J. Chem Soc. Nigeria, Vol. 45, No. 2, pp 253 - 258 [2020]

Synthesis, Characterization and Bioactivity of *N,N'*-bis[2-(5-bromo-7-azabenzimidazol-1-yl)-2-oxoethyl]ethylene-1,3-diamine and the –cyclohexyl-1,2-diamine analogue.

K. A. Oluwafemi

Chemical Sciences Department, Adekunle Ajasin University, Akungba-Akoko, Nigeria

Author's email- *augustusoluwafemi@yahoo.com, +2348166940423*

Received 27 January 2020; accepted 10 February 2020, published online 27 March 2020

Abstract: N,N'-*bis*[2-(5-bromo-7-azabenzimidazol-1-yl)-2-oxoethyl]ethylene-1,3-diamine and the –cyclohexyl-1,2-diamine analogue were synthesized from 7-aza-5-bromobenzimidazole. Their effect on human cervix adenocarcinoma cells was examined and the results indicated that the two novel compounds were not cytotoxic.

Keywords: Azabenzimidazoles; Akylation; HeLa cells; Cytotoxicity; NMR

1. INTRODUCTION

Azabenzimidazole-based compounds **1** have been explored for their bioactivity against diseases; examples of bioactive compounds that have azabenzimidazole as their cores includes an antimalarial 2-Aminoazabenzimidazole (1) and 2-pyridyl-7-azabenzimidazole (2) which is an inhibitor of allosteric AKT (Figure 1). Other reported bioactive azabenzimidazole cores include 2-phenyl-azabenzimidazoles - active against TANK-Binding Kinase 1, and Nuclear Factor Kinase subunit epsilon (3) and *N*-arylated azabenzimidazole - inhibitors of *Plasmodium falciparum* Calcium-Dependent Protein Kinase 1 (4).

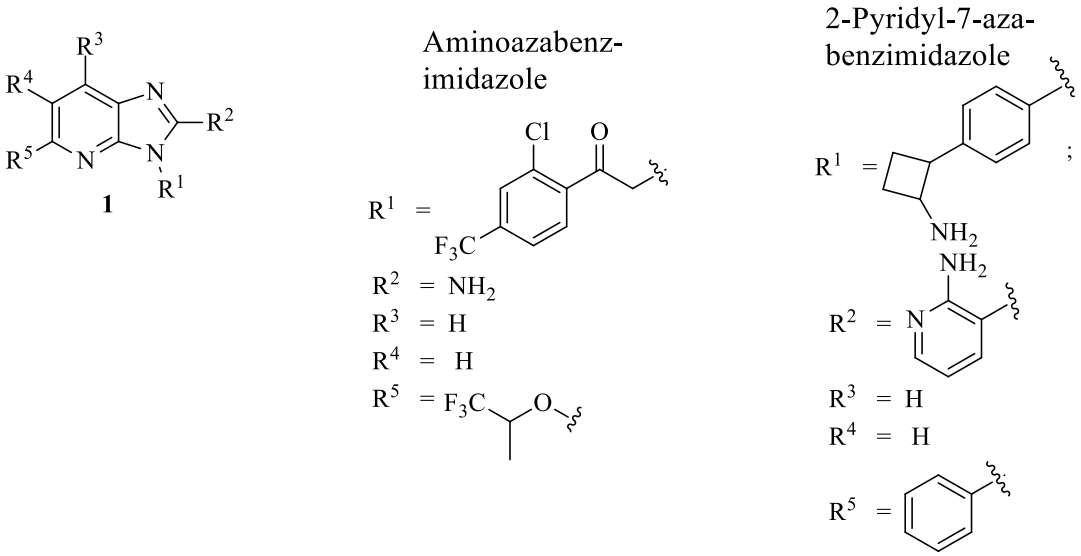


Figure 1. Bioactive azabenzimidazoles.

Based on this background, this article narrates the preparation and characterization of N,N'-*bis*[2-(5-bromo-7-azabenzimidazol-1-yl)-2-oxoethyl]ethylene-

1,3-diamine **6** and its –cyclohexyl-1,2-diamine analogue **7**; their *in-vitro* effects on human cervix adenocarcinoma (HeLa) cells was accessed – given that very little or zero toxicity is a required parameter to be considered when developing new chemotypes that could be useful in the treatment of infectious diseases.

