Discovery of Antiglioma Activity of Biaryl 1,2,3,4-Tetrahydroisoquinoline Derivatives and Conformationally Flexible Analogues

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Cultured rat astrocytes and C6 rat glioma were used as a differential screen for a variety of 1,2,3,4-tetrahydroisoquinoline (THI) derivatives. Compound **1** [1-(biphenyl-4-ylmethyl)-1,2,3,4-tetrahydroisoquinoline-6,7-diol hydrochloride] selectively blocked the growth of C6 glioma leaving normal astrocytes relatively unaffected. The potential for clinical utility of **1** was further substantiated in human gliomas and other tumor cell lines. Preliminary SAR of this activity was characterized by synthesis and testing of several THI and conformationally flexible variants.

Introduction

Gliomas are among the deadliest of tumors because surgical excision of these tumors is extremely difficult due to the invasive nature of tumors derived from glia.[1] Very few glioblastoma patients live more than one year with essentially no long-term cures.[2] The mainstay for chemotherapy is DNA alkylating agents such as BCNU, procarbazine, temozolomide, and cisplatin.[3] We report here the discovery of a series of 1,2,3,4-tetrahydroisoquinolines (THIs) and flexible analogues that have selective cytotoxic activity against rat C6 glioma cells relative to cultured rat astrocytes.[4] The C6 glioma cytotoxic activity of our lead, compound **1** (Chart 1), and a series of analogues is reported and compared to the peripheral benzodiazepine receptor (PBR) binding agents (**9**, **10**, and **11**) that possess similar cytotoxic activity against C6 glioma cells[5] (Chart 1) with IC_{50} values in C6 glioma of 73.0 μM, 95.0 μM, and 37.5 μM, respectively. Compound **1** is also compared to BCNU, 5-fluorouracil (5FU), and melphalan, chemotherapeutic agents used clinically. A broad array of THI compounds bearing a 1-monoaryl group, such as in **12**, **13**, and **14**,[6,7] lacked the cytotoxic activity of **1**, providing the initial SAR observation that directed us toward 1-biaryl analogues of **1**. Correspondingly, we have synthesized novel 1-biaryl compounds **2**, **3**, **4**, **6**, and **7** and tested **2**−**8** as analogues of **1** to develop a preliminary set of structure−activity relationships (SAR). Cytotoxicity for **1** was also demonstrated in human gliomas and diverse human tumor cell lines supporting its status as a preclinical lead for drug development.

Chemistry

The synthesis of compound **1** 1-biphenyl-4-ylmethyl-1,2,3,4-tetrahydroisoquinoline-6,7-diol hydrochloride is shown in Scheme 1. Reaction of 2-(3,4-dibenzyloxyphenyl)ethylamine hydrochloride **15** with 4-biphenylacetic acid **16** in the presence of diethyl cyanophosphonate and triethylamine yielded the amide **17**. The amide was cyclized to **18** by reaction with $POCl_3$ followed by reduction with $NaBH_4$. The lead structure **1** was synthesized by acidic deprotonation of **18**. The *N*-methyl analogue, **7**, was prepared from **18** by treatment with formaldehyde followed by sodium cyanoborohydride and zinc chloride to form **26**. Acidic deprotection of **26** to remove the *O*-benzyl groups protecting the catechol yielded **7**. Two types of analogues of **1** were synthesized and tested, substituted THI derivatives such as **2** and open chain derivatives such as **3** (Chart 1). The synthesis of 1-biphenyl-4-ylmethyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride **4** is shown in Scheme 2. Initially, the 3,4-dimethoxyphenethylamine **19** was reacted with 4-biphenylacetic acid **16** to form the intermediate amide **21**. The amide **21** was cyclized to form the 6,7-dimethoxy analogue **4** by reaction with $POCl_3$ and $NaBH_4$ followed by 2 M HCl solution in diethyl ether. Intermediate **21** was also used to synthesize conformationally flexible analogues **3** and **6**. Reaction of **21** with $BF_3{\cdot}Et_2O$ yielded the linear secondary amine **22**. Reaction of **22** with formaldehyde followed by sodium cyanoborohydride and $ZnCl_2$ yielded the tertiary amine **3**. Reaction of **22** with boron tribromide in methylene chloride yielded the secondary amine, catechol **6**. The synthesis of 2-(2-biphenyl-4-ylethyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride **2** is shown in Scheme 3. Reaction of 6,7-dimethoxytetrahydroisoquinoline hydrochloride **28** with 4-biphenylacetic acid **16** in the presence of diethyl cyanophosphonate in DMF followed by reaction with triethylamine yielded the amide intermediate **27** (Scheme 3). The amide **27** was reduced using $BF_3{\cdot}Et_2O$ in THF followed by 1 M B_2H_6 then cold 10% HCl to produce the *N*-biphenyl tertiary amine, **2** (Scheme 3). All the analogues of **1** synthesized and tested are summarized in Chart 1.

