

THE USES OF ETHYL 2-(1H-BENZO[D]IMIDAZOL-2-YL)ACETATE TO SYNTHESIS PYRAZOLE, THIOPHENE, PYRIDINE AND COUMARIN DERIVATIVES WITH ANTITUMOR ACTIVITIES

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ABSTRACT. In the present work, the ethyl 2-(1H-benzo[d]imidazol-2-yl)acetate (3) was subjected to a series of heterocyclization reactions through its reaction with different chemical reagents. The resulting molecules were thiophene, pyrazole, coumarin derivatives incorporated benzo[d]imidazole moiety. All the synthesized compounds were determined by elemental analysis, ¹H NMR, ¹³C NMR, and MS. The antitumor evaluations of the newly synthesized products toward the three cancer cell lines MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), and SF-268 (CNS cancer) showed that compounds 5a, 9b, 9c, 17, 23b and 38 were of the highest potencies among the synthesized compounds.

KEY WORDS: Oxobutanamide, Thiophene, Pyrazole, Pyran, Pyridine

INTRODUCTION

In recent years, benzimidazole derivatives have provided a large number of biologically active compounds that have been intensively used in medicinal chemistry as drugs. They are structural isomers of naturally occurring nucleotides, which allow them to interact easily with the biopolymers of the living system and different kinds of biological activity have been obtained. Some 2-aminobenzimidazoles displayed an appreciable antimicrobial effect. Their corresponding carbamate derivatives have been synthesized for their significant in vivo antifilarial activity [1]. Concerning the high affinity that they display towards a variety of enzymes and protein receptors, they could be considered as pivotal structures in drug design [2].

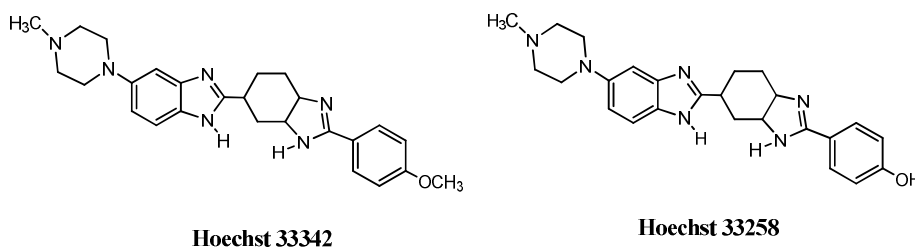


Figure 1. Examples of topoisomerase inhibitors containing benzimidazole nucleus.

Optimization of benzimidazole-based structures has resulted in marketed drugs, e.g. Omeprazole [3] and Pimobendan [4] that are therapeutically useful in the management of peptic ulcer and congestive heart failure respectively. Many derivatives of benzimidazoles are well known for their antimicrobial [5, 6] anthelmintic [7] antiviral [8, 9] and antifungal [10, 11] activities. Since 1985, benzimidazole containing compounds have been reported as well known anticancer agents [12, 13]. The role of mammalian DNA topoisomerases as molecular targets

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for anticancer drugs has been recognized. Some benzimidazoles have been reported as topoisomerase inhibitors, e.g. Hoechst 33258 and Hoechst 33342 (Figure 1) [14, 15]. On extension of this work, head to head bis-benzimidazole compounds approved high efficacy as DNA binders [16]. Some widely used anticancer drugs such as RAF265 (CHIR-265; Novartis Pharmaceuticals, Basel, Switzerland) and AZD6244 (ARRY-142886; AstraZeneca, London, England) are known to contain benzimidazole moiety. RAF265 resulted in a reduction in tumor cell growth and in tumor cell apoptosis [17].

RESULTS AND DISCUSSION

In the present work, we synthesized a series of heterocyclic compounds derived from ethyl 2-(1*H*-benzo[*d*]imidazol-2-yl)acetate together with their antitumor evaluations. Thus, the reaction of *o*-phenylenediamine (**1**) with diethylmalonate (**2**) in an oil bath at 120 °C gave ethyl 2-(1*H*-benzo[*d*]imidazol-2-yl)acetate (**3**), its structure was based on analytical and spectral data. Compound **3** was used in many acyclic and heterocyclic reactions. Thus, compound **3** reacted with either aniline (**4a**) or 4-methylaniline (**4b**) to give the anilide derivatives **5a** and **5b**, respectively. The structures of compounds **5a** and **5b** were based on their respective analytical and spectral data. Thus, the ¹H NMR spectrum **5a** showed the presence of a singlet at δ 4.93 ppm corresponding to the CH₂ group a multiplet at δ 7.25-7.41 ppm confirming the presence of the two phenyl protons and two signals at δ 8.26 and 8.32 ppm corresponding to the presence of the two NH groups. In addition the ¹³C NMR data showed beside the expected signals the presence of a signal at 54.2 for the CH₂ group and two signals at 1.72 and 164.8 indicating the C=N and CO groups, respectively.

Compound **3** reacted with any of benzaldehyde (**6a**), 4-methoxybenzaldehyde (**6b**) or 4-chlorobenzaldehyde (**6c**) to give the benzylidene derivatives **7a-c**, respectively. Either of compound **7a** with either hydrazine hydrate (**8a**) or phenylhydrazine (**8b**) or compound **7c** with hydrazine hydrate gave the pyrazole derivatives **9a-c**, respectively. Compound **7a** reacted with malononitrile (**10**) to give the ethyl 1-amino-2-cyano-3-phenylbenzo[4,5]imidazo[1,2-*a*]pyridine-4-carboxylate (**11**) (Scheme 1). The analytical and spectral data of compound **11** are in agreement with their respective analysis. Thus, the ¹H NMR spectrum **11** showed (beside the expected signals) a triplet and quartet, a singlet (D₂O exchangeable) at δ 4.83 ppm confirming the presence of the NH₂ group. In addition the ¹³C NMR showed the presence of signals at 116.8 for the CN group, signals at 144.3, 143.2, 140.8, 137.3, 128.8, 126.2, 125.9, 125.4, 124.3, 123.5, 120.1, 119.3 equivalent to the pyridine C-2, C-3, C-4, C-5 and the C₆H₅, C₆H₄ groups.

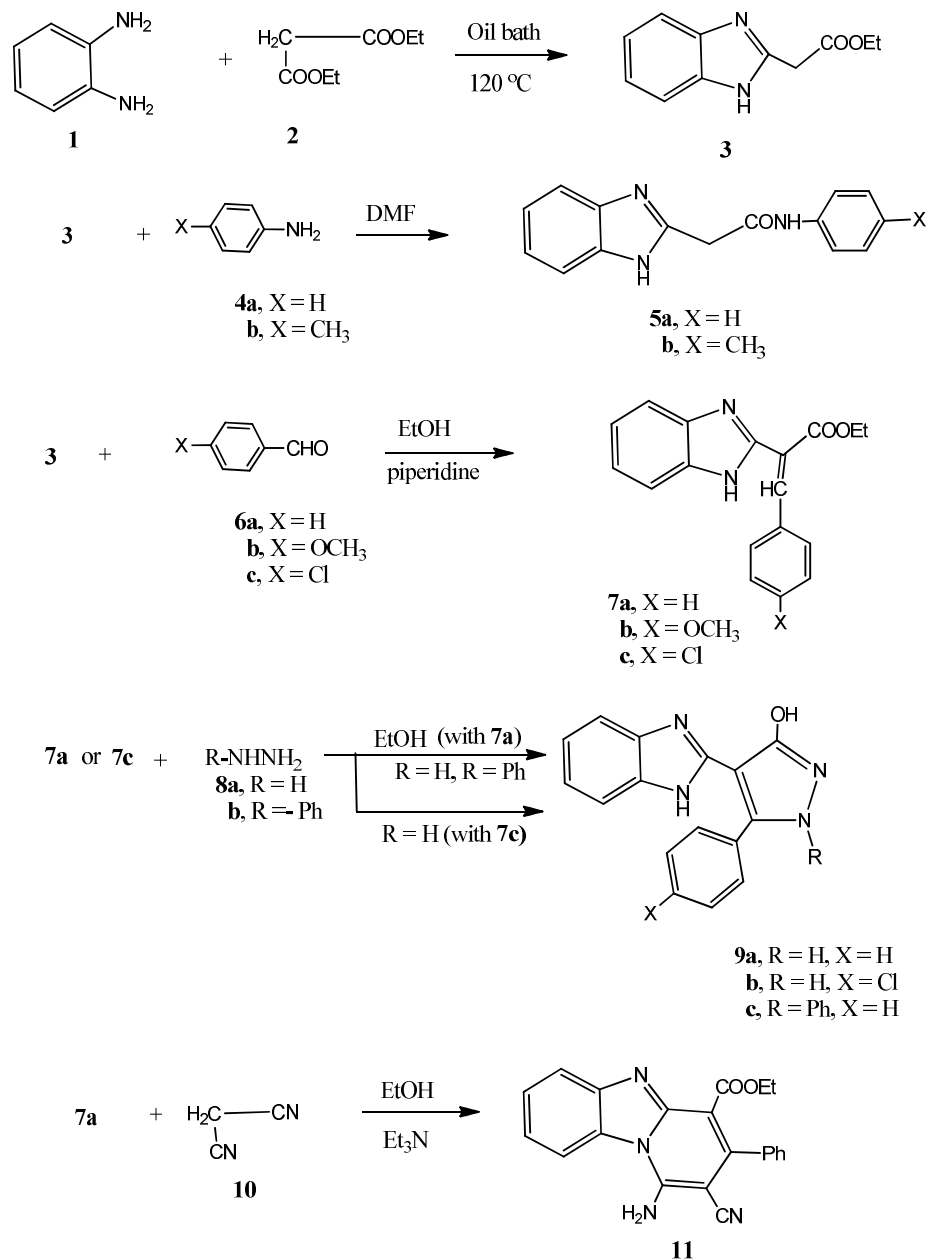
The reactivity of compound **3** towards condensation reactions was studied in order to produce biologically active products. Thus, compound **3** reacted with acetophenone (**12**) in an oil bath containing ammonium acetate to give the Knoevenagel condensation product **13**. On the other hand, the reaction of compound **3** with either cyclopentanone (**14a**) or cyclohexanone (**14b**) gave the products **15a** and **15b**, respectively.

