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Synthesis and Biological Evaluation of some Anthranilic Acid and 2-Phenylquinazoline-4(3*H*)-one Analogues

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ABSTRACT

In the present investigation a novel series of N-(phenyl) chalconyl anthranilic acids containing pyrazolines (4a–j), tetrahydropyrimidines (4k–o), tetrahydrothiopyrimidines (4p–t) and 2-phenylquinazolin-4(3*H*)-ones containing pyrazolines (8a–f), isoxazolines (8g–l), tetrahydropyrimidines (8m–r) and tetrahydrothiopyrimidines (8s–x) were synthesized and characterized by elemental analysis, FT-IR, ^{1}H NMR and mass spectroscopy. The title compounds (4a–t) and (8a–x) were investigated for their analgesic, anti-inflammatory, antimicrobial and *in vitro* protein denaturation activities. Compounds 4j and 8x were identified as lead compounds with optimum analgesic, anti-inflammatory and antimicrobial activities.

KEYWORDS

Quinazolines, antimicrobial, analgesic, anti-inflammatory, protein denaturation.

1. Introduction

The treatment of pain continues to be the subject of considerable pharmaceutical and clinical research. Microbial infections often produce pain and inflammation. Chemotherapeutic, analgesic and anti-inflammatory drugs are prescribed simultaneously in normal practice. For better patient compliance, an anti-inflammatory, antimicrobial agent with minimal side effects is highly desirable, especially in patients with impaired liver or kidney functions.

Anthranilic acid[1] derivatives such as mefenamic acid and meclofenamate are useful for clinical treatment of various pain and inflammatory disorders. They were also found to possess potent analgesic and antimicrobial activities. Compounds containing the pyrazoline[2–6] and isoxazoline[6–9] nucleus have received the attention of medicinal chemists due to a wide range of biological activities including antimicrobial, analgesic and anti-inflammatory activities. In recent years there has been an increased interest in the chemistry of quinazoline-4(3*H*)-ones because of their biological significance.[10–12] Different types of quinazoline-4(3*H*)-ones exhibited antimicrobial, analgesic and anti-inflammatory[13–15] activities. It is a well known fact that inflammation associated with diseases like arthritis is a consequence of *in vivo* denaturation of proteins.[16] In view of the above facts and in continuation of our work on pyrazolines[2] and quinazoline-4(3*H*)-ones,[10] we report herein the synthesis of new molecules possessing analgesic, anti-inflammatory and antimicrobial activities.

2. Results and Discussion

Synthesis of the title compounds (**4a–t**) and (**8a–x**) was carried as per Schemes 1 and 2. N-(phenyl) chalconyl anthranilic acid[1] (**3a–e**) and 2-phenyl-4*H*-3,1-benzoxazin-4-one (**5**)[17] were prepared from anthranilic acid according to methods from the literature. Condensation of **3a–e** separately with hydrazine hydrate (99 % m/v), phenyl hydrazine, urea and thiourea yielded N-[5(phenyl)-pyrazoline-3-yl]-anthranilic acids (**4a–e**), N-[1-phenyl-5-(substituted)-pyrazoline-3-yl]-anthranilic acids (**4f–j**), 2-[(2′-oxo-6′-phenyl-1′,2′,5′,6′-tetrahydropyrimidin-4′-yl) amino]-benzoic acids (**4k–o**), 2-[(2′-thioxo-6′-phenyl-1′,2′,5′,6′-tetrahydropyrimidin-4′-yl) amino]-benzoic acids (**4p–t**) respectively. 2-phenyl-4*H*-3,1-benzoxazin-4-one (**5**) on treatment with *p*-aminoacetophenone yielded **6** which, on condensation with araldehydes furnished 3-[4″-{3″-phenylprop-2″-enoyl} phenyl]-2-phenylquinazolin-4(3*H*)-ones (**7a–f**). Further condensation of compounds **7a–f** separately with hydrazine hydrate, hydroxylamine hydrochloride, urea and thiourea afforded 2-phenyl-3-[4′-(5″-phenyl-4,5-dihydro-1*H*-pyrazol-3′-yl)phenyl] quinazolin-4(3*H*)-ones (**8a–f**), 2-phenyl-3-[4′-(5″-phenyl-4,5-dihydroisoxazol-3′-yl)phenyl]quinazolin-4(3*H*)-ones (**8g–l**), 3-[4′{-2″-oxo-6″-phenyl-1″,2″,5″,6″-tetrahydro-pyrimidin-4″-yl} phenyl]-2-phenylquinazolin-4(3*H*)-ones (**8m–r**) and 3-[4″{-2″-thioxo-6″-phenyl-1″,2″,5″,6″-tetrahydropyrimidin-4″-yl}phenyl]-2-phenylquinazolin-4(3*H*)-ones (**8s–x**), respectively.

Structures of newly synthesized compounds were characterized by elemental analysis, FT-IR, ^{1}H NMR and mass spectroscopy. Compounds **4a–t, 8a–f** and **8m–x** showed absorption bands in the range 3229 to 3291 cm^{-1} for N-H and 1600 to 1621 cm^{-1} for C=N stretching. Compounds **8g–l** exhibited absorption bands in the range 1098 to 1121 cm^{-1} for C-O-C bending, 1601 to 1612 cm^{-1} for C=N stretching vibrations and C=O absorption bands in the range 1650 to 1672 cm^{-1}. ^{1}H NMR spectra of compounds **4a, 4f, 4j** and **8a** exhibited doublets at δ 3.21, 2.92, 2.86 and 3.06 ppm for -CH_2- and triplets at δ 3.95, 3.45, 3.21 and 4.96 ppm for -CH-, respectively. Compounds **4a, 4j, 8a** and **8x** showed singlets at δ 8.84, 8.21, 7.20 and 6.76 ppm, respectively, integrating for one proton due to -NH-, confirming the formation of the pyrazoline nucleus.

Similarly compounds **4k**, **4p**, **8m** and **8s** exhibited doublets at δ 3.33, 3.07, 2.53 and 2.31 ppm for -CH_2-, triplets at δ 4.95, 3.99, 4.62 and 4.41 ppm for -CH- and singlets at δ 6.40, 6.99, 6.14 and 6.79 ppm integrating for one proton due to -NH-, confirming the formation of the pyrimidine nucleus**.** In compound **8g** disappearance of the -NH- signal and appearance of a doublet at δ 3.53 ppm and a triplet at δ 5.62 ppm confirmed the formation of the isoxazoline nucleus. The mass spectra of compounds **4a**, **4j**,

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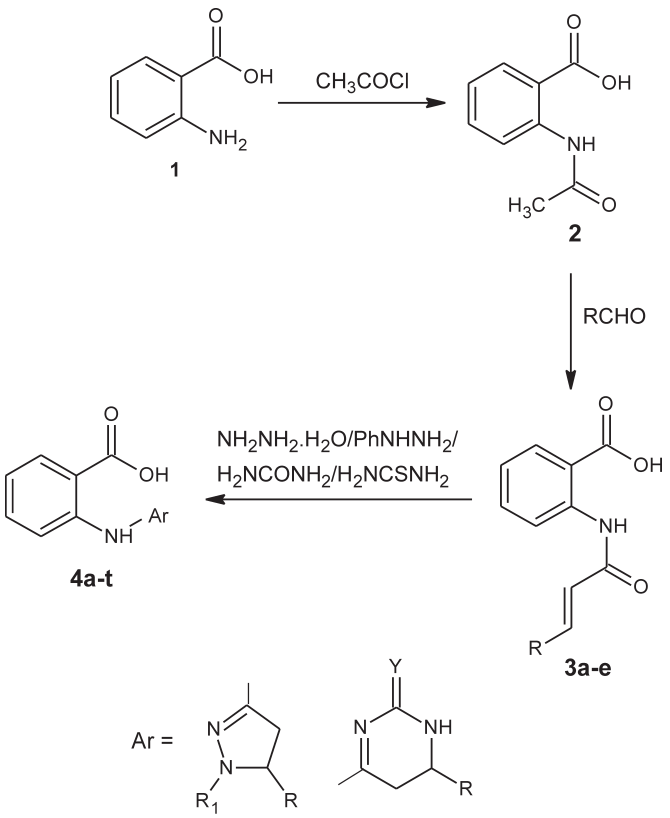


4a-e: R_1=H; **4f-j**: R_1=C_6H_5 ; **4k-o**: Y=O; **4p-t**: Y=S
R=C_6H_5: **3a, 4(a,f,k,p)**; 4-$NO_2C_6H_4$: **3b, 4(b,g,l,q)**; 4-$OCH_3C_6H_4$: **3c**, **4(c,h,m,r)**; 2-OHC_6H_4: **3d**, **4(d,i,n,s)**; 4-ClC_6H_4: **3e**, **4(e,j,o,t)**.

