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6-Nitrobenzimidazole derivatives: Potential phosphodiesterase inhibitors: Synthesis and structure–activity relationship

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ABSTRACT

6-Nitrobenzimidazole derivatives (**1**–**30**) synthesized and their phosphodiesterase inhibitory activities determined. Out of thirty tested compounds, ten showed a varying degrees of phosphodiesterase inhibition with IC_{50} values between 1.5 ± 0.043 and 294.0 ± 16.7 μM. Compounds **30** (IC_{50} = 1.5 ± 0.043 μM), **1** (IC_{50} = 2.4 ± 0.049 μM), **11** (IC_{50} = 5.7 ± 0.113 μM), **13** (IC_{50} = 6.4 ± 0.148 μM), **14** (IC_{50} = 10.5 ± 0.51 μM), **9** (IC_{50} = 11.49 ± 0.08 μM), **3** (IC_{50} = 63.1 ± 1.48 μM), **10** (IC_{50} = 120.0 ± 4.47 μM), and **6** (IC_{50} = 153.2 ± 5.6 μM) showed excellent phosphodiesterase inhibitory activity, much superior to the standard EDTA (IC_{50} = 274 ± 0.007 μM), and thus are potential molecules for the development of a new class of phosphodiesterase inhibitors. A structure–activity relationship is evaluated. All compounds are characterized by spectroscopic parameters.

1. Introduction

Benzimidazole derivatives find application in the treatment of several diseases like epilepsy, diabetes, anti-fertility, etc.[1,2] Recently several researches elucidated that biological profiles of benzimidazole analogs can suitably be modified by the introduction of different heterocyclic moieties to exhibit a broad spectrum of further biological activities, that is, anti-cancer and antifungal[3,4] antiviral,[5–8] antibacterial,[9–15] antihelmintic,[16,17] antiinflammatory,[18] antihistaminic,[19] proton pump inhibiting,[20,21] antioxidant,[22–25] antihypertensive,[26] anticoagulant,[27] antileukaemic[28] or anti-ulcer.[29]

Benzimidazole derivatives reveal also considerable biological activity against important viruses such as HIV, herpes (HSV-1), RNA influenza, and human cytomegalovirus (HCMV).[30,31] A number of benzimidazole displayed good antitumor activity.[32] The nitroimidazoles, in particular metronidazole is most commonly used and accepted as the drug of choice for the chemotherapy of anaerobic bacteria, protozoal disease and as radio sensitizer for hypoxic tumors.[33]

Nucleotide pyrophosphatases/phosphodiesterases (NPPs) (EC 3.1.4.1) are hydrolases that act on diesters of phosphoric acid. They catalyze the release of nucleoside-5′-monophosphates from various pyrophosphate bonds (e.g., nucleoside diphosphates and triphosphates, NAD, FAD, and UDP-glucose) and phospho diester bonds (in oligonucleotides and exogenous substrates like di-*p*-nitrophenyl phosphate, and *p*-nitrophenyl ester of TMP).[34] The NPP family comprises of three members known as NPP-1, NPP-2, and NPP-3. They are widely distributed in mammalian intestinal mucosa, liver cell, and serum, snake venom and in various plants. They have distinct patterns of distribution in different cells types and even within the same types of cells.[35] NPP-1 has been localized in cells of the distal convoluted tubules of the kidney, in epididymis, chondrocytes, osteoblasts, and hepatocytes. NPP1 or PC1 (plasma cell membrane glycoprotein) is a key regulator of calcification of bone and other tissues. Over-expression of NPP1 has been associated with chondrocalcinosis,[36] while underexpression causes severe periarticular calcification in mice[37] and the syndrome of idiopathic infantile arterial calcification in human.[38]

As our group is working on the lead molecules and in recent past we published our paper on the enzyme inhibition.[39] We carried out the preliminary studies on several classes of molecules randomly and 6-nitrobenzimidazole showed encouraging results therefore these molecules chose to synthesize and screened for NPPs inhibiting activities. All the synthetic compounds are characterized by spectroscopic analyses including ^{1}H NMR and EI MS. All compounds gave satisfactory elemental analysis.

2. Results and discussion

2.1. Chemistry

The syntheses of the 6-nitrobenzimidazoles derivatives **1**–**30** were carried out by reacting commercially available

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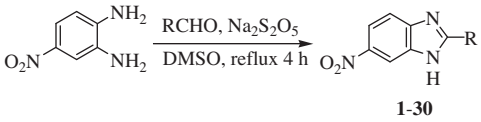


Scheme 1. Synthesis of 6-nitrobenzimidazole derivatives **1**–**30**.

4-nitrophenylenediamine with a variety of aromatic aldehydes in DMSO in high yields (Scheme 1). In a typical reaction, sodium metabisulfite ($Na_2S_2O_5$) (3 mmol) was added to a stirring solution of 4-nitrophenylenediamine (3.12 equiv) and the corresponding substituted aromatic aldehydes (3.16 equiv) in DMSO and refluxed to the completion of reaction. The progress of the reaction was monitored by TLC. After completion of the reaction it was allowed to cool to room temperature. Addition of water (30 mL) resulted in precipitation of a solid residue which was then column-chromatographed yielding the corresponding 6-nitrobenzimidazole derivative in high yields. The structures of these compounds were determined by ^{1}H NMR and EI mass spectroscopy.

