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Design, synthesis and in vitro antiprotozoal activity of benzimidazole-pentamidine hybrids

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ABSTRACT

A series of ten novel hybrids from benzimidazole and pentamidine were prepared using a short synthetic route. Each compound was tested in vitro against the protozoa *Trichomonas vaginalis, Giardia lamblia, Entamoeba histolytica, Leishmania mexicana*, and *Plasmodium berghei*, in comparison with pentamidine and metronidazole. Some analogues showed high bioactivity in the low micromolar range ($IC_{50} < 1$ μM) against the first four protozoa, which make them significantly more potent than either standard. 1,5-bis[4-(5-methoxy-*1H*-benzimidazole-2-yl)phenoxy]pentane (**2**) was 3- and 9-fold more potent against*G. lamblia* than metronidazole and pentamidine, respectively. This compound was 23-, 108-, and 13-fold more active than pentamidine against *T. vaginalis*, *E. histolytica* and *L. mexicana*, respectively. Studying further structure–activity relationships through the use of bioisosteric substitution in these hybrids should provide new leads against protozoal diseases.

Parasitic infections, such as helminthiasis and protozoosis, are still major problem in developing countries, affecting mainly the infant population.[1–3]

For the treatment of some kinds of protozoosis such as giardiasis, trichomoniasis, and amoebiasis, metronidazole is the drug of choice.[4] Recent studies have shown that this drug has several toxic effects such as genotoxicity, gastric mucus irritation, and spermatozoid damage.[5–7] Although current drug therapy for the treatment of these infections is effective, most available drugs have significant side effects that restrict their use.[8]

Benzimidazole derivatives, such as mebendazole and albendazole, are clinically anthelminthic useful drugs. More recently, antiprotozoal activity of 2- and 5-substituted benzimidazoles has been reported.[9–14] Benzimidazole core is of a wide interest because of its diverse biological activities, and it is a well-known privileged structure in medicinal chemistry.[15]

Pentamidine, an aromatic diamidine, is used at primary stage in African trypanosomiasis, antimony-resistant leishmaniasis, and AIDS associated *Pneumocystis jirovecii* pneumonia.[16–18] Pentamidine is not effective when given orally and several toxic effects including hypotension, dysglycemia, renal, pancreatic, and hepatic toxicity have been reported.[18–22] The recent increase in life-threatening *P. jirovecii* pneumonia in patients with AIDS has resulted in a revived interest in pentamidine and related derivatives.[23]

As a part of our search for basic information about structural requirements for antiprotozoal activity, we have synthesized a series of hybrids between benzimidazole and pentamidine (Fig. 1).

The in vitro antiparasitic activity of these compounds on intestinal protozoa (*Giardia lamblia, Entamoeba histolytica*), a urogenital tract parasite (*Trichomonas vaginalis*), a red blood cell parasite (*Plasmodium berghei*) and an intracellular kinetoplastid parasite (*Leishmania mexicana)*, is also reported in this letter.

Compounds **1**–**10** were prepared by alkylation of appropriate 4-hydroxybenzaldehyde, followed by conversion of the resulting bis-aldehyde to the respective benzimidazole by treatment with 1,2-phenylenediamine adequately substituted, as shown in Scheme 1. The required substituted bis-aldehydes **11** and **12** were prepared in good yields, starting from 4-hydroxybenzaldehyde (**14**) or 4-hydroxy-3-methoxybenzaldehyde (**15**) with 1,5-dibromopentane (**13**) in presence of potassium carbonate under acetonitrile reflux.[24] The cyclocondensation reaction of **11** and **12** with the

