Journal of Applied Pharmaceutical Science Vol. 3 (4 Suppl 1), pp. S28-S37, May, 2013
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2013.34.S5
ISSN 2231-3354

Synthesis of Several Novel Symmetrical Amide-Linked Tetra-Benzimidazoles as Promising DNA and/or RNA Binders

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ARTICLE INFO

Article history:
Received on: 09/04/2013
Revised on: 22/04/2013
Accepted on: 05/05/2013
Available online: 12/05/2013

Key words:
DNA and/or RNA binders, oligobenzimidazole, tetrabenzimidazoles.

ABSTRACT

Several symmetrical tetrameric benzimidazoles (dimeric-bisbenzimidazoles) were synthesized efficiently in good yields, characterized physicochemically and also by ^{1}H-NMR ^{13}C-NMR, IR, MS, and elemental analysis. Six tetrameric symmetrical benzimidazole were synthesized by varying the amide linkage between the positions C2 and C5 of the benzimidazoles as the sites for building blocks. The benzimidazole units were coupled using (EDC/HOBt) to afford amide-linked 525_L525 and $5_L25_L52_L5$ (2 and 5 are related to the numbering on benzimidazole ring while L is related to linkage between benzimidazole units) benzimidazole tetramers. Both symmetric dimers; 1,4-bis-(2-carboxybenzimidazolyl)piperazine and 1,4-bis-(2-aminobenzimidazolyl) piperazine were also prepared via condensation of the bis-(1,2-diamine) system using either trichloroacetimidate or 4-nitrobenzoate activation, followed by hydrolysis and reduction respectively. In parallel to the above syntheses, both 5-aminobenzimidazoles and 5-carboxybenzimidazoles were synthesized. Finally; the first set of tetramers was synthesized via coupling of one equivalent of 1,4-bis-(2-aminobenzimidazolyl) piperazine with two equivalents of monomeric 5-carboxybenzimidazole while the second set was achieved by coupling of one equivalent of 1,4-bis-(2-carboxybenzimidazole) piperazine with two equivalents of monomeric 5-aminobenzimidazole.

INTRODUCTION

The majority of oligomeric benzimidazole drugs act *via* binding in the minor groove of DNA, where they directly or indirectly recognize A/T base-pairing sequences. The A/T-specific binding of oligomeric benzimidazoles results in inhibition of transcription and may also lead to cytotoxicity towards tumor cells (Liu *et al.*, 1999). From a therapeutic viewpoint, the benzimidazole nucleus can be regarded as a key building block in numerous DNA intercalators. Linked to other heterocycles; benzimidazoles can intercalate with DNA and RNA thereby prevent cell growth by inhibiting the enzymes directly responsible for the formation of nucleic acids, which leads to DNA transcription inhibition and ultimately causing cell death. The medicinal chemistry of selective minor groove binders based on benzimidazole started with Hoechst 33258, an anticancer drug capable of non-covalent binding with the TTAA sequence. This intercalator was proven to inhibit topoisomerase, interfering with DNA supercoiling, as well as many other cellular processes. (Beerman *et al.*, 1992) Fig. 1.

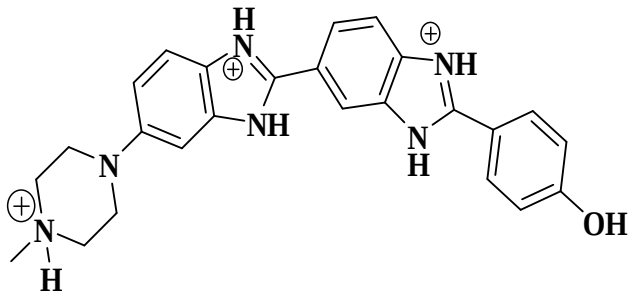

Fig. 1: The lead minor groove binder; Hoechst 3325.

Several bis, tri, tetra-benzimidazoles, linked *via* C2 and C5 are good DNA and RNA minor groove binders (Dervan *et al.*, 2001; Le Sann *et al.*, 2006; Ivanov, *et al.*, 2008). It was confirmed that oligomeric benzimidazoles, joined by amide linkages, stabilize H-bonding *via* oxygen or nitrogen of the DNA and RNA bases in the same manner as Dervan's amide-containing oligopyrroles (Dervan *et al.*, 2001).

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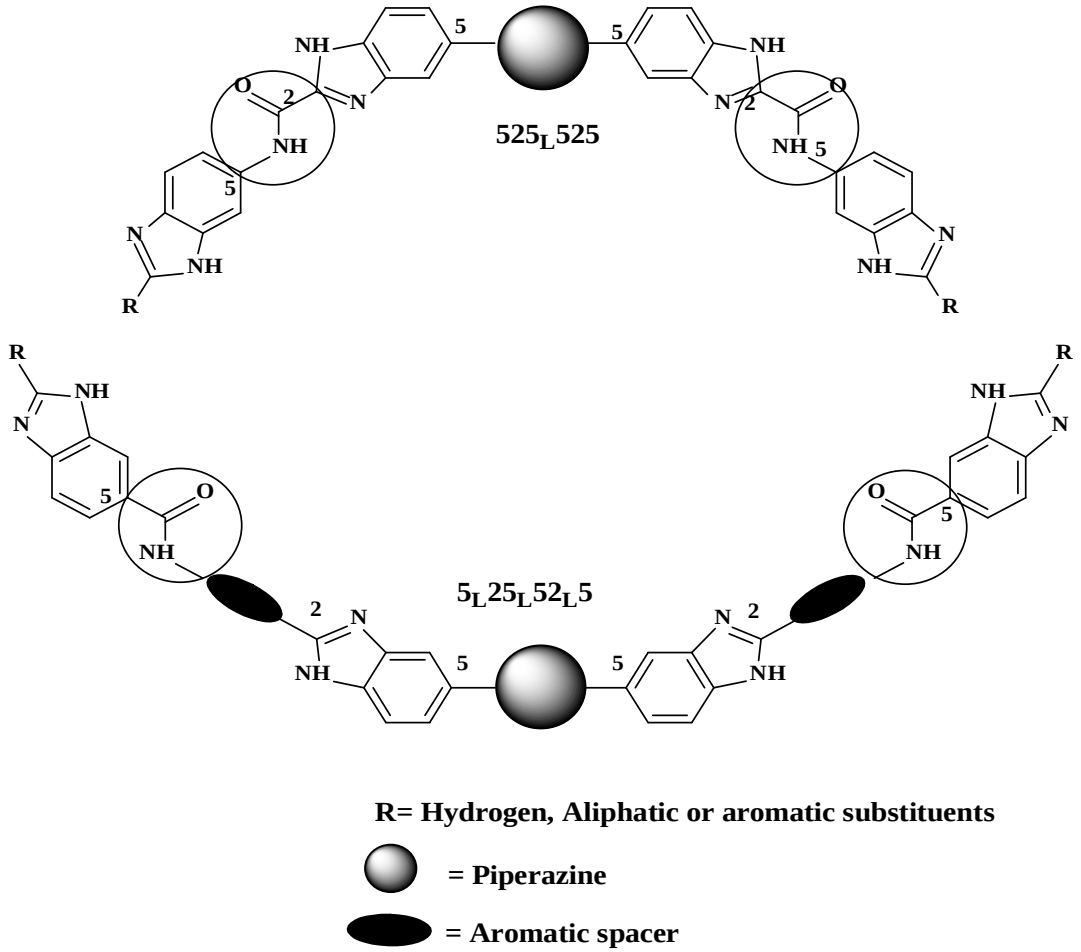


Fig. 2: Generic structures of proposed symmetric tetrameric benzimidazoles.

