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Original article

Novel benzimidazole–pyrimidine conjugates as potent antitumor agents

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ABSTRACT

As a continuation to our previous work in synthesizing antitumor benzimidazoles, a series of 2-((1*H*-benzo[*d*]imidazol-2-yl)methylthio)-4-(substituted)-6-phenylpyrimidine-5-carbonitriles was synthesized. Evaluation of the synthesized compounds for their *in vitro* cytotoxic activity against twelve cell lines namely, Cervical carcinoma (KB), Ovarial carcinoma (SK OV-3), CNS cancer (SF-268), Non small lung cancer (NCI H460), Colonadenocarcinoma (RKOP27), Leukaemia (HL60, U937, K562), Melanoma (G361, SK-MEL-28) and Neuroblastoma (GOTO, NB-1) revealed their marked potency when compared with known anti-cancer drugs.

1. Introduction

Benzimidazole moiety is a structural isostere of naturally occurring nucleotides; hence, it has been extensively utilized as a drug scaffold in the medicinal chemistry. Several promising antitumor active agents were found to contain the benzimidazole ring system. They were found to exert their antitumor activity by acting mainly as topoisomerases inhibitors [1–3], alkylating agents [4–6] and antiangiogenic agents [7–9]. In the last years, it has been demonstrated that several bi- and ter-benzimidazole derivatives act as topoisomerase I inhibitors. For example, Hoechst 33342 and Hoechst 33258 bind to the minor groove of DNA, trapping the reversible complex derived from DNA and topoisomerase I producing a limited number of highly specific single strand DNA breaks [10,11]. The benzimidazole derivatives I–VII (Fig. 1) were previously synthesized by our laboratory group, where compounds I ($IC_{50} = 4.2$ μM) and II ($IC_{50} = 8.29$ μM) were found to have high cytotoxic activity against breast cancer (MCF7) [12], compounds III and IV possess significant inhibitory activity against Burkitt's lymphoma promotion [13] and V, VI (log $GI_{50} = -5.61$) and VII have high cytotoxic activity against non small lung cancer and also breast cancer V (log $GI_{50} = -5.77$), VI (log $GI_{50} = -5.36$) [14,15].

As a continuation to our previous work in synthesizing antitumor benzimidazoles, we aimed in this manuscript to investigate the effect of replacement of the methyl group in the 2-position of the benzimidazoles I–VII (Fig. 1) by a basic moiety, which is a methylthiopyrimidine, compound **3**, (Fig. 2), on the antitumor activity. The pyrimidine ring carries a lipophilic phenyl group as well as other polar groups as OH, Cl and CN. Investigation of the effect of conversion of the 6-hydroxy group of compound **3** to chlorine to form **4** and its subsequent replacement with various amines including piperazine, as the reported cytotoxic activity of aryl piperazines [16,17]; replacement of piperazine's free *NH* of **15** by *N*-alkyl **16**, **20**, amide **17**,**18** and sulphonamides **21**–**23**, as it is known that arylsulphonamides posses a variety of mechanisms for their antitumor action and building up an additional pyrimidine nucleus in compounds **24** and **25** on the in vitro antitumor activity was also conducted. We have discovered a new class of potent antitumor benzimidazole-2-methylthiopyrimidines, as the prepared compounds were found to have pronounced in vitro activity against twelve cell lines, which will be subjected for further investigations for their in vivo activity.

2. Chemistry

A convergent synthesis was designed for the preparation of compound **3**. The first part of this synthesis involved the preparation of 2-chloro-1*H*-benzo[*d*]imidazole **1** as previously reported [18] (Scheme 1). The second part of this synthesis involved the preparation of 1,6-dihydro-2-mercapto-6-oxo-4-phenylpyrimidine-5-carbonitrile **2** by ternary condensation of benzaldehyde with thiourea and ethylcyanoacetate according to the reported procedure [19,20] (Scheme 2). Condensation of the 2 parts was then carried out

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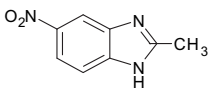
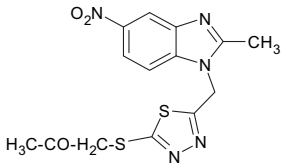


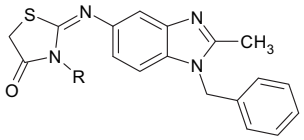
I

(IC_{50} = 4.2 μM)

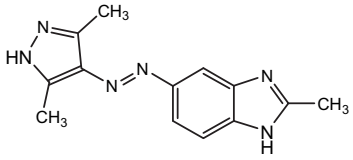


II

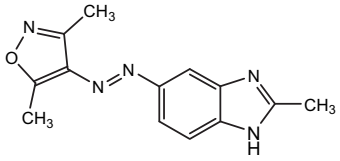
(IC_{50} = 8.29 μM)



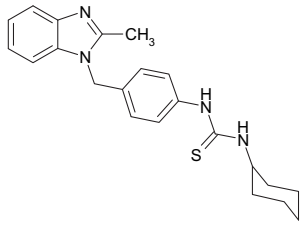
III- R=C_6H_5CO, (%EBV-EA = 12.3 (60))
IV- R= C_6H_4-OCH_3, (%EBV-EA = 13.2 (60))



V, (Breast cancer, MCF7, GI_{50}=1.82E-05)
(Breast cancer, T47D, GI_{50}= 1.68E-06)
(Non small lung cancer, HOP92, GI_{50}=1.49 E-05)

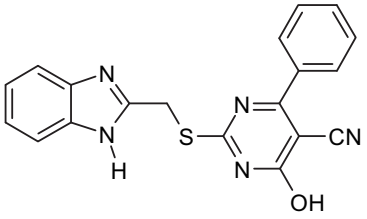


VI, (Breast cancer, MCF7, GI_{50}=2.87E-05)
(Breast cancer, T47D, GI_{50}= 1.68E-06)
(Non small lung cancer, NCI-H522, GI_{50}=1.25 E-06)



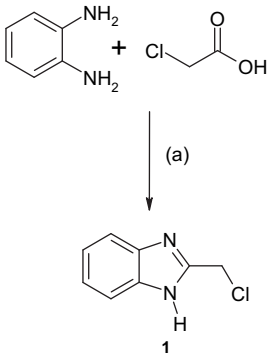
VII, (Breast cancer, MCF7, GI_{50}=1.82E-05)
(Breast cancer, T47D, GI_{50}= 4.41E-06)
(Non small lung cancer, EKVX, GI_{50}=1.49 E-05)

Fig. 1. Structures of previously synthesized antitumor benzimidazoles.



3

Fig. 2. Structure of compound **3**.



1

Reagents and conditions: (a) 4N HCl/4h reflux.

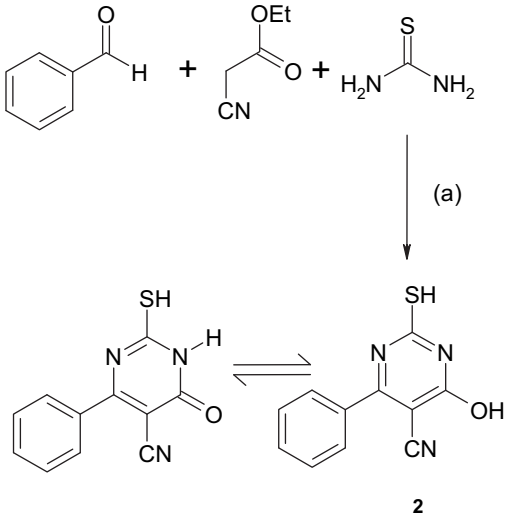
Scheme 1.

to obtain the required target compound **3**, which was subsequently halogenated by the reaction with $POCl_3$ to yield compound **4**. This highly activated intermediate was then reacted with different hydrazines, alkyl amines, aminoheterocycles, thioamides and amide to obtain compounds **5**–**14** (Scheme 3). Compounds **15** and **16** were obtained by nucleophilic substitution of the chlorine of **4** by piperazine and *N*-methylpiperazine respectively. Compound **15** was further reacted with different acyl, alkyl and sulphonyl halides to obtain compounds **17**–**23** (Scheme 2). An additional pyrimidine nucleus was built up in compounds **24** and **25** by fusing **4** with thiourea or urea respectively (Schemes 4 and 5).

