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Synthesis Of 4-prop-2-ynyl-3-butyl-benzo-[E][1,2,4]-thiadiazine-1, 1-dioxide

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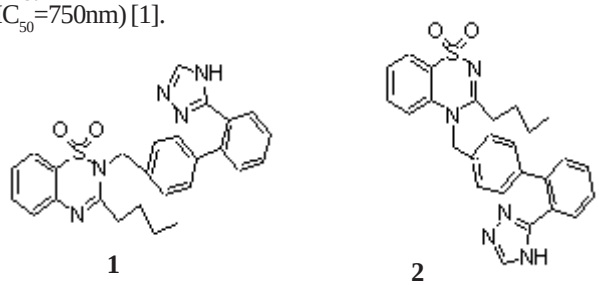
ABSTRACT

To synthesise and investigate the antimicrobial activity of 4-prop-2-ynyl-3-butyl-benzo- [e][1,2,4]-thiadiazine-1, 1-dioxide. The reported antibacterial activity of 3- butyl-4H-benzothiadiazine dioxide encouraged the investigation of the possible interaction of propynyl bromide and the corresponding antibacterial activity. The alkylation of 3-butyl-1, 2, 4-benzothiadiazine 1, 1-dioxide 5, with propynyl bromide was accomplished using anhydrous K_2CO_3 in DMF. The synthesized 4-prop-2-ynyl-3-butyl-benzo- [e][1,2,4]-thiadiazine-1, 1-dioxide was tested for antibacterial activity using clinical isolate of *Staphylococcus aureus, Bacillus subtilis, Streptococcus pneumonia, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumonia.* The spectroscopic data unequivocally characterized 4-prop-2-ynyl-butyl-4H-1,2,4-benzothiadiazine-1,1-dioxide and it possesses no antibacterial activity against all the clinical isolates compared to the commercial antibiotics used in this study. The compound showed no activity against all the tested strains of organisms.

INTRODUCTION

A survey of the literature on the synthesis and the biological activities of 3-butyl- benzothiadiazine dioxide reveals that not much has been done on the possible biological activities of the compound or its derivatives.

The biosteric replacement of the quinazoline ring of benzothiadiazine has produced compounds with improved biological activities. The 1, 2, 4-benzothiadiazine pharmacophore is present in chlorothiazide, which is used in the clinics as a diuretic. The synthesis and pharmacological evaluation of 1,2,4-benzothiadiazine 1,1-dioxide biphenyl tetrazoles as angiotensin II antagonists revealed the formation of 1 and 2. Preliminary assays showed that compound 1 (IC_{50}=87nm) exhibited stronger binding affinity than 2 (IC_{50}=750nm) [1].



1 2

Fig 1 : Compound 1 and 2

1,2,4-Thiadiazine derivatives, like 3-methyl-7-chlorobenzo-4H-1,2,4-thiadiazine 1,1-dioxide, and 7-chloro-3-iso propylamino-4H-benzo-1,2,4-thiadiazine 1,1-dioxide are potent openers of Kir6.2/SUR1 K(ATP) channels. To explore the structure-activity relationship of this series of K(ATP) openers, 4H-1,4-benzothiazine-2-carbonitrile 1,1-dioxide and N-(2-cyanomethylsulfonylphenyl) acylamide derivatives were synthesized from 2-acetylamino-5-chloro-benzenesulfonic acid pyridinium salt or 2-aminobenzenethiols [2]. The 4H-1,4-benzothiazine-2-carbonitrile 1,1-dioxide derivatives (e.g., 7-chloro-3-isopropylamino-4H-1,4-benzothiazine-2-carbonitrile 1,1-dioxide) were found to activate K(ATP) channels as indicated by their ability to hyperpolarize beta cell membrane potential, to inhibit glucose-stimulated insulin release *in vitro* and to increase ion currents through Kir6.2/SUR1 channel as measured by patch clamp. 7-Chloro-3-methyl-4H-1,4-benzothiazine-2-carbonitrile 1,1-dioxide, which inhibits insulin release *in vitro* from beta cells and rat islets, reduces plasma insulin levels and blood pressure in anaesthetized rats upon intravenous administration.

Recently, (3,4-dihydro-2H-1,2,4-benzo-thiadiazine 1,1-dioxide derivatives were synthesised as potential allosteric modulators of AMPA/kainate receptors [3].

The alkylation of 3-butyl-1, 2, 4-benzothiadiazine 1, 1-dioxide 5, with propynyl bromide was accomplished using

anhydrous K_2CO_3 in DMF. The N-4 alkylation compound 6 was the only product obtained. This confirms the generally accepted view that only the 4H-tautomer is preferred. The spectroscopic data unequivocally agrees with the above observation.

