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An efficient and economic synthesis of new quinazolin-4-(3*H*)-one derivatives and their anti-inflammatory activity

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ABSTRACT

A simple, economical and practical synthetic procedure has developed for synthesizing various 2-substituted methyl-3-substituted phenylquinazolin-4(3H)-one derivatives ***4(a,b)-8(a,b)*** *in good yields from anthranilic acid* ***(1).*** *The developed procedure is very effective in respect of handling, yields and economic point of view. The structural characterization of synthesized products was elucidated by FT-IR, ^{1}H NMR, ^{13}C NMR, mass spectral data and elemental analysis. Anti-inflammatory activity of the synthesized quinazolin-4(3H)-one derivatives was screened after the acute toxicology studies examined on the title compounds. The results revealed that* ***5a***, ***8a*** *compounds exhibited potential activity and remaining compounds were shown moderate anti-inflammatory activity on tested animals.*

Keywords: Anthranilic acid, 4(3*H*)-Quinazolinone derivatives, Organic base, Acidic conditions, Anti-inflammatory activity.

INTRODUCTION

In general, many natural and synthetic nitrogen containing heterocyclic compounds are shown promising therapeutic activity to cure various diseases. 4(3*H*)-Quinazolinone derivatives are one of the most frequently encountered heterocyclic compounds in medicinal chemistry and are reported to have a broad spectrum of biological and pharmacological activities such as antifungal [1], antibacterial [2], anticancer [3, 4], hypotensive [5], anti–HIV [6], anti-inflammatory [7], and antitumour [8] as well as synthetic intermediates in several organic reactions. Substituted-4(3*H*)-quinazolinone derivatives, particularly at 2- and 3-positions play a significant role in the central nervous system (CNS) activity [9, 10] and hypertensive activity [11, 12].

Some natural products like Febrifugine and Isofebrifugine containing quinazolinone moiety (**Figure 1**) with chiral centers are potential antimalarial agents. This class of heterocyclic derivatives also exhibited biological and pharmaceutical activities. Recently, researchers have discovered a novel potent AMPA receptor antagonist CP-465,022 based on 3-(2-chlorophenyl)-6-fluoroquinazolin-4-one as the template.[13] In the literature, few patents delineated this moiety as decanoic acid (2-dimethylamino-ethyl)-{1-[3-(4-fluorophenyl)-4-oxa-3,4-dihydroquinazolin-2-yl]-ethyl amide as $CXCR_3$ ligand with micromolar affinity.[14]

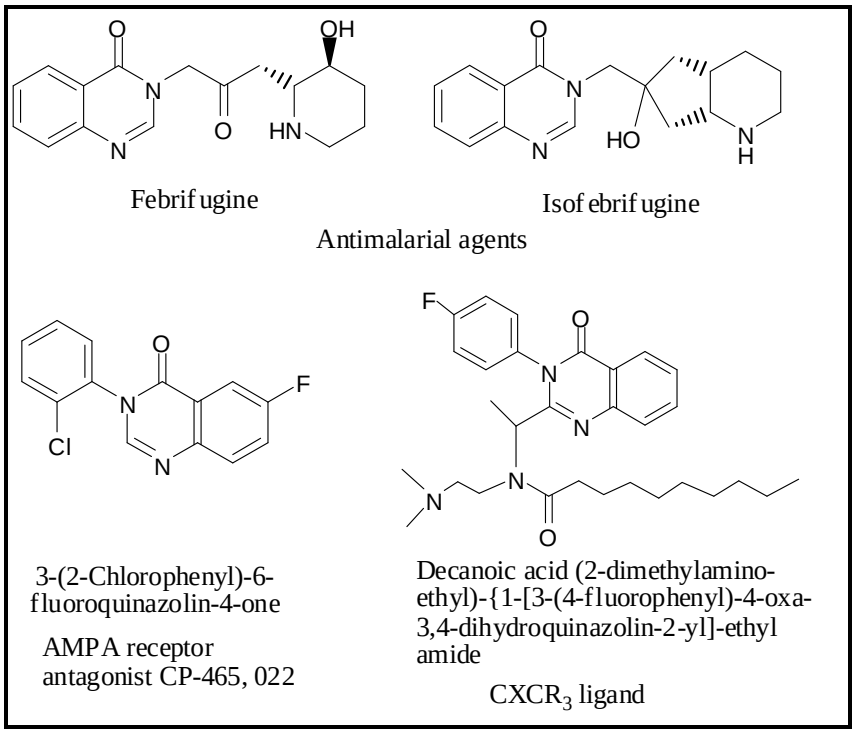


Figure 1: Some biologically active quinazolin-4(3H)-one derivatives.

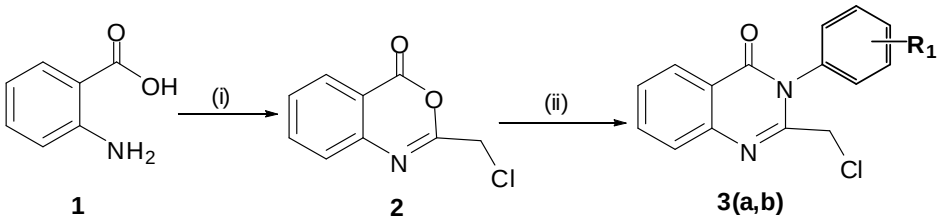
The art of organic synthesis is also enhanced by the development of improved purification techniques, parallel synthesis, automation, green-chemistry, new reagents that are cheaper, less toxic and easy to handle than the ones previously used for the same functional group transformations. Due to potential biological and pharmaceutical nature of quinazolin-4(3*H*)-one derivatives, the production of new biological active quinazolinone derivatives are needful and apart from finding short, efficient routes for the synthesis of these molecules have been accumulated a great importance.

