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Synthesis, Characterization and Pharmacological Screening of 3-[5-Substituted-1, 3, 4- Oxadiazole-2-Yl]-2-Methyl Quinazolin-4(3*h*)-Ones

P. Siddartha kumar [1], S. Subramanyam [1], S. Raja [2], T. Srivalli [3], K. Yamini[3] and R. Suthakaran*

[1]Dept. of Pharmaceutical Chemistry, Bharath Institute of Techonology (Pharmacy), Mangalpally, Ibrahimpatnam, Andhra Pradesh.
[2]Dept. of Pharmaceutical Chemistry, GITAM Institute of Pharmacy, GITAM University, Vishakapatnam, Andhra Pradesh.
[3, *]Dept. of Pharmaceutical Chemistry, Teegala Ram Reddy College of Pharmacy, Meerpet, Hyderabad, Andhra Pradesh.

ABSTRACT

The purpose of the present study was taken with a desire to prepare and evaluate the derivatives for analgesic activity and anti-inflammatory activity confirming with spectral data. The facile synthesis of 3-[5-substituted-1, 3, 4-oxadiazole-2-yl]-2-methyl quinazolin-4(3*H*)-ones has been achieved by condensation (VII a- VII e). The synthesized compounds were characterized by IR, H^1 NMR and Mass spectra. The acute oral toxicity studies were done according to OECD guidelines 423(Acute Toxic Class Method). For accurate results and to compare the results for the same activity two methods have been selected. The analgesic studies for the derivatives were studied by using two models Viz., acetic acid induced writhing model and Hot-plate latency model in mice using acetyl salicylic acid as standard. The anti-inflammatory activities were studied by using two models like carrageenin induced paw-edema method and Dextran induced paw-edema method against Phenylbutazone as standard. Compounds like 3-((5-4-chlorophenyl)-1,3,4-oxadiazol-2-yl)methylamino)-2-methyl quinazolin-4(3*H*)-one (VIIe) and 3-((5-(2-chlorophenyl)- 1,3,4-oxadiazol-2-yl)methyl amino)-2- methyl quinazolin-4(3*H*)-one (VIIc) have shown promising analgesic and anti-inflammatory activity.

Keywords: Quinazolines, Oxadiazoles, Analgesic, Anti-inflammatory.

INTRODUCTION

Quinazoline is a bicyclic compound earlier known as benzo-1,3-diazine . The name quinazolinone ws proposed for its compound by weddige, on observing that this was isomeric with the compound cinnoline and quinaoxaline. The numbering of the quinazoline ring system which is currently used was suggested by Paal and Busch. However, the name 'Quinazoline' is now universally accepted. The oxo-derivative is suffixed by one, that is quinazolinone.

Brief account of reactivity of quinazolinone. Reactions associated with tautomeric nature of the quinazolinones, are often quite complex and generally unpredictable.

1. The amide linkage in quinazolinone should not be looked on as predominantly the keto (or) the enol form but as true keto-enol tautomers, showing the reaction characteristic of both the forms.
2. Quinazolinones are always high melting crystalline solids, insoluble in water and in most organic solvents but soluble in aqueous alkali.
3. They are generally insoluble in dilute acids but are sometimes soluble in conc. Acids as well.
4. Simple quinazolinones although insoluble in dilute acids, are soluble in 6N-Hydrochloric acid.
5. The 4(3*H*)- quinazolinones are stable and form mono hydrochlorides, chloroplatinates, chloroaurates and picrates and their metal salts of silver, mercury, zinc, copper, sodium and potassium.
6. The ring system in quinazolinone is exceedingly stable to oxidation, reduction, and hydrolysis reactions and with stands other treatments designed to break the ring.

