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SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF 1-(2-METHOXYPHENYL)-(3-(2-PHTHALAMIDO)-PROPYL) PIPERAZINE AND 1-(2-METHOXYPHENYL)-(3-(2-PHTHALAMIDO)-BUTYL) PIPERAZINE

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ABSTRACT

Compounds with piperazine moiety are known to have activity against microbes. The aims of this study were to synthesize, characterize and screen 1-(2-methoxyphenyl)-(3-(2-phthalamido)-propyl) piperazine and 1-(2-methoxyphenyl)-(3-(2-phthalamido)-butyl) piperazine for antibacterial activity. 1-(2-Methoxyphenyl)-(3-(2-phthalamido)-propyl) piperazine was synthesized by refluxing 1-(2-methoxyphenyl) piperazine and anhydrous potassium carbonate in ethane nitrile with N-(3-bromopropyl) phthalimide, while 1-(2-methoxyphenyl)-(3-(2-phthalamido)-butyl) piperazine was obtained by condensing N-(bromobutyl) phthalimide and 1-(2-methoxypheyl) piperazine using acetonitrile as the solvent in anhydrous potassium carbonate in ethane nitrile the for 48 h. Purity of the synthesized compounds was ascertained by determining their melting points, characterization was done using proton nuclear magnetic resonance, carbon-13 nuclear magnetic resonance, Fourier transform infra-red spectroscopy and mass spectrometry. The synthesized compounds were tested for it antibacterial activity using standard method. 1-(2-methoxyphenyl)-(3-(2-phthalamido)-propyl) piperazine was obtained as a yellow crystal, (melting point of 100-102 ^{o}C, percentage yield 95 %) by refluxing 1-(2-methoxyphenyl) piperazine and N-(3-bromopropyl) phthalimide. 1-(2-Methoxyphenyl)-(3-(2-phthalamido)-butyl) piperazine was synthesized as yellow solid (melting point of 68-70 ^{o}C, percentage yield 90 %) by condensing N-(3-bromobutyl) phthalimide and 1-(2-methoxyphenyl) piperazine. *In vivo* antibacterial activity showed that 1-(2-methoxyphenyl)-(3-(2-phthalamido)-propyl) piperazine and 1-(2-methoxyphenyl)-(3-(2-phthalamido)-butyl) piperazine have no antibacterial activity. 1-(2-methoxyphenyl)-(3-(2-phthalamido)-propyl) piperazine and 1-(2-methoxyphenyl)-(3-(2-phthalamido)-butyl) piperazine were synthesized, characterized and showed no activity against *Escherichia coli*, *Staphylococci aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Klebsiella pneumoniae*.

KEYWORDS: **Phthalamidoalkyl piperazine; Antibacterial; Condensation.**

INTRODUCTION

Piperazine derivatives are starting materials for the synthesis of many drugs that have activity against parasitic worms [1]. Its molecules or derivatives are seen in many medicinal compounds used for the management or treatment of vertigo, erection abnormally and blood pressure. Its structure has also been observed in antipsychotics, antidepressants [2], anti-inflammatory [3], Anti-HIV [4], neurodegenerative diseases [5] and many anti-cancer agents [6]. Some of the limitations with the use of piperazine include its high price, which is related with the difficulty associated with its synthesis, poor yield due to elaborate synthetic steps and competing products or difficult work-up [7].

Gabriel synthesis is an old useful method for synthesis, it involves the addition of alkyl groups to an anion of phthalimide using a suitable reagent and further abstraction of the phthaloyl moiety to produce primary amines [8]. This synthetic path evades the difficultly associated with numerous alkylation of nitrogen by protecting the nitrogen atom of the nucleophile and in the process its reactivity is also

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modified by aldehyde and ketone molecules. The quaternary nitrogen of phthalimide salt replaces the halogen from the alkyl halides to give N-alkylated phthalimide [9].

Medicinal agents with piperazine moiety have known activity against microbes. Microbes are responsible for several infections and major risk factor for many other diseases. These microbes have developed resistance overtime due to indiscriminate use of antibiotics from over-the-counter prescription. Thus, the need to develop antibacterial agents that will be effective against resistant strain of bacteria. Phthalimido and piperazine containing moieties have been shown to inhibit the growth of some clinical isolates [10,11], thus this study aims to synthesis, characterise and screen 1-(2-methoxyphenyl)-(3-(2-phthalamido)-propyl) piperazine and 1-(2-methoxyphenyl)-(3-(2-phthalamido)-butyl)-piperazine for antibacterial activity.