The ubiquitous biological nature of 4(3*H*)-quinazolinone derivatives prompted the researchers to develop an efficient method for their synthesis for many years. Recently, Dubey *et. al* [15] reported the synthesis of (3*H*)-quinazolinone derivatives using inorganic base and maintained the basic conditions in consequent steps. The existing methods have not been entirely satisfactory owing to the number of disadvantages with respect to the long reaction time, requisite hard reaction conditions, handling, tedious work-up, yields and economic point of view. As a part of our programme, we report a new method for synthesis of 2,3-substituted-4(3*H*)-quinazolinone derivatives using organic base and maintained acidic conditions instead of tedious basic conditions in consequent synthesis at room temperature to moderate temperature. This synthetic methodology is not only interest from an economical point of view in many cases but also offer considerable synthetic advantages in terms of yield, selectivity and simplicity of the reaction procedure. By using this simple methodology, we have synthesized ten new biological active derivatives of 4(3*H*)-quinazolinone by incorporating different active aryl amines such as 2,6-difluoroaniline, 2-trifluoromethoxy aniline at 3-position as well as nitrogen nucleophiles (n-Boc piperizine, morpholine, 2-piperidine methanol, 2-piperidine ethanol, thiomorpholine) at 2-position.

MATERIALS AND METHODS

Chemistry: Melting points were determined using Guna melting point apparatus in open capillaries and are uncorrected. IR spectra were recorded using KBr pressed pellet method on FT/IR-5300 (JASCO) spectrometer. ^{1}H NMR spectra were recorded on a VARIAN 300 MHz mercury plus instrument with an internal standard of tetramethylsilane. Mass spectra were recorded on Mass instrument using (M^++1) mode. Spots on developed TLC were detected with UV-light and ninhydrine solution (as stain). Anthranilic acid, 2,6-difluoroaniline, 3-trifluoromethoxy aniline, and nitrogen nucleophiles (2^o amines) were procured from Aldrich, USA and from SD fine, Bombay were used without further purification.

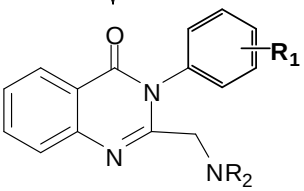
Scheme 1. Synthesis of 2,3-substituted quinazolin-4(3*H*)-one derivatives 4(a,b)-8(a,b).



1 **2** **3(a,b)**

3a) R_1= 2,6-difluoro
3b) R_1= 2-trifluoromethoxy

R_2	Compound
tert-butyl piperazine-1-carboxylate	**4(a,b)**
morpholine	**5(a,b)**
piperidin-2-ylmethanol	**6(a,b)**
2-(piperidin-2-yl)ethanol	**7(a,b)**
thiomorpholine	**8(a,b)**



4(a,b)-8(a,b)

Reagents and conditions: i) chloroacetyl chloride, DIPEA, DCM**,** rt, 2 h**,** ii) substituted anilines, PCl_3, ACN, 60 ^oC, 2 h, iii) R_2NH, DIPEA, NaI, ACN, rt, N_2, 12 h.

SYNTHESIS:

General synthetic procedure for synthesis of 2-substituted methyl-3-substituted phenylquinazolin-4(3*H*)-one derivatives 4(a,b)-8(a,b).

A mixture of anthranilic acid (36.5 mmol, l equiv.), diisopropylethylamine (DIPEA) (54.7 mmol, 1.5 equiv.) and chloroacetyl chloride (40.14 mmol, 1.1 equiv.) in dichloromethane (DCM) was stirred at room temperature for 2 h. The reaction mixture was diluted with water and extracted with ethyl acetate (2 x 50 mL). The combined organic layer was washed with water, brine and dried over with anhydrous Na_2SO_4. Solvent was evaporated under reduced pressure to obtain the product **2**. The intermediate compound **2** was treated with 2,6-difluroaniline/2-(trifluromethoxy)aniline (10.2 mmol, 1 equiv.), PCl_3 (15.30 mmol, 1.5 equiv.) in acetonitrile (ACN) at 60 ^oC for 2 h. After completion of the reaction, the reaction mixture was basified with satd. $NaHCO_3$ solution and extracted the crude product by ethyl acetate (3 × 15 mL) and dried over with anhydrous Na_2SO_4. The solvent was evaporated under vacuum to get the crude product which was purified by column chromatography with 20% ethyl acetate and hexane to afford the compounds **3a** and **3b** with percentage of 92, 89 respectively.

Synthesis of *t*-butyl-4-[(3-(2,6-difluorophenyl)-4-oxo-3,4-dihydroquinazolin-2-yl)methyl] piperazine-1-carboxylate (4a):