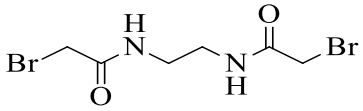
2. EXPERIMENTAL

2.1. General methods

All chemicals used were purchased from Sigma-Aldrich Chemical Co. and water used was deionized. Analytical thin layer chromatography (TLC) was performed using precoated silica gel plates and visualized under a UV lamp. Compounds were characterized using Bruker Avance 400 MHz NMR spectrometers. Chemical shifts for each experiment was reported in parts per million (ppm) relative to residual proton in deuterated solvent used for each experiment (Chloroform, 7.26 ppm and DMSO-d_6, 2.5 ppm) and the coupling constants (*J*) are reported in hertz (Hz), where s = singlet, br s = broad singlet, d =doublets and m = multiplet. The NMR spectra were analysed using mestrenova. IR spectra were recorded on a Perkin Elmer Spectrum 100 FTIR spectrometer with a diamond window. Melting points were determined using hot stage apparatus. Masses of compounds were determined by high-performance liquid chromatography-mass spectrometric (HPLC-MS) experiment was performed using Bruker daltonics compact QTOF MS with electrospray ionization probe, positive mode.

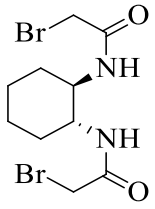
To assess anti-trypanocidal activity, compounds were added.

2.2. Method for the synthesis of *N,N'*-(ethane-1,2-diyl)bis[2-bromoacetamide] 3



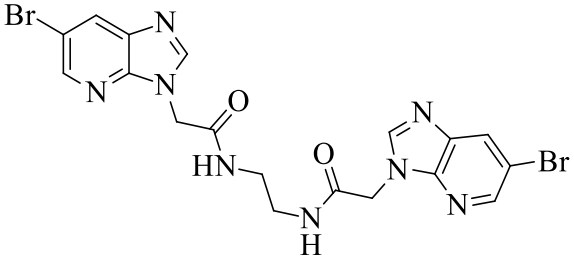
In a 50 ml round bottomed flask evacuated and filled with nitrogen, a solution of Ethylenediamine (0.2 ml, 2.9 mmol) was prepared in dry dichloromethane (25 ml) under nitrogen. Potassium carbonate (801.6 mg, 5.8 mmol) was added and the resulting mixture was stirred at 0 °C for 20 minutes under nitrogen. Bromoacetyl bromide **2** (0.5 ml, 5.8 mmol) was added slowly at 0 °C and the mixture was stirred vigorously under nitrogen for 20 minutes at room temperature. The crude product was extracted into dichloromethane (2 ×70 ml), the combined organic phase was washed with deionized water (2 ×70 ml), dried with anhydrous magnesium sulphate, filtered and concentrated *in vacuo* until the volume was reduced to about 10 ml. The residual solution was diluted with hexane (50 ml) and the precipitate formed is filtered under pressure to yield *N,N'-(ethane-1,2-diyl)bis(2-bromoacetamide)* **3** as a white solid (7851 mg, 90%); Mp: 142-144 °C [HPLC-MS: m/z calculated for $C_6H_{11}Br_2N_2O_2$ $(M+H)^+$ 300.9187. Found 301.1459]; υ_{max}/cm^{-1}: 3283, 3091 (NH), 1649 (C=O); $^{\delta}{}_H$/ppm (400 MHz; DMSO-d_6) 8.32 (s, 2H), 3.84 (4H, s, CO*CH*$_2$Br x 2), 3.16 – 3.13 (4H, m, NH*CH*$_2$ x 2); $^{\delta}{}_C$/ppm (100 MHz, DMSO-d_6) 166.2 (C=O), 38.5 (CO*C*H$_2$Br) and 29.5 (NH*C*H$_2$).

2.3. Method for the synthesis of *N,N'*-[(1R,2R)-cyclohexane-1,2-diyl]bis{2-bromoacetamide} 4.



The procedure described for **3** using (±)trans-1,2-diaminocyclohexane (0.2 ml 1.7mmol), potassium carbonate (470mg, 3.4 mmol) and bromoacetyl bromide **2** (0.3 ml, 3.4 mmol) to yield N,N'-*((1R,2R)-cyclohexane-1,2-diyl)bis(2-bromoacetamide)* **4** as a white solid (587.5 mg, 97%); Mp: 218-220 °C [HPLC-MS: m/z calculated for $C_{10}H_{17}Br_2N_2O_2$ $(M+H)^+$ 354.9657. Found 354.97868]; υ_{max}/cm^{-1}: 3242, 3076 (NH), 1641 (C=O); $^{\delta}{}_H$/ppm (400 MHz; DMSO-d_6) 8.12 (2H, d, *J* = 7.6 Hz, NH), 3.77 (4H, s, CO*CH*$_2$Br), 3.53 (2H, broad s, NH*CH* x 2), 1.77 (2H, d, *J* = 6.3 Hz,), 1.65 (2H, broad s, 2H) and 1.22 (4H, d, *J* = 5.8 Hz, 4H); $^{\delta}{}_C$/ppm (100 MHz, DMSO-d_6) 165.6 (C=O), 52.1 (CO*C*H$_2$Br), 31.5 (NH*C*H), 29.7 and 24.2 (CH_2).