3. Pharmacology

3.1. Antitumor activity

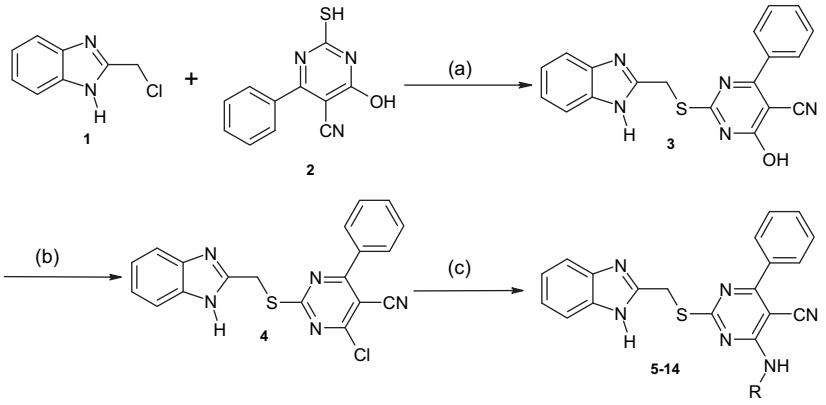
All the newly synthesized compounds were investigated for their *in vitro* antitumor activity on 12 cancer cell lines resembling 8 types of tumor using MTT assay according to Mosmann's method [21]. Table 1 reported the IC_{50} (μM) values (concentration required to achieve 50% inhibition of the tumor growth) of the tested compounds and the standards. With respect to the *in vitro* cytotoxic activity expressed in Table 1, almost all of the synthesized compounds exhibited high antitumor activity when compared with known anticancer agents. From the recorded IC_{50}



2

Reagents and conditions: (a) EtOH/K_2CO_3/6h. reflux.

Scheme 2.



Reagents and conditions: (a) THF/TEA/24h/rt. (b) $POCl_3$/30min/rt. (c) RNH_2/EtOH/TEA/2h rt/5h reflux.

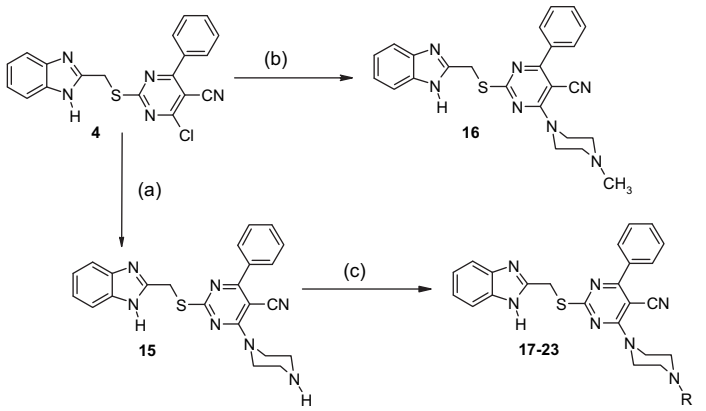
No.	R	(%) Yield
5	NH_2	81
6		82
7	CH_3	87
8	CH_2CH_3	85
9		85
10		83
11		84
12		80
13		81
14		86

Scheme 3.

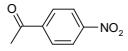
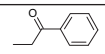
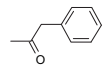
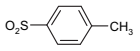
values in Table 1 it was observed that compound **3** was most active against Non small lung cancer (NCI H460), Ovarial carcinoma (SK OV-3) and against Leukaemia (HL60) but still less active than the standards. Conversion of OH group of the pyrimidine ring of **3** to Cl to obtain compound **4** does not seem to be of great interest as compound **4** was not among the most potent compounds of the series. Replacement of Cl group of **4** by other basic groups and moieties has a high effect on the activity e.g. the hydrazino compound **5** was the most active against Cervical carcinoma (KB) while the phenylhydrazino compound **6** was found to be one of the most active compounds against Melanoma (G 361) and Neuroblastoma (NB-1). The 4-aminopyridino compound **9** and the 2-aminothiazolo compound **11** were found to be the most active compounds against CNS cancer (SF-268) while the 2-aminopyridino compound **10** was the most active one against Leukaemia (U937) and Neuroblastoma (GOTO), the thiosemicarbazido compound **12** was one of the most active compounds against Neuroblastoma (GOTO), for the thiouryl compound **13** was not highly active while the uryl compound **14** was the one of the most active compounds against Colonadenocarcinoma (RKOP27). Replacement of the Cl of the pyrimidino ring by piperazine or substituted piperazine was found to have positive effect on the activity as compound **15** was the most active against Melanoma (SK-MEL-28), the *N*-methylpiperazino compound **16** was the most active among the series against Leukaemia (K562) and Neuroblastoma (NB-1), the *N*-benzoylpiperazine compound **17** and the 4-nitrobenzoylpiperazino compound **18** were found to be of the most active compounds against Melanoma (G 361), the phenylacetylpiperazino compound **19** active against Leukaemia (U937), the benzenesulfonylpiperazino compound **21** among active the most active compounds against Cervical carcinoma (KB) and Non small lung cancer (NCI H460), the tosyl piperazine compound **22** one of the most active compounds against Colonadenocarcinoma RKOP27 the 2-nitrobenzenesulfonylpiperazino compound **23** was among active the most active compounds against Neuroblastoma (NB-1). Building up additional pyrimidinethio or pyrimidinone ring fused to the pyrimidine ring of compound **3** seems to increase the activity as compound **24** was one of the most active compounds against Colonadenocarcinoma (RKOP27) and **25** was one of the most active compounds against Cervical carcinoma (KB).

3.2. The median lethal dose LD_{50}

The median lethal dose (dose required to kill half the members of the tested population) of the tested compounds was determined according to the procedure described by Lorke [22]. The geographic mean of the least dose that killed mice and the highest dose that did not kill mice was taken as the median lethal dose [23,24]. The results of the LD_{50} in mg/kg of the synthesized compounds were listed in Table 2.



Reagents and conditions: (a) Piperazine/EtOH /K_2CO_3/2h rt/8h reflux. (b) *N*-methylpiperazine/EtOH /TEA/ 1h rt. (c) DMF/ NaH/30 min/ RCl/4h rt.

No.	R	(%)Yield
17		80
18		84
19		86
20		78
21		82
22		88
23		83

Scheme 4.

4. Conclusion

Successfully we have discovered a new class of 2-((1*H*-benzo[-*d*]imidazol-2-yl)methylthio)-4-(substituted)-6-phenylpyrimidine-5-carbonitriles of potent antitumor activity against 12 cell lines namely, Cervical carcinoma (KB), Ovarial carcinoma (SK OV-3), CNS cancer (SF-268), Non small lung cancer (NCI H460), Colonadenocarcinoma (RKOP27), Leukaemia (HL60, U937, K562), Melanoma (G361, SK-MEL-28) and Neuroblastoma (GOTO, NB-1).

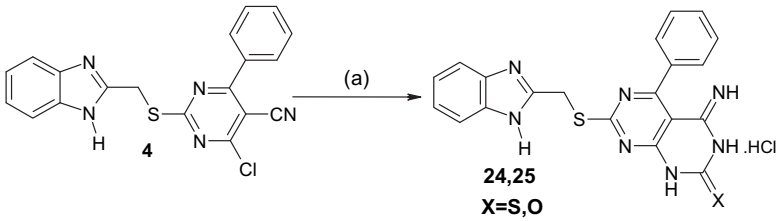
5. Experimental

5.1. Chemistry

Melting points are uncorrected and were recorded on a Gallenkamp thermometer melting point apparatus. IR spectra (KBr) were recorded on Bruker Vector 22 and Jasco FT/IR 300E Fourier transformer spectrophotometer. NMR spectra were recorded on Varian GEMINI 200 (200 MHz) and JEOL EX-270 and 500 MHz spectrometers using DMSO-d_6 as a solvent. Mass spectra were recorded on FINNGAN MAT SSQ 7000. Elemental analysis was performed in the microanalytical laboratory of the National Research Center. The synthesized compounds were named using ChemDraw Ultra software (v 10.0).