A mixture of **3a** (1equiv.), *tert*-butyl piperazine-1-carboxylate (1 equiv.), NaI (2 equiv.) and DIPEA (1.5 equiv.) in ACN was stirred at room temperature for 12 h under N_2 atmosphere. The progress of the reaction was monitored by TLC. After completion of the reaction, reaction mixture was diluted with cold water, extracted with ethyl acetate (3 x 15 mL). The combined organic layer was dried over with anhydrous Na_2SO_4 and concentrated to get crude product that was purified by 100-200 silica mesh with 40-60% ethyl acetate and pet ether to get compound **4a**. This same procedure was adopted for the preparation of remaining title products (**Table 1).** White solid, Yield 97%. mp: Mol. Wt: 456.20, mp 143-145 ^oC. IR (KBr, cm^{-1}): 3450, 2930, 1692, 1606, 1479, 1267. ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.42 (s, 9H, - C($\underline{CH_3}$)$_3$), 2.29 (m, 4H, -$\underline{CH_2}$-N-$\underline{CH_2}$- (piperazine)), 3.19 (m, 4H, -$\underline{CH_2}$-N-$\underline{CH_2}$- (piperazine)), 3.36 (s, 2H, -N–$\underline{CH_2}$- (methylene)), 7.18 (t, 2H, Ar-H), 7.41-7.59 (m, 2H, Ar-H), 7.7-7.9 (m, 2H, Ar-H), 8.32 (d, 1H, Ar-H). ^{13}C NMR (400 MHz, DMSO-d_6): δ (ppm) 28.37 (CH_3), 43.68 (CH_2), 52.57 (CH_2), 62.20 (CH_2), 79.72 (C), 111.70 (CH), 114.81 (C), 120.82 (C), 127.24 (CH), 127.60 (CH), 130.85 (CH), 134.97 (CH), 146.86 (C), 152.54 (C), 154.67 (C), 159.87 (C), 161.21 (C). MS: m/z (M^++1): 457. Anal. Calcd. for $C_{24}H_{26}F_2N_4O_3$: C, 63.15; H, 5.74; F, 8.32; N, 12.27; O, 10.51. Found: C, 63.12; H, 5.74; N, 12.26%.

3-(2,6-Difluorophenyl)-2-(morpholinomethyl)quinazolin-4(3H)-one (5a)

Brown solid, Yield 94%. Mol. Wt: 357.35, mp 168-170 ^oC. IR (KBr, cm^{-1}): 3434, 2916, 1692, 1605, 1472, 1112. ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 2.24 (m, 4H, -$\underline{CH_2}$-N-$\underline{CH_2}$-(morpholine)), 3.36 (s, 2H, -N-$\underline{CH_2}$- (methylene)),

3.49 (m, 4H, -C$\underline{H_2}$-O-C$\underline{H_2}$-(morpholine)), 7.1 (t, 2H, Ar-H), 741-7.56 (m, 2H, Ar-H), 7.74-7.84 (m, 2H, Ar-H), 8.315 (d, 1H, Ar-H). MS: m/z (M^++1): 358. Anal. Calcd. for $C_{19}H_{17}F_2N_3O_2$: C, 63.86; H, 4.79; F, 10.63; N, 11.76; O, 8.95. Found: C, 63.83; H, 4.76; N, 11.75%.

3-(2,6-Difluorophenyl)-2-[(2-(hydroxylmethyl)piperin-1-yl)methyl]quinazolin-4(3H)-one (6a)
White solid, Yield 89%. Mol. Wt: 385.16, mp 160-162 ^oC. IR (KBr, cm^{-1}): 3313, 2948, 1695, 1605, 1476, 1011 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.25-1.39 (m, 6H, piperidin), 2.3 (m, 1H, -N-C$\underline{H}$-CH_2- (piperidin)), 2.54 (m, 2H, -N-C$\underline{H_2}$- (piperidin)), 3.38-3.43 (m, 4H, -C$\underline{H_2}$-OH, -C$\underline{H_2}$- (methylene)), 3.78-3.83 (br, 1H, -O$\underline{H}$), 7.13-7.14 (t, 2H, Ar-H), 7.53 (q, 2H, Ar-H), 7.76-7.81 (m, 2H, Ar-H), 8.96 (d, 1H, Ar-H). ^{13}C NMR (400 MHz, DMSO-d_6): δ (ppm) 22.97 (CH_2), 24.89 (CH_2), 27.7 (CH_2), 53.48 (CH_2), 58.90 (CH_2), 63.58 (CH_2), 76.76 (CH), 112.18 (CH), 114.31 (C), 120.56 (C), 127.27 (CH), 127.58 (CH), 131.22 (CH), 13511 (CH), 146.62 (C), 154.84 (C), 157.31 (C), 161.11 (C). MS: m/z (M^++1): 386.

3-(2,6-Difluorophenyl)-2-[(2-(2-hydroxyethyl)piperidin-1-yl)methyl]quinazolin-4(3H)-one (7a)
Yellow solid, Yield: 90%. Mol. Wt: 399.43, mp 144-146 ^oC. IR (KBr, cm^{-1}): 3485, 2935, 1671, 1600, 1473, 1010. ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.28-1.46 (m, 6H, piperidine), 1.82 (m, 2H, -C$\underline{H_2}$), 2.2 (m, 2H, piperidine), 2.6 (s, 2H, methylene), 2.75 (m, 1H, piperidine), 3.46 (br, 1H, -O$\underline{H}$), 3.59-3.73 (m, 2H, -C$\underline{H_2}$-OH), 7.13 (t, 2H, phenyl), 7.46-7.54 (m, 2H, phenyl), 7.79 (d, 2H, phenyl), 8.29 (d, 1H, phenyl). ^{13}C NMR (400 MHz, DMSO-d_6): δ (ppm) 23.79 (CH_2), 27.91 (CH_2), 30.77 (CH_2), 49.57 (CH_2), 56.76 (CH_2), 57.34 (CH_2), 60.75 (CH), 76.77 (CH_2), 112.03 (CH), 114.20 (C), 120.54 (C), 127.35 (CH), 127.48 (CH), 131.15 (CH), 135.09 (CH), 146.75 (C), 154.06 (C), 159.66 (C), 161.15 (C). MS: m/z (M^++1): 400.