Antiglioma Activity of (1) Compared to Known Antiglioma Agents. An in vitro assay was used to screen compounds for their effects on the growth of cultured normal astrocytes and C6 glioma. We discovered that C6 glioma growth was more sensitive than normal astrocytes to certain 1,2,3,4-tetrahydroisoquinoline (THI) compounds, indicating selective cytotoxic activity in C6 glioma (rat) vs rat astrocyte. The most active and selective THI screened, compound **1**, had IC_{50} values of 2.6 μM in C6 glioma vs 29.0 μM in primary astrocytes. To

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[a] Abbreviations: 5FU, 5-fluorouracil; BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea; BME, Basal Media Eagle; DMEM, Dulbecco's Modification of Eagle's Medium; DNA, deoxyribonucleic acid; FCS, fetal calf serum; PBR, peripheral benzodiazepine receptor; PK11195 (1-(2-chlorophenyl(*N*-methyl-*N*-(1-methyl-propyl)-3-isoquinoline carboxamide; R_O5-4864, 7-chloro-5-(4-chlorophenyl)-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine; SAR, structure−activity relationship; THI, 1,2,3,4-tetrahydroisoquinoline.

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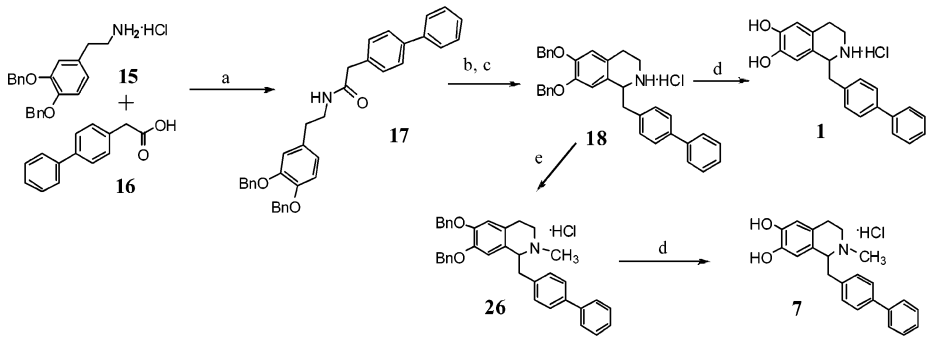
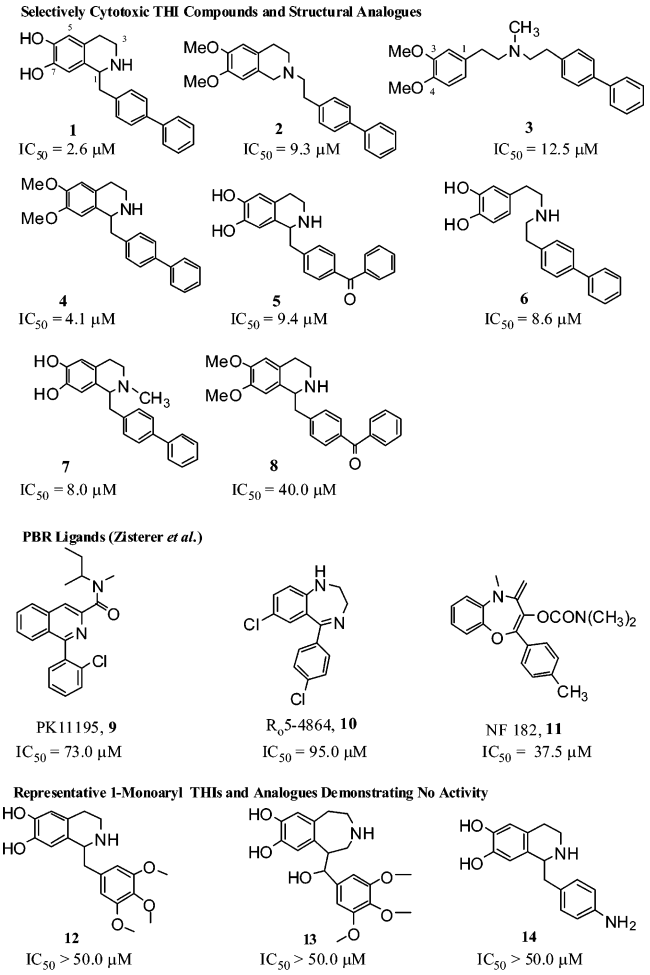
Scheme 1[a]



