Pergamon

Bioorganic & Medicinal Chemistry 8 (2000) 1383–1391

BIOORGANIC & MEDICINAL CHEMISTRY

2,3-Dihydro-Dithiin and -Dithiepine-1,1,4,4-tetroxides: Small Molecule Non-Peptide Antagonists of the Human Galanin hGAL-1 Receptor

Malcolm K. Scott,* Tina M. Ross, Daniel H. S. Lee, Hoau-Yan Wang, Richard P. Shank, Kenneth D. Wild, Coralie B. Davis, Jeffrey J. Crooke, Alexander C. Potocki and Allen B. Reitz

Drug Discovery Division, The R. W. Johnson Pharmaceutical Institute, Welsh and McKean Roads, Spring House, PA 19447, USA

Received 18 November 1999; accepted 11 February 2000

Abstract—The neuropeptide galanin modulates several physiological functions such as cognition, learning, feeding behavior, and depression, probably via the galanin 1 receptor (GAL-R1). Using an HTS assay based on ^{125}I-human galanin binding to the human galanin-1 receptor (hGAL-R1), we discovered a series of 1,4-dithiin and dithiipine-1,1,4,4-tetroxides that exhibited binding affinity IC_{50}'s to hGAL-R1 ranging from 190 to 2700 nM. Two of the dithiepin analogues, **7** and **23**, behaved pharmacologically as hGAL-R1 antagonists in secondary assays involving adenylate cyclase activity and GTP binding to G-proteins. Analogues **7** and **23** were also active in functional assays involving galanin, reversing the inhibitory effect of galanin on acetylcholine (ACh) release in rat brain hippocampal slices and electrically-stimulated guinea pig ileum twitch.

Introduction

The neuropeptide galanin, first isolated in 1983 from porcine intestine,[1] is a ubiquitous 2–9–30 amino acid peptide that is found in the mammalian central (CNS) and peripheral (PNS) nervous systems.[2–4] Human galanin (**1**) contains 30 amino acids,[5] whereas galanin found in other species contains 29 amino acids with a C-terminal amide.[6] In the CNS, galanin is distributed in axons and neurons located in the thalamus, hypothalamus, cortex, amygdala, hippocampus and spinal cord,[7,8] and in the PNS it is found in pancreatic, gastrointestinal, bladder, and genital tissue.[9]

GWTLNNSAGYLLGPHAVGNHRSFSDKNGLTS
1

Galanin has significant central effects on cognition, learning, emotional state, feeding behavior, and the modulation of pain in animals. When administered intraventricularly to rats, galanin potently impairs cognitive performance as measured by the decreased ability to carry out memory tasks in the Morris water maze,[10] the sunburst radial maze,[11] and delayed non-matching to sample tests.[12] In several studies, increases in feeding were demonstrated in rats and ground squirrels using centrally administered galanin.[13] Galanin may also be involved in emotional state via inhibition of dopaminergic cells in the ventral tegmentum as described in recent work using an animal model of depression.[14] The antinociceptive effects of galanin have been demonstrated in rats via intrathecal administration[15] and by the finding that the non-specific galanin receptor modulators M-35 and galantide reverse spinal analgesia induced by morphine.[16]

The diverse physiological effects of galanin in mammals can be attributed to specific interaction with a series of at least three G-protein coupled seven-transmembrane galanin receptors (GPCRs) including GAL-R1,[17–21] GAL-R2,[22–27] and GAL-R3.[26,28] Several recent reviews give extensive coverage to the identification, molecular biology, and function of these sub-types.[29–31] GAL-R1 is primarily localized in the brain,[21] whereas GAL-R2 is largely distributed in the periphery,[24,27] and GAL-R3 mRNA is found in some peripheral tissues, with low levels in the brain.[28,32] In the human brain, GAL-R2 and GAL-R3 are more widely distributed than GAL-R1, which is localized in greatest abundance in the cerebral

*Corresponding author. Tel.: +1-215-628-5608; fax: +1-215-628-4985; e-mail: scottm@prius.jnj.com

0968-0896/00/$ - see front matter
PII: S0968-0896(00)00062-6

cortex, amygdala, and substantia nigra.[26,33] GAL-R1 and GAL-R3 negatively regulate adenylate cyclase,[34–37] whereas GAL-R2 is only slightly inhibits the production of cAMP, and is also coupled to phospholipase C.[26,37]

Because of the high level of hGAL-R1 in human brain, this receptor is an appropriate target for the discovery of CNS drugs for treatment of various disorders. Peptide hGAL-R1 ligands such as galantide (M-15),[38] M-35,[39] M-40,[40] and galanin peptides attached to non-peptide units[41] have been evaluated. Recently, a non-peptide hGAL-R1 antagonist (**2**) was reported with a 1.7 μM hGAL-R1 IC_{50}.[42]

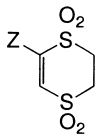
We employed an hGAL-R1 high throughput screen (HTS) of our corporate compound collection which resulted in a 0.14% hit rate of >50% inhibition at 25 μM. Confirmation, IC_{50} determinations, and secondary screening of the hits identified a variety of active chemical series. From this group, we now disclose the activity of the dithiin-1,1,4,4-tetroxide (**3**) chemical series as hGAL-R1 antagonists (Tables 1–3).

We describe here the structure activity relationships (SAR) based on **3**, ultimately leading to dithiipin-1,1,4,4-tetroxide **7**, the first non-peptidic sub-micromolar hGAL-R1 antagonist (190 nM IC_{50}).

Synthetic Chemistry

The reported synthesis of **3** and modifications therein were employed for the preparation of **3**–**42** (Scheme 1).[43] Treatment of an aryl ketone or an α-halo-aryl ketone with either ethane- or propane-dithiol and $BF_3{\cdot}OEt_2$ in CH_2Cl_2 at ambient temperature gave the corresponding dithiolanes **43**.[44] Using either bromine[45,46] or NBS,[47] these dithiolanes were converted to dithiins or dithiipins **44** via dithiolane ring opening followed by reclosure.[45] Oxidation of **44** with H_2O_2/HOAc afforded compounds **3**, **4**, **7**–**15**, **17** and **19**–**42**. The phenethyl analogues of **3**, compounds **5** and **6**, were obtained by the route depicted in Scheme 2. Chloroketone **45** was converted to the respective intermediate dithiolane **46** using $BF_3{\cdot}OEt_2$ and ethanedithiol, followed by refluxing pyridine. Oxidation of **46** provided **47** which was converted to **5** using tris-trifluoroacetate boron.[48] Compound **6** was obtained by reaction of **5** with 4-butoxybenzoyl chloride. Benzamide analogues **16** and **18** were prepared as shown in Scheme 3. Treatment of ester **48**, prepared as described above, with aqueous potassium hydroxide in DMF afforded acid **49**, which was coupled with either propyl- or phenethylamine to give amide **50**. Oxidation of **50** with 30% hydrogen peroxide/acetic acid gave **16** or **18**. The reduced analogue of **7**, compound **52**, was obtained as shown in Scheme 4. Reduction of **44** (R = 4-Me, $n = 1$) with triethysilyl hydride gave dithiipan **51**, which was oxidized with *m*-chloroperbenzoic acid (*m*CPBA), affording **52**.