Peptide nucleic acids (PNA) are classic examples of tight-binding DNA agents, which bind to complementary sequences by Watson-Crick base-pairing. Dervan's oligopyrrole ligands demonstrate that this sort of combination retains useful DNA binding ability (Dervan *et al.*, 2001; Ji *et al.*, 2001). This work focuses on building benzimidazole units at positions 2 and/or 5 via amide functionality as illustrated in Fig. 2 that includes a generic oligomers design with different head/tail to head/tail orientations that might be capable of recognizing a specific sequence in DNA or RNA. For that reason and as practical step towards synthesis of such oligomers; the 525_L525 and $5_L25_L52_L5$ oligomers were taken into consideration as synthesis targets [2 and 5 are related to the numbering on benzimidazole ring while L is related to linkage between benzimidazole units]

MATERIAL AND METHODS

^{1}H NMR spectra were recorded at 400 MHz and ^{13}C NMR at 75 MHz on a Bruker DPX 400 spectrometer. Chemical shifts are denoted in ppm (δ) relative to an internal solvent standard (Me_4Si in the case of ^{1}H). The splitting patterns for NMR- spectra are designated as follows: s (singlet), d (doublet), t (triplet), br (broad) and m (multiplet). Coupling constants (J) are designated in Hz. Mass spectra were recorded on a VG 70/70 Hybrid or a Kratos MS-50 mass spectrometer by ES. Elemental analyses were performed using a LECO CHNS-932 elemental analyzer. IR spectra were obtained using a Perkin Elmer 1605 FTIR spectrometer.

Analytical TLC was performed on Merck 60 F254 aluminium backed plates, which were visualised using a UV lamp. All chemicals were used as received from Sigma Aldrich without further purification.

Synthesis 1,4-Bis-(3,4-dinitro-phenyl)-piperazine.(3)

A mixture of 4-fluoro-1,2-dinitrobenzene **2** (5 mmol), $NaHCO_3$ (10 mmol) and methanol (30 mL) was refluxed for 30 minutes, piperazine **1** (5 mmol) was added, the colourless solution became yellow. The reaction mixture was heated under reflux for a further 12 h until TLC showed complete disappearance of the starting material. The reaction mixture was cooled in an ice bath for 2 h, and the yellow precipitate was separated from methanol by filtration. This precipitate was washed with (2x20 mL) of ethyl acetate, HCl (10%), brine (10 mL) and water (10 mL), and the organic layer was dried ($MgSO_4$). The desired product **3** was purified by recrystallization from ethanol as orange crystals (T- 1)

Synthesis of 1,4-Bis-(3,4-diamino-phenyl)-piperazine.(4)

A solution of nitrobenzimidazole (2 mmol) in conc. HCl (10 mL) was treated portion wise with $SnCl_2$ (12 mmol, 2.28 g) and the resulting solution was stirred at room temperature for several hours. The reaction mixture was poured into ice-water and neutralized with NaOH 10% solution, extracted with ethyl acetate (5x25 mL) washed with water (3x15 mL) dried over anhydrous Na_2SO_4, and then solvent removed in *vacuo* leaving an oily material. The residue was crystallized from acetone: hexane (1:10 v/v) and purified by recrystallization as off white crystals (Table 1).

General method for the synthesis of 5-carboxybenzimidazoles. (10-12)

A solution of 3,4-diamibenzoic acid (10 mmol) and 4N HCl (15 mL) was treated with appropriate aliphatic carboxylic acids (Trifluoro acetic acid, Acetic acid and Formic acid). (15 mmol) and heated under reflux (Phillips *et al.,* 1928)
. The solution was cooled and allowed to stand overnight. The mixture was neutralized with 2 N NaOH, the solid was filtered, washed with water and purified by recrystallization from suitable solvent. As known compounds **10-12**; all the synthesized products analysis data were consistent with literatures (Bougrain *et al.,* 2001; Mathias *et al.,* 1975)

Synthesis of 1,4-Bis-(2-trichloromethylbenzimidazol-5-yl)piperazine.(7)

Fresh methyl trichloroacetimidate (5 mmol, 0.88 g) was added drop wise to a cooled solution of **4** (4 mmol) in acetic acid (20 mL) (Louvet *et al.,* 1993). At the end of the addition (30 minutes), the reaction was kept at room temperature for 1 hour. And the resulting precipitate was filtered, washed with acetic acid (10 mL), dried and purified by recrystallization from methanol providing the title compound **7** as off white crystals. (**Table 1**)

Synthesis of 1,4-Bis-(2-carboxybenzimidazol-5-yl)piperazine.(8)

Bis(2-(trichloromethyl benzimidazole) **7** was added to a cooled solution of sodium hydroxide (100ml; 1N). The resulting solution was filtered and the filtrate was acidified with hydrochloric acid (4N). The resulting precipitate was filtered off, washed twice with a solution of water and acetonitrile 3:1, 30 mL ether (2x30 mL).), dried and purified by recrystallization providing the title compound **7** as pale yellow crystals (Table 1).

General coupling procedure for the synthesis of tetrameric benzimidazoles (17-19 and 20-22)

