CORE
Metadata, citation and similar papers at core.ac.uk
Provided by ZENODO

CONTINENTAL J. APPLIED SCIENCES
Osarumwense (2017) 12 (3): 29 – 39 DOI: 10.5281/zenodo.995719

Research Article

Synthesis and Analgesic Activity of 3-Amino-6-Bromo-2-Methyl Quinazolin-4(3H) – One and 6-Bromo-2-Methyl-4H-Benzo[D] [1,3]- Oxazin-4-One

Osarumwense, P. O.
Department of Chemical Science, Ondo State University of Science and Technology, Okitipupa Ondo State, Nigeria: E-mail: Osarodion.peter@yahoo.com

Abstract

The current study is aimed at the synthesis and Analgesic evaluation of quinazolinone derivatives. The condensation of Methyl-2-amino-5-bromobenzoate with acetic anhydride yielded the cyclic compound 2-methyl 6-bromo-1, 3-benzo-oxazine-4-one which further produce 3-Amino-2-Methyl 6-bromoquinazolin4(3H)-ones via the reaction with hydrazine hydrate. The compounds synthesized were unequivocally confirmed by means of Chromatography Mass Spectrophotometer and Elemental analysis. The quinazolonones were evaluated pharmacologically for their in-vivo analgesic activities by acetic acid induced writhing in mice. The two investigated compounds exhibited significant analgesic activity in the range of 74.67 - 83.80% in compared to control.

Keywords: Analgesic, 3-amino-6-bromo-2-methyl quinazolin-4(3H)-one, 6-bromo-2-methyl-4H-benzo [d] [1,3]-oxazine-4-one, Nucleophile, Quinazoline-4(3H)one.

Received: 02 July 2017 Accepted: 10 Sept. 2017

Introduction

The synthesis of quinazolinoneheterocycles has become the cornerstone for synthetic chemists and gained extensive importance in medical chemistry because of their diverse pharmacological activities including anti-mycobacterial (Ammar, 2011), Grover, 2006 and Waisser, 2001), anti-fungal (Tiwari *et al.*, 2007), antimalarial (Martin, *et al.*, 1964), antihypertensive (Alagarsamy *et al.*, 2007), Garcin *et al.*, 2000 and Jen, T *et al*, 1973), antihistaminic (Alagarsamy *et al.*, 2006, Alagarsamy *et al.*, 2005a, Alagarsamy *et al.*, 2005b, Alagarsamy *et al.*, 2005c), cardiotonic)Dempy *et al.*, 1993), anticancer (Abdel-Rahman, 1998, El- Bayouki *et al.*, 2009 and Hour *et al.*, 2000),

This work is licensed under a Creative Commons Attribution 3.0 Unported License
ISSN: 1597 – 9928 Science and Education Development Inst., Nigeria

antiviral (Alagasamy *et al.,* 2004), and thymidylate synthase inhibitory activities (Hennequin *et al.,* 1996) and Marshaszsm *et al*., 1991).

The 2,3-disubstituted quinazolones have been predicted to possess antiviral and antihypertensive activities (Pandey and other 2004).

Benzoxazneheterocylic compounds are potent non-steridalprognesterone receptor agoinsts (Zheng *et al.,* 2002), having mang other activities such as anticancer, antiangiogenic (LaDS *et al.,* 2008), antidiabetic and hypolipidemic (Madhavan *et* al., 2006), antidepressant (Zhou *et al*., 2006) and antiplatelet aggregation activities (Pritchard *et al.,* 2007).

These findings prompted the author to synthesis these quinazolinone derivatives via the interaction of the benzoxazinone derivatives with different nitrogen nucleophiles, with the aim of obtaining more precise information about the course of the reaction and determine the Analgesic properties.

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 precise 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 7-chloro 2-methyl-4H-benzo [*d*][1,3] -oxazin-4-one. The reaction of this compound with hydrazine hydrate yielded 3-amino-7-chloro-2-methyl-quinazoline-4(3H)-one.

