DESIGN AND SYNTHESIS OF A NEW CLASS OF PYRROLOBENZIMIDAZOLE BASED AGENTS TO TARGET HUMAN TUMOR HELICASES

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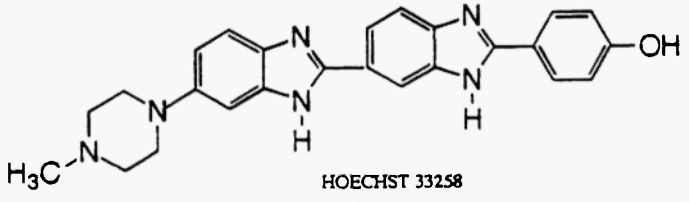
Abstract : The syntheses of pyrrolobenzimidazole based Hoechst 33258 analogues were carried out in conjunction with a design to explore the potential for selective inhibition of human tumor helicases. The structural modifications include the following : substitution of pyridine for the benzene ring of the piperazinyl benzimidazole moiety and introduction of a pyrrolo group in another benzimidazole moiety.

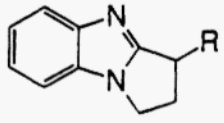
Introduction :

Hoechst 33258 readily penetrates mammalian cells and preferentially binds to double-helical DNA at rich DNA sequences nominally four or five consecutive AT base pairs in length (1-4) . Its nonintercalative mode of binding and its specificity for AT rich sequences was established by absorption spectroscopy, footprinting and x-ray studies (5). The flexible nature of this bis-benzimidazole ring system permits the ligand to adopt an optimum conformation and thus bind effectively to double-stranded DNA (5b). The bis-benzimidazole Hoechst 33258 has been used widely as a chromosomal staining agent in biochemistry because it has ready access in to cells (6). As part of our continuing efforts to develop novel anticancer agents our group synthesised and examined several types of Hoechst 33258 analogues (7).

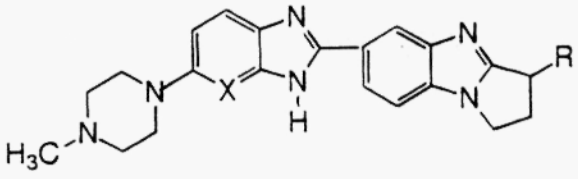
Scheme I



HOECHST 33258

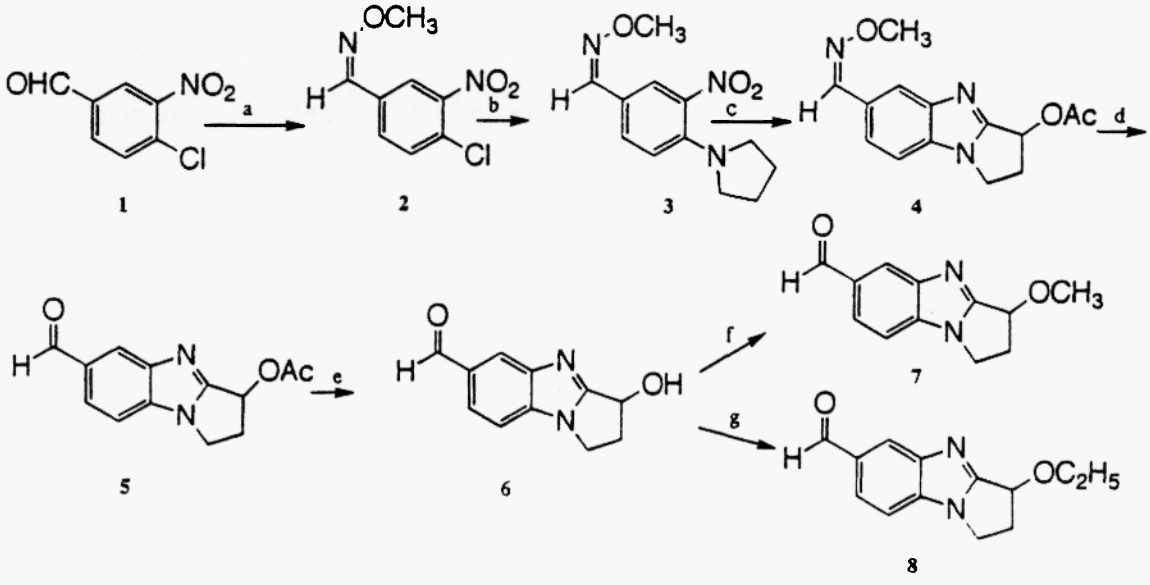


PYRROLOBENZIMIDAZOLE (PBI)



