%, 1 mL), methanol (25 mL) and piperidine (0.085 g, 0.1 mL, 0.001 mol) was stirred at room temperature for about 3 h. The solid product separated was filtered off and crystallized from dioxane. Yield, (53%), m.p.: over 300 °C. IR: 2994–2841 cm<sup>-1</sup> (CH aromatic and CH aliphatic), 1624 cm<sup>-1</sup> (C=N), 1347 cm<sup>-1</sup> (C=S). <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>): δ = 1.35–2.35 (m, 10H, piperidine protons), 5.50 (s, 2H, N-CH<sub>2</sub>), 6.91 (d, 1H, *J* = 15.6 Hz, C<sub>9</sub>H), 7.03 (dd, 1H, C<sub>8</sub>H), 7.31 (dd, 1H, C<sub>7</sub>H), 7.68 (d, 1H, *J* = 14.4 Hz, C<sub>6</sub>H), 8.62 (s, 1H, CH<sub>pyrimidine</sub>). <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>): δ = 24.03, 25.73, 50.23, 51.43 (4 CH<sub>2</sub>), 120.1, 121.7, 122.1, 128.9, 132.7, 138.3, 138.9, 140.5, 141.2, 147.6, 149.2, 150.7, 160.0 and 162.0 (Ar-C, C=C, C=N and C=S). Anal. Calcd. for C<sub>20</sub>H<sub>17</sub>N<sub>11</sub>S (443.49): C, 54.16; H, 3.86; N, 34.74; S, 7.23; Found: C, 53.83; H, 3.71; N, 34.66; S, 7.12.

[1,2,4]Triazolo[1''',5''':1'',6'']pyrimido[4'',5'':5',6']][1,2,4]triazino[3',4':3,4][1,2,4]triazino[5,6-*b*]indole (**20**). *Method A*: A mixture of compound **6** (0.30 g, 0.001 mol) and triethyl orthoformate (4 mL) in dimethylformamide (10 mL) was refluxed for 3 h. After cooling the mixture poured onto ice cold water (50 mL), the solid produced was filtered off and crystallized from ethanol to give **19** as yellowish powder. Yield, (54%), m.p.: over 300 °C. *Method B*: A mixture of compound **6** (0.30 g, 0.001 mol) and formamide (5 mL) in dimethylformamide (15 mL) was refluxed for 4 h. After cooling the solution poured onto ice/water (40 mL), the resulted solid was filtered off and crystallized from ethanol to give **19** as yellowish powder. Yield, (46%), m.p.: over 300 °C. IR: 3001–2912 cm<sup>-1</sup> (CH aromatic), 1622 cm<sup>-1</sup> (C=N). <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>): δ = 6.92 (d, 1H, *J* = 15.6 Hz, C<sub>9</sub>H), 7.04 (dd, 1H, C<sub>8</sub>H), 7.32 (dd, 1H, C<sub>7</sub>H), 7.70 (d, 1H, *J* = 14.4 Hz, C<sub>6</sub>H), 8.41 (s, 1H, CH<sub>triazole</sub>), 8.72 (s, 1H, CH<sub>pyrimidine</sub>). <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>): δ = 120.1, 121.8, 122.1, 126.2, 128.9, 132.8, 137.1, 139.5, 140.2, 149.2, 151.7, 153.2, 160.0 and 162.6 (Ar-C, C=C, and C=N). Anal. Calcd. for C<sub>14</sub>H<sub>6</sub>N<sub>10</sub> (314.26): C, 53.51; H, 1.92; N, 44.57; Found: C, 53.35; H, 1.81; N, 44.52.

[1,2,4]Triazolo[1''',5''':1'',6'']pyrimido[4'',5'':5',6']][1,2,4]triazino[3',4':3,4][1,2,4]triazino[5,6-*b*]indol-2(1H)-one (**21**). A mixture of **6** (0.30 g, 0.001 mol) and ethyl chloroformate (0.11 g, 0.001 mol) in DMF (15 mL) and Et<sub>3</sub>N was stirred for 30 min at room temperature, then the mixture refluxed for 3 h, cooled, poured onto ice water and the solid formed was filtered off and crystallized from ethanol. Yield, 64%, m.p.: 291–293 °C. IR: 3276 cm<sup>-1</sup> (NH), 1654 cm<sup>-1</sup> (C=O), and 1624 cm<sup>-1</sup> (C=N). <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>): δ = 6.94 (d, H, *J* = 15.6 Hz, C<sub>9</sub>H), 7.07 (dd, H, C<sub>8</sub>H), 7.34 (dd, H, C<sub>7</sub>H), 7.83 (d, H, *J* = 15.6 Hz, C<sub>6</sub>H), 8.65 (s, 1H, CH<sub>pyrimidine</sub>). <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>): δ = 120.1, 121.7, 122.1, 128.7, 132.5, 135.6, 137.5, 140.9, 146.2, 149.2, 149.7, 160.1, 162.4, and 163.9 (Ar-C, C=C, C=N and C=O). Anal. Calcd. for C<sub>14</sub>H<sub>6</sub>N<sub>10</sub>O (330.26): C, 50.91; H, 1.83; N, 42.41; Found: C, 50.64; H, 1.70; N, 42.31.