[a] Reagents: (a) Et_3N, $(EtO)_2P(O)CN$; (b) $POCl_3$; (c) $NaBH_4$; (d) concentrated HCl; (e) CH_2O, $NaBH_3CN$, $ZnCl_2$.

Chart 1. C6 Glioma Cytotoxic Potency of Antiglioma Agents[a]



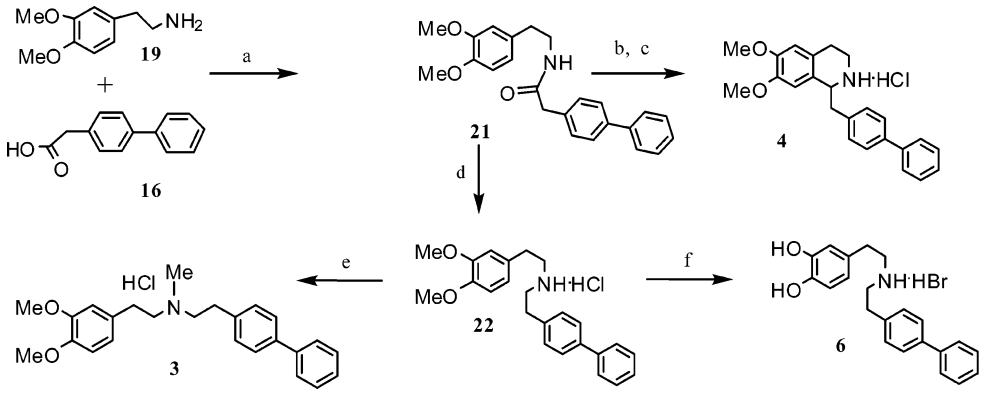
[a] The C6 glioma cytotoxic activities of several classes of agents are summarized in Chart 1. The THI and derivative compounds **1**−**8** and **12**−**14** were synthesized and tested by us using the protocol in the Supporting Information and reported in Table 1 with exception of **12**−**14** which had $IC_{50} > 50$ μM. Briefly, cells were grown and maintained in 10% FCS BME (fetal calf serum in Basal Media Eagle). The test compound was incubated with cells in 200 μL of 2% FCS BME for 96 h at 37 °C in a humid, 5% CO_2 incubator. Cells were fixed and quantitated using 0.1% cresylecth violet stain whose A_{560} was determined with a Titertek plate reader. Zisterer et al. incubated **9**, **10**, and **11** with cells in 100 μL of 10% FCS DMEM for 48 h, and then cells were quantitated spectrophotometrically (A_{570}) using MTT stain.[5] Compounds **9**, **10**, and **11** did not demonstrate any activity in our hands in a dose range comparable to **1**. Compounds **5**, **8**, **12**, **13**, and **14** were synthesized and reported by us elsewhere.[6,7,9]

