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Synthesis and cytotoxic activity of novel quinazolino-β-carboline-5-one derivatives ☆

Bipul Baruah,[a] Kavitha Dasu,[a] Balasubramanian Vaitilingam,[a] Premkumar Mamnoor,[b] Prasanthi Penubaka Venkata,[b] Sriram Rajagopal[b] and Koteswar Rao Yeleswarapu[a,*]

[a]*Discovery Chemistry, Discovery Research, Dr. Reddy's Laboratories Limited, Bollaram Road, Miyapur, Hyderabad 500050, India*
[b]*Molecular Discovery, Discovery Research, Dr. Reddy's Laboratories Limited, Bollaram Road, Miyapur, Hyderabad 500050, India*

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Abstract—A novel series of quinazolino-β-carbolinone derivatives was synthesized and evaluated for their in vitro and in vivo anticancer activity. Many compounds have shown good in vitro activity in the range 1–8 μM concentration. Three of the compounds were further tested in nude mice bearing HT-29 colon cancer xenografts.
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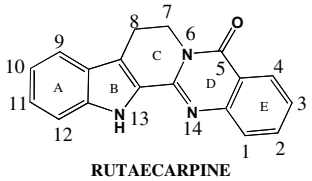
1. Introduction

Chinese herbs have been widely used as important remedies in oriental medicine. In recent past, many biologically active constituents were isolated and their pharmacological actions investigated. Evodia rutaecarpa (Chinese name: Wu-Chu-Yu) is a well-known traditional Chinese medicine and has been used for a long time in Chinese medical practice. It is used as a remedy for gastrointestinal disorders (abdominal pain, dysentery), headache, amenorrhea, and postpartum hemorrhage.[1] Rutaecarpine, a quinazolino carboline alkaloid,[2] is the major component isolated from the fruit of this plant and has been shown to possess interesting medicinal properties.



RUTAECARPINE

However, little attention was directed towards its possible antitumor activity.[3] In continuation of our studies to discover small organic molecules for different therapeutic indications,[4] we were attracted by the structural features offered by rutaecarpine. In the present study, intended to discover novel antitumor agents, we have synthesized a series of 3,10-substituted and E ring modified quinazolino-β-carbolin-5-ones, which were evaluated for their inhibitory activity and cytotoxic effects on several human tumor cell lines.

2. Chemistry

Synthetic methods for the preparation of rutaecarpine derivatives are summarized in Scheme 1. The main intermediate, substituted 1,2,3,4-tetrahydro-β-carbolinone was prepared from substituted aniline following the procedure described earlier by Abramovitch and Shapiro.[5] The β-carbolinone was then treated with substituted anthranilic acid derivatives in presence of $POCl_3$ in toluene under reflux to provide the products in almost 80–90% yield.[6] Some of quinazolino carbolinones were prepared from isatoic anhydride by treating them with tryptamine derivatives, as illustrated in Scheme 2. The isatoic anhydride **I** was dissolved in pyridine containing trifluoro acetic anhydride to give 2-(trifluoromethyl)-4-oxazinone **II**. The quinazolino carbolinones were then prepared according to the Bergman[7,8] procedure. Reaction of tryptamine derivatives **III** with oxazinone **II** gave **IV**, which were then refluxed under acidic conditions (AcOH–HCl) for

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* Corresponding author. Tel.: +91-4023045439; fax: +91-4023045438; e-mail addresses: y_koteswarrao@yahoo.com; koteswarraoy@drreddys.com