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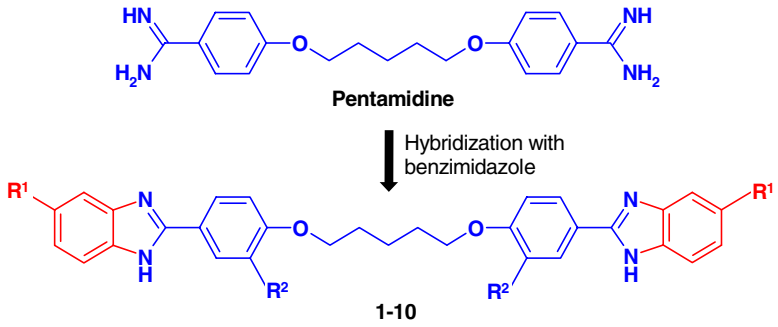
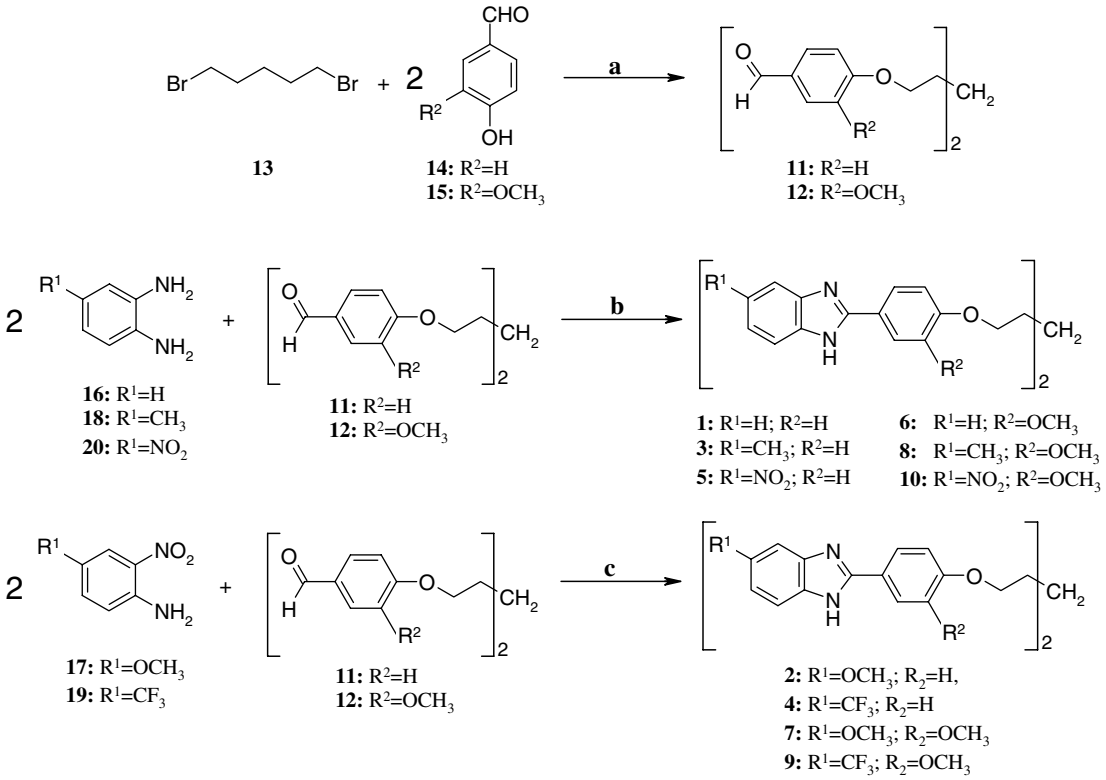


Figure 1. Drug design of hybrids **1**–**10**.



Scheme 1. Reagents and conditions: (a) MeCN, K_2CO_3, 78 °C, 17–19 h; (b) 2.1 equiv $Na_2S_2O_5$, DMF, 90 °C, 20 h; (c) 10 equiv $Na_2S_2O_4$, H_2O:EtOH, 80 °C, 10–14 h.

corresponding 4-substituted-1,2-phenylendiamines **16**, **18** and **20**, and sodium metabisulfite in DMF afforded the corresponding hybrids **1**, **3**, **5**, **6**, **8**, and **10** (Scheme 1).[25]

Compounds **2**, **4**, **7** and **9** were prepared using a one-pot reduction–cyclization reaction according to the previous described method,[26,27] where bis-aldehydes **11** and **12** reacted with the corresponding 5-substituted-2-nitroaniline (**17**, **19**) in the presence of sodium dithionite (reducing reagent) and a mixture of tap water and ethanol as solvent (Scheme 1).

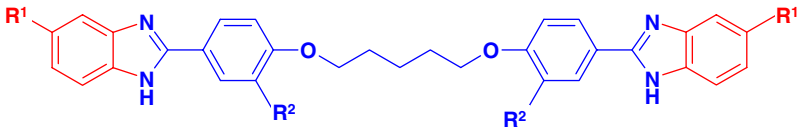
The chemical structures of the synthesized compounds were confirmed on the basis of their spectral data (NMR and mass spectra), and their purity ascertained by microanalysis. The elemental analysis was within ±0.4% of the theoretical values. Physical constants of the title compounds are shown in Table 1.

In the nuclear magnetic resonance spectra (^{1}H NMR; δ ppm), the signals of the respective protons of the compounds were verified on the basis of their chemical shifts, multiplicities and coupling constants. To elucidate the structures of the compounds, one and two-dimensional (homo- and heteronuclear correlation spectra HH and CH COSY) have been carried, in order to assign the signals of the ^{1}H and ^{13}C NMR spectra correctly.[28]

