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Preliminary communication

Design, synthesis, and anti-tumor evaluation of novel symmetrical bis-benzimidazoles

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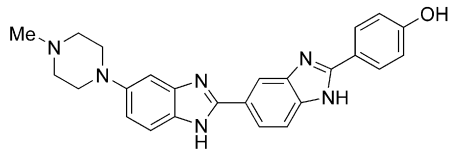
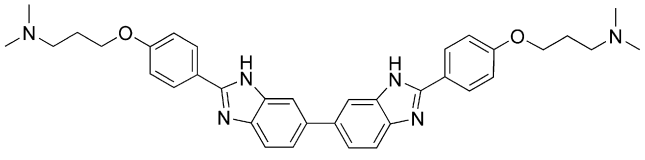
ABSTRACT

A novel symmetrical bis-benzimidazole was designed as DNA minor groove binder. Molecular modeling study showed that it could dock into the minor groove of DNA. Several derivatives were synthesized and confirmed by IR, MS, and ^{1}H NMR. All these novel compounds were screened for cytotoxic activity on SKOV-3, HeLa, and BGC-823 cell lines *in vitro*. Some compounds showed IC_{50}s in the single-digit micromolar range for cytotoxicity in several tumor cell lines.

1. Introduction



Hoechst 33258 (1)



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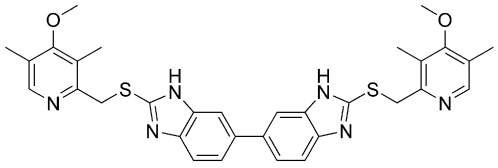

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DNA minor groove binders (MGBs) is a novel family of anti-tumor agents and some of them have entered clinical trials [1]. During the last decade, many synthetic minor groove binders have been reported, including analogues and conjugates of naturally occurring minor groove-binding agents, such as distamycin (Dst), netropsin (Net), CC-1065, anthramycin (Atm), and Hoechst 33258 [2]. Hoechst 33258 (**1**), a fluorescent reagent with a head-to-tail bis-benzimidazole structure, was initially found to be active against L1210 murine leukemia. During phase I trials in human, some responses were seen in pancreatic cancer. However, a subsequent phase II trial did not show any objective responses [3]. X-ray crystallographic and NMR studies on complexes of Hoechst 33258 with AT-containing oligonucleotides have shown that this drug fits the minor groove snugly, with the planar benzimidazole groups oriented parallel to the direction of the groove. Each inner-facing nitrogen atom hydrogen could bond in a bifurcated manner to a pair of adjacent hydrogen-bond donors on the edge of the AT base pairs [3]. Due to the synthetic accessibility and high binding affinity of Hoechst 33258, several groups have focused on a strategy to utilize the pharmacophore-like benzimidazole motif derived from Hoechst 33258 [4–7]. Among them, a series of bis-benzimidazoles were reported. Neidle et al. reported upon a series of head-to-head linked bis-benzimidazoles, such as compound **2**. This compound not only selectively recognized AT bases sequence with four base pairs but also possessed remarkably selective cytotoxicity at the micromolar level, with activity significantly greater than that shown by Hoechst 33258 in a group of ovarian carcinoma cell lines

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[8]. These symmetrical bis-benzimidazoles had favorable cellular uptake properties [9,10]. They also designed a dimeric bis-benzimidazole molecule with a high degree of sequence selectivity for AT-rich regions of DNA, which could recognize a run of almost 10 consecutive base pairs [8].

Most derivatives of Hoechst 33258 contained a phenyl group in 2-position of benzimidazole, which kept whole molecules to planar structures. To our knowledge, no literature related the alkyl chain substituents in 2-position of benzimidazole as the derivatives of Hoechst 33258 were reported so far. Occasionally, the thioether derivative of omeprazole, a compound with a substituted pyridylmethylene thioyl group at 2-position of benzimidazole, attracted our attention. On the basis of structural similarities between symmetrical bis-benzimidazole and omeprazole thioether, we combined two omeprazole thioethers into one molecule with a single bond. Therefore, a novel symmetrical bis-benzimidazole, 2,2′-di-[[(3,5-dimethyl-4-methoxy)pyrid-2-yl]methylenethio]-5,5′-bis-1*H*,1′*H*-benzimidazole (**8**) was synthesized. We hypothesized that this symmetrical bis-benzimidazole could selectively bind to the DNA minor groove. In the present paper, modeling study was performed with compound **8** and a series of derivatives were synthesized on the basis of modeling results. All of these compounds were screened for anti-tumor activity *in vitro*.

