Regular Article

Synthesis of Novel Thiophene, Thiazole and Coumarin Derivatives Based on Benzimidazole Nucleus and Their Cytotoxicity and Toxicity Evaluations

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The reactivity of compounds 2-(1-(2-chloroacetyl)-1*H*-benzo[*d*]imidazol-2-yl)acetonitrile 2 and 3-(1-(2-chloroacetyl)-1*H*-benzo[*d*]imidazol-2-yl)-2*H*-chromen-2-one 8 towards different chemical reagents were studied and a series of novel benzimidazole derivatives were obtained (2–6a–d and 8–12a–d). Moreover, *in vitro* growth inhibitory effect of the newly synthesized compounds were evaluated in term of [$IC_{50}\mu M$] against the six cancer cell lines, human lung carcinoma (A549), lung cancer (H460), human colorectal (HT29), gasteric cancer cell (MKN-45), glioma cell line (U87MG) and cellosaurus cell line (SMMC-7721) where foretinib was used as standard reference. The results showed that compounds 2 (only for A549 cell line), 3a, 4, 6c, 6d, 8, 9a, 9e and 9f were the most active compounds towards the six cancer cell lines. On the other hand, the toxicity of these most potent compounds against shrimp larvae indicated that compounds 3a, 4, 6d, 9e and 9f were non toxic while compounds 6c and 8 were very toxic and compounds 2 and 9a were harmful against the tested organisms.

Key words benzimidazole; thiophene; thiazole; coumarin; cytotoxicity; toxicity

Benzimidazole derivatives occupy an important position in medicinal chemistry and are of particular interest in the search for new bioactive compounds in the pharmaceutical industry. Thus, imidazole and benzimidazole scaffolds are extremely versatile and have featured a number of clinically used drugs such as antihistaminic,[1,2] antiulcer,[3] antihypertensive,[4] antibacterial,[5] antifungal,[6,7] anti-parasitic,[8] antiemetic,[9] anticancer,[10] antiviral[11] and other therapeutic agents with high therapeutic potency and market value.[12] In the present work, a number of benzimidazole derivatives were synthesized and evaluated their biological activity as anticancer agents. The chemical structures of some of the benzimidazole based anti-cancer drugs which are candidates in different stages of clinical trials by various pharmaceutical companies are illustrated below (Fig. 1) such as AT9283,[13] Galeterone[14] and Veliparib.[15] On the other hand, there are various methods lead to prepare benzimidazole derivatives such as a one-pot, multicomponent reaction,[16] one-pot condensation reaction,[17] addition reactions,[18] intramolecular *N*-arylations reaction,[19] a copper-catalyzed, one-pot and three-component reaction.[20] In the present work we used the 2-(1*H*-benzo[*d*]imidazol-2-yl)-acetonitrile **1** as the key starting compound for the synthesis of 1-*α*-chloroacetyl derivative followed by several heterocyclization to afford potentially cytotoxic derivatives.

Results and Discussion

Chemistry Benzimidazole structural motifs have attracted much interest in diverse areas of medicinal chemistry. These heterocycles have shown various pharmacological activities such as anti-human immunodeficiency virus (HIV),[21] poli(ADP-ribose) phosphorylase inhibitors,[22] Histamine H4 receptor binders[23] and cardiovascular.[24] In view of the tremendous biological activities of benzimidazoles, their preparation has gained considerable attention in recent years. In view of the above-mentioned facts and in continuation of our interest in the synthesis of heterocycles containing benzimidazole moiety,[25] to identify new candidates that may be value in designing new, potent, selective and less toxic antitumor agents, we report herein the synthesis and anti-tumor evaluation of some novel structure heterocycles incorporating benzo[*d*]imidazole moiety. For these reasons, we focused our effort in this work through the uses of the 2-(1*H*-benzo[*d*]-imidazol-2-yl)acetonitrile **1** as the key starting compound. The synthetic strategies adopted for the synthesis of the intermediates and target compounds are depicted in Charts 1–4. Compound **1** was obtained through the reaction of *o*-phenylenediamine with ethyl cyanoacetate at 120°C. It was reacted with chloroacetylchloride in 1,4-dioxane to give the 2-(1-(2-chloroacetyl)-1*H*-benzo[*d*]imidazol-2-yl)acetonitrile **2**.

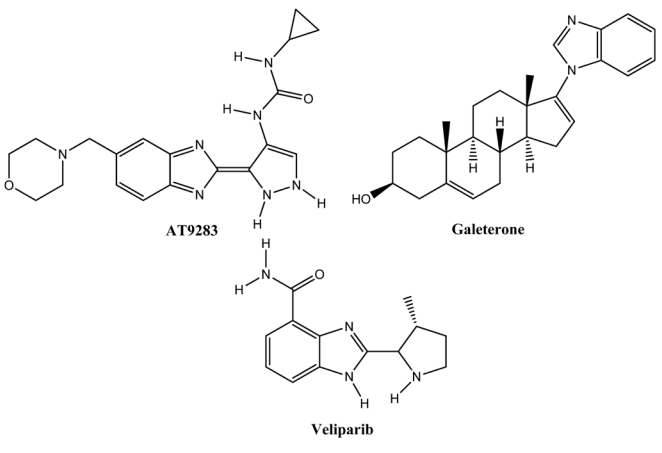

Fig. 1. Chemical Structures of Veliparib, Galeterone and AT9283

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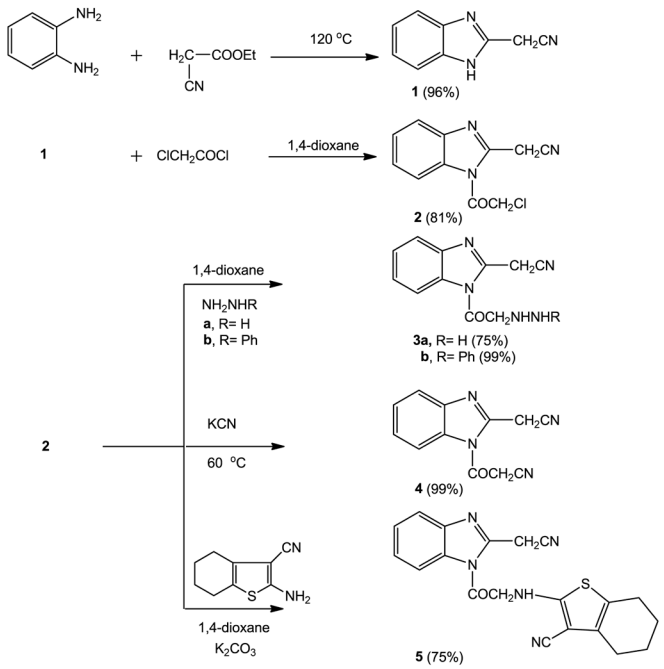


Chart 1. Synthesis of Benzimidazole Derivatives **1**; **2**; **3a**, **b**; **4** and **5**