Table 1. Binding affinities of 2-substituted-1,1,4,4-dithiintetroxides



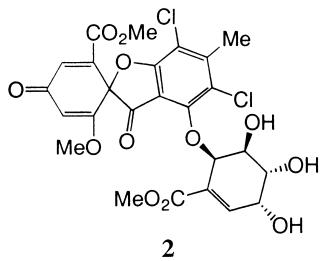
Compound	Z	hGALR1 IC_{50} (nM)
3	Phenyl	2700
4	4-Bromophenyl	1000
5	$PhenylCH_2(NH_2)CH$	8000
6	$PhenylCH_2$(4-*n*-BuOphenylCH_2OCONH)CH	1000

Biological Evaluations

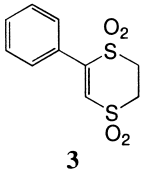
The binding affinities of compounds **3**–**42** at the hGAL-R1 were evaluated using membranes from human Bowes melanoma cells bound to wheat germ agglutin-conjugated scintillation proximity assay (SPA) beads and are reported as IC_{50} values in Tables 1–3. Determination of agonist/antagonist activity of **3**–**42** was done by measuring cAMP accumulation and GTP-γS binding. The effects of two compounds, **7** and **23**, were examined in two functional assays: galanin-induced inhibition of ACh release from rat brain hippocampal slices and the galanin-induced inhibition of ACh-stimulated guinea pig ileum twitch. The results are shown in Figures 2 and 3. The propensity of **32** and **38** to bind irreversibly to proteins was determined (Table 4) and cytotoxicity measurements were carried out on **3**, **7**, **10**, **17**,**23**, **30**, **33** and **35** (Table 5).

Results and Discussion

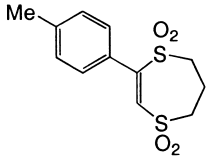
Modification of **3** to give 4-bromo derivative **4** resulted in an increase in micromolar binding affinity at the hGAL-R1, as did conversion to phenethyl analogue **6**, whereas the less complex phenethyl derivative **5** had weaker binding affinity (Table 1). Replacement of the



2

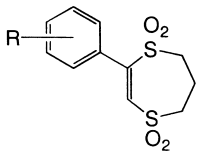


3



7

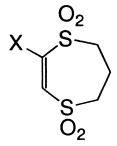
Table 2. Binding affinities of dithiipin-2-phenyl-1,1,4,4-dithiipintetroxides at hGAL-R1



Compound	R	hGAL-R1 IC_{50} (nM)
7	4-Methyl	190
8	4-Bromo	260
9	4-Fluoro	410
10	4-Phenoxy	410
11	4-Ethyl	430
12	2-Fluoro	430
13	3-Phenyloxycarbonyl	440
14	4-Trifluoromethoxy	490
15	2-Bromo	570
16	4-Propionamido	577
17	2-Trifluoromethyl	580
18	4-Phenethylamido	586
19	4-Ethoxy	590
20	3-Trifluoromethyl	600
21	4-Phenyl	600
22	2-Chloro	640
23	4-Iodo	710
24	3-Iodo	850
25	3-Bromo	890
26	3-Methoxy	910
27	3-Chloro	1000
28	2-Methoxy	1000
29	4-Ethyloxycarbonyl	1000
30	4-Methoxy	1100
31	4-Cyclohexyl	1100
32	3-Methyl	1300
33	3-Fluoro	1300
34	4-Phenylsulfonyl	1580
35	2-Methyl	1600
36	4-Chloro	1900
37	4-Trifluoromethyl	2100
38	H	2700
39	3-Trifluoromethoxy	2700

dithiintetroxide ring of **3** with a dithiipintetroxide ring (viz. **38**) had no effect on binding affinity, whereas corresponding 4-methylphenyl derivative **7** had an increased binding affinity by an order of magnitude (Table 2). Interestingly, the 2- and 3-methyl analogues, **35** and **32**, of **7** and the 4-trifluoromethyl analogue **37** were much less active, displaying binding affinities in the micromolar range. Binding affinity to the hGAL-R1 can sustain some structural variation in the 4-phenyl position as demonstrated by the 4-bromo analogue **8** which was slightly less active than **7** while other 4-substituted analogues, **9**–**11** and **14**, had significant affinity in the 400 nM range. Somewhat less active 4-substituted analogues were amides **16** and **18**, ethoxy, phenyl and iodo analogues, **19**, **21** and **23**, which ranged from 577 nM to 710 nM. Binding affinity decreases further for the 4-ethoxycarbonyl, methoxy, cyclohexyl, phenylsulfonyl, and chloro analogues, **29**, **30**, **31**,**34**, and **36**, which have micromolar activity. Halogen substitution or halogen-bearing groups in the 2-phenyl position are beneficial as shown by the binding affinities of 2-fluoro,-bromo,-trifluoromethyl, and -chloro analogues, **12**, **15**, **17** and **22**, which range from 430 nM to 640 nM whereas the 2-methoxy analogue **28** is much less active. For 3-substitution on the phenyl ring, phenoxycarbonyl analogue **13** and trifluoromethyl analogue **20** show significant binding affinity for the hGAL-R1 with IC_{50}'s of 440 nM and 600 nM respectively. The 3-iodo, bromo, methoxy, and chloro analogues, **24**–**27**, are less active with binding affinities ranging from 850 to 1 μM while the 3-fluoro and 3-trifluoromethoxy analogues, **33** and **39**, have micromolar activity. Compound **40** (Table 3), in which the phenyl ring of **7** was replaced with a 1-naphthyl group, showed good binding affinity equal to the more active 4-phenyl analogues. The 2- and 3-thienyl derivatives, **41** and **42**, were much less active than either **7** or **40**, with binding affinities in the micromolar range. Finally, the saturated analogue of **7**, dithiipan **52**, had no binding affinity for hGAL-R1.