PYRROLO BIS-BENZIMIDAZOLE

Presented herein is the synthesis of Hoechst 33258 analogues with structural modifications by introducing a pyrrolo group and changing the position of linkage of the benzimidazole ring system. Certain agents containing the pyrrolobenzimidazole ring system show marked cytotoxicity towards solid tumors but not towards leukemia (8). The mechanism of pyrrolobenzimidazole cytotoxicity implicates DNA cleavage presumably as a result of binding to the major groove followed by base or phosphate backbone alkylation (8). In addition the role of the 3-subsituent in pyrrolobenzimidazole ring systems controls both the cytotoxicity and extent and type of DNA alkylation. In particular the presence of an additional ester group at the 3-position is required for optimal cytotoxicity and an additional plausible role of this substituent is to provide liphophilicity to promote cellular uptake and a hydrogen-bonding functionality (9) to assist in DNA binding. These considerations prompted us to design the synthesis of novel pyrrolo-bisbenzimidazole ring systems.



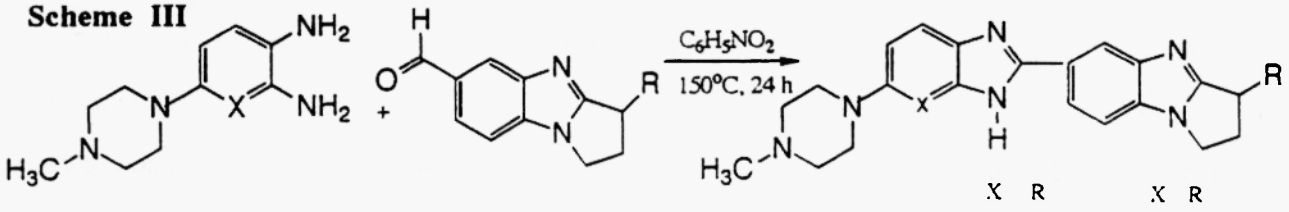
Scheme II : a) H_2N-OCH_3 / Py in MeOH; b) Pyrrolodine in EtOH; c) $ZnCl_2$ (anhydrous) /Ac_2O; d) $SnCl_2$ / HCl; e) K_2CO_3 / MeOH; f) MeI / K_2CO_3 in MeOH, g) Et I / K_2CO_3 in THF

Results and discussion :

The preparation of the pyrrolo-bisbenzimidazoles is summarised in Schemes (**II, III**). 4-Chloro-3-nitrobenzaldehyde was treated with methoxyamine hydrochloride in methanol to protect the aldehyde functionality and to give compound (**2**) in 77% yield. The aromatic aldehyde can, of course, also be blocked with simple protecting groups such as hydroxylamine hydrochloride, ethanethiol etc., but in the following steps of reductive ring cyclization these groups were affected by anhydrous $ZnCl_2$ and Ac_2O. We found that methoxyamine protecting group remained intact in the ring closure step, hence the methoxyamine hydrochloride was satisfactory as the protecting group. Heating of compound (**2**) with pyrrolidine in ethanol at 90 ^{0}C for 5 h gave compound (**3**) in 78% yield. The o-pyrrolidinonitrobenzene derivative (**3**) on cyclization with acetic anhydride in the presence of anhydrous $ZnCl_2$ Lewis acid as catalyst at 140 ^{0}C for 5 h afforded cyclized compound (**4**) in 58% yield. A $ZnCl_2$ catalysed internal redox reaction is plausibly involved leading to the o-nitroso iminium ion, which cyclizes and rearranges to the cyclized product **4** (10).

The deprotection of the methoxime function of (**4**) was surprisingly difficult using several conventional reductive and oxidative methods, such as Zn/AcOH, DIBAL, $TiCl_4$/NaI, $TiCl_3$.3THF/DIBAL (11) and NCS reagents. Finally success was realized using an anhydrous $SnCl_2$/conc.HCl reductive method to deprotect the methoxime functionality and this procedure afforded the aldehyde (**5**) in 36% yield.

