Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 2303–2307

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

# Novel Indolo[2,1-*b*]quinazoline Analogues as Cytostatic Agents: Synthesis, Biological Evaluation and Structure–Activity Relationship

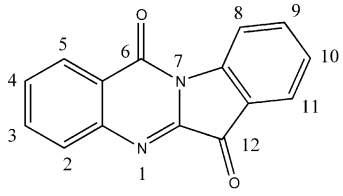
Vedula M. Sharma,[a,*] P. Prasanna,[a] K. V. Adi Seshu,[a] B. Renuka,[a] C. V. Laxman Rao,[a] G. Sunil Kumar,[a] C. Prasad Narasimhulu,[a] P. Aravind Babu,[a] R. C. Puranik,[a] D. Subramanyam,[a] A. Venkateswarlu,[a] Sriram Rajagopal,[b] K. B. Sunil Kumar,[b] C. Seshagiri Rao,[b] N. V. S. Rao Mamidi,[b] Dhanvanthri S. Deevi,[b] R. Ajaykumar[b] and R. Rajagopalan[b]

[a]*Discovery Chemistry (Natural Products), Dr. Reddy's Research Foundation, Miyapur, Hyderabad - 500 050, India*
[b]*Discovery Biology, Dr. Reddy's Research Foundation, Miyapur, Hyderabad - 500 050, India*

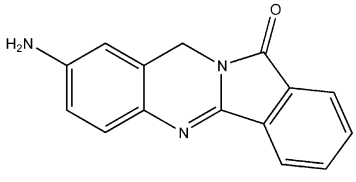
Received 22 March 2002; accepted 6 June 2002

**Abstract**—In our endeavor to design and synthesize novel anticancer agents, a new series of indoloquinazoline compounds were prepared and tested initially for anticancer activity in vitro against a panel of human cancer cell lines. Most of these compounds exhibited cytotoxic activity in in vitro screens. Compounds were selected and further evaluated using a modified Hollow Fiber Assay for their preliminary in vivo activity against 12 cell lines implanted in the subcutaneous and intraperitoneal compartments in mice. The results indicate that these compounds may constitute a new class of anticancer agents. 

In our search to identify novel anticancer compounds from Indian medicinal plants, we screened several crude plant extracts for their in vitro anticancer activity using human cancer cell lines. Many of the extracts showed cytotoxic activity in micromolar concentrations. Among the crude extracts screened, based on preliminary in vitro cytotoxic activity, the leafy extract of *Wrightia tinctoria* was further fractionated and purified and this resulted in isolation of a known alkaloid tryptanthrine (**1**), which was reported earlier in the literature from various plant sources including *W. tinctoria*.[1–5] Compound **1** is reported to possess antibacterial,[1] antifungal and weak cytotoxic activity against B-16 melanoma cells.[6] Although compound **1** is not a potent cytotoxic compound, it has a structural similarity with a known potent cytotoxic agent batracyclin (**1a**) (Fig. 1),[7] and this feature makes it an attractive lead compound for synthetic and biological studies. Since the yields of




Tryptanthrine (**1**)




Batracyclin (**1a**)

**Figure 1.** Structures of tryptanthrine (**1**) and batracyclin (**1a**).

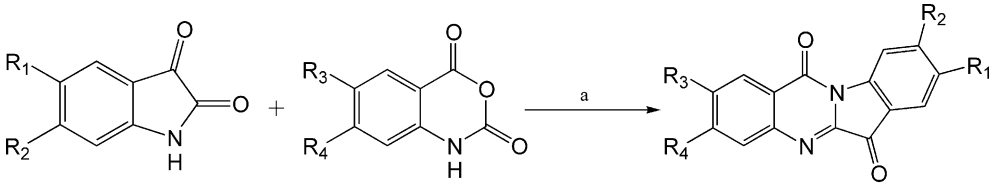
*Corresponding author. Tel.: +91-40-305439; fax: +91-40-3045438; e-mail: sarmavm@drreddys.com

