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Synthesis and Potential Trypanocidal Activity of N, N-disubstituted-3-(1-benzenesulphonylindol-2-yl and -3-yl) propagylamines

V. MUGOYELA[1*], S. MUNG'ONG'O[1] AND M. HOOPER[2]

[1]*School of Pharmacy, Muhimbili University of Health and Allied Sciences, P.O Box 65013, Dar es Salaam Tanzania*
[2]*University of Sunderland, Sunderland SR2 3SD United Kingdom*

The synthesis of N, N-disubstituted –3-(1-benzenesulphonylindol- 2-yl and 3-yl) propagylamines by cuprous catalyzed Mannich reaction of 2 and 3, 1-(1-benzenesulphonyl-indol)ethynes and secondary amines in ethanol at reflux temperature afforded the compounds 4-methylpiperazinomethyl-2-(1-benzenesulphonylindol-2-yl)ethyne (1), 4-methylpiperazinomethyl-2-(1-benzenesulphonylindol-3-yl)ethyne (2a), 4-methyl-piperazinomethyl-2-(1-benzenesulphonyl-5-methoxyindol-3yl)ethyne (2b), morpholino-2-(benzenesulphonylindol-3yl)ethyne (3a), morpholino-2-(1-benzene-sulphonyl-5-methoxy-indol-3-yl)ethyne (3b) and N-carboxyethoxymethyl-3-(1-benzenesulphonylindol-3yl)ethyne (4) in good yields. All these compounds were fully characterized by means of Infrared spectroscopy, Nuclear Magnetic Resonance (^{1}H) and Mass spectrometry, and elemental analyses. Their potential trypanocidal activity was evaluated *in vitro* against *Trypanosoma brucei brucei* (S427/118 Mi Tat 1.5) and compound 1 was found to be the most active. This study has therefore established typical synthetic procedures for the new N, N-disubstituted -3-(1-benzene-sulphonylindol-2-yl and -3-yl) propagylamines and furnished their spectral analysis.

Key words: Propagylamines, spectral analysis, synthesis, trypanocidal activity

INTRODUCTION

Human African trypanosomiasis (HAT) or sleeping sickness is classified as a neglected disease by the World Health organization (WHO). It remains an important public health problem in Tanzania and presents a serious threat to human health with an annual incidence of 400 cases [1].The disease is endemic to the central and western areas of the country [2]**.** In livestock, trypanosomiasis is equally considered to be a significant infectious disease that holds back the development of livestock production in some parts of Africa, with approximately 25 million cattle exposed to the risk of infection [3]. The medicines which are used in the treatment of sleeping sickness have been in existence since the early 20th century. Suramin sodium was introduced into clinical use in 1916 and is still the drug of choice for the early stages of *T. b. rhodesiense* infection, while pentamidine is the drug of choice for the early stages of *T. b. gambiense* infection. Melarsoprol has been used for the late stages of infections with central nervous system involvement since its introduction into treatment of human sleeping sickness [4]**.** These drugs are quite old and resistance has been encountered in several cases [5]**,** and they all lead to undesirable side effects [6, 7]. The only new drug is the amino acid DL-α-difluoromethylornithine (DMFO) which was introduced into clinical use at the turn of the 20th century [8, 9]. Therefore there is a need to develop new trypanocidal drugs either through chemical synthesis or from natural products for the treatment of the early and late stages of the infection. Several synthetic tertiary aminoalkynyl derivatives of indoles have been reported to demonstrate useful pharmacological activities [10]**.** In addition, tryptophan-related compounds have shown promising *in vitro* activity against *T. b. brucei* [10, 11]. These compounds appear to act on enzymes involved in tryptophan metabolism. The aim of this study was to synthesize tryptophan analogs with the potential to inhibit trypanocidal enzymes responsible for tryptophan metabolism.

* Author to whom correspondence may be addressed.

