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Mannich Synthesis of 2-(Thiazol-4-yl)-1h-benzimidazole-2-methylene Piperazinyl (2,5 -Diamino-1,3,4-thiadiazolyl) Oxamide and Evaluation of the Filaricidal Activity

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Abstract: The search for new biologically active agents via Mannich synthesis has continued to generate interest. And as there is now continued resistance to filaricidal drugs, synthesis of new filaricidal agents are now of neccessity. Synthesis of 2-(Thiazol-4-yl)-1H-benzimidazole -2-methylene piperazinyl (2, 5-diamino-1,3,4-thiadiazolyl) oxamide was achieved via preparation of the Mannich base of 2-(Thiazol-4-yl)-1H-benzimidazole (Thiabendazole) followed by reaction with the oxalate derivative of 2, 5-diamino-1,3,4-thiadiazole. Analysis and characterisation of both the intermediate (the Mannich Base) and the final product (2-(Thiazol-4-yl)-1H-benzimidazole -2-methylene piperazinyl (2, 5-diamino-1,3,4-thiadiazolyl) oxamide was achieved by UV, IR, ^{1}H NMR and MS Spectroscopy. The ionization constant (pKa) of the final product is also reported. *In vitro* filaricidal screening of both the intermediate Mannich base and the final product was achieved by worm motility assay against *O. gutturosa.* Both the Mannich base and 2-(Thiazol-4-yl)-1H-benzimidazole -2- methylene piperazinyl (2,5-diamino-1,3,4-thiadiazolyl) oxamide exhibited significant 85.4% and 90% chemotherapeutic efficacy while the 2-(Thiazol-4-yl)-1H-benzimidazole -2- methylene piperazinyl (2,5-diamino-1,3,4-thiadiazolyl) oxamide exhibited 92% and 96% ($P < 0.04$) chemotherapeutic efficacy on the micro and macrofilarie of *O. gutturosa* respectively. These are clearly higher than that of Thiabendazole at the same dose 500 μM/ml.

Key words: Mannich base · 2-(Thiazol-4-yl)-1H-benzimidazole · Spectroscopy · Ionization constant · Filaricidal activity

INTRODUCTION

Synthesis, now more than ever, is a vital and interesting part of organic chemistry. Organic synthesis has, rightly commanded a good deal of attention from Researchers who have to choose from different arrays of synthetic methods. Mannich reaction is one of such. It involves the introduction of a single carbon atom by the reaction of an active methylene compound with formaldehyde and an amine to form a β-aminocarbonyl compound [1a,b]. Mannich reaction consist of a simple addition step without a final elimination. This presumably reflects the fact that $-^{+}NHR_2$ is a poorer leaving group than $-^{+}OH_2$. The products of many Mannich reactions are referred to as Mannich bases and are themselves useful synthetic intermediates. Mannich reaction has been employed in the synthesis of many useful chemical substances of medicinal and industrial interest [2a,b,c,d,e]. This study aims to employ Mannich reaction to synthesize new filaricidal compounds as parasitic agents continue to pose dangerous threats to human race. Search for ideal anti parasitic agent which should at low dosage regimen be able to destroy both the adult and developmental stages of the parasite with little or no side effect on the patient still continues. Helminthiasis, or worm infestation being one of the most prevalent diseases in the world. The helminths include the Nemathelminthes e.g. Nematodes (*Ascaris lumbricoides,* Round worm, etc), Platyhelminthes, cestodes (*Taenia saginata,* beef tapeworm etc.). Drug therapy includes use of anthelmintics e.g. Thiabendazole, mebendazole, piperazine salts, ivermectin, certain antimalarials etc [3a,b,c,d,e].

2-(Thiazol-4-yl)-1H-benzimidazole (Thiabendazole), one of the antiparasitic agent in clinical use, is used as the lead compound. It is a broad spectrum anthelmintic; active against roundworms, hookworms and ascariasis. Its action against whipworm is unpredictable. It suppresses egg and larval production by inhibiting uptake of glucose. Also it binds to unpolymerised tubulin, preventing microtubule production. The resultant cellular disruption

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leads to inhibition of autolysis and cellular necrosis. Disruption of microtubule formation also accounts for the ovicidal effects of benzimidazole. Its side-effect include CNS depression and hypersensitivity reactions. Through molecular modification of Thiabendazole and assemblage of different pharmacophores, a new drug having more desirable properties than the lead in potency, toxicity, specificity, duration of action and ease of application is expected to be produced. Thiabendazole is therefore the 'lead' compound in this Mannich synthesis. Other pharmacophores used include; piperazine which is also an anthelmintic, causes narcosis, paralysis or death of the helminth with its subsequent elimination; oxalate, reduces toxicity and enhance activity; 2,5-diamino-1,3.4-thiadiazole, known to improve the activity of drugs, rapid acting with a shorter half-life in the blood [4a,b]. This study was therefore conducted to synthesize new filaricidal compounds via Mannich reaction and to investigate their filaricidal activity *in vitro* by worm motility assay against *O. gutturosa*.

