PHARMACOLOGICAL EVALUATION OF SOME 1-(SUBSTITUTEDBENZYLIDENE)-4-(4-(2-(METHYL/PHENYL)-4-OXOQUINAZOLIN-3(4*H*)-YL)PHENYL)SEMICARBAZIDE DERIVATIVES

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ABSTRACT

A series of 1-(substitutedbenzylidene)-4-(4-(2-(methyl/phenyl)-4-oxoquinazolin-3(4*H*)-yl)phenyl) semicarbazide derivatives were synthesized and evaluated for analgesic, anti-inflammatory and ulcerogenic activities. The title compounds were screened for analgesic activity by tail–flick technique using *Wistar* albino mice orally at two dose levels *i.e.* 10 and 20 mg/kg. The carrageenan-induced foot paw edema test was performed for screening anti-inflammatory activity of title compounds orally at two dose levels *i.e.* 10 and 20 mg/kg in rats. Diclofenac sodium at 10 and 20 mg/kg was administered orally as reference drug for comparison. In addition all compounds were examined for its ulcerogenicity. Results revealed that entire series of compounds exhibited mild to excellent analgesic and anti-inflammatory activity with low to moderate ulcer index. The reports indicate that all the test compounds exhibited significant activity and graded dose response. Moreover this study revealed that test compounds showed moderate analgesic and anti-inflammatory activity at 30 min of reaction time; the activity increased at 1 h, further it reached to peak level at 2 h and past its best in activity was observed at 3 h. The relationship between the functional group variation and the biological activity of the evaluated compounds were well discussed. From the results in general, it was observed that 2-phenyl quinazolinone analog exhibited better activity than corresponding 2-methyl quinazolinone analog. Based on the results obtained, compound **5k** and **5m** was found to be very active with low ulcer index compared to the rest of the compounds which were tested.

KEY WORDS : Quinazolin-4(3*H*)-one, Analgesic, Anti-inflammatory and Semicarbazide.

INTRODUCTION

Two major classes of drugs dominate in treatment of analgesia: opioids which interact with specific central receptors (μ, k, δ) mainly at spinal levels and cyclooxygenase (COX - the enzymes that synthesize prostaglandins) inhibitors, which action occurs peripherally. However the prostaglandin conception explains only a part of the effects of these drugs[1]. As a third class of analgesic agents may be considered antidepressants, anticonvulsants and anesthetics used to control neuropathic pain which is very difficult to treat[2].

Non steroidal anti-inflammatory drugs (NSAIDs) are the most commonly prescribed medications in the world. They are used for the treatment of pain, fever and inflammation, particularly arthritis. It is well known that NSAIDs are associated with several side effects such as gastrointestinal mucosal damage, bleeding, intolerance and renal toxicity[3,4]. The most prevalent one is occurrence of gastrointestinal damage with gastric upset and irritation being the major problems[5,6]. The search for safer NSAIDs continues with the failure of anticipated 'Ideal' anti-inflammatory agents, the coxibs, on long term usage[7,8]. Hence, production of safer and more active NSAIDs is still needed.

Quinazoline and quinazolinone nuclei have drawn a great attention due to their wide range of chemotherapeutic activities[9]. Additionally, different known anti-inflammatory drugs such as proquazone, fluoroquazone and tryptanthrin are bearing quinazoline nucleus[10-12]. Also, it has been reported that substitution pattern by different aryl or heteroaryl moieties at 2nd and/or 3rd position of quinazoline nucleus markedly influences the analgesic and anti-inflammatory activity[13].

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On the other hand, Schiff bases have gained importance because of the physiological and pharmacological activities associated with them. Compounds containing azomethine group (–C=N–) in the structure are known as Schiff bases, which are usually synthesized by the condensation of primary amines and active carbonyl groups. Schiff bases are well known for their pharmacological properties like analgesic, anti-inflammatory, antibacterial, antifungal, anticancer and antiviral agents[14].