Scheme III



9. X=CH

14. X=N

(5-8)

10. CH OAc 15. N OAc

11. CH OH 16. N OH

12. CH OCH_3 17. N OCH_3

13. CH OC_2H_5 18. N OC_2H_5

To our knowledge there is no previous report on deoximation of a methoxime functionality of an aromatic aldehyde system.While E.J Corey and coworkers (11) reported the deoximation of methoxime within a 3-cyclopentenone ring system, however that method was unsuccessful for aromatic ring systems. The targeted pyrrolo-bisbenzimidazole product (**10-13&15-18**) was obtained by direct condensation of aldehyde (**5-8**) and orthodiamine (**9,14**) using our previous reported method (12) in which nitrobenzene used as an oxidant. The new agents are currently undergoing biological evaluation.

EXPERIMENTAL :

Melting points were determined on an Electrothermal melting point apparatus and are uncorrected. The IR Spectra were recorded on a Nicolet 7199 FT Spectrophotometer and only the principal bands are reported. The ^{1}H-NMR Spectra were recorded on Bruker WH-200 and WH 400 spectrometers . Mass spectra were determined on Associate Electrical Industries (AEI) MS-9 and MS-50 focussing high resolution mass pectrometers. Kieselgel 60 (230-400 mesh) of E.Merck and florisil (60-100 mesh was used for chromatography , and precoated silicagel 60F-254 sheets of E.Merck were used for TLC, with the solvent system indicated in the procedure. TLC plates were visualized using UV light. All the chemicals used were of reagent grade.

4-Chloro-3-nitro-methoxybenzaldoxime (2) :

To a solution of 4-Chloro-3-nitrobenzaldehyde **1** (10g, 53.9 mmol) in methanol (200 mL) was added methoxyamine hydrochloride (6.75g, 80.83 mmol) and pyridine (5mL). The reaction mixture was stirred at room temperature for 1.5 h. The solvent was removed in vacuo and the residue was diluted with water and then extracted with dichloromethane (3x100mL). The organic solution was dried ($MgSO_4$ anhydrous) and evaporated, and the residue was crystallized from hexane to give **2** (9g, 77.8% yield). mp. 95-100 ^{0}C. ^{1}H-NMR ($CDCl_3$) : 8.2(s,1H), 8.0 (s,1H, benzylic proton), 7.7 (d, 1H, aromatic), 7.5 (d,1H,aromatic), 3.9 (s,3H, -OCH_3), Anal.calcd for $C_8H_7N_2O_3Cl$: C,44.75, H, 3.26, N, 13.05. Found: C, 44.68, H, 3,23, N, 13.00

4-Pyrrolidino-3-nitromethoxybenzaldoxime (3) :

To a solution of **2** (5g, 23.31 mmol) in 95% ethanol (50mL) was added pyrrolidine (2.5mL, 34.96 mmol) and the resulting mixture refluxed for 5 h at 90 ^{0}C. The solvent was evaporated and the residue was diluted with water and extracted with dichloromethane (3x60mL). The orange colored solution was dried (anhydrous $MgSO_4$) and evaporated to give a residue,which was chromatographed on silicagel (hexane/DCM, 1:1 to give **3** (4.5g,77.5%).mp. 75-80 ^{0}C. ^{1}H-NMR ($CDCl_3$) : 8.1 (s, 1H) , 8.0 (s, 1H, benzylic proton), 7.6 (d,1H, aromatic), 7.4 (d, 1H, aromatic), 3.9 (s,3H,-OCH3), 3.3(m,4H,-CH2-N-CH2-), 2.0(m,4H -CH_2-CH_2-). Anal.calcd for $C_{12}H_{15}N_3O_3$: C , 57.83, H, 6.02, N, 16.86. Found : C, 57.81 ; H, 6.0, n, 16.75.

6-Formylmethoxime-2,3-dihydro-1H-pyrrolo-[1,2-a]-benzimidazole-3-acetate (4):

This compound was synthesised by the reported procedure.[10] yield. 58%, mp. 145-150 ^{0}C . ^{1}H-NMR ($CDCl_3$) : 8.2 (s, 1H), 8.0 (s, 1H, benzylic proton), 7.6 (d, 1H, aromatic), 7.4 (d, 1H, aromatic), 6.2 (1H, dd, J=7.6 Hz, J=3.4 Hz, C(3) proton), 4.3 and 4.2 (2m, 2H, C (1) diastereomeric methylene), 3.9 (s, 3H, methoxy), 3.3 and 2.8 (2m, 2H, C(2) diastereomeric methylene), 2.1 (s, 3H,acetate methyl)), mass spectrum (EI mode), m/Z 273. Anal.calcd.for $C_{14}H_{15}N_3O_3$: C, 61.53, H, 5.49, N, 15.38. Found : C, 61.51; H, 5.47; N, 15.36

