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NOVEL BENZIMIDAZOLE DERIVATIVES AS EXPECTED ANTICANCER AGENTS

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Abstract: A series of 1-(1*H*-benzimidazol-2-yl)-3-(substituted)-2-propen-1-one and its 1-methyl analogues **2c-h** were synthesized and cyclized with different reagents such as ethyl cyanoacetate, thiourea, hydroxylamine hydrochloride, guanidinium sulfate, methylhydrazine, phenylhydrazine and/or hydrogen peroxide in different reactions to produce pyridones **3a**,**b,** pyrimidinethione **4a**,**b**, isoxazole **5a**,**b**, aminopyrimidine **6a,b**, pyrazoline **7i-k** and epoxy derivative **8**, respectively. Acetohydrazide **10** reacted with formic acid, acetic anhydride, carbon disulfide and/or thiosemicarbazide to yield compounds **11–19**. Also compound **21a**,**b** was condensed with different monosaccharides to yield the corresponding N-glycoside Schiff's bases derivatives **22a-h**, which upon treatment with acetic anhydride afforded **23a-h** derivatives. The anticancer activity of some of the newly synthesized compounds was evaluated against HEPG2 (human liver carcinoma cell line) and PC12 (pheochromocytoma of the rat adrenal medulla) cells. Benzimidazole-2-isoxazole **5a** derivative exhibited high potency against HEPG2 and PC12 cells. Benzimidazole chalcones **2c,e**, benzimidazole mercaptoacetohydrazide **14** and benzimidazole thiosemicarbazide **15a,b** derivatives gave high potency against PC12 cells.

Keywords: 2-acetylbenzimidazole, chalcones, acetohydrazide, glycosides, anticancer activity, HEPG2, PC12

Benzimidazole derivatives are very useful intermediates/subunits for the development of molecules of pharmaceutical or biological interest. Substituted benzimidazole derivatives have found applications in diverse therapeutical areas including antimicrobial (1–3), antioxidant (4), antiviral (5, 6), antihypertensive (7), antiprotozoal (8), anti-inflammatory (9) and molluscicidal (10) agents. Furtheremore, benzimidazoles showed anticancer activity against DNA topoisomerase I (11, 12) and colon cancer cell lines (13). The need for anticancer agents that selectively kill or inhibit the growth of neoplastic cells without affecting non-cancerous host tissues is high and persistent. Thus, the aim of the current study was the synthesis of novel benzimidazole derivatives that incorporated different heterocycles of anticancer activity, such as different compounds with the backbone of chalcones and acethydrazides, which have been found to exhibit potent cytotoxic activity against the growth of suspended leukemia (14) and lymphomas (15). They were also active in a number of solid tumor screens, e.g., HELA uterine carcinoma, PC12, SOS bone osteosarcoma, lung MB9812, lung A549 and Mcf-7 breast growth (16–18)

Also, it was of interest to prepare benzimidazole N-glycoside Schiff's bases skeleton as bioisosteric of naturally occurring molecules, hopping to produce anticancer agents (19) of high potency and selectivity. In this study some newly synthesized benzimidazole compounds **2c**, **2e**, **4b**, **5a**, **9**, **12**, **14**, **15a, 15b**, **21a**, **21b**, **21d**, **21f**, **21g**, **21h** and **22h** were evaluated as anticancer agents in HEPG2 (human liver carcinoma cell line) and PC12 (pheochromocytoma of the rat adrenal medulla) cells.

EXPERIMENTAL

All melting points are uncorrected and were taken in open capillary tubes using silicone oil on Gallenkamp apparatus. Elemental microanalyses were performed at Cairo University, Egypt. The IR spectra were recorded on FT/IR-330E, Fourier transform, Infrared spectrometer at cm^{-1} scale using KBr

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discs. ^{1}H-NMR and ^{13}C-NMR spectra were determined using A JEOL EX-270 NMR spectrometer 270 MHz and measured in δ (ppm) scale using TMS an internal standard. Mass spectra were measured using mass spectrometer Finnigan MAT SSQ-7000 and GCMS-QP 1000EX Shimadzu GC-MS spectrometer. Follow up of the reactions and checking the purity of the compounds were made by TLC on silica gel-precoated aluminium sheets (Type 60, F 254, Merck, Darmstadt, Germany) and the spots were detected by exposure to UV lamp at λ_{254} nm. The detection of N-glycosides was achieved by treatment with a solution of 15% H_2SO_4 in methanol, and heating at 150°C. The chemical names given for the prepared compounds are according to the IUPAC system.

2-Acetylbenzimidazole (**1a**) and 1-methyl-2-acetylbenzimidazole (**1b**) were prepared according to the literature (20).

1-(1*H*-Benzo[d]imidazol-2-yl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (2c), 1-(1*H*-benzo[d]imidazol-2-yl)-3-(1H-indol-3-yl)prop-2-en-1-one (2d), 1-(1*H*-benzo[d]imidazol-2-yl)-3-(5-methylfuran-2-yl)prop-2-en-1-one (2e), 3-(3,4,5-trimethoxyphenyl)-1-(1-methyl-1*H*-benzo[d]imidazol-2-yl)prop-2-en-1-one (2f), 3-(1-H-indol-3-yl)-1-(1-methyl-1*H*-benzo[d]imidazol-2-yl)prop-2-en-1-one (2g) and 1-(1-methyl-1*H*-benzo[d]imidazol-2-yl)-3-(5-methylfuran-2-yl)prop-2-en-1-one (2h)

General method: A mixture of compound **1a,b** (0.003 mol) and different aromatic aldehydes namely: 3,4,5-trimethoxybenzaldehyde, indole-3-carboxaldehyde and/or 5-methylfurfural (0.003 mol) in 5% ethanolic sodium hydroxide (30 mL) was stirred at room temperature for 24 h. Then, the reaction mixture was poured onto ice/cold water and neutralized using diluted hydrochloric acid. The formed precipitate was filtered and recrystallized to give **2c-h**, respectively.

6-(1*H*-Benzo[d]imidazol-2-yl)-1,2-dihydro-4-(3,4,5-trimethoxy phenyl)-2-oxopyridine-3-carbonitrile (3a) and 1,2-dihydro-4-(3,4,5-trimethoxyphenyl)-6-(1-methyl-1*H*-benzo[d]imidazol-2-yl)-2-oxopyridine-3-carbonitrile (3b)

General method: **A**: A mixture of compound **1a**,**b** (0.01 mol) and 3,4,5-trimethoxybenzaldehyde (2 g, 0.01 mole), ethyl cyanoacetate (1.12 g, 0.01 mole), and an excess of ammonium acetate (6.16 g, 0.08 mol) in n-butanol (20 mL) was refluxed for 8 h. The formed precipitate was filtered, washed with petrolium ether, dried and recrystallized to give **3a,b**, respectively.

B: A mixture of appropriate chalcone **2c,f** (0.01 mol), ethyl cyanoacetate (1.12 g, 0.01 mol) and an excess of ammonium acetate (6.16 g, 0.08 mol) in absolute ethanol (50 mL) was refluxed for 8 h, The formed precipitate was filtered, dried and recrystallized to give **3a,b**, respectively.

4-(1*H*-Benzo[d]imidazol-2-yl)-6-(1*H*-indol-3-yl)-pyrimidine-2(1*H*)-thione (4a) and 6-(1*H*-indol-3-yl)-4-(1-methyl-1*H*-benzo[d]imidazol-2-yl) pyrimidine-2(1*H*)-thione (4b)

General method: A mixture of compounds **2d,g** (0.003 mol), thiourea (0.23 g, 0.003 mol) in 1% ethanolic sodium hydroxide (15 mL) was refluxed for 8 h. The mixture was cooled, acidified by diluted hydrchloric acid and poured onto ice/cold water. The formed precipitate was filtered, washed several times with water, dried and recrystallized to give **4a,b**, respectively.

2-(4,5-Dihydro-5-(3,4,5-trimethoxyphenyl)isoxazol-3-yl)-1*H*-benzo[d]imidazole (5a) and 2-(4,5-dihydro-5-(3,4,5-trimethoxy phenyl) isoxazol-3-yl)-1-methyl-1*H*-benzo[d]imidazole (5b)

General method: A mixture of compound **2c**,**f** (0.01 mol) and hydroxylamine hydrochloride (0.70 g, 0.01 mol) in 5% ethanolic sodium hydroxide (30 mL) was refluxed for 8 h. The reaction mixture was cooled, poured onto ice/cold water and acidified by diluted hydrochloric acid. The formed precipitate was filtered, dried and recrystallized to give **5a,b**, respectively.

4-(1*H*-Benzo[d]imidazol-2-yl)-6-(3,4,5-trimethoxyphenyl)pyrimidin-2-amine (6a) and 4-(3,4,5-trimethoxyphenyl)-6-(1-methyl-1*H*-benzo[d]imidazol-2-yl)pyrimidin-2-amine (6b)

General method: To a refluxing mixture of compound **2c,f** (0.003 mole) and guanidinium sulfate (0.65 g, 0.003 mol) in ethanol (25 mL) an aqueous solution of sodium hydroxide (5%, 5 mL) was added portionwise during 30 min. Refluxing was continued for 6 h. After cooling, the solution was poured into ice cold/water and acidified with diluted hydrochloric acid. The formed precipitate was filtered, dried and recrystallized to give **6a,b**, respectively.

2-(4,5-Dihydro-1-methyl-5-(5-methylfuran-2-yl)-1*H*-pyrazol-3-yl)-1*H*-benzo[d]imidazole (7i)

A mixture of compound **2e** (0.50 g, 0.002 mol) and methylhydrazine (0.14 mL, 0.003 mol) in ethanol (10 mL) was refluxed for 4 h. The formed precipitate was filtered, dried and recrystallized to give **7i**.

