Syntheses, Characterisation and In-Vitro Antibacterial Studies of Aminoalkylnaphthol Derivatives against Staphylococcus Aureus & Pseudomonas Aeruginosa

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Abstract

The emergence and rise in antimicrobial resistance of *Staphylococcus aureus* and *Pseudomonas aeruginosa* has caught the attention of the medical world and they remain the leading cause of most bacterial infection worldwide. There is an urgent need to design and develop new antibacterial agents to overcome this resistance issue. Recently, the synthesis of aminoalkylnaphthol as an antibacterial agent has intensified due to their attractive biological and pharmacological properties. The present study aims to synthesise, characterise and evaluate *in-vitro* antibacterial studies of aminoalkylnaphthol derivatives. The novel molecules were synthesised using one-pot three component (aldehyde, naphthol, amine) condensation reaction catalysed by $MgSO_4$ under solvent-free condition. The reaction progress was monitored with thin layer chromatography. Four derivatives were successfully synthesised with moderate yield ranging 55% to 80%. These compounds were characterised by determining the melting point and proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy. The results show that all the synthesised compounds have melting point values which are comparable to the theoretical melting point range. The ^{1}H-NMR results showed singlet peaks which signifies -OH (δ 10.00 and 10.75 ppm) and central carbon -CH (δ 4.5 – 6.5 ppm). The doublet and triplet peaks (δ 6.20 – 7.35 ppm) showed presence of aromatic hydrogen. Also, the peaks in the range of δ 0.5 – 5 ppm signified the most shielded protons. The *in-vitro* antibacterial study was conducted by disk diffusion test to demonstrate the antibacterial activity possessed by the synthesised compounds. Compound A1 had the highest inhibition zone of 6mm for 100 µg of amount impregnated towards *S. aureus.* However, compound A3 had a zone of inhibition of 8mm for 100 µg towards *P. aeruginosa*. This study proved that the synthesised compounds have potential antibacterial activity towards *S. aureus* and *P. aeruginosa*.

Keywords: aminoalkylnaphthol, *Staphylococcus Aureus*, *Pseudomonas Aeruginosa*, One-pot Three Component Condensation Reaction, ^{1}H-NMR, Antibacterial Study.

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INTRODUCTION

The synthesis of potential new antimicrobial agent has always been a captivating goal and in particular the novel aminoalkylnaphthols. The resistance caused by *S. aureus* and *P. aeruginosa* towards wide range of antibiotics is the main reason and this has become a serious threat [1]. There is also newer method discovered to synthesise aminoalkylnaphthol and this brings great prospect for us to synthesise them in a small scale with ease [2]. As a consequence of the development of resistance recently, it is essential to develop an antibacterial agent by focusing on the target enzymes of the organisms that demonstrates a quick and broad bactericidal impact and perhaps less prone to resistance owing to the difficulty in redesigning their targets in a way that is compatible with bacterial survival [3]. The discovery and design of chemical compounds and their analogues with a specific pharmacological activity are a challenging task. The synthesis of aminoalkylnaphthols has received special attention from the scientific community because of their significant biological and catalytic properties during last few years [4]. Moreover, to the best of our knowledge, several

amino methylated 1-naphthols and 2-naphthols exhibited antibacterial activity in the sub micromolar range against both Gram-positive and Gram-negative microorganisms.

