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Preliminary communication

Novel simple thioureas with growth hormone releasing properties

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Summary — A series of thioureas containing two aromatic groups and one amino group was synthesized as potential growth hormone secretagogues. The growth hormone (GH) releasing properties of these compounds (**10–24**) were evaluated using primary rat pituitary cells. The most potent compound **20** showed potency with an EC_{50} of 1.8 μM. Two constrained analogs **23** and **24** showed similar activity.

growth hormone secretagogue / thiourea / GHRP-6 / GHRP-2 / L-629,429, MK-0677

Introduction

Several growth hormone releasing peptides (GHRP), the most interesting being GHRP-6 (**1**) and GHRP-2 (**2**), promote the release of endogenous growth hormone (GH) from the pituitary via a novel mechanism [1]. Several non-peptidyl mimics of **1** and **2**, eg, L-692,429 (**3**) have recently been reported [2] and shown to release GH in humans [3]. Also a number of penta-, tetra- and tripeptides [4, 5] and the peptidomimetic MK-0677 (**4**) [6] have been shown to release GH in various species including man (fig 1).

Based on structure–activity relationships of **1**, **2** and **3** [2, 7], a lead discovery program was established with the aim of generating new lead structures. We hypothesized that the incorporation of two aromatic groups and an amino function into a scaffold might prove sufficient to obtain compounds with GH-releasing properties. Based on this hypothesis, we set up a research program to identify simple leads which could easily be prepared and were able to release GH with affinities below 10 μmol. We here report an interesting series of compounds based on a thiourea skeleton with the proposed aromatic and amino pharmacophores. All compounds could easily be prepared in less than two steps.

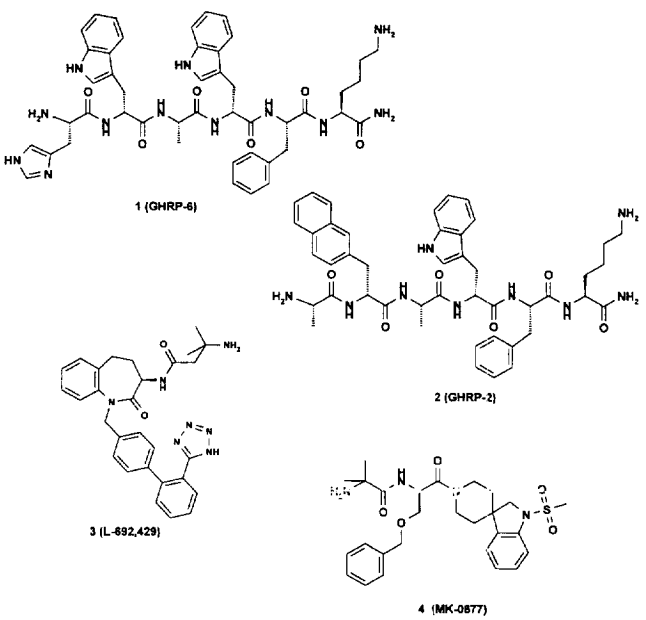

Fig 1. Structure of compounds **1–4**.

Chemistry

The GH-releasing compounds were typically constructed as depicted in scheme 1 by the addition of an amine bearing two aromatic groups to either dimethyl-

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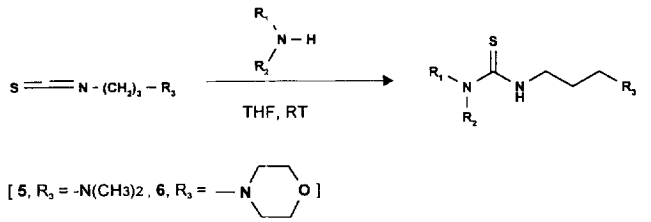


Scheme 1. Synthetic scheme for preparation of thiourea.

aminopropyl isothiocyanate **5** or morpholinopropyl isothiocyanate **6** in tetrahydrofuran at room temperature.

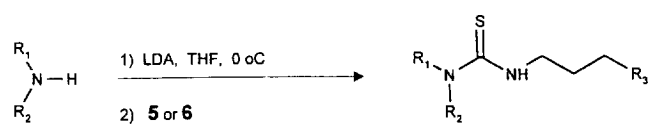
In cases where R_1 or R_2 were aromatic, addition of a base, typically lithium diisopropylamine in tetrahydrofuran at 0 °C was necessary to activate the amino group properly, as depicted in scheme 2.