Materials and methods

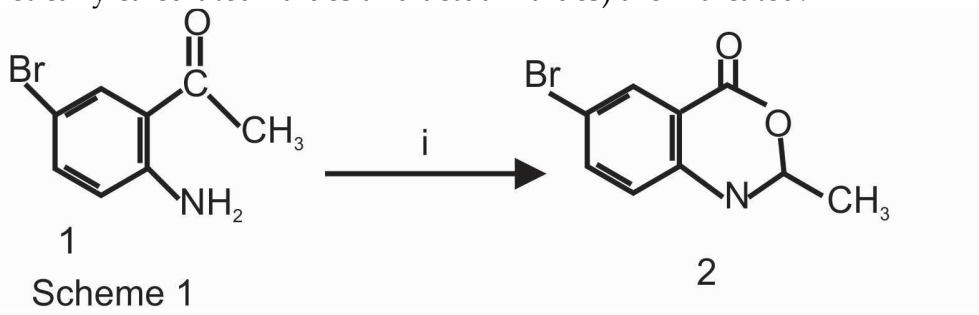
General Experimental Procedure

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.

This work is licensed under a Creative Commons Attribution 3.0 Unported License
ISSN: 1597 – 9928 Science and Education Development Inst., Nigeria

Elemental Analysis

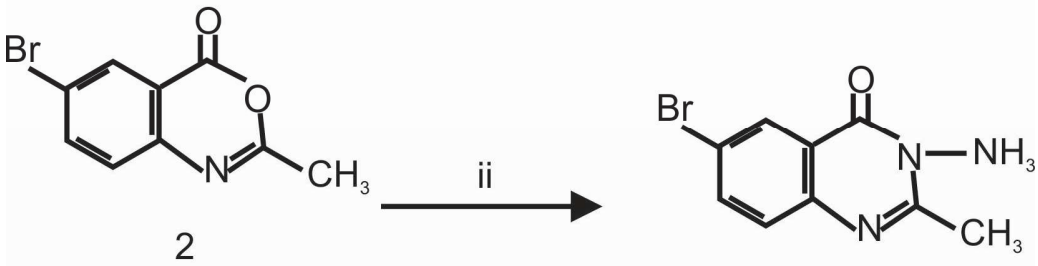
The compositions of the compounds are summarized in Table 1. The C and H contents (both theoretically calculated values and actual values) are indicated.



1 2

Scheme 1

i=Acetic anhydride, ethanol



2 3

Scheme 2

ii=Hydrazine hydrate, ethanol

General Procedure for the Synthesis of 6-bromo-2-methyl-4H-benzo [d] [1,3]-Oxazin-4-One, (1).

This involved the condensation of 0.76g (0.005mol) Methyl 2-amino-5-bromobenzoate 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^{o}C.

General Procedure for the Synthesis of 3-amino-6-bromo-2-Methyl-Quinazoline-4(3h)-One (2).

Equimolar amounts (1.61g, 0.01mol) of 6-bromo-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

This work is licensed under a Creative Commons Attribution 3.0 Unported License
ISSN: 1597 – 9928 Science and Education Development Inst., Nigeria

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-6-bromo-2-methyl-quinazolin-4 (3H) – one. Yield was 1.50g (95%) mp : 138-140^{o}C

Pharmacologcal Evaluation

Swiss mice (18-23g) of both sexes were used. The animals were maintained under standard diet Sand water. Test compounds were administered orally at dose levels. Ethics committee of the Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Analgesic activity

The acetic acid induced abdominal constriction method is widely used for the evaluation of peripheral antinociceptive activity (Gene *et al.*, 1998). It is very sensitive and able to detect antinociceptive effects of compounds at dose levels that may appear inactive in other methods like the tail-flick test (collier *et al.*, 1968. Bentley *et al.*, 1981). Local perietoneal receptors are postulated to be partly involved in the abdominal constriction response (Bentley *et al.*, 1983). The method has been associated with prostanoids in general, e.g increased levels of PGE_2 and PGE_{2a} in peritoneal fluids (Derarit *et al.*, 1980), as well as lipoxygenase products by some researchers (Levini *et al.*, 1984), Dhara *et al.*, 2000). Indomethaan (10mg/kg) was administered orally as reference drug while 10% olive oil was used as negative

Statistical analysis

All data were expressed as the mean + SEM, the students't-test was applied to determine the significance of the difference between the control group and the test compounds.