0960-894X/02/$ - see front matter 
PII: S0960-894X(02)00431-6

compound **1** from the plant are poor, we synthesized a parent compound tryptanthrine and a series of its new analogues. These compounds were evaluated initially for cytotoxic activity in vitro against human cancer cell lines. Compounds with good $GI_{50}$ values were further tested in a rapid preliminary in vivo model using modified Hollow Fibre Assay (HFA) in Swiss Albino Mice (SAM), followed by xenograft of HT-29 colon adenocarcinoma in nude mice.

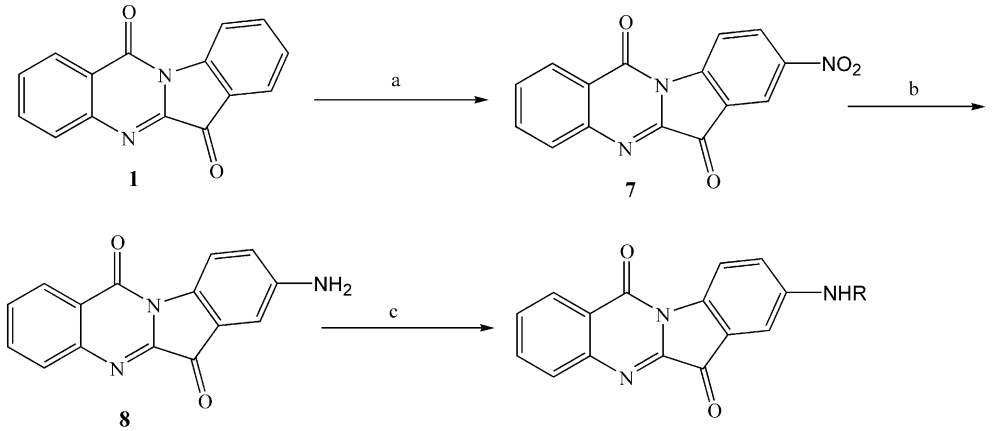
As outlined in Scheme 1, 6,12-dihydro-6,12-dioxoindolo[2,1-*b*]quinazoline compounds (**1**–**12**) were prepared by condensation of substituted isatoicanhydrides[8] with substituted isatins[9–11] in toluene by refluxing in the presence of triethylamine. The commercially available isatin and isatoicanhydride were used to prepare unsubstituted indoloquinazolines and other analogues, and known methods in the literature were followed. Additional derivatives (**13**–**18**) containing sulfonyl and alkanoyl substituents of compound **8** were also made as shown in Scheme 2 by treating with alkanoylhalides or alkylsulfonylhalides. To investigate the role of the 12-keto functionality in the biological activity of these quinazolines, compounds **19**–**22** were synthesized by exploiting 12-keto functionality through Grignard reaction. Isatin was reacted with alkylmagnesiumbromides in THF at 0 °C to obtain 3-hydroxy-3-alkyl-2-indanones. These intermediates were condensed further with isatoicanhydrides as depicted in Scheme 3.

The final synthetic sequence employed in this study is shown in Scheme 4 for the preparation of compounds **23**–**36**. Compounds **1**, **6** and **7** were reacted with hydroxylaminehydrochloride to provide the corresponding




**1** $R_1$, $R_2$, $R_3$, $R_4$ = H
**2** $R_3$ = Br; $R_1$, $R_2$, $R_4$ = H
**3** $R_3$ = $NO_2$; $R_1$, $R_2$, $R_4$ = H
**4** $R_3$, $R_4$ = OMe; $R_1$, $R_2$ = H
**5** $R_3$ = $NH_2$; $R_1$, $R_2$, $R_4$ = H
**6** $R_1$ = Br; $R_2$, $R_3$, $R_4$ = H
**9** $R_1$, $R_2$ = -$OCH_2O$-; $R_3$, $R_4$ = H
**10** $R_1$= OMe; $R_2$, $R_3$, $R_4$ = H
**11** $R_1$ = Me; $R_2$, $R_3$, $R_4$ = H
**12** $R_1$ = Cl; $R_2$, $R_3$, $R_4$ = H