2-(4,5-Dihydro-1-methyl-5-(5-methylfuran-2-yl)-1*H*-pyrazol-3-yl)-1-methyl-1*H*-benzo[d]imidazole (7j) and 2-(4,5-dihydro-5-(5-methylfuran-2-yl)-1-phenyl-1*H*-pyrazol-3-yl)-1-methyl-1*H*-benzo[d]imidazole (7k)

A mixture of compound **2h** (0.53 g, 0.002 mol), methylhydrazine and/or phenylhydrazine (0.003 mole) in ethanol (10 mL) was refluxed for 4 h. The formed precipitate was filtered, dried and recrystallized to give **7j**,**k**, respectively.

(3-(3,4,5-Trimethoxyphenyl)oxiran-2-yl)(1-methyl-1*H*-benzo[d]imidazol-2-yl)methanone (8)

A solution of **2f** (2.10 g, 0.006 mol) in acetone (50 mL) and methanol (15 mL) was mixed with 8% aqueous sodium hydroxide (12 mL) followed by the addition of hydrogen peroxide (30%, 5 mL). The solution was warmed for 1 h and allowed to stand overnight at room temperature (25°C). Then water was added and extracted with ether, evaporated and the residue was recrystallized to yield **8**.

Ethyl-2-(3-cyano-4-(3,4,5-trimethoxyphenyl)-6-(1-methyl-1*H*-benzo[d]imidazol-2-yl)pyridin-2-yloxy)acetate (9)

A mixture of compound **3b** (0.40 g, 0.001 mol), ethyl chloroacetate (0.12 mL, 0.001 mol) and anhydrous potassium carbonate (0.21 g, 0.0015 mol) in dry acetone (20 mL) was refluxed for 6 h. The reaction mixture was cooled and poured onto ice/cold water. The formed precipitate was filtered, dried and recrystallized to give **9**.

2-(3-Cyano-4-(3,4,5-trimethoxyphenyl)-6-(1-methyl-1*H*-benzo[d]imidazol-2-yl)pyridin-2-yloxy)acetohydrazide (10)

A mixture of compound **9** (1.0 g, 0.002 mole) and hydrazine hydrate 98% (0.2 mL, 0.004 mole) in ethanol (30 mL) was refluxed for 6 h. The formed precipitatewas filtered, dried and recrystallized to give **10**.

2-(3-Cyano-4-(3,4,5-trimethoxyphenyl)-6-(1-methyl-1*H*-benzo[d]imidazol-2-yl)pyridin-2-yloxy)-N'-formylacetohydrazide (11)

A mixture of acetohydrazide **10** (0.98 g, 0.002 mol) and formic acid (10 mL) was refluxed for 1 h. The formed precipitate was filtered, washed with petrolium ether, dried and recrystallized to give **11**.

2-(3-Cyano-4-(3,4,5-trimethoxyphenyl)-6-(1-methyl-1*H*-benzo[d]imidazol-2-yl)pyridin-2-yloxy)-N'-acetylacetohydrazide (12)

A mixture of acetohydrazide **10** (0.50 g, 0.001 mol) and acetic anhydride (5 mL) was refluxed for 1 h. The formed precipitate was filtered, washed with petrolium ether, dried and recrystallized to give **12**.

2-[(5-Thioxo-1,3,4-oxadiazol-2-yl)methoxy]-4-(3,4,5-trimethoxyphenyl)-6-(1-methyl-1*H*-benzo-[d]imidazol-2-yl)pyridine-3-carbonitrile (13)

Compound **10** (2.50 g, 0.005 mol), was dissolved in a hot solution of (0.28 g, 0.005 mol) potassium hydroxide in ethanol (50 mL), then carbon disulfide (30 mL) was added and the reaction mixture was gently heated until the evolution of hydrogen sulfide ceased. The excess carbon disulfide was evaporated under reduced pressure and the reaction mixture was cooled and treated with 5 mL of acetic acid. The resulting solid was collected by filtration, dried and recrystallized to give compound **13**.

2-(3-Cyano-4-(3,4,5-trimethoxyphenyl)-6-(1-methyl-1*H*-benzo[d]imidazol-2-yl)pyridin-2-yloxy)-N'-methylthiocarbonyl-mercapto-acetohydrazide (14)

To a mixture of acetohydrazide **10** (0.50 g, 0.001 mol) and triethylamine (0.10 mL, 0.001 mol) in ethanol (15 mL) carbon disulfide (0.80 mL, 0.001 mol) was added dropwise, then methyl iodide (0.015 mL, 0.001 mol) was added and the reaction mixture was kept at room temperature for 30 min. Then water (50 mL) was added and the formed precipitate was filtered, washed with water, dried and recrystallized to give **14**.

(2-(3-Cyano-4-(3,4,5-trimethoxyphenyl)-6-(1-methyl-1*H*-benzo[d]imidazol-2-yl)pyridin-2-yloxy)acetyl)-4-methylthiosemicarbazide (15a) and (2-(3-cyano-4-(3,4,5-trimethoxyphenyl)-6-(1-methyl-1*H*-benzo[d]imidazol-2-yl)pyridin-2-yloxy)acetyl)-4-phenylthiosemicarbazide (15b)

General method: A mixture of acetohydrazide **10** (0.50 g, 0.001 mol), methyl isothiocyanate and/or phenyl isothiocyanate (0.001 mol) in ethanol (20 mL) was refluxed on water bath for 8 h. The formed precipitate was filtered, washed with petrolium ether, dried and recrystallized to give **15a,b**.

2-((5-(Phenylamino)-1,3,4-oxadiazol-2-yl)methoxy)-4-(3,4,5-trimethoxyphenyl)-6-(1-methyl-1*H*-benzo[d]imidazol-2-yl)pyridine-3-carbonitrile (16)

To a mixture of **15b** (0.60 g, 0.001 mol) in ethanol (20 mL) and aqueous sodium hydroxide (5M, 1 mL), iodine in potassium iodide solution (5%) was added gradually with stirring till color of iodine persisted at room temperature. Then, the reaction mixture was refluxed on water bath for 1 h,

Table 1. Physical and analytical data of all new compounds **2c-22h**.

Comp. No.	Yield %	M.p. °C (cryst. solvent)	Mol. formula (M. Wt.)	Analysis % Calcd./ Found		
				C	H	N
2c	45	178–180 (CH_3OH)	$C_{19}H_{18}N_2O_4$ (338.36)	67.44 67.26	5.36 5.46	8.28 8.44
2d	50	105–107 (CH_3OH)	$C_{18}H_{13}N_3O$ (287.32)	75.25 75.78	4. 56 4.23	14.63 14.22
2e	70	181–183 (CH_3OH)	$C_{15}H_{12}N_2O_2$ (252.27)	71.42 71.14	4.79 4.45	11.10 10.98
2f	50	202–204 (CH_3OH)	$C_{20}H_{20}N_2O_4$ (352.38)	68.17 68.34	5.72 6.12	7.95 8.12
2g	65	140–142 (CH_3OH)	$C_{19}H_{15}N_3$ (301.34)	75.73 75.48	5.02 4.84	13.94 14.13
2h	70	233–235 (CH_3OH)	$C_{16}H_{14}N_2O_2$ (266.29)	72.16 72.02	5.30 5.64	10.52 10.31
3a	85	303–305 (CH_3COOH)	$C_{22}H_{18}N_4O_4$ (402.4)	65.66 65.41	4.51 4.23	13.92 13.66
3b	66	291–293 (CH_3COOH)	$C_{23}H_{20}N_4O_4$ (416.43)	66.34 66.22	4.84 4.65	13.45 13.10
4a	74	210–212 (C_2H_5OH)	$C_{19}H_{13}N_5S$ (343.41)	66.45 66.20	3.82 3.64	20.39 20.14
4b	68	192–194 (C_2H_5OH)	$C_{20}H_{15}N_5S$ (357.43)	67.21 66.94	4.23 3.90	19.59 19.31
5a	60	210–212 (C_2H_5OH)	$C_{19}H_{19}N_3O_4$ (353.37)	64.58 64.84	5.42 5.45	11.89 11.78
5b	72	246–248 (C_2H_5OH)	$C_{20}H_{21}N_3O_4$ (367.40)	65.38 65.48	5.76 5.50	11.44 11.47
6a	75	155–157 (C_2H_5OH)	$C_{20}H_{19}N_5O_3$ (377.4)	63.65 63.29	5.07 4.88	18.56 18.44
6b	64	180–182 (C_2H_5OH)	$C_{21}H_{21}N_5O_3$ (391.42)	67.44 67.40	5.41 5.33	17.89 17.42
7i	60	164–166 (C_2H_5OH)	$C_{16}H_{16}N_4O$ (280.32)	68.55 68.11	5.75 5.82	19.99 19.56
7j	65	180–182 (C_2H_5OH)	$C_{17}H_{18}N_4O$ (294.35)	69.37 69.11	6.16 5.89	19.03 19.32
7k	58	174–176 (C_2H_5OH)	$C_{22}H_{20}N_4O$ (356.42)	74.14 74.55	5.66 5.57	15.72 15.45
8	45	100–102 ($CH_3CO_2C_2H_5$)	$C_{20}H_{20}N_2O_5$ (368.38)	65.21 65.10	5.47 5.12	7.60 7.45
9	84	70–72 (C_2H_5OH)	$C_{27}H_{26}N_4O_6$ (502.52)	64.53 64.47	5.22 5.38	11.15 10.96
10	78	178–180 (CH_3COOH)	$C_{25}H_{24}N_6O_5$ (488.5)	61.47 61.31	4.95 4.99	17.20 17.45
11	80	206–208 (C_2H_5OH)	$C_{26}H_{24}N_6O_6$ (516.51)	60.46 60.83	4.68 4.69	16.27 16.10
12	88	212–214 (C_2H_5OH)	$C_{27}H_{26}N_6O_6$ (530.53)	61.13 61.05	4.94 4.82	15.84 16.07
13	68	180–182 (C_2H_5OH)	$C_{26}H_{22}N_6O_5S$ (530.56)	58.86 58.72	4.18 4.12	15.84 15.76
14	68	215–217 (C_2H_5OH)	$C_{27}H_{26}N_6O_5S_2$ (578.66)	56.04 56.22	4.53 4.31	14.52 14.27
15a	70	216–218 (CH_3OH)	$C_{27}H_{27}N_7O_5S$ (561.61)	57.74 57.66	4.85 4.66	17.46 17.88

Table 1. Cont.

