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Synthesis and Biological Evaluation of Some Novel Formazans

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ABSTRACT

A series of novel 1-substituted phenyl-3-(4-nitrophenyl)-4-[benzamido-(2-methyl-3-quinazolin)-4-one] formazans (**6a-h**) have been synthesized from corresponding Schiff base (**5**) which was coupled with various aryl diazonium chloride in pyridine produced 1-substituted phenyl-3-(4-nitrophenyl)-4-[benzamido-(2-methyl-3-quinazolin)-4-one] formazans (**6a-h**). The structures of the synthesized compounds were determined by IR, ^{1}HNMR, mass spectra and elemental analysis. The compounds synthesized were screened for antimicrobial and analgesic activity. Some of the synthesized compounds exhibited prominent antimicrobial and analgesic activities.

Key words: Synthesis, Quinazolinone, formazans, Antimicrobial, Analgesic.

INTRODUCTION

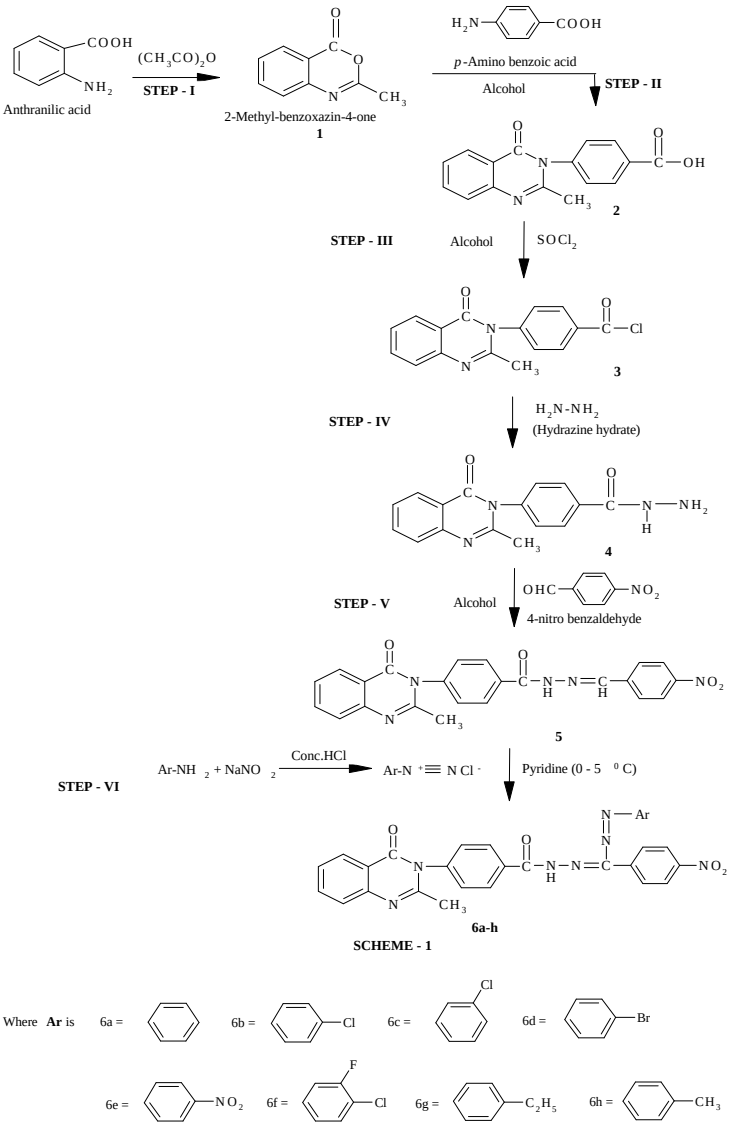
Quinazoline nucleus has wide range of importance in medicinal chemistry, due to wide spectrum of biological activities exhibited by them. Quinazolines and formazans have exhibited a variety of biological activities. Literature survey revealed that various substituted quinazolinones are known to possess antimicrobial [1], analgesic [2], anti-inflammatory [3], anticonvulsant [4], anti-tubercular [5], anticancer [6], anti-HIV [7] and other miscellaneous activities while formazans show promising analgesic [8], anti-inflammatory [8], antitubercular [9], anti cancer [10], antiHIV [10] activities. All these valid observations led us to synthesize some new quinazolinone formazan derivatives to explore their possible biological activities.