**Scheme 1.** (a) $Et_3N$ (5 equiv), toluene, reflux, 2–4 h, 70–85%.

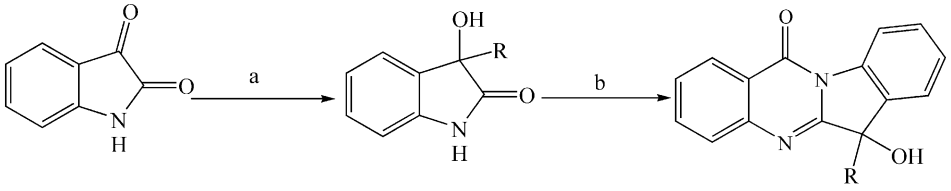



**13** R = $C(O)CH_3$
**14** R = $C(O)CH_2CH_2CH_3$
**15** R = $C(O)CH_2CH_3$
**16** R = $C(O)CH_2Cl$
**17** R = C(O)-$CH_2$—N N-$CH_3$
**18** R = $SO_2CH_3$

**Scheme 2.** (a) Concd $HNO_3/H_2SO_4$, 0 °C, 0.5 h, 95%; (b) $SnCl_2{\cdot}2H_2O$ (2 equiv), ethanol/concd HCl, 80 °C, 2 h, 38%; (c) RCOCl or $RSO_2Cl$, $Et_3N$, DCM, 0 °C, 0.5–1 h, 65–70%.

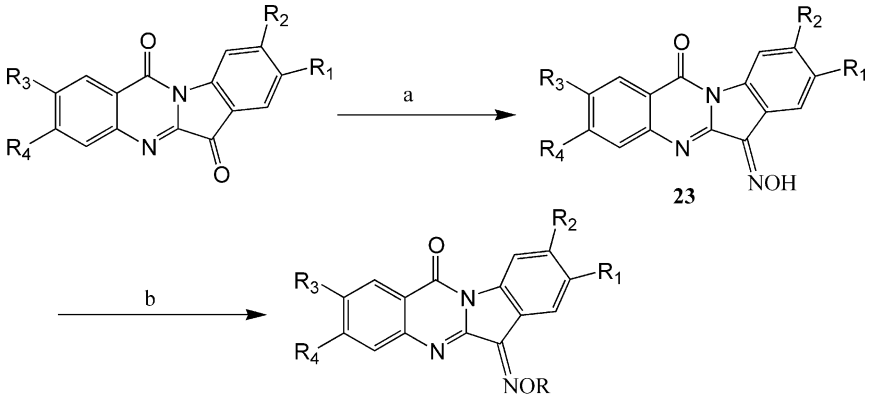
oximes. These *E* and *Z* isomeric oximes on treatment with acid halides and alkyl halides yielded corresponding oxime ester (**27**) and ethers (**24–26**, **28–36**) respectively. The mixtures of *E* and *Z* isomers were evaluated for their biological activity without separation.

The indoloquinazolines prepared in this study were tested in vitro for anticancer activity on eight cancer cell lines, and the active compounds were further screened rapidly for the in vivo activity using the modified HFA.[12] To establish the SAR of this series of compounds, each variable of general structures in Schemes 1, 2 and 4 was systematically modified, and the $GI_{50}$ values obtained were tabulated in Table 1. Average $GI_{50}$ values of eight cell lines were used in the following discussion. The parent compound **1** without any

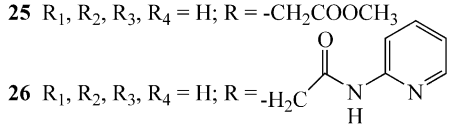


**19** R = -$CH_3$
**20** R = -CH=$CH_2$
**21** R = -$CH_2CH_2CH_2CH_3$
**22** R = -$OCH_2CH_2O$-Ph

**Scheme 3.** (a) RMgBr (2 equiv), THF, 0 °C–rt, 1–4 h, 45–50%; (b) isatoicanhydride (2 equiv), $Et_3N$ (5 equiv), toluene, reflux, 5–10 h, 25–30%.