In this study Mannich reaction derivatives, the N, N-disubstituted –3-(1-benzenesulphonyl-indol- 2- and 3-yl) propagylamines synthesized from the benzenesulphonyl protected ethynylindoles were used to screen for trypanocidal activity against *T. b. brucei*. The starting compounds namely 1-1(1-benzenesulphonylindol-2-yl and -3-yl) ethynes were previously synthesized as described previously [12]. The structures of all novel compounds were assigned by Infrared (IR) spectroscopy, Nuclear Magnetic Resonance (NMR) and Mass spectrometry (MS).

EXPERIMENTAL

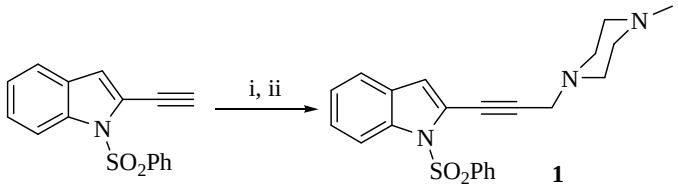
The melting points of the compounds were determined by open capillary method and are reported uncorrected. IR spectra were recorded on a Unicam S.P.157G spectrophotometer as KBr pellets. ^{1}H NMR spectra were recorded on a Perkin-Elmer R 24b (60 Hz) spectrometer. Chemical shifts are measured in ppm relative to tetramethylsilane. Mass spectra were acquired on VG Macromass 16F spectrophotometer operating at 70eV and 200 °C. Elemental analyses were in agreement with calculated values. Thin layer chromatography (TLC) was used to monitor the reactions.

Synthesis of N, N-disubstituted –3-(1-benzenesulphonylindol- 2-yl and 3-yl) propagylamines (1,2a,2b,3a,3b,4)

The structures and general pathway for synthesis of compounds **1-4** is depicted in Figures 1 and 2 while their physical characteristics are listed in Table 1.

General procedure

To a solution of the respective 1-(1-benzenesulphonyl) ethynylindole in ethanol (10 ml) were added formaldehyde (37 %w/w solution in water), the appropriate secondary amine and copper (I) chloride in catalytic amount as previously described [13]. The reaction mixture was refluxed for 3-5 h on a water bath. Water (100 ml) was added and the mixture was acidified with 5 M HCl (10 ml) before extracting with diethyl ether (2 x 20 ml). The aqueous phase was made alkaline using aqueous ammonia solution (33 % w/w) and extracted with chloroform (3 x 50 ml). The chloroform extract was dried (anhydrous magnesium sulphate, $MgSO_4$) and evaporated *in vacuo* to yield a white solid. The purity of the compounds was determined by thin-layer chromatography (TLC) using several solvent systems of different polarity.



i: HCHO, CuCl, EtOH, reflux 3-5h
ii: N-methylpiperazine

Figure 1: Structure and synthesis of compound 1

4-methylpiperazinomethyl-2-(1-benzenesulphonylindol-2-yl) ethyne (1)

To a stirred solution of 1-(1-benzenesulphonylindol-2-yl)ethyne (0.798 g, 2.84 mmol) in ethanol (10 ml) were added 37 % w/w aqueous formaldehyde (0.105 g, 3.50 mmol), 1-methylpiperazine (0.350g, 3.50 mmol) and copper (I) chloride in catalytic amounts. The reaction mixture was refluxed for 4 h on a water bath and treated as described in the general procedure to give **1**. Recrystallization from petroleum ether (b.p. 40-60 °C) and ethylacetate afforded white crystals with a yield of 0.85g (78%).

IR(KBr,cm^{-1}): 2930, 1590, 1460, 1380, 1250, 1190, 1140, 1100, 1070, 900cm^{-1}; ^{1}HNMR(60 Hz, $CDCl_3$); 2.30 (3H, s, N-CH_3); 2.40-2.90 (8H, m, N-$(CH_2)_4$); 3.63 (2H, s, CH_2); 6.85 (1H, s, indole C(3)H); 7.15-8.35 (9H, m, aromatic protons). MS m/z: 393 [M^+] (5%); 251(28); 152(21), 140(4).