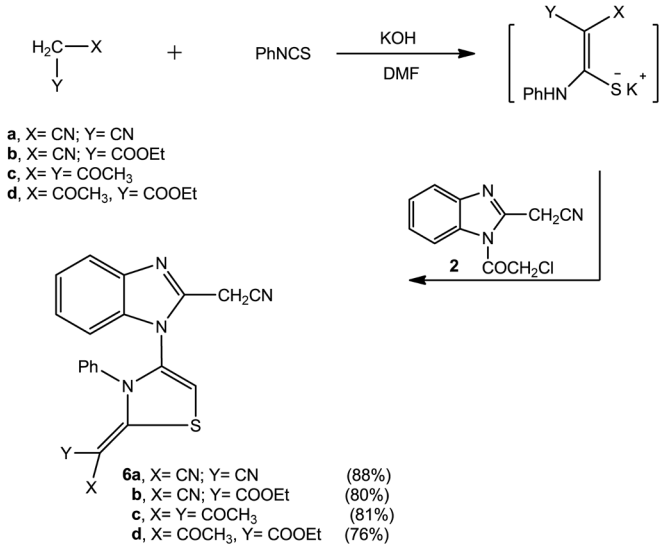


Chart 2. Synthesis of Thiazole Derivatives **6a**–**d**

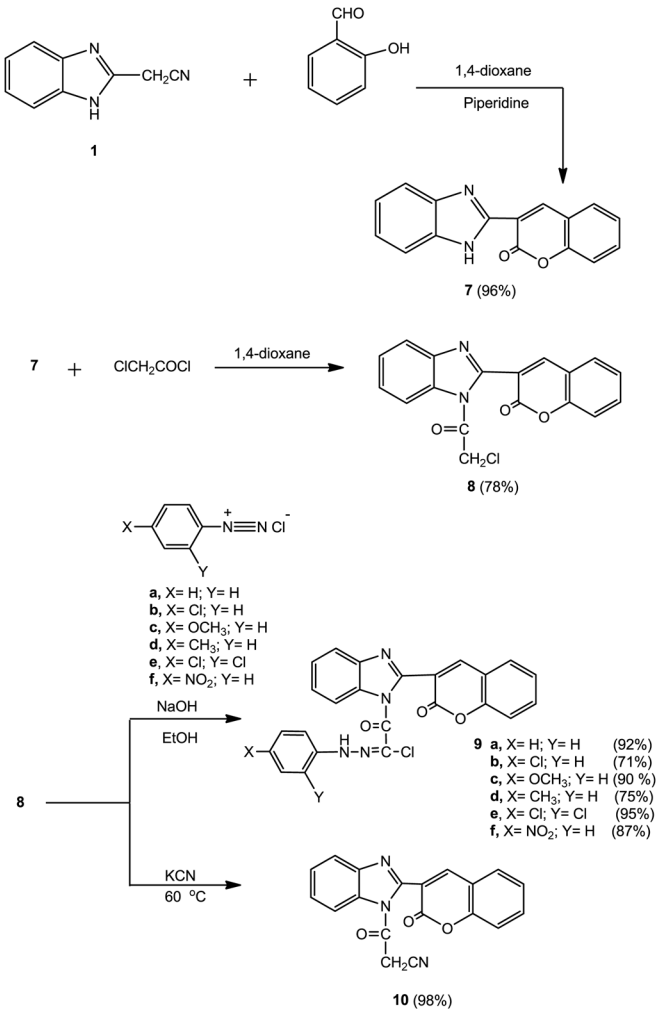


Chart 3. Synthesis of Coumarin Derivatives **7**; **8**; **9a**–**f** and **10**

Structure of compound **2** was established on the basis of the obtained analytical and spectral data. Thus, the MS showed molecular ion peak [M^+] (*m/z* 233) corresponding to molecular formula $C_{11}H_8N_3OCl$ and ^{1}H-NMR spectrum showed two singlets at δ 3.55 and 4.38 ppm for the two CH_2 groups and a multiplet at δ 7.25–7.54 ppm corresponding to the C_6H_4 group. In addition ^{13}C-NMR spectrum exhibited signals at δ 14.5, 46.1 indicating the two CH_2 groups, a signal at δ 119.5 eq to the CN group, signals at δ 112.1, 113.0, 123.5, 123.7, 130.4, 138.0 eq to the benzene ring, a signal at δ 164.1 eq to the C=O group and a signal at δ 174.0 corresponding to the C=N imidazole.

The reaction of compound **2** with either of hydrazine hydrate or phenylhydrazine gave the hydrazine derivatives **3a** and **3b**, respectively. On the other hand, treatment of compound **2** with potassium cyanide at 60°C afforded the 3-(2-(cyanomethyl)-1*H*-benzo[*d*]imidazol-1-yl)-3-oxopropanenitrile **(4)**. The structure of the latter compound was elucidated on the basis of their spectral data (IR, MS and ^{1}H-NMR). In addition, the reaction of compound **2** with the 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile gave the 2-((2-oxoacetyl)amino)-4,5,6,7-tetrahydrobenzo[*b*]-thiophene derivative **5** (Chart 1).

Recently, our research group was directed to study the reaction of active methylene reagents with phenylisothiocyanate in basic dimethylformamide/potassium hydroxide (DMF/KOH) solution followed by hetero-cyclization of the intermediate potassium sulphide salt with α-halocarbonyl compounds.[26,27] The reaction gave either thiophene or thiazole derivatives depending on the nature of the used active methylene reagent and the α-halocarbonyl compound. In continuation of this program, we studied the reactivity of compound **2** as an α-halocarbonyl derivative to form thiazole derivatives (Chart 2). Thus, any of the malononitrile, ethyl cyanoacetate, acetylacetone or ethyl acetoacetate reacted with phenyl isothiocyanate in DMF/KOH solution form the intermediate potassium sulphide salt. The reaction of the latter compound with compound **2** forms the thiazole derivatives **6a**–**d**, respectively (Chart 2). The analytical and spectral data of compounds **6a**–**d** were consistent

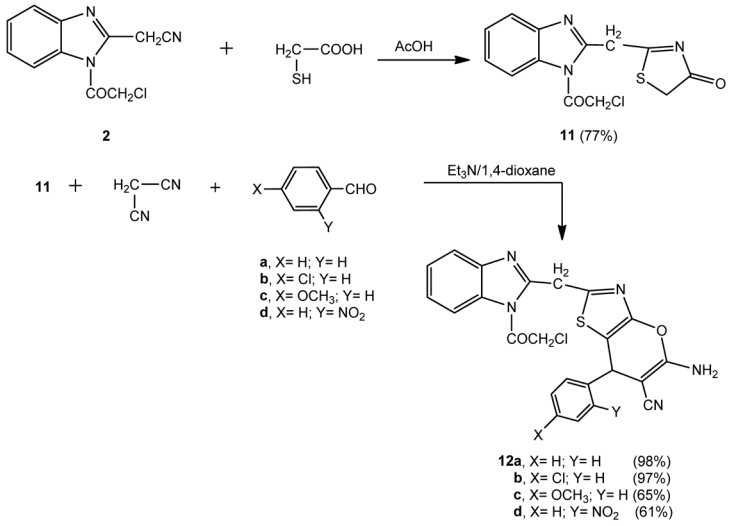


Chart 4. Synthesis of Thiazole Derivative **11** and Pyrano[2,3-*d*]thiazole Derivatives **12a**–**d**

with their respective structures. Thus, the ^{1}H-NMR spectrum of compound **6a** (as an example) revealed the presence of a singlet at δ 4.35 ppm for the CH_2 group, a singlet at range of δ 6.40 ppm for CH thiazole and a multiplet at δ 7.15–7.63 ppm for the C_6H_4 and C_6H_5 groups.

Next, the reaction of compound **1** with salicylaldehyde afforded the coumarin derivative **7**. The reaction of compound **7** with chloroacetylchloride gave the *N*-chloroacetamido derivative **8**. The latter compound showed reactivities toward some chemical reagents to form bioactive molecules. Thus, the reaction of compound **8** with any of the diazonium salts namely benzenediazonium chloride, 4-chlorobenzenediazonium chloride, 4-methoxybenzenediazonium chloride, 4-tolyldiazonium chloride, 2,4-dichlorobenzene-diazonium chloride or 4-nitrobenzenediazonium chloride afforded the hydrazonyl chloride derivatives **9a**–**f**, respectively. On the other hand, the nucleophilic displacement of compound **8** with potassium cyanide gave the *N*-cyanoacetyl derivative **10** (Chart 3).

Compound **2** reacted with thioglycollic acid to give the thiazole derivative **11**, its structure was confirmed on the basis of analytical and spectral data. The ^{1}H-NMR spectrum showed three singlets at δ 3.38, 3.70 and 4.38 ppm for the three CH_2 groups and a multiplet at δ 7.26–7.55 ppm for C_6H_4 moiety.

Multi-component reaction (MCR) is a procedure wherein three or more, commercially accessible or easily available components are gathered together through one pot method to provide an expected target; demonstrating features of starting materials, hence provide better chances for molecular diversity per step in short reaction time and attaining better yields. With a narrow set of starting precursors, a wide range of libraries can be formed in a short reaction time through MCRs.[28–32] It is quite remarkable that many top-selling pharmaceuticals contain 4*H*-pyran derivatives[33–36] encouraged us to synthesis 4*H*-pyran derivative through the MCRs of compound **11**. Thus, the reaction of compound **11** with any of the aromatic aldehydes like benzaldehyde, 4-chlorobenzaldehyde, 4-methoxybenzaldehyde or 2-nitrobenzaldehyde gave the pyran derivatives **12a**–**d**, respectively (Chart 4). The structures of compounds **12a**–**d** were established on the basis of their respective analytical and spectral data (see Experimental).

Biology. Cell Proliferation Assay The anti-proliferative activities of the newly synthesized compounds (Table 1) were evaluated against the six cancer cell lines, human lung carcinoma (A549), human colorectal (HT29), gasteric cancer cell (MKN-45), glioma cell line (U87MG), and cellosaurus cell line (SMMC-7721) and lung cancer (H460) using the standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay *in vitro*, with foretinib as the positive control.[37–39] The cancer cell lines were cultured in minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS). Approximate 4×10^3 cells, suspended in MEM medium, were plated onto each well of a 96-well plate and incubated in 5% CO_2 at 37°C for 24 h. The compounds tested at the indicated final concentrations were added to the culture medium and the cell cultures were continued for 72 h. Fresh MTT was added to each well at a terminal concentration 5 μg/mL, and incubated with cells at 37°C for 4 h. The formazan crystals were dissolved in 100 μL of dimethyl sulfoxide (DMSO) each well, and the absorbency at 492 nm (for absorbance of MTT formazan) and 630 nm (for the reference wavelength) was measured with an enzyme-linked immunosorbent assay (ELISA) reader. All of the compounds were tested three times in each cell line. The results expressed as half maximal inhibitory concentration (IC_{50}) were the averages of three determinations and calculated by using the Bacus Laboratories Incorporated Slide Scanner (Bliss) software. The mean values of three independent experiments, expressed as IC_{50} values, were presented in Table 1. Most of the synthesized compounds exhibited potent anti-proliferative activity with IC_{50} values less than 30 μM. Generally, the variations of substituents within the thienopyridine moiety together with the heterocycle ring being attached have a notable influence on the anti-proliferative activity.