Comp. No.	Yield %	M.p. °C (cryst. solvent)	Mol. formula (M. Wt.)	Analysis % Calcd./ Found		
				C	H	N
15b	82	228–230 (CH_3OH)	$C_{32}H_{29}N_7O_5S$ (623.68)	61.62 60.55	4.69 4.12	15.72 15.22
16	84	168–170 (C_2H_5OH)	$C_{32}H_{27}N_7O_5$ (589.20)	65.19 66.56	4.62 4.36	16.63 16.93
17	70	145–147 (C_2H_5OH)	$C_{32}H_{27}N_7O_4S$ (605.18)	63.46 63.22	4.49 4.32	16.19 15.95
18	64	174–176 (C_2H_5OH)	$C_{32}H_{27}N_7O_4S$ (605.67)	63.46 63.11	4.49 4.25	16.19 16.44
21a	61	180–182 ($CHCl_3$)	$C_{23}H_{22}ClN_5O_5$ (483.9)	57.09 57.18	4.58 4.61	14.47 14.52
21b	58	170–172 ($CHCl_3$)	$C_{23}H_{22}ClN_5O_5$ (483.9)	57.09 56.78	4.58 4.32	14.47 14.40
21c	52	150–152 ($CHCl_3$)	$C_{22}H_{20}ClN_5O_4$ (453.88)	58.22 58.02	4.44 4.22	15.43 15.21
21d	48	161–163 ($CHCl_3$)	$C_{22}H_{20}ClN_5O_4$ (453.88)	58.22 58.47	4.44 4.10	15.43 15.33
21e	42	155–157 ($CHCl_3$)	$C_{24}H_{24}ClN_5O_5$ (497.93)	57.89 57.56	4.86 4.74	14.06 13.84
21f	63	138–140 ($CHCl_3$)	$C_{24}H_{24}ClN_5O_5$ (497.93)	57.89 57.67	4.86 4.91	14.06 14.21
21g	48	166–168 ($CHCl_3$)	$C_{23}H_{22}ClN_5O_4$ (467.90)	59.04 58.93	4.74 4.56	14.97 14.84
21h	73	141–143 ($CHCl_3$)	$C_{23}H_{22}ClN_5O_4$ (467.90)	59.04 59.23	4.74 4.91	14.97 14.77
22a	48	92–94 (CH_2Cl_2)	$C_{33}H_{32}ClN_5O_{10}$ (694.09)	57.10 56.94	4.65 4.37	10.09 9.84
22b	53	83–85 (CH_2Cl_2)	$C_{33}H_{32}ClN_5O_{10}$ (694.09)	57.10 57.23	4.65 4.72	10.09 10.14
22c	50	78–80 ($CHCl_3$)	$C_{30}H_{28}ClN_5O_8$ (622.03)	57.93 58.10	4.54 4.33	11.26 11.01
22d	58	72–74 ($CH_3CO_2C_2H_5$)	$C_{30}H_{28}ClN_5O_8$ (622.03)	57.93 57.77	4.54 4.62	11.26 11.18
22e	40	76–68 (CH_2Cl_2)	$C_{34}H_{34}ClN_5O_{10}$ (708.11)	57.67 57.61	4.84 4.91	9.89 10.04
22f	46	80–82 (CH_2Cl_2)	$C_{34}H_{34}ClN_5O_{10}$ (708.11)	57.67 57.82	4.84 4.62	9.89 9.78
22g	45	71–73 (EtOH)	$C_{31}H_{30}ClN_5O_8$ (636.05)	58.54 58.60	4.75 4.56	11.01 10.84
22h	40	95–97 ($CH_3CO_2C_2H_5$)	$C_{31}H_{30}ClN_5O_8$ (636.05)	58.54 58.42	4.75 4.88	11.01 11.21

cooled and poured onto ice/cold water. The formed precipitate was filtered, dried and recrystallized to give **16**.

2-((5-(Phenylamino)-1,3,4-thiadiazol-2-yl)methoxy)-4-(3,4,5-trimethoxyphenyl)-6-(1-methyl-1*H*-benzo[d]imidazol-2-yl)pyridine-3-carbonitrile (17)

Compound **15b** (0.60 g, 0.001 mole) was added gradually with stirring to concentrated sulfuric acid (10 mL) at 0–5°C and stirring continued for 4 h. The reaction mixture was poured onto ice/cold

Table 2. Spectral data of the newly synthesized compounds.

Comp. no.	IR (KBr, cm^{-1})	^{1}H-NMR (solvent, δ, ppm)	MS, m/z (%)
2c	3060 (NH), 1681 (C=O, α,β unsaturated ketone)	(DMSO d_6): 3.73 (s, 3H, OCH_3), 3.88 (s, 6H, $2OCH_3$), 7.22–8.10 (m, 8H, CH=CH, Ar-H, benzimidazole), 13.48 (s, 1H, NH ex. D_2O)	338 [M^+] (100), 339 [M+1] (23)
2d	3166, 3105 (2NH), 1635 (C=O, α,β unsaturated ketone)	(DMSO d_6): 7.18–8.36 (m, 10H, CH=CH, benzimidazole), 8.91 (s, 1H, indolyl), 10.94, 11.69 (2s, 2H, (2 NH ex. D_2O)	286 [M-1] (5), 143.90 [$C_9H_7N_2$] (100)
2e		(DMSO d_6): 2.42 (s, 3H, CH_3), 6.39, 7.07(d, d, 1H, 1H, furyl), 7.32–7.87 (m, 6H, CH=CH, benzimidazole), 13.44 (s, 1H, NH ex. D_2O)	252 [M^+] (100)
2f			352 [M^+] (77), 309 [$C_{18}H_{17}N_2O_3$] (100)
2g	3399 (NH), 1684 (C=O, α,β-unsaturated ketone)		301 [M^+] (86), 115 [C_8H_5N] (100)
2h	1655 (C=O, α,β-unsaturated ketone)	(DMSO d_6): 2.55 (s, 3H, CH_3), 4.16 (s, 3H, N-CH_3), 6.37, 7.04 (d,d, 1H,1H, furyl), 7.37–7.89 (m, 6H, CH=CH, benzimidazole)	266 [M^+] (100)
3a	3280 (NH), 2219 (C≡N), 1662 (C=O, amide)	(DMSO d_6): 3.71 (s, 6H, $2OCH_3$), 3.85 (s, 3H, OCH_3), 7.2–7.72 (m, 6H, Ar-H, benzimidazole protons), 7.91 (s, 1H, pyridone), 8.55 (s, 1H, NH, pyridone ex. D_2O), 12.98 (s, 1H, NH, benzimidazole ex. D_2O)	402 [M^+] (40), [M-1] 401 (100%)
3b	2217 (C=N), 1650 (C=O, amide)	(DMSO d_6): 3.76 (s, 3H, OCH_3), 3.87 (s, 6H, $2OCH_3$), 3.88 (s, 3H, N-CH_3), 7.06–7.72 (m, 6H, Ar-H, benzimidazole), 7.76 (s, 1H, pyridone), 13.00 (s, 1H, NH, pyridone ex. D_2O)	416 [M^+] (34), [M-1] 415 (100)
4a	3369 (2NH), 3196 (NH), 1126 (C=S)	(DMSO d_6): 7.14–8.31 (m, 10H, benzimidazole, indolyl and thiopyrimidine), 8.98, 11.65 (2s, 1H,1H (2NH) indolyl and benzimidazole ex. D_2O)	
4b		(DMSO d_6): 4.14 (s, 3H, CH_3), 6.75–7.63 (m, 10H, benzimidazole, indolyl and thiopyrimidine), 9.69 (s, 1H, (NH) indolyl ex. D_2O)	358 [M+1] (18), 320 [$C_{20}H_{12}N_5$] (100)
5a	3187 (NH)	(DMSO d_6): 3.94 (m, 1H,1H, CH_2-isoxazole), 4.06 (m, 1H, CH- isoxazole), 4.19 (s, 3H, OCH_3), 4.48 (s, 6H, $2OCH_3$), 7.28–8.18 (m, 7H, Ar-H, benzimidazole), 12.05 (s, 1H, NH ex. D_2O)	353 [M^+] (2), 57 [C_4H_9] (100)
5b		(DMSO d_6): 3.65–3.76 (m, 1H,1H, CH_2-isoxazole), 3.84 (m, 1H, CH- isoxazole), 3.95 (s, 3H, OCH_3), 4.07 (s, 6H, $2OCH_3$), 4.12 (s, 3H, N-CH_3), 7.49–8.16 (m, 7H, Ar-H, benzimidazole)	365 [M-2] (5), 149 [$C_9H_{13}N_2$] (100)
6a	3362 (NH), 3192 (NH_2)	(DMSO d_6): 3.75 (s, 3H, OCH_3), 3.92 (s, 6H, $2OCH_3$), 7.20–7.95 (m, 7H, Ar-H, benzimidazole and aminopyrimidine) ^{13}C-NMR (DMSO d_6): 56.03, 60.13, 102.48, 104.21, 112.44, 119.48, 122.21, 123.67, 132.29, 134.71, 139.82, 143.63, 149.60, 153.09, 157.00, 163.60, 164.74	378 [M+1] (2), 128 [$C_8H_4N_2$] (100)
6b	3369 (NH_2)	(DMSO d_6): 3.62 (s, 3H, OCH_3), 3.83 (s, 6H, $2OCH_3$), 4.12 (s, 3H, N-CH_3), 5.89 (s, 1H, aminopyrimidine), 6.65–7.54 (m, 6H, Ar-H, benzimidazole)	377 [M-CH_2] (100)
7i		(DMSO d_6): 2.27–2.39 (m, 1H, 1H, CH_2-pyrazoline), 2.86 (s, 3H, CH_3-furyl), 4.10 (s, 3H, CH_3-pyrazoline), 4.35 (m, 1H, CH-pyrazoline), 6.08, 633 (d,d, 1H, 1H, furyl proton), 6.64–7.62 (m, 4H, benzimidazole), 12.83 (s, H, NH ex. D_2O)	280 [M^+] (45), [M-2] 278 (100)
7j		(DMSO d_6): 2.80 (s, 3H, CH_3-furyl), 3.34–3.66 (m, 1H,1H, CH_2-pyrazoline), 4.00 (m, 1H, CH-pyrazoline), 4.31 (s, 3H, N-CH_3), 6.99–8.19 (m, 4H, benzimidazole)	

Table 2. Cont.

