Scientific paper

New Approaches for the Synthesis, Cytotoxicity and Toxicity of Heterocyclic Compounds Derived from 2-Cyanomethylbenzo[c]imidazole

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Abstract

The reaction of ethyl cyanoacetate with *o*-phenylenediamine gave the 2-cyanomethylbenzo[*c*]imidazole (**1**). The latter compound was used as the key starting material to synthesise biologically active heterocyclic derivatives. Thus, the reaction of **1** with cyclohexanone and either of benzaldehyde, 4-methoxybenzaldehyde or 4-chlorobenzaldehyde gave the annulated derivatives **2a–c**, respectively. The antitumor evaluations of the newly synthesized products against the three cancer cell lines MCF-7 (breast adeno-carcinoma), NCI-H460 (non-small cell lung cancer) and SF-268 (CNS cancer) showed that compounds **2b**, **6**, **11b**, **11c**, **12b**, **16a**, **16b** and **18a** exhibited optimal cytotoxic effect against cancer cell lines, with IC_{50} values in the nM range. 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 was used. Compounds **11b, 12b** and **16b** showed no toxicity against the tested organisms.

Keywords: benzimidazole, thiophene, thiazole, synthesis, anti-tumor, toxicity

1. 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 isosteres of naturally occurring nucleotides, which allow them to interact easily with the biopolymers of the living systems and various kinds of biological activities have been obtained. Some 2-aminobenzimidazoles display 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] 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,[5–10] anthelmintic,[11] antiviral,[12–16] and antifungal[17,18] activities. Since 1985 benzimidazole containing compounds have been reported as well known anticancer agents.[19–25] The role of mammalian DNA topoisomerases as molecular targets for anticancer drugs has been recognized. Some benzimidazoles have been reported as topoisomerase inhibitors, *e.g.* Hoechst 33258 and Hoechst 33342 (Fig. 1).[26,27] As the extension of this work, head to head bisbenzimidazole compounds proved high efficacy as DNA binders.[28] 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.[29]

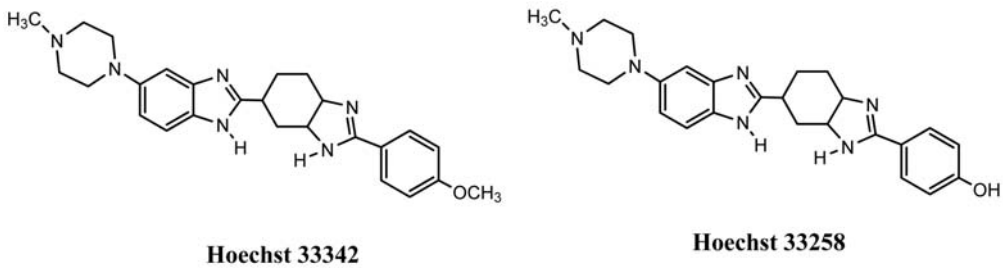
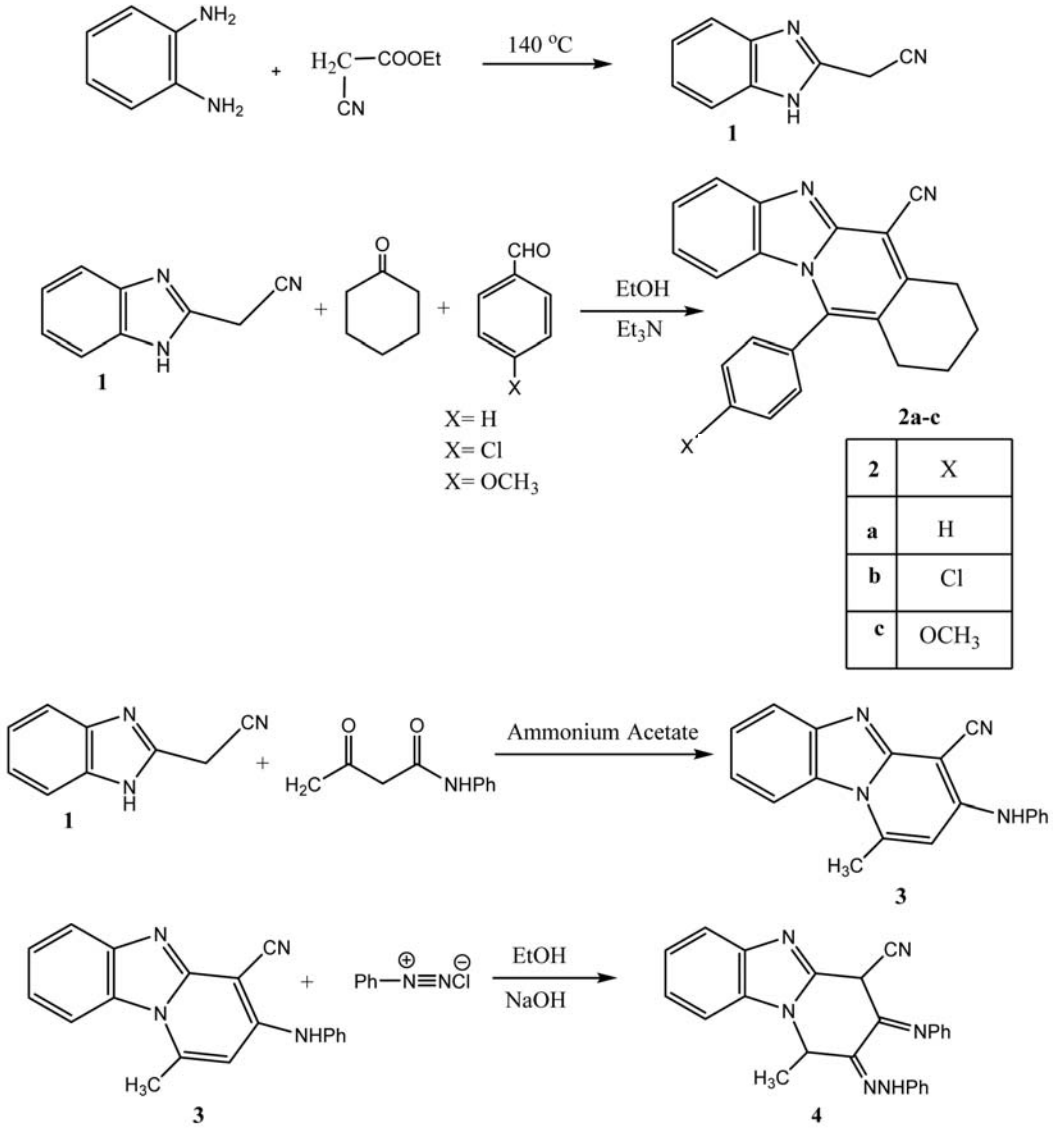


Fig. 1. Examples of topoisomerase inhibitors containing benzimidazole nucleus.

2. Results and Discussion

2. 1. Chemistry

The 2-cyanomethylbenzo[*c*]imidazole (**1**) obtained from the reaction of ethyl cyanoacetate with *o*-phenylenediamine was used as the key starting material to synthesise biologically active heterocyclic derivatives. Thus, the reaction of **1** with cyclohexanone and any of benzaldehyde, 4-methoxybenzaldehyde or 4-chlorobenzaldehyde gave the annulated derivatives **2a**–**c**, respectively. The analy-

