ejpmr, 2021,8(5), 120-123

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

SYNTHESIS AND ANTIBACTERIAL INVESTIGATION OF SOME AMIDES

***Unuigbe Osaherunmwen Isonah Pharm D and Emmanuel Eimiomodebheki Odion PhD**

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City.

***Corresponding Author: Unuigbe Osaherunmwen Isonah**

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City.

Article Received on 05/03/2021 **Article Revised on 25/03/2021** **Article Accepted on 15/04/2021**

ABSTRACT

The purpose of this work was to synthesize amide derivatives with possible antibacterial activities. The method involves the reaction of amines with pamitoyl chloride in the presence of trimethylamine. The products were purified using chromatographic techniques and were characterized using Fourier Transform Infra-Red Spectrophotometry (FT-IR), Nuclear Magnetic Resonance (^{1}HNMR, ^{13}CNMR), Mass Spectroscopy (MS) and Elemental Analyses. Agar diffusion technique was used to screen for the antibacterial activity, by dabbing the Mueller–Hinton agar plates with bacteria suspension and subsequently applying of 1000 µg/ml of the synthesized compounds. It was observed that as the bulkiness of the aromatic group increases, the melting point also increases. The antibacterial screening results show no zone of inhibition suggesting that none of the synthesized compound has antibacterial property against the tested strain.

KEYWORDS: Propargylamine, Benzylamine, 1-Naphthyl methylamine, Pamitoyl chloride, Triethylamine.

INTRODUCTION

Increased resistance of bacteria against available antibiotics and the emergence and re-emergence of both new and old diseases have cause synthetic chemists to continue to research into newer compounds with either new mechanism of action or improve efficacy (Tanvir *et al*., 2018). This search have lead us to look at existing compounds with antibacterial property, such as fatty acid and modifying them into fatty acid amide that are active against pathogenic bacteria (Gopalakrishnan *et al*. 2015; Russel, 1991) and they are formed through amidation of long chain fatty acids (Dembitsky *et al*. 2000). The reaction between fatty acid and amine can take place at normal pressure, in the presence of oxygen without an organic solvent, though in the presence of catalyst (Bilyk *et al*., 1992). This reaction can be hastened by using excess amine and increasing the temperature slightly above 60 ^{o}C with a purposeful synthesis of pure amide (Betancourt-Jimenez *et al*., 2020). Its formation has been observed to largely enhanced *in-vitro* and *in-vivo* efficiency of these starting materials but the level of its improvement is subject to the ionic and lipophilic property of the ensuing products (Malabarba *et al*., 1992; Asegbeloyin *et al*., 2018). Hence this study aims to synthesize amides from the reaction between amines and pamitoyl chloride and screen it against selected bacteria.

MATERIALS AND METHODS

The purchased reagents were used without extra purification. 3-Amino-1-propyne, benzylamine, naphthylamine, triethylamine (TEA), and palmitoylchloride were obtained from Sigma Aldrich (Germany). Dichloromethane, dimethyl formamide, ethylacetate, methanol, acetone, n-hexane, were purchased from Scharlau laboratries, Spain. Melting points were determined with Kofler electrothermal (CAT Number 1A 6304 England) and were not corrected. IR spectra were recorded from the Buck IR M500 instrument (Buck Scientific Inc. Norwalk, Connecticut, USA). ^{1}H and ^{13}C Nuclear Magnetic Resonance (^{1}H NMR and ^{13}CNMR) spectra were acquired to study the chemical composition of the fatty acid amides by utilising deuterated chloroform and analyzed using Varian Gemini 200 spectrometer. Analysis was conducted at 250 MHz and 63 MHz for ^{1}H and ^{13}C respectively. Mass spectrometer (MS) peaks were obtained to determine the molecular weight the synthesized compounds by scanning at full mode (512) using a Finnigan MAT 44S. Elemental analysis was evaluated to analyze the carbon, hydrogen and oxygen content in each compound using Perkin-Elmer 2400. Pre-coated thin layer chromatography plates was used to monitor the reactions (Merck, Germany). Silica gel (70-230 mesh) was utilized for purification of the compounds (Sigma Aldrich, Germany).

