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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

New approaches for the synthesis of pyrazole, thiophene, thieno[2,3-*b*]pyridine, and thiazole derivatives together with their anti-tumor evaluations

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Abstract The reaction of cyanoacetylhydrazine **(1)** with acetylchloride **(2)** gave the *N*-acyl derivative **3**. The latter underwent ready cyclization in sodium ethoxide to give the pyrazole derivative **4** which was the key compound for the synthesis of thiophene, thieno[2,3-*b*]pyridine, and thiazole derivatives. The anti-tumor evaluations of the newly synthesized products against the three human tumor cell lines, namely, breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-H460), and CNS cancer (SF-268), were studied. Some of these compounds were found to exhibit much higher inhibitory effects toward the three tumor cell lines than the reference doxorubicin. Molecular modeling of the four compounds **12c**, **12f**, **16a**, and **16d**, which showed the maximum inhibitory effect, were done.

Keywords Pyrazole · Thiophene · Thieno[2,3-*b*]pyridine · Thiazole · Anti-tumor

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Introduction

The pyrazole scaffold represents a common motif in many pharmaceutical active and remarkable compounds demonstrating a wide range of pharmacological activities; the most important activities are the anti-inflammatory (Elguero *et al*., 2002; Szabo *et al*., 2008); antibacterial and antifungal (Tanitame *et al*., 2005; Tanitame *et al*., 2004); hypoglycemic (Cho *et al*., 2009; Dugi *et al*., 2009); anti-hyperlipidemic (Momose *et al*., 2001); inhibition of cyclooxigenase-2 (Rida *et al*., 2009), p38 MAP kinase (Regan *et al*., 2002), and CDK2/Cyclin A (Brasca *et al*., 2007; Pevarello *et al*., 2006); and antiangiogenic activities (Abadi *et al*., 2003). Heterocyclic rings and, in particular, the pyrazole ring represent an advantageous choice for the synthesis of pharmaceutical compounds with different activities and good safety profiles (Michaelides, 2010; Hubbard and Till, 2000). Different pyrazole derivatives have also been tested for their antiproliferative activities in vitro and anti-tumor activity in vivo, often resulting in promising lead compounds (Ludwig *et al*., 2004; Perchellet *et al*., 2006; Insuasty *et al*., 2009; Labbozzetta *et al*., 2009; Pevarello *et al*., 2004; Sanchez and Cobo, 2010). In the view of the facts mentioned above and as part of our initial efforts to discover potentially active new agents, we have synthesized some new pyrazole derivatives. The novel derivatives were characterized by spectral data and elemental analysis. These compounds were screened against the three cancer cell lines, breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-H460), and CNS cancer (SF-268). Our data indicate that these novel pyrazole derivatives target the tumor cells. In particular, four compounds **12c**, **12f**, **16a**, and **16d** emerged as potent anti-tumor compounds, which may be used as lead compounds, but deserve further study in order to obtain insight into their mechanism of action.

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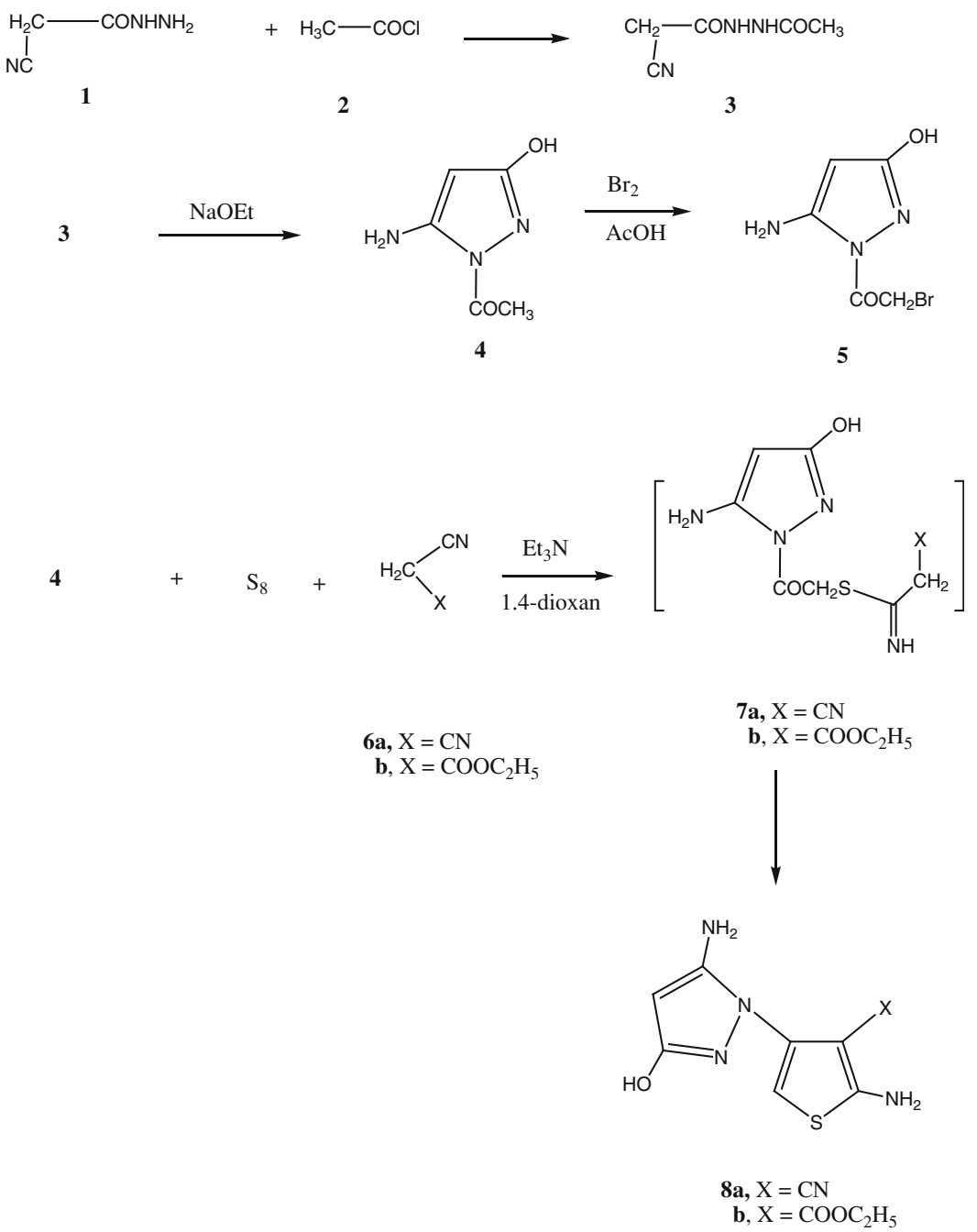
Results and discussion

Herein, in order to extend our research on anticancer heterocyclic derivatives with high inhibitory effects toward some cancer cell lines, we report the synthesis of new pyrazole derivatives starting from the *N*-acetyl-2-cyanoacetohydrazide **3**; the latter is formed through acetylation of the 2-cyanohydrazide **(1)**. Thus, compound **3** underwent cyclization in sodium ethoxide solution to give the pyrazole derivative **4**. The structure of compound **4** was based on analytical and spectral data. Thus, the ^{1}H-NMR showed a singlet at δ 2.77 ppm indicating one CH_3 group, a singlet at δ 4.79 ppm (D_2O exchangeable) indicating the presence of one NH_2 group, a singlet at δ 6.86 ppm for the pyrazole H-4, and a singlet at δ 10.22 ppm indicating one OH group. Moreover, the ^{13}C-NMR spectrum showed the presence of δ 29.6 (CH_3), 104.8, 152.3, 154.6 (pyrazole C), and 168.9 (C=O). Compound **4** reacted with bromine in acetic acid solution to give the 1-(5-amino-3-hydroxy-1*H*-pyrazol-1-yl)-bromoethanone **5**. It is of great value to note that the reaction occurred in acetic acid solution; thus, the acetyl group is an active moiety toward bromination (Sunil Kumar *et al*., 2007). However, one might expect that the C-4 of the pyrazole derivative **4** is activated by the 3-OH and the 5-NH_2 group; to our knowledge, such activity requires basic catalyzed conditions.

The *N*-acetyl group in compound **4** showed interesting reactivity towards Gewald's thiophene synthesis. Thus, its reaction with either malononitrile **(6a)** or ethyl cyanoacetate **(6b)** and elemental sulfur in the presence of triethylamine gave the thiophene derivatives **8a** and **8b**, respectively (Scheme 1). Their respective ^{1}H-NMR and ^{13}C-NMR spectra were the tools of their structural elucidation. The 2-amino group of compounds **8a** and **8b** was condensed with ethyl cyanoacetate **(6b)** in dimethylformamide to give the thiophen-2-acetamide derivatives **9a** and **9b**, respectively. The IR spectrum of compound **9a** showed the presence of two CN groups stretching at υ 2,227 and 2,220 cm^{-1} and the presence of one C=O stretching at υ 1,688 cm^{-1}. Moreover, the ^{1}H-NMR spectrum showed δ at 4.77 ppm (D_2O exchangeable) indicating the presence of one NH_2 group, a singlet at δ 4.81 ppm indicating the presence of CH_2 group, two singlets at δ 6.83 and 6.84 ppm corresponding to the pyrazole H-4 and the thiophene H-5, and two singlets at δ 8.21 and 10.08 ppm for the NH and the OH groups, respectively. The amide formation occurred at the 2-aminothiophene moiety, not at the 3-aminopyrazole moiety; this was established not only via the spectral data, indicated above, but also through the ready cyclization of the products **9a** and **9b** through the Michael addition of the CH_2 group to the 3-cyano group. Thus, both of compounds **9a** and **9b** underwent ready cyclization in sodium ethoxide solution in a boiling water bath to give the 6-hydroxythieno[2,3-*b*]pyridine-5-carbonitrile derivatives **10a** and **10b**, respectively. Such cyclization reactions will not proceed if the amide is being formed through the 2-aminopyrazole moiety present in both of compounds **8a** and **8b**.

Based on the optimized reaction conditions established above, a series of novel aryl hydrazono derivatives were obtained with the aim of improving the inhibitory effect against the tested cancer cell lines. Moreover, in order to study the relationship between the structure and activity for this type of compounds, we have studied the affects of substitution on the aryl azo compounds as well as the effect of CN group in the case of **9a** and the ester group in the case of **9b**. Thus, the reaction of either **9a** or **9b** with any of the aryldiazonium salts, namely, the benzenediazonium chloride **(11a)**, the 4-methylphenyldiazonium salt **(11b)**, or the 4-chlorophenyl diazonium salts **(11c)**, in 0–5 °C gave the aryl hydrazono derivatives **12a–f** (Scheme 2).