Scheme 1
Synthesis of anthranilic acid derivatives.

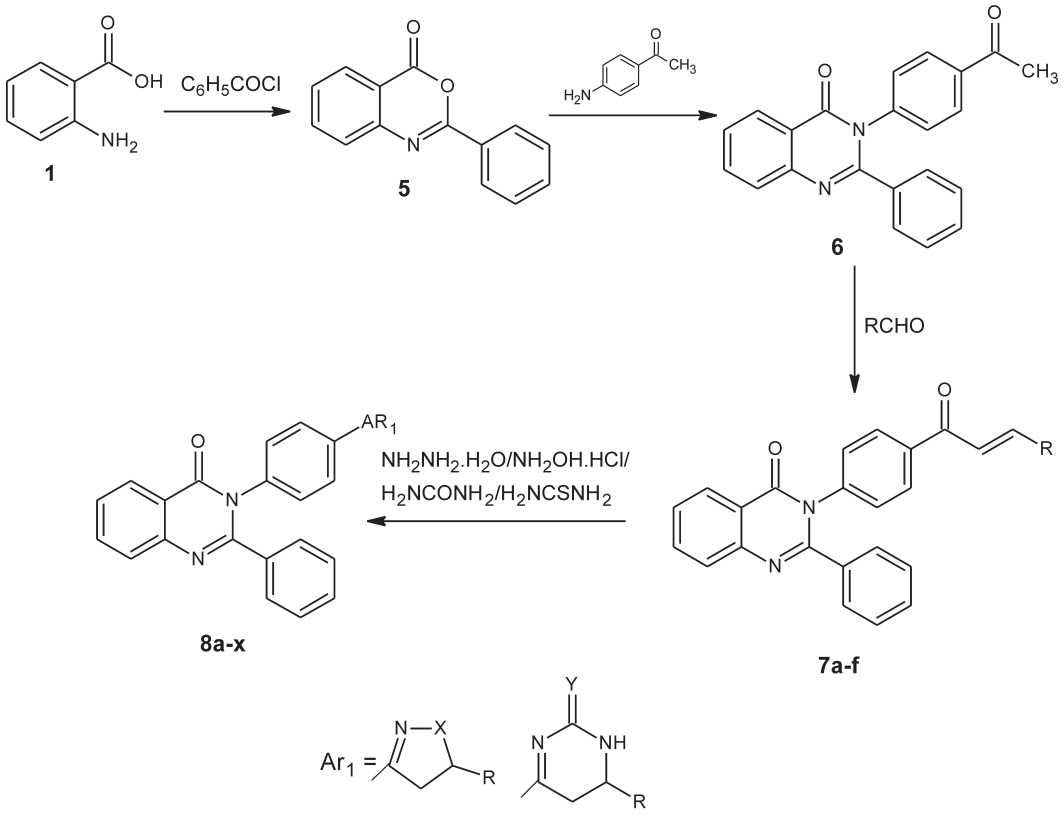
4k, 8a, 8g, 8m and **8x** showed molecular ion peaks corresponding to their molecular formulae.

Synthesized compounds **4a–e** and **8a–x** were evaluated for analgesic and anti-inflammatory activities. Student's *t*-test was performed to ascertain the significance of the exhibited activities. The test compounds (100 mg kg^{-1}) and the standard drugs paracetamol (100 mg kg^{-1}) and mefenamic acid (10 mg kg^{-1}) were administered in the form of a suspension (1 % carboxymethyl cellulose as vehicle) by oral route. Analgesic activity of the test compounds was carried out by the tail flick method[18] using wistar albino mice. The percentage analgesic activity was calculated in comparison with paracetamol (Table 1). Compounds **4g, 4j, 8a, 8b** and **8q** exhibited significant analgesic activity after 2 h of oral administration. Moreover a decrease in analgesic activity was observed after 3 h of oral administration. In general the quinazolinone derivatives (**8a–x**) were found to be more potent in comparison with pyrazoline/substituted pyrazoline and tetrahydropyrimidine/tetrahydrothiopyrimidine/substituted anthranilic acid derivatives (**4a–e).**

Anti-inflammatory activity of test compounds **4a–t** and **8a–x** in acute conditions was investigated using the carrageenan induced rat paw oedema method of Winter *et al.*[19] Compounds **4d, 4e, 4g, 4i, 4j, 4l, 4n, 4o, 4q, 4s, 4t** and **8x** exhibited significant anti-inflammatory activity after 2 h of oral administration. It is also evident from the results that anti-inflammatory activity decreased appreciably at 3 h after oral administration. In general pyrazoline/substituted pyrazoline and tetrahydropyrimidine/tetrahydrothiopyrimidine/substituted anthranilic acid derivatives of **4a–e** were found to be more potent in comparison with quinazolinone derivatives **8a–x.** Compounds **4d, 4e, 4n** and **4t** showed maximum anti-inflammatory activity. It is evident from the findings that pyrazoline and tetrahydropyrimidine/thiopyrimidine analogues of anthranilic acid bearing an electron withdrawing *para*-Cl or *ortho*-OH respectively in the phenyl ring at positions 5 and 6′ of the pyrazoline and tetrahydropyrimidine/thiopyrimidine rings, exhibited significant analgesic and anti-inflammatory activities. A similar pattern was observed in the case of 2-phenyl-quinazoline-4(3*H*)-ones bearing pyrazoline, 4,5-dihydroisoxazole, tetrahydropyrimidine at 4′ of phenyl ring substituents at the 3 position of the 2-phenyl-quinazoline-4(3*H*)-one ring. In general, replacement of either 2-OH or 4-Cl substituents with 4-NO_2, 4-OCH_3 and 2-furyl groups resulted in a marked decrease of analgesic and anti-inflammatory activities (Tables 1 and 2).

In vitro anti-inflammatory activity of test compounds **4a–t** and **8a–x** was investigated using the bovine serum albumin denaturation method[20] and the mean percentage inhibition of protein denaturation was calculated (Table 3). Compounds **4e, 4g, 4j, 4m** and **8f** exhibited significant activity compared with the reference standard mefenamic acid. However, no correlation was observed between different substituent groups and *in vitro* percentage inhibition of denaturation of bovine serum albumin.

All newly synthesized compounds were assayed by the cup plate method[21] against different species of Gram-positive and Gram-negative bacteria of clinical relevance and against two strains of pathogenic fungi in order to identify those with maximum activity as lead compounds. The data obtained for all the tested species (Table 4) showed varying degrees of activity.



Scheme 2
Synthesis of 2-phenylquinazoline-4(3*H*)-one derivatives.

Compounds **4r**, **8c**, **8e**, **8f**, **8k**, **8l**, **8r**, and **8x** exhibited significant antibacterial and antifungal activities. In general quinazolinone derivatives were found to be more active against the entire tested microorganism.

3. Experimental

3.1. General Synthetic Procedure

Melting points were determined in open capillaries and are uncorrected. IR spectra were recorded in KBr on a Jasco FT/IR 5300 spectrophotometer (Tokyo, Japan). ^{1}H NMR ($CDCl_3$) spectra were recorded on a Bruker DPX-300 MHz spectrometer (Karslruhe, Germany) using TMS as internal standard. C, H and N analyses were carried out on a Euro EA (Milan, Italy) analyzer. Mass spectra were recorded on a Perkin-Elmer Hitachi RMU-6L MS 30 instrument (Austin, TX, USA). The progress of the reaction was judged by TLC analysis of the reaction mixture.

3.2. Preparation of N-[5-(phenyl)-pyrazoline-3-yl]-anthranilic acid (4a)/N-[1-phenyl/substituted phenyl-5-phenylpyrazoline-3-yl]-anthranilic acid (**4f**)

A mixture of N-(phenyl) chalconyl anthranilic acid (**3a**) (0.01 mol) and hydrazine hydrate/phenyl hydrazine (0.01 mol) in ethanol (30 mL) was refluxed for 6 h on a water bath. The reaction mixture was concentrated, cooled and poured into ice-cold water. The solid thus separated was filtered and recrystallized from ethanol. Compounds **4b–e and 4g–l** were prepared by adopting the same procedure.

4a: Yield 54 %, m.p. 180–182 °C. IR (KBr) ν: 600 (C=N), 1712 (COOH), 3234 cm^{-1} (NH). Anal. calcd. for $C_{16}H_{15}N_3O_2$ (281): C, 68.35; H, 5.41; N, 14.91 %). Found: C, 68.31; H, 5.37; N, 14.94 %. MS (*m/z*): 281, 263, 255, 211, 205, 177, 161, 145, 120, 118, 104, 96, 52.