5.1.1. 2-((1H-Benzo[d]imidazol-2-yl)methylthio)-1,6-dihydro-6-oxo-4-phenylpyrimidine-5-carbonitrile (*3*)

A solution of the thiouracil derivative **2** (42.17 mmol) in dry tetrahydrofuran (60 mL) containing triethylamine (0.5 mL) was stirred for 1 h. A solution of **1** (7 g, 42.17 mmol) in dry tetrahydrofuran was then added portionwise and the reaction mixture was stirred at room temperature for additional 24 h, poured onto crushed ice with stirring. The precipitated product was filtered off



Reagents and conditions: (a) Thiourea or urea /fusion/250°C/15min.

Scheme 5.

Table 1
IC_{50} (μM) values of compounds **3–25** against 12 cancer cell lines.

Compound no.	IC_{50} (μM)											
						Leukaemia			Melanoma		Neuroblastoma	
	KB[a]	SK OV-3[b]	SF-268[c]	NCI H460[d]	RKOP27[e]	HL60	U937	K562	G361	SK-MEL-28	GOTO	NB-1
3	6.20×10^{-3}	2.55×10^{-3}	3.20×10^{-3}	2.5×10^{-3}	6.09×10^{-3}	2.55×10^{-3}	3.28×10^{-3}	4.63×10^{-3}	3.43×10^{-3}	7.70×10^{-3}	7.81×10^{-3}	6.57×10^{-3}
4	3.7×10^{-3}	3.37×10^{-3}	3.20×10^{-3}	3.28×10^{-3}	4.38×10^{-3}	$4.46 \times \times 10^{-3}$	4.46×10^{-3}	1.13×10^{-02}	1.25×10^{-02}	3.28×10^{-3}	8.62×10^{-3}	2.87×10^{-3}
5	2.55×10^{-3}	2.7×10^{-3}	2.77×10^{-3}	2.6×10^{-3}	2.55×10^{-3}	11.9×10^{-3}	3.9×10^{-3}	14.7×10^{-3}	14.7×10^{-3}	3×10^{-3}	2.68×10^{-3}	5.4×10^{-3}
6	2.9×10^{-3}	4.46×10^{-3}	4.46×10^{-3}	4.54×10^{-3}	4.9×10^{-3}	4.46×10^{-3}	3.28×10^{-3}	3.20×10^{-3}	2.4×10^{-3}	8.9×10^{-3}	6.41×10^{-3}	2.50×10^{-3}
7	2.7×10^{-3}	3×10^{-3}	3.20×10^{-3}	3.20×10^{-3}	3.7×10^{-3}	6.57×10^{-3}	1.92×10^{-02}	1.32×10^{-02}	1.25×10^{-02}	2.87×10^{-3}	3.28×10^{-3}	4.46×10^{-3}
8	7.35×10^{-3}	7.35×10^{-3}	7.35×10^{-3}	2.87×10^{-3}	7.35×10^{-3}	3.96×10^{-3}	2.63×10^{-3}	4.38×10^{-3}	4.38×10^{-3}	5.3×10^{-3}	3.96×10^{-3}	2.63×10^{-3}
9	3.20×10^{-3}	4.46×10^{-3}	2.4×10^{-3}	2.77×10^{-3}	5.81×10^{-3}	2.77×10^{-3}	7.35×10^{-3}	10×10^{-3}	2.55×10^{-3}	4.46×10^{-3}	2.55×10^{-3}	6.75×10^{-33}
10	7.35×10^{-3}	5.81×10^{-3}	6.75×10^{-3}	2.80×10^{-3}	3.80×10^{-3}	20×10^{-3}	2.55×10^{-3}	7×10^{-3}	8.9×10^{-3}	8.9×10^{-3}	2.50×10^{-3}	3.9×10^{-3}
11	14.7×10^{-3}	5.81×10^{-3}	2.55×10^{-3}	3.37×10^{-3}	3.20×10^{-3}	11.9×10^{-3}	3.80×10^{-3}	14.7×10^{-3}	11.9×10^{-3}	8.9×10^{-3}	5.1×10^{-3}	3.20×10^{-3}
12	3×10^{-3}	3×10^{-3}	$3.37 \times 1^{0-3}$	3.37×10^{-3}	3.20×10^{-3}	4.54×10^{-3}	3×10^{-3}	8.9×10^{-3}	3.96×10^{-3}	3.20×10^{-3}	2.55×10^{-3}	5.81×10^{-3}
13	3.20×10^{-3}	7.81×10^{-3}	3.37×10^{-3}	3.37×10^{-3}	2.63×10^{-3}	5.81×10^{-3}	20×10^{-3}	11.9×10^{-3}	5.81×10^{-3}	4.80×10^{-3}	4.80×10^{-3}	11.9×10^{-3}
14	9.6×10^{-3}	6.75×10^{-3}	3.37×10^{-3}	14.7×10^{-3}	2.50×10^{-3}	2.9×10^{-3}	3.37×10^{-3}	4.54×10^{-3}	2.55×10^{-3}	4.80×10^{-3}	$1.13 \times \times 10^{-02}$	7.35×10^{-3}
15	2.87×10^{-3}	2.63×10^{-3}	20	7.35×10^{-3}	5.81×10^{-3}	4×10^{-3}	5.81×10^{-3}	4.38×10^{-3}	2.50×10^{-3}	2.6×10^{-3}	3.7×10^{-3}	6.75×10^{-3}
16	2.70×10^{-3}	1.60	6.90×10^{-3}	2.84×10^{-3}	5.40×10^{-3}	2.80×10^{-3}	6.75×10^{-3}	2.4×10^{-3}	14.7×10^{-3}	20×10^{-3}	3.37×10^{-3}	2.4×10^{-3}
17	3×10^{-3}	4×10^{-3}	2.80×10^{-3}	3.20×10^{-3}	2.55×10^{-3}	6.75×10^{-3}	2.68×10^{-3}	3.4×10^{-3}	2.4×10^{-3}	8.9×10^{-3}	8.62×10^{-3}	7.35×10^{-3}
18	7.35×10^{-3}	8.62×10^{-3}	5.81×10^{-3}	4.46×10^{-3}	7.81×10^{-3}	2.9×10^{-3}	5.2×10^{-3}	4.38×10^{-3}	2.4×10^{-3}	2.77×10^{-3}	5.81×10^{-3}	1.32×10^{-02}
19	6.09×10^{-3}	3.20×10^{-3}	4.46×10^{-3}	9.6×10^{-3}	4.80×10^{-3}	4.38×10^{-3}	2.55×10^{-3}	14.7×10^{-3}	14.7×10^{-3}	14.7×10^{-3}	8.9×10^{-3}	2.9×10^{-3}
20	2.5×10^{-3}	2.77×10^{-3}	2.6×10^{-3}	2.4×10^{-3}	2.6×10^{-3}	4.23×10^{-3}	2.77×10^{-3}	1.13×10^{-02}	3.37×10^{-3}	3.37×10^{-3}	8.9×10^{-3}	4.54×10^{-3}
21	3×10^{-3}	2.77×10^{-3}	2.87×10^{-3}	4.6×10^{-3}	7.35×10^{-3}	4.23×10^{-3}	6.20×10^{-3}	3×10^{-3}	8.62×10^{-3}	6.41×10^{-3}	5.81×10^{-3}	3.28×10^{-3}
22	3.28×10^{-3}	4.54×10^{-3}	7.81×10^{-3}	22.7×10^{-3}	2.5×10^{-3}	4.23×10^{-3}	4.1×10^{-3}	1.13×10^{-02}	4.38×10^{-3}	4.38×10^{-3}	4.38×10^{-3}	6.75×10^{-3}
23	4.44×10^{-3}	3.20×10^{-3}	3.00×10^{-3}	3.70×10^{-3}	5.60×10^{-3}	2.77×10^{-3}	3.28×10^{-3}	14.7×10^{-3}	9.6×10^{-3}	8.9×10^{-3}	3×10^{-3}	2.50×10^{-3}
24	7.30×10^{-3}	3.50×10^{-3}	3.50×10^{-3}	3.00×10^{-3}	2.50×10^{-3}	6.75×10^{-3}	2.80×10^{-3}	14.7×10^{-3}	3.37×10^{-3}	3.37×10^{-3}	11.9×10^{-3}	3.28×10^{-3}
25	2.50×10^{-3}	3.20×10^{-3}	2.84×10^{-3}	2.84×10^{-3}	2.70×10^{-3}	3.1×10^{-3}	2.87×10^{-3}	14.7×10^{-3}	3.20×10^{-3}	8.9×10^{-3}	8.9×10^{-3}	1.13×10^{-02}
Fluorouracil	4.46×10^{-3}											
Doxorubicin		4.16×10^{-3}										
Cytarabine			7.68×10^{-3}									
GemcitabineHCl				2.13×10^{-3}								
Capecitabine					4.33×10^{-3}							
Doxorubicin						1.13×10^{-3}	4.45×10^{-3}	6.12×10^{-3}				
Aldesleukin									6.66×10^{-3}	3.45×10^{-3}		
Doxorubicin											4.73×10^{-3}	5.15×10^{-3}

