# SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF 5,6-DIFLUORO -2-METHYL -4H-BENZO [D] [[1,3] – OXAZIN-4-ONE AND 3-AMINO-6,7-DIFLUORO -2-METHYL--QUINAZOLIN 4(3H) –ONE

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## ABSTRACT

The current study was aimed at the synthesis and antibacterial evaluation of quinazolinone derivatives. The condensation of methyl-2-amino-5,6-diflorobenzoate with acetic anhydride yielded the cyclic compound 2-methyl 5,6-diflorobenzo [d] [1,3]-oxazine-4-one which further produce 3-amino-2-methyl 5,6-difloro quinazolin-4(3H)-ones via the reaction with hydrazine hydrate was carried out. The compounds synthesized were unequivocally confirmed by means of Infrared, Nuclear Magnetic Resonance ($^{1}$H and $^{13}$C), Gas Chromatography Mass Spectrophotometry and Elemental analysis. The synthesized compounds were screened against various strains of microorganism; *Staphylococcus aureus*, *Bacillus* species, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Candida albicans*. The compounds 1 (5, 6-difloro-2-methyl-4H-benzo [d] [1,3]-oxazine-4-one) and 2 (3-amino-5,6-difloro-2-methyl-quinazoline-4(3h)-one) showed significant activities against *Staphylococcus aureus, Bacillus species, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae,* and *Candida albicans.*

## INTRODUCTION

Some synthetic quinazolinones, such as ispinesib, rallitrexed, haloguginone, tempostatin, etc. have been in the market or are currently inclinical trials for various cancer treatments. (Yahia *et al.*, 2014). Quinazoline derivatives and heterocyclic annelated quinazolines are reported to be physiologically and pharmacologically active (Ram *et al*., 2013), exhibiting a wide range of activities as anticonvulsant, anti-inflammatory, antifungal, anti-malarial and sedative (Dandia *et al.*, 2005 and Jin *et al*., 2006).

A brief survey on the biological activities of quinazolin – 4(3H)- one derivatives showed anti-inflammatory (Chandrika *et al* . , 2008 and Giri *et al*., 2009), anti tumor (Park *et al.* , 2004, Jin *et al*. , 2006, Kundu *et al*., 2008 and El-Azab *et al.* , 2010), anti HIV (Alagarsamy *et al.* , 2007), antibacterial (Jessy *et al*., 2007, Mohamed *et al* ., 2010, Patel and Barat, 2010), as well as CNS depressant and anticonvulsant (Georgey *et al*., 2008 and Kashaw *et al*., 2009) activities. In the present work, 5,6-difluoro 2-methyl substituted benzo-oxazine -4-one and 3 – Amino – 6 , 7-difluoro -2-methyl-quinazoline – 4(3H) – one and the antibacterial property of these compounds were investigated.

These findings prompted the authors to synthesize 3-amino-5,6-difloro-2-methyl-quinazolinone derivatives via the interaction of the benzoxazine derivatives with nitrogen nucleophiles, with the aim of obtaining more precise information about the course of the reaction and determine the antibacterial activity.

## MATERIALS AND METHOD

### General Experimental Procedure

All reagents and solvents were purchased from Sigma-Aldrich, in Germany. Melting points were determined on a Kofler hot stage apparatus and were uncorrected. IR spectra were recorded on a Buck scientific IR M500 instrument. The $^{1}$H and $^{13}$C NMR spectra were recorded in DMSO-*d*6

at 400 $MH_z$ with HAZ Volatile V2. M Chemical shifts Sare reported in ppm relative to tetramethylsilane. Gas chromatography mass spectra were obtained on a Finingan MAT 44S Mass Spectrophotometer operating at 70eV. Elemental analysis agreed favourably with the calculated values. Analytical Thin Layer Chromatography (TLC) was used to monitor the reactions.

**General Procedure for the Synthesis of 5,6-difloro-2-methyl-4H-benzo [d] [1,3]-oxazine-4-one (1)**

This involved the condensation of 0.76g (0.005mol) Methyl 2-amino-5,6-diflorobenzoate with 10ml, 1.02g (0.01mol) acetic anhydride in 30ml ethanol medium. The reaction was heated under reflux with stirring using a magnetic stirrer until the reaction mixture showed no trace of starting material when the TLC was developed (2 hours). Yield was 2.01g (96%), mp: 149-151$^oC$.