MATERIALS AND METHODS

Materials

1-(2-Methoxyphenyl) piperazine, N-(3-bromopropyl) phthalimide, 1,3-dibromopropane and 1,4-dibromobutane were obtained from Sigma, Germany. Acetonitrile and anhydrous potassium carbonate were obtained from Scharlau, Spain. IR spectra were recorded as KBr disc on Buck Infra-Red M500 instrument (Buck Scientific Inc., Norwalk, Connecticut, USA). ^{1}H NMR spectra were recorded on Varian Germini 200 (250 MHz) (Varian Inc., Palo Alto, California, USA). Chemical shifts are reported in ppm relative to tetramethylsilane as a reference standard. ^{13}C NMR spectra were recorded on Varian Germini 200 (63 MHz), Varian Inc., Palo, California, USA). Chemical shift value (ppm) was recorded relative to tetramethylsilane as reference standard. The multiplicities are: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = double doublet, J = coupling constant, Hz = hertz. Mass spectra were acquired on a Finnigan MAT44S mass spectrometer (Thermo Finnian San Jose, California, USA) at 70 eV. Melting points were determined on a Kofler Electrothermal® melting point apparatus CAT No 1A6304, England. Weighting was done using analytical weighing balance (B 15 Matler, Toledo, Switzerland). Analytical Thin Layer chromatography (TLC) on silica gel 60 F254 pre-coated plates from Merck Damstardt, Germany. The bacteria *Escherichia coli* (EC), *Staphylococci aureus* (SA), *Pseudomonas aeruginosa* (PA), *Bacillus subtilis* (BS) and *Klebsiella pneumoniae* used in this study were obtained from University of Benin Teaching Hospital and Department of Pharmaceutical Microbiology, University of Benin.

Synthetic methods

Synthesis of N- (4-bromobutyl) phthalimide

To 30 mmol of potassium phthalimide in 50 ml of Dimethylformamide (DMF) was added 100 mmol of 1,4-dibromobutane and the reaction medium was stirred for 2 h at 60 ° C. The excess solvent and reagent were removed under reduced pressure and 100 ml water added. This was extracted with $CHCl_3$ to give pale yellow oily compound which slowly solidify and was then recrystallized from ethanol [3].

Synthesis of 1-(2-methoxy phenyl)-(3-(2-phthalimido)-propyl)-piperazine

To a mixture of 1-[2-methoxyphenyl] piperazine (8.3 g, 43.2 mmol) and anhydrous potassium carbonate (12 g, 86.8 mmol) in 150 ml acetonitrile was added N-[3-bromopropyl]-phthalimide (9.8 g, 36.0 mmol) and reflexed for 24 h to yield the desired product which was recrystallized from ethanol.

Synthesis of 1-(2-methoxy phenyl)-(3-(2-phthalamido)-butyl)-piperazine

Ten (10) g (35.5 mmol) of N-(bromobutyl)-phthalimide and 8.19 g (42.6 mmol) of 1-[2-methoxyphenyl] piperazine were condensed in the presence of 11.78 g (85.2 mmol) anhydrous potassium carbonate in 150 ml of acetonitrile for 48 h.

Antibacterial activity

Zone of inhibition was evaluated for the synthesized compounds using 25 ml of melted agar aseptically transferred into a germ-free Petri dish, then allowed to cool. A 4 h culture of the organisms *Escherichia coli* (EC), *Staphylococci aureus* (SA), *Pseudomonas aeruginosa* (PA), *Bacillus subtilis* (BS) and *Klebsiella pneumoniae* (KP) were flooded on each of the 5 nutrient agar plates respectively. Using a sterile cork borer, 4 wells were made on each of the nutrient agar plates. Into each of the wells, 0.1ml of molten nutrient agar was pipette to cover up the base of the wells. 0.2 ml of 1 mg in DMF of each of the test compounds made by the dilution, were carefully placed aseptically in each well, while the controls (positive and negative) were poured into the other wells. These were maintained on the bench for 30 minutes and then incubated right side up for 18 h.

These strains of bacteria used in this study were cultured and sub-cultured from the standard into sterile nutrient agar plate at 37°C for 48 h and standardized to 10^6 CFU/mL in 12 h sterile broth before use. 0.2 ml of synthesized (1 mg/mL) were

dissolved in DMF as diluent (negative control), while 10 mcg/ml ciprofloxacin was used as positive control [12].

RESULTS

Figures 2-7 showed the IR spectrum, ^{1}HNMR, ^{13}CNMR and MS spectrum of 1-(2-Methoxyphenyl)-(3-(2-phthalamido)-propyl) piperazine: 13.2 g (95 %), Melting point: 100-102 ^{o}C; IR (KBr): υ 2935, 2810 (C-H); 1760, 1705 (C=O), 1586 (C=C). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.9 (m, 2H, H-e), 2.5 (t, 2H, H-f), 2.6 (s, 4H, H-g), 2.9 (s, 4H, H-h), 3.7 (t, 2H, H-d), 3.8 (s, 3H, H-n), 6.8 (m, 4H, Ar-H); 7.7 (m, 2H, Ar-H(b)); 7.9 (m, 2H, Ar-H(a)); ^{13}C NMR (50 MHz, $CDCl_3$): δ 25.2 (C-e), 36.8 (C-d), 50.5 (C-h), 53.4 (C-g), 55.3 (C-n), 56.1 (C-f), 111.3 (C-k), 118.1 (C-m), 120.9 (C-j), 122.7 (C-l), 123.0 (C-a), 132.4 (C-i), 133.7 (C-b), 141.4 (C-p), 152.3 (C-c), 168.4 (C-q). MS: 381 (3)(M^{+}+2), 380 (17)(M^{+}+1), 379 (66)(M^{+}), 378 (2)(M^{+}-1), 364 (26), 351 (1), 231 (4), 219 (12), 205 (100), 190 (37), 177 (47), 160 (11), 149 (10), 134 (9), 120 (8), 106 (3), 83 (4), 70 (16); Elemental analysis $C_{22}H_{25}N_3O_3$ (379.42). Cal.: C. 69.64 H. 6.64 N. 11.07, Found C. 69.55 H. 6.53 N. 11.15.