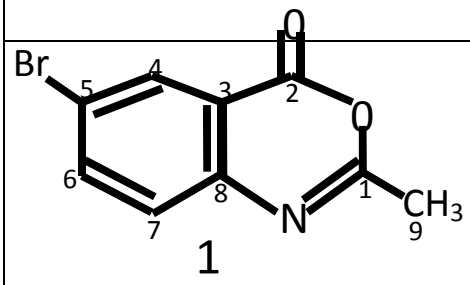
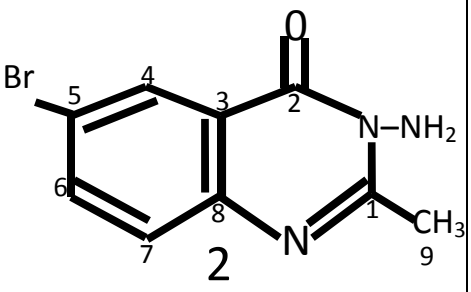
Results and discussion

Table 1: Characterization and Physical data of Synthesized Compounds

Compound No	Solvent	Formula M. wt	Analysis% Calc/Found C	H
1	Ethanol	$C_9H_6BrN0_2$ (240.053)	55.22 55.21	3.08 3.07
2	Ethanol	$C_9H_8BrN_30$ (254.083)	51. 53 51. 52	3.83 3.82

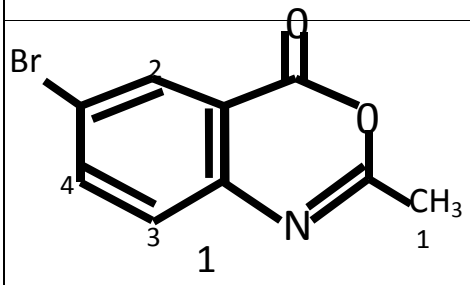
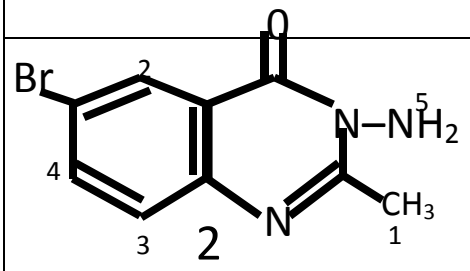
This work is licensed under a Creative Commons Attribution 3.0 Unported License
ISSN: 1597 – 9928 Science and Education Development Inst., Nigeria

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

Compound No	δ (ppm) Carbon atom number
 1	153.12(C-1),168.18(C-2),120.90(C-3), 128.28 (C-4), 113.37 (C-5), 133.77 (C-6), 122.10 (C-7), 149.28 (C-8), 25.10 (C-9)
 2	154.53 (C-1), 160.10 (C-2), 120.25 (C-3), 128.07 (C-4), 133.50 (C-5) 113.53 (C-6), 122.14 (C-7), 148.22 (C – 8), 22.30 (C- 9).

This work is licensed under a Creative Commons Attribution 3.0 Unported License
ISSN: 1597 – 9928 Science and Education Development Inst., Nigeria

Table 3: ^{1}H-NMR of Synthesized Compounds

Compound No	δ (ppm)
	8.23 (s, 1H), 8.14 (s, 1H), 6.40 (s, 1H), 2.50 (s, 3H)
	8.21 ($_{2}$s, 2H), 8.11 (Ar-1H), 7.10 (s, 1H), 5.80 (s, 1H), 2.40 (s, 3H)

Characterization of 6-bromo 2-Methyl-4H-benzo [*d*][1,3] -Oxazin-4-One.(1).
^{1}H NMR (400MHz, DMSO) δ 8.23 (s, 1H), 8.14 (s, 1H), 6.40 (s, 1H), 2.50 (s, 3H), ^{13}(NMR (400MHz, DMSO) δ 168.18, 153.12, 149.28, 133.77, 128.28, 122.10, 120.90, 113.37, 25.10. 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 of3-amino- 6-bromo 2-methyl-quinazoline-4(3H)-one. (2).
^{1}H NMR (400 MHz, DMSO) δ 8.21 ($_{2}$s, 2H), 8.11 (Ar-H), 7.10 (s,1H),5.80 (s, 1H), 2.40 (s, 3H), ^{13}C NMR (400MHz, DMSO) δ 160.10, 154.53, 148.22, 133.50, 128.07, 122.14, 120.25, 113.53, 22.30, 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.