3-Acetoxy-6-formyl-2,3-dihydro-1H-pyrrolo [1,2-a] benzimidazole (5) :

A solution of **4**, 3g (10.98 mmol) in 4 mL of conc.hydrochloric acid was cooled in an ice bath and then to this was added a solution of anhydrous stannous chloride (6.2g,32.94 mmol) in 5 mL of conc.hydrochloric acid drop by drop over 10 min, then the resultant mixture was stirred for one hour at room temperature. The reaction mixture was then placed in ice bath and was then brought to pH 6.5 with 5% sodium hydroxide solution. The cloudy solution was filtered through a Buckner funnel and the residue washed with ethyl acetate. The filtrate was thoroughly extracted with ethyl acetate (3x50mL). The ethyl acetate extracts were combined and dried (anhydrous $MgSO_4$), Evaporation of solvent gave a colorless residue, which was recrystallized from dichloro-

methane and hexane to give **5** (920 mg, 36.1% yield), mp. 115-120 ^{0}C. ^{1}H-NMR (CDCl3) : 10.1 (s, 1H), 8.3 (s,1H), 7.8 (d, 1H, aromatic), 7.5 (d, 1H, aromatic), 6.2 (1H, dd, J=7.6 Hz, J=3.4Hz, C(3) proton), 4.3 and 4.2 (2m, 2H, C(1) diastereomeric methylene), 3.3 and 2.8 (2m, 2H, C(2) diastereomeric methylene) , 2.1 (s, 3H, acetate methyl); mass spectrum (EI mode), m/z. 244, (201, 100%), 230, 185,156. Anal.calcd.for $C_{13}H_{12}N_2O_3$: C, 63.06; H, 4.91; N, 11.47. Found : C, 63.04; H, 4.92; N, 11.41.

3-Hydroxy-6-formyl-2,3-dihydro-1H-pyrrolo-[1,2-a]-benzimidazole (6) : To a solution of **5** (2.5 g, 10.77 mmol) in methanol (50 mL) was added anhydrous potassium carbonate (2.1 g, 16.15 mmol) and the resulting mixture was stirred for 1 h at room temperature. The solvent was removed in vacuo. The residue was diluted with water and then extracted with ethyl acetate (3x75 mL). The organic phase was washed with water, dried ($MgSO_4$) and evaporated to give **6** (1.6 g, 73% yield). mp.137-140 ^{0}C . ^{1}H-NMR (CDCl3) : 10.1 (s, 1H), 8.3 (s, 1H), 7.8 (d, 1H, aromatic), 7.5 (d, 1H, aromatic), 6.2 (1H, dd, J=7.6 Hz, J=3.4Hz, C(3) proton), 5.2 (s, 1H, -OH), 4.3 and 4.2 (2m, 2H, C(1) diastereomeric methylene), 3.3 and 2.8 (2m, 2H, C(2) diastereomeric methylene) : mass spectrum (EI mode) m/z. 202. Anal.calcd for $C_{11}H_{10}N_2O_2$: C,65.34 ; H,, 4.95 ; N, 13.86. Found C,65.29 ; H, 4.86 ; N, 13.80.

3-Methoxy-6-formyl-2,3-dihydro-1H-pyrrolo-[1,2-a]-benzimidazole (7) :

To a solution of **6** (1.2 g, 5.94 mmol) in methanol (25 mL) was added anhydrous potassium carbonate (800 mg) and methyl iodide (1.1 g, 17.82 mmol) and the resulting mixture was refluxed for 4 h at 70 ^{0}C . The solvent was removed in vacuo. The residue was diluted with water and then extracted with ethyl acetate (3x50 mL). The organic phase was washed with water,dried (anhydrous MgSO4) and evaporated to give **7** (850 mg,66% yield). mp.129-132 ^{0}C. ^{1}H-NMR ($CDCl_3$) : 10.1 (s, 1H), 8.3 (s,1H), 7.8 (d, 1H, aromatic), 7.5 (d, 1H, aromatic), 6.2 (1H, dd, J=7.6 Hz, J=3.4Hz, C(3) proton), 4.3 and 4.2 (2m, 2H, C(1) diastereomeric methylene), 3.7 (s, 3H, -OCH_3), 3.3 and 2.8 (2m, 2H, C(2) diastereomeric methylene) : mass spectrum (EI mode)

m/z. 216. Anal.calcd for $C_{12}H_{12}N_2O_2$: C,66.66 ; H, 5.55 ; N, 12.96. Found C, 66.58 ; H, 5.50 ; N, 12.92.

