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Short communication

Synthesis and pharmacological evaluation of 3-cyclohexyl-2-substituted hydrazino-3*H*-quinazolin-4-ones as analgesic and anti-inflammatory agents

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A series of novel 3-cyclohexyl-2-substituted hydrazino--quinazolin-4(3*H*)-ones were synthesized by reacting the amino group of 3-cyclohexyl-2-hydrazino quinazolin--4(3*H*)-one with a variety of aldehydes and ketones. The starting material, 3-cyclohexyl-2-hydrazino quinazolin--4(3*H*)-one, was synthesized from cyclohexyl amine. Title compounds were investigated for analgesic, anti-inflammatory and ulcerogenic behavior. The compound 3-cyclohexyl-2-(1-methylbutylidene-hydrazino)-3*H*-quinazolin-4-one (**4c**) emerged as the most active compound of the series and is moderately more potent in its analgesic and anti-inflammatory activities compared to the reference standard diclofenac sodium. Interestingly, test compounds showed only mild ulcerogenic potential when compared to acetylsalicylic acid.

Keywords: quinazolin-4(3*H*)-one, analgesic activity, anti--inflammatory activity, ulcerogenicity

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Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly prescribed for the treatment of acute and chronic inflammation, pain and fever. However, long-term clinical usage of NSAIDs is associated with significant side effects such as gastrointestinal lesions, bleeding, and nephrotoxicity. Therefore, discovery of new safer anti-inflammatory drugs represents a challenging goal in a research area (1–4). In our ongoing medicinal chemistry research program, we found that quinazolines and condensed quinazolines exhibit potent central nervous system (CNS) activity, including analgesic, anti-inflammatory (5) and anticonvulsant behavior (6). Quinazolin-4(3*H*)-ones with 2,3-substitution are reported to possess significant analgesic, anti-inflammatory (7, 8) and anticonvulsant

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activitiy (9). We have earlier documented that some lead 2-phenyl-3-substituted quinazolines (10) (Fig. 1, I), 2-methyl-3-substituted quinazolines (11) (Fig. 1, II), 2-methylthio--3-substituted quinazaolines (12) (Fig. 1, III), and 2,3-disubstituted quinazolines (13) exhibited good analgesic and anti-inflammatory properties. The present work is an extension of our ongoing efforts towards the development and identification of new molecules for analgesic and anti-inflammatory activities with minimal gastrointestinal ulceration side effects. Against this background, in the present study we have synthesized a series of 3-cyclohexyl-2-substituted amino-quinazolin-4(3*H*)-ones. The synthesized compounds were tested for their analgesic, anti-inflammatory and ulcerogenic behavior.

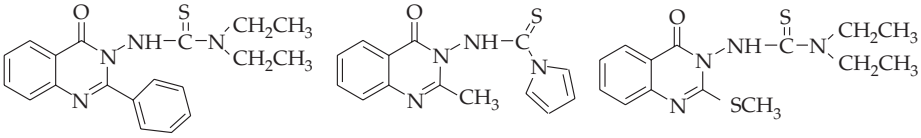

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Fig. 1. Lead molecules of quinazolin-4-ones.

EXPERIMENTAL

Melting points were taken in open capillaries on a Thomas Hoover melting point apparatus (Thomas Hoover, USA) and are uncorrected. IR spectra were recorded in film or in potassium bromide disks on a Perkin-Elmer 398 spectrometer (Perkin-Elmer, USA). ^{1}H spectra were recorded on a DPX-300 MHz Bruker FT-NMR spectrometer (Bruker, USA). Chemical shifts were reported as parts per million (δ ppm) with tetramethylsilane (TMS) as an internal standard. Mass spectra were obtained on a Jeol-SX-102 instrument (Jeol, Japan) using fast atom bombardment (FAB positive). Elemental analysis was performed on a Perkin-Elmer 2400 CHN analyzer (Perkin-Elmer) and values were within acceptable limits of the calculated values (± 0.4%). Spectral data (IR, NMR and mass spectra) and elemental analysis data are presented in Tables I and II. The progress of the reaction was monitored on ready-made silica gel plates (Merck, Norway) using chloroform/methanol (9:1) as a solvent system. Iodine was used as a developing agent. All chemicals and reagents used in the synthesis were obtained from Aldrich (USA), Lancaster (USA) or Spectrochem (India) and were used without further purification.

