Original Article

DOI: 10.5582/ddt.2012.v6.2.78

Synthesis, analgesic, anti-inflammatory and ulcerogenic properties of some novel *N*'-((1-(substituted amino)methyl)-2-oxoindolin-3-ylidene)-4-(2-(methyl/phenyl)-4-oxoquinazolin-3(4*H*)-yl)benzohydrazide derivatives

Govindaraj Saravanan[1,*], Veerachamy Alagarsamy[2], Chinnasamy Rajaram Prakash[3]

[1] *Medicinal Chemistry Research Laboratory, Bapatla College of Pharmacy, Jawaharlal Nehru Technological University, Hyderabad, Andhra Pradesh, India;*
[2] *Medicinal Chemistry Research Laboratory, M.N.R. College of Pharmacy, Sangareddy, Andhra Pradesh, India;*
[3] *Department of Medicinal Chemistry, DCRM Pharmacy College, Inkollu, Andhra Pradesh, India.*

ABSTRACT: A new series of *N*'-((1-(substituted amino)methyl)-2-oxoindolin-3-ylidene)-4-(2-(methyl/phenyl)-4-oxoquinazolin-3(4*H*)-yl)benzohydrazide derivatives 4a-4l were designed and synthesized from anthranilic acid. All the synthesized compounds were characterized by spectroscopic means and elemental analyses. The tail-flick technique and the carrageenan-induced foot paw edema test were performed for screening analgesic and anti-inflammatory activity, respectively. All of the compounds were also examined for their ulcerogenicity. Some of the compounds showed significant activity. Among the test compounds, 4b exhibited 53% and 69% analgesic activity at a dose of 10 and 20 mg/kg, respectively. It also displayed 47% (10 mg/kg) and 65% (20 mg/kg) anti-inflammatory activity with one-fourth of ulcer index of the reference drugs diclofenac and aspirin.

Keywords: Quinazolinone, isatin, schiff base, mannich base, analgesic, anti-inflammatory

1. Introduction

Inflammation is a defensive but exaggerated local tissue reaction in response to exogenous or endogenous insult. It is a fundamental physiological process that is not only essential for survival but at the same time is one of the major causes of human morbidity and mortality (*1,2*). A large number of non-steroidal anti-inflammatory drugs (NSAIDs) are available clinically to treat inflammatory disorders. NSAIDs are one of the most widely used drug categories against inflammation, mild to moderate pain and fever. In the past decade, numerous advances have taken place in the understanding of pathogenesis and as a result, significant progress has been made and is still being explored for the development of novel NSAIDs (*3*). Prostaglandin synthetase or cyclooxygenase (COX) is an enzyme which catalyzes the rate limiting steps in the biosynthesis of cyclic endoperoxides from arachidonic acid to form prostaglandins (PGs). The most important mechanism of NSAIDs is considered to be primarily by inhibition of PGs synthesis; specifically competitive inhibition of COX (*4*). Generally, the NSAIDs inhibit both isoforms COX-1 and COX-2. Most NSAIDs are mainly COX-1 selective (*e.g.,* indomethacin, aspirin, ketoprofen, piroxicam, and sulindac). The mechanism of action of celecoxib and rofecoxib is primarily selective inhibition of COX-2 (*5*). Others are considered to have mixed action on COX-1 and -2 (*e.g.,* ibuprofen, naproxen, diclofenac, etodolac, nabumetone, and meloxicam). Other mechanisms that may contribute to NSAID mediated anti-inflammatory activity include the reduction of superoxide radicals, induction of apoptosis, inhibition of adhesion molecule expression, decrease of nitric oxide synthase, decrease of proinflammatory cytokine levels (tumornecrosis factor-α, interleukin-1), modification of lymphocyte activity, and alteration of cellular membrane functions (*6*). However, long term clinical usages of NSAIDs are associated with significant side effects such as severe gastrointestinal ulceration, bleeding, intolerance and nephrotoxicity (*7,8*). Therefore, investigation of new NSAIDs is still a major challenge and production of safer and more active NSAIDs and analgesic drugs are needed.

Quinazoline and quinazolinone nuclei have drawn great attention due to their wide range of chemo-therapeutic activities (*9-15*). Additionally, different known anti-inflammatory drugs such as proquazone I, fluoroquazone II, and tryptanthrin III contain the quinazoline nucleus (Figure 1) (*16-19*). Also, it has been

**Address correspondence to:*
Mr. Govindaraj Saravanan, Medicinal Chemistry Research Laboratory, Bapatla College of Pharmacy, Jawaharlal Nehru Technological University, Hyderabad, Andhra Pradesh, India.
E-mail: sarachem1981@gmail.com

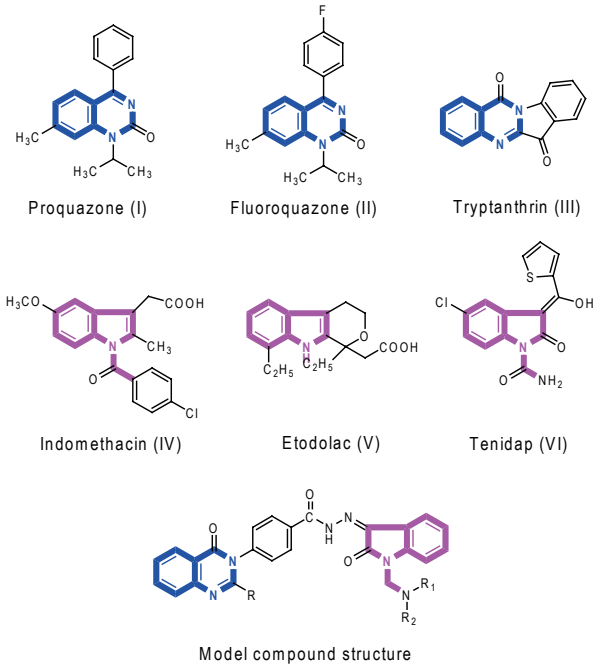


Figure 1. Some of the well established structures of NSAID and our model compound with its common pharmacophore features.

reported that the substitution pattern by different aryl or heteroaryl moieties at 2/3 position of the quinazoline nucleus markedly influences analgesic and anti-inflammatory activity (*20*).

On the other hand the indole skeleton exists in a variety of natural products and is the precursor for many pharmaceuticals, such as indomethacin IV, etodolac V, and tenidap VI (Figure 1). In recent decades, the literature has been enriched with progressive findings about synthesis and pharmacological activities of isatin (oxidized form of indole) ring, which is a core structure in various synthetic pharmaceuticals displaying a wide variety of biological activities (*21-27*).

Based on the above observations and in continuation of our anti-inflammatory and analgesic drug research program (*28,29*), it was of interest to synthesize a novel series of quinazolinone derivatives with structure modifications involving incorporation of isatin moieties at the 3rd position and a methyl/phenyl group at the 2nd position of the quinazolinone ring as a trial to obtain safer and potent anti-inflammatory and analgesic agents. The ulcerogenic activity of the compounds was also determined.