Comp. no.	IR (KBr, cm^{-1})	^{1}H-NMR (solvent, δ, ppm)	MS, m/z (%)
7k		(Acetone): 2.20 (s, 3H, CH_3-furyl), 3.60–3.88 (m, 1H,1H, CH_2-pyrazoline), 4.28 (s, 3H, N-CH_3), 5.61 (m, 1H, CH-pyrazolin), 5.96, 6.32 (d,d, 1H,1H, furyl), 6.85–7.68 (m, 9H, Ar-H, benzimidazole)	356 [M^+] (3.54), 270 [$C_{15}H_{18}N_4O$] (100)
8	1682 (C=O)	(Acetone): 3.59 (s, 6H, 2OCH_3), 3.73 (s, 3H, OCH_3), 3.99 (s, 3H, N-CH_3), 4.12 (d, 1H, CH-epoxy), 4.47 (d, 1H, CH-epoxy), 5.79-7.61 (m, 6H, Ar-H, benzimidazole)	368 [M^+] (4), [M-1] 367 (9.63), 262 [$C_{17}H_{14}N_2O$] (100)
9	2221 (C≡N), 1720 (C=O, ester)	(DMSO d_6): 1.16 (t, 3H, CH_2**CH_3**), 3.85 (s, 3H, OCH_3), 3.86 (s, 6H. 2OCH_3), 4.12 (s, 3H, N-CH_3), 4.14 (q, 2H, **CH_2**CH_3), 5.21 (s, 2H, O-CH_2), 7.07–7.73 (m, 6H, Ar-H, benzimidazole), 8.16 (s, 1H, pyridine)	502 [M^+] (100)
10	3316, 3191(NH, NH_2), 2202 (C≡N), 1583 (C=O, amide)	(DMSO d_6): 3.77 (s, 3H, OCH_3), 3.93 (s, 6H. 2OCH_3), 4.20 (s, 3H, N-CH_3), 5.17 (s, 2H, O-CH_2), 7.08–7.80 (m, 6H, Ar-H, benzimidazole), 8.23 (s, 1H, pyridine), 9.36, 12.54 (s, 3H, NH, NH_2 ex. D_2O)	490 [M+2] (18), 400 [$C_{23}H_{20}N_4O_3$] (100)
11	3438, 2928 (2NH), 2219 (C≡N), 1689 (C=O)		514 [M-2] (5), 146 [$C_9H_{10}N_2$] (100)
12	3412, 3190 (NH, NH), 2221 (C=N), 1683 (C=O, amide)	(DMSO d_6): 1.84 (s, 3H, NHCOCH_3), 3.78 (s, 3H, OCH_3), 3.89 (s, 6H, 2OCH_3), 4.23 (s, 3H, N-CH_3), 5.18 (s, 2H, O-CH_2), 7.08–7.75 (m, 6H, Ar-H benzimidazole), 8.20 (s, 1H, pyridine), 9.85, 10.25 (s, s, 1H,1H, NH, NH ex. D_2O)	531 [M+1] (3), 78 [C_6H_6] (100)
13	2218 (C≡N), 1090 (C=S)	(DMSO d_6): 3.76 (s, 3H, OCH_3), 3.87 (s, 6H, 2OCH_3), 4.26 (s, 3H, N-CH_3), 5.19 (s, 2H, O-CH_2), 7.07–7.79 (m, 6H, Ar-H, benzimidazole), 8.23 (s, 1H, pyridine)	
14	3396, 3170 (NH, NH), 2219 (C≡N), 1692 (C=O, amide), 1123 (C=S)	(DMSO d_6): 2.57 (s, 3H, S-CH_3), 3.77 (s, 6H, 2OCH_3), 3.88 (s, 3H, OCH_3), 4.12 (s, 3H, N-CH_3), 5.23 (s, 2H, O-CH_2), 7.00–7.78 (m, 6H, Ar-H, benzimidazole), 8.16 (s, 1H, pyridine), 10.74, 10.82 (2s, 1H,1H, NH, NH ex. D_2O)	578.5 [M^+] (2), 564 [M-CH_2] (2), 141 [$C_9H_5N_2$] (89)
15a		(DMSO d_6): 2.49 (s, 3H, NH-CH_3), 3.78 (s, 3H, O-CH_3), 3.89 (s, 6H, 2(OCH_3)), 4.18 (s, 3H, N-CH_3), 5.28 (s, 2H, O-CH_2), 7.09–7.76 (m, 6H, Ar-H, benzimidazole), 8.14 (s, 1H, pyridine), 8.22, 10.45 (2s, 1H,1H,1H, 3NH ex. D_2O)	561 [M^+] (4), 416 [$C_{23}H_{20}N_4O_4$] (100)
15b	3388 (3NH), 2222 (C≡N), 1586 (C=O, amide), 1125 (C=S)	(DMSO d_6): 3.74 (s, 3H, O-CH_3), 3.85 (s, 6H, 2(O-CH_3)), 4.18 (s, 3H, N-CH_3), 5.19 (s, 2H, O-CH_2), 7.05–7.71 (m, 6H, Ar-H, benzimidazole), 8.17 (s, 1H, pyridine), 9.62, 9.69, 10.35 (3s, 1H, 1H, 1H, 3NH, ex. D_2O)	623 [M^+] (1), 549 [$C_{26}H_{27}N_7O_5S$] (2)
16		(DMSO d_6): 3.77 (s, 3H, OCH_3), 3.88 (s, 6H, 2(O-CH_3)), 4.20 (s, 3H, N-CH_3), 5.26 (s, 2H, O-CH_2), 6.94–7.78 (m, 11H, Ar-H, benzimidazole), 8.15 (s, 1H, pyridine)	573 [M-1] (0.25), 266 [$C_{15}H_{14}N_4O$] (100)
17	2223 (C≡N)	(DMSO d_6): 3.75 (s, 3H, O-CH_3), 3.85 (s, 6H, 2(O-CH_3)), 4.33 (s, 3H, N-CH_3), 5.98 (s, 2H, O-CH_2), 7.11–7.85 (m, 11H, Ar-H, benzimidazole), 8.23 (s, 1H, pyridine)	
18		(DMSO d_6): 3.49 (s, 3H, O-CH_3), 3.58 (s, 6H, 2(O-CH_3)), 4.11 (s, 3H, N-CH_3), 5.55 (s, 2H, O-CH_2), 6.69–7.60 (m, 11H, Ar-H, benzimidazole), (s, 1H, pyridine)	604 [M-1] (0.89), 416 [$C_{23}H_{20}N_4O_4$] (100)
21a	3388 (OH) and 3207 (NH)	(DMSO d_6): 3.33–3.42 (m, 4H, H-6', H-6''), 3.48 (m, 3H, H-5', H-4', OH-6'), 3.59 (d, 1H, OH-5'), 4.35 (d, 1H, OH-4'), 4.40 (m, 3H, H-2, H-3', OH-3'), 4.83 (d, 1H, OH-2'), 7.26–8.26 (m, 10H, Ar-H, pyrimidine proton, benzimidazole protons, H-1'), 12.93 (s, 1H, NH ex. D_2O)1	

Table 2. Cont.