The analytical and spectral data of **15a,b** were in agreement with their respective structures. Thus, the ¹H NMR spectrum of **15a** showed (beside the expected signals) the presence the two multiplets at δ 1.18-1.24 and 2.9-2.21 ppm indicating the presence of the four cyclohexene CH₂ groups and a singlet at δ 8.32 ppm (D₂O exchangeable) indicating the presence of the NH group.

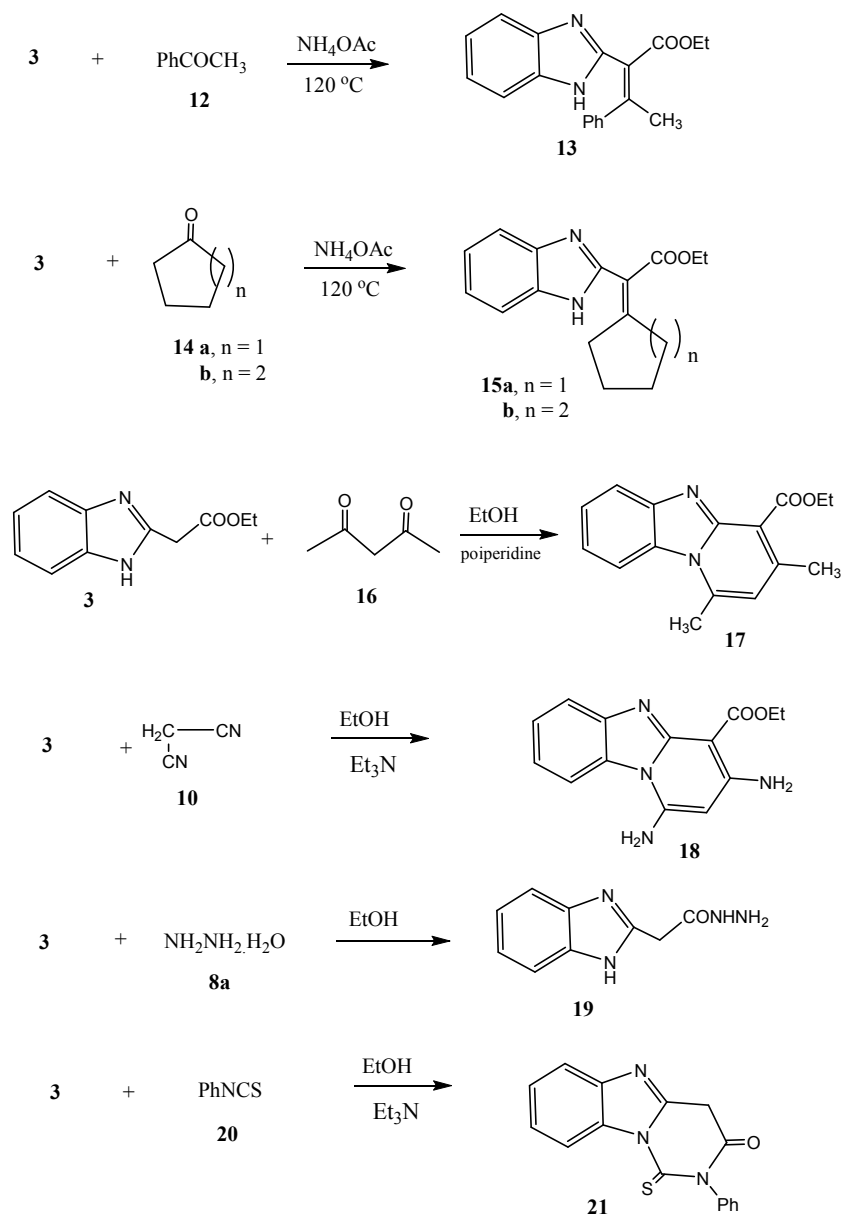
The reaction of compound **3** with acetylacetone (**16**) gave the benzo[4,5]imidazo[1,2-*a*]pyridine derivative **17**. Similarly, the reaction of compound **3** with malononitrile (**10**) gave the benzo[4,5]imidazo[1,2-*a*]pyridine derivative **18**.

Compound **3** reacted with hydrazinehydrate (**8a**) to give the hydrazide derivative **19**. On the other hand the reaction of compound **3** with phenylisothiocyanate (**20**) gave the 1,2-dihydrobenzo[4,5]imidazo[1,2-*c*]pyrimidin-3(4*H*)-one derivative **21** (Scheme 2). The structure of compound **21** was based on its analytical and spectral data. Thus, the ¹H NMR spectrum **21**

showed the presence of a singlet at δ 5.90 ppm indicating the presence of the pyrimidine CH_2 group and a multiplet at δ 7.26-7.38 ppm for the C_6H_5 and C_6H_4 groups.