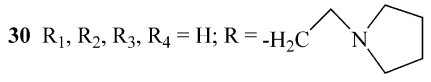


**23** $R_1$, $R_2$, $R_3$, $R_4$ = H
**24** $R_1$, $R_2$, $R_3$, $R_4$ = H; R = -$CH_2CH_2OH$
**25** $R_1$, $R_2$, $R_3$, $R_4$ = H; R = -$CH_2COOCH_3$

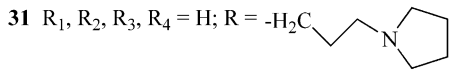


**26** $R_1$, $R_2$, $R_3$, $R_4$ = H; R =

**27** $R_1$, $R_2$, $R_3$, $R_4$ = H; R = -C(O)CH=CH-Ph
**28** $R_1$, $R_2$, $R_3$, $R_4$ = H; R = -$CH_2CH_2CH_2$-$NMe_2$
**29** $R_1$, $R_2$, $R_3$, $R_4$ = H; R = -$CH_2CH_2$-$NMe_2$

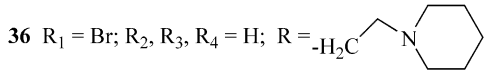


**30** $R_1$, $R_2$, $R_3$, $R_4$ = H; R =



**31** $R_1$, $R_2$, $R_3$, $R_4$ = H; R =

**32** $R_1$ = $NO_2$; $R_2$, $R_3$, $R_4$ = H; R = -$CH_2CH_2$-$NMe_2$
**33** $R_1$ = $NO_2$; $R_2$, $R_3$, $R_4$ = H; R = -$CH_2CH_2CH_2$-$NMe_2$
**34** $R_1$ = $NO_2$; $R_2$, $R_3$, $R_4$ = H; R = -$CH_2CH_2OH$
**35** $R_1$ = Br; $R_2$, $R_3$, $R_4$ = H; R = -$CH_2CH_2$-$NMe_2$



**36** $R_1$ = Br; $R_2$, $R_3$, $R_4$ = H; R =

**Scheme 4.** (a) $(H_2NOH)_2H_2SO_4$ (2 equiv), pyridine, MeOH, reflux, 4 h, 65%; (b) RX, $K_2CO_3$, DMF, 90 °C, 12–20 h, 40–50%.