2. Molecular modeling

A molecular modeling study was carried out by the Sybyl/FlexX program to determine the binding ability of compound **8** to bind to the minor groove of DNA. Usually, electrostatic, van der Waals, hydrophobic, and hydrogen bonding forces dominate binding ability. Sequence specificity is often attributed to key hydrogen bonds between a base pair and the small molecule. Another crucial structural requirement is that the ligand has a crescent shape and can adopt an "isohelical" conformation to fit the minor groove [11]. In our model, computational calculations were performed by the Sybyl 6.9.1 package (Tripos Ltd.) [12] on a Fuel Workstation (SGI Ltd.) with the Irix 6.5 platform. The X-ray crystallographic structure of the DNA dodecamer d(CGCAAATTTGCG) with a bifurcated hydrogen-bonded conformation of the AT base pairs and its complex with distamycin A was selected from the Protein Data Bank (PDB code: 2DND) for the docking study [13]. During the docking process, the binding site atoms and the ligand atoms were set to be flexible. An incremental construction algorithm was applied to generate and minimize the possible pose. A representative model of the dodecamer complexed to compound **8** is depicted in Fig. 1 with DS Visualizer v1.5 (Accelrys Ltd.) [14]. There are van der Waals contacts between compound **8** and the narrow minor groove. Compound **8** adopts a concave shape, which exactly fits into the convex minor groove in the model. These two factors indicate that compound **8** is able to penetrate deep into the minor groove of DNA. In addition, two hydrogen bonds between compound **8** and DNA are formed between one benzimidazole NH group and T20-O2 (2.20 Å), and the other benzimidazole NH group and A18-N3 (1.87 Å). Therefore, compound **8** can effectively bind at the central AT region in the minor groove.

3. Chemistry

The synthesis of these bis-benzimidazoles is outlined in Scheme 1. The common intermediate was 2,2′-dithiol-5,5′-bis-1*H*,1′*H*-benzimidazole (**7**), which reacted with sodium hydroxide and various substituted chloromethylpyridine analogues in ethanol at reflux to synthesize the desired compounds **8**–**17**. The intermediate **7** was prepared from the condensation of tetraminobiphenyl (TAB) with disulfide carbon in ethanol. The synthesis of TAB was carried out according to Vogel's method [15], with minor improvements. The overall yield of **7** was 49%.

4. Biological investigation

Preliminary cytotoxic activity of these bis-benzimidazoles on SKOV-3, HeLa, BGC-823 cell lines was investigated *in vitro*. Briefly, tumor cell lines in RPMI1640 medium with 10% fetal bovine serum were plated in 96-well microtiter plates (4.0×10^4 cells/well), and allowed to adhere at 37 °C with 5% CO_2 for 4 h. The test compound was then added, and the cells were incubated at 37 °C with 5% CO_2 for 72 h. The cell viability was assessed using standard MTT assay [16].

5. Results and discussion

As shown in Table 1, for SKOV-3 cell line, compounds **8**, **15,** and **16** showed cytotoxicity below a concentration of 10 μM. Compounds **10** and **14** were less cytotoxic with concentrations below 50 μM. The other five compounds showed smaller effects under the same conditions. For HeLa cell line, compound **14** was as effective as cisplatin. Compounds **10**, **11**, and **16** were less potent with concentrations below 50 μM and the other six compounds were much less effective. For BGC-823, only compounds **14** and **16** showed cytotoxicity with concentrations below 50 μM.

For pyrid-2-yl compounds, bulky substituents at the 3- and 4-positions in pyridine reduce activity, but a methyl at the 5-position can evidently improve activity. For compounds **14**, **15,** and **16**, they