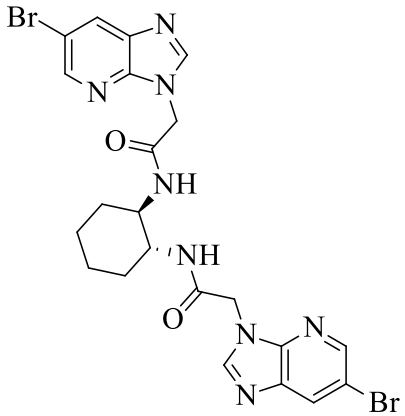
2.4. Method for the synthesis of *N,N'*-bis[2-(5-bromo-7-azabenzimidazol-1-yl)acetamido]-1,2-ethylenediamine 6.



A mixture of 5-bromo-7-azabenzimidazole **5** (200.0 mg, 1 mmol)) and caesium carbonate (488.73 mg, 1.5 mmol) in 1-methylpyrrolidinone (20 ml) was stirred at room temperature for 15 minutes. *N,N'*-(ethane-1,2-diyl)bis(2-bromoacetamide) **3** (151.0 mg, 0.5 mmol) was added to the solution. The resulting mixture was stirred vigorously at room temperature for 30 minutes while the progress of the reaction was monitored by thin layer chromatography. At the completion of the reaction, the organic crude product was extracted into ethyl acetate (2 × 70 ml). The combined organic phase

was thoroughly washed with deionized water (4 × 140 ml), dried with anhydrous sodium sulphate and filtered. The dried crude solution was concentrated to about 10 ml *in-vacuo* and diluted with hexane (40 ml) to precipitate the product. The precipitate was filtered and dried to give N,N'-*bis[2-(5-bromo-7-azabenzimidazol-1-yl)acetyl]-1,2-ethylenediamine* **6** was isolated as a white solid (386.1 mg, 72%); Mp: 284-286 °C [HPLC-MS: m/z calculated for $C_{18}H_{17}Br_2N_8O_2$ $(M+H)^+$ 534.9841. Found 534.9836]; υ_{max}/cm^{-1}: 3282, 3086 (NH), 1659 (C=O); δ_H/ppm (400 MHz; DMSO-d_6) 8.46 (2H, s, Ar-H), 8.44 (2H, d, *J* = 1.7 Hz, Ar-H), 8.38 (d, *J* = 1.5 Hz, 2H), 6.52 (2H, s, Ar-H), 4.96 (4H, s, NH*CH*$_2$), 3.19 (4H, s, CO*CH*$_2$); δ_C/ppm (100 MHz; DMSO-d_6) 165.1 (C=O), 148.1, 146.1, 143.9, 135.9, 129.6 and 112.9 (Ar-C), 59.8 NH*C*H$_2$) and 14.1 CO*C*H$_2$).

2.5. Method for the synthesis of (±)-*Trans-N,N'*-bis[2-(5-bromo-7-azabenzimidazol-1-yl)acetamido]cyclohexane 7.



The procedure described for the synthesis of **6** was followed using 5-bromo-7-azabenzimidazole **5** (200 mg, 1 mmol), caesium carbonate (488.7 mg, 1.5 mmol) 1-methylpyrrolidinone (20 ml) and N,N'-[(1*R*,2*R*)-cyclohexane-1,2-diyl]bis(2-bromoacetamide) **4** (178.0 mg, 0.5 mmol). *Trans(±)-1,2-bis[2-(5-bromo-7-azabenzimidazol-1-yl)acetamido]cyclohexane* **7** was isolated as a white solid (507.6 mg, 86%); Mp: 288-300°C [HPLC-MS: m/z calculated for $C_{22}H_{23}Br_2N_8O_2$ $(MH+2)^+$ 591.0310. Found 591.0309]; υ_{max}/cm^{-1}: 3273, 3083(NH), 1653 (C=O); δ_H/ppm (400 MHz; DMSO-d_6) 8.53 – 8.51 (m, 2H), 8.47 – 8.30 (m, 6H), 5.08 – 4.86 (m, 4H), 3.57 (s, 2H), 1.80 (d, *J* = 9.0 Hz, 2H), 1.64 (s, 2H), 1.35 – 1.15 (m, 4H); δ_C/ppm (100 MHz; DMSO-d_6) 165.97 – 165.70 (m, C=O), 154.4, 148.2, 148.1 (x 2), 146, 144.3, 143.9 (x 2), 135.9 (x 2), 129.6 (x 2), 127.9, 121.8, 113.1, 112.9 [ArC], 52.5 – 51.9 (m, CO*C*H$_2$), 47.3, 45.15 (x 2), 31.85 – 31.29 (m) and 24.24.

