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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and evaluation of new 1,2,3,4-tetrahydroisoquinoline analogs as antiglioma agents

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Abstract Novel tetrahydroisoquinoline (THI) analogs were designed, synthesized, and their antiglioma activity was evaluated. The results showed that 6,8-dimethoxy-1-(2′-methoxybiphenyl-4-ylmethyl)-1,2,3,4-tetrahydroisoquinoline hydrochloride (**25**) demonstrated improved potency, and selectivity on C6 rat glioma vs cultured rat astrocytes (EC_{50} 0.63 μM vs. 10.85 μM) compared to our recent lead molecule **EDL-155** (EC_{50} 1.5 μM vs. 27.4 μM). The isomers of **25** were isolated using a semi-preparative high-performance liquid chromatography (HPLC) method, and their in vitro biological evaluation revealed that (+) **25** was the most active, and it was nearly 21 fold more potent than (−) **25**, suggesting the antiglioma profile is influenced by stereochemical factors.

Keywords Glioblastoma multiforme (GBM) · Tetrahydroisoquinoline (THI) · Isomers · EDL-155 · Antiglioma activity

Introduction

Gliomas constitute the most frequent and malignant form of primary brain tumors. They account for more than 50% of all brain tumors, and are the most common form of primary brain tumors that arise within the central nervous system in adults (Kleihues *et al*., 2000; Lefranc *et al*., 2005). These are the deadliest of the brain cancers because surgical excision of these tumors is extremely difficult due to the invasive nature of tumors derived from glia (Barinaga, 1997). Glioblastoma Multiforme (GBM, WHO grade IV) is the most aggressive form of the gliomas, and accounts for nearly 60–70% of malignant gliomas (Maciej *et al*., 2004). They occur most commonly in adults, ages 45–55, and affect more men than women. In the United States, approximately 18,000 patients are diagnosed with GBM each year (Kang *et al*., 2008), and the mean life expectancy of these patients is approximately 1 year (Grossman and Batara, 2004; Kanzawa *et al*., 2003; Lam and Breakefield, 2001; Lopez-Gonzalez and Sotelo, 2000). A common approach used in the treatment of GBM involves surgery (Berger, 1994), radiation therapy (Shaw, 2000), and various chemotherapeutic regimens (Lesser and Grossman, 1994). The mainstay for chemotherapy is a DNA alkylating agent such as Carmustine (BCNU, **1**), Lomustine (CCNU, **2**), Temozolamide (TMZ, **3**), Melphalan (**4**), Fluorouracil (**5**) (Fig. 1). Despite the combined treatment approach of surgery, radiotherapy, and chemotherapy, there has been very little improvement in outcome for patients with GBM. Among the recent therapeutic approaches, only the combined therapy of TMZ and radiation treatment in a limited number of patients produced encouraging clinical results in long-term survival. Thus, there is a critical need for new and more effective GBM treatments. Hence, new and more effective chemotherapeutic drugs are essential to add to the existing multimodal GBM treatments.

Our group has discovered (Mohler *et al*., 2006) a series of new biaryl 1,2,3,4-THI derivatives, and conformationally flexible analogs that have potent antiglioma activity in

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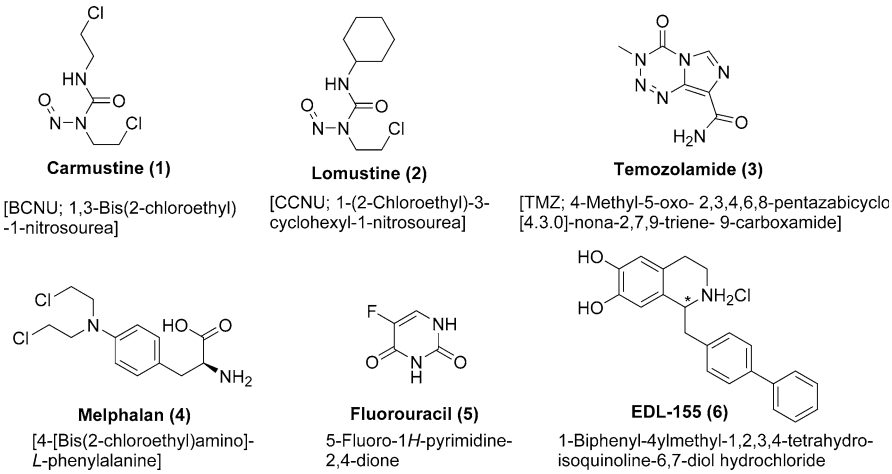