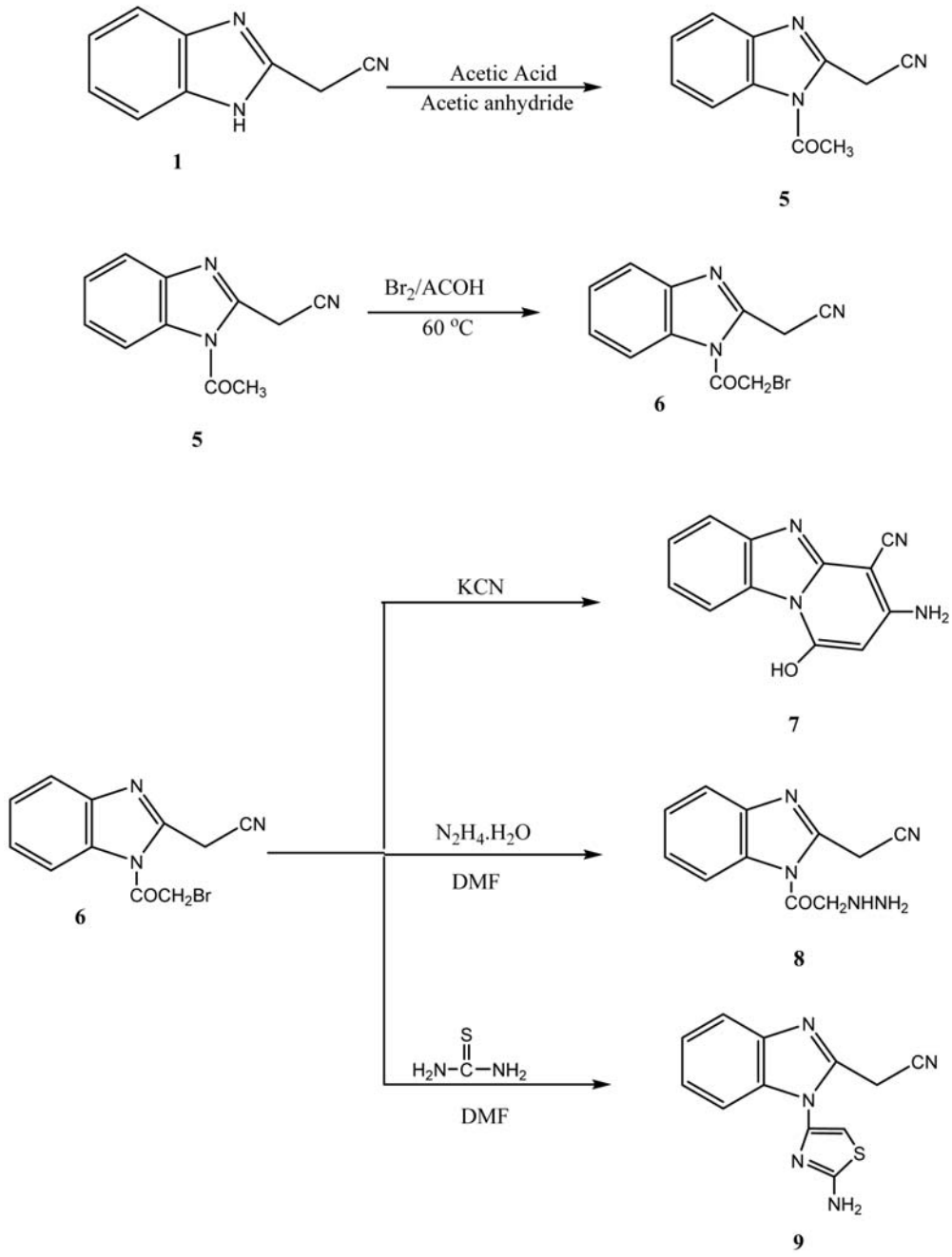


2	X
a	H
b	Cl
c	OCH_3

Scheme 1.

tical and spectral data of **2a-c** were consistent with their respective structures. Thus, the ^{1}H NMR spectrum of **2a** (as an example) showed 2.49–2.88 (CH_2-cyclohexanone), 7.13–8.01 (m, 9H, C_6H_4, C_6H_5). Moreover, the ^{13}C NMR data revealed 38.6, 39.0, 40.2, 40.6 (4 × CH_2-cyclohexanone), 116.2 (CN), 120.3, 122.8, 124.9, 127.8, 128.0, 129.8, 131.2, 132.6, 134.5, 134.8, 144.3, 146.8, 150.4 (2 × C_6H_4, pyridine C), 164.8 (C=N).

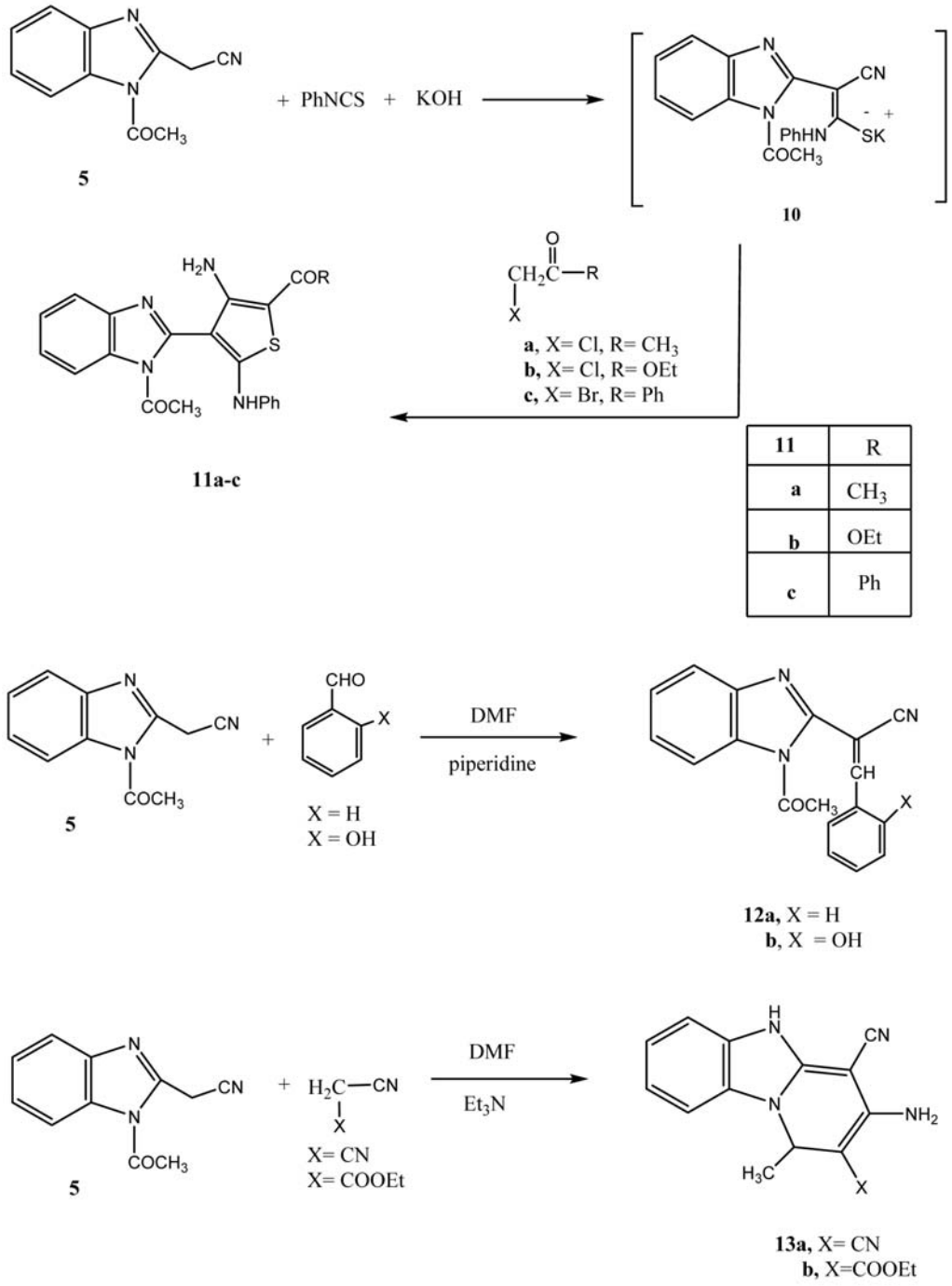
The reaction of **1** with acetoacetanilide gave the benzo[*c*]pyrazolo[3,2-*a*]pyridine derivative **3**. The latter compound reacted with benzene diazonium chloride to give the phenylazo derivative **4** (Scheme 1). On the other hand, the reaction of **1** with acetic acid/acetic anhydride mixture gave the *N*-acetyl derivative **5**. Compound **5** readily underwent bromination when treated with bromine in acetic acid solution at 60 °C to give the *N*-α-bromoacetylbenzo[*c*]imidazole derivative **6**. The latter compound as α-bromocarbonyl compound showed interesting chemical reactivity when treated with some chemical reagents. Thus, the reaction of **6** with potassium cyanide gave the benzo[*c*]imidazo[2,3-*b*]pyridine derivative **7**. On the other hand, the reaction of **6** with hydrazine hydrate afforded the hydrazine de-



Scheme 2.

rivative **8**. Compound **6** reacted with thiourea in ethanol to give the thiazole derivative **9** (Scheme 2).

Recently, our research group was involved in a comprehensive program involving the reaction of active methylene reagents with phenylisothiocyanate in basic (KOH) dimethylformamide to form the intermediate potassium sulphide salt. The latter undergoes heterocyclization when reacted with α-halocarbonyl compounds to give either thiophene or thiazole derivatives depending on the nature of the α-halocarbonyl compound and the reaction conditions.[30–32] Thus, the reaction of **5** with phenylisothiocyanate in DMF/KOH solution gave the intermediate potassium sulphide salt **10**. The latter intermediate underwent heterocyclization when reacted with any of α-chloroacetone, ethyl chloroacetate or α-bromoacetophenone to give the thiophene derivatives **11a–c**, respectively. The analytical and spectral data of **11a–c** are consistent with their respective structures.

