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Synthesis of novel pyrrolobenzodiazepine-quinoxaline hybrid molecule

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ABSTRACT

*Synthesis of novel pyrrolobenzodiazepine-quinoxaline hybrid molecule is described. This compound was prepared by linking C-9 of (**9**) with an 3-(quinoxalin-2-yl)aniline (**5**) through Buchwald–Hartwig reaction in good yields. Chemical structures were established by IR, ^{1}H NMR, ^{13}C NMR and mass spectral studies.*

Keywords: Pyrrolobenzodiazepine, Quinoxaline, Buchwald-Hartwig

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INTRODUCTION

Cancer is a group of more than 200 different diseases sharing the common characteristic of abnormal cellular division that is not subject to normal growth controls. Pyrrolobenzodiazepines (PBD) are a class of compound that can have antibiotic or anti-tumor properties and sequence-selective DNA minor-groove binding cross linking agents originally discovered in Streptomyces species. They are significantly more potent than systemic chemotherapeutic drugs. Novel results demonstrate that PBDs can be effectively used for antibody-targeted therapy[1-3]. A highly cytotoxic DNA cross-linking pyrrolobenzodiazepine (PBD) dimer with a valine-alanine dipeptide linker was conjugated to the anti-CD70 h1F6 mAb either through endogenous interchain cysteines or, site-specifically, through engineered cysteines at position 239 of the heavy chains.[4] They are characterized by an electrophilic N10–C11 imine group (or the hydrated equivalent) which forms a reversible covalent aminal linkage from their C11-position to the C2-NH2 group of a guanine in the DNA minor groove [5], [6]. Crucially, the molecules have (S)-chirality at their C11a-position, and this provides them with the appropriate 3-dimensional shape (i.e., isohelicity) to fit perfectly into the DNA minor groove. PBD/DNA adduct formation has been shown to inhibit a number of biological processes, including the binding of transcription factors to DNA [7-10] and the function of enzymes such as endonucleases [11-12] and RNA polymerase [13]. Many PBD molecules also have significant antimicrobial activity [14-19].

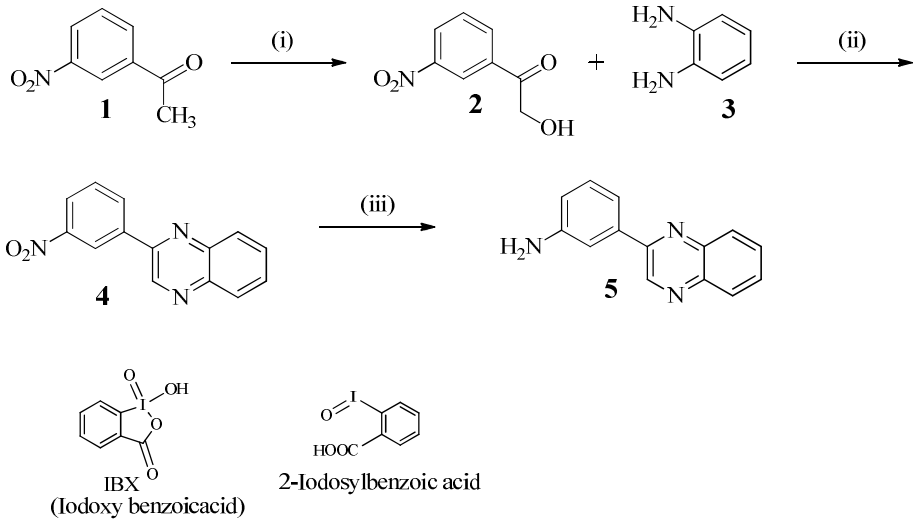
In view of the above reports, we herein report the synthesis of novel pyrrolobenzodiazepine-quinoxaline by Buchwald-Hartwig amination. Their structures were established by IR, ^{1}H-NMR, ^{13}C NMR and mass spectral data.