Fig. 1 Structures of antiglioma chemotherapeutic agents

comparison with clinically used antiglioma chemotherapeutic agents such as BCNU, 5-fluorouracil, and melphalan (Fig. 1). As a result we found a lead molecule racemic 1-biphenyl-4-ylmethyl-1,2,3,4-tetrahydroisoquinoline-6,7-diol hydrochloride (**EDL-155**, Fig. 1), which demonstrated selective cytotoxic activity against C6 rat glioma cells relative to cultured rat astrocytes. We compared antiglioma activity of **EDL-155** with standard chemotherapeutic agents such as BCNU and TMZ in in vitro experiment on C6 rat glioma versus normal rat astrocytes (**EDL-155** EC_{50} 1.5 μM vs. 27.4 μM; **BCNU** EC_{50} 4.8 μM vs. 54.8 μM; **TMZ** EC_{50} 16.5 μM vs. 51.8 μM) (Kang *et al*., 2008). The higher potency of racemic **EDL-155** prompted us to study this class of compounds in more detail, and we have focused on design, syntheses, and chiral resolution of novel THI analogs. Toward this end, we designed, synthesized, and screened a series of new 6,8-dihydroxy-THI analogs for antiglioma activity. Among all these newly synthesized compounds, THI analog **25** was the most potent antiglioma agent. We also report here the chiral resolution of the most potent compound **25** by a semi-preparative high-performance liquid chromatography (HPLC) method with the aim of elucidating the pharmacological profile of the pure isomers.

Results and discussion

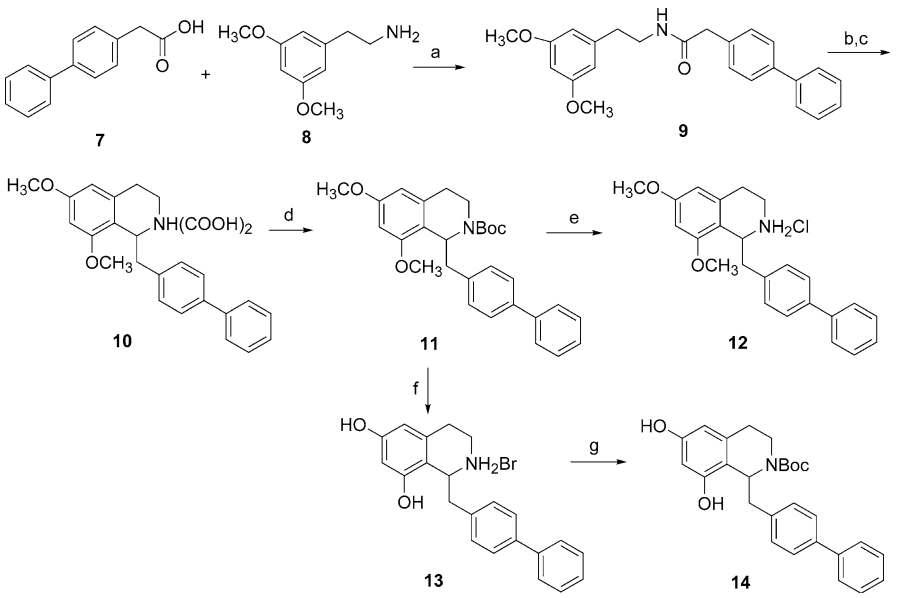
Chemistry

Target molecules were synthesized according to the sequence of reactions shown in Schemes 1 and 2 as racemic mixtures. 2-Biphenyl-4-yl-*N*-2-(3,5-dimethoxyphenyl) ethylacetamide (**9**) was prepared by reported procedure (Mohler *et al*., 2006). Biphenyl-4-yl-acetic acid (**7**) was reacted with 2-(3,5-dimethoxyphenyl)ethylamine (**8**) in presence of diethylcyanophosphonate, and triethyl amine in anhydrous DMF to obtain compound **9** in excellent yield (94%). The amide **9** was cyclized using the Bischler–Napieralski reaction with $POCl_3$ in CH_3CN followed by reduction with $NaBH_4$ to obtain the free amine, which was then treated with oxalic acid in MeOH to afford the oxalyl salt (**10**). Compound **10** was treated with 1 N NaOH in DCM, and the resulting free amine was allowed to react with di-*tert*-butyldicarbonate to give *N*-Boc-6,8-dimethoxy-1,2,3,4-THI derivative (**11**). The hydrochloride salt (**12**) separated as solid when compound **11** was stirred with 2 M HCl in ether at 0°C and allowed to warm to room temperature. Reaction of **11** with 1 M BBr_3 in DCM furnished 6,8-dihydroxy-1,2,3,4-THI hydrobromide salt (**13**), which was further reacted with di-*tert*-butyldicarbonate to obtain the *N*-Boc analog of 6,8-dihydroxy-1,2,3,4-THI (**14**).