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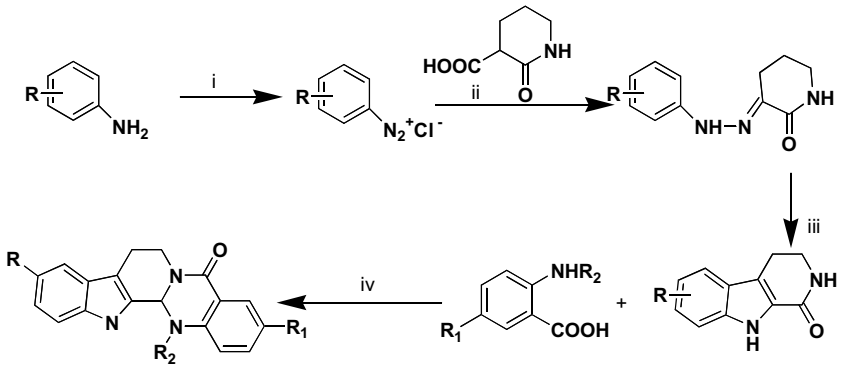
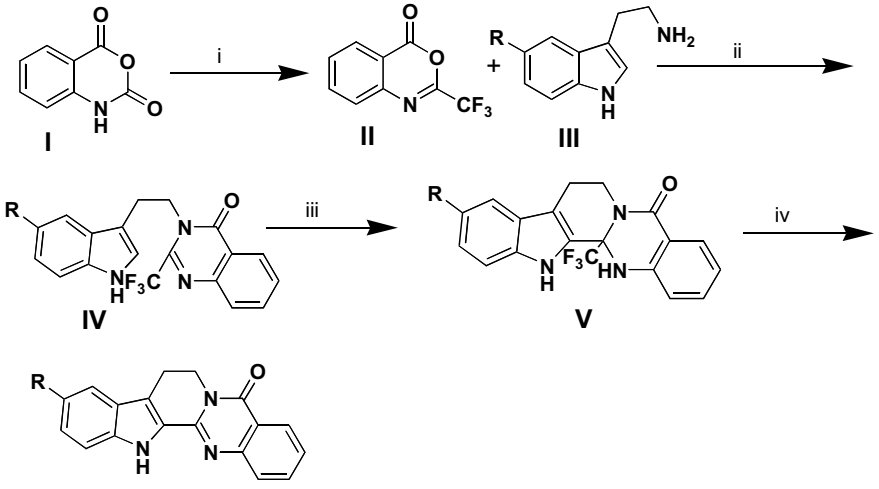


1. R, R_1, R_2 = H; 2. R = SCH_3, R_1, R_2 = H
3. R = $SOCH_3$, R_1, R_2 = H; 4. R = SO_2CH_3, R_1, R_2 = H.
5. R = Br, R_1, R_2 = H; 6. R = Cl, R_1, R_2 = H.
7. R = Cl, R_1 = H, R_2 = CH_3; 8. R = Br, R_1 = H, R_2 = CH_3.
9. R = H, R_1 = H, R_2 = CH_3; 10. R = F, R_1 = H, R_2 = CH_3.
11. R = R_2 = H, R_1 = NO_2; 12. R = OCH_3, R_1, R_2 = H.
13. R = OCF_3, R_1, R_2 = H; 14. R = CF_3, R_1, R_2 = H.
15. R = SO_2NH_2, R_1, R_2 = H.

Scheme 1. Reagents: (i) $NaNO_2$/HCl, 0 °C; (ii) AcOH, 0 °C, 8–10 h; (iii) 90% formic acid, 100 °C, 1 h; (iv) $POCl_3$/toluene, reflux, 1 h.



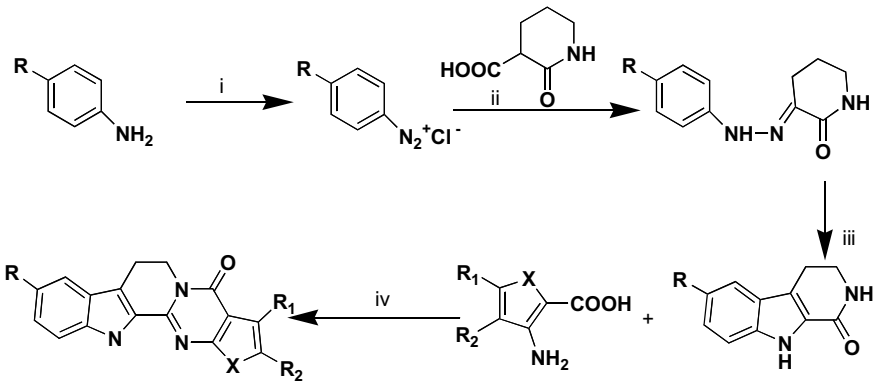
Scheme 2. Reagents: (i) $(CF_3CO)_2O$, pyridine, 25 °C, 15 min; (ii) 115 °C, 3 h; (iii) HCl, AcOH, reflux, 1 h; (iv) KOH, H_2O, EtOH.