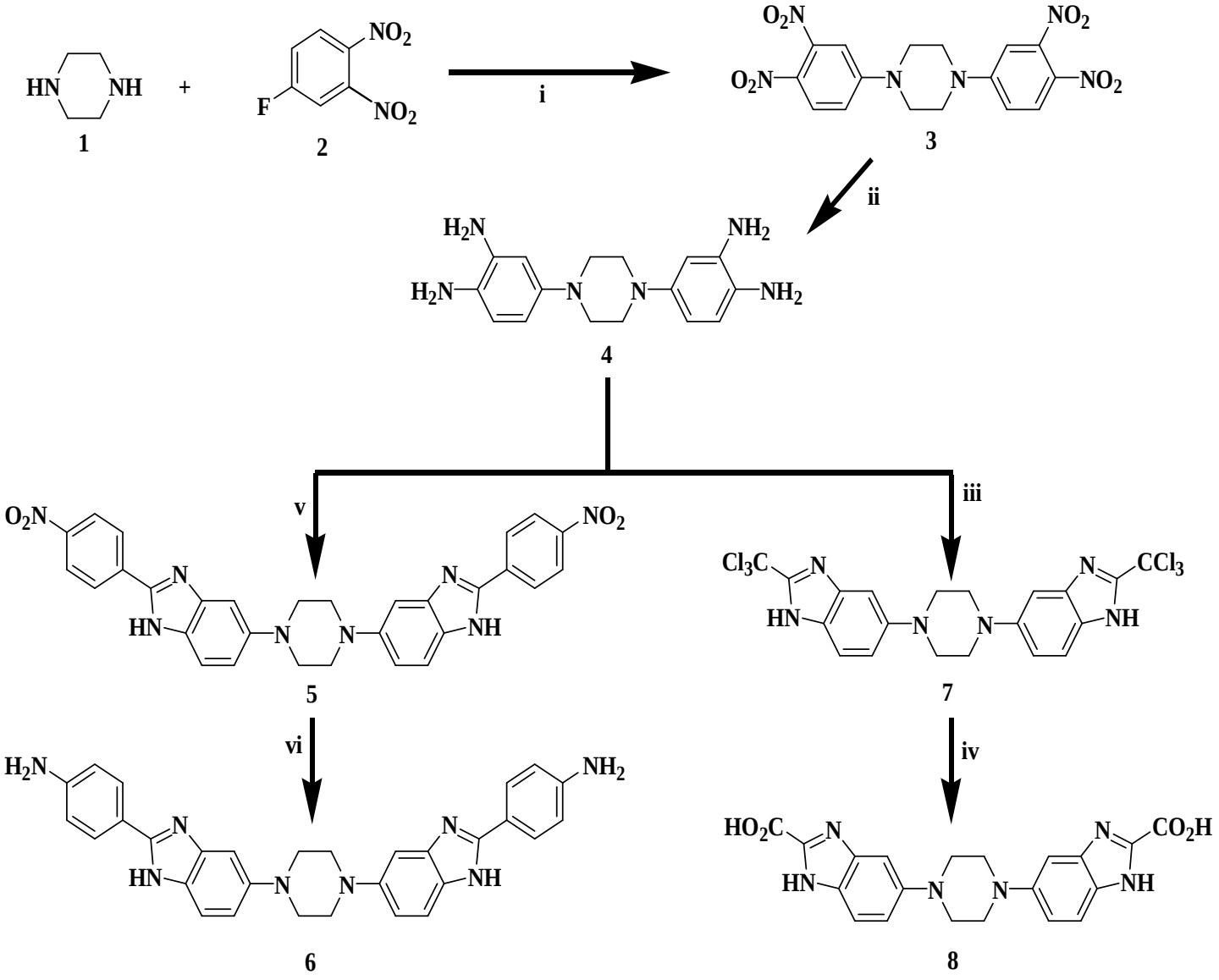
For the tetramers **20-22**; (2.2 mmol) of 5-carboxybenzimidazole derivatives **10-12** were dissolved in dry DMF (10 mL) with HOBt (2.2 mmol) and EDC (2.2 mmol). The reaction was then cooled to 0 °C and after 30 minutes, the bis(2-aminobenzimidazoles **6** (1 mmol) was added and the reaction left stirring at room temperature. For the tetramers **17-19**; (2.2 mmol) of bis(2-carboxybenzimidazole derivatives were dissolved in dry DMF (10 mL) with HOBt (2.2 mmol) and EDC (2.2 mmol). The reaction was then cooled to 0 °C and after 30 minutes. (2 mmol) of 5-aminobenzimidazole derivatives **14, 15** and **16** were added and the reaction left stirring at room temperature. Once the TLC showed no starting material the solvent was removed in *vacuo*, then (25 mL) water and ethyl acetate (50 mL) were added and the organic layer was washed with HCl 5% (5mL) then with $NaHCO_3$ (10%) (5mL) and finally with brine (5mL). The organic layers combined, dried ($MgSO_4$), concentrated and left over night on the high *vacuo*. The crude product was purified by recrystallization from suitable solvent as off white crystals for all tetramers as illustrated in Table 1.

RESULT AND DISCUSSION

The work focused on synthesis of monomeric benzimidazoles with 5-amino and 5-carboxy functionalities as precursors for coupling with novel bis(diamino) and bis(dicarboxy) benzimidazoles affording the higher blocks of novel bidirectional amide-linking symmetrical tetra-benzimidazoles of the types 525_L525 and $5_L25_L52_L5$. As the first step towards symmetrical dimerization; 1 equivalent of piperazine **1** was refluxed with 2 equivalents of 4-Fluoro-1,2-dinitrobenzene **2** in the presence of Na_2CO_3 in methanol. The compound **3** was formed and confirmed by spectroscopic tools as well as elemental analysis.

Up on reducing of **3** by using $SnCl_2$/HCl system; compound **4** was formed and confirmed by all spectroscopic tools, since the appearance of significant stretching in the 3333 cm^{-1} and the D_2O-exchangeable broad peak using ^{1}H-NMR in the range of (5.34-6.01 δ) and the m/z base peak of 298 are clear cut evidences about NH_2 presence. The compound **4**- as a precursor for benzimidazole ring synthesis -was cyclized through two different routes according to the functionality needed. The first one includes cyclization with 4-nitrobenzoic acid affording the bis(dinitrobenzimidazole) **5** which was confirmed by spectroscopic tools such as mass spectroscopy through detection of m/z at 560. The IR stretching has showed C=N as the imino group for the new formed imidazole five memebred ring at 1587 cm^{-1}. The appearance of two doublet peaks at 7.77 and 8.01 δ with, J = 8.2 Hz, which are related to the four protons in the 2(4-nitrophenyl) ring, also the appearance of stretching at 3194 cm^{-1} is related to N=O. Extendable functionality towards reduction to afford the bis-1,2-diamino **6** was accomplished using $SnCl_2$/HCl in 63 % yield. The structure of **6** was confirmed through appearance of D_2O-exchangeable broad peak using ^{1}H-NMR in the range of (5.29-6.43 δ). The disappearance of N=O stretching in the IR spectroscopy and the appearance of new stretching at 3334 cm^{-1} is related to the new N-H functionality. Also the mass spectroscopy m/z base peak at 500 is evidence about the assigned structure of **6**. The second route includes cyclization using methyl-trichloroacetimidate which was added to compound **4** in acetic acid at room temperature for 1.5 hour affording 1,4-bis(2-trichloromethyl benzimidazolyl) piperazine **7** in 71 % yield.

The dimeric benzimidazole **7** was confirmed by spectroscopic tools through the disappearance of NH_2 broad peak and stretching in both ^{1}H-NMR and IR respectively. Mass spectroscopy m/z base peak at 549 provided the evidence about the assigned structure. Hydrolysis of **7** using 1N NaOH followed by neutralization with 4N of HCl providing the bisdicarboxylic acid **8** in 79 % yield. The structure of **8** was confirmed using the mass spectroscopy through appearance of m/z base peak at 406. The ^{13}C-NMR also showed the C=O peak at 166.8 ppm. The IR spectroscopy was clear and diagnostic for C=O through the streching at 1679 cm^{-1}. The ^{1}H-NMR showed broad paek at 11.98-13.99 δ related to the carboxylic acid protons. **Figure 3** illustrates all synthetic routes. With both **6** and **8** dimers in hand; the aminolysis steps was carried out through coupling one equivalent of **6** with two equivalents of different three 5-carboxybenzimidazoles **10**,**11** and **12** monomers which were synthesized according to philips method; 3,4-diaminobenzoic acid was refluxed with trifluoroacetic acid, formic acid and acetic acid seperately in 4N HCl as illustrated in **Fig 4.**



i] Na_2CO_3, MeOH, (reflux, 7 h)
ii] $SnCl_2$/HCl, MeOH, (RT, 6 h)
iii] CCl_3CN,CH_3CO_2H,DCM, (RT, 1.5 h)
iv] a)NaOH(1N); (RT, 1/2 h). b) HCl (4N)
v] 4-Nitrobenzoic acid, HCl (4N) (reflux, 12 h)
vi] $SnCl_2$/HCl, MeOH (RT, 6 h)

Fig. 3: Synthetic routes of both 6 and 8 bis-benzimidazoles .