MATERIALS AND METHODS

Melting points were determined in an open capillary tube on Buchi R-535 and were uncorrected. Sigma-Aldrich, Merck and Lancaster Chemicals were used as such. Solvents used for spectroscopic and other physical studies were reagent grade and were further purified by standard procedures and techniques. Infrared spectra were recorded on Perkin-Elmer infrared-683 spectrophotometer with NaCl optics. Mass measurements were carried out on CEC-21-110B double focusing mass spectrometer operating at 70 eV using direct inlet systems and are given in mass units (m/z). 1H NMR spectra Varian Gemini-200, Avance 300 Varian Unity-400 and Varian FT-80A. Most of the samples were made in CCl_4/chloroform-d (1:1) using tetramethylsilane (Me_4Si) as the internal standard and are

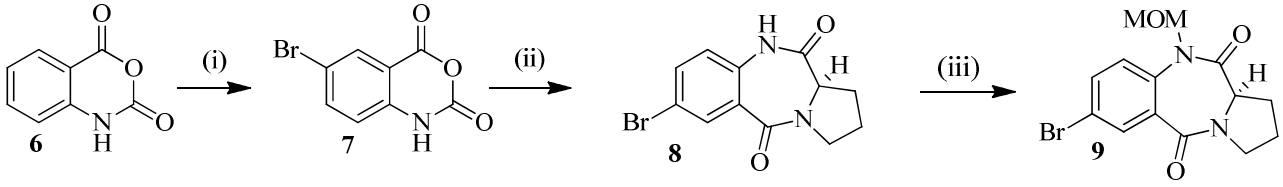
given in the δ scale. The standard abbreviations s, d, t, q, m, dd, dt, br s, refer to singlet, doublet, triplet, quartet, multiplet, double doublet, doublet triplet, broad singlet respectively.

Scheme 1



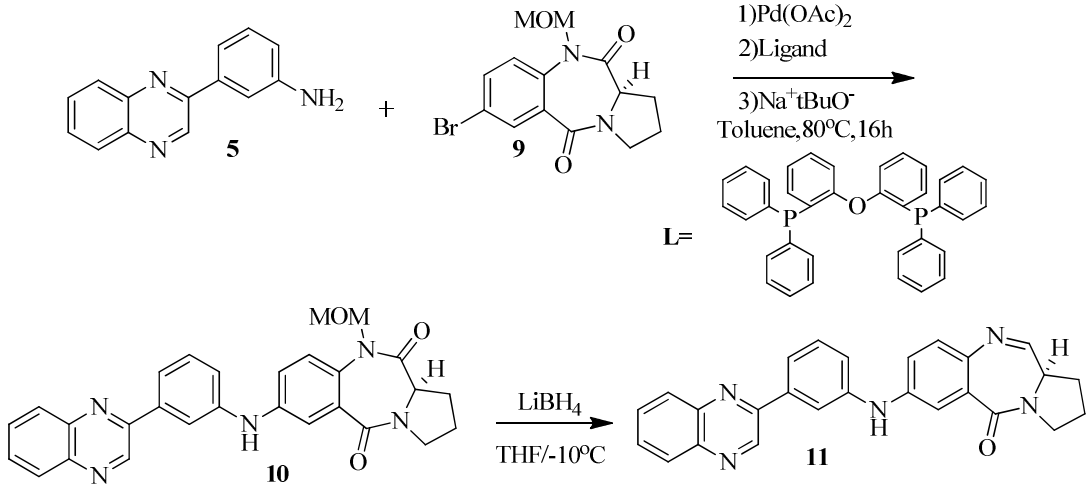
[a] *Reagent and conditions: (i) (1) KOH (3 equiv.), Iodosyl Benzoic acid, MeOH, 5°C-10°C. (2) 5% H_2SO_4, CH_2Cl_2. (ii) IBX, THF+1drop of DMSO, 80°C, 50min. (iii) $SnCl_2.2H_2O$, MeOH, 70°C, 2h.*

Scheme 2



[a] Reagent and conditions: (i) Br_2, H_2O, 50°C,2h. (ii) L-Proline, DMSO, 130°C, 6h.(iii) MOM-Cl, CH_2Cl_2, 0°C-r.t.