2.2. In vitro phosphodiesterase activity

All synthetic compounds **1**–**30** were screened for their in vitro phosphodiesterase activity. Out of thirty tested compounds, ten showed a varying degree of phosphodiesterase inhibition with IC_{50} values between 1.5 ± 0.043 and 294.0 ± 16.7 μM. Compounds **30** (IC_{50} = 1.5 ± 0.043 μM), **1** (IC_{50} = 2.4 ± 0.049 μM), **11** (IC_{50} = 5.7 ± 0.113 μM), **13** (IC_{50} = 6.4 ± 0.148 μM), **14** (IC_{50} = 10.5 ± 0.51 μM), **9** (IC_{50} = 11.49 ± 0.08 μM), **3** (IC_{50} = 63.1 ± 1.48 μM), **10** (IC_{50} = 120.0 ± 4.47 μM), and **6** (IC_{50} = 153.2 ± 5.6 μM) showed IC_{50} values superior to the standard EDTA (IC_{50} = 274 ± 0.007 μM) and thus are considered excellent inhibitors against phosphodiesterase. Only compound **5** (IC_{50} = 294.0 ± 16.7 μM) showed a slightly less IC_{50} value than the standard (EDTA). Compounds **2**, **4**, **7**, **8**, **12**, and **15**–**29** had less than 50% inhibition power, therefore, were not further evaluated for their IC_{50} values.

Some preliminary conclusions concerning the SAR of the 6-nitrobenzimidazole series and their phosphodiesterase inhibiting activity can be drawn from the data collected in Table 1.

Compound **30** (IC_{50} = 1.5 ± 0.043 μM) showed the remarkably highest inhibitory activity presumably caused by the presence of two hydroxyl groups at positions 2 and 3 of the phenyl residue. Surprisingly, compound **16** where the hydroxyl group at position 3 is replaced by an ethoxy residue was found to be completely inactive suggesting that the activity in **30** results from the combined effect of the two neighboring hydroxyls and/or the sterically less hindered orientation of one of the hydroxyl groups needed for interaction with the enzyme. In fact this hypothesis is further supported by the inactivity of compound **12** in which the ethoxy residue of compound **16** is replaced by a methoxy moiety at the same position. The supporting effect of the two neighboring hydroxyls in compound **30** is further confirmed by the inactivity of compounds **18**, bearing a 2-hydroxyphenyl, **28**, substituted by a 2,5-dihydroxyphenyl, **17** with an attached 2-hydroxy-5-chloro, and **2**, substituted by a 2-hydroxy-3,5-dibromophenyl residue.

Surprisingly compound **5** (IC_{50} = 294 ± 16.7 μM) with a 2-hydroxy-3,5-dicholrophenyl residue showed comparable activity to the standard EDTA (IC_{50} = 274 ± 0.007 μM). This enhancement in activity of compound **5** may be due to 3-chloro-residue which might provide assistance to the neighboring hydroxyl group for a significant interaction with the enzyme being smaller in size as compared to the dibromo substitution at same position as in compound **2**.

Compounds **11** (IC_{50} = 5.7 ± 0.113 μM) and **9** (IC_{50} = 11.49 ± 0.08 μM), 3,4- and 2,4-dihydroxy analogs exhibited less activity as compared to **30** caused by the change in position of the hydroxyl groups. However, again compound **11** with neighboring hydroxyl substitution in the phenyl ring is far most active than compound **9** with 2,4-dihydroxyl substitution in the phenyl ring.

Compound **1**, (IC_{50} = 2.4 ± 0.049 μM), the 4-hydroxyphenyl analog is the second most active compound of the series, however, the 2-hydroxyphenyl analog **18** was found to be completely inactive suggesting that the hydroxyl group at position 4 of the phenyl residue provides a sterically-favored orientation for a fruitful and significant interaction with the enzyme.

Compound **13**, (IC_{50} = 6.4 ± 0.148 μM), a 2,4,6-trihydroxyphenyl analog, is the fourth most active structure of the series and the 2,3,4-trihydroxyl analog **14** (IC_{50} = 10.5 ± 0.51 μM) was found to be slightly less active.

3. Materials and methods

Phosphodiesterase from Bothrops atrox crude dried venom (P4631), substrate bis(*p*-nitrophenyl) phosphate sodium salt (N-3002), and magnesium acetate were purchased from Sigma, while ammonium acetate was from Merck. Tris buffer was a product from Scharlau (TR 0423). The standard inhibitor EDTA was purchased from Avon Chem limited.

3.1. In vitro phosphodiesterase assay

The phosphodiesterase I inhibition assay was performed using snake venom according to the previously reported method[40] with minute variations. Briefly, 33 mM tris–HC1 buffer (pH 8.8), 30 mM magnesium acetate with enzyme concentration of 0.000742 U/well, and 0.33 mM bis(*p*-nitropheny1) phosphate (Sigma N-3002) were taken as substrate. EDTA with IC_{50} ± SEM = 274 ± 0.007 μM was used as positive control.[41,42]

After a pre-incubation period of 30 min, the enzyme with the test samples was observed spectrophotometrically for the enzyme activity on a microtitre plate reader at 37 °C by following release of *p*-nitrophenol from *p*-nitrophenyl phosphate registering the O.D/min at 410 nm. The assay was processed in triplicate.[43]

4. Experimental

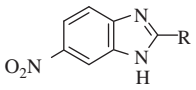
4.1. General experimental

Melting points were determined on a Büchi 434 melting point apparatus and are uncorrected. NMR experiments were performed on Avance Bruker AM 300, 400, and 500 MHz instruments. Electron impact mass spectra (EI MS) were recorded on a Finnigan MAT-311A, Germany. Thin layer chromatography (TLC) was performed on pre-coated silica gel aluminum plates (Kieselgel 60, 254, E. Merck, Germany). Chromatograms were visualized by UV at 254 and 365 nm.