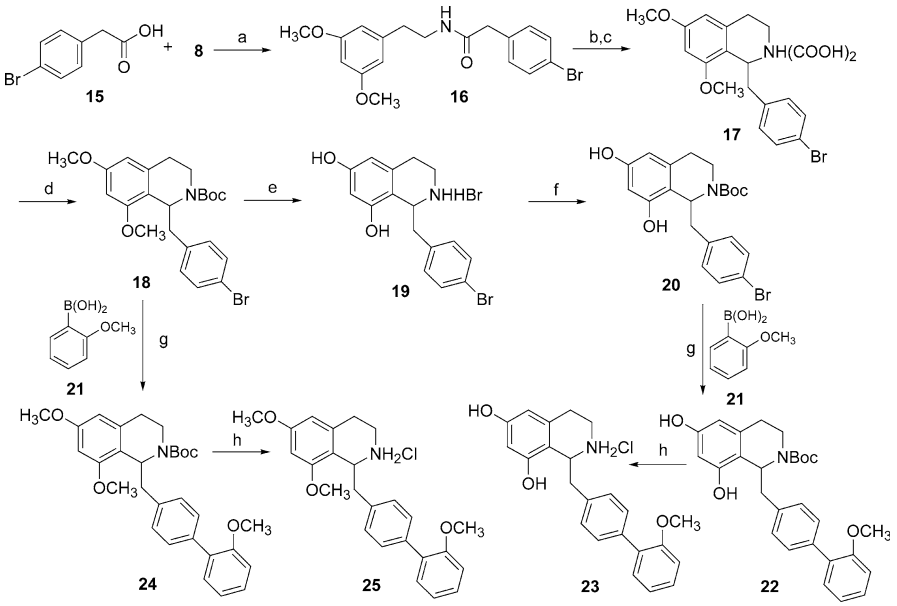
Scheme 2 shows the synthesis of D-ring-modified 6,8-dihydroxy-1,2,3,4-THI (resorcinol) analogs (compounds **23** and **25**). Compounds **16**–**18** were synthesized according to the procedure of compounds **9**–**11** using 4-bromophenylacetic acid **15**, and amine **8**. Compound **18** was treated with 1 M BBr_3 in DCM to get hydrobromide salt (**19**), which was then reacted with di-*tert*-butyl dicarbonate to give *N*-Boc-THI derivative (**20**) in good yield. Compounds **20** and **18** were coupled with 2-methoxyphenylboronic acid (**21**) using Suzuki–Miyaura reaction conditions to obtain the desired compounds **22** and **24**, respectively. Finally, the compounds **22** and **24** were stirred with 2 M HCl in ether to afford the required hydrochloride salts **23** and **25** in good yields.

Biological evaluation

An in vitro assay was used to screen all the newly synthesized racemic 1,2,3,4-THI analogs for their effects on the growth of C6 rat glioma cells relative to cultured rat astrocytes. In this study, we focused on modification of the



Scheme 1 Reagents and conditions: **a** Et_3N, $(EtO)_2P(O)CN$, DMF; **b** $POCl_3$, CH_3CN; **c** $NaBH_4$, $(COOH)_2{\cdot}2H_2O$, MeOH; **d** 1 N NaOH, DCM, $[(CH_3)_2C{\cdot}CO_2]_2O$, THF; **e** 2 M HCl, Ether; **f** 1 M BBr_3, DCM; **g** Et_3N, $[(CH_3)_2C{\cdot}CO_2]_2O$, THF



Scheme 2 Reagents and conditions: **a** Et_3N, $(EtO)_2P(O)CN$, DMF; **b** $POCl_3$, CH_3CN; **c** $NaBH_4$, $(COOH)_2{\cdot}2H_2O$, MeOH; **d** 1 N NaOH, DCM, $[(CH_3)_2C{\cdot}CO_2]_2O$, THF; **e** 1 M BBr_3, DCM; **f** Et_3N, $[(CH_3)_2C{\cdot}CO_2]_2O$, THF; **g** $Pd(OAc)_2$, PPh_3, Na_2CO_3, i-PrOH; **h** 2 M HCl, Ether