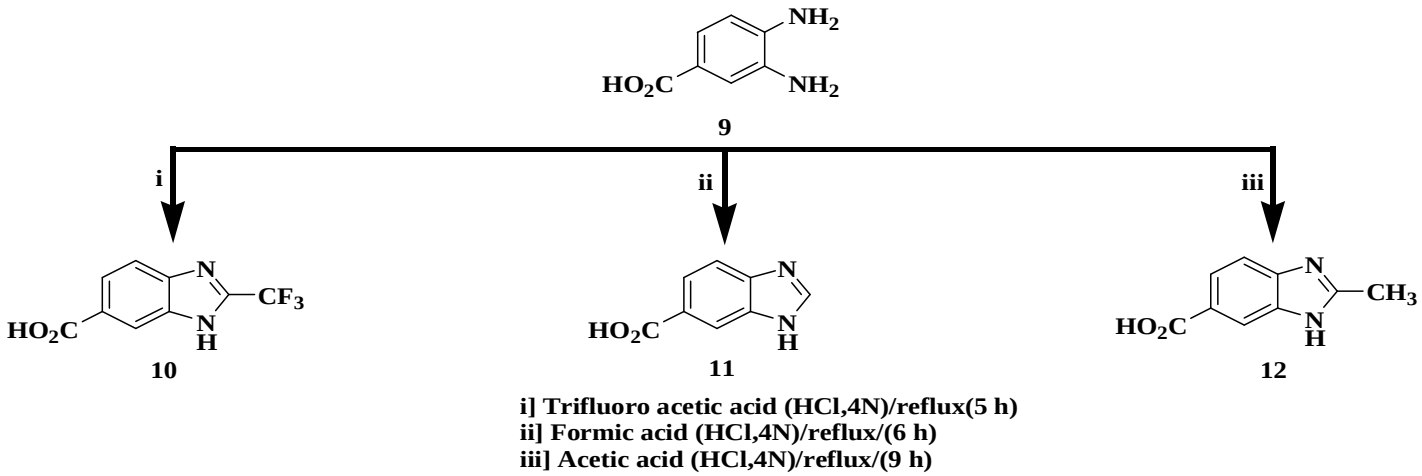


Fig. 4: Synthetic routes of 5-carboxybenzimidazoles 10-12.

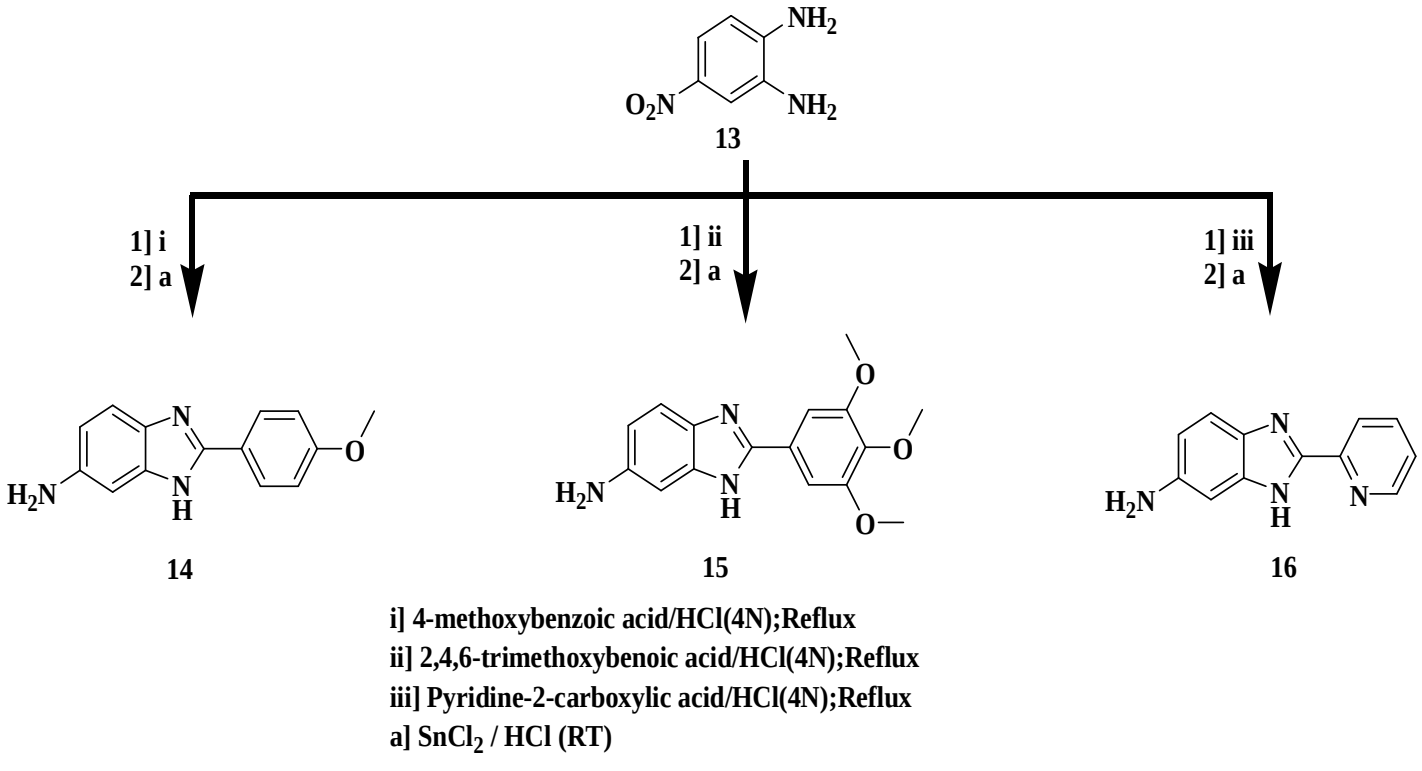


Fig. 5: Synthetic routes of 5-aminobenzimidazoles 14-16.

The required products were confirmed using spectroscopic tools and the obtained data for these known monomers were cosistent with literatures (Bougrain *et al.,* 2001; Mathias *et al.,* 1975). 5-aminobenzimidazoles **14**, **15** and **16** monomers were also synthesized according to philips method; since 4-nitro-1,2-diaminobenzene was refluxed with 4-methoxybenzoic acid, 3,4,5-trimethoxybenzoic acid and Pyridine-2-carboxylic acid in 4N HCl seperately. This step was followed by reduction using $SnCl_2$/HCl as illustrated in **Figure 5**. The required products were confirmed using spectroscopic tools and the obtained data for these known monomers were cosistent with literatures (Kim *et al.,* 1996; Haugwitz *et al.,* 1979; Misra *et al.,* 1980). The first set of symmetrical tetramers were obtained when the 1 equivalent of bis (dicarboxybenzimidazole) **8** was coupled with 2 equivalents of the 5-aminobenzimidazole derivatives. These tetramers were characterized by 525_L525 orientation system that describes the benzimidazole orientation. However three tetramers were synthesized; when **8** was added to the coupling reagents (EDC and HOBt) in DMF as a solvent under ice bath for 30 minutes followed by adding the monomers 5-aminobenzimidazoles; **14**,**15** and **16** as illustrated in **Figure 6**. The yields of these tetramers were convenient. (Bougrain *et al.,* 2001; Mathias *et al.,* 1975)