2. Materials and Methods

2.1. *Chemistry*

The chemicals and reagents used were obtained from various chemical units Merck India Ltd., Qualigens, CDH, and SD Fine Chem. All solvents used were of laboratory research (LR) grade and purified before use. All reaction steps were monitored until completion using thin layer chromatography (TLC). An iodine chamber and UV lamp were used for visualization of TLC spots. All melting points were performed in open glass capillary tubes and were uncorrected. ^{1}H-NMR spectra were performed on a Bruker ultra shield (300 MHz) NMR spectrometer in $CDCl_3$ using tetramethylsilane [$(CH_3)_4Si$] as an internal standard. Chemical shifts (δ) were expressed as parts per million (ppm). The multiplicities of the signals in the ^{1}H-NMR spectra were abbreviated by s (singlet), t (triplet), q (quartet), and m (multiplet). The *J* constant was given in Hz. Mass spectra were obtained on a JEOL-SX-102 instrument using electron impact ionization. All IR spectra were recorded in KBr pellets on a Jasco FT-IR 410 spectrometer. Elemental analyses were performed on a PerkinElmer model 240C analyzer and were within ± 0.4% of the theoretical values.

2.1.1. *Synthesis of 2-(methyl/phenyl)-4H-benzo[1,3]oxazin-4-one (**1a, 1b**)*

For the synthesis of the 2-methyl derivative: A mixture of anthranilic acid (1.37 g, 0.01 mol) and acetic anhydride (10.2 mL, 0.1 mol) was refluxed on a gentle flame for 1 h. The excess acetic anhydride was distilled off under reduced pressure and the residue was dissolved in petroleum ether and was kept aside for 1 h. The light brown solid **1a** obtained was filtered and dried (*28*).

For synthesis of the 2-phenyl derivative: To a solution of anthranilic acid 13.7 g (0.1 mol) dissolved in pyridine (60 mL), benzoyl chloride 28 g (0.2 mol) was added and the mixture was stirred for 30 min at room temperature followed by treatment with 5% $NaHCO_3$ (15 mL). The solid thus obtained **1b** was recrystallized from ethanol (*29*).

2.1.2. *Synthesis of 4-(2-(methyl/phenyl)-4-oxoquinazolin-3(4H)-yl)benzohydrazide (**2a, 2b**)*

A mixture of 2-(methyl/phenyl)-4*H*-benzo[1,3]oxazin-4-one **1a**/**1b** (1.61/2.23 g, 0.01 mol) and *p*-aminobenzohydrazide (1.51 g, 0.01 mol) was dissolved in anhydrous pyridine (50 mL) and heated on a sand bath for 10 h. The resulting solution was cooled in an ice bath and treated with dilute hydrochloric acid (100 mL). The product separated **2a**/**2b** was filtered, washed with water, and crystallized from ethanol.

2.1.3. *Synthesis of 4-(2-(methyl/phenyl)-4-oxoquinazolin-3(4H)-yl)-N'-(2-oxoindolin-3-ylidene)benzohydrazide (**3a, 3b**)*

Equimolar quantities of 4-(2-(methyl/phenyl)-4-oxoquinazolin-3(4*H*)-yl)benzohydrazide **2a**/**2b** (2.94/3.56 g, 0.01 mol) and isatin (1.47 g, 0.01 mol) were dissolved in warm ethanol (30 mL) and heated on

a steam bath for 1 h. After standing for approximately 24 h at room temperature, the crystalline products **3a**/**3b** were separated by filtration, dried under vacuum pressure and recrystallised from ethanol.

2.1.4. *General procedure for the synthesis of N'-((1-(substituted amino)methyl)-2-oxoindolin-3-ylidene)-4-(2-(methyl/phenyl)-4-oxoquinazolin-3(4H)-yl)benzohydrazide (**4a-4l**)*

To a solution of the derivative **3a**/**3b** (4.23/4.85 g, 0.01 mol) in glacial acetic acid (50 mL) containing 37% formalin (1 mL), the appropriate secondary amine derivative (0.02 mol) was added. The reaction mixture was refluxed on a water bath for 1-3 h. The reaction mixture was concentrated to approximately half the initial volume, and the resulting precipitate was recrystallized from ethanol to get a pure form of the product. The method used for the preparation and isolation of the compounds gave materials of good purity, as evidenced by their spectral analyses and by TLC. The spectral data for the title compounds **4a-4l** are presented in the appendix.

2.2. *Animals*

The animals used in the present study were Swiss albino mice weighing 20-25 g and Wistar rats weighing 150-200 g were procured from Bapatla College of Pharmacy, Bapatla, India. Animals were maintained in colony cages at 25 ± 2°C, relative humidity of 45-55%, maintained under 12 h light and dark cycle and were fed standard animal feed (*30*) and water *ad libitum*. Animals were maintained under standard conditions in an animal house approved by the committee for the purpose of control and supervision on experiments on animals (CPCSEA). The protocol was approved by the Institutional Animal Ethics Committee. All animals were acclimatized to laboratory conditions for a week before use.

The synthesized compounds were evaluated for analgesic, anti-inflammatory and ulcerogenic activities. One-way analysis of variance (ANOVA) was performed to obtain significance for all exhibited activities. Test compounds and standard drugs were administered in the form of a suspension (1% carboxy methyl cellulose as a vehicle) by the oral route for analgesic and anti-inflammatory studies and as a suspension of 10% (v/v) Tween-80 by the intraperitoneal route of administration for ulcerogenicity studies.

2.3. *Analgesic activity*

The analgesic activity was performed by the tail-flick technique using Wistar albino mice (25-35 g) of either sex selected by a random sampling technique (*31,32*). Diclofenac sodium at a dose level of 10 and 20 mg/kg was administered orally as a reference drug for comparison. The test compounds at two dose levels (10 and 20 mg/kg) were administered orally. The reaction times were recorded at 30 min, 1, 2, and 3 h after treatment and the cut-off time was 10 sec. The percent analgesic activity (PAA) was calculated by the following formula: $PAA = [T_2 - T_1/10 - T_1] \times 100$; where T_1 is the reaction time (sec) before treatment, and T_2 is the reaction time (sec) after treatment.

2.4. *Anti-inflammatory activity*

Anti-inflammatory activity was evaluated by the carrageenan-induced paw edema test in rats (*33*). Diclofenac sodium at 10 and 20 mg/kg was administered as a reference drug for comparison. The test compounds were administered at two dose levels (10 and 20 mg/kg). The paw volumes were measured using the mercury displacement technique with the help of a plethysmograph immediately before and 30 min, 1, 2, and 3 h after carrageenan injection. The percent inhibition of paw edema was calculated according to the following formula: percent inhibition $I = 100 \times [1 - (a - x)/(b - y)]$ where x is the mean paw volume of rats before the administration of carrageenan and the test compounds or the reference drug (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.