2.6. Method for Cytotoxicity Determination

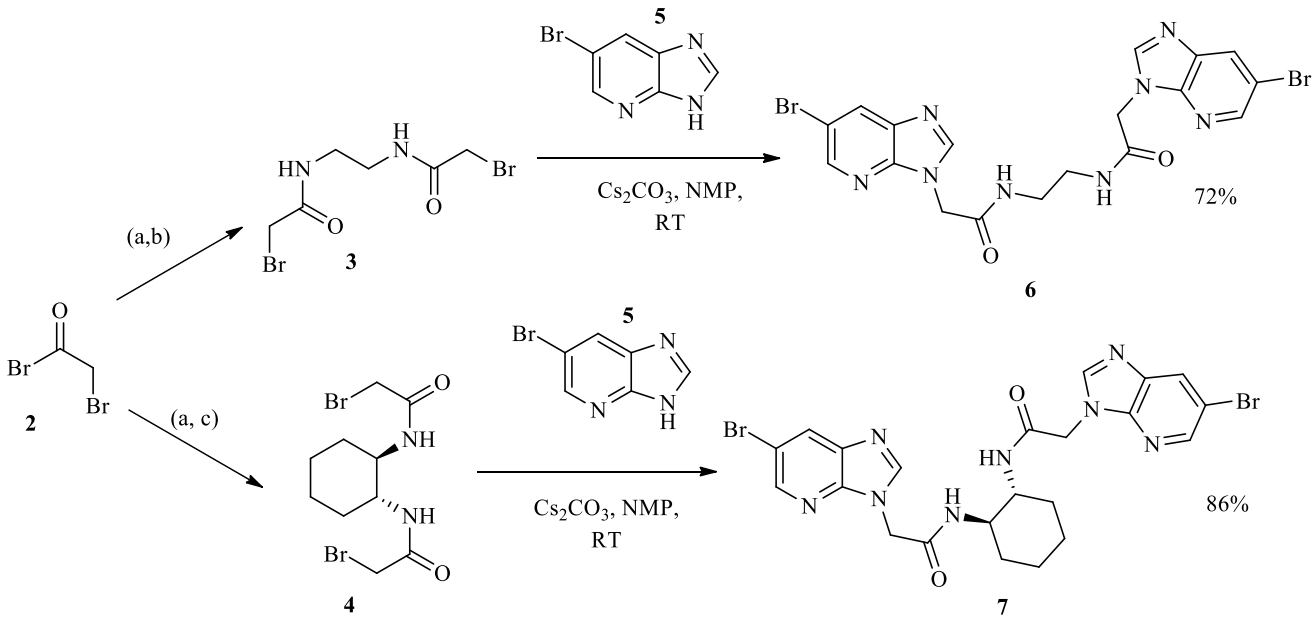
To assess the overt cytotoxicity of the compounds, they were incubated at a fixed concentration of 20 μM in 96-well plates containing HeLa (human cervix adenocarcinoma) cells for 48 hours. The numbers of cells surviving drug exposure were also determined by using the resazurin based reagent and reading resorufin fluorescence in a multiwell plate reader.

Results were expressed as % cell viability – the resorufin fluorescence in compound-treated wells relative to untreated controls. Compounds were tested in duplicate wells, and a standard deviation (SD) was also included. For the cytotoxicity assay, results were also expressed as % cell viability, based on fluorescence reading in treated wells vs. untreated control well. Emetine (which induces cell apoptosis) was used as a control drug standard.

3. RESULTS AND DISCUSSION

3.1. Chemistry

In an attempt to investigate the preliminary cytoxicity of bis-azabenzimidazole analogues, compounds **6** and **7** were prepared. The key intermediates in these reactions were prepared under nitrogen. In dry dichloromethane (DCM), ethylenediamine and (±)-*trans*-1,2-diaminocyclohexane were each treated with two equivalents of anhydrous potassium carbonate to abstract the amino protons for the generation of a nucleophilic centres.



Reagents and conditions. (a) K_2CO_3, DCM, r.t., N_2; (b) Ethylenediamine; (c) (±)-Trans-1,2-diaaminocyclohexane.

Scheme 1. Synthesis of bis-[2-(5-bromo-7-azabenzimidazol-1-yl)acetamides].

