Synthesis, characterisation and biological screening of some 2-substituted benzimidazole derivatives as potential anticancer agents

Mohammed Abed-Elrahman Shaaban[a], Aliaa Mohammed Kamal[a]* and Heba El-Sayed Teba[b]

[a]*Pharmaceutical Organic Chemistry Department, Faculty of Pharmacy, Cairo University, 11561 Cairo, Egypt*
[b]*Pharmaceutical Organic Chemistry Department, Faculty of Pharmacy, October University for Modern Sciences and Arts (MSA), Cairo, Egypt*

Twenty two new polycyclic compounds containing a 2-substituted benzimidazole core were synthesised, characterised and evaluated *in vitro* against the three most widespread cancers. Seven compounds showed outstanding antitumor activity towards breast, lung or central nervous system cancer cell lines.

Keywords: heterocyclic synthesis, 2-substituted benzimidazole derivatives, nucleophilic reactions, anticancer agents

Cancer is a worldwide health problem according to the American Cancer Society report 2014, with lung, breast and central nervous system cancers being the most widespread. According to the Brain and Central Nervous System Cancer Agency (BC Cancer Agency), the incidence of central nervous system cancer is mostly due to metastasis from other sites *e.g.* lung and breast. That is why we focused on developing new antitumor agents against these types of cancers. Existing anticancer agents are not able to treat cancer effectively because of limited activity, rapid development of resistance and adverse effects.[1]

To discover and synthesise novel compounds as anti-proliferative agents, scaffold hopping theory suggests that modifying the central core structure of the biologically active molecules by combining two or more biologically active entities could be effective.[2] The benzimidazole ring system features in many bioactive heterocyclic compounds which exhibit diverse biological and clinical applications. Since benzimidazole derivatives are structural bioisosteres of naturally occurring nucleotides, they can interact with biological macromolecules such as proteins, enzymes and receptors.[3–12] Even quite simple 2-substituted derivatives of benzimidazole **I**–**IV** (Fig. 1) are known inhibitors of type I DNA topoisomerase.[5,7,13–15]

Furthermore, some benzimidazole derivatives cause down-regulation of androgen receptor (AR) protein expression *in vitro* and *in vivo*. Recently, some benzimidazoles showed antiproliferative activity *via* destruction of DNA.[16–18]

Therefore, we devised target compounds that were comprised of a 2-substituted benzimidazole and either a pyridazinyl, thienyl, thienopyridazinyl, thienopyrimidinyl, thienooxazinyl, thiazolyl or a guanidinyl moiety, each of which has featured structurally in some anticancer agents. In this paper, we report the synthesis and anticancer testing of twenty two new polycyclic compounds that have a core of a 2-substituted benzimidazole.

Results and discussion

The synthesis of the novel compounds is shown in Schemes 1–4. All of the new compounds described below were characterised by their IR, ^{1}H NMR, ^{13}C NMR and mass spectra as well as microanalyses. The key precursor compound **1** was synthesised by reacting 2-(2-(1-ethoxy-1,3-dioxobutan-2-ylidene) hydrazinyl) benzoic acid[19] with *o*-phenylenediamine (Scheme 1).[20] The ^{1}H NMR spectrum of compound **1** showed a singlet signal at δ 2.44 ppm attributed to three protons of the CH_3 group. D_2O exchangeable signals appeared at δ 11.63 and 13.71 ppm due to the protons of 2 NH groups. Also the triplet quartet signals of the ethyl group of the former compound disappeared. In addition, its mass spectrum displayed a molecular ion peak at *m/z* 322 ($M^{+\cdot}$).

Bromination of compound **1** with bromine in acetic acid afforded the target compound **2**. The ^{1}H NMR of compound **2** showed a singlet at δ 3.38 ppm due to a CH_2 group. Compound **3** was synthesised *via* reacting compound **1** and cyanoacetohydrazide. The IR spectrum of compound **3** showed a strong peak due to the cyano group at 2240 cm^{-1}. The ^{1}H NMR

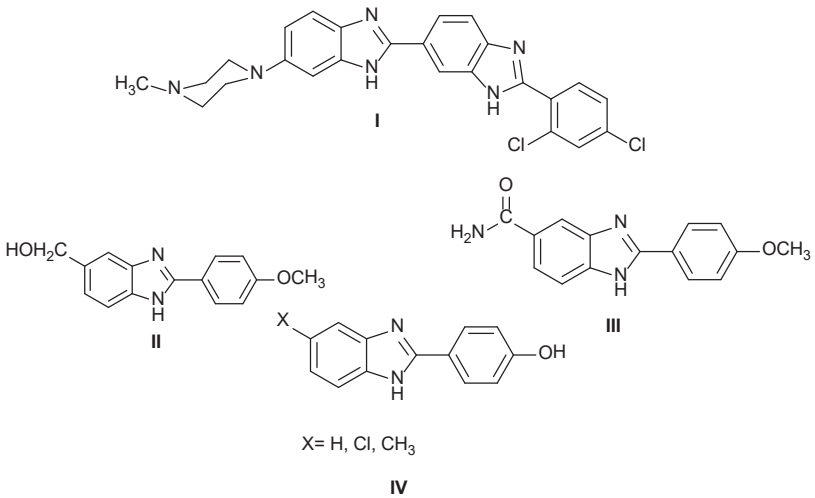
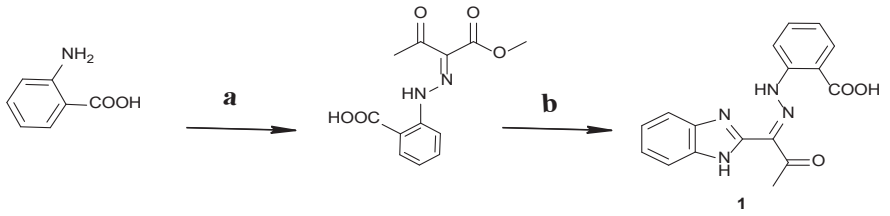


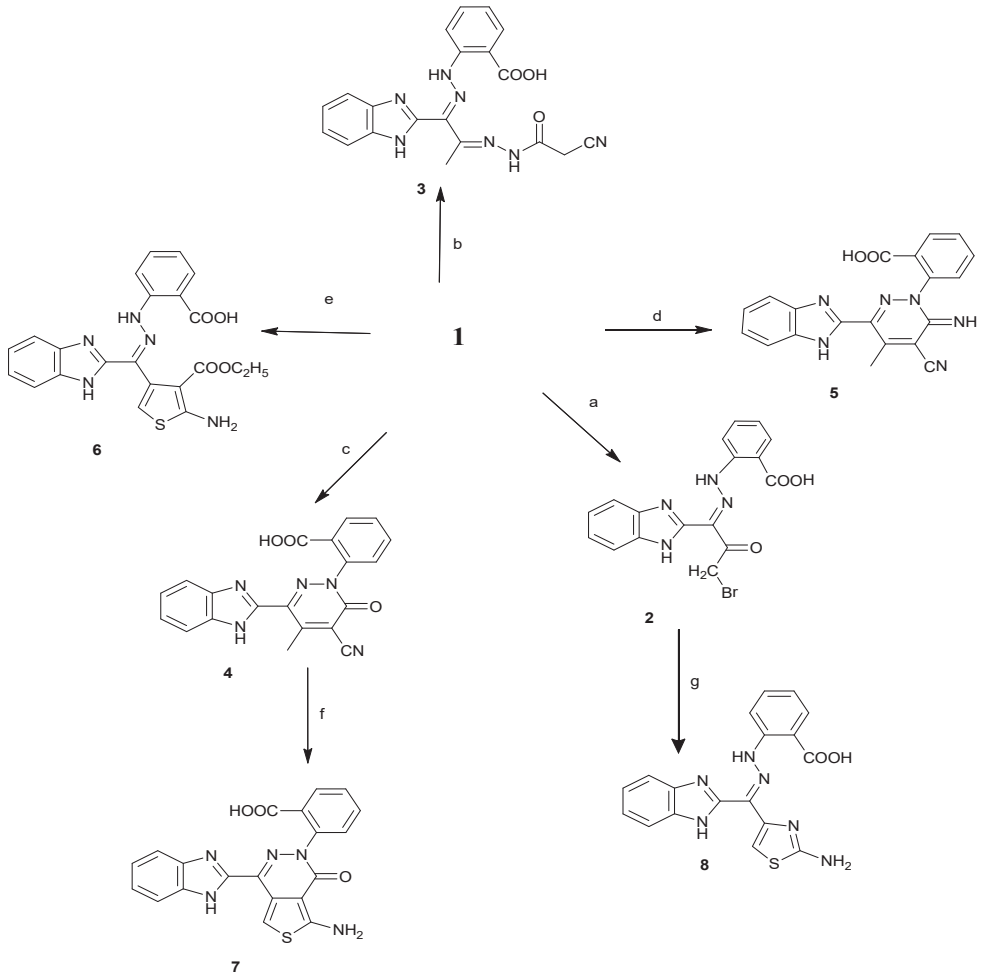
Fig. 1 Structures of some 2-substituted benzimidazoles possessing DNA topo isomerase I and II inhibition activity.