Chemistry:
Compounds were prepared as shown in Scheme I. Anthranilic acid on treatment with acetic anhydride gave 2-methylbenzoxazin-4-one (**1**) which on reaction with *p*-amino benzoic acid afforded 3-(4-carboxyphenyl)-2-methyl-3-quinazolin-4-one(**2**). The carboxylic acid was converted to corresponding acid chloride (**3**) which on reflux with hydrazine obtained 3-(4-hydrazinobenzoyl)-2-methyl-3-quinazolin-4-one (**4**). The resultant hydrazine was converted to respective Schiff base (**5**) by condensing **4** with 4-nitro benzaldehyde, which was coupled with various aryl diazonium chloride in pyridine produced 1-substituted phenyl-3-(4-nitrophenyl)-4-[benzamido-(2-methyl-3-quinazolin) -4-one] formazans (**6a-h**) **(Scheme I)**.

MATERIALS AND METHODS

Melting points of the newly synthesized compounds were determined by open capillary method and uncorrected. IR spectra (KBr, cm^{-1}) were recorded on Perkin Elmer FTIR spectrophotometer. ^{1}H NMR spectra were recorded on Brucker AMX 400 MHz instrument. Chemical shifts are reported in parts per million (?) units relative to internal standard tetramethylsilane (TMS) (Table No.2). Elemental analyses were performed and the analyses indicted by the symbols of the elements were within ±0.5% of theoretical values (Table No.1). Mass spectra were recorded on Agilent LC-MSD spectrometer.

Synthesis of 2-Methyl benzoxazin-4-one (1):
The method described by Pandey et al, was followed to synthesize compound **1**. A mixture of anthranilic acid (13.8 g, 0.1 moles) and acetic anhydride (25 ml) were refluxed for 2hrs and the reaction was monitored by TLC for the completion of reaction. The reaction mixture was poured into ice-cold water. The resulting mass was filtered and dried. Compound 1 was recrystallized from DMSO, m.p. 164^{0}C.



SCHEME - 1

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Table No.1: Physical characterization of compounds 6a-h

Compd. Name	Mol. Formula	m.p (°C)	Yield	Elemental analysis (%) (calcd./found) C	H	N	Nomenclature
2	$C_{16}H_{13}N_2O_3$	183	67	68.32 67.94	4.66 4.34	9.96 10.14	3-(4-Carboxyphenyl)-2-methyl-3-quinazolin-4-one
6a	$C_{29}H_{21}N_7O_4$	182	55	67.53 67.14	8.50 8.13	14.51 14.01	1-Phenyl-3-(4-nitrophenyl)-4-[benzamido-(2-methyl-3-quinazolin)-4-one]formazan
6b	$C_{29}H_{20}N_7O_4Cl$	122	71	64.25 63.65	7.95 8.03	13.80 14.00	1-(4-Chlorophenyl)-3-(4-nitrophenyl)-4-[benzamido-(2-methyl-3-quinazolin)-4-one] formazan
6c	$C_{29}H_{20}N_7O_4Cl$	246	66	64.25 64.59	7.95 8.15	13.80 13.22	1-(3-Chlorophenyl)-3-(4-nitrophenyl)-4-[benzamido-(2-methyl-3-quinazolin)-4-one] formazan
6d	$C_{29}H_{20}N_7O_4Br$	242	75	60.47 61.05	7.48 7.12	12.99 13.26	1-(4-Bromophenyl)-3-(4-nitro phenyl)-4-[benzamido-(2-methyl-3-quinazolin)-4-one] formazan
6e	$C_{29}H_{20}N_8O_6$	310	69	63.31 63.59	7.83 7.45	15.54 15.11	1-(4-Nitrophenyl)-3-(4-nitro phenyl)-4-[benzamido-(2-methyl-3-quinazolin)-4-one] formazan
6f	$C_{29}H_{19}N_7O_4FCl$	194	60	62.66 63.11	7.61 7.43	13.46 13.45	1-(3-Fluoro-4-chlorophenyl)-3-(4-nitrophenyl)-4-[benzamido-(2-methyl-3-quinazolin)-4-one]formazan
6g	$C_{31}H_{25}N_7O_4$	284	62	67.89 68.10	8.62 8.41	14.21 13.88	1-(4-Ethylphenyl)-3-(4-nitro phenyl)-4-[benzamido-(2-methyl-3-quinazolin)-4-one] formazan
6h	$C_{30}H_{23}N_7O_4$	168	59	67.89 67.65	8.62 8.46	14.21 14.01	1-(4-Methylphenyl)-3-(4-nitrophenyl)-4-[benzamido-(2-methyl-3-quinazolin)-4-one]formazan