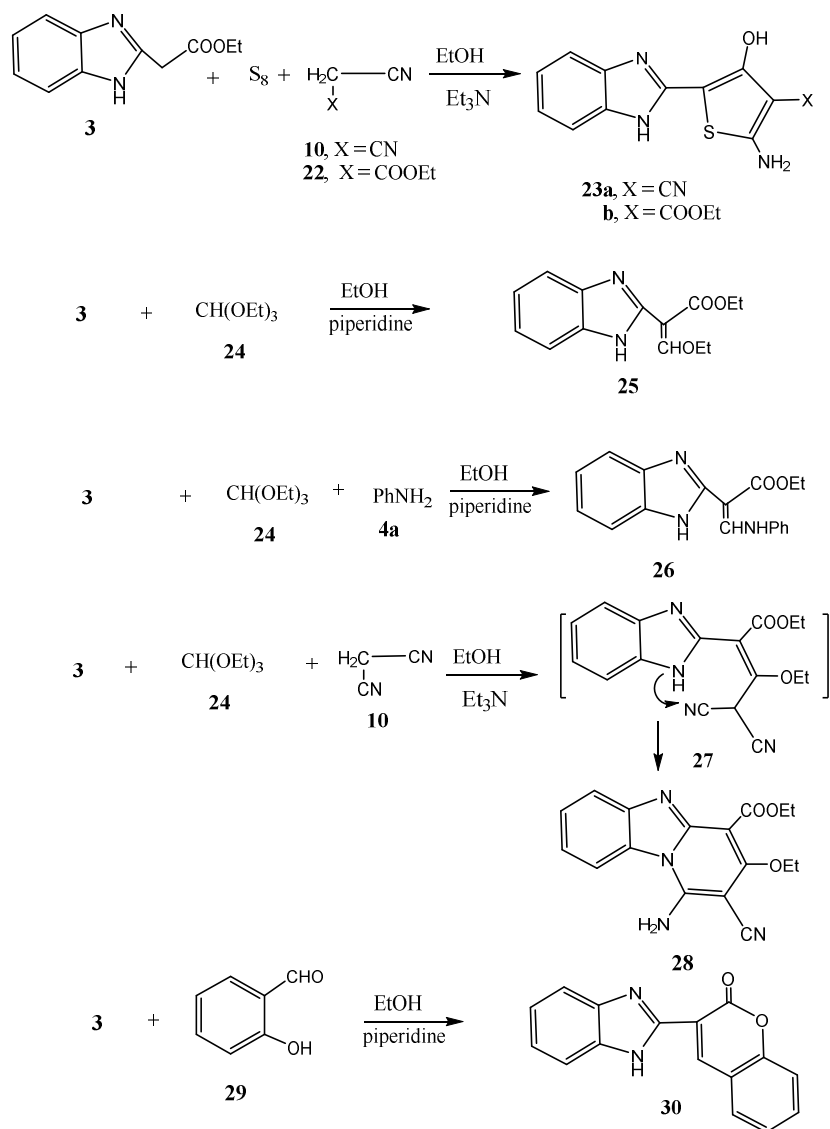
Scheme 1



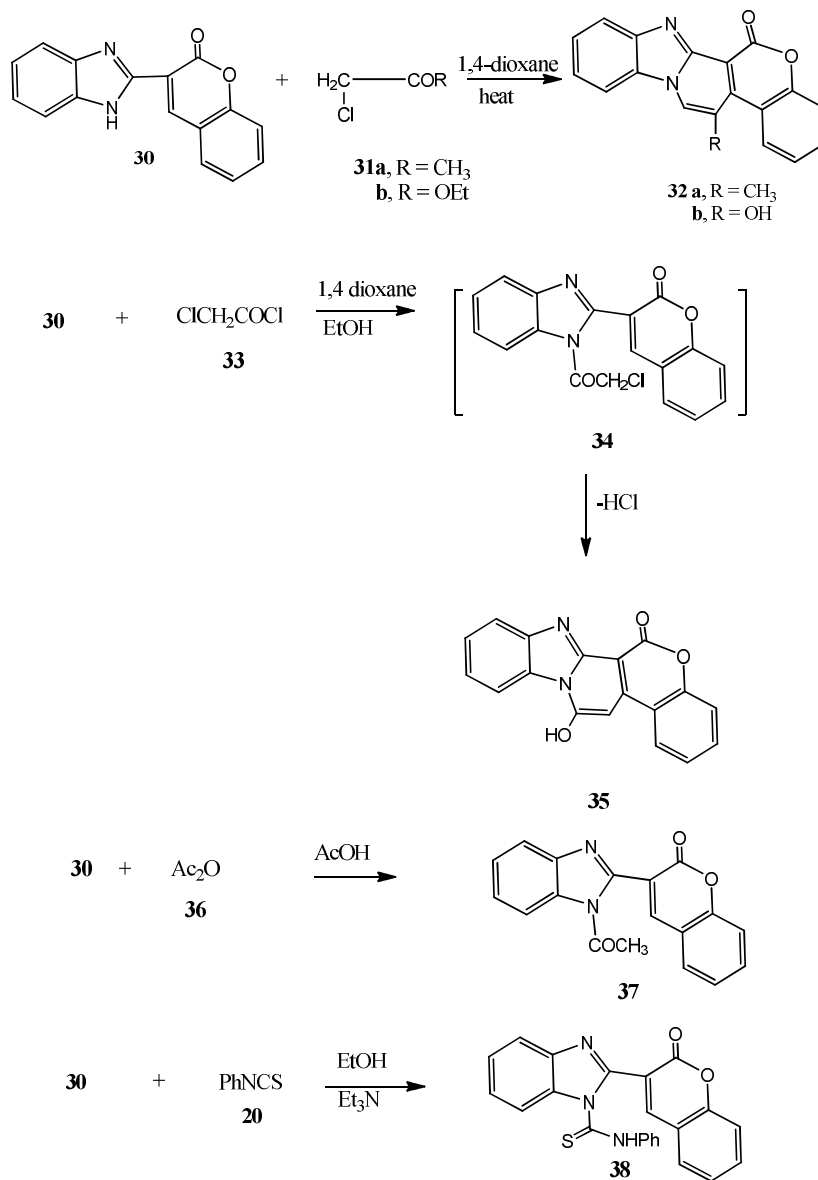
Scheme 2

The reactivity of compound **3** towards thiophene synthesis using the Gewald's thiophene synthesis was studied. Thus, the reaction of compound **3** with elemental sulfur and either of malononitrile (**10**) or ethyl cyanoacetate (**22**) gave the thiophene derivatives **23a** and **23b**, respectively. Moreover, the reaction of compound **3** with ethylorthoformate (**24**) gave the ethoxymethylene derivatives **25**. On the other hand, the multi-component reaction of

compound **3** with ethyl orthoformate (**24**) and aniline (**4a**) gave the phenylaminomethylene derivative **26**. The analytical and spectral data of compounds **25** and **26** were consistent with their respective structures (see experimental section). The multi-component reaction (MCRs) of compound **3** with ethyl orthoformate (**24**) and malononitrile (**10**) gave the ethyl 1-amino-2-cyano-3-ethoxybenzo[4,5]imidazo[1,2-*a*]pyridine-4-carboxylate **28**. Formation of the latter product took place through the intermediate formation of **27** followed by the Micheal type addition of the NH to the terminal CN group.



Scheme 3



Scheme 4

The reaction of compound **3** with salicylaldehyde (**29**) in ethanol containing piperidine gave the coumarin derivative **30**. The structure of compound **30** (Scheme 3) was based on analytical and spectral data. Thus, the ¹H NMR spectrum **30** showed a singlet at δ 6.09 corresponding to the coumarin *H*-4 and a singlet at δ 8.09 (D₂O exchangeable) indicating the presence of the NH group. In addition the ¹³C NMR spectrum showed the presence of signals at 131.5, 130.3, 129.4, 128.2, 127.3, 126.8, 126.4, 125.3, 124.1, 123.6, 122.4, 121.6 equivalent to the two phenyl

groups and two signals at 173.4 and 164.6 indicating the presence of the C=N and C=O groups, respectively.

Further confirmations for the structure of compound **30** were obtained through studying its reactivity towards some chemical reagents. Thus, compound **30** reacted with either α -chloroacetone (**31a**) or ethyl α -chloroacetate (**31b**) to give the 1*H*-benzo[4,5]imidazo[1,2-*a*]chromeno[3,4-*c*]pyridin-1-one derivatives **32a** and **32b**, respectively. Formation of the latter product was explained in terms of the first reaction of the NH of compound **30** with the α -chloro group of **32a** or **32b** followed by either elimination of water or ethanol, respectively. Moreover, the reaction of compound **30** with chloroacetylchloride (**33**) gave the 8-hydroxy-1*H*-benzo[4,5]imidazo[1,2-*a*]chromeno[3,4-*c*]pyridin-1-one **35** its analytical and spectral data confirm the proposed structure. The reaction of compound **30** with acetic anhydride (**36**) gave the *N*-acetyl derivative **37**. Our trials to make cyclization of **37** under different conditions were failed. Finally, the reaction of compound **30** with phenylisothiocyanate (**20**) gave the *N*-phenylthiourea derivative **38** (Scheme 4).

Antitumor and normal cell line 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 the European Collection of Cell Cultures (ECACC, Salisbury, UK), NCI-H460, SF-268 and normal fibroblast cells (WI 38) were kindly provided by the National Cancer Institute (NCI, Cairo, Egypt). They grow as monolayer and routinely maintained in RPMI-1640 medium supplemented with 5% heat inactivated FBS, 2 μ M glutamine and antibiotics (penicillin 100 U/mL, streptomycin 100 μ g/mL), at 37 °C in a humidified atmosphere containing 5% CO₂. Exponentially growing cells were obtained by plating 1.5 x 10⁵ cells/mL for MCF-7 and SF-268 and 0.75 x 10⁴ 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 the synthesized compounds 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 μ M. 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., Power wave XS, Wincoski, USA). For each test compound and cell line, a dose-response curve was obtained and the growth inhibition of 50% (GI₅₀), 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.

Results are given in concentrations that were able to cause 50% of cell growth inhibition (GI₅₀) after a continuous exposure of 48 h and show means \pm SEM of three-independent experiments performed in duplicate.

Structure activity relation ship

It is clear from Table 1 that many of the synthesized compounds showed high activity against the three cancer cell lines. It is obvious that compound **5a** showed higher potency than compound **5b** as it seemed that the presence of the 4-methylphenyl group responsible for the decreasing of compound **5b**. On the other hand the compounds **7a-c** showed low potencies against the three cancer cell lines. Considering the pyrazole derivatives **9a-c**, it is obvious that compounds **9b** (R = H, X = Cl) and compound **9c** (R = Ph, X = H) revealed high potencies against the three cancer cell lines whereas compound **11** showed low inhibitions. Considering compounds **13**, **5a,b** and **17** it is clear that the ethyl 1,3-dimethylbenzo[4,5]imidazo[1,2-*a*]pyridine-4-carboxylate (**17**) showed the highest inhibitions among the four compounds. The same also for compounds **18**, **19**, **21**, **23a** and **23b**, it is clear that compound **23b** showed the highest inhibitions and its reactivity was due to the presence the COOEt group attached to the thiophene ring. Considering compounds **25**, **26**, **28** and **30**, it is clear that compound **30** was the only one with high potencies. For compounds **32a**, **32b**, **37** and **38** it is obvious from Table 1 that the 2-(2-oxo-2H-chromen-3-yl)-N-phenyl-1H-benzo[*d*]imidazole-1-carbothioamide **38** was of the highest potency among these four compounds. Its reactivity is attributed to the presence of the coumarin and the N-phenylthiourea moieties.

Table 1. Effect of the newly synthesized compounds on growth of three human tumor cells.