* Correspondent. E-mail: aliaa.kamal@pharma.cu.edu.eg; k_aliaa2511@hotmail.com



Reagents and reaction conditions: a) $NaNO_2$, HCl, ethyl acetoacetate, 0–5 °C; b) Orthophenylenediamine, HCl, EtOH, reflux

Scheme 1

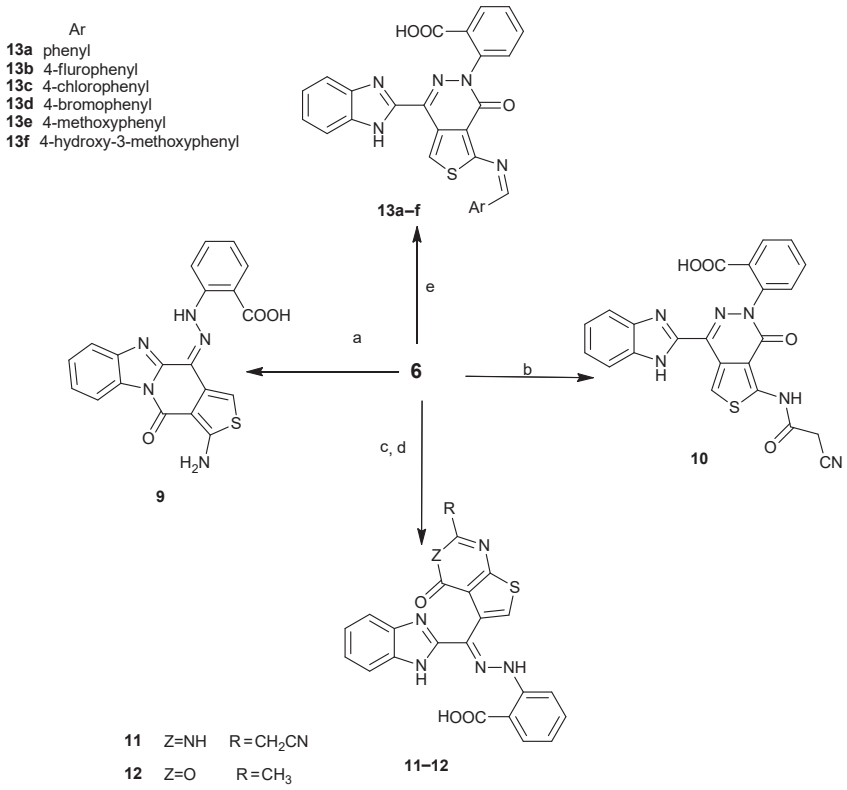


Reagents and conditions: a) Br_2, glacial acetic acid; b) 2-cyanoacetohydrazide, 1,4-dioxane, reflux; c) malononitrile, ammonium acetate; d) malononitrile, TEA, 1,4-dioxane, reflux; e) ethyl cyanoacetate, TEA, S, ethanol, 1,4-dioxane, reflux; f) TEA, S, EtOH, 1,4-dioxane, reflux; g) thiourea, EtOH, reflux;

Scheme 2

spectrum showed a singlet at δ 3.73 ppm due to a CH_2 group. Condensation of compound **1** with malononitrile under different reaction conditions afforded different products. Compound **4** was synthesised *via* an improved Knoevenagel condensation reaction. Fusion of a mixture of compound **1**, malononitrile and anhydrous ammonium acetate in the absence of solvent was carried out. Compound **4** showed a signal at δ 179.5 ppm in its ^{13}C NMR spectrum due to a carbonyl group. Compound **5** was synthesised by condensation of compound **1** with malononitrile in 1,4-dioxane using triethylamine as a catalyst**.** The major difference between compound **4** and **5** was observed in their ^{13}C NMR spectra where compound **4** showed a signal at δ 179.5 ppm corresponding to a CO group. Compounds **6** and **7** were prepared *via* condensation of either compound **1** or compound **4** with elemental sulphur in the presence of triethylamine respectively (Scheme 2).

The ^1H NMR of compound **6** showed the characteristic triplet and quartet of the ester group with the same *J* value. Compound **8** was prepared by refluxing thiourea with compound **2** in ethanol (Scheme 2). The amino group of compound **8** appeared in its ^1H NMR spectrum at δ 3.42 ppm as a D_2O exchangeable broad signal. Heating compound **6** alone in DMF and triethylamine or reacting it with ethyl cyanoacetate or malononitrile afforded compounds **9**, **10** or **11** respectively (Scheme 3). The ^1H NMR spectrum of each compound confirmed the cyclisation by the absence of triplet and quartet signals of the ethyl protons of the ester group of its precursor. The IR spectrum of compound **10** showed the appearance of an absorption band at 2250 cm^{-1}



Reagents and conditions: a) DMF, TEA, reflux; b) ethyl cyanoacetate, DMF, reflux; c) malononitrile, 1,4-dioxane, TEA, reflux; d) acetic anhydride, glacial acetic acid, reflux; e) aldehyde, piperidine, 1,4-dioxane, reflux.

Scheme 3

corresponding to a CN group. The ^{1}H NMR spectrum of **10** showed the appearance of a deshielded singlet at δ 3.94 ppm corresponding to the two protons of $COCH_2$. The CH_2 group of compound **11** was more shielded and appeared at δ 3.64 ppm.

Thienooxazine derivative **12** was synthesised *via* refluxing compound **6** in a mixture of acetic anhydride and acetic acid under anhydrous conditions. The IR spectrum showed the appearance of absorption bands at 1730, 1681 cm^{-1} attributed to C=O groups. The absence of a NH_2 singlet at δ 3.92 in addition to absence of the triplet and quartet signals attributed to ethyl group protons in its ^{1}H NMR spectrum confirmed the structure. The synthesis of compounds **13a–f** was accomplished *via* refluxing compound **6** with the appropriate aromatic aldehyde. The ^{1}H NMR spectra of **13a–f** revealed the absence of the singlet δ 3.92 of NH_2 at compound **6** and the appearance of an arylidene proton in the range δ 7.36–7.72 ppm. During the course of this work, many attempts were carried out for the preparation of compound **14**. Reaction of anthranilic acid diazonium salt with ethyl cyanoacetate followed by refluxing the hydrazono product with *o*-phenylenediamine gave no reaction according to the physical and spectral data of the product. Another attempt was carried out using 2-(1*H*-benzo[*d*] imidazol-2-yl) acetonitrile as a coupling agent with anthranilic diazonium salt. The ^{1}H NMR spectrum showed the presence of three D_2O exchangeable singlet signals at δ 12.92, 13.13, 13.78 ppm corresponding to protons of two NH groups and one OH group, respectively. Aminocyanopyridazinonyl benzimidazole **15** was prepared by refluxing a mixture of ethyl cyanoacetate and compound **14**. The IR spectrum showed a strong absorption band at 1678 cm^{-1} indicating the presence of two carbonyl groups. The ^{1}H NMR spectrum revealed the appearance of a D_2O exchangeable broad singlet at δ 3.64 corresponding to the protons of an NH_2 group. The formation of hydrazono nitrile derivatives **16** and **17** were assumed to proceed *via* nucleophilic addition of hydrazine hydrate to the nitrile moiety. The hydrazone derivatives **16** and **17** were synthesised *via* heating either compound **15** or **14**, respectively, with hydrazine hydrate (Scheme 4).