IC_{50} values were estimated by logistic regression analysis. One-way ANOVA ($P < 0.01$) was used to test treatment difference in IC_{50}. After significant factor by ANOVA, individual group differences were analyzed using Holm–Sidak's procedure for multiple comparisons versus control.

[a] Cervical carcinoma.
[b] Ovarial carcinoma.
[c] CNS cancer.
[d] Non small lung cancer.
[e] Colonadenocarcinoma.

Table 2
The results of the LD_{50} in mg/kg of the synthesized compounds.

Compound no.	LD_{50}[a] mg/kg	Compound no.	LD_{50}[a] mg/kg
1	253.36 ± 3	**14**	347 ± 5
3	344.87 ± 11	**15**	300 ± 6
4	313.34 ± 5	**16**	384 ± 4
5	344.26 ± 5	**17**	327.15 ± 3
6	387.26 ± 3	**18**	327.38 ± 2
7	302.28 ± 4	**19**	344.45 ± 3
8	325.36 ± 6	**20**	326.78 ± 5
9	316.12 ± 4	**21**	326.76 ± 5
10	367.22 ± 3	**22**	344.78 ± 6
11	348.58 ± 4	**23**	324.78 ± 4
12	345 ± 4	**24**	389 ± 4
13	327.48 ± 5	**25**	346 ± 3

Data expressed as means ± SD for three independent experiments.
[a] Dose required to kill half the members of the tested population.

and recrystallized from the appropriate solvent. Yield 73%; mp 153–155 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 4.77 (s, 2H, CH_2–S), 7.27–7.31 (m, 2H, H5 and H6 benzimidazole), 7.52–7.64 (m, 5H, phenyl H), 7.87–7.92 (m, 2H, H4 and H7 benzimidazole), 10.50 (br, 1H, OH enolic, D_2O exchangeable), 11.65 (br, 1H, NH benzimidazole, D_2O exchangeable); IR (KBr) 3393.8 (NH benzimidazole), 3200.0–2500.0 (br, enolic OH), 3079.0 (CH arom.), 2974.5 (CH aliph.), 2222.4 (CN), 1629.6 (C=N benzimidazole), 1602.7 and 1550.1 (C=N pyrimidine, C=C); MS (EI) *m/z* 359 ($M^{\bullet+}$, 9.43%), *m/z* 361 ($M^+ + 2$, 1.57%), *m/z* 57 (100%). Anal. Calc. for ($C_{19}H_{13}N_5OS$): C, 63.49; H, 3.65; N, 19.49; S, 8.92. Found: C, 63.16; H, 3.30; N, 19.85; S, 8.78.

5.1.2. 2-((1H-Benzo[d]imidazol-2-yl)methylthio)-4-chloro-6-phenylpyrimidine-5-carbonitriles (**4**)

In an ice bath, phosphorous oxychloride (10 mL) was added dropwise with gentle stirring to compound **3** (4 g, 11.14 mmol). The reaction mixture was stirred at room temperature for 30 min, and then refluxed on water bath at 70 °C for 6 h. After cooling, the reaction mixture was poured with continuous stirring onto crushed ice. The formed solid was collected by vacuum filtration, washed with ether (4.1 g, yield 97.61%) and recrystallized from methylene chloride; mp 160–162 °C. δ 4.52 (s, 2H, CH_2–S), 7.34–7.42 (m, 2H, H5 and H6 benzimidazole), 7.67–7.88 (m, 5H, phenyl H), 7.92–8.01 (m, 2H, H4 and H7 benzimidazole), 12.50 (br, 1H, NH benzimidazole, D_2O exchangeable); MS (EI) *m/z* 375/373 ($M^+ - 2$, 69%/23% Cl pattern); *m/z* 342 ($M^+ - Cl$, 11.37%), *m/z* 229 (100%). Anal. Calc. for ($C_{19}H_{12}ClN_5S$): C, 60.39; H, 3.21; N, 18.54; S, 8.49; Cl, 9.38. Found: C, 60.68; H, 3.25; N, 18.67; S, 8.71; Cl, 9.12.

5.1.3. Preparation of 2-((1H-benzo[d]imidazol-2-yl)methylthio)-4-(substituted)-6-phenyl pyrimidine-5-carbonitriles (**5**–**14**)

5.1.3.1. General method. To a well stirred solution of the appropriate amine (5.30 mmol) and triethylamine (0.5 mL) in absolute ethanol (10 mL), equimolar amount of a solution of compound **4** (2 g, 5.30 mmol) in absolute ethanol (10 mL) was added portionwise. The reaction mixture was stirred for 2 h at room temperature then heated under reflux for additional 5 h, the solvent was then removed by distillation under reduced pressure and the remained solid was washed with cold water and purified either by recrystallization or by silica gel column chromatography.

Compounds **5** and **6** were prepared from the appropriate hydrazine derivative using the same method but the reaction mixture was left to stand overnight at room temperature and the precipitated product was filtered off, washed with ether and recrystallized from the appropriate solvent.

*5.1.3.1.1. 2-((1H-Benzo[d]imidazol-2-yl)methylthio)-4-hydrazinyl-6-phenylpyrimidine-5-carbonitrile (**5**).* Yield 81%; mp 160–162 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.17 (br, 1H, NH hydrazine, D_2O exchangeable), 4.79 (s, 2H, CH_2–S), 5.10 (br, 2H, NH_2 hydrazine, D_2O exchangeable), 7.25–7.30 (m, 2H, H5, H6 benzimidazole), 7.54–7.62 (m, 5H, phenyl H), 7.83 (m, 2H, H4, H7 benzimidazole), 12.60 (br, 1H, NH benzimidazole, D_2O exchangeable); IR (KBr) 3422.2 (NH benzimidazole), 3230.0 and 3152.7 (NH–NH_2), 3077. (CH arom.), 2933 (CH aliph.), 2221.0 (CN), 1628.0 (C=N benzimidazole), 1549.7 and 1507.2 (C=N pyrimidine, C=C); MS (EI) *m/z* 373 ($M^{\bullet+}$, 1.19%), *m/z* 375 ($M^{\bullet+} + 2$, 6.9%), *m/z* 91 (100%). Anal. Calc. for ($C_{19}H_{15}N_7S$): C, 61.10; H, 4.07; N, 26.26; S, 8.59. Found: C, 61.55; H, 4.32; N, 26.45; S, 8.67.