The secondary amines bearing the two aryl groups were prepared by reductive amination with sodium borohydride in ethanol unless they were commercially available.

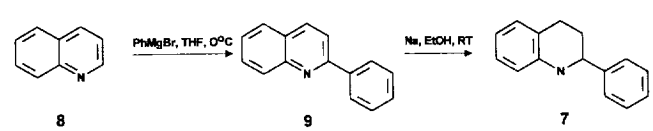
The 2-phenyl-1,2,3,4-tetrahydro-2*H*-quinoline **7** was prepared by Grignard reaction of phenylmagnesium bromide with quinoline **8** to give 2-phenylquinoline **9** [8] which could be reduced with sodium in ethanol. The reduction of **9** gave moderate to low yield, probably due to a competing disproportionation of the intermediate 2-phenyldihydroquinoline.

Results and discussion

GH release in vitro was determined in primary rat pituitary cells as described by Sartor et al [9] and Heimann et al [10]. The potencies of L-692,429,



Scheme 2. Synthetic scheme for preparation of thiourea in the presence of base.

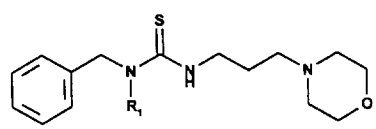


Scheme 3. Synthetic scheme for preparation of 2-phenyl-1,2,3,4-tetrahydro-2*H*-quinoline 7.

MK-0677 and GHRP-6 were determined at 125 ± 18 nM, 0.4 ± 0.2 nM and 2.0 ± 0.3 nM, respectively; all three compounds were similar in efficacy. Tables I–III show the potencies of 15 simple thioureas containing two aromatic groups and one tertiary amine.

The calculated EC_{50} values for **10–24** reported in tables I–III show that all compounds except **16** and **17** exhibited weak GH releasing ability in the range from 1 to 10 μM. The thioureas **16** and **17** were both inactive, indicating a necessity for aromatic groups without heteroatoms. The most potent compound **20** which was constructed of benzyl, (naphth-2-yl)methyl and a dimethyl amine showed an EC_{50} in the range 1.8–1.8 μM. When the dimethyl amino group was replaced by a morpholinogroup as in **14**, a slight reduction in potency occurred (EC_{50} 3.5–4.0 μM). This could indicate that the dimethyl amino function was more suitable; however, when the aromatic system is constrained, as in **23** (EC_{50} 1.7–3.0 μM) and **24** (EC_{50} 4.0–6.0 μM), the opposite observation is made. All the active compounds showed efficacies varying from 30 to 55% of GHRP-6. This low efficacy may suggest that the compounds were partial

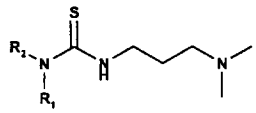
Table I. GH potency and efficacy of thioureas.



Thiourea	R_1	EC_{50} (μM)	E_{max} (% E_{max} of GHRP-6)
10	Phenyl	4.0	45
11	4-Methoxyphenyl	9.0	30
12	4-[1,2,3]-(Thiadiazol-4-yl)phenyl	4.5*	45*
13	Benzyl	4.8	45
14	(Naphth-2-yl)methyl	3.8	40
15	Phenethyl	4.0	50
16	Quinolin-2-yl	Inactive	
17	Pyridin-3-yl	Inactive	
18	4-(2-(Tetrazol-5-yl)-phenyl)benzyl	9.0	45

Data are shown as means (n = 2),*(n = 4). Assay conducted as described in *Materials and methods*.

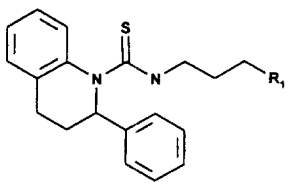
Table II. GH potency and efficacy of thioureas.



Thiourea	R_1	R_2	EC_{50} (μM)	E_{max} (% E_{max} of GHRP-6)
19	Benzyl	Phenyl	9.0	55
20	Benzyl	(Naphth-2-yl)methyl	1.6*	50*
21	Phenyl	(Naphth-1-yl)methyl	7.0	45
22	Phenyl	(Naphth-2-yl)methyl	3.0	45

Data are shown as means (n = 2), *(n = 4). Assay conducted as described in *Materials and methods.*

Table III. GH potency and efficacy of thioureas.