The aromatic region of the ^{1}H NMR spectrum contained an ABX pattern signals ranging from δ 7.01 to 8.34 (dd, J_{meta} = 1.2–2.2; J_{para} = 0.0–1.0 Hz), 6.84 to 8.06 (dd, J_{ortho} = 8.4–8.9; J_{meta} = 1.2–2.2 Hz), and 7.11 to 7.69 (dd, J_{meta} = 1.2–2.2; J_{para} = 0.0–1.0 Hz) attributable to H-4, H-6, and H-7, of the benzimidazole 5-substituted core, respectively. For compounds **1**–**5** the aromatic region of the ^{1}H NMR spectrum also contained an A_2B_2 pattern signals ranging from *δ 7.15 to 8.12 (d, J_{ortho}* = 8.1–8.8 Hz) and 7.09 to 8.11 (d, J_{ortho} = 8.1–8.9 Hz) attributable to the equivalents H-2′, H-6′ and H-3′, H-5′, respectively, of the benzene ring. For the compounds **6**–**10**, another ABX pattern signals ranging from *δ 7.11 to 7.83 (d, J_{meta}* = 0.0–1.6 Hz), 7.11 to 7.18 (d, J_{ortho}= 8.4–8.8 Hz) and 7.69 to 7.77 (dd, J_{ortho} = 8.4–8.8; J_{meta} = 0.0–1.6 Hz) attributable to H-2′, H-5′ and H-6′, respectively, of the benzene ring.

The new hybrids from benzimidazole and pentamidine (**1**–**10**) were tested in vitro as antiprotozoal agents. Biological assays results against the five protozoa tested are summarized in Table 1.

Table 1
Physicochemical data and antiprotozoal bioactivity of compounds **1–10**



Compound	R^1	R^2	MW	Mp (°C)	Yield (%)	IC_{50} (μM)				
						T. vaginalis	*G. lamblia*	*E. histolytica*	*P. berghei*	*L. mexicana*
1	–H	–H	488	180 (dec)	90	3.176	1.838	0.436	100(37%)[a]	1.065
2	$-OCH_3$	–H	548	185 (dec)	68	0.164	0.435	0.109	34.641	0.712
3	$-CH_3$	–H	516	215 (dec)	90	4.161	4.888	0.153	100(43%)[a]	0.368
4	$-CF_3$	–H	624	174.9 (dec)	90	4.96	9.601	7.288	6.531	>50
5	$-NO_2$	–H	578	174.3 (dec)	92	3.583	7.412	10.223	100(46%)[a]	>50
6	–H	$-OCH_3$	548	138.5 (dec)	90	6.153	12.739	5.186	165.951	>50
7	$-OCH_3$	$-OCH_3$	608	187.3 (dec)	64	6.293	5.192	0.284	23.981	>50
8	$-CH_3$	$-OCH_3$	576	168 (dec)	68	1.017	0.372	4.503	NI	>50
9	$-CF_3$	$-OCH_3$	685	125 (dec)	76	NT	NT	NT	63.091	>50
10	$-NO_2$	$-OCH_3$	638	183 (dec)	98	0.794	3.397	12.053	25.942	16.849
Pentamidine						3.815	4.079	11.801	CD[b]	9.568
Metronidazole						0.286	1.286	0.771	NT	NT

(dec), Compound melt with decomposition.
NI, no inhibition; NT, not tested.
[a] % Inhibition presented at maximum tested concentration (100 μM).
[b] CD: Cell damage, due to cytophatic effect caused by pentamidine.

Comparison was made among new compounds and the antiprotozoal drug of choice: metronidazol. In order to compare bioactivities, pentamidine was also tested. In vitro susceptibility assays were performed using a method previously described.[28,29] In general, all the screened compounds showed high bioactivity (<6.3 μM) against *T. vaginalis*. Compound **2**, with a methoxy group at position 5 of benzimidazole ring, was two times more active than metronidazole and 23-fold more potent than pentamidine. Compounds **8** ($-CH_3$) and **10** ($-NO_2$), with a methoxy group attached at position 2 of benzene ring, were 4- and 5-fold more potent than pentamidine, respectively. Compounds **1, 3–5** showed the same activity than pentamidine.

Against *G. lamblia*, compounds **2** and **8** were three and four times more active than metronidazole, respectively. Pentamidine was nine and 11-fold less active in comparison with these compounds.

Against *E. histolytica*, compounds **1–3** and **7** showed high bioactivity in the low micromolar range (<0.5 μM). They were two, seven, five, and three times more active than metronidazole, respectively. Compound **2** was 108-fold more potent than pentamidine, whereas compounds **1, 3**, and **7** were 27-, 77-, and 41-fold more active than this drug.

Cultured schizonts of *Plasmodium berghei* were used to assess antimalarial activity of all compounds synthesized and were prepared following the protocol described previously.[30] Biological assay results against *P. berghei* is also indicated in Table 1. Only compound **4**, with a $-CF_3$ at position 5 of benzimidazole ring, showed moderated antimalarial activity with IC_{50} of 6.53 μM. Figure 2 shows the optical microscopy studies from erythrocytes infected with this protozoan. Pentamidine caused marked cell damage, and the IC_{50} could not be calculated, due to the cytopathic effect presented by this drug. On the other hand, we did not observe any damage produced by screened compounds to erythrocytes. However, they reduced the number of schizonts compared with the negative control, without showing cytopathic effect.