Syntheses

*3-Cyclohexyl-2-thioxo-2,3-dihydro-1*H-*quinazolin-4-one* ***(1)***. – A solution of cyclohexyl amine (0.02 mol) in dimethyl sulphoxide (10 mL) was stirred vigorously. To this, carbon disulphide (1.6 mL) and aqueous sodium hydroxide 1.2 mL (2 mol L^{-1}) were added dropwise during 30 min under stirring. Dimethyl sulfate (0.02 mol) was added gradually keeping the reaction mixture stirred in a freezing mixture for 2 h. The reaction mixture was then poured into ice water. The solid obtained was *N*-cyclohexyl-methyl dithiocarbamic acid, which was filtered, washed with water, dried and recrystallized from ethanol. Methyl anthranilate (0.01 mol) and the prepared *N*-cyclohexyl-methyl dithiocarbamic

Table I. Physical and analytical data of synthesized compounds

Compd.	Mol. formula	Molecular mass[a]	M.p. (°C)	Yield (%)	Elemental analysis calcd./found (%)		
					C	H	N
1	$C_{14}H_{16}N_2OS$	260	250–251	88	64.58 64.56	6.19 6.23	10.76 10.78
2	$C_{15}H_{18}N_2OS$	274	180–182	88	65.66 65.69	6.61 6.64	10.21 10.20
3	$C_{14}H_{18}N_4O$	258	220–221	81	65.09 65.05	7.02 7.03	21.69 21.64
4a	$C_{18}H_{24}N_4O$	312	238–239	73	69.20 69.23	7.74 7.79	17.93 17.89
4b	$C_{19}H_{26}N_4O$	326	219–221	78	68.07 68.02	8.03 8.08	17.16 17.19
4c	$C_{19}H_{26}N_4O$	326	247–248	72	68.07 68.11	8.03 8.00	17.16 17.14
4d	$C_{20}H_{26}N_4O$	360	281–282	72	70.98 70.99	7.74 7.77	16.55 16.49
4e	$C_{22}H_{24}N_4O$	338	228–230	71	73.31 73.33	6.71 6.77	15.54 15.51
4f	$C_{22}H_{21}N_5O_2$	387	298–299	73	68.20 68.27	5.46 5.48	18.08 18.06
4g	$C_{21}H_{22}N_4O$	346	248–250	73	72.80 72.83	6.40 6.43	16.17 16.12
4h	$C_{21}H_{21}N_4OCl$	381	221–222	77	66.22 66.28	5.56 5.54	14.71 14.74
4i	$C_{21}H_{21}N_4OCl$	381	285–286	72	66.22 66.20	5.56 5.52	14.71 14.77
4j	$C_{21}H_{21}N_5O_3$	391	251–253	79	64.44 64.48	5.41 5.43	17.89 17.82
4k	$C_{21}H_{21}N_5O_3$	391	210–212	74	64.44 64.39	5.41 5.42	17.89 17.90
4l	$C_{22}H_{24}N_4O_2$	376	278–279	80	70.19 70.11	6.43 6.44	14.88 14.93
4m	$C_{22}H_{24}N_4O$	360	247–248	71	73.30 73.34	6.71 6.68	15.54 15.51
4n	$C_{22}H_{24}N_4O$	360	281–283	70	73.30 73.28	6.71 6.69	15.54 15.49
4o	$C_{27}H_{26}N_4O$	422	253–255	73	76.75 76.71	6.20 5.23	13.26 13.29

[a] Molecular mass determination by mass spectral analysis (M^+).

Table II. Spectral data of synthesized compounds

Compd.	IR (KBr) (cm^{-1})	^1H NMR ($CDCl_3$) (δ, ppm)	^{13}C NMR ($CDCl_3$) (δ, ppm)
1	3298 (NH), 1690 (C=O), 1218 (C=S)	0.90–2.11 (m, 10H, CH_2-cyclohexyl), 7.32–7.84 (m, 4H, ArH), 10.35 (br s, 1H, NH D_2O exchangeable)	
2	1670 (C=O), 1619 (C=C)	1.11–2.22 (m, 10H, CH_2-cyclohexyl), 2.53 (s, 3H, SCH_3), 7.24–7.75 (m, 4H ArH)	
3	3330, 3261 ($NHNH_2$), 1680 (C=O)	0.80–1.91 (m, 10H, CH_2-cyclohexyl), 4.32 (s, 2H, NH_2), 7.23–7.64 (m, 4H, ArH), 9.75 (s, 1H, NH D_2O exchangeable)	20.2 (2C), 26.5, 30.2 (2C), 49.3, 119.2, 121.3, 126.5, 127.7, 132.7, 145.9, 158.5, 162.7
4a	3310 (NH), 1683 (C=O), 1610 (C=N)	0.61–1.33 (m, 10H, CH_2-cyclohexyl), 1.54–1.64 (q, 2H, CH_2CH_3), 1.74–1.80 (t, 3H, CH_2CH_3), 2.35 (s, 3H, CH_3), 7.01–7.33 (m, 4H, ArH), 8.33 (br s, 1H, NH D_2O exchangeable)	6.7, 12.7, 21.7 (2C), 25.6, 27.3, 30.7 (2C), 49.3, 118.5, 121.1, 126.9, 127.7, 131.9, 146.7, 157.9, 160.5, 162.3
4b	3273 (NH), 1685 (C=O), 1610 (C=N)	0.55–1.21 (m, 10H, CH_2-cyclohexyl), 1.35 – 1.56 (m, 4H, $(CH_2CH_3)_2$), 1.77–1.96 (m, 6H, $(CH_2CH_3)_{2)}$, 7.34–7.66 (m, 4H, ArH), 8.15 (br s, 1H, NH D_2O exchangeable)	
4c	3311(NH), 1671 (C=O), 1612 (C=N)	0.80–1.43 (m, 10H, CH_2-cyclohexyl), 1.51–1.64 (t, 2H, $CH_2CH_2CH_3$), 1.95–2.00 (sext, 2H, $CH_2CH_2CH_3$), 2.10–2.23 (t, 3H, $CH_2CH_2CH_3$), 2.42 (s, 3H, CH_3), 7.41–7.74 (m, 4H, ArH), 8.34 (br s, 1H, NH D_2O exchangeable)	
4d	3266 (NH), 1692 (C=O), 1615 (C=N)	0.57–1.91 (m, 20H, CH_2-cyclohexyl), 7.21–7.63 (m, 4H, ArH), 8.73 (br s, 1H, NH D_2O exchangeable)	
4e	3316 (NH), 1680 (C=O), 1612 (C=N)	0.81–1.53 (m, 10H, CH_2-cyclohexyl), 1.94 (s, 3H, CH_3), 7.22–7.83 (m, 9H, ArH), 8.54 (br s, 1H, NH D_2O exchangeable)	12.3, 21.4 (2C), 26.7, 30.7 (2C), 49.7, 119.5, 121.1, 127.2, 127.7, 128.7 (2C), 129.2 (2C), 131.9, 132.1, 133.7, 145.9, 159.7, 162.7, 167.9
4f	3285 (NH), 1690 (C=O), 1610 (C=N)	0.60–1.31 (m, 10H, CH_2-cyclohexyl), 7.42–8.04 (m, 8H, ArH), 8.64 (br s, 1H, NH D_2O exchangeable), 9.25 (br s, 1H, NH D_2O exchangeable)	