The ^{1}H NMR spectra of **16** and **17** displayed D_2O exchangeable singlets corresponding to protons of three different NH_2 groups, a NH group and an OH group.

Discussion of the cytotoxic activity of the target compounds

Activity against the breast cancer (MCF-7) cell line

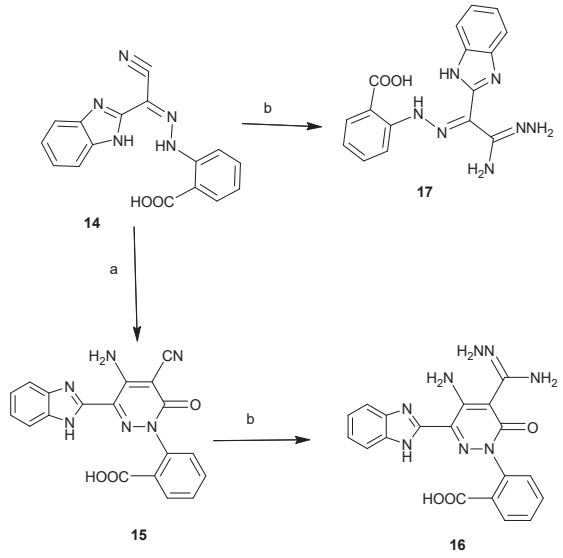
Compounds **2** and **11** with IC_{50} 0.01 μM were four times as active as doxorubicin (IC_{50} 0.04 μM), while compounds **9**, **13f** and **17** were double the activity of the reference drug with IC_{50} 0.02 μM. Compound **4** was slightly more potent than the reference drug with IC_{50} 0.03 μM, while compound **13b** was as potent as the reference drug with IC_{50} 0.04 μM (Table 1).

Activity against non-small cell lung cancer (NCI-H460)

Compounds **2**, **17** and **11** showed the highest anticancer activity with IC_{50} values of 0.01 μM and 0.02 μM in comparison to the doxorubicin (IC_{50} 0.09 μM). Compounds **4** and **13f** were three times more potent than the reference drug with IC_{50} 0.03 μM. Compound **13b** was one and half the activity of doxorubicin. Lastly compound **9** was nearly as potent as the reference drug with IC_{50} 0.08 μM (Table 1).

Activity against the central nervous system cancer cell line SF-268

Again compounds **2** and **17** were the most potent compounds in comparison to doxorubicin (IC_{50} 0.09 μM) with IC_{50} 0.02 μM. Once more compound **4** was three times as potent as the



Reagents and conditions: a) ethyl cyanoacetate, 1,4-dioxane, TEA, reflux, b) hydrazine hydrate, 1,4-dioxane, reflux

Scheme 4

reference drug with IC_{50} 0.03 μM while compounds **11**, **13b** and **13f** were nearly double the potency of the reference drug with IC_{50} 0.05 μM. Compound **9** was one and half the activity of the reference drug with IC_{50} 0.06 μM (Table 1).

It was suggested that the higher potency of compounds **2** and **17** might be due to the high flexibility of these compounds due to the presence of many single bonds compound to other compounds, *e.g.* the more rigid compound **3**. Replacing the methyl group of compound **1** with a CH_2Br group (compound **2**) altered the activity completely from a virtually inactive compound to the most active compound described herein. Increasing the rigidity of the compounds by increasing the number of the heterocyclic rings and the aromatic carbocyclic rings, *e.g.* compounds **4**, **11**, **13b** and **13f** decreased the potency of these compounds in comparison to compounds **2** and **17**, but still they were more potent than doxorubicin. It is worth mentioning that, even the replacement of the imino group in compound **4** by a carbonyl group in compound **5** diminished its biological activity.

Experimental

Melting points were determined on a Griffin apparatus and are uncorrected. IR spectra were recorded on a Shimadzu 435 Spectrometer at the Faculty of Pharmacy, Cairo University, Egypt using KBr discs. NMR spectra were obtained on a Varian Gemini 300 spectrometer (^{1}H NMR at 300 Hz, ^{13}C NMR at 75 Hz) in DMSO-*d6* using TMS as an internal standard at the Microanalytical Center, Cairo University, Giza, Egypt. Chemical shifts (δ) are given in ppm and coupling constants (*J*) in Hz. GCMS were run on Shimadzu QP-2010 spectrometer and mass spectra were run on a Hewlett Packard 5988 spectrometer at the Microanalytical Center, Cairo University, the National Research Center, Giza. Elemental analyses were carried out at the Regional Center for Microbiology and Biotechnology, Al-Azhar University, Cairo. Progress of reaction was monitored using thin layer chromatography (TLC) sheets that were precoated with UV fluorescent silica gel MERCK 60 F 254; visualisation was by UV lamp. Solvent systems were chloroform:methanol mixtures in different ratios.

2-(2-(1-(1H-Benzo[d]imidazol-2-yl)-2-ox-propylidene)hydrazinyl) benzoic acid (**1**)

A mixture of hydrazone benzoic acid derivative (2.78 g, 0.01 mol), *o*-phenylenediamine (1.08 g, 0.01 mol), ethanol (30 mL) and hydrochloric acid (4N, 40 mL) was heated under reflux for 4 h. The solid that formed while hot was filtered and crystallised from ethanol to give compound **1** as a white solid (95%); m.p. 220–22 °C; IR (KBr, cm^{-1}) ν = 3410, 3053, 2937, 1728, 1685; ^{1}H NMR: δ 2.35 (s, 3H, CH_3), 6.98–7.29 (m, 2H, ArH), 7.54–7.69 (m, 3H, ArH), 7.17–8.01 (m, 3H, ArH), 11.63 (s, 1H, NH, D_2O exchangeable), 13.71 (s, 1H, NH, D_2O exchangeable), 14.45 (s, 1H, OH, D_2O exchangeable); ^{13}C NMR: δ 202.0, 172.0, 157.2, 148.4, 141.5, 137.8, 134.7, 130.9, 122.8, 118.7, 117.4, 115.3, 115.1, 19.6; ESI-GCMS (*m/z*, 100%): 322 (M^+, 80); Anal. calcd for $C_{17}H_{14}N_4O_3$: C, 63.35; H, 4.38; N, 17.38; found: C, 63.48; H, 4.36; N, 17.53%.

