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SYNTHESIS AND ANXIOLYTIC ACTIVITY OF 2-METHYL-3-AMINO-4-QUINAZOLINONE ACETAMIDE DERIVATIVES

J. P. Patil[1]*, S. V. Amrutkar[2], R.N. Borole[3]

[1]Dept of Pharmaceutical Chemistry, AIKTC School of Pharmacy, New Panvel, Navi Mumbai, India. E-mail: jayshreepatil76@yahoo.com
[2] Sir Dr. M. S. Gosavi College of Pharmaceutical Education and Research, Nasik, India.
[3]Qsafe Consultants (India), Mumbai.

Abstract
Medicinal Chemistry plays a vital role in the eradication of fatal diseases in human being. Quinazolin-4-one is versatile lead molecule. The synthesis of its derivatives has been the focus of great interest for the development of newer bioactive agent. The present study involves the synthesis, purification and characterisation of various acetamide derivatives of 3-aminoquinazolinone. Methyl Anthranilate on condensation with acetic anhydride in presence of pyridine and hydrazine hydrate form 2-methyl-3-amino-4-quinazolinone. The purity of synthesized compound was checked by Thin Layer Chromatography. The melting point of synthesized compounds were analysed with an open ended capillary tube method. Structures of synthesized compounds were elucidated by FT-IR, GC-MS, and NMR spectral analysis. The synthesized compounds were evaluated for anxiolytic activity by Elevated plus Maze Test and Light and Dark Test using Diazepam as standard.

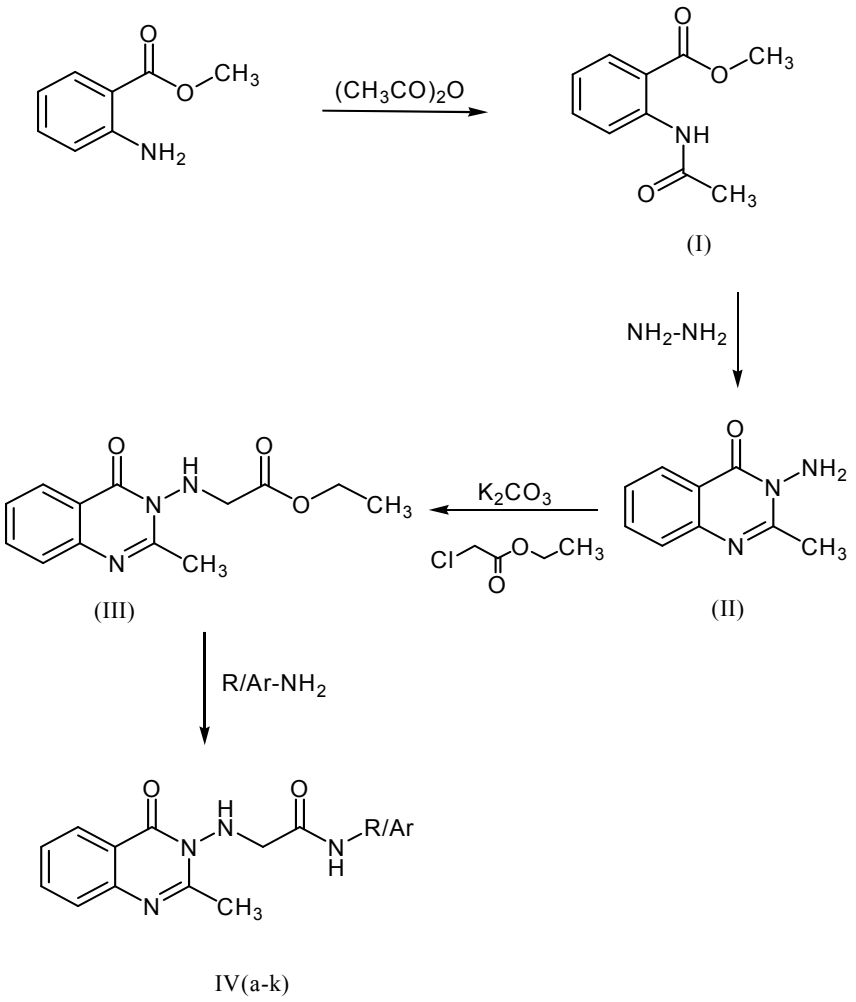
Key words: Quinazolinone, acetamide, anxiolytic