Encouraged by the excellent results, we next investigated the ability of compounds **9a,b** to form thiazoles and thiophene derivatives. Several studies (Bondock *et al*., 2010; Al-Said *et al*., 2011) have described the synthesis of thiazole and thiophene derivative, their importance, and applications as intermediates for the synthesis of heterocyclics (Gouda *et al*., 2010; Fadda *et al*., 2009). The present study aimed to contribute with the chemical and pharmacological studies of such group of compounds. Thus, the reaction of compound **9a** or **9b** with phenylisothiocyanate **(13)** in the presence of basic dimethylformamide gave the intermediate potassium sulfide salts **14a**,**b**, respectively. Heterocyclization of the latter intermediate with α-halocarbonyl compounds gave, interestingly, either thiazole or thiophene derivatives depending on the nature of the α-halocarbonyl compound. Thus, their reaction with either phenacyl bromide **(15a)** or chloroacetone **(15b)** gave the thiophene derivatives **16a–d**. Their ^{1}H-NMR and ^{13}C-NMR data are in agreement with their respective structures. Thus, the ^{1}H-NMR spectrum of **16a** (as an example) showed the presence of a singlet at δ 2.88 indicating the CH_3 group, two singlets of δ 4.77 and 4.82 ppm (D_2O exchangeable) indicating the presence of the two NH_2 groups, two singlets at δ 6.80 and 6.85 ppm corresponding to the pyrazole H-4 and the thiophene H-5, respectively, a multiplet at δ 7.29–7.38 ppm corresponding to the phenyl group, and three singlets at δ 8.26, 8.80, and 10.20 ppm (D_2O exchangeable) corresponding to the two NH groups and the OH group, respectively. Moreover, its ^{13}C-NMR spectrum showed δ: 31.2 (CH_3), 104.3, 153.7, 154.3 (pyrazole C), 116.9 (CN), 119.0, 120.4, 120.9, 121.8, 124.8, 130.9, 133.6, 134.0, 139.4, 141.5, 143.8, 144.6 (two thiophene, C_6H_5), 163.9, 164.8 (2C=O). On the other hand, the reaction of the intermediate potassium sulfide salts **14a** or **14b** with ethyl chloroacetate **(17)** gave the thiazole



Scheme 1 Synthesis of compounds **3–8a,b**

derivatives **18a** and **18b**, respectively. The analytical and spectral data of the latter products are in agreement with their respective structures.

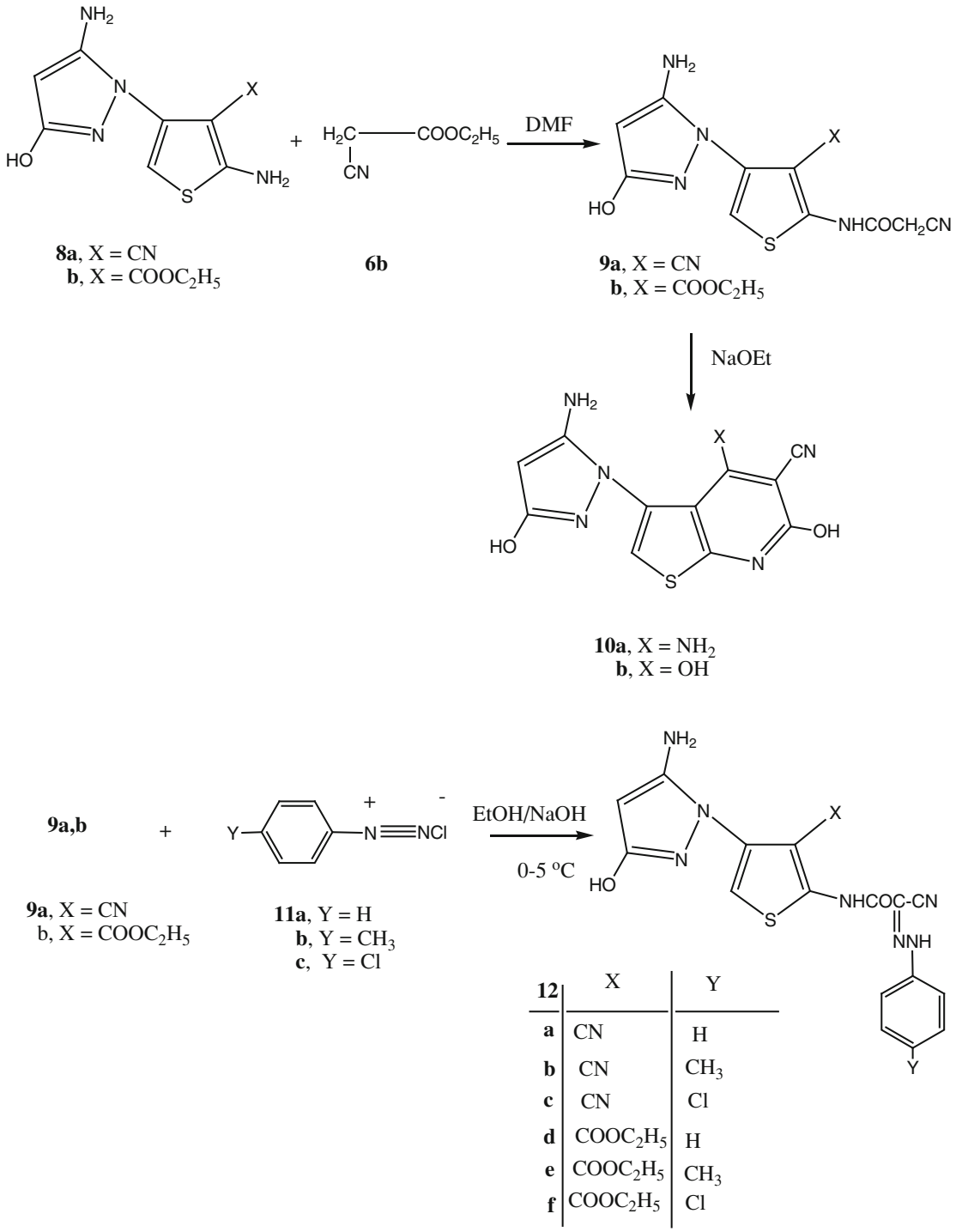
Anti-tumor activity tests

Reagents

Fetal bovine serum (FBS) and L-glutamine were from Gibco Invitrogen Co. (Scotland, UK). RPMI-1640 medium was from Cambrex (New Jersey, USA). Dimethyl sulfoxide (DMSO), doxorubicin, penicillin, streptomycin, and sulforhodamine B (SRB) were from Sigma Chemical Co. (Saint Louis, USA).

Cell cultures

Three human tumor cell lines, MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), and SF-268 (CNS cancer), were used. MCF-7 was obtained from



12	X	Y
a	CN	H
b	CN	CH_3
c	CN	Cl
d	$COOC_2H_5$	H
e	$COOC_2H_5$	CH_3
f	$COOC_2H_5$	Cl

Scheme 2 Synthesis of compounds **9a,b–12a–f**

the European Collection of Cell Cultures (ECACC, Salisbury, UK), and NCI-H460 and SF-268 were kindly provided by the National Cancer Institute (NCI, Cairo, Egypt). They grow as a monolayer and routinely maintained in RPMI-1640 medium supplemented with 5 % heat-inactivated FBS, 2 mM glutamine, and antibiotics (penicillin 100 U/mL, streptomycin 100 μg/mL) at 37 °C in a humidified atmosphere containing 5 % CO_2. Exponentially growing cells were obtained by plating 1.5×10^5 cells/mL for MCF-7 and SF-268 and 0.75×10^4 cells/mL for NCI-H460, followed by 24 h of incubation. The effect of the vehicle solvent (DMSO) on the growth of these cell lines

was evaluated in all the experiments by exposing untreated control cells to the maximum concentration (0.5 %) of DMSO used in each assay.

Tumor cell growth assay

The effects of synthesized compounds **4–18a,b** on the in vitro growth of human tumor cell lines were evaluated according to the procedure adopted by the National Cancer Institute (NCI, USA) in the "In vitro Anticancer Drug Discovery Screen" that uses the protein-binding dye sulforhodamine B to assess cell growth. Briefly, exponentially, cells growing in 96-well plates were then exposed for 48 h to five serial concentrations of each compound, starting from a maximum concentration of 150 μM. Following this exposure period, adherent cells were fixed, washed, and stained. The bound stain was solubilized and the absorbance was measured at 492 nm in a plate reader (Bio-Tek Instruments Inc., Powerwave XS, Wincoski, USA). For each test compound and cell line, a dose–response curve was obtained and the growth inhibition of 50 % (GI_{50}), corresponding to the concentration of the compounds that inhibited 50 % of the net cell growth, was calculated as described elsewhere. Doxorubicin was used as a positive control and tested in the same manner.

Effect on the growth of human tumor cell lines

The effect of compounds **4–18a,b** was evaluated on the in vitro growth of three human tumor cell lines representing different tumor types, namely, breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-H460), and CNS cancer (SF-268), after a continuous exposure for 48 h. The results are summarized in Table 1. All of the tested compounds were able to inhibit the growth of the tested human tumor cell lines in a dose-dependant manner (data not shown). The results indicated through Table 1 revealed that "compounds **8a**, **9a**, **10a**, **10b**, **12a**, **12c**, **12f**, **16a**, **16b**, **16d** and **18a** showed the highest inhibitory effect against all the three tumor cell lines." On the other hand, compounds **12c**, **12f**, **16a**, and **16d** showed high inhibitory effects against all the three human tumor cell lines, which were much higher than the reference doxorubicin. Compounds **5**, **7a**, **9b**, **12b**, **12d**, **12e, 16c**, and **18b** showed the lowest inhibitory effect. The rest of the compounds **4**, **7b**, and **8b** showed a moderate growth inhibitory effect. Comparing compound **8a**, **8b** and **9a**, **9b**, it is obvious that the presence of the CN groups in **8a**, **9a** showed a higher inhibitory effect than the CO_2Et groups in **8b**, **9b**, and **10b**. Comparing compounds **12a**, **12b**, **12c**, **12d**, **12e**, and **12f**, it is obvious that compound **12c** with $X = CN$ and $Y = Cl$ showed the highest inhibitory effect among the six compounds. Moreover, **12f** with $X = CO_2Et$ and $Y = Cl$ showed a high inhibitory effect, but it is still lower than **12c**. Similarly, for compounds **16a–d**, it is obvious that compound **16a** with the $X = CN$, $R = CH_3$ showed the highest inhibitory effect among the four compounds and **16d** with $X = CO_2Et$, $R = Ph$ showed the maximum inhibitory effect toward breast adenocarcinoma (MCF-7) among the four compounds. The cytotoxicity effects of the newly synthesized compounds toward the three cancer cell lines are indicated through Figs. 1, 2, and 3.