Compound	GI ₅₀ (μmol L ⁻¹)			
	MCF-7	NCI-H460	SF-268	WI 38
3	23.6 ± 4.1	34.6 ± 12.1	45.4 ± 2.2	>100
5a	0.21 ± 0.04	0.12 ± 0.04	0.08 ± 0.006	40.0 ± 1.3
5b	36.6 ± 10.2	33.0 ± 8.6	38.6 ± 8.0 8	>100
7a	23.2 ± 2.4	26.6 ± 2.8	22.8 ± 8.5	38.2 ± 2.6
7b	22.4 ± 5.8	26.7 ± 8.2	31.4 ± 2.4	18.6 ± 4.0
7c	20.8 ± 8.30	22.8 ± 4.32	22.8 ± 6.23	>100
9a	38.2 ± 3.6	36.3 ± 12.5	40.6 ± 8.8	>100
9b	0.01 ± 0.001	0.02 ± 0.006	0.06 ± 0.002	>100
9c	0.02 ± 0.001	0.03 ± 0.008	0.06 ± 0.008	>100
11	30.0 ± 1.4	20.8 ± 4.3	20.3 ± 2.8	>100
13	12.6 ± 2.01	18.6 ± 6.06	30.4 ± 2.36	30.6 ± 10.2
15a	32.6 ± 1.3	29.7 ± 1.6	20.2 ± 8.9	55.8 ± 8.7
15b	22.4 ± 2.10	10.4 ± 3.0	8.63 ± 2.8	>100
17	0.08 ± 0.002	0.08 ± 0.003	0.02 ± 0.002	>100
18	38.1 ± 1.0	27.8 ± 4.3	12.3 ± 2.4	>100
19	38.4 ± 8.3	29.3 ± 10	36.40 ± 2.13	60.1 ± 2.3
21	13.8 ± 2.6	12.1 ± 4.2	29.2 ± 4.1	>100
23a	2.82 ± 2.8	6.9 ± 2.2	3.0 ± 1.8	77.5 ± 5.1
23b	0.06 ± 0.007	0.06 ± 0.006	0.02 ± 0.008	>100
25	21.2 ± 4.2	30.0 ± 8.0	20.59 ± 4.01	>100
26	21.2 ± 6.1	24.0 ± 4.7	10.39 ± 6.80	>100
28	32.0 ± 7.3	26.7 ± 2.8	30.4 ± 2.9	32.6 ± 6.4
30	1.2 ± 0.4	0.3 ± 0.16	2.8 ± 0.06	22.4 ± 1.6
32a	26.6 ± 8.5	29.3 ± 12.3	18.4 ± 2.8	68.2 ± 2.0
32b	24.1 ± 10.4	30.8 ± 10.8	26.1 ± 2.8	25.2 ± 0.8
35	44 ± 4.31	29.03 ± 8.01	20.6 ± 4.0	28.2 ± 8.2
37	22.7 ± 6.2	36.5 ± 6.4	31.5 ± 8.0	>100
38	0.08 ± 0.004	0.05 ± 0.002	0.06 ± 0.001	28.0 ± 4.9
Doxorubicin	0.04 ± 0.008	0.09 ± 0.008	0.09 ± 0.007	> 100

From Table 1 it is clear that the cytotoxic effect of the cyclic compounds. Compounds **5a**, **9a**, **9b**, **17**, **23a**, **23b**, **30**, **38** exhibited optimal cytotoxic effect against cancer cell lines, with $GI_{50,s}$ in the μM range. On the other hand, compounds **11**, **13**, **15a**, **15b**, **18**, **19**, **21**, **25**, **27**, **29**, **32a**, **32b**, **35** and **37** showed low cytotoxicity effect toward the three cancer lines. It is clear from the demonstrated "SAR" that the presence of electronegative groups together with the nature of the heteroatom enhances the antitumor effect of the target molecules.

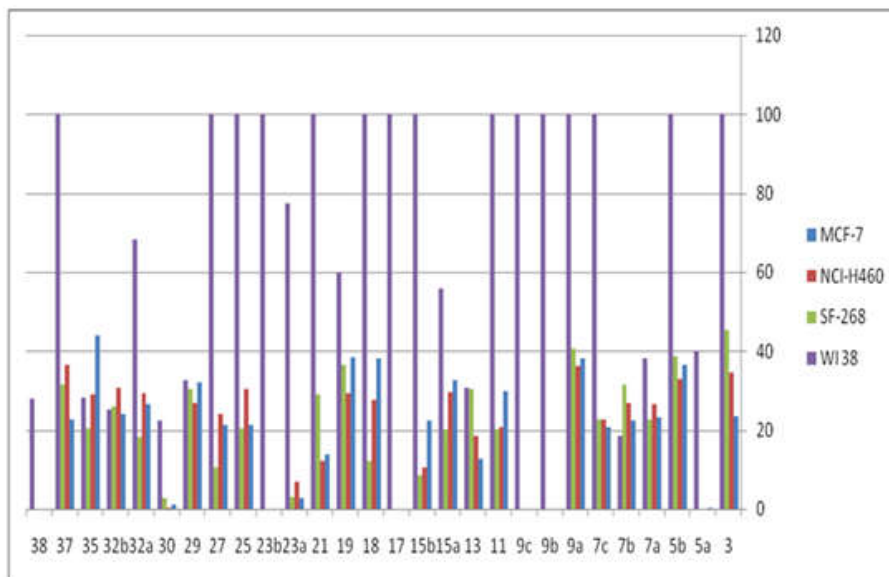


Figure 2. GI_{50} of the new synthesized compounds against MCF-7, NCI-H460, SF-268 and WI 38.

EXPERIMENTAL

General

All melting points were determined on an electrothermal apparatus (Büchi 535, Switzerland) in an open capillary tube and are uncorrected. ^{13}C -NMR and 1H -NMR spectra were recorded on Bruker DPX200 instrument in DMSO with TMS as internal standard for protons and solvent signals as internal standard for carbon spectra. Chemical shift values are mentioned in δ (ppm). Mass spectra were recorded on EIMS (Shimadzu) and ESI-esquire 3000 Bruker Daltonics instrument. Elemental analyses were carried out by the Microanalytical Data Unit Ludwig-Maximilians-Universität-München, Germany. The progress of all reactions was monitored by TLC on 2 x 5 cm pre-coated silica gel 60 F254 plates of thickness of 0.25 mm (Merck).

Ethyl 2-(1H-benzo[d]imidazol-2-yl)acetate (3)

To the dry solid of *o*-phenylene diamine (1.08 g, 0.01 mol) diethylmalonate (1.60 g, 0.01 mol) was added. The reaction mixture was heated in an oil bath at 120 °C for 1 h then left to cool. The remaining product was triturated with ethanol and the formed solid product was collected by filtration. Brown crystals from ethanol; yield: 1.73 g (85%); m.p. 130 °C. IR, ν : 3465-3328 (NH), 3058 (CH aromatic), 2973, 2848 (CH₃, CH₂), 1703 (C=O), 1640 (C=N), 1615 (C=C). 1H -

NMR, δ : 1.14 (t, 3H, J = 6.85 Hz, CH₃), 4.26 (q, 2H, J = 6.85 Hz), 4.82 (s, 2H, CH₂), 7.28-7.39 (m, 4H, C₆H₄), 8.30 (s, 1H, NH, D₂O exchangeable). ¹³C NMR (DMSO): 16.2 (ester CH₃), 52.8 (ester CH₂), 54.3 (CH₂), 121.4, 122.8, 123.6, 124.3, 126.4, 126.8 (C₆H₄), 172.8 (C=N); Anal. calcd for C₁₁H₁₂N₂O₂: C, 64.69; H, 5.92; N, 13.72%. Found: C, 64.48; H, 6.20; N, 13.80%. MS: *m/z*: (%) 204 (M⁺, 33%).

General procedure for the synthesis of the anilide derivatives 5a,b

To a solution of compound **3** (2.04 g, 0.01 mol) in dimethylformamide (30 mL) either aniline (0.93 g, 0.01 mol) or 4-methylaniline (1.08 g, 0.01 mol) was added. The reaction mixture, in each case, was heated under reflux for 4 h then poured onto ice/water mixture containing few drops of hydrochloric acid and the formed solid product was collected by filtration.

2-(1H-Benzo[d]imidazol-2-yl)-N-phenylacetamide (5a). Light brown crystals from ethanol; yield: 2.08 g (80%); m.p. 220-222 °C. IR, ν : 3472-3324 (2NH), 3050 (CH aromatic), 1680 (C=O), 1648 (C=N), 1629 (C=C). ¹H-NMR, δ : 4.93 (s, 2H, CH₂), 7.25-7.41 (m, 9H, C₆H₅, C₆H₄), 8.26, 8.32 (2s, 2H, 2NH, D₂O exchangeable). ¹³C NMR (DMSO): 54.8 (CH₂), 119.2, 120.8, 122.8, 123.6, 124.3, 125.2, 126.1, 127.3, 128.3, 129.2, (C₆H₅, C₆H₄), 164.8 (CO), 172.4 (C=N); Anal. calcd for C₁₅H₁₃N₃O: C, 71.70; H, 5.21; N, 16.72%. Found: C, 71.83; H, 5.08; N, 16.82%. MS: *m/z*: (%) 251 (M⁺, 24%).

2-(1H-Benzo[d]imidazol-2-yl)-N-(p-tolyl)acetamide (5b). Yellow crystals from ethanol; yield: 1.93 g (73%); m.p. 92 °C. IR, ν : 3480-3349 (2NH), 1686 (C=O), 1644 (C=N), 1632 (C=C). ¹H-NMR: δ : 3.17 (s, 3H, CH₃), 4.89 (s, 2H, CH₂), 7.27-7.36 (m, 8H, 2C₆H₄), 8.29, 8.30 (2s, 2H, 2NH, D₂O exchangeable). ¹³C NMR (DMSO): 24.8 (CH₃), 54.6 (CH₂), 120.1, 120.2, 121.8, 122.7, 123.3, 123.9, 126.6, 127.2, 129.1, 129.5, (C₆H₅, C₆H₄), 164.6 (CO), 172.3 (C=N); Anal. calcd for C₁₆H₁₅N₃O: C, 72.43; H, 5.70; N, 15.84%. Found: C, 72.38; H, 5.66; N, 15.69%. MS: *m/z*: (%) 265 (M⁺, 24%).