4g	3310 (NH), 1685 (C=O), 1611 (C=N)	0.55–1.11 (m, 10H, CH_2-cyclohexyl), 6.57 (s, 1H, CH), 7.24–7.92 (m, 9H, ArH), 8.55 (br s, 1H, NH D_2O exchangeable)	
4h	3056 (NH), 1690 (C=O), 1612 (C=N)	0.71–1.44 (m, 10H, CH_2-cyclohexyl), 6.33 (s, 1H, CH), 7.42–8.04 (m, 8H, ArH), 8.54 (br s, 1H, NH D_2O exchangeable)	
4i	3274 (NH), 1678 (C=O), 1613 (C=N)	0.71–1.34 (m, 10H, CH_2-cyclohexyl), 6.04 (s, 1H, CH), 7.11–7.27 (m, 8H, ArH), 8.32 (br s, 1H, NH D_2O exchangeable)	21.7 (2C), 27.1, 30.7 (2C), 49.1, 118.7, 121.9, 126.3, 127.5, 128.7 (2C), 129.9, 130.2 (2C), 131.9, 135.7, 141.7, 145.7, 159.9, 162.7
4j	3326 (NH), 1690 (C=O), 1612 (C=N)	0.56–1.26 (m, 10H, CH_2-cyclohexyl), 6.45 (s, 1H, CH), 7.33–7.88 (m, 8H, ArH), 8.34 (br s, 1H, NH D_2O exchangeable)	
4k	3260 (NH), 1681 (C=O), 1611 (C=N)	0.91–1.64 (m, 10H, CH_2-cyclohexyl), 6.35 (s, 1H, CH), 7.34–7.84 (m, 8H, ArH), 8.25 (br s, 1H, NH D_2O exchangeable)	21.7 (2C), 27.4, 29.3 (2C), 49.5, 118.9, 120.9 (2C), 125.5, 127.4, 127.9, 129.5 (2C), 132.9, 138.5, 141.9, 145.9, 149.7, 160.7, 162.8
4l	3332 (NH), 1690 (C=O), 1617 (C=N)	1.15–1.74 (m, 10H, CH_2-cyclohexyl), 3.43 (s, 3H, OCH_3), 6.45 (s, 1H, CH), 7.44–7.95 (m, 8H, ArH), 8.77 (br s, 1H, NH D_2O exchangeable)	
4m	3310 (NH), 1690 (C=O), 1617 (C=N)	0.77–1.33 (m, 10H, CH_2-cyclohexyl), 1.95 (s, 3H, CH_3), 6.44 (s, 1H, CH), 7.12–7.74 (m, 8H, ArH), 8.74 (s, 1H, NH D_2O exchangeable)	
4n	3354 (NH), 1688 (C=O), 1615 (C=N)	0.62–1.22 (m, 10H, CH_2-cyclohexyl), 1.64 (s, 3H, CH_3), 6.00 (s, 1H, CH), 7.52–8.01 (m, 8H, ArH), 8.34 (br s, 1H, NH D_2O exchangeable)	21.4 (2C), 23.7, 27.1, 29.9 (2C), 49.1, 118.6, 119.9 (2C), 125.9, 126.9, 127.9, 129.5 (2C), 132.9, 138.5, 141.9, 145.9, 149.7, 160.1, 161.9
4o	3310 (NH), 1687 (C=O), 1611 (C=N)	0.94–1.64 (m, 10H, CH_2-cyclohexyl), 3.33 (s, 3H, OCH_3), 7.22–8.03 (m, 14H, ArH), 8.34 (br s, 1H, NH D_2O exchangeable)	

acid (0.01 mol) were dissolved in ethanol (20 mL). To this, anhydrous potassium carbonate (100 mg) was added and refluxed for 20 h. The reaction mixture was cooled in ice and the solid separated was filtered and purified by dissolving in 10 % alcoholic sodium hydroxide solution and re-precipitated by treatment with dilute hydrochloric acid. The solid obtained was filtered, washed with water, dried and recrystallized from ethanol.

