SYNTHESIS OF SOME NEW *N*-ISOPROPYLAMIDINOBIBENZIMIDAZOLES AS POTENTIAL TOPOISOMERASE INHIBITORS

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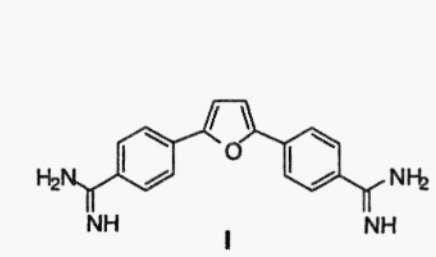
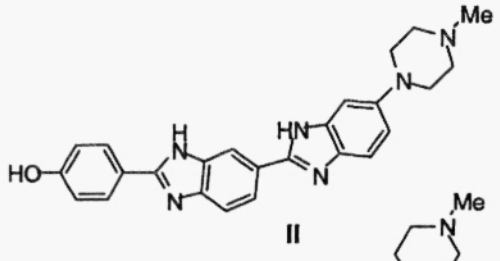
Abstract: *N*-Isopropyl-2-[4-(1-methyl-1*H*-benzimidazol-2-yl)-phenyl]-1*H*-benzimidazole-5-carboxamidine (**3**), 2'-(4-fluorophenyl)-*N*-isopropyl-1'-methyl-1*H*,1'*H*-[2,5']-bibenzimidazolyl-5-carboxamidine(**7**) and 1'-butyl-*N*-isopropyl-2'-{4-[5-(*N*-isopropyl-carbamimidoyl)-1*H*-benzimidazol-2-yl]phenyl}-1*H*,1'*H*-[2,5'] bibenzimidazolyl-5-carboxamidine (**9**) have been synthesized.

Introduction

The use of small molecules which reversibly bind to the minor groove of DNA is a well established strategy for developing potential new therapeutic agents.[1] Potent activity against *Pneumocystis carinii*, *Cryptococcus neoformans*, *Candida* and *Aspergillus* species, all opportunistic pathogens which cause serious life-threatening illness in immunocompromised individuals, have been reported for furamidine (I) and analogs.[2-6] Topoisomerases are DNA



I



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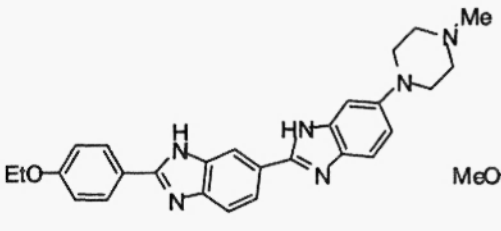

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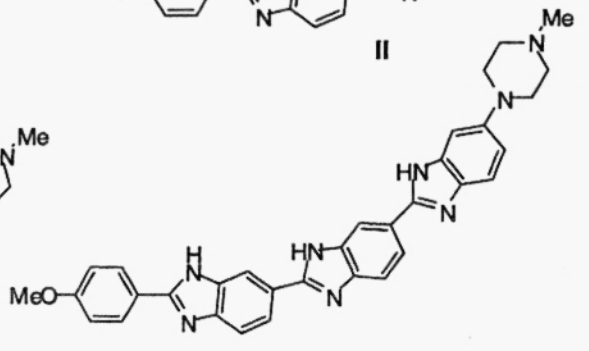