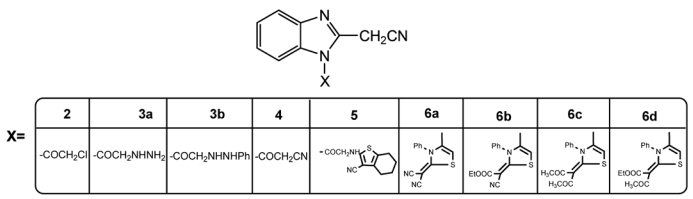
Structure Activity Relationship of the Newly Synthesized Compounds It is clear from Table 1 that most of the synthesized compounds showed from moderate to high potency against the six cancer cell lines. Compound **2** showed high cytotoxicity only against A549 with IC_{50} 0.80 μM. Moreover, compound **3a** with (R=H) showed higher cytotoxicity than **3b** (R=Ph). It is worthy to mention that the high nitrogen content in compound **3a** was responsible for its high potency.

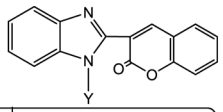
Table 1. *In Vitro* Growth Inhibitory Effect IC_{50}±S.E.M. (μM) of the Newly Synthesized Compounds against Cancer Cell Lines

Compound No.	IC_{50}±S.E.M. (μM)[a]					
	A549	H460	HT29	MKN-45	U87MG	SMMC-7721
2	0.80±0.12	9.59±2.83	8.48±3.21	6.49±2.49	8.66±2.52	7.27±2.97
3a	0.38±0.09	0.42±0.09	0.29±0.07	0.88±0.29	1.83±0.29	0.72±0.09
3b	6.29±2.04	8.80±3.05	6.28±2.36	5.77±2.18	8.69±2.59	6.32±1.52
4	0.83±0.26	0.39±0.05	0.61±0.27	0.80±0.36	0.36±0.46	1.53±0.29
5	8.29±2.33	8.42±2.29	9.18±1.44	6.62±1.93	8.58±2.69	8.51±2.64
6a	8.36±4.69	2.66±1.58	8.73±2.66	6.83±2.69	8.40±2.59	4.66±1.50
6b	8.28±3.69	7.29±2.64	8.59±4.39	9.38±2.72	9.42±2.93	6.36±2.82
6c	1.55±0.49	2.56±0.52	0.49±0.16	0.94±0.59	3.28±0.63	0.25±0.64
6d	0.38±0.05	0.53±0.04	0.66±0.04	0.23±0.06	0.82±0.14	0.49±0.09
7	6.63±2.69	8.79±2.39	6.70±1.41	2.90±0.93	1.69±0.72	2.59±0.83
8	0.29±0.03	0.59±0.28	0.57±0.08	0.80±0.39	0.34±0.09	0.69±0.40
9a	0.48±0.23	0.70±0.58	0.69±0.42	0.66±0.39	1.27±0.74	0.59±0.32
9b	8.87±2.69	6.21±1.42	8.69±1.69	9.69±2.57	9.28±2.55	6.59±2.38
9c	8.66±2.29	9.29±1.97	8.73±2.79	6.63±2.68	6.28±0.39	8.93±2.52
9d	8.28±3.27	8.60±2.80	1.35±0.59	1.43±0.61	10.04±3.17	10.79±4.51
9e	0.25±0.19	0.36±0.16	0.89±0.27	0.73±0.06	0.29±0.08	0.22±0.05
9f	0.28±0.04	0.61±0.49	0.88±0.33	1.38±0.59	3.33±0.06	2.52±0.16
10	8.31±1.88	9.21±2.60	7.42±1.09	9.31±2.72	8.59±2.97	8.53±2.29
11	2.39±0.85	1.08±0.69	2.68±0.86	8.69±2.03	8.48±2.93	9.59±3.49
12a	6.23±1.25	6.09±1.27	9.58±1.59	8.32±2.42	8.22±1.49	7.58±2.83
12b	1.26±0.84	1.75±0.32	8.59±2.73	4.68±1.48	9.45±2.58	7.29±2.19
12c	8.35±2.69	8.53±3.70	7.84±2.69	6.38±1.42	8.68±2.80	8.48±1.69
12d	9.47±0.14	8.30±1.69	9.08±1.58	7.34±2.48	9.20±1.84	6.22±2.80
Foretinib	0.08±0.01	0.18±0.03	0.15±0.023	0.03±0.0055	0.90±0.13	0.44±0.062

a) Data are expressed as means±S.E.M. of three independent experiments performed in duplicates.

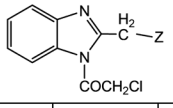
Structures of the Newly Synthesized Compounds





Y=

7	H
8	-$COCH_2Cl$
9a	$C_6H_5NHN{=}C(Cl)C(O)$-
9b	(4-Cl)$C_6H_4NHN{=}C(Cl)C(O)$-
9c	(4-OCH_3)$C_6H_4NHN{=}C(Cl)C(O)$-
9d	(4-CH_3)$C_6H_4NHN{=}C(Cl)C(O)$-
9e	(2,4-Cl_2)$C_6H_3NHN{=}C(Cl)C(O)$-
9f	(4-NO_2)$C_6H_4NHN{=}C(Cl)C(O)$-
10	-$COCH_2CN$



Z=

11	12a	12b	12c	12d

Compound **4** showed high cytotoxicity against the six cancer cell lines. It is obvious that the CN group present in compound **4** was responsible for it high activity. The reaction of compound **2** with the 2-amino-4,5,6,7-tetrahydrobenzo[*b*]-thiophene to give compound **5**, surprising with moderate cytotoxicity against the six cancer cell lines. Considering the thiazole derivatives **6a**–**d** it is obvious that compounds **6c** (X=Y=$COCH_3$) and **6d** (X=$COCH_3$, Y=COOEt) showed most activities among such series of compounds. It is very common between compounds **6c** and **6d** that the presence of the $COCH_3$ was responsible for the high potency of these two compounds. Moreover, the presence of the high oxygen content COOEt moiety was responsible for the extremely high potency of compound **6d**. In addition, compound **6c** showed higher potency than the reference foretinib against the SMMC-7721 cell line. On the other hand, the coumarin derivative **7** showed moderate cytotoxicity. Moreover, alkylation of compound **7** to give compound **8** showed high potency against the six cancer cell lines. Such reactivity of compound **8** was attributed to the presence of the $COCH_2Cl$ moiety. Considering the hydrazidic halide derivatives **9a**–**f**, compounds **9a** (X=Y=H), **9e** (X=Y=Cl) and **9f** (X=NO_2, Y=H) showed high cytotoxicity against the six cancer cell lines. Very common between compounds **9a**–**c** the presence of the –NH–N=C–(CO)Cl moiety which was responsible of their high potencies. In addition the presence of the two Cl groups in **9e** and the NO_2 group in **9f** extremely increase their potencies. It was surprisingly that compound **10**, which was produced through the nucleophilic displacement of the Cl in compound **8** into CN showed moderate potencies towards the six cancer cell lines. Similarly, compounds **11** and **12a**–**d** showed moderate potency, although compound **12b** showed slight activities against A549 and H460 cell lines with IC_{50}'s 1.26 and 1.75 μM. The cytotoxicity evaluation of the most active newly synthesized compounds toward the six cancer cell lines are illustrated through Fig. 2.

Toxicity Bioactive compounds are often toxic to shrimp larvae. Thus, in order to monitor these chemicals *in vivo* lethality to shrimp larvae (*Artemia salina*), Brine-Shrimp Lethality Assay[40] was used. Results were analyzed with LC_{50} program to determine LC_{50} values and 95% confidence intervals.[41,42] Results are given in Table 2 for the compounds which exhibited optimal cytotoxic effect against the cancer cell lines which are the nine compounds **2**, **3a**, **4**, **6c**, **6d**, **8**, **9a**, **9e** and **9f**.