Moreover, the data described through Table 1 were studied through statistical ANOVA program. The statistical data are presented through Tables 2 and 3 and the statistical ANOVA diagram Fig. 4.

Experimental

All melting points were determined on an electrothermal apparatus (Büchi 535, Switzerland) in an open capillary

Table 1 Effects of the synthesized compounds **4–18a,b** on the growth of three human tumor cell lines

Compound	GI_{50} (μMmol/L)		
	MCF-7	NCI-H460	SF-268
4	8.6 ± 1.4	4.9 ± 0.8	3.8 ± 0.8
5	55.4 ± 12.2	42.1 ± 8.0	38.9 ± 8.8
7a	38.1 ± 0.6	17.3 ± 1.4	24.3 ± 1.5
7b	22.6 ± 12.2	12.6 ± 8.6	51.4 ± 14.6
8a	0.6 ± 0.04	0.3 ± 0.08	0.1 ± 0.08
8b	11.8 ± 0.6	14.5 ± 0.8	16.7 ± 1.6
9a	4.3 ± 0.8	2.0 ± 0.8	1.5 ± 0.1
9b	20.0 ± 0.2	22.6 ± 1.4	28.4 ± 0.6
10a	0.7 ± 0.50	0.2 ± 0.08	1.0 ± 0.02
10b	2.0 ± 0.6	2.0 ± 0.4	1.5 ± 8.0
12a	0.9 ± 0.2	0.1 ± 0.02	0.3 ± 0.05
12b	38.0 ± 1.8	44.0 ± 0.8	20.5 ± 1.1
12c	0.01 ± 0.003	0.02 ± 0.001	0.01 ± 0.001
12d	20.0 ± 0.2	32.6 ± 1.4	34.4 ± 0.6
12e	26.7 ± 17.5	22.2 ± 12.8	30.0 ± 9.0
12f	0.07 ± 0.05	0.02 ± 0.008	0.01 ± 0.001
16a	0.03 ± 0.007	0.02 ± 0.008	0.01 ± 0.004
16b	2.0 ± 1.2	3.6 ± 1.4	2.4 ± 0.8
16c	32.0 ± 1.8	12.0 ± 0.8	14.5 ± 4.1
16d	0.01 ± 0.006	0.03 ± 0.002	0.06 ± 0.005
18a	4.2 ± 2.6	2.2 ± 0.04	0.5 ± 0.2
18b	20.9 ± 4.9	18.6 ± 2.6	18.3 ± 2.6
Doxorubicin	0.04 ± 0.008	0.09 ± 0.008	0.09 ± 0.007

Results are given in concentrations that were able to cause 50 % of cell growth inhibition (GI_{50}) after a continuous exposure of 48 h and show mean ± SEM of three independent experiments performed in duplicate

CELL NO 1 MCF-7

CELL NO 2 NCI-H460

CELL NO 3 SF-268

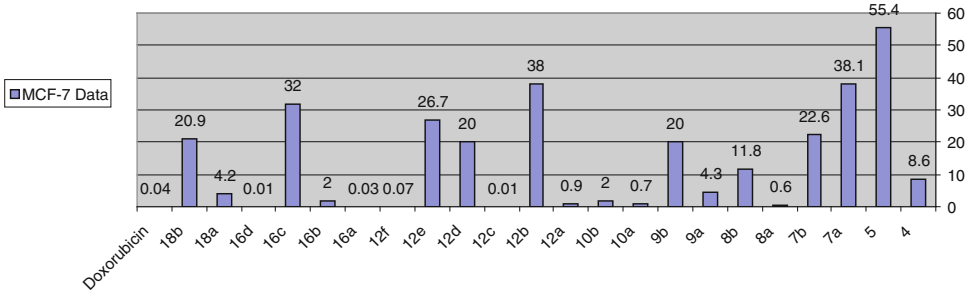


Fig. 1 Cytotoxicity of compounds **4–18a**,**b** toward MCF-7

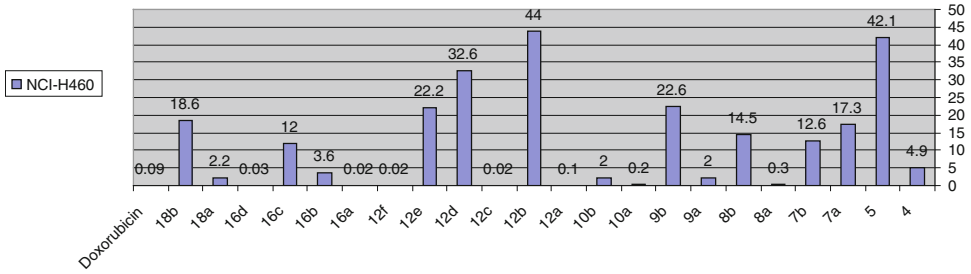


Fig. 2 Cytotoxicity of compounds **4–18a**,**b** toward NCI-H460

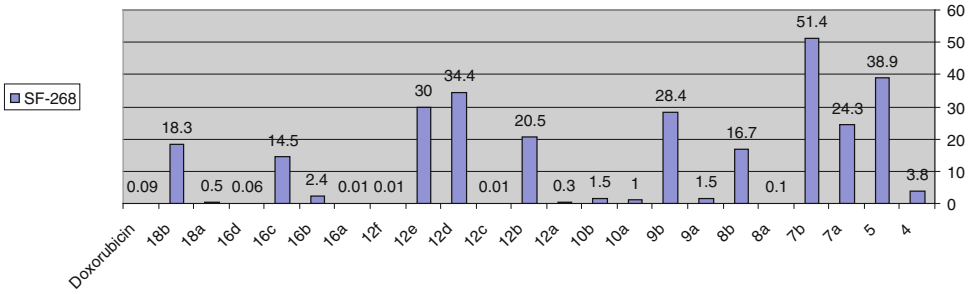


Fig. 3 Cytotoxicity of compounds **4–18a**,**b** toward SF-268

Table 2 ANOVA

Cells	Sum of squares	*df*	Mean square	*F*	Sig.
Between groups	67.220	2	33.610	0.148	0.863
Within groups	15018.954	66	227.560		
Total	15086.174	68			

Table 3 Test of homogeneity of variances

Levene statistic	*df*1	*df*2	Sig.
Cells			
0.551	2	66	0.579

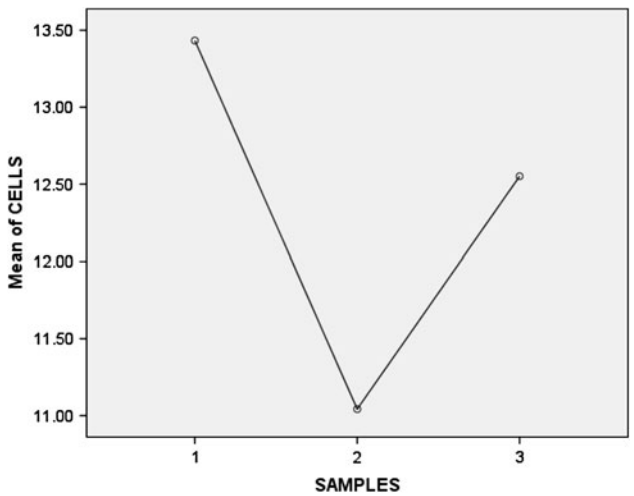
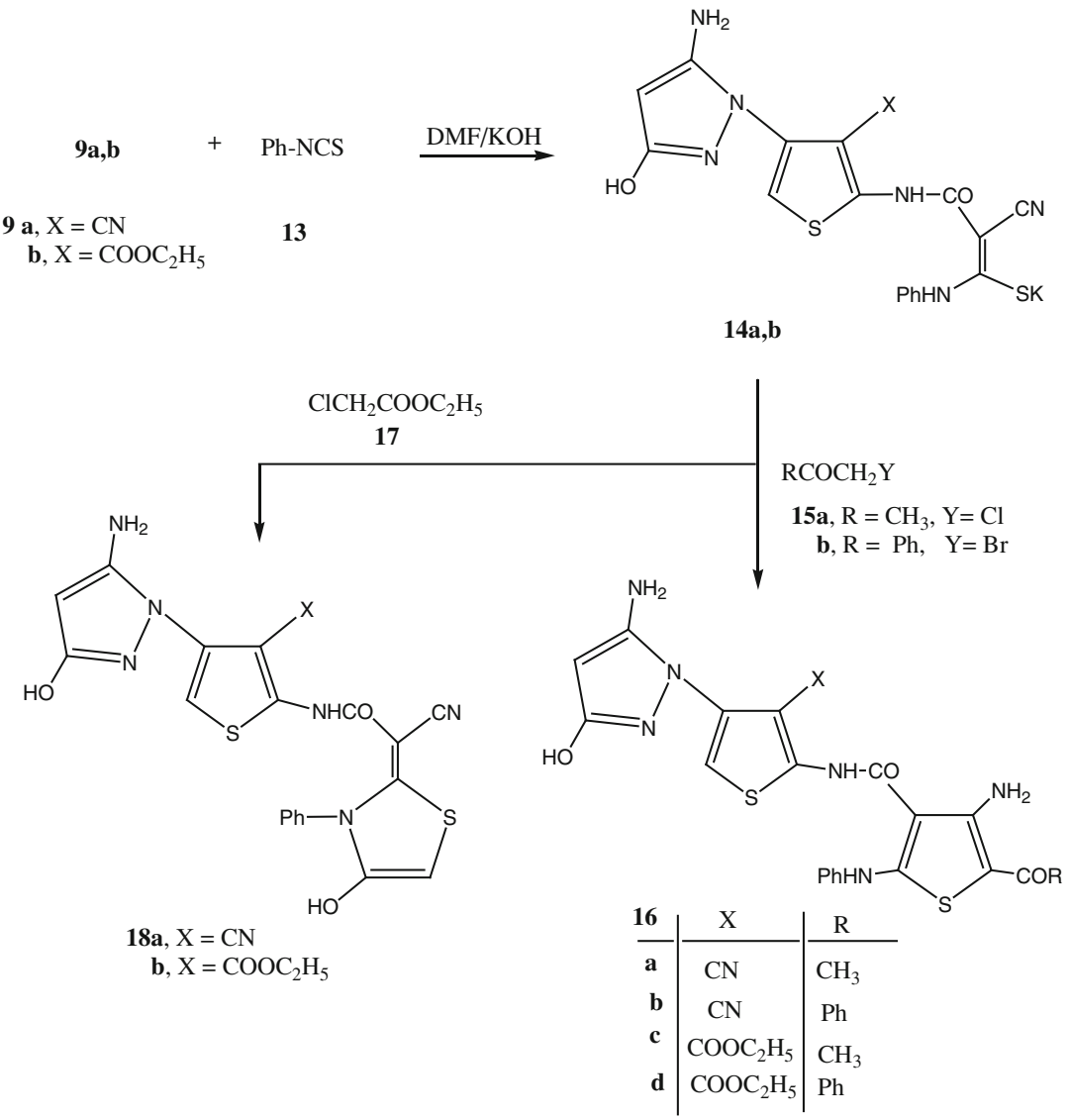