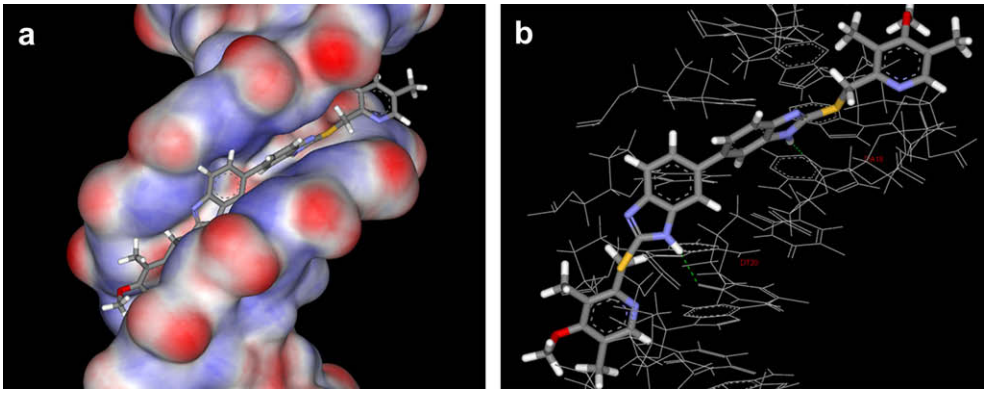
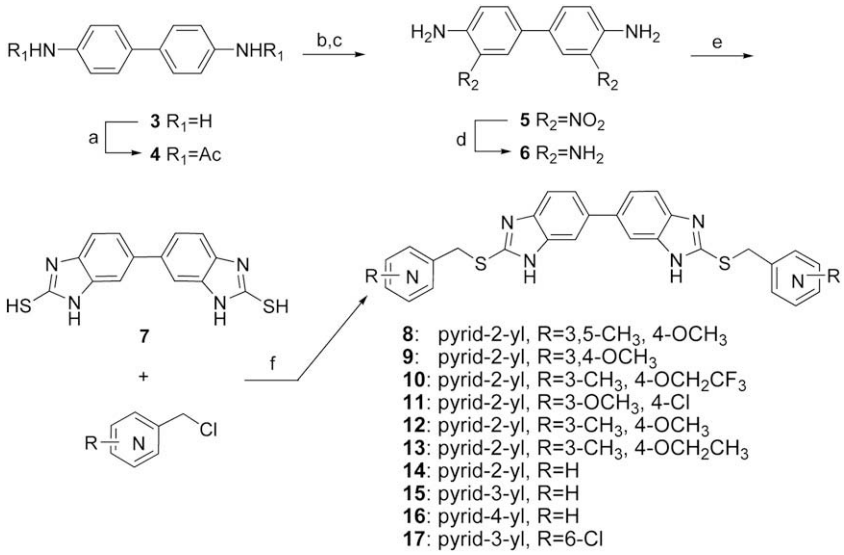


Fig. 1. Two views of compound **8** binding to the sequence d(CGCAAATTTGCG) (PDB code 2DND). (a) Close-up view of binding in the minor groove, highlighting the electrostatic potential surface of the oligonucleotide. (b) Close-up view of hydrogen bonds between compound **8** and the DNA minor groove. The hydrogen bond distances were measured to be 2.20 Å between T20-O2 and one benzimidazole NH group of compound **8**, 1.87 Å between A18-N3 and the other benzimidazole NH. Additionally, the corresponding hydrogen bond angles are 159° and 165°, respectively.



Scheme 1. *Reagents and conditions*: (a) $(CH_3CO)_2O/NEt_3$, acetone, 0 °C; (b) Conc. HNO_3, acetic acid, 0–50 °C; (c) 42% KOH aq, ethanol, reflux; (d) H_2/Pd-C, methanol, r.t.; (e) KOH, CS_2, ethanol, reflux; (f) various substituted pyridines, NaOH, ethanol, reflux.

almost shared same structures, except the position of nitrogen in pyridyl group. Pharmacological results in SKOV-3 cell line demonstrated that pyrid-3-yl and pyrid-4-yl compounds showed higher activity than pyrid-2-yl compound. However, bioevaluation in HeLa cell line, pyrid-2-yl compound was more effective than pyrid-4-yl compound, and pyrid-3-yl compound was least effective. In BGC-823 cell line, pyrid-2-yl compound was equally effective as pyrid-4-yl compound, and pyrid-3-yl compound was least effective. Nevertheless, further research is still needed in order to investigate further aspects of these effective compounds.