Thiourea	R_1	EC_{50} (μM)	E_{max} (% E_{max} of GHRP-6)
23	Morpholin-4-yl	2.2*	40*
24	Dimethylamine	5.0	50

Data are shown as means (n = 2), *(n = 3). Assay conducted as described in *Materials and methods.*

agonists; compound **15** was therefore tested together with GHRP-6. As seen in table IV, the GH releasing effect of GHRP-6 was 75% inhibited by 100 μM **15**, indicating a similar site of action.

Conclusion

A series of novel simple thioureas containing two aromatic groups and one tertiary amino group have been prepared and tested for GH-releasing properties in a primary rat pituitary assay. The most active compound was **20**, bearing a benzyl- and a 2-naphthylmethyl group on N1 and 3-dimethylaminopropyl on N3 of the thiourea skeleton and showed an EC_{50} of 1.8 μM. A less flexible compound **23** also showed comparable potency, with an EC_{50} of 2.2 μM. The compounds all showed partial agonism to GHRP-6.

Although the thioureas do not show potency in the low nanomolar range like compounds **1–4**, they represent a new class of simple compounds that may be optimized to show high affinity to the GHRP receptor [11].

Experimental protocols

Materials and methods

All tissue culture reagents were purchased either from Gibco Laboratories or Sigma. Trypsin was obtained from Worthington.

Rat pituitary culture

The method was a modification of that of Heiman et al [10]. Briefly, the rats were decapitated and pituitaries dissected. The neurointermediate lobes were removed and the remaining tissue was immediately placed in ice-cold isolation buffer (Gey's medium supplemented with 0.25% D-glucose, 2% non-

Table IV. Inhibition of GHRP-6 GH releasing properties.

Compound	*% Inhibition*
GHRP-6 (10 nM)	0
GHRP-6 (10 nM) + **15** (100 μM)	70–80

Data are shown as means (n = 2). Assay conducted as described in *Materials and methods.*

essential amino acids and 1% BSA). The tissue was cut into small pieces and transferred to isolation buffer supplemented with trypsin and DNase. This mixture was incubated at 70 rotations/min for 35 min at 37 °C. Using a standard Pasteur pipette, the tissue was then aspirated into single cells. After dispersion, cells were filtered through a nylon filter (160 mm) to remove undigested tissue. The cell suspension was washed with isolation buffer supplemented with trypsin inhibitor and finally resuspended in culture medium; DMEM supplemented with 25 mM HEPES, 4 mM glutamine, 0.075% sodium bicarbonate, 0.1% non-essential amino acid, 2.5% foetal calf serum, 3% horse serum, 10% fresh rat serum, 1 nM T_3 and 40 mg/L dexamethazone, to a density of 2 x 10^5 cells/mL. The cells were seeded into microtiter plates (Nunc, Denmark), 200 mL/well, and cultured for 3 days at 37 °C and 8% CO_2.

Following the culture period, the cells were washed with stimulation buffer (Hanks balanced salt solution supplemented with 1% BSA, 0.25% D-glucose and 25 mM HEPES), and preincubated for 1 h. Afterwards, the buffer was exchanged and different concentrations of the compound were added, and the plates were incubated for 15 min. The medium was decanted and analyzed for GH content in a rGH SPA test system. The compounds were tested in concentrations ranging from 10 nM to 100 μM each with triple determinations. Each compound being tested in two to four separate experiments.

Data analysis

Dose–response curves were constructed using non-linear regression, sigmoidal dose–response equation with variable slope (fig P, Biosoft). From the dose–response curves, the efficacy (maximal GH released, E_{max}) was calculated and expressed in percentage of the E_{max} of GHRP-6 tested in the same experiment. The potency (EC_{50}) was determined as the concentration inducing half-maximal stimulation of the GH release. All values given are mean values.