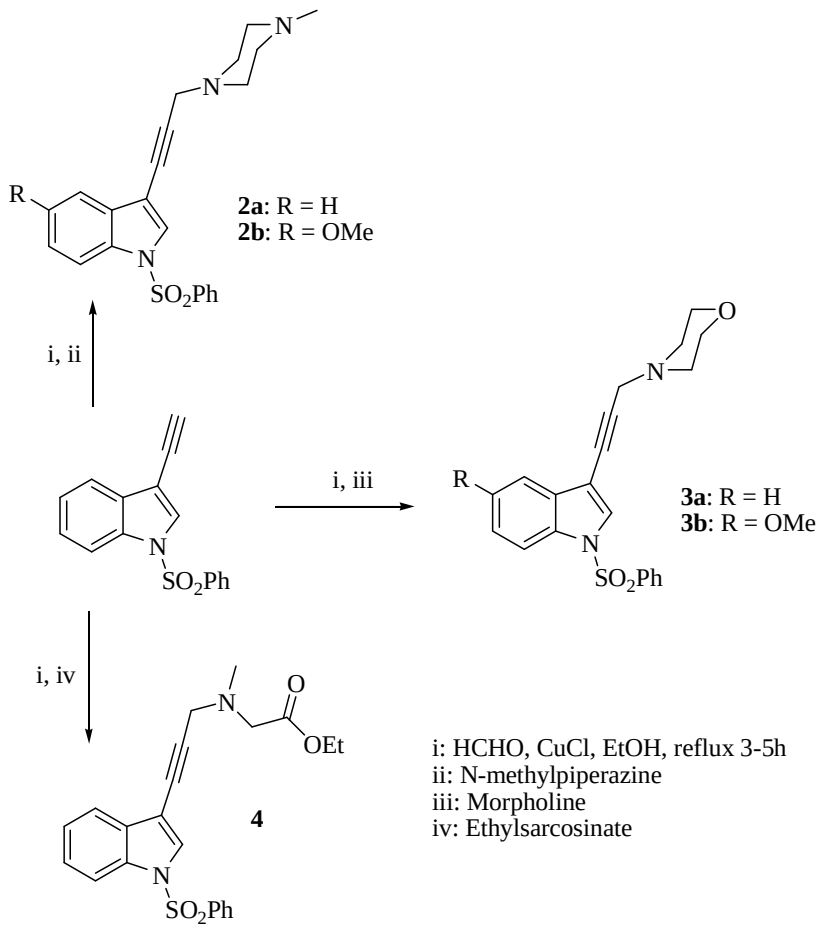


Figure 2: Chemical structures and synthesis of compounds 2a, 2b, 3a, 3b and 4

4-methylpiperazinomethyl-2-(1-benzenesulphonylindol-3-yl) ethyne (2a)

To a stirred solution of 1-(1-benzenesulphonyl-3-yl) ethyne (0.7 g, 2.48 mmol) in ethanol (10 ml) were added 37 % w/w aqueous formaldehyde (0.102g, 3.40 mmol), 1-methylpiperazine (0.34 g, 3.40 mmol) and copper (I) chloride in catalytic amount. The reaction mixture was refluxed for 2 h on a water bath and treated as described in the general procedure to give **2a**. Recrystallization from petroleum ether and ethyl acetate afforded white crystals with a yield of 0.75g (77%).

IR ($CHCl_3$) 3160, 2950, 2220, 1610, 1450, 1380, 1350, 1340, 1285, 1210, 1180, 1110, 1090, 930; ^{1}HNMR ($CDCl_3$) 2.31, (3H, s, CH_3); 2.42 – 2.82 (8H, m, N – $(CH_2)_4$); 2.58 (2H, s, CH_2); 7.15 – 8.08 (10H, m, aromatic protons); MS m/z: 373 [M^+] (4%); 251(50), 152(28), 140(13).