1. Introduction

Quinazoline-4(3*H*)-ones and its derivatives are versatile nitrogen heterocyclic compounds which have long been known as a promising class of biologically active compounds[i, ii]. Quinazolinone are excellent reservoir of bioactive substances. The stability of the Quinazoline nucleus has inspired medicinal chemists to introduce many bioactive moieties to this nucleus to synthesize new potential medicinal agents. Quinazoline is 1, 3-diazanaphthalene. It is also known as 5, 6-benzopyrimidine [iii], and its 4-oxo derivative is called 4(3*H*)-quinazolinone [iv-vi] Quinazolin-4(3H)-ones are also important building blocks in the synthesis of natural and pharmacological compounds[vii]. The Quinazoline skeleton, when selectively functionalized, is a building block for the preparation of numerous alkaloids and substances with pronounced biological activities.

2. Chemistry

Methyl Anthranilate was refluxed with acetic anhydride to form Methyl 2-Acetamidobenzoate (I). Compound (I) treated with hydrazine hydrate to form 2-methyl-3-amino-4-quinazolinone (II) which on treatment with ethyl chloroacetate in presence of K_2CO_3 using DMF as solvent form ethyl 2-(2-methyl-4-oxoquinazolin-3-ylamino) acetate (III). (III) on reaction with different aliphatic and aromatic amines produces 2-methyl-3-amino-4-quinazolinone acetamide derivatives



(I) (II) (III) IV(a-k)

3. Experimental

3.1 Chemistry

Methyl 2-Acetoamidobenzoate (I)

In 100 ml RBF, a solution of Methyl Anthranilate (0.016 mol) in acetic anhydride (0.127 mol) was refluxed for 15 minutes. The reaction mixture was cooled, poured into cold water (50 ml) containing a drop of pyridine and stirred until the oil solidifies. Crude product was filtered, washed with cold water and dried it at 100^0C. The product was recrystallised from ethanol.

Yield 73.70%, mp 98-100^0C, and IR (KBr, cm^{-1}): 1697.41 (C=O in ester), 1234.48 (C-O), 1593.25 (C=O in amide), 1527.67 (N-H), 1089.82 (C-N). Mass spectrum [M^+], m/z 193 (100%).

3-amino-2-methyl-4-quinazolinone (II)

Method I (Conventional)

In 100 ml RBF, a solution of hydrazine hydrate (10 ml) and Methyl 2-Acetamidobenzoate (2 gm) in ethanol was refluxed for 2 hours. The reaction mixture was cooled and stirred into cold water (50 ml). Crude product was filtered, washed with cold water and dried it at 100^0C. Crude product was recrystallised from ethanol.

Method II (Microwave)

In 100 ml RBF, a solution of hydrazine hydrate (10 ml) and 2 gm of Methyl 2-Acetoamidobenzoate (I) in ethanol was irradiated at 140 W for 3 min. The reaction mixture was cooled and stirred into cold water (50 ml). Crude product was filtered, washed with cold water and dried it at 100^0C. The product was recrystallised from ethanol.

Yield 66.54% (Conventional); 77.93% (Microwave)

mp 150-152^{0}C, and IR (KBr, cm^{-1}): 1666.91 (C=O), 1597.11 (N-H), 1257.63 (C-N), 3300.31 (N-H str.); mass spectrum [M^++2], m/z 177. ^{1}H NMR ($CDCl_3$, δ, ppm): 7.4-8.2 (Ar-H), 1.25 (s, 3H, CH_3), 1.98 (s, 2H, NH_2)

Ethyl 2-(2-methyl-4-oxoquinazolin-3-ylamino)acetate (III)