Materials and experimental procedures

All reactions were carried out under a nitrogen atmosphere with magnetic stirring. Organic phases were dried over anhydrous magnesium sulfate and the solvent was evaporated under vacuum. Column chromatography was carried out on Merck silica gel 60 (art 9385). HPLC analysis was performed using a 5-mm C18 4 x 250 mm column, eluting with a 20–80% gradient of 0.1% trifluoroacetic acid/acetonitrile and 0.1% trifluoroacetic acid/water for 30 min at 35 °C. All NMR spectra were recorded on a Bruker AMX2 spectrometer operating at 400 MHz for ^{1}H and shifts (δ) are given in parts per million (ppm). Melting point (mp) is given in °C and is not corrected. Compounds used as starting material are either known compounds or compounds which can readily be prepared by methods known per se.

General procedure for the preparation of thioureas

A stirred solution of the primary amine (47 mmol) and the aldehyde (47 mmol) in 99.9% ethanol (200 mL) was refluxed overnight and cooled to room temperature. Then sodium borohydride (1.8 g, 47 mmol) was added in small portions over a period of 15–30 min. After 30 min, water (400 mL) was added and the solution was concentrated in vacuo to a minimum and extracted four times with ethyl acetate/THF 1:1 (200 mL). The combined organic layer was dried, filtered and concentrated in vacuo to give an oil which was dissolved in ethyl acetate (100 mL) and treated with 3 M hydrogen chloride in ethyl acetate (50 mL). The white solid which precipitated out was collected, washed four times with ethyl acetate (50 mL) and dried overnight in vacuo to give the corresponding secondary amine as a hydrochloride salt. The hydrochloride was dissolved water/methanol 1:1 (600 mL) and saturated sodium bicarbonate (200 mL) was added. The solution was concentrated in vacuo to a minimum and extracted four times with ethylacetate (200 mL), dried and concentrated in vacuo to the corresponding secondary amine as a free amine. The resulting amine in THF (20 mL) was added to a solution of 3-(morpholin-4-yl)propyl isothiocyanate (1.5 eq) or (3-(dimethylamino)propyl) isothiocyanate in THF (30 mL) at –78 °C over a period of 10 min. The mixture was stirred at room temperature overnight and the solvent was removed in vacuo and the resulting oil chromatographed on silica in 10% methanol/methylene chloride to give an oil. The oil, dissolved in ethyl acetate (20 mL) was treated with 3 M HCl in ethyl acetate (10 mL). The solvent was removed in vacuo to give the desired product.

1-Benzyl-3-(3-(morpholin-4-yl)propyl)-1-phenylthiourea hydrochloride ***10***. Yield 17%; ^{1}H-NMR ($CDCl_3$, free base): δ 2.00 (t, 2H), 3.07 (t, 2H), 3.25 (m, 4H), 3.70 (t, 2H), 3.85 (m, 4H), 5.45 (s, 2H), 7.00–7.5 (m, 10H); reverse phase HPLC: 19 min.

1-Benzyl-3-(3-(morpholin-4-yl)propyl)-1-(4-methoxy-phenyl)-thiourea hydrochloride ***11***. Yield 8%; ^{1}H-NMR ($CDCl_3$, free amine): δ 1.70 (t, 2H), 2.25 (m, 6H), 2.45 (t, 2H), 3.50 (t, 2H), 3.70 (t, 2H), 3.80 (s, 3H), 5.50 (s, 2H), 5.80 (b, 1H), 6.80–6.95 (m, 4H), 7.20–7.35 (m, 5H); anal ($C_{22}H_{29}N_3O_2S$•HCl•$2H_2O$) C, H, N; reverse phase HPLC: 20 min.

1-Benzyl-3-(3-(morpholin-4-yl)propyl)-1-(4-([1,2,3]thiadiazol-4-yl)benzyl)thiourea hydrochloride ***12***. Yield 81%; ^{1}H-NMR ($CDCl_3$, free amine): δ 2.00 (t, 2H), 3.00 (t, 4H), 3.45 (d, 2H), 3.75 (t, 4H), 4.00 (d, 2H), 5.05 (s, 2H), 5.10 (s, 2H), 7.20–7.45 (m, 7H), 8.05 (d, 2H), 9.20 (s, 1H); anal ($C_{24}H_{29}N_5OS_2$• HCl•H_2O) C, H, N; reverse phase HPLC: 23 min.