General procedure for the synthesis of the benzylidene derivatives 7a-c

To a solution of compound **3** (2.04 g, 0.01 mol) in ethanol (30 mL) containing piperidine (0.50 mL) either benzaldehyde (1.06 g, 0.01 mol) or 4-methoxy benzaldehyde (1.31 g, 0.01 mol) or 4-chlorobenzaldehyde (1.34 g, 0.01 mol) was added. The reaction mixture, in each case, was heated under reflux for 3 h then poured onto ice/water mixture containing few drops of hydrochloric acid and the formed solid product was collected by filtration.

Ethyl 2-(1H-benzo[d]imidazol-2-yl)-3-phenylacrylate (7a). Gray crystals from ethanol; yield: 2.45 g (77%); m.p. > 300 °C. IR, ν : 3478-3329 (NH), 3057 (CH aromatic), 2962, 2873 (CH₃, CH₂), 1688 (C=O), 1646 (C=N), 1630 (C=C). ¹H-NMR: δ : 1.14 (t, 3H, J = 7.66 Hz, CH₃), 4.21 (q, 2H, J = 7.66 Hz, CH₂), 6.24 (s, 1H, CH=C), 7.29-7.38 (m, 9H, C₆H₅, C₆H₄), 8.26 (s, 1H, NH, D₂O exchangeable). ¹³C NMR (DMSO): 16.3 (ester CH₃), 52.5 (ester CH₂), 89.3, 90.2 (CH=C), 120.6, 122.1, 123.2, 123.9, 124.1, 124.6, 125.8, 126.2, 127.8, 128.2, (C₆H₅, C₆H₄), 164.8 (CO), 172.6 (C=N); Anal. calcd for C₁₈H₁₆N₂O₂: C, 73.95; H, 5.52; N, 9.58%. Found: C, 72.73; H, 5.49; N, 9.37%. MS: *m/z*: (%) 292 (M⁺, 64%).

Ethyl 2-(1H-benzo[d]imidazol-2-yl)-3-(4-methoxyphenyl)acrylate (7b). Light brown crystals from ethanol; yield: 2.22 g (69%); m.p. 151 °C. IR, ν : 3484-3362 (NH), 3055 (CH aromatic), 2949, 2868 (CH₃, CH₂), 1686 (C=O), 1640 (C=N), 1626 (C=C). ¹H-NMR: δ : 1.16 (t, 3H, J = 7.08 Hz, CH₃), 3.14 (s, 3H, CH₃), 4.23 (q, 2H, J = 7.08 Hz, CH₂), 6.26 (s, 1H, CH=C), 7.27-7.36 (m, 8H, 2 C₆H₄), 8.26 (s, 1H, NH, D₂O exchangeable). ¹³C NMR (DMSO): 16.4 (ester CH₃), 53.8 (OCH₃), 52.3 (ester CH₂), 89.1, 90.4 (CH=C), 120.3, 122.8, 123.0, 123.7, 124.4, 125.8,

126.3, 127.6, 128.2, 129.4 (C₆H₅, C₆H₄), 164.6 (CO), 172.6 (C=N); Anal. calcd for C₁₉H₁₈N₂O₃: C, 70.79; H, 5.63; N, 8.69%. Found: C, 70.82; H, 5.73; N, 8.59%. MS: *m/z*: (%) 322 (M⁺, 83%).

Ethyl 2-(1H-benzo[d]imidazol-2-yl)-3-(4-chlorophenyl)acrylate (7c). Yellow crystals from ethanol; yield: 2.61 g (80%); m.p. 103 °C. IR, *v*: 3477-3349 (NH), 3054 (CH aromatic), 2933, 2863 (CH₃, CH₂), 1689 (C=O), 1644 (C=N), 1631 (C=C). ¹H-NMR: *δ*: 1.14 (t, 3H, J = 7.27 Hz, CH₃), 4.21 (q, 2H, J = 7.27 Hz, CH₂), 6.24 (s, 1H, CH=C), 7.24-7.39 (m, 8H, 2 C₆H₄), 8.29 (s, 1H, NH, D₂O exchangeable). ¹³C NMR (DMSO): 16.3 (ester CH₃), 52.2 (ester CH₂), 89.5, 90.1 (CH=C), 120.3, 122.7, 124.8, 125.2, 126.4, 127.4, 127.9, 128.3, 128.6 (C₆H₅, C₆H₄), 164.5 (CO), 172.4 (C=N); Anal. calcd for C₁₈H₁₅ClN₂O₂: C, 66.16; H, 4.63; N, 8.57%. Found: C, 66.09; H, 4.83; N, 8.59%. MS: *m/z*: (%) 326 (M⁺, 60%).

General procedure for the synthesis of the pyrazole derivatives 9a-c

To a solution of either compound **7a** (3.22 g, 0.01 mol) with either of hydrazine hydrate (0.50 g, 0.01 mol) or phenylhydrazine (1.08 g, 0.01 mol) or compound **7c** (3.26 g, 0.01 mol) with hydrazine hydrate (0.50 g, 0.01 mol) was added. The reaction mixture, in each case, was heated under reflux for 6 h then poured onto ice/water mixture containing few drops of hydrochloric acid and the formed solid product was collected by filtration.

4-(1H-Benzo[d]imidazol-2-yl)-5-phenyl-1H-pyrazol-3-ol (9a). Light brown crystals from ethanol; yield: 2.32 g (84%); m.p. 106 °C. IR, *v*: 3569-3329 (OH, 2NH), 3055 (CH aromatic), 1644 (C=N), 1630 (C=C). ¹H-NMR: *δ*: 7.27-7.42 (m, 9H, C₆H₅, C₆H₄), 8.27, 8.31 (2s, 2H, 2NH, D₂O exchangeable), 10.28 (s, 1H, OH, D₂O exchangeable). ¹³C NMR (DMSO): 142.6, 143.8 (pyrazole C-4, C-5), 119.8, 120.5, 122.8, 123.6, 124.1, 124.5, 126.8, 127.1, 129.4, 130.6 (C₆H₅, C₆H₄), 172.6, 176.2 (2C=N); Anal. calcd for C₁₆H₁₂N₄O: C, 69.55; H, 4.38; N, 20.28%. Found: C, 69.73; H, 4.51; N, 20.37%. MS: *m/z*: (%) 276 (M⁺, 38%).

4-(1H-Benzo[d]imidazol-2-yl)-5-(4-chlorophenyl)-1H-pyrazol-3-ol (9b). Light brown crystals from ethanol; yield: 2.45 g (79%); m.p. 208-211 °C. IR, *v*: 3523-3321 (OH, 2NH), 3055 (CH aromatic), 1646 (C=N), 1630 (C=C). ¹H-NMR: *δ*: 7.22-7.39 (m, 8H, 2C₆H₄), 8.29, 8.33 (2s, 2H, 2NH, D₂O exchangeable), 10.26 (s, 1H, OH, D₂O exchangeable). ¹³C NMR (DMSO): 143.9, 142.4 (pyrazole C-4, C-5), 119.6, 120.8, 123.3, 123.9, 124.2, 124.8, 126.8, 128.9, 129.4, 130.8 (C₆H₅, C₆H₄), 176.4, 172.5 (2C=N); Anal. calcd for C₁₆H₁₁ClN₄O: C, 61.84; H, 3.57; N, 18.03%. Found: C, 61.95; H, 3.84; N, 17.89%. MS: *m/z*: (%) 310 (M⁺, 30%).

4-(1H-Benzo[d]imidazol-2-yl)-1,5-diphenyl-1H-pyrazol-3-ol (9c). Yellow crystals from 1,4-dioxane; yield: 2.32 g (66%); m.p. 152-154 °C. IR, *v*: 3520-3349 (OH, NH), 3053 (CH aromatic), 1642 (C=N), 1633 (C=C); ¹H-NMR: *δ*: 7.26-7.37 (m, 14H, 2C₆H₅), 8.25 (s, 1H, NH, D₂O exchangeable), 10.29 (s, 1H, OH, D₂O exchangeable). ¹³C NMR (DMSO): 144.2, 142.6 (pyrazole C-4, C-5), 119.9, 120.3, 123.1, 123.6, 124.1, 124.3, 125.2, 125.3, 126.2, 126.8, 127.0, 127.6, 128.2, 130.8 (C₆H₄, 2C₆H₅), 176.2, 172.7 (2C=N); Anal. calcd for C₂₂H₁₆N₄O: C, 74.98; H, 4.58; N, 15.90%. Found: C, 75.22; H, 4.72; N, 15.73%. MS: *m/z*: (%) 352 (M⁺, 21%).