*5.1.3.1.2. 2-((1H-Benzo[d]imidazol-2-yl)methylthio)-4-(2-phenylhydrazinyl)-6-phenyl pyrimidine-5-carbonitrile (**6**).* Yield 82%; mp 242–244 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 4.79 (s, 2H, CH_2–S), 5.50 (br, 2H, NH–NH phenyl, D_2O exchangeable), 7.13–7.17 (m, 2H, H5, H6 benzimidazole), 7.25–7.28 (m, 2H, H2″, H6″ phenylhydrazine), 7.45–7.53 (m, 3H, H3″, H4″, H5″ phenylhydrazine), 7.62–7.64 (m, 3H, H3′, H4′, H5′ phenyl), 7.85–7.90 (m, 2H, H2′, H6′ phenyl), 8.11–8.15 (m, 2H, H4, H7 benzimidazole), 12.75 (br, 1H, NH benzimidazole, D_2O exchangeable); IR (KBr) 3463.1 (NH benzimidazole), 3360.0 and 3263.0 (NH–NH phenyl), 3059.1 (CH arom.), 2970 (CH aliph.), 2219.1 (CN), 1611.1 (C=N benzimidazole), 1550.3 and 1511.1 (C=N pyrimidine, C=C); MS (EI) *m/z* 449 ($M^{\bullet+}$, 100%) *m/z* 451 ($M^{\bullet+} + 2$, 15%). Anal. Calc. for ($C_{25}H_{19}N_7S$): C, 66.79; H, 4.27; N, 21.81; S, 7.13. Found: C, 66.50; H, 4.03; N, 21.56; S, 7.22.

*5.1.3.1.3. 2-((1H-Benzo[d]imidazol-2-yl)methylthio)-4-(methylamino)-6-phenyl pyrimidine-5-carbonitrile (**7**).* Yield 87%; mp 191–193 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.87 (s, 3H, NH–$\underline{CH_3}$), 3.35 (s, 2H, CH_2–S), 7.29–7.34 (m, 2H, C^5H, C^6H benzimidazole), 7.48–7.57 (m, 3H, C^{3a}H, C^{4a}H, C^{5a}H), 7.65–7.70 (m, 2H, C^{2a}H, C^{6a}H), 7.73–7.76 (m, 2H, C^4H, C^7H benzimidazole), 10.97 (br, 1H, $\underline{NH}$–CH_3, D_2O exchangeable), 12.90 (br, 1H, NH benzimidazole, D_2O exchangeable); IR (KBr) 3381.8 (NH benzimidazole), 3248.1 (NH–CH_3), 3030. (CH arom.), 2923.5 (CH aliph.), 2199.1 (CN), 1612.4 (C=N benzimidazole), 1560.9 and 1585.2 (C=N pyrimidine, C=C); MS (EI) *m/z* 372 ($M^{\bullet+}$, 11.5%), *m/z* 374 ($M^{\bullet+} + 2$, 5.5%), *m/z* 239 (100%). Anal. Calc. for ($C_{20}H_{16}N_6S$): C, 64.49; H, 4.34; N, 22.57; S, 8.61. Found: C, 64.72; H, 4.52; N, 22.85; S, 8.75.

*5.1.3.1.4. 2-((1H-Benzo[d]imidazol-2-yl)methylthio)-4-(ethylamino)-6-phenylpyrimidine-5-carbonitrile (**8**).* Yield 85%; mp 173–175 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.20 (t, 3H, CH_2–$\underline{CH_3}$), 3.35 (q, 2H, NH–$\underline{CH_2}$–CH_3), 4.65 (s, 2H, CH_2–S), 7.01–7.30 (m, 2H, C^5H, C^6H benzimidazole), 7.35–7.60 (m, 5H, phenyl H), 7.74 (m, 2H, C^4H, C^7H benzimidazole), 8.15 (br, 1H, $\underline{NH}$–CH_2CH_3, D_2O exchangeable), 12.90 (br, 1H, NH benzimidazole, D_2O exchangeable); IR (KBr) 3392.6 (NH benzimidazole), 3294.1 (NH–ethyl), 3056.8 (CH arom.), 2933.8 (CH aliph.), 2208.7 (CN), 1625.0 (C=N benzimidazole), 1562.0 and 1545.1 (C=N pyrimidine, C=C); MS (EI) *m/z* 386 ($M^{\bullet+}$, 29%), *m/z* 388 ($M^{\bullet+} + 2$, 8%), *m/z* 267 (100%). Anal. Calc. for ($C_{21}H_{18}N_6S$): C, 65.25; H, 4.70; N, 21.75; S, 8.30. Found: C, 65.57; H, 4.78; N, 21.65; S, 8.41.

*5.1.3.1.5. 2-((1H-Benzo[d]imidazol-2-yl)methylthio)-4-phenyl-6-(pyridin-4-ylamino)pyrimidine-5-carbonitrile (**9**).* Yield 85%; mp 223–225 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 4.80 (s, 2H, CH_2–S), 6.95 (d, $J = 7.2$ Hz, 2H, H3″, H5″ pyridine), 7.12–7.29 (m, 2H, H5, H6 benzimidazole), 7.49–7.65 (m, 5H, phenyl H), 7.92–8.02 (m, 2H, H4, H7 benzimidazole), 8.72 (br, 1H, $\underline{NH}$–pyridine, D_2O exchangeable), 9.22 (d, $J = 7.2$ Hz, 2H, H2″, H6″ pyridine), 12.88 (br, 1H, NH benzimidazole, D_2O exchangeable); IR (KBr) 3378.8 (NH benzimidazole), 3303.0 ($\underline{NH}$–pyridine), 3073.6 (CH arom.), 2930 (CH aliph.), 2215.6 (CN), 1621.2 (C=N benzimidazole), 1551.5 and 1533.8 (C=N, C=C groups); MS (EI) *m/z* 435 ($M^{\bullet+}$, 1.06%), *m/z* 437 ($M^{\bullet+} + 2$, 0.25%), *m/z* 260 (100%). Anal. Calc. for ($C_{24}H_{17}N_7S$): C, 66.18; H, 3.94; N, 22.51; S, 7.36. Found: C, 66.47; H, 4.00; N, 22.87; S, 7.47.

*5.1.3.1.6. 2-((1H-Benzo[d]imidazol-2-yl)methylthio)-4-phenyl-6-(pyridin-2-ylamino)pyrimidine-5-carbonitrile (**10**).* Yield 83%; mp 152–154 °C; IR (KBr) 3384.5 (NH benzimidazole), 3194.3 ($\underline{NH}$–pyridine), 3059.8 (CH arom.), 2954.1 (CH aliph.), 2204.0 ($\underline{CN}$), 1626.0 (C=N benzimidazole), 1567.4 and 1533.6 (C=N, C=C

groups); *m/z* 432 ($M^{\bullet+}-3$, 0.28%), *m/z* 55 (100%). Anal. Calc. for ($C_{24}H_{17}N_7S$): C, 66.18; H, 3.94; N, 22.51; S, 7.36. Found: C, 66.49; H, 4.09; N, 22.27; S, 7.39.

*5.1.3.1.7. 2-((1H-Benzo[d]imidazol-2-yl)methylthio)-4-phenyl-6-(thiazol-2-ylamino)pyrimidine-5-carbonitrile (**11**).* Yield 84%; mp 207–209 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 4.63 (s, 2H, CH_2–S), 7.16–7.31 (m, 2H, H5, H6 benzimidazole), 7.41 (d, $J = 7.2$ Hz, 1H, H5′thiazole), 7.50–7.55 (m, 5H, phenyl H), 7.95–8.04 (m, 3H, H4, H7 benzimidazole, H4′ thiazole), 8.55 (br, 1H, NH–thiazole, D_2O exchangeable), 12.54 (br, 1H, NH benzimidazole, D_2O exchangeable); IR (KBr) 3421.3 (NH benzimidazole), 3171.0 (NH aminothiazole), 3062.0 (CH arom.), 2929.7 (CH aliph.), 2210.9 (CN), 1611.0 (C=N benzimidazole), 1590.0 and 1542.7 (C=N, C=C groups); MS (EI) *m/z* 441 ($M^{\bullet+}$, 0.56%), *m/z* 443 ($M^{\bullet+}+2$, 0.23%), *m/z* 103 (100%). Anal. Calc. for ($C_{22}H_{15}N_7S_2$): C, 59.84; H, 3.43; N, 22.21; S, 14.52. Found: C, 59.90; H, 3.56; N, 22.35; S, 14.62.