30 min to give **V**. Elimination of CF_3H was accomplished by treating **V** with alcoholic KOH to form the quinazolino-β-carbolinone derivatives. Scheme 3 illustrates synthesis of compounds having different heterocyclic rings as E ring in the overall structure.

3. Discussion

The quinazolino-β-carbolin-5-ones prepared in this study were tested for in vitro anticancer activity on eight cancer cell lines and the active compounds were further screened for their in vivo activity. To establish the SAR of this series of compounds, each variable of general structures in Schemes 1–3 was systematically modified and the GI_{50} values obtained are tabulated in Table 1.

The parent compound **1**, without any substituent has been reported to possess no significant cytotoxic activity against different cell lines.[3] However, in the present in vitro eight panel cancer cell lines,[9] the compound exhibited some cytotoxic activity. We initially started our SAR studies on quinazolino-β-carbolinone bearing different substituents on A ring at position C-10. Compounds **3**, **4**, and **12**–**15** did not have significant anticancer activity in human cancer cell lines. However, compounds **2**, **5**, and **6** with SCH_3, Br, and Cl substituent at C-10 position showed better activity than the parent compound. To optimize activity of these compounds, different N-1 methyl substituted quinazolino-β-carbolinones were synthesized (**7**–**10**) and tested for anticancer activity. Compound **7** with Cl at C-10 was found to be potent.



16. X = S, R, R_1, R_2 = H.
17. X = S, R = SCH_3, R_1=CH_3, R_2 = COOEt.
18. X = S, R = Cl, R_1 = H, R_2 = H.
19. X = S, R = F, R_1 = H, R_2 = H.
20. X = N, R = Br, R_1 = CH_3, R_2 = H.
21. X = O, R = Cl, R_1 = t-Butyl, R_2 = H.

Scheme 3. Reagents: (i) $NaNO_2$/HCl, 0 °C; (ii) AcOH, 0 °C, 8–10 h; (iii) 90% formic acid, 100 °C, 1 h; (iv) $POCl_3$/toluene, reflux, 1 h.

Table 1. In vitro cytotoxic activities of quinazolino-β-carbolin-5-ones (GI_{50} values in μM)

Compound	Breast MCF7/ADR	CNS U251	Colon SW620	Lung H522	Melanoma UACC62	Ovarian SKOV3	Prostate DU145	Renal ACHN
1	21	0.02	25	37	>100	3	0.2	20
2	23	3	26	35	17	13	15	0.08
3	>100	7	>100	91	>100	27	0.1	2
4	>100	>100	>100	>100	>100	>100	32	1
5	62	5	33	59	>100	3	3	>100
6	40	2.5	12	9	>100	2	2	2
7	1	6	11	10	7	11	2	1
8	15	0.3	14	18	>100	3	3	0.4
9	4	5	1	1	6	2	15	11
10	35	0.9	11	2	>100	80	32	27
11	2	3	2	3	2	12	3	3
12	>100	51	>100	>100	>100	>100	17	1
13	>100	>100	6	18	>100	>100	57	>100
14	>100	>100	24	28	>100	>100	>100	>100
15	19	>100	>100	94	>100	>100	—	16
16	>100	>100	>100	>100	7	10	>100	>100
17	>100	>100	>100	>100	7	20	2	13
18	>100	53	>100	74	2	12	12	2
19	>100	>100	>100	>100	7	17	1	46
20	54	26	44	33	9	14	34	20
21	40	11	18	—	14	8	15	2.4

At this juncture, to see the effect of substituent on the E ring, we synthesized few compounds with a heterocycle fused to E ring (**16**–**21**). These compounds were found to be inactive. However, introducing a NO_2 group in the E ring imparted good anticancer activity, with an average GI_{50} value 2 (compound **11**). It was subsequently observed that this compound was also highly stable in both human and mouse plasma.