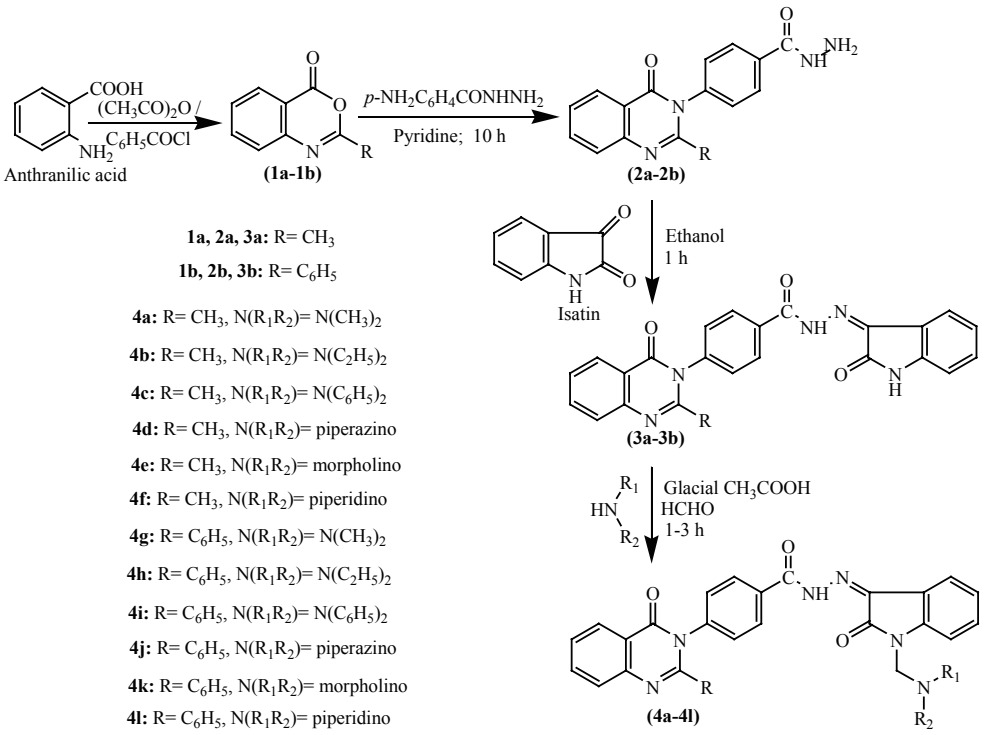
2.5. *Ulcerogenicity*

Ulceration in rats was induced as reported by Goyal *et al.* (*34*). Albino Wistar rats weighing 150-200 g of either sex were divided into various groups of six animals each. The control group of animals was only given 10% (v/v) Tween-80 suspension intraperitoneally. One group was administered with aspirin intraperitoneally at a dose of 200 mg/kg once daily for three days. Diclofenac was also administered as a standard drug at 20 mg/kg once daily for three days to another group of animals by the same route. The remaining group of animals was given the test compounds intraperitoneally at a dose of 20 mg/kg. On the fourth day, the pylorus was ligated using the method of Shay *et al.* (*35*). Animals were fasted for 36 h before the pylorus ligation procedure. The animals were sacrificed four hours post ligation. The stomach was removed and opened along with the greater curvature. Ulcer index was determined by the method of Ganguly and Bhatnagar (*36*).

3. Results and Discussion

3.1. *Chemistry*

The synthetic pathway giving access to the titled compounds **4a-4l** is represented in Scheme 1. Initially, 2-(methyl/phenyl)-4*H*-benzo[1,3]oxazin-



Scheme 1. Synthetic protocol of title compounds.

4-one **1a**/**1b** were synthesized from anthranilic acid using acetic anhydride/benzoyl chloride by a simple acetylation/benzoylation followed by a ring closure reaction. In the subsequent step, 4-(2-(methyl/phenyl)-4-oxoquinazolin-3(4*H*)-yl)benzohydrazide **2a**/**2b** were synthesized by a simple reaction of compounds **1a**/**1b** with *p*-aminobenzohydrazide with elimination of a water molecule. Before the final step, Schiff bases **3a**/**3b** were synthesized by nucleophilic addition of the amino derivatives **2a**/**2b** with the carbonyl compound isatin in ethanol. This reaction was followed by dehydration to generate compounds **3a**/**3b** by forming a stable imine. In the last step, the title compounds **4a**-**4l** were synthesized through a Mannich reaction by treating compound **3a**/**3b** with formaldehyde and secondary amines like dimethylamine, diethylamine, diphenylamine, piperazine, morpholine, and piperidine. The physicochemical parameters of all the synthesized compounds are summarized in Table 1. The structures of the synthesized compounds were confirmed by spectral (IR, ^{1}H-NMR, and Mass) and elemental analyses data.

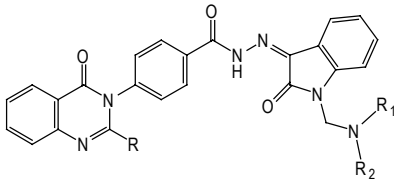
3.2. *Analgesic activity*

Using Wistar albino mice analgesic activity for title compounds **4a**-**4l** was carried out by the tail-flick technique. The results obtained from the above study are summarized in Table 2. The results of analgesic activity indicate that all the test compounds exhibited a graded dose response and not all of them are significant but some of them gave significant activity. Moreover, this study revealed that test compounds showed moderate analgesic activity at 30 min of reaction time; the activity increased at 1 h, further it reached to peak level at 2 h and it decreased again at 3 h. Compound **4a** and **4g** with dimethyl substitution showed good activity. With the increased lipophilicity (diethyl group), compounds **4b** and **4h** showed an increase in activity. Substitution with alicyclic amine (piperidine) in **4f** and **4l** further increases the lipophilicity and retains the activity of diethyl substitution. The presence of an additional hetero atom such as nitrogen and oxygen in the alicyclic amine rings such as: (piperazine and morpholine) in **4d**, **4e**, **4j**, and **4k** led to a decrease in activity which might be due to a fall in lipophilicity. Aromatic substitution (diphenyl group) in **4c** and **4i** showed lower activity. The compounds with aliphatic substitution **4a**, **4b**, **4g**, and **4h** had shown better activity than the rest of the compounds **4c**-**4f** and **4i**-**4l** with alicyclic amine or aromatic substitution. Compounds **4b**, **4f**, **4h**, and **4l** were found to be the most active analgesic agents and they are almost equal or moderately more potent when compared to the reference standard diclofenac sodium.

3.3. *Anti-inflammatory activity*

Anti-inflammatory activity was performed by the carrageenan-induced paw edema test in rats. The anti-inflammatory activity results (Table 3) showed that all test compounds protected rats from carrageenan-induced inflammation reasonably at 30 min of reaction time; the activity increased at 1 h and it reached the maximum level at 2 h. Declining activity was observed at 3 h. The compounds possessing dimethyl amino substituents **4a**

Table 1. Synthesized compounds 4a-4l



Compound	-R	-N(R_1R_2)	Mol. formula	% Yield	Mp (°C)	R_f[a]
4a	$-CH_3$	$-N(CH_3)_2$	$C_{27}H_{24}N_6O_3$	75	223-225	0.65
4b	$-CH_3$	$-N(C_2H_5)_2$	$C_{29}H_{28}N_6O_3$	72	256-258	0.72
4c	$-CH_3$	$-N(C_6H_5)_2$	$C_{37}H_{28}N_6O_3$	78	280-282	0.49
4d	$-CH_3$		$C_{29}H_{27}N_7O_3$	70	237-239	0.84
4e	$-CH_3$		$C_{29}H_{26}N_6O_4$	74	244-246	0.58
4f	$-CH_3$		$C_{30}H_{28}N_6O_3$	81	219-221	0.78
4g	$-C_6H_5$	$-N(CH_3)_2$	$C_{32}H_{26}N_6O_3$	78	265-268	0.68
4h	$-C_6H_5$	$-N(C_2H_5)_2$	$C_{34}H_{30}N_6O_3$	72	252-254	0.61
4i	$-C_6H_5$	$-N(C_6H_5)_2$	$C_{42}H_{30}N_6O_3$	71	229-231	0.70
4j	$-C_6H_5$		$C_{34}H_{29}N_7O_3$	75	286-288	0.45
4k	$-C_6H_5$		$C_{34}H_{28}N_6O_4$	77	248-251	0.54
4l	$-C_6H_5$		$C_{35}H_{30}N_6O_3$	70	212-214	0.80

[a] Solvent system used was ethylacetate/hexane/formic acid (4:2:4, v/v).