Tetrazolo[1''',5''':1'',6'']pyrimido[4'',5'':5',6']][1,2,4]triazino[3',4':3,4][1,2,4]triazino[5,6-*b*]indole (**22**). Compound **6** (0.30 g, 0.001 mol) dissolved in acetic acid and conc. HCl mixture (1:1, 8 mL), the solution cooled to 4 °C, sodium nitrite (0.069 g, 0.001 mol, in 5 mL H<sub>2</sub>O) was added and the mixture stirred for 1 h, the solid formed filtered, washed with water, dried, and crystallized from dioxane. Yield, 42%, m.p.: over 300 °C. IR: 1619 cm<sup>-1</sup> (C=N). <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>): δ = 6.92 (d, H, *J* = 15.6 Hz, C<sub>9</sub>H), 7.03 (dd, H, C<sub>8</sub>H), 7.31 (dd, H, C<sub>7</sub>H), 7.79 (d, H, *J* = 15.6 Hz, C<sub>6</sub>H), 8.69 (s, 1H, CH<sub>pyrimidine</sub>). <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>): δ = 120.1, 121.7, 122.1, 128.9, 131.6, 132.5, 136.9, 138.2, 140.1, 149.0, 152.8, 160.0, and 162.4 (Ar-C, C=C, C=N and C=O). Anal. Calcd. for C<sub>13</sub>H<sub>5</sub>N<sub>11</sub> (315.25): C, 49.53; H, 1.60; N, 48.87; Found: C, 49.39; H, 1.48; N, 48.76.

### 5.2. Antitumor Activity Assay

The tested human carcinoma cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). The cells were grown on RPMI-1640 medium supplemented with 10% heat inactivated fetal calf serum, 1% L-glutamine, and 50 µg/mL gentamycin at 37 °C in a humidified atmosphere with 5% CO<sub>2</sub> incubator (Shel lab 2406, Candler, NC, USA).

For antitumor assays, the tumor cell lines were suspended in medium at concentration  $5 \times 10^4$  cell/well in Corning® 96-well tissue culture plates, then incubated for 24 h. The tested compounds were then added into 96-well plates (three replicates) to achieve ten concentrations for each compound (started from 500 to 1 µg/mL). Six vehicle controls with media or 0.1% DMSO were run for each 96 well plate as a control. After incubating for 24 h, the numbers of viable cells were determined by the MTT assay [35]. Briefly, the media was removed from the 96 well plate and replaced with 100 µL of fresh culture RPMI 1640 medium without phenol red then 10 µL of the 12 mM MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (Sigma, Taufkirchen, Germany) was added to each well including the untreated controls. The 96 well plates were then incubated at 37 °C and 5% CO<sub>2</sub> for 4 h. An 85 µL aliquot of the media was removed from the wells, and 50 µL of DMSO was added to each well and mixed thoroughly with the pipette and incubated at 37 °C for 10 min. Then, the optical density was measured at 590 nm with the microplate reader (SunRise, TECAN, Inc., Männedorf, Switzerland) to determine the number of viable cells and the percentage of viability was calculated as  $[(OD_t/OD_c) \times 100\%]$  where OD<sub>t</sub> is the mean optical density of wells treated with the tested sample and OD<sub>c</sub> is the mean optical density of untreated cells. The relation between surviving cells and drug concentration is plotted to get the survival curve of each tumor cell line after treatment with the specified compound. The 50% inhibitory concentration (IC<sub>50</sub>), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose response curve for each conc. using Graphpad Prism software (San Diego, CA, USA) [36].