The shrimp lethality assay is considered as a useful tool for preliminary assessment of toxicity, and it has been used for the detection of fungal toxins, plant extract toxicity, heavy metals, cyanobacteria toxins, pesticides, and cytotoxicity testing of dental materials.[43] It has also been shown that, *A. salina* toxicity test results have a correlation with rodent and human acute oral toxicity data. Generally, a good correlation was obtained between *A. salina* toxicity test and the rodent data. Likewise, the predictive screening potential of the aquatic invertebrate tests for acute oral toxicity in man, including *A. salina* toxicity test, was slightly better than the rat test for test compounds.

In order to prevent the toxicity results from possible false effects originated from solubility of compounds and DMSO's possible toxic effect, the tested compounds were prepared by dissolving in DMSO in the suggested DMSO volume ranges. It is clear from Table 2 that compounds **3a**, **4**, **6d**, **9e** and **9f** showed non toxicity against the tested organisms. On the other hand, compounds **6c** and **8** were very toxic; in addition, compounds **2, 9a** were harmful.

Conclusion

The present work was to discuss a variety and novel benzimidazole derivatives which were synthesized *via* the reaction of compounds **2** and **8** with different chemical reagents. The results of reactivity of the newly synthesized compounds towards the six cancer cell lines showed that compounds **2** (only for A549 cell line), **3a**, **4**, **6c**, **6d**, **8**, **9a**, **9e** and **9f** revealed the optimal cytotoxic effect against all the cancer cell lines where foretinib was used as the reference standard. Also, the toxicity of the most cytotoxic nine compounds was evaluated and the result indicated that compounds **3a**, **4**, **6d**, **9e** and **9f** were non toxic.

Experimental

General All melting points (mp) were uncorrected and determined on an electrothermal apparatus (Büchi 535, Switzerland) in an open capillary tube. IR spectra (KBr discs) were recorded on a FTIR plus 460 IR spectrophotometer (Shimadzu, Japan). ^{13}C-NMR and ^{1}H-NMR spectra were recorded on Varian Gemini-200 (200 MHz) (Japan) spectrometer in DMSO-d_6 as solvent, using tetramethylsilane (TMS) as internal reference, and chemical shifts (δ, ppm). Mass spectra were recorded using Hewlett Packard 5988 (U.S.A.). A GC/MS system and GCMS-QP 1000 Ex Shimadzu (Japan) using EI (electron impact method). Elemental analyses were carried out on Vario EL III Elemental CHNS analyzer (Japan).

Synthetic pathways are presented in Charts 1–4, cytotoxicity and toxicity of the newly synthesized products was expressed through Tables 1 and 2.

Chemistry

Preparation of the 2-(1*H*-Benzo[*d*]imidazol-2-yl)acetonitrile (**1**)[44]

To the dry solid of *o*-phenylene diamine (1.08 g, 0.01 mol), ethyl caynoacetate (1.13 g, 0.01 mol) was added. The reaction mixture was heated in an oil bath at 120°C for 30 min then was left to cool. The solid product produced upon triturating with diethyl ether was collected by filtration and crystallized from ethanol.

Preparation of 2-(1-(2-Chloroacetyl)-1*H*-benzo[*d*]imidazol-2-yl)acetonitrile (**2**)

To a compound **1** (1.57 g, 0.01 mol) in 1,4-dioxane (30 mL), chloroacetyl chloride (1.12 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 15 min then poured into a beaker containing ice/water mixture. The formed solid product was collected by filtration, dried and crystallized from 1,4-dioxane.

Pale yellow crystals; mp:>300°C; IR (KBr, cm^{-1}): 3080, 2962, 2196, 1591, 1470. ^{1}H-NMR (DMSO-d_6) δ: 3.55 (s, 2H, CH_2), 4.38 (s, 2H, CH_2), 7.25–7.54 (m, 4H, C_6H_4). ^{13}C-NMR (DMSO-d_6) δ: 14.5, 46.1, 119.5, 112.1, 113.0, 123.5, 123.7, 130.4, 138.0, 164.1, 174.0. MS (EI): *m*/*z* (%) 235 $[M+2]^+$ (10.13), 234 $[M+1]^+$ (4.85), 233 $[M]^+$ (30.17), 232 $[M-1]^+$ (0.88), 231 $[M-2]^+$ (0.34), 184 (100.00). *Anal.* Calcd. for $C_{11}H_8N_3OCl$ (233.65): C, 56.54; H, 3.45; N, 17.98. Found: C, 56.69; H, 3.70; N, 18.30.

General Procedure for the Preparation of 2-(1-(2-Hy-

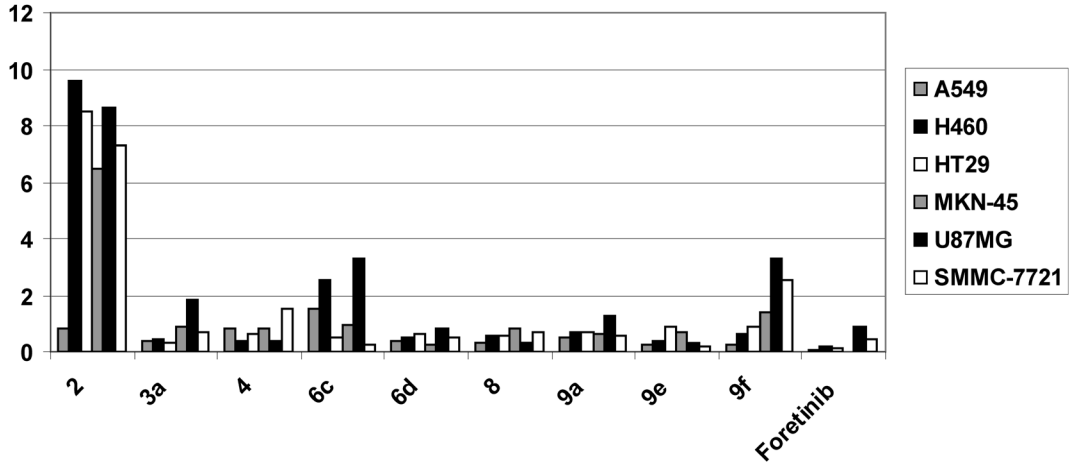


Fig. 2. The Cytotoxicity Evaluation of Most Active Newly Synthesized Compounds against the Six Cancer Cell Lines

drazinylacetyl)-1*H*-benzo[*d*]imidazol-2-yl)acetonitrile Derivatives (3a, b) To a solution of compound **2** (2.33 g, 0.01 mol) in 1,4-dioxane (40 mL), either of hydrazine hydrate (0.50 g, 0.01 mol) or phenyl hydrazine (1.08 g, 0.01 mol) was added. The reaction mixture, in each case, was heated under reflux for 5 h and then poured onto a beaker containing ice/water mixture. The formed solid product, in each case, was collected by filtration, dried and crystallized from 1,4-dioxane.

2-(1-(2-Hydrazinylacetyl)-1*H*-benzo[*d*]imidazol-2-yl)-acetonitrile (**3a**)

Brown crystals; mp: 273–275°C; IR (KBr, cm^{-1}): 3393, 3229, 3090, 2933, 2192, 1590, 1469. ^{1}H-NMR (DMSO-d_6) δ: 2.73 (s, 2H, NH_2), 3.90 (s, 2H, CH_2), 4.45 (s, 2H, CH_2), 7.07–7.67 (m, 4H, C_6H_4), 12.95 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ: 16.2, 52.3, 114.7, 115.0, 117.3, 123.4, 123.4, 130.2, 139.1, 167.1, 177.2. MS (EI): *m/z* (%) 229 $[M]^+$ (11.21), 228 $[M-1]^+$ (48.07), 227 $[M-2]^+$ (12.31), 157 (100.00). *Anal.* Calcd for $C_{11}H_{11}N_5O$ (229.24): C, 57.63; H, 4.84; N, 30.55. Found: C, 58.01; H, 4.99; N, 30.80.

2-(1-(2-(2-Phenylhydrazinyl)acetyl)-1*H*-benzo[*d*]imidazol-2-yl)acetonitrile (**3b**)

Brown crystals; mp: 183–185°C; IR (KBr, cm^{-1}): 3427, 3200, 3060, 2928, 2197, 1597, 1446. ^{1}H-NMR (DMSO-d_6) δ: 3.40 (s, 2H, CH_2), 3.55 (s, 2H, CH_2), 6.99–7.94 (m, 9H, C_6H_4, C_6H_5), 12.70, 13.30 (2s, 2H, 2NH). ^{13}C-NMR (DMSO-d_6) δ: 16.1, 52.1, 114.4, 114.4, 114.8, 115.5, 117.1, 122.3, 123.0, 123.2, 129.3, 129.3, 130.1, 139.0, 141.1, 168.2, 178.1. MS (EI): *m/z* (%) 307 $[M+2]^+$ (1.40), 306 $[M+1]^+$ (1.19), 305 $[M]^+$ (2.27), 304 $[M-1]^+$ (0.85), 303 $[M-2]^+$ (1.91). *Anal.* Calcd for $C_{17}H_{15}N_5O$ (305.33): C, 66.87; H, 4.95; N, 22.94. Found: C, 66.99; H, 5.20; N, 23.11.