3-(2,6-Difluorophenyl)-2-(thiomorpholinomethyl)quinazolin-4(3H)-one (8a)
Brown solid, Yield 95%. Mol. Wt: 373.11, mp 114-116 ^oC, IR (KBr, cm^{-1}): 3392, 2951, 1684, 1600, 1469, 986 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 2.40-2.43 (t, 4H, -C$\underline{H_2}$-S-C$\underline{H_2}$- (thiomorpholin)), 2.47-2.50 (t, 4H, -C$\underline{H_2}$-N-C$\underline{H_2}$- (thiomorpholin)), 3.35 (S, 2H, methylene), 7.07-7.12 (t, 2H, phenyl), 7.47-7.53 (m, 2H, phenyl), 7.76-7.81 (m, 2H, phenyl), 8.3 (d, 1H, phenyl). MS: m/z (M^++1): 374.

t-butyl-4-[(4-oxo-3-(3-trifluoromethoxy)phenyl)-3,4-dihydroquinazolin-2-yl)methyl] piperazine-1-carboxylate (4b)
Brown solid, Yield 94%. Mol. Wt: 504.20, mp 85-87 ^oC. IR (KBr, cm^{-1}): 3445, 2977,
1694, 4596, 1473, 1268, 1164. ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.43 (s, 9H, - C($\underline{CH_3}$)$_3$), 2.24 (m, 4H, -C$\underline{H_2}$-N-C$\underline{H_2}$- (piperazine)), 2.4 (s, 2H, methylene), 3.27 (m, 4H, -C$\underline{H_2}$-N-C$\underline{H_2}$- (piperazine)), 7.26-7.38 (m, 3H. Phenyl), 7.55 (q, 2H, phenyl), 7.74-7.78 (m, 2H, phenyl), 8.29 (d, 1H, phenyl). ^{13}C NMR (400 MHz DMSO-d_6): δ (ppm) 29.70 (CH_3), 53.29 (CH_2), 57.99 (CH_2), 60.415 (CH_2), 77.397 (C), 114.14 (CH), 119.48 (CH), 120.61 (CH), 121.63 (C), 127.08 (CH), 127.51 (CH), 128.905 (CH), 130.69 (CH), 134.07 (CH), 134.98 (C), 146.45 (C), 154.63 (C), 154.775 (C), 162.136 (C), 163.702 (C). MS: m/z (M^++1): 505.

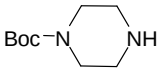
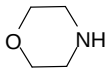
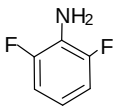
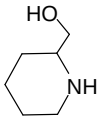
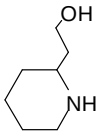
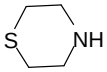
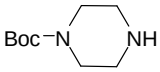
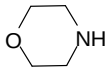
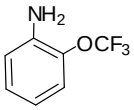
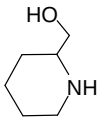
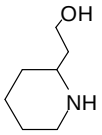
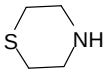
2-(Morpholinomethyl)-3-(3-(trifluoromethoxy)phenyl)quinazolin-4(3H)-one (5b)
White solid, Yield 96%. Mol. Wt: 405.13, mp 103-105 ^oC. IR (KBr, cm^{-1}): 3435, 2937, 1682, 1596, 1474, 1273, 1165. ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 2.29-2.31 (m, 2H, -C$\underline{H_2}$-N-C$\underline{H_2}$-(morpholine)), 3.53-3.56 (m, 4H, -C$\underline{H_2}$-O-C$\underline{H_2}$- (morpholine)), 2.37 (s, 2H, methylene), 7.28-7.38 (m, 3H, phenyl), 7.57 (q, 2H, phenyl), 7.74-7.78 (m, 2H, phenyl), 9.29 (d, 1H, phenyl). MS: m/z (M^++1): 406. Anal. Calcd for $C_{20}H_{18}F_3N_3O_3$: C, 59.26; H, 4.48; F, 14.06; N, 10.37; O, 11.84. Found: C, 59.25; H, 4.47; N, 10.36%.

2-[(2-Hydroxymethyl)piperidin-1-yl)methyl]-3-(3-(trifluoromethoxy)phenyl) quinazolin-4(3H)-one (6b)
Crystalline white solid, Yield 85%. Mol. Wt: 449.16, mp 98-100 ^oC. IR (KBr, cm^{-1}): 3406, 2932, 1677, 1592, 1475, 1252, 1168, 1040. ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.29-1.62 (m, 6H, -C$\underline{H_2}$-C$\underline{H_2}$-C$\underline{H}$2- (piperidine)), 2.43-2.46 (m, 2H, -N-C$\underline{H_2}$- (piperidine)), 2.52-2.56 (m, 1H, -C$\underline{H}$-N-, (piperidine)), 2.62-2.78 (m, 2H, methylene), 3.38-3.5 (m, 2H, -C$\underline{H_2}$-OH), 3.7 (t, 1H, -O$\underline{H}$), 6.83-6.93 (m, 1H, phenyl), 7.22-7.28 (m, 1H, Phenyl) 7.38-7.63 (m, 4H, phenyl), 7.73-7.98 (m, 2H, phenyl), 8.29 (d, 1H, phenyl). ^{13}C NMR (400 MHz DMSO-*d6)*: δ (ppm) 27.12 (CH_2), 29.36 (CH_2), 30.02 (CH_2), 49.31 (CH_2), 56.41 (CH_2), 57.55 (CH_2), 59.54 (CH_2), 60.93 (CH_2), 113.01 (CH), 116.55 (CH), 120.76 (C), 121.90 (CH), 123.15 (C), 126.97 (CH), 127.11 (CH), 128.60 (CH), 129.86 (CH), 132.01 (CH), 134.93 (C), 146.67 (C), 153.88 (C), 162.18 (C), 171.52 (C). MS: m/z (M^++1): 450.