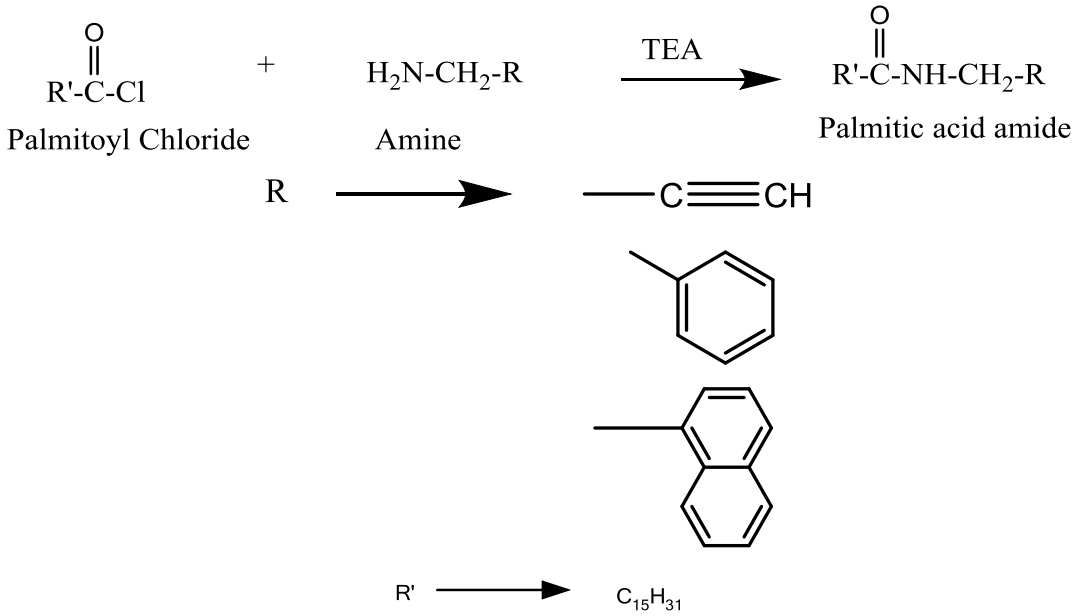
Preparation of Palmitic acid prop-2-ynylamide. To a stirring mixture of propargylamine (0.48ml, 7.28 mmol.) in dichloromethane (20 ml) was added 1mL triethylamime (TEA) in a round bottom flask in an ice-cold chamber (0-5ºC), 2.2 ml of palmitoylchloride was added drop wisely. The ice was removed and the resulting whitish syrupic mixture was stirred for 4 h at room temperature, then diluted with 20 ml of

dichloromethane and washed with 10 ml of 1N HCl and water respectively. Organic extract was dried over anhydrous sodium sulphate and the resultant pale brownish crude product after evaporation in *vacuo* was purified with silica using n-hexane-ethyl acetate (4:1) solvents and then a whitish crystalline compound which was obtained from recrystallization by methanol-water (1:1).

Preparation of Palmitic acid benzylamide. To a stirring mixture of benzylamine (0.80 ml, 7.28 mmol.) in dichloromethane (20 ml) was added 1mL triethylamime (TEA) in a round bottom flask in an ice-cold chamber (0-5ºC), 2.2 ml Palmitoylchloride was added drop wisely. The ice was removed and the resulting whitish syrupic mixture was stirred for 4 h at room temperature and treated as in the synthetic procedure in section (i).

iii. Preparation of Palmitic acid (naphthalen-1-ylmethyl)-amide. To a stirring mixture of 1-naphthyl methylamine (1.06ml, 7.28 mmol.) in dichloromethane (20 ml) was added 1 ml triethylamime (TEA) in a round bottom flask in an ice-cold chamber (0-5ºC), 2.2 ml Palmitoylchloride was added dropwisely and finally washed down with another 10 ml dichloromethane. The ice was removed and the resulting whitish syrupy mixture was stirred for 4 h at room temperature and treated as in the section (i).

SCHEME 1



Antibacterial activity

The bacteria species were obtained from the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Benin, Nigeria and included *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa and Escherichia coli.* The isolates were standardised to give concentration of 1.5 x 108 CFU/ml. Susceptibility testing was evaluated by using modified Kirby-Bauer diffusion method through swabbing the Mueller–Hinton agar plates with suspension of each bacteria strain. A cork borer was used to make four wells, which was sealed at the bottom with the aid of 2 ml of molten agar, 200 µl solutions of the synthesized compounds representing 1000 µg/ml were then aseptically dispensed into the labelled wells and the plates were incubated at 37 °C for 18 hours uprightly. The zone of inhibition made by each of the compounds were measured and recorded (CLSI, 2008).