Table 3. Binding affinities of 2-(naphthy)l- and 2-(thienyl)-1,1,4,4 dithiipintetroxides at hGALR1

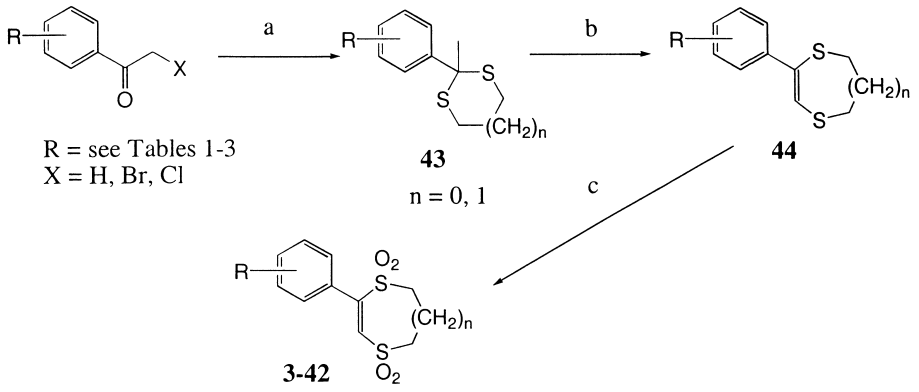


Compound	X	hGAL-R1 IC_{50} (nM)
40	1-Naphthyl	371
41	2-Thienyl	1300
42	3-Thienyl	1300

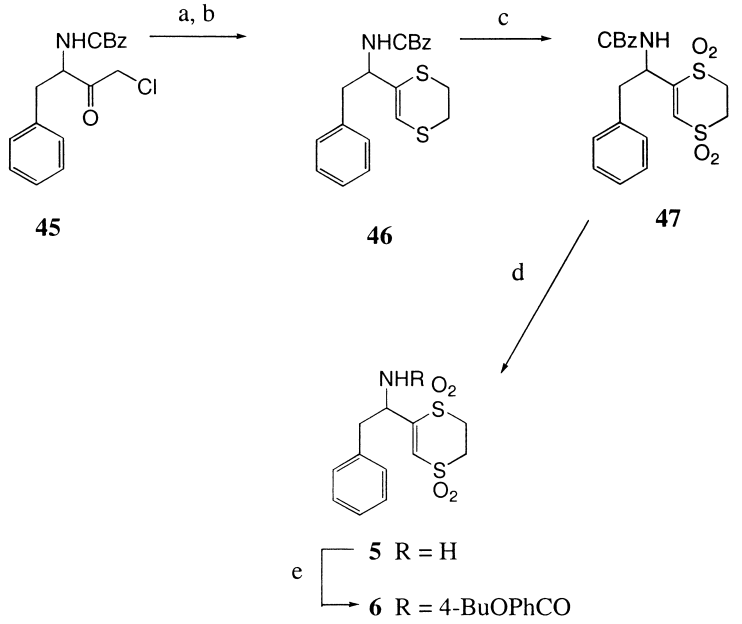
Using methods previously described to measure cAMP accumulation[49] and GTP-γS binding,[49] it was determined that compounds **3**–**42** are full antagonists at hGAL-R1 (data not shown). Since **7** had the best binding affinity of the analogues of **3** at the hGAL-R1, it was evaluated in our general receptor screening program. Compound **7** appears to be relatively specific for the hGAL-R1, with no binding observed at 30 μM to hGAL-R2 and weak or no affinity for hGAL-R2, NPY1, NPY2, NPY5, MC4R, δ-opioid receptor and other GPCRs.

Since galanin inhibits acetylcholine (ACh) release in mammalian brain,[49–51] presumably via a pertussis toxin-sensitive G_I protein,[49] and impairs performance in rodent learning in a cholinergically related manner,[52] we studied the effects of **7** and **23** on the release of ACh from rat cortical brain slices and cortical synaptosomes in the presence of galanin.[49] For comparative purposes, we employed the galanin antagonist galantide [38] in the cortical synaptosome study. As shown in Figures 1 and 2, **7** and **23** blocked the inhibitory effect of galanin on ACh release in a potent, dose-dependent manner in both experiments. At 10 μM, **7** almost totally blocked the effects of galanin in rat cortical brain slices. Furthermore, in another assay involving the effect of galanin on ACh modulation, the galanin-induced inhibition of electrically-induced contraction of guinea pig ileum, both **7** and **23** significantly blocked galanin inhibition as depicted in Figure 3.

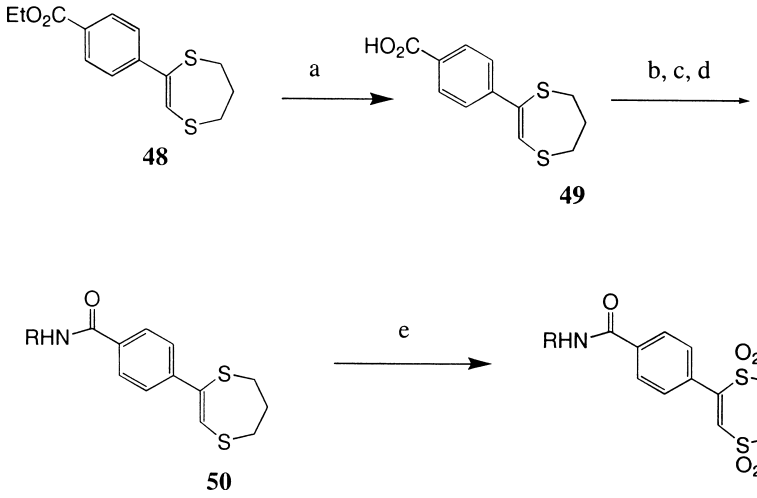
The dithiin and dithiepine-1,1,4,4-tetroxides described above contain a reactive segment, namely a 1,4-α,β-unsaturated sulfone, that is capable of undergoing



Scheme 1. Reagents and conditions: (a) $BF_3{\cdot}OEt_2$, $HSCH_2(CH_2)nCH_2SH$; (b) Br_2 or NBS; (c) H_2O_2, HOAc.

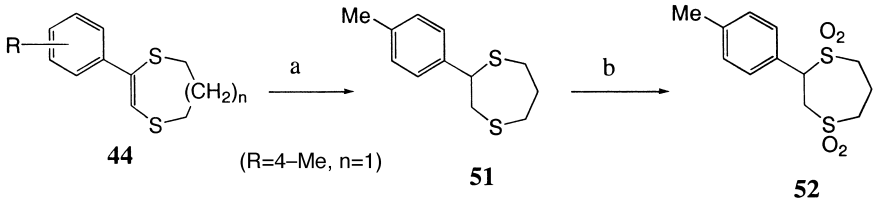


Scheme 2. Reagents and conditions: (a) $BF_3{\cdot}OEt_2$, $HSCH_2CH_2SH$; (b) pyridine; (c) H_2O_2, HOAc; (d) TFA, $B(CF_3CO_2)_3$; 4-BuOPhCOCl, TEA.