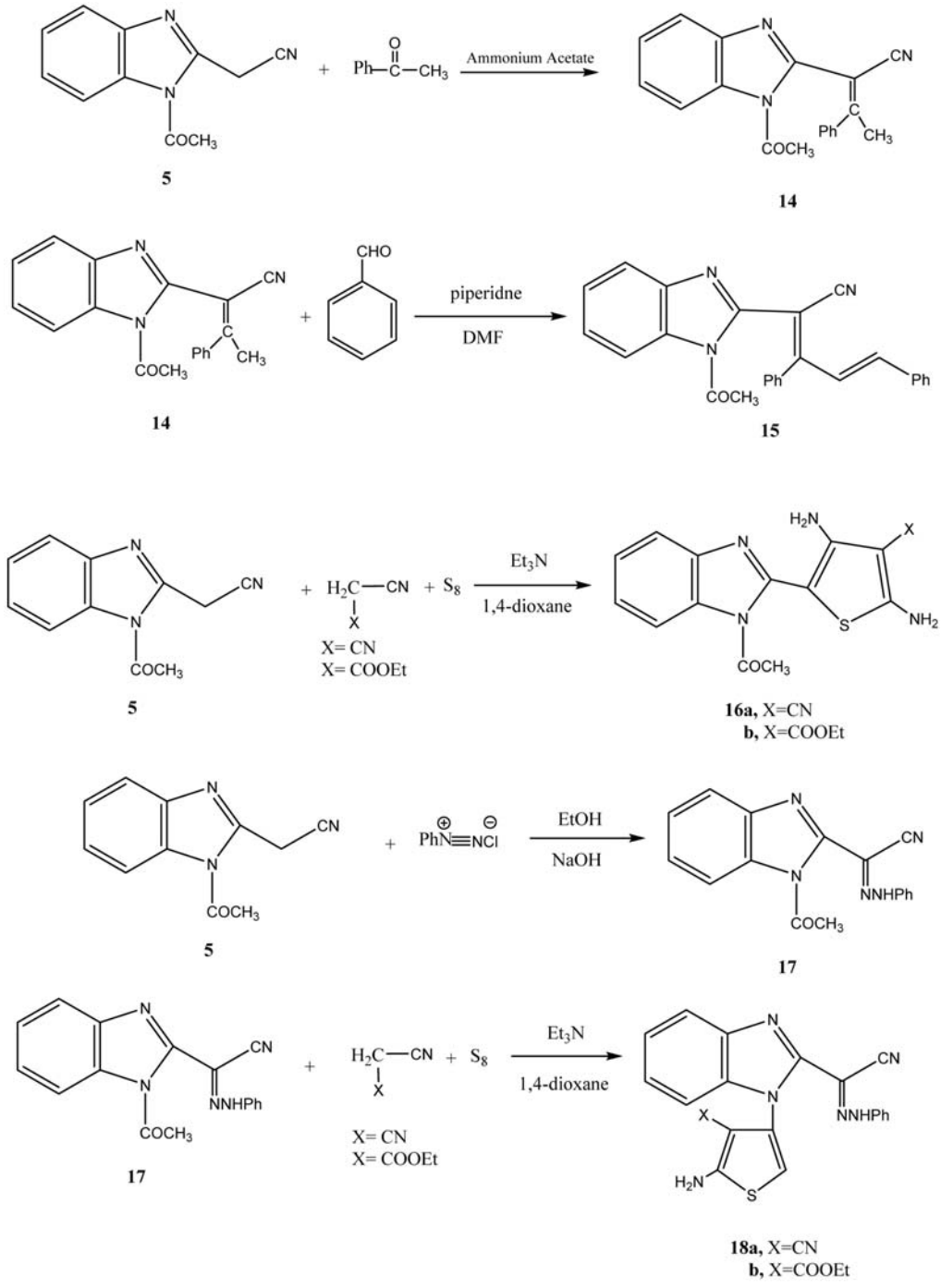


Scheme 3.

The reaction of **5** with either benzaldehyde or salicylaldehyde gave the benzylidene derivatives **12a** and **12b**, respectively. On the other hand, the reaction of **5** with either malononitrile or ethyl cyanoacetate in DMF containing triethylamine gave the 1,5-dihydrobenzo[4,5]imidazo[1,2-*a*]pyridine derivatives **13a** and **13b**, respectively (Scheme 3).

Compound **5** reacted with acetophenone in an oil bath at 120 °C to give the Knoevenagel condensation product **14**. The latter compound reacted with benzaldehyde to give the benzylidene derivative **15**.

The reactivity of **5** towards the well-known Gewald's thiophene synthesis was studied to give biologically active thiophene derivatives. Thus, the reaction of **5** with elemental sulfur and either of malononitrile or ethyl cyanoacetate gave the thiophene derivatives **16a** and **16b**, respectively. On the other hand, the reaction of **5** with benzenediazonium chloride in ethanol/sodium hydroxide solution affor-



Scheme 4.

ded the phenylhydrazo derivative **17**. Compound **17** underwent the Gewald's thiophene synthesis through the *N*-acetyl moiety when reacted with elemental sulfur and either of malononitrile or ethyl cyanoacetate in 1,4-dioxane containing triethylamine under reflux to give the thiophene derivatives **18a** and **18b**, respectively (Scheme 4).

3. Anti-tumor and Normal Cell Line Activity Tests

3. 1. Chemicals

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).

3. 1. 1. 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 were grown as monolayer and routinely maintained in RPMI-1640 medium supplemented with 5% heat inactivated FBS, 2 mM glutamine and antibiotics (penicillin 100 U/mL, streptomycin 100 μg/mL), at 37 °C in a humidified atmosphere containing 5% CO_2. Exponentially growing cells were obtained by plating 1.5×10^5 cells/mL for MCF-7 and SF-268 and 0.75×10^4 cells/mL for NCI-H460, followed by 24 h of incubation. The effect of the vehicle solvent (DMSO) on the growth of these cell lines was evaluated in all the experiments by exposing untreated control cells to the maximum concentration (0.5%) of DMSO used in each assay.

3. 1. 2. Tumor Cell Growth Assay

The effects of **2a–c** to **18a**,**b** on the *in vitro* growth of human tumor cell lines were evaluated according to the procedure adopted by the National Cancer Institute (NCI, USA) in the žIn vitro Anticancer Drug Discovery Screen' that uses the protein-binding dye sulforhodamine B to assess cell growth.[33] Briefly, exponentially, cells growing in

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

Compound	GI_{50} (μ mol L^{-1})			
	MCF-7	NCI-H460	SF-268	WI 38
2a	33.0 ± 1.4	20.8 ± 4.3	20.3 ± 2.8	38.4 ± 2.90
2b	0.8 ± 0.04	0.5 ± 0.02	0.06 ± 0.001	20.0 ± 4.94
2c	22.1 ± 10.4	30.8 ± 10.8	26.1 ± 2.8	28.2 ± 0.8
3	33.6 ± 10.2	40.0 ± 8.6	38.6 ± 8.0 8	>100
4	32.2 ± 3.6	36.3 ± 12.5	40.6 ± 8.8	50.7 ± 8.2
5	22.8 ± 8.30	22.8 ± 4.32	22.8 ± 6.23	44.8 ± 6.0
6	0.01 ± 0.001	0.02 ± 0.004	0.06 ± 0.002	>100
7	28.4 ± 5.8	22.7 ± 8.2	30.4 ± 2.4	18.6 ±4.0
8	23.55 ± 4.06	34.6 ± 12.06	45.41 ± 2.16	>100
9	33.6 ± 8.5	40.3 ± 12.3	30.4 ± 2.8	62.2 ± 2.0
11a	26.4 ± 2.10	12.42 ± 3.01	10.63 ± 2.83	>100
11b	0.81 ± 0.04	0.52 ± 0.04	0.08 ± 0.006	40.0 ± 1.3
11c	1.6 ± 0.4	0.6 ± 0.16	1.8 ± 0.06	22.4 ±1.6
12a	26.2 ± 2.4	28.6 ± 2.8	26.8 ± 8.5	30.2 ± 2.6
12b	0.02 ± 0.001	0.03 ± 0.006	0.06 ± 0.008	> 100
13a	30.22 ± 6.12	28.99 ± 4.70	10.39 ± 6.80	> 100
13b	12.6 ± 2.01	18.6 ± 6.06	30.4 ± 2.36	30.6 ± 10.2
14	12.33 ± 2.16	16.36 ± 2.26	18.20 ± 5.28	55.5 ±8.3
15	30.7 ± 6.2	38.5 ± 6.4	37.5 ± 8.0	66.0 ± 18.4
16a	2.6 ± 2.8	6.6 ± 2.2	5.0 ± 1.81	0.5 ± 5.1
16b	0.06 ± 0.006	0.06 ± 0.006	0.02 ± 0.008	>100
17	38 ± 4. 18	39.03 ± 8.01	22.59 ± 4.01	20.20 ± 8.2
18a	0.08 ± 0.002	0.08 ± 0.003	0.02 ± 0.002	>100
18b	36.0 ± 7.3	26.7 ± 2.8	30.4 ± 2.9	32.6 ± 6.4
Doxorubicin	0.04 ± 0.008	0.09 ± 0.008	0.09 ± 0.007	> 100

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

96-well plates were then exposed for 48 h to five serial concentrations of each compound, starting from a maximum concentration of 150 μM. Following this exposure period adherent cells were fixed, washed, and stained. The bound stain was solubilized and the absorbance was measured at 492 nm in a plate reader (Bio-Tek Instruments Inc., Power wave XS, Winooski, USA). For each test compound and cell line, a dose-response curve was obtained and the growth inhibition of 50% (GI_{50}), corresponding to the concentration of the compounds that inhibited 50% of the net cell growth was calculated as described elsewhere. Doxorubicin was used as a positive control and tested in the same manner.