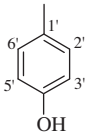
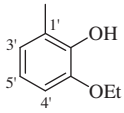
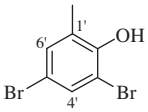
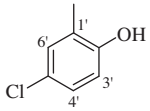
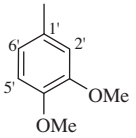
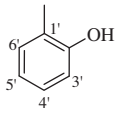
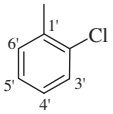
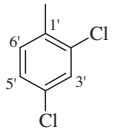
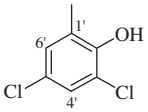
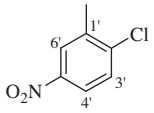
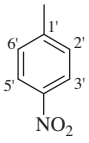
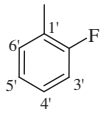
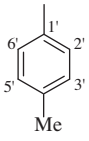
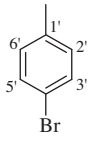
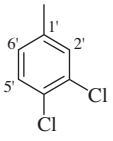
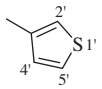
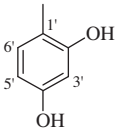
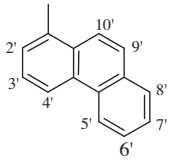
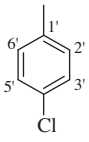
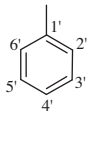
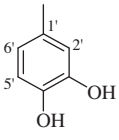
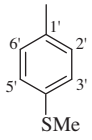
4.2. Synthetic procedure of compounds 1–30

6-Nitrobenzimidazole derivatives were synthesized by refluxing 4-nitrophenylenediamine (3.12 equiv) and substituted aromatic aldehydes (3.16 equiv) in 15 mL DMSO in a round bottom flask (100 mL). Sodium metabisulfite ($Na_2S_2O_5$) (3 mmol) was added to the stirring solutions. The reaction mixture was heated until the reaction completion and the progress was observed by TLC. When the reactions were completed they were cooled to room temperature. Addition of water (30 mL) resulted in the precipitation of crude solid residues. The crude mixtures were chromatographed on silica gel columns to afford the 6-nitrobenzimidazole derivatives **1–30** in high yields. The structures of the synthesized compounds **1–30** were confirmed by ^{1}H NMR and EI mass spectroscopy.

Table 1
Phosphodiesterase activity of 6-nitrobenzimidazole derivatives **1–30**

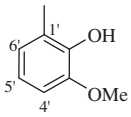
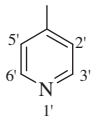
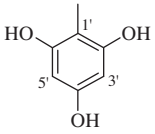
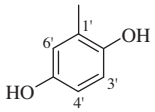
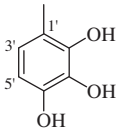
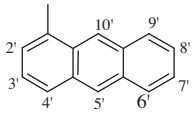
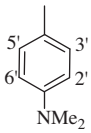
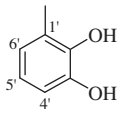


1-30

Compound No.	R	IC_{50} (μM ± SEM)[a]	Compound No.	R	IC_{50} (μM ± SEM)[a]
1		2.4 ± 0.049	**16**		NA[b]
2		NA[b]	**17**		NA[b]
3		63.1 ± 1.48	**18**		NA[b]
4		NA[b]	**19**		NA[b]
5		294.0 ± 16.7	**20**		NA[b]
6		153.2 ± 5.6	**21**		NA[b]
7		NA[b]	**22**		NA[b]
8		NA[b]	**23**		NA[b]
9		11.49 ± 0.08	**24**		NA[b]
10		120.0 ± 4.47	**25**		NA[b]
11		5.7 ± 0.113	**26**		NA[b]

(*continued on next page*)

Table 1 (*continued*)

Compound No.	R	IC_{50} (μM ± SEM)[a]	Compound No.	R	IC_{50} (μM ± SEM)[a]
12		NA[b]	**27**		NA[b]
13		6.4 ± 0.148	**28**		NA[b]
14		10.5 ± 0.51	**29**		NA[b]
15		NA[b]	**30**		1.5 ± 0.043
EDTA[c]		274 ± 0.007			

[a] SEM is the standard error of the mean.
[b] NA–not active.
[c] EDTA standard inhibitor for phosphodiesterase activity.

4.2.1. 4-(6-Nitro-1*H*-benzimidazol-2-yl)phenol (1)

Yield: 0.65 g (81%); ^{1}H NMR: (300 MHz, DMSO-d_6): δ_H 8.38 (d, 1H, $J_{7,5}$ = 2.0 Hz, H-7), 8.10 (dd, 1H, $J_{5,7}$ = 2.0, $J_{5,4}$ = 9.0 Hz, H-5), 8.04 (d, 2H, $J_{2',3'/6',5'}$ = 8.7 Hz, H-2′/6′), 7.69 (d, 1H, $J_{4,5}$ = 9.0 Hz, H-4), 6.94 (d, 2H, $J_{3',2'/5',6'}$ = 8.7 Hz, H-3′/5′); MS: *m/z* (rel. abund.%), 255 (M^+, 100), 209 (37), 182 (25). Anal. Calcd for $C_{13}H_9N_3O_3$: C, 61.18; H, 3.55, N, 16.46; Found: C, 60.83, H, 3.34, N, 16.73.

4.2.2. 2,4-Dibromo-6-(6-nitro-1*H*-benzimidazol-2-yl)phenol (2)

Yield: 0.82 g (82%); ^{1}H NMR: (300 MHz, DMSO-d_6): δ_H 8.59 (br s, 1H, H-7), 8.34 (d, 1H, $J_{4',6'}$ = 2.5 Hz, H-4′), 8.21 (dd, 1H, $J_{5,7}$ = 2.5, $J_{5,4}$ = 9.0 Hz, H-5), 7.98 (d, 1H, $J_{6',4'}$ = 2.5 Hz, H-6′), 7.88 (d, 1H, $J_{4,5}$ = 9.0 Hz, H-4); MS: *m/z* (rel. abund.%), 412 (M^+, 100), 367 (36), 169 (10). Anal. Calcd for $C_{13}H_7Br_2N_3O_3$: C, 37.80; H, 1.71, N, 10.17; Found: C, 37.83, H, 1.14, N, 10.13.