***Corrosponding author**

R. Suthakaran

Email id: drsutha.2010@gmail.com

7. If a methyl group is present at 3-position, prohibiting the usual tautomerism, the methyl group is lost during the chlorination.
8. The position of alkylation of quinazolinones is similar to all the aromatic nitrogen heterocyclic systems in which a hydroxyl group is found ortho (or) para to the nitrogen position. Such compounds exist in tautomeric mixture, the two structures being inter-convertible by the shift of a proton and a pair of electrons.
9. Reactivity of the 2-methyl group, the methyl group in 2-position of 4(3*H*)- quinazolinone system was found to be quite reactive since it is linked to an azo-methine carbon and condenses with aldehydes to give aryl compounds.

These studies interestingly revealed that quite a few of such quinazolinone derivatives posses a wide variety of pharmacological activities.

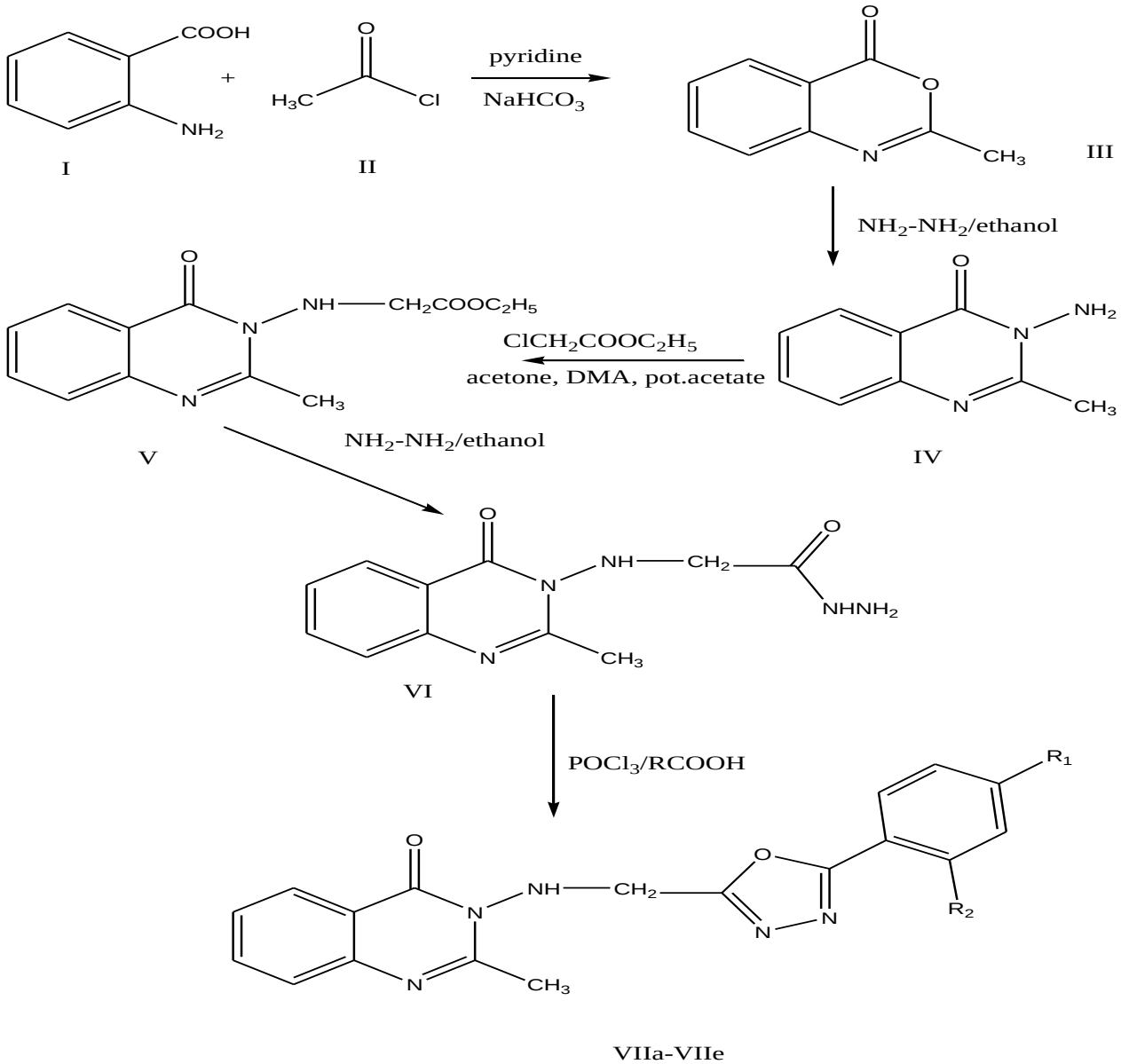
Oxadiazole

Oxadiazoles are five memberd heterocyclic compounds with two nitrogen atoms and one oxygen atom. They are synthesized by ring condensation and rearrangements. Depending on the position of heteroatoms, oxadiazoles are classified as 1,2,3-, 1,2,4-, 1,2,5- and 1,3,4- oxadiazoles.

MATERIALS AND METHODS

All the reactions were carried out under prescribed laboratory conditions. The solvents and reagents used in the synthetic work were of laboratory reagent grade and were purified by distillation and crystallization techniques (Sondhi *et al.,* 2007). Melting points of synthesized compounds were determined by open capillary method and were uncorrected. IR spectra of compounds were recorded on FT-IR spectrometer using KBr pellet. NMR spectra were recorded in JMR spectrometer using TMS as internal standard. Mass spectra were recorded in LC-MS spectrometer.

SCHEME



Experimental

Preparation of 2-methyl 3-amino quinazolin-4(3*H*)-one: (IV)

Step-1: Preparation of 2-methyl-4H-benzo[d] [1,3]oxazin-4-one: (III)

