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Preliminary communication

Synthesis and biological properties of 4-(3*H*)-quinazolone derivatives

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Abstract

A new quinazolone series has been designed, and synthesized by the anthranilic acid and different acid derivatives. Their structures have been elucidated on the basis of elemental analyses and spectral studies (IR, ^{1}H NMR, FT-IR and FAB-MS). A preliminary radiolabelling study with technetium has shown a very good future prospect for further evaluation in vivo. The biological activities (antifungal, antibacterial as well as anticancerous) of the evaluated compounds are discussed in this article.

Keywords: Analogues; Spectroscopic; Antifungal; Antibacterial; Anticancerous

1. Introduction

Quinazolone and their derivatives are building block for approximately 150 naturally occurring alkaloids isolated from a number of families of the plant kingdom, from microorganisms and animals [1–4] and are now known for a wide range of biological properties including hypnotic, sedative, analgesic, anticonvulsant, antibacterial, antidiabetic, anti-inflammatory, anti-tumor and several other useful and interesting properties [5–13]. In addition, some derivatives are calcium antagonists and share the common property of interfering with the influx of extracellular calcium via the calcium L channel [14]. Recently quinozolone chemistry has got new direction due to some resemblance with folic acid [15–17]. So keeping this fact in mind that quinazolone as very important moiety, we have synthesized a bis series of quinazolone having different substituents (according to our previous work [12]) in good yield and after that screened them for in vivo microbial activity.

2. Results and discussion

All the final compounds **4A–D** were prepared in good yields. The physical parameters of these compounds are mentioned in Table 1 and the synthetic route is shown in Scheme 1. All intermediate as well as final quinazolone analogues are analyzed by different spectroscopic technique such as UV, IR, NMR, mass spectroscopy and by elemental analysis. The spectral evidences confirm the presence of –N–C=O, –NH–N–C and fused ring system (IR at 3330, 1685, 1460 cm^{-1}). Similarly, NMR multiplet in the range of 6.7–8 ppm of 15–25 hydrogens also confirms the presence of aromatic rings. Aminobenzoic acid with excess equivalent of benzoyl chloride in the presence of a base particularly an organic base like pyridine affords 2-phenyl-4-oxo-3,1-benzoxazine (**2**) in excellent yield. It is thought that initially *N*-benzoyl anthranilic acid is formed which undergoes tautomerization and subsequent cyclization in the presence of pyridine .In the presence of

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Table 1
Physical parameters of 3-(2′-phenyl-quinazolin-4′-yl-amino)2-(phenyl/styryl/benzamidomethyl/phthalimidomethyl)-4(3*H*)-quinazolone

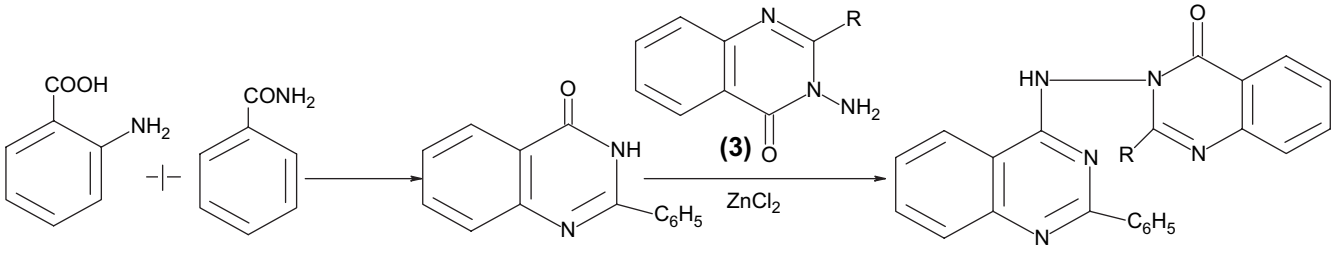
Compound	R	Mp (°C)	Yield	Colour	Molecular formula	Molecular weight	Analysis					
							Carbon %		Hydrogen %		Nitrogen %	
							Calcd.	Found	Calcd.	Found	Calcd.	Found
4A	Phenyl	110	80	Brown	$C_{28}H_{19}N_5O$	441	76.19	76.01	4.31	4.10	15.87	15.54
4B	Styryl	138	75	Grey	$C_{30}H_{21}N_5O$	466	77.25	76.97	4.51	4.32	15.02	14.82
4C	Phthalimidomethyl	121	85	Grey	$C_{31}H_{20}N_6O_3$	524	70.99	70.45	3.82	3.51	16.03	15.79
4D	Benzamidomethyl	105	89	Pinkish	$C_{30}H_{22}N_6O_2$	498	72.29	72.10	4.42	4.21	16.87	16.39