Our efforts to improve the cytotoxic activity of compound **1** resulted in compounds **6**, **7**, and **11**, which showed promising in vitro cytotoxic activity with GI_{50} values ranging from 1 to 2 μM. These compounds were tested for their antitumor activity against human xenografts in nude mice. In this antitumoral assay, HT-29 human tumors were initiated by implantation of tumor fragments (~60 mm^3) from established tumors in athymic nude mice. Tumor fragments were implanted by s.c. in the right flank. The s.c. tumors were measured with calipers, and mice were weighed every alternate day. When tumors reached a volume of ~100 mm^3, mice were randomized to control and treated groups. Test compounds were administered orally at specified doses, once daily for 20 days. Tumor size was measured every alternate day and the volume was calculated using the equation $V = (D \times d^2)/2$ where V (mg) is tumor volume, D is longest diameter in mm and d is shortest diameter in mm. The results of the xenograft studies are tabulated in Table 2.

The antitumor activity is expressed as the optimum $T/C\%$ (median tumor volume of test/control $\times 100$).

Table 2. Result of HT-29 xenograft studies

Compound	MTD, PO in athemic nude mice	Schedule	Dose (mg/kg/day)	Total dose (mg/kg)	Max. mean% weight loss (day)	% T/C (day)
1	>1000	QD×20	300	6000	6 (20)	132 (20)[10]
6	>1000	QD×20	100	2000	10 (20)	94 (20)[11]
7	100	QD×20	25	500	3 (20)	94 (20)[12]
11	600	QD×20	100	2000	12 (20)	75 (20)[13]

Compound showing an optimum T/C% value of <43% was considered as active in this xenograft model. Though compounds **6**, **7**, and **11** showed very good in vitro activity, the activity did not translate in the xenograft model, probably because of their poor bioavailability.

In conclusion, in this communication, we have described the synthesis, in vitro and in vivo anticancer activity of a new series of quinazolino-β-carbolinone derivatives. Many compounds have shown good in vitro activity, in the range 1–8 μM concentration. Further work to impart the desired in vivo activity among these novel compounds is presently under progress.

