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Synthesis and Anti-inflammatory Activity of 5, 6-difluoro-2-Methyl-4H-benzo (d) (1, 3)-Oxazin-4-one(1) and 3-Amino-5,6-difluoro-2-Mehtyl-quinzolin 4(3H)-One(2)

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Abstract

Background: Inflammation is one of the means the human body uses to defend itself in the event of infection, trauma, or exposure to toxic substances and it is closely associated with a number of disease symptoms. Steroidal and non-steroidal anti-inflammatory agents have been the drugs of choice for managing inflammation. However, reports of unpleasant side effects have necessitated a search for new anti-inflammatory agents which have minimal side effects. Inflammation is an adaptive response of body tissues to external challenge or cellular injury. It is generally thought that inflammation is a beneficial host response defense system, but it can become harmful if dysregulated. The complex mediators including proinflammatory and cytotoxic cytokines, growth factors, bioactive lipids, and nitric oxide in the uncontrolled inflammation reaction can sustain or induce a pathologic process involved in different diseases, such as allergy, sepsis, arthritis, the metabolic syndrome, autoimmune diseases, and cancer anti-inflammatory activities of 101 extracts from different parts of 84 traditional medicinal plants were evaluated by a panel of *in vitro* and *in vivo* assays. Nuclear factor-kappa B (NF-κB) inhibitory effects were determined by luciferase assay in stably transfected Hela cells. The inhibition of inflammatory mediators on the transcriptional level has emerged as a promising approach for the development of anti-inflammatory drugs

Method: The current study is aimed at the synthesis and Anti–inflammatory 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. 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 for their Anti–inflammatory activity.

Result: The two compounds 1 and 2 showed significant activity as an Anti–inflammatory agent.

Conclusion: The investigated Compounds exhibited significant Anti–inflammatory activity in the range of 70.56 – 83.80% compared to control.

Keywords: Antiinflammatory Activity; 5,6-difloro-2-methyl-4H-benzo [d] [1,3]-oxazine-4-one; 3-amino-5,6-difloro-2-methyl-quinazolin-4(3H)-one; Nucleophile; Quinazoline-4(3H)-one; Analgesic

1 Introduction

Heterocyclic chemistry comprises at least half of all organic chemistry research worldwide. In particular, heterocyclic structures form the basis of many pharmaceutical, agrochemical and veterinary products. Among a wide variety of

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nitrogen heterocycles that have been explored for developing role in medicinal chemistry and subsequently have emerged as a pharmacophore [1].

Quinazolinone derivatives represent one of the most active classes of compounds possessing a wide spectrum of biological activity. They are widely used in pharmaceutical and agrochemicals. Several reports have been published on the biological activities of quinazolinone dervatives, including their anti-inflammatory [1-7], antimalarial [8, 9], anticonvulsant [17 - 20], and antitumor [21, 22], activities.

Quinazolinone peptides were reported for their anti-inflammatory, antioxidant, anthelminthic, antibacterial and antifugal activities [23].

This research was aimed at synthesis of 5,6-difluoro-2-Methyl-4H-benzo(d) (1,3)-Oxazin-4-one and 3-Amino-5,6-difluoro-2-Mehtyl-quinzolin 4(3H)-One, investigation them for their analgesic activity and to obtain more precise information about the course of reaction.

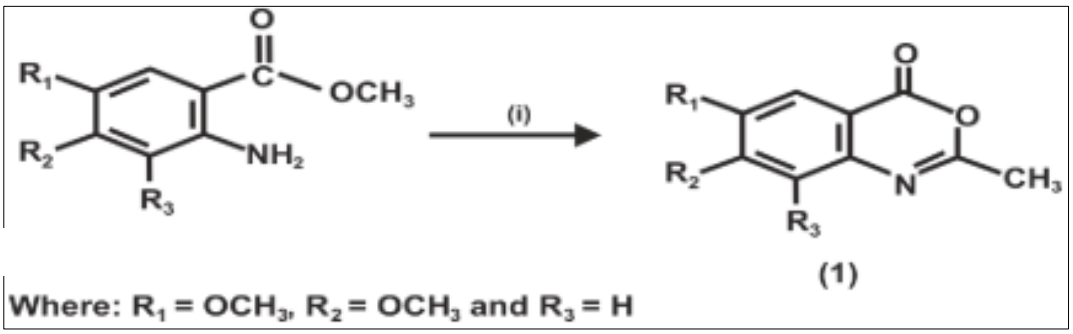
1.1 Chemistry

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 pricise information about the course of the reaction and some interesting pharmaceutical compounds. The reaction of 4, 5-disubstituted derivatives of methylanthranilate 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.