Comp. no.	IR (KBr, cm^{-1})	^{1}H-NMR (solvent, δ, ppm)	MS, m/z (%)
21b	3444 (OH), 3293 (NH)		483 [M^+] (5), 60 [C_4H_{12}] (100)
21c	3351 (OH) and 3100 (NH)	(DMSO d_6): 3.72–3.93 (m, 4H, H-5', H-5'', H-4', OH-5'), 4.00 (m, 1H, H-2'), 4.80–4.89 (m, 3H, H-3', OH-3', OH-4'), 4.91 (d, 1H, OH-2'), 7.26–8.20 (m, 10H, Ar-H, pyrimidine, benzimidazole, H-1'), 11.92 (s, 1H, NH ex. D_2O)	
21d	3414 (OH), 3337 (NH)	(DMSO d_6): 3.32–3.83 (m, 4H, H-5', H-5'', H-4', OH-5'), 4.30 (m, 1H, H-2'), 4.35–4.48 (m, 3H, H-3', OH-3', OH-4'), 4.80 (d, 1H, H-2'), 7.03–8.25 (m, 10H, Ar-H, pyrimidine, benzimidazole, H-1')	
21e		(DMSO d_6): 3.26–3.30 (m, 4H, H-6', H-6''), 3.68 (m, 3H, H-5', H-4', OH-6'), 3.71 (d, 1H, OH-5'), 4.21 (s, 3H, N-CH_3), 4.56 (d, 1H, OH-4'), 4.87 (m, 3H, H-2, H-3', OH-3'), 4.94 (d, 1H, OH-2'), 7.28–8.21 (m, 10H, Ar-H, pyrimidine, benzimidazole, H-1')	
21f	3368 (OH)	(DMSO d_6): 3.30–3.50 (m, 4H, H-6', H-6''), 3.70 (m, 3H, H-5', H-4', OH-6'), 3.85 (d, 1H, OH-5'), 4.30 (s, 3H, N-CH_3), 4.45 (d, 1H, OH-4'), 4.80 (m, 3H, H-2, H-3', OH-3'), 5.00 (d, 1H, OH-2'), 7.00–8.30 (m, 10H, Ar-H, pyrimidine, benzimidazole, H-1') ^{13}C-NMR (DMSO d_6): 33.07, 61.24, 66.99, 70.57, 74.12, 78.78, 79.79, 106.04, 111.07, 119.77, 122.75, 123.95, 128.79, 128.97, 135.82, 137.22, 141.90, 147.94, 158.61	
21g	3368 (OH)	(DMSO d_6): 3.32–3.65 (m, 4H, H-5', H-5'', H-4', OH-5'), 3.71 (m, 1H, H-2'), 4.29 (s, 3H, N-CH_3), 4.96–4.99 (m, 3H, H-3', OH-3', OH-4'), 5.00 (d, 1H, OH-2'), 7.28–8.20 (m, 10H, Ar-H, pyrimidine, benzimidazole, H-1')	468 [M+1] (5),118 [$C_7H_2N_2$] (100)
21h	3389 (OH)	(DMSO d_6): 3.35–3.50 (m, 4H, H-5', H-5'', H-4', OH-5'), 3.73 (m, 1H, H-2'), 4.30 (s, 3H, N-CH_3), 4.91–4.96 (m, 3H, H-3', OH-3', OH-4'), 5.00 (d, 1H, OH-2'), 7.24–8.22 (m, 10H, Ar-H, pyrimidine, benzimidazole, H-1')	467 [M^+] (5), 349 [$C_{13}H_{13}ClN_5$] (100)
22a		(DMSO d_6): 1.75, 2.05, 2.15, 2.22, 2.48 (5s, 15H, 5($COCH_3$)), 3.33–3.44 (m, 2H, H-6', H-6''), 4.04 (d, 1H, H-2'), 5.10 (m, 2H, H-3', H-4'), 5.42 (m, 1H, H-5'), 7.31–8.48 (m, 10H, Ar-H, pyrimidine, benzimidazole, H-1'), 12.88 (s, 1H, NH ex. D_2O)	
22b	3363 (NH)	(DMSO d_6): 1.82, 1.91, 1.98, 2.06, 2.25, 2.51 (5s, 15H, 5($COCH_3$)), 3.30–3.38 (m, 2H, H-6', H-6''), 4.10 (d, 1H, H-2'), 5.10 (m, 2H, H-3', H-4'), 5.50 (m, 1H, H-5'), 7.25–8.50 (m, 10H, Ar-H, pyrimidine, benzimidazole, H-1') ^{13}C-NMR (DMSO d_6): 20.37, 20.45, 20.56, 21.31, 62.35, 65.96, 69.40, 71.57, 72.63, 78.20, 112.43, 119.93, 122.58, 124.30, 129.01, 134.57, 134.86, 135.22, 136.04, 141.88, 143.87, 149.26, 161.45, 169.67, 169.81, 170.01, 170.65	
22c	3345 (NH)	($CDCl_3$): 2.00, 2.07, 2.11, 2.17 (4s, 12H, 4($COCH_3$)), 3.86–3.89 (m, 2H, H-5', H-5''), 4.07 (d, 1H, H-2'), 5.38 (m, 2H, H-3'), 5.61 (m, 1H, H-4'), 7.33–8.19 (m, 10H, Ar-H, pyrimidine, benzimidazole, H-1')	
22d	3345 (NH)	(DMSO d_6): 1.86, 1.93, 2.05, 2.21 (4s, 12H, 4($COCH_3$)), 3.98–4.10 (m, 2H, H-5', H-5''), 4.30 (d, 1H, H-2'), 5.10 (m, 2H, H-3'), 5.51 (m, 1H, H-4'), 7.31–8.40 (m, 10H, Ar-H, benzimidazole, H-1')	
22e		($CDCl_3$): 2.04–2.14 (m, 15H, 5($COCH_3$)), 3.30–3.40 (m, 2H, H-6', H-6''), 4.12 (d, 1H, H-2'), 4.32 (s, 3H, N-CH_3), 5.33 (m, 2H, H-3', H-4'), 5.50 (m, 1H, H-5'), 7.27–8.23 (m, 10H, Ar-H, pyrimidine, benzimidazole, H-1')	

Table 2. Cont.

Comp. no.	IR (KBr, cm^{-1})	^{1}H-NMR (solvent, δ, ppm)	MS, m/z (%)
22f		($CDCl_3$): 1.88, 1.92, 1.95, 2.08, 2.23 (5s, 15H, 5($COCH_3$)), 3.30–3.40 (m, 2H, H-6', H-6''), 4.04 (d, 1H, H-2'), 4.30 (s, 3H, N-CH_3), 5.12 (m, 2H, H-3', H-4'), 5.54 (m, 1H, H-5'), 7.33–8.25 (m, 10H, Ar-H, pyrimidine, benzimidazole, H-1')	
22g		($CDCl_3$): 2.08–2.28 (m, 12H, 4($COCH_3$)), 3.48–3.51 (m, 2H, H-5', H-5''), 4.12 (d, 1H, H-2'), 5.32 (m, 2H, H-3'), 5.44 (m, 1H, H-4'), 7.28–8.19 (m, 10H, Ar-H, pyrimidine, benzimidazole, H-1')	
22h		($CDCl_3$): 2.00–2.30 (m, 12H, 4($COCH_3$)), 3.40–3.65 (m, 2H, H-5', H-5''), 4.20 (d, 1H, H-2'), 4.34 (s, 3H, N-CH_3), 5.13 (m, 2H, H-3'), 5.43 (m, 1H, H-4'), and at 7.29–8.25 (m, 10H, Ar-H, pyrimidine, benzimidazole, H-1')	

ex. D_2O = exchangeable by D_2O

water. The formed precipitate was filtered, washed with water dried and recrystallized to give **17**.

2-((4-Phenyl-5-thioxo-1,2,4-triazol-3-yl)methoxy)-4-(3,4,5-trimethoxyphenyl)-6-(1-methyl-1*H*-benzo[d]imidazol-2-yl)pyridine-3-carbonitrile (18)

Compound **15b** (0.60 g, 0.001 mol) dissolved in (5%) ethanolic sodium hydroxide solution (20 mL) was refluxed for 8 h .The reaction mixture was cooled, poured onto ice/cold water and acidified by hydrochloric acid. The formed precipitate was filtered, dried and recrystallized to give **18**.

1-(1*H*-Benzo[d]imidazol-2-yl)-3-(4-chlorophenyl)prop-2-en-1-one (**19a**), 3-(4-chlorophenyl)-1-(1-methyl-1*H*-benzo[d]imidazol-2-yl)prop-2-en-1-one (**19b**), 4-(1*H*-benzo[d]imidazol-2-yl)**-6-(**4-chlorophenyl)pyrimidin-2-amine **(20a)** and 4-(4-chlorophenyl)-6-(1-methyl-1*H*-benzo[d]imidazol-2-yl)pyrimidin-2-amine (**20b**) were prepared according to literature (21).

2-N-(1-(E)-Polyhydroxyalkylidine)imino-4-(4-chlorophenyl)-6-(1*H*-benzo[d]imidazol-2-yl)pyrimidine (21a-d) and 2-N-(1-(E)-polyhydroxyalkylidine)imino-4-(4-chlorophenyl)-6-(1-methyl-1*H*-benzo [d] imidazol-2-yl)pyrimidine (21e-h)

General method: A mixture of **20a,b** (0.001 mol) in ethanol (20 mL), few drops of acetic acid and the respective monosacaride, namely: galactose, mannose, arabinose, and/or xylose (D-series) (0.001 mol) dissolved in 1 mL of water were heated on a water bath at 60°C for 6 h. The formed precipitate-was filtered while hot, dried and recrystallized to give **21a–h**, respectively.

2-N-(1-(E)-Polyacetoxyalkylidine)imino-4-(4-chlorophenyl)-6-(1*H*-benzo[d]imidazol-2-yl)-pyrimidine (22a-d) and 2-N-(1-(E)-polyacetoxy alkylidine)imino-4-(4-chlorophenyl)-6-(1-methyl-1*H*-benzo [d] imidazol-2-yl)pyrimidine (22e–h)

General method: A mixture of **21a–h** (0.01 mol) and acetic anhydride (0.015 mole) in 10 mL of pyridine was stirred for 4 h at room temperature. The reaction mixture was poured onto ice/cold water, the formed precipitate was filtered, washed several times with water, dried and recrystallized to give **22a–h**, respectively.

***In vitro* cytotoxicity screening**

Materials and methods

HEPG2 cell line

HEPG2 cells were maintained in DMEM medium supplemented with 10% FBS and 100 U/mL penicillin/streptomycin. To quantify cytotoxicity of test compounds, cells were seeded in 96-well plates at a density of 2×10^4 cells/well and cultured for 24 h. Trypan blue exclusion test was used to ensure the cell viability was greater than 99%.

At the end of 24 h, the medium was removed, cell layer washed using Hank's balanced salt solution and replenished with fresh reduced serum medium containing the test compounds at the indicated concentrations (Fig. 1). Each concentration was repeated in triplicates. The plate was then incubated at 37°C for 72 h.

The following controls were run on every plate: positive control: indicated doses of acetaminophen (a known hepatotoxin); blank: no treatment added; solvent control: 0.2% DMSO.

At the end of 72 h, the media containing the test compounds were aspirated out, the cell layer was washed again with Hank's balanced salt solution and replaced with media containing CellTiter 96® AQueous One Solution Reagent (Promega) to assess the cytotoxicity.

The plates were incubated for 3 h at 37°C and the absorbance was read at 490 nm and the viability of the cells was calculated as follows:

$$\%\text{Viability} = \frac{(\text{average triplicate absorbance of test well}) - \text{b. a.}}{\text{verage triplicate absorbance of blank}) - \text{b. a.}} \times 100$$

b.a. = background absorbance

PC12 cell line

PC12 cells were derived from a pheochromocytoma of the rat adrenal medulla. These cells stop dividing and terminally differentiate when treated with nerve growth factor.