*3-Cyclohexyl-2-methylsulfanyl-3*H*-quinazolin-4-one (**2**).* – 3-Cyclohexyl-2-thioxo-2,3-dihydro-1*H*-quinazolin-4-one (**1**) (0.01 mol) was dissolved in 40 mL of 2 % alcoholic sodium hydroxide solution. To this, dimethylsulfate (0.01 mol) was added dropwise under stirring. The stirring was continued for 1 h, the reaction mixture was then poured into ice water. The solid obtained was filtered, washed with water, dried and recrystallized from the ethanol/chloroform (75:25) mixture.

*3-Cyclohexyl-2-hydrazino-3*H*-quinazolin-4-one (**3**).* – 3-Cyclohexyl-2-methylsulfanyl-3*H*-quinazolin-4-one (**2**) (0.01 mol) was dissolved in ethanol (25 mL). To this, hydrazine hydrate (99 %) (0.1 mol) and anhydrous potassium carbonate (100 mg) were added and refluxed for 29 h. The reaction mixture was cooled and poured into ice water. The solid so obtained was filtered, washed with water, dried and recrystallized from the chloroform/benzene (25:75) mixture.

*General synthetic procedure for 3-cyclohexyl-2-substituted hydrazino-3*H*-quinazolin-4-ones (**4a-o**).* – A mixture of 3-cyclohexyl-2-hydrazino-3*H*-quinazolin-4-one (**3**) (0.004 mol) and appropriate ketone/aldehyde (0.004 mol) in glacial acetic acid was refluxed for 41 h. The reaction mixture was poured into ice water. The solid obtained was filtered, washed with water, dried under high vacuum and recrystallized from ethanol.

Pharmacology

The synthesized compounds were evaluated for analgesic, anti-inflammatory and ulcerogenic activity. Test compounds and standard drugs were administered in the form of a suspension (1 % carboxymethylcellulose as a vehicle) by oral route of administration for analgesic and anti-inflammatory studies, but for ulcerogenicity studies 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; they were fed standard animal feed. All the animals were acclimatized for a week before the experiment. The Institutional Animal Ethics Committee approved the protocol adopted for experimentation on animals.

Analgesic activity. – Test for analgesic activity was performed by the tail-flick technique (14, 15) using Wistar albino mice (25–35 g) of either sex selected by random sampling technique. Diclofenac sodium, at dose levels of 10 and 20 mg kg^{-1}, was administered orally as reference drug for comparison. The test compounds at two dose levels (10 and 20 mg kg^{-1}) were administered orally. The reaction time was recorded at 30 min, 1, 2 and 3 h after the treatment, and the cut-off time was 10 s.

Anti-inflammatory activity. – Anti-inflammatory activity was evaluated by the carrageenean-induced paw oedema method (16). Sixteen groups of albino Wistar rats of either sex weighing 150–200 g, 6 animals each, were orally dosed with the tested compounds at two dose levels (10 and 20 mg kg^{-1}). Diclofenac sodium, 10 and 20 mg kg^{-1}, was

administered as standard drug for comparison. Paw volumes were measured using the mercury displacement technique with the help of a plethysmograph immediately after and 30 min, 1, 2 and 3 h after carrageenean injection.

Ulcerogenicity index. – Wistar Albino rats weighing 150–200 g, of either sex, were divided into sixteen groups of six animals each (17). Animals were administered the test compounds intraperitoneally at a dose of 20 mg kg^{-1} (17). The control group of animals was administered only 10 % (*V*/*V*) Tween 80 suspension intraperitoneally. One group was administered acetylsalicylic acid (Ranbaxy, India) intraperitoneally at a dose of 20 mg kg^{-1} once daily for three days. On the fourth day, pylorus was ligated as per the reported method (18). 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 the greater curvature. Ulcer index was determined by the reported method (19).

Statistical analysis

Statistical analysis of the biological activity of synthesized compounds on animals was evaluated using a one-way analysis of variance (ANOVA). In all cases, *posthoc* comparisons of the means of individual groups were performed using Tukey's test. All values were expressed as mean ± SD. The GraphPad Prism 3.0 version was used for statistical analysis.