anhydrous zinc chloride as Lewis acid catalyst, the enol form of **2** reacts with **3** to give a secondary amino compound.

Some conclusions can be drawn regarding the antifungal activity of quinazolyl-quinazolones (Table 2). Thus, compound **4C** bearing R = phthalimidomethyl was found most active at a lower concentration of 10 μg/ml. This compound caused an inhibition of *Aspergillus flavus* to the extent of 83.3% at 10 μg/ml while benzamidomethyl substituted compound showed a decrease of about 23% in activity at the same dose level (10 μg/ml). Interestingly, a phenyl-substituted compound was found much superior than a styryl-substituted compound at a lower dose level since percentage inhibition was 76.7% with the former compound while the latter compound had inhibition of only 53.3%.

Antibacterial activity shows that compound **4C** (Table 3) was found highly active against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* but showed only slight activity against *Staphylococcus aureus*. High activity was demonstrated by compound **4A** (Table 3) against *K. pneumoniae* but moderate order of activity was observed against *P. aeruginosa* and lower order of activity against *S. aureus*. The compounds **4B** and **4A** showed high order of antibacterial activity against *S. aureus*. Since all the three bacteria are highly pathogenic and all four compounds have shown high activity against one or two strains of bacteria, it is speculated that such compounds can be considered as potential candidate molecules as far as antibacterial activity is concerned. It is of interest that compound **4C** has also showed pronounced antifungal activity against *A. flavus* at lower concentration level in addition to exhibiting high activity against pathogenic bacterial strains. In order to confirm the potentiality of these compounds for their antimicrobial and antifungal activities, more intensive bioassay programme leading to involve more strains of bacteria and fungi are needed.

Two quinazolyl-quinazolines (Table 4) were found to show positive results while two showed negative results. Thus, compound **4B** bearing R = styryl was found to cause a reduction in cell multiplication to the extent of 8.69 ± 1.15 compared to the control 10.21 ± 1.01 for the normal cell. Another compound (**4D**) having R′ = benzamidomethyl showed a positive result but to a lesser extent since decrease in multiplication was less pronounced as compared to the control. This compound caused the decrease in the multiplication to the magnitude of 9.85 ± 0.61 as compared to the control (10.21 ± 1.01). It is obvious from the general molecular structure that the substitution has been made at only one position and therefore the activity seems totally dependent upon the nature of the substituent. Thus, compound **4A** bearing R = phenyl caused a negative result since cell no. $\times 10^{14}$ value was more than 10.21 ± 1.01 the value for normal cell. This compound increased the multiplication of the cells (11.79 ± 1.06). It is therefore interpreted that a styryl ($C_6H_5CH{=}CH-$) substituent finds a better fit at the receptor site than a phenyl group (C_6H_5-). In the same manner,



Where

Comp. No.	(R)
4(A)	Phenyl
4(B)	Styryl
4(C)	Phthalimido methyl
4(D)	Benzamidomethyl

Scheme 1.

Table 2
Antifungal activity data of 3-(2′-phenyl-quinazolin-4′-yl-amino)2-(phenyl/styryl/benzamidomethyl/phthalimidomethyl)-4(3*H*)-quinazolone

