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Research Article

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Synthesis of 2, 3-disubstituted quinazolone derivatives for analgesic and antimicrobial activities

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ABSTRACT

In view of the effective range of biological activities exhibited by quinazolinones, a series of 2, 3-disubstituted quinazolin-4-(3H)-ones have been synthesized and evaluated for analgesic, antibacterial and antifungal activities. Five derivatives of quinazolones were synthesized by incorporating the heterocyclic moieties of substituted Indole, Benzothiazole and Pyridine at third position of the ring. The structures of the compounds were confirmed on the basis of IR, ^{1}H NMR and Mass spectroscopy. The analgesic and antimicrobial activity of the compounds was screened by Eddy's hot plate method and Disc diffusion method respectively. All the five compounds could show analgesic and antimicrobial activities. The compound II showed better analgesic activity than other compounds. Significant activity was shown by compound IV against gram positive organisms and by compound I against gram negative organisms. The best antifungal activity was shown by compound IV.

Keywords: Quinazolones, Analgesic, Antibacterial and Antifungal activity.

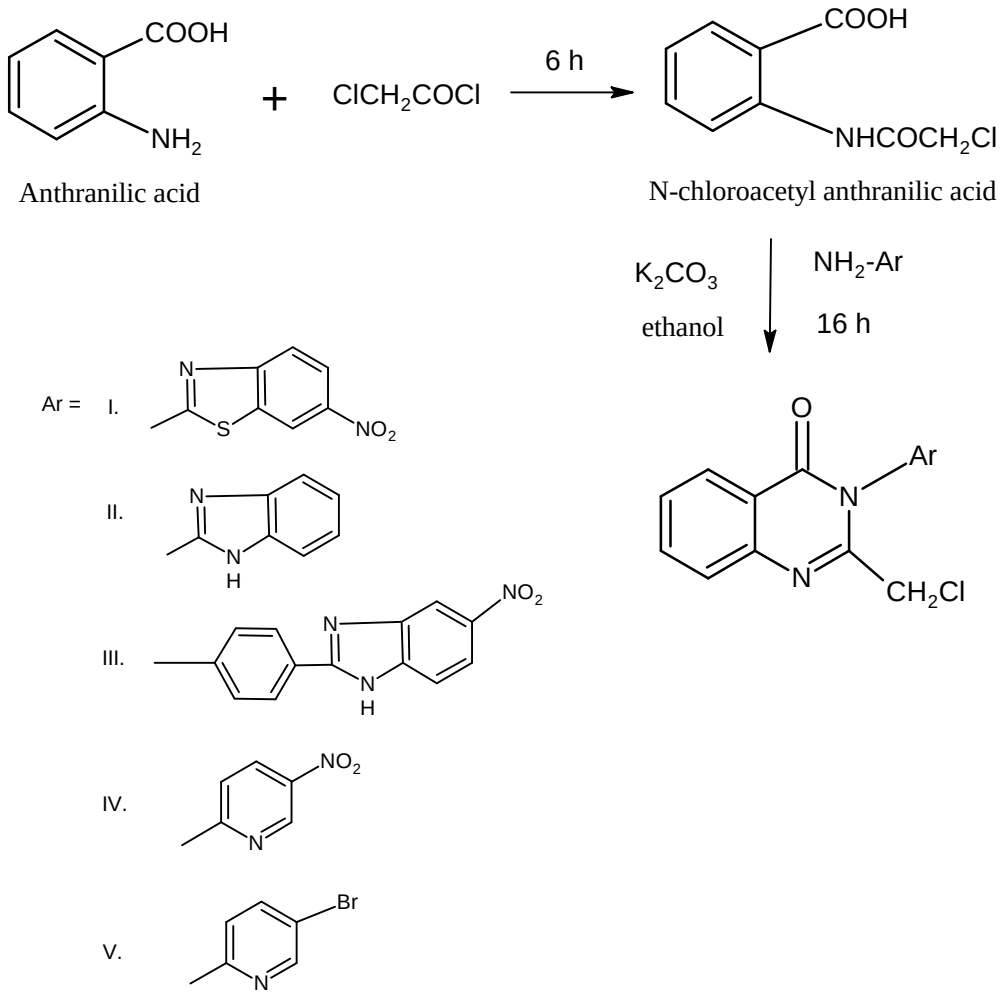
INTRODUCTION

Quinazolin-4(3H)-one is a versatile heterocyclic moiety for the medicinal chemists for molecular modification due to its wide spectrum of biological activities. They have established a variety of biological activities like analgesic [1], anti-inflammatory [1], antihypertensive [2], antihistaminic, anticancer [3-4], sedative- hypnotic and antimicrobial activities [5-6]. The second and third positions are the most favourable positions for making substitutions on the quinazolone ring and many research works have evidenced this concept [7]. This prompted us to synthesize a new series of quinazolone derivatives by incorporating the heterocyclic rings at the third position. The 2-chloro methyl-3-heterocycle substituted quinazolones were synthesized by cyclizing the N-chloro acetyl anthranilic acid with amino substituted indole, benzthiazole and pyridine. The N-chloro acetyl anthranilic acid was prepared by acetylating the anthranilic acid. The structures of all the synthesized compounds were confirmed by IR, ^{1}H NMR and mass spectroscopy. All the compounds have been screened for their analgesic, antibacterial and antifungal activities.

