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Synthesis and pharmacological screening of various new quinazolin-4-one derivatives as anti-inflammatory and antifungal agents

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ABSTRACT

A series of 2-substituted-3H-quinazolin-4-one derivatives were designed and synthesized from anthranilic acid. All these compounds were characterized by IR, ^{1}HNMR, mass spectroscopy and elemental analysis. The newly synthesized compounds were screened for their anti-inflammatory and antifungal activities. Anti-inflammatory activity was assessed by using carrageenan induced rat paw oedema method and antifungal activity against *Candida albicans*. by cup plate method.

Keywords: *anthranilic acid, quinazoline, quinazolinone, anti-inflammatory activity, antifungal activity*

1. Introduction

Quinazoline is a compound made up of two fused six-membered aromatic rings, that is a benzene ring and a pyrimidine ring. The benzene ring modifies the chemistry of pyrimidine ring in a number of ways and thus it shows a profound effect on the properties of the pyrimidine structure. It causes the delocalization of π electrons of the 3, 4-double bond making its reactivity like that of an isolated double bond. As a consequence quinazoline is very reactive toward nucleophiles. Quinazoline and quinazolinone nuclei have a wide range of biological activities including antiviral [1], antibacterial [2,3], antifungal [4], antimalarial [5], anticancer [6-8], antihypertensive [9], diuretic [10,11], inhibitors of derived growth factor receptor phosphorylation [12], anticonvulsant [13], anti-inflammatory, analgesic and selective COX-II inhibitors[14-16]. Many derivatives of quinazolines for example keto-quinazolines are known as quinazolinone. These are the most important compounds and depending upon the position of the keto or oxo group, these compounds may be classified into two types: "2-(1H) quinazolinones" or "1, 2-dihydro-2- oxo quinazolines" and "4-(3H)-quinazolines" or "3, 4-dihydro-4-oxoquinazolines". Quinazolin-4-one forms the largest group of quinazoline derivatives because they are easily prepared and it is found in various quinazoline alkaloids. Quinazolinones are reported as high melting crystalline solids, insoluble in water and in most of the organic solvents but soluble in aqueous alkali. They are generally insoluble in diluted acids but are sometimes soluble in concentrated acids. Simple 4-(3H)-quinazolinones, although insoluble in diluted acids but soluble in 6N hydrochloric acid. 4-(3H)-quinazolinones form stable mono hydrochlorides, chloroplatinate, chloroaurates, picrates and their metal salts of silver, mercury, zinc, copper, sodium and potassium [17].

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2. Experimental section

2.1. Material and methods. Most of the chemicals used were of L.R. grade and obtained from Merck India, Emerck Germany, Central Drug House Ltd and S.D. Fine chemicals Ltd etc. Melting points were recorded in open glass capillary using Kjeldahl flask containing liquid paraffin and are uncorrected. Infra red (IR) spectra were recorded by KBr palattes technique using Hitachi IR spectrometer 270-230 (Vmax in cm^{-1}). Proton Magnetic resonance (^1H NMR) spectra were obtained on Bruker Model DPX-300 (300 MHz NMR) spectrometer (chemical shift in δ ppm) in $CDCl_3$ and DMSO using Tetramethylsilane (TMS) as standard. The purity of compounds was controlled by thin layer chromatography (Silicagel-G, 0.25mm) using analytical grade solvents.

2.2. Synthetic procedure.

2.2.1. General procedure for synthesis of 2-substituted-3H-quinazolin-4-one: (Ia-Ib) Equimolar quantities of various amide and anthranilic acid were refluxed with absolute ethanol under anhydrous condition for 6-8 hrs. On completion of reaction 10% NH_3 solution was added to it and the product extracted with chloroform. Chloroform layer was evaporated on water bath to dryness and residue was recrystallized from ethanol.

2.2.2. General procedure for the synthesis of 2-substituted-3H-quinazolin-4-one: (IIa-IIc) Equimolar quantities of ester and various amides were refluxed in absolute ethanol under anhydrous condition. On completion of reaction, the product was concentrated by evaporation on water bath. Finally the product was poured over crushed ice and the precipitate obtained. A final product was recrystallized with benzene.

2.2.3. General procedure for the synthesis of 2-substituted-6-nitro-3H-quinazolin-4-one: (IIIa-IIIc) 2-Amino-benzoic acid ethyl ester was treated with mixture of equal amount of concentrated nitric acid and sulphuric acid by maintaining the temperature of the reaction mixture below 10ºC. Thus 2-Amino-4 nitro benzoic acid ethyl ester was prepared. 2-Amino-4-nitro benzoic acid ethyl ester and substituted amides were taken in round bottom flask and reaction is carried out in the presence of absolute ethanol for 5-6 hrs. Final product was recrystallized from ethanol.