Fig. 4 Statistics ANOVA diagram

tube and are uncorrected. Elemental analysis was performed on a Yanaco CHNS Corder elemental analyzer (Japan). IR spectra (υ, cm^{-1}) were recorded in KBr pellets on a PA-9721 IR spectrophotometer (Shimadzu, Japan). ^{1}H-NMR and ^{13}C-NMR spectra were obtained on a Jeol 300 MHz (Japan) spectrometer with DMSO-d_6 as a solvent using TMS as internal reference, and chemical shifts (δ) are expressed in ppm. Mass spectra were recorded on Kratos (75 eV) MS equipment (Germany). Synthetic pathways are presented in Schemes 1, 2, and 3. The anti-tumor evaluations of the newly synthesized compounds on the growth of three human tumor cell lines are given in Table 1.



Scheme 3 Synthesis of compounds **14a,b–18a,b**

1-(5-Amino-3-hydroxy-1*H*-pyrazol-1-yl)ethanone **4**

A suspension of compound **3** (1.41 g, 0.01 mol) in sodium ethoxide solution [prepared by dissolving metallic sodium (0.64 g, 0.01 mol) in absolute ethanol (30 mL)] was boiled in a boiling water bath for 8 h. The reaction mixture was left to cool and then poured onto ice/water containing few drops of hydrochloric acid. The formed solid product was collected by filtration.

*Compound **4***

Crystallized from ethanol to give white crystals, yield 1.27 g (90 %), m.p. 210–213 °C. *Anal*. Calculated for $C_5H_7N_3O_2$ (141.13): C, 42.55; H, 5.00; N, 29.77. Found: C, 42.43; H, 4.81; N, 30.01. MS: *m/e* = 141 (M^+, 10 %). IR, υ: 3577–3323 (OH, NH_2), 1690 (C=O), 1653 (C=N). ^{1}H-NMR, δ: 2.77 (s, 3H, CH_3), 4.79 (s, 2H, NH_2), 6.86 (s, 1H, pyrazole H-4), 10.22 (s, 1H, OH). ^{13}C-NMR, δ: 29.6 (CH_3), 104.8, 152.3, 154.6 (pyrazole C), 168.9 (C=O).

1-(5-Amino-3-hydroxy-1*H*-pyrazol-1-yl)-bromoethanone **5**

To a solution of compound **4** (1.41 g, 0.01 mol) in acetic acid (40 mL) at 50 °C, bromine (1.80 g, 0.01 mol) was added dropwise. The reaction mixture was kept at room temperature for 1 h with continuous stirring. The solid product was formed upon pouring onto ice/water and the formed solid product was collected by filtration.

Compound ***5***

Crystallized from acetic acid to give white crystals, yield 1.49 g (68 %), m.p. 160–163 °C. *Anal*. Calculated for $C_5H_6BrN_3O_2$ (220.02): C, 27.29; H, 2.75; N, 19.10. Found: C, 27.39; H, 2.88; N, 19.01. MS: *m/e* = 220 (M^+, 23 %), 222 ($M^+ + 2$, 12 %). IR, υ: 3547–3363 (OH, NH_2), 1688 (C=O), 1650 (C=N). ^{1}H-NMR, δ: 4.07 (s, 2H, CH_2), 4.81 (s, 2H, NH_2), 6.82 (s, 1H, pyrazole H-4), 10.02 (s, 1H, OH). ^{13}C-NMR, δ: 38.2 (CH_2), 104.3, 153.0, 154.8 (pyrazole C), 167.3 (C=O).

2-Amino-4-(5-amino-3-hydroxy-1H-pyrazol-1-yl)thiophene-3-carbonitrile **8a** and ethyl 2-amino-4-(5-amino-3-hydroxy-1*H*-pyrazol-1-yl)thiophene-3-carboxylate **8b**

General procedure

To a solution of compound **4** (1.41 g, 0.01 mol) in 1,4-dioxan containing triethylamine (0.50 mL) and elemental sulfur (0.32 g, 0.01 mol), either malononitrile **(6a)** (0.66 g, 0.01 mol) or ethyl cyanoacetate **(6b)** (1.13 g, 0.01 mol) was added. The reaction mixture in each case was heated under reflux for 1 h, then poured onto ice/water containing few drops of hydrochloric acid, and the formed solid product, in each case, was collected by filtration.

Compound ***8a*** Crystallized from acetic acid to give pale yellow crystals, yield 1.49 g (68 %), m.p. 160–163 °C. *Anal*. Calculated for $C_8H_7N_5OS$ (221.24): C, 43.43; H, 3.19; N, 31.66; S, 14.49. Found: C, 43.29; H, 3.28; N, 31.85; S, 14.57. MS: *m/e* = 221 (M^+, 33 %). IR, υ: 3529–3342 (OH, $2NH_2$), 2222 (CN), 1632 (C=N). ^{1}H-NMR, δ: 4.55, 4.83 (2s, 4H, $2NH_2$), 6.86, 6.99 (2s, 2H, pyrazole H-4, thiophene H-5), 10.12 (s, 1H, OH). ^{13}C-NMR, δ: 116.8 (CN), 104.2, 153.3, 154.6 (pyrazole C), 117.8, 125.6, 133.2, 138.1 (Thiophene C).

Compound ***8b*** Crystallized from acetic acid to give yellow crystals, yield 2.09 g (78 %), m.p. 148–151 °C. *Anal*. Calculated for $C_{10}H_{12}N_4O_3S$ (268.29): C, 44.77; H, 4.51; N, 20.88; S, 11.95. Found: C, 44.69; H, 4.58; N, 20.95; S, 11.83. MS: *m/e* = 268 (M^+, 18 %). IR, υ: 3544–3337 (OH, $2NH_2$), 1688 (C=O), 1644 (C=N). ^{1}H-NMR, δ: 1.14 (t, 3H, J = 7.02 Hz, CH_3), 4.22 (q, 2H, J = 7.02 Hz, CH_2), 4.53, 4.86 (2s, 4H, $2NH_2$), 6.84, 6.92 (2s, 2H, pyrazole H-4, thiophene H-5), 10.23 (s, 1H, OH). ^{13}C-NMR, δ: 16.2 (CH_3), 42.3 (CH_2), 104.0, 153.1, 154.4 (pyrazole C), 118.3, 125.2, 133.6, 138.4 (Thiophene C), 164.2 (C=O).

N-(4-(5-amino-3-hydroxy-1*H*-pyrazol-1-yl)-3-cyanothiophen-2-yl)-2-cyano-acetamide **9a** and ethyl 2-(2-cyanoacetamido)-4-(5-amino-3-hydroxy-1*H*-pyrazol-1-yl)thiophene-3-carboxylate **9b**

General procedure

A solution of either **8a** (2.21 g, 0.01 mol) or **8b** (2.68 g, 0.01 mol) in dimethylformamide (30 mL), ethyl cyanoacetate **(6b)** (1.13 g, 0.01 mol) was added. The reaction mixture, in each case, was heated under reflux for 3 h and then left to cool on reaching room temperature. The solid product formed upon pouring onto ice/water was collected by filtration.

Compound ***9a*** Crystallized from 1,4-dioxan to give orange crystals, yield 2.27 g (79 %), m.p. 266–269 °C. *Anal*. Calculated for $C_{11}H_8N_6O_2S$ (288.29): C, 45.83; H, 2.80; N, 29.15; S, 11.12. Found: C, 45.73; H, 3.18; N, 29.05; S, 11.33. MS: *m/e* = 288 (M^+, 68 %). IR, υ: 3572–3366 (OH, NH, NH_2), 2227, 2220 (2 CN), 1688 (C=O), 1636 (C=N). ^{1}H-NMR, δ: 4.77 (s, 2H, NH_2), 4.81 (s, 2H, CH_2), 6.83, 6.84 (2s, 2H, pyrazole H-4, thiophene H-5), 8.21 (s, 1H, NH), 10.08 (s, 1H, OH). ^{13}C-NMR, δ: 39.2 (CH_2), 116.5, 117.9 (2 CN), 104.4, 152.9, 154.8 (pyrazole C), 118.0, 124.9, 134.5, 138.8 (thiophene C), 164.5 (C=O).

Compound ***9b*** Crystallized from 1,4-dioxan to give orange crystals, yield 2.88 g (86 %), m.p. 230–233 °C. *Anal*. Calculated for $C_{13}H_{13}N_5O_4S$ (335.34): C, 46.56; H, 3.91; N, 20.88; S, 9.56. Found: C, 46.63; H, 4.02; N, 20.92; S, 9.63. MS: *m/e* = 335 (M^+, 46 %). IR, υ: 3576–3337 (OH, NH, NH_2), 1693, 1684 (2C=O), 2220 (CN), 1648 (C=N). ^{1}H-NMR, δ: 1.12 (t, 3H, J = 7.26 Hz, CH_3), 4.24 (q, 2H, J = 7.26 Hz, CH_2), 4.73 (s, 2H, NH_2), 4.88 (s, 2H, CH_2), 6.79, 6.92 (2s, 2H, pyrazole H-4, thiophene H-5), 8.22 (s, 1H, NH), 10.19 (s, 1H, OH). ^{13}C-NMR, δ: 16.3 (CH_3), 39.7, 42.6 ($2CH_2$), 104.3, 153.2, 154.6 (pyrazole C), 117.9 (CN), 118.4, 125.0, 133.46, 138.74 (thiophene C), 164.0, 166.3 (2C=O).