Mario Betti in 1900 first synthesised aminoalkylnaphthols and they were called as Betti bases [2] via the condensation of three-components namely secondary amines, aromatic aldehydes, and 2-naphthols. A single product is formed from three or more starting materials with the multicomponent reaction (MCR), that is a convergent reaction in which all or most of the molecules contribute to the formation of new product [5]. Organic multicomponent reactions in recent years have gained much importance since these syntheses produce the desired product in a single operation without the need for isolating the intermediate compounds [6]. The dominance of MCRs are one pot reactions, less amount of solvents consumption or even solventless (solvent free or neat reaction), less time compared to divergent reactions, lower cost, environmentally friendly and generally take simpler procedures [7-9]. Combined component condensation of aldehydes, β-naphthol and different amides is required for the preparation of aminoalkylnaphthols in the presence of various acids and other catalysts. These include cyanuric chloride, montmorillonite K10, sulfamic acid, thiamine hydrochloride, $FeCl_3{\cdot}SiO_2$, $K_5CoW_{12}O{\cdot}3H_2O$, $HClO_4–SiO_2$, ionic liquid, P_2O_5, $Sr(OTf)_2$, silica sulfuric acid, $Yb(OTf)_2$, $Ce(SO_4)_2$, *p*-TSA, $Fe(HSO_4)_3$, molybdophosphoric acid, cation-exchange resins and Pentafluorophenylammonium Triflate (PFPAT) [10, 11]. Though, there are some methods reported that have certain disadvantages. These include highly acidic and expensive catalysts, use of carcinogenic solvents, use of toxic compounds, unsatisfactory yield, high temperature, prolonged reaction time, and the use of additional microwave [12] or ultrasonic irradiation [13].

Characterisation of the synthesised compound is necessary to validate the compound by recognising the chemical structure, functional groups, physical properties, elementary **composition and purity.** Thin Layer Chromatography (TLC) is used to monitor the progress of a reaction**. It** is one of the easiest and the most versatile technique because of its low cost, simplicity, quick development time, high sensitivity, and good reproducibility [14]. Nuclear Magnetic Resonance (^{1}H-NMR) spectroscopy has turned into the preeminent technique for determining the structure of organic compounds. The signal that matches the energy transfer is measured in many ways and processed in order to yield an ^{1}H-NMR spectrum for the nucleus concerned. Additionally, the determination of the melting point of a compound is one way to help in the identification of a sample or establish its purity.

Moderate to satisfactory antimicrobial activity was demonstrated by aminoalkylnaphthols. This includes *in vitro* antibacterial activity against *E. coli, S. aureus, K. pneumonia* and *P. aeruginosa* [4]. In order to test the antimicrobial activity, possessed by the synthesised aminoalkylnaphthol derivatives, the disk diffusion method was used for its convenience, efficiency, and cost. Agar disk-diffusion is a test that has been standardised for certain critical bacterial pathogens like *Streptococci, Haemophilus influenzae, Haemophilus parainfluenzae, Neisseria gonorrhoeae and Neisseria meningitidis* using specific culture media. However, the limitation of this method being, it cannot distinguish bacteriostatic and bactericidal effects since the bacterial growth inhibition does not mean the bacterial death, [15].

Materials and Methods

1. Synthesis of Aminoalkylnaphthol derivatives

The procedure used to synthesise was referred from a study conducted by K. Chinna and J. Nageshwar and it was adapted to comply with the apparatus available and overall conditions of the lab [9]. The derivatives of aminoalkylnaphthol were synthesised by one-pot three component condensation reaction using $MgSO_4$ as the catalyst. Every starting material (aldehyde, naphthol, amine) and the catalyst were weighed in carefully and mixed together in a round-bottomed flask. The mixture was stirred at 100 ºC with a magnetic bar. The chemical reaction was monitored by TLC. After completion of the reaction, the crude product was cooled to 25 ºC using ice bath. The solid residue was then dissolved in boiling ethanol and stirred for 5 min. Then again, the solution was cooled to 25 ºC, and the solid obtained was filtered. Subsequently, the solid were recrystallised from aqueous ethanol. The crystallised product was scraped from the round-bottomed flask. The crystals were used for determining the melting point, further characterisation analysis and antibacterial studies.

2. Characterisation of the synthesised compounds

The characterisation using TLC was carried out by preparing the developing chamber. It consists of a beaker with a watch glass on the top of it. Ethyl acetate and hexane mixture was used as the mobile phase. Small amount of the reaction mixture was placed on the TLC plate in 30-minute interval. The TLC plate was then placed in a chamber containing the mobile phase. The plate was then observed in the UV hood. The UV light can be used to shine the spots that fluoresce under UV light, and it signifies the materials that were used in the reaction. The reaction is considered complete when there is a change in the previous observation.

The compounds were characterised further by determining its melting points. A glass capillary melting point tube is packed with crystals about 3 mm high. The capillary tube is then placed into the sample chamber of the Stuart® Digital Melting Point Apparatus SMP10 and was heated. The sample was observed through the magnifying lens until the melting of the crystals occurs. As soon as the crystals melts, the temperature was recorded from the LED display.