1,1-Dibenzyl-3-(3-(morpholin-4-yl)propyl)thiourea-hydrochloride ***13***. Yield 88%; ^{1}H-NMR (CD_3OD): δ 2.00 (t, 2H), 3.00 (t, 4H), 3.45 (t, 2H), 3.7 (t, 4H), 4.05 (t, 2H), 5.00 (s, 4H), 7.2–7.4 (m, 10H); anal ($C_{22}H_{30}N_3OS$•HCl) C, H, N; reverse phase HPLC: 20 min.

1-Benzyl-3-(3-(morpholin-4-yl)propyl)-1-((naphth-2-yl)-methyl)thiourea hydrochloride ***14***. Yield 77%; ^{1}H-NMR ($CDCl_3$): δ 1.70 (t, 2H), 2.15 (d, 4H), 2.25 (t, 2H), 3.25 (d, 2H), 3.80 (q, 2H), 4.90 (s, 2H), 5.10 (s, 2H), 7.10–7.90 (m, 12H); anal ($C_{26}H_{31}N_3OS$•HCl•H_2O) C, H, N; reverse phase HPLC: 28 min.

1-Benzyl-3-(3-(morpholin-4-yl)propyl)-1-(pheneth-2-yl)thiourea hydrochloride ***15***. Yield 40%; mp = 159–160 °C; ^{1}H-NMR ($CDCl_3$, free amine): δ 1.70 (t, 2H), 2.30 (t, 4H), 2.35 (t, 2H), 3.00 (t, 2H), 3.40 (t, 4H), 3.70 (t, 2H), 3.95 (t, 2H), 4.70 (s, 2H), 6.80 (b, 1H), 7.1–7.4 (m, 10H); reverse phase HPLC: 22 min.

1-Benzyl-3-(3-(morpholin-4-yl)propyl)-1-(quinolin-3-yl)thiourea dihydrochloride ***16***. Yield 62%; ^{1}H-NMR ($CDCl_3$, free amine): δ 1.70 (t, 2H), 2.15 (t, 4H), 2.45 (t, 2H), 3.15 (t, 4H), 3.70 (m, 4H), 5.60 (s, 2H), 6.45 (s, 1H), 7.2–7.8 (m, 9H), 8.05 (d, 1H), 8.60 (s, 1H); anal ($C_{24}H_{28}N_4OS$•2HCl•H_2O) C, H, N; reverse phase HPLC: 15 min.

1-Benzyl-3-(3-(morpholin-4-yl)propyl)-1-(pyridin-2-yl)thiourea hydrochloride ***17***. Yield 79%; ^{1}H-NMR ($CDCl_3$, free amine):

δ 1.95 (t, 2H), 2.43 (t, 4H), 2.46 (t, 2H), 3.65 (t, 4H), 3.85 (t, 2H), 5.85 (s, 2H), 6.95–7.10 (m, 2H), 7.20–7.35 (m, 5H), 7.55 (t, 1H), 8.25 (d, 1H); anal ($C_{20}H_{26}N_4OS$•HCl•$2\frac{1}{2}H_2O$) C, H, N; reverse phase HPLC: 15 min.

3-(3-(Morpholin-4-yl)propyl)-1-((naphth-2-yl)methyl)-1-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]thiourea hydrochloride ***18***. Yield 54%; ^{1}H-NMR ($CDCl_3$, free amine): δ 1.75 (t, 2H), 2.65 (t, 2H), 3.10 (t, 4H), 3.60 (t, 4H), 3.75 (t, 2H), 4.65 (s, 2H), 5.35 (s, 2H), 7.10–7.60 (m, 13H); reverse phase HPLC: 21 min.

1-Benzyl-3-(3-(dimethylamino)propyl)-1-phenylthiourea-hydrochloride ***19***. Yield 38%; mp = 180–181 °C; ^{1}H-NMR ($CDCl_3$, free base): δ 2.10 (t, 2H), 2.55 (s, 6H), 2.85 (t, 2H), 3.75 (t, 2H), 5.49 (s, 2H), 6.3 (b, 1H), 6.9–7.5 (m, 10H); anal ($C_{19}H_{25}N_3S$•HCl) C, H, N; reverse phase HPLC: 19 min.