A- and D-rings of THI to see the effect on potency. The A-ring-modified analogs (**12** and **13)** have shown moderate activity against C6 rat glioma as well as selectivity toward astrocytes (**12**: EC_{50} 2.31 μM vs. 8.95 μM; **13**: EC_{50} 2.09 μM vs. 11.92 μM), whereas compound **11** displayed lower potency (EC_{50} 7.54 μM), and no effect on astrocytes but compound **14** showed small decrease in cytotoxicity (EC_{50} 4.97 μM). Overall the *N*-substitution lowered the cytotoxicity as compared to corresponding amine salts. We next turned our attention to modify the D-ring of THI. The *N*-Boc-6,8-dihydroxy-THI **22** lowered activity, and selectivity (EC_{50} 7.34 μM vs. 67.51 μM), whereas corresponding methoxy derivative **24** was much less active (EC_{50} 59.71 μM), and showed no effect on astrocytes. Compound **23** demonstrated the moderate activity, and selectivity (EC_{50} 2.61 μM vs. 15.73 μM), and it was most comparable to compound **13** (Table 1). However, compound **25** displayed marked increase in activity, and selectivity (EC_{50} 0.63 μM vs. 10.85 μM). This compound was the most potent, and selective THI analog in this study, and it showed nearly 2.4-fold increase in the activity in comparison with our previous lead molecule **EDL-155** (EC_{50} 1.5 μM vs. 27.4 μM). The antiglioma studies were carried out for all newly synthesized THI analogs including compound **25** as racemic mixture, but stereoisomers often show different pharmacological activities. Therefore, we resolved the racemic mixture to investigate the biological properties of each enantiomer of compound **25**.

Table 1 C6 glioma cytotoxic activity and selectivity of **THI** analogs

Compound no.	EC_{50} μM C6 glioma	EC_{50} μM astrocyte
11	7.54	No effect
12	2.31	8.95
13	2.09	11.92
14	4.97	8.58
18	10.13	28.13
19	12.61	23.90
20	7.04	9.97
22	7.34	67.51
23	2.61	15.73
24	59.71	No effect
25	0.63	10.85
(+) **25**	0.26	13.08
(−) **25**	5.39	15.73
EDL-155	1.5	27.4

Chiral resolution by HPLC and optical rotation

It is well known that the enantiomers of chiral drugs generally show significant differences in their pharmacokinetics (PK) and pharmacodynamics (PD) and adverse reactions. Therefore, with the aim of elucidating enantiopharmacological profile of compound **25**, we performed the chiral resolution of its precursor *N*-Boc-THI analog **24** by HPLC method. We resolved the individual isomers by (R, R) WHELK-01 chiral HPLC column (Regis technologies, Morton Grove, IL), which enabled us to directly, and efficiently obtain both enantiomers in nearly 100% optical purity with 85:15 ratio of hexane and isopropanol (Fig. 2). The first fraction eluted at 8.12 min, and second fraction eluted at 15.00 min. The collected fractions from each isomer were pooled and evaporated under vacuum to give the residues as first fraction called isomer-I and second fraction called as isomer-II. Purity of isomers-**I** and **II** were 100% (Fig. 2d, e). After resolution, both the isomers, **I** and **II** of **24,** were treated with 2 M HCl in ether to afford corresponding hydrochloride salts **25-isomers-I** & **II**, respectively. We examined the specific rotations of **25-isomers-I** & **II** in MeOH solution using DigiPol 781 automatic polarimeter, and their specific rotations were $[\alpha]_D = -35.0$ and $[\alpha]_D = +40.2$ ($t = 25°C$), respectively. Biological studies revealed that (+) **25** was the most active, and it was nearly 21 fold more potent than (−) **25** on C6 rat glioma cell lines [(+) **25**: EC_{50} 0.26 μM; (−) **25**: EC_{50} 5.39 μM]. This confirmed that the antiglioma activity of **racemic-25** (0.63 μM) was due to primarily the (+) **25**.

In summary, we successfully synthesized novel A-ring-, and D-ring-modified 6,8-dihydroxy THI (resorcinol) analogs, and evaluated for their antiglioma activity on C6 rat glioma vs normal rat astrocytes. The D-ring-modified resorcinol derivative **25** demonstrated improved potency and selectivity in our assay when compared to **EDL-155**. Resolution of **25** by a semi-preparative chiral HPLC method enabled us directly, and efficiently to obtain both enantiomers with 100% optical purity. The high potency and selectivity of (+) **25** suggests that the stereochemical properties influence the pharmacobiological profile of this class of compounds.

Experimental

Chemistry

All the reagents and solvents were purchased from Aldrich, and used without further purification. Melting points were determined on a Fisher apparatus, and are uncorrected. Proton NMR spectra were recorded on a Bruker ARX 300 spectrometer (300 MHz), and spectral data were consistent with assigned structures. Chemical shifts were expressed in ppm (δ); coupling constants (J) are given in Hz. Mass spectra were collected on a Brucker ESQUIRE electrospray/ion trap instrument in the positive and negative modes. Elemental analysis (C, H, N) was performed by Atlantic Microlab, Inc. (Norcross, GA), and results were within ±0.4% of the theoretical values for the formula given.