6. Conclusion

A series of novel bis-benzimidazoles were synthesized as novel DNA minor groove binders and evaluated for anti-tumor activity. Molecular modeling was used to predict the binding of compound **8** with the DNA minor groove, and the results showed that compound **8** could effectively fit into the minor groove. Preliminary anti-tumorogenic activities indicated that compounds **8** and **16** were most effective on SKOV-3 cell line. And compounds **14** and **16** showed broad activities on these tumor cell lines. This is the first paper focused on a series of alkyl chain substituted bis-benzimidazoles as novel DNA minor groove binders. This work enriched the structural types of bis-benzimidazoles and provided references for the further development of minor groove binders. Further studies in this area are in progress and will be reported upon in the future.

7. Experimental protocols

All commercially available reagents and solvents were employed without further purification unless specified. Solvents were dried and re-distilled prior to use using standard methods. Melting points are uncorrected and were determined in a Büchi Melting Point B-540 apparatus. Infrared spectra were recorded in a Bruker IFS-55 spectrophotometer. ^{1}H NMR spectra were recorded in Bruker ARX 300 MHz, using TMS as the internal standard if not specifically mentioned (chemical shifts in *d* values, *J* in Hz). Mass spectra were obtained on Shimazu GCMS-QP5050A and Waters Micromass® Quattro micro™ API mass spectrometer. Column chromatography was performed on silica gel H and analytical TLC data on silica gel HF254. Hydrogen gas was produced by a QL-300 hydrogen generator.

7.1. Synthesis of N,N′-diacetyl-4,4′-diamino-p-biphenyl (4)

To a stirred solution of benzidine (**3**) (5.0 g, 27.2 mmol) in acetone (50 ml) with an ice-bath, acetic anhydride (10.0 ml, 105.8 mmol) was slowly added dropwise and reaction was carried out for 3 h. Then, triethylamine (25 ml, 179.4 mmol) was added dropwise. The reaction was monitored by TLC. A white solid was obtained after filtration, and washed with acetone. After drying, the yield of compound **4** was 6.8 g (93%), m.p. 339–342 °C. GC–MS (*m*/*z*): $[M]^+$ 268.

7.2. Synthesis of 3,3′-dinitro-4,4′-diamino-p-biphenyl (5)

Compound **4** (6.8 g, 25.4 mmol) in solution with acetic acid (50 ml) was stirred in an ice-bath. Fuming nitric acid (8 ml, 171.7 mmol) was slowly added dropwise for 2 h. Then, the ice-bath was removed and the reaction mixture was incubated at r.t. for 2 h. The mixture was poured onto ice (100 ml) and stirred. The resultant yellow solid was filtered and washed with water. After drying as much as possible, the yellow compound was mixed with a solution of potassium hydroxide (7.3 g, 106.7 mmol) in water (10 ml) and ethanol (30 ml), and then heated to reflux for 4 h. Then the mixture was poured onto ice (100 ml) and stirred. The resultant red solid was filterated and washed with water. After drying, the yield of the compound **5** was 5.3 g (76%, two steps), m.p. 280–282 °C. GC–MS (*m*/*z*): $[M]^+$ 274. ^{1}H NMR (300 MHz, DMSO-d_6, ppm): 8.15 (d, $J = 2.0$, 1H, 2-H), 7.76 (dd, $J = 8.8$, $J = 2.1$, 1H, 6-H), 7.51 (s, 2H, NH_2), 7.11 (d, $J = 8.8$, 1H, 5-H).

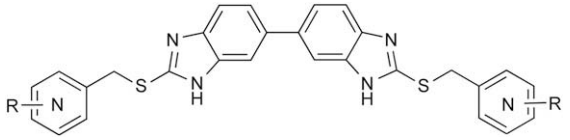
7.3. Synthesis of 3,3′,4,4′-tetraamino-p-biphenyl (6)

Hydrogen gas was passed through a stirred mixture of compound **5** (5.3 g, 19.3 mmol), Pd-C (0.3 g, 10%) and methanol (50 ml) at a flow rate of 10 ml/min for 3 h. After filtration, the filtrate was evaporated under reduced pressure to give 3.7 g of compound **6** with a yield of 89%, m.p. 174–176 °C. GC–MS (*m*/*z*): $[M]^+$ 214.