To a solution of anthranilic acid (0.1 mole) is taken in a beaker and pyridine,acetyl chloride (0.2 mole) was added. The reaction mixture is stirred continously further followed by 5 % of sodium bicarbonate. The solid obtainded is recrystallized from ethanol and dried.

Step-2: Preparation of 2-methyl 3-amino quinazolin-4(3*H*)-one: (IV)

A mixture of 2-methyl-4H-benzo[d] [1,3]oxazin-4-one (0.01 mole) compound was taken in round bottomed flask and treated with hydrazine hydrate in ethanol was refluxed for 3 hrs and thus resulting solution was poured into the crushed ice. A white precipitate was obtained and recrystallised with ethanol and dried.

Preparation of ethyl 2-(4-oxo-2-methyl quinazolin-4(3*H*)-yl-amino) acetate: (V)

A mixture of 2-methyl 3-amino quinazolin-4(3*H*)-one compound (0.01mole) was taken in round bottomed flask and treated with chloro ethyl acetate (0.01mole), dimethyl amine (DMA), acetone, potassium acetate, and refluxed for 6 hrs and the resulting solution was poured into crushed ice, precipitate was obtained, filtered and recrystallized from ethanol for two times and dried.

Preparation of 2-(4-oxo-2-methylquinazoli-4(3*H*)-yl-amino acetohydrazide: (VI)

A mixture of ethyl2-(4-oxo-2-methyl quinazolin-4(3*H*)-ylamino) acetate compound (0.01mole) was taken in round bottomed flask and treated with hydrazine hydrate (0.01mole),in ethanol refluxed for 3 hrs and the resulting solution was poured into crushed ice, precipitate was obtained, filtered and recrystallized from ethanol for two times and dried.

Preparation of 3-((5-phenyl-1,3,4-oxadiazol-2-yl)methylamino)-2-methyl quinazolin-4(3*H*) one: (VII a)

A mixture of 2-(4-oxo-2-methylquinazolin-4(3*H*)-yl-amino) acetohydrazide compound (0.01mole) was taken in round bottomed flask and treated with benzoic acid in $POCl_3$ refluxed for 5 hrs and the contents were cooled and poured into crushed ice. Then it was neutralized with sodium bicarbonate solution and resulting solid was filtered and recrystallized from ethanol and dried.