### 5.3. Theoretical Molecular Docking Techniques

The docking calculations were carried out using Docking Server [32]. The MMFF94 force field [31,32] was used for energy minimization of compound molecules and protein models using Docking Server. Gasteiger partial charges were added to the compound atoms. Non-polar hydrogen atoms were merged, and rotatable bonds were defined. Docking calculations were carried out on 2q7k and 3hb5 protein models. Essential hydrogen atoms, Kollman united atom type charges and solvation parameters were added with the aid of AutoDock tools [27]. Affinity (grid) maps of 20 µg/mL × 20 µg/mL × 20 Å<sup>3</sup> grid points and 0.375 Å spacing were generated using the Autogrid program [34]. AutoDock parameter set- and distance-dependent dielectric functions were used in the calculation of van der Waals and electrostatic terms, respectively. Docking simulations were performed using the Lamarckian genetic algorithm and the Solis and Wets local search method [37–40]. Initial position, orientation and torsions of the compound molecules were set randomly. Each docking experiment was derived from 10 different runs that were set to terminate after a maximum of 250,000 energy evaluations. The population size was set to 150. During the search, a translation step of 0.2 Å and quaternion and torsion steps of 5 were applied.

## 6. Conclusions

In conclusion, the anticancer screening showed not all the tested compounds exhibited a good result, except compound **16** which exhibited good cytotoxic activities against the three tested carcinoma cell lines (HepG2, MCF-7, and HCT-116) compared with the reference drug cisplatin. This result confirms that increasing of the compound volume is not necessarily effective in producing more active anticancer compounds. The effectiveness of compound **16** may be due to the octa-CH<sub>2</sub> chain separating two bulky five-ring compounds. Molecular docking modeling was performed on these studied fused

triazino compounds against the receptor of prostate cancer 2q7k and breast cancer 3hb5 to get a chance to compare between theoretical and practical results.

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## References

1. Saad, H.A.; Youssef, M.M.; Mosselhi, M.A. Microwave Assisted Synthesis of Some New Fused 1,2,4-Triazines Bearing Thiophene Moieties with Expected Pharmacological Activity. *Molecules* **2011**, *16*, 4937–4957. [[CrossRef](#)] [[PubMed](#)]
2. Saad, H.A.; Moustafa, A.H. Synthesis and Anticancer Activity of Some New S-Glycosyl and S-Alkyl 1,2,4-Triazinone Derivatives. *Molecules* **2011**, *16*, 5682–5700. [[CrossRef](#)] [[PubMed](#)]
3. Saad, H.A. Synthesis of novel fused heterocyclic compounds from 7-amino-[1,2,4]triazino [3,4-*b*][1,3,4]thiadiazine-8-carbonitrile. *Curr. Org. Synth.* **2012**, *9*, 573–582. [[CrossRef](#)]
4. Aly, M.R.E.; Saad, H.A.; Hafez, S.H.A. Three-Component Process for the Synthesis of Pyrimido[2,1-*c*] [1,2,4]triazine Derivatives via Knoevenagel Condensation under Thermal Aqueous Conditions. *Curr. Org. Synth.* **2015**, *12*, 208–219.
5. Aly, M.R.E.; Gobouri, A.A.; Hafez, S.H.A.; Saad, H.A. Synthesis, Reactions and Biological Activity of some Triazine Derivatives Containing Sulfa Drug Moieties. *Russ. J. Bioorg. Chem.* **2015**, *4*, 491–504.
6. Amin, M.A.; Saad, H.A. Synthesis and Biological Activity of Fused Heteropolycyclic Systems Containing an Indole Moiety. *Curr. Org. Synth.* **2016**, *13*, 116–125. [[CrossRef](#)]
7. AlHarthi, R.R.; Ali, R.S.; Amin, M.A.; Saad, H.A. Synthesis of Some New Fused 1,2,4-Triazines of Expected Antimicrobial Activity. *Russ. J. Gen. Chem.* **2016**, *86*, 2906–2913.
8. El-Nahass, M.M.; Ashour, A.; Atta, A.A.; Saad, H.A.; Hassanien, A.M.; AlBaradi, A.M.; El-Zaidia, E.F.M. Dielectric relaxation and optical properties of 4-amino-3-mercapto-6-(2-(2thienyl)vinyl)-1,2,4-triazin-5(4*H*)-one donor. *Pramana J. Physics* **2017**, *88*, 6. [[CrossRef](#)]
9. Shehab, W.S.; Saad, H.A.; Mouneir, S.M. Synthesis and Antitumor/Antiviral Evaluation of 6-Thienyl-5-cyano-2-thiouracil Derivatives and Their Thiogalactosides Analogs. *Curr. Org. Synth.* **2017**, *14*, 291–298. [[CrossRef](#)]
10. Al Osaimi, A.G.; Ali, R.S.; Saad, H.A.; El Sayed Aly, M.R. Synthesis, Reactions and Biological Activity of Some New Derivatives of Fused [1,2,4]triazino[5,6-*b*]indole. *Russ. J. Gen. Chem.* **2017**, *87*, 1246–1255. [[CrossRef](#)]
11. Anderson, E.A. Cascade polycyclisations in natural product synthesis. *Org. Biomol. Chem.* **2011**, *9*, 3997–4006. [[CrossRef](#)] [[PubMed](#)]
12. Dancik, V.; Seiler, K.P.; Young, D.W.; Schreiber, S.L.; Clemons, P.A. Distinct Biological Network Properties between the Targets of Natural Products and Disease Genes. *J. Am. Chem. Soc.* **2010**, *132*, 9259–9261. [[CrossRef](#)] [[PubMed](#)]
13. Liu, W.; Khedkar, V.; Baskar, B.; Schürmann, M.; Kumar, K. Branching Cascades: A Concise Synthetic Strategy Targeting Diverse and Complex Molecular Frameworks. *Angew. Chem. Int. Ed.* **2011**, *50*, 6900–6905. [[CrossRef](#)] [[PubMed](#)]
14. Salem, M.S.; Sakr, S.I.; El-Senousy, W.M.; Madkour, H.M.F. Synthesis, antibacterial, and antiviral evaluation of new heterocycles containing the pyridine moiety. *Arch. Pharm. (Weinheim)* **2013**, *346*, 766–773. [[CrossRef](#)] [[PubMed](#)]
15. El-salam, N.M.A.; Mostafa, M.S.; Ahmed, G.A.; Alothman, O.Y. Synthesis and Antimicrobial Activities of Some New Heterocyclic Compounds Based on 6-Chloropyridazine-3 (2*H*)-thione. *J. Chem.* **2013**, *2013*, 1–8. [[CrossRef](#)]
16. Azab, M.E.; Youssef, M.M.; El-Bordany, E.A. Synthesis and antibacterial evaluation of novel heterocyclic compounds containing a sulfonamido moiety. *Molecules* **2013**, *18*, 832–844. [[CrossRef](#)] [[PubMed](#)]