In vitro antileishmanial assays were performed as described previously.[31] Compounds **1–3** exhibited high bioactivity. They were 9-, 13-, and 26-fold more active than pentamidine, respectively. This is a promising result since pentamidine is used in the treatment of antimony-resistant leishmaniasis.

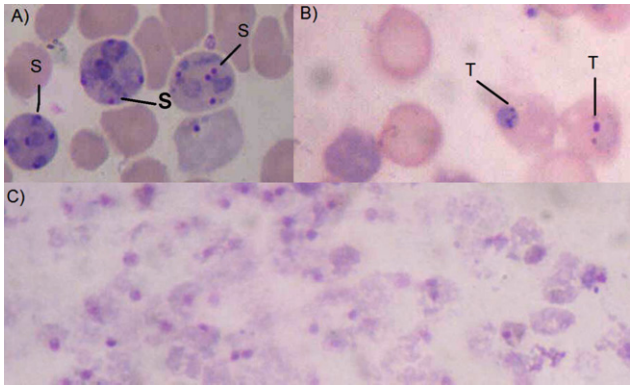


Figure 2. Optical microscopy studies: (A) Culture only with vehicle; erythrocyte infected with *P. berghei* developed schizonts (S) after 16 h. (B) Treated culture with compound **4**, showing erythrocytes infected with *P. berghei* where development of shcizont did not progress after 16 h culture. Parasite remain at the trophozoite stage (T), and no cell damage is observed. (C) Culture with pentamidine, cell damage is observed (cytopathic effect).

The bioactivity observed against these five protozoa suggests that the introduction of benzimidazole core into the pentamidine structure, and the inclusion of electron-donating moieties (Fig. 3), enhances the antiprotozoal activity.

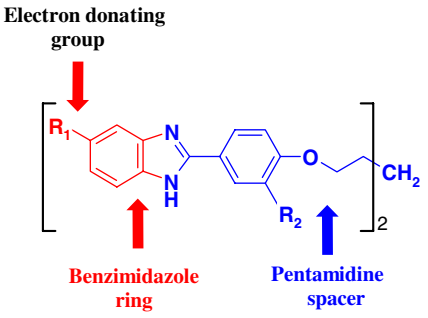


Figure 3. Proposed pharmacophoric group, based on the bioactivity pattern against *T. vaginalis, G. lamblia, E. histolytica* and *L. mexicana*.

In conclusion, we have synthesized and screened the vitro antiprotozoal activity of new hybrids of pentamidine, in which the central pentyldioxyphenyl spacer was retained and the terminal amidine groups were replaced with 5-substituted benzimidazole scaffold. The obtained results are very promising since many of the compounds showed activity comparable with the current used antiprotozoal drugs metronidazole and pentamidine, whereas compounds **1–3** exhibited even higher bioactivity, especially toward *E histolytica* and *L. mexicana*. This study demonstrated that the bioisosteric replacement of benzimidazole ring instead of amidine moiety in pentamidine, results in an enhancement of antiprotozoal bioactivity. Further optimization and pharmacokinetic characterization of this series are in progress in our laboratory.[32]

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.05.009.