Two equivalents of bromoacetyl bromide were then added and the required products were isolated after 20 minutes, completion of the reaction having been determined by thin layer chromatography. Proton and carbom-13 (^{1}H and ^{13}C) NMR analysis indicated the success of this step, and the isolated diamides were pure enough for use in the second step.

Using compound 3 as a representative example, the ^{1}H NMR spectrum of the symmetrical diamide (Figure 2) shows the amido N-H signal at 8.32 ppm, the ethylene proton signal as a multiplet (due to rotational isomerism) at 3.17-3.13 ppm and the bromomethylene as singlet at 3.84 ppm (Figure 2). The ^{13}C NMR spectrum of shows the carbonyl carbon signal at 166.7 ppm, the ethylene carbon signals at 30.0 ppm and the bromomethylene signal almost overlapping the DMSO multiplets at 39.0 ppm (Figure 3).

The second step in this synthetic sequence involved the use of caesium carbonate as a base and *1*-methylpyrrolidone (NMP) as the solvent. The two reactants involved were soluble in NMP – a solvent that can easily be extracted into water at the end of the reaction process (during work-up). However, the products **6** and **7** were difficult to purify because of their polarity. Consequently, care was taken to make sure that exactly two equivalent of 5-bromo-7-azabenzimidazole and two equivalent of caesium carbonate were used to eliminate the possibility of having excess starting materials in the crude product mixture. The formation and purity of the desired products were confirmed by NMR analysis and 72 and 86% yields were obtained for the two compounds, respectively. The HPLC-MS analysis for **6** ($C_{18}H_{17}Br_2N_8O_2$ 534.9841) indicated the presence of $[M+H]^+$ 534.9838 peak.

The COSY experiment showed that the aliphatic proton signals appeared as singlets - no crosspeaks between the protons, as expected (Figure 4). The ^{1}H NMR spectrum of *N,N'*-bis[2-(5-bromo-7-azabenzimidazol-1-yl)acetyl]-1,2-ethylenediamine **6** indicated that the ethylene protons (1- and 2-CH_2) resonated at 3.19 ppm (Figure 5) while the HSQC spectrum assisted in deducing that these protons correlate with the ^{13}C signal resonating at 38.4 ppm, thus confirming that the signal corresponds to C-1 and C-2 (Figures 6 and 7). The methylene protons 5- and 5'-CH_2 correspond to the NMR signal at 4.96 ppm while HSQC spectrum correlates this signal to the ^{13}C signal at 45.6 ppm – evidently corresponding to C-5 and C-5'.

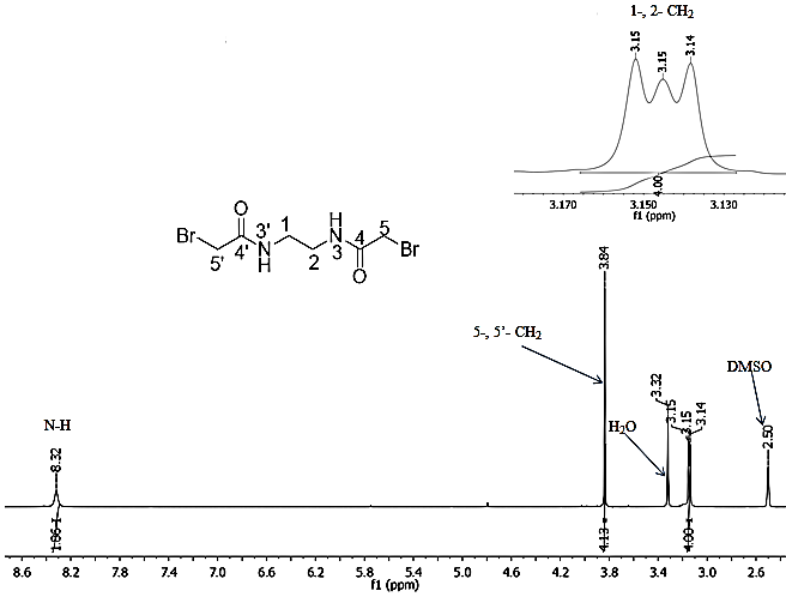


Figure 2. 400 MHz ^{1}H NMR spectrum of **3** in DMSO-d_6.

