Bioorganic & Medicinal Chemistry Letters 19 (2009) 4308–4311

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Potent agonists of the Hedgehog signaling pathway

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ARTICLE INFO

Article history:
Received 23 April 2009
Revised 18 May 2009
Accepted 20 May 2009
Available online 29 May 2009

Keywords:
Hedgehog signaling pathway
Agonist
SAR study
Small molecule

ABSTRACT

A family of biaryl substituted 1,4-diaminocyclohexanamides of 3-chlorobenzothiophene-2-carboxylic acid is reported as picomolar modulators of Hedgehog protein function. SAR for the 1,4-diaminocyclohexane group is shown to be exquisitely sensitive to substitution on the 4-amino group, and SAR for the 3-chlorobenzothiophene group is highly specific. Preliminary SAR studies of the biaryl substituent led to a picomolar compound with in vivo activity.

The Hedgehog (Hh) family of proteins plays an essential role in embryonic development.[1–3] The protein ligands for the receptor, Sonic (SHh), Indian (IHh), and Desert (DHh), as well as the other protein components of the Hedgehog cascade, show very high interspecies homology, from species as diverse as *Drosophila* and human. The Hh protein receptor, Patched-1 (Ptch-1), is a transmembrane protein with a structure reminiscent of that found in ion channels. Ptch-1 inhibits Smoothened (Smo), a receptor structurally similar to G protein coupled receptors. Upon binding of an Hh protein ligand to Ptch-1, the normally inhibited Smo is disinhibited and it activates the nuclear transcription factor Gli-1 via a signaling cascade.

The three Hedgehog ligands, SHh, IHh, and DHh, are morphogens which have different roles in embryogenesis. Thus, IHh affects cartilage and bone development, SHh influences neuronal development in the CNS, and DHh regulates peripheral neuronal development.

The Hh pathway is essential for the formation of normal nerves in the central and peripheral nervous system. It has been shown that treatment with a Hh protein accelerates the restoration of nerve function in models of trauma and disease.[4] This indicates that the Hh pathway may have a potential therapeutic effect in treating certain neurological disorders (e.g., stroke, Parkinson's disease, spinal cord injury and diabetic neuropathy).

While several small molecule Hh pathway inhibitors have been reported,[5] small molecule activators of this pathway are less well known.[6] We previously disclosed the structures of three compounds from a family of biaryls[7] (which are also described in this Letter; **8**, **11** and **21i**) which were shown to activate the Hh pathway by binding to Smo and we now wish to report an SAR study which led to an improved compound (**21k**) with picomolar potency and in vivo activity.

Biaryl **8** was an HTS hit[8] whose structure was confirmed by resynthesis. This compound has three obvious regions for structural manipulation as indicated in Figure 1: the 3-chlorobenzothiophene, the 1,4-diaminocyclohexane, and the biaryl group. Here we report initial results which demonstrate that both the 1,4-diaminocyclohexane and the 3-chlorobenzo thiophene regions are exquisitely sensitive to structural variation. Further, preliminary SAR at the biaryl group led to compounds with picomolar potency and in vivo activity.

Initially, we investigated the importance of the central diamine functionality for activity. A series of compounds was made with

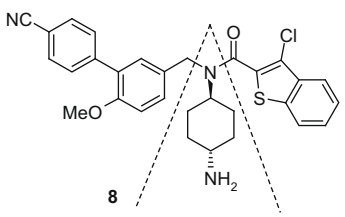

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Figure 1. Initial Hh agonist screening hit.

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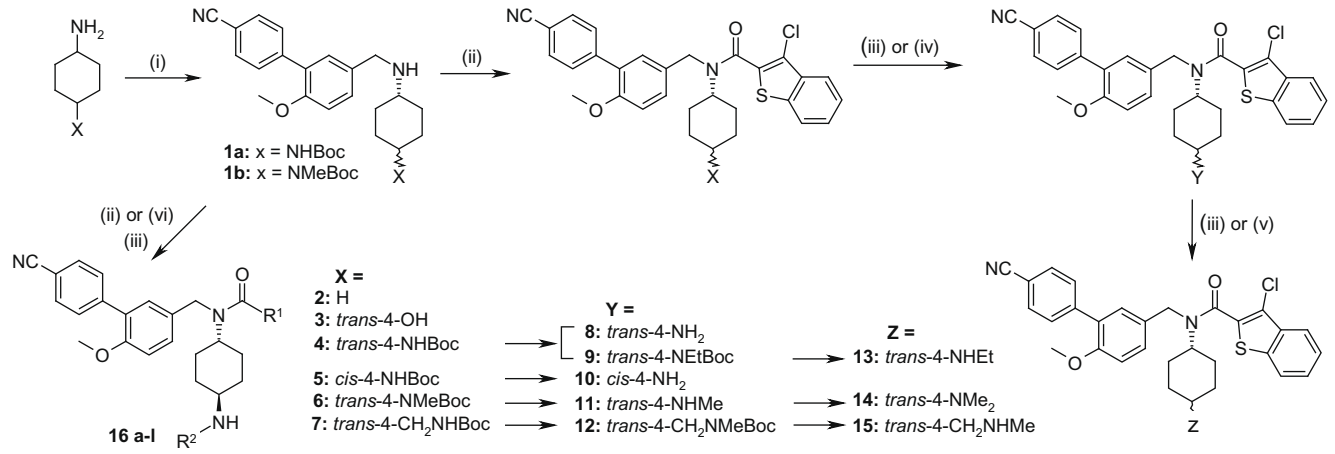
doi:10.1016/j.bmcl.2009.05.096



