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SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL INVESTIGATION OF SOME ISATOIC ANHYDRIDE DERIVED AMIDES

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ABSTRACT

Amide is a class of organic compounds with diverse biomedical utilities. The purpose of this work was to synthesize amide derivatives with possible antibacterial activities. The method involves the reaction of each of the amines (propargylamine, benzylamine and 1-naphthy methylamine) with isatoic anhydride in DMF resulting in opening of isatoic anhydride ring with evolution of carbon dioxide. The compounds were purified using column chromatography and were characterized with the combination of Fourier transform infra-red, nuclear magnetic resonance, mass spectroscopy and elemental analyses. These compounds were then screened for the antibacterial activity using agar wells plate incubated for 24 hours at 37 °C and zone of inhibition were measured and recorded. The antibacterial screening of the synthesised and characterized compounds showed no zone of inhibition suggesting that none of the synthesized compound had antibacterial property against the tested bacteria strains.

KEYWORDS: Antibacterial; Propargylamine; Benzylamine; 1-naphthy methylamine; Isatoic anhydride.

INTRODUCTION

Chemical synthesis is the implementation of reactions with the aim of obtaining products (Vogel *et al*., 1996). This happens by manipulations usually involving a single or multiple steps. Discovery and development of new organic compounds of biomedical importance is a critical component of medicinal chemistry. Organic synthesis thus occupies a central role in any pharmaceutical development endeavor. Amides are compounds with the functional group $RCONH_2$, which is a combination of ketone and amine functional groups. It may also referred to as the conjugate base of ammonia or organic amine (McNaught and Wilkinson, 1997), with diverse biomedical utilities which include antibacterial activity (Priya *et al*., 2005; Narasimhan *et al*., 2004).

Amides can be simply made by coupling a carboxylic acid with amine, which is thermodynamically favorable, though it has reduced reactivity from high activation energy, largely as a result of deprotonation of carboxylic acid by amine, hence such direct reaction more often requires moderate temperatures. This reaction converts the carboxylic acid group to an improve electrophile (White *et al*., 2012). Recently, other methods of preparation have seen an increase in the development amide bond formation using Boron reagent (Sabatini *et al., 2017*; Todorovic and Perrin, 2020*).*

The emergence of bacterial resistance strain is a global phenomenon, with frequent studies being conducted by pharmaceutical companies to develop compounds with modified functional groups that could improve or solve this worldwide problem. The causes of this upsurge in resistance include; few option of antibiotics, long duration treatment regimens and patient non-adherance (Nguyen and Thompson, 2006). Re-established interest in the search of newer antibacterial drugs led to the discovery of new classes (Aminov, 2010), which include fluoroquinolones, oxazolidionone, nitroimidazo-oxazole/oxazine and diarylquinoline at various phases of development (Laughon, 2001). However, new cases of extensively drug resistance antibacteria may appear and as such, there is a vital need for new and more effective antibacterial agents having new modes of action (Mohite and Bhasker, 2012). Thus this study aim the aim to synthesize and characterize amide derivatives from isatoic anhydride and investigate the possible antibacterial activity of the synthesized product.

MATERIALS AND METHODS

Reagents and chemicals used were obtained from reputable manufacturer. Melting points were determined with Kofler electrothermal (England). Infra-red spectra were recorded in KBr discs on Buck IR M500 instrument (Connecticut, USA). Proton and carbon-13 spectra were recorded on varian Gemini 200 (California, USA). Mass spectra were gotten from finnigan MAT 44S (California, USA) and operating at 70 eV. The elemental analysis was on Perkin-Elmer 2400 CHN (Massachusetts USA). Pre-coated thin layer chromatography plates used to monitor the reaction (Darmstadt, Germany). Silica gel