Preparation of 3-((5-phenylhydroxyl-1,3,4-oxadiazol-2-yl)methylamino)-2-methyl quinazolin-4(3*H*) one: (VIIb)

A mixture of 2-(4-oxo-2-methylquinazolin-4(3*H*)-yl-amino) acetohydrazide compound (0.01mole) was taken in round bottomed flask and treated with salicylic acid in $POCl_3$ refluxed for 5 hrs and the contents were cooled and poured into crushed ice. Then it was neutralized with sodium bicarbonate solution and resulting solid was filtered and recrystallized from ethanol and dried.

Preparation of 3-((5-(2-chlorophenyl)-1,3,4-oxadiazol-2-yl)methylamino)-2-methyl quinazolin-4(3*H*) one: (VIIc)

A mixture of 2-(4-oxo-2-methylquinazoli-4(3*H*)-yl-amino) acetohydrazide compound (0.01mole) was taken in round bottomed flask and treated with o-chloro benzoic acid in $POCl_3$ refluxed for 5 hrs and the contents were cooled and poured into crushed ice. Then it was neutralized with sodium bicarbonate solution and resulting solid was filtered and recrystallized from ethanol and dried.

Preparation of 3-((5-(2-nitrophenyl)-1,3,4-oxadiazol-2-yl)methylamino)-2-methyl quinazolin-4(3*H*) one: (VIId)

A mixture of 2-(4-oxo-2-methylquinazoli-4(3*H*)-yl-amino) acetohydrazide compound (0.01mole) was taken in round bottomed flask and treated with o-nitro benzoic acid in $POCl_3$ refluxed for 5 hrs and the contents were cooled and poured into crushed ice. Then it was neutralized with sodium bicarbonate solution and resulting solid was filtered and recrystallized from ethanol and dried.

Preparation of 3-((5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)methylamino)-2-methyl quinazolin-4(3*H*) one: (VIIe)

A mixture of 2-(4-oxo-2-methylquinazoli-4(3*H*)-yl-amino) acetohydrazide compound (0.01mole) was taken in round bottomed flask and treated with p-chloro benzoic acid in $POCl_3$ refluxed for 5 hrs and the contents were cooled and poured into crushed ice. Then it was neutralized with sodium bicarbonate solution and resulting solid was filtered and recrystallized from ethanol and dried.

Table.1 Physico-Chemical data of synthesized compounds

Derivative	Molecular formula	R'	R''	Molecular Weight(gm)	% yield	M.P (° C)	R_f value (cm)
VIIa	$C_{18}H_{15}N_5O_2$	H	--	333.12	65	154	0.69
VIIb	$C_{18}H_{15}N_5O_3$	OH	--	349.34	59	274	0.44
VIIc	$C_{18}H_{14}ClN_5O_2$	Cl	--	367.79	51	195	0.73
VIId	$C_{18}H_{13}N_6O_4$	NO_2	--	378.34	65	173	0.68
VIIe	$C_{18}H_{14}ClN_5O_2$	--	Cl	367.79	58	198	0.72

*Solvent system: n-hexane: ethyl acetate (1:1).

PHARMACOLOGICAL SCREENING

Acute toxicity study

Acute toxicity was carried out in vivo. All solutions were prepared using 2ml of 0.9% saline solution and administered per os using gastric tube. The acute oral toxicity study was conducted using the limit dose test of up and down procedure according to OECD/OCDE Test Guide lines on Acute Oral Toxicity under a computer guided Statistical Programe-AOT425 stat Pgm, version 1.0 (AOT, 2001), at a limit dose of 3000mg/kg body weight oral route and default of sigma at 0.5.