The reported antibacterial activity of 3- butyl-4H-benzothiadiazine dioxide encouraged the investigation of the possible interaction of propynyl bromide and the corresponding antibacterial activity.

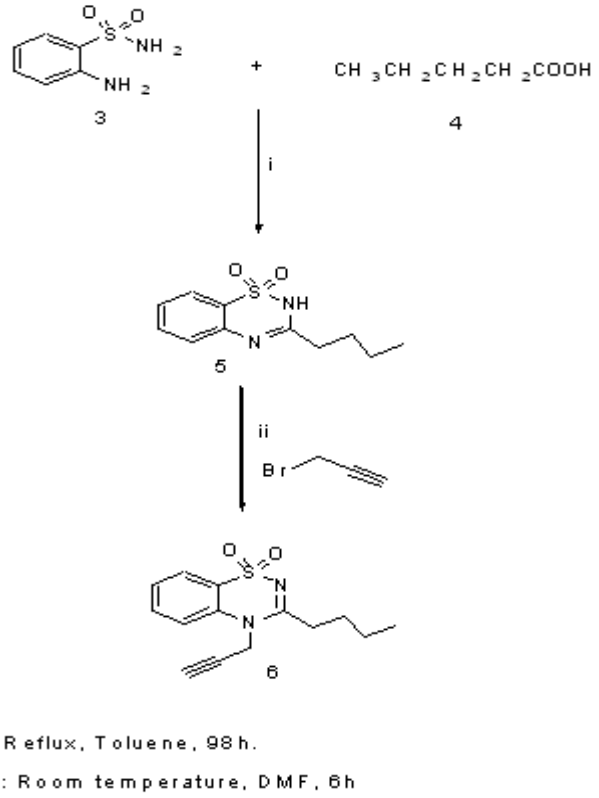


Fig 2: Synthesis of 4-Prop-2-ynyl-butyl-4H-1, 2 , 4-benzothiadiazine-1,1-dioxide.

MATERIALS AND METHODS

Melting points were determined with a Kofler hot stage microscope and were uncorrected. The reactions and purity of the products were monitored by TLC using pre-coated silica gel plates (Merck 60 F_{254}). Silica gel Merck 60 (70-230 mesh) was used for column chromatography. NMR (^1H and ^{13}C) were recorded on a Varian Gemini 200 (TMS), IR were measured on a Perkin-Elmer type 457 and the MS were determined using Varian MAT 44S, EI: 70 eV.

3-Butyl-2H-1, 2, 4-benzothiadiazine 1,1-dioxide:

2-Aminobenzenesulfonamide (4g, 23.2mmol) was added to a flask containing pentanoic acid (25mmol) and toluene-4-sulphonic acid in toluene (60ml) and refluxed for 98 hours. The reaction mixture was cooled to 0^0C, the solid collected and crytallised from ethyl acetate to give 3-butyl-2H-1,2,4-benzothiadiazine 1,1-dioxide (3.87g, 70%), mp159-161^{0}C (158-160^{0}C Chern and co-worker). IR (KBr) 3440, 3000, 1630, 1600, 1410, 1340, 770. ^1H NMR ($CDCl_3$) d : 1.86 (t, 3H, J=7.3Hz, CH_3); 1.34 (sext, 2H, J= 7.6Hz, CH_2); 1.68 (quint, 2H, J=7.7Hz,CH_2); 2.52 (t, 2H, J=7.2Hz, CH_2); 7.31(d, 1H, J=8.0Hz, Ar-H); 7.39 (t, 1H, J=7.2Hz, Ar-H); 7.50-7.60 (m, 1H, Ar-H); 9.88 (d, 1H, J=7.0Hz, Ar-H); 10.05 (br, 1H, NH); ^{13}C NMR ($CDCl_3$) d: 13.7, 22.0, 28.6, 36.1, 117.7, 120.8 123.8, 133.3, 135.2, 161.4; ms: m/z: 239 (M^++1). Anal. Calc. for $C_{11}H_{14}N_2O_2S$ (238.30): C 55.44, H 5.92, N 11.75 found $C_1$55.40, H 5.82, N 11.60.

