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Synthesis and pharmacological study of novel pyrido-quinazolone analogues as anti-fungal, antibacterial, and anticancer agents

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Abstract—A versatile method for novel pyrido-quinazolones was described here and tested for anti-fungal, antibacterial, and anti-cancerous activities. These synthesized compounds were characterized on the basis of spectroscopic techniques and evaluated for specific radiopharmaceuticals. Preliminary radiolabeling results with ^{99m}Tc and biological evaluation studies showed promising results for further evaluation in vivo. The efficiency of labeling was more than 98% and complexes were stable for about 18 h at 25 °C in the presence of serum.

Novel pyrido-quinazolone derivatives are a privileged structure present in many biological active compounds. Pyrido derivatives constitute an important class of compounds possessing diverse type of biological properties including antibacterial, antidiabetic,[1–6] antifungal,[7] and antiarrhythmic.[8] In addition some pyrido derivatives are calcium antagonists and share the common property of interfering with the influx of extracellular calcium via the calcium L channel.[9] In addition, quinazolines/quinazolones are associated with various biological properties ranging from anti-convulsant[10] and antibacterial[11] to anti-diabetic.[12] Interest in quinazolone chemistry has increased recently because of their association with anticancer property.[13] The strategy adopted was the synthesis of quinazolone derivatives that has some resemblance to folic acid.[14–16] These compounds were mainly evaluated for inhibition of enzyme dihydrofolate reductase and were found to be inhibitors of dihydrofolate reductase in human leukaemia cells.[17,18]

A general method for the synthesis of pyrido-quinozolones is being required in order to effect more comprehensive exploration of the chemical space through the variation of different substituents and fusion of other rings. These changes should also minimize the side effects found in other triazole drugs.[19–22] In conclusion, the requirement is to synthesize novel molecules having good potential with high therapeutic index.

Keeping in view the diverse therapeutic activities of quinazolones and as part of our ongoing development of efficient protocols for the preparation of bioactive heterocycles, the present study describes a simple, novel, high yielding synthesis of the title compounds and to screen them for their in vitro microbial activity.

All intermediate products and final pyrido-quinazolone derivatives of this synthesis process were analyzed by spectral evidences obtained from IR, NMR, Mass spectra and by elemental analysis.

The spectral evidences confirms the presence of –N–C=O and –NH–C=O and fused benzene ring (IR at 3465, 815, and 840 cm^{-1}). Similarly NMR multiplet in the range of (6.7–8) ppm of 15-25H also confirms the presence of aromatic rings. The synthesis of **4(A–E)** and **2(X–Y)** is mentioned in Scheme 1 and their reaction parameters are given in Table 1 and Table 2.

The synthesized compounds **4(A–E)** were tested for their antitumor activity against breast cancer cell line viz.

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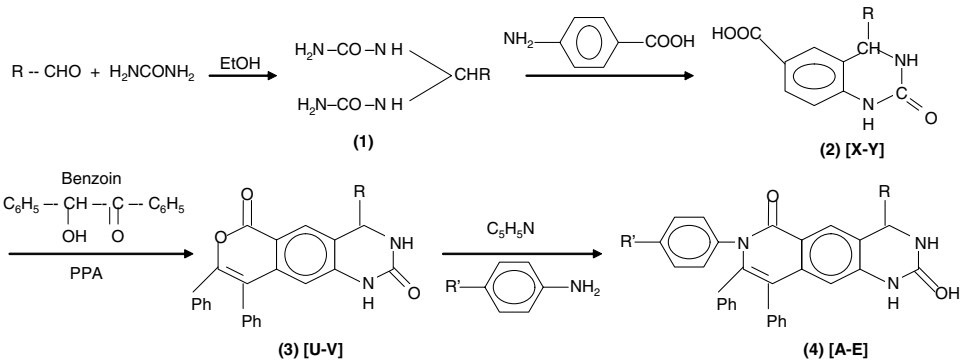

Scheme 1.

Table 1. Reaction parameters of **2(X–Y)** compounds

Compound	R	Yield (%)	mp (°C)
2X	H	72	249
2Y	Ph	65	284

Table 2. Reaction parameters of **4(A–E)** compounds

Compound	R	R′	Yield (%)	mp (°C)
4A	Ph	H	70	128
4B	Ph	Cl	62	140
4C	Ph	OCH_3	87	170
4D	H	Cl	92	194
4E	H	H	84	220

Table 3. In vitro affinity determination cytotoxicity of compounds **4(A–E)** against human breast adenocarcinoma cell line

Compound	IC_{50} (μm)
4A	4.4
4B	25.6
4C	11.4
4D	3.7
4E	8.5

(MCF-7) human breast adenocarcinoma cell line (as mentioned in Table 3). The IC_{50} value has exhibited significant inhibitory activity against this cell line.