assess the significance of this observation we decided to determine the IC_{50} values in our assay for commonly used chemotherapeutic agents and compare them to **1**. The IC_{50} values for melphalan, BCNU, and 5FU were 12.6 μM, 9.3 μM, and 6.1 μM, respectively, for C6 glioma; thus **1** demonstrated greater than 2-fold higher potency and similar to improved selectivity (Table 1), suggesting that **1** may have advantages over currently used antiglioma agents. Next we wanted to compare **1** to reported compounds that have similar structure and activity to see if compounds such as **1** were known. The closest structural analogues that reported C6 glioma cytotoxicity were the PBR ligands. Two commercially available examples, **9** and **10**, were purchased and characterized in our assay but did not exhibit any cytotoxicity in the dose range used, revealing that **1** and analogues were much more potent than the PBR ligands. Thus **1** was more potent and at least as selective as all the known cytotoxic agents that were characterized as reference materials. These observations suggested to us that THI compounds such as **1** should be explored for their potential as antiglioma chemotherapeutic agents and prompted us to try to establish a preliminary SAR.

Activity in Human Gliomas and Other Human Tumor Cell Lines. Compound **1** was tested for cytotoxic activity in T98G human glioblastoma cell line as a proof of principle that cytotoxicity in C6 glioma was indicative of cytotoxicity in human glioma cells. No normal fetal human astrocyte cell lines were readily available, so this experiment did not have a negative control. Nonetheless, **1** did exhibit activity in T98G cells with an $IC_{50} = 10.2$ μM $\pm$ 1.8 μM. Despite the decline in activity relative to C6 glioma (approximately 4-fold less active), this experiment suggests that rat C6 glioma activity is indicative of cytotoxicity in human gliomas. Given that gliomas are known to be widely variable,[8] we decided to determine the IC_{50} values for **1** in an additional human glioma cell line, U87, with an IC_{50} value of 25.9 μM (Table 1). Compound **1** was further characterized with regard to diverse cancer cell type cytotoxicity. The IC_{50} values for **1** in A549 lung cancer, MCF7 breast cancer, LNCaP prostate cancer, and HeLa cervical cancer cell lines were 10.2 μM, 3.8 μM, 10.7 μM, and 3.0 μM, respectively (Table 2). These six cell lines demonstrated that compound **1** has cytotoxicity in a broad array of cancer cell lines, suggesting that **1** and analogues may have therapeutic potential in a variety of cancers.

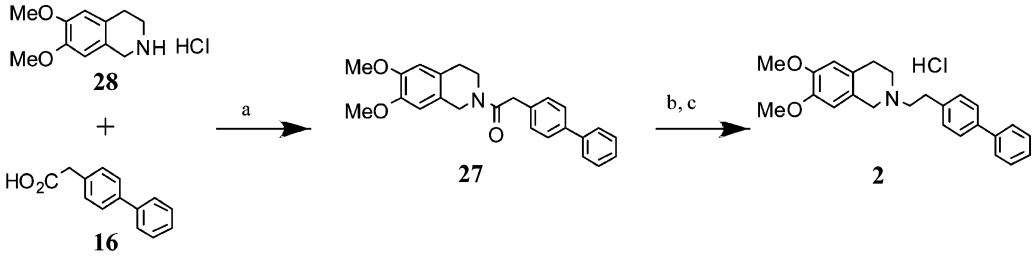
Synthesis and Testing of THI and Non-THI Analogues. To expand on **1** as a lead structure, other readily obtainable THI analogues were tested for activity. Compounds **12**, **13**, and **14** are examples of compounds that were screened that did not have activity (Chart 1). The prominent difference between these molecules and **1** was the biaryl group in **1** which seemed to be the key structural component that conferred activity to **1**. Thus the biaryl component was taken to be critical to the antiglioma activity, and this initial SAR observation led us to compile and characterize a series of biaryl compounds including biaryl

Scheme 2[a]



[a] Reagents: (a) toluene, reflux; (b) $POCl_3$; (c) $NaBH_4$; (d) $BF_3{\cdot}Et_2O$, 1 M B_2H_6; (e) CH_2O, $NaBH_3CN$, $ZnCl_2$; (f) BBr_3.