2 Material and method

2.1 Experimental

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.

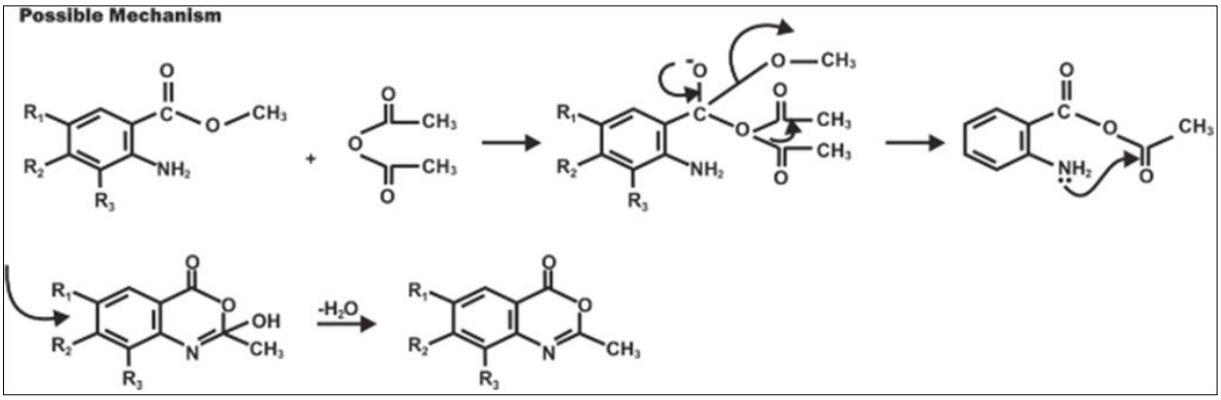


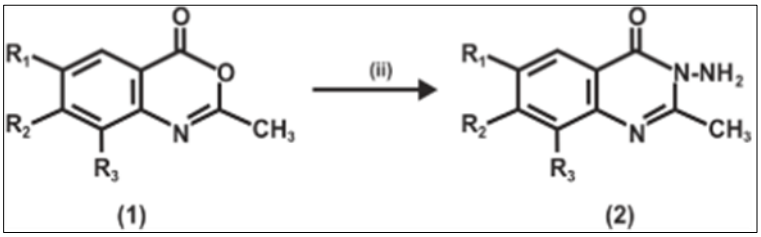
Scheme 1

i = Acetic anhydride

a = Ethanol

Where: R_1 = F, R_2 = F, and R_3 = H





Scheme 2

i = Hydrazine hydrate

a = Ethanol

Where: R_1 = F, R_2 = F, and R_3 = H

Possible Mechanism

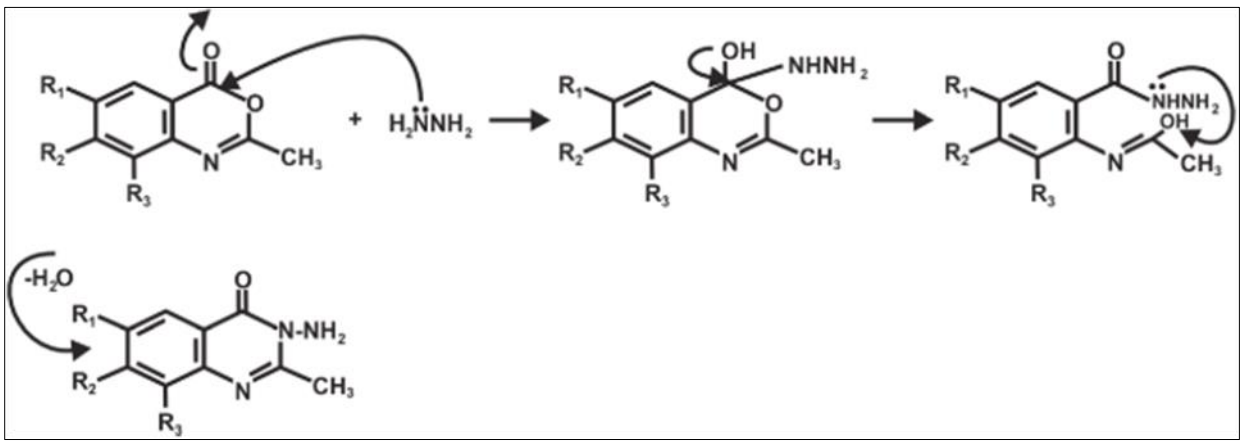


Figure 1 Possible Mechanism of Scheme 2

2.2 Elemental Analysis

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

2.2.1 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°C.

2.2.2 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 recrystallized from dimethylformamide (DMF) to give pure 3-amino-5,6-difloro-2-methyl-quinazolin-4(3H)–one. Yield was 1.50g(95%) mp : 138-140°C

2.3 Pharmacological evaluation

Wistar rats (180-230g) of either sex kept in the laboratory animal house of the Faculty of pharmacy, University of Benin, Benin City, Nigeria were used. The animals were maintained under standard environmental conditions and had free access to standard diet and water(test compounds were administered orally by gavage in 10% olive oil suspensions at different dose levels). Ethical approval was obtained from the Animal Use and Ethics committee of the Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

2.4 Anti-inflammatory Activity