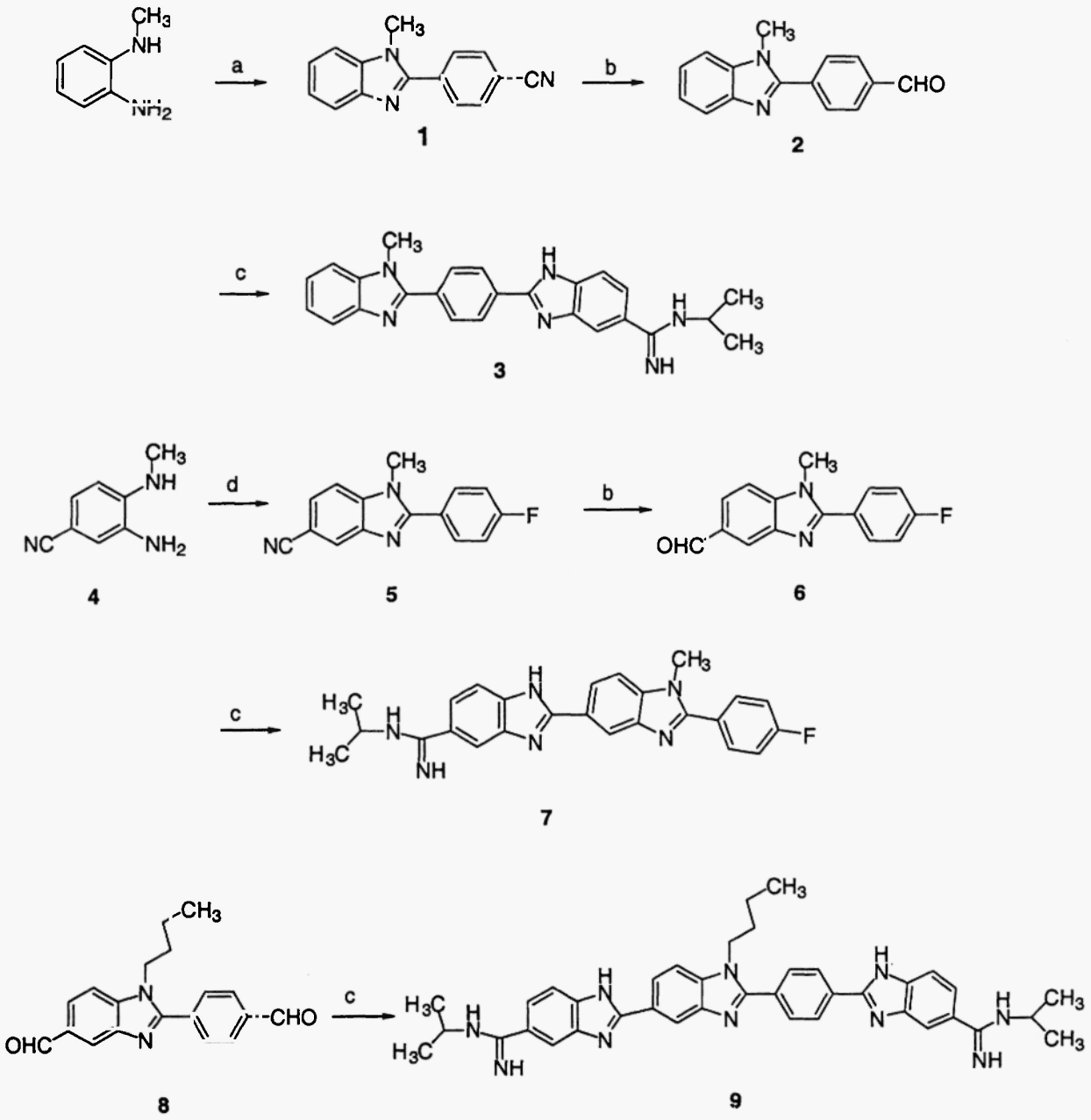
IV

dependent enzymes, which play important roles for topological and conformational changes in DNA during transcription and translation. Bibenzimidazole minor groove binding molecules show promise as antitumor agents.[7] Several bibenzimidazoles and terbenzimidazoles have recently shown to be topoisomerase I poisons. Two well studied dyes, Hoechst 33258 and 33342 (II and III)[8,9] and their new analogs[10] represent a structurally-unique class of topoisomerase I poisons. These agents bind to the minor groove of DNA and have been shown to interfere with topoisomerase I function. Recently, a new tris benzimidazole derivative (IV)[11] also has been designed and synthesized as a topoisomerase I poison.[12] As part of our continuing program of synthesis of minor groove binding molecules we report the synthesis of three new *N*-isopropylamidinobibenzimidazoles.

Results and Discussion

The target amidines **3**, **7** and **9** were synthesized (Scheme 1) by condensing the appropriate benzimidazolcarboxaldehydes **2**, **6** and **8**, with 3,4-diamino-*N*-isopropylbenzamidine hydrochloride in the presence of 1,4-benzoquinone. Using this approach benzimidazole formation occurred rapidly, (monitored by tlc), however, complete removal of all of the hydroquinone by-product was difficult and the yields for this reaction were modest. For the synthesis of **1** and **5** the sodium metabisulfite approach was used [13] and very good yields were obtained. However, for the synthesis of **7** the sodium metabisulfite[13] method was attempted but failed to produce product and the use of the benzoquinone method was required. The nitrile groups of **1** and **5** were converted to corresponding benzimidazolecarboxaldehydes **2** and **6** using DIBAL. Generally, the yields in these reductions rarely exceed 50-60 %, however, the desired aldehydes are obtained in one step.[14] The synthesis of **8** was reported by us earlier.[14] Reduction of the bis-benzimidazolenitrile precusor of **8** with DIBAL gave some ring cleavage yielding 4-butylamino-3-(4-formylbenzylamino) benzaldehyde along with **8**. However, during the reduction of mono-nitriles to form **2** and **6** no ring cleavage was observed, despite using DIBAL in excess .