This work is licensed under a Creative Commons Attribution 3.0 Unported License
ISSN: 1597 – 9928 Science and Education Development Inst., Nigeria

Table 4: Effect of the test compounds on acetic acid induced writhing in mice.

Compound No	Does mg/kg (p.o)	Numbers of writhing (per 20 min)	% Inhibition
1	20 40	36.11 ± 0.18 20.42 ± 2.45	74.67 77.41
2	20 40	27.56 ± 1.16 16.01 ± 0.22	79.06 83.80
TWEEN 80	0.2ML	69.00 ± 0.12	
Acetylsalicylic acid		22.50 ± 3.07	67.39
Indomethacin	10	14.80 ± 4.95	78.55

Values are meant ± S.E.M; P<0.001, significantly different from control, paired t-test (n=5), P.O = per oral.

Statistical analysis

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

Discussion

The present study reported the synthesis of two derivatives of quinazolinone, 6-bromo-2-methyl-4H-benzo [d] [1,3]-oxazine-4-one,(1) and 3-amino-6-bromo-2-methyl quinazolin-4(3H)-one(2).The compounds were investigated for their Analgesic activity.

The structures of the compounds were confirmed based on their elemental and spectra analyses, thus IR spectrum of the compounds. Compound 1 reveals strong absorption band at 1730, 2871, 2718, and 3135 attributed to Umax for C=O, CH aliphatic and NH, while compound 2 reveals strong absorption band at 1685, 1620 and 3350, attributed to C = O, C=N and NH. The in-vivo analgesic activity of compounds synthesized were determined using mouse writhing assay and the results obtained are summarized in table 4. Compound 2 showed the highest activity at 40mg.kg compared to the other compound 1, acetylsalicylic acid and indomethacin. . It may be that the substitution of Amino group at position three increase the activity. These compounds synthesized have a higher activity than acetylsalicyclic acid, which is a standard analgesic drug.

This work is licensed under a Creative Commons Attribution 3.0 Unported License
ISSN: 1597 – 9928 Science and Education Development Inst., Nigeria

Conclusion

The compounds have high Analgesic activity. Compound 2 has a higher Analgesic activity compared to Compound 1.

Acknowledgement

The author appreciates the assistance of Dr. Marvis E. in England for running the spectra and the Ethics committee of the Faculty of Pharmacy, University of Benin, Benin City, Nigeria. Department of Pharmacology at the University of Benin who assisted in Animal supplied.

References

Abdel-Rahman, T (1998). Synthesis of some new biologically active 2, 3-disubstituted quinazolin-4-ones. *Boll. Chim. Farm*. 137; 43-47.

Alagarsamy, V.; Giridhar, R.; Yadav, M (2006). Synthesis and pharmacological investigation of novel 1-substituted-4-(4-substituted phenyl)-4H -[1, 2, 4] triazolo [4, 3-a] quinazolin-5-ones as a new class of hl-antihistamine agents. *J Pharm Pharmacol*. 58:1249-1255.

Alagarsamy, V.; Giridhar, R.; Yadav, MR (2005a). Corrigendum to synthesis and pharmacological investigation of novel-substituied-4-phenyl-l, 2, 4-triazolo [4, 3-a] quinazolin-5 \4H)-ones as a new class of hl-antihistaminic agents. *Bioorg Med ChemLett*. 2005; 15:3316.

Alagarsamy, V.; Giridhar, R.; Yadav, MR (2005b). Synthesis and hl-antihistaminic activity' of some novel 1-substituted-4-(3-methylphenyl)-l, 2, 4-triazolo [4, 3-a] quinazolin-5 (4H)-ones. *Biol. Pharm. Bull*. 28:1531.