Based on the above observations and in continuation of our anti-inflammatory and analgesic drug research program, it was of interest to synthesize a novel series of quinazolinone derivatives with structure modifications involving at 2nd and 3rd position of quinazolinone ring as a trial to obtain safer and potent anti-inflammatory and analgesic agents. The ulcerogenic activity of the compounds was also determined.

MATERIALS AND METHODS

Chemistry

All solvents used were of laboratory grade and were obtained from SD fine chemicals (Mumbai, India), and Merck (Mumbai, India). Melting points were determined in open glass capillary tubes and were uncorrected. Compounds were routinely checked for their purity on Silica gel G (Merck) thin layer chromatography (TLC) plates. Iodine chamber and UV lamp were used for visualization of TLC spots. The IR spectra were recorded in KBr pellets on (BIO–RAD FTS) FT–IR spectrophotometer. ^{1}H NMR spectra were recorded on Bruker DPX–300 NMR spectrometer in $CDCl_3$ using tetramethylsilane (TMS) as an internal standard. The chemical shifts were reported in ppm scale. Mass spectra were obtained on a JEOL–SX–102 instrument using electron impact ionization. Elemental analyses for C, H and N were performed on a Perkine Elmer model 240C analyzer and were within ±0.4 % of the theoretical values.

Synthesis of title compounds 5a-5n

Title compounds and other intermediate compounds are synthesized as per our earlier reported method[15].

Biological activities

The synthesized compounds were evaluated for analgesic, anti-inflammatory and ulcerogenic activities. One-way analysis of variance (ANOVA) was performed to certain the significance of all the exhibited activities. The test compounds and the standard drugs were administered in the form of a suspension (1% carboxy methyl cellulose as a vehicle) by oral route of administration for analgesic and anti-inflammatory but for ulcerogenicity studies by intraperitoneally as suspension in 10% v/v Tween-80. Each group consisted of six animals. The animals were maintained in colony cages at 25 ± 2°C, relative humidity of 45–55%, under a 12 h light and dark cycle; were fed standard animal feed[16]. Animals were maintained under standard conditions in an animal house approved by committee for the purpose of control and supervision on experiments on animals (CPCSEA). Institutional Animal Ethics Committee approved the experimental protocol. All the animals were acclimatized for a week before use.

Analgesic activity

The analgesic activity was performed by tail-flick technique using *Wistar* albino mice (25-35 g) of either sex selected by random sampling technique[17,18]. Diclofenac sodium at a dose level of 10 and 20 mg/kg was administered orally as reference drug for comparison. The test compounds at two dose levels *i.e.* 10 and 20 mg/kg were administered orally. The reaction times were recorded immediately before and 30 min, 1, 2 and 3 h after the treatment and cut-off time was 10 s. The percent analgesic activity (PAA) was calculated by the following formula. PAA = $[T_2\text{-}T_1/10\text{-}T_1]$ x 100; where T_1 is the reaction time (s) before treatment and T_2 is the reaction time (s) after treatment.

Anti-inflammatory activity

Anti-inflammatory activity was evaluated by carrageenan-induced paw oedema test in rats[19]. Diclofenac sodium 10 and 20 mg/kg was administered as standard drug for comparison. The test compounds were administered at two dose levels of 10 and 20 mg/kg. The paw volumes were measured using the mercury displacement technique with the help of plethysmograph immediately before and 30 min, 1, 2 and 3 h after carrageenan injection. The percent inhibition of paw oedema was calculated according to the following formula. Percent inhibition I = 100[1-(a - x)/(b - y)] where x is the mean paw volume of rats before the administration of carrageenan and test compounds or reference compound (test group), a is the mean paw volume of rats after the administration of carrageenan in the test group (drug treated), b is the mean paw volume of rats after the administration of carrageenan in the control group, y is the mean paw

volume of rats before the administration of carrageenan in the control group.

