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SYNTHESIS, SPECTROSCOPIC CHARACTERIZATION AND ANTIBACTERIAL SCREENING OF NOVEL N-(BENZOTHIAZOL-2-YL)ETHANAMIDES

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Abstract

Synthesis, electronic, infrared, elemental micro analytical studies were carried on N-(benzothiazol-2-yl)trichloroethanamide **[4]** and N-(benzothiazol-2-yl)chloroethanamide **[5]**. They were also screened *in vitro* and *in vivo* for antibacterial activity. The results indicate that the compounds are very stable and that they show high antibacterial activities against both gram-positive and gram-negative bacteria tested. Both derivatives of 2-aminobenzothiazole were active against the multiresistant bacteria with IZD ranging from 9 -18 mm **[5]** and 9 – 20mm **[4]**. From the MIC results it is observed that the **[5]** derivative produced a better antibacterial activity than the **[4]** derivative. The lethal concentrations (LC_{50}) of the compounds were also determined. Their solubilities and melting points were also determined.

Keywords: Lethal Concentration, derivatives, ethanamides, *in vitro, invivo,* bioactivity.

*For correspondence

Introduction

Thiazole and some of its derivatives have been found to be of physiological significant. Vitamin B1 (thiamin), a heat-labile, water-soluble vitamin responsible for the proper metabolism of carbohydrate and its coenzyme cocarboxylate contain the thiazole ring [1]. There has been a lot of interest in the chemistry of thiazole derivatives. This is due to their high possession of antimicrobial activities. Thiazole are known to posses antitubercular [2], antibacterial [3-4], fungicidal [5], hypotensive and hypothermic [6] activities.

Resistance to antibiotics is growing and has become a global concern [7]. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug resistant pathogens [8]. In an era of decreasing microbial susceptibilities to current available antimicrobials, there is a pressing need to develop new agents and therapeutic strategies for the treatment of infectious diseases [9-11]. This work reports the synthesis, characterization and antibacterial activity of N-(benzothiazol-2-yl)trichloroethanamide **[4]** and N-(benzothiazol-2-yl)chloroethanamide **[5]**.

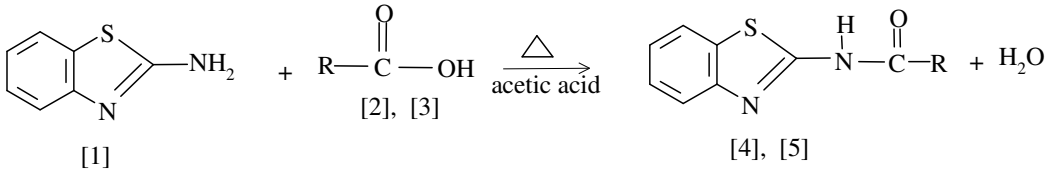
Experimental

All reagents were of analytical grade and were used as supplied except otherwise stated. The compounds, N-(benzothiazol-2-yl)trichloroethanamide **[4]** and N-(benzothiazol-2-yl)chloroethanamide **[5]** were prepared following the methods described by Allen and Vanallan [12]. Electronic spectra of TCAABT and CAABT were generated using UNICAN SP 800 UV-Visible spectrophotometer. IR spectra were performed in nujol using Puck specific model 500 spectrophotometer. The elemental microanalyses were done using LECO-CHNS 932 microanalysis apparatus at the Department of Pharmaceutical Sciences, University of Strathchlyde, Glasgow, Scotland.

Synthesis of N-(benzothiazol-2-yl)trichloroethanamide

To a hot solution of 2-aminobenzothiazole **[1]** (3.00g, 20mmol) in acetic acid(30ml), trichloroacetic acid **[2]** (3.39g, 21mmol) dissolved in 40ml acetic acid was added drop wise with continuous stirring. The mixture was further stirred and refluxed for two hours at 90^0C. The mixture was allowed to cool. After twenty minutes, white precipitate formed. The precipitate was filtered and recrystallized in absolute ethanol.