^{1}H-NMR was carried out by preparing aminoalkylnaphthol crystals (5-10 mg) and they were solubilised in deuterated solvent, dimethyl sulfoxide (DMSO). The solution is then transferred into sample tubes where subsequently analyses were done using the Bruker Ascend™ 700 ^{1}H-NMR machine. The results obtained were then analysed and interpreted for further evaluation.

3. In-vitro Antibacterial Study

To prepare the different concentrations of aminoalkylnaphthol analogues, the stock solution was prepared by using 10 mg of the compound. this solution was prepared with 10 ml of 95% ethanol to get a final concentration of 1000 µg/ml. The stock solution was then used to obtain the different concentrations with serial dilution technique, 750 µg/ml, 500 µg/ml and 250µg/ml respectively. The solutions were stored at room temperature until further use. On the other hand, Mueller Hinton agar powder (38.0 g) was weighed and mixed with distilled water (1 L). Homogenous solution was obtained by heating and stirring the mixture constantly. The sterilization of the agar solution was done using the autoclave at 121 ℃ for 20 minutes. As soon as the sterilisation process was completed, the agar solution was kept to cool and was poured into sterile agar plates. Single colony of *S. aureus* and *P. aeruginosa* were obtained from culture plates. These were then transferred into two separate nutrient broths and was allowed for incubation at 37 ℃ for 24 hours to allow bacterial growth. Spectrophotometric measurements were done for both bacterial broths after two hours to ensure the bacterial broths are within the range of McFarland Standard 0.5 (measurement of absorbance between 0.08 to 0.10).

4. Antibacterial study – Disk diffusion test

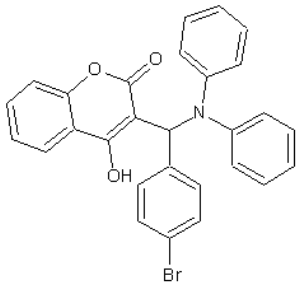
The test was carried out by using micropipettes where 100 µl of the bacterial broths (*S. aureus* and *P. aeruginosa).* Bacterial broths were pipetted out onto the sterile plates of Muller-Hinton Agar (MHA). Broth was evenly spread on agar plates using a sterile hockey stick. Agar plates were then left to dry. The hockey stick was passed over the flame to ensure sterility after each spreading, Approximately 100, 75, 50 and 25 µg of aminoalkylnaphthol analogue was impregnated onto the discs. Negative and positive control used include 95% ethanol and gentamicin 10 µg respectively. The impregnated discs with defined concentrations of the aminoalkylnaphthol derivatives were placed onto the surface of the agar. Zone of inhibition was measured on the agar plates which were incubated at 37 ℃ for at least 24 hours. The zone of inhibition was measured from the center of the antibiotic disk to a point on the circumference of the zone where a distinct edge is present to the nearest millimeter.

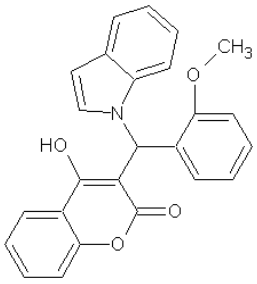
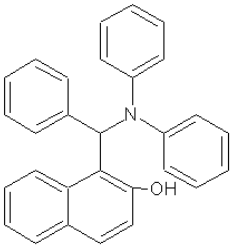
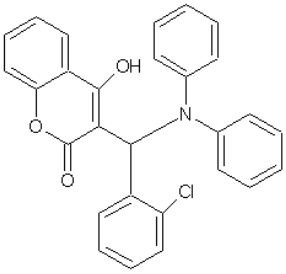
RESULTS AND DISCUSSION

1. Synthesised Aminoalkylnaphthol Derivatives

Four aminoalkylnaphthol derivatives were synthesised as summarised in Table 3.1. All of them showed a reasonable desired percentage yield ranging from 55% to 80 %. Similar findings showed the percentage yield ranging between 74-95% [2].