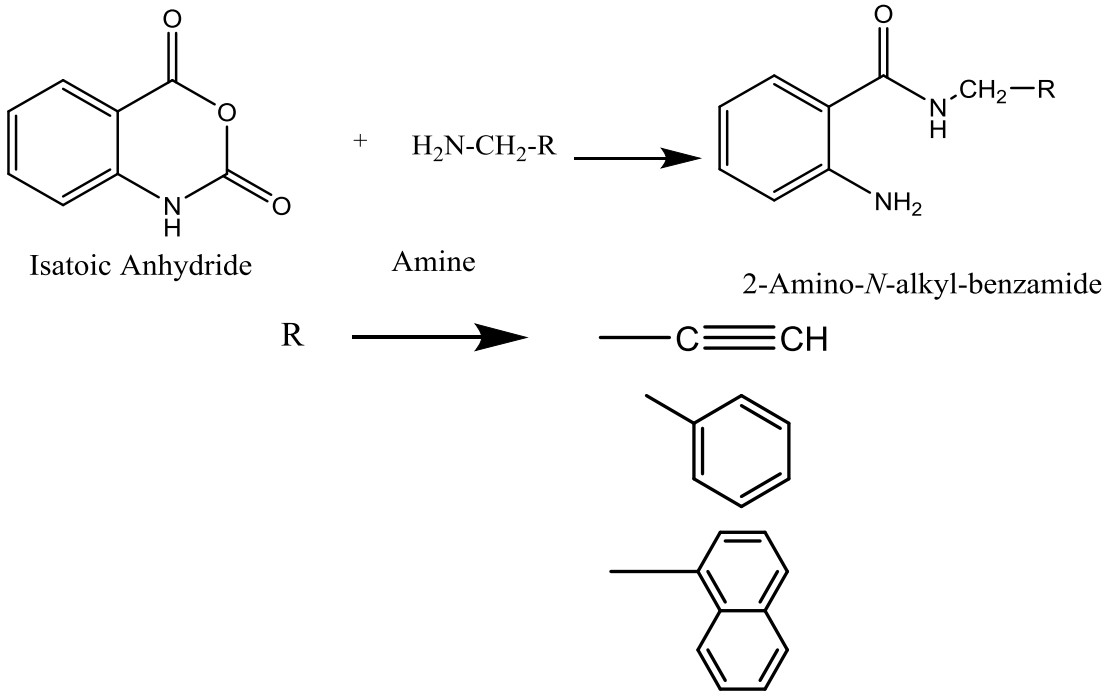
(70-230 mesh) was used to purify the compounds (Sigma Aldrich, Germany).

Synthesis of 2-Amino-N-prop-2-ynyl-benzamide: Isatoic anhydride (1g, 6.1 mmol), placed in round bottom flask was mixed with 5 ml of dried DMF, which was then warmed to 30-40 ^{o}C in an oil bath while propargylamine (0.40ml, 6.1 mmol) was added drop wisely within 15 minutes, this resulted in the evolution of carbon dioxide and it was maintained until the release of the gas ceased. The reaction mixture was poured into a 25 ml of cold water and it pH was adjusted to 9 with 50 % sodium hydroxide solution. The product was filtered, washed and air dried to give a crude product which was purified by silica using n-hexane/ethyl acetate (4:1) to give brownish compound.

Synthesis of 2-amino-N-benzyl-benzamide: Isatoic anhydride (1g, 6.1 mmol) was mixed with 5 ml of dried DMF and maintained at a temperature of 35-40 ^{o}C, benzylamine (0.67 ml, 6.1 mmol) was added drop wisely over a period of 1hr, while the reaction temperature was maintained and evolution of carbon dioxide was observed. The mixture was then poured into 25 ml of cold water following the cessation of carbon dioxide and the pH adjusted to 9 with 50 % sodium hydroxide solution. The product was treated as stated above.

Synthesis of 2-amino-N-naphthalen-1-ylmethyl-benzamide: Isatoic anhydride (1g, 6.1 mmol) was mixed with 5 ml of dried DMF and maintained at a temperature of 45-50 ^{o}C while 1-naphthylmethylamine (0.89 ml, 6.1 mmol) was added drop wisely in a period of 1.5hr. The reaction mixture was treated as stated above.

SCHEME 2



General synthetic scheme of 2-amino-N-alkyl-benzamide

Antibacterial evaluation: The bacteria species used for this study were collected from the Department of Pharmaceutical Microbiology, University of Benin. The bacteria include Gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis)* and Gram negative *(Pseudomonas aeruginosa and Escherichia coli)*. The isolates used were standardised using colony suspension method to attain a concentration of 1.5 x 10^{8} CFU/ml. Antibacterial susceptibility testing was evaluated by agar diffusion plate by sponging Mueller-Hinton agar plates with the already prepared saline suspension for each strain of bacteria and with the aid of cork borer four wells were made in the agar. These holes were covered at the base with the aid of sterilized molten agar. Each synthesized compound were dissolve in dimethyl sulfoxide (DMSO) at 200 µl solutions for each, representing 1000 µg/ml were aseptically dispensed into the labelled wells. The plates were then incubated at for 24 hours at 37 °C. The diameters of zone of inhibition produced by each of the compounds were measured and recorded (CLSI, 2008).