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24. General method of synthesis of bis-aldehydes **11** and **12**. The corresponding 4-hydroxybenzaldehyde **14** or **15** (0.041 mol) and potassium carbonate (6.7 g, 0.049 mol) were dissolved in acetonitrile (100 mL) and the mixture was heated at 78 °C. After an hour, 1,5-dibromopentane (9.4 g, 5.6 mL, 0.041 mol) was added dropwise. TLC was used to monitor the reaction. When almost all the starting material was reacted (7–9 h), a second charge (1.9 equiv) of the corresponding 4-hydroxybenzaldehyde (0.0697 mol) and potassium carbonate (10.8 g, 0.0779 mol) was added. After the completion of the reaction (10 h +), the solvent was removed under reduced pressure and the resulting solid washed with water to extract the formed KBr. The crude solid product was then recrystallized from acetonitrile. 1,5-bis(4-carboxyaldehy dephenoxy) pentane (**11**). Yield 13.56 g (78.63%) of a crystalline white solid. Mp 79–81 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ: 1.56 (m, 2H, $OCH_2CH_2CH_2$), 1.80 (m, 4H, $OCH_2CH_2CH_2$), 4.09 (t, 4H, OCH_2), 7.11 (d, 4H, H-2, H-6, J = 8.5 Hz), 7.85 (d, 4H, H-3, H-5, J = 8.5 Hz), 9.85 (s, 2H, CHO) ppm. MS (IE) m/z (% rel. int.) 190 (70), 121 (100), 68 (49), 41 (76). 1,5-bis(2-methoxy-4-carboxyaldehy dephenoxy)pentane (**12**) Yield 5.95 g (81%) of a white solid. Mp 107.4–109 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ: 1.55 (m, 2H, $OCH_2CH_2CH_2$), 1.81 (m, 4H, $OCH_2CH_2CH_2$), 3.81 (s, 6H, 3′-OCH_3), 4.09 (t, 4H, OCH_2), 7.16 (d, 2H, H-6, J = 8.1 Hz), 7.36 (d, 2H, H-3, J = 1.5 Hz), 7.53 (dd, 2H, H-5, J = 1.47, J = 8.06 Hz), 9.82 (s, 2H, H-3) ppm. MS (IE) m/z (% rel. int.) 372 (M^+, 20), 221 (80), 152 (70), 69 (47), 41 (47).
25. General method of synthesis of 5-substituted bis(*1H*-benzimidazoles)**1, 3, 5, 6, 8**, and **10**. The corresponding bis-aldehyde **11** or **12** (0.0006 mol) and sodium metabisulfite (0.29 g, 0.0015 mol) were dissolved in 5 mL of DMF. The mixture was stirred and heated under reflux during 1 h. After this time, the appropriate 4-substituted-1,2-phenylendiamine (0.0007 mol) in DMF (3 mL) was added. The mixture was heated at 90 °C. When all starting materials were reacted (2–8 h), a second charge of the corresponding 4-substituted-1,2-phenylendiamine (0.0007 mol) was added, and the temperature maintained during 20 h. After the completion of the reaction, the mixture was cooled to room temperature and washed with tap water (20 mL). The crude solid product was filtered and recrystallized from Ethanol-acetone. 1,5-bis[4-(*1H*-benzimidazole-2-yl)phenoxy] pentane (**1**). Yield 0.723 g (90.1%) of a white solid. Mp 180 °C (dec). ^{1}H NMR (200 MHz, DMSO-d_6): δ: 1.58 (m, 2H, $OCH_2CH_2CH_2$), 1.80 (m, 4H, $OCH_2CH_2CH_2$), 4.06 (t, 4H, OCH_2), 7.10 (d, 4H, H-5′, H-3′, J = 8.79 Hz), 7.17 (dd, 4H, H-5, H-6, J = 3.3, J = 6.1), 7.55 (dd, 4H, H-4, H-7, J = 3.3, J = 6.1), 8.09 (d, 4H, H-2′, H-6′, J = 8.8) ppm. ^{13}C NMR (50 MHz, DMSO-d_6) δ: 22.25 ($OCH_2CH_2CH_2$), 28.41 ($OCH_2CH_2CH_2$), 67.72 (OCH_2), 114.46 (C-3′, C-5′), 114.90 (C-4, C-7), 121.95 (C-1′, C-5′, C-6′), 128.47 (C-2′, C-6′), 137.41 (C-3a, C-7a), 150.68 (C-2), 160.59 (C-4′) ppm. MS (FAB^+) m/z 489 $(M+H)^+$; Anal. Calcd for $C_{31}H_{28}N_4O_2$: C, 76.21; H, 5.80; N, 10.21. Found: C, 76.45; H, 5.71; N, 9.85. 