Ulcerogenicity

Ulceration in rats was induced as reported method[20]. Albino rats of Wistar strain weighing 150-200 g of either sex were divided into various groups each of six animals. Control group of animals were administered only with 10% v/v Tween-80 suspension intraperitoneally. One group was administered with Aspirin intraperitoneally in a dose of 200 mg/kg once daily for three days. Diclofenac was also administered as standard drug at 20 mg/kg once daily for three days to another group of animals in the same route. The remaining group of animals was administered with test compounds

TABLE 1. PHYSICAL DATA AND ANALGESIC ACTIVITIES OF SYNTHESIZED COMPOUNDS 5a-5n

Comp. code	Molecular Formula	Mp	Dose (mg/kg)	Percentage analgesic activity[a]			
				30 min	1 h	2 h	3 h
5a	$C_{25}H_{19}N_3O_3$	261-263	10	25±0.76*	34±1.33*	40±0.81*	24±1.60*
			20	39±0.99*	43±2.34*	48±0.78**	29±1.01*
5b	$C_{24}H_{18}N_4O_2$	228-230	10	17±2.02*	25±1.15*	28±0.69*	15±1.34*
			20	28±2.07*	34±1.62*	37±0.55**	20±1.79*
5c	$C_{24}H_{17}N_3O_3$	284-286	10	20±1.85*	28±0.78**	33±1.32*	19±0.94**
			20	31±1.58*	37±0.86*	41±1.21**	24±2.16*
5d	$C_{24}H_{16}N_4O_4$	257-259	10	26±1.07*	37±2.21*	42±0.95*	27±1.38*
			20	40±1.16*	45±0.53*	51±2.08*	31±1.64*
5e	$C_{25}H_{16}F_3N_3O_2$	272-274	10	16±0.93*	23±1.06*	27±1.52**	13±0.66**
			20	25±0.89*	31±1.27**	35±0.96*	17±2.20*
5f	$C_{24}H_{16}ClN_3O_2$	183-185	10	32±1.42*	41±1.50*	47±0.79**	30±1.05**
			20	45±0.97*	51±2.05*	58±1.74*	39±0.79*
5g	$C_{25}H_{19}N_3O_3$	246-248	10	19±1.25*	26±0.83*	30±1.46*	18±0.89**
			20	29±1.71*	36±0.67*	38±2.53*	21±1.33*
5h	$C_{24}H_{18}N_4O_2$	198-200	10	36±0.68*	45±1.34*	51±1.03*	34±0.76*
			20	49±0.75*	55±1.58*	65±1.02**	44±0.87*
5i	$C_{24}H_{17}N_3O_3$	292-294	10	30±1.19*	41±0.86*	45±0.62*	29±1.58*
			20	43±1.83*	48±0.60**	57±1.34*	36±0.99**
5j	$C_{24}H_{16}N_4O_4$	226-228	10	35±0.94*	45±0.43*	50±1.38*	34±1.22*
			20	48±1.09*	55±0.72*	63±1.47*	43±0.65*
5k	$C_{25}H_{16}F_3N_3O_2$	229-231	10	38±1.01*	50±0.97*	55±0.56*	37±1.13**
			20	51±1.27*	59±0.64**	68±0.86**	45±1.12*
5l	$C_{24}H_{16}ClN_3O_2$	235-237	10	29±2.17*	38±1.02*	44±0.89*	27±0.50*
			20	42±2.05*	46±0.91**	54±1.29*	32±1.80*
5m	$C_{25}H_{16}F_3N_3O_2$	229-231	10	39±0.73*	52±0.55*	58±1.04*	39±1.29***
			20	55±0.82*	60±1.35*	71±1.60**	47±0.96*
5n	$C_{25}H_{16}F_3N_3O_2$	229-231	10	33±1.15*	44±2.07*	48±0.62*	31±1.78*
			20	46±1.40*	52±0.49*	60±1.35**	39±0.88*
Control	-	-		3±0.46	5±0.67	6±0.51	4±0.72
Diclofe	-	-	10	35±0.58*	46±1.23**	51±0.79**	33±1.05*
	-	-	20	49±1.14*	56±0.67***	65±0.51*	43±1.39**

[a] Each value represents the mean ± SEM (n=6); Significance levels *p < 0.5, **p < 0.01, ***p < 0.001 as compared with the respective control.

intraperitoneally in a dose of 20 mg/kg. On fourth day, pylorus was ligated as per previous reported method[21]. Animals were fasted for 36 h before the pylorus ligation procedure. Four hours after the ligation, animals were sacrificed. The stomach was removed and opened along with the greater curvature. Ulcer index was determined by earlier reported method[22].