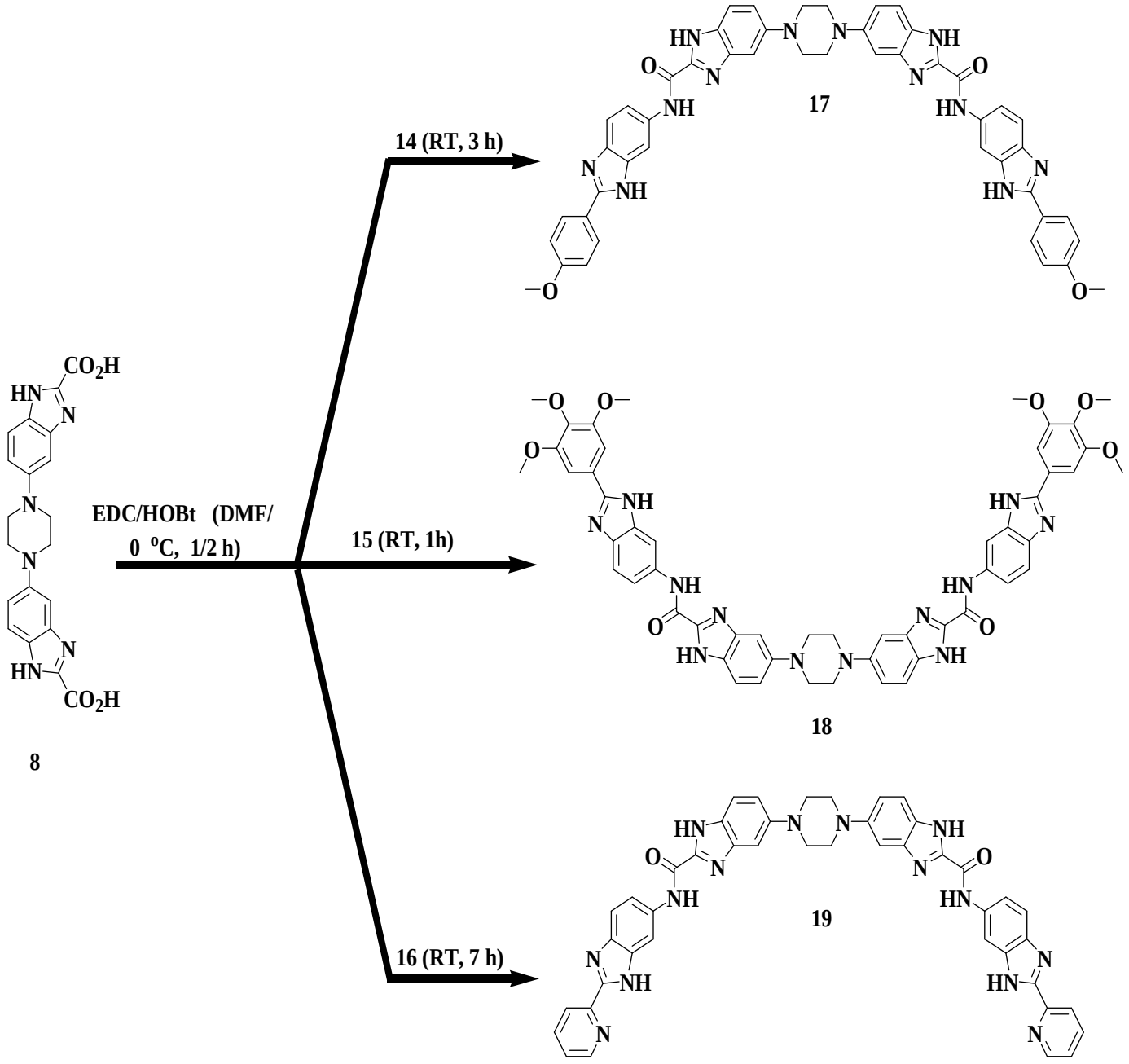


Fig. 6: Coupling of 8 with 14, 15 and 16 towards tetramers 17, 18 and 19 synthesis.

The target tetramers **17**,**18** and **19** were confirmed through spectroscopic tools; since ^{1}H-NMR showed all the amide N-H proton as broad peaks in the range between (9.48 and 11.23 ppm) for the three tetramers. The ^{13}C-NMR confirmed the carbonyl streching in the range between 164.5 and 168.8 ppm. The IR showed significant streching that confirmed the N-H amide through having broad absorption in the range between 3309 and 3329 cm^{-1} coinciding with indicative peaks towards confirming the C=O through streching appearance in the range (1663-1673 cm^{-1}). All compounds were confirmed by elemental analysis as illustrated in **Tables 1**. The second set of symmetrical tetramers were obtained when 1 equivalent of bis (diaminobenzimidazolyl) piperazine **6** was coupled with 2 equivalents of the monomers 5-carboxybenzimidazole derivatives **10**, **11** and **12** through their stirring seperately with the reagents (EDC and HOBt) in DMF as a solvent in ice bath for 30 minutes.

6 was added and the mixture was stirred at room temperature for several hours. The synthesized tetramers were characterized by $5_L25_L52_L5$ orientation system that describes benzimidazole ring orientation to its neighbouring benzimidazole unit, which is different from the first set of tetramers as illustrated in **Fig. 7**. The yields of these tetramers were convenient. The target tetramers **20**, **21** and **22** were confirmed through spectroscopic tools; since ^{1}H-NMR had showed all the amide N-H proton as broad peaks in the range between (9.18 and 11.24 ppm) for the three tetramers. The ^{13}C-NMR confirmed the carbonyl streching in the range between 164.7 and 169.3 ppm. The IR showed significant streching that confirmed the N-H amide through having broad absorption in the range between 3311 and 3322 cm^{-1} coinciding with indicative peaks towards confirming the C=O through streching appearance in the range (1664-1677 cm^{-1}). All tetramers were confirmed by elemental analysis as illustrated in **Tables 1**