**General Procedure for the Synthesis of 3-amino-5, 6-difloro-2-methyl-quinazoline-4(3h)-one (2)**

Equimolar amounts (1.61g, 0.01mol) of 5,6-diflöro-2-methyl-4H-benzo [d] [1,3]-oxazine-4-one, and 0.51g (0.01mol) hydrazine hydrate were heated under reflux in 30ml ethanol with stirring using a magnetic stirrer until the reaction mixture showed no trace of starting material when the TLC was developed (3 hours). At the end of the reaction, the reaction mixture was concentrated in vacuum under reduced pressure using rotary evaporator. The white precipitate formed was then filtered, washed three times with 20ml of distilled water (20ml x 3). The white crystals were dried and re-crystallized from dimethyl formamide (DMF) to give pure 3-amino-5,6-difloro-2-methyl-quinazolin-4(3H)–one. Yield was 1.50g (95%) mp : 138-140$^oC$

**Chemistry of the Synthesized Compounds**

The introduction of 2-amino substituent is a successful strategy to improve the chemical stability of benzoxazinone. Due to the pharmacological activities of 4(3H)-quinazolinone derivatives, 2,3-disubstituted derivative of quinazoline-4-one were synthesized via the interaction of the benzoxazinone derivative with nitrogen nucleophile with the aim of obtaining more precise information about the course of the reaction and some interesting pharmaceutical compounds. The reaction of 4, 5-disubstituted derivatives of methyl anthranilate and acetic anhydride yielded the cyclic compound 5,6-diforo-2-methyl-4H-benzo[*d*][1,3]-oxazin-4-one. The reaction of this compound with hydrazine hydrate yielded 3-amino-5,6-difloro-2-methyl-quinazoline-4(3H)-one.

**Evaluation of Antimicrobial activity**

Agar well diffusion method described by Okeke *et al.* (2001) was utilized for the antimicrobial activity. Six species; *Staphylococcus aureus* (ATCC10145), *Bacillus species* (NCTC 8236), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (NCTC 10418), *Pseudomonas aeruginosa* (ATCC 14756) and *Candida albicans* (ATCC24433) stock cultures were used. The test organisms were supplied by the Pharmaceutical Microbiology Department of the University of Benin. The test organisms were cultured overnight in nutrient broth (for bacterial isolates) and Sabouraud dextrose broth (for fungal isolate), diluted to the turbidity of 0.5 McFarland standard. 0.2 ml of broth cultures were seeded on nutrient agar (for bacterial organisms) or Sabouraud dextrose agar (for the fungus) and allowed to dry. The various concentrations of the compounds (20 – 640 mg/ml) were introduced. The culture plates were incubated at 37$^oC$ for 24hours (for bacterial organisms) or at room temperature (28$^oC$) for 48 hours (for the fungus). The results were taken by considering the zone of inhibition by the test compounds. Precisely 20 mg/ml of Ciprofloxacin (CPX) and Ketanaxol (PEX) were used as positive controls for antibacterial and antifungal assays respectively, while the vehicle (10% DMSO) was used as negative control. Activity and inactivity were observed in accordance with the standard and accepted method (Balouiri *et al*., 2016).

**Statistical Analysis**

All data were expensed as means ± SEM; the student's t-test was applied to determine the significance of the difference between the control group and the test compounds.

## RESULTS AN DISCUSSION

The elemental composition of the compounds are summarized in Table 1. The C and H contents (both theoretically calculated values and actual values) are indicated.