17. El-Sawy, E.R.; Ebaid, M.S.; Abo-Salem, H.M.; Al-Sehemi, A.G.; Mandour, A.H. Synthesis, anti-inflammatory, analgesic and anticonvulsant activities of some new 4,6-dimethoxy-5(heterocycles)benzofuran starting from naturally occurring visnagin. *Arab. J. Chem.* **2013**, *7*, 914–923. [[CrossRef](#)]
18. Cao, X.; Sun, Z.; Cao, Y.; Wang, R.; Cai, T.; Chu, W.; Hu, W.; Yang, Y. Design, Synthesis, and Structure—Activity Relationship Studies of Novel Fused Heterocycles-Linked Triazoles with Good Activity and Water Solubility. *J. Med. Chem.* **2014**, *57*, 3687–3706. [[CrossRef](#)] [[PubMed](#)]
19. Chen, Y.; Yu, K.; Tan, N.Y.; Qiu, R.H.; Liu, W.; Luo, N.L.; Tong, L.; Au, C.T.; Luo, Z.Q.; Yin, S.F. Synthesis, characterization and anti-proliferative activity of heterocyclic hypervalent organoantimony compounds. *Eur. J. Med. Chem.* **2014**, *79*, 391–398. [[CrossRef](#)] [[PubMed](#)]
20. El-Sawy, E.R.; Mandour, A.H.; El-Hallouty, S.M.; Shaker, K.H.; Abo-Salem, H.M. Synthesis, antimicrobial and anticancer activities of some new *N*-methylsulphonyl and *N*-benzenesulphonyl-3-indolyl heterocycles. 1st Cancer Update. *Arab. J. Chem.* **2013**, *6*, 67–78. [[CrossRef](#)]
21. Mabkhot, Y.N.; Barakat, A.; Al-Majid, A.M.; Alshahrani, S.; Yousuf, S.; Choudhary, M.I. Synthesis, reactions and biological activity of some new *bis*-heterocyclic ring compounds containing sulphur atom. *Chem. Cent. J.* **2013**, *7*, 112–120. [[CrossRef](#)] [[PubMed](#)]
22. Bergman, J.; Koch, E.; Pelcman, B. 2,2'-Biindolyl revisited. Synthesis and reactions. *Tetrahedron* **1995**, *51*, 5631–5642. [[CrossRef](#)]
23. Gribble, G.W.; Pelcman, B. Total syntheses of the marine sponge pigments faspaplysin and homofaspaplysin B and C. *J. Org. Chem.* **1992**, *57*, 3636–3642. [[CrossRef](#)]
24. Carter, D.S.; Vranken, D.L.V. Synthesis of Homofaspaplysin C and Indolo[2,3-*a*]carbazole from Dityryptophans. *J. Org. Chem.* **1999**, *64*, 8537–8545. [[CrossRef](#)]
25. Segraves, N.L.; Robinson, S.J.; Garcia, D.; Said, S.A.; Fu, X.; Schmitz, F.J.; Pietraszkiewicz, H.; Valeriote, F.A.; Crews, P. Comparison of Faspaplysin and Related Alkaloids: A Study of Structures, Cytotoxicities, and Sources. *J. Nat. Prod.* **2004**, *67*, 783–792. [[CrossRef](#)] [[PubMed](#)]
26. Dubovitskii, S.V. Method for synthesis of 12*H*-pyrido[1,2-*a*:3,4-*b'*]diindoles. Total synthesis of homofaspaplysin C. *Tetrahedron Lett.* **1996**, *37*, 5207–5208. [[CrossRef](#)]
27. Lawrie, A.M.; Noble, M.E.M.; Tunnah, P.; Brown, N.R.; Johnson, L.N.; Endicott, J.A. Protein kinase inhibition by staurosporine revealed in details of the molecular interaction with CDK2. *Nat. Struct. Mol. Biol.* **1997**, *4*, 796–801. [[CrossRef](#)]