The mixture of 3-amino-2-methylquinazolin-4(3H)-one (0.01 mole), ethyl chloroacetate (0.01 mole) was irradiated under microwave at 700 W for 23-25 minutes in presence of anhydrous K_2CO_3 using DMF as solvent. The reaction mixture was cooled and poured into ice-cold water. The resulting solid was filtered, washed with water and recrystallised from ethanol/water. Yield 34.22%, mp 124-128^{0}C, and IR (KBr, cm^{-1}): 1728.28 (C=O), 1257.63 (C-O), 1197.83 (C-N), 3302.24 (N-H str.); mass spectrum [M^+], m/z 246. ^{1}H NMR ($CDCl_3$, δ, ppm): 7.4-8.2 (Ar-H), 1.62 (s, 3H, CH_3), 2.56 (s, 1H, NH), 2.71 (s, 2H, CH_2COO), 4.23 (q, 2H, $COOCH_2CH_3$), 1.25 (t, 3H, $COOCH_2CH_3$).

2-(2-methyl-4-oxoquinazolin-3(4H)-ylamino)acetamide (IVa)

It was obtained from (III) and ammonia in crystalline form. Yield 65%, mp 145-146^{0}C, IR (KBr, cm^{-1}): 1660.77 (amide I), 1599.04 (amide II), 1195.91(amide III), 3302.24 (N-H), 2949.26 (C-H alkyl).

2-(2-methyl-4-oxoquinazolin-3(4H)-ylamino)-N,N-diethylacetamide (IVb)

It was obtained from (III) and diethylamine in crystalline form. Yield 53%, mp 140-142^{0}C, IR (KBr, cm^{-1}): 1658.84 (amide I), 1597.11 (amide II), 1141.90(amide III), 3302.24 (N-H), 2951.19 (C-H alkyl).

2-(2-methyl-4-oxoquinazolin-3(4H)-ylamino)-N-phenylacetamide (IVc)

It was obtained from (III) and aniline in crystalline form. Yield 72%, mp 147-148^{0}C, IR (KBr, cm^{-1}): 1689.70 (amide I), 1600.97 (amide II), 1111.30 (amide III), 3311.98 (N-H), 2931.90 (C-H alkyl).

2-(2-methyl-4-oxoquinazolin-3(4H)-ylamino)-N-(3-chlorophenyl) acetamide (IVd)

It was obtained from (III) and m-chloroaniline in crystalline form. Yield 63%, mp 156-158^{0}C, IR (KBr, cm^{-1}): 1691.63 (amide I), 1599.04 (amide II), 1193.96 (amide III), 3306.10 (N-H), 2931.90 (C-H alkyl), 771.56 (C-Cl), 696.33, 771.56 (C-H bend m-substitution).

2-(2-methyl-4-oxoquinazolin-3(4H)-ylamino)-N-(4-chlorophenyl) acetamide (IVe)

It was obtained from (III) and p-chloroaniline in crystalline form. Yield 65%, mp 142-144^{0}C, IR (KBr, cm^{-1}): 1689.70 (amide I), 1600.97 (amide II), 1192.05 (amide III), 3311.89 (N-H), 2929.97 (C-H alkyl), 771.55 (C-Cl).

2-(2-methyl-4-oxoquinazolin-3(4H)-ylamino)-N-(3-nitrophenyl) acetamide (IVf)
It was obtained from (III) and m-nitroaniline in crystalline form. Yield 87%, mp 68-70^{0}C, IR (KBr, cm^{-1}): 1658.84 (amide I), 1597.11 (amide II), 1197.83 (amide III), 3302.24 (N-H), 2985.91 (C-H alkyl), 875.71 (C-N Nitro-aromatic), 1475.59 (N=O symmetric), 1429.30 (N=O asymmetric).

2-(2-methyl-4-oxoquinazolin-3(4H)-ylamino)-N-p-tolylacetamide (IVg)
It was obtained from (III) and p-toluidine in crystalline form. Yield 73%, mp 135-136^{0}C, IR (KBr, cm^{-1}): 1668.48 (amide I), 1602.90 (amide II), 1190.12 (amide III), 3309.96 (N-H), 2929.97 (C-H alkyl), 869.92 (C-H bend p-disubstituted).