3. 1. 3. Structure Activity Relationship:

From Table 1 it is clear that the benzimidazole moiety was found to be crucial for the cytotoxic effect of the cyclic compounds **2a–c** to **18a**,**b**. Compounds **2b**, **6**, **11b**, **11c**, **12b**, **16a**, **16b** and **18a** exhibited optimal cytotoxic effect against cancer cell lines, with IC_{50} values in the nM range. Comparing the cytotoxicity of the benzimidazothiophenes **11b** and **11c**, it is obvious that the cytotoxicity of **11b** is higher than that of **11c**. The presence of the 2-EtO group in **11b** is responsible for its high potency. Considering the 7,8,9,10-tetrahydrobenzo[4,5]imidazo[1,2-*b*]isoquinoline derivatives **2a–c**, it is clear that the cytotoxicity of **2b** is higher than those of **2a** and **2c**. Such high cytotoxicity of **2b** is attributed to the presence of the 4-chlorophenylisoquinoline moiety together with the benzimidazole moiety. The high cytotoxicity of **16b** relative to **16a** is also explained in terms of the presence of the 3-EtO moiety. On the other hand, by considering the (1*H*-benzo[*d*]imidazol-1-yl)-2-aminothiophene derivatives **18a** and **18b** it is clear that the presence of the 2-carbonitrile group present in **18a** is responsible for its high potency. The bromo-1*H*-benzo[*d*]imidazole derivative **6** showed the maximum cytotoxicity effect towards the three cancer cell lines followed by acetyl-1*H*-benzo[*d*]imidazolhydroxyphenyl derivative **12b**.

3. 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[34] was used. Results were analyzed with LC_{50} program to determine LC_{50} values and 95% confidence intervals.[35] Results are given in Table 2 for the compounds which exhibited optimal cytotoxic effect against cancer cell lines, being the eight compounds **2b**, **6**, **11b**, **11c**, **12b**, **16a**, **16b** and **18a**. The shrimp lethality assay is considered as a useful tool for preliminary assessment of toxicity, and it has

Table 2. Toxicity of the most potent compounds against the cancer cell lines

Compound	Conc. (μg/mL)	Mortality[a]	Toxicity	LC_{50}	Upper 95% lim	Lower 95% lim
2b	10	6	Very toxic	12.05	–	–
	100	8				
	1000	10				
6	10	1	Very toxic	18.38	–	–
	100	6				
	1000	10				
11b	10	0	Non toxic	982.15	–	–
	100	0				
	1000	2				
11c	10	0	Harmful	420.28	112.23	90.55
	100	5				
	1000	10				
12b	10	0	Non toxic	880.42	–	–
	100	1				
	1000	4				
16a	10	2	Very toxic	14.88	–	–
	100	8				
	1000	10				
16b	10	0	Non toxic	999.33	–	–
	100	0				
	1000	5				
18a	10	0	Harmful	22.70	210.59	160.22
	100	6				
	1000	8				

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

been used for the detection of fungal toxins, plant extract toxicity, heavy metals, cyano bacteria toxins, pesticides, and cytotoxicity testing of dental materials,[36] natural and synthetic organic compounds.[34] 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.[37]

In order to prevent the toxicity results from possible false effects originated from solubility of compounds and DMSO's possible toxicity effect, compounds were prepared by dissolving in DMSO in the suggested DMSO volume ranges. It is clear from Table 2 that **11b**, **12b**, and **16b** showed no toxicity against the tested organisms. On the other hand, **2b**, **6** and **16a** are very toxic, in addition, **11c** and **18a** are harmful.

3. 2. 1. Toxicity Method

All toxicity tests were 96-h static renewal tests and water quality measurements (dissolved oxygen, pH, temperature, salinity) were taken in the control containers each day. Tests were run in a Revcos Environmental Chamber at 25 °C, 20% salinity, and a 16-h light : 8-h dark cycle. A media change was made every 24 h. Larvae used for all tests were one to two days old and exposed in 600-mL glass beakers containing 400 mL of media with 10 larvae/beaker and three replicates/concentration. Larvae were fed newly hatched *Artemia* after daily media change. The concentration of each compound was taken in terms 10, 100 and 1000 mg/mL. Adult shrimp toxicity tests were also run to complete the grass shrimp toxicity profile. Adult shrimp (acclimated for two weeks before testing) were exposed in 4-L wide-mouth glass jars containing 2 L of media and 10 shrimp/jar with two replicates/concentration and were run under conditions as described above for larvae.[38]

4. Experimental

All melting points are uncorrected. IR spectra were recorded on KBr discs on a PyeUnicam SP-1000 spectrophotometer. ^{1}H and ^{13}C NMR spectra were measured on a Varian EM-390-200 MHz in DMSO as solvent using TMS as internal standard, and chemical shifts are expressed as δ. Analytical data were obtained from the Microanalytical Data Unit at Cairo University, Giza, Egypt.

General procedure for the synthesis of the imidazo [1,2-*b*]isoquinolines 2a–c

To a solution of **1** (1.57 g, 10 mmol) in ethanol (25 mL) containing triethylamine (0.5 mL), cyclohexanone (0.98 g, 10 mmol) and any of benzaldehyde (1.06 g, 10 mmol), 4-chlorobenzaldehyde (1.40 g, 10 mmol) or 4-methoxybenzaldehyde (1.36 g, 10 mmol) were added. The reaction mixture was heated under reflux for 3 h, then poured into a beaker containing ice/water mixture containing a few drops of hydrochloric acid. The solid product formed was collected by filtration and dried. The obtained product was crystallized from ethanol to give greenish brown crystals.

11-Phenyl-7,8,9,10-tetrahydrobenzo[4,5]imidazo[1,2-*b*]isoquinoline-6-carbonitrile (2a).

Yield 2.26 g (70%); m.p. 223–225 °C; Anal. Calcd. for $C_{22}H_{17}N_3$ (323.39): C, 81.71; H, 5.30; N, 12.99%; found: C, 81.60; 4.85; N, 13.20%. IR (KBr) ν/cm^{-1} 3092–3030 (CH aromatic), 2887 (CH-alph.), 2222 (CN), 1523 (C=N), 1589, 1437 (C=C). ^{1}H NMR (DMSO-d_6) δ 2.49–2.88 (m, 8H, 4CH_2-cyclohexanone), 7.13–8.01 (m, 9H, C_6H_4, C_6H_5). ^{13}C NMR (DMSO-d_6) δ 38.6, 39.0, 40.2, 40.6 (4×CH_2), 116.2 (CN), 120.3, 122.8, 124.9, 127.8, 128.0, 129.8, 131.2, 132.6, 134.5, 134.8, 144.3, 146.8, 150.4 (2×C_6H_4, pyridine C), 164.8 (C=N). MS (*m/z*) 323 (M$^+$, 23%).

11-(4-Chlorophenyl)-7,8,9,10-tetrahydrobenzo[4,5] imidazo[1,2-*b*]isoquinoline-6-carbonitrile (2b).

Yield 2.32 g (65%); m.p. 267–269 °C; Anal. Calcd. for $C_{22}H_{16}ClN_3$ (357.84): C, 73.84; H, 4.51; N, 11.74%; found: C, 73.61; H, 4.38; N, 11.94%. IR (KBr) ν/cm^{-1} 3091– 3027 (CH aromatic), 2900 (CH-alph.), 2222 (CN), 1584, 1488 (C=C), 1523 (C=N). ^{1}H NMR (DMSO-d_6) δ 2.49–2.51 (m, 8H, 4CH_2-cyclohexanone), 7.26–8.01 (m, 8H, 2C_6H_4). ^{13}C NMR (DMSO-d_6) δ 38.8, 39.12, 39.7, 39.9 (4×CH_2), 115.9 (CN), 120.0, 122.9, 125.8, 126.2, 130.2, 131.1, 134.5, 136.1, 138.4, 140.2, 144.9, 145.6, 150.3 (2×C_6H_4, pyridine-C), 165.2 (C=N). MS (*m/z*) 357 (M$^+$, 80%).

11-(4-Methoxyphenyl)-7,8,9,10-tetrahydrobenzo[4,5] imidazo[1,2-*b*] isoquinoline-6-carbonitrile (2c).

Yield 2.58 g (73%); m.p. 243–246 °C; Anal. Calcd. for $C_{23}H_{19}N_3O$ (353.42): C, 78.16; H, 5.42; N, 11.89%; found: C, 77.33; H, 4.05; N, 11.06%. IR (KBr) ν/cm^{-1} 3103–3015 (CH aromatic), 2901–2838 (CH-alph.), 2212 (CN), 1643 (C=O), 1512 (C=N), 1589, 1446 (C=C). ^{1}H NMR (DMSO-d_6) δ 1.30 (s, 3H, CH_3), 2.49–2.51 (m, 8H, 4×CH_2-cyclohexanone), 6.91–8.02 (m, 8H, 2C_6H_4). ^{13}C NMR (DMSO-d_6) δ 38.55, 38.84, 39.39, 39.67, 39.95 (4-CH_2, cyclohexanone), 55.5 (CH_3), 116.7 (CN), 120.3, 123.6, 124.6, 125.3, 130.8, 131.1, 134.5, 136.6, 138.8, 140.6, 143.7, 146.8, 150.2 (2×C_6H_4, pyridine-C), 165.8 (C=N). MS (*m/z*) 353 (M$^+$, 36%).