Scheme I



Reagents **a** : $Na_2S_2O_5$ adduct of 4-cyanobenzaldehyde, **b** : DIBAL (1.0 *M* solution in CH_2Cl_2), **c** : 1,4-benzoquinone and 3,4-diamino-*N*-isopropylbenzamidine HCl, **d** : $Na_2S_2O_5$ adduct of 4-fluorobenzaldehyde

Experimental

Uncorrected melting points were measured on a Mel Temp 3.0 capillary melting point apparatus. ^{1}H-NMR spectra were recorded employing a Varian GX400 spectrometer; chemical shifts (δ) are in ppm relative to TMS and coupling constants (J) are reported in hertz. Mass spectra were recorded by EI and ESI methods at Georgia Institute of Technology, Atlanta, GA. Microanalyses were performed by Atlantic Microlab Inc, GA. All chemicals and solvents were purchased from Aldrich Chemical Co. or Fischer Scientific.

4-(1-Methyl-1*H*-benzimidazol-2-yl)benzonitrile (1)

4-Cyanobenzaldehyde (1.96 g, 15 mmol) was dissolved in EtOH (50 ml) and sodium metabisulfite (1.6 g) in water (10 ml) was added in portions. The mixture was kept in a refrigerator for several hours. The precipitate was filtered and dried to yield 3.06 g (87 %). A mixture of this adduct (1.93 g, 8.2 mmol) and *N*-methyl-1,2-phenylenediamine (1 g, 8.2 mmol) in DMF (5 ml) was heated at 130 °C for 4 h. The mixture was cooled, poured into water and the resultant solid was filtered. Crystallization of crude product from aqueous EtOH (80 %) gave **1**; yield 1.34 g (70 %); mp 208-210 °C; ^{1}H-NMR (DMSO-d_6) δ 3.9 (s, 3H), 7.25 (m, 2H), 7.65 (m, 2H), 8.0 (m, 4H). ES-MS *e/z* 234 (M+1, 100).

4-(1-Methyl-1*H*-benzimidazol-2-yl)benzaldehyde (2)

To a solution of **1** (0.3 g, 1.29 mmol) in dry CH_2Cl_2 (30 ml), 3 ml of DIBAL (1.0 M solution in CH_2Cl_2, 3 mmol) was added and the mixture was heated at reflux for 10 h under a nitrogen atmosphere. Cold dilute H_2SO_4 (20 ml) was added and the mixture was stirred overnight. The mixture was extracted with CH_2Cl_2 and the extract was concentrated. Crystallization of the crude product from EtOH gave **2**; yield 0.11 g (36 %); mp 225 – 226 °C; ^{1}H-NMR (DMSO-d_6) δ 3.9 (s, 3H), 7.3 (m, 2H), 7.7 (m, 2H), 8.1 (4H), 10.1 (s, 1H). ES-MS *e/z* 237 (M+1, 100).

N-Isopropyl-2-[4-(1-methyl-1*H*-benzimidazol-2-yl)-phenyl]-1*H*-benzimidazole-5-carboxamidine Hydrochloride (3)

A mixture of **2** (0.124 g, 0.525 mmol), 3,4-diamino-*N*-isopropylbenzamidine . HCl . $^1/_2$ H_2O (0.125 g, 0.525 mmol) and 1,4-benzoquinone (0.057 g, 0.525 mmol) in absolute EtOH (10 ml) was heated under reflux for 8 h. After EtOH was removed, dilute sodium hydroxide solution (1 M, 30 ml) was added and the mixture was stirred 3 h at room temperature. The solid was filtered, washed with hot water, and then chromatographed using $CHCl_3$ isopropanol ammonia (50 : 25 : 4) as eluent. The HCl salt of **3** was crystallized from a mixture of ethanolic HCl and ether to yield 0.055 g (18 %); mp >290 °C; ^{1}H-NMR (DMSO-d_6) δ 1.3 (d, 6H, J = 6.3), 4.1 (m, overlapping 4H), 7.62 (m, 3H), 7.87 (m, 2H), 8.04 (d, 1H,