Alagarsamy, V.; Giridhar, R.; Yadav, MR (2005c). Synthesis and pharmacological investigation of novel 1-substituted-4-phenyl-l, 2, 4-triazolo [4, 3-a] quinazolin-5 (4H)-ones as a new class of hl-antihistaminic agents. *Bioorg. Med. ChemLett*. 15:1877-1880.

Alagarsamy, V.; Pathak, US (2007). Synthesis and antihypertensive activity of novel 3-benzyl-2-substituted-3H-[1, 2, 4] triazolo [5, 1-b] quinazolin-9-ones. *Bioorg. Med. Chem*. 15:3457-3462.

This work is licensed under a Creative Commons Attribution 3.0 Unported License
ISSN: 1597 – 9928 **Science and Education Development Inst., Nigeria**

Alagarsamy, V.; Revathi, R.; Meena, S.; Ramaseshu, K.; Rajasekaran, S.; De Clercq, E (2004). Anti-HIV, antibacterial and antifungal activities of some 2, 3-disubstituted quinazolin-4(3H)-ones. *Indian J. Pharm. Sci.* 66:459-462.

Alagarsamy, V.; Yadav, MR.; Giridhar, R (2006). Synthesis and pharmacological investigation of novel 1-alkyl-4-(4-substituted aryLheteroaryl)-1, 2,. 4-triazolo [4, 3- a] quinazolin-5 (4H)-ones as a new class of hl-antihistaminic agents. *Arzneimittelforschung*. 56:834-841.

Ammar, YA.; Mohamed, Y.; El-Sharief, A.; El-Gaby M.; Abbas, S (2011). Synthesis of some biologically active 4 (3H)-quinazolinones derived from 2, 3-pyridine dicarboxylic anhydride. *ChemSci. J.* 2:15.

Dempcy, RO.;Skibo, EB (1993). Kinetic studies of 2-(2'-haloethyl) and 2-ethenyl substituted quinazolinone alkylating agents Acid-catalyzed dehydrohalogenation and alkylation involving a quinazolinoneprototropic tautomer. *Bioorg. Med.. ChemLett*. 1:39-43.

El-Bayouki, KA.; Aly, MM.; Mohamed, YA.; Basyouni, W.; Abbas, SY (2009). Novel 4 (3H)-quinazolinone containing biologically active thiazole, pyrazole, 1, 3-dithiazole, pyridine, chromene, pyrazolopyrimidine and pyranochromene of expected biological activity. *World J. Chem*. 4:161-170.

Garcia, J.; Somanathan, R.; Rivero, I.; Aguirre, G.; Hellberg, L (2000). Synthesis of deuterium-labeled antihypertensive 3-(4-phenyl-l'-piperazinyl)-propyl-2,4-quinazolinedione. *Synthetic Communication*. 30: 2707-2711.

Grover, G.; Kini, SG (2006). Synthesis and evaluation of new-quinazolone derivatives of nalidixic acid as potential antibacterial and antifungal agents. *Eur J Med Chem*. 41:256-262.

Hennequin, LF.; Boyle, FT.; Wardleworth, JM.; Marsham, PR.; Kimbell, R.; Jackman, AL (1996). Quinazolineantifolatesthymidylate synthase inhibitors: Lipophilic analogues with modification to the C2-methyl substituent. *J. Med. Chem*. 39:695-704.

Hour, M-J.; Huang, L-J.;Kuo, S-C.; Xia, Y.;Bastow, K.; Nakanishi, Y.; Hamel, E.; Lee, K-H (2000). 6-alkylamino-and 2, 3-dihydro-3'-methoxy-2-phenyl-4-quinazolinones and related compounds: Their synthesis, cytotoxicity, and inhibition of tubulin polymerization. *J. Med. Chem*. 43:44.79-4487.