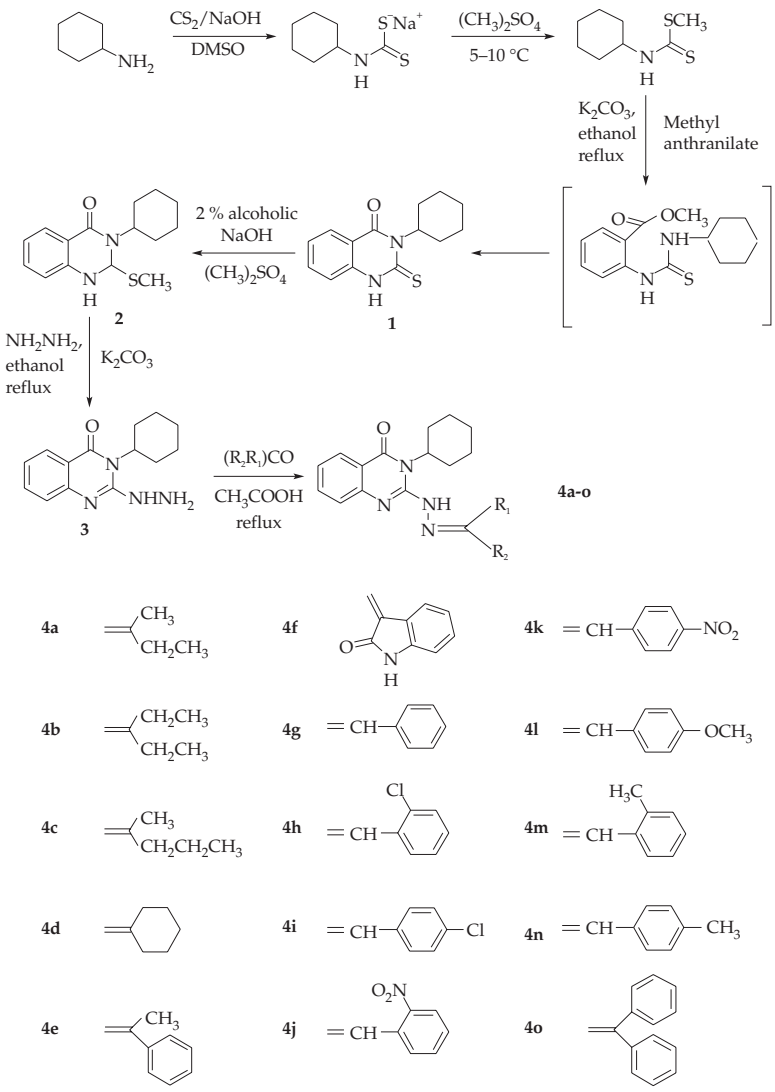
RESULTS AND DISCUSSION

The key intermediate 3-cyclohexyl-2-thioxo-2,3-dihydro-1*H*-quinazolin-4-one (**1**) was prepared from cyclohexyl amine with carbon disulphide and sodium hydroxide in dimethyl sulphoxide to give sodium dithiocarbamate, which was methylated with dimethyl sulfate to afford the dithiocarbamic acid methyl ester. The later refluxed with methyl anthranilate in ethanol yielded the desired compound **1** *via* the thiourea intermediate in a good yield. IR spectra of compound **1** showed intense peaks at 3298 cm^{-1} for cyclic thiourea (NH), 1690 cm^{-1} for carbonyl (C=O) and 1218 cm^{-1} for thioxo (C=S) stretching. ^{1}H NMR spectrum of **1** showed a multiplet at δ 0.91–2.02 and 7.33–7.84 ppm for cyclohexyl (10H) protons and aromatic (4H) protons, respectively, and a singlet at δ 10.35 ppm, indicating the presence of NH. Further, the molecular ion recorded (*m*/*z* 260 [M^+]) in the mass spectrum is also in agreement with the molecular mass of the compound.

3-Cyclohexyl-2-methysulfanyl-3*H*-quinazolin-4-one (**2**) was obtained by dissolving **1** in 2 % alcoholic sodium hydroxide solution and methylating with dimethyl sulfate under stirring at room temperature. The IR spectrum of compound **2** showed disappearance of NH and C=S stretching signals of cyclic thiourea. It showed a peak for carbonyl (C=O) stretching at 1690 cm^{-1}. The ^{1}H NMR spectrum of compound **2** showed singlets due to SCH_3, a multiplet at δ 1.10–2.21 and 7.22–7.73 ppm for cyclohexyl (10H) protons and aromatic (4H) protons, respectively. Molecular ion recorded in the mass spectrum (*m*/*z* 274 [M^+]) further confirmed the assigned structure.

Nucleophilic displacement of the methylthio group of **2** with hydrazine hydrate was carried out using ethanol as solvent to afford 3-cyclohexyl-2-hydrazino-3*H*-quinazolin-

-4-one (**3**). The long duration of reaction (29 h) required might be due to the presence of the bulky cyclohexyl ring at position **3**, which might have reduced the reactivity of quinazoline ring system at the C-2 position. The formation of **3** was confirmed by the ^{1}H NMR spectrum singlets at δ 4.32 and 9.73 ppm due to NH_2 and NH, respectively, a multiplet at δ 0.80–1.91 and 7.24–7.65 ppm for cyclohexyl (10H) protons and aromatic (4H) protons, respectively. The NH and NH_2 signals at 3330–3261 cm^{-1} appeared in the IR



Scheme 1

spectrum. It also showed a peak for carbonyl (C=O) at 1680 cm^{-1}. Further, the molecular ion recorded in the mass spectrum (*m*/*z* 258 [M^+]) is also in agreement with the molecular mass of the compound.