All the reactions were carried out using little modification of reported procedures (Mohler *et al*., 2006; Nikulin *et al*., 2006).

*General procedure for the Suzuki–Miyaura coupling reactions (Compounds **22, 24**)*

To a solution of compound (**18/20**) (1 mmol) in anhydrous i-PrOH (15 ml), palladium (II)acetate (4 mol%), and triphenylphosphine (8 mol%) were added, and the mixture was stirred under argon at room temperature for 30 min. To this mixture, 2-methoxy phenylboronic acid **21** (1.5 mmol), and Na_2CO_3 (3 mmol) were added successively, and the reaction mixture was refluxed for 16 h. After being cooled and concentrated, the obtained residue was partitioned between EtOAc and saturated $NaHCO_3$ aqueous solution. Two layers were separated, and the aqueous phase was extracted with EtOAc. The combined organic layers were washed with water followed by brine, and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure, and the crude product was purified by flash column chromatography (Table 2).

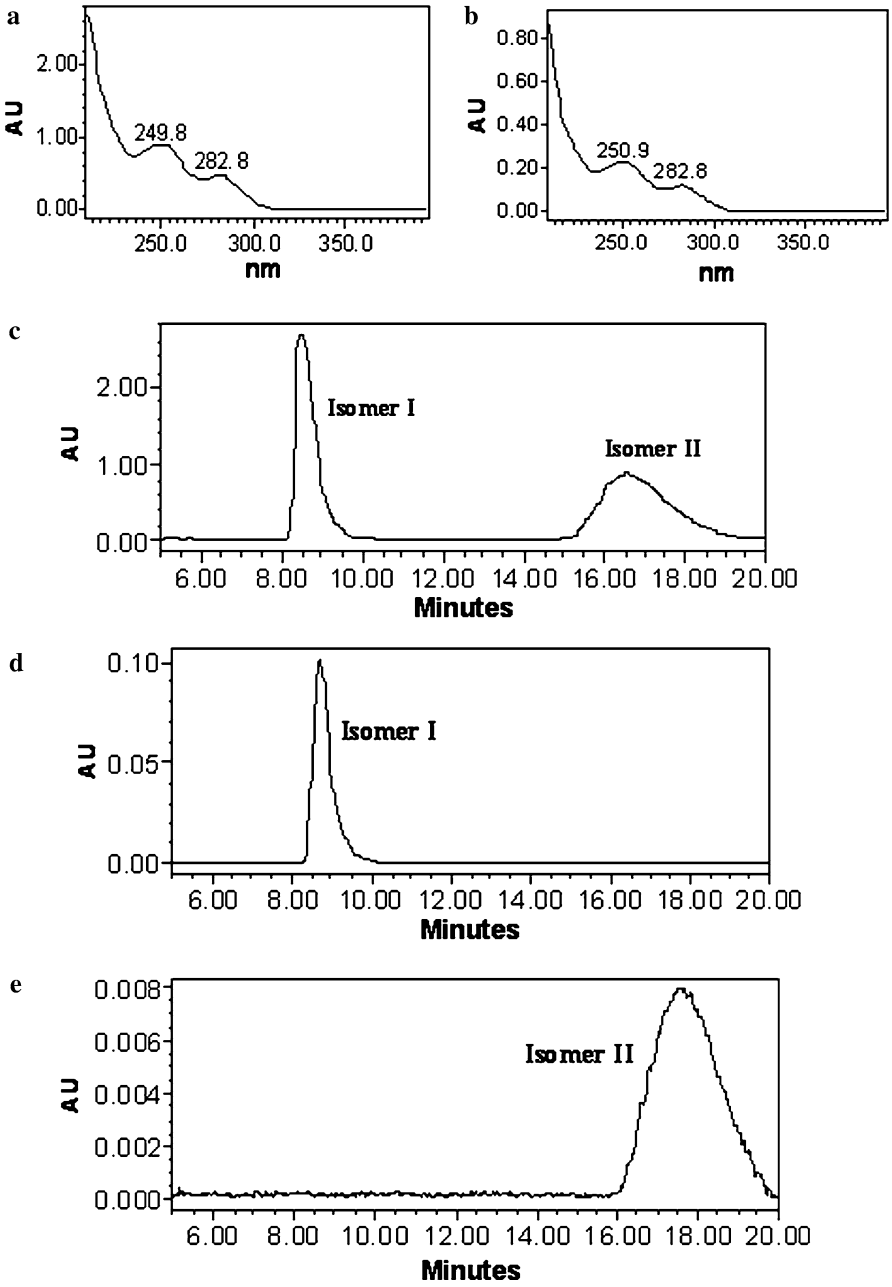


Fig. 2 Chiral separation of **24** using LC/DAD. UV spectrum of isomer-**I** (**a**) and isomer-**II** (**b**). Separation of racemic mixture (**c**), separated isomer-**I** (**d**), and separated isomer-**II** (**e**)