5.1.4. 1-(2-((1H-Benzo[d]imidazol-2-yl)methylthio)-5-cyano-6-phenylpyrimidin-4-yl)thiosemicarbazide (**12**), thiourea or urea (**13**,**14**)

5.1.4.1. General method. To a well stirred solution of the appropriate thioamide or amide (5.30 mmol) and triethylamine (0.5 mL) in absolute ethanol (15 mL), equimolar amount of a solution of **4** (2 g, 5.30 mmol) in absolute ethanol (10 mL) was added portionwise. The reaction mixture was stirred at room temperature for 2 h then heated under reflux for additional 10 h, after cooling the solvent was removed by distillation under reduced pressure and the remained solid was washed with cold water and purified by recrystallization from the appropriate solvent.

*5.1.4.1.1. 1-(2-((1H-Benzo[d]imidazol-2-yl)methylthio)-5-cyano-6-phenylpyrimidin-4-yl)thiosemicarbazide (**12**).* Yield 80%; mp 129–132 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 4.50 (br, 1H, NH, D_2O exchangeable), 4.71 (s, 2H, CH_2–S), 5.05 (br, 2H, NH_2, D_2O exchangeable), 7.38–7.44 (m, 2H, H5, H6 benzimidazole), 7.53–7.62 (m, 5H, phenyl H), 7.68–7.72 (m, 2H, H4, H7 benzimidazole), 9.63 (br, 1H, NH, D_2O exchangeable), 12.50 (br, 1H, NH benzimidazole, D_2O exchangeable); IR (KBr) 3420.3 (br, NH and NH_2 groups), 3061.9 (CH arom.), 2929.4 (CH aliph.), 2219.1 (CN), 1620.0 (C=N benzimidazole), 1583.0 and 1525.6 (C=N pyrimidine, C=C); MS (EI) *m/z* 432 ($M^{\bullet+}$, 15%), *m/z* 434 ($M^{\bullet+}+2$, 6%), *m/z* 213 (100%). Anal. Calc. for ($C_{20}H_{16}N_8S_2$): C, 55.53; H, 3.74; N, 25.91; S, 14.83. Found: C, 55.85; H, 3.56; N, 26.04; S, 14.78.

*5.1.4.1.2. 1-(2-((1H-Benzo[d]imidazol-2-yl)methylthio)-5-cyano-6-phenylpyrimidin-4-yl)thiourea (**13**).* Yield 81%; mp 118–120 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 4.40 (br, 2H, NH_2, D_2O exchangeable), 4.90 (s, 2H, CH_2–S), 7.38–7.44 (m, 2H, H5, H6 benzimidazole), 7.52–7.60 (m, 3H, H3′, H4′, H5′), 7.66–7.79 (m, 4H, H4, H7 benzimidazole, H2′, H6′), 9.82 (br, 1H, NH, D_2O exchangeable), 12.91 (br, 1H, NH benzimidazole, D_2O exchangeable); IR (KBr) 3396.8 (NH benzimidazole), 3286.1 and 3184.9 (NH, NH_2), 3050 (CH arom.), 2921.6 (CH aliph.), 2213.9 (CN), 1607.6 (C=N benzimidazole), 1563.9 and 1531.2 (C=N pyrimidine, C=C); MS (EI) *m/z* 417 ($M^{\bullet+}$, 2.32%), *m/z* 419 ($M^{\bullet+}+2$, 0.92), *m/z* 56 (100%). Anal. Calc. for ($C_{20}H_{15}N_7S_2$): C, 57.53; H, 3.63; N, 23.49; S, 15.36. Found: C, 57.75; H, 3.47; N, 23.64; S, 15.21.

*5.1.4.1.3. 1-(2-((1H-Benzo[d]imidazol-2-yl)methylthio)-5-cyano-6-phenylpyrimidin-4-yl)urea (**14**).* Yield 86%; mp 190–192 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 4.22 (br, 2H, NH_2, D_2O exchangeable), 4.80 (s, 2H, CH_2–S), 7.10–7.25 (m, 2H, H5, H6 benzimidazole), 7.40–7.65 (m, 5H, phenyl H), 7.70–7.90 (m, 2H, H4, H7 benzimidazole), 9.63 (br, 1H, NH, D_2O exchangeable), 12.65 (br, 1H, NH benzimidazole, D_2O exchangeable); IR (KBr) 3421.6 (br, NH and NH_2 groups), 3059.8 (CH arom.), 2930.0 (CH aliph.), 2217.7 (CN), 1663.3 (C=O), 1627.6 (C=N benzimidazole), 1550.0 and 1533.3 (C=N pyrimidine, C=C); MS (EI) *m/z* 399 ($M^{\bullet+}-2$, 100%). Anal. Calc. for ($C_{20}H_{15}N_7OS$): C, 59.83; H, 3.77; N, 24.43; S, 7.99. Found: C, 59.48; H, 3.85; N, 24.52; S, 7.75.

5.1.5. 2-((1H-Benzo[d]imidazol-2-yl)methylthio)-4-phenyl-6-(piperazin-1-yl)pyrimidine-5-carbonitrile (**15**)

A mixture of piperazine (6.81 g, 79.57 mmol) and anhydrous potassium carbonate (1.82 g, 13.26 mmol) in absolute ethanol (10 mL) was stirred for 30 min, a solution of compound **2** (5 g, 13.26 mmol) in absolute ethanol (15 mL) was then added portionwise with continuous stirring. The reaction mixture was stirred for 2 h at room temperature then heated under reflux for additional 8 h, after cooling it was filtered to remove insoluble material and the filtrate was concentrated under vacuum then poured onto ice and neutralized with dilute hydrochloric acid. Yield 44%; mp 182–184; IR (KBr) 3421.9 (NH benzimidazole), 3300.0 (NH piperazine), 3058.5 (CH arom.), 2930.5 (CH aliph.), 2198.2 (CN), 1619.9 (C=N benzimidazole), 1584.1 and 1546.9 (C=N pyrimidine, C=C); MS (EI) *m/z* 427 ($M^{\bullet+}$, 0.8%), *m/z* 429 ($M^{\bullet+}+2$, 0.16%), *m/z* 350 ($M^{\bullet+}$ – phenyl, 24.74%), 281 (100%). Anal. Calc. for ($C_{23}H_{21}N_7S$): C, 64.61; H, 4.96; N, 22.93; S, 7.50. Found: C, 64.23; H, 4.86; N, 22.75; S, 7.56.

5.1.6. 2-((1H-Benzo[d]imidazol-2-yl)methylthio)-4-(methylpiperazin-1-yl)-6-phenylpyrimidine-5-carbonitrile (**16**)

A mixture of compound **2** (2 g, 5.3 mmol), 4-methylpiperazine (0.53 g, 5.30 mmol) and triethylamine (0.5 mL) in absolute ethanol (50 mL) was stirred for 1 h. The formed precipitate was filtered off, washed with water and recrystallized from water/acetone. Yield 68%; mp 222–224 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.35 (s, 3H, CH_3), 2.55–2.59 (m, 4H, piperazine H), 3.98–4.04 (m, 4H, piperazine H), 4.78 (s, 2H, CH_2–S), 7.19–7.24 (m, 2H, H5, H6 benzimidazole), 7.55–7.66 (m, 5H, phenyl H), 7.84–7.92 (m, 2H, H4, H7 benzimidazole), 12.52 (br, 1H, NH benzimidazole, D_2O exchangeable); IR (KBr) 3421.3 (NH benzimidazole), 3054.6 (CH arom.), 2946.5 (CH aliph.), 2209.9 (CN), 1621.9 (C=N benzimidazole), 1575.0 and 1526.8 (C=N pyrimidine, C=C); MS (EI) *m/z* 441 ($M^{\bullet+}$, 9.65%), *m/z* 443 ($M^{\bullet+}+2$, 3.63%), *m/z* 310 (100%). Anal. Calc. for ($C_{30}H_{25}N_7OS$): C, 67.77; H, 4.75; N, 18.44; S, 6.03. Found: C, 67.82; H, 4.87; N, 18.65; S, 6.15.