4.2.3. 2-(3,4-Dimethoxyphenyl)-6-nitro-1*H*-benzimidazole (3)

Yield: 0.79 g (85%); ^{1}H NMR: (300 MHz, MeOD): δ_H 8.47 (d, 1H, $J_{7,5}$ = 2.0 Hz, H-7), 8.18 (dd, 1H, $J_{5,7}$ = 2.5, $J_{5,4}$ = 9.0 Hz, H-5), 7.70 (m, 3H, H-4/2′/6′), 7.15 (d, 1H, $J_{5',6'}$ = 8.5 Hz, H-5′), 3.96 (s, 3H, –OMe), 3.92 (s, 3H, –OMe); MS: *m/z* (rel. abund.%), 299 (M^+, 100), 253 (30), 210 (49). Anal. Calcd for $C_{15}H_{13}N_3O_4$: C, 60.20; H, 4.35, N, 14.04; Found: C, 61.02, H, 4.44, N, 13.53.

4.2.4. 2-(2-Chlorophenyl)-6-nitro-1*H*-benzimidazole (4)

Yield: 0.688 g (80%); ^{1}H NMR: (400 MHz, DMSO-d_6): δ_H 8.54 (br s, 1H, H-7), 8.16 (d, 1H, $J_{5,4}$ = 8.5 Hz, H-5), 7.94 (d, 1H, $J_{6',5'}$ = 7.0 Hz, H-6′), 7.81 (d, 1H, $J_{4,5}$ = 8.5 Hz, H-4), 7.70 (d, 1H, $J_{3',4'}$ = 7.0 Hz, H-3′), 7.70 (m, 2H, H-4′/5′); MS: *m/z* (rel. abund.%), 273 (M^+, 100), 243 (28), 227 (29). Anal. Calcd for $C_{13}H_8ClN_3O_2$: C, 57.05; H, 2.95, N, 15.35; Found: C, 56.92, H, 3.04, N, 15.33.

4.2.5. 2,4-Dichloro-6-(6-nitro-1*H*-benzimidazol-2-yl)phenol (5)

Yield: 0.83 g (83%); ^{1}H NMR: (300 MHz, DMSO-d_6): δ_H 8.58 (d, 1H, $J_{7,5}$ = 1.5 Hz, H-7), 8.18 (m, 2H, H-5/6′), 7.84 (d, 1H, $J_{4,5}$ = 9.0 Hz, H-4), 7.72 (d, 1H, $J_{4',6'}$ = 2.0 Hz, H-4′); MS: *m/z* (rel. abund.%), 323 (M^+, 100), 293 (23), 277 (47). Anal. Calcd for $C_{13}H_7Cl_2N_3O_3$: C, 48.17; H, 2.18, N, 12.96; Found: C, 48.12, H, 2.14, N, 12.93.

4.2.6. 6-Nitro-2-(4-nitrophenyl)-1*H*-benzimidazole (6)

Yield: 0.71 g (80%); ^{1}H NMR: (300 MHz, MeOD): δ_H 8.59 (d, 1H, $J_{7,5}$ = 2.0 Hz, H-7), 8.44 (d, 2H, $J_{3',2'/5',6'}$ = 9.0 Hz, H-3′/5′), 8.38, (d, 2H, $J_{2',3'/6',5'}$ = 9.0 Hz, H-2′/6′), 8.25 (dd, 1H, $J_{5,7}$ = 2.0, $J_{5,4}$ = 9.0 Hz, H-5), 7.79 (d, 1H, $J_{4,5}$ = 9.0 Hz, H-4); MS: *m/z* (rel. abund.%), 284 (M^+, 100), 238 (15), 192 (15). Anal. Calcd for $C_{13}H_8N_4O_4$: C, 54.93; H, 2.84, N, 19.71; Found: C, 53.98, H, 2.82, N, 19.72.

4.2.7. 2-(4-Methylphenyl)-6-nitro-1*H*-benzimidazole (7)

Yield: 0.61 g (81%); ^{1}H NMR: (300 MHz, DMSO-d_6): δ_H 8.44 (d, 1H, $J_{7,5}$ = 2.0 Hz, H-7), 8.12 (m, 3H, H-5/2′/6′), 7.72 (d, 1H, $J_{4,5}$ = 9.0 Hz, H-4), 7.40 (d, 2H, $J_{3',2'/5',6'}$ = 8.0 Hz, H-3′/5′); MS: *m/z* (rel. abund.%), 253 (M^+, 100), 209 (23), 180 (13). Anal. Calcd for $C_{13}H_8N_4O_4$: C, 66.40; H, 4.38, N, 16.59; Found: C, 66.38, H, 4.32, N, 16.42.

4.2.8. 4.10.2-(3,4-Dichlorophenyl)-6-nitro-1*H*-benzimidazole (8)

Yield: 0.76 g (79%); ^{1}H NMR: (300 MHz, MeOD): δ_H 8.58 (d, 1H, $J_{7,5}$ = 2.0 Hz, H-7), 8.25 (dd, 1H, $J_{5,7}$ = 2.5, $J_{5,4}$ = 9.0 Hz, H-5), 7.90 (d, 1H, $J_{5',6'}$ = 8.4 Hz, H-5′), 7.78 (d, 1H, $J_{4,5}$ = 9.0 Hz, H-4), 7.74 (d, 1H, $J_{2',6'}$ = 2.0 Hz, H-2′), 7.56 (dd, 1H, $J_{6',2'}$ = 2.0, $J_{6',5'}$ = 8.4 Hz, H-6′); MS: *m/z* (rel. abund.%), 307 (M^+, 100), 277 (33), 261 (33). Anal. Calcd for $C_{13}H_7Cl_2N_3O_2$: C, 50.67; H, 2.29, N, 13.64; Found: C, 50.90, H, 2.27, N, 13.62.