4-Prop-2-ynyl-butyl-4H-1, 2, 4-benzothiadiazine-1, 1-dioxide. (6)

To a mixture of 3-butyl-1, 2, 4-benzothiadiazine 1, 1-dioxide (1.0g, 4.3mmol) and propargyl bromide (0.507g, 4.3mmol) and potassium bromide (0.426g, 4.3mmol) in dimethylforamide (20ml) was stirred at room temperature for 6 hours. The mixture was poured into water (60ml) and the solid collected and dried (Fig 2). Crystallisation from dichloromethane-hexane mixture afforded colourless plates of 4-prop-2-butyl 4H-1, 2, 4-benzothiadiazine-1, 1-dioxide. 0.82g (70%), mp 158-159^{0}C; IR (KBr). 2990, 2100, (C^{o}CH), 1630, 1590, 1420, 1330, 770; ^1H NMR ($CDCl_3$). d=0.94-1.01 (t, 3H, J=7.3Hz, CH_3); 1.38-1.53 (sext, 2H, J=7.3Hz, CH_2-CH_3); 1.78-1.93 (quint, 2H, J=7.6Hz, -CH_2-CH_2-CH_2-CH_3); 2.55 (t, 1H, J=2.3Hz, a"CH); 2.79-2.86 (t, 2H, J=7.3Hz, CH_2-C_3H_7); 4.67 (d, 2H, J=2.4Hz, N-CH2-Ca"CH); 7.45-7.51 (M, 2H, ArH); 7.66 (t, 1H, J=8.3Hz, ArH); 8.01 (dd, 1H, J=1.6, 8.3Hz, ArH).

^{13}C NMR ($CDCl_3$): 14.2, 22.6, 28.4, 35.6, 40.8, 58.2, 76.5, 115.9, 125.5, 126.9, 133.6, 137.7, 160.6, 162.7. ms: 276(8%)[M+], 275 (8%)[M+-1], 261(3), 247(32), 234(100), 211(5), 183(6), 169(55), 129(5), 102(6), 77(6). $C_{14}H_6N_2O_2S$ (276.294) Cal. C60.86, H 5.84, N 10.14, 11.58 Found C, 60.78, H.

ANTIBACTERIAL ACTIVITY

Clinical isolates of *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus pneumonia*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* were all supplied by the Pharmaceutical Microbiology Department of the University of Benin. The Agar-well diffusion method was used to determine the antibacterial activity of the compound [4-6].

Inocula of test organisms obtained were prepared by growing each pure isolate in Nutrient broth for 18 hours at 37^{0}C. The overnight broth culture was matched with McFarland turbidity standard to give an approximate of 10^8cfu/ml. 0.2ml was then used to seed a molten Nutrient agar medium which has cooled to 45^{0}C to obtain approximately 10^6cfu/ml. This was poured unto sterile Petri dishes and used for analysis.

A stock solution of the compound was made by dissolving 10mg in 5mls DMSO to give a concentration of 2mg/ml. 0.1ml of the stock was delivered into wells (6mm in diameter) bored unto the surface of the already seeded Nutrient agar plates. Equal volume of DMSO was assayed along as control. Commercial discs containing gentamycin (10µg) and ciprofloxacin (5µg) were used in parallel in the agar-well diffusion method. *Staphylococcus aureus* (NCTC 10788) was set up along with test organisms as a check on media and inherent sensitivity of isolates produced by the compound. The plates were allowed to stand on the bench for 30 minutes and incubated at 37^{0}C for 24 hours

Table 1: Antibacterial activity

Microorganism	Diameter of zones of Inhibition (mm)			
	4-Prop-2-ynyl-butyl-4H-1, 2, 4-benzothiadiazine-1, 1-dioxide) (200µg)	Gentamycin (10µg)	Ciprofloxacin (5µg)	DMSO
Staphylococus aureus	-	30	26	-
Bacillus subtilis	-	26	21	-
Streptococcus pneumonia	-	22	20	-
Escherichia coli	-	22	30	-
Pseudomonas aeruginosa	-	24	28	-
Klebsiella pneumonia	-	26	27	-
Staphylococus	-	25	27	-

- = No zone of inhibition

RESULTS AND DISCUSSION

Table 1 shows that 4-prop-2-ynyl-butyl-4H-1,2,4-benzothiadiazine-1,1-dioxide possesses no antibacterial activity against all the clinical isolates compared to the commercial antibiotics used in this study. This shows that the introduction of prop-2-ynyl destroys the antibacterial activity of butyl-4H-1, 2, 4-benzothiadiazine-1, 1-dioxide instead of enhancing it. One possible reason for this inactivity could be the increase in the lipophilicity of the resulting compound (4-prop-2-ynyl-butyl-4H-1, 2, 4-benzothiadiazine-1, 1-dioxide). Increase in lipophilic nature of the compound decreases the absorption of the compound in aqueous medium. This makes the compound unavailable at the site where the action is needed. The minimum inhibitory concentration was not determined since there was no zone of inhibition observed.

CONCLUSION

The organisms were all resistant to 4-prop-2-ynyl-butyl-4H-1, 2, 4-benzothiadiazine-1, 1-dioxide, 6 indicating the absence of antibacterial properties as shown in the table 1.

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