**Table 1.** In vitro cytotoxic activities of indolo [2,1-*b*] quinazolines

| Compd | $GI_{50}$ values in μM | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Breast MCF7/ADR | CNS U251 | Colon SW620 | Lung H522 | Melanoma M14 | Ovarian SKOV3 | Prostate DU145 | Renal A498 |
| **1** | 30.0 | 100 | 5.0 | 15.0 | 15.0 | 2.5 | 0.4 | 0.95 |
| **2–4** | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **5** | 40.0 | 70.0 | 60.0 | 60.0 | 60.0 | 40.0 | 60.0 | 80.0 |
| **6** | 0.7 | >100 | 4.0 | 0.7 | 0.8 | 5.0 | 0.15 | 3.0 |
| **7** | 1.0 | 4.5 | 25.0 | 4.8 | 15.0 | 25.0 | 8.0 | 2.0 |
| **8** | 5.0 | 0.7 | 4.8 | 5.0 | 5.0 | 5.0 | 2.0 | 3.0 |
| **9–10** | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **11** | 0.1 | 20.0 | >100 | 0.15 | 60.0 | >100 | >100 | 60.0 |
| **12** | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **13** | 0.4 | 20.0 | 30.0 | 5.0 | 10.0 | 20.0 | 0.5 | 0.4 |
| **14** | 10.0 | 12.0 | 10.5 | 18.2 | 18.5 | 6.0 | 20.0 | 20.0 |
| **15** | 20.0 | >100 | 22.0 | 18.0 | 10.0 | 8.0 | 10.0 | 7.0 |
| **16** | 0.06 | 0.2 | 0.6 | 4.0 | 0.9 | 0.05 | 0.25 | 0.4 |
| **17** | 20.0 | >100 | 10.0 | 15.0 | 8.0 | 6.0 | 10.0 | 20.0 |
| **18–19** | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **20** | 20.0 | 12.0 | 10.0 | 15.0 | 8.0 | 6.0 | 10.0 | 12.0 |
| **21–22** | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **23** | 15.0 | 15.0 | 8.0 | 50.0 | 40.0 | 30.0 | 50.0 | 60.0 |
| **24** | 0.01 | 1.0 | 20.0 | 7.0 | 30.0 | 20.0 | 3.0 | 40.0 |
| **25** | 0.01 | >100 | >100 | 80.0 | 40.0 | 50.0 | 30.0 | >100 |
| **26** | 8.0 | 8.5 | >100 | 25.0 | 95.0 | 52.0 | 10.0 | 20.0 |
| **27** | 7.5 | 20.0 | 60.0 | 45.0 | 90.0 | 6.0 | >100 | 10.0 |
| **28** | 7.5 | 0.7 | 4.0 | 0.6 | 5.0 | 2.0 | 3.5 | 4.0 |
| **29** | 0.4 | 0.8 | 0.75 | 0.7 | 2.0 | 2.0 | 2.5 | 0.6 |
| **30** | 6.0 | 25.0 | 0.85 | 3.0 | 2.0 | 3.0 | 3.0 | 5.0 |
| **31** | 5.0 | 6.0 | 4.5 | 4.0 | 6.0 | 4.0 | 5.0 | 7.0 |
| **32** | 4.0 | 7.5 | 0.75 | 9.0 | 7.5 | 9.0 | >100 | >100 |
| **33** | 4.0 | 2.5 | 0.5 | 0.4 | 0.4 | 0.6 | 2.0 | 0.09 |
| **34** | 0.4 | 0.8 | 0.75 | 0.7 | 2.0 | 2.0 | 2.5 | 0.6 |
| **35** | 0.06 | 0.45 | 3.0 | 2.0 | 2.0 | 3.0 | 0.2 | 1.5 |
| **36** | 0.3 | 0.095 | 3.0 | 2.0 | 2.5 | 1.0 | 0.55 | 2.0 |

substituents exhibited cytotoxic activity against B-16 melanoma cell lines as reported in the literature,[6] however, in the present in vitro eight panel cancer cell lines, the compound exhibited cytotoxic activity at 20 μM. We started our SAR studies from indoloquinazolines bearing different substituents on A ring. Compounds **2–5** did not have significant anticancer activity in human cancer cells; however, compounds **6** and **8** with bromo and amino substituents on the C-10 position showed better activity. Particularly, compound **6** exhibited cytotoxic activity at less than 1 μM concentration in four out of eight cell lines. Compounds **7**, **10**, **11** and **12** with nitro, methoxy, methyl and chloro substituents on the same position exhibited weak activity. To optimize activity of **8**, different amides **13–17** and sulfonamide **18** were synthesized and tested for anticancer activity. Compound **16** with terminal halo functionality was more potent with an average $GI_{50}$ of 1 μM. However, when this compound was tested for its in vitro stability in plasma of both human and mouse, it was observed to be less stable, contrary to the parent compound **8** which was more stable.