Compound	R	hGAL IC_{50}
16	Propyl	577 nM
18	Phenethyl	586 nM

Scheme 3. Reagents and conditions: (a) 3 N KOH, DMF; (b) *N*-methylmorpholine; (c) *i*-BuOCOCl; (d) RNH_2; (e) H_2O_2, HOAc.



Scheme 4. Reagents and conditions: (a) Et_3SiH, CH_2Cl_2; (b) *m*CPBA, CH_2Cl_2.

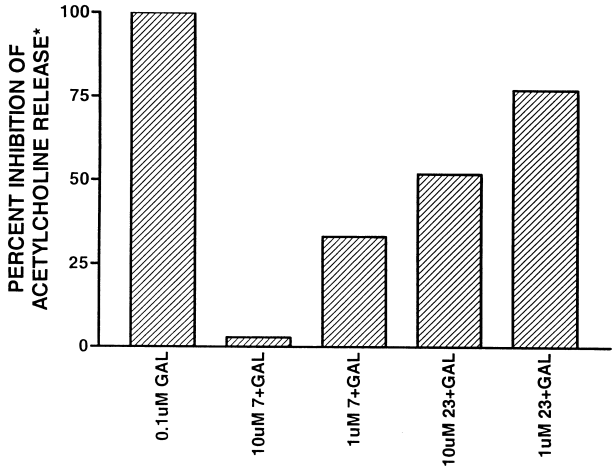


Figure 1. Effect of **7** and **23** on the galanin (GAL)-induced inhibition of ACH release from rat cortical brain slices.

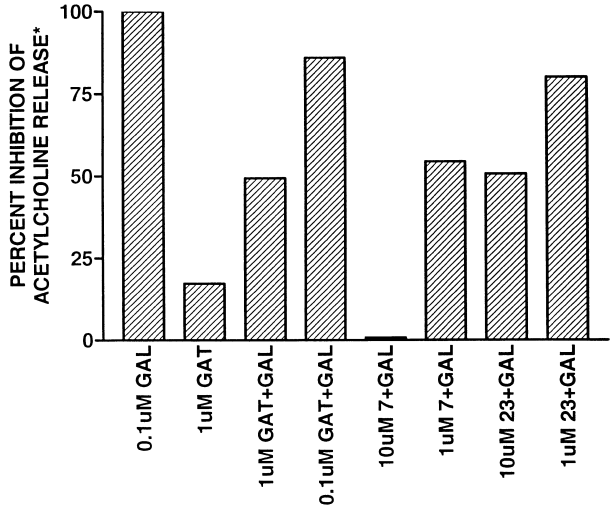


Figure 2. Effect of **7**, **23** and galantide (GAT) on the galanin(GAL)-induced inhibition of ACH release from rat cortical synaptosomes.

Michael-type addition by a nucleophile. The propensity of these compounds as Michael acceptors was confirmed by the disappearance of both **3** and **7** within 1 or 5 h respectively after treatment with propylamine and diethylamine (data not shown).

Because of the chemically reactive nature of these compounds, two studies were performed using selected analogues to determine if they bound irreversibly to proteins and, more specifically, to the hGAL-R1. In the first study, compounds **3**, **7**, **10**, **23**, **30**, **32**, **33**, **35** and **38**

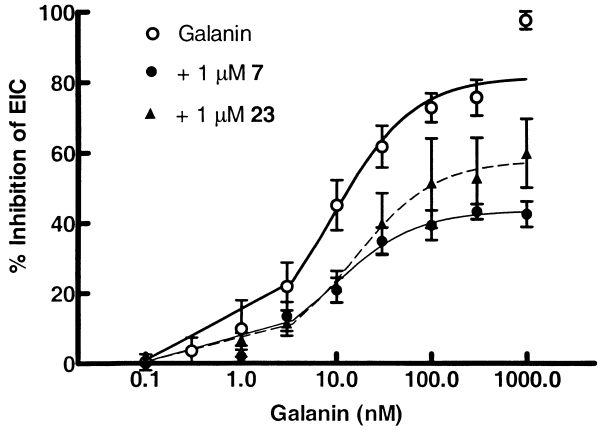


Figure 3. Effect of **7** and **23** on the galanin-induced inhibition of guinea pig ileum twitch.

were preincubated with BSA prior to the hGAL-R1 binding experiments. The results, which are reported in Table 4 as the ratio of the IC_{50} without incubation with BSA to the IC_{50} following preincubation with BSA, indicate that the potencies of **3**, **7**, **10**, **23**, **30**, **32**, **33**, **35** and **38** were only slightly altered, showing that preincubation of the compounds with proteins does not affect their activity at the hGAL-R1. In the second study, Bowes melanoma membranes containing the hGAL-R1 were incubated with **32** and **38**, washed with a low salt buffer, and then treated again with **32** and **38**. Both compounds generated similar dose–response curves compared with untreated Bowes melanoma membranes, suggesting that these compounds do not bind irreversibly to proteins or the hGAL-R1. In addition, the effect of several of these analogues was evaluated on cell viability. When **7**, **10**, **23**, **30**, **33** and **35** were incubated with Bowes melanoma cells, there was no effect on cell viability and the cells multiplied and maintained in culture, whereas **17** and **28** caused a decrease (Table 5).

Conclusions

The hGAL-R1 antagonistic activity of the title compounds is an important finding in the discovery process for CNS therapeutic agents based on hGAL-R1, demonstrating that non-peptidic small molecules can bind to the receptor with sub-micromolar affinity and elicit responses in functional assays antagonizing the effect of galanin. While these compounds are precluded as hGAL-R1 antagonist drug candidates due to their chemically reactive nature and solubility, they have

Table 4. Binding of dithiipine analogues to BSA

Compound	IC_{50} (nM)/IC_{50} (nM) BSA treated
3	0.70
7	0.60
10	2.02
23	0.95
30	1.31
32	0.88
33	1.19
35	0.86
38	0.83

Table 5. Effect on cell viability of dithiin and dithiipine 1,1,4,4-tetroxides

Compound	% Cell viability of Bowes melanoma cells at 18 h (5 μM)
3	100
7	95
10	95
17	30
23	100
28	60
30	100
33	100
35	90

served a purpose in providing SAR information for other related chemical series.

Experimental

Chemistry

General remarks. The ^{1}H NMR spectra were obtained on a 300 MHz Bruker AC 300 NMR spectrometer with Me_4Si as an internal standard. The mass spectrum of each compound was generated using Hewlett–Packard 1050 Series electrospray or chemical ionization mass spectrometers. The spectral data for each compound supported the assigned structure. Chromatographic separations were performed using either a Chromatotron Model 7924T radial chromatography system or a Biotage Flash 40 flash chromatography system. Most reagents and solvents were purchased and used without further purification.