4b: Yield 63 %, m.p. 225–227 °C. IR (KBr) ν: 1586 (NO_2), 1602 (C=N),1716 (COOH), 3229 cm^{-1} (NH). Anal. calcd. for $C_{16}H_{14}N_4O_4$ (312): C, 58.94; H, 4.28; N, 17.21 %. Found: C, 58.89; H, 4.32; N, 17.17 %.

4c: Yield 57 %, m.p. 145–148 °C. IR (KBr) ν: 1098 (C-O-C), 1608 (C=N), 1710 (COOH), 3236 cm^{-1} (NH). Anal. calcd. for $C_{17}H_{17}N_3O_3$ (311): C, 65.62; H, 5.51; N, 13.49 %. Found: C, 65.58; H, 5.50; N, 13.50 %.

4d: Yield 74 %, m.p. 178–180 °C. IR (KBr) ν: 1601 (C=N), 1704 (COOH), 3241 (NH), 3452 cm^{-1} (OH). Anal. calcd. for $C_{16}H_{15}N_3O_3$ (297): C, 64.67; H, 5.12; N, 14.09 %. Found: C, 64.64; H, 5.09; N, 14.13 %.

4e: Yield 65 %, m.p. 158–160 °C. IR (KBr) ν: 732 (C-Cl), 1610 (C=N), 1698 (COOH), 3237 cm^{-1} (NH). Anal. calcd. for $C_{16}H_{14}N_3O_2Cl$ (316): C, 60.83; H, 4.51; N, 13.29 %. Found: C, 60.86; H, 4.47; N, 13.31 %.

4f: Yield 71 %, m.p. 110–112 °C. IR (KBr) ν: 732 (C-Cl), 1610 (C=N), 1698 (COOH), 3237 cm^{-1} (NH). Anal. calcd. for $C_{22}H_{19}N_3O_2$ (357): C, 73.89; H, 5.41; N, 11.80 %. Found: C, 73.93; H, 5.36; N, 11.76 %. ^{1}H NMR ($CDCl_3$, 300 MHz), δ: 2.92 (d, 2H, CH_2 of pyrazoline), 3.45 (t,1H, CH of pyrazoline), 6.81–7.84 (m, 14H, aromatic proton), 8.25 (s, 1H, NH), 9.65 ppm (s, 1H, COOH).

4g: Yield 67 %, m.p. 280–283 °C. IR (KBr) ν: 1587 (NO_2), 1621 (C=N), 1723 cm^{-1} (COOH). Anal. calcd. for $C_{22}H_{18}N_4O_4$ (402): C,

Table 1 Analgesic activity of synthesized compounds by tail flick method. Dose: 100 mg kg^{-1}.

Compound	Percentage analgesic activity[a]		
	1 h	2 h	3 h
4a	16.4 ± 0.3[c]	17.3 ± 0.8[c]	5.2 ± 0.5[c]
4b	42.2 ± 0.5[c]	42.6 ± 0.8[c]	16.3 ± 0.6[c]
4c	2.4 ± 0.4[c]	9.2 ± 0.9[b]	10.4 ± 0.5[b]
4d	3.3 ± 0.6[c]	43.1 ± 0.9[d]	17.4 ± 0.6[c]
4e	24.3 ± 0.3[c]	32.4 ± 0.8[c]	23.7 ± 0.6[c]
4f	4.6 ± 0.6[b]	8.1 ± 0.9[b]	7.8 ± 0.6[b]
4g	39.8 ± 0.5[c]	43.4 ± 0.9[c]	23.2 ± 0.8[c]
4h	1.4 ± 0.7[c]	2.2 ± 0.6[c]	2.2 ± 0.6[b]
4i	33.2 ± 0.4[c]	40.4 ± 0.6[c]	33.1 ± 0.6[c]
4j	25.4 ± 0.3[c]	43.9 ± 0.4[c]	32.8 ± 0.6[c]
4k	4.4 ± 0.2[b]	9.3 ± 0.4[b]	10.4 ± 0.6[b]
4l	23.9.±0.4[c]	42.1 ± 0.4[c]	23.8 ± 0.3[c]
4m	2.4 ± 0.5[b]	5.4 ± 0.5[b]	7.2 ± 0.4[b]
4n	29.3±0.6[c]	43.2±.0.5[c]	35.2 ± 0.4[c]
4o	27.2 ± 0.6[c]	43.4 ± 0.4[c]	37.1 ± 0.6[c]
4p	4.2 ± 0.5[b]	11.5 ± 0.5[b]	6.2 ± 0.5[b]
4q	26.7 ± 0.5[c]	43.4 ± 0.5[c]	25.2 ± 0.5[c]
4r	2.3 ± 0.5[b]	6.2 ± 0.6[b]	8.4 ± 0.5[b]
4s	25.4 ± 0.6[c]	41.8 ± 0.7[c]	31.8 ± 0.5[c]
4t	26.8 ± 0.8[c]	44.6 ± 0.8[c]	35.2 ± 0.6[c]
8a	12.6 ± 0.7[c]	44.2 ± 1.1[c]	41.4 ± 0.6[c]
8b	16.9 ± 0.6[c]	41 ± 0.8[c]	4.3 ± 0.6[c]
8c	18.4 ± 0.9[c]	41.9 ± 0.5[c]	41.2 ± 0.6[c]
8d	14.8 ± 0.3[c]	43.2 ± 0.5[c]	42.3 ± 0.4[c]
8e	17.9 ± 0.5[c]	41.1 ± 0.2[c]	39.4 ± 0.4[c]
8f	18.1 ± 0.6[c]	40.2 ± 0.4[c]	42.4 ± 0.4[c]
8g	18.3 ± 0.6[c]	40.2 ± 0.4[c]	43.2 ± 0.5[c]
8h	14.4 ± 0.8[c]	35.7 ± 0.3[c]	35.3 ± 0.5[c]
8i	15.7 ± 0.7[c]	42.2 ± 0.3[c]	41.3 ± 0.5[c]
8j	15.6 ± 0.5[c]	39.9 ± 0.3[c]	42.2 ± 0.5[c]
8k	17.3 ± 0.6[c]	42.3 ± 0.4[c]	41.8 ± 0.5[c]
8l	18.2 ± 0.4[c]	40.4 ± 0.3[c]	42.3 ± 0.6[c]
8m	16.6 ± 0.6[c]	40.1 ± 0.6[c]	31.4 ± 0.6[c]
8n	19.3 ± 0.9[c]	40.8 ± 0.5[c]	25.2 ± 0.5[c]
8o	21.8 ± 0.6[c]	33.2 ± 0.5[c]	19.1 ± 0.6[c]
8p	20.9 ± 0.6[c]	40.2 ± 0.9[c]	24.4 ± 0.8[c]
8q	25.1 ± 0.7[c]	45.3 ± 0.7[c]	35.9 ± 0.8[c]
8r	19.3 ± 0.7[c]	39.4 ± 1.0[c]	25.7 ± 0.6[c]
8s	18.8 ± 0.4[c]	33.4 ± 0.7[c]	20.4 ± 0.6[c]
8t	17.4 ± 0.3[c]	43.1 ± 0.7[c]	25.1 ± 0.3[c]
8u	1.3 ± 0.6[d]	3.2 ± 0.5[b]	2.9 ± 0.4[c]
8v	17.3 ± 0.5[c]	42.4 ± 0.5[c]	31.4 ± 0.5[c]
8w	17.2 ± 0.4[c]	40.4 ± 0.6[c]	25.2 ± 0.6[c]
8x	11.7 ± 0.3[c]	35.1 ± 0.7[c]	28.8 ± 0.5[c]
Paracetamol	45.2 ± 0.2[c]	52.8 ± 0.7[c]	46.2 ± 0.5[c]

[a] Average of three readings; results are expressed as mean ± SEM (n = 6); significance levels [b] $P < 0.05$, [c] $P < 0.01$ and [d] $P < 0.001$ compared with the respective control.

Table 2 Anti-inflammatory activity of synthesized compounds by carrageenan-induced rat paw oedema method.