7.4. Synthesis of 2,2′-dithiol-5,5′-bis-1H,1′H-benzimidazole (7)

Compound **6** (3.7 g, 17.3 mmol) was dissolved in ethanol (10 ml) and the resulting solution was added to a mixture of potassium hydroxide (8.4 g, 122.8 mmol), water (10 ml), carbon disulfide

Table 1
Structure and anti-tumor activity of bis-benzimidazoles against SKOV-3, HeLa, and BGC-823 tumor cell lines



Compound	Substituents		IC_{50} (μM)[a]		
	Pyridyl	R	SKOV-3	HeLa	BGC-823
Taxol			0.00134	–	–
Cisplatin			–	1.6	1.3
8	Pyrid-2-yl	3,5-CH_3, 4-OCH_3	2.95	>50	>50
9	Pyrid-2-yl	3,4-OCH_3	>50	>50	>50
10	Pyrid-2-yl	3-CH_3, 4-OCH_2CF_3	22.96	18.3	>50
11	Pyrid-2-yl	3-OCH_3, 4-Cl	>50	45.4	>50
12	Pyrid-2-yl	3-CH_3, 4-OCH_3	>50	>50	>50
13	Pyrid-2-yl	3-CH_3, 4-OCH_2CH_3	>50	>50	>50
14	Pyrid-2-yl	H	38.60	7.1	16.4
15	Pyrid-3-yl	H	8.73	>50	>50
16	Pyrid-4-yl	H	2.81	32.4	11.0
17	Pyrid-3-yl	6-Cl	>50	>50	>50

[a] The drug concentrations that inhibited cell growth by 50% (IC_{50}) were determined from semilogarithmic dose-response plots.

(5.0 ml, 82.7 mmol), and ethanol (30 ml) dropwise. The solution was heated to reflux for 4 h and monitored by TLC. The mixture was evaporated under reduced pressure. Water (50 ml) was added to the residue, and conc. HCl was added to adjust the pH to about 3. The solution was filterated to yield the solid and dried. The yield of compound **7** was 4.0 g (78%), with an m.p. >400 °C. GC–MS (*m*/*z*): $[M]^+$ 298; ^{1}H NMR (300 MHz, DMSO-d_6, ppm): 12.61 (d, 2H, 1-NH and 2-SH), 7.40 (dd, $J = 8.3$, $J = 1.6$, 1H, Bz-6-H), 7.29 (s, 1H, Bz-4-H), 7.21 (d, $J = 8.3$, 1H, Bz-7-H).

7.5. General process for the synthesis of compounds **8**–**17**

After a mixture of compound **7** (1.0 g, 3.36 mmol), ethanol (30 ml), sodium hydroxide (1.0 g, 24.0 mmol), and water (10 ml) was stirred for 30 min, substituted chloromethylpyridine hydrochloride (6.90 mmol) was added and heated to reflux for 8 h. The mixture was evaporated under reduced pressure. Water (50 ml) was added to the residue, and glacial acetic acid was added to adjust the pH to about 5. The solution was filterated to yield the solid and dried. Compounds **8**–**17** were purified by gel column chromatography (fluent $CHCl_3$:CH_3OH = 90:1).

Compounds **8**–**17** were characterized as follows.

7.5.1. Data for 2,2′-di-[[(3,5-dimethyl-4-methoxy)prid-2-yl]methylenethio]-5,5′-bis-1H,1′H-benzimidazole (**8**)

Yield: 80%; m.p.: 109–112 °C; ^{1}H NMR (300 MHz, DMSO-d_6, ppm): 12.68 (1H, br s, Bz-NH), 8.19 (1H, s, Py-6-H), 7.67 (1H, s, Bz-4-H), 7.50 (1H, d, $J = 8.3$, Bz-7-H), 7.42 (1H, dd, $J = 8.3$, $J = 1.5$, Bz-6-H), 4.71 (2H, s, SCH_2), 3.72 (3H, s, OCH_3), 2.30 (3H, s, Py-3-CH_3), 2.21 (3H, s, Py-5-CH_3); ESI-MS (*m*/*z*): 597.2 $[M + H]^+$; IR (KBr, cm^{-1}): 3439, 1624, 1569, 1475, 1433, 1384, 1273, 1078, 1000, 805.

7.5.2. Data for 2,2′-di-[[(3,4-dimethoxy)prid-2-yl]methylenethio]-5,5′-bis-1H,1′H-benzimidazole (**9**)

Yield: 89%; m.p.: 202–205 °C; ^{1}H NMR (300 MHz, DMSO-d_6, ppm): 12.68 (1H, br s, Bz-NH), 8.17 (1H, d, $J = 5.7$, Py-6-H), 7.40–7.70 (3H, m, Bz-4-H, Bz-7-H, Bz-6-H), 7.09 (1H, d, $J = 5.7$, Py-5-H), 4.69 (2H, s, SCH_2), 3.89 (3H, s, OCH_3), 3.82 (3H, s, OCH_3); ESI-MS (*m*/*z*): 601.1 $[M + H]^+$; IR (KBr, cm^{-1}): 3441, 1626, 1587, 1489, 1428, 1384, 1302, 1281, 1227, 1071, 995, 788.