2-(2-methyl-4-oxoquinazolin-3(4H)-ylamino)-N-m-tolylacetamide (IVh)
It was obtained from (III) and m-toluidine in crystalline form. Yield 64%, mp 144-146^{0}C, IR (KBr, cm^{-1}): 1691.63 (amide I), 1599.04 (amide II), 1196.91 (amide III), 3304.17 (N-H), 2931.90 (C-H alkyl), 696.33, 773.40 (C-H bend m-substituted).

2-(2-methyl-4-oxoquinazolin-3(4H)-ylamino)-N-morpholinoacetamide (IVi)
It was obtained from (III) and morpholine in crystalline form. Yield 87%, mp 132-134^{0}C, IR (KBr, cm^{-1}): 1689.70 (amide I), 1599.04 (amide II), 1196.91 (amide III), 3304.17 (N-H), 2963.98 (C-H alkyl), 1031.95 (Symmetric C-O-C stretch).

2-(2-methyl-4-oxoquinazolin-3(4H)-ylamino) acetohydrazide (IVj)
It was obtained from (III) and hydrazine hydrate in crystalline form. Yield 61%, mp 130-132^{0}C, IR (KBr, cm^{-1}): 1656.91 (amide I), 1595.18 (amide II), 1199.76 (amide III), 3302.24 (N-H), 2985.91 (C-H alkyl), 3551.47 (N-H free amine).

2-(2methyl-4-oxoquinazolin-3(4H)-ylamino)-N'-phenylacetohydrazide (IVk)
It was obtained from (III) and phenyl hydrazine in crystalline form. Yield 82%, mp 138-140^{0}C, IR (KBr, cm^{-1}): 1658.84 (amide I), 1597.11 (amide II), 1197.83 (amide III), 3302.24 (N-H), 2910.68 (C-H alkyl).

3.2 Anxiolytic Activity:

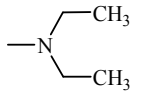
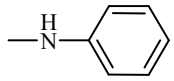
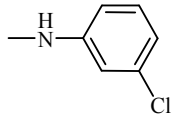
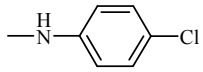
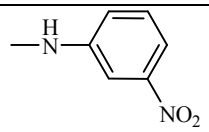
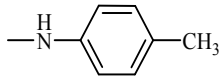
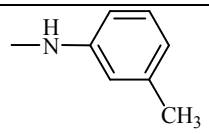
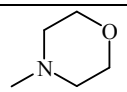
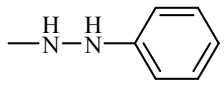
Anxiolytic activity was performed using Elevated plus maze (EPM) and Light and dark model. Male Swiss albino mice weighing between 18-22 gm were divided into 15 groups containing 5 animals each. The mice were treated with acetamide derivatives (30 mg/kg) 30 min before intraperitonial or 60 min before oral administration. The mice were placed centrally facing toward one of the closed arm of EPM and number of entries into open arms and time spent in open arms were evaluated. Similarly time spent in the light area of light and dark paradigm was taken as a measure of anxiety.

4. Results:

4.1 Chemistry

Table 1: Physical Characteristics of synthesized Acetamide Derivatives

Sr. No.	R/Ar	IUPAC name	Code	mp (^{0}C)	% Yield	R_F
1.	—NH_2	2-(2-methyl-4-oxoquinazolin-3-ylamino)acetamide	AA1	145-146	65	0.70
2.		2-(2-methyl-4-oxoquinazolin-3-ylamino)-N,N-	AA2	140-142	53	0.50