RESULTS

2-Amino-N-prop-2-ynyl-benzamide: 1.42g (89%), melting point: 92-94 ^{o}C, R_f value: 0.29 (n-Hexane: Ethylacetate 4:1), IR (KBr) 3429 (N-H str.), 3287 (NH_2), 3049 (aromatic CH stretch), 2109 (C≡C), 1617 (C=O), 1528 (aromatic C=C bending), ^{1}H NMR ($CDCl_3$, DMSO) 2.13(s, 1H, ≡CH, C_1), 3.99(d, J=3H_Z, 2H, CH_2, C_3), 6.51(s, 2H, NH_2, N_{12}), 6.58(d, J=10H_Z, 1H, Ar-H, C_{10}),

7.05(d,1H, Ar-H C_8), 7.35(d,1H, Ar-H C_9), 7.56(d, 1H, Ar-H, C_7), 8.20(t, J=8H_Z, 1H, NH, N_4), ^{13}C NMR ($CDCl_3$, DMSO) 30.0, 72.1, 81.3, 116.6, 117.7, 118.5, 128.9, 133.2, 148.8, 169.9, MS 174 (M^+, 7%), 145 (10), 120 (100), 92 (44). Elemental analysis; Molecular formula: $C_{10}H_{10}N_2O$ (174.203), Cal.: C. 68.95 H. 5.79 N. 16.08, Found: C. 68.87 H. 5.86 N. 16.21.

2-Amino-N-benzyl-benzamide: 1.94g (70%), melting point: 122-124 ^oC, Rf value: 0.37 (n-Hexane: Ethylacetate 4:1), IR (KBr) 3473 (N-H str.), 3302, 3358 (NH_2), 3030 (aromatic CH stretch), 1577 (C=0), 1536 (aromatic C=C bending), ^{1}H NMR (DMSO) 4.45(d, J=6H_Z, 2H, CH_2, C_7), 6.43(s, 2H, NH_2, N_{16}), 6.55(s, 1H, Ar-H, C_{12}), 6.72(d, J=1H_Z, 1H, Ar-H, C_{14}), 7.13(d,1H, Ar-H C_{13}), 7.24(d,2H, Ar-H $C_{1,5}$) 7.25(d, 1H, Ar-H, C_3), 7.31(d,2H, Ar-H $C_{2,4}$) 7.56(d,1H, Ar-H C_{11}) 8.78(t, J=6H_Z, 1H, NH), ^{13}C NMR (DMSO) 42.6, 114.9, 115.0, 115.1, 116.9, 127.1, 127.6, 128.5, 132.2, 140.4, 150.3, 169.3, MS 226 (M^+, 100%), 120 (90), 92 (10). Elemental analysis; Molecular formula: $C_{14}H_{14}N_2O$ (226.279), Calculated: C. 74.31 H. 6.24 N. 12.38; Found: C. 74.44 H. 6.32 N. 12.40.

2-Amino-N-naphthalen-1-ylmethyl-benzamide: 0.79g (95%), melting point: 182-184 ^oC, R_f value: 0.53 (n-Hexane: Ethylacetate 4:1), IR (KBr) 3473 (N-H str.), 3306, 3358 (NH_2), 3052 (aromatic CH stretch), 1628 (C=0), 1528 (aromatic C=C bending), ^{1}H NMR (DMSO) 4.92(d, J=6H_Z, 2H, CH_2, C_{11}), 6.50(s 2H, NH_2, N_{19}), 6.52(s, 1H, Ar-H, C_{16}), 6.54(d, J=1H_Z, 1H, Ar-H, C_{18}), 6.73(d,1H, Ar-H C_1), 7.15(d, 2H, Ar-H C_{17}) 7.48(d, 1H, Ar-H, C_7), 7.57(d,2H, Ar-H C_{15}) 7.59(d,1H, Ar-H C_2), 7.85(d,1H, Ar-H C_8), 7.95(d,1H, Ar-H C_5) 8.83(t, J=6H_Z, 1H, NH, N_{12}), ^{13}C NMR (DMSO) 40.7, 114.9, 115.0, 116.9, 123.9, 125.6, 125.9, 126.2, 126.6, 127.8, 128.6, 131.4, 132.2, 133.8, 135.4, 150.3, 169.3**.** MS 276 (M^+, 48%), 205(30), 149(95). Elemental analysis; molecular formula: $C_{18}H_{16}N_2O$ (276.339), Cal.: C. 78.24 H. 5.84 N. 10.14, Found: C. 78.12 H. 5.79 N. 10.06.

Tab 1. Bacteria susceptibility to synthesized compounds.