Scheme 1. Synthesis of 1,4-diaminocyclohexane analogs. Reagents and conditions: (i) $Na(OAc)_3BH$, **20a**, EtOH; (ii) acid chloride, Et_3N, CH_2Cl_2, rt, 18 h; (iii) concd HCl, EtOH (1:2), rt, 3 h; (iv) NaH, alkyl halide, THF, rt, 2 h; (v) alkyl aldehyde, $Na(OAc)_3BH$, toluene, EtOH, rt, 18 h; (vi) carboxylic acid, TBTU, CH_2Cl_2, rt, 18 h.

Table 1
SAR at the 1,4-diaminocyclohexane ring

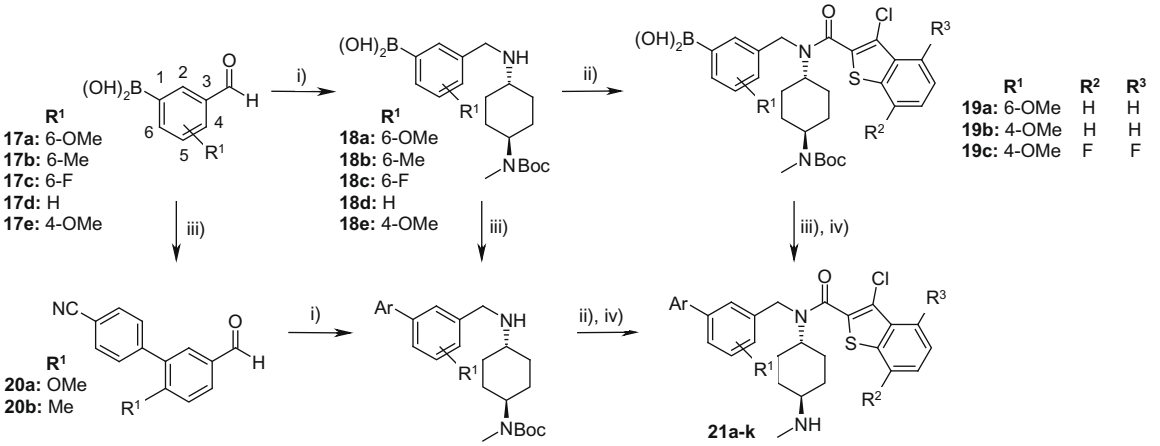
X	Entry	EC_{50}[a] (nM)	Y	Entry	EC_{50}[a] (nM)	Z	Entry	EC_{50}[a] (nM)
H	**2**	>10,000	*trans*-4-NH_2	**8**	5000	*trans*-4-NHEt	**13**	>10,000
trans-4-OH	**3**	5000	*cis*-4-NH_2	**10**	>10,000	*trans*-4-NMe_2	**14**	>10,000
			trans 4-NHMe	**11**	40 (±20), A_{max} = 80%	*trans*-4-CH_2NHMe	**15**	>10,000

[a] EC50 and A_{max} values of final product defined according to Ref. 8.

modifications to this part of the molecule (Scheme 1, Table 1). The general route to the target compounds (Scheme 1) involved the reductive amination of aldehyde **20a** (Scheme 2) with a variety of Boc protected amines to give the corresponding secondary amines. Amide formation from these amines with 3-chlorobenzothiophene-2-carbonyl chloride gave final compounds **2** and **3** and intermediates **4–7**. Boc-deprotection of intermediates **4–6** gave target amines **8, 10–11**. Target amines **13** and **15** were prepared by alkylation of the corresponding Boc-protected amines (**4** and **7**) using the relevant alkyl halide, followed by Boc-deprotection. An alternative procedure was used to prepare the tertiary amine **14**, by reductive alkylation of **11** with formaldehyde.