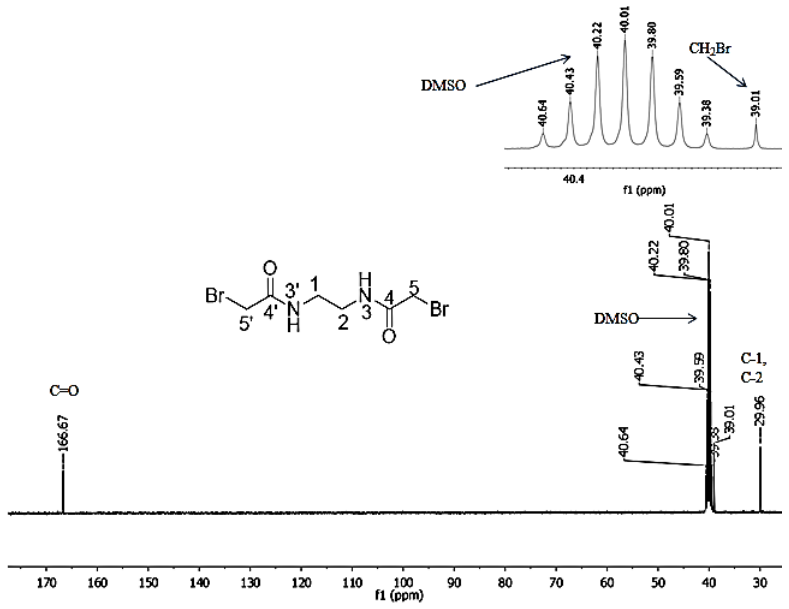


Figure 3. 100 MHz ^{13}C NMR spectrum of **3** in DMSO- d_6.

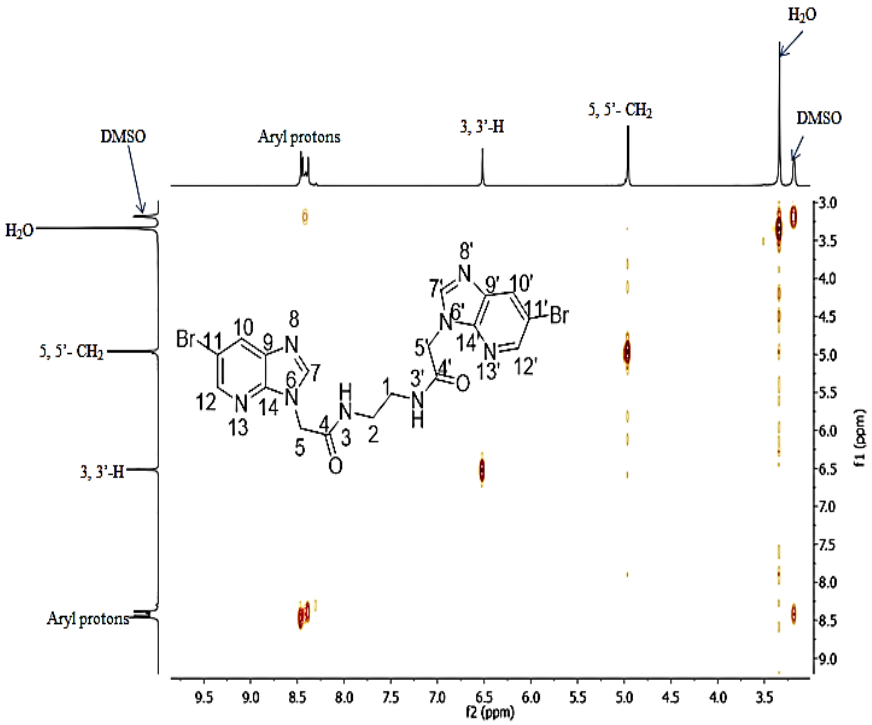


Figure 4. 400 MHz COSY NMR spectrum of **6** in DMSO-d_6.