1-Methyl-3-(phenylamino)benzo[4,5]imidazo[1,2-*a*] pyridine-4-carbonitrile (3).

To a mixture of **1** (1.57 g, 10 mmol) and acetoacetanilide (1.77 g, 10 mmol), ammonium acetate (0.77 g, 10

mmol) was added. The reaction mixture was heated in oil bath at 140 °C for 1 h then left to cool. The semisolid formed was triturated with ethanol (40 mL) and the formed solid product was collected by filtration and dried. The obtained product was crystallized from ethanol to give light amber crystals.

Yield 2.27 g (76%); m.p. > 300 °C; Anal. Calcd. for $C_{19}H_{14}N_4$ (298.34): C, 76.49; H, 4.73; N, 18.78%; found: C, 76.33; H, 4.44; N, 18.63%. IR (KBr) ν/cm^{-1} 3423–3106 (NH), 3056 (CH aromatic), 2955–2829 (CH-alph.), 2208 (CN), 1569, 1489 (C=C), 1526 (C=N). ^{1}H NMR (DMSO-d_6) δ 1.91 (s, 3H, CH_3), 5.94 (s, 1H, pyridine C_5), 7.05–7.55 (m, 9H, C_6H_4, C_6H_5), 13.19 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 20.24 (CH_3), 116.2 (CN), 111.1, 115.9, 120.7, 120.7, 122.1, 126.2, 127.5, 128.7, 131.5, 104.2, 146.6, 150.8, 154.0, 155.1 (C_6H_4, C_6H_5, pyridine-C), 164.1 (C=N). MS (*m/z*) 298 (M$^+$, 18%).

1-Methyl-2-(2-phenylhydrazono)-3-(phenylimino)-1,2,3,4-tetrahydrobenzo-[4,5]imidazo-[1,2-*a*]pyridine-4-carbonitrile (4)

To a cold (0–5 °C) solution of **3** (1.20 g, 40.2 mmol) in ethanol (50 mL) containing sodium hydroxide solution (10 mL, 10%) and a solution of benzenediazonium chloride (40.2 mmol) [which was prepared by dissolving sodium nitrite (0.60 g, 80.4 mmol) in water (2 mL) was added to a cold solution of aniline (0.4 mL, 40.2 mmol) containing the appropriate amount of hydrochloric acid with continuous stirring] was added with continuous stirring. The reaction mixture was stirred at room temperature for 3 h and the solid product formed was collected by filtration and dried. The obtained product was crystallized from ethanol to give red crystals.

Yield 2.67 g (66%); m.p. 245–248 °C; Anal. Calcd. for $C_{25}H_{20}N_6$ (404.47): C, 74.24; H, 4.98; N, 20.78%; found: C, 74.05; H, 4.88; N, 19.82%. IR (KBr) ν/cm^{-1} 3419–3200 (NH), 3058 (CH aromatic), 2961–2854 (CH-alph.), 2210 (CN), 1600 (C=N), 1450 (C=C). ^{1}H NMR (DMSO-d_6) δ 1.91 (s, 3H, CH_3), 5.82 (s, 1H, pyridine C_6), 7.17 (s, 1H, pyridine C_3), 7.25–7.79 (m, 14H, C_6H_4, 2×C_6H_5), 8.53 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 19.9 (CH_3), 115.8 (CN), 118.1, 120.7, 120.7, 120.7, 121.4, 122.0, 122.9, 123.1, 123.9, 124.5, 124.9, 125.83, 126.1, 127.3, 128.7, 141.3, 156.1 (C_6H_4, 2×C_6H_5, pyridine-C), 173.2, 186.9 (2×C=N). MS (*m/z*) 404 (M$^+$, 66%).

2-(1-Acetyl-1*H*-benzo[*d*]imidazol-2(3*H*)-ylidene) acetonitrile (5)

A solution of **1** (1.57 g, 10 mmol) in acetic acid (15 mL) and acetic anhydride (35 mL) was heated under reflux till a precipitate is formed after 30 min, then poured into a beaker containing ice/water mixture. The solid product formed was collected by filtration and dried. The obtained product was crystallized from ethanol to give gold crystals.

Yield 1.47 g (74%); m.p. > 300 °C; Anal. Calcd. for $C_{11}H_9N_3O$ (199.21): C, 66.32; H, 4.55; N, 21.09%; found: C, 64.48; H, 4.33; N, 21.63%. IR (KBr) ν/cm^{-1} 3069 (CH aromatic), 2965–2871 (CH-alph.), 2192 (CN), 1630 (C=O), 1594, 1410 (C=C), 1513 (C=N). ^{1}H NMR (DMSO-d_6) δ 2.21 (s, 3H, CH_3), 3.30 (s, 2H, CH_2), 7.2-7.51 (m, 4H, C_6H_4). ^{13}C NMR (DMSO-d_6) δ 27.0 (CH_3), 65.3 (CH_2), 115.8 (CN), 121.1, 123.1, 124.0, 125.3, 126.0, 130.3 (C_6H_4), 151.1 (C=O), 189.2 (C=N). MS (*m/z*) 199 (M$^+$, 49%).

2-(1-Bromo-1*H*-benzo[*d*]imidazol-2-yl)acetonitrile (6)

A solution of **5** (1.99 g, 10 mmol) in glacial acetic acid (10 mL) was warmed to 60 ºC, then bromine (0.08 g, 10 mmol) in acetic acid (10 mL) was added drop-wise with continuous stirring. The reaction mixture was stirred for 1.5 h then poured into ice/water and the solid product formed was collected by filtration. The obtained product was crystallized from ethanol to give brownish orange crystals.

Yield 1.95 g (70%); m.p. > 300 °C; Anal. Calcd. for $C_{11}H_8BrN_3O$ (278.10): C, 47.51; H, 2.90; N, 15.11%; found: C, 47.33; H, 3.89; N, 13.49%. IR (KBr) ν/cm^{-1} 3050 (CH aromatic), 2971 (CH-alph.), 2193 (CN), 1630 (C=O), 1602, 1474 (C=C). ^{1}H NMR (DMSO-d_6) δ 3.30, 4.00 (2s, 4H, 2×CH_2), 7.20–7.63 (m, 4H, C_6H_4). ^{13}C NMR (DMSO-d_6) δ 55.8, 62.7 (2×CH_2), 116.4 (CN), 119.2, 121.3, 124.8, 129.3, 133.6 (C_6H_4), 168.8 (C=O), 172.7 (C=N). MS (*m/z*) 278 (M$^+$, 28%).

3-Amino-1-hydroxybenzo[4,5]imidazo[1,2-*a*]pyridine-4-carbonitrile (7)

To a solution of **6** (2.78 g, 10 mmol) in dimethylformamide (5 mL) heated on a water bath at 60 °C potassium cyanide (0.65 g, 10 mmol), dissolved in a least amount of water, was added while stirring. The reaction mixture was left in the water bath for 30 min at 60 °C then poured into a beaker containing ice/water mixture and a few drops of hydrochloric acid. The solid product formed was collected by filtration and dried. The obtained product was crystallized from ethanol to give reddish brown crystals.

Yield 1.93 g (86%); m.p. > 300 °C; Anal. Calcd. for $C_{12}H_8N_4O$ (224.22): C, 64.28; H, 3.60; N, 24.99%; found: C, 63.88; H, 3.88; N, 24.69%. IR (KBr) ν/cm^{-1} 3436–3233 (OH, NH_2), 3050 (CH aromatic), 2193 (CN), 1513 (C=N), 1597 (C=C). ^{1}H NMR (DMSO-d_6) δ 5.21 (s, 2H, D_2O exchangeable, NH_2), 7.22 (s, 1H, pyridine C_5), 7.21–7.88 (m, 4H, C_6H_4), 12.91 (s, 1H, OH). ^{13}C NMR (DMSO-d_6) δ 116.8 (CN), 119.2, 121.3, 124.8, 129.3, 133.6, 136.2, 136.8, 140.1, 142.4, 144.1, 146.7 (C_6H_4). MS (*m/z*) 224 (M$^+$, 60%).

2-(1-(2-Hydrazinylacetyl)-1*H*-benzo[*d*]imidazol-2-yl) acetonitrile (8)

To a solution of **6** (2.78 g, 10 mmol) in dimethylformamide (5 mL) hydrazine hydrate (0.50 g, 10 mmol) was added. The reaction mixture was stirred for 3 h at room temperature then poured into a beaker containing acidi-

fied ice/water mixture. The solid product formed was collected by filtration and dried. The obtained product was crystallized from ethanol to give light brown crystals.

Yield 2.01 g (88%); m.p. > 300 °C; Anal. Calcd. for $C_{11}H_{11}N_5O$ (229.24): C, 57.63; H, 4.84; N, 30.55%; found: C, 57.80; H, 4.69; N, 30.79%. IR (KBr) ν/cm^{-1} 3414–3214 (NH, NH_2), 3100 (CH aromatic), 2900 (CH-alph.), 2194 (CN), 1668 (C=O), 1600, 1470 (C=C), 1519 (C=N). ^{1}H NMR (DMSO-d_6) δ 3.34 (s, 2H, CH_2), 4.40 (s, 2H, CH_2), 7.21–7.63 (m, 4H, C_6H_4), 5.76 (s, 2H, NH_2), 12.74 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 56.8, 65.9 (2×CH_2), 120.8, 122.4, 126.7, 128.3, 133.6, 145.9 (C_6H_4), 164.2 (C=O), 172.8 (C=N). MS (*m/z*) 229 (M^+, 18%).