Acknowledgements

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References and notes

1. Li, S. C. In *Pentssu Kang Mu*, 1976; Chapter 32, p 1064; Lia, J. F.; Chen, C. F.; Chow, S. Y. *J. Formosan Med. Assoc.* **1981**, *79*, 0–38; Chow, S. Y.; Chen, S. M.; Yang, C. M. *J. Formosan Med. Assoc.* **1976**, *75*, 349–357; Anon. In *Encyclopedia of Chinese Medicine*; People's: Shangai, People's Republic of China, 1977; p 1118.
2. Asahina, Y.; Ishimasa, S. *Synthese des Rutaecarpins. Yakugaku Zasshi* **1926**, *534*, 61–63; Bergman, J. In *The Alkaloids*; Brossi, A. R., Ed.; Academic: New York, 1983; Vol. 21, pp 29–54.
3. Yang, L. M.; Lin, S. J.; Lin, L. C.; Kuo, Y. H. *The Chin. Pharm. J.* 199, *51*, 219–225; Yang, L. M.; Chen, C. F.; Lee, K. H. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 465–468.
4. Pal, M.; Madan, M.; Padakanti, S.; Pattabiraman, V. R.; Kalleda, S.; Vanguri, A.; Mullangi, R.; Casturi, S. R.; Yeleswarapu, K. R. *J. Med. Chem.* **2003**, *46*, 3975; Pal, M.; Veeramaneni, V. R.; Nagabelli, M.; Misra, P.; Casturi, S. R.; Yeleswarapu, K. R. *Biorg. Med. Chem. Letts.* **2003**, *13*, 1639; Barua, B.; Dasu, K.; Vaitalingam, B.; Misra, P.; Casturi, S. R.; Yeleswarapu, K. R. *Biorg. Med. Chem. Lett.* **2004**, *14*, 445; Pal, M.; Veeramaneni, V. R.; Padakanti, S.; Nagabelli, M.; Vanguri, A.; Mamnoor, P.; Misra, P.; Mullangi, R.; Casturi, S. R.; Yeleswarapu, K. R. *Ind. J. Chem.* **2003**, *42B*, 593.
5. Abramovitch, R. A.; Shapiro, D. *J. Chem. Soc.* **1954**, 4263; Abramovitch, R. A.; Shapiro, D. *J. Chem. Soc.* **1956**, 4589; Phillips, R. R.. In: *Organic Reactions*; John Wiley & Sons: New York, 1959; Vol. X, Chapter 2, p 143.
6. Rahman, A. V.; Ghazala, M. *Synthesis* **1980**, 372–374.
7. Bergman, J.; Bergman, S. *Heterocycles* **1986**, *16*, 347.
8. Bergman, J.; Bergman, S. *J. Org. Chem.* **1985**, *50*, 1246.
9. In vitro cell growth assay: Cells were seeded on a 96-well cell culture plates at a concentration of 10,000 cells/well in a volume of 100 μL of RPMI medium with 10% fetal bovine serum and were incubated at 37 °C in a CO_2 incubator. After 24 h cells were treated with varied concentrations of compounds in 100 μL of medium and the control wells received vehicle. The plates were further incubated at 37 °C in a CO_2 incubator for 48 h. The assay was terminated by addition of 50% cold TCA to a final concentration of 10% to the cells, followed by incubation at 4 °C for 1 h. At the time of compound addition, a separate set of cells having a 24 h growth were terminated for time zero growth (T_0). The TCA fixed plates were washed thrice with distilled water, air dried, and stained with 0.4% SRB in 1% acetic acid for 30 min at room temperature. The plates were washed with 1% acetic acid, dried, and the protein bound dye was dissolved in 100 μL of 10 mM Tris buffer and read at 515 nm. The percentage growth of treated cells is calculated using the following formulae: if T is greater than or equal to T_0, percentage growth = $100[(T - T_0)/(C - T_0)]$ and if T is less than T_0, percentage growth = $100[(T - T_0)/T_0]$, where C, T and T_0 are optical densities of control, test, and time zero, respectively. From the growth curves, the GI_{50} values were extrapolated.
10. Compound **1**: mp 160–161 °C; (lit.[7] 159 °C) IR: (KBr) 3325, 1655 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.23 (2H, t, $J = 7$ Hz), 4.60 (2H, t, $J = 7$ Hz), 7.30–7.80 (8H, m) and 8.30 (1H, s); CIMS m/z 288 (MH^+, 100%).
11. Compound **6**: mp 308–309 °C; IR: (KBr) 3335, 1653 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.23 (2H, t, $J = 6.9$ Hz), 4.60 (2H, t, $J = 7$ Hz), 7.20–7.80 (7H, m) and 8.30 (1H, s); CIMS m/z 322 (MH^+, 100%).
12. Compound **7**: mp 300–301 °C; IR: (KBr) 3332, 1652 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.99 (3H, s), 3.23 (2H, t, $J = 7$ Hz), 4.65 (2H, t, $J = 7$ Hz), 7.40–7.84 (7H, m) and 8.40 (1H, s); CIMS m/z 338 (MH^+, 100%).
13. Compound **11**: mp 294–295 °C; IR: (KBr) 3325, 1655 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.13 (2H, t, $J = 7$ Hz), 4.70 (2H, t, $J = 7$ Hz), 7.30–8.50 (8H, m) and 8.90 (1H, s); CIMS m/z 333 (MH^+, 100%).