The efficiency of radiolabeling with ^{99m}Tc was more than 98% for all the five compounds **4(A–E)**. Radiolabeling of pyridozolone derivatives was carried out by simple reduction method (as mentioned in previous different reviews)[23–26] by using $SnCl_2{\cdot}2H_2O$ under nitrogen atmosphere. In brief, 100 ml of each derivative was mixed with $SnCl_2{\cdot}2H_2O$ (1 mg/ml in 10% acetic acid) in order of 10^{-2} M and followed by addition of 0.5 ml $NaHCO_3$ for maintaining their pH within 6–6.5. Radiochemical purity was determined by ITLC-SG strips. The result has shown minimal inhibitory concentration for compounds **4(A)** and **4(D)**, while maximum for **4(B)** compound.

R_f is determined by ITLC-SG strips using 0.8% NaCl aqueous solution (saline) as developing solvent and simultaneously in acetone. Each ITLC was cut in 0.1 cm segment and counts of each segment were taken. By this appropriate method, the percentage of complex formed between ^{99m}Tc and pyrido-quinazolone derivatives could be calculated. Complex was stable for about 18 h at 25 °C and has confirmed that the synthesized compounds have some heteroatom to donate the lone pair to ^{99m}Tc.

Preliminary biodistribution studies in albino mice strains as animal model have shown that the activity of radiometal has reached to all the organs which are susceptible to CCK and benzodiazepines receptors[27,28] because these are the binding area of pyrido-quinazolone derivatives.

The microbial activity analysis such as anti-fungal activity of compounds was determined by agar plate method[29] using the concentrations of 10, 20, 50, and 100 μg/ml of the test compounds. In order to perform the anti-fungal 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, Petridishes were inoculated separately with the fungal isolates and kept at 27 °C for 7 days. Percent inhibition in fungal zones was recorded after that. The solutions of the test compounds were prepared in dimethyl sulfoxide (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. Anti-fungal activity data are recorded in Table 4.

Out of five, only two compounds (**4(A–B)**) have shown good potentials against these fungi (*Aspergillus flavus* and *Aspergillus niger*). Others have shown less inhibition ($\leqslant$25%). The control is taken from amphotericin B.

Antibacterial activity was determined by disk diffusion method. In this technique, the filter paper (Whatmann No. 1) sterile disks of 5 mm diameter impregnated with the test compounds (10 μg/ml of dimethyl sulfoxide, DMSO) were placed in the nutrient agar plate at 37 °C for 24 h. The inhibition zones around the dried impregnated disks were measured after 24 h. The antibacterial activity was classified as highly active (>14 mm), moderately active (10–14 mm), and slightly active (6–10 mm),

Table 4. Anti-fungal activity data of *N*-aryl-8,9-diphenyl-2-oxopyrido[*c*] 4-aryl-2-oxo-1,3-dihydro-quinazolones

Compound	R	R′	Concentration (μg/ml)	*A. flavus*		*A. niger*	
				Colony diameter	Inhibition (%)	Colony diameter	Inhibition (%)
4A	Phenyl	Hydrogen	10	1.4	53.3	0.8	60
			20	1.2	60.0	0.5	75
			50	0.8	73.3	0.2	90
			100	0.4	86.7	0.1	95
4B	Phenyl	Chloro	10	1.2	60.0	1.0	50
			20	0.7	76.7	0.6	70
			50	0.5	83.3	0.4	80
			100	0.2	93.3	0.2	90
Control	—	—	—	3.0	—	2.0	

Table 5. Antibacterial activity data of *N*-aryl-8,9-diphenyl-2-oxo-pyrido [*c*] 4-aryl-2-oxo-1,3-dihydro-quinazolones

Compound	R	R′	Control	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Klebsiella pneumoniae*
4A	Phenyl	Hydrogen	–	++	+++	+
4B	Phenyl	Chloro	–	++	++	++
4C	Phenyl	Methoxy	–	+	+	+
4D	Phenyl	Chloro	–	+	+	+
4E	Hydrogen	Hydrogen	–	+	+	+

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

and less than 6 mm was taken as inactive. All the samples were tested in triplicate. The antibacterial activity data are recorded in Table 5.