		diethylacetamide				
3.		2-(2-methyl-4-oxoquinazolin-3-ylamino)-N-phenylacetamide	AA3	147-148	72	0.53
4.		2-(2-methyl-4-oxoquinazolin-3-ylamino)-N-(3-chloro-phenyl)acetamide	AA4	156-158	63	0.47
5.		2-(2-methyl-4-oxoquinazolin-3-ylamino)-N-(4-chloro-phenyl)acetamide	AA5	142-144	65	0.52
6.		2-(2-methyl-4-oxoquinazolin-3-ylamino)-N-(3-nitrophenyl)acetamide	AA6	68-70	87	0.52
7.		2-(2-methyl-4-oxoquinazolin-3-ylamino)- N-p-tolylacetamide	AA7	135-136	73	0.40
8		2-(2-methyl-4-oxoquinazolin-3-ylamino)- N-m-tolylacetamide	AA8	144-146	64	0.41
9		2-(2-methyl-4-oxoquinazolin-3-ylamino)-N-morpholinoacetamide	AA9	132-134	87	0.39
10		2-(2-methyl-4-oxoquinazolin-3-ylamino) acetohydrazide	AA10	130-132	61	0.47
11		2-(2-methyl-4-oxoquinazolin-3-ylamino)-N'-phenylacetohydrazide	AA11	138-140	82	0.51

4.2 Anxiolytic Activity

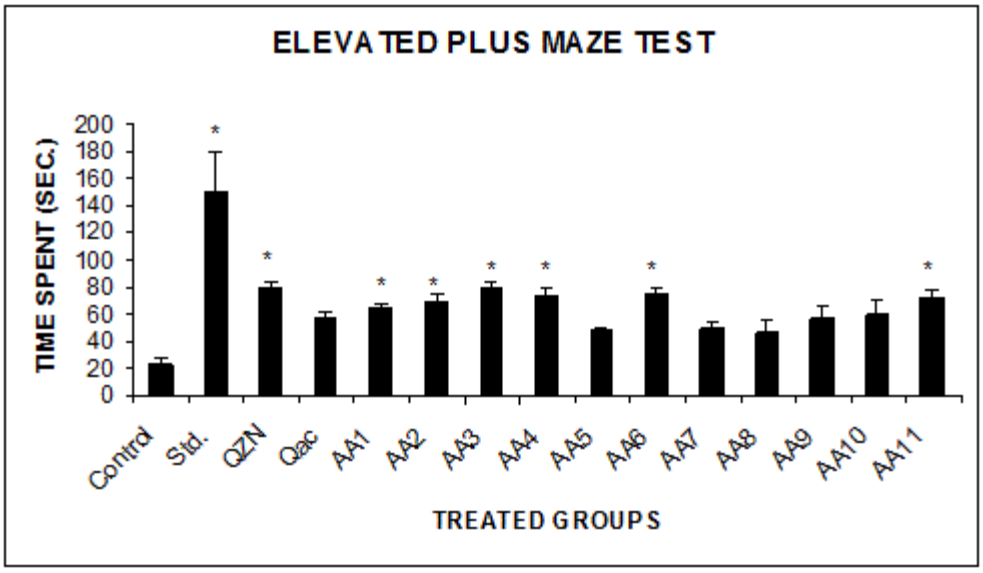


Figure 1: Effect of synthesized compound in elevated plus-maze test

All values are expressed as mean ± SEM, n=5, *p<0.05 compared with control. Statistical analysis was performed with One-way ANOVA followed by Dunnett's test. p<0.05 was considered as statistical significant.