2.3. Anti-inflammatory activity. Anti-inflammatory activity was carried out by using carrageenan induced paw oedema method in albino rats [18]. Indomethacin was used as standard drugs for comparison. Drug and test compounds were given orally by preparing with 1% CMC suspension. Freshly prepared aqueous suspension of carrageenan (1% w/v, 0.1ml) was injected in the right hind paw of each rat. The foot volume was measured at 2 hrs and 4 hrs (**Table 1**). The mean increase in the paw volume in each group was calculated. The paw volume was measured by mercury displacement (plethysmograph). The difference in volume gave the amount of oedema developed.

2.4. Antifungal activity. Antifungal activity was done by Cup-plate diffusion method. The fungi *Candida albican*s ((NCIM 3471) was used in the fungicidal bioassay [19]. The medium used were double strength malt yeast extract (Hi-Media) for antifungal activity.

3. Results section

3.1. Synthesis and spectral characterization. A series of 2-substituted-3H-quinazolin-4-one derivatives were designed and synthesized from anthranilic acid. All synthesis steps are presented in scheme 1. In the IR spectra of all the synthesized compounds showed the absorption band in the region of 1664-1672 cm^{-1} and 3294-3443 cm^{-1} assigned for CO group and NH group respectively

providing strong evidence for the formation of quinazoline-4-one derivatives. ^{1}H NMR spectrum showed most important signal at range of δ 7.5-10.3 ppm for NH moiety and different other signals were observed at the expected chemical shift.

2-methylquinazolin-4(3H)-one (Ia): Yield: 70 %; mp: 218-220°C; MW: 160.17, R_f value: 0.73; I.R. (KBr) 3294 cm^{-1} (N-H), 1664 cm^{-1} (C=O), 1321 cm^{-1} (C=N), 757 cm^{-1} (Ar-CH_3)., ^{1}HNMR: δ 8.29, δ 7.49 and δ 7.78 (d, for three aryl protons of 8th, 5th and 6th positions, respectively), δ 7.70 (m, 7th aryl position), δ 11.9 (s,-NH); MS (ESI): m/e: 160.06 (100.0%),161.07 (9.9%). Found: C, 67.49; H, 5.03; N, 17.49; O, 9.99; Calculated: C, 67.44; H, 5.00; N, 17.45; O, 9.95.

2-vinylquinazolin-4(3H)-one (Ib): Yield: 63 %; mp: 160-162 °C; MW: 172.18, R_f value: 0.34; I.R. (KBr): 3348 cm^{-1} (-N-H), 1655 cm^{-1} (-C=O), 1249 cm^{-1} (-C=N); ^{1}HNMR: δ 7.8, δ 6.7,(d, for three aryl protons of 5th and 8th positions), δ7.04(t), δ7.3(m) for three aryl protons of 6th and 7th positions; MS (ESI):m/e: 172.06 (100.0%) 173.07 (10.9%) Found: C, 69.76; H, 4.68; N, 16.27; O, 9.29; Calculated: C, 69.74; H, 4.65; N, 16.25; O, 9.25.

2-phenylquinazolin-4(3H)-one (IIa): Yield: 50 %; mp: 110-112 °C; MW: 222.24, R_f value: 0.51; I.R. (KBr) 3367cm^{-1} (N-H), 1658 cm^{-1} (-C=O), 1402 cm^{-1} (C=N, Ar); ^{1}HNMR: δ 7.8 (d,), δ7.9 (d,), δ 8.1 (d,) for 8th, 5th and 6th position; δ7.5 (t), δ7.2 (t), δ 7.4 (t) for the aryl portion of quin-7, quin-6,3-Ar and 4-Ar. MS(ESI): m/e: 222.08 (100.0%) 223.08 (15.9%), 224.09 (1.1%), Found: C, 75.66; H, 4.54; N, 12.60; O, 7.20; Calculated: C, 75.64; H, 4.52; N, 12.57; O, 7.15.