3-Ethoxy-6-formyl-2,3-dihydro-1H-pyrrolo-[1,2-a]-benzimidazole (8) :

This was synthesised similarly as compound **7** by using ethyl iodide in THF to give compound **8** (800 mg, 86% yield). mp.118-120 ^{0}C. ^{1}H NMR ($CDCl_3$): 10.1 (s,1H), 8.3 (s,1H), 7.8 (d, 1H, aromatic), 7.5 (d, 1H, aromatic), 6.2 (1H, dd, J=7.6 Hz, J=3.4Hz, C(3) proton), 4.3 and 4.2 (2m, 2H, C(1) diastereomeric methylene), 3.8 (q,2H, -CH_2-CH_3),1.9 (t, 3H, -O-CH_2-CH_3), 3.3 and 2.8 (2m, 2H, C(2) diastereomeric methylene) : mass spectrum (EI mode) m/z. 230. Anal.calcd for $C_{13}H_{14}N_2O_2$: C,67.82 ; H, 6.08 ; N, 12.17. Found : C, 67.79 ; H, 5.98 ; N, 12.04.

General Procedure for the Preparation of Pyrrolo-bisbenzimidazoles (10-13 and 15-18) :

A mixture of 4-(4-methyl-1-piperazinyl)-1,2-diaminobenzene (0.43 mmol) and the appropriate aldehyde (0.43 mmol) in 4 mL of nitrobenzene was heated at 150 ^{0}C for 24 h. The nitrobenzene was removed under reduced pressure, and the resulting residue was chromatographed on florisil by using ethyl acetate and methanol (7:3) to give the pyrrolo-bisbenzimidazoles as a yellow solid. The ^{1}H NMR of compounds 10-13 and 15-18 were described in a table.

5-(4-Methyl-1-piperazinyl)-[2,3-dihydro-1H-pyrrolo-[1,2-a]-3-acetylbenzimidazole-6'-yl] benzimidazole (10) : Mass spectrum (EI ode) m/z 430 (M^+ of base), 360, 300, 299, 71. Anal.calcd for $C_{24}H_{26}N_6O_2$: C,66.97; H,6.04: N,19.53. Found : C, 66.95; H,6.01; N,19.49. (70 mg, 38% yield). mp. 165-170 ^{0}C.

5-(Methyl-1-piperazinyl)-[2,3-dihydro-1H-pyrrolo-[1,2-a]-3-hydroxybenzimidazole-6'-yl] benzimidazole (11) : (Mass spectrum EI mode) m/Z 388 (M^+ of base), 372, 310, 269, 217. Anal.calcd.for $C_{22}H_{24}N_6O$: C, 68.04; H, 6.18; N, 21.64. Found : C, 68.00; H, 6.13; N, 21.61. (80 mg, 40% yield). mp.200-205 ^{0}C.

5-(4-Methyl-1-piperazinyl)-[2,3-dihydro-1H-pyrrolo-[1,2-a]-3-methoxybenzimidazole-6'-yl] benzimidazoie (12) : Mass spectrum (EI mode) m/z 402 (M^+ of base). Anal calcd for $C_{23}H_{26}N_6O$: C, 68.65; H, 6.46; N,20.89. Found : C, 68.58; H, 6.38; N, 20.67. (75 mg, 43% yield). mp.175-180 ^{0}C

5-(4-Methyl-1-piperazinyl)-[2,3-dihydro-1H-pyrrolo-[1,2-a]-3-ethoxybenzimidazole-6'-yl] benzimidazoie (13) : Mass spectrum (EI mode) m/z 416 (M^+ of base). Anal calcd for $C_{24}H_{28}N_6O$: C, 69.23; H,6.73; N,20.19. Found : C, 69.03; H, 6.68; N, 20.01.(80mg, 44% yield).mp.168-170 ^{0}C.