5.1.7. 2-((1H-Benzo[d]imidazol-2-yl)methylthio)-4-(aryl or alkyl piperazin-1-yl)-6-phenylpyrimidine-5-carbonitriles (**17**–**23**)

5.1.7.1. General method. To a solution of compound **13** (4.68 mmol) in dimethylformamide (15 mL), sodium hydride (0.11 g, 4.68 mmol) was added gradually under ice cooling. The reaction mixture was stirred for 30 min, and then the appropriate halide (4.68 mmol) was added portionwise. The whole mixture was warmed to room temperature and stirred for 4 h, poured onto ice-water with continuous stirring. The precipitated product was filtered off and purified either by recrystallization or by silica gel column chromatography.

*5.1.7.1.1. 2-((1H-Benzo[d]imidazol-2-yl)methylthio)-4-(4-benzoylpiperazin-1-yl)-6-phenylpyrimidine-5-carbonitrile (**17**).* Yield 80%; mp 164–166 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.39–3.38 (m, 4H, piperazine H), 3.61–3.79 (m, 4H, piperazine H), 4.03 (s, 2H, CH_2–S), 7.11–7.43 (m, 6H, H3′, H4′, H5′ phenyl, H3″, H4″, H5″ benzoyl), 7.52–7.67 (m, 4H, H2′, H6′ phenyl, H2″, H6″ benzoyl), 7.84 (m, 2H, H5, H6 benzimidazole), 7.97 (m, 2H, H4, H7 benzimidazole), 9.95 (br, 1H, NH benzimidazole, D_2O exchangeable); IR (KBr) 3423.5 (NH benzimidazole), 3057.9 (CH arom.), 2930.5 (CH aliph.), 2198.5 (CN), 1634.9 (C=O), 1625.0 (C=N benzimidazole), 1581.4 and 1547.6 (C=N pyrimidine, C=C); MS (EI) *m/z* 531 ($M^{\bullet+}$, 0.38%), *m/z* 104 (100%). Anal. Calc. for ($C_{30}H_{25}N_7OS$): C, 67.77; H, 4.75; N, 18.44; S, 6.03. Found: C, 67.82; H, 4.87; N, 18.65; S, 6.15.

*5.1.7.1.2. 2-((1H-Benzo[d]imidazol-2-yl)methylthio)-4-(4-(4-nitrobenzoyl)piperazin-1-yl)-6-phenylpyrimidine-5-carbonitrile (**18**).* Yield 84%; mp 127–129 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.12–3.85 (m, 8 H, piperazine H), 4.21 (s, 2H, CH_2–S), 7.21–8.27 (m, 13H, arom. H), 12.30 (br, 1H, NH benzimidazole, D_2O exchangeable); IR (KBr) 3410.49 (NH benzimidazole), 3060.0 (CH arom.), 2920.6 (CH aliph.),

2199.42 (CN), 1641.12 (C=O), 1610 (C=N benzimidazole), 1590.0 and 1545.67 (C=N pyrimidine, C=C), 1530.6 and 1373.2 (NO_2); MS (EI) *m/z* 576 ($M^{\bullet+}$, 0.52%), *m/z* 578 ($M^{\bullet+}+2$, 0.13%), *m/z* 150 (100%). Anal. Calc. for ($C_{30}H_{24}N_8O_3S$): C, 62.48; H, 4.20; N, 19.43; S, 5.56. Found: C, 61.77; H, 4.45; N, 19.65; S, 5.68.

*5.1.7.1.3. 2-((1H-Benzo[d]imidazol-2-yl)methylthio)-4-phenyl-6-(4-(2-phenylacetyl)piperazin-1-yl)pyrimidine-5-carbonitrile (**19**).* Yield 86%; mp 162–164 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.09–3.70 (m, 10H, piperazine H + CO–$\underline{CH_2}$), 4.05 (s, 2H, CH_2–S), 7.00–8.13 (m, 14H, arom. H), 12.51 (br, 1H, NH benzimidazole, D_2O exchangeable); IR (KBr) 3447.6 (NH benzimidazole), 3035.6 (CH arom.), 2923.8 (CH aliph.), 2229.9 (CN), 1676.3 (C=O), 1628.1 (C=N benzimidazole), 1596.1 and 1547.3 (C=N pyrimidine, C=C); MS (EI) *m/z* 546 ($M^{\bullet+}+1$, 0.05%), *m/z* 105 (100%). Anal. Calc. for ($C_{31}H_{27}N_7OS$): C, 68.23; H, 5.00; N, 17.97; S, 5.88. Found: C, 68.52; H, 4.77; N, 17.76; S, 5.90.

*5.1.7.1.4. 2-((1H-Benzo[d]imidazol-2-yl)methylthio)-4-(4-(2-oxo-2-phenylethyl)piperazin-1-yl)-6-phenylpyrimidine-5-carbonitrile (**20**).* Yield 78%; mp 120–122 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.28–3.69 (m, 4H, piperazine H), 3.7–4.02 (m, 6H, $\underline{CH_2}$–CO, piperazine H), 4.10 (m, 2H, CH_2–S), 7.01–7.38 (m, 7H, H5, H6 benzimidazole, H3′, H4′, H5′ phenyl, H3″, H5″ 2-oxo-2-phenylethyl), 7.38–7.65 (m, 5H, H2′, H6′ phenyl, H2″, H4″, H6″ 2-oxo-2-phenylethyl), 7.69–7.92 (m, 2H, H4, H7 benzimidazole), 9.50 (br, 1H, NH benzimidazole, D_2O exchangeable); IR (KBr) 3386.4 (NH benzimidazole), 3058.5 (CH arom.), 2928 (CH aliph.), 2199.4 (CN), 1656.5 (C=O), 1627.6 (C=N benzimidazole), 1587.1 and 1540.8 (C=N pyrimidine, C=C); MS (EI) *m/z* 544 ($M^{\bullet+}-1$, 0.77%), *m/z* 249.97 (100%). Anal. Calc. for ($C_{31}H_{27}N_7OS$): C, 68.23; H, 5.00; N, 17.97; S, 5.88. Found: C, 68.45; H, 4.66; N, 17.85; S, 5.89.

*5.1.7.1.5. 2-((1H-Benzo[d]imidazol-2-yl)methylthio)-4-phenyl-6-(4-(phenylsulphonyl)piperazin-1-yl)pyrimidine-5-carbonitrile (**21**).* Yield 82%; mp 90–92 °C; ^{1}H NMR(200 MHz, DMSO-d_6) δ 3.04 (m, 4H, piperazine H), 3.97 (m, 4H, piperazine H), 4.61 (s, 2H, CH_2–S), 7.20–8.10 (m, 14H, arom. H), 13.14 (br, 1H, NH benzimidazole, D_2O exchangeable); IR (KBr) 3394.1 (NH benzimidazole), 3055.2 (CH arom.), 2950.8 (CH aliph.), 2199.4 (CN), 1615.1 (C=N benzimidazole), 1545.0 and 1520.0 (C=N pyrimidine, C=C), 1336.6 and 1145.0 (SO_2); MS (EI) *m/z* 567 ($M^{\bullet+}$, 3.60%), *m/z* 569 ($M^{+}+2$, 0.82%), *m/z* 218 (100%). Anal. Calc. for ($C_{29}H_{25}N_7O_2S_2$): C, 61.35; H, 4.45; N, 17.27; S, 11.29. Found: C, 61.53; H, 4.35; N, 17.53; S, 11.32.