Figures 8-11 reveal the IR spectrum, ^{1}H NMR, ^{13}C NMR and MS spectrum of 1-(2-Methoxyphenyl) -(3-(2-phthalamido)-butyl)-piperazine: 12.6g, 90 %, Melting point: 68-69 ^{o}C. IR (KBr): υ 2980, 2795 (C-H), 1760, 1694 (C=O); 1587 (C=C). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.5 (m, 2H, H-f), 1.7 (m, 2H, H-g), 2.4 (t, 2H, H-h), 2.6 (s, 4H, H-i), 3.0 (s, 4H, H-j), 3.7 (t, 2H, H-e), 3.8 (s, 3H, H-r), 3.9 (m, 4H, Ar-H), 7.7 (m, 2H, Ar-H(b)), 7.8 (m, 2H, Ar-H(a)). ^{13}C NMR (50 MHz, $CDCl_3$): δ 24.1 (C-h), 26.6 (C-f), 37.3 (C-e), 50.6 (C-j), 53.4 (C-i), 55.3 (C-g), 58.0 (C-r), 111.4 (C-m), 118.2 (C-n), 121.0 (C-l), 122.7 (C-p), 123.1 (C-a), 132.1 (C-k), 133.8 (C-b), 141.4 (C-p), 152.3 (C-c), 168.3 (C-d). MS: 71 (13), 82 (1), 134 (5), 149 (4), 160 (9), 177 (21), 190 (28), 205 (100), 231 (1), 257 (2), 365 (1), 378 (9), 393 (23) (M^{+}), 394 (6) (M^{+}+1); Elemental analysis $C_{23}H_{27}N_3O_3$ (393.446). Cal.: C. 70.21 H. 6.92 N. 10.67; Found C.70.19 H. 6.80 N. 10.58.

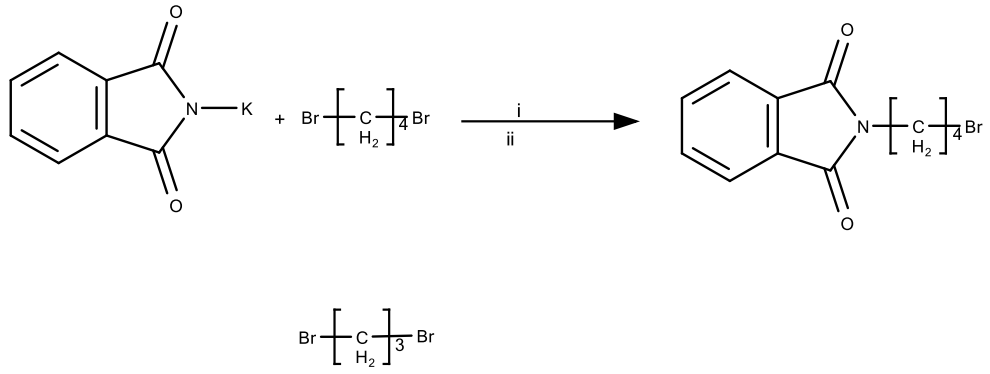
DISCUSSION

1-[2-Methoxyphenyl]-[3-[2-phthalamido]-propyl] piperazine was prepared in good yield (95 %) by reaction between 1-[2-methoxyphenyl] piperazine and N-[3-bromopropyl]-phthalimide (Figure 1), which was purified by column chromatography and recrystallized in ethanol-ether mixture (1:1). IR spectrum showed C-H stretches at 2935 cm^{-1} and 2810 cm^{-1}, C=O stretches at 1760 cm^{-1} and 1705 cm^{-1} while C=C vibration at 1586 cm^{-1}. ^{1}H NMR divulge the piperazine protons at 2.60 ppm and 2.90 ppm as singlet protons, while the propyl protons were seen at 1.9 ppm as multiplet, 2.50 ppm triplet, and 3.7 ppm triplet. ^{13}C NMR revealed the diagnostic carbonyl carbon 168.41 ppm. Mass spectrometry showed the molecular ion peak (M^{+}) at m/z 379 corresponding to the molecular weight of 1-(2-methoxyphenyl)-(3-(2-phthalamido)-propyl) piperazine.

1-(2-Methoxyphenyl)-(3-(2-phthalamido)-butyl)-piperazine was synthesized in high yield (90 %) from reaction between N-(bromobutyl)-phthalimide and 1-(2-methoxyphenyl)-piperazine (Figure 1). IR spectrum showed C-H stretches at 2980, 2795 and C=O stretches was seen at 1760, 1694, while C=C vibration was observed at 1587. In the ^{1}H NMR spectrum, the chemical shift of butyl moiety were at 1.5 ppm as multiplet, 1.7 ppm as multiplet, 2.4 ppm as triplet and 3.7 ppm as triplet. ^{13}C NMR spectrum revealed a diagnostic carbonyl carbon at 168.30 ppm. The molecular ion peak (M^{+}) in the mass spectrum was seen at 393 which is the molecular weight of 1-(2-methoxyphenyl)-(3-(2-phthalamido)-butyl)-piperazine.