Compound	Dose/mg kg^{-1}	Percentage anti-inflammatory activity[a]		
		1 h	2 h	3 h
4a	100	29.4 ± 0.4[b]	40.3 ± 0.3[c]	41.1 ± 0.3[c]
4b	100	24.2 ± 0.9[b]	49.4 ± 0.4[c]	29.2 ± 0.3[b]
4c	100	18.3 ± 0.8[b]	29.4 ± 0.3[b]	29.2 ± 0.5[b]
4d	100	50.8 ± 0.5[c]	66.2 ± 0.3[c]	41.2 ± 0.5[c]
4e	100	38.1 ± 0.5[c]	58.9 ± 0.3[c]	50.3 ± 0.5[c]
4f	100	17.9 ± 0.5[c]	26.2 ± 0.5[b]	24.4 ± 0.6[b]
4g	100	37.8 ± 0.8[c]	54.7 ± 0.5[b]	55.4 ± 0.6[b]
4h	100	12.7 ± 0.5[b]	17.6 ± 0.5[b]	13.3 ± 0.8[c]
4i	100	35.2 ± 0.5[c]	55.3 ± 0.8[c]	42.3 ± 0.6[c]
4j	100	3.41 ± 0.7[c]	55.1 ± 0.8[b]	26.2 ± 0.6[b]
4k	100	18.2 ± 0.7[b]	33.4 ± 0.8[c]	14.8 ± 0.5[b]
4l	100	39.7 ± 0.7[b]	52.6 ± 0.7[c]	37.2 ± 0.9[c]
4m	100	39.9 ± 0.7[b]	20.2 ± 0.7[b]	21.4 ± 0.6[b]
4n	100	44.3 ± 0.5[c]	60.1 ± 0.7[c]	58.9 ± 0.9[c]
4o	100	35.2 ± 0.5[c]	54.7 ± 0.5[c]	41.6 ± 0.6[c]
4p	100	4.2 ± 0.7[c]	19.3 ± 0.5[b]	4.4 ± 0.8[b]
4q	100	42.1 ± 0.2[c]	57.2 ± 0.5[c]	43.2 ± 0.8[c]
4r	100	11.3 ± 0.8[b]	11.3 ± 0.6[c]	1.3 ± 0.6[c]
4s	100	35.3 ± 0.7[c]	51.8 ± 0.5[c]	46.4 ± 0.6[c]
4t	100	42.1 ± 0.7[c]	60.3 ± 0.5[c]	54.4 ± 0.8[b]
8a	100	28.3 ± 0.8[c]	31.2 ± 0.8[c]	23.2 ± 0.8[b]
8b	100	22.4 ± 0.8[c]	25.4 ± 0.6[c]	23.3 ± 0.7[c]
8c	100	28.4 ± 0.5[b]	31.4 ± 0.6[b]	18.4 ± 0.9[b]
8d	100	27.8 ± 0.5[c]	37.5±0.6[c]	23.2 ± 0.7[c]
8e	100	32.6 ± 0.5[c]	37.5 ± 0.7[c]	28.8 ± 0.6[c]
8f	100	17.6 ± 0.6[c]	19.2 ± 0.6[b]	17.9 ± 0.9[c]
8g	100	33.1 ± 0.6[c]	37.5 ± 0.6[b]	18.2 ± 0.9[b]
8h	100	11.3 ± 0.6[b]	31.4 ± 0.9[b]	29.2 ± 0.9[c]
8i	100	33.2 ± 0.6[c]	37.5±.0.4[c]	35.1 ± 0.8[c]
8j	100	17.4 ± 0.6[c]	25.4 ± 0.4[b]	6.2 ± 0.8[b]
8k	100	28.4 ± 0.7[b]	37.5±.0.8[b]	35.3 ± 0.8[b]
8l	100	17.1 ± 0.6[b]	31.3 ± 0.8[b]	18.3 ± 1.1[b]
8m	100	16.8 ± 0.7[c]	43.7 ± 0.7[c]	35.4 ± 0.8[c]
8n	100	6.4 ± 0.4[c]	30.8 ± 0.7[b]	22.7 ± 0.5[b]
8o	100	17.3 ± 0.5[c]	12.4 ± 0.6[b]	6.1 ± 0.5[b]
8p	100	6.3 ± 0.2[c]	19.1 ± 0.6[c]	5.9 ± 0.8[c]
8q	100	17.2 ± 0.6[b]	31.2 ± 0.5[b]	29.2 ± 0.5[c]
8r	100	17.1 ± 0.6[c]	44.1 ± 0.5[c]	34.6 ± 0.5[c]
8s	100	21.8 ± 0.4[c]	31.2 ± 0.5[b]	22.9 ± 0.7[b]
8t	100	4.8 ± 0.4[b]	12.4 ± 0.7[b]	6.2 ± 0.4[b]
8u	100	6.1 ± 0.9[c]	19.3 ± 0.7[c]	6.4 ± 0.6[c]
8v	100	11.3 ± 0.9[c]	18.6 ± 0.7[c]	12.3 ± 0.4[c]
8w	100	21.7 ± 0.5[c]	44.4 ± 0.6[c]	35.1 ± 0.4[c]
8x	100	21.8 ± 0.5[c]	50.3 ± 0.5[c]	41.2 ± 0.4[c]
Mefenamic acid	10	56.2 ± 0.5[d]	54.2 ± 0.5[d]	45.4 ± 0.3[d]

[a] Average of three readings; results are expressed as mean ± SEM (n = 6); significance levels [b] $P < 0.05$, [c] $P < 0.01$ and [d] $P < 0.001$ compared with the respective control.

65.62; H, 4.48; N, 13.90 %. Found: C, 65.66; H, 4.51; N, 13.92 %.

4h: Yield 65 %, m.p. 278–281 °C. IR (KBr) ν: 1112 (C-O-C), 1614 (C=N), 1725 cm^{-1} (COOH). Anal. calcd. for $C_{23}H_{21}N_3O_3$ (387): C, 71.34; H, 5.51; N, 10.90 %. Found: C, 71.30; H, 5.46; N, 10.85 %.

4i: Yield 63 %, m.p. 149–151 °C. IR (KBr) ν: 1618 (C=N), 1731 (COOH), 3462 cm^{-1} (OH). Anal. calcd. for $C_{22}H_{19}N_3O_3$ (373): C, 70.75; H, 5.12; N, 11.28 %. Found: C, 70.76; H, 5.13; N, 11.25 %.

4j: Yield 78 %, m.p. 157–159 °C. IR (KBr) ν: 742 (C-Cl), 1621 (C=N), 1711 cm^{-1} (COOH). Anal. calcd. for $C_{22}H_{18}N_3O_2Cl$ (392): C, 67.41; H, 4.59; N, 10.77 %. Found: C, 67.43; H, 4.63; N, 10.72 %. ^{1}H NMR ($CDCl_3$, 300 MHz), δ: 2.86 (d, 2H, CH_2 of pyrazoline), 3.21 (t, 1H, CH of pyrazoline), 6.92–7.69 (m, 14H, aromatic proton), 8.21 (s, 1H, NH), 10.11 ppm (s, 1H, COOH). MS (m/z): 391, 373, 321, 315, 281, 253, 229, 138, 120, 96, 86, 52.

4k: Yield 65 %, m.p. 188–190 °C. IR (KBr) ν: 1607 (C=N), 1667 (C=O), 1723 (COOH), 3256 cm^{-1} (NH). Anal. calcd. for $C_{17}H_{15}N_3O_3$ (309): C, 66.06; H, 4.93; N, 13.55 %. Found: C, 66.01; H, 4.89; N, 13.58 %. ^{1}H NMR ($CDCl_3$, 300 MHz), δ: 3.33 (d, 2H, -CH_2- oxypyrimidine), 4.95 (t, 1H, -CH- oxypyrimidine), 6.40 (d, 1H, NH of oxypyrimidine), 6.81–7.84 (m, 9H, aromatic proton), 8.81 (s, 1H, NH), 9.60 ppm (s, 1H, COOH). MS (m/z): 309, 291, 281, 266, 205, 189, 172, 161, 145, 120, 118, 114, 96, 52.

4l: Yield 62 %, m.p. 142–144 °C. IR (KBr) ν: 1578 (NO_2), 1611 (C=N), 1671 (C=O), 1728 (COOH), 3245 cm^{-1} (NH). Anal. calcd. for $C_{17}H_{14}N_4O_5$ (354): C, 57.62; H, 3.40; N, 15.79 %. Found: C, 57.63; H, 3.98; N, 15.81 %.

Table 3 *In vitro* anti-inflammatory screening of synthesized compounds by inhibition of bovine serum albumin denaturation.