EXPERIMENTAL SECTION

The melting points were determined in open capillary tubes in a Hicon melting point apparatus. Infra-red spectrum was recorded in Jasco FT/IR 410 spectrometer by KBr pellet method and the ^{1}H NMR spectra was taken on a Bruker Avance II (400 MHz) NMR spectrometer using dimethylsulfoxide as solvent and chemical shifts are expressed in parts per million relative to tetra methyl silane as an internal standard. Mass spectra was recorded on shimadzu 2010A LC-MS. The completion of the reactions was checked by thin layer chromatography on silica gel G coated plates using chloroform: methanol (7:3) solvent system and the spots were detected by Iodine vapours. The animal study protocol was approved by Institutional Animal Ethical Committee. (Reg No: 409/01/CPCSEA).

Scheme



Synthesis of N-chloroacetyl anthranilic acid: [8]
0.1M (13.7gm) of anthranillic acid was taken in a 250 ml round bottom flask followed by added 0.15 M (16.95ml) of chloroacetyl chloride in 80ml of benzene and two or three drops of pyridine under cold condition. The reaction mixture is refluxed for 6 h, cooled and filtered. The solid was purified by recrystallization using acetone-ethanol mixture (1:1). Yield 72%, m.p. 170°C. IR (KBr): 3500 (OH str), 3300 (NH str), 3015 (C-H Ar str), 2967 (C-H alkyl str), 1687 (C=O str), 1588 (C=C Ar str) cm^{-1}.

Synthesis of 2-chloro methyl-3- substituted quinazolin-4(3H)-one: [8]
N-chloroacetyl anthranilic acid (0.01M) was refluxed separately with (0.01M) 2-amino benzthiazole, 2- amino benzimidazole, 2-amino 5-nitro phenyl benzimidazole, 2-amino 5-nitro pyridine and 2-amino 5-bromo pyridine in the presence of 10g of K_2CO_3 in 100ml of dry ethanol. The ethanol was evaporated and the mixture was neutralized with 0.1M HCl under ice cold condition. Then the residue was washed thoroughly with boiling water and recrystallized from acetone: ethanol mixture (1:1).

Table 1: Physical data of the Compounds

Code	Molecular formula	Molecular weight	% yield	Melting point (°C)	R_f value
I	$C_{16}H_9N_4O_3SCl$	372	70.16	286	0.60
II	$C_{16}H_{11}N_4OCl$	310	60.20	194	0.50
III	$C_{22}H_{15}N_5O_3Cl$	432	65.00	274	0.80
IV	$C_{14}H_9N_4O_3Cl$	316	52.00	254	0.70
V	$C_{14}H_9N_3OClBr$	350	46.00	164	0.64