The synthesis of 1-(2-methoxyphenyl) -(3-(2-phthalamido]-propyl) piperazine and 1-(2-methoxyphenyl) -(3-(2-phthalamido)-butyl) piperazine are illustrated in scheme 1 and 2. The reaction of potassium phthalimide (1) and 1,4-dimethylbutane produced N-(3-propylbromide) phthalamido took place in dimethylformamide (DMF). This reaction can also occur *insitu* by reacting anhydrous potassium carbonate or potassium potassium hydroxide with phthalimide. The reflux of N-(3-propylbromide)-phthalamido with 1-(2-methoxyphenyl) piperazine using acetonitrile as solvent and anhydrous potassium carbonate resulted in 1-(2-methoxy phenyl)-(3-(2-phthalimido]propyl)-piperazine.

Screening of these compounds against Gram-positive (*E. coli, B. subtilis* and *S. aureus*) and negative bacteria (*K. pneumoniae* and *P. aeruginosa*) revealed the absent of activity due inability of the compounds to inhibit the growth of the bacteria, when compared to ciprofloxacin which was used as the positive control, which showed zone of inhibition ranging from 21-23 mm. DMSO which was used as the solvent for dissolving the compounds also showed no zone of inhibition against the bacteria. This lack of activity could be linked to antagonizing effect of the individual moieties forming the newly synthesized molecules which have been previously reported to be effective against these bacteria [10,11].



i = DMF, ii= anhydrous K_2CO_3, CH_3CN/ Reflux (24-48 h)

Figure 1. The synthetic pathway for phthalamidoalkyl piperazine.

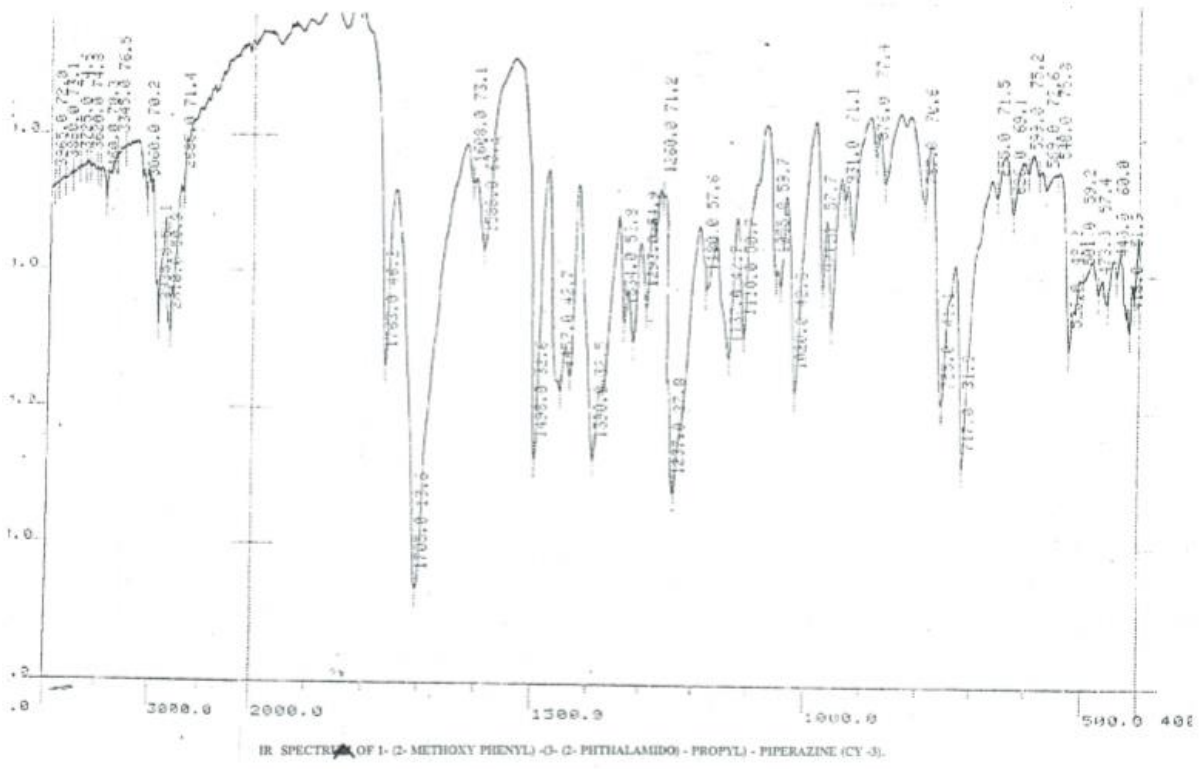


Figure 2. IR spectrum of 1-(2-methoxyphenyl)-(3-(2-phthalamido)-propyl) piperazine.