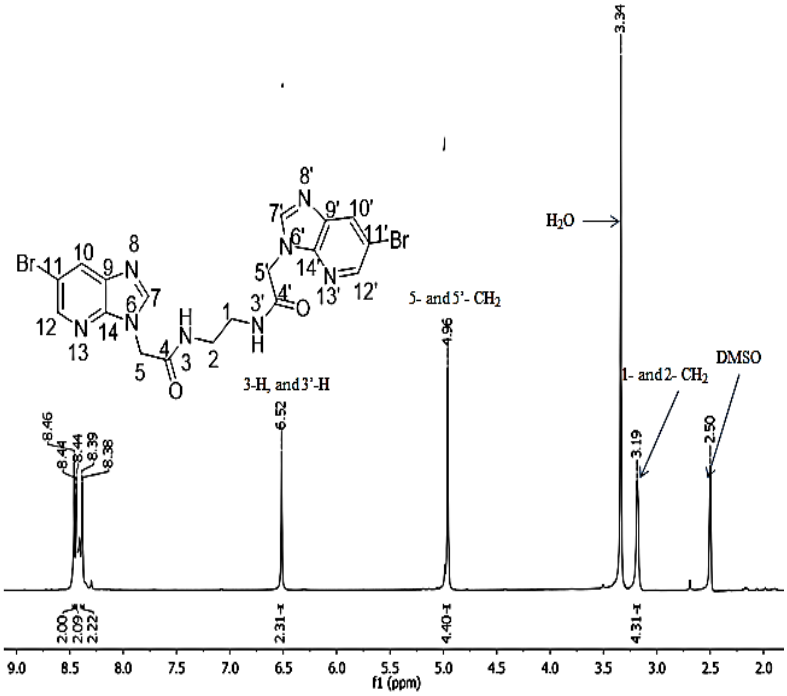


Figure 5. 400 MHz ^{1}H NMR spectrum of **6** in DMSO- d_6.

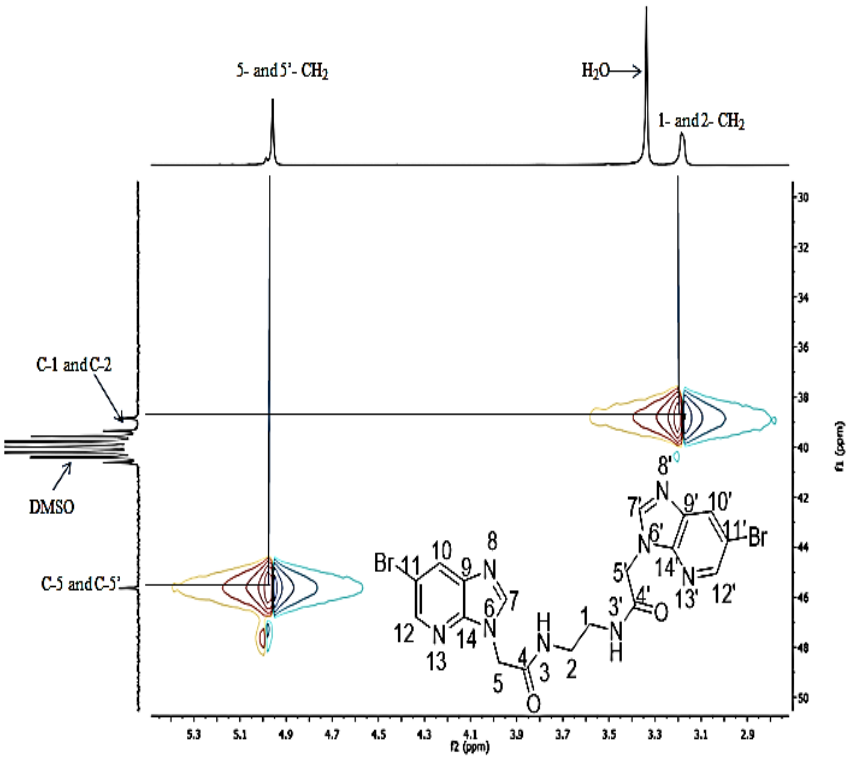


Figure 6. Partial HSQC NMR spectrum of **6** in DMSO-d_6.

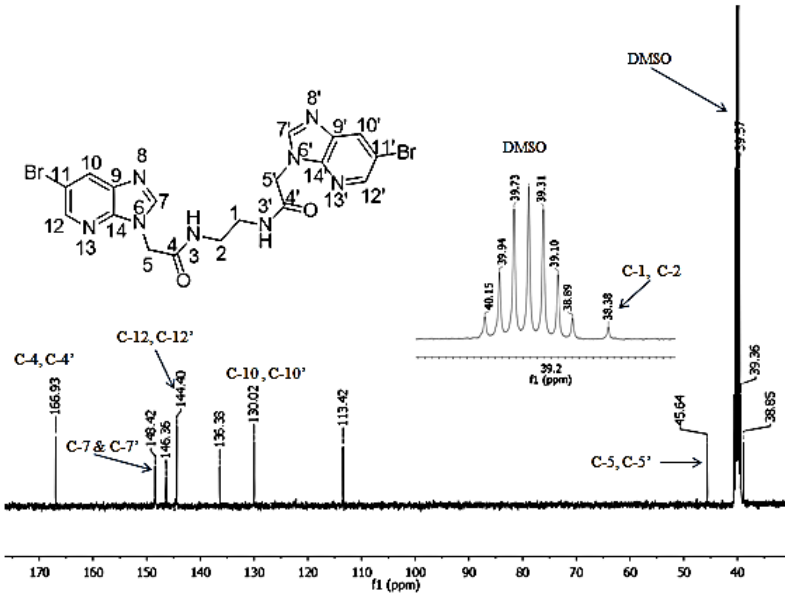


Figure 7. 100 MHz ^{13}C NMR spectrum of **6** in DMSO- d_6.