Cytotoxicity assay

The normal astrocytes for negative control testing were cultured from the cerebral cortex of 3–4 day old Sprague-Dawley rat pups as described (McCarthy and DeVellis, 1978), and modified (Geisert and Stewart, 1991) procedure. The animals were anesthetized by cold, and decapitated, after which their brains were immediately removed. Placed the cortices in a Petri-dish containing 10 ml of Hank's Balanced Salt Solution (HBSS), and the tissue in 20 ml of 0.1% trypsin in HBSS for 10 min. The astrocytes were plated at a density of 5×10^3 cells/cm^2 into T-75 culture flasks. C6 rat glioma cell line was purchased from ATCC (American Type Culture Collection). Cells were grown in BME with 10% fetal calf serum culturing media in a 37°C incubator containing a humid 5% CO_2 atmosphere. For the compounds testing, the cells were trypsinized, and transferred to 96-well microtiter plates at approximate cell density of 10^3 cells/well of C6 glioma, and 10^4 cells/well of astrocytes. The cells were grown overnight in 200 µl of 10% FCS BME in a 37°C incubator containing a humid 5% CO_2 atmosphere. All newly synthesized compounds were dissolved completely to make a 100 µM stock solution, and diluted to produce a series of concentrations. Immediately

Table 2 Physical properties and spectroscopic data of THI analogs

Comp. no.	Yield (%)	M.P. (°C)	^{1}H NMR (d_6-DMSO)	Mass *m/z*
9	94	108–10	δ 8.11 (bs, 1*H*, –NH), 7.64 (d, *J* = 7.8 Hz, 2*H*, ArH), 7.56 (d, *J* = 7.8 Hz, 2*H*, ArH), 7.48–7.43 (m, 2*H*, ArH), 7.36 (d, *J* = 6.9 Hz, 1*H*, ArH), 7.30 (d, *J* = 7.5 Hz, 2*H*, ArH), 6.36–6.33 (m, 3*H*, ArH), 3.70 (s, 6*H*, 2*OCH_3), 3.43 (s, 2*H*, –CH_2), 3.28–3.26 (m, 2*H*, –CH_2), 2.65 (t, *J* = 7.2 Hz, 2H, –CH_2)	398 $[M + Na]^+$
10	73	181–83	δ 8.44–8.21 (bs, 1*H*, NH), 7.68–7.65 (m, 5*H*, ArH), 7.50–7.44 (m, 2*H*, ArH), 7.39–7.36 (m, 2*H*, ArH), 6.49 (s, 1*H*, ArH), 6.45 (s, 1*H*, ArH), 4.77–4.73 (m, 1*H*, –CH), 3.77 (s, 3*H*, OCH_3), 3.73 (s, 3*H*, OCH_3), 3.52–3.44 (m, 2*H*, CH_2), 3.28–3.05 (m, 4*H*, 2*–CH_2)	360 $[M–(CO_2H)_2 + H]^+$
11[a]	80	109–11	δ 7.62–7.18 (m, 9*H*, ArH), 6.47 and 6.44 (s, 1*H*, ArH), 6.35 and 6.33 (s, 1*H*, ArH), 5.40–5.38 and 5.24–5.22 (m, 1*H*, –CH), 3.89, 3.80 and 3.75 (s, 6*H*, 2*OCH_3), 3.06–3.02 and 2.89–2.70 (m, 6*H*, 3*–CH_2), 1.26 and 1.00 (s, 9*H*, $[CH_3]_3$)	482 $[M + Na]^+$
12	78	124–26	δ 9.29 (s, 1*H*, –NH_2), 8.54 (s, 1H, –NH_2), 7.62 (d, *J* = 7.2 Hz, 4*H*, ArH), 7.50–7.45 (m, 2*H*, ArH), 7.38 (d, *J* = 7.8 Hz, 3*H*, ArH), 6.50 (s, 1*H*, ArH), 6.45 (s, 1*H*, ArH), 4.76 (s, 1H, –CH), 3.77 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 3.21–3.14 (m, 4H, 2*–CH_2), 3.02–2.94 (m, 2H, –CH_2)	395 $[M–(HCl) + H]^+$
13	92	238–40	δ 9.99 (bs, 1H, ArOH), 9.38 (bs, 1H, ArOH), 9.08 (bs, 1H, –NH_2), 8.22 (bs, 1H, –NH_2), 7.70–7.67 (m, 4H, ArH), 7.50–7.26 (m, 5H, ArH), 6.30 (d, *J* = 2.1 Hz, 1H, ArH), 6.11 (d, *J* = 2.1 Hz, 1H, ArH), 4.70 (d, *J* = 8.1 Hz, 1H, –CH), 3.43–3.33 (m, 2H, –CH_2), 3.22–3.04 (m, 2H, –CH_2), 2.93–2.80 (m, 2H, –CH_2)	332 $[M–(HBr) + H]^+$