Screening of Analgesic activity [9]
Albino mice of either sex (20 - 25g) were grouped as control, standard and test (I-V) containing six numbers. The animals were acclimated to laboratory conditions for one week prior to the experiment. Eddy's hot plate with the temperature of 55° ± 1°C used for the study. Mice were placed on hot plate and the reaction time that is licking of

paw or jumping response was recorded in seconds. The cut off time is 15 seconds and the animals not showing any response after 15 seconds were removed from the study. The test compounds were administered intraperitonially with a dose of 50 mg/kg in 1% polyethylene glycol. Standard group was administered with pentazocine intraperitonially in a dose of 50mg/kg whereas the control group was given only with 1% v/v polyethylene glycol. The reaction time was measured at the interval of 0, 30, 60, 90 and 180 minutes. The results are presented in Table 2.

Table 2: Analgesic activity

Compound	Paw licking or jumping in seconds				
	0 min	30 min	60 min	90 min	180 min
Control	7.02 ±0.58	8.06 ±0.58	7.08 ±0.58	7.01 ±0.58	6.67 ±0.67
Standard	10.04 ±1.15	10.21 ±0.58	11.03 ±0.58	9.67 ±0.33	9.67 ±0.88
I	8.33 ±0.88	8.33 ±0.88	9.67 ±0.33	8.45±0.78	8.08 ±1.15
II	11.06 ±0.58	10.33 ±0.33	8.33 ±0.33*	8.33 ±0.33*	7.02 ±0.58***
III	8.03 ±0.58	7.67 ±0.33	7.07 ±0.58**	6.05 ±0.00	5.03 ±0.58*
IV	8.02 ±0.58	9.01 ±0.58	7.05 ±0.58	7.67 ±0.33	6.08 ±0.58
V	7.06 ±0.58	8.04 ±0.58	9.02 ±0.58	7.67 ±0.88	7.02 ±0.58

**P<0.05, **P<0.01. ***P<0.001 compare to 0 h response. One-way ANOVA followed by Bonferroni test.*

Screening of Antimicrobial activity [10]

The synthesized compounds were tested for antibacterial and antifungal activity by disc diffusion method at the concentration of 25, 50 and100 µg/ml. The antibacterial activity was screened against each of two gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and positive organisms (*Bacillus subtilis* and *Staphylococcus aureus)* and the antifungal activity was screened against *Aspergillus niger.* Whatmann filter paper (grade-1) disc of 5 mm diameter and 2 mm width was sterilized by autoclaving for 15 min at 121°C. The steriled agar petridish was seeded with test bacteria and the impregnated discs were placed on the medium with suitable space between the discs. The plates were incubated at 37°C ± 1°C for 18-24 h for bacterial medium and 25°C ± 1°C for 72 h for fungal medium. The inhibition of zones caused by various synthesized compounds and standard drugs ciprofloxacin and ketoconazole on the bacterial and fungal microorganisms were examined and results are given in the Table 3.

Table 3: Antimicrobial Activity

Code	Zone of Inhibition (in mm)														
	Gram positive						Gram negative						Fungal		
	S. aureus			*B. subtilis*			*E. coli*			*P. aeruginosa*			*A. niger*		
I	15	18	27	14	17	18	20	23	29	19	20	26	15	19	23
II	19	21	27	18	19	24	16	21	27	14	20	24	13	16	18
III	16	19	29	12	16	19	17	22	28	17	22	28	21	24	28
IV	22	26	28	21	25	28	20	22	28	20	22	29	22	26	28
V	20	22	28	20	23	25	15	18	24	14	19	23	15	13	24
Ciprofloxacin	36	38	39	37	37	39	35	37	38	38	39	39			
Ketoconazole													38	38	39

RESULTS AND DISCUSSION

The 2-chloromethyl -3-heterocycle substituted quinazolones were synthesised successfully from anthranilic acid with good yield and their structures were confirmed through the spectral data. The synthesized compounds shown moderate analgesic and antimicrobial activity.