2-styrylquinazolin-4(3H)-one (IIb): Yield: 45%; mp: 186-188 °C; Mol. Wt.: 248.28, R_f value: 0.57; I.R. (KBr) 3443 cm^{-1} (N-H), 1634 cm^{-1} (-C=O), 1218 cm^{-1} (C=N); ^{1}HNMR: δ 6.5 (t), δ 7.2 (t) to the aryl portions of 3,4,5,6 and 7 th positions; δ 2.0 (d), δ2.2 (d), δ6.7 (d), δ 7.0 (d), for 2,7,5 and 8th positions; MS (ESI): m/e: 248.09 (100.0%), 249.10 (17.5%), 250.10 (1.8%); Found: C, 77.40; H, 4.87; N, 11.28; O,6.44; Calculated: C, 77.37; H, 4.85; N, 11.25; O, 6.40.

2-(4-chlorophenyl) quinazolin-4(3H)-one (IIc): Yield: 55 %; mp: 210-212°C; Mol. Wt.: 256.69, R_f value: 0.58; I.R. (KBr) 3362 cm^{-1} (-N-H), 1683 cm^{-1} (-C=O), 1227 cm^{-1} (-C=N), 2851 cm^{-1} (C-H, Ar); ^{1}HNMR: δ 6.7 (d,), δ 7.2 (d) for 5th and 8th position, δ 7.3 (d), δ 7.8 (d) for 6,3, and 5th positions, δ 10.3 (s) for –NH group; MS (ESI): m/e: 256.04 (100.0%), 258.04 (32.3%), 257.04 (15.9%), 259.04 (4.9%), 258.05 (1.1%); Found: C, 65.51; H, 3.53; Cl, 13.81; N, 10.91; O, 6.23; Calculated: C, 65.58; H, 3.50; N,10.87; O, 6.20.

2-methyl-6-nitroquinazolin-4(3H)-one (IIIa): Yield: 58 %; mp: 160-162°C; Mol. Wt.: 205.17, R_f value: 0.78; I.R. (KBr) 1305 cm^{-1} (-NO_2), 1676 cm^{-1} (-C=O), 1420 cm^{-1} (-C=N), 3390 cm^{-1} (N-H); ^{1}HNMR: δ 6.7 (d), δ 7.7 (d) for 8th and 7th position, δ 7.24 (s), δ 7.8 (d) for -NH, δ 2.75 (s) for CH_3; MS (ESI): m/e: 205.05 (100.0%), 206.05 (11.0%); Found: C, 52.69; H, 3.44; N, 20.48; O, 23.39; Calculated: C, 52.65; H, 3.42; N, 20.45; O, 23.35.

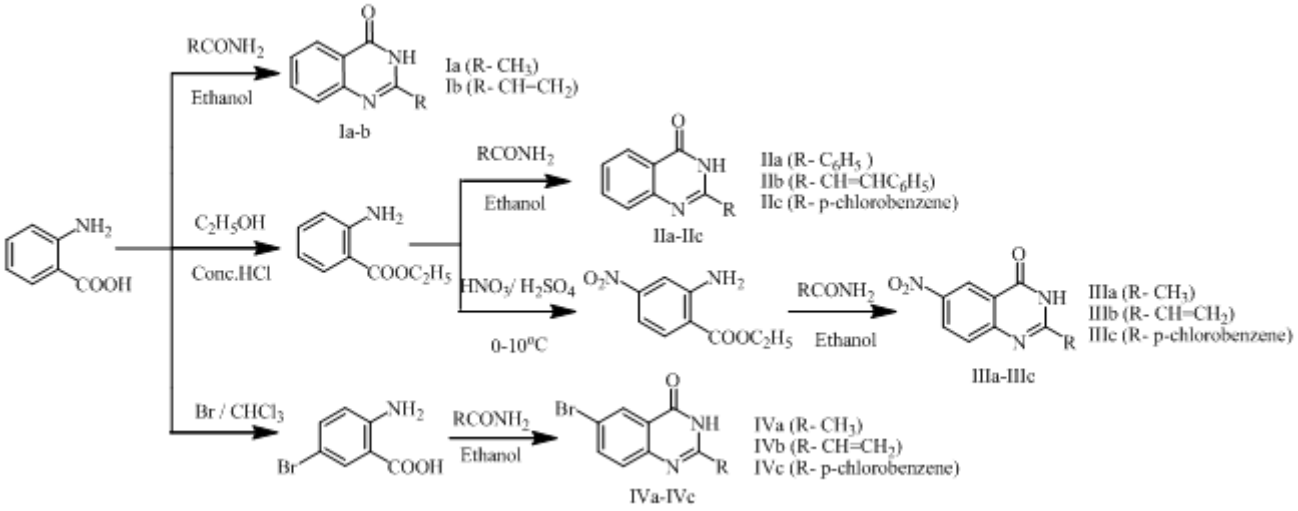
6-nitro-2-vinylquinazolin-4(3H)-one (IIIb): Yield: 45 %; mp: 88-90°C; Mol. Wt.: 217.18, R_f value: 0.66; I.R. (KBr) 1680 cm^{-1} (-C=O), 1280 cm^{-1} (-C=N), 3353 cm^{-1} (N-H); ^{1}HNMR: δ 7.52 (s), δ 7.08 (d) for 5th and 7th position, δ 6.2-6.0 (m) for vinyl protons, δ 7.28 (s) for –NH; MS (ESI): m/e: 217.05 (100.0%), 218.05 (12.0%); Found: C, 55.30; H, 3.25; N, 19.35; O, 22.10; Calculated: C, 55.27; H, 19.32; N, 19.33; O, 22.07.