Compound	Absorbance at 660 nm	Mean percentage inhibition of denaturation [a]
4a	0.123	39.77
4b	0.143	62.50
4c	0.097	10.23
4d	0.139	57.95
4e	0.152	72.72
4f	0.119	35.23
4g	0.147	67.04
4h	0.101	14.77
4i	0.138	56.82
4j	0.148	68.18
4k	0.118	34.09
4l	0.136	54.55
4m	0.101	14.77
4n	0.136	54.55
4o	0.147	67.07
4p	0.121	37.05
4q	0.139	57.95
4r	0.108	22.72
4s	0.137	56.81
4t	0.144	68.18
8a	0.122	38.63
8b	0.142	61.36
8c	0.116	31.81
8d	0.136	54.55
8e	0.128	45.45
8f	0.152	72.73
8g	0.119	35.23
8h	0.146	65.90
8i	0.126	43.18
8j	0.133	51.14
8k	0.142	61.36
8l	0.142	61.36
8m	0.121	37.50
8n	0.133	51.14
8o	0.109	23.86
8p	0.118	34.09
8q	0.121	37.50
8r	0.139	57.95
8s	0.111	26.14
8t	0.123	39.77
8u	0.114	29.55
8v	0.136	54.54
8w	0.142	61.36
8x	0.139	57.95
Mefenamic acid	0.158	79.54
Control	0.088	0.00

[a]Average of three readings.

4m: Yield 81 %, m.p. 166–168 °C. IR (KBr) ν: 1118 (C-O-C), 1612 (C=N), 1669 (C=O), 1731 (COOH), 3255 cm^{-1} (NH). Anal. calcd. for $C_{18}H_{17}N_3O_4$ (339): C, 63.74; H, 5.09; N, 12.41 %. Found: C, 63.71; H, 5.05; N, 12.38 %.

4n: Yield 69 %, m.p. 171–173 °C. IR (KBr) ν: 1609 (C=N), 1672 (C=O), 1732 (COOH), 3253 (NH), 3484 cm^{-1} (OH). Anal. calcd. for $C_{17}H_{15}N_3O_4$ (664): C, 62.80; H, 4.67; N, 12.96 %. Found: C, 62.76; H, 4.65; N, 12.92 %.

4o: Yield 65 %, m.p. 156–158 °C. IR (KBr) ν: 746 (C-Cl), 1616 (C=N), 1664 (C=O), 1733 (COOH), 3248 cm^{-1} (NH). Anal. calcd. for $C_{17}H_{14}N_3O_3Cl$ (344): C, 59.44; H, 4.15; N, 12.19 %. Found: C, 59.40; H, 4.10; N, 12.22 %.

3.3. Preparation of 2-[(2′-oxo-6′-phenyl-1′,2′,5′,6′-tetrahydropyrimidin-4′-yl) amino]-benzoic acid (4k)/2-[(2′-thioxo-6′-phenyl-1′,2′,5′,6′-tetrahydropyrimidin-4′-yl) amino] benzoic acid (4p)

To a mixture of N-(phenyl) chalconyl anthranilic acid (**3a**) (0.01 mol) and urea/thiourea (0.01 mol) in methanol (50 mL), a few drops of potassium hydroxide solution (2 %) were added and the mixture refluxed for 9 h on a water bath. The reaction mixture was concentrated under vacuum and poured into ice-cold water and allowed to stand overnight. The solid thus separated was filtered and recrystallized from methanol:water (1:1). Compounds **4l–o** and **4q–t** were prepared by adopting the same procedure.

4p: Yield 72 %, m.p. 184–186 °C. IR (KBr) ν: 1608 (C=N), 1721 (COOH), 3254 cm^{-1} (NH). Anal. calcd. for $C_{17}H_{15}N_3O_2S$ (325): C, 62.74; H, 4.61; N, 12.89 %. Found: C, 62.75; H, 4.65; N, 12.91 %. ^{1}H NMR ($CDCl_3$, 300 MHz), δ: 3.07 (d, 2H, -CH_2- of oxypyrimidine), 3.99 (t, 1H, CH thiopyrimidine), 6.99 (d, 1H, NH thiopyrimidine), 7.29–7.84 (m, 9H, aromatic proton), 8.78 (s, 1H, NH), 9.65 ppm (s, 1H, COOH).

4q: Yield 67 %, m.p. 144–146 °C. IR (KBr) ν: 1582 (NO_2), 1613 (C=N), 1727 (COOH), 3244 cm^{-1} (NH). Anal. calcd. for $C_{17}H_{14}N_4O_4S$ (370): C, 55.09; H, 3.78; N, 15.17 %. Found: C, 55.13; H, 3.81; N, 15.13 %.

4r: Yield 68 %, m.p. 165–167 °C. IR (KBr) ν: 1110 (C-O-C), 1609 (C=N), 1722 (COOH), 3263 cm^{-1} (NH). Anal. calcd. for $C_{18}H_{17}N_3O_3S$ (355): C, 60.81; H, 4.78; N, 11.87 %. Found: C, 60.83; H, 4.82; N, 11.82 %.

4s: Yield 74 %, m.p. 169–170 °C. IR (KBr) ν: 1612 (C=N), 1728 (COOH), 3257 (NH), 3479 cm^{-1} (OH). Anal. calcd. for $C_{17}H_{15}N_3O_3S$ (341): C, 59.78; H, 4.41; N, 12.33 %. Found: C, 59.81; H, 4.43; N, 12.31 %.

4t: Yield 59 %, m.p. 162–163 °C. IR (KBr) ν: 741 (C-Cl), 1610 (C=N), 1741 (COOH), 3253 cm^{-1} (NH). Anal. calcd. for $C_{17}H_{14}N_3O_2SCl$ (360): C, 56.72; H, 3.96; N, 11.71 %. Found: C, 56.74; H, 3.92; N, 11.68 %.

3.4. Preparation of 3-(4′-acetophenyl)-2-phenylquinazolin-4(3*H*)-one (6)

A mixture of 2-phenyl-4*H*-3,1-benzoxazin-4-one (**5**) (0.01 mol) and 4-aminoactophenone (0.01 mol) in dry pyridine (60 mL) was refluxed for 5 h. The reaction mixture was concentrated, cooled and poured into ice-cold water. The solid thus separated was filtered, dried and recrystallized from ethanol.

3.5. Preparation of 3-[4′{-3″-phenylprop-2″-enoyl}phenyl]-2-phenylquinazolin-4(3*H*)-one (7a)

To a mixture of 3-(4′-acetophenyl)-2-phenylquinazolin-4(3*H*)-one (**6**) (0.01 mol) and benzaldehyde (0.01 mol) in methanol (50 mL), 5 mL potassium hydroxide solution (2 %) was added and the resulting reaction mixture was stirred for 10 h and then refluxed for 8 h on a water bath. The reaction mixture was concentrated under vacuum, cooled and poured into ice-cold water and allowed to stand overnight. The solid thus separated was filtered, dried and recrystallized from methanol:water (1:1). Compounds **7b–f** were prepared by adopting the same procedure.

7a: Yield 56 %, m.p. 156–157 °C IR (KBr) ν: 1602 (C=N), 1657 cm^{-1} (C=O). Anal. calcd. for $C_{31}H_{26}N_2O_2$ (818): C, 80.23; H, 7.69; N, 6.08 %. Found: C, 81.20; H, 5.72; N, 6.11 %. ^{1}H NMR ($CDCl_3$, 300 MHz), δ: 6.20–6.95 (m, 5H, aromatic proton), 7.41 (d, 1H, -CH- chalcone), 7.87 (d, 1H, -CH- chalcone), 8.12–8.39 ppm (m, 13H, aromatic proton). MS (*m*/*z*): 818 (M^+).

7b: Yield 63 %, m.p. 162–163 °C. IR (KBr) ν: 1123 (C-O-C), 1608 (C=N), 1666 cm^{-1} (C=O). Anal. calcd. for $C_{27}H_{28}N_2O_3$ (428): C,

Table 4 Antimicrobial screening of synthesized compounds by the cup plate method.

Compound	Zone of inhibition/mm [a]					
	S.a.	*S.f.*	*E.c.*	*S.t.*	*C.a.*	*A.n.*
4a	14	10	13	11	16	12
4b	16	16	15	17	18	18
4c	14	12	10	11	10	12
4d	16	15	17	18	17	17
4e	17	17	16	19	18	19
4f	13	12	13	11	14	14
4g	18	18	17	17	21	20
4h	16	12	12	17	10	12
4i	18	17	18	19	20	21
4j	18	17	19	20	21	19
4k	10	11	11	12	10	09
4l	16	15	16	15	17	16
4m	12	10	11	10	09	08
4n	17	16	17	17	18	17
4o	18	19	18	17	18	18
4p	10	09	08	10	08	08
4q	17	17	16	17	18	17
4r	11	10	10	09	10	08
4s	16	17	17	17	19	16
4t	20	19	19	20	21	22
8a	16	15	16	16	14	12
8b	17	16	16	17	15	14
8c	21	20	22	22	19	18
8d	14	15	14	13	12	10
8e	19	20	20	18	19	17
8f	22	21	22	18	19	19
8g	17	15	15	14	13	13
8h	17	16	16	18	17	16
8i	19	18	20	21	19	18
8j	15	15	16	15	17	13
8k	22	21	20	19	20	19
8l	24	23	22	24	22	21
8m	14	14	13	12	10	09
8n	16	13	12	15	11	11
8o	20	19	17	17	18	16
8p	17	16	15	14	11	09
8q	19	18	18	21	17	16
8r	22	23	24	20	19	21
8s	16	14	15	15	11	10
8t	16	12	13	11	12	10
8u	24	23	21	20	20	18
8v	16	14	14	13	12	15
8w	17	18	19	17	18	16
8x	21	20	22	19	20	21
Ciprofloxacin	29	31	32	26	–	–
Clotrimazole	–	–	–	–	28	27
DMSO	–	–	–	–	–	–

[a] Average of three readings.
S. a.: *Staphylococcus aureus; S.f.*: *Staphylococcus faecalis; E.c.*: *Escherichia coli; S.t.*: *Salmonella typhi; C.a.*: *Candida albicans; A.s.*: *Aspergillus niger.*

77.46; H, 4.30; N, 6.73 %. Found: C, 81.20; H, 5.72; N, 6.11 %. MS (*m*/*z*): 428 (M^+).