Ethyl 1-amino-2-cyano-3-phenylbenzo[4,5]imidazo[1,2-a]pyridine-4-carboxylate (11)

To a solution of compound **6a** (3.33 g, 0.01 mol) in ethanol (40 mL) containing triethylamine (0.50 mL), malononitrile (0.66 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 6 h then poured onto ice/water mixture containing few drops of hydrochloric acid and the formed solid product was collected by filtration. Yellow crystals from 1,4-dioxane; yield: 2.42 g (80%); mp: 82 °C. IR, *v*: 3488-3343 (NH₂), 3056 (CH aromatic), 2927, 2860 (CH₃, CH₂), 2225 (CN), 1687 (CO), 1640 (C=N), 1636 (C=C). ¹H-NMR: *δ*: 1.15 (t, 3H, J = 6.89 Hz, CH₃),

4.23 (q, 2H, J = 6.89 Hz, CH₂), 4.83 (s, 2H, NH₂, D₂O exchangeable), 7.28-7.42 (m, 9H, C₆H₅, C₆H₄). ¹³C NMR (DMSO): 16.4 (ester CH₃), 52.5 (ester CH₂), 116.8 (CN), 119.3, 120.1, 123.5, 124.3, 125.4, 125.9, 126.2, 128.8, 137.3, 140.8, 144.3, 143.2, (pyridine C-2, C-3, C-4, C-5, C₆H₅, C₆H₄), 164.8 (CO), 172.4 (C=N); Anal. calcd for C₂₁H₁₆N₄O₂: C, 70.77; H, 4.53; N, 15.72%. Found: C, 70.93; H, 4.70; N, 15.63%. MS *m/z* (%) 356 (M⁺, 38%).

Ethyl 2-(1H-benzo[d]imidazol-2-yl)-3-phenylbut-2-enoate (13)

To the dry solid of compound **3** (2.04 g, 0.01 mol) ammonium acetate (0.50 g) and acetophenone (1.20 g, 0.01 mol) were added. The reaction mixture was heated in an oil bath at 120 °C for 30 min then left to cool. The product was triturated with ethanol and the formed solid product was collected by filtration. Pale yellow crystals from 1,4-dioxane; yield: 1.69 g (69%); m.p. 240-243 °C. IR, *v*: 3463-3379 (NH), 3057 (CH aromatic), 2955, 2860 (CH₃, CH₂), 1689 (CO), 1638 (C=N), 1633 (C=C). ¹H-NMR: δ : 1.14 (t, 3H, J = 6.07 Hz, CH₃), 2.87 (s, 3H, CH₃), 4.22 (q, 2H, J = 6.07 Hz, CH₂), 7.25-7.40 (m, 9H, C₆H₅, C₆H₄), 8.30 (s, 1H, NH, D₂O exchangeable). ¹³C NMR (DMSO): 16.3 (ester CH₃), 52.2 (ester CH₂), 86.8, 90.4 (C=C), 120.6, 121.2, 123.2, 123.8, 124.1, 125.3, 128.6, 129.4 (C₆H₅, C₆H₄), 164.5 (CO), 173.1 (C=N); Anal. calcd for C₁₉H₁₈N₂O₂: C, 74.49; H, 5.92; N, 9.14%. Found: C, 74.53; H, 5.88; N, 9.08%. MS: *m/z*: (%) 306 (M⁺, 28%).

General procedure for the synthesis of the ylidene derivatives 15a, b

To the dry solid of compound **3** (2.04 g, 0.01 mol) ammonium acetate (0.50 g) either cyclopentanone (0.78 g, 0.01 mol) or cyclohexanone (0.92 g, 0.01 mol) were added. The reaction mixture was heated in an oil bath at 120 °C for 1 h then left to cool. The product was triturated with ethanol and the formed solid product was collected by filtration.

Ethyl 2-(1H-benzo[d]imidazol-2-yl)-2-cyclopentylideneacetate (15a). Pale yellow crystals from ethanol; yield: 2.53 g (83%); m.p. 155 °C. IR, *v*: 3459-3322 (NH), 3053 (CH aromatic), 2929, 2863 (CH₃, CH₂), 1686 (CO), 1636 (C=N), 1630 (C=C). ¹H-NMR: δ : 1.13 (t, 3H, J = 6.93 Hz, CH₃), 1.18-1.24 (m, 4H, 2CH₂), 2.19-2.21 (m, 4H, 2CH₂), 4.22 (q, 2H, J = 6.93 Hz, CH₂), 7.27-7.38 (m, 4H, C₆H₄), 8.32 (s, 1H, NH, D₂O exchangeable). ¹³C NMR (DMSO): 16.2 (ester CH₃), 1.89-2.13 (2m 4CH₂), 52.6 (ester CH₂), 86.4, 90.6 (C=C), 120.3, 121.2, 123.2, 125.6, 128.6 (C₆H₄), 164.8 (CO), 173.2 (C=N); Anal. calcd for C₁₆H₁₈N₂O₂: C, 71.09; H, 6.71; N, 10.36%. Found: C, 70.93; H, 6.93; N, 10.47%. MS: *m/z*: (%) 270 (M⁺, 36%).

Ethyl 2-(1H-benzo[d]imidazol-2-yl)-2-cyclohexylideneacetate (15b). Gray crystals from ethanol; yield: 2.33 g (77%); m.p. 120 °C. IR, *v*: 3463-3319 (NH), 3048 (CH aromatic), 2929, 2868 (CH₃, CH₂), 1688 (CO), 1632 (C=N), 1628 (C=C). ¹H-NMR: δ : 1.15 (t, 3H, J = 7.16 Hz, CH₃), 1.13-1.18 (m, 4H, 2CH₂), 2.08-2.16 (m, 6H, 3CH₂), 4.22 (q, 2H, J = 7.16 Hz, CH₂), 7.23-7.36 (m, 4H, C₆H₄), 8.30 (s, 1H, NH, D₂O exchangeable). Anal. calcd for C₁₇H₂₀N₂O₂: C, 71.81; H, 7.09; N, 9.85%. Found: C, 71.63; H, 6.87; N, 9.62%. MS: *m/z*: (%) 284 (M⁺, 19%).

Ethyl 1,3-dimethylbenzo[4,5]imidazo[1,2-a]pyridine-4-carboxylate (17)

To a solution of compound **3** (2.04 g, 0.01 mol) in ethanol (40 mL) containing piperidine (0.50 mL), acetylacetone (1.00 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 2 h then poured onto ice/water mixture containing few drops of hydrochloric acid and the formed solid product was collected by filtration. Brown crystals from ethanol; yield: 1.67 g (73%); m.p. 233 °C. IR, *v*: 3056 (CH aromatic), 2925, 2863 (CH₃, CH₂), 1684 (CO), 1644 (C=N), 1631 (C=C). ¹H-NMR: δ : 1.13 (t, 3H, J = 7.22 Hz, CH₃), 2.89, 2.93 (2s, 6H, 2CH₃), 4.22 (q, 2H, J = 7.22 Hz, CH₂), 6.14 (s, 1H, pyridine H-3), 7.25-7.39 (m, 4H, C₆H₄). ¹³C NMR

(DMSO): 16.6 (ester CH₃), 23.8, 24.6 (2CH₃), 52.5 (ester CH₂), 121.2, 123.4, 124.8, 126.5, 128.6, 129.4, 139.3, 141.8, 142.2, 142.6 (pyridine, C₆H₄), 164.3 (CO), 173.3 (C=N); Anal. calcd for C₁₆H₁₆N₂O₂: C, 71.62; H, 6.01; N, 10.44%. Found: C, 71.80; H, 5.82; N, 10.63%. MS: *m/z*: (%) 268 (M⁺, 37%).

Ethyl 1,3-diaminobenzo[4,5]imidazo[1,2-a]pyridine-4-carboxylate (18)

To a solution of compound **3** (2.04 g, 0.01 mol) in ethanol (40 mL) containing piperidine (0.50 mL), malononitrile (0.66 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 4 h then poured onto ice/water mixture containing few drops of hydrochloric acid and the formed solid product was collected by filtration. Light brown crystals from ethanol; yield: 2.32 g (80%); m.p. 235-238 °C. IR, *v*: 3880-3268 (2NH₂), 3058 (CH aromatic), 2965, 2872 (CH₃, CH₂), 1689 (CO), 1642 (C=N), 1630 (C=C). ¹H-NMR: *δ*: 1.12 (t, 3H, J = 7.42 Hz, CH₃), 4.22 (q, 2H, J = 7.42 Hz, CH₂), 4.72, 5.04 (2s, 4H, 2NH₂, D₂O exchangeable), 6.13 (s, 1H, pyridine H-3), 7.26-7.37 (m, 4H, C₆H₄). ¹³C NMR (DMSO): 16.6 (ester CH₃), 52.3 (ester CH₂), 121.6, 126.4, 127.1, 127.2, 128.8, 129.2, 139.2, 141.4, 142.0, 142.9 (pyridine, C₆H₄), 164.5 (CO), 173.6 (C=N); Anal. calcd for C₁₄H₁₄N₄O₂: C, 62.21; H, 5.22; N, 20.73%. Found: C, 62.38; H, 5.41; N, 20.92%. MS: *m/z*: (%) 270 (M⁺, 46%).