At this juncture, additional analogues of **1** were prepared to know the importance of the 12-keto functionality in the biological activities of indoloquinazolines. Several analogues lacking C-12 carbonyl functional group were synthesized. The alterations that were tested included (i) 12-hydroxy-12-alkyl/aryl disubstituted derivatives **19–22** (Scheme 3). These compounds were found to be inactive. (ii) 12-Ketoxime ethers/12-ketoxime ester derivatives (**24–36**) (Scheme 4). It was observed that 12-ketoxime ethers (**24–26**, **28–36**) are more active than 12-ketoxime ester (**27**) with an exception of compounds **25** and **26**. Among the ether derivatives, compound **24** containing a terminal hydroxy group in alkyl chain is less active than side chains with terminal *N*,*N*-dialkylamino groups **28–31**. Compound **28** having a terminal alkylamino side chain with three carbon atoms is slightly less active when compared to two carbon atoms **29**, likewise compounds **30**, **31** with cyclicamino side chains are also less active than *n*-alkylamino side chain **28**, **29**.[14] All these findings are similar even with 10-bromo (**35**, **36**) and 10-nitro (**32**, **33**,[15] **34**) derivatives.

Our efforts to improve the cytotoxic activity of compound **1** resulted in compounds **28–36** which showed promising in vitro cytotoxic activity with $GI_{50}$ values ranging from 1 to 5 μM. Compounds **29** and **33** with good in vitro activity were tested for their in vivo activity in HFA using two different doses (75 and 150 mg/kg) in QD×4 schedule.[12,13] The actively growing different types of cancer cells in hollow fibre capsules were implanted in SAM aspectically under light ether anaesthesia in to the subcutaneous (sc) and intraperitonial (ip) compartments. The anticancer activity in this model was assessed based on percentage net growth in both the

**Table 2.** Results of xenograft studies

| Compd | MTD in SAM (mg/kg) | Schedule | Dose (mg/kg/day) | Total dose (mg/kg) | Max. mean% weight loss (day) | Optimum T/C% (day) |
|---|---|---|---|---|---|---|
| **29** | 400 | QD×10[a] | 40 | 400 | 14 (24) | 75 (24) |
| **33** | 400 | QD×10[a] | 40 | 400 | 14 (26) | 23 (17) |

[a]QD×10, single dose for 10 days.

compartments. Each compound was tested against 12 human cancer cell lines. Compounds were selected for further in vivo testing in xenografts by using the HFA criteria, that is a %T/C of 50 or less in 10 of the 48 possible test concentrations (12 cell lines × 2 sites × 2 compound doses). To simplify evaluation, a points system has been adopted which allows rapid viewing of the activity of a given compound. For this a value of 2 is assigned for each compound dose which results in a 50% or greater reduction in a viable cell mass. The ip and sc samples were scored separately. Compounds with a total score $\geq 20$ in IP + SC were referred for xenograft testing. Compounds **29** and **33** scored 30/48, and 36/48, respectively. These compounds were further evaluated in HT-29 human colon adeno carcinoma xenografts in male nu/nu mice (18–24 g, 10–14 weeks old). Tumor-bearing athymic mice were given 40 mg/kg dose of the test compounds through the intraperitoneal route in QD×10 schedule. The tumor volume of each mouse was determined by measuring two dimensions with vernier calipers using the formula tumor volume = (length × width$^2$)/2. The antitumor activity was expressed as the optimum T/C% (median tumor volume of control × 100) (Table 2). Compounds showing an optimum T/C% value of $<43\%$ are considered as active. Compound **33** has shown a T/C% of 23 on day 17, this compound is to be tested in other xenograft models. Compound **33** has shown 76% bioavailability at 20 mg/kg dose whereas compound **29** has shown only 14%. Eventhough compound **29** has shown activity in HFA, it did not translate in xenograft studies may be because of its poor bioavailability.