*5.1.7.1.6. 2-((1H-Benzo[d]imidazol-2-yl)methylthio)-4-phenyl-6-(4-tosylpiperazin-1-yl)pyrimidine-5-carbonitrile (**22**).* Yield 88%; mp 179–181 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.42 (s, 3H, *p*-CH_3), 2.96–3.01 (m, 4H, H_2, piperazine H), 3.86–3.97 (m, 6H, piperazine H, CH_2–S), 7.27–7.82 (m, 13H, arom. H), 12.84 (br, 1H, NH benzimidazole, D_2O exchangeable); IR (KBr) 3421.7 (NH benzimidazole), 3033.0 (CH arom.), 2963.2 (CH aliph.), 2221.9 (CN), 1628.6 (C=N benzimidazole), 1549.8 and 1507.5 (C=N pyrimidine, C=C), 1300.6 and 1157.7 (SO_2); MS (EI) *m/z* 581 ($M^{\bullet+}$, 1.2%), *m/z* 583 ($M^{+}+2$, 0.42%), *m/z* 91 (100%). Anal. Calc. for ($C_{30}H_{27}N_7O_2S_2$): C, 61.94; H, 4.69; N, 16.86; S, 11.02. Found: C, 61.80; H, 4.57; N, 16.97; S, 11.10.

*5.1.7.1.7. 2-((1H-Benzo[d]imidazol-2-yl)methylthio)-4-(4-(2-nitrobenzensulphonyl)piperazin-1-yl)-6-phenylpyrimidine-5-carbonitrile (**23**).* Yield 83%; mp 117–119 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.20–3.60 (m, 4H, piperazine H), 3.79–4.20 (m, 6H, 4 piperazine H, CH_2–S), 7.36–7.54 (m, 3H, H5 and H6 benzimidazole, H4′), 7.79–7.93 (m, 5H, H2′, H3′, H5′, H6′ phenyl, H4″ 2-nitrobenzensulphonyl), 7.96–8.03 (m, 5H, H4 and H7 benzimidazole, H3″, H5″, H6″2-nitrobenzensulphonyl), 12.95 (br, 1H, NH benzimidazole, D_2O exchangeable); IR (KBr) 3396.7 (NH benzimidazole), 3035.4 (CH arom.), 2953.6 (CH aliph.), 2212.7 (CN), 1626.8 (C=N benzimidazole), 1583.0 and 1529.0 (C=N pyrimidine, C=C), 1512.2 and 1392.1 (NO_2), 1330.0 and 1153.5 (SO_2); MS (EI) *m/z* 535 ($M^{\bullet+}$ – phenyl, 10.44%), *m/z* 174 (100%). Anal. Calc. for ($C_{29}H_{24}N_8O_4S_2$): C, 56.85; H, 3.96; N, 18.29; S, 10.47. Found: C, 56.95; H, 3.65; N, 18.18; S, 10.38.

*5.1.8. 7-((1H-Benzo[d]imidazol-2-yl)methylthio)-3,4-dihydro-4-imino-5-phenylpyrimido[4,5-d]pyrimidin-2(1H)-(thione or one) (**24**,**25**)*

A mixture of compound **4** and thiourea or urea was fused together at 250 °C for 15 min. The formed mass was triturated with ethanol and the remained solid was recrystallized from methylene chloride to obtain **24** and **25** respectively.

*5.1.8.1. 7-((1H-Benzo[d]imidazol-2-yl)methylthio)-3,4-dihydro-4-imino-5-phenylpyrimido[4,5-d]pyrimidin-2(1H)-thione (**24**).* Yield 71%; mp 210–212 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 4.75 (s, 2H, CH_2–S), 7.25–7.35 (m, 2H, H5, H6 benzimidazole), 7.55–7.70 (m, 5H, phenyl H), 7.78–7.83 (m, 2H, H4, H7 benzimidazole), 8.22, 8.52, 8.60 (br, 3H, 3NH, D_2O exchangeable), 12.50 (br, 1H, NH benzimidazole, D_2O exchangeable); IR (KBr) 3396.9 (NH benzimidazole), 3196.9 (br, NH groups), 3055.3 (CH arom.), 2922.3 (CH aliph.), 1624.1 (C=N benzimidazole), 1598.0 and 1548.9 (C=N, C=C groups), 1240 (C=S); MS (EI) *m/z* 454 ($M^{\bullet+}$, 1.21%), *m/z* 84 (100%). Anal. Calc. for ($C_{20}H_{15}N_7S_2\cdot HCl$): C, 52.91; H, 3.56; N, 21.60; S, 14.13. Found: C, 52.75; H, 3.68; N, 21.23; S, 14.52.

*5.1.8.2. 7-((1H-Benzo[d]imidazol-2-yl)methylthio)-3,4-dihydro-4-imino-5-phenylpyrimido[4,5-d] pyrimidin-2(1H)-one (**25**).* Yield 78%; mp 177–179 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 4.56 (s, 2H, CH_2–S), 7.21–7.28 (m, 2H, H5, H6 benzimidazole), 7.42–7.63 (m, 5H, phenyl H), 7.82–7.95 (m, 2H, H4, H7 benzimidazole), 8.31, 8.56, 8.75 (br, 3H, 3NH, D_2O exchangeable), 12.88 (br, 1H, NH benzimidazole, D_2O exchangeable); IR (KBr) 3370.0 (NH benzimidazole), 3134.7 (br, NH groups), 3053.0 (CH arom.), 2924.0 (CH aliph.), 1670.0 (C=O), 1626.6 (C=N benzimidazole), 1562.06 and 1520.0 (C=N, C=C groups); MS (EI) *m/z* 437 ($M^{\bullet+}$, 0.48%), *m/z* 143 (100%). Anal. Calc. for ($C_{20}H_{15}N_7OS\cdot HCl$): C, 54.85; H, 3.69; N, 22.39; S, 7.32. Found: C, 54.76; H, 3.23; N, 22.52; S, 7.12.

5.2. Pharmacology

5.2.1. Antitumor activity

5.2.1.1. In vitro cytotoxicity. The in vitro cytotoxicity of the synthesized compounds against 12 different cancer cell lines was performed with the MTT assay according to the Mosmann's method [21]. The MTT assay is based on the reduction of the soluble 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) into a blue–purple formazan product, mainly by mitochondrial reductase activity inside living cells.

The cells used in cytotoxicity assay were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum. Cells suspended in the medium (2Ý 104/mL) were plated in 96-well culture plates and incubated at 37 °C in a 5% CO_2 incubator. After 12 h, the test sample (2 mL) was added to the cells (2Ý 104) in 96-well plates and cultured at 37 °C for 3 days. The cultured cells were mixed with 20 mL of MTT solution and incubated for 4 h at 37 °C. The supernatant was carefully removed from each well and 100 mL of DMSO was added to each well to dissolve the formazan crystals which were formed by the cellular reduction of MTT. After mixing with a mechanical plate mixer, the absorbance of each well was measured by a microplate reader using a test wavelength of 570 nm. The results were expressed as the IC_{50}, which is the concentration of the drugs inducing a 50% inhibition of cell growth of treated cells when compared to the growth of control cells. Each experiment was performed at least 3 times. There was a good reproducibility between replicate wells with standard errors below 10%.

5.2.2. Median lethal dose LD_{50}

The median lethal dose of the tested compounds was determined according to the procedure described by Lorke [22]. The experiment was carried out on two phases; the first phase involved three groups of three animals per each group. One dose was given to each group intraperitoneally and the treated mice were monitored for 24 h for mortality and general behaviors. The second phase comprises 3–4 groups of one mouse per group were given doses, based on the findings of phase 1, the mice were again monitored for 24 h. The geographic mean of the least dose that killed mice and the highest dose that did not kill mice was taken as the median lethal dose [23,24].

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