1,5-bis[4-(5-methyl-*1H*-benzimidazole-2-yl) phenoxy]pentane (**3**) Yield 0.162 g (90%) of beige solid. Mp 215 °C (dec). ^{1}H NMR (400 MHz, DMSO-d_6): δ: 1.58 (m, 2H, $OCH_2CH_2CH_2$), 1.81 (m, 4H, $OCH_2CH_2CH_2$), 2.41 (s, 6H, 5-CH_3), 4.05 (t, 4H, OCH_2), 7.01 (dd, 2H, H-6, J = 1.1, J = 8.2 Hz), 7.09 (d, 4H, H-3′, H-5′, J = 8.7 Hz), 7.35 (s, 2H, H-4), 7.45 (d, 2H, H-7, J = 8.2 Hz), 8.08 (d, 4H, H-2′, H-6′, J = 8.7 Hz) ppm. ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.41 (5-CH_3), 22.31 ($OCH_2CH_2CH_2$), 28.48 ($OCH_2CH_2CH_2$), 67.65(OCH_2), 113.99 (C-4), 114.60 (C-6), 114.80 (C-3′, C-5′), 121.98 (C-1′), 124.48 (C-7), 127.97 (C-2′, C-6′), 131.24 (C-5), 137.25 (C-7a), 138.49 (C-3a), 150.76 (C-2), 160.02 (C-4′) ppm. MS (FAB^+) m/z 517 $(M+H)^+$; Anal. Calcd for $C_{33}H_{32}F_6N_4O_2$: C, 76.72; H, 6.24; N, 10.84. Found: C, 76.41; H, 6.45; N, 10.11. 1,5-bis{4-[5-nitro-*1H*-benzimidazole-2-yl] phenoxy}pentane (**5**). Yield 0.339 g (91.62%) of orange solid. Mp 174.3 °C (dec). ^{1}H NMR (200 MHz, DMSO-d_6): δ: 1.61 (m, 2H, $OCH_2CH_2CH_2$), 1.81 (m, 4H, $OCH_2CH_2CH_2$), 4.09 (t, 4H, OCH_2), 7.12 (d, 4H, H-3′, H-5′, J = 8.8 Hz), 7.69 (d, 2H, H-7, J = 9.2 Hz), 8.05 (d, 2H, H-4, J = 1.8 Hz), 8.12 (d, 4H, H-2′, H-6′, J = 8.8 Hz), 8.36 (dd, 2H, H-6, J = 1.8, J = 9.2 Hz) ppm. ^{13}C NMR (50 MHz, DMSO-d_6) δ: 22.17 ($OCH_2CH_2CH_2$), 28.29 ($OCH_2CH_2CH_2$), 67.66 (OCH_2), 114.13 (C-4), 114.9 (C-3′, C-5′), 115.28 (C-7), 117.65 (C-6), 121.13 (C-1′), 128.59 (C-2′, C-6′), 128.96 (C-3a, C-7a), 142.29 (C-5), 155.78 (C-2), 160.75 (C-4′) ppm. MS (FAB^+) m/z 579 $(M+H)^+$; Anal. Calcd for $C_{31}H_{26}N_6O_6$: C, 64.35; H, 4.53; N, 14.53. Found: C, 65.59; H, 5.02; N, 13.91. 1,5-bis[4-(*1H*-benzimidazole-2-yl)-2-methoxy-phenoxy] pentane (**6**). Yield 0.358 g (90.1%) of a white solid. Mp 138.5 °C (dec). ^{1}H NMR (200 MHz, DMSO-d_6): δ: 1.61 (m, 2H, $OCH_2CH_2CH_2$), 1.83 (m, 4H, $OCH_2CH_2CH_2$), 3.89 (s, 6H, 3′-OCH_3), 4.08 (t, 4H, OCH_2), 7.16 (dd, 2H, H-5′, J = 1.6, J = 8.4 Hz), 7.23 (dd, 4H, H-5, H-6, J = 2.8, J = 9.2 Hz), 7.61 (dd, 4H, H-4, H-7, J = 2.8, J = 9.2 Hz), 7.75 (dd, 2H, H-6′, J = 1.2, J = 8.8 Hz), 7.79 (d, 2H, H-2′, J = 1.6 Hz) ppm. ^{13}C NMR (50 MHz, DMSO-d_6) δ: 22.29 ($OCH_2CH_2CH_2$), 28.41 ($OCH_2CH_2CH_2$), 55.72 (3′-OCH_3), 67.27 (OCH_2), 110.12 (C-2′), 112.88 (C-5′), 114.39 (C-5, C-6), 119.72 (C-6′). 121.05 (C-1′), 122.392 (C-4, C-7), 137.75 (C-3a, C-7a), 149.00 (C-4′), 150.11 (C-3′), 159.83 (C-2) ppm. MS (FAB^+) m/z 549 $(M+H)^+$; Anal. Calcd for $C_{33}H_{32}N_4O_4$: C, 72.24; H, 5.88; N, 10.21. Found: C, 71.99; H, 5.93; N, 10.29. 1,5-bis[2-methoxy-4-(5-methyl-*1H*-benzimidazole-2-yl)phenoxy] pentane (**8**). Yield 0.31 g (68.4 %) of brown solid. Mp 168 °C (dec). ^{1}H NMR (200 MHz, DMSO-d_6): δ: 1.59 (m, 2H, $OCH_2CH_2CH_2$), 1.80 (m, 4H, $OCH_2CH_2CH_2$), 2.43 (s, 6H, 5-CH_3), 3.89 (s, 6H, 3′-OCH_3), 4.05 (t, 4H, OCH_2), 7.11 (d, 2H, H-7, J = 8.0 Hz), 7.17 (d, 2H, H-5′, J = 8.4 Hz), 7.41 (s, 2H, H-4), 7.52 (d, 2H, H-6, J = 8.0 Hz), 7.77 (d, 2H, H-6′, J = 10.2 Hz), 7.83 (s, 2H, H-2′) ppm. ^{13}C NMR (50 MHz, DMSO-d_6) δ: 21.95 ($OCH_2CH_2CH_2$), 22.98 (5-CH_3), 29.04