Table 2. Analgesic activity of the synthesized compounds (Tail-flick method)

Compound	Dose (mg/kg)	Analgesic activity (%)			
		30 min	1 h	2 h	3 h
4a	10	35 ± 0.93*	41 ± 1.36*	45 ± 0.90*	33 ± 0.26*
	20	46 ± 0.51*	54 ± 0.51**	62 ± 0.33**	39 ± 0.62*
4b	10	40 ± 1.07**	48 ± 1.16*	53 ± 0.71*	37 ± 1.42**
	20	53 ± 0.90*	62 ± 0.48***	69 ± 0.79**	46 ± 1.53*
4c	10	25 ± 1.64*	29 ± 0.26*	34 ± 1.45*	24 ± 1.72*
	20	32 ± 1.55*	41 ± 1.21*	44 ± 1.86*	25 ± 1.89*
4d	10	30 ± 0.78*	36 ± 0.79*	41 ± 1.74*	29 ± 1.40*
	20	41 ± 1.22*	52 ± 1.37*	56 ± 1.42**	33 ± 1.19*
4e	10	28 ± 1.63*	35 ± 0.56*	39 ± 1.21*	27 ± 1.82*
	20	38 ± 0.81*	46 ± 1.16*	50 ± 1.64*	28 ± 1.36**
4f	10	37 ± 0.96***	47 ± 1.52*	49 ± 0.58**	33 ± 1.18*
	20	50 ± 1.94*	58 ± 2.02*	66 ± 1.22**	44 ± 0.61**
4g	10	33 ± 1.69**	38 ± 0.56*	44 ± 0.95*	32 ± 1.29*
	20	45 ± 0.67*	54 ± 1.27**	60 ± 0.74*	36 ± 1.62**
4h	10	38 ± 1.60*	47 ± 1.52**	50 ± 1.61*	35 ± 1.18*
	20	51 ± 0.51**	59 ± 1.41**	68 ± 1.73***	44 ± 1.10*
4i	10	22 ± 2.12*	27 ± 0.68*	30 ± 1.09*	20 ± 1.76*
	20	30 ± 1.39*	38 ± 1.67*	43 ± 1.28*	23 ± 0.61*
4j	10	29 ± 0.67*	34 ± 2.43*	38 ± 1.46*	27 ± 1.39*
	20	40 ± 0.52*	49 ± 1.16**	53 ± 0.87*	29 ± 1.67*
4k	10	27 ± 1.38*	33 ± 1.38*	36 ± 0.56**	26 ± 1.57*
	20	37 ± 1.09*	48 ± 0.69*	51 ± 2.10*	28 ± 0.75*
4l	10	34 ± 0.81**	45 ± 1.47*	49 ± 1.35**	31 ± 1.80*
	20	48 ± 0.64*	55 ± 1.59**	65 ± 0.57**	40 ± 1.52*
Control	--	3 ± 0.39	6 ± 0.52	5 ± 0.63	4 ± 0.43
Diclofenac	10	36 ± 1.52**	43 ± 1.31*	47 ± 1.87**	34 ± 1.16*
	20	48 ± 0.68*	57 ± 1.47***	63 ± 0.55*	39 ± 0.74**

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.

Table 3. Antiinflammatory activity (Carrageenan induced paw edema test in rats) and ulcer index of the synthesized compounds

Compound	Dose (mg/kg)	Anti-inflammatory activity (%)				Ulcer index
		30 min	1 h	2 h	3 h	
4a	10	31 ± 0.91*	38 ± 0.64**	42 ± 1.41*	30 ± 1.53*	0.55 ± 0.32
	20	42 ± 1.62*	50 ± 1.35*	61 ± 1.25**	39 ± 0.58*	
4b	10	36 ± 1.78*	44 ± 1.05*	47 ± 1.64**	39 ± 1.39*	0.46 ± 0.24
	20	47 ± 0.63*	58 ± 0.79***	65 ± 1.57*	48 ± 1.27**	
4c	10	16 ± 1.53*	20 ± 1.28*	29 ± 1.24*	19 ± 1.65*	0.64 ± 0.29
	20	25 ± 0.39*	36 ± 0.62*	41 ± 1.66*	25 ± 1.14*	
4d	10	24 ± 1.50*	30 ± 1.64*	34 ± 1.51*	25 ± 0.73*	0.88 ± 0.57
	20	33 ± 1.07**	42 ± 1.90*	50 ± 0.74*	31 ± 1.45*	
4e	10	21 ± 0.72*	26 ± 0.77*	32 ± 1.73*	24 ± 1.41*	0.70 ± 0.44
	20	31 ± 0.59*	39 ± 1.26*	46 ± 1.29*	28 ± 1.27*	
4f	10	25 ± 0.96*	33 ± 1.39*	36 ± 1.41*	27 ± 1.55**	0.76 ± 0.50
	20	36 ± 1.35*	45 ± 1.71*	54 ± 1.78**	34 ± 0.65*	
4g	10	28 ± 1.72**	36 ± 1.42***	39 ± 1.65*	28 ± 1.03*	0.56 ± 0.39
	20	40 ± 0.61*	49 ± 1.89*	58 ± 1.93**	39 ± 2.07**	
4h	10	34 ± 1.46**	40 ± 0.51*	42 ± 0.83*	38 ± 1.28*	0.49 ± 0.27
	20	45 ± 1.22*	54 ± 1.31**	63 ± 1.26***	48 ± 1.19*	
4i	10	15 ± 0.37*	18 ± 1.54*	26 ± 1.52*	17 ± 1.75*	0.68 ± 0.24
	20	23 ± 0.72*	32 ± 1.82*	39 ± 1.18*	20 ± 0.71*	
4j	10	22 ± 1.55*	27 ± 1.71**	33 ± 1.47*	23 ± 1.19*	0.92 ± 0.60
	20	30 ± 0.44*	41 ± 0.94*	48 ± 1.82*	28 ± 1.42*	
4k	10	19 ± 0.80*	23 ± 1.65*	30 ± 1.44*	21 ± 1.51*	0.72 ± 0.41
	20	28 ± 1.45*	37 ± 1.37**	42 ± 1.34*	26 ± 1.64*	
4l	10	24 ± 0.71*	31 ± 1.84*	34 ± 1.62*	26 ± 1.36*	0.77 ± 0.35
	20	34 ± 1.52*	44 ± 1.13**	51 ± 0.93*	30 ± 1.15**	
Control	--	4.1 ± 0.62	6.4 ± 0.95	4.9 ± 0.43	3.2 ± 0.58	0.13 ± 0.07
Diclofenac	10	30 ± 0.82**	36 ± 0.99*	40 ± 1.40**	32 ± 1.62*	1.61 ± 0.53
	20	42 ± 1.27*	52 ± 1.28***	59 ± 1.28**	41 ± 1.29*	
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.

and **4g** exhibited equipotent anti-inflammatory activity to the reference standard diclofenac sodium. With increased lipophilicity, compounds with diethyl amino substituents **4b** and **4h** showed moderately more potent activity than that of diclofenac sodium. Unlike analgesic activity, the replacement of these alkyl groups with the alicyclic amine (piperidine) in **4f** and **4l** led to a sharp decrease in activity. Placement of an additional hetero atom such as nitrogen and oxygen with the alicyclic amine (piperazine and morpholine) in **4d**, **4e**, **4j**, and **4k** showed a further decrease in activity. Among all the synthesized compounds, **4c** and **4i** with aromatic substitution (diphenyl amino group) exhibited the least activity. Moreover among the tested compounds, 2-methyl quinazolinone derivatives showed greater analgesic and anti-inflammatory activity than the corresponding 2-phenyl quinazolinone analogs.