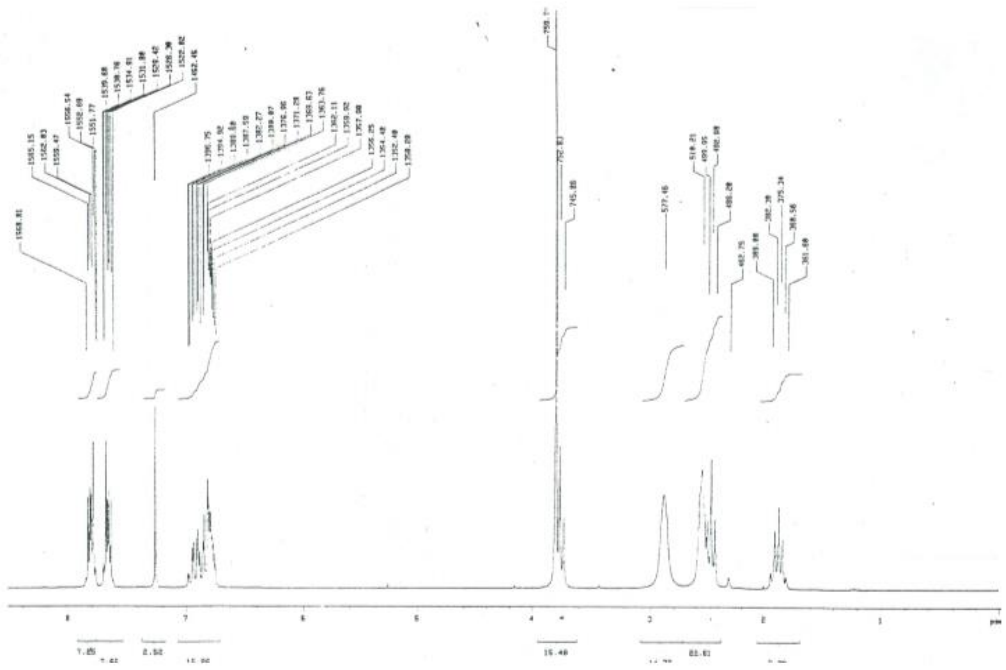


Figure 3. [1]H-NMR of 1-(2-methoxyphenyl)-(3-(2-phthalamido)-propyl) piperazine.

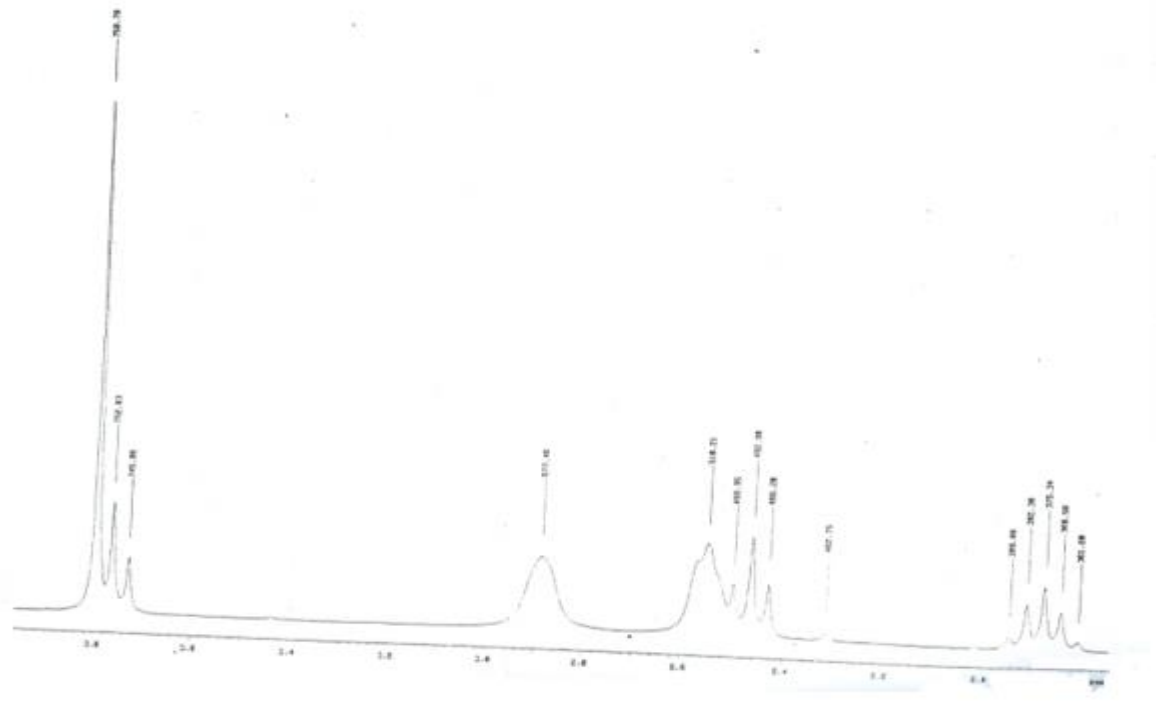


Figure 4. Expanded [1]H-NMR of 1-(2-methoxyphenyl)-(3-(2-phthalamido)-propyl) piperazine.