The yield was 79.40% and the melting point is 118-120^0C. TCAABT is soluble in DMSO at room temperature. It is also soluble in absolute ethanol, chloroform, diethyl ether and acetic acid at elevated temperature. The equation of reaction is shown in scheme 1.



[2], [4]; $R = CCl_3$
[3], [5]; $R = CH_2Cl$

[1]**=** 2-aminobenzothiazole

[2]= trichloroacetic acid

[3]= chloroacetic acid

[4]= N-(benzothiazol-2-yl)trichloroethanamide

[5]= N-(benzothiazol-2-yl)chloroethanamide

Scheme 1: Synthesis of some N-(benzothiazol-2-yl)ethanamide

Synthesis of N-(benzothiazol-2-yl)chloroethanamide

To a hot solution of 2-aminobenzothiazole **[1]** (3.00g, 20mmol) in acetic acid(30ml), chloroacetic acid **[3]** (1.89g, 20mmol) dissolved in 20ml acetic acid was added drop wise with continuous stirring. The mixture was further stirred and refluxed for 40 minutes at 120^0C and left overnight. The white precipitate formed was filtered, washed with cold ethanol several times and

dried. The yield was 87.40% and the melting point is 106-108^{0}C. The equation of reaction is shown in scheme 1.

Antibacterial assay

In vitro Test:

Multi-resistant bacterial strains isolated from clinical conditions were used in the study. The bacterial strains used were *Escherichia coli* (5 strains), *Proteus* species (2 strains), *Pseudomonas aeroginosa* (2 strains) and *Staphylococcus aureus* (1 strain). The bacterial strains were resistant to ampicillin (10µg), Chloramphenicol (30 µg), Streptomycin (10 µg), trimethoprim-sulphamethoxazole (25 µg) and tetracycline (30 µg). The antibacterial activities of N-(benzothiazol-2-yl)chloroethanamide **[5]** and N-(benzothiazol-2-yl)trichloroethanamide **[4]** against these multi-resistant bacteria were determined using the agar well diffusion method as described by Chah *et al*[13]. Mueller-Hinton agar plates were inoculated with 0.1 ml of 3 hr broth culture of the test bacteria. Using a cork borer, wells (7 mm in diameter and 2.5 mm deep) were bored into the inoculated agar. The test compounds were solubilised in 20 % v/v dimethyl sulfoxide (DMSO) and 50 µl of each compound at a concentration of 20 mg/ml were delivered into the wells. One of the wells contained 20% v/v DMSO and served as control. The plates were incubated at 37^{o}C for 24 hours and assessment of antibacterial activity was based on the measurement of the diameter of inhibition zone (IZD) around the wells. The test was performed in triplicates mean IZD was recorded to the nearest whole millimetre.

The minimum inhibitory concentrations (MICs) of the test compounds were determined using the agar dilution method as described by Ojo *et al* [14]. Two-fold serial dilutions of test compounds were made in 20% v/v DMSO. One millilitre of each serial dilution was added to 19 ml of sterile Mueller-Hinton agar maintained at 45^{o}C, thoroughly mixed and poured into a sterile plate and the medium allowed to solidify. The final concentrations of the compounds ranged from 20mg/ml to 1.25mg/ml. Amended media were incubated overnight at 37^{o}C to check for sterility. Overnight nutrient broth cultures of the test bacteria were adjusted to contain approximately 10^8cfu/ml and 25µl of each of the test organisms was spot-inoculated on the amended culture media. Inoculated plates were incubated at 37^{o}C for 24 hours and observed for presence of visible growth. The minimum inhibition concentration was determined as the value of the lowest concentration that completely suppressed growth of the organisms.

