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THIAZOLE-DERIVED POTENT, HIGHLY BIOAVAILABLE SHORT DURATION GROWTH HORMONE SECRETAGOGUES

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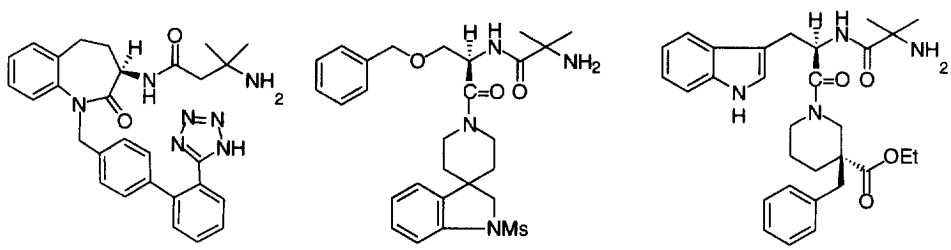
Abstract: Replacement of the phenyl in **3** with a 2-pyridyl or 4-thiazolyl group resulted in increased potency in the rat pituitary cell GH release assay and in beagles.

Introduction Growth hormone secretagogues (GHS's) are growth hormone releasing peptides (GHRP) and their small organic molecule mimetics that cause GH secretion from the pituitary somatotroph.[1] They exert their stimulatory effect through a recently identified orphan G-protein coupled receptor (GHSr).[2] Clinical studies have shown that these GHS are efficacious in promoting GH release in man.[3] Several structural types of small molecule GHS have been reported, among them three series from these laboratories that have been studied extensively. These are the biphenyl benzolactam class typified by **1**;[4] the 4-spiropiperidine class represented by **2**;[5] and the 3,3-disubstituted piperidine class typified by **3**.[6] Compound **1** is short-acting and lacks good oral bioavailability; on the other hand, compound **2** has a long duration of action after oral administration. Chronic dosing of **2** results in considerable down regulation of GH release but prolonged elevation in IGF-1 (insulin-like growth factor-1) levels.[5] Compound **3** was developed as an orally active short duration compound that, like the previous short duration injectable secretagogues, causes only moderate down regulation of GH release after chronic administration in beagles.[7] The metabolism of both the indole and 3-ester groups of **3** is thought to contribute to the short duration of **3** relative to **2**. Herein we report some of our structure-activity relationship (SAR) studies in which the phenyl group has been replaced with various heterocycles. These studies resulted in the identification of another short duration compound (1-[2(*R*)-(2-amino-2-methylpropionylamino)-3-(1*H*-indol-3-yl)propionyl]-3-(4-thiazolyl)piperidine-3(*S*)-carboxylic acid ethyl ester, which is bioavailable and more potent than **3**.



1 **2** **3**

Figure 1

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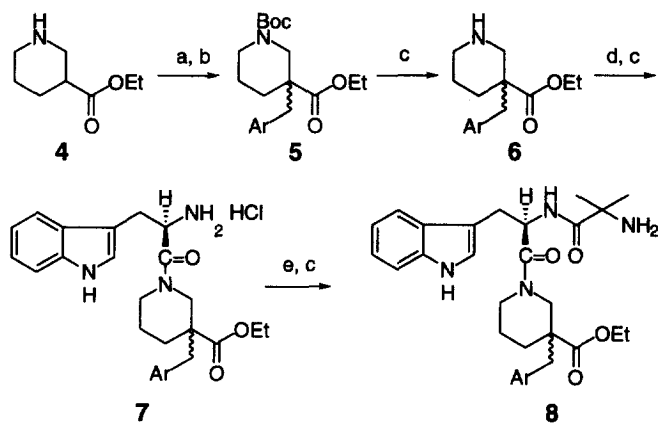
Chemistry The phenyl ring in **3** was replaced with heteroaryls to achieve potentially useful alterations in its physical properties. Among the many small heteroaryls possible, we chose pyridine and thiazole since they are proven phenyl replacements and are present in various drugs. All regioisomers were synthesized for a full comparison. The preparation of GHS of general structure **8** was carried out in nine linear steps from the commercially available (±)-ethyl nipecotate, as described in Scheme 1.[6] Treatment of (±)-ethyl nipecotate (**4**) with di-t-butyl dicarboxylate followed by alkylation with the halide ($ArCH_2Br$ or $ArCH_2Cl$) gave the intermediate **5**. The three isomers of picolyl chloride are commercially available as their hydrochloride salts, and were converted to free base by partitioning between dichloromethane and dilute sodium hydroxide immediately before use. The thiazolylmethyl bromides were prepared from their corresponding methyl compounds with *N*-bromosuccinimide in carbon tetrachloride under radical reaction conditions. Removal of the Boc protecting group yielded the racemic ethyl 3-arylmethylnipecotate **6**. Incorporation of D-Trp and Aib was carried out successively by EDC/HOBT coupling of the Boc amino acid followed by HCl removal of the Boc protecting group to afford the final product **8** as a mixture of diastereomers. The acids **10** and **11** (Table 2) were prepared by direct saponification of their corresponding ethyl esters.