PC12 cells (passage 25) were cultured on collagen-coated 96-well plates (BD Biosciences) using DMEM medium supplemented with 10% horse serum, 5% FBS, 1% penicillin/streptomycin and 50 ng/mL of nerve growth factor. The cells were seeded at a density of 2×10^4 cells/well and viability was ensured to be greater than 99% using the trypan blue exclusion assay.

The cells were allowed to differentiate for 72 h, after which the compounds to be tested were added to the culture media. Stock solutions of the test compounds were prepared fresh on the day of the experiment and diluted in culture media to reach final concentrations as indicated in Figure 2. The plates were then incubated for 24 h, the media removed and cell layer washed with Hank's balanced salt solution, and fresh media with CellTiter 96® AQueous One Solution Cell Proliferation Assay reagent (Promega) was added to assess the cell viability.

The plate was incubated for 3–4 h, after which the absorbance was recorded at 492 nm using a plate reader. The viability of the cells was calculated and expressed as a percentage of control (no treatment). Glutamate, which is known to cause excitotoxic death in PC12 cells at 5 mM and 10 mM concentrations, 24 h incubation, was used as a positive control on every plate. DMSO was tested at a final concentration of 0.2%, to confirm its non-cytotoxic effect.

RESULTS AND DISCUSSION

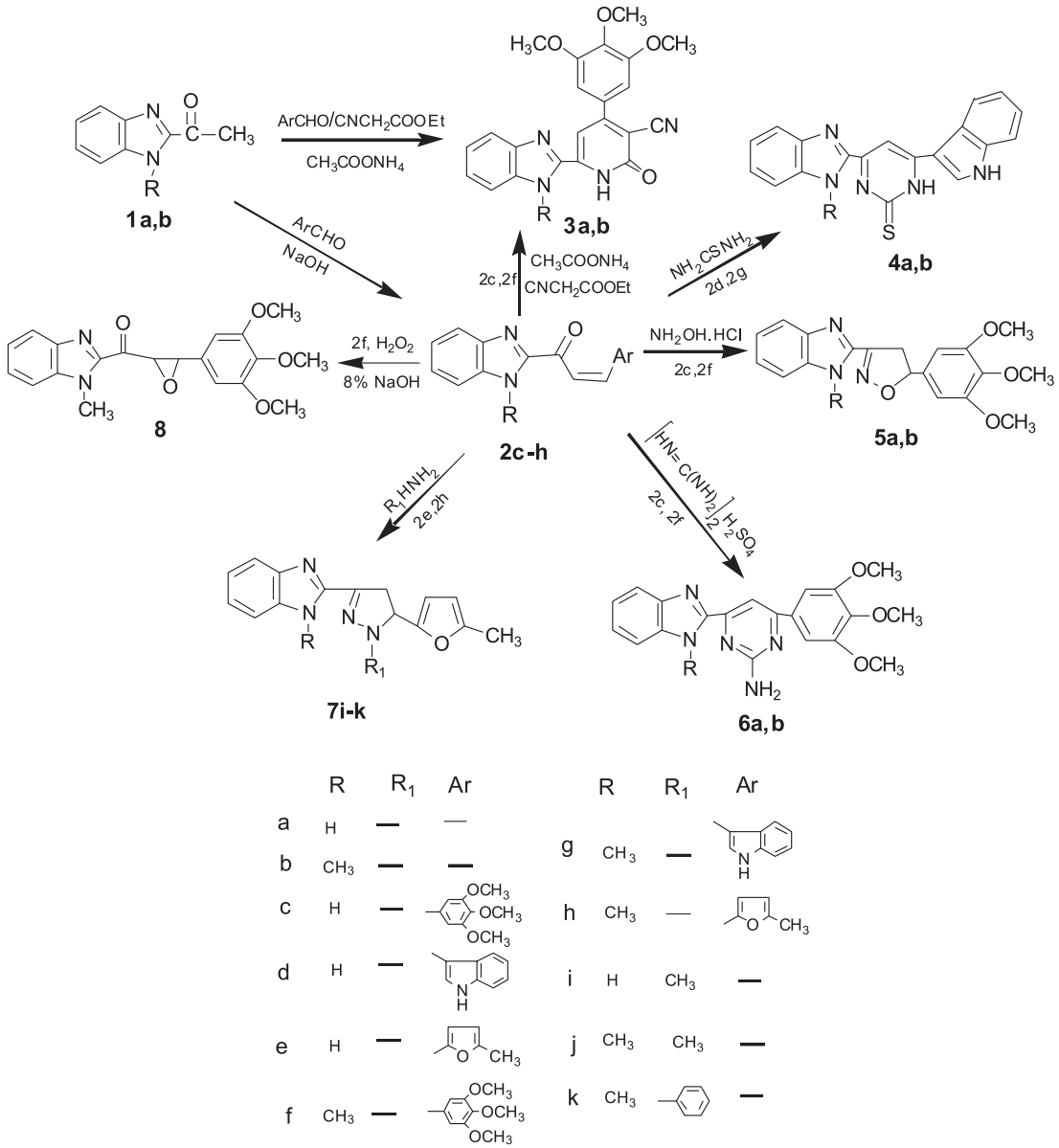
Chemistry

Starting from 2-acetylbenzimidazole (**1a**) and 2-acetyl-1-methylbenzimidazole (**1b**), which were prepared according to the reported method (20), Claisen-Schmidt condensation of **1a,b** with appropriate aromatic aldehydes namely, 3,4,5-trimethoxybenzaldehyde, indole-3-carboxaldehyde and/or 5-methyl furfural in (5%) ethanolic sodium hydroxide solution afforded 1-(1*H*-benzo[d]imidazol-2-yl)-3-(substituted)prop-2-en-1-one **2c-e** and 3-(substituted)-1-(1-methyl-1*H*-benzo[d]imidazol-2-yl)prop-2-en-1-one **2f-h**, respectively.

One pot reaction (22) of **1a,b** with 3,4,5-trimethoxybenzaldehyde and ethyl cyanoacetate in excess of anhydrous ammonium acetate afforded 6-(1*H*-benzo[d]imidazol-2-yl)-1,2-dihydro-4-(3,4,5-trimethoxyphenyl)-2-oxopyridine-3-carbonitrile (**3a**) and 1,2-dihydro-4-(3,4,5-trimethoxyphenyl)-6-(1-methyl-1*H*-benzo[d]imidazol-2-yl)-2-oxopyridine-3-carbonitrile (**3b**). Also **3a,b** were prepared by cyclocondensation of chalcone **2c**,**f** with ethyl cyanoacetate and excess ammonium acetate in ethanol. Cyclization of α,β-unsaturated ketone **2d,g** with thiourea in ethanolic sodium hydroxide (1%) afforded the corresponding pyrimidine-2-thione derivative: 4-(1*H*-benzo[d] imidazol-2-yl)-6-(1*H*-indol-3-yl)pyrimidine-2(1*H*)-thione (**4a**) and 6-(1*H*-indol-3-yl)-4-(1-methyl-1*H*-benzo[d]imidazol-2-yl) pyrimidine-2(1*H*)-thione (**4b**), respectively (Scheme 1).

Furtheremore, cyclocondensation of **2c,f** with hydroxylamine hydrochloride in 5% ethanolic sodium hydroxide yielded 2-(4,5-dihydro-5-(3,4,5-trimethoxyphenyl)isoxazol-3-yl)-1*H*-benzo[d]imidazole (**5a**) and 2-(4,5-dihydro-5-(3,4,5-trimethoxyphenyl)isoxazol-3-yl)-1-methyl-1*H*-benzo[d]imidazole (**5b**), respectively. Moreover, cyclocondensation of **2c**,**f** with guanidinium sulfate in ethanolic sodium hydroxide (5%) gave 4-(1*H*-benzo[d]imidazol-2-yl)-6-(3,4,5-trimethoxyphenyl) pyrimidin-2-amine (**6a**) and 4-(3,4,5-trimethoxyphenyl)-6-(1-methyl-1*H*-benzo[d]imidazol-2-yl)pyrimidin-2-amine (**6b**), respectively. Reaction of **2e**,**h** with methylhydrazine and/or phenylhydrazine in ethanol produced the corresponding pyrazole derivatives **7i-k**, respectively. Treatment of **2f** with hydrogen peroxide (30%) in acetone yielded (3-(3,4,5-trimethoxyphenyl)oxiran-2-yl)(1-methyl-1*H*-benzo[d]imidazol-2-yl)methanone (**8**) (Scheme 1).

Condensation of **3b** with ethyl chloroacetate in dry acetone using sodium carbonate as an acid scavenger gave ethyl-2-(3-cyano-4-(3,4,5-trimethoxyphenyl)-6-(1-methyl-1*H*-benzo[d]imidazol-2-yl)pyridin-2-yloxy) acetate (**9**) which condensed with hydrazine hydrate (98%) in ethanol according to Padhyay and Basu (23) to give 2-(3-cyano-4-



Scheme 1.

(3,4,5-trimethoxyphenyl)-6-(1-methyl-1*H*-benzo-[d]imidazol-2-yl)pyridin-2-yloxy) acetohydrazide (**10**), which is very useful starting material for the synthesis of all target compounds in this work.