Preparation of 3-(2-(Cyanomethyl)-1*H*-benzo[*d*]imidazol-1-yl)-3-oxopropanenitrile (**4**)

A solution of compound **2** (2.33 g, 0.01 mol) in 1,4-dioxane (25 mL) was heated on a water bath at 60°C then potassium cyanide (0.65 g, 0.01 mol) in a least amount of water, was added while stirring. The whole reaction mixture was heated in a water bath at 60°C for 30 min then poured into a beaker containing ice/water mixture with a few drops of hydrochloric acid. The solid product formed was collected by filtration, dried and crystallized from ethanol.

White crystals; mp: 298–300°C; IR (KBr, cm^{-1}): 3009, 2952, 2886, 2210, 2197, 1622, 1593, 1467. ^{1}H-NMR (DMSO-d_6) δ: 3.59 (s, 2H, CH_2), 4.38 (s, 2H, CH_2), 7.26–7.55 (m, 4H, C_6H_4). ^{13}C-NMR (DMSO-d_6) δ: 16.0, 19.1, 112.1, 113.1, 116.1, 117.0, 123.4, 124.0, 130.3, 139.0, 162.2, 174.1. MS (EI): *m/z* (%) 226 $[M+2]^+$ (0.67), 225 $[M+1]^+$ (1.21), 224 $[M]^+$ (5.51), 223 $[M-1]^+$ (0.67), 57 (100.00). *Anal.* Calcd for $C_{12}H_8N_4O$ (224.22): C, 64.28; H, 3.60; N, 24.99. Found: C, 64.67; H, 3.93; N, 25.30.

Preparation of 2-((2-(2-(Cyanomethyl)-1*H*-benzo[*d*]imidazol-1-yl)-2-oxoethyl)-amino)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile (**5**)

To a solution of compound **2** (2.33 g, 0.01 mol) in 1,4-dioxane (25 mL), potassium carbonate (0.50 g) and 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile (1.78 g, 0.01 mol) were added. The reaction mixture was heated in a boiling water bath for 5 h then poured onto ice/water mixture containing few drops of hydrochloric acid. The solid product formed, was collected by filtration, dried and crystallized from 1,4-dioxane.

Faint brown crystals; mp: 259–261°C; IR (KBr, cm^{-1}): 3445, 3327, 2926, 2841, 2205, 2194, 1617, 1585, 1470. ^{1}H-NMR (DMSO-d_6) δ: 1.69–1.71 (m, 4H, $2CH_2$ cyclohexene), 2.49–2.50 (m, 4H, $2CH_2$ cyclohexene), 3.56 (s, 2H, CH_2), 4.54 (s, 2H, CH_2), 6.85–7.64 (m, 4H, C_6H_4), 12.96 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ: 16.0, 21.7, 22.8, 23.4, 23.9, 51.9, 83.3, 114.6, 115.9, 116.8, 118.9, 123.4, 123.7, 130.3, 134.5, 139.0, 150.6, 160.0, 162.6, 172.1. MS (EI): *m/z* (%) 375 $[M]^+$ (59.77), 374 $[M-1]^+$ (72.41), 314 (100.00). *Anal.* Calcd for $C_{20}H_{17}N_5OS$ (375.45): C, 63.98; H, 4.56; N, 18.65; S, 8.54. Found: C, 64.20; H, 4.90; N, 18.90; S, 8.91.

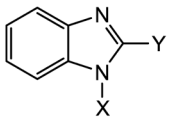
General Procedure for the Preparation of 2-(1-(2-Methylene-3-phenyl-2,3-dihydrothiazol-4-yl)-1*H*-benzo[*d*]imidazol-2-yl)acetonitrile (6a–d) To a solution of any of malononitrile (0.66 g, 0.01 mol), ethyl cyanoacetate (1.13 g, 0.01 mol), acetyl acetone (1.00 g, 0.01 mol) or ethyl acetoacetate (1.30 g, 0.01 mol) in dimethylformamide (20 mL), phenyl isothiocyanate (1.35 g, 0.01 mol) containing potassium hydroxide (0.56 g, 0.01 mol) was added, with continuous stirring overnight at room temperature. In the second day, compound **2** (2.33 g, 0.01 mol) was added to each reaction mixture with stirring for another night at room temperature. The whole reaction mixture, in each case, was poured onto ice/water mixture containing a few drops of hydrochloric acid. The solid product formed, in each case, was collected by filtration, dried and

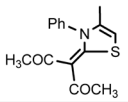
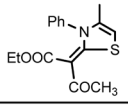
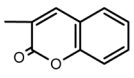
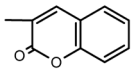
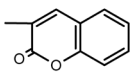
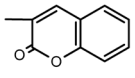
Table 2. Toxicity of the Most Potent Compounds against the Cancer Cell Lines

Compound No.	Cons. (μg/mL)	Mortality[a)]	Toxicity	LC_{50}	Upper 95% lim	Lower 95% lim
2	10	0	Harmful	119.35	70.38	26.79
	100	5				
	1000	10				
3a	10	0	Non toxic	972.31	—	—
	100	0				
	1000	4				
4	10	0	Non toxic	860.20	—	—
	100	2				
	1000	4				
6c	10	5	Very toxic	138.32	—	—
	100	8				
	1000	10				
6d	10	2	Non toxic	890.11	—	—
	100	6				
	1000	10				
8	10	2	Very toxic	44.8	—	—
	100	6				
	1000	10				
9a	10	0	Harmful	140.29	70.40	18.90
	100	3				
	1000	8				
9e	10	0	Non toxic	888.28	—	—
	100	2				
	1000	4				
9f	10	0	Non-toxic	856.28	—	—
	100	0				
	1000	4				

a) Ten organisms (*A. salina*) tested for each concentration.

Structures of the Most Potent Compounds



	X	Y		X	Y
2	-$COCH_2Cl$	-CH_2CN	**3a**	-$COCH_2NHNH_2$	-CH_2CN
4	-$COCH_2CN$	-CH_2CN	**6c**		-CH_2CN
6d		-CH_2CN	**8**	-$COCH_2Cl$	
9a	$C_6H_5NHN{=}C(Cl)C(O)$-		**9e**	$(2,4\text{-}Cl_2)C_6H_3NHN{=}C(Cl)C(O)$-	
9f	$(4\text{-}NO_2)C_6H_4NHN{=}C(Cl)C(O)$-				

crystallized from1,4-dioxane.

2-(4-(2-(Cyanomethyl)-1*H*-benzo[*d*]imidazol-1-yl)-3-phenylthiazol-2(3*H*)-ylidene)malononitrile (**6a**)

Faint brown crystals; mp: 205–207°C; IR (KBr, cm^{-1}): 3100, 3070, 2924, 2260, 2202, 2170, 1627, 1435. ^{1}H-NMR (DMSO-d_6) δ: 4.35 (s, 2H, CH_2), 6.40 (s, 1H, CH thiazole), 7.15–7.63 (m, 9H, C_6H_4, C_6H_5). ^{13}C-NMR (DMSO-d_6) δ: 16.0, 50.1, 82.0, 114.0, 115.2, 118.9, 118.9, 119.9, 122.3, 122.6, 122.6, 123.2, 123.6, 129.5, 129.5, 131.0, 133.3, 139.0, 141.1, 165.1, 174.7. MS (EI): *m/z* (%) 382 $[M+2]^+$ (1.52), 381 $[M+1]^+$ (2.48), 380 $[M]^+$

(0.82), 379 $[M-1]^+$ (1.05), 378 $[M-2]^+$ (0.49), 57 (100.00). *Anal.* Calcd for $C_{21}H_{12}N_6S$ (380.43): C, 66.30; H, 3.18; N, 22.09; S, 8.43. Found: C, 66.60; H, 3.40; N, 22.30; S, 8.80.