4-Amino-3-(5-amino-3-hydroxy-1*H*-pyrazol-1-yl)-6-hydroxythieno[2,3-b]-pyridine-5-carbonitrile **10a** and 3-(5-amino-3-hydroxy-1*H*-pyrazol-1-yl)-4,6-dihydroxythieno[2,3-b]pyridine-5-carbonitrile **10b**

General procedure

A suspension of either **9a** (2.88 g, 0.01 mol) or **9b** (3.35 g, 0.01 mol) in sodium ethoxide solution [prepared by dissolving metallic sodium (0.64 g, 0.01 mol) in absolute ethanol (30 mL)] was boiled in a boiling water bath for 8 h. The reaction mixture was left to cool and then poured onto ice/water containing few drops of hydrochloric acid. The formed solid product was collected by filtration.

Compound ***10a*** Crystallized from 1,4-dioxan to give orange crystals, yield 2.47 g (86 %), m.p. 170–173 °C. *Anal*. Calculated for $C_{11}H_8N_6O_2S$ (288.29): C, 45.83; H, 2.80; N, 29.15; S, 11.12. Found: C, 45.62; H, 2.92; N, 29.32; S, 11.33. MS: *m/e* = 288 (M^+, 16 %). IR, υ: 3576–3333 (2OH, $2NH_2$), 2222 (CN), 1640 (C=N). ^{1}H-NMR, δ: 4.79, 4.86 (2s, 4H, $2NH_2$), 6.75, 6.89 (2s, 2H, pyrazole H-4, thiophene H-5), 10.16, 10.22 (2s, 2H, 2OH). ^{13}C-NMR, δ: 117.8 (CN), 104.5, 153.6, 154.9 (pyrazole C), 118.2, 124.7, 133.3, 138.8, 140.2, 143.8, 148.2 (thiophene, pyridine C).

Compound ***10b*** Crystallized from 1,4-dioxan to give pale brown crystals, yield 1.90 g (66 %), m.p. 180–183 °C. *Anal*. Calculated for $C_{11}H_7N_5O_3S$ (289.27): C, 45.67; H, 2.44; N, 24.21; S, 11.08. Found: C, 45.86; H, 2.66; N; 24.02; S, 10.83. MS: *m/e* = 289 (M^+, 100 %). IR, υ: 3555–3320 (3OH, NH_2), 2227 (CN), 1630 (C=N). ^{1}H-NMR, δ: 4.83 (s, 2H, NH_2), 6.86, 6.94 (2s, 2H, pyrazole H-4, thiophene H-5), 10.09, 10.27, 10.29 (3s, 3H, 3OH). ^{13}C-NMR, δ: 117.8 (CN), 104.3, 152.6, 155.7 (pyrazole C), 118.8, 124.8, 134.0, 138.8, 140.7, 141.5, 149.6 (thiophene, pyridine C).

N-(4-(5-amino-3-hydroxy-1*H*-pyrazol-1-yl)-3-cyanothiophen-2-yl)-α-phenyl-hydrazono-2-cyanoacetamide **12a**, N-(4-(5-amino-3-hydroxy-1*H*-pyrazol-1-yl)-3-cyanothiophen-2-yl)-α-(p-methylphenylhydrazono)-2-cyanoacetamide **12b**, N-(4-(5-amino-3-hydroxy-1*H*-pyrazol-1-yl)-3-cyanothiophen-2-yl)-α-(p-chlorophenylhydrazono)-2-cyanoacetamide **12c,** ethyl 2-(α-phenylhydrazono-2-cyanoacetamido)-4-(5-amino-3-hydroxy-1*H*-pyrazol-1-yl)thiophene-3-carboxylate **12d**, ethyl 2-α-(p-methylphenylhydrazono)-2-cyanoacetamido)-4-(5-amino-3-hydroxy-1*H*-pyrazol-1-yl)thiophene-3-carboxylate **12e** and ethyl 2-α-(p-chlorophenylhydrazono)-2-cyanoacetamido)-4-(5-amino-3-hydroxy-1*H*-pyrazol-1-yl)thiophene-3-carboxylate **12f**

General procedure

To a cold solution (0–5 °C) of either **9a** (2.88 g, 0.01 mol) or **9b** (3.35 g, 0.01 mol) in ethanol (40 mL) containing sodium hydroxide (5 mL, 10 %),benzenediazonium chloride **(11a)**, p-methylbenzene-diazonium chloride **(11b)**, or p-chlorobenzenediazonium chloride **(11c)** [prepared by adding sodium nitrite (0.70 g, 0.01 mol) solution to a cold solution (0–5 °C) of aniline (0.94 g, 0.01 mol), p-methylaniline (1.04 g, 0.01 mol), or p-chloroaniline (1.24 g, 0.01 mol) in the appropriate quantity of concentrated hydrochloric acid with continuous stirring] was added. The whole reaction mixture, in each case, was stirred at room temperature for an additional 1 h and the formed solid product was collected by filtration.

Compound ***12a*** Crystallized from ethanol to give reddish brown crystals, yield 3.21 g (82 %), m.p. 133–135 °C. *Anal*. Calculated for $C_{17}H_{12}N_8O_2S$ (392.39): C, 52.03; H, 3.08; N, 28.56; S, 8.17. Found: C, 51.93; H, 3.18; N, 28.45; S, 8.23. MS: *m/e* = 392 (M^+, 42 %). IR, υ: 3537–3321 (OH, 2NH, NH_2), 2225, 2220 (2 CN), 1685 (C=O), 1638 (C=N). ^{1}H-NMR, δ: 4.82 (s, 2H, NH_2), 6.82, 6.88 (2s, 2H, pyrazole H-4, thiophene H-5), 7.29–7.38 (m, 5H, C_6H_5), 8.20–825 (2s, 2H, 2NH), 10.18 (s, 1H, OH). ^{13}C-NMR, δ: 116.8, 117.3 (2 CN), 104.5, 153.2, 154.6 (pyrazole C), 118.2, 122.0, 123.4, 124.6, 130.8, 133.9, 138.9 (thiophene, C_6H_5, C), 164.8 (C=O), 173.8 (C=N).

Compound ***12b*** Crystallized from ethanol to give red crystals, yield 3.57 g (88 %), m.p. 190–193 °C. *Anal*. Calculated for $C_{18}H_{14}N_8O_2S$ (406.42): C, 53.19; H, 3.47; N, 27.57; S, 7.89. Found: C, 52.92; H, 3.32; N, 27.62; S, 7.93. MS: *m/e* = 406 (M^+, 18 %). IR, υ: 3555–3340 (OH, 2NH, NH_2), 2222, 2220 (2 CN), 1689 (C=O), 1643 (C=N). ^{1}H-NMR, δ: 2.88 (s, 3H, CH_3), 4.79 (s, 2H, NH_2), 6.80, 6.84 (2s, 2H, pyrazole H-4, thiophene H-5), 7.28–7.36 (m, 4H, C_6H_4), 8.22, 8.25 (2s, 2H, 2NH), 10.23 (s, 1H, OH). ^{13}C-NMR, δ: 24.8 (CH_3), 116.6, 117.8 (2 CN), 104.6, 154.6, 155.0 (pyrazole C), 118.8, 122.2, 123.6, 125.6, 128.9, 133.6, 138.7 (thiophene, C_6H_4, C), 164.6 (C=O), 173.4 (C=N).

Compound ***12c*** Crystallized from ethanol to give Orange crystals, yield 3.07 g (72 %), m.p. 144–147 °C. *Anal*. Calculated for $C_{17}H_{11}ClN_8O_2S$ (426.84): C, 47.84; H, 2.60; N, 26.25; S, 7.51. Found: C, 47.97; H, 2.52; N, 26.32; S, 7.63. MS: *m/e* = 426 (M^+, 8 %). IR, υ: 3571–3320 (OH, 2NH, NH_2), 2226, 2220 (2 CN), 1687 (C=O), 1642 (C=N). ^{1}H-NMR, δ: 4.80 (s, 2H, NH_2), 6.78, 6.82 (2s, 2H, pyrazole H-4, thiophene H-5), 7.26–7.39 (m, 4H, C_6H_4), 8.24, 8.26 (2s, 2H, 2NH), 10.33 (s, 1H, OH). ^{13}C-NMR, δ: 116.8, 117.9 (2 CN), 104.2, 154.4, 155.2 (pyrazole C), 118.3, 122.0, 123.9, 125.1, 129.2, 132.8, 139.5 (thiophene, C_6H_4, C), 163.8 (C=O), 172.8 (C=N).

Compound ***12d*** Crystallized from 1,4-dioxan to give red crystals, yield 2.89 g (66 %), m.p. 196–199 °C. *Anal*. Calculated for $C_{19}H_{17}N_7O_4S$ (439.45): C, 51.93; H, 3.90; N, 22.31; S, 7.30. Found: C, 52.23; H, 4.11; N, 22.42; S, 7.60. MS: *m/e* = 439 (M^+, 12 %). IR, υ: 3571–3332 (OH, 2NH, NH_2), 1689, 1682 (2C=O), 2223 (CN), 1638 (C=N). ^{1}H-NMR, δ: 1.11 (t, 3H, J = 6.53 Hz, CH_3), 4.23 (q, 2H, J = 6.53 Hz, CH_2), 4.80 (s, 2H, NH_2), 6.74, 6.98 (2s, 2H, pyrazole H-4, thiophene H-5), 7.28–7.41 (m, 5H, C_6H_5), 8.20, 8.31 (2s, 2H, 2NH), 10.21 (s, 1H, OH). ^{13}C-NMR, δ: 16.1 (CH_3), 42.5 (CH_2), 104.6, 153.8, 154.0 (pyrazole C),

116.8 (CN), 118.2, 122.3, 123.9, 125.0, 133.46, 134.8, 138.7 (thiophene, C_6H_5, C), 164.0, 166.6 (2C=O), 172.5 (C=N).