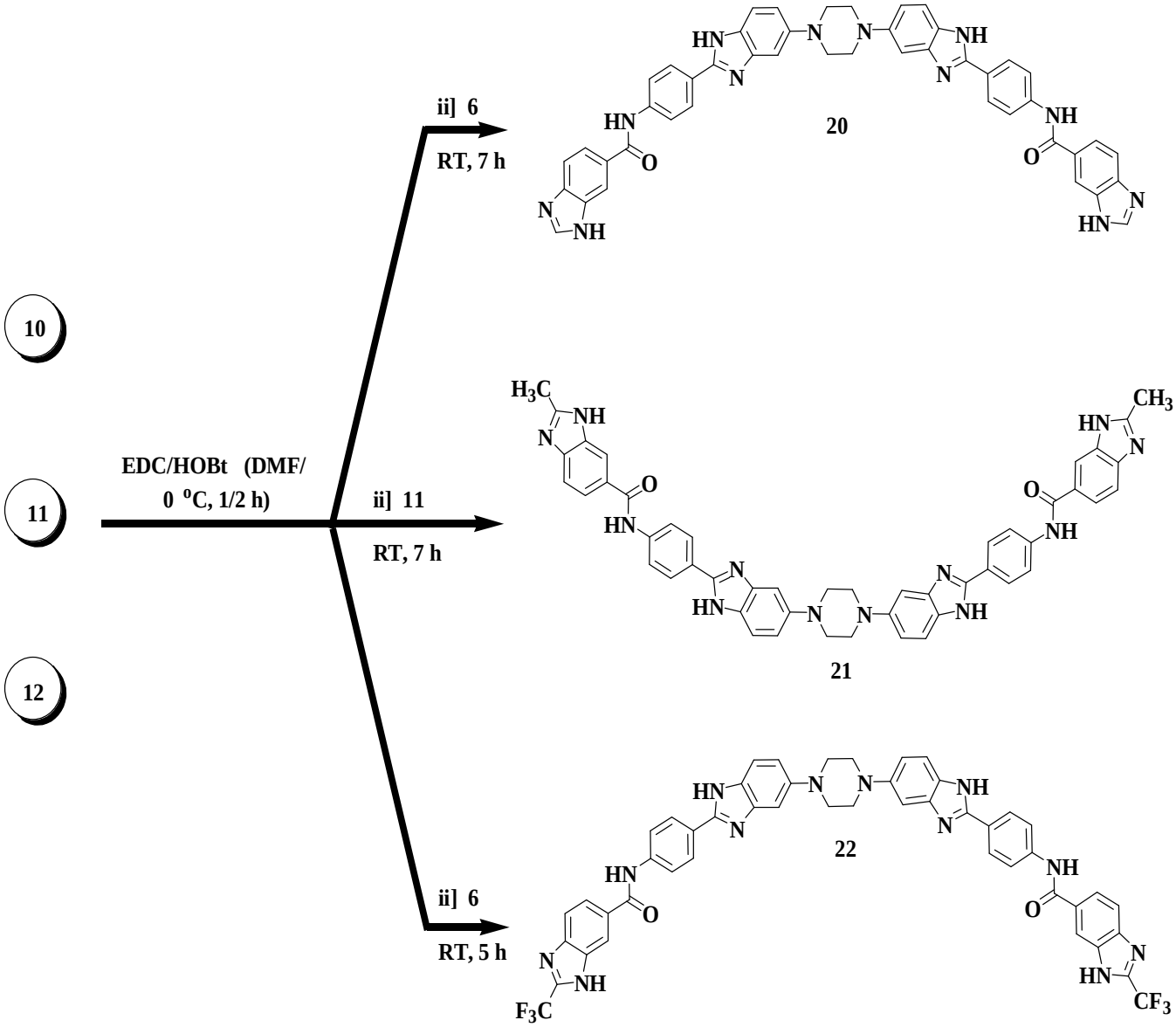


Fig. 7: Coupling of 6 with 3, 4 and 5 towards tetramers 20, 21 and 22 synthesis.

Table. 1: Physiochemical parameters of the novel synthesized bis- and tera-benzimidazoles.

Sr. No	Molecular Formula and Molecular Mass	Yield %	Melting point (^{0}C)	(Cryst. Solvent)	Elemental Analysis % Calcd./ Found
3	$C_{16}H_{14}N_6O_8$ 418	81	248-251	Ethanol	C, 45.94/45.90 H, 3.37/3.41 N, 20.09/20.10 O, 30.60/30.59
4	$C_{16}H_{22}N_6$ 298	78	237-240	Methanol	C, 64.40/64.36 H, 7.43/7.45 N, 28.16/28.18
5	$C_{30}H_{24}N_8O_4$ 560	77	279-282	Ethanol/ Hexane (1:1)	C, 64.28/64.31 H, 4.32/4.29 N, 19.99/20.00 O, 11.42/11.40
6	$C_{30}H_{28}N_8$ 500	63	289-290	Ethanol/ Hexane (1:2)	C, 71.98/71.95 H, 5.64/5.63 N, 22.38/22.42
7	$C_{20}H_{16}Cl_6N_6$ 549	71	293-296	Methanol	C, 43.43/43.40 H, 2.92/2.90 Cl, 38.46/38.51 N, 15.19
8	$C_{20}H_{18}N_6O_4$ 406	79	>300	Ethanol/Water(2:1)	C, 59.11/59.09 H, 4.46/4.46 N, 20.68/20.70 O, 15.75/15.75
17	$C_{48}H_{40}N_{12}O_4$ 848	83	>300	Ethanol/Ethylacetate(3:1)	C, 67.91/67.88 H, 4.75/4.72 N, 19.80/19.85 O, 7.54/7.55
18	$C_{52}H_{48}N_{12}O_8$ 968	89	>300	Ethanol/Ethylacetate(3:1)	C, 64.45/64.43 H, 4.99/4.97 N, 17.35/17.40 O, 13.21/13.20
19	$C_{44}H_{34}N_{14}O_2$ 790	87	>300	Ethanol/ Ethylacetate(3:1)	C, 66.82/66.80 H, 4.33/4.35 N, 24.80/24.84 O, 4.05/4.01
20	$C_{46}H_{36}N_{12}O_2$ 788	74	>300	Ethanol/ Hexane(1:2)	C, 70.04/70.02 H, 4.60/4.62 N, 21.31/21.33O, 4.06/4.03
21	$C_{48}H_{40}N_{12}O_2$ 816	79	>300	Ethanol/ Ethylacetate(3:1)	C, 70.57/70.52 H, 4.94/4.90 N, 20.58/20.62 O, 3.92/3.96

1,4-Bis-(3,4-dinitro-phenyl)-piperazine.(3)

^{1}H NMR (400MHz, $CDCl_3$) δ 3.76 (s, 4xCH_2, piperazine), 7.96 (d,2H, *J* = 8.0 Hz, 2xH), 8.41 (d,2H, *J* = 8.0 Hz, 2xH). 8.96 (s, 2H, 2xH). ^{13}C NMR (75MHz, $CDCl_3$) δ 54.3 (4xCH_2), 107.5 (2xCH), 123.8 (2xCH), 129.1 (2xCH), 136.8 (2xC), 148.6 (2xC), 155.5 (2C) IR υ_{max} cm $^{-1}$ 779 (N-O), 1275 (C-N), 1453 (C=C), 2821 (C-H, aliphatic), 3052 (C-H, aromatic), 3188 (N=O).

1,4-Bis-(3,4-diamino-phenyl)-piperazine.(4)

^{1}H NMR (400MHz, $CDCl_3$) δ 3.49 (s, 4xCH_2), 5.34-6.01 (br, 8H, 4xNH_2), 6.16 (d,2H, *J* = 7.8 Hz, 2xH), 7.10 (d,2H, *J* = 7.8 Hz, 2xH). 8.96 (s, 2H, 2xH). ^{13}C NMR (75MHz, $CDCl_3$) δ 49.9 (4xCH_2), 100.1 (2xCH), 111.8 (2xCH), 120.1 (2xCH), 122.5 (2xC), 140.1 (2xC), 145.3 (2C) IR υ_{max} cm $^{-1}$ 1279 (C-N), 1466 (C=C), 2835 (C-H, aliphatic), 3064 (C-H, aromatic), 3333 (N-H).

1,4-Bis-{2-(4-Nitro- phenyl)- 1H- benzoimidazol-5-yl]}-piperazine. (5)

^{1}H NMR (400MHz, DMSO) δ 3.77 (s, 4xCH_2, piperazine), δ 7.77 (d, 2H, *J* = 8.2 Hz, 2x2H), 7.89 (d, 1H, *J* = 8.0 Hz), 8.01 (d, 1H, *J* = 8.0 Hz), 8.12 (d, 2H, *J* = 8.2 Hz). 10.45-12.00 (br, 2xNH imidazole). ^{13}C NMR (75MHz, DMSO) δ 51.4 (4xCH_2), 113.9 (4xCH), 117.8 (2xC), 120.3 (4xCH), 121.4 (2xCH), 125.7 (4xCH), 130.1 (2xC), 132.4 (2xCH), 139.1 (2xC), 140.5 (2xC), 148.8, 150.1 (2xC), 151.8 (2xC). IR υ_{max} cm^{-1} 770 (N-O), 1264 (C-N), 1439 (C=C), 1587 (C=N), 2901 (C-H, aliphatic), 3078 (C-H, aromatic), 3194 (N=O), 3317 (N-H).