J = 8.5), 8.1 (s, 1H), 8.2 (d, 2H, J = 8), 8.6 (d, 2H, J = 8), 9.06 (s), 9.46 (s), 9.6 (br s). ES-MS *e/z* 409 (M+1, 2.5), 205(100). Anal. Calcd. for $C_{25}H_{24}N_6$. 3.0 HCl . 3.5 H_2O: C, 51.69 ; H, 5.85 ; N, 14.46. Found : C, 51.93 ; H, 6.06 ; N, 14.45.

2-(4-Fluorophenyl)-1-methyl-1*H*-benzimidazole-5-carbonitrile (5)

In similar manner as for **1**, 3-amino-4-methylaminobenzonitrile (0.64 g, 4.35 mmol) and the sodium metabisulfite adduct of *p*-fluorobenzaldehyde (1 g, 4.35 mmol) were allowed to react. Crystallization of the crude product from aqueous EtOH gave **5** ; yield 0.78 g (72 %); mp 160 °C; ^{1}H-NMR (DMSO-d_6) δ 3.9 (s, 3H), 7.4 (m, 2H), 7.68 (d, 1H, J = 8.4), 7.8 (d, 1H, J = 8.4), 7.93 (m, 2H), 8.2 (s, 1H). MS *e/z* 251 ($M^{+\cdot}$, 13), 223(5), 149 (100), 121 (9.5), 104 (17,5), 76 (27).

2-(4-Fluorophenyl)-1-methyl-1*H*-benzimidazole-5-carbaldehyde (6)

In similar manner as for **2**, **5** (0.3 g, 1.2 mmol) was allowed to react with DIBAL. After extracting with CH_2Cl_2, the solvent was evaporated and the residue chromatographed using EtOAc n-Hexane (1:1) as eluent to yield 0.13 g (43 %) of **6**; mp 168-170 °C;^{1}H-NMR (DMSO-d_6) δ 3.9 (s, 3H), 7.45 (m, 2H), 7.82 (d, 1H, J = 9), 7.86 (d, 1H, J = 9), 7.95 (m, 2H), 8.26 (s, 1H), 10.07 (s, 1H). ES-MS *e/z* 255 (M+1, 100).

2'-(4-Fluorophenyl)-*N*-isopropyl-1'-methyl-1*H*,1'*H*-[2,5']-bibenzimidazolyl-5-carboxamidine Hydrochloride (7)

In similar manner as for **3**, 1,4-benzoquinone (0.098 g, 0.91 mmol), **6** (0.23 g, 0.91 mmol), and 3,4-diamino-*N*-isopropylbenzamidine.HCl.0.5H_2O (0.215 g, 0.91 mmol) were allowed to react. The residue was chromatographed using CH_2Cl_2 isopropanol ammonia (50 : 25 : 4) as eluent. The HCl salt of **7** was crystallized from the mixture of ethanolic HCl and ether to yield 0.089 g (17%); mp: >290 °C; ^{1}H-NMR (DMSO-d_6) δ 1.3 (d, 6H), 4.04 (s, 3H), 4.12 (m, 1H), 7.57 (m, 2H), 7.74 (d, 1H, J = 8.5), 7.93 (d, 1H, J = 8.5), 8.07 (m, 2H), 8.16 (s, 1H), 8.2 (d, 1H, J = 9), 8.6 (d, 1H, J = 9), 8.97 (s, 1H), 9.2 (s), 9.6 (s), 9.76 (br s). ES-MS *e/z* 427 (M+1 , 2.6), 214(100). Anal. Calcd. for $C_{25}H_{23}FN_6$. 3 HCl . 2.0 H_2O: C, 52.51 ; H, 5.29 ; N, 14.69 Found : C, 52.65 ; H, 5.45 ; N, 14.25 .