2-(1H-Benzo[d]imidazol-2-yl)acetohydrazide (19)

To a solution of compound **3** (2.04 g, 0.01 mol) in ethanol (40 mL) hydrazine hydrate (0.50 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 6 h then poured onto ice/water mixture containing few drops of hydrochloric acid and the formed solid product was collected by filtration. Brown crystals from ethanol; yield: 1.15 g (80%); m.p. > 300 °C. IR, *v*: 3488-3429 (NH₂, NH), 3054 (CH aromatic), 2983 (CH₂), 1684 (CO), 1640 (C=N), 1630 (C=C). ¹H-NMR: *δ*: 4.93 (s, 2H, CH₂), 4.89 (s, 2H, NH₂, D₂O exchangeable), 7.29-7.39 (m, 4H, C₆H₄), 8.28, 8.32 (2s, 2H, 2NH, D₂O exchangeable). ¹³C NMR (DMSO): 52.8 (CH₂), 120.3, 123.8, 124.6, 126.4, 127.3, 129.8 (C₆H₄), 163.8 (CO), 173.4 (C=N); Anal. calcd for C₉H₁₀N₄O: C, 56.83; H, 5.30; N, 29.46%. Found: C, 56.66; H, 5.28; N, 29.69%. MS: *m/z*: (%) 190 (M⁺, 36%).

2-Phenyl-1-thioxo-1,2-dihydrobenzo[4,5]imidazo[1,2-c]pyrimidin-3(4H)-one (21)

To a solution of compound **3** (2.04 g, 0.01 mol) in ethanol (40 mL) containing triethylamine (0.50 mL) phenylisothiocyanate (1.30 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 4 h then poured onto ice/water mixture containing few drops of hydrochloric acid and the formed solid product was collected by filtration. Yellow crystals from ethanol; yield: 2.67 g (87%); m.p. 202 °C. IR, *v*: 3057 (CH aromatic), 2980 (CH₂), 1689 (CO), 1643 (C=N), 1632 (C=C). ¹H-NMR: *δ*: 5.90 (s, 2H, pyrimidine CH₂), 7.26-7.38 (m, 9H, C₆H₄, C₆H₅). ¹³C NMR (DMSO): 54.2 (CH₂), 120.3, 123.7, 124.1, 124.6, 124.9, 125.8, 129.3, 126.7, 127.2, 128.2 (C₆H₅, C₆H₄), 163.4 (CO), 173.6 (C=N), 180.3 (C=S); Anal. calcd for C₁₆H₁₁N₃OS: C, 65.51; H, 3.78; N, 14.32; S, 10.93%. Found: C, 65.33; H, 4.02; N, 14.59; S, 11.27%. MS: *m/z*: (%) 293 (M⁺, 22%).

General procedure for the synthesis of the thiophene derivatives 23a,b

To a solution of compound **3** (2.04 g, 0.01 mol) in ethanol (40 mL) containing triethylamine (0.50 mL) elemental sulfur or either of malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) were added. The reaction mixture was heated under reflux for 4 h then poured onto ice/water mixture containing few drops of hydrochloric acid and the formed solid product was collected by filtration.

2-Amino-5-(1H-benzo[d]imidazol-2-yl)-4-hydroxythiophene-3-carbonitrile (23a). Dark brown crystals from ethanol; yield: 2.61 g (87%); m.p. 252 °C. IR, ν : 3572-3341 (OH, NH₂, NH), 3054 (CH aromatic), 2220 (CN), 1646 (C=N), 1628 (C=C). ¹H-NMR: δ : 5.21 (s, 2H, NH₂, D₂O exchangeable), 7.31-7.39 (m, 4H, C₆H₄), 8.22 (s, 1H, NH, D₂O exchangeable), 10.15 (s, 1H, OH, D₂O exchangeable). ¹³C NMR (DMSO): 116.8 (CN), 122.1, 121.3, 123.2, 125.3, 127.2, 128.7 (C₆H₄), 173.4 (C=N); Anal. calcd for C₁₂H₈N₄O₂S: C, 56.24; H, 3.15; N, 21.86; S, 12.51%. Found: C, 56.08; H, 3.28; N, 21.79; S, 12.73%. MS: m/z : (%) 256 (M⁺, 42%).

Ethyl 2-amino-5-(1H-benzo[d]imidazol-2-yl)-4-hydroxythiophene-3-carboxylate (23b). Black crystals from ethanol; yield: 2.89 g (87%); m.p. 249 °C. IR, ν : 3559-3321 (OH, NH₂, NH), 3058 (CH aromatic), 2977, 2857 (CH₃, CH₂), 1688 (CO), 1643 (C=N), 1629 (C=C). ¹H-NMR: δ : 1.14 (t, 3H, J = 7.42 Hz, CH₃), 4.22 (q, 2H, J = 7.42 Hz, CH₂), 5.14 (s, 2H, NH₂, D₂O exchangeable), 7.27-7.37 (m, 4H, C₆H₄), 8.24 (s, 1H, NH, D₂O exchangeable), 10.32 (s, 1H, OH, D₂O exchangeable). Anal. calcd for C₁₄H₁₃N₃O₃S: C, 55.43; H, 4.32; N, 13.85; S, 10.57%. Found: C, 55.22; H, 4.62; N, 14.09; S, 10.64%. MS: m/z : (%) 303 (M⁺, 52%).

Ethyl 2-(1H-benzo[d]imidazol-2-yl)-3-ethoxyacrylate (25)

To a solution of compound **3** (2.04 g, 0.01 mol) in ethanol (40 mL) containing piperidine (0.50 mL) ethyl orthoformate (1.48 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 6 h then poured onto ice/water mixture containing few drops of hydrochloric acid and the formed solid product was collected by filtration. Yellow crystals from ethanol; yield: 2.22 g (68 %); m.p. >300 °C. IR, ν : 3489-3351 (NH), 3057 (CH aromatic), 2983, 2866 (CH₃, CH₂), 1704 (CO), 1646 (C=N), 1624 (C=C). ¹H-NMR: δ : 1.11, 1.13 (2t, 6H, J = 7.03, 6.67 Hz, 2CH₃), 3.83, 4.20 (2q, 4H, J = 7.03, 6.67 Hz, 2CH₂), 6.08 (s, 1H, CH=C), 7.23-7.39 (m, 4H, C₆H₄), 8.21 (s, 1H, NH, D₂O exchangeable). ¹³C NMR (DMSO): 16.3, 16.5 (two OCH₂CH₃), 52.3, 52.8 (two OCH₂CH₃), 86.4, 90.8 (CH=C), 121.5, 122.9, 125.1, 126.4, 127.2, 128.6 (C₆H₄), 164.5 (CO), 173.8 (C=N); Anal. calcd for C₁₄H₁₆N₂O₃: C, 64.60; H, 6.20; N, 10.76%. Found: C, 64.81; H, 5.93; N, 10.59%. MS: m/z : (%) 260 (M⁺, 42%).

Ethyl 2-(1H-benzo[d]imidazol-2-yl)-3-(phenylamino)acrylate (26)

To a solution of compound **3** (2.04 g, 0.01 mol) in ethanol (40 mL) containing piperidine (0.50 mL) ethyl orthoformate (1.48 g, 0.01 mol) and aniline oil (0.93 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 3 h then poured onto ice/water mixture containing few drops of hydrochloric acid and the formed solid product was collected by filtration. Pale yellow crystals from ethanol; yield: 2.35 g (79%); m.p. >300 °C. IR, ν : 3471-3329 (NH), 3055 (CH aromatic), 2980, 2873 (CH₃, CH₂), 1699 (CO), 1643 (C=N), 1629 (C=C). ¹H-NMR: δ : 1.15 (t, 3H, J = 7.13 Hz, CH₃), 4.23 (q, 2H, J = 7.13 Hz, CH₂), 6.03 (s, 1H, CH=C), 7.26-7.42 (m, 9H, C₆H₅, C₆H₄), 8.23, 8.34 (2s, 2H, 2NH, D₂O exchangeable). ¹³C NMR (DMSO): 16.2 (ester CH₃), 52.5 (ester CH₂), 88.4, 90.3 (C=C), 120.6, 121.6, 123.6, 123.8, 124.1, 125.3, 126.4, 127.3, 128.8, 129.5 (C₆H₅, C₆H₄), 164.8 (CO), 173.3 (C=N), Anal. calcd for C₁₈H₁₇N₃O₂: C, 70.34; H, 5.58; N, 13.67%. Found: C, 70.29; H, 5.73; N, 13.82%. MS: m/z : (%) 307 (M⁺, 22%).