1,4-Bis-{2- (4-amino-phenyl) -1H- benzoimidazol-5-yl]-piperazine.(6)

^{1}H NMR (400MHz, DMSO) δ 3.37 (4xCH_2), 5.29-6.43 (br, 4H, 2xNH2), 6.73 (d, 4H, *J* = 8.2 Hz, 2x2H), 6.76 (d, 2H, *J* = 8.0 Hz, 2xH), 6.85 (s, 2H), 7.38 (d, 2H, *J* = 8.2 Hz), 8.12 (d, 2H, *J* = 8.2 Hz). 9.79-10.65 (br, 2xNH imidazole). ^{13}C NMR (75MHz, DMSO) δ 48.9 (4xCH_2), 100.0 (4xCH), 109.3 (2xC), 116.3 (4xCH), 119.8 (2xCH), 120.7 (4xCH), 126.2 (2xC), 132.5 (2xCH), 136.3 (2xC), 139.5 (2xC), 144.4, 150.0 (2xC), 151.2 (2xC). IR υ_{max} cm $^{-1}$, 1236 (C-N), 1429 (C=C), 1590 (C=N), 2896 (C-H, aliphatic), 3066 (C-H, aromatic), 3334, (N-Hamine).

1,4-Bis-(2-trichloromethyl-benzimidazol-5-yl)piperazine.(7)

^{1}H NMR (400MHz, DMSO) δ 3.66 (s, 4xCH_2), 6.88 (d,2H, *J* = 8.0 Hz), 7.38 (d,2H, *J* = 8.0 Hz). 8.13 (s, 2H), 10.13-10.78 (br, 2NH).^{13}C NMR (75MHz, DMSO) δ 51.9 (4xCH_2), 101.6 (2xCH), 120.0 (2xCH), 120.9 (2xCH), 125.4 (2xC), 146.3 (2xC), 146.9 (2C), 155.7 (2C) IR υ_{max} cm $^{-1}$ 769 (C-Cl), 1288 (C-N), 1463 (C=C), 1653 (C=N), 2833 (C-H, aliphatic), 3068 (C-H, aromatic), 3481 (N-H, amine).

1,4-Bis-(2-carboxybenzimidazol-5-yl)piperazine.(8)

^{1}H NMR (400MHz, DMSO) δ 3.99 (s, 4xCH_2), 8.37 (d,2H, *J* = 7.9 Hz), 8.89 (d,2H, *J* = 7.9 Hz). 9.09 (s, 2H). 10.89-11.24 (br, 2xNH).11.98-13.99 (br, 2xCOOH). ^{13}C NMR (75MHz, DMSO) δ 55.9 (4xCH_2), 104.7 (2xCH), 122.1 (2xCH), 124.5 (2xCH), 129.4 (2xC), 136.3 (2xC), 146.9 (2C), 155.7 (2C), 166.8 (2C=O), IR υ_{max} cm $^{-1}$ 769 (C-Cl), 1280 (C-N), 1462 (C=C), 1675 (C=N), 1679(C=O), 2823 (C-H, aliphatic), 3071 (C-H, aromatic), 3198 (O-H), 3479 (N-H, amine).

1,4-Bis-{2- [2- (4- methoxy- phenyl)- 3H-benzimidazol-5-ylcarbamoyl]-1H-benzoimidazol-5-yl)}-piperazine.(17)

(^{1}H NMR (400MHz, DMSO) δ 3.56 (s, 4xCH_2, piperazine), δ 3.86 (s, 6H, 2xCH_3), 6.79 (d, 2H, J = 8.1 Hz), 7.08 (s, 2H), 7.28 (d,4H, *J* = 8.0 Hz, 4XH), 7.52 (d, 2H, *J* = 8.1 Hz), 7.69 (d,2H, *J* = 7.9 Hz), 7.89 (d,2H, *J* = 7.9 Hz), 8.23 (s, 2H), 8.39 (d,2H, *J* = 8.0 Hz), 9.76-10.78 (br, 2xNH amide), 10.98-12.28 (br, 4xNH imidazole). ^{13}C NMR (75MHz, DMSO) δ 51.4 (4xCH_2),

55.7 (2x CH_3) 100.2 (2xCH), 112.7 (2xCH), 119.1 (2xCH), 127.8 (2xCH), 128.3 (2xC), 129.3 (2xC), 133.6 (2xCH), 137.1 (2xCH), 138.6 (2xC), 139.3 (2xC), 139.8 (2xCH), 141.5 (2xCH), 144.2 (2xCH), 149.9 (2xC), 150.1 (2xCH), 152.0 (2C), 156.6 (2xC), 157.3 (2xC), 158.0 (2xC), 159.1 (2xC), 162.9 (2C), 164.5 (2xC=O). IR υ_{max} cm^{-1} 239 (C-N), 1033 (C-O), 1442 (C=C), 1667 (C=N), 1666 (C=O), 2900 (C-H, aliphatic), 3008 (C-H, aromatic), 3311(N-H, amide), 3466 (N-H, amine).

1,4-Bis-{(2-[2-(3,4,5-Trimethoxy-phenyl)-3H-benzimidazol-5-ylcarbamoyl]-1H-benzimidazol-5-yl)}-piperazine.(18)

^{1}H NMR (400MHz, DMSO) δ 3.62(s, 4xCH_2, piperazine), δ 3.82(s, 6H, 2xCH_3), 3.96 (s, 12H, 4xCH_3), 6.66 (s, 4H, 2x2H), 6.85 (d, 2H, J = 8.1 Hz, 2xH), 7.08 (s, 2xH), 7.52 (d, 2H, *J* = 8.1 Hz, 2xH), 7.78 (d,2H, *J* = 7.9 Hz, 2xH), 7.80 (s, 2xH),7.99 (d,2H, *J* = 7.9 Hz, 2xH), 8.59 (s, 2xH), 9.48-10.88 (br, 2xNH amide), 11.79-12.28 (br, 4xNH imidazole). ^{13}C NMR (75MHz, DMSO) δ 51.4 (4xCH_2), 56.9 (4xCH_3) 61.3 (2xCH_3) 109.9 (2xCH), 115.8 (2xCH), 129.0 (2xCH), 131.8 (2xC), 132.3 (2xC), 133.6 (2xCH), 138.6 (2xC), 139.3 (2xC), 139.8 (2xCH), 141.5 (2xCH), 144.2 (2xCH), 149.9 (2xC), 150.1 (2xCH), 152.0 (2xC), 156.6 (2xC), 157.3 (2xC), 158.0 (2xC), 153.7 (2xC), 159.1 (2C), 159.8(2C), 165.7 (2xC=O). IR υ_{max} cm^{-1} 245 (C-N), 1067(C-O), 1450 (C=C), 1655 (C=N), 1673 (C=O), 2912 (C-H, aliphatic), 3000 (C-H, aromatic), 3309(N-H, amide),3487 (N-H, amine).

1,4-Bis- {2- [2- pyridyl- 3H-benzimidazol-5-ylcarbamoyl]-1H-benzimidazol-5-yl)}-piperazine. (19)

^{1}H NMR (400MHz, DMSO) δ 3.61 (s, 4xCH_2, piperazine), 6.70 (d, 2H, *J* = 8.1 Hz, 2xH), 6.93 (s, 2xH), 7.47-7.53 (m, 6H, pyridine), 7.52 (d, 2H, , *J* = 8.1 Hz, 2xH), 7.69 (d,2H, *J* = 7.9 Hz, 2xH), 7.89 (d,2H, *J* = 7.9 Hz, 2xH), 7.93-8.18 (m, 2H, pyridine), 8.37 (s, 2xH), 10.53-11.23 (br, 2xNH amide), 11.71-12.09 (br, 4xNH imidazole). ^{13}C NMR (75MHz, DMSO) δ 56.0 (4xCH_2), 107.1 (2xCH), 118.9 (2xCH), 119.2 (2xCH), 125.2 (2xCH), 127.3 (2xC), 127.5 (2xCH), 129.9 (2xC), 130.6 (2xCH), 133.1 (2xCH), 133.8 (2xC), 133.9 (2xC), 140.3 (2xCH), 140.9 (2xCH), 144.5 (2xCH), 145.0 (2xC), 146.9 (2xCH), 147.1 (2C), 150.0 (2xC), 154.2 (2xC), 155.9 (2xC), 168.8 (2xC=O). IR υ_{max} cm^{-1} 1241 (C-N), 1033 (C-O), 1431 (C=C), 1659 (C=N), 1663 (C=O), 2911 (C-H, aliphatic), 3066 (C-H, aromatic), 3329 (N-H, amide), 3411 (N-H, amine).