Compound ***12e*** Crystallized from 1,4-dioxan to give brown crystals, yield 3.17 g (70 %), m.p. 120–122 °C. *Anal*. Calculated for $C_{20}H_{19}N_7O_4S$ (453.47): C, 52.97; H, 4.22; N, 21.62; S, 7.07. Found: C, 52.73; H, 4.30; N, 21.82; S, 7.14. MS: *m/e* = 453 (M^+, 33 %). IR, υ: 3562–3330 (OH, 2NH, NH_2), 1688, 1684 (2C=O), 2223 (CN), 1634 (C=N). ^{1}H-NMR, δ: 1.13 (t, 3H, *J* = 6.88 Hz, CH_3), 2.89 (s, 3H, CH_3), 4.22 (q, 2H, *J* = 6.88 Hz, CH_2), 4.76 (s, 2H, NH_2), 6.76, 6.98 (2s, 2H, pyrazole H-4, thiophene H-5), 7.28–7.37 (m, 4H, C_6H_4), 8.19, 8.29 (2s, 2H, 2NH), 10.25 (s, 1H, OH). ^{13}C-NMR, δ: 16.4 (CH_3), 27.5 (CH_3), 42.6 (CH_2), 104.2, 152.9, 153.8 (pyrazole C), 116.9 (CN), 118.4, 122.7, 123.6, 125.0, 133.46, 134.3, 137.5 (thiophene, C_6H_4, C), 163.6, 166.8 (2C=O), 172.2 (C=N).

Compound ***12f*** Crystallized from 1,4-dioxan to give brown crystals, yield 3.17 g (70 %), m.p. 120–122 °C. *Anal*. Calculated for $C_{19}H_{16}ClN_7O_4S$ (473.89): C, 48.16; H, 3.40; N, 20.69; S, 6.77. Found: C, 48.01; H, 3.52; N, 20.88; S, 6.93. MS: *m/e* = 473 (M^+, 20 %). IR, υ: 3551–3322 (OH, 2NH, NH_2), 1688, 1684 (2C=O), 2223 (CN), 1634 (C=N). ^{1}H-NMR, δ: 1.11 (t, 3H, *J* = 6.88 Hz, CH_3), 4.26 (q, 2H, *J* = 6.88 Hz, CH_2), 4.88 (s, 2H, NH_2), 6.72, 6.89 (2s, 2H, pyrazole H-4, thiophene H-5), 7.24–7.36 (m, 4H, C_6H_4), 8.20, 8.30 (2s, 2H, 2NH), 10.25 (s, 1H, OH). ^{13}C-NMR, δ: 27.8 (CH_3), 42.8 (CH_2), 104.5, 152.8, 153.9 (pyrazole C), 116.9 (CN), 118.6, 122.2, 123.8, 124.8, 133.8, 134.6, 137.7 (thiophene, C_6H_4, C), 163.4, 166.9 (2C=O), 172.0 (C=N).

5-Acetyl-4-amino-N-(4-(5-amino-3-hydroxy-1*H*-pyrazol-1-yl)-3-cyanothiophen -2-yl)-2-(phenylamino)thiophene-3-carboxamide **16a**, 4-amino-5-benzoyl-N-(4-(5-amino-3-hydroxy-1*H*-pyrazol-1-yl)-3-cyanothiophen-2-yl)-2-(phenylamino)thiophene-3-carboxamide **16b**, 5-acetyl-4-amino-N-(4-(5-amino-3-hydroxy-1*H*-pyrazol-1-yl)-3-ethoxycarbonyl-thiophen-2-yl)-2-(phenylamino)thiophene-3-carboxamide **16c**, 4-amino-5-benzoyl-N-(4-(5-amino-3-hydroxy-1*H*-pyrazol-1-yl)-3-ethoxycarbonylthiophen-2-yl)-2-(phenylamino)thiophene-3-carboxamide **16d**, N-(4-(5-amino-3-hydroxy-1*H*-pyrazol-1-yl)-3-cyanothiophen-2-yl)-2-cyano-2-(4-hydroxy-3-phenylthiazol-2(3H)-ylidene)acetamide **18a** and ethyl 2-(2-cyano-2-(4-hydroxy-3-phenylthiazol-2-(3H)-ylidene)acetamido)-4-(5-amino-3-hydroxy-1*H*-pyrazol-1-yl)thiophene-3-carboxylate **18b**

General procedure

To a solution of either **9a** (2.88 g, 0.01 mol) or **9b** (3.35 g, 0.01 mol) in dimethylformamide (30 mL) containing potassium hydroxide (0.56 g, 0.01 mol), phenylisothiocyanae **(13)** (1.30 g, 0.01 mol) was added. The whole reaction mixture was stirred at room temperature overnight. The next day, chloroacetone **(15a)** (0.92 g, 0.01 mol), phenacylbromide **(15b)** (2.0 g, 0.01 mol), or ethyl chloroacetate **(17)** (1.22 g, 0.01 mol) was added with continuous stirring overnight at room temperature and then poured onto ice/water containing few drops of hydrochloric acid (till pH 6). The solid product, formed in each case, was collected by filtration.

Compound ***16a*** Crystallized from ethanol to give yellow crystals, yield 3.64 g (76 %), m.p. 120–122 °C. *Anal*. Calculated for $C_{21}H_{17}N_7O_3S_2$ (479.53): C, 52.60; H, 3.57; N, 20.45; S, 13.37. Found: C, 52.43; H, 3.41; N, 20.49; S, 13.26. MS: *m/e* = 479 (M^+, 15 %). IR, υ: 3555–3312 (OH, 2NH, $2NH_2$), 2223, 2227 (2CN), 1683 (C=O), 1634 (C=N). ^{1}H-NMR, δ: 2.88 (s, 3H, CH_3), 4.77, 4.82 (2s, 4H, $2NH_2$), 6.80, 6.85 (2s, 2H, pyrazole H-4, thiophene H-5), 7.29–7.38 (m, 5H, C_6H_5), 8.26, 8.80 (2s, 2H, 2NH), 10.20 (s, 1H, OH). ^{13}C-NMR, δ: 31.2 (CH_3), 116.9 (CN), 104.3, 153.7, 154.3 (pyrazole C), 119.0, 120.4, 120.9, 121.8, 124.8, 130.9, 133.6, 134.0, 139.4, 141.5, 143.8, 144.6 (two thiophene, C_6H_5, C), 163.9, 164.8 (2C=O).

Compound ***16b*** Crystallized from ethanol to give pale yellow crystals, yield 3.35 g (62 %), m.p. 190–193 °C. *Anal*. Calculated for $C_{26}H_{19}N_7O_3S_2$ (541.60): C, 57.66; H, 3.54; N, 18.10; S, 11.84. Found: C, 57.48; H, 3.42; N, 18.29; S, 11.66. MS: *m/e* = 541 (M^+, 20 %). IR, υ: 3512–3357 (OH, 2NH, $2NH_2$), 2220, (CN), 1685 (C=O), 1636 (C=N). ^{1}H-NMR, δ: 4.78, 4.84 (2s, 4H, $2NH_2$), 6.83, 6.89 (2 s, 2H, pyrazole H-4, thiophene H-5), 7.26–7.39 (m, 10H, $2C_6H_5$), 8.25, 8.86 (2s, 2H, 2NH), 10.24 (s, 1H, OH). ^{13}C-NMR, δ: 117.2 (CN), 104.6, 153.8, 154.2 (pyrazole C), 118.8, 120.2, 120.9, 121.8, 124.8, 126.0, 128.5, 129.9, 133.6, 134.2, 139.4, 142.1, 143.8, 144.8 (two thiophene, $2C_6H_5$, C), 163.2, 166.2 (2C=O).

Compound ***16c*** Crystallized from ethanol to give pale brown crystals, yield 4.67 g (89 %), m.p. 190–193 °C. *Anal*. Calculated for $C_{23}H_{22}N_6O_5S_2$ (526.59): C, 52.46; H, 4.21; N, 15.96; S, 12.18. Found: C, 52.59; H, 4.50; N, 16.22; S, 12.28. MS: *m/e* = 526 (M^+, 22 %). IR, υ: 3524–3318 (OH, 2NH, $2NH_2$), 1693, 1683,1680 (3C=O), 1636 (C=N). ^{1}H-NMR, δ: 1.14 (t, 3H, *J* = 6.89 Hz, CH_3), 2.69 (s, 3H, CH_3), 4.22 (q, 2H, *J* = 6.89 Hz, CH_2), 4.79, 4.86 (2s, 4H, $2NH_2$), 6.80, 6.85 (2s, 2H, pyrazole H-4, thiophene H-5), 7.29–7.38 (m, 5H, C_6H_5), 8.26, 8.80 (2s, 2H, 2NH), 10.24 (s, 1H, OH). ^{13}C-NMR, δ: 16.8, 26.8 ($2CH_3$), 42.8 (CH_2), 104.5, 152.9, 154.8 (pyrazole C), 118.3, 120.4, 121.6, 121.8, 124.8, 130.9, 134.8, 133.6, 139.4, 141.5, 143.8, 144.6 (two thiophene, C_6H_5, C), 164.1, 165.4, 168.0 (3C=O).