The 7-azabenzimidazole protons 7-H and 7'-H (which are far removed from other aromatic protons) gave rise to the singlet at 8.46 ppm (Figure 8). With the aid of an HSQC experiment, it was shown that this proton signal correlates with the ^{13}C signal at 148.4 ppm on the ^{13}C spectrum; corresponding to C-7 and C-7' (Figures 7 and 9). Protons 10-H/ 10'-H and protons

12-H/ 12'-H however have a *meta*-arrangement and this results in a small coupling between the pairs. The 10-H/ 10'H signal appears at 8.38 ppm with a coupling constant of 1.5 Hz. The C-10/ C-10' correspond to the signal at 130.0 ppm on the ^{13}C NMR spectrum. The 12-H, 12'-H are represented by the proton signal at 8.44 ppm in the ^{1}H NMR spectrum and C-12 and C-12' correspond to the signal at 140.4 ppm on the ^{13}C NMR spectrum. The carbonyl carbons C-4/ C-4' correspond to the signal at 166.9 ppm on the ^{13}C NMR spectrum (Figure 7).

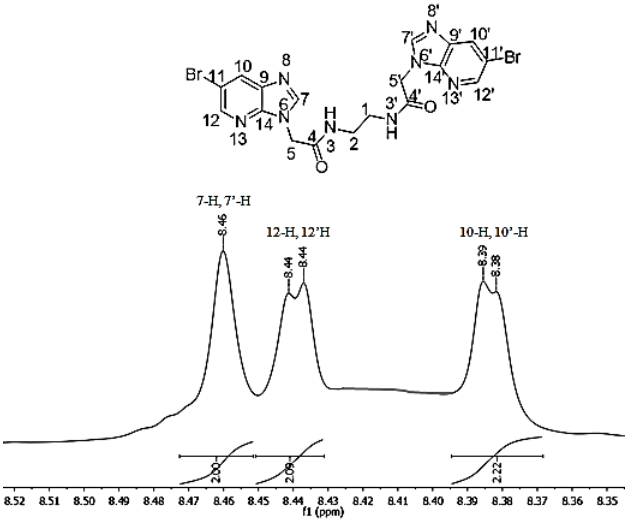


Figure 8. 400 MHz ^{1}H NMR spectrum of **6** in DMSO-$_{d6}$ (expanded).

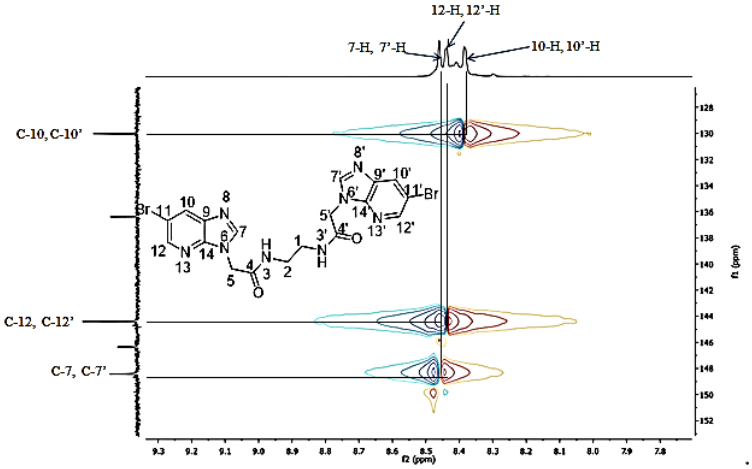


Figure 9. Partial HSQC NMR spectrum of **6** in DMSO- d_6 (expanded).

3.2. Bioactivity

The compounds **6** and **7** were assessed against HeLa (human cervix adenocarcinoma) cells at 20 μM in order to determine the overt cytotoxicity. A resazurin-based assay was used to determine the percentage of surviving cells by reading the resorufin fluorescence data using a multiplate reader. The cytotoxicity results showed that compound **6** had cell viability of 96% while the cell viability of compound **7** was 92%.

Conclusively, two bis-azabenzimidazoles were prepared in a convenient two-step; the compounds were structurally characterized and profiled for their level of cytoxicity. Results obtained indicated that none of the tested synthetic compounds was significantly cytotoxic.

ACKNOWLWDGEMENT

The author is indebted to Dr. Rosalyn Klein and Prof. Perry Kaye and Mrs Michelle Isaacs (Rhodes University, Grahamstown, South Africa); the Tertiary Education Trust fund (TETFund) for a bursary; Adekunle Ajasin University, Akungba-Akoko, Nigeria

for study leave and Rhodes University, Grahamstown South Africa.

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