2-(1-(2-Aminothiazol-4-yl)-1*H*-benzo[*d*]imidazol-2-yl) acetonitrile (9)

To a solution of **6** (2.78 g, 10 mmol) in dimethylformamide (20 mL), thiourea (0.76 g, 10 mmol) was added. The reaction mixture was heated under reflux for 3 h then left to cool. The solid product formed was collected by filtration and dried. The obtained product was crystallized from ethanol to give brown crystals.

Yield 2.12 g (83%); m.p. > 300 °C; Anal. Calcd. for $C_{12}H_9N_5S$ (255.30): C, 56.45; H, 3.55; N, 27.43; S, 12.56%; found: C, 56.78; H, 3.69; N, 27.39; S, 12.48%. IR (KBr) ν/cm^{-1} 3428–3227 (NH_2), 3050 (CH aromatic), 2967–2922 (CH-alph.), 2195 (CN), 1513 (C=N), 1597, 1474 (C=C). ^{1}H NMR (DMSO-d_6) δ 3.30 (s, 2H, CH_2), 5.21 (s, 2H, NH_2), 6.23 (s, 1H, thiazole C_5), 7.24–7.63 (m, 4H, C_6H_4). ^{13}C NMR (DMSO-d_6) δ 55.6, (CH_2), 116.8 (CN), 120.8, 122.4, 126.7, 128.3, 133.6, 134.3, 138.5, 145.9 (C_6H_4, thiazole C), 168.2, 172.8 (2×C=N). MS (*m/z*) 255 (M^+, 25%).

General procedure for the synthesis of the thiophene derivatives 11a–c

To a solution of **5** (1.99 g, 10 mmol) in dimethylformamide (20 mL) containing finely divided potassium hydroxide (0.56 g, 10 mmol), phenylisothiocyanate (1.35 g, 10 mmol) was added. The reaction mixture was stirred at room temperature for 24 h, then any of chloroacetone (0.92 g, 10 mmol), ethyl chloroacetate (1.22 g, 10 mmol) or α-bromoacetophenone (1.99 g, 10 mmol) was added. The whole reaction mixture was stirred at room temperature for additional 24 h. The solid product formed upon dilution with ice/water mixture containing hydrochloric acid (till pH 6) was collected by filtration and dried. The obtained product was crystallized from ethanol to give copper-coloured crystals for **11a** and **11b** and brown crystals for **11c.**

1-(4-(1-Acetyl-1*H*-benzo[*d*]imidazol-2-yl)-3-amino-5-(phenylamino)thiophen-2-yl)ethanone (11a)

Yield 3.36 g (86%); m.p. > 300 °C; Anal. Calcd. For $C_{21}H_{18}N_4O_2S$ (390.46): C, 64.60; H, 4.65; N, 14.35; S, 8.21%; found: C, 64.88; H, 4.67; N, 14.72; S, 8.40%. IR (KBr) ν/cm^{-1} 3456–3210 (NH), 3064 (CH aromatic), 2966–2875 (CH-alph.), 2192 (CN), 1730 (C=O), 1598, 1470 (C=C), 1516 (C=N). ^{1}H NMR (DMSO-d_6) δ 1.84, 1.89 (2s, 6H, 2×CH_3), 5.48 (s, 2H, NH_2), 7.12–7.51 (m, 9H, C_6H_4, C_6H_5), 12.71 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 27.0 (CH_3), 38.8 (CH_2), 115.8 (CN), 121.4, 123.1, 124.9, 125.1, 126.2, 127.9, 128.2, 129.4, 130.3, 133.2, 134.8, 136.8, 140.2, 142.1 (C_6H_4, C_6H_5, thiophene C), 163.8, 166.2 (2 C=O), 182.5 (C=N). MS (*m/z*) 390 (M^+, 38%).

Ethyl 4-(1-acetyl-1*H*-benzo[*d*]imidazol-2-yl)-3-amino-5-(phenylamino)-thiophene-2-carboxylate (11b)

Yield 2.94 g (70%); m.p. 140 °C; Anal. Calcd. for $C_{22}H_{20}N_4O_3S$ (420.48): C, 62.84; H, 4.79; N, 13.32; S, 7.63%; found: C, 62.73; H, 4.52; N, 13.06; S, 7.67%. IR (KBr) ν/cm^{-1} 3441–3211 (NH), 3063 (CH aromatic), 2970–2876 (CH-alph.), 2193 (CN), 1725 (C=O), 1597, 1472 (C=C), 1516 (C=N). ^{1}H NMR (DMSO-d_6) δ 1.16–1.21 (t, 3H, CH_3), 2.11 (s, 3H, CH_3), 3.97 (s, 2H, NH_2), 4.11–4.16 (q, 2H, CH_2), 6.76–7.55 (m, 9H, C_6H_4, C_6H_5), 12.74 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 16.3 (ester CH_3), 28.2 (CO-$\underline{CH_3}$), 52.8 (ester CH_2), 115.9 (CN), 120.6, 122.8, 123.4, 125.0, 125.8, 126.3, 126.8, 128.1, 130.3, 133.4, 136.1, 138.9, 141.8, 142.0 (C_6H_4, C_6H_5, thiophene C), 164.3, 166.9 (2×C=O), 172.8 (C=N). MS (*m/z*) 420 (M^+, 26%).

1-(2-(4-Amino-5-benzoyl-2-(phenylamino)thiophen-3-yl)-1*H*-benzo[*d*]imidazol-1-yl)ethanone (11c)

Yield 2.26 g (50%); m.p. > 300 °C; Anal. Calcd. for $C_{26}H_{20}N_4O_2S$ (452.53): C, 69.01; H, 4.45; N, 12.38; S, 7.09%; found: C, 68.89; H, 4.65; N, 12.09; S, 6.83%. IR (KBr) ν/cm^{-1} 3431–3212 (NH, NH_2), 3067 (CH aromatic), 2968–2879 (CH-alph.), 2195 (CN), 1690, 1627 (2 C=O), 1599, 1471 (C=C), 1516 (C=N). ^{1}H NMR (DMSO-d_6) δ 2.12 (s, 3H, CH_3), 2.73 (s, 2H, D_2O exchangeable, NH_2), 7.21–8.09 (m, 14H, C_6H_4, 2×C_6H_5), 8.74 (s, 1H, D_2O exchangeable, NH). ^{13}C NMR (DMSO-d_6) δ 28.6 (CO-$\underline{CH_3}$), 119.3, 121.9, 124.2, 125.8, 125.8, 126.0, 126.8, 127.1, 127.9, 128.1, 130.3, 133.4, 136.1, 138.9, 140.3, 141.2, 142.6, 143.8 (2×C_6H_5, thiophene C), 163.9, 165.2 (2×C=O), 170.6 (C=N). MS (*m/z*) 452 (M^+, 42%).

General procedure for the synthesis of the benzylidene derivatives 12a,b

To a solution of **5** (1.99 g, 10 mmol) in dimethylformamide (25 mL) containing piperidine (0.5 mL), either of benzaldehyde (1.06 g, 10 mmol) or salicylaldehyde (1.22 g, 10 mmol) was added. The reaction mixture was heated under reflux for 3 h then poured into a beaker containing ice/water mixture containing a few drops of hydrochloric acid. The solid product formed was collected by filtration and dried. The obtained product was crystallized from ethanol to give yellow crystals of **12a** and orange crystals of **12b**.

2-(1-Acetyl-1*H*-benzo[d]imidazol-2-yl)-3-phenylacrylonitrile (12a)

Yield 1.81 g (63%); m.p. > 300 °C; Anal. Calcd. for $C_{18}H_{13}N_3O$ (287.32): C, 75.25; H, 4.56; N, 14.63%; found: C, 74.33; H, 4.36; N, 14.49%. IR (KBr) ν/cm^{-1} 3066 (CH aromatic), 2967–2876 (CH-alph.), 2193 (CN), 1627 (C=O), 1599, 1470 (C=C), 1515 (C=N). ^{1}H NMR (DMSO-d_6) δ 2.00 (s, 3H, CH_3), 3.30 (s, 1H, CH), 7.21–7.51 (m, 9H, C_6H_4, C_6H_5). ^{13}C NMR (DMSO-d_6) δ 28.4 (CO-$\underline{CH_3}$), 88.5, 90.6 (CH=C), 121.6, 123.8, 124.2, 124.9, 125.0, 127.1, 130.3, 133.4, 138.9, 139.0 (2×C_6H_5), 165.8 (C=O), 171.2 (C=N). MS (*m/z*) 287 (M^+, 23%).