RESULTS

Palmitic acid prop-2-ynylamide: 1.63g (76%), melting point: 98-100 ^{o}C. IR (KBr) 3291 (N-H str.), 2847, 2918 (C-H str.[saturated], 3071 (≡C-H), 1640 (C=O). ^{1}H NMR (DMSO, $CDCl_3$): 0.70(t, J=6H_Z, 3H, CH_3, C_{20}), 1.01(brs 24H, $(CH_2)_{12}$, C_{8-19}), 1.41(t, J=7H_Z, 2H, CH_2, C_7), 2.07(t, J=2H_Z, 2H, CH_2, C_6), 2.41(s, 1H, ≡CH, C_1), 3.82(d, 2H, CH_2, C_3), 7.27(t, 1H, NH, N_4). ^{13}C NMR (DMSO, $CDCl_3$): 23.4, 26.1, 26.4, 29.5, 30.0, 30.2, 32.6, 36.9, 64.4, 71.8, 81.1, 174.0 (C=O). MS: 293 (M^+, 1%), 250 (10), 152 (10), 110 (45), 97 (100). Elemental analysis; $C_{19}H_{35}NO$ (293.495), Cal.: C. 77.76 H. 12.02 N. 4.77, Found: C. 77.66 H. 12.00 N. 4.57.

Palmitic acid benzylamide: 1.92g (69%), melting point 92-94 ^{o}C. R_f value 0.43 (n-Hexane: Ethylacetate 4:1); IR (KBr) 3306 (N-H str.), 3034 (aromatic C=C), 2847, 2918 (C-H str.[saturated], 1636 (C=O); ^{1}H NMR ($CDCl_3$) 0.91(t, J=5H_Z, 3H, CH_3, C_1), 1.28 (brs 24H, $(CH_2)_{12}$, C_{2-13}), 1.65(t, J=8H_Z, 2H, CH_2, C_{14}), 2.27(t, J=8H_Z, 2H,CH_2,

C_{15}), 4.47(d, J=5H_Z, 2H, CH_2, C_{18}), 6.22(t, 1H, NH, N_{17}), 7.33(brs, 5H, (Ar-H) $C_{1'-5'}$). ^{13}C NMR ($CDCl_3$) 15.0, 23.6, 26.8, 30.5, 32.8, 37.5, 44.6, 128.5, 129.6, 139.1,174.2(C=O). MS 345 (M^+, 0.03%), 302(1), 176(3), 162(19), 149(100), 91(51). Elemental analysis: $C_{23}H_{39}NO$ (345.571), Cal.: C. 79.94 H. 11.38 N. 4.05 Found: C. 79.91 H. 11.39 N. 4.04.

Palmitic acid (naphthalen-1-ylmethyl)-amide: 2.11 g (73 %); melting point: 100 - 102 ^oC. R_f value: 0.46 (n-Hexane: Ethylacetate 4:1)**;** IR (KBr) 3302 (N-H str.), 3056 (aromatic C=C), 2970 (C-H str.[saturated], 1625 (C=O). ^1H NMR ($CDCl_3$, DMSO) 0.80 (t, J=6H_Z, 3H, CH_3, C_1), 1.17 (brs 26H,$(CH_2)_{13}$, C_{2-14}), 2.13 (t, J=8H_Z 2H, CH_2, C_{15}), 4.75 (d, J=5H_Z, 2H, CH_2, C_{18}), 7.36 (brs 5H, (Ar-H) $C_{2'-6'}$), 7.69 (brs 2H, (Ar-H) $C_{1',7'}$) 7.79 (t, 1H, NH, N_{17}), ^{13}C NMR ($CDCl_3$, DMSO) 14.9, 23.4, 26.6, 30.2, 32.6, 37.0, 42.1, 124.5, 126.1, 126.6, 127.1, 127.2, 128.9, 129.3, 132.2, 134.4, 135.0, 174.1 (C=O). MS 395 (M^+, 6%), 256 (54), 213 (25), 185 (17), 129 (30). Elemental analysis; $C_{27}H_{41}NO$ (395.631), Cal.: C. 81.97 H. 10.45 N. 3.54, Found: C. 81.95 H. 10.43 N. 3.55.

Antibacterial screening results

Table 1: Antibacterial activity of the synthesized compounds.

Compounds	***S. aureus***	***B. subtilis***	***E. coli***	***P. aeruginosa***
Palmitic acid prop-2-ynylamide	-	-	-	-
Palmitic acid benzylamide	-	-	-	-
Palmitic acid (naphthalen-1-ylmethyl)-amide	-	-	-	-
Ciprofloxacin 5µg	20	20	24	16
Dimethyl sulphoxide	-	-	-	-

- = No inhibition.