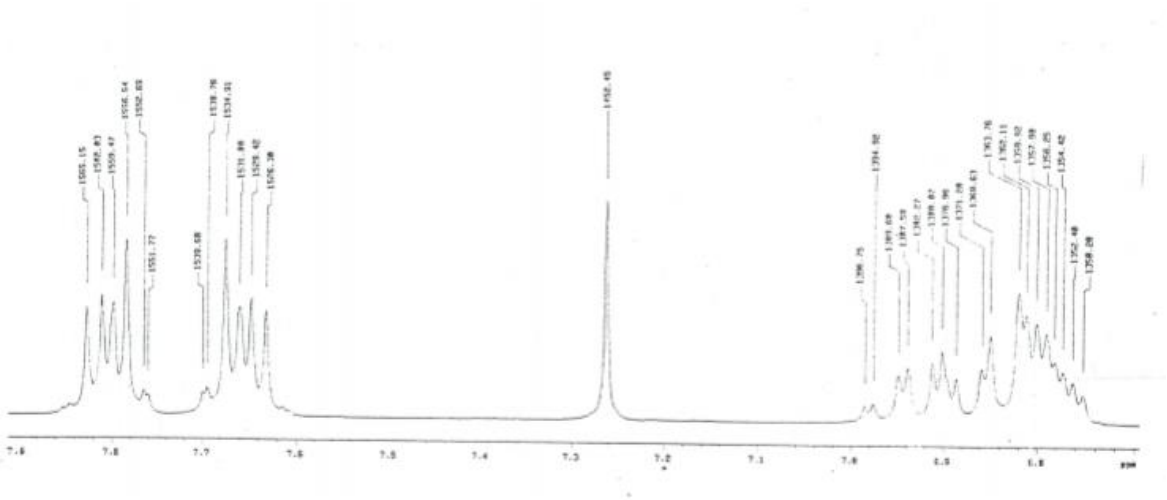


Figure 5. Expanded ^{1}H-NMR of 1-(2-methoxyphenyl)-(3-(2-phthalamido)-propyl) piperazine.

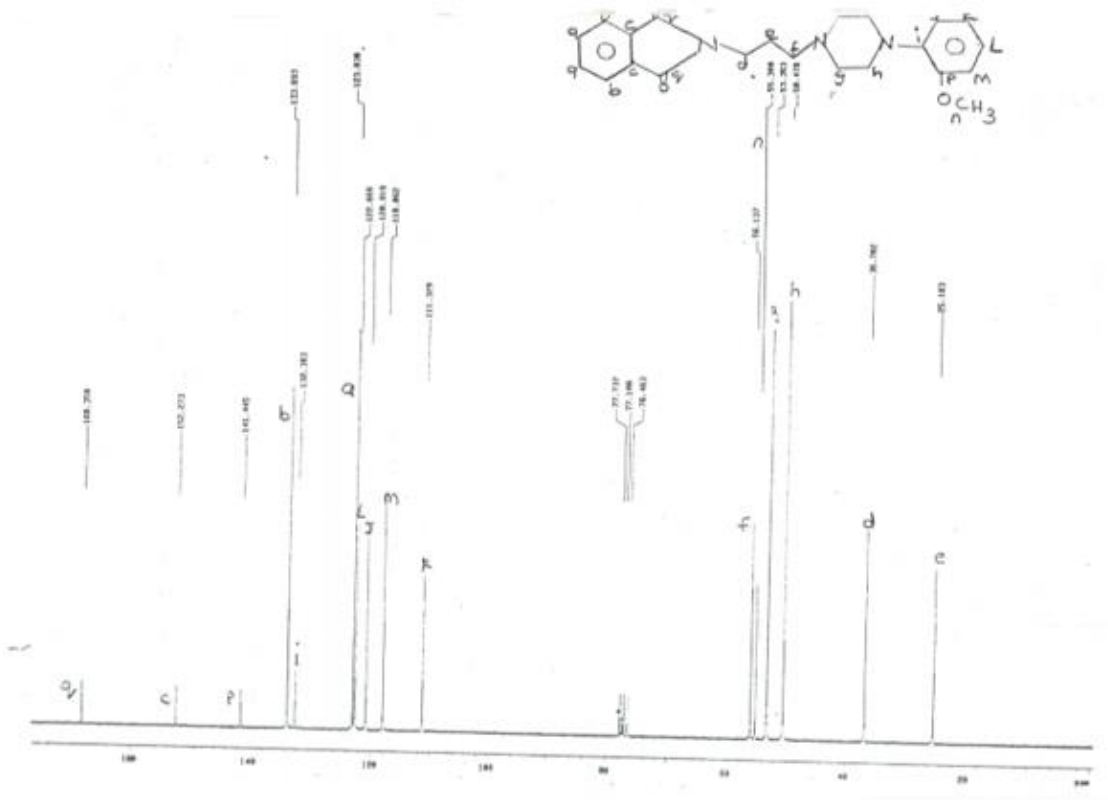


Figure 6. ^{13}C-NMR of 1-(2-methoxyphenyl)-(3-(2-phthalamido)-propyl) piperazine.