Scheme 3[a]



[a] Reagents: (a) $(EtO)_2P(O)CN$, Et_3N; (b) $BF_3{\cdot}Et_2O$, 1 M B_2H_6; (c) HCl.

Table 1. C6 Glioma Selective Cytotoxic Activities of 1 and Derivatives

	IC_{50} (μM)[a–c]	
compound	C6 glioma	astrocytes
1	2.6 ± 0.6	29.0 ± 0.6
	10.2 ± 5.8 (human T98G)[d]	n/a
	25.9 ± 3.3 (human U87)[d]	
4	4.1 ± 0.2	83.8 ± 73.2
5[e]	9.4 ± 0.9	21.0 ± 7.2
8[e]	40.0 ± 2.6	40.0 ± 0.8
7	8.0 ± 0.9	15.0 ± 1.3
2	9.3 ± 0.6	15.5 ± 1.7
6	8.6 ± 0.5	>100
3	12.5 ± 0.8	39.0 ± 30.7
melphalan	12.6 ± 2.6	34.0 ± 1.6
BCNU	9.3 ± 3.9	155.6 ± 104.8
5FU	6.1 ± 3.9	>100

[a] IC_{50} values were determined in dose−response experiments with incubation periods of 4 days. [b] The reported IC_{50} values and standard deviation for all compounds except **1** and BCNU were calculated using WinNonlin as discussed in the Experimental Section. [c] Note that **1** and BCNU were further characterized using the computer program Scientist to derive the reported IC_{50} values and standard deviations. [d] Values for IC_{50} determined in T98G and U87 human glioblastoma cell lines which were purchased from ATCC. [e] The synthesis of compounds **5** and **8** was previously reported.[9]

Table 2. Activity of **1** in Diverse Human Tumor Cell Lines[a,b]

A549 (lung)	MCF7 (breast)	LNCaP (prostate)	HeLa (cervical)
10.2 ± 0.9	3.8 ± 2.5	10.7 ± 3.4	3.0 ± 0.05

[a] A549, MCF7, LNCaP, and HeLa tumor cell lines were purchased from ATCC cell lines were purchased from ATCC. [b] The reported IC_{50} values and standard errors were derived using the computer program Scientist.

variants (**5** and **8**), methoxylated variants (**2**, **3**, **4**, and **8**), a biaryl attachment point variant (**2**), flexible non-THI variants (**3** and **6**), and tertiary amine variants (**3** and **7**).

Replacement of the biphenyl group at the 1-position of **1** with the benzophenone substituent as in **5** resulted in an almost 4-fold decrease in activity and decreased selectivity (Table 1). This suggests that C6 cytotoxic activity has some degree of tolerance to modification of the biaryl group at the C-1 position. The observation of activity in the benzophenone suggested a specific molecular recognition event that mediates the cytotoxic activity, suggesting that optimization of this activity should be possible.

Other biaryl structural analogues of **1** explored included O-methylated **1** yielding **4** (Scheme 2), which is less than 2-fold lower activity than **1**. The effect on selectivity is hard to discern since the astrocyte error is large for this compound. The *O*-methyl benzophenone analogue **8** also demonstrated diminished activity and surprisingly abolished selectivity compared to **5**. The N-methylation of **1** to form **7** resulted in a 3-fold decrease in activity and significantly reduced selectivity. Varying the point of attachment of the aromatic moiety also decreased the activity of **2** which is 2-fold less active than **4**. Significant activity was retained for all these THI variants discussed thus far, suggesting we could expand our SAR probe to include analogues that were very different than the lead compound **1**.