Table No.2: Spectral data of compounds 6a-h

Compd.	IR (KBr) (cm^{-1})	^{1}HNMR ($CDCl_3$) (d ppm)
2	1521.95 (C=C), 1681.14 (C=O, ketone), 1601.54 (C=O, aryl acid), 2926.79 (Ar-CH_3), 3363.50 (-OH)	11.175 (s, 1H, -COOH), 6.504-8.494 (m, 8H, Ar-H), 2.068-2.138 (s, 3H, -CH_3).
6a	1410.77 (C=C), 1571.10 (C=N), 1511.14 (N=N of formazan), 1340.68 (Ar-NO_2)	7.251-8.76 (m, 9H, Ar-H), 3.502 (s, 1H, N-H),1.52 (s, 3H, -CH_3)
6b	1423.64 (C=C), 1596.10 (C=N), 1521.74 (N=N of formazan), 1343.68 (Ar-NO_2), 842.90(C-Cl)	7.261-8.716 (m, 9H, Ar-H), 3.495 (s, 1H, N-H),1.548 (s, 3H, -CH_3)
6c	1427.36 (C=C), 1591.22 (C=N), 1523.57 (N=N of formazan), 1342.79 (Ar-NO_2), 843.07(C-Cl)	7.252-8.644 (m, 9H, Ar-H), 3.489 (s, 1H, N-H),1.542 (s, 3H, -CH_3)
6d	1456.64 (C=C), 1588.13 (C=N), 1520.53 (N=N of formazan), 1344.08 (Ar-NO_2), 739.14 (C-Br)	7.615-8.334 (m, 9H, Ar-H), 3.480 (s, 1H, NH),1.581
6e	1416.64 (C=C), 1591.03 (C=N), 1516.50 (N=N of formazan), 1342.38 (Ar-NO_2)	7.662-8.311 (m, 9H, Ar-H), 3.491 (s, 1H, NH), 1.534 (s, 3H, -CH_3).
6f	1479.02 (C=C), 1646.24 (C=N), 1527.12 (N=N of formazan), 1344.53 (Ar-NO_2), 844.11 (C-Cl), 1013.34 (C-F)	. 7 261-8.715 (m, 8H, Ar-H), 3.495 , (s, 1H, NH)1.549 (s, 3H, -CH_3).
6g	1458.02 (C=C), 1600.18 (C=N), 1518.53 (N=N of formazan), 1343.55 (Ar-NO_2)	7.391-8.816 (m, 9H, Ar-H), 3.512 (s, 1H, NH),1.554 (s, 3H, -CH_3).
6h	1495.20 (C=C), 1603.82 (C=N), 1520.13 (N=N of formazan), 1341.66 (Ar-NO_2), 2815.21 (Ar-CH_3)	7.485-8.956 (m, 9H, Ar-H), 3.472 (s, 1H, NH),1.542 (s, 3H, -CH_3).