Ethyl 1-amino-2-cyano-3-ethoxybenzo[4,5]imidazo[1,2-a]pyridine-4-carboxylate (28)

To a solution of compound **3** (2.04 g, 0.01 mol) in ethanol (40 mL) containing piperidine (0.50 mL) ethyl orthoformate (1.48 g, 0.01 mol) and malononitrile (0.66 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 3 h then poured onto ice/water mixture containing few drops of hydrochloric acid and the formed solid product was collected by filtration. Yellow crystals from ethanol; yield: 2.22 g (83%); m.p. >300 °C. IR, ν : 3488-3337 (NH₂), 3056 (CH aromatic), 2988, 2893 (CH₃, CH₂), 2220 (CN), 1688 (CO), 1643 (C=N), 1629 (C=C). ¹H-NMR:

δ : 1.13 (t, 3H, J = 6.79 Hz, CH₃), 4.21 (q, 2H, J = 6.79 Hz, CH₂), 4.93 (s, 2H, NH₂, D₂O exchangeable), 6.14 (s, 1H, pyridine H-4), 7.28-7.39 (m, 4H, C₆H₄). ¹³C NMR (DMSO): 16.3, 16.8 (two OCH₂CH₃), 52.4, 52.9 (two OCH₂CH₃), 116.4 (CN), 121.5, 122.9, 125.1, 127.2, 128.6, 138.4, 140.1, 142.6, 142.7 (pyridine, C₆H₄), 164.2 (CO), 173.6 (C=N); Anal. calcd for C₁₇H₁₆N₄O₂: C, 62.95; H, 4.97; N, 17.27%. Found: C, 64.32; H, 4.60; N, 19.83%. MS: *m/z*: (%) 324 (M⁺, 41%).

3-(1H-Benzo[d]imidazol-2-yl)-2H-chromen-2-one (30)

To a solution of compound **3** (2.04 g, 0.01 mol) in ethanol (30 mL) containing piperidine (0.50 mL) salicylaldehyde (1.22 g, 0.01 mol) was added. The reaction mixture, in each case, was heated under reflux for 3 h then poured onto ice/water mixture containing few drops of hydrochloric acid and the formed solid product was collected by filtration. Yellow crystals from 1,4-dioxane; yield: 1.41 g (89%); m.p. 273 °C. IR, ν : 3458-3323 (NH), 3054 (CH aromatic), 1690 (CO), 1641 (C=N), 1633 (C=C). ¹H-NMR: δ : 6.09 (s, 1H, coumarin H-4), 7.26-7.42 (m, 8H, 2C₆H₄), 8.09 (s, 1H, NH, D₂O exchangeable). ¹³C NMR (DMSO): 121.6, 122.4, 124.1, 125.3, 126.4, 126.8, 128.2, 129.4, 130.3, 131.5, (C₆H₅, C₆H₄), 164.6 (CO), 173.4 (C=N); Anal. calcd for C₁₆H₁₀N₂O₂: C, 73.27; H, 3.84; N, 10.68%. Found: C, 73.40; H, 3.94; N, 10.42%. MS: *m/z*: (%) 262 (M⁺, 16%).

General procedure for the synthesis of the annulated derivatives 32a, b

To a solution of compound **31** (2.62 g, 0.01 mol) in 1,4-dioxane containing triethylamine (0.50 mL) either α -chloroacetone (**32a**) (0.92 g, 0.01 mol) or ethyl α -chloroacetate (**32b**) (1.22 g, 0.01 mol) was added. The reaction mixture in each case was heated under reflux for 4 h then evaporated under vacuum. The remaining product, in each case, was triturated with ethanol and formed solid product was collected by filtration.

7-Methyl-1H-benzo[4,5]imidazo[1,2-a]chromeno[3,4-c]pyridin-1-one (32a). Green crystals from 1,4-dioxane; yield: 0.95 g (67%); m.p. 195 °C. IR, ν : 3056 (CH aromatic), 1687 (CO), 1640 (C=N), 1629 (C=C). ¹H-NMR: δ : 2.93 (s, 3H, CH₃), 5.93 (s, 1H, pyridine H-4), 7.29-7.38 (m, 8H, 2C₆H₄). Anal. calcd for C₁₉H₁₂N₂O₂: C, 75.99; H, 4.03; N, 9.33%. Found: C, 75.72; H, 3.83; N, 9.59%. MS: *m/z*: (%) 300 (M⁺, 36%).

7-Hydroxy-1H-benzo[4,5]imidazo[1,2-a]chromeno[3,4-c]pyridin-1-one (32b). Yellow crystals from acetic acid; yield: 1.40 g (82%); m.p. 217 °C. IR, ν : 3522-3341 (OH), 3057 (CH aromatic), 1689 (CO), 1642 (C=N), 1631 (C=C). ¹H-NMR: δ : 5.90 (s, 1H, pyridine H-4), 7.26-7.41 (m, 8H, 2C₆H₄), 10.26 (s, 1H, OH, D₂O exchangeable). Anal. calcd for C₁₈H₁₀N₂O₃: C, 71.52; H, 3.33; N, 9.27%. Found: C, 71.69; H, 3.46; N, 9.51%. MS: *m/z*: (%) 302 (M⁺, 40%).

8-Hydroxy-1H-benzo[4,5]imidazo[1,2-a]chromeno[3,4-c]pyridin-1-one (35)

To a solution of compound **31** (2.62 g, 0.01 mol) in 1,4-dioxane containing triethylamine (0.50 mL) either chloroacetylchloride (1.12 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 4 h then evaporated under vacuum. The remaining product was triturated with ethanol and formed solid product was collected by filtration. Yellow crystals from acetic acid; yield: 2.55 g (69%); m.p. 263 °C. IR, ν : 3563-3319 (OH), 3055 (CH aromatic), 1688 (CO), 1640 (C=N), 1629 (C=C). ¹H-NMR (DMSO-*d*₆) δ : 5.93 (s, 1H, pyridine H-4), 7.24-7.35 (m, 8H, 2C₆H₄), 10.28 (s, 1H, OH, D₂O exchangeable). ¹³C NMR (DMSO): 119.9, 120.3, 123.1, 123.6, 124.1, 124.3, 125.2, 125.3, 127.6, 128.3, 129.4, 130.8, 140.6, 142.8, 143.2 (pyran, pyridine, 2C₆H₄), 163.8 (CO), 176.6 (C=N); Anal. calcd for C₁₈H₁₀N₂O₃: C, 71.52; H, 3.33; N, 9.27%. Found: C, 71.72; H, 3.36; N, 9.66%. MS: *m/z*: 302 (M⁺, 40%).

3-(1-Acetyl-1H-benzo[d]imidazol-2-yl)-2H-chromen-2-one (**37**)

A solution of compound **31** (2.62 g, 0.01 mol) in acetic acid (10 mL) and acetic anhydride (5 mL) was heated under reflux for 3 h then evaporated under vacuum. The remaining product was triturated with diethyl ether and the formed solid product was collected by filtration. Yellow crystals from acetic acid; yield: 1.56 g (74%); m.p. 292 °C. IR, ν : 3055 (CH aromatic), 1690, 1678 (2CO), 1638 (C=N), 1632 (C=C). ¹H-NMR: δ : 2.80 (s, 3H, CH₃), 6.23 (s, 1H, coumarin H-4), 7.26-7.39 (m, 8H, 2C₆H₄). ¹³C NMR (DMSO): 24.6 (CH₃), 119.3, 121.8, 123.1, 124.3, 125.0, 126.1, 127.6, 128.0, 129.4, 130.8, 140.3, 142.3, 143.8 (pyran, 2C₆H₄), 165.2, 164.4 (2CO), 176.6 (C=N); Anal. calcd for C₁₈H₁₂N₂O₃: C, 71.05; H, 3.97; N, 9.21%. Found: C, 71.33; H, 3.76; N, 9.38%. MS: m/z : (%) 304 (M⁺, 28%).

2-(2-Oxo-2H-chromen-3-yl)-N-phenyl-1H-benzo[d]imidazole-1-carbothioamide (**38**)

To a solution of compound **31** (2.62 g, 0.01 mol) in ethanol (40 mL) containing triethylamine (0.50 mL) phenyl isothiocyanate (1.30 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 6 h then evaporated under vacuum. The remaining product was triturated with ethanol and formed solid product was collected by filtration. Pale yellow crystals from acetic acid; yield: 2.85 g (70%); m.p. 194 °C. IR, ν : 3488-3349 (NH), 3055 (CH aromatic), 1689 (CO), 1638 (C=N), 1632 (C=C). ¹H-NMR: δ : 6.33 (s, 1H, coumarin H-4), 7.26-7.38 (m, 13H, C₆H₅, 2C₆H₄), 8.31 (s, 1H, NH, D₂O exchangeable). ¹³C NMR (DMSO): 119.3, 121.8, 122.6, 123.2, 123.8, 124.3, 125.3, 125.8, 126.4, 127.6, 128.5, 129.4, 130.8, 140.3, 142.6, 142.9, (pyran, C₆H₅, 2C₆H₄), 164.4 (CO), 176.3 (C=N), 180.3 (C=S); Anal. calcd for C₂₃H₁₅N₃O₂S: C, 69.50; H, 3.80; N, 10.57; S, 8.07%. Found: C, 69.79; H, 3.68; N, 10.41; S, 8.19%. MS: m/z : (%) 397 (M⁺, 32%).

CONCLUSION

Ethyl 2-(1H-benzo[d]imidazol-2-yl)acetate (**3**) was used for several heterocyclic transformation reactions. The antitumor evaluations of the newly synthesized products toward the three cancer cell lines MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), and SF-268 (CNS cancer) showed that compounds **5a**, **9b**, **9c**, **17**, **23b** and **38** were of the highest potencies among the synthesized compounds. The work showed that benzimidazole derivatives were excellent compounds that can be used as anticancer agents.

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