Experimental Section

Materials: Trioxonitrate (V) acid, methanol, Diethyloxalate, sodium metal, hydrochloric acid, sodium hydroxide, piperazine anhydrous, formaldehyde, 1,4-dioxan, ammonia solution, dimethylformamide, glacial acetic acid and ethylacetate (BDH). Buffer powders (Gallenkamp). Thiabendazole (Mintezol,Merck SD). Perchloric acid (standardised), 2,5-diamino-1,3,4-thiadiazole. All solvents used were analar grade and were used without further purification. Assesment of the degree of purity of intermediate and final products obtained was achieved by means of Gallemkamp Melting point Apparatus Model MFB 595 for the determination of melting point and also analytical Thin Layer Chromatography. Thin Layer Chromatography was carried out using Silica Gel F_{254}, chloroform, methanol as the mobile phase. Also the pH and pKa were also determined via non-aqueous titration using the pH meter 7020 (Electronic Instrument Ltd London). These compounds were further characterized by spectroscopic analysis, UV-Visible, Infra-red and NMR spectrometry. The UV/Visible Spectra of 0.01% w/v of the fractions were determined with the aid of P W Allen and Co. 425 UV/Visible spectrophotometer Model LCF 750 Q. The samples were scanned between 190nm and 1100nm. Data from chart/recorder gave a graph of Absorbance against wavelength (nm). Vmax (cm^{-1}) from IR data also confirmed the structures. The Infrared spectra of the synthesized compounds were determined using a Nicolet Avatar 330 Fourier Transform (FT) Infrared Spectrophotometer. The Kbr disc method was used for the preparation of the sample.

The spectrophotometer determines the relative strength and position of all absorption in the infrared region and plots the intensity (Transmittance against wave number). The ^{1}H NMR spectra of the synthesized compounds were determined using a 300MHz machine for 10% (w/v) solutions in deuterochloroform. Pulse irradiation technique employed was FT NMR at ambient temperature. The ^{1}H NMR signals of the compounds appeared at δ scale relative to TMS. ESI-HRMS spectra were obtained on Bruker micro TOF instrument with an ESI source. Microscope was used for the filaricidal activity.

Worms: The worms of *O. gutturosa (micro and macrofilariae)* identified by the Superintendent Veterinary Officer were obtained from Nigerian Veterinary Research Institute Jos, Nigeria.

Media: 20% of 50ml of heat activated calf medium (GILBCO). Dissecting medium was made up of Medium 199 and 10% calf serum (GILBCO).

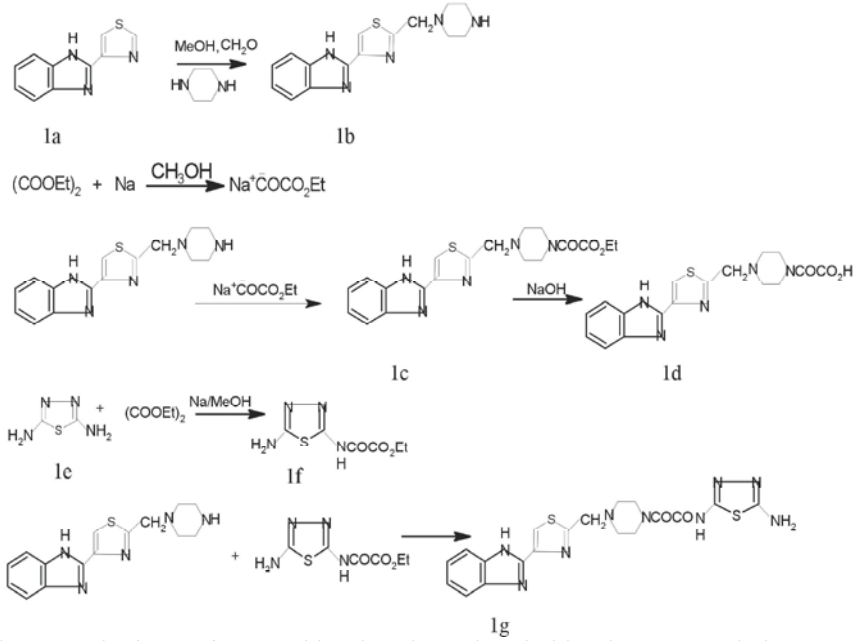
Drug: 2-(Thiazol-4-yl)-1H-benzimidazole (Thiabendazole) standard (MSD).