A total of five rats of either sex (three females, two males) were randomly selected out of a population of 38 rats by systematic randomization techniques. The population sample was selected such that the weight differences do not exceed ±10% of the mean initial weight of the sample population. The rats were fasted of rat chow overnight prior to dosing on each occasion. A rat was picked at a time, weighed and dosed with equivalent 3000mg/kg body weight of synthesized compunds VIIa, VIIb, VIIc, VIId and VIIe dissolved in 1ml of 0.9% saline used as the vehicle was given. Feeding was done using gastric feeding tube. Each animal was observed each time for signs of regurgitation and then kept in a metabolic cage. Each was watched for every 15 min in the first four hours dosing, then every 30 min for the short-term outcome and the remaining 12 days for the long-term possible lethal outcome which in this case was "death". Behavioral manifestations of acute oral toxicity were also noted. All observations were systematically recorded with individual records being maintained for each rat.

Analgesic activity

Animals

Albino mice weighing 200-250 gm, supplied by M/s: B.N. Ghosh & Co., Kolkatta, India, were placed in cages with wire-net floors in a controlled room temperature 29°C, Relative humidity 60-70% and provided with food and water ad libitum. The animals were deprived of food for 24 hrs before experimentation but allowed free access to tap water throughout. All studies were carried out by using six rats in each group.

Determination of Analgesic activity

Hot plate latency assay in Mice

Experiments were carried out according to method described (Rani *et al.*, 2004). Mice that showed nociceptive responses within 20 s when placed on hot plate maintained at 55± 0.5 °C were selected and grouped in to seven groups of (n=6). Group 1 was treated with saline groups 2 to 6 received 200 mg/kg p.o resp., while group 7 received 100 mg/kg of acetyl salicylic acid (standard drug) p.o., each mice was placed singly on the hot plate and the latency to exhibit thermal stimulus were determined at 0 hr, 0.5 hr, 1 hr and 2 hr before and after the treatment. Licking of paws and jumping were the parameters evaluated. Sixty seconds was taken as the cut-off time to avoid mouse tissue damage. Analgesic activity was expressed as mean percent maximal effect calculated as (Table 3).

% MPE= Post drug latency-Pre drug latency/cut off time pre drug latency.

Anti-inflammatory activity

Animals

Male albino Wister rats weighing 200-250 gm, supplied by M/s: B.N. Ghosh & Co., Kolkatta, India, were placed in cages with wire-net floors in a controlled room temperature 29° c, Relative humidity 60-70% and provided with food and water ad libitum. The animals were deprived of food for 24 hrs before experimentation but allowed free access to tap water throughout. All studies were carried out by using six rats in each group.

Determination of anti-inflammatory activity

Carrageenan induced rat paw oedema

Oedema was induced by subplanter injection of 0.1ml of 1% freshly prepared suspension of carrageenan in to the right hind paws of the rats of four groups of six animals each. The volume of the injected and contra-lateral paws were measured at 1hr,3hr and 5hr after induction of inflammation using a plethysmometer according to the method described by (Winter *et al.*, 1962). The test groups received the synthesized compounds at dose of 200mg/kg, the standard group received phenylbutazone at dose of 100mg/kg, and control animals received the vehicle only. All the treatments were given intraperitoneally 30 min prior to the injection of carrageenan except for the synthesized compounds. Increase of paw oedema thickness was calculated (Table 4).