Compound	R	Concentration (μg/ml)	*A. flavus*		*A. niger*	
			Colony diameter	Inhibition (%)	Colony diameter	Inhibition (%)
4A	Phenyl	10	0.7	76.7	1.4	30
		20	0.5	83.3	1.2	40
		50	0.2	93.3	0.8	60
		100	0.1	96.7	0.5	75
4B	Styryl	10	1.4	53.3	0.8	60
		20	0.6	80.0	0.6	70
		50	0.4	86.7	0.2	90
		100	0.1	96.7	0.1	95
4C	Phthalimidomethyl	10	0.5	83.3	1.5	25
		20	0.4	86.7	0.6	70
		50	0.4	86.7	0.5	75
		100	0.1	96.7	0.3	85
4D	Benzamidomethyl	10	1.2	60.0	1.0	50
		20	1.0	66.6	0.8	60
		50	0.7	76.7	0.5	75
		100	0.4	86.7	0.2	90
Control	−	−	3.0	−	2.0	−

a benzamidomethyl and not a phthalimidomethyl substituent is more appropriate in eliciting the desired biological effects since the compound containing the former substituent (compound **4D**) was found to show a positive result and the latter substituent (compound **4C**) showed a negative cytotoxic effect.

3. Experimental

3.1. Materials and methods

All chemicals used in present study were of analytical grade purchased from Sigma, Aldrich and Merck chemical Co. All the solvents were used after distillation, TLC was run on the silica get coated aluminum sheets (silica gel 60 F_{254}, E Merck, Germany) and visualized in UV light. Melting points were determined by using Thomas Hoover apparatus and are uncorrected. IR spectra were recorded on the FT-IR Perkin–Elmer spectrum BX spectrophotometer. NMR spectra were obtained by using Bruker NMR instrument 300 MHz. The FAB-MS spectra were recorded from JEOL SX 102/DA-6000 spectrometer using *m*-nitrobenzyl alcohol as matrix. EI-MS spectra were recorded on a JEOL SX102/DA (KV 10 mA) instrument. Elemental analysis was done on elementar analysensysteme GmbH vario EL system. Radio complexation and radio chemical purity was checked by instant strip chromatography (silica gel impregnated paper chromatography) with IILC-SG (Gelman sciences, Ann Arbor, MI, USA).

3.1.1. Synthesis of 2-phenyl-4(3H)-quinazolone (*2*)

A mixture of anthranilic acid (0.01 mol) and benzamide (0.01 mol) was heated on an oil bath at 120–130 °C for 4 h. Subsequently, the melt was allowed to cool for 30 min at room temperature. During this period, the melted mass solidified. It was treated with an aqueous solution of sodium bicarbonate (10%) in order to dissolve any unreacted acid into the cyclized product. An additional quantity of sodium bicarbonate solution was added to ensure the complete dissolution of the acid (until there was no effervescence of carbon dioxide). The solid was filtered off and washed with water in order to remove any inorganic materials. It was dried under vacuum over night and recrystallized from ethanol as white crystalline mass, mp 122–123 °C [124 °C] [12], yield 1.87 g (85%).

3.1.2. Synthesis of 2-aryl-3-amino-4(3H)-quinazolones (*3*)

Anthranilic acid (0.01 mol) was dissolved in dry pyridine (30 ml) by stirring slowly at room temperature. The solution was cooled to 0 °C and a solution of an aromatic acid chloride (0.02 mol, different for all four products according to their

Table 3
Antibacterial activity data of 3-(2′-phenyl-quinazolin-4′-yl-amino)2-(phenyl/styryl/benzamidomethyl/phthalimidomethyl)-4(3*H*)-quinazolone

Compound	R	Control	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Klebsiella pneumoniae*
4A	Phenyl	−	++	+	+++
4B	Styryl	−	++	+++	+
4C	Phthalimidomethyl	−	+++	+	+++
4D	Benzamidomethyl	−	++	++	++

+ = 6–10 mm (slightly active); ++ = 10–14 mm (moderately active); +++ = >14 mm (highly active); − = Control.

Table 4
Cytotoxic activity data of 3-(2′-phenyl-quinazolin-4′-yl-amino)2-(phenyl/styryl/benzamidomethyl/phthalimidomethyl)-4(3*H*)-quinazolone

Compound	R	Cell no. $\times 10^{14}$	Activity
4A	Phenyl	11.79 ± 1.06	Negative
4B	Styryl	8.69 ± 1.15	Positive
4C	Phthalimidomethyl	11.88 ± 1.15	Negative
4D	Benzamidomethyl	9.85 ± 0.61	Positive
Control		10.21 ± 1.01	Normal

subsituent) in dry pyridine (30 ml) was added to this solution slowly with constant stirring. When the addition was complete, the reaction mixture was further stirred for half an hour at room temperature and set aside for 1 h. The pasty mass obtained was diluted with water (~50 ml) and treated with aqueous sodium bicarbonate solution to remove the unreacted acid. When the effervescence ceased, it was filtered off and washed with water to remove the inorganic materials and the adhered pyridine. The crude benzoxazine thus obtained was dried and recrystallized from diluted ethanol, yield 2.23 g (81%).