Compound ***16d*** Crystallized from ethanol to give yellow crystals, yield 2.94 g (56 %), m.p. 110–113 °C. *Anal*. Calculated for $C_{28}H_{24}N_6O_5S_2$ (588.66): C, 57.13; H, 4.11; N, 14.28; S, 10.89. Found: C, 57.42; H, 4.38; N, 14.30; S, 10.93. MS: *m/e* = 588 (M^+, 52 %). IR, υ: 3583–3338 (OH, 2NH, $2NH_2$), 1688, 1685, 1680 (3C=O), 1636 (C=N). ^{1}H-NMR, δ: 1.12 (t, 3H, J = 6.29 Hz, CH_3), 4.24 (q, 2H, J = 6.29 Hz, CH_2), 4.73, 4.88 (2s, 4H, $2NH_2$), 6.82, 6.84 (2s, 2H, pyrazole H-3, thiophene H-5), 7.26–7.41 (m, 10H, $2C_6H_5$), 8.28, 8.82 (2s, 2H, 2NH), 10.26 (s, 1H, OH). ^{13}C-NMR, δ: 16.6 (CH_3), 42.3 (CH_2), 117.0 (CN), 104.6, 152.6, 154.3 (pyrazole C), 118.0, 120.6, 121.8, 124.8, 126.0, 126.9, 127.4, 131.6, 133.6, 134.5, 139.9, 141.8, 142.0, 143.8,

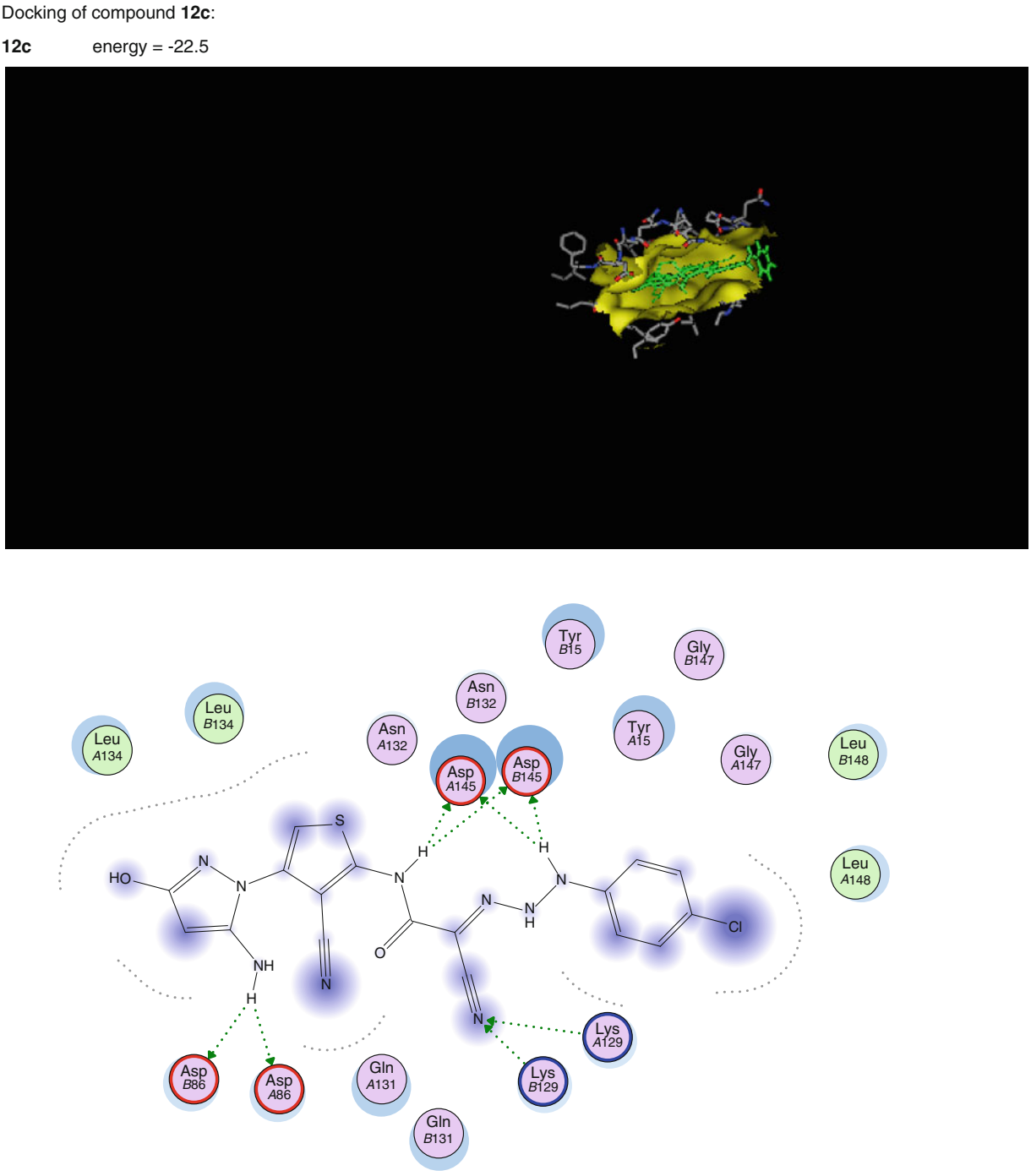


Fig. 5 Docking of compound **12c**

144.8 (two thiophene, two C_6H_5, C), 163.7, 165.4, 166.2 (3C=O).

Compound ***18a*** Crystallized from 1,4-dioxan to give yellow crystals, yield 3.38 g (73 %), m.p. 266–269 °C. *Anal*. Calculated for $C_{20}H_{13}N_7O_3S_2$ (463.49): C, 51.83; H, 2.83; N, 21.15; S, 13.84. Found: C, 52.03; H, 2.61; N, 20.89; S, 13.76. MS: $m/e = 463$ (M^+, 35 %). IR, υ: 3571–3349 (2OH, NH, NH_2), 2226, 2221 (2CN), 1687 (C=O), 1632 (C=N). ^{1}H-NMR, δ: 4.79 (s, 2H, NH_2), 6.78,

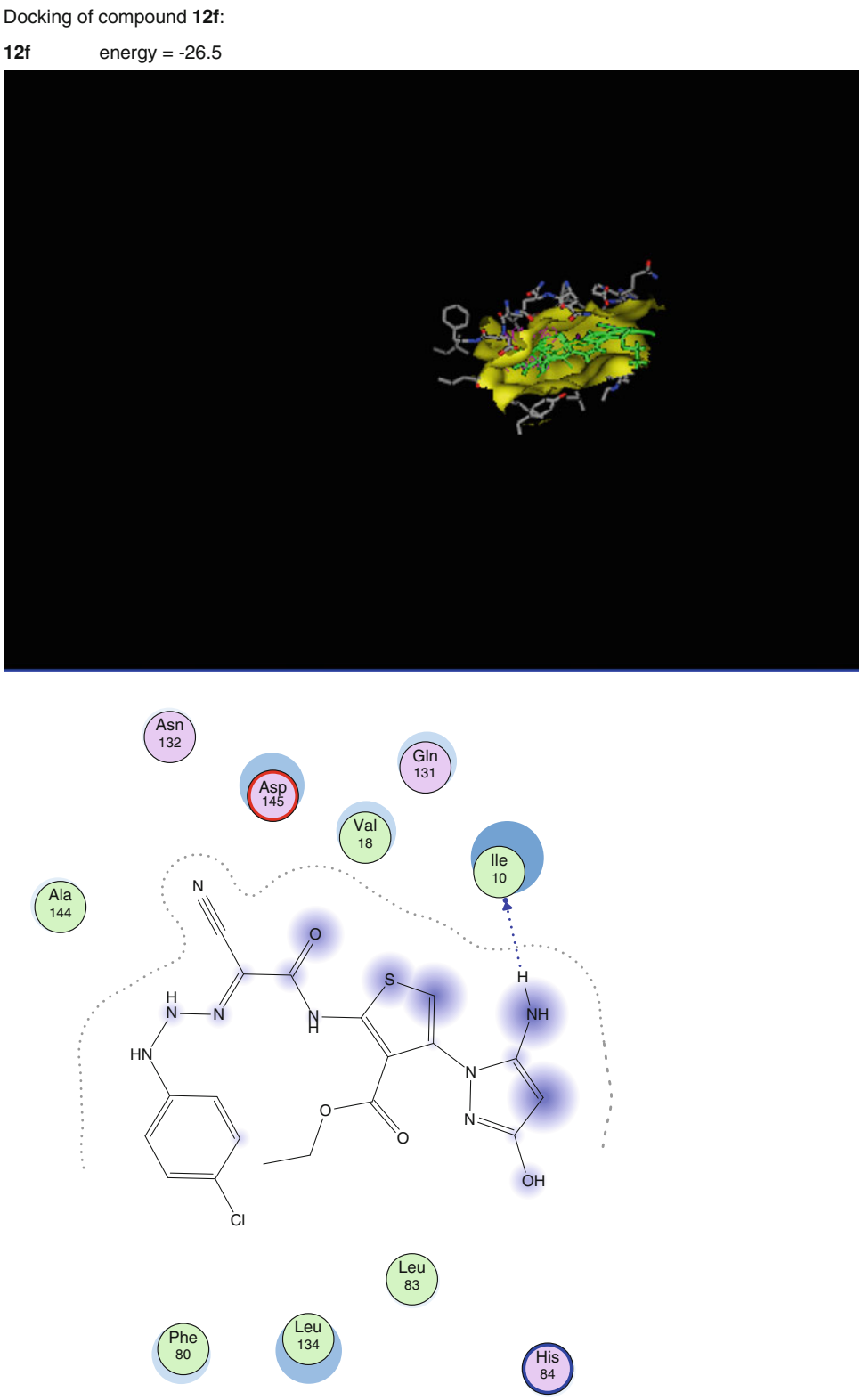


Fig. 6 Docking of compound **12f**

6.83 6.88 (3s, 3H, pyrazole H-4, thiophene H-5, thiazole H-5), 7.31–7.42 (m, 5H, C_6H_5), 8.29 (s, 1H, NH), 10.26, 10.29 (2s, 2H, 2OH). ^{13}C-NMR, δ: 116.6, 117.3 (2CN), 119.0, 120.3 (C=C), 104.6, 153.8, 154.8 (pyrazole C), 118.0, 121.3, 121.7, 121.8, 126.8, 130.0, 130.9, 139.4, 142.8, 143.8, 148.0, 150.0, 151.6 (thiophene, thiazole, C_6H_5, C), 164.0 (C=O).