RESULTS AND DISCUSSION

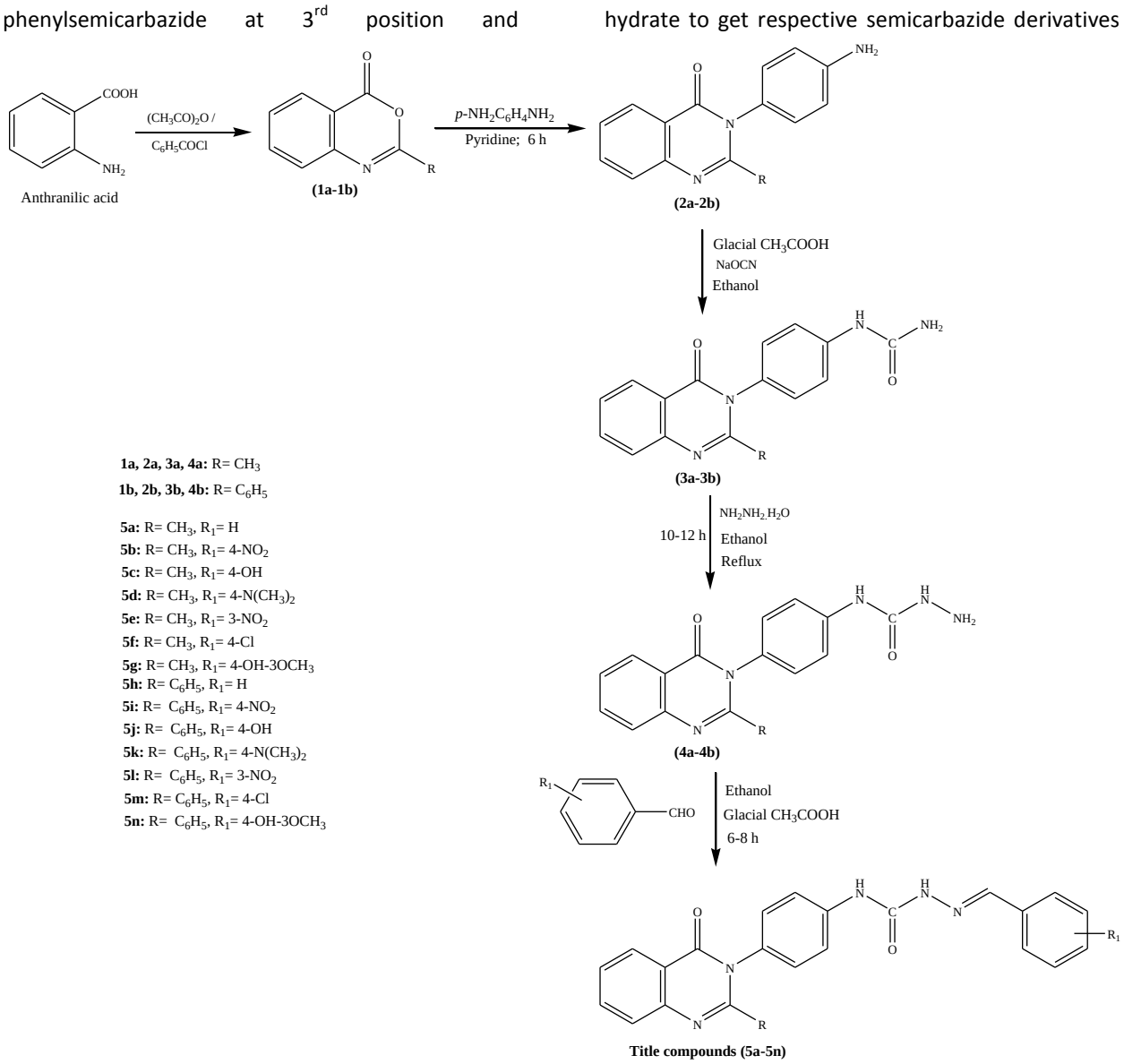
Chemistry

In this study, a series of novel quinazolin-4(3*H*)-one derivative was synthesized by substituting different 1-(substitutedbenzylidene)-4-