Ethyl 2-Cyano-2-(4-(2-(cyanomethyl)-1*H*-benzo[*d*]imidazol-1-yl)-3-phenyl-thiazol-2(3*H*)-ylidene)acetate (**6b**)

Faint brown crystals; mp: 218–220°C; IR (KBr, cm^{-1}): 3062, 2979, 2928, 2201, 2190, 1751, 1623, 1470. ^{1}H-NMR (DMSO-d_6) δ: 1.16–1.21 (t, 3H, CH_3), 4.14–4.21 (q, 2H, CH_2), 4.38 (s, 2H, CH_2), 7.38 (s, 1H, CH thiazole), 7.40–7.53 (m, 9H, C_6H_4, C_6H_5). ^{13}C-NMR (DMSO-d_6) δ: 14.0, 16.6, 60.9, 82.5, 103.6, 114.2, 115.3, 117.0, 117.8, 122.3, 122.7, 122.7, 125.3, 125.3, 129.1, 129.1, 130.3, 134.7, 138.5, 141.1, 164.9, 172.2, 173.3. MS (EI): *m*/*z* (%) 427 $[M]^+$ (15.33), 426 $[M-1]^+$ (15.33), 425 $[M-2]^+$ (19.81), 57 (100.00). *Anal.* Calcd for $C_{23}H_{17}N_5O_2S$ (427.48): C, 64.62; H, 4.01; N, 16.38; S, 7.50. Found: C, 64.99; H, 4.40; N, 16.60; S, 7.80.

2-(1-(2-(2,4-Dioxopentan-3-ylidene)-3-phenyl-2,3-dihydrothiazol-4-yl)-1*H*-benzo[*d*]imidazol-2-yl)acetonitrile (**6c**)

Yellow crystals; mp: 228–230°C; IR (KBr, cm^{-1}): 3070, 2925, 2192, 1651, 1645, 1587, 1460. ^{1}H-NMR (DMSO-d_6) δ: 2.49–2.51 (s, 3H, CH_3), 2.55–2.57 (s, 3H, CH_3), 4.39 (s, 2H, CH_2), 7.15 (s, 1H, CH thiazole), 7.27–7.57 (m, 9H, C_6H_4, C_6H_5). ^{13}C-NMR (DMSO-d_6) δ: 17.7, 30.8, 30.8, 82.3, 112.1, 114.2, 115.3, 119.3, 121.0, 122.8, 122.8, 123.3, 123.3, 129.6, 129.6, 130.4, 136.1, 138.0, 140.2, 163.1, 179.4, 179.4, 183.2. MS (EI): *m*/*z* (%) 416 $[M+2]^+$ (3.10), 415 $[M+1]^+$ (11.25), 414 $[M]^+$ (37.64), 413 $[M-1]^+$ (3.98), 412 $[M-2]^+$ (0.97), 77 $[C_6H_5]^+$ (84.23). *Anal.* Calcd for $C_{23}H_{18}N_4O_2S$ (414.48): C, 66.65; H, 4.38; N, 13.52; S, 7.74. Found: C, 66.98; H, 4.63; N, 13.80; S, 8.10.

Ethyl 2-(4-(2-(Cyanomethyl)-1*H*-benzo[*d*]imidazol-1-yl)-3-phenylthiazol-2(3*H*)-ylidene)-3-oxobutanoate (**6d**)

Faint brown crystals; mp: 267–269°C; IR (KBr, cm^{-1}): 3006, 2884, 2195, 1719, 1622, 1590, 1472. ^{1}H-NMR (DMSO-d_6) δ: 1.30–1.35 (t, 3H, CH_3), 2.03 (s, 3H, CH_3), 4.16–4.21 (q, 2H, CH_2), 4.39 (s, 2H, CH_2), 6.90 (s, 1H, CH thiazole), 7.26–7.55 (m, 9H, C_6H_4, C_6H_5). ^{13}C-NMR (DMSO-d_6) δ: 14.1, 16.1, 32.7, 60.6, 82.3, 105.2, 112.0, 113.1, 119.4, 122.1, 123.4, 123.4, 124.0, 124.0, 129.5, 129.5, 130.3, 137.2, 138.2, 141.0, 150.5, 164.0, 165.2, 183.1. MS (EI): *m*/*z* (%) 445 $[M+1]^+$ (0.68), 444 $[M]^+$ (0.86), 184 (100.00), 77 $[C_6H_5]^+$ (31.32). *Anal.* Calcd for $C_{24}H_{20}N_4O_3S$ (444.51): C, 64.85; H, 4.54; N, 12.60; S, 7.21. Found: C, 65.10; H, 4.88; N, 12.90; S, 7.50.

Preparation of 3-(1*H*-Benzo[*d*]imidazol-2-yl)-2*H*-chromen-2-one (**7**)

To a solution of compound **1** (1.57 g, 0.01 mol) in 1,4-dioxane (30 mL) containing piperidine (0.50 mL), salicylaldehyde (1.22 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 5 h then poured onto ice/water mixture containing a few drops of hydrochloric acid. The formed solid product was collected by filtration, dried and crystallized from1,4-dioxane.

Canary yellow crystals; mp: 228–230°C; IR (KBr, cm^{-1}): 3341, 3051, 2929, 1707, 1602, 1451. ^{1}H-NMR (DMSO-d_6) δ: 6.77–8.00 (m, 8H, C_6H_4), 8.59 (s, 1H, pyran C4), 12.93 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ: 116.1, 116.6, 116.6, 122.1, 122.7, 122.7, 125.0, 127.1, 129.4, 129.5, 134.8, 134.8, 142.2, 145.7, 153.2, 159.2. MS (EI): *m*/*z* (%) 261 $[M-1]^+$ (37.1), 244 (100.00). *Anal.* Calcd for $C_{16}H_{10}N_2O_2$ (262.26): C, 73.27; H, 3.84; N, 10.68. Found: C, 73.50; H, 4.02; N, 10.40.

Preparation of 3-(1-(2-Chloroacetyl)-1*H*-benzo[*d*]imidazol-2-yl)-2*H*-chromen-2-one (**8**)

To a solution of compound **7** (2.62 g, 0.01 mol) in 1,4-dioxane (30 mL), chloroacetyl chloride (1.12 g, 0.01 mol) was added. The reaction mixture was heated under reflux for about 30 min. and then poured onto ice/water mixture. The solid product formed was collected by filtration, dried and crystallized from 1,4-dioxane.

Cannary yellow crystals; mp: 284–286°C; IR (KBr, cm^{-1}): 3058, 2949, 1729, 1609, 1448. ^{1}H-NMR (DMSO-d_6) δ: 4.36 (s, 2H, CH_2), 7.22–7.73 (m, 8H, $2C_6H_4$), 8.00–8.03 (s, 1H, pyran C4). ^{13}C-NMR (DMSO-d_6) δ: 46.0, 114.5, 115.0, 116.6, 120.4, 123.1, 123.3, 125.7, 127.0, 128.0, 130.0, 132.5, 135.0, 146.5, 153.7, 162.0, 169.1, 175.0. MS (EI): *m*/*z* (%) 336 $[M-2]^+$ (18.8), 173 (100.00), 77 $[C_6H_5]^+$ (31.32). *Anal.* Calcd for $C_{18}H_{11}N_2O_3Cl$ (338.74): C, 63.82; H, 3.27; N, 8.27. Found: C, 64.10; H, 3.50; N, 8.50.

General Procedure for the Preparation of 2-Oxo-2-(2-(2-oxo-2*H*-chromen-3-yl)-1*H*-benzo[*d*]imidazol-1-yl)-*N*′-phenylacetohydrazonoyl Chloride Derivatives (9a–f) To a cold solution (0–5°C) of **8** (3.38 g, 0.01 mol) in ethanol (50 mL) containing sodium hydroxide solution (10 mL, 10%) a solution (0.01 mol) of any of benzenediazonium chloride, *p*-chlorobenzenediazonium chloride, *p*-methoxybenzenediazonium chloride, *p*-methylbenzenediazonium chloride, 2,4-dichlorobenzenediazonium chloride or *p*-nitrobenzenediazonium chloride [which was prepared by the addition of sodium nitrite (0.69 g, 0.01 mol) in water (2 mL) to a cold solution (0–5°C) of the corresponding arylamine (0.01 mol) containing the appropriate amount of hydrochloric acid (3 mL) with continuous stirring] was added with continuous stirring. The reaction mixture was stirred at room temperature and the solid product formed was collected by filtration and dried. The obtained product was crystallized from ethanol.

2-Oxo-2-(2-(2-oxo-2*H*-chromen-3-yl)-1*H*-benzo[*d*]-imidazol-1-yl)-*N*′-phenylacetohydrazonoyl Chloride (**9a**)

Dark yellow crystals; mp: 213–215°C; IR (KBr, cm^{-1}): 3410, 3053, 1711, 1650, 1606, 1567, 1510, 1445. ^{1}H-NMR (DMSO-d_6) δ: 7.21–7.91 (m, 13H, $2C_6H_4$, C_6H_5), 8.11 (s, 1H, pyran C4), 9.25 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ: 113.0, 113.0, 114.0, 115.0, 120.5, 122.6, 123.0, 123.4, 124.8, 125.4, 126.8, 128.0, 129.5, 129.6, 129.6, 130.5, 134.5, 142.0, 142.4, 145.6, 162.2, 168.1, 175.0, 179.3. MS (EI): *m*/*z* (%) 445 $[M+2]^+$ (0.68), 444 $[M+1]^+$ (1.92), 443 $[M]^+$ (4.04), 442 $[M-1]^+$ (11.04), 441 $[M-2]^+$ (4.78), 262 (100.00), 77 $[C_6H_5]^+$ (86.99). *Anal.* Calcd for $C_{24}H_{15}N_4O_3Cl$ (442.85): C, 65.09; H, 3.41; N, 12.65. Found: C, 65.30; H, 3.71; N, 12.99.