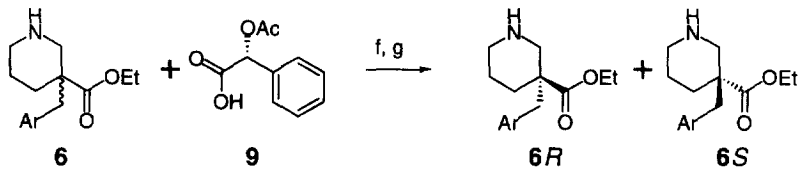
Scheme 1. Synthesis **8**



Reagents: (a) Boc_2O/DCM; (b) KHMDS, $ArCH_2Br$ DMF-THF, -78–0 °C; (c) HCl/EtOAc; (d) Boc-D-Trp-OH, HOBT, EDC, DCM; (e) Boc-Aib-OH, HOBT, EDC, DCM.

The enantiomericaly pure final products (where Ar is 2-pyridyl or 4-thiazolyl) were prepared starting with chiral nipecotates. Attempts to resolve the racemic nipecotates **6** with chiral acids failed under various conditions. However, coupling with (*R*)-(-)-*O*-acetylmandelic acid **9** gave two separable diastereomers.[8] Removal of the chiral auxiliaries was carried out under acidic conditions to give both pure enantiomers **6*R*** and **6*S***. The absolute stereochemistry of the more potent final products (**8-1*S*** and **8-4*S***) was assigned *S* based on an X-ray crystallographic analysis of the crystalline amide intermediates.

Scheme 2. Resolution of **6**



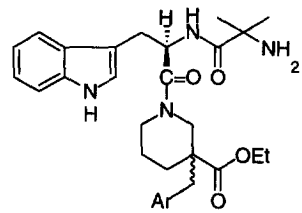
Reagents: (f) HOBt, EDC, DCM; separated by silica column; (g) reflux, HCl/EtOH.

Results and Discussion Our analogs of **3** were first tested in vitro for their ability to stimulate GH release from rat pituitary cells under the previously described conditions.[9] Selected compounds were then evaluated in the beagle dog model.[3] The minimum effective dose that caused at least a fourfold increase in serum GH levels was considered a positive response and was used to compare in vivo potencies.

Table 1 summarizes the results from SAR studies that were carried out by replacing the phenyl with all three regioisomers of pyridine and thiazole. Properties of **3** are given for comparison. As can be seen from the table, the 2-pyridyl isomer is preferred for in vitro potency over its 3- and 4- regioisomers, although the latter two also had low nanomolar activities. As a mixture of two diastereomers, the 2-pyridyl analog **8-1** demonstrated an EC_{50} of 0.5 nM in the rat pituitary GH release assay. All three compounds (**8-1**, **8-2**, **8-3**) were effective in releasing GH in beagles when dosed at 0.5 mg/kg po. The two diastereomers of **8-1** (**8-1*S***, **8-1*R***) were subsequently synthesized for further evaluation. As expected, the resultant diastereomers differ in potency with the *S*-epimer **8-1*S*** being more potent, as was found with **3**. To our best knowledge, **8-1*S***, with an EC_{50} of 0.2 nM, has the most potent GHS activity in the rat pituitary assay for small molecules reported to date. **8-1*S*** was active in beagles when administered iv at 0.025 mg/kg (lowest dose tested). However, its high in vitro potency and iv potency did not translate into unusually high oral activity in beagle dogs. Although both dogs responded well at 0.25 mg/kg po, neither of the two dogs responded when tested at 0.125 mg/kg. The acid **10** (Table 2), hydrolysis product of **8-1*S***, was only weakly active in the rat pituitary assay as observed previously with **12**.[6] Nonetheless, the sub-nanomolar potency of **8-1*S*** encouraged additional heterocylic substitutions at this position.