To a cold solution of 2-aryl-4-oxo-3,1-benzoxazine (0.05 mol) in anhydrous pyridine (25 ml) was added a solution of hydrazine hydrate (0.1 mol) in anhydrous pyridine (25 ml) dropwise with constant stirring. When the addition was complete, the resultant reaction mixture was stirred vigorously for 30 min at room temperature and subsequently heated under reflux for 6 h under anhydrous reaction conditions. It was allowed to cool to room temperature and poured into ice cold water containing diluted hydrochloric acid (50 ml diluted HCl and 100 ml water). On standing for 1 h solidification occurred which was allowed to settle down. It was filtered off, washed repeatedly with water and dried in vacuo. Recrystallization from diluted ethanol afforded 2-aryl-3-amino-4(3*H*)-quinazolones in analytically pure form. The spectral analysis of intermediate compounds are given in Table 5.

3.1.3. Synthesis of 3-(2′-phenyl-quinazolin-4′-yl-amino)-2-(phenyl/styryl/benzamidomethyl/phthalimidomethyl)-4(3H)-quinazolones (4A–D)

The target compounds (**4A–D**) were synthesized by heating equimolar quantities of 2-phenyl-4(3*H*)-quinazolone (**2**) and 2-aryl-3-amino-4(3*H*)-quinazolone (**3**) containing anhydrous zinc chloride (1.0 g) at 130–140 °C for 4 h. During heating, the contents were stirred occasionally. Subsequently, the hot melt was cooled to room temperature, treated with diluted hydrochloric acid (~100 ml) and then stirred vigorously. The solid was filtered off and washed with cold water. After removing water, it was dissolved in ethanol and treated by charcoal. The solvent was evaporated in vacuo and the crystalline product was washed with ethanol and dried.

Compound **2**: mp 214 °C, IR (KBr pellets, cm^{-1}) 3330, 1685, 1460 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 7.3–8.1 (m, 9H, ArH), 4.1 (s, 1H, NH). MS (EI) *m/z* 221 (M^+, 90.1).

Compound **4A**: mp 110 °C, IR (KBr pellets, cm^{-1}) 3140, 2134, 1645, 1324, 915 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 7.1–84 (m, 18H, ArH), 4.0 (s, 1H, NH). MS (EI) *m/z* 440 (M^+, 86.4), 221, 116.

Compound **4B**: mp 138 °C, IR (KBr pellets, cm^{-1}) 3425, 2954, 1670, 1456, 815 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 7.3–8.5 (m, 18H, ArH), 4.0 (s, 1H, NH), 5.6 and 6.1 (s, 1H, –HC=CH–). MS (EI) *m/z* 493, 465 (M^+, 98.0).

Compound **4C**: mp 121 °C, IR (KBr pellets, cm^{-1}) 3210, 2156, 1595, 1394, 992 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 75–8.1 (m, 17H, ArH), 4.1 (s, 1H, NH), 3.7 (s, 2H, CH_2). MS (EI) *m/z* 523 (M^+, 79.1), 398.

Compound **4D**: mp 105 °C, IR (KBr pellets, cm^{-1}) 3315, 2834, 1645, 1184 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 7.0–8.4 (m, 17H, ArH), 3.9 (s, 1H, NH). MS (EI) *m/z* 497 (M^+, 80.8).

The activity analysis such as antifungal activity of compounds was determined by agar plate method [18] using the concentrations of 10, 20, 50 and 100 μg/ml of the test compounds. In order to perform the antifungal activity 1 ml of each test compound was poured into a petri dish having about 20–25 ml of molten potato dextrose agar medium. As the medium solidified, Petri dishes were inoculated separately with the fungal isolates and kept at 27 °C for seven days. Percentage inhibition in fungal zones was recorded after that. The solutions of the test compounds were prepared in dimethylsulphoxide (DMSO) and the required concentrations were achieved by diluting the solutions and stirring. Any turbidity if obtained was removed by quick filtration through fluted filter paper. Amphotericin is taken as the control (7.5 μg/ml), antifungal activity data are recorded in Table 2.