7c: Yield 69 %, m.p. 116–117 °C. IR (KBr) *ν*: 1587 (NO_2), 1602 (C=N), 1672 cm^{-1} (C=O). Anal. calcd. for $C_{31}H_{25}N_3O_4$ (503): C, 73.89; H, 4.97; N, 8.29 %. Found: C, 73.94; H, 5.00; N, 8.34 %. MS (*m*/*z*): 503 (M^+).

7d: Yield 71 %, m.p. 148–149 °C. IR (KBr) *ν*: 1098 (C-O-C), 1611 (C=N), 1668 cm^{-1} (C=O). Anal. calcd. for $C_{32}H_{28}N_2O_3$ (488): C, 78.63; H, 5.81; N, 5.71 %. Found: C, 78.67; H, 5.78; N, 5.73 %. MS (*m*/*z*): 488 (M^+).

7e: Yield 75 %, m.p. 146–148 °C. IR (KBr) *ν*: 1607 (C=N), 1659 (C=O), 3465 cm^{-1} (OH). Anal. calcd. for $C_{31}H_{26}N_2O_3$ (474): C, 78.50; H, 5.49; N, 5.88 %. Found: C, 78.46; H, 5.52; N, 5.90 %. MS (*m*/*z*): 474 (M^+).

7f: Yield 78 %, m.p. 154–156 °C. IR (KBr) *ν*: 749 (C-Cl), 1601 (C=N), 1672 cm^{-1} (C=O). Anal. calcd. for $C_{31}H_{25}N_2O_2Cl$ (493): C, 75.49; H, 5.08; N, 5.71 %. Found: C, 75.52; H, 5.11; N, 5.68 %. MS (*m*/*z*): 493 (M^+).

3.6. Preparation of 2-phenyl-3-[4′-(5″-phenyl-4,5-dihydro-1*H*-pyrazol-3′-yl)phenyl] quinazolin-4(3*H*)-one (8a)

A mixture of 3-[4′-{3″-phenylprop-2″-enoyl}phenyl]-2-phenylquinazolin-4(3*H*)-one (**7a**) (0.01 mol) and hydrazine hydrate (0.01 mol) in ethanol (30 mL) was refluxed for 6 h on a

water bath. The reaction mixture was concentrated, cooled and poured into ice-cold water. The solid thus separated was filtered, dried and recrystallized from ethanol. Compounds **8b–f** were prepared by adopting the same procedure.

8a: Yield 71 %, m.p. 192–193 °C. IR (KBr) *ν*: 749 (C-Cl), 1605 (C=N), 1655 (C=O), 3388 cm^{-1} (NH). Anal. calcd. for $C_{29}H_{22}N_4O$ (442): C, 78.73; H, 4.97; N, 12.66 %. Found: C, 78.71; H, 5.01; N, 12.63 %. ^1H NMR ($CDCl_3$, 300 MHz), *δ*: 3.06 (d, 2H, -CH_2- pyrazoline), 4.96 (t, 1H, -CH- pyrazoline), 7.20 (s, 1H, NH), 7.39–8.29 ppm (m, 18H, aromatic proton). MS (*m*/*z*): 442, 416, 366, 339, 272, 220, 196, 145, 119, 77, 52.

8b: Yield 57 %, m.p. 179–180 °C. IR (KBr) *ν*: 1121 (C-O-C), 1607 (C=N), 1661 (C=O), 3386 cm^{-1} (NH). Anal. calcd. for $C_{27}H_{20}N_4O_2$ (432): C, 74.93; H, 4.62; N, 12.99 %. Found: C, 74.98; H, 4.66; N, 12.95 %. MS (*m*/*z*): 432 (M^+).

8c: Yield 63 %, m.p. 132–134 °C. IR (KBr) *ν*: 1583 (NO_2), 1612 (C=N), 1677 (C=O), 3376 cm^{-1} (NH). Anal. calcd. for $C_{29}H_{21}N_5O_3$ (487): C, 71.40; H, 4.29; N, 14.41 %. Found: C, 71.45; H, 4.34; N, 14.37 %. MS (*m*/*z*): 487 (M^+).

8d: Yield 71 %, m.p. 188–189 °C. IR (KBr) *ν*: 1090 (C-O-C), 1617 (C=N), 1662 (C=O), 3391 cm^{-1} (NH). Anal. calcd. for $C_{30}H_{24}N_4O_2$ (472): C, 76.22; H, 5.08; N, 11.90 %. Found: C, 76.25; H, 5.12; N, 11.86 %. MS (*m*/*z*): 472 (M^+).

8e: Yield 76 %, m.p. 162–163 °C. IR (KBr) *ν*: 1611 (C=N), 1656 (C=O), 3377 (NH), 3462 cm^{-1} (OH). Anal. calcd. for $C_{29}H_{22}N_4O_2$ (458): C, 73.07; H, 4.79; N, 12.19 %. Found: C, 75.97; H, 4.84; N, 11.75 %. MS (*m*/*z*): 458 (M^+).

8f: Yield 54 %, m.p. 159–161 °C. IR (KBr) *ν*: 740 (C-Cl), 1613 (C=N), 1670 (C=O), 3382 cm^{-1} (NH). Anal. calcd. for $C_{29}H_{21}N_4OCl$ (477): C, 75.92; H, 4.42; N, 11.79 %. Found: C, 73.03; H, 4.44; N, 12.22 %. MS (*m*/*z*): 477 (M^+).

3.7. Preparation of 2-phenyl-3-[4′-(5″-phenyl-4,5-dihydro-isoxazol-3′-yl)phenyl] quinazolin-4(3*H*)-one (8g)

A mixture of 3-[4′-{3″-phenylprop-2″-enoyl}phenyl]-2-phenylquinazolin-4(3*H*)-one (**7a**) (0.01 mol) and hydroxylamine hydrochloride (0.01 mol) in ethanol (30 mL) was refluxed on a water bath for 6 h. The reaction mixture was concentrated, cooled and poured into ice-cold water. The solid thus separated was filtered, dried and recrystallized from ethanol. Compounds **8h–l** were prepared by adopting the same procedure.

8g: Yield 54 %, m.p. 166–167 °C. IR (KBr) *ν*: 1108 (C-O-C), 1605 (C=N), 1655 cm^{-1} (C=O). Anal. calcd. for $C_{29}H_{21}N_3O_2$ (443): C, 78.50; H, 4.42; N, 11.79 %. Found: C, 78.54; H, 4.81; N, 9.51 %. ^1H NMR ($CDCl_3$, 300 MHz), *δ*: 3.53 (d, 2H, -CH_2- isoxazoline), 5.62 (t, 1H, -CH- isoxazoline), 7.20–8.39 ppm (m, 18H, aromatic proton). MS (*m*/*z*): 443, 417, 367, 340, 272, 221, 197, 146, 120, 77, 52.

8h: Yield 57 %, m.p. 172–173 °C. IR (KBr) *ν*: 1122 (C-O-C), 1601 (C=N), 1663 cm^{-1} (C=O). Anal. calcd. for $C_{27}H_{19}N_3O_3$ (433): C, 74.79; H, 4.44; N, 9.68 %. Found: C, 74.81; H, 4.42; N, 9.69 %. MS (*m*/*z*): 433 (M^+).

8i: Yield 63 %, m.p. 176–177 °C. IR (KBr) *ν*: 1114 (C-O-C), 1579 (NO_2), 1610 (C=N), 1671 cm^{-1} (C=O). Anal. calcd. for $C_{29}H_{20}N_4O_4$ (488): C, 71.28; H, 4.11; N, 11.51 %. Found: C, 71.30; H, 4.13; N, 1.47 %. MS (*m*/*z*): 488 (M^+).