4- Methyl piperazinomethyl – 2 – (1- benzene sulphonyl – 5- methoxy indol – 3-yl) ethyne (2b)

To a stirred solution of 1-(1- benzenesulphonyl –5- methoxyindol –3-yl) ethyne (0.6 g, 1.92 mmol) in ethanol (10ml) were added 37 % w/w aqueous formaldehyde (0.075g, 2.50 mmol), 1-methyl piperazine (0.25g, 2.50 mmol), and copper (I) chloride in catalytic amount. The reaction mixture was refluxed for 3h on a water bath and treated as described for **2a** to give **2b** with a yield of 0.740g, (91%).

IR ($CHCl_3$) 3160, 2975, 2820, 2240, 1615,1590, 1450, 1380, 1290, 1180, 1150, 1100, 1040, 1000, 950, 850; ^{1}HNMR ($CDCl_3$) 1.28 (3H, s, N-CH_3); 2.34 - 2.60 [8H, m, N-$(CH_2)_4$]; 3.52 (2H, s ,CH_2); 3.75 (3H, s, 0CH_3); 6.70 – 7.90 (9H, m, aromatic protons); MS m/z: 423 [M^+] (9%); 282 (52), 170 (32), 155 (6).

Morpholino-2-(benzene sulphonylindol-3-yl)ethyne (3a)

To a stirred solution of 1-(1-benzene suplhonyindol 3-yl) ethyne (0.696 g, 2.48 mmol) in ethanol (10 ml) were added 37 % w/w aqueous formaldehyde (0.104 g, 3.45 mmol), Morpholine (0.3 g, 3.45 mmol), and copper (I) chloride in catalytic amount. The reaction mixture was refluxed for 2h on a water bath treated as described for **2a** to give **3a** with a yield of 0.4 g (42%).

IR ($CHCl_3$) 3160, 2950, 2870, 2830, 2220, 1610, 1590, 1450, 1380, 1350, 1340, 1289, 1180, 1120, 1090, 930; ^{1}H NMR ($CDCl_3$) 2.54 - 2.84 [4H, m, N-$(CH_2)_2$]; 3.55 (2H, s, CH_2); 3.65 - 3.95 [4H, m, N$(CH_2)_2$]; 7.20 - 8.10 (10H, m, aromatic protons); MS m/z: 380 [M^+] (6%); 293 (27), 238 (100), 152 (34).

Morpholino-2-(1-benzene Sulphonyl- 5-Methoxyindol-3-yl) ethyne (3b)

To a stirred solution of 1-(-benzene sulphonyl-5-methoxy indol-3-yl) ethyne (0.399 g, 1.28 mmol) in ethanol (10 ml) were added 37 % w/w aqueous formaldehyde (0.069g, 2.30 mmol), morpholine (0.2 g, 2.30 mmol) and copper (I) chloride in catalytic amount and treated as described for compound **2b** to give **3b** with a yield of 0.44 g (83%).

IR($CHCl_3$) 2240, 1615, 1590, 1450, 1380, 1290, 1180, 1150, 1100, 920, 860; ^{1}NMR ($CDCl_3$) 2.50 - 280 [4H, m, N-$(CH_2)_2$]; 3.55 (2H, s, CH_2); 3.65 - 3.95 (7H, m, OCH_3, N-$(CH_2)_2$]; 6.80 - 8.00 (9, H, m, aromatic protons); MS m/z: 410 [M^+] (70%); 269 (20), 183 (50), 154 (5).

N- Carboethoxy methyl-3-(-benzene sulphonylindol –3-yl) propargylamine (4).

To a stirred solution of 1-(1- benzene sulphonylindol-3-yl) ethyne (0.5 g, 1.77 mmol) in ethanol (10 ml) were added 37 % w/w aqueous formaldehyde (0.07 g, 2.34 mmol), ethyl- sarcosinate (0.16 g, 2.34 mmol) and copper (I) chloride in catalytic amount and treated as described for compound **2b** to give **4** with a yield of 0.51g (70%).