Methods

Preparation of the Mannich Base of 2-(Thiazol-4-yl)-1H-benzimidazole: (0.40g, 0.002M) of piperazine (anhydrous), (2.50g, 0.01M) of 2-(Thiazol-4-yl)-1H-benzimidazole (Thiabendazole) 1a and 10ml formaldehyde were made to react in a basic medium; absolute methanol. This was refluxed at a temperature of 50-60° for 36hrs (Scheme 1).

Esterification of Mannich Base of 2-(Thiazol-4-yl)-1H-benzimidazole: Compound (1b) is the Mannich base. 15.0ml of Mannich Base of 2-(Thiazol-4-yl)-1H-benzimidazole was crystallised as the ester (1c) by using 4.0ml of diethyloxalate and 0.01g sodium metal in 10.0ml of absolute methanol. A white precipitate (1d) was formed which was air-dried.(Scheme 1)

Preparation of the Oxalate Derivative of 2, 5-diamino-1, 3, 4-thiadiazole: (0.6g, 0.005M)of 2, 5-diamino-1,3, 4-thiadiazole(1e) and 0.005M of diethyloxalate were reacted in a basic medium (absolute methanol) to give the oxalate derivative of 2,5-diamino-1,3,4-thiadiazole (1f).



Scheme 1: Mannich Synthesis of 2-(Thiazol-4-yl)-1H-benzimidazole -2-methylene piperazinyl (2,5 diamino-1,3,4-thiadiazolyl) oxamide.

Preparation of 2-(Thiazol-4-yl)-1H-benzimidazole -2-methylene piperazinyl (2, 5 diamino-1, 3, 4-thiadiazolyl) oxamide (1g): 2.50g of the preformed Mannich base(1b) was reacted with the oxalate derivative of 2, 5-diamino-1, 3, 4-thiadiazole(1f). This was refluxed in absolute methanol at 50-60°C for 36hrs. Crystals obtained were left to stand overnight and then obtained by vacuum filteration, air dried and kept for further analysis. The oxamide derivative of 2-(Thiazol-4-yl)-1H-benzimidazole (1g) was purified by recrystallisation in N, N-dimethylformamide (Scheme 1).

After the reactions proceeded for a stated period of time, respective of the reaction conditions, the insoluble products were sonicated in cold ethanol, filtered and air dried. Appearance, yield and melting points of products obtained are reported with experimental data. Qualitative test was also performed to confirm the presence of oxamide derivative in the final product (Biuret's test).

Determination of ionization constant (pKa) of 2-(Thiazol-4-yl)-1H-benzimidazole -2-methylene piperazinyl (2,5 diamino-1,3,4-thiadiazolyl) oxamide via potentiometric titration.

The pH was standardised using a standard buffer solution of pH4 (0.05M potassium hydrogenphthalate) and pH9 (0.01M borax solution). Standardized perchloric acid was used to titrate 2.5ml of solution of 2-(Thiazol-4-yl)-1H-benzimidazole -2-methylene piperazinyl (2,5 diamino-1,3,4-thiadiazolyl) oxamide in 60% 1,4-dioxan. The pKa was then determined from a graph of pH vs volume (ml) of titrant using the Henderson-Hasselbalch equation:

$$pH = pKa + \log [salt]/[acid]$$

The pH beyond the equivalence point was calculated from the excess titrant added using the formular:

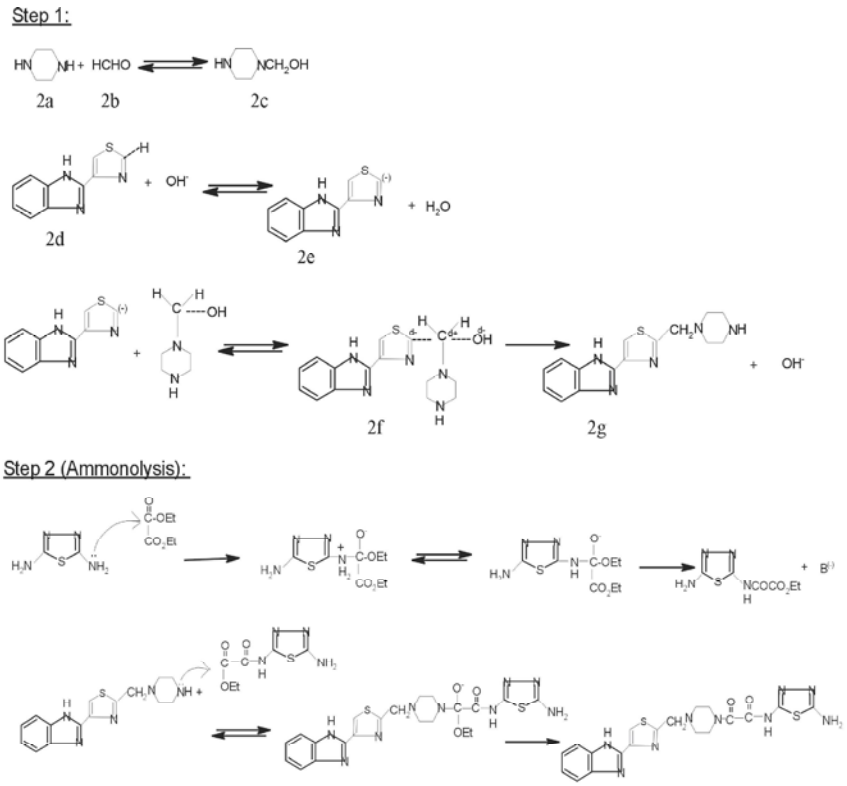
$$pH = pKw - pOH$$

$$pOH = 14 - \log [H^+]$$

$$pOH = -\log [OH]$$

(pH = -log of hydrogen ion concentration; pKa = -log ionization constant; pKw = ionic constant of water). The pKa results represent mean values of three determinations carried out near pH equivalence at 30ºC. The results were statistically analysed and the limit of experimental error found to be ±0.001.

Filaricidal Activity: Determination of maximum dose-regimen: Previously identified Microfilariae of *O.gutturosa* placed in the dissecting medium for two hours at room temperature was transferred into incubating medium at an atmosphere of 6% CO_2 in air. 150 Microfilariae of *O.gutturosa* were used while doses of drugs employed are 300, 400,500 and 550µM/ml. Six different groups of wells labelled A, B, C, D, E and F were made to contain 12,12, 12,12, 16 and 20 wells each respectively and contain the incubating medium alone (A), the synthesized compounds at doses 300, 400,500 and 550µM/ml (B), microfilariae only



Scheme 2: Mechanism of Base Catalysed Mannich Reaction

(C), incubating medium and the different dose levels of the synthesized compounds (D), incubating medium inoculated with 150 microfilariae of *O.gutturosa* and different doses (300, 400,500 and 550µM/ml) of Thiabendazole respectively (E) while the last group contain the combined contents of A,B and C wells. After 72hrs, microscopic examination of the filaricidal activity revealed that the maximum dose regimen was 500µM/ml for each of the tested compounds considering four replicate analysis.

Determination of the Filaricidal Activity of Synthesized Compounds

Microfilaricidal Activity: The same procedure was employed as in determination of maximum dose-regimen above, contents of groups A, B, E and F remained the same while that of C and D changed. 500µM/ml of each of synthesised compounds were placed in group C set of wells which already contained 150 microfilariae of *O.gutturosa,* while group D contained the incubating medium seeded with 150 microfilariae of *O.gutturosa* and 500µM/ml of Thiabendazole.. The process was repeated for three days. Larvicidal activity of the compounds were then determined as above.

Macrofilaricidal Activity: The same procedures were repeated for Macrofilaricidal activity using macrofilariae of *O.gutturosa.*

Statistical Analysis: Student's 't' test and analysis of variance were processed on SPSS 15 windows software.
Mechanism of based catalysed Mannich Reaction (Scheme 2).

Step 1: This involved the reaction of a carbanion (derived from the active hydrogen compound) with the aminomethylol by an S_N^2 mechanism. 2-(Thiazol-4-yl)-1H-benzimidazole was the active hydrogen in this case.