3.4. *Ulcerogenicity*

In addition all test compounds were examined for ulcerogenicity and the results are summarized in Table 3. All test compounds exhibited ulcer indexes less than those obtained with the standard diclofenac and aspirin. Results of the ulcer index revealed that compounds bearing alkyl substituents **4a**, **4b**, **4g**, and **4h** showed a negligible ulcer index, whereas the replacement of the methyl group with a phenyl group at the 2nd position of the quinazoline ring led to a slight increase in the ulcer index. The other test compounds, **4d**-**4f** and **4j**-**4l** possessing alicyclic amines exhibited a higher ulcer index. The test compounds exhibited 29-57% and 26-51% 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, *N*'-(1-((diethylamino) methyl)-2-oxoindolin-3-ylidene)-4-(2-methyl-4-oxoquinazolin-3(4*H*)-yl)benzohydrazide **4b** exhibited the least ulcer index (0.46 ± 0.24) which is about one-fourth of the ulcer index of the reference standards. Out all of the tested compounds, *N*'-(2-oxo-1-(piperazin-1-ylmethyl)indolin-3-ylidene)-4-(4-oxo-2-phenylquinazolin-3(4*H*)-yl)benzohydrazide **4j** was found to possess the highest ulcer index (0.92 ± 0.60) which is about 54% of the ulcer index of diclofenac and aspirin.

4. Conclusion

In summary, a series of *N*'-((1-(substituted amino)methyl)-2-oxoindolin-3-ylidene)-4-(2-(methyl/phenyl)-4-oxoquinazolin-3(4*H*)-yl)benzohydrazide derivatives **4a**-**4l** were synthesized and characterized

by IR, ^{1}H-NMR, mass spectroscopy and elemental analyses. Some of the test compounds exhibited significant analgesic and anti-inflammatory activity with a mild to moderate ulcer index. In general, it was found that 2-methyl quinazolinone analogs showed more potent activity than corresponding 2-phenyl quinazolinone derivatives. Also, quinazoline derivatives bearing alkyl amino groups exhibited the best activity followed by derivatives bearing alicyclic amines whereas derivatives having aryl amino groups showed the least activity. Among all test compounds, *N*'-(1-((diethylamino)methyl)-2-oxoindolin-3-ylidene)-4-(2-methyl-4-oxoquinazolin-3(4*H*)-yl)benzohydrazide **4b** showed the most potent analgesic and anti-inflammatory activity which is more potent than that of the reference diclofenac. From this study we concluded that incorporation of an isatin moiety at the 3rd position and a methyl group at the 2nd position of the quinazolinone ring resulted in potent analgesic and anti-inflammatory activity with a minimal ulcer index. Hence, this analog could be developed as a new class of analgesic and anti-inflammatory agents. However, further structural modification should be planned to enhance their analgesic and anti-inflammatory activities with the low ulcerogenic index.

Acknowledgements

The authors gratefully acknowledge the management of Bapatla College of Pharmacy for providing infrastructure facilities to carry out this research work. The authors also want to thank the Central Instrumentation Facility, IIT Chennai, India for the spectral analysis of the compounds used in this study.

References

1. O'Neill LA. Targeting signal transduction as a strategy to treat inflammatory diseases. Nat Rev Drug Discov. 2006; 5:549-563.
2. Cheeseright TJ, Holm M, Lehmann F, Luik S, Gottert M, Melville JL, Laufer S. Novel lead structures for p38 MAP kinase *via* field screen virtual screening. J Med Chem. 2009; 52:4200-4209.
3. Bhandari SV, Dangre SC, Bothara KG, Patil AA, Sarkate AP, Lokwani DK, Gore ST, Deshmane BJ, Raparti VT, Khachane CV. Design, synthesis and pharmacological screening of novel nitric oxide donors containing 1,5-diarylpyrazolin-3-one as nontoxic NSAIDs. Eur J Med Chem. 2009; 44:4622-4636.
4. Chao EY, Caravella JA, Watson MA, *et al*. Structure-guided design of *N*-phenyl tertiary amines as trans repression-selective liver X receptor modulators with anti-inflammatory activity. J Med Chem. 2008; 51:5758-5765.
5. Laufer SA, Hauser DRJ, Domeyer DM, Kinkel K, Liedtke AJ. Design, synthesis and biological evaluation of novel tri and tetra substituted imidazoles as highly potent and specific ATP-mimetic inhibitors of p38 MAP kinase: Focus on optimized interactions with the enzyme's surface-exposed front region. J Med Chem. 2008; 51:4122-4149.
6. Hynes JJ, Dyckman AJ, Lin S, *et al*. Design, synthesis and anti-inflammatory properties of orally active 4-(phenylamino)-pyrrolo[2,1-f][1,2,4]triazine p38α mitogen-activated protein kinase inhibitors. J Med Chem. 2008; 51:4-16.
7. Antre RV, Cendilkumar A, Goli D, Andhale GS, Oswal RJ. Microwave assisted synthesis of novel pyrazolone derivatives attached to a pyrimidine moiety and evaluation of their anti-inflammatory, analgesic and antipyretic activities. Saudi Pharma Journal. 2011; 19:233-243.
8. Amin KM, Kamel MM, Anwar MM, Khedr M, Syam YM. Synthesis, biological evaluation and molecular docking of novel series of spiro [(2*H,*3*H*)-quinazoline-2,1'-cyclohexan]-4(1*H*)-one derivatives as anti-inflammatory and analgesic agents. Eur J Med Chem. 2010; 45:2117-2131.
9. Kumar P, Shrivastava B, Pandeya SN, Stables JP. Design, synthesis and potential 6 Hz psychomotor seizure test activity of some novel 2-(substituted)-3-{[substituted] amino}quinazolin-4(3*H*)-one. Eur J Med Chem. 2011; 46:1006-1018.
10. Abdel Gawad NM, Georgey HH, Youssef RM, El-Sayed NA. Synthesis and antitumor activity of some 2,3-disubstituted quinazolin-4(3*H*)-ones and 4,6-disubstituted-1,2,3,4-tetra hydroquinazolin-2*H*-ones. Eur J Med Chem. 2010; 45:6058-6067.
11. Pattan SR, Krishna Reddy VV, Manvi FV, Desai BG, Bhat AR. Synthesis of *N*-3[4(4-chlorophenylthiazol-2-yl)-2-aminomethyl]quinazolin-4(3*H*)-one and their derivatives for antitubercular activity. Indian J Chem. 2006; 45B:1778-1781.
12. Dinakaran M, Selvam P, Declercq E, Sridhar SK. Synthesis, antiviral and cytotoxic activity of 6-bromo-2,3-disubstituted-4(3*H*)-quinazolinones. Biol Pharm Bull. 2003; 26:1278-1282.
13. Mohamed MS, Kamel MM, Kassem EMM, Abotaleb N, Abd El-moez SI, Ahmed MF. Novel 6,8-dibromo-4(3*H*)-quinazolinone derivatives of antibacterial and antifungal activities. Eur J Med Chem. 2010; 45:3311-3319.
14. Shukla JS, Shukla R. Synthesis of 4-(5-substituted arylidine-4-thiazolidinone-2-thione)-6,8-substituted quinazolines as potential antihelmintic agents. J Indian Chem Soc. 1989; 66:209-210.
15. Alagarsamy V, Rajasolomon V, Meena R, Ramseshu KV. Synthesis, analgesic, anti-inflammatory and antibacterial activities of some novel 2-butyl-3-substituted quinazolin-4(3*H*)-ones. Biol Pharmac Bull. 2005; 28:1091-1094.
16. Vanryzin RJ, Trpold JH. The toxicology profile of the anti-inflammatory drug proquazone in animals. Drug Chem Toxicol. 1980; 3:361-379.
17. Wheatly D. Analgesic properties of fluproquazone. Rheumatol Rehabil. 1982; 21:98-100.
18. Fankhauser S, Laube W, Marti HR, Schultheis HR, Vogtlin J, Graf-fenried BV. Antipyretic activity of fluproquazone in man. Arzneimittelforschung. 1981; 31:934-935.
19. Oberthur C, Hamburger M. Tryptanthrin content in Isatis tinctoria leaves – A comparative study of selected strains and post harvest treatments. Planta Med. 2004; 70:642-645.
20. Kumar A, Verma RS, Jaju BP, Sinha JN. Quinazolinylpyrazolines as anti-inflammatory agents. J Indian Chem Soc. 1990; 67:920-921.