2-(1-Acetyl-1*H*-benzo[*d*]imidazol-2-yl)-3-(2-hydroxyphenyl)acrylonitrile (12b)

Yield 2.79 g (92%); m.p. > 300 °C; Anal. Calcd. for $C_{18}H_{13}N_3O_2$ (303.31): C, 71.28; H, 4.32; N, 13.85%; found: C, 71.33; H, 4.88; N, 9.04%. IR (KBr) ν/cm^{-1} 3432–3215 (OH), 3100 (CH aromatic), 2999–2882 (CH-alph.), 2194 (CN), 1633 (C=O), 1601, 1477 (C=C), 1518 (C=N). ^{1}H NMR (DMSO-d_6) δ 1.20 (s, 3H, CH_3), 3.57 (s, 1H, CH), 7.21–7.51 (m, 8H, 2C_6H_4), 12.75 (s, 1H, D_2O exchangeable, OH). ^{13}C NMR (DMSO-d_6) δ 28.6 (CO-$\underline{CH_3}$), 88.9, 90.8 (CH=C), 120.8, 122.5, 124.2, 124.6, 125.6, 126.8, 128.7, 133.4, 136.3, 140.3 (2×C_6H_5), 164.6 (C=O), 170.8 (C=N). MS (*m/z*) 303 (M^+, 36%).

General procedure for the synthesis of the benzo[4,5]imidazo[1,2-*a*]pyridine derivatives 13a,b

To a solution of **5** (1.99 g, 10 mmol) in dimethylformamide (30 mL) containing triethylamine (0.5 mL), either malononitrile (0.66 g, 10 mmol) or ethyl cyanoacetate (1.13 g, 10 mmol) was added. The reaction mixture was heated under reflux for 4 h then poured into a beaker containing ice/water mixture containing a few drops of hydrochloric acid. The solid product formed was collected by filtration and dried. The obtained product was crystallized from ethanol to give buff crystals of **13a** and yellowish brown crystals of **13b**.

3-Amino-1-methylbenzo[4,5]imidazo[1,2-*a*]pyridine-2,4-dicarbonitrile (13a)

Yield 2.04 g (82%); m.p. > 300 °C; Anal. Calcd. For $C_{14}H_{11}N_5$ (249.27): C, 67.46; H, 4.45; N, 28.10%; found: C, 67.06; H, 4.18; N, 28.43%. IR (KBr) ν/cm^{-1} 3470–3212 (NH, NH_2), 3077 (CH aromatic), 2971–2877 (CH-alph.), 2223, 2194 (2CN), 1599, 1474 (C=C), 1517 (C=N). ^{1}H NMR (DMSO-d_6) δ 1.16 (s, 3H, CH_3), 5.51 (s, 2H, D_2O exchangeable, NH_2), 6.30 (s, 1H, pyridine C_6), 7.21–7.50 (m, 4H, C_6H_4), 12.75 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 22.7 (CH_3), 116.8, 117.3 (2×CN), 119.8, 120.7, 122.5, 124.8, 125.3, 126.4, 128.0, 130.9, 133.2, 134.8, 143.2 (C_6H_4, imidazole, pyridine C), 173.8 (C=N). MS (*m/z*) 249 (M^+, 22%).

Ethyl 3-amino-4-cyano-1-methylbenzo[4,5]imidazo[1,2-*a*]pyridine-2-carboxylate (13b)

Yield 2.01 g (68%); m.p. > 300 °C; Anal. Calcd. for $C_{16}H_{16}N_4O_2$ (296.32): C, 64.85; H, 5.44; N, 18.91%; found: C, 64.92; H, 4.27; N, 18.73%. IR (KBr) ν/cm^{-1} 3426–3214 (NH, NH_2), 3103 (CH aromatic), 2883 (CH-alph.), 2194 (CN), 1750 (C=O, ester), 1601, 1472 (C=C). ^{1}H NMR (DMSO-d_6) δ 1.10 (t, 3H, CH_3), 2.12 (s, 3H, CH_3), 3.29 (q, 2H, CH_2), 5.01 (s, 2H, D_2O exchangeable, NH_2), 6.10 (s, 1H, pyridine C_6), 7.20–7.51 (m, 4H, C_6H_4), 12.73 (s, 1H, D_2O exchangeable, NH). ^{13}C NMR (DMSO-d_6) δ 16.8 (ester CH_3), 22.4 (CH_3), 56.9 (ester CH_2), 116.5, 117.1 (2×CN), 119.9, 121.3, 122.8, 124.4, 125.1, 126.8, 127.4, 130.3, 133.6, 134.1, 143.8 (C_6H_4, imidazole, pyridine C), 162.8 (C=O). MS (*m/z*) 296 (M^+, 35%).

2-(1-Acetyl-1*H*-benzo[d]imidazol-2-yl)-3-phenylbut-2-enenitrile (14)

To a mixture of **5** (1.99 g, 10 mmol) and acetophenone (1.35 g, 10 mmol), ammonium acetate (0.77 g, 10 mmol) was added. The reaction mixture was heated in oil bath at 140 °C for 1 h then left to cool. The semisolid formed was triturated with ethanol (40 mL) and the formed solid product was collected by filtration and dried. The obtained product was crystallized from ethanol to give dark brown crystals.

Yield 1.99 g (66%); m.p. 210–213 °C; Anal. Calcd. for $C_{19}H_{15}N_3O$ (301.34): C, 75.73; H, 5.02; N, 13.94%; found: C, 74.83; H, 5.39; N, 13.85%. IR (KBr) ν/cm^{-1} 3086 (CH aromatic), 2880 (CH-alph.), 2194 (CN), 1630 (C=O), 1599, 1472 (C=C), 1517 (C=N). ^{1}H NMR (DMSO-d_6) δ 1.20, 2.40 (2s, 6H, 2×CH_3), 7.21–7.52 (m, 9H, C_6H_4, C_6H_5). ^{13}C NMR (DMSO-d_6) δ 22.4, 28.6 (CH_3, CO-$\underline{CH_3}$), 88.9, 90.6 (CH=C), 121.3, 122.5, 124.2, 124.6, 126.1, 126.8, 127.2, 133.4, 136.3, 136.9 (2×C_6H_5), 164.6 (C=O), 170.6 (C=N). MS (*m/z*) 301 (M^+, 17%).

2-(1-Acetyl-1*H*-benzo[*d*]imidazol-2-yl)-3,5-diphenylpenta-2,4-dienenitrile (15)

To a solution of **14** (3.01 g, 10 mmol) in dimethylformamide (25 mL) containing piperidine (0.50 mL), benzaldehyde (1.06 g, 10 mmol) was added. The reaction mixture was heated under reflux for 4 h then poured into a beaker containing ice/water mixture containing a few drops of hydrochloric acid. The solid product formed was collected by filtration and dried. The obtained product was crystallized from ethanol to give brown crystals.

Yield 2.57 g (66%); m.p. 150 °C; Anal. Calcd. for $C_{26}H_{19}N_3O$ (389.45): C, 80.18; H, 4.92; N, 10.79%; found: C, 80.29; H, 5.14; N, 10.59%. IR (KBr) ν/cm^{-1} 3057 (CH aromatic), 2927 (CH-alph.), 2195 (CN), 1627 (C=O), 1596 (C=N), 1489 (C=C). ^{1}H NMR (DMSO-d_6) δ 2.23 (s, 3H, CH_3), 2.72, 2.89 (2s, 2H, 2×CH), 6.50–8.35 (m, 14H, C_6H_4, 2×C_6H_5). ^{13}C NMR (DMSO-d_6) δ 24.3 (CH_3), 98.5, 104.8 (C=C), 116.4 (CN), 120.4, 121.3, 122.4, 124.9, 125.8, 126.8, 127.8, 128.0, 130.6, 133.5,

136.5, 140.8 (C_6H_4, 2×C_6H_5), 164.3 (C=O), 173.1 (C=N). MS (*m/z*) 389 (M^+, 44%).

General procedure for the synthesis of the thiophene derivatives 16a,b

To a solution of **5** (1.99 g, 10 mmol) in 1,4-dioxane (25 mL) containing triethylamine (0.50 mL) and elemental sulfur (0.32 g, 10 mmol) either malononitrile (0.66 g, 10 mmol) or ethyl cyanoacetate (1.13 g, 10 mmol) was added. The reaction mixture was heated under reflux for 4 h then poured into a beaker containing ice/water mixture containing a few drops of hydrochloric acid. The solid product was collected by filtration and dried. The obtained product was crystallized from ethanol to give dark brown crystals of **16a** and light brown crystals of **16b.**

5-(1-Acetyl-1*H*-benzo[*d*]imidazol-2-yl)-2,4-diaminothiophene-3-carbonitrile (16a) Yield 2.08 g (70%); m.p. 288–293 °C; Anal. Calcd. for $C_{14}H_{11}N_5OS$ (297.34): C, 56.55; H, 3.73; N, 23.55; S, 10.78%; found: C, 56.43; H, 3.49; N, 23.70; S, 10.63%. IR (KBr) ν/cm^{-1} 3426–3213 (2×NH_2), 3050 (CH aromatic), 2973, 2882 (CH-alph.), 2195 (CN), 1680 (C=O), 1600 (C=N), 1470 (C=C), 1518 (C=N). ^{1}H NMR (DMSO-d_6) δ 2.06 (s, 3H, CH_3), 2.73, 2.89 (2s, 4H, D_2O exchangeable, 2×NH_2), 7.20–7.95 (m, 4H, C_6H_4). ^{13}C NMR (DMSO-d_6) δ 22.6 (CH_3), 116.3 (CN), 119.8, 120.7, 122.6, 124.6, 129.5, 130.4, 133.5, 142.8, 143.2, 144.3 (C_6H_4, thiophene C), 163.2 (C=O), 172.3 (C=N). MS (*m/z*) 297 (M^+, 32%).