The title compounds, 3-cyclohexyl-2-substituted hydrazino-3*H*-quinazolin-4-ones (**4a-o**), were obtained by condensation of the amino group of 3-cyclohexyl**-**2-hydrazino-3*H*-quinazolin-4-one (**3**) with a variety of aldehydes and ketones. Formation of the title product is indicated by the disappearance of peak due to NH_2 of the starting material in IR and ^{1}H NMR spectra of all compounds **4a-o**. The IR and ^{1}H NMR spectra of these compounds showed the presence of peaks due to ($N=CR_1R_2$), carbonyl (C=O), NH and aryl groups. Mass spectra of the title compounds showed molecular ion peaks corresponding to their molecular formula. In mass spectra of compounds **4a-o**, a common peak at *m*/*z* 144 corresponding to quinazolin-4-one moiety appeared. The M^++2 peaks were observed in the spectra of compounds **4h** and **4i**, confirming the presence of a chlorine atom in the compounds. The relative intensities of these M^++2 peaks in comparison with M^+ peaks were in a ratio 1:3. Elemental analysis satisfactorily confirmed elemental composition and purity of the synthesized compounds.

The results of analgesic testing indicated that the test compounds exhibited moderate analgesic activity after 30 min of reaction time and an increase in activity after 1 h, reaching the peak level after 2 h. Decline in activity was observed after 3 h (Table III). Compound **4a** with 1-methylpropylidene substituent showed good activity; with increased lipophilicity of 1-ethylpropylidene group compound **4b** showed an increase in activity. After replacement of 1-ethylpropylidene group with its isomer 2-pentylidene group compound **4c** retained its activity. Replacement of an alkyl chain at the 2-position with a cycloalkyl group and an aralkyl group (compounds **4d** and **4e**, respectively) led to moderate decrease in activity. Placement of an aryl group at the *N*-3 position (compounds **4f, 4g** and **4m-o**) also resulted in decreased activity. Placement of an electron withdrawing group at the N-3 aryl ring (compounds **4h-l**) led to further decrease of activity.

The anti-inflammatory activity data (Table IV) indicate that all the test compounds moderately protected rats from carrageenean-induced inflammation after 30 min of reaction time, with increased activity after 1 h that reached the peak level after 2 h. Decline in activity was observed after 3 h. Compound 3-cyclohexyl-2-(1-methyl butylidene-hydrazino)-3*H*-quinazolin-4-one (**4c**) emerged as the most active anti-inflammatory agent and it is more potent compared to the reference standard diclofenac sodium.

The ulcer index of the test compounds (Table V) reveals that the compounds with open-chain aliphatic substituents (compounds **4a-c**) showed a negligible ulcer index, whereas aryl substituents (compounds **4e-g** and **4m-o**) exhibited little increase in the ulcer index and the aryl substituents containing electron withdrawing groups (compounds **4h-l**) exhibited a higher ulcer index then other test compounds. When compared to the reference standard acetylsalicylic acid (ulcer index 1.73 ± 0.41) and diclofenac (ulcer index 1.65 ± 0.59), the test compounds exhibited about 35 to 50 % of the ulcer index of reference standards. Compound 3-cyclohexyl-2-(1-methylpropylidene-hydrazino)-3*H*-quinazolin-4-one (**4a**) exhibited the lowest ulcer index (0.45 ± 1.51) among the test compounds wherease the compound 3-cyclohexyl-2-(*N'*-(2-nitro-benzylidene-hydrazino)-3*H*-quinazolin-4-one (**4j**) showed the highest ulcer index (0.79 ± 1.26) among the test compounds.

In our earlier studies, we observed that the presence of alkyl groups exhibited more analgesic and anti-inflammatory activities then aryl groups at the *N*-3 position of qui-

Table III. Analgesic activity of test compounds (tail-flick technique)