28. Sasaki, T.; Ohtani, I.I.; Tanaka, J.; Higa, T. Iheyamines, new cytotoxic bisindole pigments from a colonial ascidian, *Polycitorella* sp. *Tetrahedron Lett.* **1999**, *40*, 303–306. [[CrossRef](#)]
29. Bush, J.A.; Long, B.H.; Catino, J.J.; Bradner, W.T.; Tomita, K. Production and biological activity of rebeccamycin, a novel antitumor agent. *J. Antibiot.* **1987**, *40*, 668–678. [[CrossRef](#)] [[PubMed](#)]
30. Tomchin, A.B.; Uryupoy, O.Y.; Smirnov, A.V. Thiourea and thiosemicarbazide derivatives: Structure, transformations, and pharmacological activity. Part II. Antihypoxic activity of 1,2,4-triazino[5,6-*b*]indole derivatives. *Pharm. Chem. J.* **1997**, *31*, 125. [[CrossRef](#)]
31. Bikadi, Z.; Hazai, E. Application of the PM6 semi-empirical method to modeling proteins enhances docking accuracy of AutoDock. *J. Cheminf.* **2009**, *1*, 15. [[CrossRef](#)] [[PubMed](#)]
32. Beteringhe, A.; Racuciu, C.; Balan, C.; Stoican, E.; Patron, L. Molecular Docking Studies Involving Transitional Metal Complexes (Zn(II), Co(II), Cu(II), Fe(II), Ni(II) with Cholic Acid (AC) as Ligand against Aurora A Kinase. *Adv. Mater. Res.* **2013**, *787*, 236–240. [[CrossRef](#)]
33. Bikadi, Z.; Demko, L.; Hazai, E. Functional and structural characterization of a protein based on analysis of its hydrogen bonding network by hydrogen bonding plot. *Arch. Biochem. Biophys.* **2007**, *461*, 225–234. [[CrossRef](#)] [[PubMed](#)]
34. McDonald, I.K.; Thornton, J.M. Satisfying hydrogen bonding potential in proteins. *J. Mol. Biol.* **1994**, *238*, 777–793. [[CrossRef](#)] [[PubMed](#)]
35. Humphrey, W.; Dalke, A.; Schulten, K. VMD: Visual molecular dynamics. *J. Mol. Graph.* **1996**, *14*, 33–38. [[CrossRef](#)]
36. Halgren, T.A. Merck molecular force field. I. Basis, form, scope, parameterization, and performance of MMFF94. *J. Comput. Chem.* **1996**, *17*, 490. [[CrossRef](#)]
37. Mosmann, T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **1983**, *65*, 55–63. [[CrossRef](#)]

38. Ghorab, M.M.; Alsaied, M.S.; El-Gaby, M.S.A.; Safwat, N.A.; Elaasser, M.M.; Soliman, A.M. Biological evaluation of some new *N*-(2,6-dimethoxypyrimidinyl)thioureidobenzenesulfonamide derivatives as potential antimicrobial and anticancer agents. *Eur. J. Med. Chem.* **2016**, *124*, 299–310. [[CrossRef](#)] [[PubMed](#)]
39. Morris, G.M.; Goodsell, D.S. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. *J. Comput. Chem.* **1998**, *19*, 1639–1662. [[CrossRef](#)]
40. Solis, F.J.; Wets, R.J.B. Minimization by Random Search Techniques. *Math. Operat. Res.* **1981**, *6*, 19–30. [[CrossRef](#)]

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