We then shifted our attention to the thiazole series. As observed in the pyridine series, all three isomers (2-, 4-, and 5-) had nanomolar EC_{50} although they are somewhat less potent than the pyridines (Table 1). The 4-thiazolyl derivative **8-4** is most active in the pituitary cell assay with an EC_{50} of 1 nM as a mixture of diastereomers. More importantly, **8-4** appeared to be a very powerful GHS in beagles. Very strong responses were observed with **8-4** in two beagles at 0.5 mg/kg po (lowest dose tested) suggesting a higher bioavailability than that of **8-1*S***. The response of beagles to **8-5** was weaker reflecting its lower in vitro potency. The two diastereomers of **8-4** (**8-4*S*** and **8-4*R***) were subsequently synthesized for further evaluation. Again, the *S*-epimer **8-4*S*** is more potent with an EC_{50} of 0.5 nM in the rat pituitary assay, while that of **8-4*R*** is 15 nM. Its corresponding acid **11** was weakly active as expected.

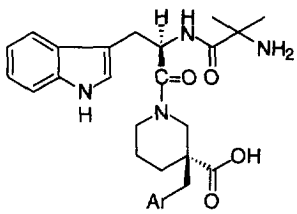
Table 1. GHS potency in the rat pituitary cell assay[a] and in Beagles[b]



Entry	Ar	rat Pituitary EC_{50} (nM)	Beagles: in vivo GH release			
			iv dose (mg/kg)	responses	po dose (mg/kg)	response
3		1.6	0.01	1/1	0.25 0.5	6/8 8/8
8-1		0.5	0.1	1/1	0.5	2/2
8-2		2.2	0.1	1/1	0.5	2/2
8-3		4.4	0.1	1/1	0.5	2/2
8-1*S*		0.2	0.025 0.05	1/1 1/1	0.125 0.25	0/2 3/3
8-1*R*		3.0	0.05	1/1	0.25	1/2
8-4		1.0	0.1	1/1	0.5 1.0	2/2 2/2
8-5		4.2	0.25	2/2	0.25 0.5	1/2 2/2
8-6		6.1	NA			
8-4*S*		0.5	0.0025	1/1	0.125 0.25	3/4 4/4
8-4*R*		15	0.1	1/1	0.5 1.0	1/2 2/2

[a]EC_{50} Values are for half-maximal release of GH in the rat pituitary cell assay, normalized against standards **1** (60 nM).[4] [b]Doses shown represent the lowest dose where fourfold elevations over basal GH levels were observed.

Table 2. GHS potency of parent acids



Entry	Ar	EC_{50} (nM)
10	2-Py	570
11	4-Thiazolyl	>500
12	Ph	190

Compound **8-4*S*** was active when administered iv to beagles at 0.0025 mg/kg, which is ten times more active than **2**. In an eight-dog cross over study,[5] **8-4*S*** was administered orally to beagles at doses of 0.125, 0.25, and 0.5 mg/kg. It increased GH levels with mean peaks of 12.8, 30.6, and 89.3 ng/mL versus 2.8 ng/mL for placebo. Peak GH levels occur between 15–45 min after dosing, and return to baseline after 60 min reflecting its short duration of action. On the other hand, GH levels were still elevated after 90 min for **2** at oral doses of 0.25 and 0.5 mg/kg. When **8-4*S*** was administered daily at 0.25 mg/kg for 7 days, the GH responses on days 1, 4, and 7 were similar suggesting little down regulation after chronic dosing.

The pharmacokinetic properties of **8-4*S*** in male beagle dogs included bioavailability of 44% (po dose: 3.95 mg/kg; iv dose: 0.49 mg/kg). Clearance rate (Cl_p), volume of distribution (V_{dss}), and half life ($t_{1/2}$) were 26.5 mL/min/kg, 0.97 L/kg, and 1.3 h, respectively. However, the pharmacokinetics of **8-4*S*** in rats could not be measured due to very rapid hydrolysis of the ethyl ester upon contact with rat plasma. For comparison purposes, the bioavailability of **3** is 29% with similar Cl_p (23 mL/min/kg), V_{dss} (1.3 L/kg), and $t_{1/2}$ (1.2 h) in beagles. It has a bioavailability of 12% in Sprague–Dawley rats.

The in vitro specificity of **8-4*S*** and its acid metabolite **11** were evaluated in over 100 receptor and enzyme assays. No significant binding was observed at 10 μM, in assays which included opiate, neurokinin, adrenergic, somatostatin, cholecystokinin, bradykinin, vasopressin, and benzodiazepine receptors.

In summary, systematic studies of pyridine and thiazole analogs of **3** have resulted in a series of potent growth hormone secretagogues. The 2-pyridyl derivative **8-1*S*** has an EC_{50} 0f 0.2 nM in the rat pituitary assay, which is the most potent small molecule GHS reported to date in that assay. The 4-thiazolyl analogue **8-4*S*** (EC_{50} = 0.5 nM) has an excellent oral bioavailability and short duration of action in beagles. Details of in vivo efficacy studies with these compounds will be reported in due course.

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