3-(4-Carboxyphenyl)-2-methyl-3-quinazolin-4-one (2):

Compound 1 was treated with equimolar amount of p-amino benzoic acid (PABA) in alcohol. The mixture was refluxed for 4 hrs and the reaction was monitored by TLC for the completion of reaction. The resulting product is added to ice-cold water, filtered and dried. The dried compound **2** was recrystallized from DMSO.

3-(4-Benzoyl chloro)-2-methyl-3-quinazolin-4-one (3):

Compound **3** was synthesized by addition of double the molar concentration thionyl chloride (0.02 mole) to compound **2** (0.01 mole) in alcohol. The mixture was vigorously stirred for 30 min and was concentrated. The solid residue formed at the bottom is collected dried and recrystallized from DMSO (yield 71%, m.p. 112^{0}C).

3-(4-Hydrazino benzoyl)-2- methyl-3-quinazolin-4-one (4):

Compound **4** was obtained from the refluxation of 3-(4-benzoyl chloro)-2-methyl-3-quinazolin-4-one (0.01 mole) and double the molar concentration (0.02 mole) of hydrazine hydride in alcohol for 4 hrs. The resulting product is concentrated and the solid residue is collected, dried and recrystallized from DMSO (yield 55%, m.p. 132^{0}C).

3-[(4'-Nitrophenyl)-4-azobenzamido)]-2-methyl-3-quinazolin-4-one (5):

Compounds (5a-5b) were synthesized by condensation of compound 4 (0.01 mole) with substituted aldehyde (0.01 mole) in alcohol, stirred well to get the respective quinazolinone derivatives. The resulting product is filtered, dried and recrystallized from DMSO (Yield 66%. m.p.168^{0}C) .

1-Substituted phenyl-3-(4-nitrophenyl-4-[benzamido-(2-methyl-3-quinazolin)-4-one] formazans (6a-6h):

The diazonium salts obtained from the respective amines (0.01 mole) were added with stirring to compound 5 in pyridine at 0-5ºC for 30 minutes and the resultant mixture was added to cold water. The resulting product is filtered, dried and recrystalized from DMSO.

Biological evaluation

The biological evaluation of the synthesized compounds was carried out in the department of pharmacology, Chalapathi Institute of Pharmaceutical Sciences and its animal facility is approved by CPCSEA. The experimental protocols for the same have been approved by the Institutional Animal Ethics Committee.

Antimicrobial activity

All the synthesized compounds of present study were screened for *in-vitro* antibacterial and antifungal activity [11] against six different strains of bacteria i.e. gram negative organisms like *Escherichia coli, Pseudomonas aeruginosa*, gram positive organisms i.e *Bacillus subtilis, Bacillus cereus, Staphylococcus aureus, Staphylococcus epidermidis* and four fungal organisms i.e *Aspergillus niger, Saccharomyces cerevisiae, Candida albicans, Candida glabrata* by paper disc method. What man filter paper grade-1 disc of 5 mm diameter was sterilized by autoclaving for 15 min at 121^{0}C. The sterile discs were impregnated with different synthesized compounds which were dissolved in DMF at the concentration 10 mg/ml. The nutrient agar of 20 ml was placed in a flat bottomed petridish. When solidified 4 ml of second nutrition solution seeded with test bacteria was poured evenly on to the first layer (40 – 48^{0}C). As soon as the second layer was solidified, the impregnated discs were placed on the medium suitably spaced apart and plates were incubated at 5^{0}C for 1 hr, to permit good diffusion and transferred to an incubator at 37^{0}C ± 1^{0}C for 18-24 hrs for bacterial medium and 25^{0}C ± 1^{0}C for 72 hrs for fungal medium. The inhibition of zones caused by various synthesized compounds and standard drugs, Ciprofloxacin and Flucanazole on the bacterial and fungal microorganisms respectively were examined and results were given in the Table No.3.