2-(2-(1-(1H-Benzo[d]imidazol-2-yl)-3-bromo-2-oxopropylidene) hydrazinyl)benzoic acid (**2**)

Compound **1** (3.22 g, 0.01 mol) was dissolved in glacial acetic acid (20 mL) then one drop of a solution of bromine in acetic acid (1:1) was added, the reaction mixture was left in sunlight till the colour disappeared then further addition of bromine solution (1 mL) was completed dropwise with continuous shaking till the colour disappeared. The reaction mixture was poured onto ice and the solid that formed was filtered off, washed with water and crystallised from acetic acid to give compound **2** as a yellow solid (80%); m.p. 234–236 °C; IR (KBr, cm^{-1}) ν = 3433, 3141, 3060, 2960, 1693; ^{1}H NMR: δ 3.38 (s, 2H, CH_2), 7.24–7.29 (m, 2H, ArH), 7.67–7.72 (m, 3H, ArH), 7.95–8.01 (m, 3H, ArH), 13.65 (s, 1H, NH, D_2O exchangeable), 14.44 (s, 1H, NH, D_2O exchangeable), 15.20 (s, 1H, OH, D_2O exchangeable); ^{13}C NMR: δ 199.5, 172.7, 156.2, 148.4, 141.6,

Table 1 Results, expressed as IC_{50} (μM) values, of the evaluation of *in vitro* cytotoxic activity of compounds **1–17** (Schemes 1, 2, 3 and 4) against three human tumour cell lines

Cpd. No.	MCF-7[b]	NCI-H460[c]	SF-268[d]	Cpd. No.	MCF-7	NCI-H460	SF-268
Dox[a]	0.04 ± 0.008	0.09 ± 0.008	0.09 ± 0.007	**12**	2.0 ± 0.4	1.3 ± 0.5	2.5 ± 1.0
1	12.0 ± 2.2	4.3 ± 2.4	3.6 ± 1.6	**13a**	30.4 ± 2.8	20.1 ± 4.6	36.3 ± 4.5
2	0.01 ± 0.009	0.01 ± 0.002	0.02 ± 0.006	**13b**	0.04 ± 0.002	0.06 ± 0.004	0.05 ± 0.001
3	0.2 ± 0.05	0.8 ± 0.02	0.2 ± 0.04	**13c**	22.8 ± 2.6	32.0 ± 0.4	36.5 ± 6.0
4	0.03 ± 0.006	0.03 ± 0.004	0.03 ± 0.008	**13d**	62.2 ± 2.4	40.6 ± 1.8	42.8 ± 0.8
5	0.2 ± 0.09	0.8 ± 0.08	0.2 ± 0.06	**13e**	18.8 ± 2.6	18.1 ± 0.6	26.3 ± 0.8
6	2.2 ± 0.9	1.7 ± 0.04	3.2 ± 1.2	**13f**	0.02 ± 0.008	0.03 ± 0.006	0.05 ± 0.001
7	10.0 ± 4.2	12.3 ± 2.6	14.0 ± 1.8	**14**	22.2 ± 4.8	10.1 ± 2.6	2.8± 0.8
8	2.0 ± 0.6	2.7 ± 0.4	1.6 ± 0.1	**15**	10.8 ± 2.0	4.2 ± 2.4	6.2 ± 2.6
9	0.02 ± 0.01	0.08 ± 0.01	0.06 ± 0.02	**16**	0.4 ± 0.1	0.2 ± 0.01	0.1 ± 0.02
10	2.2 ± 0.8	4.6 ± 0.4	1.2 ± 0.8	**17**	0.02 ± 0.002	0.01 ± 0.006	0.02 ± 0.008
11	0.01 ± 0.005	0.02 ± 0.002	0.05 ± 0.008				

[a]Dox = doxorubicin.
[b]MCF-7 = breast cancer cell line.
[c]NCI-H460 = non-small cell lung cancer cell line.
[d]SF-268 = central nervous system cancer cell line.

137.8, 134.5, 131.1, 122.8, 118.2, 117.4, 115.3, 114.5, 38.6; ESI-GCMS (*m/z*, 100%): 402 ($M + 1^+$, 100); Anal. calcd for $C_{17}H_{13}BrN_4O_3$: C, 50.89; H, 3.27; N, 13.96; found: C, 50.93; H, 3.30; N, 14.12%.

2-[2-(1-(1H-Benzo[d]imidazol-2-yl)-2-(2-(2-cyanoacetyl) hydrazono)propylidene) hydrazinyl]benzoic acid (**3**)

A mixture of compound **1** (3.22 g, 0.01 mol) and 2-cyanoacetohydrazide (0.99 g, 0.01 mol) in 1,4-dioxane (20 mL) was heated under reflux for 2 h. The solid that formed while hot was filtered and crystallised from ethanol to give compound **3** as a white solid (87%); m.p. 264–266 °C; IR (KBr, cm^{-1}) ν = 3431, 3412, 3184, 3153, 3034, 2980, 2240, 1701, 1649; ^{1}H NMR: δ 2.14 (s, 3H, CH_3), 3.73 (s, 2H, CH_2), 7.11–7.16 (m, 2H, ArH), 7.61–7.72 (m, 3H, ArH), 7.87–7.99 (m, 3H, ArH), 11.22 (s, 1H, NH, D_2O exchangeable), 11.52 (s, 1H, NH, D_2O exchangeable), 12.82 (s, 1H, NH, D_2O exchangeable), 14.43 (s, 1H, OH, D_2O exchangeable); ^{13}C NMR: δ 176.2, 172.2, 155.4, 148.4, 141.4, 137.8, 134.4, 130.8, 122.8, 118.4, 117.2, 115.8, 115.2, 114.9, 24.5, 6.7; ESI-GCMS (*m/z*, 100%): 404 ($M + 1^+$, 67); Anal. calcd for $C_{20}H_{17}N_7O_3$: C, 59.55; H, 4.25; N, 24.31; found: C, 59.59; H, 4.28; N, 24.41%.

2-[3-(1H-Benzo[d]imidazol-2-yl)-5-cyano-4-methyl-6-oxopyridazin-1(6H)-yl]benzoic acid (**4**)

A mixture of compound **1** (3.22 g, 0.01 mol) and malononitrile (0.66 g, 0.01 mol) was heated with anhydrous ammonium acetate (0.77 g, 0.01 mol) for 15 minutes; the solid product that formed was washed with cold ethanol, filtered and crystallised from ethanol to give compound **4** as a white solid (80%); m.p. 188–190 °C; IR (KBr, cm^{-1}) ν = 3332, 3186, 3090, 2974, 2222, 1685, 1670; ^{1}H NMR: δ 2.49 (s, 3H, CH_3), 3.56 (br s, 1H, NH, D_2O exchangeable), 7.30–7.43 (m, 3H, ArH), 7.67–7.77 (m, 2H, ArH), 7.98–8.25 (m, 3H, ArH), 15.61 (s, 1H, OH, D_2O exchangeable); ^{13}C NMR: δ 179.5, 172.2, 159.3, 155.6, 141.8, 139.9, 137.7, 133.8, 130.5, 124.6, 122.8, 122.3, 120.4, 117.3, 115.2, 108.9, 18.9; ESI-GCMS (*m/z*, 100%): 371 (M^+, 64); Anal. calcd for $C_{20}H_{13}N_5O_3$: C, 64.69; H, 3.53; N, 18.86; found: C, 64.82; H, 3.58; N, 18.98%.

2-[3-(1H-Benzo[d]imidazol-2-yl)-5-cyano-6-imino-4-methylpyridazin-1(6H)-yl]benzoic acid (**5**)

A mixture of compound **1** (3.22 g, 0.01 mol), malononitrile (0.66 g, 0.01 mol) and 1,4-dioxane (20 mL) was treated with triethylamine (1 mL) and heated under reflux for 2 h. The solid product that was formed upon neutralisation with ice cold water containing hydrochloric acid (2 mL) to pH 6, was collected by filtration and crystallised from ethanol to give compound **5** as a white solid (75%); m.p. 212–214 °C; IR (KBr, cm^{-1}) ν = 3483, 3448, 3055, 2978, 2230, 1660; ^{1}H NMR: δ 2.52 (s, 3H, CH_3), 7.24–7.29 (m, 2H, ArH), 7.68–7.73 (m, 3H, ArH), 7.87–8.01 (m, 3H, ArH), 11.34 (s, 1H, NH, D_2O exchangeable), 11.84 (s, 1H, NH, D_2O exchangeable), 14.42 (s, 1H, OH, D_2O exchangeable); ^{13}C NMR: δ 172.2, 168.3, 155.5, 153.9, 148.5, 141.6, 137.8, 134.8, 130.8, 122.8, 118.5, 117.2, 115.4, 115.0, 113.2, 8.2; ESI-GCMS (*m/z*, 100%): 370 (M^+, 100); Anal. calcd for $C_{20}H_{14}N_6O_2$: C, 64.86; H, 3.81; N, 22.69; found: C, 64.91; H, 3.84; N, 22.83%.