8j: Yield 57 %, m.p. 152–153 °C. IR (KBr) *ν*: 1099 (C-O-C), 1612 (C=N), 1659 cm^{-1} (C=O). Anal. calcd. for $C_{30}H_{23}N_3O_3$ (473): C, 76.12; H, 4.89; N, 8.91 %. Found: C, 76.09; H, 4.90; N, 8.87 %. MS (*m*/*z*): 473 (M^+).

8k: Yield 70 %, m.p. 168–170 °C. IR (KBr) *ν*: 1100 (C-O-C), 1612 (C=N), 1650 (C=O), 3468 cm^{-1} (OH). Anal. calcd. for $C_{29}H_{21}N_3O_3$ (459): C, 75.78; H, 4.59; N, 9.17 %. Found: C, 75.80; H, 4.61; N, 9.14 %. MS (*m*/*z*): 459 (M^+).

8l: Yield 73 %, m.p. 196–197 °C. IR (KBr) *ν*: 738 (C-Cl), 1112 (C-O-C), 1604 (C=N), 1658 cm^{-1} (C=O). Anal. calcd. for $C_{29}H_{20}N_3O_2Cl$ (478): C, 72.91; H, 4.18; N, 8.82 %. Found: C, 72.88; H, 4.22; N, 8.79 %. MS (*m*/*z*): 478 (M^+).

3.8. Preparation of 3-[4′-{2″-oxo-6″-phenyl-1″,2″,5″,6″-tetrahydropyrimidin-4″-yl}phenyl]-2-phenylquinazolin- 4(3*H*)-one (8m)/3-[4″-{2″-thioxo-6″-phenyl-1″,2″,5″,6″-tetrahydropyrimidin-4″-yl}phenyl] -2-phenylquinazolin-4(3*H*)-one (8s)

To a mixture of 3-[4′-{3″-phenylprop-2″-enoyl}phenyl]-2-phenylquinazolin-4(3*H*)-one (**7a**) (0.01 mol) and urea/thiourea (0.01 mol) in methanol, a few drops of potassium hydroxide solution (2 %) were added and the reaction mixture was refluxed for 9 h on a water bath. After completion of the reaction the mixture was poured into ice-cold water and allowed to stand overnight. The solid thus separated was filtered, dried and recrystallized from methanol:water (1:1). Compounds **8m–r** and **8t–x** were prepared by adopting the same procedure.

8m: Yield 49 %, m.p. 186–187 °C. IR (KBr) *ν*: 1602 (C=N), 1667 (C=O), 3380 cm^{-1} (NH). Anal. calcd. for $C_{30}H_{22}N_4O_2$ (470): C, 76.55; H, 4.72; N, 11.94 %. Found: C, 76.58; H, 4.71; N, 11.91 %. ^1H NMR ($CDCl_3$, 300 MHz), *δ*: 2.53 (d, 2H, -CH_2- oxypyrimidine), 4.62 (t, 1H, -CH- oxypyrimidine), 6.14 (d, 1H, -NH oxypyrimidine), 7.20–8.39 ppm (m, 18H, aromatic proton). MS (*m*/*z*): 470, 444, 427, 367, 272, 222, 197, 147, 77, 52.

8n: Yield 71 %, m.p. 190–191 °C. IR (KBr) *ν*: 1122 (C-O-C), 1600 (C=N), 1668 (C=O), 3378 cm^{-1} (NH). Anal. calcd. for $C_{28}H_{20}N_4O_3$ (460): C, 73.07; H, 4.41; N, 12.16 %. Found: C, 73.03; H, 4.38; N, 12.17 %. MS (*m*/*z*): 460 (M^+).

8o: Yield 67 %, m.p. 144–145 °C. IR (KBr) *ν*: 1579 (NO_2), 1610 (C=N), 1671 (C=O), 3375 cm^{-1} (NH). Anal. calcd. for $C_{30}H_{21}N_5O_4$ (515): C, 69.91; H, 4.07; N, 13.62 %. Found: C, 69.89; H, 4.11; N, 13.58 %. MS (*m*/*z*): 515 (M^+).

8p: Yield 68 %, m.p. 159–153 °C. IR (KBr) *ν*: 1099 (C-O-C), 1612 (C=N), 1659 (C=O), 3386 cm^{-1} (NH). Anal. calcd. for $C_{31}H_{24}N_4O_3$ (500): C, 74.41; H, 4.79; N, 11.21 %. Found: C, 74.38; H, 4.83; N, 11.19 %. MS (*m*/*z*): 500 (M^+).

8q: Yield 65 %, m.p. 155–156 °C. IR (KBr) *ν*: 1612 (C=N), 1650 (C=O), 3371 (NH), 3468 cm^{-1} (OH). Anal. calcd. for $C_{30}H_{22}N_4O_3$ (486): C, 74.02; H, 4.60; N, 11.49 %. Found: C, 74.06; H, 4.56; N, 11.52 %. MS (*m*/*z*): 486 (M^+).

8r: Yield 72 %, m.p. 162–163 °C. IR (KBr) *ν*: 738 (C-Cl), 1604 (C=N), 1658 (C=O), 3378 cm^{-1} (NH). Anal. calcd. for $C_{30}H_{21}N_4O_2Cl$ (505): C, 74.02; H, 4.21; N, 11.12 %. Found: C, 71.36; H, 4.19; N, 11.10 %. MS (*m*/*z*): 505 (M^+).

8s: Yield 74 %, m.p. 178–179 °C. IR (KBr) *ν*: 1605 (C=N), 1657 (C=O), 3380 cm^{-1} (NH). Anal. calcd. for $C_{30}H_{22}N_4OS$ (486): C, 74.09; H, 4.60; N, 11.48 %. Found: C, 74.05; H, 4.56; N, 11.51 %. ^1H NMR ($CDCl_3$, 300 MHz), *δ*: 2.31 (d, 2H, -CH_2- thiopyrimidine), 4.41 (t, 1H, -CH- of thiopyrimidine), 6.79 (d, 1H, NH of thiopyrimidine), 7.20–8.37 ppm (m, 18H, aromatic proton). MS (*m*/*z*): 486 (M^+).

8t: Yield 77 %, m.p. 189–190 °C. IR (KBr) *ν*: 1126 (C-O-C), 1604 (C=N), 1657 (C=O), 3371 cm^{-1} (NH). Anal. calcd. for $C_{28}H_{20}N_4O_2S$ (476): C, 70.61; H, 4.24; N, 11.81 %. Found: C, 70.57; H, 4.23; N, 11.5 %. MS (*m*/*z*): 476 (M^+).

8u: Yield 68 %, m.p. 147–148 °C. IR (KBr) *ν*: 1583 (NO_2), 1608 (C=N), 1667 (C=O), 3371 cm^{-1} (NH). Anal. calcd. for $C_{30}H_{21}N_5O_3S$ (531): C, 67.82; H, 3.99; N, 13.14 %. Found: C, 67.78; H, 3.98; N, 13.17 %. MS (*m*/*z*): 531(M^+).

8v: Yield 58 %, m.p. 162–163 °C. IR (KBr) *ν*: 1102 (C-O-C), 1605 (C=N), 1657 (C=O), 3385 cm^{-1} (NH). Anal. calcd. for $C_{31}H_{24}N_4O_2S$ (516): C, 72.11; H, 4.72; N, 10.81 %. Found: C, 72.07; H, 4.68; N, 10.85 %. MS (*m*/*z*): 516 (M^+).

8w: Yield 74 %, m.p. 153–154 °C. IR (KBr) *ν*: 1610 (C=N), 1662

(C=O), 3379 (NH), 3461 cm^{-1} (OH). Anal. calcd. for $C_{30}H_{22}N_4O_2S$ (502): C, 71.73; H, 4.38; N, 11.16 %. Found: C, 71.69; H, 4.41; N, 11.15 %. MS (*m*/*z*): 502 (M^+).

8x: Yield 82 %, m.p. 166–167 °C. IR (KBr) ν: 747 (C-Cl), 1611 (C=N), 1672 (C=O), 3383 cm^{-1} (NH). Anal. calcd. for $C_{30}H_{21}N_4OSCl$ (520): C, 69.20; H, 4.10; N, 10.71 %. Found: C, 69.16; H, 4.06; N, 10.75 %. ^1H NMR ($CDCl_3$, 300 MHz), δ: 2.33 (d, 2H, $-CH_2-$ thiopyrimidine), 4.74 (t, 1H, -CH- of thiopyrimidine), 6.76 (d, 1H, NH of thiopyrimidine), 7.21–8.52 ppm (m, 18H, aromatic proton). MS (*m*/*z*): 520, 494, 460, 417, 298, 272, 197, 86, 51.