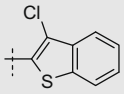
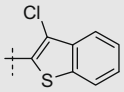
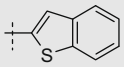
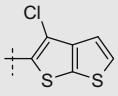
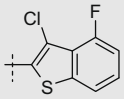
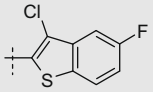
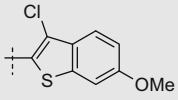
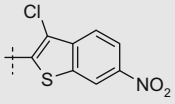
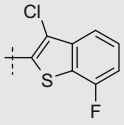
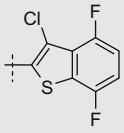
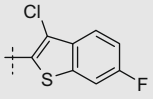
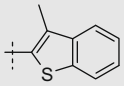
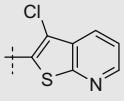
The SAR data in Table 1 demonstrate the critical dependence of activity upon the *trans*-1,4-diaminocyclohexane scaffold. The cis analog **10** was much less active than **8** (>10 μM), as was the cyclohexyl analog **2** which lacks the 4-amino group. The hydroxy analog **3** was also only weakly active. A 100-fold boost in potency over **8** occurred with the *trans*-4-NH-methyl analog **11**, whereas the larger *trans*-4-NH-ethyl analog **13** was much less active, as was the extended *trans*-4CH_2NH-methyl derivative **15**. The *trans*-4-*N,N*-dimethyl analog **14** also showed very little activity. An extended series of other much less active analogs (Table 1a, Supplementary data) further underscores the exceptional activity of the *N*-methyl analog **11**. The very specific and potent activity of **11** may result from a more basic secondary amine[9] with a very small, lipophilic substituent (methyl) which binds a receptor region that is quite intolerant of steric bulk.

SAR of the 3-chlorobenzothiophene group was next investigated and a series of structurally related amides was prepared. A few derivatives were based around the original hit **8**, but the



Scheme 2. Synthesis of biaryl analogs. Reagents and conditions: (i) *trans*-*N*-methyl-*N*-*tert*-butoxycarbonyl-1,4-diaminocyclohexane, acetic acid, $Na(OAc)_3BH$, THF, toluene; (ii) acid chloride, DIPEA, CH_2Cl_2; (iii) aryl bromide, $Pd(PPh_3)_4$, Cs_2CO_3, toluene, EtOH, MW 180 °C, 20 min; (iv) concd HCl, EtOH, 3 h, rt.

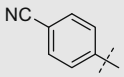
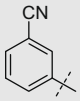
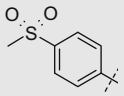
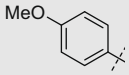
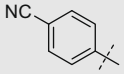
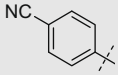
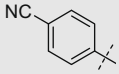
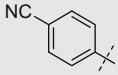
Table 2
SAR at the 3-chlorothiophenecarboxamide group

Entry	R^1	R^2	EC_{50}[a] (nM)
8		H	5000
11		Me	40
16a		H	>10,000
16b		Me	300
16c		Me	15
16d		Me	40
16e		Me	>10,000
16f		Me	>10,000
16g		Me	10
16h		Me	2 (±3), A_{max} = 80%
16i		Me	2000
16j		H	>10,000
16k		H	5000
16l		Me	>10,000

[a] EC_{50} and A_{max} values determined according to Ref. 8.

majority were derived from the more potent *N*-methyl analog **11**. Amides **16a–h** and **16j** (Scheme 1, Table 2) were prepared by reaction of the secondary amines **1a** or **1b** with the corresponding acid chlorides[10] whereas amides **16i,k,l** were prepared by coupling reactions of **1a** or **1b** to the corresponding carboxylic acids[11,12] using TBTU.

Table 3
SAR around the biaryl region

Entry	Ar	R^1	R^2	R^3	EC_{50}[a] (nm)
11		6-OMe	H	H	40
21a		6-OMe	H	H	300
21b		6-OMe	H	H	90
21c		6-OMe	H	H	15
21d		6-OMe	H	H	100
21e		6-F	H	H	150
21f		H	H	H	200
21g		6-Me	H	H	2000
21h		4-OMe	H	H	25
21i		H	H	H	30
21j		4-OMe	H	H	2
21k		4-OMe	F	F	0.3 (±0.5), A_{max} = 80%

[a] EC_{50} and A_{max} values determined according to Ref. 8.

The data in Table 2 demonstrate that removal of the chlorine atom (**16a**), or the phenyl ring (**16j**) results in a loss of activity relative to **8**, whereas replacement of chlorine with a methyl group (**16k**) retained weak activity similar to **8**. Continued SAR work resulted in a few derivatives that showed a significant increase in activity compared to **11**. Substitution of fluorine into the 3-chlorobenzothiophene in the 4-, 5-, or 7-positions gave the potent analogs **16c**,**d**,**g**, respectively. However the 6-fluoro analog **16i** was only weakly active. Additional six-substituted analogs (**16e,f**) were prepared and these were also much less active. Heterocyclic replacements (**16b,l**) of the phenyl ring in the 3-chlorobenzothiophene were prepared, but this resulted in a significant loss of activity. Combining the 4- and 7-fluorine substituents into the 4,7-difluorothiophene **16h**, led to an EC_{50} of 2 nM.