Anti-inflammatory activity was measured using carrageenan induced rat paw oedema assay (Winter et al., 1962; Adeyemiet al., 2002). Group of 5 rats of both sexes (pregnant females excluded) were given a dose of a test compound. After one-hour 0.1ml, 1% carrageenan suspension in 0.9% NaCl solution was injected into the sub-plantar tissue of the right hind paw. The linear paw circumference was measured at hourly interval for four h two groups of drug treated rats and one control group were used each test day and the mean paw oedema value for the test group being compared with its mean value for the control group for that day. Anti-inflammatory activity (Duffy et al., 2001) was measured as the percentage reduction in oedema level when drug was present, relative to control as shown in table 3. Indomethacin (10mg/kg) was administered orally as reference drug while 10% olive oil was used as negative control.

3 Results

Table 1 Characterization and physical data of synthesized compounds

Compound No	Solvent	Formula M. wt	Analysis% Calc/Found	
			C	H
1	Ethanol	C9H6F2N02 (240.053)	55.22	3.08
			55.21	3.07
2	Ethanol	C9H8F2N30 (254.083)	51. 53	3.83
			51. 52	3.82

Table 2 ^{13}C-NMR Of Synthesized Compounds

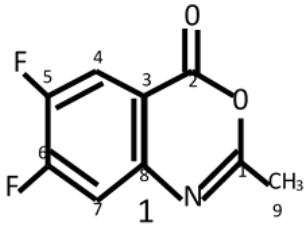
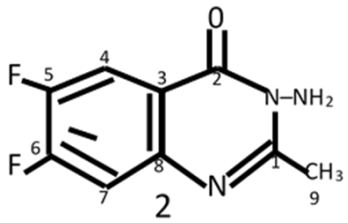
Compound No	δ (ppm) Carbon atom number
	155.15(C-1), 160.48(C-2), 120.14(C-3), 128.09(C-4), 112.71(C-5), 112.61(C-6), 122.15 (C-7), 148.10 (C-8), 24.10 (C-9)
	154.51(C-1), 160.14 (C-2), 120.28(C-3), 128.21 (C-4), 112.41 (C-5), 112.14 (C-6), 122.20 (C-7), 148.05(C – 8), 24.15 (C- 9).