TABLE 2. ANTI-INFLAMMATORY AND ULCER INDEX OF SYNTHESIZED COMPOUNDS 5a-5n

Compd. code	Dose (mg/kg)	Percentage protection in paw edema[a]				Ulcer index
		30 min	1 h	2 h	3 h	
5a	10	20±2.17*	24±0.93*	30±1.69*	19±1.02*	0.84±0.41
	20	30±0.52*	41±1.26*	48±2.05**	28±0.79*	
5b	10	12±0.74**	15±1.38*	19±0.53*	14±2.05*	0.97±0.65
	20	21±1.87*	32±2.03*	37±0.36*	20±1.51*	
5c	10	15±1.21*	18±0.84*	23±0.66*	17±0.90**	0.92±0.59
	20	24±1.01**	36±0.65**	42±1.88*	23±2.36*	
5d	10	21±0.59*	26±2.01*	30±1.25*	23±1.47*	0.83±0.32
	20	32±0.45*	43±1.01*	50±2.15**	31±0.73*	
5e	10	11±0.85*	15±0.52*	18±1.14*	12±0.57**	0.99±0.74
	20	20±2.19*	29±0.96**	36±1.42*	18±1.05*	
5f	10	26±1.40*	30±0.86*	38±1.51*	25±1.23*	0.70±0.45
	20	36±0.71*	48±1.37**	55±1.98*	37±0.80*	
5g	10	13±2.32**	17±1.35*	20±0.89**	16±1.96*	0.94±0.52
	20	23±1.53*	33±1.74*	40±0.27*	23±2.46***	
5h	10	30±0.49*	35±1.77*	43±1.33*	31±0.62*	0.60±0.36
	20	42±0.98*	53±1.44**	59±1.60*	43±0.59*	
5i	10	24±1.57*	29±0.91*	35±0.78**	25±2.15*	0.74±0.63
	20	35±0.55**	46±2.29*	54±1.41*	35±1.74*	
5j	10	29±1.14*	33±0.59**	42±1.27*	29±0.71*	0.63±0.48
	20	40±1.24*	52±0.70*	59±1.07*	41±0.51***	
5k	10	33±0.61**	38±1.64*	47±1.10**	35±0.86*	0.58±0.39
	20	45±0.60*	57±1.49***	63±0.84*	44±1.37***	
5l	10	23±0.98*	27±2.11**	33±0.65*	24±1.42*	0.79±0.60
	20	33±1.94*	45±0.58*	51±1.13*	34±1.59*	
5m	10	35±1.06*	41±0.48**	50±0.90**	37±1.39*	0.54±0.26
	20	47±1.32***	58±0.85*	66±0.69*	49±1.10***	
5n	10	27±0.58*	32±1.20*	39±2.02**	28±0.95*	0.68±0.51
	20	39±0.96*	50±1.12*	56±1.54*	37±0.78*	
Control		4.3±0.39	6.9±0.63	5.1±0.55	3.3±0.74	0.11±0.06
Diclofenac	10	30±0.73**	36±0.39*	43±1.46*	30±0.64**	1.61±0.53
	20	41±0.43*	53±1.17***	60±0.79**	42±1.62*	
Aspirin	200	-	-	-	-	1.79±0.65

Each value represents the mean ± SEM (n=6); Significance levels *p < 0.5, **p < 0.01, ***p < 0.001 as compared with the respective control.