DISCUSSION

Many compounds containing amide functionality have potential biological properties and considerable efforts have been undertaken to exploit synthetic routes to these compounds. The choice of amines for this project was premeditated to explore possible antibacterial activity. All amines have a terminal -CH_2NH_2 with propargylamine having a straight carbon chain, benzylamine having a benzene ring and 1-naphthyl methylamine having a naphthalene ring.

2-Amino-N-naphthalen-1-ylmethyl-benzamide gave the highest yield of 95% while 2-palmitoylamino-N-prop-2-ynyl-benzamide gave the lowest yield of 21%.

The reaction of palmitoyl chloride with propargylamine gave palmitic acid prop-2-ynylamide in good yield (76%). The NH and =CH stretch vibrations in the IR, were situated at 3291, 3071 cm^{-1} respectively. The ^1H NMR showed the NH proton as a triplet down field at 7.27 ppm, the CH_2 proton alpha to NH appeared as a doublet at 3.82ppm, the alkynyl CH appeared as a singlet at 2.41 ppm, methylene protons beta to the carbonyl group appeared as a triplet at 2.07 ppm, these protons are more deshielded than the protons on the terminal methyl group which appeared as a triplet at 0.70 ppm because the carbon atom bearing these protons is directly bonded to the carbonyl group, while the methylene proton beta to it appeared as triplet at 1.41 ppm. The remaining methylene groups appeared as a triplet at 1.01 ppm. The ^{13}C NMR spectrum revealed the diagnostic carbonyl peaks at 174.0 ppm, the beta alkynyl carbon at 81.1 ppm and alpha alkynyl carbon at 71.8 ppm. The mass spectrometry (MS) of the compound showed the molecular ion peak (M^+) at 294 (m/z) which corresponded to with the molecular weight.

The reaction of palmitoyl chloride with benzylamine gave palmitic acid benzylamide in good yield (68%). The NH stretch and C=O vibrations in the IR, were situated at 3306, 1636 cm^{-1} respectively. The ^1H NMR showed the NH proton as a triplet down field at 6.22 ppm, the CH_2 proton alpha to NH appeared as a doublet at 4.47 ppm, the aromatic H appeared as a doublet at 7.33 ppm, methylene protons beta to the carbonyl group appeared as a triplet at 2.27 ppm. These protons are more deshielded than the protons on the terminal methyl group which appeared as a triplet at 0.91ppm because the carbon atom bearing these protons is directly bonded to the carbonyl group, while the methylene proton beta to it appeared as triplet at 1.65 ppm. The remaining methylene groups appeared as a triplet at 1.28 ppm. The ^{13}C NMR spectrum revealed the diagnostic carbonyl peaks at 174.2 ppm, and the aromatic carbon at ppm between 128.5ppm and 139.1ppm. The mass spectrometry (MS) of the compound showed the molecular ion peak (M^+) at 346 (m/z) which corresponded to with the estimated molecular weight.

The reaction of palmitoyl chloride with 1-naphthyl methyl amine gave palmitic acid (naphthalen-1-ylmethyl)-amide in good yield (73%). The NH stretch and C=O vibrations in the IR, were situated at 3302, 1625 cm^{-1} respectively. The ^1H NMR showed the NH proton as a triplet down field at 7.79ppm, the CH_2 proton alpha to NH appeared as a doublet at 4.75ppm, the aromatic H appeared as a doublet at ppm within 7.36ppm and 7.69ppm, methylene protons beta to the carbonyl group appeared as a triplet at 2.13ppm, these protons are more deshielded than the protons on the terminal methyl group which appeared as a triplet at 0.80ppm because the carbon atom bearing these protons is directly bonded to the carbonyl group. The remaining methylene groups appeared as a triplet at 1.17ppm. The ^{13}C NMR spectrum

revealed the diagnostic carbonyl peaks at 174.1ppm, and the aromatic carbon at ppm within 124.5ppm and 135.0ppm. The mass spectrometry (MS) of the compound revealed the molecular ion peak (M^+) at 396 (m/z) which corresponded to molecular weight.