*Compound **18b*** Crystallized from ethanol to give orange crystals, yield 3.06 g (60 %), m.p. 188–190 °C. *Anal.*

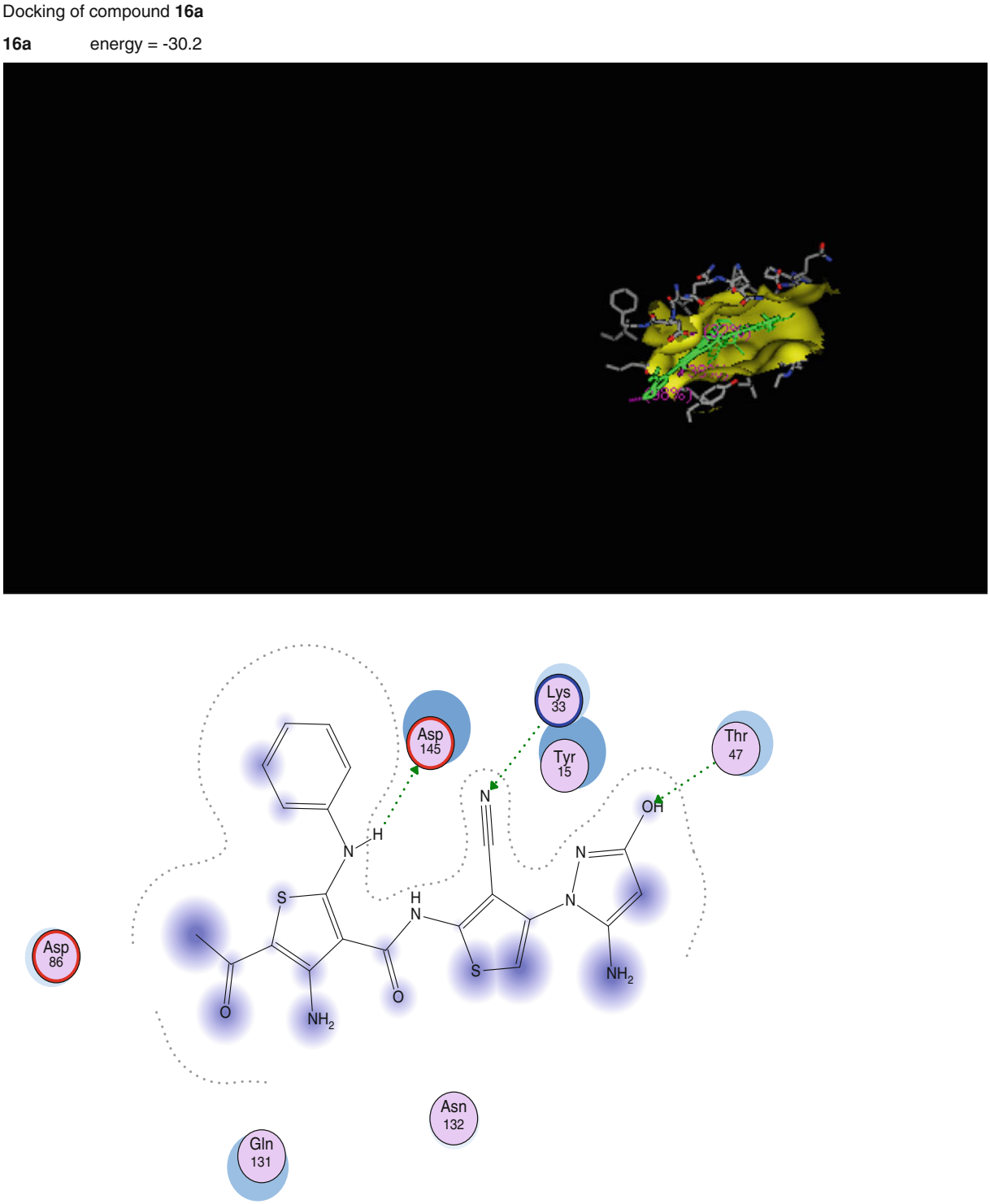


Fig. 7 Docking of compound **16a**

Calculated for $C_{22}H_{18}N_6O_5S_2$ (510.55): C, 51.76; H, 3.55; N, 16.46; S, 12.56. Found: C, 51.61; H, 3.40; N, 16.86; S, 12.72. MS: $m/e = 510$ (M^+, 30 %). IR, υ: 3563–3388 (2OH, NH, NH_2), 2222 (CN), 1690, 1688 (2C=O), 1634 (C=N). ^{1}H-NMR, δ: 1.13 (t, 3H, $J = 7.07$ Hz, CH_3), 4.26 (q, 2H, $J = 7.07$ Hz, CH_2), 4.76 (s, 2H, NH_2), 6.83, 6.86, 7.01 (3s, 3H, pyrazole H-4, thiophene H-5, thiazole H-5), 7.29–7.40 (m, 5H, C_6H_5), 8.35 (s, 1H, NH), 10.24, 10.28 (2 s, 2H, 2OH). ^{13}C-NMR, δ: 16.6 (CH_3), 42.6 (CH_2), 117.2 (CN), 104.6, 152.2, 154.8 (pyrazole C), 118.3, 120.4, 121.4, 122.5, 124.8, 126.6, 127.4, 131.6, 133.6, 139.9, 142.0, 143.8, 144.8, 148.9, 152.0, 153.3 (thiophene, thiazole, C_6H_5, C), 162.3, 163.8 (2C=O).

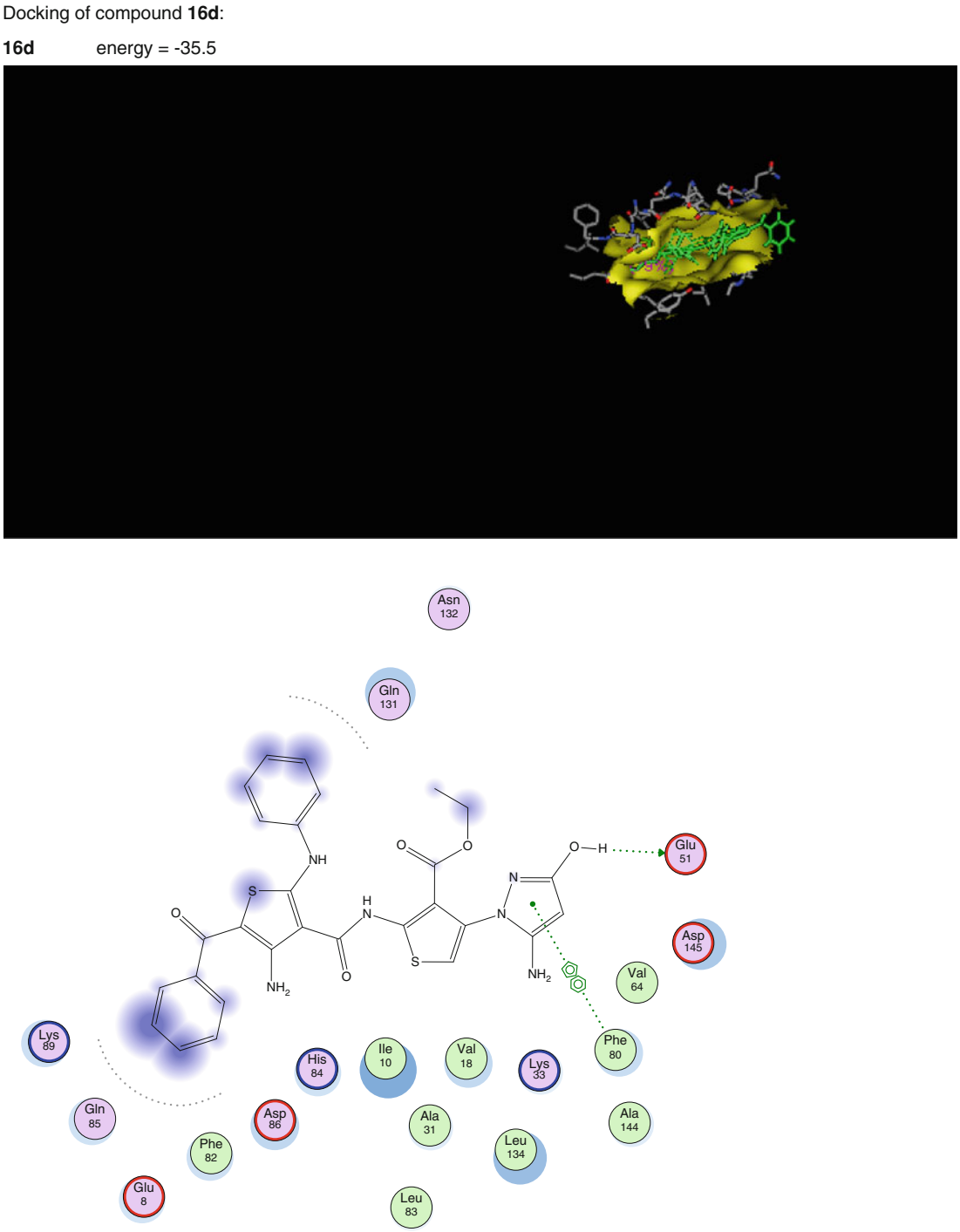


Fig. 8 Docking of compound **16d**

Preparation for docking

Docking was carried out on an Intel Pentium 1.6 GHz processor, 512 MB memory, with windows XP operating system and Molecular Operating Environment (MOE 2008.10; Chemical Computing Group, Canada) as the computational software. All the minimizations were performed with MOE until a root mean square deviation (RMSD) gradient of 0.05 kcal/mol/Å with MMFF94x force field was reached and the partial charges were automatically calculated. The 3D structure of the Protein Cyclin-Dependent Kinase2 (CDK2) complexes with (Thiophene Carboxamide) was obtained from the Protein Data Bank (PDB ID: 1EVE) at Research Collaboration for Structural Bioinformatics (RCSB), protein data bank base 60 with 2.5 Å resolution.

Scoring

Poses generated by the placement methodology were scored using the London dG scoring function implemented in MOE, which estimates the free energy of binding of the ligand from the given pose. The top 10 poses for each ligand were output in the MOE database. Each resulting ligand pose was then subjected to MMFF94x energy minimization. The minimized docking conformations were then rescored using the London dG scoring method. Validation of the function implemented in MOE was done by docking the native ligand (Thiophene Carboxamide) into its binding site; the docked results of the previous mentioned ligand were compared to the crystal structure of the bound ligand–protein complex. The RMSD of the docked ligand was 2.5 Å as it seems exactly superimposed on the native-bound one. These results indicate the accuracy of the MOE in comparison with the biological methods.

In the present work, all new compounds were docked using the rigid receptor/flexible ligand approach adopting five energy maps which are hydrophobicity, electrostatic, hydrogen bond formation, and two Van der Waal parameters. The docking scores were expressed in energy terms. The lower the binding energy, the better the binding affinity. The docking study displayed showed that most of the designed compounds have a promising affinity to inhibit CDK2. It is of great value that among our docking studies, we find that the maximum inhibitory effect has been seen toward CDK2; for that reason, we selected the thiophene derivatives **12c**, **12f**, **16a**, and **16d** to be docked against such protein kinase using thiophene carboxamide as a reference compound. See Figures 5, 6, 7, and 8

Conclusions

In this work, we succeeded in synthesizing a series of pyrazole derivatives incorporated with other heterocyclic rings. The anti-tumor evaluations of the newly synthesized products showed that compounds **12c**, **12f**, **16a**, and **16d** were the most active compounds toward the three cancer cell lines.

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