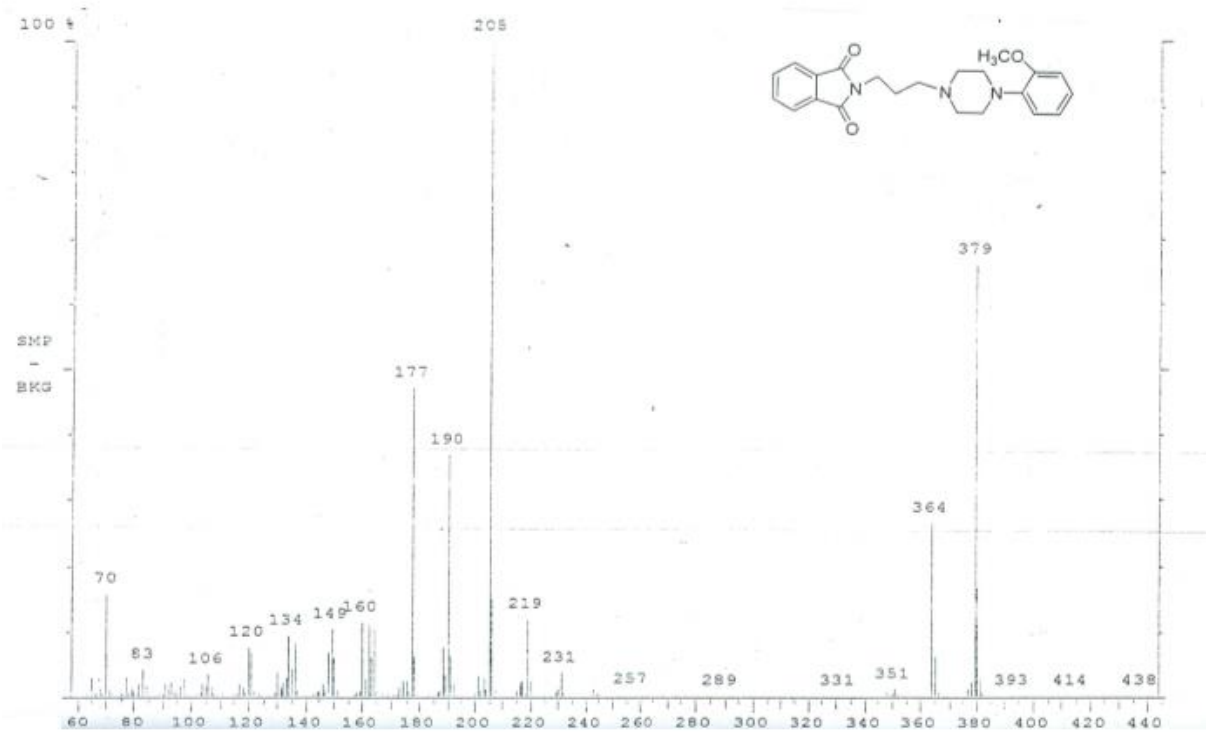


Figure 7. Mass spectrum of 1-(2-methoxyphenyl)-(3-(2-phthalamido)-propyl) piperazine.