Compounds	***S. aureus***	***B. subtilis***	***E. coli***	***P. aeruginosa***
2-Amino-N-prop-2-ynyl-benzamide	-	-	-	-
2-Amino-N-benzyl-benzamide	-	-	-	-
2-Amino-N-naphthalen-1-ylmethyl-benzamide	-	-	-	-
Ciprofloxacin 5µg	20	20	24	16
DMSO	-	-	-	-

- = No inhibition

DISCUSSION

2-Amino-N-prop-2-ynyl-benzamide was synthesized in high yield (89%) by the reaction of isatoic anhydride with propargylamine as shown above. The IR spectrum showed amide and amine NH stretches at 3,429 and3,287 cm^{-1} respectively, and C=O vibrations at 1617 cm^{-1}. The ^{1}H NMR showed the amide NH proton as a triplet down field at 8.20ppm and amine NH proton as a singlet at 6.51ppm, the CH_2 proton alpha to NH appeared as a doublet at 3.99ppm, the alkynyl CH appeared as a singlet at 2.13ppm and the aromatic H appeared as a doublet at ppm within 6.58ppm and 7.56ppm. The ^{13}C NMR spectrum revealed the diagnostic carbonyl peaks at 169.9ppm, the beta alkynyl carbon at 81.3ppm and alpha alkynyl carbon at 72.1ppm and the aromatic carbon at ppm within 116.6ppm and 148.8ppm. The mass spectrometry (MS) of the compound gave molecular ion peak (M^+) at 175 (m/z), corresponding to with the expected weight.

2-Amino-N-benzyl-benzamide was obtained in good yield (70%) by the reaction of isatoic anhydride with benzylamine as shown above. The IR spectrum showed amide and amine NH stretches at 3,473 and (3,302, 3,358) cm^{-1} respectively, and C=O vibrations at 1577 cm^{-1}. The ^{1}H NMR showed the amide NH proton as a triplet down field at 8.78ppm and amine NH proton as a singlet at 6.43ppm, the CH_2 proton alpha to NH appeared as a doublet at 4.45ppm and the aromatic H appeared as a doublet at ppm within 6.55ppm and 7.56ppm. The ^{13}C NMR spectrum revealed the diagnostic carbonyl peaks at 169.3ppm, the methylene carbon alpha to NH at 42.6ppm and the aromatic carbon at ppm within 114.9ppm and 150.3ppm. The mass spectrometry (MS) of the compound revealed M^+ at 227 (m/z) which corresponded to the predictable molecular weight.

2-Amino-N-naphthalen-1-ylmethyl-benzamide was produced in good quantity (95%) by the reaction of isatoic anhydride with 1-naphthyl methylamine as shown above. he IR spectrum showed amide and amine NH stretches at 3,473 and (3,306, 3,358) cm^{-1} respectively, and C=O vibrations at 1628 cm^{-1}. The ^{1}H NMR showed the amide NH proton as a triplet down field at 8.83ppm and amine NH proton as a singlet at 6.50ppm, the CH_2 proton alpha to NH appeared as a doublet at 4.92ppm and the aromatic H appeared as a doublet at ppm within 6.52ppm and 7.95ppm. The ^{13}C NMR spectrum revealed the diagnostic carbonyl peaks at 169.3ppm, the methylene carbon alpha to NH at 40.7ppm and the aromatic carbon at ppm within 114.9ppm and 150.3ppm. The mass spectrometry (MS) of the compound showed M^+ at 277 (m/z) and corresponded to the estimated molecular mass.

The antibacterial susceptibility testing of the synthesized compounds revealed that the tested strains of microorganism were not susceptible to the synthesized compounds (Table 1). The reason for this inactivity cannot be proffered immediately because it is well

known that many amides do exhibit anti-bacterial activity. The presence of free carbonyl functional group is necessary for antimicrobial activity (lance *et al*., 2001) probably the introduction of aromatic group in some of the compounds which increases the bulkiness of the compounds may have made it difficult for the compounds to cross the bacteria cell wall (Baba *et al*., 2013). These corroborate the findings in this study.

CONCLUSION

The synthesis of new compounds with different mechanism of action from already established compounds are very important in drug discovery and development as well as in the understanding of structure activity relationships. In this work, three compounds were synthesized; 2-Amino-N-prop-2-ynyl-benzamide, 2-Amino-N-benzyl-benzamide and 2-Amino-N-naphthalen-1-ylmethyl-benzamide. The compounds synthesized were in appreciable yield (68-95%). The spectroscopic analysis unequivocally established the structures of the compounds. The preliminary antibacterial screening showed that none of the compounds possessed antibacterial activities.

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