General procedure for the preparation of aminothiophene derivatives **6** *and* **7**

A mixture of compound **1** (3.22 g, 0.01 mol) (for compound **6**) or compound **4** (3.71 g, 0.01 mol) (for compound **7**), ethyl cyanoacetate (1.13 g, 0.01 mol) (for compound **6** only), elemental sulphur (0.32 g, 0.01 mol) in ethanol (10 mL) and 1,4-dioxane (10 mL) was treated with triethylamine (1 mL) and heated under reflux for 1 h. The solid product that formed upon neutralisation with ice-cold water containing hydrochloric acid (2 mL) to pH 6, was collected by filtration and crystallised from ethanol to give compounds **6** or **7**.

*2-(2-((5-Amino-4-(ethoxycarbonyl) thiophen-3-yl)(1*H*-benzo[d] imidazole-2-yl) methylene)hydrazinyl) benzoic acid* (**6**): White solid (78%); m.p. 220–222 °C; IR (KBr, cm^{-1}) ν = 3440, 3400, 3310, 3055, 2974, 1670, 1650; ^{1}H NMR: δ 2.39–2.41 (t, 3H, *J* = 5.1 Hz, CH_3), 3.79 (q, 2H, *J* = 5.1 Hz, CH_2), 3.92 (s, 2H, NH_2, D_2O exchangeable), 6.95–7.08 (m, 2H, ArH), 7.34 (s, 1H, thiophene), 7.45–7.64 (m, 3H, ArH), 7.82 (s, 1H, NH, D_2O exchangeable), 7.84–7.91 (m, 3H, ArH), 11.62 (s, 1H, NH, D_2O exchangeable), 14.44 (s, 1H, OH, D_2O exchangeable); ^{13}C NMR: δ 172.2, 161.3, 154.5, 145.2, 141.6, 138.8, 136.4, 134.8, 130.8, 126.9, 125.6, 123.5, 123.0, 121.1, 118.5, 117.2, 115.4, 115.0, 59.2, 13.2; ESI-GCMS (*m/z*, 100%): 449 (M^+, 64); Anal. calcd for $C_{22}H_{19}N_5O_4S$: C, 58.79; H, 4.26; N, 15.58; found: C, 58.88; H, 4.24; N, 15.65%.

*2-(7-Amino-4-(1*H*-benzo[d]imidazol-2-yl)-1-ox-thieno[3,4-d] pyridazin-2(1*H*)yl) benzoic acid* (**7**):

White solid (70%); m.p. 237–239 °C; IR (KBr, cm^{-1}) ν = 3332, 3186, 3090, 2974, 2222, 1670, 1660; ^{1}H NMR: δ 3.09 (s, 2H, NH_2, D_2O exchangeable), 7.17 (s, 1H, thiophene), 7.20–7.44 (m, 3H, ArH), 7.64–7.75 (m, 3H, ArH), 7.93–8.16 (m, 2H, ArH), 13.74 (s, 1H, NH, D_2O exchangeable), 15.31 (s, 1H, OH, D_2O exchangeable); ^{13}C NMR: δ 172.2, 168.3, 155.6, 142.0, 141.6, 139.8, 139.0, 137.8, 134.2, 133.8, 130.6, 128.9, 125.5, 122.8, 122.1, 120.5, 115.8; ESI-GCMS (*m/z*, 100%): 371 (M^+, 69); Anal. calcd for $C_{20}H_{13}N_5O_3S$: C, 59.55; H, 3.25; N, 17.36; found: C, 59.63; H, 3.22; N, 17.53%.

2-(2-((2-Aminothiazol-4-yl)(1H-benzo[d]imidazol-2-yl)methylene) hydrazinyl)benzoic acid (**8**)

A mixture of compound **2** (4.01 g, 0.01 mol) and thiourea (0.76 g, 0.01 mol) in ethanol (30 mL) was heated under reflux for 3 h. The solid that formed while hot was filtered and crystallised from *n*-butanol to give compound **8** as a white solid (65%); m.p. 227–229 °C; IR (KBr, cm^{-1}) ν = 3460, 3415, 3072, 1676; ^{1}H NMR: δ 3.42 (br s, 2H, NH_2, D_2O exchangeable), 7.24–7.29 (m, 2H, ArH), 7.54 (s, 1H, thiazole), 7.68–7.73 (m, 3H, ArH), 7.78–7.98 (m, 3H, ArH), 11.24 (s, 1H, NH, D_2O exchangeable), 11.93 (s, 1H, NH, D_2O exchangeable), 14.44 (s, 1H, OH, D_2O exchangeable); ^{13}C NMR: δ 175.1, 172.2, 155.2, 148.4, 142.4, 141.8, 137.8, 134.4, 130.8, 122.8, 118.7, 117.3, 115.8, 115.2, 110.9; ESI-GCMS (*m/z*, 100%): 378 (M^+, 70); Anal. calcd for $C_{18}H_{14}N_6O_2S$: C, 57.13; H, 3.73; N, 22.21; found: C, 57.19; H, 3.79; N, 22.38%.

2-(2-(1-amino-11-oxobenzo[4,5] imidazo[1,2-a]thieno[3,4-d]pyridin -4(11H)-ylidene)hydrazinyl)benzoic acid (**9**)

To a solution of compound **6** (4.49 g, 0.01 mol) in dimethyl formamide (DMF) (20 mL), triethylamine (1 mL) was added and then the solution was heated under reflux for 4 h. The reaction mixture was cooled and treated with a few drops of concentrated hydrochloric acid; the solid that formed was filtered off and crystallised from ethanol to give compound **9** as a white solid (60%); m.p. 226–228 °C; IR (KBr, cm^{-1}) ν = 3480, 3290, 3051, 1728, 1681; ^{1}H NMR: δ 3.33 (br s, 2H, NH_2, D_2O exchangeable), 7.24–7.29 (m, 2H, ArH), 7.68–7.71 (m, 2H, ArH), 7.95–8.01 (m, 4H, ArH), 8.03 (s, 1H, thiophene), 11.36 (s, 1H, NH, D_2O exchangeable), 14.44 (s, 1H, OH, D_2O exchangeable); ^{13}C NMR: δ 172.4, 169.3, 165.8, 155.6, 145.8, 141.5, 138.9, 134.7, 133.3, 131.1, 130.7, 127.7, 123.5, 123.5, 118.6, 115.6, 115.2, 115.0, 114.1, 110.2; Anal. calcd for $C_{20}H_{13}N_5O_3S$: C, 59.55; H, 3.25; N, 17.36; found: C, 59.62; H, 3.26; N, 17.50%.

2-(4-(1H-benzo[d]imidazol-2-yl)-7-(2-cyano acetamido)-1-oxothieno-[3,4-d]pyridazin-2(1H)-yl)benzoic acid (**10**)

A mixture of compound **6** (4.49 g, 0.01 mol) and ethyl cyanoacetate (1.13 g, 0.01 mol) in dimethyl formamide (20 mL) was heated under reflux for 4 h. The solid that formed after cooling was filtered off and crystallised from dimethyl formamide to give compound **10** as a white solid (70%); m.p. 159–161 °C; IR (KBr, cm^{-1}) ν = 3419, 3408, 3400, 3047, 2974, 2250, 1680, 1660, 1647; ^{1}H NMR: δ 3.94 (s, 2H, CH_2), 4.35 (s, 1H, NH, D_2O exchangeable), 7.20–7.34 (m, 3H, ArH), 7.44–7.63 (m, 3H, ArH), 7.82–8.00 (m, 2H, ArH), 7.68 (s, 1H, thiophene), 11.28 (s, 1H, NH, D_2O exchangeable), 13.30 (s, 1H, OH, D_2O exchangeable); ^{13}C NMR: δ 172.5, 169.3, 168.2, 157.4, 155.6, 141.5, 138.9, 138.9, 138.1, 137.7, 130.5, 125.7, 124.2, 123.2, 123.2, 119.5, 118.3, 115.7, 115.5, 115.2, 115.2, 114.6, 24.9; Anal. calcd for $C_{23}H_{14}N_6O_4S$: C, 58.72; H, 3.00; N, 17.86; found: C, 58.80; H, 3.04; N, 17.93%.