Analgesic activity

The analgesic activity was evaluated by Eddy's hot plate method Eddy et al [12] using paracetamol as standard. Albino mice of wistar strain weighing 25-30 gm of either sex were divided in to separate groups each with four animals. Tween 80

Table No.3: Antimicrobial activity of compounds 6a-h

Compound name	*6a*	*6b*	*6c*	*6d*	*6e*	*6f*	*6g*	*6h*	*Std.*
Antibacterial									
B. subtilis	-	17	12	17	19	13	17	15	21
B. cereus		17	15	13	18	14	12	15	17
E. coli		8	13	12	20	12	14	8	22
St.aureus		15	13	17	10	14	13	11	20
St. epidermidis		24	18	18	25	14	23	13	20
Ps. Aeruginosa		16	12	11	14	13	11	14	21
Antifungal									
C. albicans	-	14	11	8	12	8	7	6	19
C. glabrata		29	25	17	29	11	18	19	22
As. niger		19	16	14	28	16	12	10	17
Sa. cerveceae		18	-	10	5	5	15	6	18

Standard – Ciprofloxacin for antibacterial
- Fluconazole for antifungal

suspension (1% v/v) of the test compounds were administered intraperitonialy in a dose of 10 mg/kg. The control group was given only 1% v/v tween-80 suspension. One group was administered with Paracetamol as standard intraperitonialy in a dose of 100mg/kgAll the animals were dropped on the hot plate maintaining at 55^{0}C± 0.5^{0}C and the reaction time was taken as the interval extending from the instinct, the animal licks it's paws or jumps in an attempt to get out of the chamber. All other signs of discomfort such as licking and jumping are disregarded. The reaction time was measured before and after administration of the drug, the response was considered positive when the reaction time after injection of the test substance was longer than normal reaction time and results were reported Table No. 4.

RESULTS AND DISCUSSION

Various 1-Substituted phenyl-3-(4-nitrophenyl)-4-[benzamido-(2-methyl-3-quinazolin)-4-one] formazan derivatives were synthesized. The structures of the synthesized compounds were confirmed by IR at which the compounds **6a-6h** showed absorption bands within the range of 1571.10 to 1646.24 and 1511.14 to 1527.12 cm^{-1} for C=N and N=N of formazan nucleus respectively. ^{1}HNMR spectra of the synthesized compounds showed singlet at 1.52-1.58 due to 3 protons which

Table No.4: Analgesic activity of compounds 6a-h against thermally induced pain by Eddy's hot plate method

Compound name	Basal reaction (Sec) (Mean ± SE)		Reaction time (Sec) (Mean ± SE)	
	Paw-licking	Jump response	Paw-licking	Jump response
6a	4±0.88	6±1.48	4±0.87	6±1.48
6b	3±0.72	5±1.30	5±1.14	8±1.87
6c	4±0.59	7±1.05	4±0.59	7±1.05
6d	4±1.51	6±1.26	8±1.84	12±1.26
6e	3±0.84	5±1.48	9±0.84	12±0.89
6f	3±0.76	5±1.30	7±1.26	11±1.10
6g	3±0.66	5±1.05	6±0.84	8±1.26
6h	3±0.92	6±1.26	3±0.82	6±1.06
Paracetamol (100mg/Kg)	5±0.84	7±1.26	10±0.84	13±1.26
Control	4±0.71	6±1.05	4±1.58	6±1.30

are attached to the carbon atom of the methyl functional group. 1-H (NH) proton of the benzamido group shown a singlet at 3.48-3.51. Aromatic protons showed multiplets in the range of 7.25 –8.81. The expected signals with appropriate multiplicities for different types of protons were observed for the derivatives.
The synthesized compounds were subjected to antimicrobial and analgesic activities by the standard methods. It is evidential from Table No.3 all the compounds exhibited significant antibacterial and antifungal activities

The synthesized compounds in addition were also screened for analgesic activity by Eddy's hot plate method. However compounds 6d, 6e, 6f shown significant analgesic activity compared to standard.

In conclusion the present work provides excellent approach for the synthesis of pharmacologically active and important formazan derivatives.

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