4.2.9. 4.11.4-(6-Nitro-1*H*-benzimidazol-2-yl)-1,3-benzenediol (9)

Yield: 0.69 g (81%); ^{1}H NMR: (300 MHz, DMSO-d_6): δ_H 8.44 (br s, 1H, H-7), 8.12 (dd, 1H, $J_{5,7}$ = 2.0, $J_{5,4}$ = 9.0 Hz, H-5), 7.92 (d, 1H, $J_{6',5'}$ = 8.7 Hz, H-6′), 7.74 (d, 1H, $J_{4,5}$ = 9.0 Hz, H-4), 6.45 (m, 2H, H-3′/5′), 7.56 (dd, 1H, $J_{6',2'}$ = 2.0, $J_{6',5'}$ = 8.4 Hz, H-6′); MS: *m/z* (rel. abund.%), 271 (M^+, 100), 225 (88). Anal. Calcd for $C_{13}H_8N_3O_4$: C, 57.57; H, 3.34, N, 15.49; Found: C, 57.68, H, 3.82, N, 15.42.

4.2.10. 2-(4-Chlorophenyl)-6-nitro-1*H*-benzimidazole (10)

Yield: 0.73 g (85%); ^{1}H NMR: (300 MHz, DMSO-d_6): δ_H 8.47 (br s, 1H, H-7), 8.21 (d, 2H, $J_{3',2'/5',6'}$ = 8.5 Hz, H-3′/5′), 8.13 (dd, 1H, $J_{5,7}$ = 2.5, $J_{5,4}$ = 9.0 Hz, H-5), 7.76 (d, 1H, $J_{4,5}$ = 9.0 Hz, H-4), 7.69 (d, 2H, $J_{2',3'/6',5'}$ = 8.5 Hz, H-2′/6′); MS: *m/z* (rel. abund.%), 273 (M^+, 100), 227 (70), 200 (33). Anal. Calcd for $C_{13}H_8ClN_3O_2$: C, 57.05; H, 2.95, N, 15.35; Found: C, 57.08, H, 2.92, N, 15.36.

4.2.11. 4-(6-Nitro-1*H*-benzimidazol-2-yl)-1,2-benzenediol (11)

Yield: 0.69 g (81%); ^{1}H NMR: (300 MHz, DMSO-d_6): δ_H 8.37 (d, 1H, $J_{7,5}$ = 2.0 Hz, H-7), 8.08 (dd, 1H, $J_{5,7}$ = 2.0, $J_{5,4}$ = 9.0 Hz, H-5), 7.66 (d, 1H, $J_{5',6'}$ = 8.7 Hz, H-5′), 7.63 (d, 1H, $J_{2',6'}$ = 2.0 Hz, H-2′), 7.50 (dd, 1H, $J_{6',2'}$ = 2.0, $J_{6',5'}$ = 8.0 Hz, H-6′), 6.90 (d, 1H, $J_{4,5}$ = 9.0 Hz, H-4); MS: *m/z* (rel. abund.%), 271 (M^+, 100), 241 (38), 225 (83). Anal. Calcd for $C_{13}H_9N_3O_4$: C, 57.57; H, 3.34, N, 15.49; Found: C, 57.58, H, 3.33, N, 15.46.

4.2.12. 2-Methoxy-6-(6-nitro-1*H*-benzimidazol-2-yl)phenol (12)

Yield: 0.68 g (77%); ^{1}H NMR: (300 MHz, DMSO-d_6): δ_H 8.46 (d, 1H, $J_{7,5}$ = 2.0 Hz, H-7), 8.05 (dd, 1H, $J_{5,7}$ = 2.0, $J_{5,4}$ = 9.0 Hz, H-5), 7.73 (d, 1H, $J_{4',5'}$ = 8.0 Hz, H-4′), 7.70 (d, 1H, $J_{4,5}$ = 9.0 Hz, H-4), 7.06 (d, 1H, $J_{6',5'}$ = 8.0 Hz, H-6′), 7.50 (t, 1H, $J_{5'/4',6'}$ = 8.0 Hz, H-5′), 3.82 (s, 3H, -OMe); MS: *m/z* (rel. abund.%), 285 (M^+, 100), 267 (82), 196 (57). Anal. Calcd for $C_{14}H_{11}N_3O_2$: C, 58.95; H, 3.85, N, 14.73; Found: C, 58.98, H, 3.82, N, 14.76.

4.2.13. 2-(6-Nitro-1*H*-benzimidazol-2-yl)-1,3,5-benzenetriol (13)

Yield: 0.72 g (81%); ^{1}H NMR: (300 MHz, MeOD): δ_H 8.37 (d, 1H, $J_{7,5}$ = 2.0 Hz, H-7), 8.14 (dd, 1H, $J_{5,7}$ = 2.0, $J_{5,4}$ = 9.0 Hz, H-5), 7.68 (d, 1H, $J_{4,5}$ = 9.0 Hz, H-4), 6.00 (s, 2H, H-3′/5′); MS: *m/z* (rel. abund.%), 287 (M^+, 100), 241 (26), 203 (30). Anal. Calcd for $C_{13}H_9N_3O_5$: C, 54.36; H, 3.16, N, 14.63; Found: C, 54.35, H, 3.12, N, 14.66.