Piperazine (2a) combined with formaldehyde (2b) to set up an equilibrium with the piperazinomethylol (2c). 2-(Thiazol-4-yl)-1H-benzimidazole (2d) reacted with hydroxide ion to set up an equilibrium with the carbanion (2e) and water. Intermediates 2c and 2e reacted by an S_N^2 mechanism to form an equilibrium concentration of the activated complex or transition state (2f); which decomposed by a relatively irreversible rate controlling step to yield the Mannich base(2g) [5a.b].

Step 2 (Ammonolysis): This reaction involved nucleophilic attack by the base 2,5-diamino-1,3,4-thiadiazole on the electron deficient carbon of the diethyl oxalate followed by the reaction of the Mannich base with the oxalate derivative of 2,5-diamino-1,3,4-thiadiazole which also involved nucleophilic attack by the Mannich base on the electron-deficient oxalate derivative of 2,5-diamino-1,3,4-thiadiazole.

RESULTS AND DISCUSSION

Mannich Base of 2-(Thiazol-4-yl)-1H-benzimidazole (1d): White crystals; yield: 71%; m.p: 310-311°C. Soluble in 60% DMF, dil mineral acid and 60% 1,4-dioxan, sparingly soluble in methanol, ethanol and chloroform. Insoluble in water and ethylacetate. R_f 0.41 (Silica gel $F_{254,}$ $CHCl_3$: MeOH 9:1). Soluble in water, methanol, chloroform, ethylacetate, hexane and acetone.UV (EtOH, λmax): 631, 343, 282, 270 nm. IR (KBr) V_{max} cm^{-1}: 3250, 3400 and 3650(N-H stretch), 2900(C-H aromatic stretch), 2850(C-H aliphatic stretch), 2810-2950(O-H carboxylic, H-bonded), 1590-1640(C=O stretch), 1450(C-H aliphatic bending), 1380(C-N amines), 1290-1300(C-O carboxylic acid), 720-780(C-H aromatic out of plane bend). ^{1}H NMR (300 MHz; $CDCl_3$): δ 7.24 (s, 4H, 4x-Ar-H), 8.15 (s, 1H, NH), 5.25 (s, 1H, SCH), 3.13 (s, 2H, $-CH_2$), 4.09 (q, J=7.7Hz, 4H,2x-NCH_2), 5.35 (q, J=7.5Hz, 4H, 2x-NCH_2),13.20 (s, 1H, 1x-OH). ESI HRMS (amu): measured for $C_{17}H_{17}N_5O_3S$ [M+H]+ 372.1093; actual [M+H]+ 372.1098.

2-(Thiazol-4-yl)-1H-benzimidazole-2-methylene piperazinyl (2, 5 diamino-1,3,4-thiadiazolyl) oxamide (1g): White crystals; yield: 69.9%; m.p: 325-330°C. Soluble in 60% DMF, dil mineral acid and 60% 1,4-dioxan, sparingly soluble in methanol, ethanol and chloroform. Insoluble in water and ethylacetate. R_f 0.32 (Silica gel $F_{254,}$ $CHCl_3$: MeOH 9:1). UV (EtOH, λmax): 588, 346, 284, 269 nm. IR (KBr) V_{max} cm^{-1}: 3300- 3400 (N-H stretch), 2900(C-H aromatic stretch), 2850(C-H aliphatic stretch), 1600-1630(C=O stretch), 1450(C-H aliphatic bending), 1355-1310(C-N amines), 715-825(C-H aromatic out of plane bend).^{1}H NMR (300 MHz; $CDCl_3$): δ 7.44 (s, 4H, 4x-Ar-H), 8.12 (s, 1H, NH), 5.15 (s, 1H, SCH), 3.12 (s, 2H, $-CH_2$), 4.19 (q, J=7.7Hz, 4H,2x-NCH_2), 5.15 (q, J=7.5Hz, 4H, 2x-NCH_2), 6.69 (s, 1H, NH), 5.83 (s, 2H, 1x-NH_2). ESI HRMS (amu): measured for $C_{19}H_{19}N_9O_2S_2$ [M+H]+ 470.2693; actual [M+H]+ 470.2699. pKa=10.5 and 8.1.