2,3-Dihydro-1,4-dithiin-1,1,4,4-tetroxide (3). A mixture of α-chloroacetophenone (2.50 g, 16.0 mmol) and ethanedithiol (1.69 g, 17.9 mmol) at ambient temperature was treated dropwise with boron trifluoroetherate (1.13 g, 8.0 mmol). A mild exotherm and gas evolution ensued. After 5 min, Et_2O (10 mL) was added followed by water with thorough mixing. The organic layer was separated and washed three times with 15% NH_4OH solution, dried over anhyd. Na_2SO_4, filtered, and evaporated to a yellow oil. A solution of this material and pyridine (20 mL) was refluxed for 0.5 h. The reaction mixture was cooled, diluted with water (20 mL), and mixed thoroughly with 50:50 EtO_2:hexane. The organic layer was separated, washed with 1 N HCl solution until acidic, water and 15% NH_4OH solution. Separation of the organic layer, drying, and filtering afforded a crude orange solid. This material was passed through flash silica using CH_2Cl_2 as eluant to give **44** (R = H, $n = 0$) as a white crystalline solid. A slurry of **44** (R = H, $n = 0$, 1.0, 5.15 mmol) and HOAc (3.0 mL) was added in portions to a refluxing solution of 30% H_2O_2 (2.97 mL) and HOAc (2.97 mL) and the resulting light yellow solution was refluxed for 15 min. On cooling, a crystalline solid formed which was filtered and washed with water, affording **3** as a light yellow crystalline solid (0.80 g, 61%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42–7.66 (m, 5H, aromatic), 6.67 (s, 1H, vinyl), 4.00 (s, 4H, SO_2CH_2). MS (EI): $m/e = 258$.

Compound **4** was prepared in a similar fashion to give a white powder (0.037, 3%). ^{1}H NMR (DMSO-d_6) δ 32;7.81 (s, 1H), 7.8 (d, 2H), 7.55 (d, 2H), 4.3 (q, 4H). MS (ES+) = 335.19, 313.13, 292.14, 277.17, 275.16, 255.2, 253.2, 242.24, 232.18, 191.16, 169.19.

2,3-Dihydro-2-(1-(amino)-phenethyl)-1,4-dithiin-1,1,4,4-tetroxide (5). A solution of alpha-chloro (*N*-carboxybenzyl) phenylalanine (**45**, 0.7 g, 2.1 mmol) in tetrahydrofuran (10 mL) and ethanedithiol (0.18 mL, 2.1 mmol) was treated dropwise with boron trifluoroetherate (0.26 mL, 2.1 mmol) at room temperature. The resulting reaction was stirred overnight at room temperature, quenched carefully with 10% NH_4OH, and extracted with CH_2Cl_2. The organic layer was separated, dried with $MgSO_4$, filtered and evaporated. The residual oil was taken up in pyridine (5 mL) and heated to 100 °C for 2 h. Evaporation of the solvent yielded dithiane **46** as an oil which was treated with a solution of 30% H_2O_2 and acetic acid (5 mL) and stirred at ambient temperature for 72 hours. Filtration of the reaction mixture afforded **47** (0.175 g, 19%) as a crystalline solid.

This material (0.14 g, 0.32 mmol) was dissolved in trifluoroacetic acid (3 mL), treated with tris-trifluoroacetate boron[48] (0.56 g, 1.6 mmol) and stirred for one hour at room temperature. Methanol was added to the reaction mixture followed by evaporation in vacuo (3×). The residue was taken up in 10% MeOH/dichloromethane solution and washed with sodium carbonate solution. The organic layer was separated, dried with $MgSO_4$, filtered and evaporated to a residue which was purified via silica prep plate (10% MeOH/dichloromethane) to yield **5** (0.017 g, 18%). ^{1}H NMR ($CDCl_3$) δ 7.4 (m, 5H), 6.95 (s, 1H), 4.35 (dd, $J = 1.5$ Hz, 0.5 Hz, 1H), 3.85 (m, 4H), 3.4 (dd, $J = 1.5$ Hz, 0.5 Hz, 1H), 2.6 (q, 1H), 1.4 (bs, 2H). MS (ES+) = 302.1 (H+), 209.1.

2,3-Dihydro-2-(1-(4-(butoxyphenylcarbonyl)amino)-phenethyl)-1,4-dithiin-1,1,4,4-tetroxide (6). Triethylamine (89 mL, 0.64 mmol) was added to a solution of **5** (0.11 g, 0.32 mmol) in dioxane (1 mL) and cooled to 0 °C followed by addition of 4-butoxybenzoyl chloride (61 mL, 0.32 mmol). The reaction was stirred for 48 h at ambient temperature, diluted with dichloromethane, and washed with sodium bicarbonate solution. The organic layer was separated, dried with $MgSO_4$, filtered, and solvent evaporated in vacuo to a crude oil. Purification by silica radial chromatography (10% MeOH/dichloromethane)

afforded **6** (0.038, 25%) as a crystalline solid. ^{1}H NMR (DMSO-d_6) δ 8.6 (d, 1H), 7.75 (d, 2H), 7.4 (s, 1H), 7.25 (d, 2H), 7.15 (m, 3H), 6.8 (d, 2H), 5.3 (bt, 1H), 4.2 (m, 4H), 4.0 (t, 2H), 3.8 (bs, 1H), 3.3 (m, 1H), 3.0 (dd, J = 6 Hz, 4.5 Hz, 1.7 (m, 2H), 1.4 (m, 2H), 0.8 (t, 3H). MS (ES+) = 478.1 (H+), 191.1, 169.1.

2,3-Dihydro-2-(4-methylphenyl)-1,4-dithiepine-1,1,4,4-tetroxide (7). A solution of 4′-methylacetophenone (10 g, 74.5 mmol) and CH_2Cl_2 (200 mL) was treated dropwise with boron trifluoroetherate (12.5 mL, 104 mmol) at room temperature. To the resulting solution was added 1,3-propanedithiol (7.45 mL, 74.5 mmol) and the reaction mixture was stirred for 4 h at room temperature. The reaction was quenched carefully with saturated $NaHCO_3$ solution and extracted with dichloromethane. The organic layer was separated and evaporated in vacuo. The residue was dissolved in ethyl acetate and washed with 1 N NaOH, water, and saturated NaCl solution. The organic layer was separated, dried with $MgSO_4$, filtered and solvent evaporated in vacuo to yield a crude oil. Purification of the crude oil via flash chromatography (50% CH_2Cl_2/hexane) afforded 15.2 g of dithiolane **43** (R = 4-Me, n = 1) as a crystalline solid. A solution of this material (2.00 g, 8.9 mmol), N-bromosuccinimide (1.59 g, 8.9 mmol), and $CHCl_3$ (150 mL) was stirred at room temperature for 20 min followed by the addition of 1,8-dimethylamino naphthalene (1.91 g, 8.9 mmol). The reaction was stirred for 10 min, filtered, and the organic solvent was evaporated in vacuo. The residue was dissolved in 1:1 CH_2Cl_2:hexane and filtered to yield a crude oil. Purification of the crude oil by flash chromatography (30% dichloromethane/hexane) yielded 0.41 g of dithiin **44** (R = 4-Me, n = 1) as an oil.