2-(2-((1H-Benzo[d]imidazol-2-yl)(2-(cyano methyl)-4-oxo-3,4-dihydro-thieno [2,3-d] pyrimidin-5-yl) methylene)hydrazinyl)benzoic acid (**11**)

A mixture of compound **6** (4.49 g, 0.01 mol) and malononitrile (0.66 g, 0.01 mol) in 1,4-dioxane (20 mL) was treated with triethylamine (1 mL) and heated under reflux for 4 h. The solid that formed after cooling was filtered and crystallised from 1,4-dioxane to give compound **11** as a white solid (72%); m.p. 194–196 °C; IR (KBr, cm^{-1}) ν = 3583, 3332, 3224, 3078, 2981, 2210, 1710, 1681; ^{1}H NMR: δ 3.64 (s, 2H, CH_2), 7.37 (s, 1H, thiophene), 7.51–8.27 (m, 8H, ArH), 11.10 (s, 1H, NH, D_2O exchangeable), 11.32 (s, 1H, NH, D_2O exchangeable), 12.43 (s, 1H, NH, D_2O exchangeable), 14.44 (s, 1H, OH, D_2O exchangeable); ^{13}C NMR: δ 172.5, 167.9, 163.3, 155.6, 148.5, 141.5, 140.5, 137.7, 134.5, 130.8, 128.1, 125.7, 125.0, 122.8, 118.3, 117.4, 115.7, 115.2, 114.9, 22.9; ESI-GCMS (*m/z*, 100%): 470 ($M + 1^+$, 65); Anal. calcd for $C_{23}H_{15}N_7O_3S$: C, 58.84; H, 3.22; N, 20.88; found: 58.97; H, 3.19; N, 21.04%.

2-(2-((1H-Benzo[d]imidazol-2-yl)(2-methyl-4-oxo-4H-thieno[2,3-d] [1,3]oxazin-5-yl)methylene) hydrazinyl)benzoic acid (**12**)

A mixture of compound **6** (4.49 g, 0.01 mol) and acetic anhydride (4 mL, 0.04 mol) in glacial acetic acid (10 mL) was heated under reflux for 2 h. The solid that formed after cooling was filtered off and crystallised from ethanol to give compound **12** as a white solid (59%); m.p. 233–235 °C; IR (KBr, cm^{-1}) ν = 3479, 3441, 3055, 2985, 1730, 1681; ^{1}H NMR: δ 2.65 (s, 3H, CH_3), 7.25–7.29 (m, 3H, ArH), 7.31 (s, 1H, thiophene), 7.34 (s, 2H, NH, D_2O exchangeable), 7.66–7.71 (m, 2H, ArH), 7.98–8.19 (m, 3H, ArH), 15.28 (s, 1H, OH, D_2O exchangeable); ^{13}C NMR: δ 172.5, 165.9, 162.8, 154.8, 148.5, 144.3, 141.5, 137.8, 134.5, 134.1, 131.0, 126.7, 122.8, 118.3, 117.4, 115.7, 115.2, 114.9, 18.9; ESI-GCMS (*m/z*, 100%): 445 (M^+, 68); Anal. calcd for $C_{22}H_{15}N_5O_4S$: C, 59.32; H, 3.39; N, 15.72; found: C, 59.40; H, 3.45; N, 15.81%.

General procedure for preparation of 2-(4-(1H-Benzo[d]imidazol-2-yl)-7-(arylidene-amino)-1-oxothieno [3,4-d]pyridazin-2(1H)-yl) benzoic acid **13a**–**f**

A mixture of compound **6** (4.49 g, 0.01 mol) and the respective aromatic aldehyde (0.01 mol) in 1,4-dioxane (20 mL) was treated with piperidine (1 mL) and heated under reflux for 4 h. The solid that formed while hot was filtered and crystallised from benzene to afford **13a**–**f**.

*2-[4-(1*H*-Benzo[d]imidazol-2-yl)-7-(benzylidene-amino)-1-oxo-1*H*-thieno[3,4-d] pyridazine-2-yl]benzoic acid* (**13a**): Yellow solid (75%); m.p. 244–246 °C; IR (KBr, cm^{-1}) ν = 3437, 3030, 1662; ^{1}H NMR: δ 7.23–7.29 (m, 4H, ArH), 7.36 (s, 1H, CH=N), 7.68–7.73 (m, 4H, ArH), 7.71 (S, 1H, thiophene), 7.95–8.01 (m, 5H, ArH), 14.45 (s, 1H, NH, D_2O exchangeable), 15.15 (s, 1H, OH, D_2O exchangeable); Anal. calcd for $C_{27}H_{17}N_5O_3S$: C, 65.98; H, 3.49; N, 14.25; found: C, 66.09; H, 3.52; N, 14.33%.

*2-{4-(1*H*-Benzo[d]imidazol-2-yl)-7-[(4-fluoro-benzylidene)-amino]-1-oxo-1*H*-thieno[3,4-d] pyridazin-2-yl}-benzoic acid* (**13b**): Yellow solid (30%); m.p. 215–217 °C; IR (KBr, cm^{-1}) ν = 3460, 3446, 3059, 1666; ^{1}H NMR: δ 6.88–6.99 (m, 4H, ArH), 7.52 (s, 1H, CH=N), 7.44–7.61 (m, 4H, ArH), 7.75–7.91 (m, 4H, ArH), 7.98 (S, 1H, thiophene), 14.45 (s, 1H, NH, D_2O exchangeable), 15.15 (s, 1H, OH, D_2O exchangeable); ^{13}C NMR: δ 172.5, 165.2, 164.5, 163.8, 155.8, 142.2, 141.5, 139.7, 137.1, 137.8, 134.1, 130.5, 130.1, 128.0, 126.9, 126.7, 124.3, 122.8, 122.3, 120.3, 115.7, 115.2; Anal. calcd for $C_{27}H_{16}FN_5O_3S$: C, 63.65; H, 3.17; N, 13.75; found: C63.76; H, 3.21; N, 13.82%.

*2-{4-(1*H*-Benzo[d]imidazol-2-yl)-7-[(4-chloro-benzylidene)-amino]-1-oxo-1*H*-thieno[3,4-d] pyridazin-2-yl}-benzoic acid* (**13c**): Yellow solid (45%); m.p. 190–192 °C; IR (KBr, cm^{-1}) ν = 3446, 3419, 3057, 1670; ^{1}H NMR: δ 7.23–7.29 (m, 4H, ArH), 7.36 (s, 1H, CH=N), 7.68–7.73 (m, 4H, ArH), 7.71 (S, 1H, thiophene), 7.95–8.01 (m, 4H, ArH), 11.34 (s, 1H, NH, D_2O exchangeable), 12.43 (s, 1H, OH, D_2O exchangeable); Anal. calcd for $C_{27}H_{16}ClN_5O_3S$: C, 61.66; H, 3.07; N, 13.32; found: C, 61.74; H, 3.12; N, 13.40%.

*2-{4-(1*H*-Benzo[d]imidazol-2-yl)-7-[(4-bromo-benzylidene)-amino]-1-oxo-1*H*-thieno[3,4-d]pyridazin-2-yl}-benzoic acid* (**13d**): White solid (45%); m.p. 148–150 °C; IR (KBr, cm^{-1}) ν = 3446, 3431, 3049, 1672; ^{1}H NMR: δ 6.86–6.99 (m, 4H, ArH), 7.47 (s, 1H, CH=N), 7.55–7.58 (m, 4H, ArH), 7.75 (S, 1H, thiophene), 7.89–7.91 (m, 4H, ArH), 14.20 (s, 1H, NH, D_2O exchangeable), 14.75 (s, 1H, OH, D_2O exchangeable); Anal. calcd for $C_{27}H_{16}BrN_5O_3S$: C, 56.85; H, 2.83; N, 12.28; found: C, 56.96; H, 2.86; N, 12.36%.