Compd.	Dose (mg kg^{-1})	Analgesic activity (%)[a]			
		30 min	1 h	2 h	3 h
4a	10	61 ± 2[c]	66 ± 2[c]	68 ± 2[c]	36 ± 1[d]
	20	73 ± 2[c]	77 ± 2[c]	80 ± 2[c]	45 ± 2[c]
4b	10	65 ± 1[c]	69 ± 1[c]	74 ± 1[c]	38 ± 1[c]
	20	74 ± 1[c]	78 ± 1[c]	83 ± 2[c]	47 ± 1[c]
4c	10	67 ± 1[c]	70 ± 2	77 ± 2[c]	41 ± 1[c]
	20	78 ± 2[c]	81 ± 2[c]	85 ± 1[c]	50 ± 1[c]
4d	10	45 ± 1[c]	51 ± 1[c]	54 ± 1[c]	31 ± 1[e]
	20	57 ± 1[c]	60 ± 2[c]	65 ± 2[e]	39 ± 1
4e	10	52 ± 1[c]	56 ± 2[c]	58 ± 1[c]	34 ± 1
	20	63 ± 2[c]	67 ± 2[c]	70 ± 2[c]	41 ± 1[e]
4f	10	48 ± 1[c]	51 ± 1[c]	52 ± 2[c]	29 ± 1[c]
	20	59 ± 1[c]	62 ± 2[c]	63 ± 1	37 ± 1[e]
4g	10	45 ± 2[c]	49 ± 1[c]	52 ± 1[c]	30 ± 1[d]
	20	57 ± 1[c]	62 ± 1[c]	64 ± 2	39 ± 2
4h	10	36 ± 1	44 ± 2	48 ± 2[e]	29 ± 1[c]
	20	45 ± 1	50 ± 1[c]	56 ± 1[c]	35 ± 1[c]
4i	10	38 ± 1	43 ± 2	50 ± 2[d]	28 ± 1[c]
	20	51 ± 1[c]	55 ± 1	59 ± 2	36 ± 1[d]
4j	10	40 ± 2	48 ± 1[c]	52 ± 2[c]	29 ± 1[c]
	20	54 ± 1[c]	57 ± 1[e]	62 ± 1	38 ± 1
4k	10	40 ± 2	44 ± 1	47 ± 2	27 ± 2[c]
	20	49 ± 1[d]	52 ± 1[d]	58 ± 1[e]	33 ± 1[c]
4l	10	41 ± 2[e]	46 ± 1[d]	51 ± 2[c]	29 ± 1[c]
	20	49 ± 2[d]	55 ± 2	59 ± 2	39 ± 1
4m	10	45 ± 1[c]	48 ± 1[c]	52 ± 2[c]	31 ± 1[c]
	20	54 ± 1[c]	57 ± 2[e]	61 ± 2	38 ± 1
4n	10	47 ± 2[c]	52 ± 1[c]	55 ± 2[c]	30 ± 1[d]
	20	59 ± 1[c]	60 ± 1[c]	63 ± 1	39 ± 2
4o	10	48 ± 1[c]	50 ± 2[c]	51 ± 2[c]	32 ± 1
	20	56 ± 2[c]	58 ± 1[d]	63 ± 2	39 ± 2
Control[b]	–	–	–	–	–
Diclofenac	10	37 ± 2	43 ± 1	45 ± 1	33 ± 1
	20	46 ± 1	55 ± 1	62 ± 2	39 ± 1

[a] Mean ± SD from six experiments done in duplicate.
[b] Control animals were administered orally 1 % CMC.
Significant difference relative to diclofenac at the same time point and the same dosage: [c] $p < 0.0001$, [d] $p < 0.001$, [e] $p < 0.01$.

Table IV. Anti-inflammatory activity of test compounds (carrageenean-induced paw oedema test)

Compd.	Dose (mg kg^{-1})	Anti-inflammatory protection (%)[a]			
		30 min	1 h	2 h	3 h
4a	10	48 ± 2[c]	51 ± 1[c]	55 ± 1[c]	30 ± 2[e]
	20	56 ± 1[c]	62 ± 2[c]	65 ± 2[c]	39 ± 2[e]
4b	10	49 ± 1[c]	53 ± 1[c]	59 ± 2[c]	35 ± 2
	20	62 ± 1[c]	66 ± 1[c]	71 ± 2[c]	43 ± 2
4c	10	50 ± 2[c]	57 ± 1[c]	63 ± 2[c]	36 ± 1[d]
	20	65 ± 2[c]	68 ± 2[c]	74 ± 2[c]	47 ± 1[c]
4d	10	40 ± 2[c]	42 ± 1[c]	48 ± 2[c]	30 ± 2[e]
	20	49 ± 2[e]	55 ± 1[d]	59 ± 1	37 ± 1[c]
4e	10	44 ± 2[c]	47 ± 1[c]	48 ± 1[c]	30 ± 2[e]
	20	52 ± 1[c]	58 ± 1[c]	61 ± 1	38 ± 1[c]
4f	10	38 ± 2[c]	42 ± 2[c]	47 ± 1[c]	30 ± 2[e]
	20	49 ± 1[e]	51 ± 1	55 ± 1[d]	39 ± 1[c]
4g	10	37 ± 2[c]	40 ± 1[e]	46 ± 1[c]	29 ± 1[c]
	20	48 ± 1[e]	50 ± 2[e]	55 ± 1[d]	35 ± 1[c]
4h	10	32 ± 1	35 ± 1[d]	39 ± 1	27 ± 2[c]
	20	41 ± 2[e]	42 ± 1[c]	49 ± 1[c]	33 ± 1[c]
4i	10	36 ± 1[c]	38 ± 1	42 ± 1[e]	29 ± 1[c]
	20	42 ± 1[e]	49 ± 1[d]	51 ± 1[c]	36 ± 1[c]
4j	10	37 ± 1[c]	40 ± 1[e]	45 ± 1[c]	27 ± 1[c]
	20	45 ± 1	48 ± 1[c]	52 ± 1[c]	35 ± 2[c]
4k	10	32 ± 2	39 ± 1	39 ± 1	28 ± 2[c]
	20	43 ± 2	47 ± 2[c]	52 ± 2[c]	36 ± 2[c]
4l	10	35 ± 2[d]	38 ± 2	39 ± 2	28 ± 1[c]
	20	44 ± 2	47 ± 2[c]	48 ± 1[c]	35 ± 1[c]
4m	10	41 ± 1[c]	46 ± 1[c]	49 ± 1[c]	29 ± 1[c]
	20	52 ± 2[c]	52 ± 2	58 ± 2	36 ± 2[c]
4n	10	37 ± 2[c]	42 ± 1[c]	46 ± 1[c]	30 ± 2[e]
	20	48 ± 1[e]	54 ± 2[e]	57 ± 1[e]	40 ± 1[e]
4o	10	36 ± 1[c]	40 ± 1	42 ± 1[e]	31 ± 2[e]
	20	51 ± 1[c]	56 ± 1[c]	58 ± 2	39 ± 1[e]
Control[b]		–	–	–	–
Diclofenac		32 ± 1	38 ± 1	39 ± 2	33 ± 1
		45 ± 2	52 ± 1	60 ± 2	42 ± 1