N′-(4-Chlorophenyl)-2-oxo-2-(2-(2-oxo-2*H*-chromen-3-yl)-1*H*-benzo[*d*]imidazol-1-yl)acetohydrazonoyl Chloride (**9b**)

Pale orange crystals; mp: 280–282°C; IR (KBr, cm^{-1}): 3410, 3302, 3035, 1709, 1606, 1577, 1511, 1475. ^{1}H-NMR (DMSO-d_6) δ: 7.24–7.93 (m, 12H, $3C_6H_4$), 8.13 (s, 1H, pyran C4), 9.26 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ: 114.3, 115.3, 116.2, 117.4, 117.4, 119.1, 123.2, 123.2, 125.2, 127.6, 127.8, 128.4, 129.4, 129.4, 129.5, 130.3, 137.1, 142.7, 148.2, 150.0, 155.1, 161.5, 167.2, 175.1. MS (EI): *m*/*z* (%) 478 $[M+1]^+$ (0.11), 477 $[M]^+$ (0.15), 476 $[M-1]^+$ (0.15), 475 $[M-2]^+$ (0.26), 261 (100.00). *Anal.* Calcd for $C_{24}H_{14}N_4O_3Cl_2$ (477.30): C, 60.39; H, 2.96; N, 11.74. Found: C, 60.69; H, 3.31; N, 11.90.

N′-(4-Methoxyphenyl)-2-oxo-2-(2-(2-oxo-2*H*-chromen-3-yl)-1*H*-benzo[*d*]imidazol-1-yl)acetohydrazonoyl Chloride (**9c**)

Brown crystals; mp: 213–215°C; IR (KBr, cm^{-1}): 3335, 3056, 2928, 1707, 1601, 1570, 1499, 1452. ^{1}H-NMR (DMSO-d_6) δ: 3.87 (s, 3H, CH_3), 7.13–8.01 (m, 12H, $3C_6H_4$), 8.10 (s, 1H, pyran C4), 9.14 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ: 55.6, 114.4, 115.2, 115.5, 115.5, 116.0, 117.2, 117.2, 119.4, 123.9, 123.9, 124.9, 127.8, 128.3, 130.2, 129.5, 132.9, 138.5, 145.5, 153.2, 154.2, 158.7, 161.7, 167.5, 175.3. MS (EI): *m*/*z* (%) 475 $[M+2]^+$ (0.28), 474 $[M+1]^+$ (0.70), 473 $[M]^+$ (0.70), 472 $[M-1]^+$ (0.14), 471 $[M-2]^+$ (0.08), 396 (100.00). *Anal.* Calcd for $C_{25}H_{17}N_4O_4Cl$ (472.88): C, 63.50; H, 3.62; N, 11.85. Found: C, 63.81; H, 3.91; N, 12.10.

2-Oxo-2-(2-(2-oxo-2*H*-chromen-3-yl)-1*H*-benzo[*d*]-imidazol-1-yl)-*N*′-(*p*-tolyl)acetohydrazonoyl Chloride (**9d**)

Orange crystals; mp: 223–225°C; IR (KBr, cm^{-1}): 3394, 3053, 2922, 1709, 1603, 1570, 1508, 1453. ^{1}H-NMR (DMSO-d_6) δ: 2.40 (s, 3H, CH_3), 7.21–8.01 (m, 12H, $3C_6H_4$), 8.14 (s, 1H, pyran C4), 9.16 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ: 55.5, 114.5, 115.3, 115.9, 115.9, 116.5, 117.3, 117.3, 119.4, 122.5, 122.5, 125.0, 127.8, 128.5, 129.4, 130.3, 132.8, 138.2, 145.6, 153.1, 154.4, 158.7, 161.5, 167.3, 176.2. MS (EI): *m*/*z* (%) 456 $[M-1]^+$ (0.29), 455 $[M-2]^+$ (0.78), 91 (100.00), 77 $[C_6H_5]^+$ (12.31). *Anal.* Calcd for $C_{25}H_{17}N_4O_3Cl$ (456.88): C, 65.72; H, 3.75; N, 12.26. Found: C, 65.99; H, 4.01; N, 12.30.

N′-(2,4-Dichlorophenyl)-2-oxo-2-(2-(2-oxo-2*H*-chromen-3-yl)-1*H*-benzo[*d*]imidazol-1-yl)acetohydrazonoyl Chloride (**9e**)

Faint brown crystals; mp: 278–280°C; IR (KBr, cm^{-1}): 3385, 3059, 1706, 1600, 1573, 1510, 1457. ^{1}H-NMR (DMSO-d_6) δ: 7.14–8.07 (m, 11H, $2C_6H_4$, C_6H_3), 8.14 (s, 1H, pyran C4), 9.20 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ: 113.3, 114.6, 118.0, 118.4, 120.4, 123.2, 123.2, 125.2, 125.8, 126.1, 126.7, 127.2, 128.2, 130.1, 132.8, 135.6, 137.1, 145.5, 146.0, 155.3, 161.5, 167.3, 170.3, 172.2. MS (EI): *m*/*z* (%) 511 $[M]^+$ (0.14), 510 $[M-1]^+$ (0.29), 509 $[M-2]^+$ (0.84), 261 (100.00). *Anal.* Calcd for $C_{24}H_{13}N_4O_3Cl_3$ (511.74): C, 56.33; H, 2.56; N, 10.95. Found: C, 56.69; H, 2.82; N, 11.30.

N′-(4-Nitrophenyl)-2-oxo-2-(2-(2-oxo-2*H*-chromen-3-yl)-1*H*-benzo[*d*]imidazol-1-yl)acetohydrazonoyl Chloride (**9f**)

Brown crystals; mp: 243–245°C; IR (KBr, cm^{-1}): 3408, 3060, 1716, 1601, 1570, 1519, 1456. ^{1}H-NMR (DMSO-d_6) δ: 7.21–7.98 (m, 12H, $3C_6H_4$), 8.17 (s, 1H, pyran C4), 9.17 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ: 113.5, 113.5, 114.2, 115.6, 116.6, 120.1, 123.2, 123.2, 124.0, 124.0, 125.2, 127.0, 127.3, 130.0, 131.3, 137.3, 138.6, 145.7, 148.9, 153.7, 161.5, 167.1, 170.3, 173.5. MS (EI): *m*/*z* (%) 490 $[M^+ +2]$ (0.03), 489 $[M+1]^+$ (0.04), 488 $[M]^+$ (0.04), 487 $[M-1]^+$ (0.03), 486 $[M-2]^+$ (0.08), 261 (100.00). *Anal.* Calcd for $C_{24}H_{14}N_5O_5Cl$ (487.85): C, 59.09; H, 2.89; N, 14.36. Found: C, 59.29; H, 3.11; N, 14.60.

Preparation of 3-Oxo-3-(2-(2-oxo-2*H*-chromen-3-yl)-1*H*-benzo[*d*]imidazol-1-yl)propanenitrile (**10**)

A solution of compound **8** (3.38 g, 0.01 mol) in 1,4-dioxane (25 mL) was heated on water bath at 60°C, then potassium cyanide (0.65 g, 0.01 mol) in a least amount of water (2 mL), was added with continuous stirring. The reaction mixture was left in the water bath for 30 min. at 60°C then poured onto ice/water mixture containing a few drops of hydrochloric acid. The solid product, so formed, was collected by filtration and crystallized from ethanol.

Brown crystals; mp: 210–212°C; IR (KBr, cm^{-1}): 3065, 2930, 2201, 1714, 1647, 1610, 1457. ^{1}H-NMR (DMSO-d_6) δ: 3.56 (s, 2H, CH_2), 7.12–7.18 (s, 1H, pyran C4), 7.20–7.95 (m, 8H, $2C_6H_4$). ^{13}C-NMR (DMSO-d_6) δ: 113.5, 114.2, 115.1, 116.7, 120.7, 123.8, 123.8, 125.0, 126.2, 127.5, 130.5, 133.5, 138.5, 142.1, 150.2, 161.2, 165.8, 172.3. MS (EI): *m*/*z* (%) 331 $[M+2]^+$ (0.19), 330 $[M+1]^+$ (0.25), 329 $[M]^+$ (0.37), 327 $[M-2]^+$ (0.25), 57 (100.00). *Anal.* Calcd for $C_{19}H_{11}N_3O_3$ (329.31): C, 69.30; H, 3.37; N, 12.76. Found: C, 69.68; H, 3.71; N, 12.95.