1,4-Bis-{2-{4-[(3H-Benzimidazole-5-carbonyl)-amino]-phenyl}-1H-benzimidazol-5-yl)-piperazine.(20)

^{1}H NMR (400MHz, DMSO) δ 3.56 (s, 4xCH_2, piperazine), 6.96 (d, 2H, *J* = 8.0 Hz, 2xH), 7.34 (d, 2H, *J* = 8.0 Hz, 2xH), 7.46 (s, 2H,2xH), 7.46 (d, 4H, *J* = 7.9 Hz, 2x2H), 7.5 (s, 2H, 2xH), 7.70 (d, 2H, *J* = 7.9 Hz, 2x2H), 7.78 (d,2H, , *J* = 8.1 Hz, 2x2H) 8.22 (s, 2xH, 2H), 8.39 (s, 2xH, 2H), 9.33-10.93 (br, 2H, 2xNH amide), 11.23-11.98 (br, 4H, 4xNH imidazole). ^{13}C NMR (75MHz, DMSO) δ 55.5 (4xCH_2), 105.9 (2xCH), 114.0 (2xCH), 118.8 (2xCH), 129.1 (2xCH), 129.9 (2xC), 129.3 (2xCH), 129.9 (2xC), 131.1 (4xCH), 137.4 (2xC), 138.6 (2xC), 139.2(2xCH), 146.8 (2xCH), 147.3 (2xC), 148.9 (4xCH), 149.0 (2C), 153.0 (2xC), 155.3 (2xC), 156.4 (2xC), 156.9 (2xC), 167.8 (2xC=O). IR υ_{max} cm^{-}1223 (C-N), 1045 (C-O), 1440 (C=C), 1678 (C=N), 1677 (C=O), 2923 (C-H, aliphatic), 3099 (C-H, aromatic), 3319 (N-H, amide), 3473 (N-H, amine).

1,4-Bis- {2- {4- [(2-methyl- 3H-Benzimidazole-5-carbonyl)-amino]-phenyl}-1H-benzimidazol-5-yl)-piperazine.(21)

^{1}H NMR (400MHz, DMSO) δ 2.81 (s, 3H, CH_3), 3.44 (s, 4xCH_2, piperazine), 6.82 (d, 2H, *J* = 8.0 Hz, 2xH), 7.13 (d, 2H, *J* = 8.0 Hz, 2xH), 7.46 (s,2H, 2xH), 7.22 (d, 4H, *J* = 7.9 Hz, 2x2H), 7.34 (s, 2H, 2xH), 7.58 (d,2H, *J* = 7.9 Hz, 2x2H), 7.45 (d,2H, , *J* = 8.1 Hz, 2x2H) 8.23 (s, 2xH, 2H), 8.46 (s, 2xH, 2H), 9.18-10.55 (br, 2H, 2xNH amide), 11.02-11.86 (br, 4H, 4xNH imidazole). ^{13}C NMR (75MHz, DMSO) δ 13.8 (2xCH), 53.1 (4xCH_2), 103.4 (2xCH), 110.2 (2xCH), 115.8 (2xCH), 123,5 (2xCH), 124.6 (2xC), 129.3 (2xCH), 130.3 (2xC), 133.5 (4xCH), 135.2 (2xC), 133.6 (2xC), 137.8 (2xCH), 143.2 (2xCH), 145.3 (2xC), 147.2 (4xCH), 148.4 (2C), 150.1 (2xC), 152.9 (2xC), 156.4 (2xC), 159.9 (2xC), 164.7 (2xC=O). IR υ_{max} cm^{-}1211 (C-N), 1067 (C-O), 1436 (C=C), 1675 (C=N), 1673(C=O), 2919 (C-H, aliphatic), 3079 (C-H, aromatic), 3322 (N-H, amide), 3444 (N-H, amine).

1,4-Bis- {2- {4- [(2- trifluoromethyl-3H-Benzimidazole-5-carbonyl)- amino]- phenyl}- 1H- benzimidazol-5-yl)-piperazine.(22)

^{1}H NMR (400MHz, DMSO) δ 3.73 (s, 4xCH_2, piperazine), 6.90 (d, 2H, *J* = 8.0 Hz, 2xH), 7.36 (d, 2H, *J* = 8.0 Hz, 2xH), 7.65 (s,2H,2xH), 7.71 (d, 4H, *J* = 7.9 Hz, 2x2H), 7.85 (s, 2H, 2xH), 7.82 (d,2H, *J* = 7.9 Hz, 2x2H), 8.17 (d,2H, , *J* = 8.1 Hz, 2x2H) 8.37 (s, 2xH, 2H), 8.66 (s, 2xH, 2H), 10.11-11.24 (br, 2H, 2xNH amide), 11.89-12.69 (br, 4H, 4xNH imidazole). ^{13}C NMR (75MHz, DMSO) δ 58.4 (4xCH_2), 111.0 (2xCH), 119.4 (2xCH), 122.7 (2xCH), 129.2 (2xCH), 134.8 (2xC), 136.0 (2xCH), 138.9 (2xC), 141.5 (4xCH), 143.3 (2xC), 147.9 (2xC), 150.1 (2xCH), 151.8 (2xCH), 155.6 (2xC), 157.0 (4xCH), 158.1 (2C), 158.3 (2xC), 158.9 (2xC), 159.0 (2xC), 159.7 (2xC), 169.3 (2xC=O). IR υ_{max} cm^{-1} 591(C-F), 1256(C-N), 1059 (C-O), 1422 (C=C), 1658(C=N), 1664 (C=O), 2977 (C-H, aliphatic), 3065 (C-H, aromatic), 3311 (N-H, amide), 3492 (N-H, amine).

CONCLUSION

Several symmetrical tetrameric 2 and/or 5 orientations amide-linked benzimidazoles were synthesized in a new methodology that lead for getting more blocks of benzimidazoles in simple way with very good yields. These oligomeric benzimidazoles are very important in the field of biological chemistry that deal with nucleic acids binding. The diversity of orientation might provide change in the intermolecular hydrogen bonding that are formed between the targeted DNA or RNA and amide-linked oligo-benzimidazoles drugs. After syntheses were

ccomplished; the biological actvity will be carried out to check how things go, and what does the future hold for such new drugs as we think; they are strongly candidate to act *via* DNA or RNA grooves and can directly or indirectly recognize sequences in these grooves resulting in inhibition of transcription at A/T sites consequently an effect drug to fight cancer.

ACKNOWLEDGMENT

Author thanks; prof. Zackaria Fataftah; Chemistry Department; Yarmouk University-Irbid (Jordan) for analysis facilities.

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How to cite this article:

Kanan M. Wahedy., Synthesis of Several Novel Symmetrical Amide-Linked Tetra-Benzimidazoles as Promising DNA and/or RNA binders. J App Pharm Sci, 2013; 3 (4 Suppl 1): S28-S37.