This material (0.41 g, 1.83 mmol) was dissolved in dichloromethane (10 mL) and slowly added to a mixture of 30% H_2O_2 (2 mL)/acetic acid (2 mL) at 100 °C and stirred at 100 °C for 5 min. The reaction was cooled and stored in the freezer overnight. Filtration afforded **7** (0.680 g, 11%) as a crystalline solid. This material (0.100 g) was purified using a Model 215 Gilson Automated Preparative HPLC (reverse phase column 100×20 mm, stationary phase: C-18 on 5.4 μm beads with 80 Å pore size, mobile phase: acetonitrile/water containing 0.1% TFA, 30–90% acetonitrile gradient) to give **7** as a white solid; mp (sinter 182 °C) 184.5–186.5 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.32–7.40 (m, 3H, aromatic, vinyl), 7.23–7.32 (m, 2H, aromatic), 3.90–3.98 (t, 2H, SO_2CH_2), 3.78–3.88 (t, 2H, SO_2CH_2), 2.5 (m, 2H, CH_2), 2.331 (s, 3H, CH_3); MS (CI): m/z 304 ($M+NH_4^+$). Anal. calcd for $C_{12}H_{14}O_4S_2$: C 50.33, H 4.93, S 22.39; obsd: C 50.31, H 4.92, S 22.10. Using the procedure described above, compounds **8**–**15**, **16**, **19**–**26** and **28**–**42** were prepared.

2,3-Dihydro-2-(4(propylaminocarbonyl)-phenyl)-1,4-dithiepine-1,1,4,4-tetroxide (16). A stirred mixture of **48** (1.40 g, 5.0 mmol), DMF (100 mL), and 3 N aqueous KOH was heated at 100 °C for 0.5 h. The reaction was cooled to room temperature followed by the addition of CH_2Cl_2 (100 mL) and ice. The mixture was acidified with 1 N HCl and the organic layer was separated, dried over anhyd. $MgSO_4$, filtered and evaporated to yield acid **49** (R = 4-CO_2H, n = 1, 0.44 g, 35%). N-Methylmorpholine (90 μL, 0.8 mmol) was added to a solution of **49** (0.1 g, 0.4 mmol) in chloroform (20 mL) cooled to 0 °C. After stirring for 15 min, isobutylchloroformate (60 mL, 0.4 mmol) was added and stirred for an additional 20 min followed by addition of propylamine (24 mL, 0.4 mmol). The reaction was stirred for 2 h, diluted with saturated bicarbonate solution, and extracted with dichloromethane. The organic layer was separated, dried with $MgSO_4$, filtered and evaporated to yield an oil. Purification by silica radial chromatography (2% MeOH/dichloromethane) yielded **50** (0.07 g, 60%).

A solution of **50** (0.07 g, 0.24 mmol) in dichloromethane (1 mL) was added to a solution of refluxing 30% H_2O_2 (1 mL) and acetic acid (1 mL) and refluxed for 5 min followed by cooling in the freezer for 18 h. Filtration afforded **16** (0.032 g, 37%) as a crystalline solid. ^{1}H NMR (DMSO-d_6) δ 7.9 (d, 2H), 7.58 (s, 1H), 7.52 (d, 1H), 3.95 (t, 2H), 3.8 (t, 2H), 3.2 (q, 2H), 2.35 (m, 2H), 1.5 (m, 2H), 1.9 (t, 3H). MS (CI) = 358 (H+), 299, 274, 262, 246, 230, 206, 189.

2,3-Dihydro-2-(4(phenethylaminocarbonyl)-phenyl)-1,4-dithiepine-1,1,4,4-tetroxide (18). Using the procedure described for **16**, **18** (0.011 g, 19%) was obtained as a crystalline solid. ^{1}H NMR (DMSO-d_6) δ 8.7 (t, 1H), 7.78 (d, 2H), 7.55 (d, 2H), 7.52 (s, 1H), 7.3 (m, 5H), 4.0 (t, 2H), 3.75 (t, 2H), 3.52 (q, 2H), 2.75 (t, 2H), 2.4 (m, 2H). MS (CI) = 388 (H+), 356, 324, 308, 292, 251.

2,3-Dihydro-2-(2-methylphenyl)-1,4-dithiepine-1,1,4,4-tetroxide (32). Using the procedure described for the preparation of **7**, a solution of dithiolane **43** (R = 2-Me, n = 1, 1.49 g, 6.7 mmol) and $CHCl_3$ (136 mL), cooled in an ice bath, was treated dropwise with a solution of Br_2 (0.22 g, 7.6 mmol) and $CHCl_3$ (34 mL). After the addition was complete, the ice bath was removed and the reaction was stirred at room temperature for 15 min. Excess 5% $NaHCO_3$ was added with thorough mixing. The organic layer was separated, dried over anhyd. Na_2SO_4, filtered, and evaporated to an oily residue. The addition of Et_2O caused a white solid to form, which was filtered. The filtrate was evaporated and the residue was passed through flash silica using 75:25 hexane: CH_2Cl_2 to give 0.276 g (19%) of **44** (R = 2-Me, n = 1) as an oil. A solution of this material (0.110 g, 0.495 mmol) and HOAc (0.20 mL) was added to a refluxing solution of 30% H_2O_2 (0.297 mL) and HOAc (0.297 mL). The solution was refluxed 15 min, cooled in ice, and a white crystalline solid formed that was filtered, washed with water and Et_2O, and dried to afford **32**, 70 mg (50%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.22–7.40 (m, 5 H, aromatic, vinyl), 3.90–4.00 (t, 2H, SO_2CH_2), 3.74–3.84 (t, 2H, SO_2CH_2), 2.26–2.39 (m, 2H, CH_2), 2.31 (s, 3 H, CH_3). MS (EI): m/e = 286.

Prepared similarly was compound **27**.