4.2.14. 4-(6-Nitro-1*H*-benzimidazol-2-yl)-1,2,3-benzenetriol (14)

Yield: 0.75 g (84%); ^{1}H NMR: (400 MHz, DMSO-d_6): δ_H 8.45 (br s, 1H, H-7), 8.13 (dd, 1H, $J_{5,7}$ = 2.5, $J_{5,4}$ = 9.0 Hz, H-5), 7.73 (d, 1H, $J_{6',5'}$ = 8.5 Hz, H-6′), 7.41 (d, 1H, $J_{4,5}$ = 9.0 Hz, H-4), 7.73 (d, 1H, $J_{5',6'}$ = 8.5 Hz, H-5′); MS: *m/z* (rel. abund.%), 287 (M^+, 100), 241 (69), 212 (13). Anal. Calcd for $C_{13}H_9N_3O_5$: C, 54.36; H, 3.16, N, 14.63; Found: C, 54.35, H, 3.12, N, 14.66.

4.2.15. *N,N*-Dimethyl-4-(6-nitro-1*H*-benzimidazol-2-yl)aniline (15)

Yield: 0.37 g (82%); ^{1}H NMR: (400 MHz, DMSO-d_6): δ_H 8.33 (br s, 1H, H-7), 8.06 (dd, 1H, $J_{5,7}$ = 2.0, $J_{5,4}$ = 9.0 Hz, H-5), 8.04 (d, 2H, $J_{2',3'/6',5'}$ = 8.8 Hz, H-2′/6′), 7.63 (d, 1H, $J_{4,5}$ = 9.0 Hz, H-4), 6.94 (d, 2H, $J_{3',2'/5',6'}$ = 8.8 Hz, H-3′/5′), 3.26 (s, 3H, NCH_3), 3.01 (s, 3H, NCH_3); MS: *m/z* (rel. abund.%), 282 (M^+, 100), 236 (73). Anal. Calcd for $C_{15}H_{14}N_4O_2$: C, 63.82; H, 5.00, N, 19.85; Found: C, 64.01, H, 4.97, N, 19.86.

4.2.16. 2-Ethoxy-6-(6-nitro-1*H*-benzimidazol-2-yl)phenol (16)

Yield: 0.73 g (78%); ^{1}H NMR: (400 MHz, DMSO-d_6): δ_H 8.53 (br s, 1H, H-7), 8.16 (dd, 1H, $J_{5,7}$ = 2.5, $J_{5,4}$ = 9.0 Hz, H-5), 7.81 (d, 1H, $J_{4,5}$ = 9.0 Hz, H-4), 7.68 (d, 1H, $J_{6',5'}$ = 8.0 Hz, H-6′),7.14 (d, 1H, $J_{4',5'}$ = 8.0 Hz, H-4′), 6.97 (t, 1H, $J_{5'/4',6'}$ = 8.0 Hz, H-5′), 4.10 (q, 2H, J = 7.0 Hz, -OCH_2), 1.37 (t, 3H, J = 7.0 Hz, -CH_3); MS: *m/z* (rel. abund.%), 299 (M^+, 78), 284 (100), 196 (48). Anal. Calcd for $C_{15}H_{13}N_3O_4$: C, 60.20; H, 4.38, N, 14.04; Found: C, 60.21, H, 4.37, N, 14.06.

4.2.17. 4-Chloro-2-(6-nitro-1*H*-benzimidazol-2-yl)phenol (17)

Yield: 0.73 g (81%); ^{1}H NMR: (300 MHz, DMSO-d_6): δ_H 8.53 (d, 1H, $J_{7,5}$ = 2.0 Hz, H-7), 8.16 (m, 2H, H-5/6′), 7.82 (d, 1H, $J_{4,5}$ = 9.0 Hz, H-4), 7.66 (dd, 1H, $J_{4',6'}$ = 2.5, $J_{4',5'}$ = 8.7 Hz, H-4′), 7.11 (d, 1H, $J_{5',4'}$ = 8.7 Hz, H-5′); MS: *m/z* (rel. abund.%), 289 (M^+, 100), 243 (53), 179 (09). Anal. Calcd for $C_{13}H_8ClN_3O_3$: C, 53.90; H, 2.78, N, 14.15; Found: C, 53.91, H, 2.77, N, 14.52.

4.2.18. 2-(6-Nitro-1*H*-benzimidazol-2-yl)phenol (18)

Yield: 0.66 g (82%); ^{1}H NMR: (300 MHz, DMSO-d_6): δ_H 8.53 (br s, 1H, H-7), 8.16 (m, 2H, H-5/6′), 7.82 (d, 1H, $J_{4,5}$ = 9.0 Hz, H-4), 7.43 (t, 1H, $J_{4'/5',6'}$ = 7.5 Hz, H-4′), 7.06 (m, 2H, H-3′/5′); MS: *m/z* (rel. abund.%), 255 (M^+, 100), 209 (94), 182 (12). Anal. Calcd for $C_{13}H_9N_3O_4$: C, 61.18; H, 4.38, N, 14.04; Found: C, 61.19, H, 4.37, N, 14.06.

4.2.19. 2-(2,4-Dichlorophenyl)-6-nitro-1*H*-benzimidazole (19)

Yield: 0.78 g (81%); ^{1}H NMR: (300 MHz, DMSO-d_6): δ_H 8.55 (d, 1H, $J_{7,5}$ = 1.5 Hz, H-7), 8.18 (dd, 1H, $J_{5,7}$ = 2.5, $J_{5,4}$ = 9.0 Hz, H-5), 7.98 (d, 1H, $J_{6',5'}$ = 8.5 Hz, H-6′), 7.90 (d, 1H, $J_{3',5'}$ = 2.0 Hz, H-3′), 7.41 (d, 1H, $J_{4,5}$ = 9.0 Hz, H-4), 7.73 (d, 1H, $J_{5',3'}$ = 2.0, $J_{5',6'}$ = 8.5 Hz, H-5′); MS: *m/z* (rel. abund.%), 307 (M^+, 100), 261 (49), 159 (42). Anal. Calcd for $C_{13}H_7Cl_2N_3O_3$: C, 50.67; H, 2.29, N, 13.64; Found: C, 50.66, H, 2.27, N, 13.62.