In the preparation of the Mannich Base of 2-(Thiazol-4-yl)-1H-benzimidazole, the quantity of the active hydrogen compound used was determined by the number of α-carbons which had hydrogen attached. The reaction medium was very basic and a secondary amine was used (since it has been observed that 2° amine unlike 1° amine go to specific reaction site). The IR spectra of compounds 1d and 1g showed absorption bands at ν = 3250 - 3650 cm^{-1} and 3300-3400cm^{-1} respectively which are assignable to the N-H stretching frequency. The V_{max} at 2900 and 2850 cm^{-1} are due to C-H (aromatic) and C-H (aliphatic) stretch respectively. Compound 1d at V_{max} 2810 - 2950 cm^{-1} is indicative of O-H due to carboxylic acid which are strongly hydrogen bonded. This is absent in compound 1g. V_{max}at 1590-1640 cm^{-1} for 1d and 1600-1630 cm^{-1} for 1g are due to the C=O stretching frequency. Both compounds have absorption peaks at 1450 cm^{-1} due to aliphatic C-H bending which also confirmed the $-CH_2$- in Mannich bases. The C-N bond can be seen at V_{max} 1380cm^{-1} for 1d and 1310-1355 cm^{-1} for 1g. Carboxylic acid C-O seen at 1290-1300 cm^{-1} in 1d is absent in 1g. Absorption peaks at 720 and 780 cm^{-1} for 1d and 715 and 825 cm^{-1} for 1g are indicative of the aromatic C-H out of plane bend. C-C stretch is not interpretatively useful. The bands vibrational frequency are affected by factors like intra- or inter-molecular hydrogen bonding, conjugation effects and ring size effects. The UV-Visible absorption spectra for the two products showed maximum absorption at 631nm and 588nm for 1d and 1g respectively, indicating that the compounds are highly conjugated. The ð > ð* transition of benzene appear in the region 270 -282 and 269-284nm for 1d and 1g respectively while the absorption in the region 343 and 346nm for 1d and 1g confirmed the n > ð* of the heterocyclic ring, such as those lone pair of electrons present in O, N and S all of which are present in the compounds. As for the proton NMR, the most characteristic signals in the ^{1}H NMR spectra of 1d and 1g are the 4H singlet aromatic protons occurring at δ 7.24 and 7.44 respectively. These facts were also supported by characteristic peaks in NMR data at δ 8.15 (1d) and 8.12 (1g) for NH protons,

$-CH_2$- singlets occurred at δ 3.13(1d) and 3.12 (1g) characteristic of the methylene protons. The 2,5-diamino-1,3,4-thiadiazole moiety in (1g) is clearly indicated by absorptions bands at 6.69 and 5.83 occuring as singlets for 1H of NH and 2H of NH_2. The peak at δ 13.20, a singlet corresponds to the hydrogen of the carboxylic acid present in the recrystallised Mannich base (1d). This is distinctly absent in 2-(Thiazol-4-yl)-1H-benzimidazole -2-methylene piperazinyl (2, 5 diamino-1,3,4-thiadiazolyl) oxamide (1g).

Table 1: Filaricidal activity of the Mannich base and 2-(Thiazol-4-yl)-1H-benzimidazole -2-methylene piperazinyl (2,5 diamine-1,3,4-thiadiazolyl) oxamide on *O.gutturosa**

		Viable Microfilariae Count N=5				Viable Macrofilariae Count N=5			
Compound	Dose µM/ml	BT	AT	Df.	% Chem. Efficacy	BT	AT	Df.	% Chem. Efficacy
Thiabendazole	500	138±0.03	18±0.02	88	63.71	138±0.03	45±0.02	93	67.39
Mannich base	500	150±0.04	22±0.03	128	85.4	150±0.02	15±0.03	135	90.00
Oxamide derivative	500	150±0.03	12±0.03	138	92.00	150±0.03	6±0.03	144	96.00

*Each value represents the mean ± standard deviation of five replicate analysis.P < 0.04 compared with control Student's t – test