2-[(2-(2-Hydroxyethyl)piperidin-1-yl)methyl)-3-(trifluoromethoxy)phenyl]quinazolin-4(3H)-one (7b)
Yellow solid, Yield 91%. Mol. Wt: 447.18, mp 154-156 ^oC. IR (KBr, cm^{-1}): 3338, 2930, 1676, 1594, 1476, 1258, 1164, 1056. ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.28-1.58 (m, 8H, piperidine and –$\underline{CH_2}$-CH_2-OH), 2.21-2.23 (m, 1H, -N-C$\underline{H}$–CH_2-CH_2-OH (2-hydroxyethyl piperidine)), 2.74-2.84 (m, 2H,-N-C$\underline{H_2}$- (2-hydroxyethyl piperidine)), 3.34-3.42 (s, 2H, methylene), 3.56-3.66 (m, 2H, -C$\underline{H_2}$-OH), 3.77-3.82 (br, 1H, -CH2-O$\underline{H}$), 7.18-7.3 (m, 2H, phenyl), 7.39 (d, 1H, phenyl), 7.52 (m, 1H, phenyl), 7.59 (t, 1H, phenyl), 7.95 (d, 2H, phenyl), 8.27 (d, 1H, phenyl). MS: m/z (M^++1): 448.

2-(Thiomorpholinomethyl)-3-(3-(trifluoromethoxy)phenyl)quinazolin-4(3H)-one (8b)
White solid, Yield 94%. Mol. Wt: 421.11, mp 116-118 ^{o}C. IR (KBr, cm^{-1}): 3410, 2955, 1685, 1610, 1473, 686 cm^{-1}.^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 2.43-2.45 (t, 4H, -C$\underline{H_2}$-S-C$\underline{H_2}$- (thiomorpholin)), 2.46-2.48 (t, 4H, -C$\underline{H_2}$-N-C$\underline{H_2}$- (thiomorpholin)), 3.3 (s, 2H, methylene), 7.24-7.29 (m. 2H, phenyl), 7.365 (d, 1H, phenyl), 7.5-7.59 (m, 2H, phenyl), 7.76-7.83 (m, 2H, phenyl), 8.29 (d, 1H, phenyl). MS: m/z (M^{+}+1): 422.

Table 1: Physical characterization data of 2,3-substituted quinazolin-4(3*H*)-one derivatives 4(a,b)-8(a,b)

Aniline derivatives	Nitrogen nucleophiles	Product	Reaction time (h)	Yield (%)	M.P (°C)
		**4a**	12	97	143-145
		**5a**	12	94	168-170
		**6a**	12	89	160-162
		**7a**	12	90	144-146
		**8a**	12	95	114-116
		**4b**	12	94	85-87
		**5b**	12	96	103-105
		**6b**	12	85	98-100
		**7b**	12	91	154-156
		**8b**	12	94	116-118

Biological Activity
All the experiments were carried out using Wister rats. The animals had free access to food and water and they were housed under natural (12 h each) light-dark cycle with access to standard to pellet chow and water. Although the relative humidity should be atleast 30 % and preferably not exceed 70 % other than during room cleaning. The optimum aim should be 50-60 %. The animals were acclimatized for 7 days to the laboratory conditions before performing the experiments. Animals were group-caged by dose, but the number of animals per cage must not interfere with clear observation of each animal.

Acute toxicology: The newly synthesized compounds **4(a,b)-8(a,b)** were assayed for their anti-inflammatory activity. Doses were selected based upon the acute toxicity studies. Acute toxicology study was performed on the title compounds according to Organization for Economic Co-operation and Development (OECD) guidelines for testing of chemicals, Number 423 "Acute Oral Toxicity – Acute Toxic Class Method", adopted 17th December 2001. Each group consists of 3 Wister rats (overnight fasted) was kept in the colony cage at 25 ± 2°C with 55 % relative humidity and 12 h light/dark cycle was maintained. A specified fixed dose of 250, 500, 750, 1000, 1500, 2000, 3000 and 4000 mg/kg was selected and administered orally as a single dose as fine suspension prepared in saline using gum acacia powder. The acute toxic symptoms and the behavioral changes such as dullness, piloerection and recumbency produced by the test compounds were observed continuously for 4 h, 8 h, 12 h and 24 h onset of toxic symptoms and gross behavioral changes were also recorded. For the compounds **4a**, **6a**, **7a**, **4b**, **5b**, **6b**, **7b** and **8b** mortalities were found at 3000 mg/kg bw during the 24 h of observation, **5a** and **8a**, the mortalities were found at 2000 mg/kg bw during the 24 h of observation. Lacrimation was observed in addition to dullness, piloerection and

recumbency in treated animals with **5a** and **8a** compounds (300 mg/kg bw) during first 4 h of observation. No abnormalities were detected during the observation of gross pathology for all the tested animals.

Carrageenan induced paw oedema model[21]**.** The test was used to determine the anti-inflammatory activity of the title compounds. The animals were divided into four groups of six animals each and were fasted for a period of 24 h prior to the study. Group 1 was treated as control and Group 2 received diclofenac sodium 100 mg/Kg/mL, suspended in 0.5 % sodium carboxymethyl cellulose. Group 3 and 4 were treated with title compounds 5 and 50 mg/Kg/mL, in which the concentration of the title compounds were taken based on acute toxicology studies of the test solutions. Oedema was induced by injecting l00 µL of a 1 % solution of carrageenan in saline into the subplantar region of the right hind paw of the Wister rats. The vehicle, test solutions and the standard drugs were administered 60 min. prior to the injection of the phlogestic agent. The volumes of oedema of the injected and contralaterals paws were measured at 0, 1, 3, 6 and 12 h after the induction of inflammation using a plethysomgraph. The data is represented in **Table 2**.