The two compounds **4(A–B)** have shown high order of antibacterial activity against *Staphylococcus aureus*. Since all the three bacteria are highly pathogenic and other compounds have shown lesser activity against these three strains of bacteria. Therefore, first two compounds **4(A)** and **4(B)** can be considered as potential candidate molecules as far as antibacterial activity is concerned. The control is taken from tetracycline.

Two pyrido[*c*] quinazolones out of four have been demonstrated to exhibit definite positive cytotoxic activity (Table 6). Both these compounds **4(A–B)** slightly decreased the multiplication of the cells as compared to the normal. Thus, the compound **4(A)** having R = Phenyl and R′ = Hydrogen had the cell no. $\times 10^{14}$ = 9.17 ± 0.90 while the compound **4(B)** having R = Phenyl and R′ = Chloro had the cell no. $\times 10^{14}$, 9.27 ± 0.87. On the basis of these positive results shown by these compounds it can be anticipated that the substitutions in the phenyl ring attached with the nitrogen atom are not of much significance. However, substitution by an electronegative group (−I effect) seems more appropriate than a group like methyl (+I effect) since presence of such a group has been found to increase the multiplication of the cell as is evident from the biological activity data recorded in Table 6. The compounds **4(C)** and **4(D)** having R = Phenyl and R′ = Methoxy, and R = Hydrogen and R′ = Chlorine, respectively, were found to increase the multiplication of cells as compared to the control (normal). Both these compounds had cell no. $\times 10^{14}$, 12.34 ± 1.05 and 11.85 ± 1.05, respectively.

Table 6. Cytotoxic activity data (breast cancer cell line (MCF-7)) of 2-phenyl-4-[3′-(2′-aryl-4′(3′*H*) quinazolyl) amino] quinazolones

Compound	R	R′	Cell no. $\times 10^{14}$	Activity
4A	Phenyl	Hydrogen	9.17 ± 0.90	Positive
4B	Phenyl	Chloro	9.27 ± 0.87	Positive
4C	Phenyl	Methoxy	12.34 ± 1.05	Negative
4D	Hydrogen	Chlorine	11.85 ± 1.05	Negative
Control			10.21 ± 1.01	Normal

*Synthesis of alkylideno/arylideno-bis urea (**A**).* A mixture of an aldehyde RCHO (0.1 mol) and urea (0.25 mol) was dissolved in methanol (50 ml). The reaction mixture was heated under reflux, after refluxing for 4 h. Subsequently, ethanol was distilled off and the residual thick oily material was cooled to 0 °C. It solidified in about 1 h. The solid product so obtained was washed with cold methanol to give crude condensed product (**A**), solvent from the filtrate was removed under reduced pressure, and the crude condensed product was purified by column chromatography over silica gel. Elution with $CHCl_3$ removed side products and further elution with $CHCl_3$/MeOH (9.5–0.5) gave pure condensed product.

*Synthesis of 4-aryl-6-carboxylato-1,2,3,4-tetrahydroquinazolones (**B**).* Alkylideno/Arylideno-bis urea (0.06 mol) and *p*-aminobenzoic acid (0.08 mol) were mixed together and heated at 145–150 °C for 4 h on oil bath. A clear liquid was obtained on heating, which on cooling to room temperature solidified, and it was added hydrochloric acid (50 ml) and stirred very well. On complete neutralization a solid separated out which was filtered off and washed with water.

*Synthesis of 4-aryl-8,9-diphenyl-1,4-dihydro-3H-7-oxa-1,3-diazanthracine-2,6-diones (**C**).* A mixture of 4-aryl-6-carboxylate-1,2,3,4-tetrahydroquinazolone (0.02 mol)

and Benzoin (0.03 mol) in polyphosphoric acid (10 ml) was heated at 100 °C on oil bath for 5 h. The crude compound was obtained by separating solvent at reduced pressure and purified by column chromatography.

*Synthesis of N-Aryl-8,9-diphenyl-2-oxo-pyrido-[g]-4-aryl-2-oxo-1,3-dihydro-quinazolones (**D**)*. A mixture of compound C (0.01 mol) and para substituted Aniline (0.02 mol) in anhydrous pyridine (30 ml) refluxed for 6 h. The solution was cooled to room temperature and acidified with diluted HCl (50 ml). A solid separated out which was filtered off and washed with water successively. It was dried at 100 °C and recrystallized from glacial acetic acid.

Compound **2(X)**: mp 249 °C, IR (KBr pellets, cm^{-1}) 3032, 1665, 3465, 815. ^1H NMR (200 MHz, $CDCl_3$) δ ppm; 4.12 (d, 2H, CH_2), 6.13 (s, 1H, –NH), 6.01 (t, 1H, –NH), 7.1–7.4 (m, 8H, ArH). Anal. Calcd for $C_9H_8N_2O_3$ C 56.25, H 4.17, N 14.58, O 25.01. Found: *m/z* [M+1] 193.