[a] Mean ± SD from six experiments done in duplicate.
[b] Control animals were administered orally 1 % CMC.
Significant difference relative to diclofenac at the same time point and the same dosage: [c] $p < 0.0001$, [d] $p < 0.001$, [e] $p < 0.01$.

Table V. Ulcerogenicity

Compd.	Ulcer index[a]
4a	0.45 ± 1.51
4b	0.50 ± 1.82
4c	0.50 ± 1.75
4d	0.48 ± 1.64
4e	0.61 ± 1.23
4f	0.66 ± 1.42
4g	0.64 ± 1.82
4h	0.71 ± 1.21
4i	0.73 ± 1.54
4j	0.79 ± 1.26
4k	0.74 ± 1.71
4l	0.77 ± 1.21
4m	0.58 ± 1.23
4n	0.60 ± 1.84
4o	0.59 ± 1.65
Control[b]	0.15 ± 0.33[c]
Diclofenac	1.65 ± 0.59
Acetylsalicylic acid	1.73 ± 0.41

[a] Mean ± SD from six experiments done in duplicate.
[b] Control animals were administered 10 % (*V*/*V*) Tween 80 (*i.p.*).
Significant difference relative to diclofenac: [c] $p < 0.0001$.

nazolin-3-yl-4(3*H*)-one (10-13). Hence, we also made a substitution at the C-2 position in such a way as to increase lipophilicity of the molecule. Placement of such a group enhanced the analgesic and anti-inflammatory activities. In the present study, the most active compound **4c** exhibited 77 and 85 % analgesic activity at 10 and 20 mg kg^{-1}, dose levels respectively, after 2 h. In contrast, diclofenac sodium showed 45 and 62 % analgesic activity at 10 and 20 mg kg^{-1} dose levels, respectively, after 2 h. The most active compound **4c** showed 63 and 74 % anti-inflammatory activity at doses of 10 and 20 mg kg^{-1}, respectively, after 2 h, whereas diclofenac sodium revealed 39 and 60 % anti-inflammatory activity at doses of 10 and 20 mg kg^{-1}, respectively, after the same time. Interestingly, compound **4c** showed 35 % of ulcer index of the reference NSAID's acetylsalicylic acid and diclofenac.

CONCLUSIONS

In the present study, synthesis of a new series of 3-cyclohexyl-2-substituted hydrazino-3*H*-quinazolin-4-ones (**4a-o**) has been described. Among the series, 3-cyclohexyl-2--(1-methylbutylidene-hydrazino)-3*H*-quinazolin-4-one (**4c**) emerged as the most active

compound and it is moderately more potent in its analgesic and anti-inflammatory activities compared to the reference standard diclofenac sodium. Although this series could be developed as a novel class of analgesic and anti-inflammatory agents, further structural modifications are planned to increase the analgesic and anti-inflammatory activities with reduced ulcerogenicity.

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S A Ž E T A K

Sinteza i farmakološka evaluacija 3-cikloheksil-2-supstituiranih hidrazino-3*H*-kinazolin-4-ona kao analgetika i antiinflamatorika

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Reakcijom amino skupine 3-cikloheksil-2-hidrazino kinazolin-4(3*H*)-ona s različitim aldehidima i ketonima sintetizirani su novi 3-cikloheksil-2-supstituirani hidrazino-kinazolin-4(3*H*)-oni. Početni spoj 3-cikoheksil-2-hidrazino kinazolin-4(3*H*)-on pripravljen je iz cikloheksilamina. Sintetizirani spojevi testirani su na analgetsko i protuupalno djelovanje te ulcerogena svojstva. Spoj 3-cikloheksil-2-(1-metilbutiliden-hidrazino)-3*H*-kinazolin-4-on (**4c**) imao je najjače analgetsko i protuupalno djelovanje, nešto jače nego referentni spoj diklofenak natrij. Osim toga, testirani spojevi imaju samo blago ulcerogeno djelovanje u usporedbi s acetilsalicilnom kiselinom.

Ključne riječi: kinazolin-4(3H)-on, analgetsko djelovanje, protuupalno djelovanje, ulcerogenost

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