1'-Butyl-*N*-isopropyl-2'-{4-[5-(*N*-isopropyl-carbamimidoyl)-1*H*-benzimidazol-2-yl]phenyl}-1*H*,1'*H*-[2,5']bibenzimidazolyl-5-carboxamidine Hydrochloride (9)

A mixture of **8**[14] (0.19 g, 0.62 mmol), 3,4-diamino-*N*-isopropylbenzamidine .HCl .0.5 H_2O (0.294g, 1.24 mmol) and 1,4-benzoquinone (0.134 g, 1.24 mmol), in 10 ml of absolute EtOH was heated under reflux for 12 h. The mixture was cooled to room temperature, and the blue solid was collected by filtration,

washed with cold EtOH, and then stirred in dilute sodium hydroxide solution for 3 h. The precipitate was washed with water (until the filtrate was colorless) and dried. The free base was treated with charcoal in boiling EtOH and filtered. The filtrate was concentrated to 50 ml and acidified with HCl gas to give a blue solid; yield 0.09 g (15 %); mp > 290 °C; ^{1}H-NMR (DMSO-d_6) δ 0.8 (t, 3H, J = 7.2), 1.22 (m, 2H, J = 7.2), 1.35 (d, 12H, J = 6), 1.8 (m, 2H, J = 7.1), 4.15 (m, 2H), 4.58 (t, 2H), 7.6-8.95 (m, 13H), 9.05 (s), 9.1 (s), 9.5 (s), 9.6 (s), 9.7 (br s), 9.8 (br s). ES-MS *e/z* 651 (M+1, 100). Anal. Calcd. for $C_{39}H_{42}N_{10}$. 5.0 HCl . 4.0 H_2O : C, 51.75 ; H, 6.13 ; N, 15.47. Found : C, 52.03 ; H, 6.40 ; N, 14.96.

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References

1. S. Neidle, *Nat. Prod. Rep.* **18**, 291 (2001).
2. D. W. Boykin, A. Kumar, J. Spychala, M. Zhou, R. J. Lombardy, W. D. Wilson, C. C. Dykstra, S. K. Jones, J. E. Hall, R. R. Tidwell, C. Laughton, C. M. Nunn and S. Neidle, *J. Med. Chem.* **38**, 912 (1995)
3. J. O. Trent, G. R. Clark, A. Kumar, W. D. Wilson, D. W. Boykin, J. E. Hall, R. R. Tidwell, B. L. Blagburn and S. Neidle, *J. Med. Chem.* 39, 4554 (1996)
4. D. W. Boykin, A. Kumar, G. Xiao, W. D. Wilson, B. C. Bender, D. R. McCurdy, J. E. Hall and R. R. Tidwell, *J. Med. Chem.* **41**, 124 (1998)
5. I. Francesconi, W. D. Wilson, F. A. Tanious, J. E. Hall, B. C. Bender, R. R. Tidwell, D. McCurdy and D. W. Boykin, *J. Med. Chem.* **42**, 2260 (1999)
6. M. D. Poeta, W. A. Schell, C. C. Dykstra, S. Jones, R. R. Tidwell, A. Czarny, M. Bajic, M. Bajic, A. Kumar, D. W. Boykin, and J. R. Perfect, *Antimicrob. Agents and Chemother.* **42**, 2495 (1998)
7. J. Mann, A. Baron, Y. Opoku-Boahen, E. Johansson, G. Parkinson, L. R. Kelland, and S. Neidle, *J. Med. Chem.* **44**, 138 (2001)
8. T. Stokke and H. B. Steen, *J. Histochem. Cytochem.* **33**, 333 (1985)
9. R. P. Haugland, In Handbook of Fluorescent Probes and Research Chemicals, 6th ed. M.T.Z. Spence, Ed. Molecular Probes: Eugene, OR, 1996
10. T. G. Minehan, K. Gottwald, and P.B. Dervan, *Helv. Chim. Acta* **83**, 2197 (2000)
11. Y.-H. Ji, D. Bur, W. Hasler, V. R. Schmitt, A. Dorn, C. Bailly, M. J. Waring, R. Hochstrasser and W. Leupin, *Bioorg. Med. Chem.* 9, 2905 (2001)
12. M. Rangarajan, J. S. Kim, S.-P. Sim, A. Liu, L. F. Liu, and E. J. LaVoie, *Bioorg. Med. Chem.* **8**, 2591 (2000)
13. H. F. Ridley, R. G. W. Spickett, and G. M. Timmis, *J. Heterocycl. Chem.* *2*, 453 (1965)
14. D. W. Boykin and H. Göker, *Heterocyclic Comm.*, (in press)