21. Muthukumar VA, George S, Vaidhyalingam V. Synthesis and pharmacological evaluation of 1-(1-((substituted)methyl)-5-methyl-2-oxoindolin-3-ylidene)-4-(substitutedpyridin-2-yl)thio semicarbazide. Biol Pharm Bull. 2008; 31:1461-1464.
22. Sridhar SK, Ramesh A. Synthesis and pharmacological activities of Schiff bases and hydrazones of Isatin derivatives. Indian Drugs. 2001; 38:174-180.
23. Kang IJ, Wang LW, Hsu TA, Yueh A, Lee CC, Lee YC, Lee CY, Chao YS, Shih SR, Chern JH. Isatin-β-thiosemicarbazones as potent herpes simplex virus inhibitors. Bioorg Med Chem Lett. 2011; 21:1948-1952.
24. Gudipati R, Anreddy RNR, Sarangapani M. Synthesis, characterization and anticancer activity of certain 3-{4-(5-mercapto-1,3,4-oxadiazole-2-yl)phenylimino}indolin-2-one derivatives. Saudi Pharma Journal. 2011; 19:153-158.
25. Banerjee D, Yogeeswari P, Bhat P, Thomas A, Srividya M, Sriram D. Novel isatinyl thiosemicarbazones derivatives as potential molecule to combat HIV-TB co-infection. Eur J Med Chem. 2011; 46:106-121.
26. Singh DP, Kumar K, Sharma C. New 14-membered octaazamacrocyclic complexes: Synthesis, spectral, antibacterial and antifungal studies. Eur J Med Chem. 2010; 45:1230-1236.
27. Hans RH, Wiid IJF, Van Helden PD, Wan B, Franzblau SG, Gut J, Rosenthal PJ, Chibale K. Novel thiolactone-isatin hybrids as potential antimalarial and antitubercular agents. Bioorg Med Chem Lett. 2011; 21:2055-2058.
28. Alagarsamy V, Muruganantham G, Venkateshaperumal R. Synthesis, analgesic, anti-inflammatory and antibacterial activities of some novel 2-methyl-3-substituted quinazolin-4(3*H*)-ones. Biol Pharm Bull. 2003; 26:1711-1714.
29. Alagarsamy V, Rajasolomon V, Vanikavitha G, Paluchamy V, Ravichandran M, Arnoldsujin A, Thangathirupathi A, Amuthalakshmi S, Revathi R. Synthesis, analgesic, anti-inflammatory and anti-bacterial activities of some novel 2-phenyl-3-substituted quinazolin-4(3*H*)-ones. Biol Pharm Bull. 2002; 25:1432-1435.
30. Guide to the care and use of experimental animals (Olfert ED, Cross BM, McWilliam AA, eds.), Vol. 1. 2nd ed., Canadian Council of Animal Care, 1993.
31. Kulkarni SK. Heat and other physiological stress-induced analgesia: Catecholamine mediated and naloxone reversible response. Life Sci. 1980; 27:185-188.
32. D'Amour FE, Smith DL. A method for determining loss of pain sensation. J Pharmacol Exp Ther. 1941; 72:74-79.
33. Winter CA, Risley EA, Nuss GW. Carrageenin induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc Soc Exp Biol Med. 1962; 111:544-547.
34. Goyal RK, Chakrabarti A, Sanyal AK. The effect of biological variables on the anti ulcerogenic effect of vegetable plantain banana. Planta Med. 1985; 29:85-88.
35. Shay M, Komarov SA, Fels D, Meranze D, Grunstein H, Siplet H. A simple method for the uniform production of gastric ulceration in the rats. Gastroenterology. 1945; 5:43-61.
36. Ganguly AK, Bhatnagar OP. Effect of bilateral adrenalectomy on production of restraint ulcers in the stomach of albino rats. Can J Physiol Pharmacol. 1973; 51:748-750.

(Received January 7, 2012; Revised February 20, 2012; Re-revised April 16, 2012; Accepted April 16, 2012)

Appendix

Characterization data of the synthesized compounds in the current study

*2-Methyl-4H-benzo[1,3]oxazin-4-one (**1a**)*. Yield: 71%; mp = 182°C; IR (KBr) ν_{max} cm^{-1}: 3,096 (Ar-CH_{str}), 2,882 (CH_3-CH_{str}), 1,712 (C=O), 1,636 (C=N), 1,055 (C-O-Cstr); ^{1}H-NMR (300 MHz, $CDCl_3$): δ ppm: 2.38 (s, 3H, CH_3), 6.92-7.40 (m, 4H, Ar-H); MS (EI) *m/z:* 161 [M^+]; Anal. Calcd. for $C_9H_7NO_2$: C, 67.07; H, 4.38; N, 8.69. Found: C, 67.16; H, 4.40; N, 8.66.

*2-Phenyl-4H-benzo[1,3]oxazin-4-one (**1b**)*. Yield: 80%; mp = 120°C; IR (KBr) ν_{max} cm^{-1}: 3,077 (Ar-CH_{str}), 1,751 (C=O), 1,625 (C=N), 1,038 (C-O-C_{str}); ^{1}H-NMR (300 MHz, $CDCl_3$): δ ppm: 6.95-7.78 (m, 9H, Ar-H); MS (EI) *m/z*: 223 [M^+]; Anal. Calcd. for $C_{14}H_9NO_2$: C, 75.33; H, 4.06; N, 6.27. Found: C, 75.42; H, 4.05; N, 6.29.

*4-(2-Methyl-4-oxoquinazolin-3(4H)-yl)benzohydrazide (**2a**)*. Yield: 74 %; mp = 174-176°C; IR (KBr) ν_{max} cm^{-1}: 3,363 (NH_{str}), 3,038 (Ar-CH_{str}), 2,932 (CH_3-CH_{str}), 1,725 (C=O of quinazoline), 1,650 (C=O of amide); ^{1}H-NMR (300 MHz, $CDCl_3$): δ ppm: 2.53 (s, 3H, CH_3), 3.86 (s, 2H, NH_2), 6.85-7.92 (m, 8H, Ar-H), 9.82 (s, 1H, CONH); MS (EI) *m/z*: 294 [M^+]; Anal. Calcd. for $C_{16}H_{14}N_4O_2$: C, 65.30; H, 4.79; N, 19.04. Found: C, 65.54; H, 4.78; N, 18.97.