Conformationally flexible derivatives were synthesized as linear secondary or tertiary amines linking the disubstituted phenyl and biaryl moieties (**6** and **3**). The rationale of synthesizing these compounds was to determine whether these linear analogues could adopt a conformation similar to the THI ring system and therefore bind to the same target site. These open chain analogues demonstrate significant albeit diminished antiglioma activity compared to **1** but in line with the other analogues. The linear analogue **6** of **1** is more than 3-fold less active than **1**. However, it is noteworthy that the selectivity of **6** is preserved or potentially even enhanced. The tertiary amine **3** demonstrated that the cumulative effect of *O*- and *N*-methyl groups in the open chain analogues such as **6** was a relatively small decrease in cytotoxicity (only approximately 50%). O-Methylation alone generally decreases cytotoxicity by approximately two- to 4-fold. This suggests that the *N*-methyl group may contribute favorably to cytotoxic activity (i.e.

partially counteracts the loss of cytotoxicity associated with the *O*-methyl group). However the cumulative O- and N-methylation has a detrimental effect on selectivity. The synthesis and testing of these variants of **1** allowed the observation of the structural determinants necessary for the cytotoxic activity.

Conclusions

Normal cultured rat astrocytes and C6 rat glioma were used as a differential screen for a variety of 1,2,3,4-tetrahydroisoquinoline (THI) derivatives. Compound **1** selectively blocked the growth of C6 glioma, leaving normal astrocytes relatively unaffected. Compound **1** also demonstrated improved potency and improved to equivocal selectivity in our assay, when compared to other C6 cytotoxic agents such as **9** and **10**, and established chemotherapeutic agents such as BCNU, 5FU, and melphalan. To establish the potential for clinical utility of this activity, we demonstrated that **1** also had cytotoxic activity in human gliomas and other diverse human cancers. Biaryl analogues were synthesized and tested to develop an initial SAR including the following observations concerning glioma selective cytotoxicity: (1) a substituted THI is superior to comparable conformationally mobile analogues, (2) a biaryl moiety confers significant cytotoxicity, and (3) a tertiary amine group in linear compounds is more toxic but perhaps less selective. All the derivatives of **1** tested in this study were more potent than **9** and **10**, suggesting that these molecules represent a new class of antiglioma molecules worthy of further exploration, and these preclinical studies have supported **1** as the most potent of these antiglioma agents.

Experimental Section

1-Biphenyl-4-ylmethyl-1,2,3,4-tetrahydroisoquinoline-6,7-diol hydrochloride (1). 6,7-Bis-benzyloxy-1-biphenyl-4-ylmethyl-1,2,3,4-tetrahydroisoquinoline hydrochloride, compound **18** (2.5 g, 4.56 mmol), was stirred in 10 mL of concentrated aqueous HCl solution and 10 mL of methanol. The reaction mixture was refluxed for 10 h, concentrated under reduced pressure, and then treated with 10 mL of ether to give a solid. The solid was collected on a glass filter funnel, washed with ether (2 × 10 mL), and recrystallized from ether/methanol to give 1.3 g (78%) of **1** as off-white crystals: mp = 208−210 °C; ^{1}H NMR (d_6-DMSO) δ 9.17 (s, 1H, ArOH), 9.09 (s, 1H, NH), 8.90 (s, 1H, ArOH), 7.68 (d, J = 7.9 Hz, 4H, ArH), 7.47 (t, J = 7.3 Hz, 4H, ArH), 7.36 (t, J = 7.3 Hz, 1H, ArH), 6.58 (d, J = 4.0 Hz, 2H, ArH), 4.63 (s, 1H, CH), 3.32 (t, J = 9.9 Hz, 2H, CH_2), 3.17 (q, J = 7.3 Hz, 2H, CH_2), 2.98−2.73 (m, 2H, CH_2); ^{13}C NMR (d_6-DMSO) δ 145.1, 144.0, 139.8, 138.8, 135.4, 130.2, 128.9, 127.4, 126.8, 126.5, 122.7, 122.4, 115.2, 113.5, 55.0, 39.1, 24.2; MS (ES) *m*/*z* 332 $[M + H]^+$. Anal. ($C_{22}H_{21}NO_2 \cdot HCl \cdot 0.25\ H_2O$) C, H, N.

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Supporting Information Available: Characterization of **1**-**4**, **6**, **7, 17**, **18**, **21**, **22**, **26**, and **27** and description of biological assays[10] are available free of charge via the Internet at http://pubs.acs.org.

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