2-(4-chlorophenyl)-6-nitroquinazolin-4(3H)-one (IIIc): Yield: 20 %; mp: 210-212°C; Mol. Wt.: 301.68, R_f value: 0.60; I.R. (KBr) 3362 cm-1 (N-H), 1680 cm^{-1} (-C=O), 1224 cm^{-1} (C=N),2851 cm^{-1} (C-H, Ar), 1453 cm^{-1} (NO_2); ^{1}HNMR: δ 6.7 (d) 8th position, δ 7.2 (s) to the aryl portions of 5th positions; δ 7.3 (d), δ 7.8 (d) to the 2,6 & 3,5 th position, δ6.7 (d) of 7th position, δ 10.5(d) for NH group; MS (ESI): m/e: 301.03 (100.0%), 303.02 (32.0%), 302.03 (15.3%), 304.03 (5.0%), 303.03 (1.9%), 302.02 (1.1%); Found:C, 55.74; H, 2.67; Cl, 11.75; N, 13.93; O, 15.91; Calculated: C, 55.70; H, 2.65; N, 13.90; O, 15.88

6-bromo-2-methylquinazolin-4(3H)-one (IVa): Yield: 53 %; mp: 88-90 °C; Mol. Wt.: 239.07, R_f value: 0.77; I.R. (KBr) 3435 cm^{-1} (N-H), 1658 cm^{-1} (-C=O), 1402 cm^{-1} (C=N),1577 cm^{-1} (Ar-Br); ^{1}HNMR: δ 7.6 (d) 8th position, δ 8.2 (d) to the aryl portions of 5th positions; δ 2.5 (s) –CH_3 group at 2nd position, δ 7.75 (t) to the 7th position, δ 12 (d) for NH group; MS (ESI): m/e: 237.97 (100.0%), 239.97 (97.4%), 238.98 (9.9%), 240.98 (9.6%); Found: C, 45.22; H, 2.95; Br, 33.42; N, 11.72; O, 6.69; Calculated: C, 45.20; H, 2.93; N, 11.70; O, 6.65.

6-bromo-2-vinylquinazolin-4(3H)-one (IVb): Yield: 65 %; mp: 190-192 °C; Mol. Wt.: 251.08, R_f value: 0.75; I.R. (KBr) 3396 cm^{-1} (N-H), 1674 cm^{-1} (-C=O), 1252 cm^{-1} (C=N), 750 cm^{-1} (Ar-Br); ^{1}HNMR: δ 1.07(d and δ 6.1 for the protons at 2-(2'=CH_2)and 8th position, δ 7.08 (s) to the aryl portions of 5th positions; δ 2.5 (s) CH_3 group at 2nd position, δ 7.5 (s) for NH group; MS (ESI): m/e: 249.97 (100.0%), 251.97 (97.4%), 250.98 (10.9%), 252.98 (10.7%); Found:C, 47.84; H, 2.81; Br, 31.82; N, 11.16; O, 6.37; Calculated: C, 47.81; H, 2.79; N, 11.13; O, 6.35.

6-bromo-2-(4-chlorophenyl) quinazolin-4(3H)-one (IVc): Yield: 34 %; mp: 200-202 °C; Mol. Wt.: 335.58, R_f value: 0.63; I.R. (KBr) 3362 cm-1 (N-H), 1683 cm^{-1} (-C=O), 1227 cm^{-1} (C=N), 798 cm^{-1} (Ar-Br), 2851 cm^{-1} (Ar-C-H), ^{1}HNMR: δ 6.7(d) 8th position, δ 7.2 (s) to the aryl protons of 5th positions; δ 2.5 (s) –CH_3 group at 2nd position, δ 10.6 (s) for NH group; MS (ESI): m/e: 335.95 (100.0%), 333.95 (77.3%), 337.95 (24.6%), 336.95 (15.8%), 334.95 (12.3%), 338.95 (3.7%); Found: C, 50.11; H, 2.40; Br, 23.81; Cl, 10.56; N, 8.35; O, 4.77; Calculated: C, 50.09; H, 2.37; N, 8.30; O, 4.75.