Scheme 3



Chemistry

Synthesis of *m*-amino quinoxaline **5** was started with α-hydroxylation of 3-nitro acetophenone with KOH using Iodosylbenzoic acid in dichloromethane with 83% yield of compound **2.** 2-hydroxy-1-(3-nitrophenyl)ethanone **2** treated with *o*-phenylenediamine **3** in presence of IBX at 80°C in THF+DMSO(9:1) gave the resulted compound **4** was reduced in the presence of $SnCl_2.2H_2O$ in MeOH to afford *m*-amino quinoxaline **2 (Scheme 1).** Bromination of isatoic anhydride (**6)** by treating it with bromine in water at 50°C for 2h. Compound **7** was treated with L-proline in DMSO at 130°C for 12h to afford (S)-7-bromo-2,3-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-5,11(10H,11aH)-dione**(8).** The dilactam **8** was treated with methoxy methyl chloride(MOM-Cl) in the presence of NaH in dry THF for 10h at r.t. to afford compound **(9) (Scheme 2).** Synthesis of compound **11** involving the

Buchwald–Hartwig reaction for the formation of carbon–nitrogen bonds *via* the palladium-catalyzed cross-coupling of aryl halides **9** with amines **5.** It was confirmed by its ^{1}H NMR showed characteristic peak at 9.25 (s, 1H) and ESI-MS showed 480 corresponding to $(M+H)^+$. Finally the key step of this synthesis is hydride reduction of MOM-protected dilactam **6**. After careful studies, we found that MOM-protected dilactam **13** was successfully converted to target compound in the form of imine (**11**) in 55% yield as yellow colour oil, by treating with $LiBH_4$ (1 molar equiv, prepared *insitu* from each 1.0 equiv of $NaBH_4$ and LiCl) in THF at -10^{o}C for 7h (**Scheme 3).**

Analytical data

Synthesis of compound 2-(3-Nitrophenyl)quinoxaline (4)

Light orange colour solid, mp 185-187^{o}C, ^{1}H NMR (300MHz, $CDCl_3$) δ (ppm) 7.72-7.90 (m, 3H, Ar-H), 8.11-8.25 (m, 2H, Ar-H), 8.30 (td, 1H, $J_{(1,2)}$ = 8.3, $J_{(1,3)}$ =2.3, $J_{(1,3)}$ =1.5, , Ar-H), 8.6(d, 1H, *J* =8.3, Ar-H), 9.09-9.15(m, 1H, Ar-H), 9.41(s, 1H, Ar-H), ^{13}C NMR ($CDCl_3$, 75MHz) δ (ppm) 122.5, 124.7, 129.2, 129.8, 130.2, 130.5, 130.9, 133.1, 142.5, MS (ESI) m/z: 252 $(M+H)^+$, HRMS (ESI) Calcd for $C_{14}H_9N_3O_2$ $(M+H)^+$ 252.1652 found 252.1657.

Synthesis of compound 3-(Quinoxalin-2-yl) benzenamine (5)

Yellow colour Solid, mp 163-165^{o}C, ^{1}H NMR (500MHz, $CDCl_3$) δ (ppm) 3.80 (bs, 2H, -NH_2), 6.76 (dd, 1H, $J_{(1,2)}$ =2.9, $J_{(1,3)}$=8.9, Ar-H), 7.20-7.33(m, 1H, Ar-H), 7.45-7.59 (m, 2H, Ar-H), 7.64-7.80(m, 2H, Ar-H), 8.09(s, 2H, *J*=10.9, Ar-H), 9.25 (s, 1H, Ar-H), ^{13}C NMR ($CDCl_3$, 75MHz) δ (ppm) 113.8, 117.0, 117.8, 129.1, 129.4, 129.5, 130.1, 130.2, 137.8, 141.6, 142.2, 143.5, 147.3, 152.0, MS (ESI) m/z :222 $(M+H)^+$, HRMS (ESI) Calcd for $C_{16}H_{11}N_3$ $(M+H)^+$ 222.1249, found 222.1258.