5-(4-Methyl-1-piperazinyl)-[2,3-dihydro-1H-pyrrolo-[1,2-a]-3-acetylbenzimidazole-6'-yl]-3H-imidazo[4,5-b]-pyridine (15) : Mass spectrum (EI mode) m/z 431 (M^+ of base), 412, 390.2, 334.2, 25.2. Anal calcd for $C_{23}H_{25}N_7O_2$: C, 64.03; H,5.80; N22.73. Found : C, 63.89; H, 5.6; N, 22.68. (90mg, 48% yield). mp.145-150 ^{0}C

5-(4-Methyl-1-piperazinyl)-[2,3-dihydro-1H-pyrrolo-[1,2-a]-3-hydroxybenzimidazole-6'-yl]-3H-imidazo[4,5-b]-pyridine (16) : Mass spectrum (EI mode) m/z 389 (M^+ of base), 334.2, 25.2. Anal calcd for $C_{21}H_{23}N_7O$: C, 64.78; H,5.91; N,25.19. Found : C, 64.35; H, 5.86; N, 25.02. (95mg, 56% yield). mp.180-185 ^{0}C

5-(4-Methyl-1-piperazinyl)-[2,3-dihydro-1H-pyrrolo-[1,2-a]-3-methoxybenzimidazole-6'-yl]-3H-imidazo[4,5-b]-pyridine (17) : Mass spectrum (EI mode) m/z 403 (M^+ of base). Anal calcd for $C_{22}H_{25}N_7O$: C, 65.50; H,6.20; N24.31. Found : C, 65.42; H, 6.16; N, 24,26. (85mg, 49% yield). mp. 160-165 ^{0}C.

5-(4-Methyl-1-piperazinyl)-[2,3-dihydro-1H-pyrrolo-[1,2-a]-3-ethoxybenzimidazole-6'-yl]-3H-imidazo[4,5-b]-pyridine (18) : Mass spectrum (EI mode) m/z 417 (M^+ of base). Anal .calcd. for $C_{23}H_{27}N_7O$: C, 66.18; H,6.47; N23.50. Found : C, 66.08; H, 6.40; N, 23.39. (80mg, 45% yield). mp.170-175 ^{0}C.