Scheme 1: Synthesis of the various Quinazolin-4-one derivatives

3.2. Anti-inflammatory activity. Results of anti-inflammatory activity are shown in **Figure 1.** Among all the synthesized compounds, those having electron withdrawing group increases the activity. Compounds **Ib** and **IIa** exhibited significant anti-inflammatory activity after both 2hrs and 4hrs. Indomethacin was used as standard.

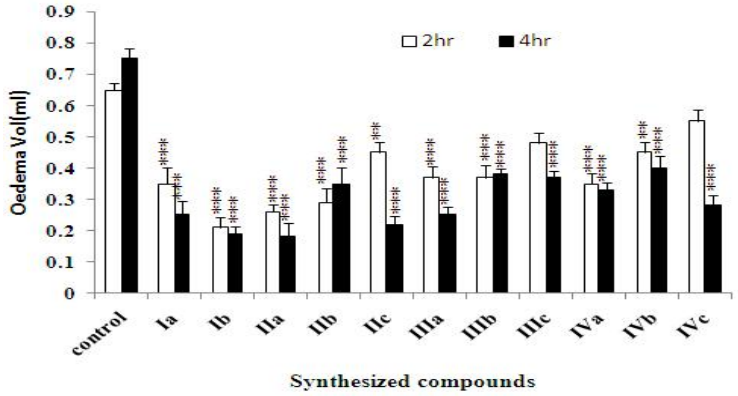


Figure 1: The mean oedema volume of anti-inflammatory activity of the tested compounds

Data represent mean values±SEM of six animals per group and data were analyzed by using one-way ANOVA followed by Tukey test ***p< 0.001, **p< 0.01 compared to control.

Table 1: Anti-Inflammatory activity of tested compounds.

Compounds	Oedema Vol (ml) (mean ± SEM)	
	2 hrs	4 hrs
Control	0.65±0.021	0.75±0.031
Ia	0.35±0.051***	0.25±0.043***
Ib	0.21± 0.033***	0.19±0.023***
IIa	0.26± 0.024***	0.18±0.043***
IIb	0.29±0.044***	0.35±0.053***
IIc	0.45± 0.034**	0.22±0.026***
IIIa	0.37± 0.037***	0.25±0.025***
IIIb	0.36± 0.040***	0.38±0.020***
IIIc	0.48±0.031	0.37±0.021***
IVa	0.35±0.032***	0.33±0.024***
IVb	0.45±0.032**	0.40±0.038***
IVc	0.55±0.036	0.28±0.034***

3.3. Antifungal activity. The results of preliminary bioassays were compared with standard drug Voriconazole. Most of the synthesized compounds showed antifungal activity against the tested fungi. Test solution of each compound was given at two concentrations such as 100 and 200µg/ml. Among all the synthesized compounds **IIa**, **IIc, IIIb** and **IIIc** were found to exhibit good antifungal activity at the concentration of 200 µg/ml. The compound **IVc** was found to be most active at both the concentrations, but less than the standard drug Voriconazole (**Table 2**). Chloro substitution in **IVc** showed maximum inhibition of value 13mm on 100µg/ml and 17mm on 300µg/ml concentration.

Table 2: Antifungal activity of synthesized compounds

Compounds	Zone of inhibition (mm)		Compounds	Zone of inhibition (mm)	
	100µg/ml	200µg/ml		100µg/ml	200µg/ml
Ia	7	14	IIIc	10	14
Ib	8	10	IVa	9	10
IIa	10	13	IVb	8	9
IIb	9	11	IVc	13	17
IIc	10	15	DMSO	3	3
IIIa	8	9	Voriconazole	15	18
IIIb	11	16			

4. Conclusions

Quinazoline-4-ones an important group of compounds reported to have different biological activities and hence the present studies were undertaken in order to synthesize new derivatives and to investigate them for their anti-inflammatory and antifungal activity. Compounds with methyl and phenyl substituents exhibited significant anti-inflammatory activity after both 2hrs and 4hrs when compared with control. The compounds with substituents chloro and phenyl groups showed significant activity when compared to standard drug voriconazole.

5. References

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