(S)-7-bromo-2,3-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-5,11(10H,11aH)-dione (8) : yellow solid (6.043 g, 93 %). M.p. 128^{o}C, $[\alpha]_D^{20}$=+415 (c=1.0 in $CHCl_3$) , ^{1}H NMR ($CDCl_3$, 500 MHz, ppm) δ 1.81-2.14 (bs, 3H), 2.66-2.90 (bs, 1H), 3.40-3.67 (m, 1H), 3.71-3.91 (m, 1H), 3.96-4.15 (m, 1H), 6.95 (d, 1H, $J_{(1,2)}$ = 9.01), 7.48-7.59 (dd, 1H, $J_{(1,2)}$=9.01, $J_{(1,3)}$=3.01), 8.07 (d, 1H, $J_{(1,3)}$=3.01), 9.40-9.55 (bs, 1H). MS (ESI) *m/z* 317$(M+H)^+$, 319$(M+3H)^+$.

(S)-7-bromo-10-(methoxymethyl)-2,3-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-5,11(10H,11aH)-dione (9):

^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm) 1.91-1.22 (m, 4H), 2.62-2.79 (m, 1H), 3.38-3.63 (m, 4H), 3.70-3.87 (m, 1H), 4.05-4.18 (m, 1H), 4.66 (d, 1H, *J*=9.82), 5.47 (d, 1H, *J*=9.82), 7.49-7.65 (m, 2H), 7.99-8.05 (m, 1H), ^{13}C NMR ($CDCl_3$, 300MHz, ppm) δ 23.56, 26.53, 46.75, 57.02, 57.28, 79.50, 119.63, 124.13, 131.17, 132.66, 135.12, 138.53, 163.69, 169.89, MS (ESI) *m/z* 339$(M+H)^+$, 341$(M+3H)^+$.

(S)-10-(methoxymethyl)-7-((3-(quinoxalin-2-yl)phenyl)amino)-2,3-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4] diazepine-5,11(10H,11aH)-dione (10): ^{1}H NMR ($CDCl_3$, 300 MHz, ppm) δ 1.73-1.92 (m, 3H), 2.22-2.35 (m, 1H), 3.18 (s, 3H), 3.35-3.38 (m, 1H), 3.42-3.55 (m, 1H), 3.81-3.87 (m, 1H), 4.63-4.69 (d, 1H), 5.41-5.45 (d, 1H), 6.49-6.57 (d, 1H), 6.79-6.99 (m, 5H), 7.57-7.69 (m, 2H), 8.21-8.38 (m, 2H), 9.25 (s, 1H), 23.42, 29.25, 46.75, 51.43, 67.91, 73.95, 106.26, 109.83, 117.85, 120.12, 121.54, 128.61, 129.05, 129.62, 141.23, 141.38, 141.97, 143.65, 150.26,150.67, 155.62, 163.45, 170.56, MS (ESI) *m/z* 480 $(M+H)^+$,

(S)-7-((3-(quinoxalin-2-yl)phenyl)amino)-2,3-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-5(11aH)-one (11)

^{1}H NMR ($CDCl_3$, 500 MHz, ppm) δ 1.90-2.14(m, 3H), 2.58-2.68(m, 1H), 3.92-4.16(m, 1H), 4.18-2.29(m, 2H), 6.82(d, 1H), 7.01-7.24(m, 4H), 7.25-7.39(m, 3H), 7.65-7.74(m, 1H), 7.84-7.92(d, 2H), 8.18-8.21(d, 2H), 9.24(s, 1H), ^{13}C NMR ($CDCl_3$, 300 MHz, ppm) δ 22.53, 29.62, 53.56, 56.24, 117.02, 118.43, 119.89, 129.43, 129.93, 130.01, 130.74, 131.13, 133.58, 140.09, 141.26, 142.82, 146.83, 149.34, 154.43, 162.73, 164.03, EI-MS 53, 65, 77, 91, 109, 139, 300, 325, 419.

RESULTS AND DISCUSSION

In summary, a simple, convenient and general method has been developed for the preparation of hybrid molecule utilizing easily accessible and inexpensive starting materials. This synthetic approach includes some important aspects such as high yields and mild reaction conditions, which make this synthetic protocol a useful and an attractive procedure for the synthesis of novel pyrrolobenzodiazepine-quinoxaline hybrid molecule. And the identity and purity of the compounds were confirmed on the basis of their sharp m.p., TLC, IR, ^{1}H-NMR and ^{13}C-NMR data.

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