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Synthesis, Characterization and Anti-Inflammatory, Analgesic, and Antimicrobial activities of substituted 4-(4- oxo-2-phenylquinazolin-3(4H)-yl) N-aryl-methylene benzenesulfonamide derivatives

Dhansay Dewangan[1]*, D. K. Tripathi[1], Tekeshwar Kumar[1], Kushagra Nagori[1], Vipin Shah[2] , R.Rajavel[2]

[1] Rungta College of Pharmaceutical Sciences and Research Bhilai,India.

[2]Department of Pharmaceutical Chemistry, Nandha College of Pharmacy and Research Center, Erode-52 Tamilnandu, India

*Corres.author: danu_drugs@yahoo.com

Abstract: Reaction of anthranilic acid with benzyl chloride in pyridine afforded 2- Phenyl-3-1- Benzoxazine-4-one **(1),** which on condensation reaction with the sulphanilamide in alcoholic KOH solution yield 4-(4-oxo-2-phenylquinazolin-3(4H)-yl) benzenesulfonamide (**2**). When **(2)** was reacted with different aromatic aldehydes in alcoholic KOH solution, different substituted4-(4-oxo-2-phenylquinazolin-3(4H)-yl) N-aryl-methylene benzenesulfonamide derivatives was obtained (**3**). The structures of the compounds were confirmed by M.P., TLC, IR, ^{1}H NMR, MASS spectral analysis. All these synthesized compounds shown to have significant analgesic, anti-inflammatory and anti-bacterial activities.

Keywords- Anthranilic acid, Benzyl chloride, Sulphanilamide, Analgesic, Anti-Inflammatory,Anti-Bacterial activities.

Introduction:

Among the wide variety of heterocycles that have been explored for developing pharmaceutically important molecule, quinazolinone derivatives have played an vital role in the medicinal chemistry.There are large number of synthetic compounds with quinazolinone nucleus used for antbacterial and antifungal[1-4], analgesic and anti-inflammatory activities[5-8] , antihyperglycemic activity[9] , anticonvulsant activity[10-11],antitubercular activity[12-14],anxiolytic and antidopaminergic activity[15], when properly substituted in quinazolinone nucleus.The broad spectrum of therapeutic values of quinazolinone ring system prompted us to synthesize the title compounds and screen them for pharmacological activites.The sequence of reactions is as shown in **Scheme I.**

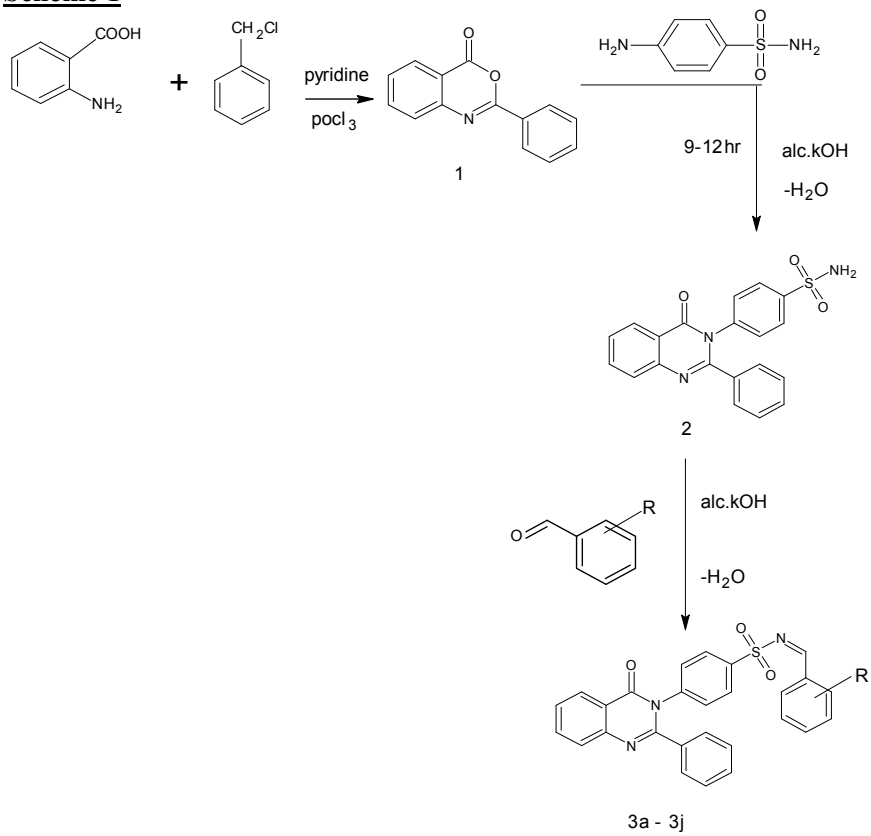
The reaction of starting compound, anthranilic acid and benzyl chloride in pyridine afforded the corresponding 2- Phenyl-3,-1-Benzoxazine-4-one **(1)** .Their IR spectra showed carbonyl peak in the region 1666-1700 cm^{-1}.The structure were further confirmed by recording ^{1}H NMR spectra. Condensation reaction of these derivative with the sulphanilamide gave the desired4-(4-oxo-2-phenylquinazolin-3(4*H*)-yl)benzene sulfonamide **(2).**The assigned structure have been confirmed by the further IR and ^{1}H NMR spectra.The