In vivo Test: [Brine Shrimps Lethality Test (BSLT)]

The method of McLaughlin and coworkers was used to study the bioactivity of the synthesized compounds [15]. *Artemia salina* eggs obtained from a pet shop in Davis California was incubated in natural sea water (from Bar Beach, Lagos, Nigeria) in a dam-well under room condition. About ten (10) 48h- shrimp nauplii in 1mL of autoclaved sea water were put into Bijou bottles using a Pasteur pipette under a stereo-microscope with a light source. They were separated into 7 groups in triplicate. Increasing concentrations (10, 100, 1000 ppm) of the synthesized compounds (N-(benzothiazol-2-yl)trichloroethanamide **[4]** and N-(benzothiazol-2-yl)chloroethanamide **[5])** were added into each of the triplicate and distilled water was added into the control group. The nauplii were incubated at room temperature (37^oC) for 24h after which the survivors in each well were counted. The results were analysed using Finney Probit Analysis (MS-DOS-Computer-Program) to determine the LC_{50} at 95% confidence interval. Weak nauplii were noted as an indication of central nervous system depression.

Results and discussion

Electronic spectra Analysis:

N-(benzothiazol-2-yl)trichloroethanamide [4]

The Uv spectra of **[4]** in DMSO shows a single peak at 271nm ($36900cm^{-1}$). This is indicative of $\pi \rightarrow \pi^*$ transition in the aromatic ring. The compound has ϵ_{max} of $1.8x10^4$.

N-(benzothiazol-2-yl)chloroethanamide

The Uv spectra of **[5]** in ethanol shows two very strong bands at 227nm ($44053cm^{-1}$) and 263nm ($38023cm^{-1}$). These are indicative of $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transition in the aromatic ring and carbonyl group respectively. It can be said that the $n- \pi^*$ transition did not appear in **[4]** because of the three chloride atoms present in the compound which can draw lone pairs of electron from the carbonyl group to themselves (this can deactivate the lone pair of electron in the carbonyl group) since they are highly electronegative compared to the **[5]** which has only one chlorine atom in the compound.

Infrared Spectra Analysis:

Vibrational frequencies of **[4]** and **[5]** displayed in Table I:

Table **I:** The Infrared spectra result of N-(benzothiazol-2-yl)trichloroethanamide **[4]** and N-(benzothiazol-2-yl)chloroethanamide **[5]**

N-(benzothiazol-2-yl)trichloroethanamide	N-(benzothiazol-2-yl)chloroethanamide	Assignment
cm^{-1}	cm^{-1}	
3397 (br)	3404 (br)	N-H stretching frequency
2980 (s)	2925 (s)	C-H stretching frequency
2750 (sh)	2853 (sh)	
1644 (s)	1600 (m)	C=O stretching frequency
1565 (s)	1583 (s)	Aromatic ring C=C stretching frequency
1485 (s)	1457 (s)	Thiazole ring C=N stretching frequency
1376 (m)	1377 (s)	
743 (s)	720 (s)	C-S-C stretching frequency
627 (s)	631 (s)	C-Cl stretching frequency

Legend: br= broad; m= medium; s=strong; sh= shoulder

The broad peak of medium intensities around 3397cm^{-1} in **[4]** and 3404cm^{-1} in **[5]** are due to N-H stretching frequency. The peak around 1644cm^{-1} and 1600cm^{-1} in **[4]** and **[5]** respectively are assigned C=O stretching frequency. The benzene ring vibration which occurred at 1565cm^{-1} in **[4]** and 1583cm^{-1} in **[5]** are assigned C=C stretching frequency. The peaks around 1485cm^{-1} and 1376cm^{-1} in **[4]** and 1457cm^{-1} 1377cm^{-1} in **[5]** which are peculiar to thiazole ring C=N

stretching frequency were so assigned. The peaks at 1067cm^{-1} and 1097cm^{-1} in **[4]** and **[5]** respectively are assigned to C-Cl stretching frequency. However, 743cm^{-1} and 720cm^{-1} are assigned C-S-C stretching frequencies for **[4]** and **[5]** respectively.