Percentage Anti-inflammatory activity = 1–Vt /Vc × 100

Where, Vc and Vt are the volume of the paw oedema in control and drug treated resp

RESULTS AND DISCUSSION

Spectral data of *3-((5-phenyl-1,3,4-oxadiazol-2-yl)methylamino)-2-methyl quinazolin-4(3H) one* VIIa

I.R data **(cm^{-1}):** 3402.70(NH s), 3044.50(aromatic CH s, 1687.50(C=O), 1590.20(C=N imine), and1183(C-O-C). ***H^1 NMR(∂)*** **:** 2.6 (3H,S,-CH_3), 5.2 (2H,S,CH_2), 7.2 (1H,S,NH), 7.4-8.3(9H,M,Ar-H). ***Mass spectra*****:** 333 M^+

Spectral data of *3-((5-phenylhydroxyl-1, 3, 4-oxadiazol-2-yl) methylamine)-2-methyl quinazolin-4(3H) one* VIIb

I.R data **(cm^{-1}):** 3405.50 (NH s), 3120.10 (aromatic CH s), 1700.20 (C=O), 3575.30(OH s), 1593.30 (C=N imine), and 1107.20 (C-O-C).***H^1 NMR(∂)*** **:** 2.6 (3H,S,-CH_3), 5.4 (1H,S,OH), 4.9 (2H,S,-CH_2), 6.7 (1H,S,-NH), 7.1-8.1(8H,M,Ar-H).***Mass spectra*****:** 349 M^+

Spectral data of *3-((5-(2-chlorophenyl)-1, 3, 4-oxadiazol-2-yl)methylamino)-2-methyl quinazolin-4(3H) one* VIIc

I.R data **(cm^{-1}):** 3425.70 (NH stretch), 3043.50 (aromatic CH stretch), 1695.20 (C=O), 755.10 (C-Cl stretch),1600.30 (C=N imine), and 1123.30 (C-O-C). ***H^1 NMR(∂)*** **:** 2.53 (3H,S,-CH_3), 5.1 (2H,S,-CH_2), 6.8 (1H,S,-NH), 7.0-8.0 (8H,M,Ar-H).***Mass spectra*****:** 367 M^+

Spectral data of *3-((5-(2-nitrophenyl)-1, 3, 4-oxadiazol-2-yl)methylamino)-2-methyl quinazolin-4(3H) one* VIId

I.R data **(cm^{-1}):** 3413.70 (NH stretch), 3154.50 (aromatic CH stretch), 1681.20 (C=O), 912.50 (C-NO_2 stretch), 1601.50 (C=N imine), and 1116.20 (C-O-C).***H^1 NMR(∂)***

: 2.85 (3H,S,-CH_3), 4.85 (2H,S,-CH_2), 6.7 (1H,S,-NH), 7.1-7.9(8H,M,Ar-H).***Mass spectra*:** 378 M^+

Spectral data of *3-((5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)methylamino)-2-methyl quinazolin-4(3H) one* VIIe

***I.R data* (cm^{-1}):** 3410.70 (NH s), 3065.10 (aromatic CH s), 1680.20 (C=O), 757.10 (C-Cl stretch),1595.50 (C=N imine), and 1168.11 (C-O-C). ***H^1 NMR(∂)* :** 2.5 3H,S,-CH_3), 5.2 (2H,S,-CH_2), 6.8 (1H,S,-NH), 7.0-8.0(8H,M,Ar-H). ***Mass spectra*:** 367 M^+

Acute toxicity studies

The acute oral toxicity study was done according to the OECD guide lines 423 (Acute Toxic Class Method). There were no deaths of rats administered 300mg/kg of all the synthesized compounds VIIa,VIIb,VIIc,VIId, and VIIe with in short and long term outcome of the limit dose test up and down procedure. However, the observed behavioral signs of toxicity include irritation, restlessness, tachypnoea, anorexia, bilateral narrowing of the eyelids and abnormal posture (which was characterized by tugging of the head in between the hind limbs). The LD_{50} was calculated to be greater than 3000 mg/kg oral route (Table 2).

According to (Clarke and Clarke 1997) substances with LD_{50} of 1000mg/kg body weight/oral route are regarded as being safe or of low toxicity. The high LD_{50} obtained is an indication that the extract could be administered with a high degree of safety where the absorption might be incomplete due to inherent factors impeding the absorption in GIT. In the present study, acute toxicity study of all the synthesised compounds VIIa, VIIb, VIIc, VIId, VIIe showed that no mortality of rats occurred at a limit dose of 3000 mg/kg and 5mg/kg body weight given per os. This is an indication that all the synthesized compounds have low acute toxicity when administered per os.