4.2.20. 2-(2-Chloro-5-nitrophenyl)-6-nitro-1*H*-benzimidazole (20)

Yield: 0.77 g (77%); ^{1}H NMR: (300 MHz, DMSO-d_6): δ_H 8.79 (d, 1H, $J_{6',4'}$ = 2.5, H-6′), 8.60 (d, 1H, $J_{7,5}$ = 1.5 Hz, H-7), 8.41 (dd, 1H, $J_{5,7}$ = 2.5, $J_{5,4}$ = 9.0 Hz, H-5), 7.73 (dd, 1H, $J_{4',6'}$ = 2.0, $J_{4',3'}$ = 8.5 Hz, H-4′), 8.01 (d, 1H, $J_{3',4'}$ = 8.5 Hz, H-3′), 7.88 (d, 1H, $J_{4,5}$ = 9.0 Hz, H-4); MS: *m/z* (rel. abund.%), 318 (M^+, 100), 272 (75), 226 (47). Anal. Calcd for $C_{13}H_7ClN_4O_4$: C, 49.00; H, 2.21, N, 17.58; Found: C, 48.91, H, 2.27, N, 17.52.

4.2.21. 2-(2-Fluorophenyl)-6-nitro-1*H*-benzimidazole (21)

Yield: 0.645 g (79%); ^{1}H NMR: (400 MHz, DMSO-d_6): δ_H 8.52 (d, 1H, $J_{7,5}$ = 2.0 Hz, H-7), 8.27 (td, 1H, $J_{4',6'}$ = 1.5, $J_{4'/3',5'}$ = 8.5 Hz, H-4′), 8.14 (dd, 1H, $J_{5,7}$ = 2.5, $J_{5,4}$ = 9.0 Hz, H-5), 7.81 (d, 1H, $J_{4,5}$ = 9.0 Hz, H-4); 7.62 (m, 1H, H-3′), 7.48 (m, 2H, H-5′/6′); MS: *m/z* (rel. abund.%), 257 (M^+, 100), 211 (57), 184 (34). Anal. Calcd for $C_{13}H_8FN_3O_2$: C, 60.70; H, 3.13, N, 16.34; Found: C, 60.60, H, 3.14, N, 16.32.

4.2.22. 2-(4-Bromophenyl)-6-nitro-1*H*-benzimidazole (22)

Yield: 0.79 g (80%); ^{1}H NMR: (300 MHz, DMSO-d_6): δ_H 8.47 (br s, 1H, H-7), 8.15 (d, 2H, $J_{3',2'/5',6'}$ = 8.5 Hz, H-3′/5′), 8.13 (dd, 1H, $J_{5,7}$ = 2.5, $J_{5,4}$ = 9.0 Hz, H-5), 7.80 (d, 2H, $J_{2',3'/6',5'}$ = 8.5 Hz, H-2′/6′), 7.76 (d, 1H, $J_{4,5}$ = 9.0 Hz, H-4); MS: *m/z* (rel. abund.%), 317 (M^+, 100), 289 (29), 192 (21). Anal. Calcd for $C_{13}H_8BrN_3O_2$: C, 49.08; H, 2.53, N, 13.21; Found: C, 49.01, H, 2.51, N, 13.22.

4.2.23. 6-Nitro-2-(3-thienyl)-1*H*-benzimidazole (23)

Yield: 0.65 g (84%); ^{1}H NMR: (300 MHz, DMSO-d_6): δ_H 8.44 (d, 1H, $J_{7,5}$ = 2.1, H-7), 8.38 (m, 1H, H-2′), 8.12 (dd, 1H, $J_{5,7}$ = 2.1, $J_{5,4}$ = 8.7 Hz, H-5), 7.79 (m, 3H, H-4/4′/5′);MS: *m/z* (rel. abund.%), 245 (M^+, 100), 199 (61), 172 (37). Anal. Calcd for $C_{11}H_7N_3O_2S$: C, 53.87; H, 2.88, N, 17.13; Found: C, 53.91, H, 2.77, N, 14.52.

4.2.24. 6-Nitro-2-(1-phenanthryl)-1*H*-benzimidazole (24)

Yield: 0.85 g (86%); ^{1}H NMR: (300 MHz, DMSO-d_6): δ_H 8.98 (d, 2H, $J_{4',3'/5',6'}$ = 8.1, Hz, H-4′/5′), 8.95 (d, 1H, $J_{2',3'}$ = 8.4 Hz, H-2′), 8.60 (br s, 1H, H-7), 8.44 (m, 1H, H-10′), 8.20 (dd, 1H, $J_{5,7}$ = 2.1, $J_{5,4}$ = 8.7 Hz, H-5), 8.15 (d, 1H, $J_{9',10'}$ = 8.2 Hz, H-9′), 7.78 (m, 5H, H-4/3′/6′/7′/8′); MS: *m/z* (rel. abund.%), 339 (M^+, 100), 292 (86), 203 (25). Anal. Calcd for $C_{21}H_{13}N_3O_2$: C, 74.33; H, 3.86, N, 12.38; Found: C, 73.98, H, 3.87, N, 12.36.

4.2.25. 6-Nitro-2-phenyl-1*H*-benzimidazole (25)

Yield: 0.57 g (76%); ^{1}H NMR: (400 MHz, DMSO-d_6): δ_H 8.46 (d, 1H, $J_{7,5}$ = 2.0, H-7), 8.21 (dd, 2H, $J_{5,7/2',4'}$ = 2.0, $J_{5,4/2',3'}$ = 8.0 Hz, H-5/2′), 8.12 (dd, 1H, $J_{6',4'}$ = 2.4, $J_{6',5'}$ = 9.0 Hz, H-6′), 7.75 (d, 1H,

$J_{4,5}$ = 9.0 Hz, H-4), 7.58 (m, 3H, H-3′/4′/5′);MS: *m/z* (rel. abund.%), 239 (M^+, 100), 209 (38), 166 (19). Anal. Calcd for $C_{13}H_9N_3O_2$: C, 65.27; H, 3.79, N, 17.56; Found: C, 64.97, H, 3.77, N, 17.55.