7.5.3. Data for 2,2′-di-[[[3-methyl-4-(2,2,2-trifluoroethyl)]prid-2-yl]methylenethio]-5,5′-bis-1H,1′H-benzimidazole (**10**)

Yield: 80%; m.p.: 177–180 °C; ^{1}H NMR (300 MHz, DMSO-d_6, ppm): 12.68 (1H, br s, Bz-NH), 8.31 (1H, d, $J = 5.7$, Py-6-H), 7.40–7.80 (3H, m, Bz-4-H, Bz-6-H, Bz-7-H), 7.09 (1H, d, $J = 5.7$, Py-5-H), 4.90 (2H, q, $J = 8.7$, OCH_2CF_3), 4.75 (2H, s, SCH_2), 2.26 (3H, s, Py-3-CH_3); ESI-MS (*m*/*z*): 705.6 $[M + H]^+$; IR (KBr, cm^{-1}): 3430, 2936, 1626, 1580, 1474, 1431, 1384, 1282, 1256, 1170, 1110, 979, 857, 803, 663, 578.

7.5.4. Data for 2,2′-di-[[(3-methoxy-4-chloro)prid-2-yl]methylenethio]-5,5′-bis-1H,1′H-benzimidazole (**11**)

Yield: 69%; m.p.: 124–127 °C; ^{1}H NMR (300 MHz, DMSO-d_6, ppm): 12.66 (1H, br s, Bz-NH), 8.25 (1H, d, $J = 5.2$, Py-6-H), 7.66 (1H, s, Bz-4-H), 7.55 (1H, d, $J = 5.2$, Py-5-H), 7.49 (1H, d, $J = 8.3$, Bz-7-H), 7.41 (1H, d, $J = 8.5$, Bz-6-H), 4.80 (2H, s, SCH_2), 3.92 (3H, s, Py-3-OCH_3); ESI-MS (*m*/*z*): 609.3 $[M + H]^+$; IR (KBr, cm^{-1}): 3438, 1626, 1564, 1461, 1401, 1384, 1281, 1233, 1082, 897, 818, 664.

7.5.5. Data for 2,2′-di-[[(3-methyl-4-methoxy)prid-2-yl]methylenethio]-5,5′-bis-1H,1′H-benzimidazole (**12**)

Yield: 79%; m.p.: 159–162 °C; ^{1}H NMR (300 MHz, DMSO-d_6, ppm): 12.67 (1H, br s, Bz-NH), 8.26 (1H, d, $J = 5.6$, Py-6-H), 7.67 (1H, s, Bz-4-H), 7.50 (1H, d, $J = 8.3$, Bz-7-H), 7.42 (1H, dd, $J = 8.3$, $J = 1.0$, Bz-6-H), 6.96 (1H, d, $J = 5.7$, Py-5-H), 4.71 (2H, s, SCH_2), 3.85 (3H, s, OCH_3), 2.21 (3H, s, Py-3-CH_3); ESI-MS (*m*/*z*): 569.5 $[M + H]^+$; IR (KBr, cm^{-1}): 3439, 1625, 1584, 1467, 1431, 1384, 1297, 1097, 807, 600.

7.5.6. Data for 2,2′-di-[[(3-methyl-4-ethoxy)prid-2-yl]methylenethio]-5,5′-bis-1H,1′H-benzimidazole (**13**)

Yield: 75%; m.p.: 125–128 °C; ^{1}H NMR (300 MHz, DMSO-d_6, ppm): 12.68 (1H, br s, Bz-NH), 8.24 (1H, d, $J = 5.6$, Py-6-H), 7.69 (1H, s, Bz-4-H), 7.52 (1H, d, $J = 8.3$, Bz-7-H), 7.44 (1H, dd, $J = 8.3$, $J = 1.2$, Bz-6-H), 6.94 (1H, d, $J = 5.7$, Py-5-H), 4.71 (2H, s, SCH_2), 4.11 (2H, q, $J = 6.9$, OCH_2CH_3), 2.21 (3H, s, Py-3-CH_3), 1.35 (3H, t, $J = 6.9$, OCH_2CH_3); ESI-MS (*m*/*z*): 597.4 $[M + H]^+$; IR (KBr, cm^{-1}): 3439, 1625, 1583, 1467, 1384, 1299, 1279, 1092, 809.