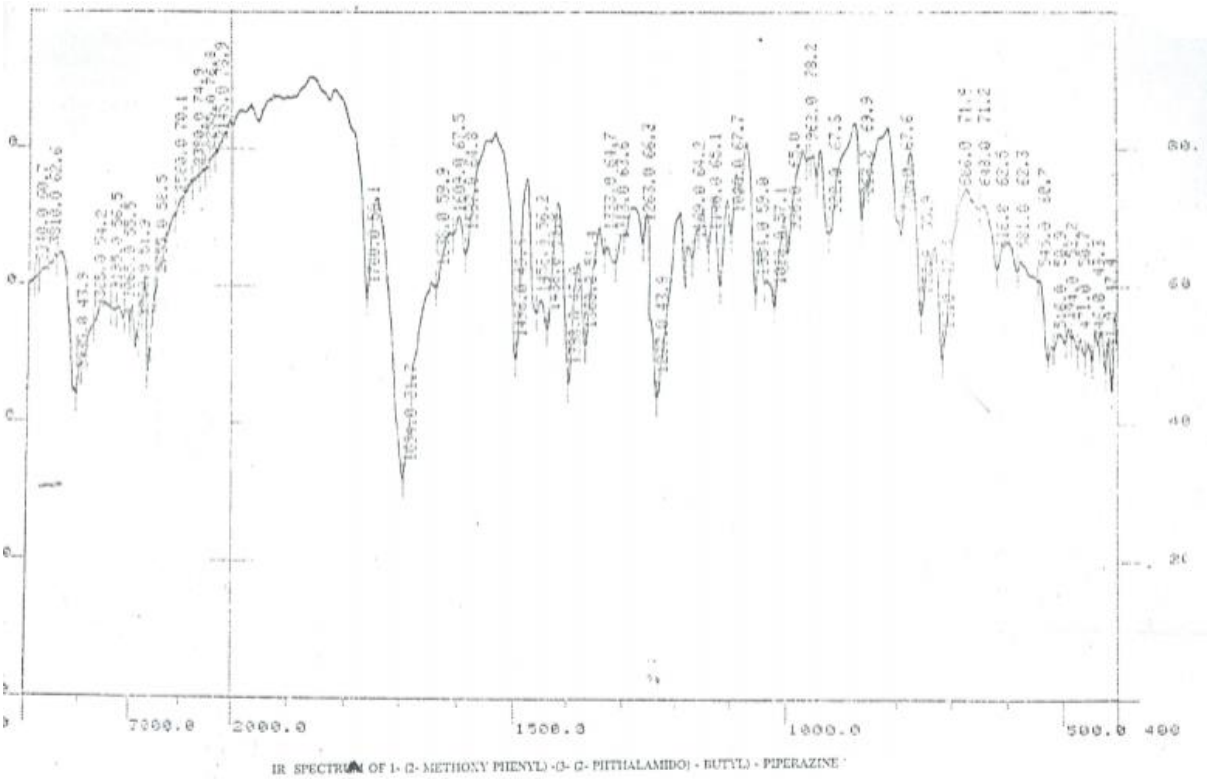


Figure 8. IR spectrum of 1-(2-methoxyphenyl)-(3-(2-phthalamido)-butyl) piperazine.

Figure 9. [1]H-NMR of 1-(2-methoxyphenyl)-(3-(2-phthalamido)-butyl) piperazine.

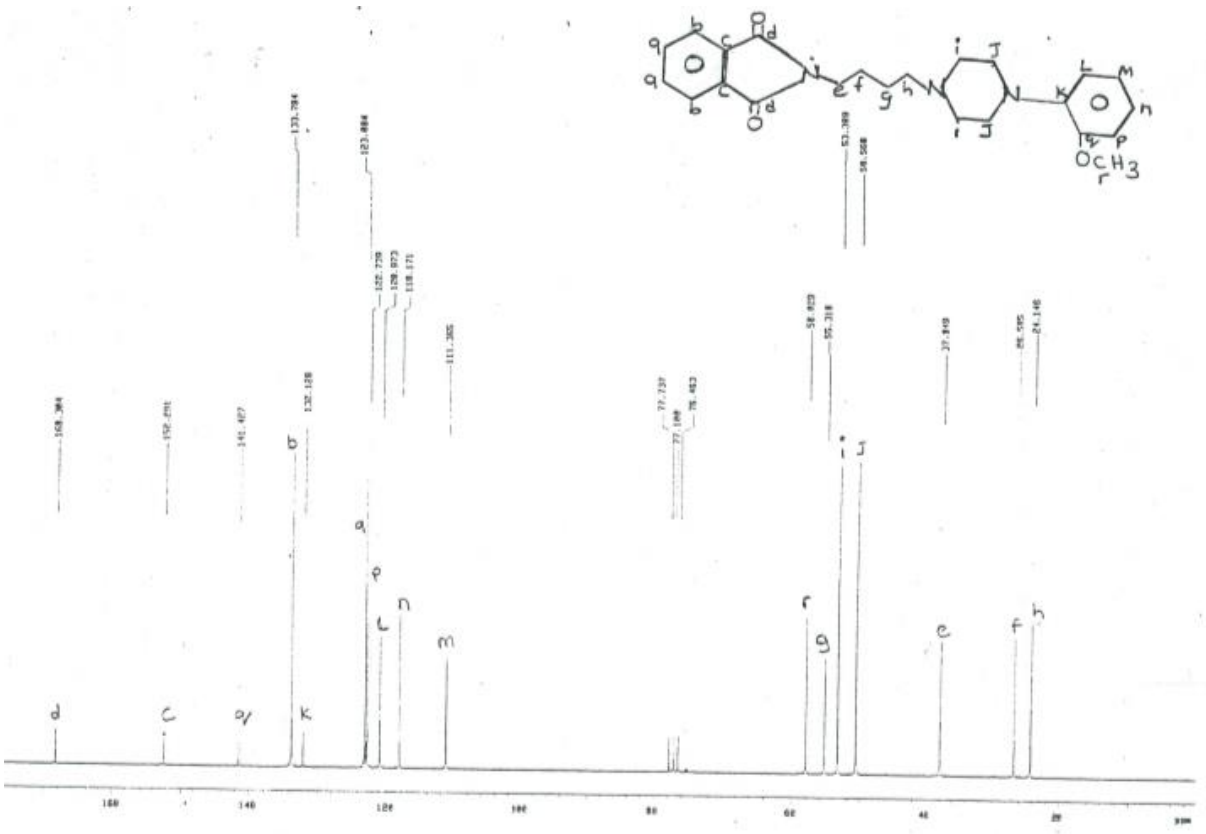


Figure 10. [13]C-NMR of 1-(2-methoxyphenyl)-(3-(2-phthalamido)-butyl) piperazine.

CONCLUSION

This study shows the synthesis of 1-(2-methoxyphenyl)-(3-(2-phthalamido)-propyl) piperazine and 1-(2-methoxyphenyl)-(3-(2-phthalamido)-butyl) piperazine from its starting materials phthalamadoalkyl and arylpiperazine by reflux and condensation in a single step reaction. They were physically characterized by melting point determination and spectroscopically using ^{1}HNMR, ^{13}CNMR, Fourier transform IR and MS. Antibacterial activity was absent for these compounds.

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