Table 1: Characterization and Physical data of Synthesized Compounds

| Compound No | Solvent | Formula M. wt | Analysis% Calc/Found C | H |
|---|---|---|---|---|
| 1 | Ethanol | $C_9H_6F_2N0_2$ | 55.22 | 3.08 |
| | | (240.053) | 55.21 | 3.07 |
| 2 | Ethanol | $C_9H_8F_2N_30$ | 51. 53 | 3.83 |
| | | (254.083) | 51. 52 | 3.82 |

The present study reported the synthesis of two derivatives of quinazolinone, 5,6-difluoro-2-methyl-4H-benzo [d] [1,3]-oxazin-4-one,(1) and 3-amino-6,7-difluoro-2-methyl quinazolin-4(3H)-one(2).The compounds were investigated for their antimicrobial activity. Structural elucidations of compounds synthesized were characterized by correct elemental analysis and careful inspections of spectral data. Looking at the $^1$H NMR spectra of the compounds synthesized (Table 2), compound 1 displayed a singlet at δ 3.68 which was due to methyl group. Other singlets appeared at δ7.16 and 6.41 attributed to aromatic protons. Also, $^1$H NMR spectrum of compound 2 showed a characteristic signal at δ 2.58 (singlet) corresponding to methyl group. Two singlets appeared at δ7.41 and 7.10 attributed to aromatic protons. Another signal appeared at 5.80 which was attributed to the protons of the amino group. For the IR spectra, compound 1 were characterized by absence of υ $NH_2$and presence of υ C-O stretch in 1102cm$^{-1}$ region of the compound. Compound 2 was characterized by absence of υ C-0 and presence of υ$NH_2$ in 3301cm$^{-1}$ region of the compound.

The $^{13}$C NMR spectrum of compound 1 (Table 3), revealed signals at δ16.95, attributed to methyl group, while the aromatic carbon atoms appeared between δ values 100.05-168.28 with the carbonyl carbon atom appearing as the highest δ value of 168.28. Similarly, compound 2 showed signals at δ22.58, attributed to methyl group, while the aromatic carbon atoms appeared between δ values 105.64-160.28, with the carbonyl carbon atom appearing as the highest δ value of 160.28.

Table 2: $^{13}$C-NMR of Synthesized Compounds

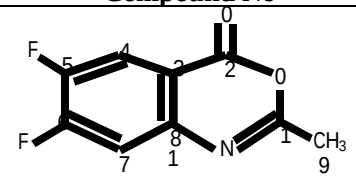
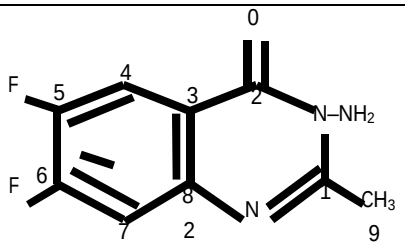
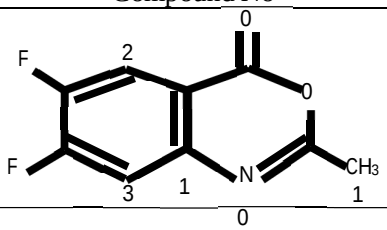
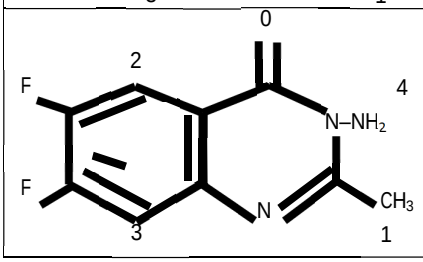
| **Compound No** | **δ (ppm) Carbon atom number** |
|---|---|
|  | 155.15(C-1), 160.48(C-2), 120.14(C-3), 128.09(C-4), 112.71(C-5), 112.61(C-6), 122.15 (C-7), 148.10 (C-8), 24.10 (C-9) |
|  | 154.51(C-1), 160.14 (C-2), 120.28(C-3), 128.21 (C-4), 112.41 (C-5), 112.14 (C-6), 122.20 (C-7), 148.05(C – 8), 24.15 (C- 9). |

Table 3: $^{13}$C-NMR of Synthesized Compounds

| Compound No | δ (ppm) |
|---|---|
|  | 7.21 – 7.96 (m, 3H, ArH), 2.52 (s, 3H $CH_§$) |
|  | 7.51 – 7.14 ($_m$, 3H, Ar-H), 5.03 (s, 2H), 2.53 (s, 3H, $CH_3$) |

**Characterization of 5,6-difloro 2-methyl-4H-benzo [*d*][1,3] -oxazin-4-one.(1).**
$^1$H NMR (400MHz, DMSO) δ 7.21 – 7.96 (m, 3H, ArH ), 2.52 (s, 3H $CH_§$), $^{13}$CNMR (400MHz, DMSO) δ 160.48, 155.15,148.10, 128.09, 120.14, 122.15, 112.71, 112.61, 24.10,. IR (KBr,cm$^1$) 3135, ($NH_2$), 3018 (CH aromatic), 2951, 2871, 2718 (CH aliphatic),1730(C=0),1150 (C-0).Anal. Cal for $C_9H_6BrN0_2$; C 55.21; H 3.07. Found: C 55.22, H 3.08. Yield was 2.01g (96%), mp: 149-151$^o$C.