Scheme 1. Synthetic protocols of intermediates and title compounds

phenylsemicarbazide at 3rd position and hydrate to get respective semicarbazide derivatives methyl/phenyl group at 2nd position. Initially, anthranilic acid and acetic anhydride/benzoyl chloride were used as starting materials to synthesize 2-(methyl/phenyl)-4*H*-benzo-[1,3]-oxazin-4-one **1a/1b** by a simple acetylation/benzoylation followed by ring closure reaction. In the subsequent step, 3-(4-aminophenyl)-2-(methyl/phenyl)-quinazolin-4(3*H*)-one **2a/2b** was synthesized through simple reaction by treating compound **1a/1b** with *p*-phenylenediamine with the elimination of water molecule. On stirring with sodium cyanate and glacial acetic acid, compounds **2a/2b** gets converted to its respective urea derivatives **3a/3b**. Before final step, the compounds **3a/3b** treated with hydrazine **4a/4b**. In the last step, the compounds **5a-5n** were synthesized by a Schiff base reaction, in which a different aromatic aldehydes (carbonyl compound) and amino derivative (quinazolinone analog) undergoes nucleophilic addition, forming a hemiaminal. This reaction followed by a dehydration results in title compounds **5a–5n** by forming a stable imine according to the synthetic Scheme 1.

Biological activities

Entire test compounds **5a-5n** was tested for their analgesic activity and anti-inflammatory activity by tail-flick technique using *Wistar* albino mice and Carrageenan-induced paw edema test using *Wistar*

rats, respectively. The results of analgesic and anti-inflammatory studies are summarized in Table 1 and 2 respectively. The reports indicate that test compounds showed moderate activity at 30 min of reaction time; the activity increased at 1 h, further it reached to peak level at 2 h and past its best in activity was observed at 3 h. Moreover this study revealed that all compounds exhibited significant activity and graded dose response. From these studies, in general it was observed that 2-phenyl quinazolinone analog exhibited better activity than corresponding 2-methyl quinazolinone analog. Within 2-phenyl quinazolinone derivatives, compound **5h** with unsubstituted phenyl derivative showed good activity which is almost equal to standard drug Diclofenac sodium. With the increased lipophilicity *p*-dimethylamino **5k** and *p*-chloro **5m** phenyl derivative showed an increase in activity. Compounds **5k** and **5m** were found to exhibit more potent analgesic and anti-inflammatory activity than Diclofenac sodium. Replacement of chlorine or dimethylamino group with hydroxy and/or methoxy group (**5j** and **5n**) decreases the lipophilicity results in decreased activity. Exchange of hydroxy and/or methoxy group with nitro group (**5i** and **5l**) further decreases the lipophilicity led to reasonable decrease in activity.

Further all the test compounds were examined for its ulcerogenicity and the results are summarized in Table 2. Entire test compounds exhibited less ulcer index compared to standard Diclofenac and Aspirin. Results of ulcer index revealed that the compounds bearing 2-phenyl quinazolin-4(3*H*)-one nucleus **5h-5n** showed negligible ulcer index, whereas replacement of 2-phenyl by 2-methyl moiety leads to slight increases in ulcer index. The test compounds exhibited 34-61% and 30-55% of the ulcer index when compared to the reference drug Diclofenac (1.61±0.53) and Aspirin (1.79±0.65) respectively. Among the tested compounds, 1-(4-chlorobenzylidene)-4-(4-(4-oxo-2-phenylquinazolin-3(4*H*)-yl)phenyl)semicarbazide **5m** exhibited least ulcer index (0.54±0.26) which is about one-third of the ulcer index of reference standards. Out of entire compounds tested, 1-(3-nitrobenzylidene)-4-(4-(2-methyl-4-oxoquinazolin-3(4*H*)-yl)phenyl)semicarbazide **5e** exhibited higher ulcer index (0.99±0.74) which is about 58% of the ulcer index of Diclofenac and Aspirin.

From the present work, it was found that compound **5m** exhibited 58 and 71% analgesic activity at 10 and 20 mg/kg dose level, respectively, at the reaction time of 2 h. The compound **5m** also showed 50 and 66% anti-inflammatory activity at the dose 10 and 20 mg/kg, respectively, at the reaction time of 2 h. Interestingly the above mentioned compound exhibited less ulcer index. Hence this analog could be developed as a new class of analgesic and anti-inflammatory agents. However, further structural modification is planned to enhance the analgesic and anti-inflammatory activities with the decreased ulcerogenic index.

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