This work is licensed under a Creative Commons Attribution 3.0 Unported License
ISSN: 1597 – 9928 **Science and Education Development Inst., Nigeria**

Jen, T.; Dienel, B.; Dowalo, F.; Van Hoeven, H.; Bender, P.; Love, B (1973). Synthesis of pyrrolo[2, 3,-b] [2, 3-b] isoquinolineimidazofl, 2-bisoquinoline, pyrrolo[2, 1-b] quinazoline, and 1, 3-thiazino [2, 3-b] quinazoline derivatives and related heterocycles as potential antihypertensive agents. *J. Med. Chem*. 16; 633-637.

La, DS.; Belzile, J.; Bready, JV.; Coxon, A.; DeMelfi, J.; Doerr, N.; Estrada, J.; Flynn. JC (2008). Flynn, SR.; Grace; ffaRF. Novel 2, 3-dihydro-l,4-benzoxazines as potent and orally bioavailable inhibitors of tumor -driven angiogenesis. *J. Med. Chem*. 51:1695-1705.

Madhavan, GR.;Chakrabarti, R.;AnanthaReddy, K.; Rajesh, B.;Balraju, V.;BheemaRao, P.;Rajagopalan, R.;Iqbal, J (2006). Dual ppar-a and -y activators derived from novel benzoxazinonecontainingthiazolidinediones having antidiabetic and hypolipidemic potential. *BioorgMed Chem*. 14:584-591.

Marsham PR, Hughes LR, Jackman AL, Hayter AJ, Oldfield J, Wardleworth JM, Bishop JA, O'Connor BM, Calvert AH (1991). Quinazolineantifolatethymidylate synthase inhibitors: Heterocyclic benzoyl ring modifications. *J Med Chem*. 34: 1594-1605.

Martin, TA.; Wheeler, AG.; Majewski, RF.; Corrigan, JR (1964). Sulfanilamidoquinazolines. *J. Med. Chem*. 1964; 7:812-814.

Pandey, V. K.; Tusi, S; Tusi, Z.; Raghubir, R.; Dixit, M.; Joshi, M. N. (2004). Heterocyclic compounds Thiazolyl quinazolinoes and potential antiviral and antihypertensive agent. *Indian J. Chem*. 43, 180-184.

Pritchard, KM.; Al-Rawi, J.; Bradley, C (2007). Synthesis, identification and antiplatelet evaluation of 2-morpholino substituted benzoxazines. *Eur J Med Chem*. 2007; 42:1200-1210.

Tiwari, AK.; Singh, VK.; Bajpai, A.; Shukla, G.; Singh, S.; Mishra, AK (2007). Synthesis and biological properties of 4-(3H)-quinazolone derivatives. *Eur. J. Med. Chem.* 42:1234-1238.

Waisser, K.; Gregor, J.; Dostal, H.; Kunes, J.; Kubicova, L.; Klimesova, V.; Kaustovd, J (2001). Influence of the replacement of the oxo function with the thioxo group on the antimycobacterial activity of 3-aryl-6, 8- dichloro-2H-l, 3-benzoxazine-2, 4 (SH)-diones and 3-aryl quinazoline-2, *4* (1H, 3H)-diones. *Farmaco*, 56:803-807.

This work is licensed under a Creative Commons Attribution 3.0 Unported License
ISSN: 1597 – 9928 **Science and Education Development Inst., Nigeria**

Zhang, P.; Terefenko, EA.; Fensome, A.; Zhang, Z.; Zhu, Y.; Cohen, J.; Winneker, R.;Wrobel, J.; Yardley, J (2002). Potent nonsteroidal progesterone receptor agonists: Synthesis and sar study of 6-aryl benzoxazines. *Bioorg. Med. ChemLett*. 12:787-790.

Zhou, D.; Harrison, BL.; Shah, U.; Andree, TH.; Hornby, GA.; Scerni, R.; Schechter, LE.; Smith, DL.; Sullivan, KM.;Mewshaw, RE (2006). Studies toward the discovery of the next generation of antidepressants. Part 5: 3,4-dihydro-2H -benzo [1, 4] oxazine derivatives with dual 5-htla receptor and serotonin; transporter affinity. *Bioorg. Med. ChemLett*. 16:1338-1341.

This work is licensed under a Creative Commons Attribution 3.0 Unported License
ISSN: 1597 – 9928 **Science and Education Development Inst., Nigeria**