Preliminary SAR of the biaryl region was explored last. Target compounds **21a–k** were prepared from the boronic acid benzaldehydes[13] **17a–e** in a four step synthesis (Scheme 2). The first three steps involved a reductive amination with *trans*-*N*-methyl-*N*-*tert*-butoxycarbonyl-1,4-diaminocyclohexane,[14] a Suzuki coupling reaction with the corresponding aryl bromide and an amide formation but the order of these steps varied between compounds. Target compound **21g** was obtained by a Suzuki coupling reaction on **17b** to give the biaryl aldehyde **20b** followed by reductive amination then amide formation followed by Boc-deprotection, while target compounds **21c,j,k** were obtained by reductive aminations on aldehydes **17a** and **17e** to give amines **18a** and **18e** respectively. The amide formations then gave **19a–c** and Suzuki coupling reactions followed by Boc-deprotections gave the desired compounds. Finally, targets **21a–b, d–f, h–i** were obtained by reductive aminations on aldehydes **17a,c–e** to give amines **18a–d** followed by Suzuki coupling reactions then the amide formation followed by Boc-deprotection.

The proximal aryl ring of the biaryl group was first chosen for exploration. Deletion of the methoxyl group (**21f**) (Table 3) resulted in loss of activity, as did replacement with methyl (**21g**) or fluoro (**21e**). The isomeric methoxyl analog (**21h**), showed slightly improved activity relative to the parent (**11**).

Changes to the distal aryl group demonstrated that deletion of the *para*-cyano group (**21a**) or replacement by methoxyl (**19d**) both led to some reduction of activity, as did the isomeric cyano analog (**19b**). Replacement of the *para*-cyano group by sulfone (**21c**) led to an enhancement of activity. A number of heterocyclic analogs of the distal ring were prepared, of which the 4-pyridyl compound (**21i**) was notable in potency. Combining this preferred structure with the potent 4,7-difluoro substituent pattern on the 3-chlorobenzothiophene led to **21k**, a compound of exceptional, picomolar activity.

The compounds in Tables 1 and 2 were tested for in vitro Hh agonist activity in a Gli-luc reporter assay using mouse cell lines.[7,8,15] Compound **21k** was also tested using daoy (human) cell lines and gave an EC_{50} = 0.4 nM (±0.8), A_{max} = 86%. The pharmacokinetic (PK) profile of **21k** was then measured in mouse; after an oral dose of the hydrochloride salt at 5 mg/Kg administered as a suspension in 0.5% methyl cellulose **21k** showed a C_{max} of 50 ng/ml and $T_{1/2}$ of 4 h in plasma. The compound also showed a good distribution into the brain, where a C_{max} of 81 ng/ml and $T_{1/2}$ of 4 h was achieved.[16]

In conclusion, we have described the optimization of a micromolar hit **8** to afford compound **21k**, a potent Hh agonist with a good PK profile. As such, **21k** has potential as a novel therapy for stroke and other neurological disorders.

Acknowledgements

The authors thank Dane Springer, Warren Hirst and the team at Wyeth for their helpful discussions and support in publishing this work.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.05.096.

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8. Screening for agonists was done in an isolated stable clone of the C_3H_{10} $T_{1/2}$ cell line transfected with a luciferase reporter downstream of multimerized Gli binding sites and a minimal promoter as previously reported (US Patent 6,613,798). In this system a 10–20-fold up-regulation of luciferase activity was observed upon 24 h stimulation by Hh protein. The EC_{50}, the concentration of half maximal stimulation by small molecule, as well as the A_{max}, the maximal response relative to the Hh protein control was determined for selected compounds. For a more detailed description of the GLi-luc assay see: Brunton, S. A.; Stibbard, J. H. A.; Rubin, L. L.; Kruse, L. I.; Guicherit, O. M.; Boyd, E. A.; Price, S. *J. Med. Chem.* **2008**, *51*, 1108.
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15. A member of this class of compounds was shown to activate Shh signaling using a primary astrocyte model. See: Atkinson, P. J.; Dellovade, T.; Albers, D.; Von Schack, D.; Saraf, K.; Needle, E.; Reinhart, P. H.; Hirst, W. *J. Neurochem.* **2009**, *108*, 1539.
16. Compound **21K** showed a high volume of distribution (Vss = 23.4 L/kg at 3 mg/kg iv in rats) and the compound was detected in skin (data not shown).