($OCH_2CH_2CH_2$), 56.44 (3′-OCH_3), 68.90 (OCH_2), 110.82 (C-2′), 114.25 (C-4), 114.46 (C-7), 114.73 (C-5′), 120.34 (C-6′), 120.70 (C-1′), 125.22 (C-6), 133.22 (C-5), 135.43 (C-7a), 136.95 (C-3a), 149.65 (C-4′), 150.68 (C-3′), 151.14 (C-2) ppm. MS (FAB^+) *m/z* 577 $(M+H)^+$; Anal. Calcd for $C_{35}H_{36}N_4O_4$: C, 72.90; H, 6.29; N, 9.72. Found: C, 73.61; H, 6.42; N, 9.55. 1,5-bis[2-methoxy-4-(5-nitro-1*H*-benzimidazole-2-yl)phenoxy] pentane (**10**). Yield 0.343 g (97.9%) of orange solid. Mp 183 °C (dec). ^1H NMR (200 MHz, DMSO-d_6) δ: 1.59 (m, 2H, $OCH_2CH_2CH_2$), 1.81 (m, 4H, $OCH_2CH_2CH_2$), 3.88 (s, 6H, 3′-OCH_3), 4.05 (t, 4H, OCH_2), 7.11 (m, 4H, H-2′, H-5′), 7.68 (d, 2H, H-7, *J* = 8.8 Hz), 7.69 (dd, 2H, H-6′, *J* = 2.2, *J* = 8.8 Hz) 8.06 (dd, 2H, H-6, *J* = 2.2, *J* = 8.8 Hz), 8.34 (d, 2H, H-4, *J* = 1.8 Hz) ppm. ^{13}C NMR (50 MHz, DMSO-d_6) δ: 22.98 ($OCH_2CH_2CH_2$), 29.05 ($OCH_2CH_2CH_2$), 56.41 (3′-OCH_3), 68.93 (OCH_2), 110.85 (C-4), 111.73 (C-2′), 113.37 (C-5′), 11.91 (C-7), 118.89 (C-6), 120.49 (C-6′), 121.19 (C-1′), 138.53 (C-3a), 142.74 (C-5), 143.38 (C-7a), 149.68 (C-4′), 151.59 (C-3′), 156.02 (C-2) ppm. MS (FAB^+) *m/z* 639 $(M+H)^+$; Anal. Calcd. for $C_{33}H_{30}N_6O_8$: C, 62.06; H, 4.73; N, 13.16. Found: C, 63.60; H, 5.00; N, 13.03.