Table

Compound	^{1}H NMR(DMSO d_6) in δ ppm
10	12.8 (bs, 1H, NH), 8.4 (d, J=2.4Hz, 1H), 8.2 (d, J=8.7 Hz, 1H), 7.8 (dd, J=2.4 Hz,8.7 Hz, 1H), 7.4 (dd, J=2.3 Hz, 8.7 Hz, 1H), 7.0 (d, =2.4 Hz, 1H), 6.9 (d, J=8.7 Hz, 1H), 6.2 (dd, J=7.6 Hz, J=3.4 Hz, C(3) proton, H), 4.3 and 4.2 (2m, 2H, C(1) diastereomeric methylene), 3.3 and 2.8 (2m, H, C(2) diastereomeric methylene), 3.29 (m, 4H, 2xCH_2N), 2.6 (m, 4H, 2x CH_2N), 2.3 (s, 3H,-N-CH_3), 2.1 (s, 3H, acetate methyl)
11	12.8 (bs, 1H, NH), 8.4 (d, J=2.4Hz, 1H), 8.2 (d, J=8.7 Hz, 1H), 7.8 (dd, J=2.4 Hz, 8.7 Hz, 1H), 7.4 (dd, J=2.3 Hz, 8.7 Hz, 1H), 7.0 (d, =2.4 Hz, 1H), 6.9 (d, J=8.7 Hz, 1H), 6.2 (dd, J=7.6 Hz, J=3.4 Hz, C(3) proton,1H),5.4(bs, OH, 1H), 4.3 and 4.2 (2m, 2H, C(1) diastereo meric methylene), 3.3 and 2.8 (2m, H, C(2) diastereomeric methylene), 3.29 (m, 4H, 2x CH_2N), 2.6 (m, 4H, 2xCH_2N), 2.3 (s, 3H,-N-CH_3).
12	12.7 (bs, 1H, NH), 8.4 (d, J=2.4Hz, 1H), 8.2 (d, J=8.7 Hz, 1H), 7.8 (dd, J=2.4 Hz, 8.7 Hz, 1H), 7.4 (dd, J=2.3 Hz, 8.7 Hz, 1H), 7.0 (d, =2.4 Hz, 1H), 6.9 (d, J=8.7 Hz, 1H), 6.2 (dd, J=7.6 Hz, J=3.4 Hz, C(3) proton, 1H), 4.3 and 4.2 (2m, 2H, C(1) diastereomeric methylene), 3.3 and 2.8 (2m, H, C(2) diastereomeric methylene), 3.29 (m, 4H, 2xCH_2N), 2.6 (m, 4H, 2x CH_2N),3.3 (s, 3H, OCH_3),2.3 (s, 3H,-N-CH_3).
13	12.8 (bs, 1H, NH), 8.4 (d, J=2.4Hz, 1H), 8.2 (d, J=8.7 Hz, 1H), 7.8 (dd, J=2.4 Hz, 8.7 Hz, 1H), 7.4 (dd, J=2.3 Hz, 8.7 Hz, 1H), 7.0 (d, =2.4 Hz, 1H), 6.9 (d, J=8.7 Hz, 1H), 6.2 (dd, J=7.6 Hz, J=3.4 Hz, C(3) proton, 1H), 4.3 and 4.2 (2m, 2H, C(1) diastereomeric methylene), 3.3 and 2.8 (2m, H, C(2) diastereomeric methylene), 3.29 (m, 4H, 2xCH_2N), 2.6 (m, 4H, 2x CH_2N), 3.8(q,2H, OCH_2-CH_3), 1.9 (t, 3H, O-CH_2-CH-3),2.3 (s, 3H,-N-CH_3).
15	13.0 (bs, 1H, NH), 8.4 (d, J=2.4Hz, 1H), 8.1 (d, J=8.7 Hz, 1H), 7.8 (d, 1H), 7.6 (dd, J=2.3 Hz, 8.7 Hz, 8.7 Hz, 1H), 6.8 (d, J=8.7 Hz, 1H), 6.1 (dd, J=7.6 Hz, J=3.4 Hz, C(3) proton, 1H), 4.3 and 4.2 (2m, 2H, C(1) diastereomeric methylene), 3.3 and 2.8 (2m, H, C(2) diastereo meric methylene), 3.29 (m, 4H, 2xCH_2N), 2.6 (m, 4H, 2xCH_2N), 2.3 (s, 3H,-N-CH_3), 2.1 (s, 3H, acetate methyl)
16	13.0 (bs, 1H, NH), 8.4 (d, J=2.4Hz, 1H), 8.1 (d, J=8.7 Hz, 1H), 7.8 (d,1H), 7.6 (dd, J=2.3 Hz, 8.7 Hz, 8.7 Hz,1H), 6.8 (d, J=8.7 Hz, 1H), 6.1 (dd, J=7.6 Hz, J=3.4 Hz, C(3) proton, 1H), 4.3 and 4.2 (2m, 2H, C(1) diastereomeric methylene), 3.3 and 2.8 (2m, H, C(2) diastereo meric methylene), 3.29 (m, 4H, 2xCH_2N), 2.6 (m, 4H, 2xCH_2N), 5.2 (bs, 1H, OH),2.3 (s, 3H,-N-CH_3).
17	13.1 (bs, 1H, NH), 8.4 (d, J=2.4Hz, 1H), 8.1 (d, 1H), 7.8 (d 1H), 7.6 (dd, J=2.3 Hz, 8.7 Hz, 1H), 6.7 (d, J=8.7 Hz, 1H), 6.2 (dd, J=7.6 Hz, J=3.4 Hz, C(3) proton, 1H), 4.3 and 4.2 (2m, 2H, C(1) diastereomeric methylene), 3.3 and 2.8 (2m, H, C(2) diastereomeric methylene), 3.3(s,3H, OCH_3) 3.29 (m, 4H, 2xCH_2N), 2.6 (m, 4H, 2xCH_2N), 2.3 (s, 3H,-N-CH_3).
18	13.0 (bs, 1H, NH), 8.4 (d, J=2.4Hz, 1H), 8.1 (d, 1H), 7.8 (d, 1H), 7.6 (dd,J=2.3 Hz, 8.7 Hz, 1H), 7.0 (d, 6.7 (d, J=8.7 Hz, 1H), 6.2 (dd, J=7.6 Hz, J=3.4 Hz, C(3) proton, H), 4.3 and 4.2 (2m, 2H, C(1) diastereomeric methylene), 3.3 and 2.8 (2m, H, C(2) diastereomeric methylene), 3.3 (m, 4H, 2xCH2N), 2.6 (m, 4H, 2xCH2N), 2.3 (s, 3H,-N-CH3), 3.8 (q, 2H, CH_2-CH_3), 1.9 (t, 3H, CH_2-CH_3).

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