IR ($CHCl_3$); 3160, 3070, 2990, 2940, 2800, 2220, 1740, 1610, 1590, 1450, 1380, 1340, 1280, 1180, 1130, 1050; ^{1}HNMR ($CDCl_3$) 1.25 (3H, t, CH_3); 250 (3H, s, N-CH_3); 3.50 (2H, s, CH_2); 3.75 (2H, s, N-CH_2); 4.21 (2H, q, OCH_2); 7.10 - 8.05 (10H, m, aromatic protons); MS m/z: 410 [M^+] (7%); 294 (100), 269 (16), 153 (36), 196 (11).

RESULTS AND DISCUSSION

A total of six new N,N-disubstituted-3-(1-benzene sulphonylindol-2yl and-3yl)propagyl-amines **1, 2a, 2b, 3a, 3b** and **4** were synthesized and three of them were screened for trypanocidal activity. The results for i*n vitro* trypanocidal activity against *T. b. brucei* of compounds **1, 2a** and **2b** were previously reported [14]**.** All three compounds were active, with compound **1** being the most active with an MIC of less than 1.0 µmol/L and an ED_{50} of 2.88 µmol/L, completely inhibiting growth at 10 µmol/L. The other three compounds **3a, 3b** and **4** were not tested for activity due to resource constraints. The present work is a report of the typical synthetic procedures of all the six new compounds and their spectral data.

The preparation of these compounds (Figure 1) involved the Mannich reaction as described by Nilson *et al*. [13] using Cu (I) salts. This method was preferred because it was found to be very simple to set up and work up. The reaction was carried out at reflux temperature in ethanol in the presence of catalytic amount of cuprous chloride for about 1 - 4 h. Subsequent work-up involved acidification of the reaction mixture and extraction of the non-basic components using ether followed by basification and isolation of the reaction products. All the reactions provided solid products in good yields.

However Nilsson's method failed to give compound **4**, which was consequently prepared in low yield using Salvador's Copper (II) procedure [15]. It is not clear why the Cu (II) method was more effective than the more generally applicable procedure of using Cu (I). Copper salts assist formation of the incipient acetylide ion from the terminal ethynyl group of the 1-(1-benzenesulphonylindol-2-yl or -3-yl) ethyne.

The structures of the synthesized compounds were confirmed by IR, ^{1}HNMR and MS data. Elemental analysis of the new compounds was performed for carbon, hydrogen and nitrogen content and results obtained were comparable with the calculated data (Table 1). The IR spectra of all compounds clearly indicated the presence of a triple bond at 2220 - 2240 cm^{-1} and the absence of a terminal ethynyl triple bond at 3300 cm^{-1} which was in the spectra of the starting materials. ^{1}HNMR spectra of compounds **2a, 2b, 3a, 3b** and **4** showed singlets at 3.50 - 3.65 ppm corresponding to the hydrogens of the methylene bridge between the indolyl ring and the amine side chain. The chemical shift for the methylene bridge protons for compound **1** resonated upfield at 2.58 ppm due to shielding. The ^{1}HNMR spectra of aromatic hydrogens in all compounds appeared as multiplets of 10H between 6.70 - 8.10 ppm. Compound **4**, further showed another singlet at 3.75 ppm corresponding to the deshielded hydrogens of the methylene group adjacent to the -C=O [16]**.** With regard to MS data, a loss of the benzenesulphonyl group (M-141) was observed in all compounds with exception of compound **4** with a trifuoroacetyl group (M-97).

CONCLUSION

The present study demonstrates successful synthesis of the title compounds in good yields through the Mannich reaction. The reaction has proved to be an effective method of introducing a propagylamine side chain among the 1-(1-benzenesulphonylindole-2yl and -3yl)ethynes using various secondary amines. The chemical structures of the new synthesized compounds were elucidated using various spectral data and elemental analysis with respect to carbon, hydrogen and nitrogen.

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