14[a]	67	198–200	δ 9.58 and 9.52 (bs, 1H, ArOH), 9.08 and 9.05 (bs, 1H, ArOH), 7.61–7.19 (m, 9H, ArH), 6.23 and 6.21 (s, 1H, ArH), 6.03 and 6.00 (s, 1H, ArH), 5.36–5.33 and 5.20–5.17 (m, 1H, –CH), 3.16–3.12 and 2.79–2.61 (m, 6H, 3*–CH_2), 1.24 and 0.99 (s, 9H, $[CH_3]_3$)	454 $[M + Na]^+$
16	93	86–88	δ 8.10 (t, *J* = 5.1 Hz, 1H, –NH), 7.46 (d, *J* = 8.4 Hz, 2H, ArH), 7.16 (d, J = 8.1 Hz, 2H, ArH), 6.33 (s, 3H, ArH), 3.70 (s, 6H, 2*OCH_3), 3.36 (s, 2H, –CH_2), 3.27 (q, J = 7.2, 6.9 Hz, 2H, –CH_2), 2.63 (t, J = 7.2 Hz, 2H, –CH_2)	400 $[M + Na]^+$
17	85	168–70	δ 8.45–8.20 (bs, 1H, NH), 7.53 (d, J = 8.1 Hz, 2H, ArH), 7.21 (d, *J* = 8.4 Hz, 2H, ArH), 6.46 (s, 1H, ArH), 6.42 (s, 1H, ArH), 4.70 (t, *J* = 6.6 Hz, 1H, –CH), 3.76 (s, 3H, –OCH_3), 3.68 (s, 3H, –OCH_3), 3.42–3.35 (m, 2H, –CH_2), 3.11–2.93 (m, 4H, 2*–CH_2)	362 $[M–(CO_2H)_2 + H]^+$
18[a]	85	94–96	δ 7.49–7.06 (m, 4H, ArH), 6.45 and 6.43 (s, 1H, ArH), 6.34 and 6.32 (s, 1H, ArH), 5.32–5.29 and 5.15–5.12 (m, 1H, –CH), 3.87, 3.79 and 3.74 (s, 6H, 2*OCH_3), 2.99–2.94 and 2.80–2.61 (m, 6H, 3*–CH_2), 1.26 and 1.04 (s, 9H, $[CH_3]_3$)	484 $[M + Na]^+$
19	75	252–54	δ 9.94 (s, 1H, ArOH), 9.38 (s, 1H, ArOH), 9.06 (bs, 1H, –NH_2), 8.26 (bs, 1H, –NH_2), 7.56 (d, *J* = 7.5 Hz, 2H, ArH), 7.28 (d, *J* = 7.2 Hz, 2H, ArH), 6.27 (s, 1H, ArH), 6.10 (s, 1H, ArH), 4.64 (d, *J* = 7.8 Hz, 1H, –CH), 3.28–3.16 (m, 2H, –CH_2), 3.09–3.01 (m, 2H, –CH_2), 2.84–2.78 (m, 2H, –CH_2)	334 $[M–(HBr) + H]^+$
20[a]	77	222–24	δ 9.57 and 9.50 (s, 1H, ArOH), 9.08 and 9.05 (s, 1H, ArOH), 7.48–7.06 (m, 4H, ArH), 6.19 and 6.17 (s, 1H, ArH), 6.03 and 5.99 (s, 1H, ArH), 5.28–5.25 and 5.11–5.09 (m, 1H, –CH), 3.28–3.04 and 2.77–2.57 (m, 6H, 3*–CH_2), 1.25 and 1.04 (s, 9H, $[CH_3]_3$)	456 $[M + Na]^+$
22[a]	55	200–202	δ 9.57 and 9.50 (s, 1H, ArOH), 9.07 and 9.04 (s, 1H, ArOH), 7.38–6.99 (m, 8H, ArH), 6.22 and 6.20 (s, 1H, ArH), 6.03 and 6.01 (s, 1H, ArH), 5.38–5.35 and 5.22–5.19 (m, 1H, –CH), 3.80 and 3.74 (s, 3H, OCH_3), 3.26–3.08 and 2.80–2.58 (m, 6H, 3*–CH_2), 1.26 and 1.02 (s, 9H, $[CH_3]_3$)	484 $[M + Na]^+$
23	75	210–12	δ 10.05 (s, 1H, ArOH), 9.41 (s, 1H, ArOH), 9.25 (bs, 1H, –NH_2), 8.45 (bs, 1H, –NH_2), 7.48 (d, *J* = 7.8 Hz, 2H, ArH), 7.39 (d, *J* = 7.8 Hz, 2H, ArH), 7.35–7.27 (m, 2H, ArH), 7.12 (d, *J* = 8.1 Hz, 1H, ArH), 7.06–7.01 (m, 1H, ArH), 6.33 (s, 1H, ArH), 6.11 (s, 1H, ArH), 4.67 (d, *J* = 8.7 Hz, 1H, –CH), 3.77 (s, 3H, OCH_3), 3.35 (bs, 4H, 2*–CH_2), 3.18–3.03 (m, 2H, –CH_2)	362 $[M–(HCl) + H]^+$
24[a]	51	126–28	δ 7.40–7.00 (m, 8H, ArH), 6.45 and 6.43 (s, 1H, ArH), 6.35 and 6.33 (s, 1H, ArH), 5.41–5.39 and 5.26–5.22 (m, 1H, –CH), 3.89, 3.79 and 3.74 (s, 9H, 3*OCH_3), 3.04–3.00 and 2.86–2.71 (m, 6H, 3*–CH_2), 1.28 and 1.03 (s, 9H, $[CH_3]_3$)	512 $[M + Na]^+$
25	80	140–42	δ 9.31 (s, 1H, –NH_2), 8.57 (s, 1H, –NH_2), 7.47 (d, *J* = 7.8 Hz, 2H, ArH), 7.38–7.27 (m, 4H, ArH), 7.12 (d, *J* = 8.1 Hz, 1H, ArH), 7.06–7.01 (m, 1H, ArH), 6.50 (s, 1H, ArH), 6.45 (s, 1H, ArH), 4.70 (bs, 1H, –CH), 3.76 (s, 6H, 2*OCH_3), 3.73 (s, 3H, OCH_3), 3.18–3.06 (m, 4H, 2*–CH_2), 3.04–2.95 (m, 2H, –CH_2)	390 $[M–(HCl) + H]^+$