These compounds in this study have melting points around 100 ± 8 ^oC, this could be linked to the presence of the long carbon chain of palmitoyl moiety due to low or absence of hydrogen bonding in the molecules synthesized, the bonds in these compounds are mainly van de Waals force which are weak bonds by nature (Asegbeloyin *et al*., 2018). The compounds are all soluble in chloroform while Palmitic acid prop-2-ynylamide and Palmitic acid (naphthalen-1-ylmethyl)-amide are soluble in DMSO. The data from the mass spectra are in agreement with the expected molecular weight of the compounds and the elemental analysis were satisfactory. The *in-vitro* antibacterial screening of the synthesised compounds was carried out by agar well diffusion method with selected bacteria from both Gram negative (*E. coli* and *P. aeruginosa*) and Gram positive (*S. aureus and B. subtilis*) classes. The solvent used was DMSO which have no activity against these bacteria. It was found that the compounds were inactive against the tested strains of bacteria (Table 1). The reason for this inactivity cannot be immediately proffered because it is a common knowledge that amides do exhibit reasonable anti-microbial activity. It has been reported that the presence of free carbonyl functional group is necessary for antimicrobial activity (lance *et al*., 2001). Additionally, the presence of long chain (hexadecane) and introduction of aromatic group in palmitic acid benzylamide and palmitic acid (naphthalen-1-ylmethyl)-amide was expected to increase the lipophilicity of the resultant molecule which was expected to enhance the solubility and permeability of the compounds (Liu *et al*., 2013).

CONCLUSION

In this study, palmitic acid prop-2-ynylamide, palmitic acid benzylamide and palmitic acid (naphthalen-1-ylmethyl)-amide were synthesize at 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.

REFERENCES

1. Liu, H., Yang, W., Zhou, W., Xu, Y., Xie, J. and Li, M. (2013). Crystal structures and antimicrobial activities of copper (II) complexes of fluorine thioureido ligands. Inorganic Chimica Acta, 405: 387-397.
2. Asegbeloyin, J.N., Oyeka, E.E., Babahan I and Okpareke O. (2018). Novel synthesis of metal complexes of palmitoyl thioureas and their antimicrobial activities. J. Chem Soc. Nigeria, 43(3): 550 -560.
3. Jagessar, R.C. and Rampersaud, D. (2008). Amides as antimicrobial agents. New York Science Journal, 1, 1, 1, 22-55.
4. Lance R. Peterson (2001). Quinolone molecular structure-activity relationships" clinical infectious diseases, 33, 3, 15, S180-S186.
5. Narasimhan, B., Belsare, D., Dhake, A. (2004). Eur.J.Med. Chem., 39(10): 827-34
6. Malabarba, A., Ciabatti, R., Kettenring, J., Scotti, R., Candiani, G., Pallanza, R., Goldstein, B. P. (1992). Synthesis and antibacterial activity of a series of basic amides of teicoplanin and deglucoteicoplanin with polyamines. Journal of Medicinal Chemistry, 35(22): 4054–4060. doi:10.1021/jm00100a010.
7. Nguyen, H.T., Ho, D.V., Vo, H.Q., Le, A.T., Nguyen, H.M., Kodama, T., Ito, T., Morita, H., Raal, A. (2017). Antibacterial activities of chemical constituents from the aerial parts of *Hedyotis pilulifera.* Pharmaceutical Biology, 55, 1. 787-799.
8. Tanvir, R., Javeed, A., Rehman, Y. (2018). Fatty acids and their amide derivatives from endophytes: new therapeutic possibilities from a hidden source, *FEMS Microbiology Letters*, 365: 12.
9. Gopalakrishnan S, Srinivas V, Alekhya G. (2015). Evaluation of broad-spectrum *Streptomyces* sp. for plant growth promotion traits in Chickpea (*Cicer arietinum* L.). Philipp Agric Sci., 98: 270–8.
10. Dembitsky V. M, Shkrob I, Rozentsvet O A. (2000). Fatty acid amides from freshwater green alga Rhizoclonium hieroglyphicum. Phytochemistry, 54: 965–7.
11. Russel, A.D. (1991). Mechanisms of bacterial resistance to non-antibiotics: food additives and food pharmaceutical preservatives. J. Appl. Bacteriol., 71: 191-201.
12. Bilyk, A., Bistline, R.G. Jr., Piazza, G.J., Feairheller, S.H., Haas, M.J. (1992). A novel technique for the preparation of secondary fatty amides. J. Am. Oil Chem. Soc., 69: 488−491.
13. Betancourt-Jimenez, D., Youngblood, J. P., Martinez, C. J. (2020). Synthesis and Characterization of Fatty Acid Amides from Commercial Vegetable oils and Primary Alkyl Amines for Phase Change Material (PCM) Applications. ACS Sustainable Chemistry & Engineering. doi:10.1021/acssuschemeng.0c03626.