*2-{4-(1*H*-Benzo[d]imidazol-2-yl)-7-[(4-methoxy-benzylidene)-amino]-1-oxo-1*H*-thieno[3,4-d] pyridazin-2-yl}-benzoic acid* (**13e**): White solid (65%); m.p. 198–200 °C; IR (KBr, cm^{-1}) ν = 3446, 3421 (OH, NH), 3032, 2974, 1672; ^{1}H NMR: δ 3.84 (s, 3H, CH_3), 7.04–8.03 (m, 14H, 12ArH + 1H thiophene + 1H, CH=N), 8.12 (s, 1H, NH, D_2O exchangeable), 11.35 (s, 1H, OH, D_2O exchangeable); ^{13}C NMR: δ 172.5, 165.2, 164.5, 163.8, 155.8, 142.2, 141.5, 139.7, 137.1, 137.8, 134.1, 130.5, 130.1, 128.0, 125.2, 124.2, 123.3, 122.8, 122.3, 120.3, 115.7, 114.2, 56.8; Anal. calcd for $C_{28}H_{19}N_5O_4S$: C, 64.48; H, 3.67; N, 13.43; found: C64.59; H, 3.75; N, 13.49%.

*2-{4-(1*H*-Benzo[d]imidazol-2-yl)-7-[(4-hydroxy-3-methoxy-benzylidene)-amino]-1-oxo-1*H*-thieno[3,4-d]pyridazin-2-yl}-benzoic acid* (**13f**): White solid (40%); m.p. 188–190 °C; IR (KBr, cm^{-1}) ν = 3444, 3433, 3030, 2936, 1677; ^{1}H NMR: δ 3.54 (s, 3H, CH_3), 3.78 (s, 1H, OH, D_2O exchangeable), 7.27–7.36 (m, 3H, ArH), 7.72 (s, 1H, CH=N), 7.74–7.96 (m, 4H, ArH), 7.99 (S, 1H, thiophene); 7.99–8.02 (m, 4H, ArH), 14.41 (s, 1H, NH, D_2O exchangeable), 15.15 (s, 1H, OH carboxylic, D_2O exchangeable); ^{13}C NMR: δ 172.5, 165.2, 163.8, 155.6, 149.5, 145.3, 142.2, 141.5, 139.7, 137.8, 137.1, 134.1, 130.1, 128.0, 125.2, 124.7, 124.2, 122.8, 122.5, 122.3, 120.3, 116.9, 116.3, 115.7, 56.9; Anal. calcd for $C_{28}H_{19}N_5O_5S$: C, 62.56; H, 3.56; N, 13.03; found: C, 62.65; H, 3.61; N, 13.11%.

2-(2-((1H-Benzo[d]imidazol-2-yl)(cyano) methylene)hydrazinyl) benzoic acid (**14**)

An ice-cold solution of sodium nitrite (6.90 g, 0.1 mol) in water (20 mL) was added dropwise to a solution of anthranilic acid (13.71 g, 0.1 mol) in hydrochloric acid (25 mL) at 0–5 °C. After complete addition of sodium nitrite solution, a cold solution of sodium acetate (24.60 g, 0.3 mol) in water (100 mL) was then added to the diazonium salt solution at 5 °C. A solution of sodium acetate (8.20 g, 0.1 mol) in water (30 mL) was added to a stirred solution of 2-benzimidazolyl acetonitrile (1.57 g, 0.1 mol) in a mixture of water (150 mL) and ethanol (100 mL), and then the previously prepared diazonium salt was added portion wise. The mixture was stirred for 2 h, allowed to stand in the refrigerator overnight, filtered, washed with water and crystallised from ethanol to give compound **14** as a white solid (89%); m.p. >300 °C; IR (KBr, cm^{-1}) ν = 3357, 3062, 2206, 1690; ^{1}H NMR: δ 7.17–7.37 (m, 3H, ArH), 7.64–7.95 (m, 2H, ArH), 7.988.02 (m, 3H, ArH), 8.14 (s, 1H, NH, D_2O exchangeable), 8.16 (s, 1H, NH, D_2O exchangeable), 13.78 (s, 1H, OH, D_2O exchangeable); ^{13}C NMR: δ 172.5, 155.1, 148.3, 141.5, 137.8, 134.4, 130.8, 122.8, 118.5, 117.7, 117.4, 115.7, 115.1; ESI-GCMS (*m/z*, 100%)*:* 306 ($M + 1^+$, 89); Anal. calcd for $C_{16}H_{11}N_5O_2$: C, 62.95; H, 3.63; N, 22.94; found: C, 62.98; H, 3.60; N, 23.18%.

2-(4-Amino-3-(1H-benzo[d]imidazol-2-yl)-5-cyano-6-oxopyridazin-1(6H)-yl)benzoic acid (**15**)

A mixture of compound **14** (3.05 g, 0.01 mol) and ethyl cyanoacetate (1.13 g, 0.01 mol) in 1,4-dioxane (20 mL) was treated with triethylamine (1 mL) and heated under reflux for 4 h. The solid product formed upon dilution with ice-cold water containing hydrochloric acid (2 mL) till pH 6, was collected by filtration and crystallised from ethanol to give compound **15** as a yellow solid (70%); m.p. > 300 °C; IR (KBr, cm^{-1}) ν = 3305, 3059, 2222, 1678; ^{1}H NMR: δ 3.64 (br s, 2H, NH_2, D_2O exchangeable), 7.16–7.36 (m, 3H, ArH), 7.59–7.70 (m, 3H, ArH), 7.87–8.04 (m, 2H, ArH), 12.84 (s, 1H, NH, D_2O exchangeable), 15.37 (s, 1H, OH, D_2O exchangeable); ^{13}C NMR: δ 172.5, 170.9, 164.1, 155.6, 139.8, 137.8, 134.1, 130.5, 124.2, 122.8, 122.3, 120.3, 117.2, 115.7, 88.5; Anal. calcd for $C_{19}H_{12}N_6O_3$: C, 61.29; H, 3.25; N, 22.57; found: C, 61.36; H, 3.28; N, 22.64%.

General procedure for hydrazinolysis of the cyano group of compounds **14** *and* **15**

A mixture of compound **15** (3.72 g, 0.01 mol) (for compound **16**) or compound **14** (3.05 g, 0.01 mol) (for compound **17**) and 99% hydrazine

hydrate (0.05 g, 0.01 mol) in 1,4-dioxane (15 mL) and ethanol (15 mL) was heated under reflux for 2 h. The solid that formed while hot was filtered and washed with hot ethanol to give compound **16** or **17**.

*2-(4-Amino-3-(1*H*-benzo[d]imidazol-2-yl)-5-(hydrazonomethyl)-6-oxopyridazin-1(6*H*)-yl) benzoic acid* (**16**): Yellow solid (82%); m.p. 286–288 °C; IR (KBr, cm^{-1}) ν = 3421, 3346, 3051, 1710; ^{1}H NMR: δ 3.30 (s, 2H, NH_2, D_2O exchangeable), 7.12–7.36 (m, 2H, ArH), 7.65–7.87 (m, 3H, ArH), 7.95–8.34 (m, 3H, ArH), 8.93 (s, 2H, NH_2, D_2O exchangeable), 11.24 (s, 1H, NH, D_2O exchangeable), 12.64 (s, 2H, NH_2, D_2O exchangeable), 14.23 (s, 1H, OH, D_2O exchangeable); ^{13}C NMR: δ 172.5, 164.1, 157.3, 155.6, 154.6, 141.5, 139.8, 137.8, 134.1, 130.5, 124.0, 122.8, 122.3, 120.3, 115.7, 110.2; Anal. calcd for $C_{19}H_{16}N_8O_3$: C, 56.43; H, 3.99; N, 27.71; found: C, 56.52; H, 4.00; N, 27.85%.