Table 3 ^{1}H-NMR Of Synthesized Compounds

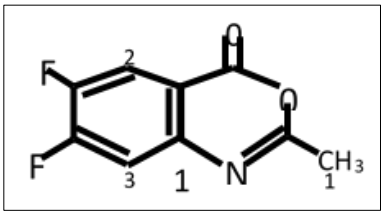
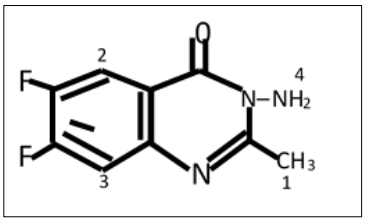
Compound No	δ (ppm)
	7.21 – 7.96 (m, 3H, ArH), 2.52 (s, 3H CH§)
	7.51 – 7.14 (m, 3H, Ar-H), 5.03 (s, 2H), 2.53 (s, 3H, CH3)

Table 4 Effect of the synthesized compounds on carrageenan induced paw edema (anti–inflammatory activity of the compounds synthesized relative to control)

Diameter of inflammation (mm)				
GROUPS	0 min	60 min	120 min	180 min
Control	2.37±0.29	1.98±0.17	1.60±0.31	2.19±0.30
100 (mg/kg)	1.36±0.21	0.81±0.27*	1.24±0.34	1.62±0.19
200 (mg/kg)	1.25±0.57	0.89±0.40*	0.84±0.21	0.95±0.26*
400 (mg/kg)	1.46±0.31	1.46±0.19	1.38±0.24	1.02±0.25*
Diclofenac (25 mg/kg)	1.22±0.19	0.63±0.09*	0.65±0.36	0.35±0.08***

Values are mean ± S.E.M. where number of replicates, n=4 per group, (*p<0.05, ***p<0.001)

3.1 Anti–inflammation activity

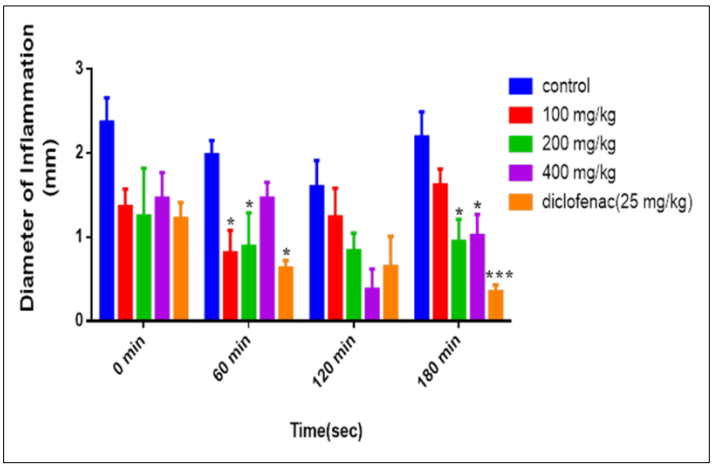


Figure 2 Carrageenan induced paw edema in rat. Values are mean ± S.E.M. where number of replicates, n=4 per group, (*p<0.05, ***p<0.001)

3.2 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.

3.3 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.

4 Discussion

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 Anti–inflammatory 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, 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 υNH_2 in 3301cm^{-1} region of the compound.

The ^{13}C NMR spectrum of compound 1, 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. These compounds synthesized exhibited promising Anti–inflammatory activities. The in-vivo anti–inflammatory activity of compounds synthesized were determined using carrageenan induced paw edema and the results obtained are summarized in table 4. Compound 2 showed the highest activity at 40mg.kg compared to the other compound 1, and indomethacin a standard drug. It may be that the substitution of amino group at position three increases the activity. These compounds synthesized have a higher activity than indomethacin, which is a standard anti–inflammatory drug.

5 Conclusion

The present study has showed that the quinazolinone derivatives 1 and 2 have high anti–inflammatory activity. Compound 1 has Anti–inflammatory activity of 70.56% and 71.33% at 20mg/kg and 40mg/kg dose levels, while Compound 2 has Anti–inflammatory activity of 81.66% and 83.80% at 20mg/kg and 40mg/kg dose levels. Compound 2 has a higher Anti–inflammatory activity compared to Compound 1 and Indomethacin a standard Anti–inflammatory drug. From this result, Compound 2 could be a potential Anti–inflammatory and a tool to pharmaceutical drug delivery.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The author declares no conflict of interest.

Statement of ethical approval

Ethic approval, consent to participate and the procedure used was approved by the Ethic approval committee of Ondo State University of Science and Technology, Okitipupa, Ondo State, Nigeria.

Author declaration

The author hereby declares that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by me.

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