**Characterization of 3-amino- 5,6-difloro 2-methyl-quinazoline-4(3H)-one. (2).**
$^1$H NMR (400 MHz, DMSO) δ 7.51 – 7.14 ($_m$, 3H, Ar-H), 5.03 (s, 2H), 2.53 (s, 3H, $CH_3$), $^{13}$C NMR (400MHz, DMSO) δ 160.14, 154.51, 148.08, 128.21, 122.20, 120.28, 112.41, 112.14, 24.15, IR (KBr,cm$^1$)3350($NH_2$),1685 (C=0),1620 (C=N), Anal. Cal. for $C_9H_8BrN_30$; C 51. 52, H 3.82; Found, C 51.53, H 3.83.Yield was 1.00g (95%) mp: 98-100$^o$C.

When compared with the activities of the standard drugs; Ciprofloxacin (CPX) and Ketanaxol (PEX) (Figure 1), the results showed that the compounds synthesized exhibited promising antimicrobial activities against *Staphylococcus aureus*, *Bacillus species*, *Pseudomonas aeruginosa* ,*Escherichia coli, Klebsiella pneumoniae,* and *Candida albicans* stock cultures (Table 4).

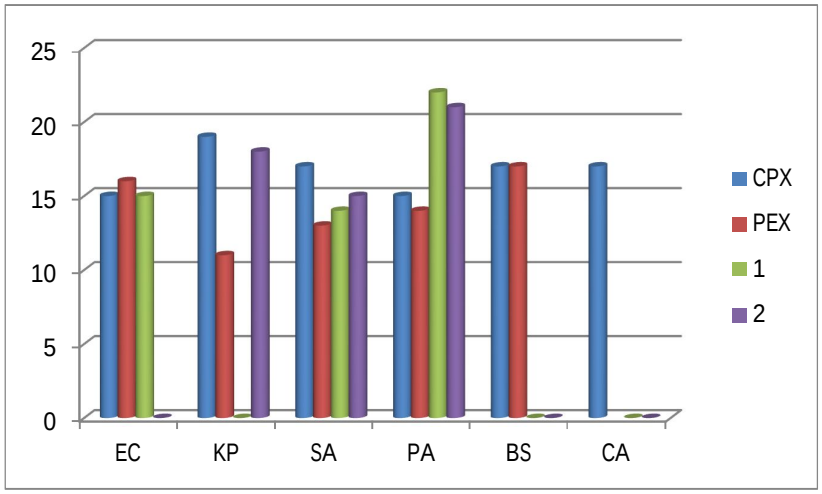



Figure 1. The effect of the standard drugs towards studied bacteria

SA=Staphylococcus aureus, BS = *Bacillus* species, EC = *Escherichia coli*, KP = *Klebsiella pneumoniae*, PA= *Pseudomonas aeruginosa* and CA= *Candida albicans*
Significantly different From Ligand at P<O.05, Values are in mm.

Table 4: Minimum inhibitory concentrations (MIC) in mg/mL of tested compounds against tested standard microorganisms

| Test Organism | Compound | |
|---|---|---|
| | 1 | 2 |
| *Escherichia coli* | 6.00 | - |
| *Bacillus* species | - | - |
| *Staphylococcus aureus* | 6.00 | 6.00 |
| *Klebsiella pneumoniae* | - | 6.00 |
| *Pseudomonas aeruginosa* | 7.00 | 7.00 |
| *Candida albicans* | - | - |

## CONCLUSION

The research findings have shown that quinazolinone derivatives 1 and 2 have potent antibacterial activity. Compound 2 has a higher activity against *Klebsiella pneumoniae aeruginosa* compared to Compound 1. The later was more potent against *Escherichia coli.*

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