Table 2 Anti-inflammatory activity of 2,3-substituted quinazolin-4(3*H*)-one derivatives 4(a,b)-8(a,b) by Carrageenan induced paw oedema model.

Group	Dose mg/kg	Paw volume				
		0 hour	1 hour	3 hour	6 hour	12 hour
Control	CMC	1.917 ± 0.117	1.950 ± 0.084	2.000 ± 0.126	1.833 ± 0.137	1.667 ± 0.103
PC	100	1.833 ± 0.082	1.567 ± 0.082[b]	1.350 ± 0.105[b]	1.167 ± 0.121[b]	1.083 ± 0.075[b]
4a	5	1.800 ± 0.089	1.683 ± 0.117[b]	1.483 ± 0.147[b]	1.317 ± 0.075[b]	1.300 ± 0.063[b]
5a	50	1.817 ± 0.075	1.617 ± 0.075[b]	1.433 ± 0.082[b]	1.233 ± 0.082[b]	1.183 ± 0.041[b]
6a	5	1.783 ± 0.075[a]	1.717 ± 0.075[b]	1.517 ± 0.075[b]	1.300 ± 0.063[b]	1.250 ± 0.055[b]
7a	5	1.850 ± 0.084	1.767 ± 0.052[b]	1.533 ± 0.052[b]	1.350 ± 0.055[b]	1.150 ± 0.055[b]
8a	50	1.800 ± 0.063	1.600 ± 0.063[b]	1.383 ± 0.075[b]	1.200 ± 0.089[b]	1.133 ± 0.052[b]
4b	5	1.867 ± 0.052	1.817 ± 0.041[a]	1.633 ± 0.052[b]	1.433 ± 0.052[b]	1.267 ± 0.052[b]
5b	5	1.850 ± 0.055	1.700 ± 0.089[b]	1.483 ± 0.117[b]	1.350 ± 0.084[b]	1.217 ± 0.075[b]
6b	5	1.883 ± 0.041	1.717 ± 0.075[b]	1.517 ± 0.075[b]	1.367 ± 0.052[b]	1.193 ± 0.041[b]
7b	5	1.321 ± 0.053	1.756 ± 0.058[b]	1.471 ± 0.051[b]	1.425 ± 0.048[b]	1.231 ± 0.045[b]
8b	5	1.524 ± 0.056	1.722 ± 0.042[a]	1.466 ± 0.042[b]	1.424 ± 0.056[b]	1.255 ± 0.048[b]

**P values a = P < 0.05, b = P < 0.01, c = P < 0.001, Control = 0.5 % CMC (Carboxymethyl cellulose) and PC = Positive Control (Dichlofenac).*

Cotton pellet-induced granuloma model. The test was performed on the Wister rats using the cotton pellet-induced granuloma method. The rats were anesthetized under light ether, and an incision was made on the lumbar region by curved scissors, a subcutaneous tunnel was made and a sterilized cotton pellet (100 ± 1 mg) was inserted using blunted forceps in that area. All the animals received either title compounds or diclofenac sodium or vehicle (0.5% CMC) orally depending upon their respective group for seven consecutive days from the day of cotton pellet insertion. On 8th day, the animals were anesthetized again and cotton pellets were removed, dried and weighed. The data is shown in **Table 3** and % of inhibition was calculated by using the following formula.

$$\% \text{ of inhibition} = \left(1 - \frac{\text{Wt}}{\text{Wc}}\right) \times 100$$

$$\text{Wt} = \text{Weight of the cotton pellet of the test solution}$$

$$\text{Wt} = \text{Weight of the cotton pellet of the control}$$

Table 3 Anti-inflammatory activity of 2,3-substituted quinazolin-4(3*H*)-one derivatives 4(a,b)-8(a,b) by Cotton pellet – induced granuloma model

Group	Dose (mg/kg bw)	Granuloma dry weight (mg)	% inhibition
Control	0.5 % CMC	63.667 ± 1.366	-
PC	100	29.667 ± 1.211[b]	53.40
4a	5	35.833 ± 0.753[b]	43.71
5a	50	34.500 ± 1.049[b]	45.81
6a	5	38.500 ± 1.517[b]	39.52
7a	5	39.333 ± 1.506[b]	38.22
8a	50	31.833 ± 1.941[b]	50.00
4b	5	41.000 ± 1.673[b]	35.60
5b	5	35.500 ± 1.225[b]	44.24
6b	5	37.167 ± 1.169[b]	41.62
7b	5	34.667 ± 1.211[b]	45.54
8b	5	32.623 ± 1.225[b]	43.32

**P values a = P < 0.05, b = P < 0.01, c = P < 0.001 when compared with normal control*
Control = 0.5 % CMC (Carboxymethyl cellulose), PC = Positive Control (Dichlofenac).
** Highlighted values are statistically significant from control (P < 0.05)*

Carrageenan induced air-pouch model. The rats were divided into 11 groups (n = 6) and air-pouch was produced. Rats were anesthetized and air cavities were produced by subcutaneous injection of 20 mL of sterile air into the

intrascapular area of the back on 0 day by using 0.22 µm filter. An additional 10 mL of air was injected into the cavity for every 3rd day (3rd and 6th day) to keep the space open. On 7th day, 2 mL of 1% solution of carrageenan dissolved in saline was injected directly into the pouch to induce an inflammatory response. The rats were orally pre-treated with either vehicle or title compounds or diclofenac sodium 2 h prior to the injection of carrageenan. The second dose of treatment was repeated after 24 h of the first treatment. After 48 h of carrageenan injection, the rats were anesthetized with ether and the pouch was carefully opened by a small incision. The volume of exudates was collected and measured. An aliquot of the exudates was used for quantification of leukocyte concentration using a haemocytometer and differential cell count was performed using a manual cell counter after staining with Wright's stain. Neutrophils are segmented white blood cells. The cytoplasm contains both primary and secondary granules that take up both acidic and basic dyes of the Wright stain. Monocytes are mononuclear white blood cells that remove debris and micro-organisms by phagocytises and possess antigen for recognition by immune lymphocytes. The results were expressed as the total number of neutrophils and monocytes. The data is presented in **Table 4**.