2-(chloromethyl)-3-(6-nitro-1, 3-benzothiazol-2-yl) quinazolin-4(3*H*)-one: (I)

IR (KBr)**:** 3094 (C-H Ar str), 1647 amide (C=O str), 1530 (NO_2 str), 1570 (C=N str), 1330 (C-N), 720(C-Cl str), 616 (C-S bending).cm^{-1}. ^{1}H NMR: (DMSO) ppm: 8.29 - 8.05 (Protons of aromatic ring in benzthiazole), 8.1-7.6 (Protons of aromatic ring in quinazolone), 5.36 (s-2H of CH_2-Cl). (m/z): 325.4 (M+), 275.3, 141.4, 133.5, 119.4, 176.5, 151.4.

3-(1*H*-benzimidazol-2-yl)-2-(chloromethyl) quinazolin-4(3*H*)-one: (II)

IR (KBr)**:** 3483 (N-H str), 3132 (C-H Ar str), 1666 amide (C=O str), 1570 (C=N str), 1566 (C=C str), 1313 (C-N), 697 (C-Cl str) cm^{-1}.^{1}H NMR: (DMSO): ppm: 8.1-7.6 (Protons of aromatic ring in Quinazolone), 7.1-7.0 (Protons of aromatic ring in Benzimidazole), 5.26 (s-2H of CH_2-Cl), 5 (s-1H of NH). (m/z): 310.72 (M+), 274.6, 261, 236, 186 145.

2-(chloromethyl)-3-[4-(5-nitro-1*H*-benzimidazol-2-yl) phenyl]quinazolin-4(3*H*)-one. (III)
IR (KBr)**:** 3446 (N-H str), 3102 (C-H Ar str), 1676 cyclic amide (C=O str), 1646 (C=C str), 1528 (C=N str),1343 (C-N str), 758 (C-Cl str) cm^{-1}. ^{1}H NMR: (DMSO): ppm: 8.1-7.6(Protons of aromatic ring in Quinazolone), 8.10-7.32(m-2H of phenyl-H), 7.66-7.0 (Protons of aromatic ring in benzimidazole), 5.26 (s-2H of CH_2-Cl). (m/z):432.83(M+), 389.53, 358.78, 325.72, 312.74. 284.72.

2-(chloromethyl)-3-(5-nitropyridin-2-yl)quinazolin-4(3*H*)-one. (IV)
IR (KBr)**:** 3446 (N-H str), 3102 (C-H Ar str), 1676 amide (C=O str), 1606 (C=C str), 1588 (C=N str),1243 (C-N str), 752 (C-Cl str) cm^{-1}. ^{1}H NMR: (DMSO): ppm: 9.36-8.0 ((Protons of aromatic ring in pyridine), 8.1-7.6 ((Protons of aromatic ring in Quinazolone), 5.26 (s-2H of CH_2-Cl). (m/z): 316.7 (M+), 281.4, 268.22, 257.24, 246.2, 232.23. 1173.25, 107.15.

3-(5-bromopyridin-2-yl)-2-(chloromethyl)quinazolin-4(3*H*)-one. (V)
IR (KBr)**:** 3242 (C-H Ar str), 1656 amide (C=O str), 1602 (C=C str), 1586 (C=N str), 752 (C-Cl str), 650 (C-Br bending) cm^{-1}. ^{1}H NMR: (DMSO): ppm: 8.6- 7.4 (Protons of aromatic ring in pyridine), 8.1-7.6 ((Protons of aromatic ring in Quinazolone), 5.25 (s-2H of CH_2-Cl). (m/z): 350.59 (M+), 318.59, 306.62, 296.47, 282.4, 268.34, 253.23.150.22, 130.31, 122.25.

Among the compounds screened for analgesic activity, compound II shown better activity whereas the remaining compounds could show only moderate activity. The statistical method one-way ANOVA followed by Bonferroni test was applied to find out whether the test is statistically significant; the values are within the confidence level.

The better antimicrobial activity resides in the compound I and IV, the compound I could show better activity against gram negative organism whereas the compound IV could show better activity against gram positive organism. The fungus strain *Aspergillus niger* was better inhibited by the compound IV followed by compound III.
It is concluded that the electron withdrawing group containing compounds showed better activity against bacterial and fungus strains, probably the nitro group on the heterocyclic ring plays important role in the inhibition of the microorganisms.

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