Results of the non-aqueous titration of 2-(Thiazol-4-yl)-1H-benzimidazole -2-methylene piperazinyl (2,5 diamino-1,3,4-thiadiazolyl) oxamide (1g) using standardized perchloric acid as titrant gave two Ionization constant (pKa) values 8.1 and 10.5. Ionization constant (pKa) is one of the physicochemical properties that must be determined to provide information that can be used to predict biological availability, metabolism and excretion of drugs, since it influences both the absorption and passage of the drug through cell membranes. It can be used to predict the biological activity of a new drug in a series of compounds. It has also been observed that certain drugs are absorbed in their un - dissociated state either directly or by ion pair or complex formation. Weak bases for instance are reabsorbed in the renal tubules, depending on their degree of ionization and are eliminated at a rate depending on their pKa values. The rate is faster when the pH of urine is low and slower when the pH is higher. The biological half-lives of drugs may therefore increase or decrease with changes in pH of urine (Olaniyi, 1989). 1g at pKa of 8.1 and 10.5 may enable the nitrogen lone pair in the compound to be protonated at physiological pH and then be able to interact with the anionic site which is a clear indication of the effectiveness of the product and its biological availability (1g). Higher melting point of the products is on account of the increased molecular weight and H-bonding. In aqueous alkali suspension, the oxamide moiety gave a pink colouration with dilute copper (ll) salt solution which is a positive test confirming the presence of the oxamide derivative in the final product.

Filaricidal Activity: Complete inhibition of worm motility and subsequent mortality observed in the *in vitro* micro/macro filaricidal activities by worm motility assay against *O. gutturosa* showed that the two synthesized compounds are more active than the lead drug, Thiabendazole.

The Mannich base exhibited significant 85.4% and 90% chemotherapeutic efficacy while 2-(Thiazol-4-yl)-1H-benzimidazole -2-methylene piperazinyl (2,5 diamino-1,3,4-thiadiazolyl) oxamide exhibited 92% and 96% chemotherapeutic efficacy ($P < 0.04$ in both cases) on the micro and macrofilarie of *O. gutturosa* respectively (Table 1). These are clearly higher than that of Thiabendazole at the same dose 500 µM/ml.

CONCLUSION

Preparation of 2-(Thiazol-4-yl)-1H-benzimidazole -2-methylene piperazinyl (2,5 diamino-1,3,4-thiadiazolyl) oxamide(1g) was achieved via a reaction involving preparation of Mannich base of thiabendazole using formaldehyde and piperazine followed by reaction with the oxalate derivative of 2,5-diamino-1,3,4-thiadiazole. Both the intermediate Mannich base and the final product 2-(Thiazol-4-yl)-1H-benzimidazole -2-methylene piperazinyl (2,5 diamino-1,3,4-thiadiazolyl) oxamide are white crystals, having solubility profile typical of weak drugs, practically insoluble in water but soluble in dilute mineral acid. The Mannich reaction affords an exceptionally useful synthetic route for the synthesis of the products giving a good yield 71 and 69.9% for the Mannich base and 2-(Thiazol-4-yl)-1H-benzimidazole -2-methylene piperazinyl (2,5 diamino-1,3,4-thiadiazolyl) oxamide respectively. The results of this study also showed that the Mannich base, despite being an intermediate product in this synthesis and the final product 2-(Thiazol-4-yl)-1H-benzimidazole -2-methylene piperazinyl (2,5 diamino-1,3,4-thiadiazolyl) oxamide have potent filaricidal activity. Since these compounds are effective in the *in vitro* worm motility assay against *O. gutturosa*, these compounds can be effective in therapy that involves treatment of micro and macro filaricides. It was also concluded that differences in the chemotherapeutic efficacies of the synthesized compounds are to a large extent dependent on

the relationship between their chemical structures being a factor of the pharmacophores reacted.The synthesized compounds therefore showed greater pharmacological activity than the lead on account of the following assumptions; The thiadiazole moiety itself is an antiparasitic agent and easily metabolised, piperazine is an antihelminthic, causes flaccid paralysis while oxalate is an activity enhancer (increases potency) and it also reduces toxicity. The oxalate combined with 2,5-diamino-1,3,4-thadiazole are known to reduce blood glucose, has prolonged activity and therefore metabolised last, a state of sustained release activity is achieved whereby, the oxamide portion will be released last. This assumption is hypothetical if the pharmacophores work synergistically therefore reducing or eliminating the side effects of Thiabendazole which include hypersensitivity (vomiting, anorexia, nausea etc.), CNS depression among others. The mechanism of action of these compounds is not unconnected with the interference of carbohydrate metabolism in *O.gutturosa.* More *in vivo* assays are also essential to classify them as biological filaricides and studies are still on to compare their mode of action to some filaricides in clinical use.

Lastly, incidences of drug combination in therapy has made the study of medicine more interesting and has brought a tremendous improvement to chemotherapy which is also a fact clearly demonstrated by the results of this study.

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