7.5.7. Data for 2,2′-di-[(prid-2-yl)methylenethio]-5,5′-bis-1H,1′H-benzimidazole (**14**)

Yield: 81%; m.p.: 91–94 °C; ^{1}H NMR (300 MHz, DMSO-d_6, ppm): 12.68 (1H, br s, Bz-NH), 8.52 (1H, d, $J = 4.7$, Py-6-H), 7.75 (1H, t, $J = 7.5$, Py-4-H), 7.60–7.72 (1H, m, Bz-4-H), 7.54 (1H, d, $J = 7.7$, Py-3-H), 7.40–7.60 (2H, m, Bz-6-H, Bz-7-H), 7.29 (1H, dd, $J = 7.2$, $J = 5.1$, Py-5-H), 4.69 (2H, s, SCH_2); ESI-MS (*m*/*z*): 481.4 $[M + H]^+$; IR (KBr, cm^{-1}): 3427, 1623, 1593, 1475, 1435, 1384, 1341, 1276, 1004, 804, 748, 585.

7.5.8. Data for 2,2′-di-[(prid-3-yl)methylenethio]-5,5′-bis-1H,1′H-benzimidazole (**15**)

Yield: 74%; m.p.: 103–106 °C; ^{1}H NMR (300 MHz, DMSO-d_6, ppm): 12.70 (1H, br s, Bz-NH), 8.66 (1H, d, $J = 1.6$, Py-2-H), 8.43 (1H, dd, $J = 4.8$, $J = 1.1$, Py-6-H), 7.87 (1H, dt, $J = 7.8$, $J = 1.6$, Py-4-H), 7.67 (1H, s, Bz-4-H), 7.51 (1H, d, $J = 8.3$, Bz-7-H), 7.43 (1H, dd, $J = 8.4$, $J = 0.8$, Bz-6-H), 7.33 (1H, dd, $J = 7.8$, $J = 4.8$, Py-5-H), 4.59 (2H, s, SCH_2); ESI-MS (*m*/*z*): 481.4 $[M + H]^+$; IR (KBr, cm^{-1}): 3423, 1623, 1479, 1430, 1384, 1343, 1278, 1126, 1030, 975, 803, 709, 668.

7.5.9. Data for 2,2′-di-[(prid-4-yl)methylenethio]-5,5′-bis-1H,1′H-benzimidazole (**16**)

Yield: 87%; m.p.: 126–129 °C; ^{1}H NMR (300 MHz, DMSO-d_6, ppm): 12.69 (1H, br s, Bz-NH), 8.45 (2H, m, $J = 4.5$, Py-2-H and Py-6-H), 7.54 (1H, s, Bz-4-H), 7.45 (2H, m, Py-3-H and Py-5-H), 7.36 (1H, d, $J = 8.3$, Bz-7-H), 7.22 (1H, dd, $J = 8.3$, $J = 1.4$, Bz-6-H), 4.52 (2H, s, SCH_2); ESI-MS (*m*/*z*): 481.4 $[M + H]^+$; IR (KBr, cm^{-1}): 3424, 3077, 1605, 1561, 1498, 1417, 1385, 1338, 1279, 1227, 1068, 1004, 977, 803, 754, 714, 665.

*7.5.10. Data for 2,2′-di-[(6-chloro-prid-3-yl)methylenethio]-5,5′-bis-1*H*,1′*H*-benzimidazole (**17**)*

Yield: 71%; m.p.: 214–217 °C; ^{1}H NMR (300 MHz, DMSO-d_6, ppm): 12.66 (1H, s, Bz-NH), 8.50 (1H, s, Py-2-H), 7.40–8.10 (5H, m, Bz-4-H, Bz-7-H, Bz-6-H, Py-4-H, and Py-5-H), 4.58 (2H, s, SCH_2); ESI-MS (m/z): 549.2 $[M+H]^+$; IR (KBr, cm^{-1}): 3446, 1626, 1587, 1461, 1435, 1384, 1280, 1106, 1027, 806, 668.

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