Ethyl 5-(1-acetyl-1*H*-benzo[*d*]imidazol-2-yl)-2,4-diaminothiophene-3-carboxylate (16b)

Yield 2.38 g (69%); m.p. > 300 °C; Anal. Calcd. for $C_{16}H_{16}N_4O_3S$ (344.39): C, 55.80; H, 4.68; N, 16.27; S, 9.31%; found: C, 55.89, H, 4.29; N, 16.60; S, 8.04%. IR (KBr) ν/cm^{-1} 3430–3216 (2 NH_2), 3107 (CH aromatic), 2978, 2884 (CH-alph.), 1633 (C=O), 1750 (C=O, ester), 1601 (C=N), 1471 (C=C), 1518 (C=N). ^{1}H NMR (DMSO-d_6) δ 1.04 (t, 3H, CH_3), 2.33 (s, 3H, CH_3), 4.28 (q, 2H, CH_2), 5.80, 5.95 (2s, 4H, D_2O exchangeable, 2 NH_2), 7.21–7.51 (m, 4H, C_6H_4). ^{13}C NMR (DMSO-d_6) δ 16.2 (ester CH_3), 22.8 (CH_3), 120.3, 120.8, 121.2, 123.8, 124.8, 125.8, 134.8, 142.8, 143.6, 144.9 (C_6H_4, thiophene C), 164.8 (C=O), 172.0 (C=N). MS (*m/z*) 344 (M^+, 48%).

2-(2-Phenylhydrazono)-2-(1-acetyl-1*H*-benzo[*d*]imidazol-2-yl) acetonitrile (17)

To a cold solution (0–5 °C) of **5** (1.99 g, 10 mmol) in ethanol (50 mL) containing sodium hydroxide solution (10 mL, 10%) and a solution of benzenediazonium chloride (10 mmol) [which was prepared by dissolving sodium nitrite (0.70 g, 10 mmol) in water, 2 mL was added to a cold solution of aniline (0.93 g, 10 mmol) containing appropriate amount of hydrochloric acid and with continuous stirring] was added with continuous stirring. The solid product formed was collected by filtration and dried. The obtained product was crystallized from ethanol to give brown crystals.

Yield 2.24 g (74%); m.p. 265 °C; Anal. Calcd for $C_{17}H_{13}N_5O$ (303.32): C, 67.32; H, 4.32; N, 23.09%; found: C, 67.29; H, 4.09; N, 23.27%. IR (KBr) ν/cm^{-1} 3423–3214 (NH), 3098 (CH aromatic), 2976–2883 (CH-alph.), 2194 (CN), 1631 (C=O), 1521 (=N-NH), 1601, 1475 (C=C). ^{1}H NMR (DMSO-d_6) δ 2.21 (s, 3H, CH_3), 7.21–7.51 (m, 9H, C_6H_4, C_6H_5), 12.76 (s, 1H, D_2O exchangeable, NH). ^{13}C NMR (DMSO-d_6) δ 22.8 (CH_3), 115.9 (CN), 120.4, 120.7, 123.2, 124.6, 126.5, 128.6, 133.5, 142.8, 143.2, 144.3 (C_6H_5, C_6H_4), 164.0 (C=O), 172.8 (C=N). MS (*m/z*) 303 (M^+, 20%).

General procedure for the synthesis of the phenylhydrazone derivatives 18a,b

To a solution of **17** (3.03 g, 10 mmol) in 1,4-dioxane (30 mL) containing triethylamine (0.50 mL) and elemental sulfur (0.32 g, 10 mmol) either malononitrile (0.66 g, 10 mmol) or ethyl cyanoacetae (1.13 g, 10 mmol) was added. The reaction mixture was heated under reflux for 4 h then poured into a beaker containing ice/water mixture containing a few drops of hydrochloric acid. The solid product was collected by filtration and dried. The obtained product was crystallized from ethanol to give yellow crystals of **18a** and light green crystals of **18b**.

4-(2-((2-Phenylhydrazono)(cyano)methyl)-1*H*-benzo[*d*]imidazol-1-yl)-2-aminothiophene-3-carbonitrile (18a)

Yield 3.45 g (90%); m.p. 198 °C; Anal. Calcd. for $C_{20}H_{13}N_7S$ (383.43): C, 62.65; H, 3.42; N, 25.57; S, 8.36%; found: C, 62.88; H, 3.59; N, 25.19; S, 8.07%. IR (KBr) ν/cm^{-1} 3352–3209 (NH, NH_2), 3100 (CH-aromatic), 2922 (CH-alph.), 2196 (CN), 1547 (C=N), 1463 (C=C). ^{1}H NMR (DMSO-d_6) δ 5.60 (s, 2H, D_2O exchangeable, NH_2), 7.20 (s, 1H, thiophene C_5), 7.21–7.67 (m, 9H, C_6H_4, C_6H_5), 12.75 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 116.3. 116.8 (2×CN), 120.0, 120.6, 123.2, 125.1, 126.5, 127.2, 136.5, 138.1, 139.2, 140.6, 140.8, 141.3, 142.6 (C_6H_5, C_6H_4, thiophene C), 164.0 (C=O), 172.8 (C=N). MS (*m/z*) 383 (M^+, 18%).

Ethyl 4-(2-((2-phenylhydrazono)(cyano)methyl)-1*H*-benzo[*d*]imidazol-1-yl)-2-aminothiophene-3-carboxylate (18b)

Yield 2.54 g (59%); m.p. > 300 °C; Anal. Calcd. for $C_{22}H_{18}N_6O_2S$ (430.48): C, 61.38; H, 4.21; N, 19.52; S, 7.45%; found: C, 61.03; H, 4.58; N, 19.71; S, 7.66%. IR (KBr) ν/cm^{-1} 3427–3215 (NH, NH_2), 3100 (CH aromatic), 2900 (CH-alph.), 2194 (CN), 1632 (C=O), 1520 (C=N), 1601, 1475 (C=C). ^{1}H NMR (DMSO-d_6) δ 1.10 (t, 3H, CH_3), 4.21 (q, 2H, CH_2), 5.01 (s, 2H, D_2O exchangeable, NH_2), 7.20 (s, 1H, thiophene C_5), 7.21–7.51 (m, 9H, C_6H_4, C_6H_5), 12.77 (s,1H, D_2O exchangeable, NH). ^{13}C NMR (DMSO-d_6) δ 16.3 (ester CH_3), 54.8 (ester

CH_2), 116.5 (CN), 120.3, 121.8, 123.8, 125.1, 127.2, 128.6, 130.2, 138.1, 139.2, 139.9, 140.4, 142.9, 143.8 (C_6H_5, C_6H_4, thiophene C), 164.0 (C=O), 172.8 (C=N). MS (*m/z*) 430 (M^+, 22%).

5. Conclusions

In the present study we have synthesized a series of heterocyclic derivatives of 2-cyanomethylbenzo[*c*]imidazole **1**. The newly synthesized products were tested against MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), and SF-268 (CNS cancer) and the results showed that compounds **2b**, **6**, **11b**, **11c**, **12b**, **16a**, **16b** and **18a** exhibited optimal cytotoxic effect against cancer cell lines. The toxicity of these optimal cytotoxic compounds was monitored via *in vivo* lethality to shrimp larvae (*Artemia salina*). The results showed that compounds **11b, 12b**, and **16b** are non toxic towards shrimp larvae.

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Povzetek

Pri reakciji etil cianoacetat z *o*-fenilendiaminom je nastal 2-cianometilbenzo[*c*]imidazol (**1**). To spojino smo uporabili kot ključno izhodno snov za sintezo biološko aktivnih heterocikličnih derivatov. Pri reakciji **1** s cikloheksanonom ter benzaldehidom, 4-metoksibenzaldehidom oz. 4-klorobenzaldehidom so nastali pripojeni derivati **2a**–**c**. Testiranje antitumorske aktivnosti novopripravljenih produktov proti trem rakastim celičnim linijam, t.j. MCF-7 (adeno-carcinom dojke), NCI-H460 (nemikrocelični karcinom pljuč) in SF-268 (rak centralnega živčnega sistema), je pokazalo, da spojine **2b**, **6**, **11b**, **11c**, **12b**, **16a**, **16b** in **18a** kažejo optimalno citotoksično učinkovitost proti tem rakastim linjam z IC_{50} vrednostmi v nM območju. Ker so bioaktivne spojine pogosto strupene za ličinke morskih rakcev, smo se odločili preveriti še *in vivo* strupenost teh spojin na ličinke *Artemia salina.* Spojine **11b, 12b** in **16b** niso pokazale nobene strupenosti za testirane organizme.