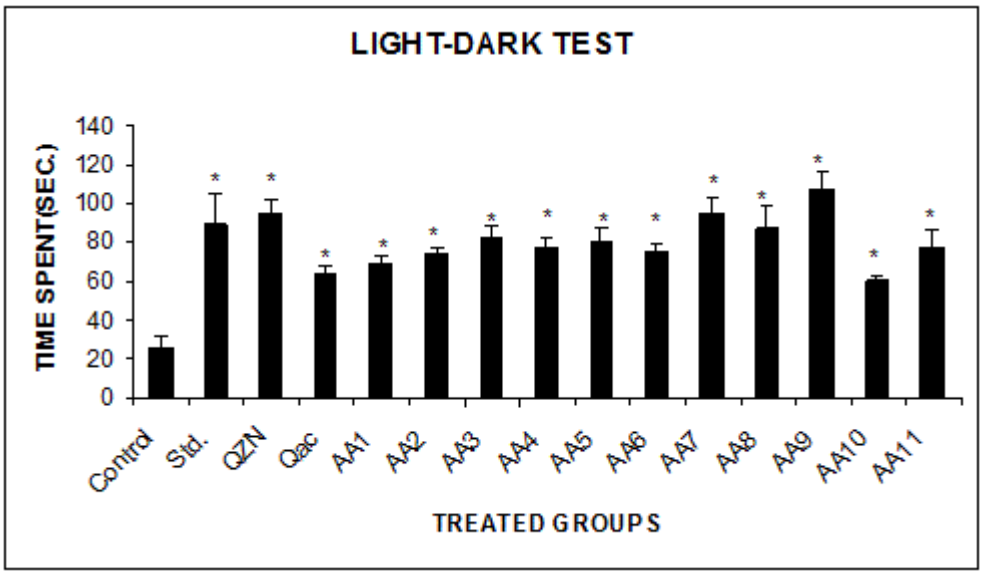


Figure 2: Effect of synthesized compound in light and dark test

All values are expressed as mean ± SEM, n=5, *p<0.05 compared with control. Statistical analysis was performed with One-way ANOVA followed by Dunnett's test. p<0.05 was considered as statistical significant.

5. Discussion

5.1 Chemistry

Our work is initiated with the reaction between Methyl Anthranilate (I) and acetic anhydride. To optimize the reaction conditions, the irradiation power and reaction time were variably investigated. We are pleased to find that the reaction provided of 3-amino-2-methyl-4-quinazolinone (III) in 77.93% yield after 3 min of irradiation at 140 W. In comparison, a conventional thermal heating of this reaction at reflux in ethanol for 2h gave 66.54%.

Mechanistically, the reaction proceeds via a methyl 2-acetamidobenzoate (I) intermediate. Appearance of molecular ion m/z 193 (M^+) in mass spectrum confirmed the intermediate (I).

Encouraged by this result, reaction of 3-amino-2-methyl-4-quinazolinones with ethyl chloroacetate in DMF in presence of potassium carbonate yielded 34.22% Ethyl 2-(2-methyl-4-oxoquinazolin-3-ylamino) acetate (III). Appearance of a quartet $COOCH_2CH_3$ at δ 4.2, triplet $COOCH_2CH_3$ at δ 1.2, singlet CH_2COO at δ 2.7 in ^1H NMR confirmed its formation.

Next, attention was focused on the reaction of various amines toward the Ethyl 2-(2-methyl-4-oxoquinazolin-3-ylamino) acetate. From IR spectral studies, disappearance of peak of ester functional group and appearance of characteristic peaks of amide I, amide II and amide III confirmed the acetamide derivatives. All Physical characteristics of acetamide library were given in table 1.

5.2 Anxiolytic Activity

In elevated plus maze model, QZN, AA1, AA2, AA3, AA4, AA6, AA11 showed a anxiolytic profile at dose 30 mg/kg as that of the standard anxiolytic diazepam. Among them **QZN** and **AA3** has shown the potent activity. (Figure 1)

On the other hand AA5, AA7, AA8, AA9, AA10 failed to alter any behavioural profile. The reason for the differences in the behavioural effects of these synthesized compounds is not clear.

In the light and dark Test, among acetamide library tested all compounds QZN, QAc, AA1, AA2, AA3, AA4, AA5, AA6, AA7, AA8, AA9, AA10 and AA11 showed an anxiolytic profile at dose 30 mg/kg as that of the standard anxiolytic diazepam. Among them **QZN** and **AA9** has shown the potent activity.

6. Conclusion

Considering the environment friendly role of neat reaction under microwaves, the bio potential of quinazolinone and our ongoing endeavours towards green synthesis, we have thus developed a facile, rapid and environmentally benign **microwave-assisted synthesis** of quinazolinone moieties like 3-amino-2-methyl-4-quinazolinone. Therefore, it has been demonstrated that the synthesis of a variety of heterocyclic compounds can be carried out safely in microwave reactors with remarkable rate enhancements with the reduction of time, improved yields and purity of the products.

According to pharmacological view, it is concluded highest anxiolytic activity has been observed with **aromatic** and **heteroaromatic acetamide** compounds.

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