Table.2 OECD guidelines 423 (Acute Toxic Class Method)

S. No	Treatment	Signs of toxicity	Onset of toxicity	Weight variation	Duration of observation
1	Compound VII a	Nil	Nil	Negligible	12 days
2	Compound VII b	Nil	Nil	Negligible	12 days
3	Compound VII c	Nil	Nil	Negligible	12 days
4	Compound VII d	Nil	Nil	Negligible	12 days
5	Compound VII e	Nil	Nil	Negligible	12 days

Table.3 Analgesic activity of synthesized compounds determined by Hot plate latency test on mice

Compound	Dose (mg/kg)	Percent analgesic activity			
		0 min	30 min	60 min	120 min
VIIa	100	27.39 ± 0.9	44.64 ± 0.6	59.53 ± 0.5	21.13 ± 0.9
VIIb	100	23.81 ± 0.2	38.99 ± 0.7	52.08 ± 0.7	17.26 ± 0.6
VIIc	100	33.63 ± 0.8	59.23 ± 0.7	75.30 ± 0.9	24.40 ± 0.1
VIId	100	16.47 ± 0.6	30.16 ± 0.2	46.73 ± 0.5	14.88 ± 0.5
VIIe	100	36.56 ± 0.2	62.59 ± 0.7	75.89 ± 0.3	30.32 ± 0.4
Standard (Aspirin)	100	39.25 ± 0.8	66.47 ± 0.7	77.25 ± 0.4	65.09 ± 0.8
Control	-	1.46 ± 0.7	2.27 ± 0.9	3.02 ± 0.5	2.09 ± 0.67

*Each value represents the mean ± SD (n=6). Significant difference relative to control at same time points and comparison were measured: $p < 0.001$, $p < 0.01$, $P < 0.05$

Table.4 Anti-inflammatory activity of synthesized compounds determined by Carrageenan induced paw oedema test in rats

Compound	Dose (mg/kg)	Percent Protection		
		1 hr	3 hr	5 hr
VIIa	200	38.78 ± 0.2	59.32 ± 0.8	44.84 ± 0.7
VIIb	200	35.38 ± 0.8	56.70 ± 0.5	40.05 ± 0.3
VIIc	200	52.38 ± 0.2	69.81 ± 0.9	56.32 ± 0.8
VIId	200	28.58 ± 0.2	43.52 ± 0.9	38.85 ± 0.7
VIIe	200	60.27 ± 0.3	70.32 ± 0.8	65.83 ± 0.5
Standard (Phenyl butazone)	200	62.58 ± 0.2	73.75 ± 0.3	70.02 ± 0.2
Control (Carageenan treated)	-	1.17 ± 0.1	1.95 ± 0.05	2.07 ± 0.8

*Each value represents the mean ± SD (n=6). Significant difference relative to control at same time points and comparison were measured: $p < 0.001$, $p < 0.01$, $P < 0.05$

CONCLUSION

The synthesis of 2-methyl 3-amino quinazolin-4(3H) one (IV) was carried out in step-I and Step-II. All the compounds were screened for analgesic activity using acetyl salicylic acid as the standard and the compounds showing significant activity were carried forward for anti-inflammatory screening using phenyl butazone as the standard. It can be concluded from our present work that compound 3-((5-4-chlorophenyl)-1,3,4-oxadiazol-2-yl)methylamino)-2-methyl quinazolin-4(3*H*)-one (VIIe) and 3-((5-(2-chlorophenyl)- 1,3,4-oxadiazol-2-yl)methyl amino)-2- methyl quinazolin-4(3*H*)-one (VIIc) have good analgesic and anti-inflammatory activity.

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