1-Benzyl-3-(3-(dimethylamino)propyl)-1-((naphth-2-yl)-methyl)thiourea hydrochloride ***20***. Yield 33%; ^{1}H-NMR ($CDCl_3$, free amine): δ 1.65 (t, 2H), 1.70 (s, 6H), 2.30 (t, 2H), 3.70 (t, 2H), 4.90 (s, 2H), 5.05 (s, 2H), 7.2–7.9 (m, 12H); anal ($C_{22}H_{29}N_3S$•HCl•1/3 H_2O) C, H, N; reverse phase HPLC: 23 min.

3-(3-(Dimethylamino)propyl)-1-(naphth-1-yl)methyl-1-phenyl-thiourea hydrochloride ***21***. Yield 38%; ^{1}H-NMR ($CDCl_3$, free base): δ 2.00 (t, 2H), 2.90 (s, 6H), 3.12 (t, 2H), 3.72 (t, 2H), 5.90 (s, 2H), 6.8–8.3 (m, 12H); anal ($C_{23}H_{27}N_3S$•HCl.3/2 H_2O) C, H, N; reverse phase HPLC: 23 min.

3-(3-Dimethylaminopropyl)-1-((naphth-2-yl)methyl)-1-phenyl-thiourea hydrochloride ***22***. Yield 20%; ^{1}H-NMR ($CDCl_3$, free amine): δ 1.60 (t, 2H), 1.70 (s, 6H), 2.20 (t, 2H), 3.75 (t, 2H), 5.65 (s, 2H), 6.9–7.9 (m, 12H); anal ($C_{23}H_{29}N_3S$•HCl) C, H, N; reverse phase HPLC: 25 min.

General procedure for the preparation of quinoline carbothioic amides

To a solution of 2-phenylquinoline (10.0 g, 50 mmol) in 1-propanol (250 mL) under reflux was added sodium (11.5 g, 0.5 mol) in portions over a 30-min period. After 2 h at reflux, the solution was cooled and water (50 mL) slowly added. The mixture was concentrated in vacuo to an oil which was dissolved in methylene chloride (300 mL) and washed four times with water (200 mL). The organic layer was dried over magnesium sulphate, filtered and concentrated in vacuo to give 8.2 g (78%) of 2-phenyl-1,2,3,4-tetrahydrohydro-2*H*-quinoline.

To a solution of 2-phenyl-1,2,3,4-tetrahydrohydro-2*H*-quinoline (0.5 g, 2.4 mmol) in THF (15 mL) at –78 °C was added lithium diisopropylamide (1.3 mL of a 2.0 M solution in THF). After 10 min, the appropriate isothiocyanate (2.6 mmol) in THF (10 mL) was added and the mixture was stirred overnight. The mixture was concentrated in vacuo to an oil, which was chromatographed on silica gel with 20% methanol/methylene chloride. The product thus obtained was dissolved in ethyl acetate (20 mL) and treated with 3 M HCl in ethyl acetate added (10 mL). The mixture was concentrated in vacuo to give the desired compound.

2-Phenyl-3,4-dihydro-2H-quinoline-1-carbothioic acid (3-(morpholin-4-yl)propyl)amide hydrochloride ***23***. Yield 29%; ^{1}H-NMR ($CDCl_3$, free base): δ 1.70 (m, 2H), 2.15 (m, 1H), 2.25 (t, 4H), 2.35 (m, 1H), 2.55 (m, 1H), 2.65 (t, 2H), 3.30 (t, 4H), 3.65 (m, 2H), 3.95 (m, 1H), 6.60 (t, 1H), 7.1–7.4 (m, 9H); anal ($C_{23}H_{29}N_3OS$•HCl) C, H, N; reverse phase HPLC: 21 min.

2-Phenyl-3,4-dihydro-2H-quinoline-1-carbothioic acid (3-(dimethylamino)propyl)amide hydrochloride ***24***. Yield 32%; ^{1}H-NMR (CD_3OD): δ 2.00 (m, 2H), 2.15 (m, 1H), 2.55 (m, 1H), 2.70 (m, 2H), 2.85 (s, 3H), 2.90 (s, 3H), 3.15 (t, 2H), 3.75 (m, 2H), 6.55 (t, 1H), 7.1–7-4 (m, 9H); anal ($C_{21}H_{27}N_3S$•HCl) C, H, N; reverse phase HPLC: 21 min.

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