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27. General method of synthesis of 5-substituted bis(*1H*-benzimidazoles) **2, 4, 7** and**9** via one-step reduction–cyclization reaction. A mixture of bis-aldehyde **11** or **12** (0.0006 mol), the corresponding 5-substituted-2-nitroaniline **17, 19** (0.0017 mol), and an excess of sodium dithionite (2.96 g, 0.017 mol, 10 equiv) were dissolved in a mixture of ethanol–water 2:1 (30 mL). Then the mixture was heated at 80 °C during 10–14 h. After the completion of the reaction, the solvent was removed under reduced pressure and the resulting solid washed with water. The crude solid product was then recrystallized from Ethanol–acetone. 1,5-bis[4-(5-methoxy-*1H*-benzimidazole-2-yl) phenoxy]pentane (**2**). Yield 0.12 g (68%) of yellow solid. Mp 185 °C (dec). ^1H NMR (200 MHz, DMSO-d_6) δ: 1.61 (m, 2H, $OCH_2CH_2CH_2$), 1.83 (m, 4H, $OCH_2CH_2CH_2$), 3.81 (s, 6H, 5-OCH_3), 4.11 (t, 4H, OCH_2), 6.88 (dd, 2H, H-6, *J* = 2.2, *J* = 9.3 Hz), 7.08 (d, 2H, H-4, *J* = 2.2 Hz), 7.15 (d, 4H, H-2′, H-6′, *J* = 8.8 Hz), 7.49 (d, 2H, H-7, *J* = 9.3 Hz), 8.07 (d, 4H, H-3′, H-5′, *J* = 8.8 Hz) ppm. ^{13}C NMR (50 MHz, DMSO-d_6) δ: 22.31 ($OCH_2CH_2CH_2$), 29.05 ($OCH_2CH_2CH_2$), 56.23 (5-OCH_3), 68.39 (OCH_2), 97.67 (C-4), 112.89 (C-6), 115.65 (C-3′, C-5′), 115.95 (C-7), 121.16 (C-1′), 128.86 (C-2′, C-6′), 132.62 (C-7a), 138.19 (C-3a), 150.84 (C-2), 156.81 (C-5), 161.11 (C-4′) ppm. MS (FAB^+) *m/z* 549 $(M+H)^+$; Anal. Calcd for $C_{33}H_{32}N_4O_4$: C, 72.24; H, 5.88; N, 10.21. Found: C, 72.63; H, 5.86; N, 9.35. 1,5-bis{4-[5-(trifluoromethyl)-*1H*-benzimidazole-2-yl]phenoxy} pentane (**4**). Yield 0.35 g (87.6%) of a light yellow solid. Mp 174.9 °C (dec). ^1H NMR (200 MHz, DMSO-d_6) δ: 1.58 (m, 2H, $OCH_2CH_2CH_2$), 1.79 (m, 4H, $OCH_2CH_2CH_2$), 4.06 (t, 4H, OCH_2), 7.11 (d, 4H, H-3′, H-5′, *J* = 9.3 Hz), 7.46 (dd, 2H, H-6, *J* = 8.2, *J* = 1.2 Hz), 7.70 (d, 2H, H-7, *J* = 8.2 Hz), 7.86 (s, 2H, H-4), 8.11 (d, 4H, H-2′, H-6′, *J* = 8.8 Hz) ppm. ^{13}C NMR (50 MHz, DMSO-d_6) δ: 22.17 ($OCH_2CH_2CH_2$), 28.32 ($OCH_2CH_2CH_2$), 67.73 (OCH_2), 96.86 (C-4), 112.34 (C-7), 115.01 (C-5′), 119.05 (5-CF_3), 120.5(C-6), 120.53 (C-1′), 123.17 (C-5), 128.68 (C-2′, C-6′), 132.90, (C-7a), 139.90 (C-3a), 153.51 (C-2), 160.87 (C-4′) ppm. MS (FAB^+) *m/z* 625 $(M+H)^+$; Anal. Calcd for $C_{33}H_{26}F_6N_4O_2$: C, 63.46; H, 4.20; N, 8.97. Found: C, 64.60; H, 4.55; N, 8.23. 1,5-bis[2-methoxy-4-(5-methoxy-1*H*-benzimidazole-2-yl)phenoxy] pentane (**7**). Yield 0.209 g (63.9%) of a dark red solid. Mp 187.3 °C (dec). ^1H NMR (400 MHz, DMSO-d_6) δ: 1.58 (m, 2H, $OCH_2CH_2CH_2$), 1.81 (m, 4H, $OCH_2CH_2CH_2$), 2.41 (s, 6H, 5-CH_3), 4.05 (t, 4H, OCH_2), 7.01 (dd, 2H, H-6, *J* = 1.1, *J* = 8.2 Hz), 7.09 (d, 4H, H-3′, H-5′, *J* = 8.7 Hz), 7.35 (s, 2H, H-4), 7.45 (d, 2H, H-7, *J* = 8.2 Hz), 8.08 (d, 4H, H-2′, H-6′, *J* = 8.7 Hz) ppm. ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.41 (5-CH_3), 22.31 ($OCH_2CH_2CH_2$), 28.48 ($OCH_2CH_2CH_2$), 67.65 (OCH_2), 113.99 (C-4), 114.60 (C-6), 114.80 (C-3′, C-5′), 121.98 (C-1′), 124.48 (C-7), 127.97 (C-2′, C-6′), 131.24 (C-5), 137.25 (C-7a), 138.49 (C-3a), 150.76 (C-2), 160.02 (C-4′) ppm. MS (FAB^+) *m/z* 609 $(M+H)^+$; Anal. Calcd for $C_{35}H_{36}N_4O_6$: C, 69.06; H, 5.96; N, 9.20. Found: C, 69.28; H, 5.95; N, 8.61. 1,5-bis{2-methoxy-4-[5-(trifluoromethyl)-1*H*-benzimidazole-2-yl]phenoxy}pentane (**9**). Yield 0.35 g (76%) of a light yellow solid. Mp 125 °C (dec). ^1H NMR (400 MHz, DMSO-d_6) δ: 1.56 (m, 2H, $OCH_2CH_2CH_2$), 1.80 (m, 4H, $OCH_2CH_2CH_2$), 3.85 (s, 6H, 3′-OCH_3), 4.05 (t, 4H, OCH_2), 7.15 (d, 2H, H-5′, *J* = 8.4 Hz), 7.54 (dd, 2H, H-6, *J* = 1.6, *J* = 8.8 Hz), 7.75 (d, 2H, H-7, *J* = 8.4 Hz), 7.76 (d, 2H, H-6′, *J* = 8.4 Hz), 7.77 (d, 2H, H-2′, *J* = 1.6 Hz), 7.89 (s, 2H, H-4) ppm. ^{13}C NMR (100 MHz, DMSO-d_6) δ: 22.52 ($OCH_2CH_2CH_2$), 28.29 ($OCH_2CH_2CH_2$), 55.85 (3′-OCH_3), 68.32 (OCH_2), 110.47 (C-2′), 112.05 (C-4), 112.90 (C-5′), 114.69 (C-7), 119.24 (C-5), 119.59 (C-6), 120.61 (C-6′), 123.30 (5-CF_3), 136.96 (C-3a), 138.64 (C-7a), 149.06 (C-4′), 150.99 (C-3′), 153.11 (C-2) ppm. MS (FAB^+) *m/z* 686 $(M+H)^+$; Anal. Calcd for $C_{35}H_{30}F_6N_4O_4$: C, 61.40; H, 4.42; N, 8.18. Found: C, 62.41; H, 4.45; N, 8.11.
28. For more details, see supporting information.
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32. At the same time that this manuscript was been written and submitted, another group published one compound closely related to our work, see: Mayence, A.; Pietka, A.; Collins, M. S.; Cushion, M. T.; Tekwani, B. L.; Huang, T. L.; Vanden Eynde, J. J. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2658.