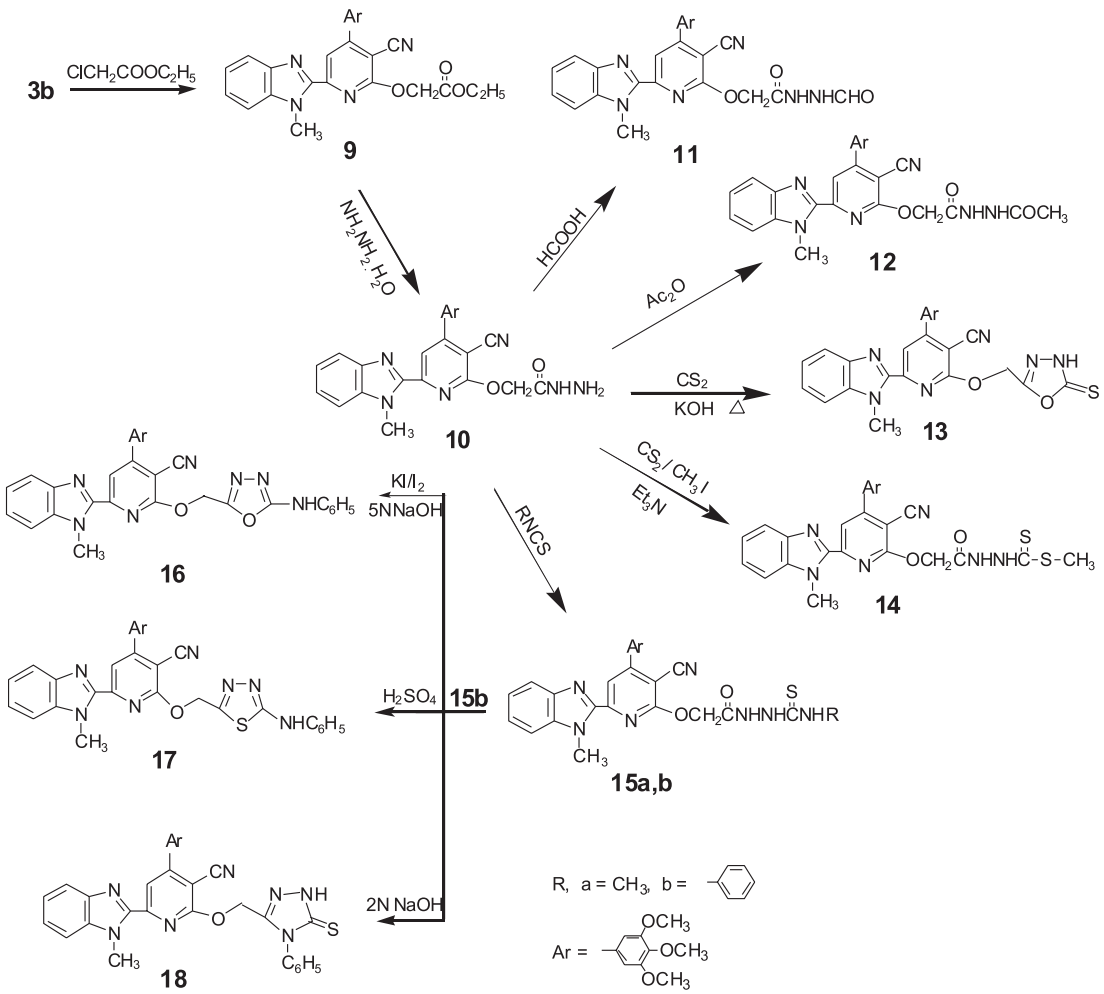
Refluxing the acetohydrazide **10** with formic acid gave 2-(3-cyano-4-(3,4,5-trimethoxyphenyl)-6-(1-methyl-1*H*-benzo[d]imidazol-2-yl)pyridin-2-yloxy)-N'-formylacetohydrazide (**11**), whereas the treatment with acetic anhydride on a water bath yielded 2-(3-cyano-4-(3,4,5-trimethoxyphenyl)-6-(1-methyl-1*H*-benzo[d]imidazol-2-yl)pyridin-2-yloxy)-N'-acetylacetohydrazide (**12**). Also, 1.3,4-oxadiazole-2-thione derivative (**13**) was achieved by cyclization of **10** using carbon disufide in alcoholic potassium hydroxide (24). Reacting of acetohydrazide **10** with carbon disulfide, triethylamine and methyl iodide afforded 2-(3-cyano-4-(3,4,5-trimethoxyphenyl)-6-(1-methyl-1*H*-benzo[d]imidazol-2-yl)pyridin-2-yloxy)-N'-methylthiocarbonyl-mercapto-acetohydrazide (**14**) according to published method (25) (Scheme 2).

Treatment of **10** with the appropriate thiosemicarbazide, namely: methyl and phenyl isothiocyanate, using the reported method (26), afforded

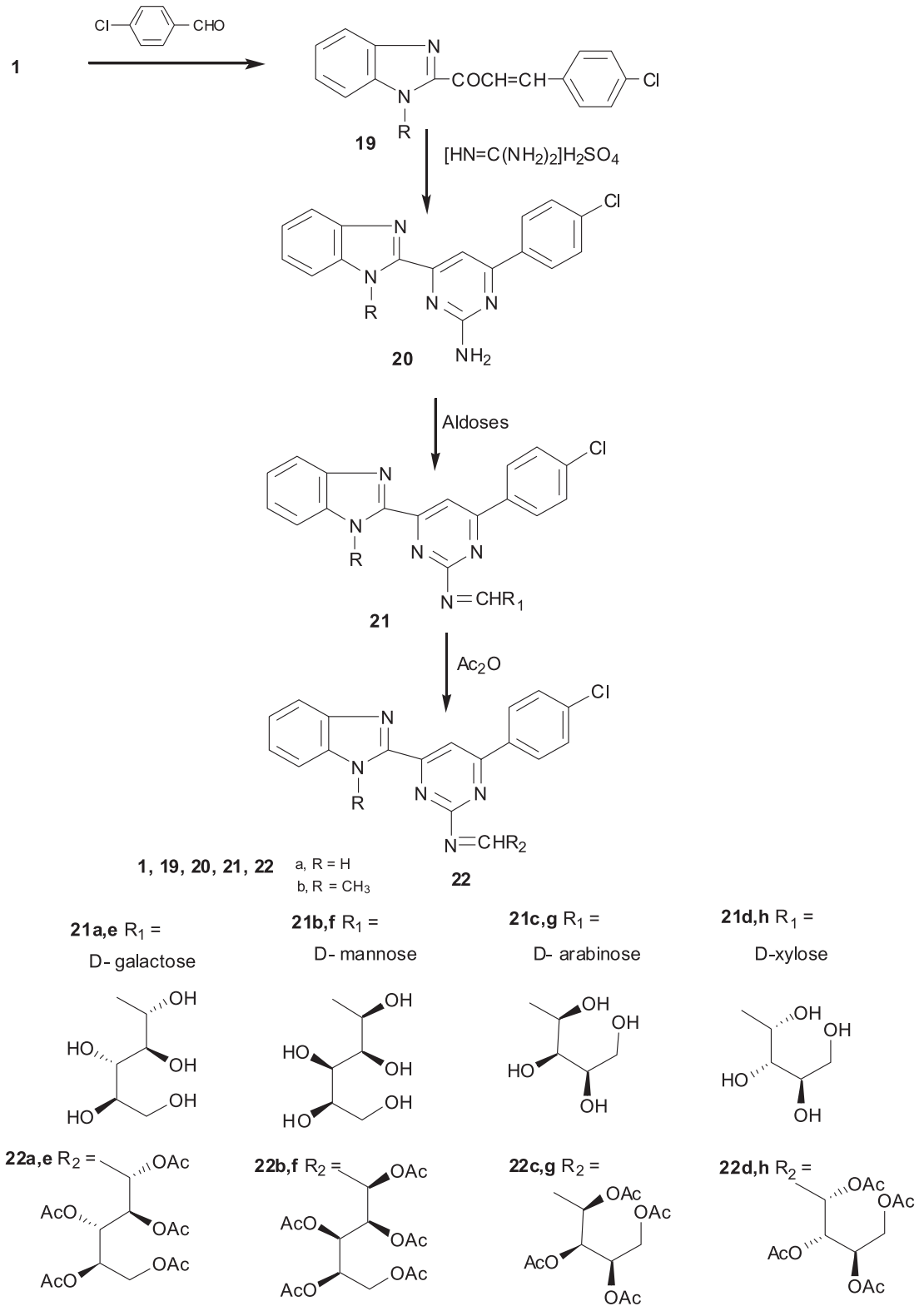
the corresponding 4-methyl (or phenyl)-1-[2-(pyridin-2-yloxyacetyl)] thiosemicarbazide derivatives **15a,b**, respectively.

On the other hand, according to the known chemotherapeutic activities of 1,3,4-oxadiazoles, 1,3,4- thiadiazoles and 1,2,4-triazoles as antiviral, antifungal (27) and anticancer agents (28, 29), it was of interest to incorporate such moiety into the parent benzimidazol-6-yl pyridine backbone to obtain more active and less toxic anticancer agents. So **15b** was allowed to react with potassium iodide and iodine to give 2-((5-phenyl-1,3,4-oxadiazol-2-yl)methoxy)-4-(3,4,5-trimethoxyphenyl)-6-(1-methyl-1*H*-benzo[d]imidazol-2-yl)pyridine-3-carbonitrile (**16**). Cyclization of **15b** by sulfuric acid at 0–5°C yielded 2-((5-phenyl-1,3,4-thiadiazol-2-yl)methoxy)-4-(3,4,5-trimethoxyphenyl)-6-(1-methyl-1*H*-benzo[d]imidazol-2-yl)pyridine-3-carbonitrile (**17**) while cyclization by 2M ethanolic sodium hydroxide afforded 2-((4-phenyl-5-thioxo-1,2,4-triazol-3-yl)methoxy)-4-(3,4,5-trimethoxyphenyl)-6-(1-methyl-1*H*-benzo[d]imidazol-2-yl)pyridine-3-carbonitrile (**18**), respectively (Scheme 2).