Preparation of 2-((1-(2-Chloroacetyl)-1*H*-benzo[*d*]imidazol-2-yl)methyl)thiazol-4(5*H*)-one (**11**)

To a solution of compound **2** (2.33 g, 0.01 mol) in acetic acid (15 mL), thioglycollic acid (0.92 g, 0.01 mol) was added. The reaction mixture was heated under reflux system for about 5 h and then poured onto ice/water mixture. The solid product formed was collected by filtration, dried and crystallized from 1,4-dioxane.

Pale yellow crystals; mp: 295–297°C; IR (KBr, cm^{-1}): 3082, 3010, 2955, 2885, 1713, 1624, 1590, 1469. ^{1}H-NMR (DMSO-d_6) δ: 3.38 (s, 2H, CH_2), 3.70 (s, 2H, CH_2), 4.38 (s, 2H, CH_2), 7.26–7.55 (m, 4H, C_6H_4). ^{13}C-NMR (DMSO-d_6) δ: 38.7, 39.8, 45.8, 112.1, 113.0, 123.1, 123.5, 130.4, 139.0, 164.1, 168.0, 172.3, 178.1. MS (EI): *m*/*z* (%) 310 $[M+2]^+$ (5.61), 309 $[M+1]^+$ (4.43), 57 (100.00). *Anal.* Calcd for $C_{13}H_{10}N_3O_2SCl$ (307.76): C, 50.73; H, 3.28; N, 13.65; S, 10.42. Found: C, 50.99; H, 3.51; N, 13.90; S, 10.66.

General Procedure for the Preparation of 5-Amino-2-((1-(2-chloroacetyl)-1*H*-benzo[*d*]imidazol-2-yl)methyl)-7-phenyl-7*H*-pyrano[2,3-*d*]thiazole-6-carbonitrile Derivatives (12a–d) To a solution of compound **11** (3.07 g, 0.01 mol) in 1,4-dioxane (20 mL) containing a catalytic amount of triethylamine (0.50 mL) any of benzaldehyde (1.06 g, 0.01 mol), *p*-chlorobenzaldehyde (1.40 g, 0.01 mol), *p*-methoxybenzaldehyde (1.36 g, 0.01 mol) or 2-nitrobenzaldehyde (1.51 g, 0.01 mol) and malononitrile (0.66 g, 0.01 mol) were added. The reaction mixture, in each case, was heated under reflux system for 5 h then poured onto ice/water mixture containing a few drops of hydrochloric acid. The solid products formed, in each case, was collected by filtration, dried and crystallized from1,4-dioxane.

5-Amino-2-((1-(2-chloroacetyl)-1*H*-benzo[*d*]imidazol-2-yl)-methyl)-7-phenyl-7*H*-pyrano[2,3-*d*]thiazole-6-carbonitrile (**12a**)

Yellow crystals; mp: 290–292°C; IR (KBr, cm^{-1}): 3428, 3231, 3077, 2953, 2884, 2198, 1621, 1589, 1470. ^{1}H-NMR (DMSO-d_6) δ: 3.56 (s, 2H, CH_2), 4.38 (s, 2H, CH_2), 4.60 (s, 1H, pyran C4), 7.23–7.93 (m, 9H, C_6H_4, C_6H_5), 8.53 (s, 2H, NH_2). ^{13}C-NMR (DMSO-d_6) δ: 29.0, 30.0, 45.8, 59.0, 114.1, 115.0, 119.5, 123.1, 123.5, 125.0, 128.0, 128.0, 129.4, 129.4, 130.4, 134.3, 139.0, 141.0, 144.0, 150.6, 160.2, 164.0, 183.2. MS (EI): *m*/*z* (%) 462 $[M]^+$ (0.30), 461 $[M-1]^+$ (0.31), 233 (100.00). *Anal.* Calcd for $C_{23}H_{16}N_5O_2SCl$ (461.92): C, 59.80; H, 3.49; N, 15.16; S, 6.94. Found: C, 59.91; H, 3.81; N, 15.30; S, 7.20.

5-Amino-2-((1-(2-chloroacetyl)-1*H*-benzo[*d*]imidazol-2-yl)methyl)-7-(4-chlorophenyl)-7*H*-pyrano[2,3-*d*]thiazole-6-carbonitrile (**12b**)

Brown crystals; mp: 264–266°C; IR (KBr, cm^{-1}): 3429, 3226, 3097, 2952, 2884, 2196, 1621, 1587, 1470. ^{1}H-NMR (DMSO-d_6) δ: 3.57 (s, 2H, CH_2), 4.38 (s, 2H, CH_2), 4.40 (s, 1H, pyran C4), 7.26–7.97 (m, 8H, $2C_6H_4$), 8.54 (s, 2H, NH_2). ^{13}C-NMR (DMSO-d_6) δ: 29.8, 30.5, 45.8, 64.0, 114.2, 115.5, 119.4, 123.4, 123.4, 125.8, 125.8, 129.9, 129.9, 130.2, 131.9, 133.3, 139.0, 141.3, 143.5, 150.4, 160.0, 164.5, 183.0. MS (EI): *m*/*z* (%) 498 $[M+2]^+$ (0.13), 497 $[M+1]^+$ (0.42), 496 $[M^+]$ (1.98), 495 $[M-1]^+$ (5.83), 494 $[M-2]^+$ (2.02), 57 (100.00).

Anal. Calcd for $C_{23}H_{15}N_5O_2SCl_2$ (496.37): C, 55.65; H, 3.05; N, 14.11; S, 6.46. Found: C, 55.99; H, 3.41; N, 14.30; S, 6.80.

5-Amino-2-((1-(2-chloroacetyl)-1*H*-benzo[*d*]imidazol-2-yl)-methyl)-7-(4-methoxyphenyl)-7*H*-pyrano[2,3-*d*]thiazole-6-carbonitrile (**12c**)

Brown crystals; mp: 271–273°C; IR (KBr, cm^{-1}): 3423, 3224, 3074, 2946, 2196, 1625, 1569, 1467. ^{1}H-NMR (DMSO-d_6) δ: 3.55 (s, 3H, CH_3), 3.81 (s, 2H, CH_2), 4.37 (s, 2H, CH_2), 4.90 (s, 1H, pyran C4), 7.11–7.95 (m, 8H, $2C_6H_4$), 8.32 (s, 2H, NH_2). ^{13}C-NMR (DMSO-d_6) δ: 29.7, 30.5, 45.8, 55.7, 64.0, 113.7, 114.6, 114.6, 115.0, 119.4, 123.3, 123.3, 127.2, 130.2, 130.2, 130.7, 138.4, 141.3, 143.6, 150.4, 157.2, 160.2, 164.2, 183.0. MS (EI): *m/z* (%) 493 $[M^+ +1]$ (0.18), 492 $[M^+]$ (0.18), 184 (100.00). *Anal.* Calcd for $C_{24}H_{18}N_5O_3SCl$ (491.95): C, 58.59; H, 3.69; N, 14.24; S, 6.52. Found: C, 58.69; H, 3.82; N, 14.30; S, 6.73.

5-Amino-2-((1-(2-chloroacetyl)-1*H*-benzo[*d*]imidazol-2-yl)methyl)-7-(2-nitrophenyl)-7*H*-pyrano[2,3-*d*]thiazole-6-carbonitrile (**12d**)

Dark brown crystals; mp: 280–282°C; IR (KBr, cm^{-1}): 3429, 3200, 3100, 2925, 2196, 1624, 1590, 1466. ^{1}H-NMR (DMSO-d_6) δ: 3.56 (s, 2H, CH_2), 4.38 (s, 2H, CH_2), 4.90 (s, 1H, pyran C4), 7.25–7.54 (m, 8H, $2C_6H_4$), 8.01 (s, 2H, NH_2). ^{13}C-NMR (DMSO-d_6) δ: 25.6, 30.2, 45.8, 64.0, 114.3, 115.2, 119.4, 123.4, 123.4, 124.2, 126.1, 129.7, 130.2, 132.5, 134.1, 138.7, 141.5, 143.2, 149.1, 150.4, 160.7, 164.9, 183.1. MS (EI): *m/z* (%) 509 $[M+2]^+$ (0.24), 508 $[M+1]^+$ (0.17), 507 $[M]^+$ (0.25), 184 (100.00). *Anal.* Calcd for $C_{23}H_{15}N_6O_4SCl$ (506.92): C, 54.49; H, 2.98; N, 16.58; S, 6.33. Found: C, 54.80; H, 3.21; N, 16.90; S, 6.65.

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