substitution reaction of (**2**) with different aromatic aldehydes in KOH solution furnished substituted 4-(4-oxo-2-phenylquinazolin-3(4H)-yl) N-aryl-methylene benzenesulfonamide derivatives **(3a-3j).** The characterization data of all the newly synthesized compounds are tabulated in **Table I.** The structures of the above compounds were confirmed by IR, ^{1}H NMR and mass spectral studies depicted in **Table II**. These compounds were screened for their Anti-Inflammatory Analgesic, Antimicrobial activities.

Results and Discussion:

IR data of compounds clearly shows a strong C=N stretching band around 1540.21 cm^{-1} and C=O absorption band around 1750.31 cm^{-1} which indicates ring closure of quilazolinone ring.All final compounds have strong absorption around 3200 cm^{-1} and around 1590 cm^{-1} which are evidence for aromatic C-H and aromatic C-C bonds respectively.IR data also confirms the presence of specific functional groups present in final synthesized compounds.^{1}HNMR data also confirms the presence of specific functional groups present in final synthesized compounds.The presence of shift value 4.1, 3.2, and 10.64-11.52 shown the presence of methoxy, hydroxyl group and Schiff base respectively in synthesized compounds. Exhaustive pharmacological studies have been conducted with the quilazolinone derivative. The 2-position and 3-position is an extremely important site of molecular modification,which play a dominant role in determining the pharmacological activites of quilazolinone derivatives.. The synthesized compounds were screened *in-vitro* anti-bacterial with *E.Coli ,P.aeuriginosa, S.aureus, B.subtilis* which is cause for common cold and cough. Few compounds like compound **3a,3c, 4g** and **4i** were shows good antibacterial activity against standard. So the compound contain **3**-position with an substituted 2-OH, 3-OCH_3-4-OH, 4-Cl and 3,4,5-$(OCH_3)_3$ with phenyl ring in 2- position enhance the antimicrobial activity of quinazolinone derivatives. Of the all the compounds **3c** and **3g** were shown comparatively significant activity. How ever, still need some more novel approach towards the functional group at the SAR to explore the pharmacological activities.

Scheme 1



(3a): R = 2-OH	**(4f): R** = 2-NO_2
(3b): R = 4-OCH_3	**(4g): R** = 4Cl
(3c): R = 3-OCH_3-4-OH	**(4h): R** = 4-CH_3
(3d): R = 3-NO_2	**(4i): R** = 3,4,5-$(OCH_3)_3$
(3e): R = -H	**(4j): R** = 4-N-$(CH_3)_2$

Table I:Characterization data of Quinazolinone Derivatives:

Comp.	R	Mol.formula	M.P.(°c)	Mol. wt.	App.	Rf	Solibility	%yield (w/w)	Chemical name
3a	2-OH	$C_{27}H_{19}N_3O_4S$	163	481	Brown	0.57	DMF	66.666	4-(4-oxo-2-phenylquinazolin-3(4*H*)-yl)N-(2hydroxy-phenyl)methylene benzenesulfonamide
3b	4-OCH_3	$C_{28}H_{21}N_3O_4S$	172	495	Brown	0.53	DMF	48.898	4-(4-oxo-2-phenylquinazolin-3(4*H*)-yl)N-(4 methoxy phenyl)-methylenebenzenesulfonamide
3c	3-OCH_3-4-OH	$C_{28}H_{21}N_3O_5S$	143	511	Yellow brown	0.65	DMF	67.171	4-(4-oxo-2-phenylquinazolin-3(4*H*)-yl)N-(3methoxy-4 hydroxy phenyl) -methylene benzenesulfonamide
3d	3-NO_2	$C_{27}H_{18}N_4O_4S$	163	510	Yellow white	0.56	DMF	46.177	4-(4-oxo-2-phenylquinazolin-3(4*H*)-yl)N-(3 nitro phenyl)-methylene benzenesulfonamide
3e	H	$C_{27}H_{19}N_3O_3S$	133	465	Grey	0.57	DMF	54.75	4-(4-oxo-2-phenylquinazolin-3(4*H*)-yl)N-(phenyl)-methylene benzenesulfonamide
3f	2-NO_2	$C_{27}H_{18}N_4O_4S$	162	510	Yellow white	0.63	DMF	42.033	4-(4-oxo-2-phenylquinazolin-3(4*H*)-yl)N-(2 nitro phenyl)-methylene benzenesulfonamide
3g	4Cl	$C_{27}H_{18}ClN_3O_3$	157	499	Brown white	0.66	DMF	67.738	4-(4-oxo-2-phenylquinazolin-3(4*H*)-yl)N-(4 chloro- phenyl)-methylene benzenesulfonamide
3h	4-CH_3	$C_{28}H_{21}N_3O_3S$	154	479	Yellow white	0.55	DMF	61.729	4-(4-oxo-2-phenylquinazolin-3(4*H*)-yl)N-(3-methyl phenyl)-methylene benzenesulfonamide
3i	3,4,5-(OCH_3)	$C_{30}H_{25}N_3O_6S$	153	555	Brown	0.61	DMF	57.455	4-(4-oxo-2-phenylquinazolin-3(4*H*)-yl)N-(3,4,5-trimethoxy phenyl)-methylene benzenesulfonamide
3j	4-N-(CH_3)$_2$	$C_{29}H_{24}N_4O_3S$	168	508	Grey	0.67	DMF	80.00	4-(4-oxo-2-phenylquinazolin-3(4*H*)-yl)N-(4 dimethyl amino phenyl)-methylene benzenesulfonamide

Experimental Section:

Melting points were determined in open capillaries and were uncorrected. Purity of the compounds were checked by TLC. IR spectra (KBr, cm^{-1}) were recorded on Perkins Elmer Infrared-283 FTIR. ^{1}HNMR ($CDCl_3$) on a Bruker 300MHz spectrometer using TMS as an internal reference.The mass spectra were recorded on a API 3000 LC-MS.

2- Phenyl-3,-1-Benzoxazine-4-one (1).

A mixture of Anthranilic Acid (0.1 mol) and Benzyl chloride (0.1mol) in pyridine was stirred mechanically for 30 min. The reaction mixture was cooled and poured into crushed ice and the solid mass, which separated out was filtered and washed with water.It is dried and recrystallized from methanol to give crystalline compound.

4-(4-oxo-2-phenylquinazolin-3(4*H*)-yl)benzenesulfonamide (2).

A mixture of 2- Phenyl-3,-1-Benzoxazine-4-one (0.01 mole) and sulphanilamide (0.01) was taken in a 250 ml round bottom flask and add 100 ml of alcoholic KOH solution to the flask.The mixture was refluxed for 9-12 hours.Then the refluxed mixture was poured into crushed ice. The solid mass which was separated out was filtered and washed with water.It is dried and recrystallized from methanol

4-(4-oxo-2-phenylquinazolin-3(4*H*)-yl)N-aryl-methylene benzenesulfonamide 3(a-j).

A mixture of 4-(4-oxo-2-phenylquinazolin-3(4*H*)-yl)benzenesulfonamide(0.01mole) and different aromatic aldehydes(0.01mole) were taken in round bottom flask and added 100 ml of alcoholic KOH to the flask . The mixture was refluxed for 9-12 hours.Then the refluxed mixture was poured into crushed ice. The solid mass which was separated out filtered and washed with water.It is dried and recrystallized from methanol.