Compound **2(Y)**: mp 284 °C, IR (KBr pellets, cm^{-1}) 3045, 1672, 865, 840. ^1H NMR (200 MHz, $CDCl_3$) δ ppm; 4.07 (d, 2H, CH_2), 6.31 (s, 1H, –NH), 7.1–7.6 (m, 8H, ArH). Anal. Calcd for $C_{15}H_{12}N_2O_3$ C 60.23, H 4.72, N 10.45, O 23.79. Found: *m/z* [M+1] 269.

Compound **3(U)**: mp 136 °C, IR (KBr pellets, 1742, 3036, 1125, 1670). ^1H NMR (200 MHz, $CDCl_3$) δ ppm; 6.0 (d, 1H, –C*H*), 6.11 (s, 1H, –N*H*), 6.29 (d, 1H, –NH), 7.09–7.90 (m, 17H, ArH). Anal. Calcd for $C_{23}H_{16}N_2O_3$; C 72.21, H 4.51, N 7.31, O 14.32. Found: *m/z* [M+1] 339.

Compound **3(V)**: mp 145 °C, IR (KBr pellets, 1740, 3032, 1125, 1665, 3465). ^1H NMR (200 MHz, $CDCl_3$) δ ppm; 4.40 (d, 2H, –CH_2), 6.14 (s, 1H, –NH), 6.3 (d, 1H, N*H*), 7.10–7.9 (m, 11H, ArH). Anal. Calcd for $C_{29}H_{20}N_2O_3$; C 77.32, H 4.11, N 5.99, O 11.21. Found: *m/z* [M+1] 415.

Compound **4(A)**: mp 128 °C, IR (KBr pellets, cm^{-1}) 3470, 1565, 1695, 1640, 3020. ^1H NMR (200 MHz, $CDCl_3$) δ ppm; 6.1–7.2 (m, 22H, –ArH), 8.36 (s, 1H, –NH). Anal. Calcd for $C_{35}H_{25}N_3O_2$ C 80.92, H 4.80, N 8.09, O 6.17. Found: *m/z* [M+1] 520.

Compound **4(B)**: mp 140 °C, IR (KBr pellets, cm^{-1}) 1568, 1640, 3022. ^1H NMR (200 MHz, $CDCl_3$) δ ppm; 7.0–8.4 (m, 21H, –ArH), 4.02 (s, 1H, –NH). Anal. Calcd for $C_{35}H_{24}N_3O_2Cl$ C 75.88, 1n, H 4.34, N 75.68, O 5.78, Cl 6.41. Found: *m/z* [M+1] 354.5.

Compound **4(C)**: mp 170 °C, IR (KBr pellets, cm^{-1}) 1360, 3470, 3020. ^1H NMR (200 MHz, $CDCl_3$) δ ppm; 6.9–7.9 (m, 21H, –ArH), 3.70 (s, 3H, OCH_3). Anal. Calcd for $C_{36}H_{27}N_3O_3C$ 78.69, H 4.12, N 8.79, O 8.74. Found: *m/z* [M+1] 550.

Compound **4(D)**: mp 194 °C, IR (KBr pellets, cm^{-1}) 2930, 1660, 3465, 1635. ^1H NMR (200 MHz, $CDCl_3$) δ ppm; 7.1–7.8 (m, 16H, –ArH), 4.38 (d, 2H, –CH_2). Anal. Calcd for $C_{29}H_{20}N_3O_2Cl$ C 72.90, H 4.19, N 8.39, O 6.70, Cl 7.43. Found: *m/z* [M+1] 478.5.

Compound **4(E)**: mp 220 °C, IR (KBr pellets, cm^{-1}) 1367, 3474, 1680, 1658. ^1H NMR (200 MHz, $CDCl_3$) δ ppm; 7.1–7.7 (m, 17H, –ArH), 6.03 (s, 1H, –NH). Anal. Calcd for $C_{29}H_{21}N_3O_2$ C 78.60, H 4.24, N 9.48, O 7.22. Found: *m/z* [M+1] 444.

Various tricyclic pyrido-quinazolone derivatives have been synthesized and evaluated for anti-fungal, antibacterial and anticancer analgesic activities. Similarly, radiolabeling and biodistribution studies have confirmed the respective receptor binding. These compounds have shown promising results for future application.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.06.015.

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