The discovery of glycosides and continuous study of their biological activities (19, 30) led us to construct compounds containg benzimidazole Schiff's bases connected with different aldoses which might have potential anticancer properties against experimental tumor cell lines. Thus the reaction of **20a,b** (21) with different monosaccharides (aldoses) namely, D-galactose, D-mannose, D-arabinose, and/or D-xylose in refluxing ethanol and few drops of glacial acetic acid afforded the corre-



Scheme 2.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N
N
COCH=CH
Cl
R
19
[HN=C(NH2)2]H2SO4
Cl
N
N
R
N
N
NH2
20
Aldoses
Cl
N
N
R
N
N
N=CHR1
21
Ac2O
Cl
N
N
R
N
N
N=CHR2
22
1, 19, 20, 21, 22
a, R = H
b, R = CH3
21a,e R1 =
D- galactose
21b,f R1 =
D- mannose
21c,g R1 =
D- arabinose
21d,h R1 =
D-xylose
OH
HO
22a,e R2 =
22b,f R2 =
22c,g R2 =
22d,h R2 =
OAc
AcO

Scheme 3.

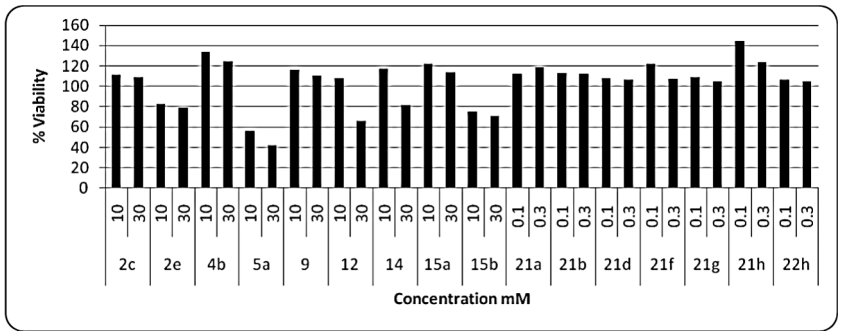

Figure 1. Cytotoxicity of tested compounds in HEPG2 cells

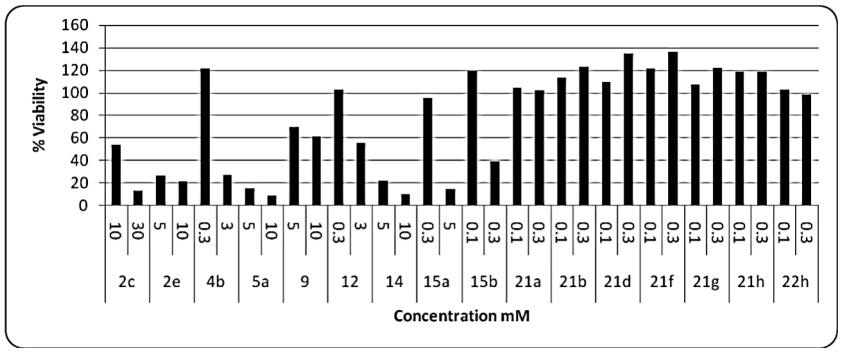

Figure 2. Cytotoxicity of tested compounds in PC12 cells

Table 3. IC_{50} (mM) of the selected compounds on human liver carcinoma cell line (HEPG2) and pheochromocytoma of the rat adrenal medulla (PC12) cells.

Compound	HEPG2–24 h	PC12–24 h
	IC50	IC50
2c	> 30 mM	0.103 mM
2e	> 30 mM	5 mM
4b	> 30 mM	1 mM
5a	2.4 mM	0.268 mM
9	> 30 mM	18 mM
12	> 30 mM	3 mM
14	> 30 mM	0.954 mM
15a	> 30 mM	0.251 mM
15b	> 30 mM	1.5 mM
21a	> 30 mM	> 30 mM
21b	> 30 mM	> 30 mM
21d	> 30 mM	> 30 mM
21f	> 30 mM	> 30 mM
21g	> 30 mM	> 30 mM
21h	> 30 mM	> 30 mM
22h	> 30 mM	> 30 mM

sponding N-glycosides 2-N-(1-(E)-polyhydroxyalkylidine)imino-4-(4-chlorophenyl)-6-(1*H*-benzo[d]imidazol-2-yl)pyrimidine (**21a-d**) and 2-N-(1-(E)-polyhydroxyalkylidine)imino-4-(4-chlorophenyl)-6-(1-methyl-1*H*-benzo[d]imidazol-2-yl)pyrimidine (**21e-h**), respectively. Compounds **21a-h** were treated with acetic anhydride in pyridine at room temperature to give the corresponding acetoxy derivatives 2-N-(1-(E)-polyacetoxyalkylidine)imino-4-(4-chlorophenyl)-6-(1*H*-benzo[d]imidazol-2-yl)pyrimidine (**22a-d**) and 2-N-(1-(E)-polyacetoxyalkylidine)imino-4-(4-chlorophenyl)-6-(1-methyl-1*H*-benzo[d]imidazol-2-yl)pyrimidine (**22e-h**), respectively (Scheme 3).

***In vitro* cytotoxicity activity**

Cytotoxicity of positive control

Acetaminophen, a known hepatotoxin used as positive control, was tested at concentrations between 5 mM and 50 mM. At 20 mM, there was a 50% reduction in cell viability (31, 32).

Glutamate, known to cause excitotoxic cell death in neurons at 5 mM and 10 mM, used as positive control, was tested for its cytotoxic effects in PC12. Greater than 50% reduction in cell viability was observed in cells treated with 10 mM of glutamate (33, 34).

Cytotoxicity of tested compounds:

Sixteen of the newly synthesized compounds: **2c**, **2e**, **4b**, **5a**, **9**, **12**, **14**, **15a, 15b**, **21a**, **21b**, **21d**, **21f**, **21g**, **21h** and **22h**, were evaluated for their anticancer potential in the two cell lines, HEPG2 and PC12.

Compounds **2c, 4b**, **9** and **15a** did not exhibit any cytotoxic effects at concentrations as high as 10 and 30 mM, when tested in HEPG2 cells (Fig. 1).

Similarly, in PC12 cells, compounds **4b**, and **15a** did not exhibit cytotoxicity when tested at 0.1 mM and 0.3 mM, but a difference in response was observed with the new chemical entities **2e**, **5a**, **14** and **15b** (Fig. 2). However, **4b** (3 mM) reduced the viability of PC12 cells by more than 70%. **2c**, **12** and **15a** were cytotoxic to PC12 cells at concentrations greater than 5 mM.

Compounds **12** and **14** reduced the viability of HEPG2 cells between 20–30% at 30 mM, but were non-toxic below this concentration. They also greatly reduced the percentage of viability of the PC12 cells at 3 mM, 5 mM and 10 mM concentration.

Compound **5a** reduced the viability of HEPG2 cells by approximately 50% at concentrations greater than 10 mM. The cytotoxic effects were comparable in PC12 cells at 5 mM.

Compounds **21a,b,d,f,g,h** and **22h** did not reduce the viability of HEPG2 and PC12 cells at concentration between 0.1 mM to 0.3 mM. (Fig. 1 and 2).

CONCLUSION

Compound **5a** had a promising anticancer activity against cell lines PC12 and HEPG2 while compounds **2e**, **4b**, **14**, **15a** and **15b** were active only in PC12 cells. Conversely, compounds **2c**, **2e**, **4b**, **5a**, **9**, **12**, **14**, **15a** and **15b** had little *in vitro* cytotoxic activity against HEPG2 cell line at low concentrations. It is also of great importance to mention that glycoside derivatives **21a**, **21b**, **21d**, **21f**, **21g**, **21h** and **22h** had little *in vitro* cytotoxic activity against HEPG2 and PC12 cell lines (Table 3). Thus, with further testing, many of the synthesized compounds have the potential to be developed into potent anticancer agents.

Structure-activity relationship (SAR)

The data of the chosen benzimidazole chalcone derivatives **2c**, **2e**, **4b** and **5a**, acetohydrazide derivatives **9**, **12**, **14**, **15a** and **15b** as well as N-glycoside derivatives **21a**, **21b**, **21d**, **21f**, **21g**, **21h** and **22h** evidenced that compound **2c** was the most active compound in PC12 cell line showing (IC_{50} = 0.103 mM), whereas compound **5a** (IC_{50} = 2.4 mM) was the most active one in HEPG2 cell line.

The activity of the selected compounds could be correlated with structure variation and modification as follows:

The tested compounds, benzimidazole chalcone and their cyclization derivatives, have little effect in HEPG2 cells at low concentration. The activity order of the compounds in PC12 was **2c** (IC_{50}= 0.103 mM) > **5a** (IC_{50} = 0.268 mM) > **4b** (IC_{50} = 1 mM)> **2e** (IC_{50} = 5 mM)

It was found that the α,β-unsaturated ketone **2c** (IC_{50} = 0.103 mM) is the most active compound in PC12 cell line, while its isoxazole derivative **5a** showed dual activity (IC_{50} = 2.4 and 0.268 mM) for liver HEPG2 and adrenal medullary PC12 cancers, respectively.

The tested acetohydrazide derivatives showed significant activity against PC12 cell line with little effect in HEPG2 cells at low concentration

The result obtained from the study of the PC12 activities revealed that **15a** (IC_{50} = 0.251 mM) showed the most prompt activity in PC12 cell line due to the presence of sulfur atoms in the attached side chains that enhances the cytotoxic spectrum > **14** (IC_{50} = 0.954 mM) > **15b** (IC_{50} = 1.5 mM), in comparison with compounds **12** (IC_{50} = 3 mM) and **9** (IC_{50} = 18 mM) which haven't sulfur atom in their side chains.

The variation of cytotoxic spectrum of activity between the two closely related isothiocyanate derivatives **15a** and **15b** indicates that the cytotoxcity can be obtained in this class of compounds by attaching small alkyl group into thiosemicarbazide side chain **15a**, rather than the larger aryl group **15b**.

Benzimidazole derivatives that have benzimidazole group attached to a series of open chain monosaccharides linked to nitrogen atom of aminopyrimidine ring to give N-glycoside derivatives, contrary to expectations, showed weak activities in HEPG2 or PC12 cell lines at low concentration.

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