(2-Methylphenyl)-1,4-dithiepan-1,1,4,4-tetroxide (43). A mixture of **44** (R = 4-Me, n = 1, 0.165 g, 0.86 mmol),

triethylsilyl hydride (0.164 mL, 1.03 mmol) and CH_2Cl_2 (10 mL) was stirred at room temperature for 15 min. Trifluoroacetic acid was added (0.132 mL, 1.72 mmol) to the reaction and the mixture was stirred overnight at room temperature. The reaction mixture was mixed thoroughly with 5% aqueous $NaHCO_3$ solution, the organic layer was separated, dried with $MgSO_4$, filtered and the solvent evaporated to yield **51** (0.11 g, 67%).

A solution of **51** (0.11 g, 0.5 mmol) and CH_2Cl_2 (5 mL) was added to a mixture of mCPBA (0.635 g, 2.21 mmol) and dichloromethane (20 mL) and stirred overnight at room temperature. The reaction mixture was filtered, diluted with ethyl acetate and washed with 1 N NaOH. The organic layer was separated, dried with $MgSO_4$, filtered and evaporated to yield **52** as a white powder (0.020 g, 7%). ^{1}H NMR (DMSO-d_6) δ 7.25 (q, 4H), 4.9 (d, 1H), 4.45 (m, 1H), 3.7 (m, 3H), 3.55 (m, 2H), 2.3 (s, 3H), 2.25 (t, 2H). MS (CI) = 306 (NH_4+), 240, 191.

Biology

Binding assays. Membranes prepared from the human Bowes melanoma cell line that expresses hGAL-R1 (50 μg) were coupled to wheat germ agglutinin coated scintillation proximity assay (SPA, 250 μg) beads in a buffer containing 50 mM HEPES, pH 7.4, 2 mM Ca^{++}, 2 mM Mg^{++}, 0.1% BSA and a protease inhibitors cocktail. ^{125}I-Human galanin (40 pM) was then added in the presence or absence of unknowns. Non-specific binding was defined by 10 μM human galanin. IC_{50} values were determined by the Graphpad software Prism (Graphpad Software Inc., San Diego, CA).

The hGAL-R2 binding study of **7** was performed using conditions identical with the hGAL-R1 study except that recombinant hGAL-R2 expressed on HEK293 cells was used as the receptor source.

Secondary assays. Determinations of ^{35}S-γGTP binding to G-proteins and cAMP measurements were carried out as described previously.[49]

Galanin-induced inhibition of acetylcholine release. Measurements of the effect of **7** and **23** on galanin-induced inhibition of ACh release from brain slices and synaptosomes were carried out as described previously.[49]

Electrical-stimulated guinea pig ileum twitch. Male, Hartley guinea pigs (300–900 g) were euthanized by CO_2 and the terminal portion of the ileum (discarding the 10 cm nearest the caecum) was quickly removed and placed into oxygenated (95:5 O_2:CO_2) Kreb's bicarbonate buffer (NaCl, 119 mM; KCl, 4.7 mM; $CaCl_2$, 2.5 mM; KH_2PO_4, 1.2 mM; $MgSO_4$, 1.2 mM; $NaHCO_3$, 25.0 mM; dextrose, 11.1 mM) at 37 °C. The ileum was cleaned of intestinal contents, blood vessels, and connective tissue, cut into several pieces (approximately 2 cm each), attached to gold chains with stainless steel clips, and then suspended between vertical platinum electrodes in 20 ml organ baths at a tension of 1.0 g, again bathed in oxygenated Kreb's bicarbonate buffer at 37 °C. Tissues were stimulated electrically (0.1 Hz, single pulses, 0.25 ms duration) at the minimum voltage required to produce a maximal tissue response (40–60 V). Agonists (such as galanin) were evaluated for their ability to inhibit the electrically-evoked twitch. Putative antagonists were evaluated for their ability to shift the concentration–effect curve of an agonist to the right. IC_{50} and maximum inhibition values were determined using Prism (Graphpad Software Inc., San Diego, CA).

Protein binding assay (BSA). In a separate experiment, 100 μM of compounds **3**, **7**, **10**, **23**, **30**, **32**, **33**, **35** and **38** were first incubated with 1% bovine serum albumin for 16 h at room temperature. Their respective affinities to hGAL-R1 were then measured in the binding assay as described above.

Protein binding assay (Bowes melanoma cell membranes). Bowes melanoma membranes were incubated with compounds **32**, **38** (10 uM), or vehicle for 1 h at 25 °C and then washed extensively with 50 mM HEPES, pH 7.4, containing 2 mM Ca, 2 mM Mg, 0.1% BSA and protease inhibitors. These two membrane preparations were then used in a receptor binding study as described above. The dose–response curves generated for galanin, **32**, or **38** using these membrane preparations were almost identical to those obtained in the normal binding assay.

Cytotoxicity measurements. Human Bowes melanoma cells were maintained in DMEM/F12 medium supplemented with 10% FCS and antibiotics in a 96-well culture plate. Test compounds **7**, **10**, **17**, **23**, **28**, **30**, **33** and **35** (1–100 μM) were added separately to each well and incubated for 24 h at 37 °C and 5% CO_2. Cell viability was determined using the MTT assay using the Cyto Tox 96 Non-radioactive cytotoxicity assay kit (Promega) according to the vendor's protocol.

Acknowledgements

The authors gratefully acknowledge helpful discussions and scientific input of Drs. Barry Dubinsky and Carlos Plata-Salamán of The R. W. Johnson Pharmaceutical Research Institute and Dr. Jacqueline N. Crawley of The National Institute of Mental Health.

References and Notes

1. Tatemoto, K.; Rokaeus, A.; Jornwall, H.; McDonald, T. J.; Mutt, V. *FEBS Lett*. **1983**, *164*, 124.
2. Bartfai, T.; Hokfelt, T.; Langel, U. *Crit. Rev. Neurobiol*. **1993**, *7*, 229.
3. Crawley, J. N. *Regulatory Neuropeptides* **1995**, *59*, 1.
4. Kask, K.; Berthold, M.; Bartfai, T. *Life Sci*. **1997**, *60*, 1523.
5. Bersani, M.; Johnsen, A. H.; Hojrup, P.; Dunning, B. E.; Andreasen, J. J.; Holst, J. *J. FEBS Lett*. **1991**, *283*, 189.
6. Bartfai, T.; Langel, U. *Eur. J. Med. Chem*. **1995**, *30*, 163s.
7. Melander, T.; Hokfelt, T.; Rokaeus, A. *J. Comp. Neurol*. **1986**, *248*, 475.
8. Skofitsch, G.; Jacobowitz, D. M. *Peptides* **1985**, *6*, 509.
9. Rokaeus, A. *Trends Neurosci*. **1987**, *10*, 158.