*2-[2-(2-Amino-1-(1*H*-benzo[d]imidazol-2-yl)-2-hydrazonoethylidene) hydrazinyl] benzoic acid* (**17**):

Yellow solid (71%); m.p. 292–294 °C; IR (KBr, cm^{-1}) ν = 3348, 3302, 3055, 1678; ^{1}H NMR: δ 3.32 (br s, 4H, $2NH_2$, D_2O exchangeable), 7.21–7.32 (m, 3H, ArH), 7.55–7.63 (m, 2H, ArH), 7.88–7.93 (m, 3H, ArH), 8.91 (s, 1H, NH, D_2O exchangeable), 12.41 (s, 1H, NH, D_2O exchangeable), 14.18 (s, 1H, OH, D_2O exchangeable); ^{13}C NMR: δ 172.5, 155.5, 154.9, 148.3, 141.5, 137.8, 134.6, 130.8, 122.8, 118.5, 117.2, 115.2, 115.7; ESI-GCMS (*m/z*, 100%): 337 (M^+, 58); Anal. calcd for $C_{16}H_{15}N_7O_2$: C, 56.97; H, 4.48; N, 29.07; found: C, 57.11; H, 4.42; N29.19%.

Cytotoxic activity

Biological screening of the newly synthesised compounds was done by a research group at the National Cancer Institute and the National Research Center, Giza, Egypt. Activity was evaluated on *in vitro* growth of three human tumour cell lines, breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-H460), and central nervous system cancer (SF-268) and was repeated three times for each compound.

The newly synthesised compounds were evaluated on the *in vitro* growth of three human tumour cell lines representing different tumour types, namely, breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-H460) and central nervous system cancer (SF-268) using Doxorubicin as reference drug using SRB assay.

Materials, methods and reagents

Foetal bovine serum (FBS) and L-glutamine were from Gibco Invitrogen Co. (Scotland, UK). RPMI-1640 medium was from Cambrex (New Jersey, USA). Dimethylsulfoxide (DMSO), doxorubicin, penicillin, streptomycin and sulforhodamine B (SRB) were from Sigma Chemical Co. (Saint Louis, USA). Stock solutions of selected compounds were prepared in DMSO and kept at –20 °C. Appropriate dilutions of the compounds were freshly prepared just prior to the assays. Final concentrations of DMSO did not interfere with the cell growth.

Cell cultures

Three human tumour cell lines, MCF-7, NCI-H460 and SF-268 were used. MCF-7 was obtained from the European Collection of Cell Cultures (ECACC, Salisbury, UK) and NCI-H460 and SF-268 were kindly provided by the National Cancer Institute (NCI, Cairo, Egypt). They grew as monolayers and when routinely maintained in RPMI-1640 medium supplemented with 5% heat inactivated FBS, 2 mM glutamine and antibiotics (penicillin 100 μ mL^{-1}, streptomycin 100 μ mL^{-1}), at 37 °C in a humidified atmosphere containing 5% CO_2. Exponentially growing cells were obtained by plating 1.5 × 10^5 cells mL^{-1} for MCF-7 and SF-268 and 0.75 × 10^4 cells mL^{-1} 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.[32]

The effect of the newly synthesised products was evaluated on the *in vitro* growth of three human tumour cell lines representing different tumour types (MCF-7, NCI-H460 and SF-268) after a continuous exposure of 48 h and the results are summarised in Table 1.

Conclusion

The aim of the present study was to synthesise novel anticancer compounds with two or more biologically active pharmacophores, one of them being 2-substituted benzimidazole. This was achieved by the synthesis, characterisation and bio-testing against three human tumour cell lines of twenty two new polycyclic 2-substituted benzimidazole derivatives. The anti-tumour activity of seven compounds **2**, **4**, **9**, **11**, **13b**, **13f** and **17** was greater than that of doxorubicin and they therefore represent promising novel lead compounds.

Received 18 February 2016; accepted 20 February 2016
Paper 1603926 doi: 10.3184/174751916X14577069096465
Published online: 21 March 2016

References

1 O.B. Patel and L.J. Patel, *Int. J. Pharm. Appl. Sci*., 2011, **2**, 15.
2 H.J. Böhm, A. Flohr and M. Stah, *Drug Discovery Today: Technol*., 2004, **1**, 217.
3 J.S. Kim, A.C. Yu, A. Liu and E.J. La Voie, *J. Med. Chem.,* 1997, 40, 2818.
4 A.S. Alpan, H.S. Gunes and Z. Topcu, *Acta Biochim. Pol*., 2007, **54**, 561.
5 A. Mahiuddin, S.C.B. David and K. Naresh, *Top Heterocycl. Chem.,* 2007, **9**, 87.
6 G. Coban, S. Zencir, I. Zupkó, B. Réthy, H.S. Gunes and Z. Topcu, *Eur. Med. Chem.,* 2009, **44**, 2280.
7 M. Singh and V. Tandon, *Eur. J. Med. Chem.,* 2011, **46**, 659.
8 A.S. Alpan, Ö. Yılmaz, O. Kılıçkaya, P. Kara, H.S. Güneş and M.Ş. Özsöz, *Marmara Pharm. J.,* 2012, **16**, 48.
9 K. Błaszczak-Świątkiewicz, M. Mirowski, K. Kaplińska, R. Kruszyński, A. Trzęsowska-Kruszyńska and E. Mikiciuk-Olasik, *Acta Biochim. Pol.,* 2012, **59**, 279.
10 K. Błaszczak-Świątkiewicz, P. Olszewska and E. Mikiciuk-Olasik, *Acta Biochim. Pol.,* 2013, **60**, 427.
11 K. Nepali, S. Sharma, M. Sharma, P.M.S. Bedi and K.L. Dhar, *Eur. Med. Chem.,* 2014, **76**, 539.
12 D. Seenaiah, P.R. Reddy, G.M. Reddy, A. Padmaja, V. Padmavathi and N.S. Krishna, *Eur. Med. Chem.,* 2014, **77**, 1.
13 P.D. Foglesong, C. Reckord and S. Swink, *Canc. Chemotherp. Pharmacol.,* 1992, **30**, 123.
14 B.T. Joseph, M. Facompré, H. Da Costa, S. Routier, J.Y. Mérour, P. Colson, C. Houssier and C. Bailly, *Bioorg. Med. Chem.,* 2001, **9**, 1533.
15 H. Ding, C. Zhang, X. Wu, C. Yang, X. Zhang, J. Ding and Y. Xie, *Bioorg. Med. Chem. Lett.,* 2005, 15, 4799.
16 S. Altamura, L. Bartholomew, A. Biroccio, A. Ceccacci, L. Pacini, F. Narjes, N. Gennari, M. Bisbocci, I. Incitti, L. Orsatti, S. Harper, I. Stansfield, M. Rowley, R. De Francesco and G. Migliaccio, *J. Virol*., 2003, **77**, 13225.
17 T. Vasaitis, A. Belosay, A. Schayowitz, A. Khandelwal, P. Chopra, L.K. Gediya, Z. Guo, H.B. Fang, V.C. Njar and A.M. Brodie, *Mol. Cancer Ther.,* 2008, **7**, 2348.
18 K. Błaszczak-S´wiatkiewicz, P. Olszewska and E. Mikiciuk-Olasik, *Pharmacol. Reports,* 2014, **66**, 100.
19 J.M.A. Al-Rawi and M.A.R. Khayat, *Magn. Reson. Chem*, 1989, **27**, 112.
20 B.A. Reddy, *J. Chem*., 2010, **7**, 222.
21 A. Monk, D.A. Scudiero, P. Skehan, R.H. Shoemaker and K.D. Paull, *J. Natl. Cancer Inst.,* 1991, **83**, 757.