Elemental Analysis:

The elemental micro analytical results are shown in Table 2:

Table 2: Elemental micro analytical data for N-(benzothiazol-2-yl)trichloroethanamide **[4]** and N-(benzothiazol-2-yl)chloroethanamide **[5]**.

Compound	Calculated %				Found %			
	C	H	N	S	C	H	N	S
[4]	36.55	1.69	9.48	-	34.69	2.22	8.94	-
[5]	47.68	3.09	12.36	14.13	42.79	3.25	11.06	14.31

Antibacterial activity of [4] and [5]

The results of the antibacterial activity of N-(benzothiazol-2-yl)trichloroethanamide **[4]** and N-(benzothiazol-2-yl)chloroethanamide **[5]** against these multi-resistant bacteria are presented in Table 3. Both derivatives of 2-aminobenzothiazole were active against the multiresistant bacteria with IZD ranging from 9 -18 mm **[5]** and 9 – 20mm **[4]**. From the MIC results it is observed that the **[5]** derivative produced a better antibacterial activity than the **[4]** derivative. Bearing in mind that the bacteria strains used in this study were highly resistant to conventional antibacterial agents the potentials of the test compounds as chemotherapeutic agents against multi-resistant bacteria can be exploited.

Table 3: Antibacterial activity of N-(benzothiazol-2-yl)trichloroethanamide **[4]** and N-(benzothiazol-2-yl)chloroethanamide **[5]**

Bacteria strain	N-(benzothiazol-2-yl)chloroethanamide		N-(benzothiazol-2-yl)trichloroethanamide	
	Inhibition zone diameter (mm)	***MIC (mg/ml)***	***Inhibition zone diameter (mm)***	***MIC (mg/ml)***
E. coli 1	14	2.5	9	5.0

E. coli 2	9	2.5	13	5.0
E. coli 3	18	2.5	13	5.0
E. coli 4	17	2.5	20	5.0
E. coli 5	10	2.5	10	5.0
Proteus spp 1	17	2.5	15	10
Proteus spp 2	15	2.5	12	10
P. aeroginosa 1	12	2.5	9	10
P. aeroginosa 2	11	2.5	9	10
Staphylococcus aureus	11	2.5	11	5.0

Lethal Concentration (LC_{50}) and Effective Concentration (EC_{50}) Results

The Cytotoxic result is shown in table 4.

Table 4:Lethal Concentration (LC_{50}) Result in ppm (Cytotoxic test)

S/N	SAMPLES	LC_{50} (ppm)	EC_{50} (ppm)
1	N-(benzothiazol-2-yl)trichloroethanamide	417.7±36.8	41.7
2	N-(benzothiazol-2-yl)chloroethanamide	567.7±40.9	56.8

BSLT is a rapid, inexpensive and single bioassay for testing bioactivity of natural and synthetic products, which in most cases correlates reasonably well with cytotoxicity and antitumour properties of the products [16]. The result of BSLT established that the compounds contain very potent bioactive compounds. The bioactivity was rated moderate due to the values of the LC_{50}. The EC_{50} value for general bioactivity is approximately one tenth of the value of the LC_{50} in BSLT [15]. Therefore the EC_{50} was estimated to be approximately 41.7 and 56.8 ppm for N-(benzothiazol-2-yl)trichloroethanamide and N-(benzothiazol-2-yl)chloroethanamide respectively. The surviving nauplii were dull and inactive, which may be a sign of central nervous system depression [15].

Conclusion:

Both derivatives of 2-aminobenzothiazole were active against the multiresistant bacteria with IZD ranging from 9-18 mm N-(benzothiazol-2-yl)chloroethanamide **[5]** and 9 – 20mm N-(benzothiazol-2-yl)trichloroethanamide **[4]**. From the MIC results it is observed that the **[5]** derivative produced a better antibacterial activity than the **[4]** derivative. Bearing in mind that the bacteria strains used in this study were highly resistant to conventional antibacterial agents, the potentials of the test compounds as chemotherapeutic agents against multi-resistant bacteria can be exploited.

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