4. Biological Activity

The synthesized compounds were evaluated for analgesic and anti-inflammatory activities. Student's *t*-test was performed to ascertain the significance of the exhibited activities. The test compounds and standard drugs were administered in the form of a suspension (1 % carboxymethyl cellulose as vehicle) by an oral route. Each group consisted of six animals. Animals were procured from Orissa University of Agriculture and Technology, Bhubaneswar, Orissa, and were maintained in colony cages at 23 ± 2 °C, and a relative humidity of 45–50 %, maintained under 12 h light and dark cycle and fed with standard rat pellet diet (Hindustan Liver Ltd., Mumbai, India). Prior approval of the local Animal Ethical Committee was obtained to carry out the experimental work on the animals.

Acute oral toxicity was performed for the compounds **4a–t** and **8a–x** following the Organization of Economic Cooperation and Development guidelines (OECD-423) (acute toxic class method). Swiss albino mice (n = 3) of either sex selected by random sampling were used for the study. The animals were fasted for 3–4 h with water *ad libitum*, after which the test compounds (1 % suspension in CMC) were administered orally at doses of 50, 100, 250, 500 and 1000 mg kg^{-1} and the mice observed for three days. In the present study, mortality was not observed even at 1000 mg kg^{-1}, indicating that the compounds are non-toxic to animals.

4.1. Analgesic Activity

The analgesic activity was determined by the tail flick method.[16] Paracetamol (100 mg kg^{-1}) was administered as a standard drug for comparison. Test compounds (100 mg kg^{-1}) were administered orally by intragastric tube. The animals were held in position by a suitable restrainer with the tail extending out and the tail (up to 5 cm) was then dipped in a beaker of water maintained at 55 ± 5 °C. The time in seconds taken to withdraw the tail clearly out of water was taken as the reaction time. Three readings for each animal were recorded at 30, 60, 120 and 180 min after administration of compounds. A cut off point of 10 s was observed to prevent damage to the tail. The results are presented in Table 1.

4.2. Anti-inflammatory Activity

Anti-inflammatory activity was determined by the carrageenan-induced rat paw oedema method[17] in albino rats (n = 6) of either sex (100–140 g). Mefenamic acid (10 mg kg^{-1}) was administered as a standard drug. The test compounds were administered (100 mg kg^{-1}) orally 30 min prior to the administration of carrageenan in the right hind paws of the rats. The paw thickness was measured three times using vernier callipers at 30, 60, 120 and 180 min after carrageenan administration. Results are presented in Table 2.

4.3. Inhibition of Protein Denaturation

The reaction mixtures (0.5 mL) consisted of 0.45 mL bovine serum albumin (5 % aqueous solution) and 0.05 mL of synthesized compound (100 μg mL^{-1} of final volume). pH was adjusted at 6.3 using a small amount of 1 mol L^{-1} HCl. The samples were incubated at 37 °C for 20 min and then heated at 57 °C for 3 min. After cooling the samples, 2.5 mL phosphate buffer saline (pH 6.3) was added to each tube. Turbidity was measured spectrophotometrically at 660 nm.[18] For control tests 0.05 mL distilled water was used instead of the synthesized compound. Mefenamic acid (10 μg mL^{-1}) was used as reference standard for comparison of the protein denaturation property. Results are presented in Table 3.

4.4. Antimicrobial/Antifungal Activity

In vitro antimicrobial study was carried on Muller Hinton agar (Hi-media) plates (37 °C, 24 h) by the agar diffusion cup plate method.[19] The test microorganisms were obtained from the Department of Microbiology, Orissa University of Agriculture and Technology, Orissa, India. All the compounds were screened for antimicrobial activity at the 100 μg mL^{-1} concentration level against the following bacterial strains: *Staphylococcus aureus*, *Staphylococcus feacalis*, *Escherichia coli* and *Salmonella typhi*. Antifungal activity was tested on Sabouraud dextrose agar (Himedia) plates (26 °C, 48–72 h) by the cup plate method[19] against *Candida albicans* and *Aspergillus niger* at a concentration level of 100 μg mL^{-1}. Ciprofloxacin and clotrimazole were used as reference standards for comparison of antibacterial and antifungal activity. DMSO was used as a solvent control for both antibacterial and antifungal activities. The results are presented in Table 4.

5. Conclusion

In summary, we have prepared a series of novel N-[substituted pyrazoline-3-yl]-anthranilic acids (**4a–t)** and 2-phenyl quinazolin-4(3*H*)-ones (**8a–x)**, from which compounds **4j** and **8x** were identified as lead compounds with optimum analgesic, anti-inflammatory and antimicrobial activities. Further investigations of the biological profiles of these selected compounds are in progress.

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References

1 P. Rani, V.K. Srivastava and A. Kumar, *Indian J. Chem.*, 2003, **42B**, 1729–1733.

2 (a) S.K. Sahu, M. Banerjee, A. Samantray, C. Behera and M.A. Azam, *Tropical J. Pharm. Res.*, 2008, **7**, 961–968; (b) S.K. Sahu, M. Banerjee, S.K. Mishra, R.K. Mohanta, P.K. Panda and P. K. Misro, *Acta Pol. Pharma-Drug Res.*, 2007, **64**, 121–126.

3 M. Amir and S. Kumar, *Indian J. Chem.*, 2005, **44B**, 2532–2537.

4 A.B. Adnan, M.A.A. Hayam and A.G. Aida, *Archiv Pharm., 2005,* **338**, 167–174.

5 T. Akihiko, O. Yoshihiro, O. Keiko, T. Hideo, K. Motoji, W. Masaaki and Y. Jun-ichi, *Bioorg. Med. Chem. Letters,* 2005, **15**, 4299–4303.

6 F.E. Goda, A.R. Maarouf and E.R. Bendary, *S. Pharm. J.,* 2003, **11**, 111–117.

7 (a) S.K. Sahu, S.K. Mishra, M. Banerjee, P.K. Panda and P.K. Mishro, *J. Indian Chem. Soc.,* 2006, **83**, 725–727; (b) S.K. Sahu, M.A. Azam, M. Banerjee, P. Choudhary, S. Sutradhar, P.K. Panda and P.K. Misro, *J. Indian Chem. Soc.*, 2007, **80**, 1011–1015.

8 A.G. Habeeb, P.N.P. Rao and E.E. Kanus, *J. Med. Chem.*, 2001, **44**, 2921–2927.

9 A.G. Habeeb, P.N.P. Rao and E.E. Kanus, *Drug Dev. Res.,* 2000, **51**, 273–286.

10 S.K. Sahu, M.A. Azam, M. Banerjee, S. Acharrya, C.C. Behera and S. Si, *J. Braz. Chem. Soc.,* 2008, **19**, 963–970.

11 M.A. Sayyed, S.S. Mokle and Y.B. Vibhute, *Arkivoc,* 2006, **11**, 221–226.

12 A.A. El-Barbary, A.Z. Abou El-Ezz and A.M. Sharaf Nielson, *Phosphorus, Sulfur, Silicon Relat. Elem.,* 2006, **18**, 1895.

13 V. Alagarsamy, V.R. Solomon and K. Dhanabal, *Bioorg. Med. Chem.,* 2007, **15**, 235–241.

14 V. Alagarsamy, V.R. Solomon, R. Meena, K.V. Ramaseshu, K. Thirumurugan and S. Murugeshan, *Med. Chem.,* 2007, **3**, 67–73.

15 S.S. Laddha, S.G. Wadodkar and S.K. Meghal, *Arkivoc,* 2006, **11**, 1–20.

16 J. Antoniou, T. Steffen, F. Nelson, N. Winterbottom, A.P. Hollander, R.A. Poole, M. Aebi and M. Alini, *J. Clin. Invest.,* 1996, **98**, 996–1003.

17 U. Ameta, S. Ojha, D. Bhambi and G.L. Talesara, *Arkivoc,* 2006, **13**, 83–89.

18 J.M. Glassman, *Agents with Analgesic Activity and Dependences Liability*, in *Screening Methods in Pharmacology,* (R.A. Turner and P. Hebborn, eds.), Academic Press, New York, USA, 1971, pp. 231–232.

19 C.A. Winter, E.A. Risley and R.H. Silber, *J. Pharmacol. Exp. Ther.,* 1968, **162**, 196–201.

20 S. Chatterjee and S.N. Das, *Indian J. Pharmacol.,* 1996, **28**, 116–119.

21 A.L.J. Barry, *The Antimicrobial Susceptibility Test, Principle and Practices,* 4th edn., ELBS, London, UK, 1999, p. 180.