Table 3.1: The structure, chemical name, molecular formula, and molecular weight of the synthesised compounds

Compound structure	Chemical Name	Molecular Formula	Molecular Weight
 A1	3-[(4-bromophenyl) -(diphenylamine-1-yl)-methyl]-4-hydroxy-chromen-2-one	$C_{28}H_{20}BrNO_3$	498.367

 A3	3-[(2-methoxyphenyl)-indole-1-yl-methyl]-4-hydroxy-chromen-2-one	$C_{25}H_{19}NO_4$	397.422
 A8	1-[phenyl-diphenylamine-1-yl-methyl] naphthalen-2-ol	$C_{29}H_{23}NO$	401.499
 A10	3-[(2-chlorophenyl) -(diphenylamine-1-yl)-methyl]-4-hydroxy-chromen-2-one	$C_{28}H_{20}ClNO_3$	453.916

2. Characterisation of Synthesised Aminoalkylnaphthol Derivatives

The TLC method was used to determine the components in the mixture. Figure 3.1 shows the separation of the starting materials which includes the aldehyde, naphthol and amine used in the synthesis. By observing the appearance of the product and the disappearance of the starting materials, the reaction progress was monitored. The visualisation was done under the UV light since most compounds were colorless. The outcome is shown in the Figure 3.1 where the starting materials have disappeared, and transition of a new fluorescent spot is observed. This indicates the reaction has completed and the product is achieved.

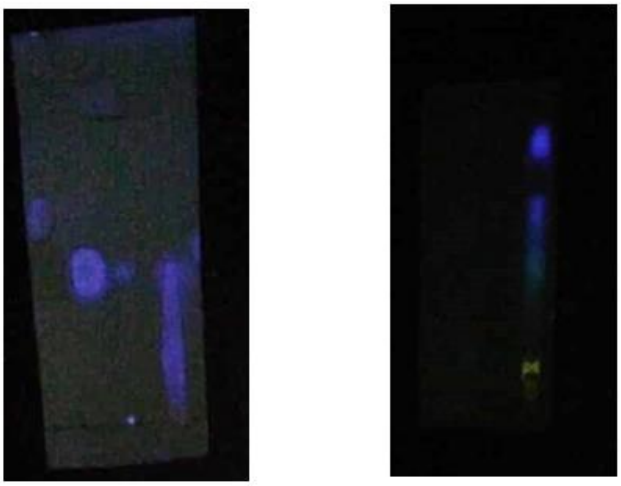


Figure 3.1: Separation of starting materials versus product after completion of reaction

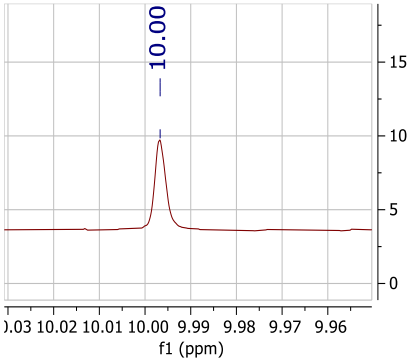
The melting point was determined for all the synthesised aminoalkylnaphthol analogues to compare the purity of these

compounds. Table 3.2 show the results obtained. The theoretical melting point from the literature is compared with the observed melting of the analogues. In this study, the synthesised compounds have melting point values which is comparable to the theoretical melting point range. However, they may contain some impurities which results in the broader melting point range by 1 – 2 ℃. These compounds can be only considered as moderately pure.

Table 3.2: Melting points of the synthesised compounds

Entry	Theoretical melting point (℃)	Melting point observed (℃)
A1	178 - 184	182 - 185
A3	180 - 185	177 - 180
A8	175 - 182	179 - 181
A10	177 - 181	180 - 183

The ^{1}H-NMR in this study was operated with 700 MHz and DMSO as the solvent. As a reference of the expected structure in **Table 3.1**, the absence of neighbouring hydrogens signifies a single peak which is known as singlet. The spectra of compound A1 and A3 confirms the presence of -OH which is observed at δ 10.00 and 10.75 ppm respectively as shown in **Figure 3.2**. The central carbon (-CH) that holds the structure of all compounds does not have any neighbouring hydrogens thus a singlet peak is expected in ranges between δ 4.5 – 6.5 ppm. The signal perceived between δ 6.5 – 8 ppm are in the region representing a hydrogen group attached to an aromatic ring. This is clearly shown in **Figure 3.3** and **3.4** where the spectra showed a number of peaks denominates this chemical shift. The doublet and triplet peaks at the range between δ 6.20 – 7.35 ppm showed the presence of aromatic hydrogen. The peaks observed in the range of δ 0.5 – 5 ppm are accounted for protons that are most shielded as shown in **Figure 3.5.** The most shielded is the amine (RNH_2) group of the compound.