*4-(4-Oxo-2-phenylquinazolin-3(4H)-yl)benzohydrazide (**2b**)*. Yield: 77%; mp = 159-161°C; IR (KBr) ν_{max} cm^{-1}: 3,384 (NH_{str}), 3,077 (Ar-CH_{str}), 1,751 (C=O of quinazoline), 1,669 (C=O of amide); ^{1}H-NMR (300 MHz, $CDCl_3$): δ ppm: 3.74 (s, 2H, NH_2), 7.13-8.09 (m, 13H, Ar-H), 9.85 (s, 1H, CONH); MS (EI) *m/z:* 356 [M^+]; Anal. Calcd. for $C_{21}H_{16}N_4O_2$: C, 70.77; H, 4.53; N, 15.72. Found: C, 70.99; H, 4.52; N, 15.67.

*4-(2-Methyl-4-oxoquinazolin-3(4H)-yl)-N'-(2-oxoindolin-3-ylidene)benzohydrazide (**3a**)*. Yield: 70%; mp = 190-192°C; IR (KBr) ν_{max} cm^{-1}: 3,368 (NH_{str}), 3,045 (Ar-CH_{str}), 2,940 (CH_3-CH_{str}), 1,733 (C=O of quinazoline), 1,647 (C=O of amide), 1,592 (C=N_{str}); ^{1}H-NMR (300 MHz, $CDCl_3$): δ ppm: 2.60 (s, 3H, CH_3), 7.06-8.20 (m, 12H, Ar-H), 8.94 (s, 1H, NH of isatin), 9.90 (s, 1H, CONH); MS (EI) *m/z:* 423 [M^+]; Anal. Calcd. for $C_{24}H_{17}N_5O_3$: C, 68.08; H, 4.05; N, 16.54. Found: C, 67.90; H, 4.06; N, 16.60.

*4-(4-Oxo-2-phenylquinazolin-3(4H)-yl)-N'-(2-oxoindolin-3-ylidene)benzohydrazide (**3b**)*. Yield: 75%; mp = 203-205°C; IR (KBr) ν_{max} cm^{-1}: 3,390 (NH_{str}), 3,034 (Ar-CH_{str}), 1,747 (C=O of quinazoline), 1,662 (C=O of amide), 1,605 (C=N_{str}); ^{1}H-NMR (300 MHz, $CDCl_3$): δ ppm: 7.28-8.31 (m, 17H, Ar-H), 9.02 (s, 1H, NH of isatin), 9.97 (s, 1H, CONH); MS (EI) *m/z:* 485

[M^+]; Anal. Calcd. for $C_{29}H_{19}N_5O_3$: C, 71.74; H, 3.94; N, 14.43. Found: C, 71.95; H, 3.93; N, 14.39.

N'-(1-((dimethylamino)methyl)-2-oxoindolin-3-ylidene)-4-(2-methyl-4-oxoquinazolin-3(4H)-yl)benzohydrazide ***(4a)***. IR (KBr) ν_{max} cm^{-1}: 3,361 (NH_{str}), 3,047 (Ar-CH_{str}), 2,935 (CH3-CH_{str}), 1,738 (C=O of quinazoline), 1,672 (C=O of amide); ^{1}H-NMR (300 MHz, $CDCl_3$): δ ppm: 2.36 (s, 3H, CH_3 of quinazoline), 2.95 (s, 6H, $N(CH_3)_2$), 4.51 (s, 2H, NCH_2N), 6.98-7.83 (m, 12H, Ar-H), 9.87 (s, 1H, CONH); MS (EI) *m/z:* 480 [M^+]; Anal. Calcd. for $C_{27}H_{24}N_6O_3$: C, 67.49; H, 5.03; N, 17.49. Found: C, 67.25; H, 5.05; N, 17.54.

N'-(1-((diethylamino)methyl)-2-oxoindolin-3-ylidene)-4-(2-methyl-4-oxoquinazolin-3(4H)-yl)benzohydrazide ***(4b)***. IR (KBr) ν_{max} cm^{-1}: 3,388 (NH_{str}), 3,020 (Ar-CH_{str}), 2,942 (CH_3-CH_{str}), 1,745 (C=O of quinazoline), 1,649 (C=O of amide); ^{1}H-NMR (300 MHz, $CDCl_3$): δ ppm: 1.62 (t, J = 5.4 Hz, 6H, CH_3 of C_2H_5), 2.55 (s, 3H, CH_3 of quinazoline), 4.49 (q, J = 5.8 Hz, 4H, CH_2 of C_2H_5), 4.79 (s, 2H, NCH_2N), 7.06-8.11 (m, 12H, Ar-H), 9.94 (s, 1H, CONH); MS (EI) *m/z:* 508 [M^+]; Anal. Calcd. for $C_{29}H_{28}N6O_3$: C, 68.49; H, 5.55; N, 16.52. Found: C, 68.71; H, 5.54; N, 16.46.

N'-(1-((diphenylamino)methyl)-2-oxoindolin-3-ylidene)-4-(2-methyl-4-oxoquinazolin-3(4H)-yl)benzohydrazide ***(4c)***. IR (KBr) ν_{max} cm^{-1}: 3,355 (NH_{str}), 3,066 (Ar-CH_{str}), 2,938 (CH_3-CH_{str}), 1,720 (C=O of quinazoline), 1,654 (C=O of amide); ^{1}H-NMR (300 MHz, $CDCl_3$): δ ppm: 2.28 (s, 3H, CH_3 of quinazoline), 4.35 (s, 2H, NCH_2N), 6.85-7.97 (m, 22H, Ar-H), 9.81 (s, 1H, CONH); MS (EI) *m/z:* 604 [M^+]; Anal. Calcd. for $C_{37}H_{28}N_6O_3$: C, 73.50; H, 4.67; N, 13.90. Found: C, 73.67; H, 4.66; N, 13.86.

4-(2-Methyl-4-oxoquinazolin-3(4H)-yl)-N'-(2-oxo-1-(piperazin-1-ylmethyl)indolin-3-ylidene)benzohydrazide ***(4d)***. IR (KBr) ν_{max} cm^{-1}: 3,376 (NH_{str}), 3,052 (Ar-CH_{str}), 2,947 (CH_3-CH_{str}), 1,741 (C=O of quinazoline), 1,645 (C=O of amide); ^{1}H-NMR (300 MHz, $CDCl_3$): δ ppm: 2.40 (s, 3H, CH_3 of quinazoline), 3.09-3.53 (m, 1H, NH of piperazine), 3.81-4.25 (m, 8H, CH_2 of piperazine), 4.47 (s, 2H, NCH_2N), 6.82-8.01 (m, 12H, Ar-H), 9.99 (s, 1H, CONH); MS (EI) *m/z:* 521 [M^+]; Anal. Calcd. for $C_{29}H_{27}N_7O_3$: C, 66.78; H, 5.22; N, 18.80. Found: C, 66.96; H, 5.21; N, 18.74.

4-(2-Methyl-4-oxoquinazolin-3(4H)-yl)-N'-(1-(morpholinomethyl)-2-oxoindolin-3-ylidene) benzohydrazide ***(4e)***. IR (KBr) ν_{max} cm^{-1}: 3,409 (NH_{str}), 3,041 (Ar-CH_{str}), 2,936 (CH_3-CH_{str}), 1,728 (C=O of quinazoline), 1,662 (C=O of amide), 1,065 (C-O-C_{str}); ^{1}H-NMR (300 MHz, $CDCl_3$): δ ppm: 2.32 (t, J = 6.0 Hz, 4H, C_3,C_5-CH_2 of morpholine), 2.69 (s, 3H, CH_3 of quinazoline), 3.45 (t, J = 5.2 Hz, 4H, C_2,C_6-CH_2 of morpholine), 4.38 (s, 2H, NCH_2N), 7.04-8.15 (m, 12H, Ar-H), 9.82 (s, 1H, CONH); MS (EI) *m/z:* 522 [M^+]; Anal. Calcd. for $C_{29}H_{26}N_6O_4$: C, 66.66; H, 5.02; N, 16.08. Found: C, 66.87; H, 5.01; N, 16.03.