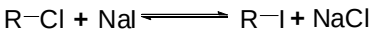
Table 4 Anti-inflammatory activity of 2,3-substituted quinazolin-4(3*H*)-one derivatives 4(a,b)-8(a,b) by Carrageenan induced air – pouch model.

Group	Dose	Excudate volume	Neutrophils 10^6	Monocytes x 10^6
Control	CMC	3.917 ± 0.194	309.333± 9.771	129.500 ± 3.082
PC	100	0.983 ± 0.133[b]	111.833±7.548[b]	60.000 ± 4.517[b]
4a	5	1.300 ± 0.063[b]	157.833±7.139[b]	83.333 ± 3.327[b]
5a	50	1.167 ± 0.052[b]	144.667±4.885[b]	74.333 ± 2.944[b]
6a	5	1.450 ± 0.105[b]	161.333±4.803[b]	89.833 ± 2.041[b]
7a	5	1.467 ± 0.082[b]	162.167±4.070[b]	91.833 ± 2.563[b]
8a	50	1.033 ± 0.121[b]	129.167±4.792[b]	68.167 ± 2.041[b]
4b	5	1.617 ± 0.117[b]	178.667±4.844[b]	93.500 ± 2.074[b]
5b	5	1.350 ± 0.105[b]	158.500±3.017[b]	84.833 ± 1.602[b]
6b	5	1.400 ± 0.089[b]	160.833±3.312[b]	83.833 ± 1.722[b]
7b	5	1.183 ± 0.098[b]	133.833±3.764[b]	80.167 ± 2.137[b]
8b	5	1.356 ± 0.088[b]	131.511±3.456[b]	80.012 ± 2.011[b]

P values a = P < 0.05, b = P < 0.01, c = P < 0.001, Control = 0.5 % CMC (Carboxymethyl cellulose) and PC= Positive Control (Dichlofenac).

RESULTS AND DISCUSSION

We have developed a new synthetic methodology under acidic, requisite room temperature (simple) conditions and using organic base for synthesizing **4(a,b)-8(a,b)** underlined in **Scheme 1,** resulting the optimum yields. Starting, 2-chloromethyl benzo[*d*][1,3]oxazin-4-one **(2)** intermediate was obtained in high yield (89%) by reaction of anthranilic acid **(1)** with chloroacetyl chloride in presence of DIPEA in DCM at room temperature. Subsequently, the intermediate compound **2** was reacted with aniline derivatives such as 2,6-difluoroaniline, 3-trifluoromethoxy aniline in ACN containing PCl_3 (acidic condition) at moderate temperature (60 ^oC) for 2 h under N_2 atmosphere and followed by work-up to afford 2-(chloromethyl)-3-(2,6-difluorophenyl)quinazolin-4(3*H*)-one **(3a)** and 2-(chloromethyl)-3-(3-trifluoromethoxy phenyl)quinazolin-4(3*H*)-one **(3b)** scaffolds in high yields 92%, 89% respectively. Finally, the targeted compounds **4(a,b)-8(a,b)** have synthesized by replacement of Cl in **3(a,b)** with secondary amines such as n-Boc piperizine, morpholine, 2-piperidine methanol, 2-piperidine ethanol, thiomorpholine in presence of DIPEA and NaI in ACN at room temperature for 12 h with quantitative yields shown in **Table 1**. It was observed that in absence of NaI, the direct substitution of chlorine with nitrogen nucleophiles in ACN using DIPEA gave low yield (42%) even applied hard conditions (95^oC). The reaction is favored in presence of NaI, because the possible explanation is that the initial replacement of chlorine of compound **3** replaced by iodine with subsequent nucleophilic substitution of iodo derivatives of compound **3** by the nitrogen nucleophile is facilitated.



Chemical structures of all the newly synthesized compounds were confirmed by their elemental analyses, IR, ^1H NMR, ^{13}C NMR and mass spectral data and are represented in the experimental section. Experimentally, the absorption bands of IR spectra for compounds **4(a,b)-8(a,b)** are appeared in the region of 1660-1700, 1590-1640 and 1415-1490 cm^{-1} for functionalities C=O, C=N and C-N respectively. In ^1H-NMR spectra, the Ar-H protons appeared at region of 7.15-8.40 ppm and the methylene protons appeared at region of 2.26-3.26 ppm. The molecular ion recorded in mass spectra for further confirmation of the structures of the title compounds. Obviously, all spectral details are mentioned in the experimental section for each compound.

Pharmacology Studies:

The newly synthesized compounds **4(a,b)-8(a,b)** were assayed for their anti-inflammatory activity. Doses were selected based upon the acute toxicity studies. For the compounds **4a**, **6a**, **7a**, **4b**, **5b**, **6b**, **7b** and **8b** mortalities were found at 300 mg/kg bw during the 24 h of observation, **5a** and **8a**, the mortalities were found at 200 mg/kg bw during the 24 h of observation. The common clinical signs observed in the treated animals were dullness, piloerection and recumbency. Lacrimation was observed in addition to dullness, piloerection and recumbency in

treated animals with **5a** and **8a** compounds (300 mg/kg bw) during first 4 h of observation. No abnormalities were detected during the observation of gross pathology for all the tested animals.