Antibacterial activity was determined by disk diffusion method [18]. In this method, the filter paper (Whatmann

Table 5
Spectral data of intermediate (**3**), having different R groups (phenyl, styryl, phthalimidomethyl, benzamidomethyl)

Intermediate (**3**)	Spectral data
When R = phenyl	MP (EtOH): 102 °C; IR (KBr) 816, 1387, 1673, 3402 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 7.1–8.2 (m, 9H, ArH), 3.4 (s, 1H, NH); MS (EI): *m/z* 237 (M^+, 92%), 220, 121.
When R = styryl	MP (EtOH): 87 °C; IR (KBr) 892, 1134, 1480, 1605, 2942, 3324 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 7.3–8.5 (m, 9H, ArH), 4.0 (s, 1H, NH), 5.4 and 6.3 (s, 1H, –HC=CH–); MS (EI): *m/z* 263 (M^+, 85%), 219, 76.
When R = phthalimidomethyl	MP (EtOH): 175 °C; IR (KBr) 846, 1397, 1743, 3205 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 7.9–8.3 (m, 9H, ArH), 4.1 (s, 1H, NH), 3.5 (s, 2H, CH_2); MS (EI): *m/z* 320 (M^+, 80%), 221, 119.
When R = benzamidomethyl	MP (EtOH): 206 °C; IR (KBr) 798, 1277, 1668, 1803, 3342 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ (ppm) 7.6–8.2 (m, 9H, ArH), 4.3 (s, 1H, NH), 3.6 (s, 2H, CH_2); MS (EI): *m/z* 321 (M^+, 72%).

no. 1) with sterile discs of 5 mm diameter impregnated with the test compounds (10 μg/ml of DMSO) were placed in the nutrient agar plate at 37 °C for 24 h.

The inhibition zones around the dried impregnated discs were measured after 24 h. The antibacterial activity was classified as highly active (>14 mm), moderately active (10–14 mm), slightly active (6–10 mm) and less than 6 mm was regarded as inactive. All the samples were tested in triplicate and tetracycline is taken as control (20 μg/ml). The antibacterial activity data are recorded in Table 3.

These compounds were tested against (MCF-7) human breast adenocarcinoma cell line (originally obtained in 1977, from the Michigan cancer foundation). Routine culture maintenance and experimental studies were carried out at 37 °C in a cell incubator with humid atmosphere at 5% CO_2. Cell propagation was achieved in minimal Eagle (MEM, with Earle's salts) with phenol red, 10% fetal bovine serum (FBS), L-glutamine, penicillin, streptomycin and gentamycin as described in all the previous literature. Before any experiment, the cells were transferred for four days to a defined medium, containing phenol red free DMEM, supplemented with 10% charcoal-stripped Estrogen (17 β-estrodiol) in concentration of up to 100 μg added to defined medium. Doxorubicin is taken as standard.

For 3-(4,5-dimethylthiazol-2-yl)-2,5-phenyltetrazolium bromide (MTT) assay MCF-7 cells (1×10^4 cells/well) were placed in a 96-well tissue culture plate and exposed to the compounds under investigation. Cells were processed with the MTT assay for 24, 48 and 72 h of incubation. In brief, 10 μL of MTT (5 mg/kg stock solution) in PBS (phosphate buffer saline) was added to every well containing 100 μL cell suspension in medium and the cultures were allowed to incubate at 37 °C for 5 h. The reaction mixture was carefully taken out and 100 μL of DMSO was added to each well and pipetted up and down several times unless it became homogenic. After 10 min, the colour was read at 540 nm using spectrophotometer plate reader (Bio-Rad, Tokyo, Japan). The activity data are recorded in Table 4.

4. Conclusion

Four different quinazoline derivatives have been synthesized and evaluated for antifungal, antibacterial and anticancer activities. These compounds have shown promising results for future application.

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