4-(2-Methyl-4-oxoquinazolin-3(4H)-yl)-N'-(2-oxo-1-(piperidin-1-ylmethyl)indolin-3-ylidene)benzohydrazide ***(4f)***. IR (KBr) ν_{max} cm^{-1}: 3,364 (NH_{str}), 3,039 (Ar-CH_{str}), 2,950 (CH_3-CH_{str}), 1,732 (C=O of quinazoline), 1,657 (C=O of amide); ^{1}H-NMR (300 MHz, $CDCl_3$): δ ppm: 1.24-1.78 (m, 6H, C_3,C_4,C_5-CH_2 of piperidine), 2.22 (s, 3H, CH_3 of quinazoline), 2.57 (t, J = 5.6 Hz, 4H, C_2,C_6-CH_2 of piperidine), 4.63 (s, 2H, NCH_2N), 7.15-8.29 (m, 12H, Ar-H), 9.76 (s, 1H, CONH); MS (EI) *m/z:* 520 [M^+]; Anal. Calcd. for $C_{30}H_{28}N_6O_3$: C, 69.22; H, 5.42; N, 16.14. Found: C, 69.05; H, 5.44; N, 16.18.

N'-(1-((dimethylamino)methyl)-2-oxoindolin-3-ylidene)-4-(4-oxo-2-phenylquinazolin-3(4H)-yl)benzohydrazide ***(4g)***. IR (KBr) ν_{max} cm^{-1}: 3,352 (NH_{str}), 3,074 (Ar-CH_{str}), 2,945 (CH_3-CH_{str}), 1,723 (C=O of quinazoline), 1,640 (C=O of amide); ^{1}H-NMR (300 MHz, $CDCl_3$): δ ppm: 2.81 (s, 6H, $N(CH_3)_2$), 4.55 (s, 2H, NCH_2N), 6.90-8.07 (m, 17H, Ar-H), 9.85 (s, 1H, CONH); MS (EI) *m/z:* 542 [M^+]; Anal. Calcd. for $C_{32}H_{26}N_6O_3$: C, 70.84; H, 4.83; N, 15.49. Found: C, 70.59; H, 4.84; N, 15.55.

N'-(1-((diethylamino)methyl)-2-oxoindolin-3-ylidene)-4-(4-oxo-2-phenylquinazolin-3(4H)-yl)benzohydrazide ***(4h)***. IR (KBr) ν_{max} cm^{-1}: 3,393 (NH_{str}), 3,038 (Ar-CH_{str}), 2,957 (CH_3-CH_{str}), 1,742 (C=O of quinazoline), 1,676 (C=O of amide); ^{1}H-NMR (300 MHz, $CDCl_3$): δ ppm: 1.75 (t, J = 5.8 Hz, 6H, CH_3 of C_2H_5), 4.32 (q, J = 5.2 Hz, 4H, CH_2 of C_2H_5), 4.70 (s, 2H, NCH_2N), 7.12-8.34 (m, 17H, Ar-H), 10.03 (s, 1H, CONH); MS (EI) *m/z:* 570 [M^+]; Anal. Calcd. for $C_{34}H_{30}N_6O_3$: C, 71.56; H, 5.30; N, 14.73. Found: C, 71.78; H, 5.28; N, 14.69.

N'-(1-((diphenylamino)methyl)-2-oxoindolin-3-ylidene)-4-(4-oxo-2-phenylquinazolin-3(4H)-yl)benzohydrazide ***(4i)***. IR (KBr) ν_{max} cm^{-1}: 3,358 (NH_{str}), 3,024 (Ar-CH_{str}), 1,750 (C=O of quinazoline), 1,665 (C=O of amide); ^{1}H-NMR (300 MHz, $CDCl_3$): δ ppm: 4.52 (s, 2H, NCH_2N), 6.99-8.03 (m, 27H, Ar-H), 9.97 (s, 1H, CONH); MS (EI) *m/z:* 666 [M^+]; Anal. Calcd. for $C_{42}H_{30}N_6O_3$: C, 75.66; H, 4.54; N, 12.60. Found: C, 75.40; H, 4.55; N, 12.64.

N'-(2-oxo-1-(piperazin-1-ylmethyl)indolin-3-ylidene)-4-(4-oxo-2-phenylquinazolin-3(4H)-yl)benzohydrazide ***(4j)***. IR (KBr) ν_{max} cm^{-1}: 3,370 (NH_{str}), 3,058 (Ar-CH_{str}), 1,736 (C=O of quinazoline), 1,643 (C=O of amide); ^{1}H-NMR (300 MHz, $CDCl_3$): δ ppm: 2.81-3.38 (m, 1H, NH of piperazine), 3.65-4.24 (m, 8H, CH_2 of piperazine), 4.34 (s, 2H, NCH_2N), 6.87-7.80 (m, 17H, Ar-H), 10.09 (s, 1H, CONH); MS (EI) *m/z:* 583 [M^+]; Anal. Calcd. for $C_{34}H_{29}N_7O_3$: C, 69.97; H, 5.01; N, 16.80. Found: C, 70.22; H, 4.99; N, 16.75.

*N'-(1-(morpholinomethyl)-2-oxoindolin-3-ylidene)-4-(4-oxo-2-phenylquinazolin-3(4H)-yl)benzohydrazide (**4k**)*. IR (KBr) ν_{max} cm^{-1}: 3,385 (NH_{str}), 3,028 (Ar-CH_{str}), 1,739 (C=O of quinazoline), 1,652 (C=O of amide), 1,053 (C-O-C_{str}); ^{1}H-NMR (300 MHz, $CDCl_3$): δ ppm: 2.56 (t, J = 5.6 Hz, 4H, C_3,C_5-CH_2 of morpholine), 3.32 (t, J = 6.0 Hz, 4H, C_2,C_6-CH_2 of morpholine), 4.46 (s, 2H, NCH_2N), 7.05-8.09 (m, 17H, Ar-H), 9.90 (s, 1H, CONH); MS (EI) *m/z:* 584 [M^+]; Anal. Calcd. for $C_{34}H_{28}N_6O_4$: C, 69.85; H, 4.83; N, 14.38. Found: C, 69.64; H, 4.84; N, 14.43.

*N'-(2-oxo-1-(piperidin-1-ylmethyl)indolin-3-ylidene)-4-(4-oxo-2-phenylquinazolin-3(4H)-yl)benzohydrazide (**4l**)*. IR (KBr) ν_{max} cm^{-1}: 3,367 (NH_{str}), 3,033 (Ar-CH_{str}), 1,724 (C=O of quinazoline), 1,670 (C=O of amide); ^{1}H-NMR (300 MHz, $CDCl_3$): δ ppm: 1.12-1.67 (m, 6H, C_3,C_4,C_5-CH_2 of piperidine), 2.30 (t, J = 5.2 Hz, 4H, C_2,C_6-CH_2 of piperidine), 4.61 (s, 2H, NCH_2N), 7.22-8.38 (m, 17H, Ar-H), 9.85 (s, 1H, CONH); MS (EI) *m/z:* 582 [M^+]; Anal. Calcd. for $C_{35}H_{30}N_6O_3$: C, 72.15; H, 5.19; N, 14.42. Found: C, 72.41; H, 5.18; N, 14.38.