[a] In the NMR spectrum, a mixture of conformers of 3:1 ratio was appeared (Nikulin *et al.*, 2006)

before treatment, the 10% FCS BME was suctioned off the cells, and replaced with the 180 µl of 2% FCS BME to which the initial solution was added. A 20-µl aliquot each of these initial solutions was added to 180 µl of 2% FCS BME to produce the test concentration (0.001, 0.01, 0.1, 1, 5, 10, 25, 100 µM). The cultured cells were incubated with testing compounds for 4 days. The cells were fixed with 4% paraformaldehyde, stained with 0.1% Cresylecth violet stain. The screening data were collected as four wells for each dose per compound (screening) or concentration (dose response curve). Also, the average growth of cells in eight wells with no treatment was used as a negative control for each plate. The cell number was determined in a manner similar to that described (Wagner *et al*., 2003). The cytotoxic character of each compound was reported as the percent of cell survival, calculated as the average A_{560} for treated cells divided by A_{560} of untreated (negative control) cells, and expressed as a percentage. Values less than or equal to 100% indicate a cytostatic or cytotoxic effect. Dose response curves and EC_{50} values were attained via plots of percent survival versus concentration.

Chiral liquid chromatography

High-performance liquid chromatography grade hexane and isopropyl alcohol (IPA) were used from Sigma-Aldrich. Waters Alliance HPLC instrument was used for the separation (Waters Corp., Milford, MA). The compound **24** was dissolved in IPA, and the solution was filtered through the 0.45-µm membrane prior to being loaded onto the column. Three eluents were used to develop the first analytical method prior to the preparative separation. Hexane:IPA 50:50, 75:25, 85:15 v/v. The eluent hexane:IPA (85:15 v/v) was used for the semi-preparative separation. WHELK-01 chiral HPLC column from Regis Technology was used. Flow rate was set 8 ml/min throughout the preparative separation.

Optical rotation

The optical rotations were measured with DigiPol 781 automatic polarimeter (Fairfield, NJ). The pure isomers (+) **25** and (−) **25**, 2 mgs each, were dissolved in 1 ml methanol to measure the rotations at 589 nm (25°C).

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