Table II:IR, NMR and Mass Spectra of Quinazolinone Derivatives

IR (cm^{-1}) (KBr)	^{1}H NMR δ (ppm)	MS
3025.45(Aromatic-C-H str), 1700.31(C=O str), 1640.31(C=C str), 983.73(aromatic C-Cstr), 1540.21(C=Nstr), 931.65(C-N str), 3003.27(O-H str).	7.27-7.74 (12H, Ar-H), 10.23(1H, -NCH-), 3.2(1H, -OH)	480^+
3135.45(Aromatic-C-H str), 1750.31(C=O str), 1631.40(C=C str), 965.47(aromatic C-Cstr), 1545.64(C=Nstr), 926.86(C-N str), 1229.66(C-O str of OCH3)	6.8-7.3(12H, Ar-H), 11.3(1H, -NCH-), 3.6-4.1(3H, -OCH_3)	494^+
3135.45(Aromatic-C-H str), 1820.31(C=O str), 1730.31(C=C str), 1003.73(aromatic C-Cstr), 1710.21(C=Nstr), 971.64(C-N str), 3303.47(O-H str), , 1219.46(C-O str of OCH3)	6.5-7.8(12H, Ar-H), 10.75(1H, -NCH-), 4.1(1H, -OH), 3.9-4.2(3H, -OCH_3)	510^+
3267.86(Aromatic-C-H str), 1678.53(C=O str), 1576.45(C=C str), 1332.43(aromatic C-Cstr), 1528.21(C=Nstr), 1085.64(C-N str), 1445.64(-NO_2 str)	7.1-7-3(12H, Ar-H), 12.76(1H, -NCH-)	509^+
3154.67(Aromatic-C-H str), 1699.75(C=O str), 1567.75(C=C str), 1132.43(aromatic C-Cstr), 1540.21(C=Nstr), 985.64(C-N str)	7.2-7.5(13H, Ar-H), 11.54(1H, -NCH-),	464^+
3345.06(Aromatic-C-H str), 1728.53(C=O str), 1654.64(C=C str), 1533.43(aromatic C-Cstr), 1654.21(C=Nstr), 1001.64(C-N str), 1335.64(-NO_2 str).	6.9-7.7(12H, Ar-H), 10.64(1H, -NCH-)	509^+
3045.06(Aromatic-C-H str), 1553.28(C=O str), 1451.45(C=C str), 1345.43(aromatic C-Cstr), 1623.21(C=Nstr), 1109.64(C-N str), 715.64(-C-Cl str).	6.8-7.9(12H, Ar-H), 11.76(1H, -NCH-)	498^+
3154.67(Aromatic-C-H str), 1699.75(C=O str), 1567.75(C=C str), 1132.43(aromatic C-Cstr), 1540.21(C=Nstr), 985.64(C-N str), 2985.64(ali C-H str)	7.3-7.8(12H, Ar-H), 10.48(1H, -NCH-), 3.11-4(3H, -CH_3)	478^+
3334.67(Aromatic-C-H str), 1709.75(C=O str), 1677.75(C=C str), 1012.43(aromatic C-Cstr), 1540.21(C=Nstr), 1085.64(C-N str), 1229.66(C-O str of OCH3)	7.1-7.7(10H, Ar-H), 10.84(1H, -NCH-),3.6-4.5(9H, -$(OCH_3)_3$)	554^+
3334.67(Aromatic-C-H str), 1709.75(C=O str), 1677.75(C=C str), 1012.43(aromatic C-Cstr), 1540.21(C=Nstr), 1085.64(C-N str), 1320.91(N-C str),	7.1-7.8(11H, Ar-H), 10.04(1H, -NCH-),3.76-4.21(6H,-N-$(CH_3)_2$)	507^+

Pharmacological activity:

Acute Toxicity**:** Animals: Swiss albino mice weighing 20 – 25 gms were used for the study. Animals were fed a standard pellet (Pranav Agro Industries Ltd., Sangli) and water ad libitum and maintained at 24 – 28 C temperature, 60 – 70% relative humidity and 12 hr day and nigh cycle. Animals described as fasted were deprived of food for 4 days, but had free access to water.

In-vitro Antibacterial Screening of Synthesized Compounds:

Plate hole diffusion method**:** The synthesized compounds were tested for their *in vitro* antibacterial activity against the gram-negative bacteria and gram-positive bacteria ,by cup-plate method.Chlotrimazole and Streptomycin were used as standard drug for antibacterial studies. Nutrient Agar(Beef extract 10 gm,Peptone 10 gm, Sodium Chloride 5 gm ,Agar 20 gm, purified water 1000ml) was employed as culture media for antibacterial studies. The ingredients were dissolved in water, and adjust the pH to 7.2 to 7.4 by using dilute alkali/dilute acid and autoclave at 120ºC for 20 min.30-35 ml of nutrient agar was transferred to the Petri dish. 1000µg/disc,500µg/disc,250µg/dish concentration of the test compounds are prepared &Dimethyl Formamide (DMF) was used as vehicle and Chlotrimazole and Streptomycin (250µg/ml) was used as standard. Nutrient agar plates were prepared aseptically to get a thickness of 5-6 mm.The plates were allowed to solidify and inverted to prevent condensate falling on the agar surface. The plates were dried at 37 ºC just before inoculation. The standard inoculums is inoculated in the plates prepared earlier aseptically by dipping a sterile swab in the inoculums,removing the excess of inoculums by pressing and rotating the swab firmly against the sides of the culture tube above the level of the liquid and finally streaking the swab all over the surface of 60 after each application.finally press the swab round the edge of the agar surface.The sterilized discs for the test drugs were placed in the Petri dishes aseptically .Incubate the Petri dish at 37 ºC ± 0.2 ºC for about 18-24 hrs, after placing them in the refrigerator for one hour to facilitate uniform diffusion.The average zone diameter of the plates were measured and recorded.All compounds synthesized were tested for antibacterial activity against 5 gram + ve & 5 gram (–) ve bacteria.

Analgesic activity:

Test for analgesic activity was performed by Acetic acid induced writhing method using Swiss albino mice (25-35g) of male sex selected by random sampling technique. Diclofenac (5mg/kg) was used as standard drug for comparison. The test compound (Quinazolinone Derivatives) were administered at dose of 5 mg/kg. The inhibition of writhing in mice by synthesized compound will be compared against to the inhibition of writhing by a standard analgesic agent the reaction time was recorded after 10 min of the administration of standard / test compounds. The percent analgesic activity (PAA) was calculated by the following formula:

PAA = 1-Treated/Control ×100

Table No A: Anti –Bacterial Activity of Quinazolinone derivatives using plate hole diffusion method:

Compound	one of inhibition (mm)			
	E.Coli	***P.aeuriginosa***	***S.aureus***	***B.Subtilis***
3a	21	20	20	22
3b	19	18	17	16
3c	20	21	21	22
3d	18	17	16	16
3e	17	16	17	18
3f	17	16	14	18
3g	22	21	21	20
3h	19	16	18	18
3i	21	22	21	21
3j	17	16	18	17
Streptomycin (250µg/ml)	23	24	--	--
Clotrimazole	--	--	23	23
DMF(solvent)	--	--	--	--

Table No B:Analgesic effect of Quinazolinone derivatives (5 mg/ kg) and standard drug Diclofenac (5 mg/kg) on Acetic acid induced writhing test in Swiss albino male mice.

Treatment group	Total number of writhes	%Inhibition
Control (Vehicle)	70.0 ± 0.3	--
Standard drug	23.67 ± 0.7	66.67
3a	46.3 ± 0.5	34.78
3b	43.33 ± 0.88	38.11
3c	37.3 ± 0.4	47.46
3d	42.16 ± 0.94	39.77
3e	42.50 ± 0.76	39.28
3f	40.33 ± 0.80	42.38
3g	38.16 ± 0.54	45.48
3h	42.50 ± 0.92	39.28
3i	41.33 ± 0.71	40.95
3j	37.16 ± 0.30	44.48

(n=3,p<0.1) The experimental groups compaired with control

Table No C :Anti-inflammatory effect of Quinazolinone derivative on Carrageenan -induced paw edema in rats.(10mg/kg),using indomethacin as standard drug (10mg/kg),

Treatment group	Edema induced by Carrageenan (mm.)	%Inhibition
Control (Vehicle)	6.41 ± 0.02	----
Standard drug	3.81 ± 0.03	40.56
3a	2.11 ± 0.03	*23.292*
3b	1.82 ± 0.03	*18.293*
3c	2.27 ± 0.02	*30.605*
3g	3.67 ± 0.03	*33.073*
3h	2.92 ± 0.02	*28.825*
3i	2.57 ± 0.03	*25.449*

(n=4,p<0.1) The experimental groups compaired with control.

Anti-inflammatory activity:

The inhibitory activity of synthesized compound on carrageenean induced rat paw edema will be determined according to mercury displacement method by using plethismograph. 8 groups of adult male albino rates (150-180gm) four animals in each will be orally dosed with synthesized compound one hour before carrageenan challenge, foot paw edema will be induced by sub planter injection of 0.05ml of 1% suspension of carrageenan in saline in to the planter tissue of one hind paw. The equal vol. of saline will be injected serve as a control group. The standard group will receive indomethacin (10mg/kg) s.c. The mercury displacement will be compared with standard for evaluation of anti-inflammatory activity of synthesized compounds.

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