4.2.26. Methyl 4-(6-nitro-1*H*-benzimidazol-2-yl)phenyl sulfide (26)

Yield: 0.74 g (74%); ^{1}H NMR: (400 MHz, DMSO-d_6): δ_H 8.43 (d, 1H, $J_{7,5}$ = 2.0 Hz, H-7), 8.13(d, 2H, $J_{2',3'/6',5'}$ = 8.5 Hz, H-2′/6′), 8.10 (dd, 1H, $J_{5,7}$ = 2.0, $J_{5,4}$ = 9.0 Hz, H-5), 7.74 (d, 1H, $J_{4,5}$ = 9.0 Hz, H-4), 7.46 (d, 2H, $J_{3',2'/5',6'}$ = 8.5 Hz, H-3′/5′); MS: *m/z* (rel. abund.%), 285 (M^+, 100), 239 (80), 212 (41). Anal. Calcd for $C_{14}H_{11}N_3O_2S$: C, 58.93; H, 3.89, N, 14.73; Found: C, 58.92, H, 3.87, N, 14.72.

4.2.27. 6-Nitro-2-(4-pyridinyl)-1*H*-benzimidazole (27)

Yield: 0.61 g (81%); ^{1}H NMR: (400 MHz, DMSO-d_6): δ_H 8.81 (d, 2H, $J_{2',3'/6',5'}$ = 8.5 Hz, H-2′/6′), 8.55 (br s, 1H, H-7), 8.16 (dd, 1H, $J_{5,7}$ = 2.0, $J_{5,4}$ = 8.8 Hz, H-5), 8.12 (d, 2H, $J_{3',2'/5',6'}$ = 8.5 Hz, H-3′/5′), 7.83 (d, 1H, $J_{4,5}$ = 8.8 Hz, H-4); MS: *m/z* (rel. abund.%), 240 (M^+, 100), 194 (48), 63 (24). Anal. Calcd for $C_{12}H_8N_4O_2$: C, 60.00; H, 3.36, N, 23.32; Found: C, 60.01, H, 3.37, N, 22.82.

4.2.28. 2-(6-Nitro-1*H*-benzimidazol-2-yl)-1,4-benzenediol (28)

Yield: 0.71 g (83%); ^{1}H NMR: (400 MHz, DMSO-d_6): δ_H 9.17 (br s, 1H, NH), 8.51 (br s, 1H, H-7), 8.13 (dd, 1H, $J_{5,7}$ = 2.5, $J_{5,4}$ = 9.0 Hz, H-5), 7.79 (d, 1H, $J_{4,5}$ = 9.0 Hz, H-4), 7.53 (d, 1H, $J_{6',4'}$ = 2.5 Hz, H-6′), 6.88 (m, 2H, H-3′/4′);MS: *m/z* (rel. abund.%), 271 (M^+, 100), 225 (65). Anal. Calcd for $C_{13}H_8N_3O_4$: C, 57.57; H, 3.34, N, 15.49; Found: C, 57.68, H, 3.82, N, 15.42.

4.2.29. 2-(9-Anthryl)-6-nitro-1*H*-benzimidazole (29)

Yield: 0.83 g (84%); ^{1}H NMR: (300 MHz, DMSO-d_6): δ_H 8.90 (s, 1H, H-6′), 8.64 (br s, 1H, H-7), 8.24 (m, 3H, H-5/2′/10′), 7.88 (d, 1H, $J_{4,5}$ = 8.7 Hz, H-4), 7.67 (d, 2H, $J_{5',4'/7',8'}$ = 8.7 Hz, H-5′/7′), 7.57 (m, 4H, H-3′/4′/8′/9′); MS: *m/z* (rel. abund.%), 339 (M^+, 100), 292 (57), 146 (10). Anal. Calcd for $C_{21}H_{13}N_3O_2$: C, 74.33; H, 3.86, N, 12.38; Found: C, 73.98, H, 3.87, N, 12.36.

4.2.30. 3-(6-Nitro-1*H*-benzimidazol-2-yl)-1,2-benzenediol (30)

Yield: 0.73 g (86%); ^{1}H NMR: (300 MHz, DMSO-d_6): δ_H 8.53 (br s, 1H, H-7), 8.17 (m, 1H, H-5), 7.80 (d, 1H, $J_{4,5}$ = 8.7 Hz, H-4), 7.53 (t, 1H, $J_{5'/4',6'}$ = 8.0 Hz, H-5′), 6.93 (m, 2H, H-4′/6′); MS: *m/z* (rel. abund.%), 271 (M^+, 100), 225 (53), 196 (8). Anal. Calcd for $C_{13}H_8N_3O_4$: C, 57.57; H, 3.34, N, 15.49; Found: C, 57.68, H, 3.82, N, 15.42.

5. Conclusion

From these SAR considerations it can be concluded that the 6-nitrobenzimidazoles with the utmost phosphodiesterase inhibitory activities bear a substituted or unsubstituted 4-hydroxyphenyl or a 2,3-dihydroxyphenyl residue. From the present study it is concluded that 6-nitrobenzimidazole derivatives are a new class of compounds with an outstanding phosphodiesterase inhibitory potential. Compounds **1, 3**, **6**, **9**, **10**, **11**, **13**, **14**, and **30**, demonstrated excellent phosphodiesterase inhibitory activities which are superior to the standard EDTA and thus are potential lead molecules for further research.

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