Anti-inflammatory activity has investigated after examined acute toxicity of the test solutions using Cotton pellet-induced granuloma, Carrageenan induced paw oedema and air-pouch models and Dichlofenac used as a reference drug. The results are represented in **Table. 2, 3** and **4**. Anti-inflammatory activity screening data from the above three methods revealed that all the tested compounds **4(a&b)-8(a&b)** are active and shown moderate to potential anti-inflammatory activity towards all the tested animals. The potency of the anti-inflammatory activity was mainly influenced by the moieties, 2,6-difluorophenyl and morpholine, thiomorpholine at 3- and 2-positions attached to quinazolin 4(3*H*)-one compounds respectively. Compounds **5a, 8a** have shown potent anti-inflammatory activity towards all the tested animals (Wister rats). Aromatic fluorine groups might be cause for exploring the potency of the anti-inflammatory activity.

CONCLUSION

In conclusion, a simple, economical and efficient method was developed for preparation of different quinazolin-4(3*H*)-one derivatives in acidic medium, requisite organic base instead of inorganic base and room temperature conditions in consequent steps. This methodology has several advantages particularly high yields and the experimental simplicity. All the synthesized compounds were characterized and evaluated for their anti-inflammatory activity by Carrageenan induced paw oedema, Cotton pellet-induced granuloma and Carrageenan induced air-pouch models. In all the test models exposed, the title compounds have shown moderate to good anti-inflammatory activity, when compared to control group (Dichlofenac). Compounds **5a** and **8a** exhibited potential anti-inflammatory activity in all three models.

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REFERENCES

[1] M. M. Ghorab, S. D. Abdel-Gawad, M. S. A. El-Gaby, *Il Farnco*, **2000**, 55, 249.
[2] H. J. Hess, T. H. Cronin, A. Scriabine, *J. Med. Chem.*, **1968**, 11, 140.
[3] N. R. El-Brollosy, M. F. Abdel-Megeed, A. Genady, R. Alexandria, *J. Pharm Sci.*, **2003**, 17 (1), 17.
[4] B. R. Shab, J. J. Bhatt, H. H. Patel, N. K. Undavia, P. B. Trivedi, N. C. Desai, *Indian. J. Chem.*, **1995**, 34b, 201.
[5] A. Kumar, M. Tyagi, V. K. Shrivasthavam, *Indian. J. Chem.*, **2003**, 42B, 2142.
[6] M. A. Khili, R. Soliman, A. M.Furghuli, A. A. Bekhitm, *Arch. Pharm.*, **1994**, 27, 327.
[7] H. B. Shivaram, M. T. Padmaja, M. K. Shivnanda, P. M. Akbarali, *Indian. J. Chem.*, **1998**, 37B, 715.
[8] D. S. Bradly, *Tetrahedron lett.*, **2001**, 42, 1851.
[9] K. H. Boltze, H. D. Dell, H. Lehwaldm, N. loranz, M. Ruberg – Schweer, *Arzneim forsch/Drug Res.*, **1963**, 13, 688.
[10] J. F. Wolfe Rathman, T. L.; Sleevi M. C.; campbellm J. A.; T. D. Greenwood, *J. Med. Chem.*, **1990**, 33, 161.
[11] M. A. Aziza, M. W. Nassar, S. G. Abdul Hamide, A. E. El-Hakim, A. S. El-Azab, *Indian. J. Heterocycle. Chem.*, **1996**, 6c, 25.
[12] (a) V. K. Pandey, L. P. Pathak, S. K. Mishra, *Indian. J. Chem.*, **2005**, 44B, 1940. (b) J. M. S. Pattanaik, M. paranaik, D. Bhatta, *Indian. J. Chem.*, **1998**, 378, 1304.
[13] B. L. Chenard, F. S. Menniti, M. J. Pagnozzi, K. D. Shenk, F. E. Ewing, W. M. Welch, *Bioorg. Med. Chem. Lett.*, **2000**, 10, 1203. (b) B. L. Chenard, W. M. Welch, J. F. Blake, T. W. Butler, A. Reinhold, F. E. Ewing, M. J. Pagnozzi, F. S. Menniti, *J. Med. Chem.*, **2001**, 44, 1710.
[14] T. J. Schall, D. J. Dairaghi, B. E. Mcmaster, *PCT Int Appl WO0116144*, **2001**.
[15] Palle V. R. Acharyulu, P. K. Dubey, P. V. V. Prasada Reddy, T. Suresh, *ARKIVOC*, **2008**, 17-26, ISSN 1551.
[16] V. Alagarsamy, V. R. Solomon, S. Murugesan, *Bioorg. Med. Chem.*, **2007**, 15, 235.
[17] N. M. Raghavendra, P. Thampi, P. M. Gurubasavarajaswamy, D. Sriram, *Chem. Pharm. Bull*., **2007**, 55, 1615.
[18] S. E. S. Abbas, A. E. M. Saafan, *Bull. Pharm. Sci*., **2007**, 30, 51.
[19] B. Das, Reddy, V. S. M. Krishnaiah, *Tetrahedron Lett.*, **2006**, 47, 8471.
[20] B. Das, M. Krishnaiah, P. Thirupathi, K. Laximinarayana, *Tetrahedron Lett.*, **2007**, 48, 4263.
[21] S. S. Antarkar, T. Chinwalla, N. Bhatt, Anti-inflammatory activity of *Rubia cordifolia* Linn. In Rats, *Indian J. Pharmacol.*, **1994**, 15(3), 185.