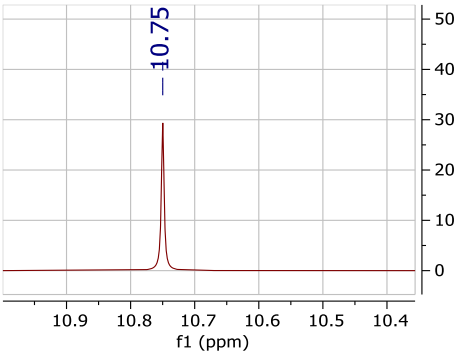


Figure 3.2: Singlet peaks from Compound A1 and A3 signifying the -OH group

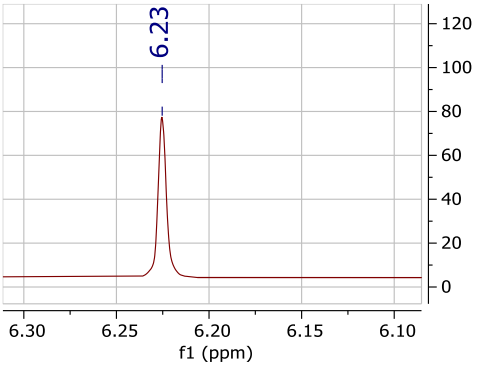
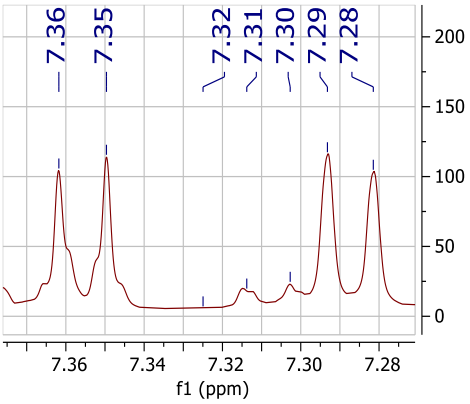


Figure 3.3: Singlet peak signifying the central carbon (-CH)



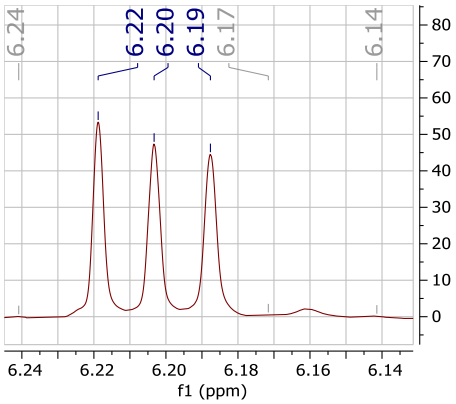


Figure 3.4: Doublet and triplet peak representing aromatic hydrogen

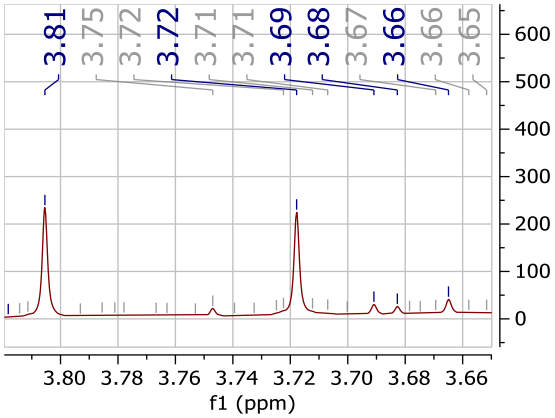


Figure 3.5: Peaks at the range of δ 0.5 – 5 ppm signifying the most shielded protons

Compound A1 and A3 shows desired peaks within the range specified despite certain inconsistency presence of hydrogen. However, Compound A8 and A10 did not show desired signal signifying the expected compound. This can be as a result of thermal degradation of the compound. Also, any signals observed at less than δ 0.5 might be due to presence of impurities. This possibly will affect the overall structure of the compound.