In conclusion, in this communication we report the synthesis of a new series of indoloquinazoline derivatives and evaluation of their in vitro and in vivo anticancer activity. Many of the compounds have shown in vitro activity in the range of 1–5 μM concentration. Two of the compounds with in vitro cytotoxic activity were further evaluated in preliminary modified hollow fibre assay to assess the anticancer property. Based on the results of hollow fibre assay, compounds **29** and **33** were tested in nude mice bearing HT-29 colon cancer xenografts and compound **33** was found to be active. Further xenograft studies are planned to assess the anticancer activity with the sensitive cell lines from a hollow fibre assay.

## Acknowledgements

We thank the analytical department for providing the spectral data.

## References and Notes

1. George, V.; Koshy, A. S.; Singh, O. V.; Nayar, M. N. S.; Pushpangadan, P. *Fitoterapia* **1996**, *LXVII*, 553.
2. Bergman, J.; Egestad, B.; Lindstrom, J. O. *Tetrahedron Lett*. **1977**, *30*, 2625.
3. Honda, G.; Tabata, M. *Plantamedica* **1979**, *36*, 85.
4. Honda, G.; Tosirisuk, V.; Tabata, M. *Plantamedica* **1980**, *38*, 275.
5. Honda, G.; Tabata, M.; Tsuda, M. *Plantamedica* **1979**, *37*, 172.
6. Zou, J.; Huang, L. *Yaoxue Xuebao* **1985**, *20*, 45.
7. Johnson, R. K.; Hertzberg, R. P. *Ann. Rep. Med. Chem.* **1990**, *25*, 129.
8. Coppola, G. M. *Synthesis* **1980**, 505.
9. Sandmeyer, T. *Helv. Chim. Acta* **1919**, *2*, 234.
10. Stolle, R. *J. Prakt. Chem.* **1922**, *105*, 137.
11. Piyasena, H.; Meanwell, N. A. *Tetrahedron Lett*. **1994**, *35*, 7303.
12. Sriram, R.; Kumar, K. B. S.; Dhanvanthri, S. D.; Shravan, K. R.; Krishna, N. S.; Sony, P.; Kumar, R. A.; Rao, C. S.; Rajagopalan, R. *Proc. AACR* **2000**, *41*, 200.
13. Melinda, G. H.; Michael, C. A.; Richard, F. C.; Betty, J. A.; Joseph, G. M.; Lousis, M.; Michael, R. G. *Life Sciences* **1995**, *57*, 131.
14. Compound **29**: mp 152–153 °C; IR: 3421, 1689, 1642 cm$^{-1}$; $^1$H NMR ($CDCl_3$) δ 2.35 (6H, s), 2.85 (2H, t, $J$ = 6.0 Hz), 4.72 (2H, t, $J$ = 6.0 Hz), 7.36 (1H, t, $J$ = 7.6 Hz), 7.55 (2H, q, $J$ = 7.6 Hz), 7.78 (1H, t, $J$ = 7.4 Hz), 7.95 (1H, d, $J$ = 8.0 Hz), 8.30 (1H, d, $J$ = 7.6 Hz), 8.40 (1H, d, $J$ = 7.4 Hz), 8.64 (1H, d, $J$ = 8.0 Hz); CIMS $m/z$ 335 ($MH^+$, 100%).
15. Compound **33**: mp 175–176 °C; IR: 3419, 2927, 1688, 1599 CM$^{-1}$; $^1$H NMR ($CDCl_3$) δ 2.20 (2H, m), 2.30 (6H, s), 2.54 (2H, t, $J$ = 8.0 Hz), 4.76 (2H, t, $J$ = 6.0 Hz), 7.59 (1H, t, $J$ = 7.8 Hz), 7.83 (1H, t, $J$ = 8.0 Hz), 7.93 (1H, d, $J$ = 7.8 Hz), 8.41 (1H, d, $J$ = 8.0 Hz), 8.50 (1H, d, $J$ = 8.4 Hz), 8.82 (1H, d, $J$ = 9.0 Hz), 9.10 (1H, s); CIMS $m/z$ 394 ($MH^+$, 100%).