3. In-Vitro Antibacterial Study of the Synthesised Compounds

The different amounts of compound used in this study influenced the zone of inhibition. The amount impregnated on to the disks ranged from 25 µg to 100 µg with an increment by 25 µg. The higher amount of compound used results in a larger zone of inhibition. This can be associated with the potency of these novel drug compounds given that the antibacterial property of the compound correlates with the bacteria growth inhibition observed on the agar plate. The negative control demonstrates about 1mm zone of inhibition while the positive control showed 8mm zone of inhibition.

In this antibacterial study, it was found that compound A1, A8 and A10 has a promising result with a reasonable zone of inhibition against *S. aureus*. This is shown in Table 4.1 where compound A1 had the highest inhibition zone of 6mm for 100 µg, 4mm for 75 µg and 2mm for 50 µg as well as 25 µg.

Table 4.1: Zone of inhibition (mm) of aminoalkylnaphthol compounds towards S. aureus

Entry	Amount of compound impregnated (µg)			
	25	**50**	**75**	**100**
A1	2	2	4	6
A3	0	0	0	0
A8	0	0	0	2
A10	0	1	2	4

On the contrary, compound A3 showed some antibacterial activity against *P. aeruginosa*. From the outcomes in **Table 4.2**, compound A3 had a zone of inhibition of 8mm for 100 µg, 5mm for 75 µg, 2mm for 50 µg and 1mm for 25 µg. As expected, the zone of inhibition improved with the increased amount of compound used.

Table 4.2: Zone of inhibition (mm) of aminoalkylnaphthol compounds towards P. aeruginosa

Entry	Amount of compound impregnated (µg)			
	25	**50**	**75**	**100**
A1	0	0	1	1
A3	1	2	5	8
A8	0	0	0	0
A10	0	0	0	1

CONCLUSIONS

With the aim of developing new antibacterial agents, four aminoalkylnaphthol derivatives were synthesised using the one-pot three component condensation reaction catalysed by $MgSO_4$ under solvent-free condition. This multicomponent reaction proved as a sophisticated and economic way to build up complex structures in a single synthetic procedure which resulted in a short reaction time, mild reaction and a moderate yield. The simplicity of this method could be due to the formation of carbon-carbon and carbon-heteroatom bonds in a single step. Plus, the solvent-less condition apparently reduce pollution and lessen the handling costs due to simplification of experimental procedure.

The characterisation of the synthesised compounds which was conducted for validation purpose showed that all of the synthesised compounds have melting point values which is comparable to the theoretical melting point range. The findings of the synthesised compounds showed diverse spectral analyses from the ^{1}H-NMR spectroscopy. The ^{1}H-NMR results showed presence of singlet peaks which signifies -OH and central carbon -CH at their respective range. Also, the doublet and triplet peaks showed presence of aromatic hydrogen and any peaks in the range of δ 0.5 – 5 ppm signified the most shielded protons. Although they were a few similarities with the expected ^{1}H-NMR spectrum, it had unveiled that the synthesised compounds may contain impure substances as well. In order to support the fact that the novel drugs possess antibacterial properties, the compounds were evaluated for their antibacterial activity by the disk diffusion method. The compound A1 had the highest inhibition zone of 6mm for 100 µg of amount impregnated towards *S. aureus.* However, compound A3 had a zone of inhibition of 8mm for 100 µg towards *P. aeruginosa*.

The zone of inhibition obtained improved with the increased amount of compound used. The substandard antibacterial activity obtained from this study showed that the compounds might contain certain impurities as stated before which to an extent disrupted the expected antibacterial properties of the synthesised compounds. Therefore, the findings of this study suggest that compounds A1 and A3 have the potential of developing as a newer antibacterial agent to overcome the resistant issue.

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Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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