10. Sundstrom, E.; Archer, T.; Melander, T.; Hokfelt, T. *Neurosci. Lett*. **1988**, *88*, 331.
11. Malin, D. H.; Novy, B. J.; Lett-Brown, A.; Plotner, R. E.; May, B. T.; Radulescu, S. J.; Crothers, M. K.; Osgood, L. D.; Lake, J. R. *Life Sci*. **1992**, *50*, 939.
12. Robinson, J. K.; Crawley, J. N. *Behav. Neurosci*. **1993**, *107*, 458.
13. Boswell, T.; Richardson, R. D.; Schwartz, M. W.; D'Allessio, D. A.; Woods, S. C.; Sipols, A. J.; Baskin, D. G.; Kenagy, G. J. *Brain Res*. **1993**, *32*, 379.
14. Weiss, J. M.; Bonsall, R. W.; Demetrikopoulos, M. K.; Emery, M. S.; West, C. H. K. *Ann. N. Y. Acad. Sci*. **1998**, *863*, 364.
15. Cridland, R. A.; Henry, J. L. *Neuropeptides* **1988**, *11*, 23.
16. Reimann, W.; Englberger, W.; Friderichs, E.; Selve, N.; Wiffert, B. *Naunyn Schmied. Arch. Pharmacol*. **1994**, *350*, 380.
17. Amiranoff, B.; Servin, A. L.; Rouyer-Fessard, C.; Couvineau, A.; Tatemoto, K.; Laburthe, M. *Galanin Endocrinology* **1987**, *121*, 284.
18. Servin, A. L.; Amiranoff, B.; Rouyer-Fessard, C.; Tatemoto, K.; Laburthe, M. *Biochem. Biophys. Res. Commun*. **1987**, *144*, 298.
19. Wynick, D.; Smith, D. M.; Ghatei, M.; Akinsanya, K.; Bhogal, R.; Purkiss, P.; Byfield, P.; Yanaihara, N.; Bloom, S. R. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 4231.
20. Fisone, G.; Langel, U.; Carlquist, M.; Bergman, T.; Consolo, S.; Hokfelt, T.; Unden, A.; Andell, S.; Bartfai, T. *Eur J. Biochem*. **1989**, *181*, 269.
21. Habert-Ortoli, E.; Amiranoff, B.; Loquet, I.; Laburthe, M.; Mayaux, J. *F. Proc. Natl. Acad. Sci. USA* **1994**, *91*, 9780.
22. Howard, A. D.; Tan, C.; Shiao, L. L. *FEBS Lett*. **1997**, *405*, 285.
23. Wang, S.; Hashemi, T.; He, C.; Strader, C.; Bayne, M. *Mol. Pharmacol*. **1997**, *52*, 337.
24. Fathi, Z.; Cunningham, A. M.; Iben, L. G.; Battaglino, P. B.; Ward, S. A.; Nichol, K. A.; Pine, K. A.; Wang, J.; Goldstein, M. E.; Iismaa, T. P.; Zimanyi, I. A. *Brain Res. Mol. Brain Res*. **1997**, *51*, 49.
25. Smith, K. E.; Forray, C.; Walker, M. W. *J. Biol. Chem*. **1997**, *272*, 24612.
26. Kolakowski, L. F., Jr.; O'Neill, G. P.; Howard, A. D.; Broussard, S. R.; Sullivan, K. A.; Feighner, S. D.; Sawzdargo, M.; Nguyen, T.; Kargman, S.; Shiao, L.; Hreniuk, D. L.; Tan, C. P.; Evans, J.; Abramovitz, M.; Chateauneuf, A.; Coulombe, N.; Ng, G.; Johnson, M. P.; Tharian, A.; Khoshbouei, H.; George, S. R.; Smith, R. G.; O'Dowd, B. F. *J. Neurochem*. **1998**, *71*, 2239.
27. Bloomquist, B. T.; Beauchamp, M. R.; Zhelnin, L.; Brown, S.; Gore-Willse, A. R.; Gregor, P.; Cornfield, L. J. *Biochem. Biophys. Res. Commun*. **1998**, *243*, 474.
28. Wang, S.; He, C.; Hashemi, T.; Bayne, M. *J. Biol. Chem*. **1997**, *272*, 31949.
29. Fathi, Z. Galanin receptors: recent developments and potential uses as therapeutic targets. In *Annual Reports in Medicinal Chemistry;* Bristol, J. A., Ed.; Academic Press: New York; 1998 Vol. 33, pp 41–50.
30. Wang, S.; Parker, E. M. *Exp. Opin. Ther. Patents* **1998**, *8*, 1225.
31. Wang, S.; Gustafson, E. L. *Drug News Perspect*. **1998**, *8*, 458.
32. Bard, J. A.; Borowsky, B.; Smith, K. E.; Branchek, T. A; Gerald, C. P.; Jones, K. A. World Patent WO 9815570, 1998.
33. Sullivan, K. A.; Shiao, L. L.; Cascieri, M. A. *Biochem. Biophys. Res. Commun*. **1997**, *233*, 823.
34. Parker, E. M.; Izzarelli, D. G.; Nowak, H. P. *Mol. Brain Res*. **1995**, *34*, 179.
35. Wang, S.; He, C.; Maguire, M. *FEBS. Lett*. **1997**, *411*, 225–230.
36. Heuillet, E.; Bouaiche, Z.; Menager, J.; Dugay, P.; Munoz, N.; Dubois, H.; Amiranoff, B.; Crespo, A.; Lavayre, J.; Blanchard, J.-C.; Doble, A. *Eur. J. Pharmacol*. **1994**, *269*, 139.
37. Wang, S.; Hashemi, T.; Fried, S.; Clemmons, A. L.; Hawes, B. E. *Biochemistry* **1998**, *37*, 6711.
38. Bartfai, T.; Bedecs, K.; Land, T.; Langel, U.; Bertorelli, R.; Girotti, P.; Consolo, S.; Xu, X. J.; Wiesenfeld-Hallin, Z.; Nilsson, S. *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 10961.
39. Wiesenfeld-Hallin, Z.; Xu, X. J.; Langel, U.; Bedecs, K.; Hoekfelt, T.; Bartfai, T. *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 3334.
40. Langel, U.; Land, T.; Bartfai, T. *Int. J. Pept. Protein Res*. **1992**, *39*, 516.
41. Pooga, M.; Jureus, A.; Rezaei, K; Hasanvan, H.; Saar, K.; Kask, K.; Kjellen, P.; Land, T.; Halonen, J.; Maeorg, U.; Uri, A.; Solyom, S.; Bertfai, T.; Langel, U. *J. Pept. Res*. **1998**, *51*, 65.
42. Chu, M.; Mierzwa, R.; Truumees, I.; King, A.; Sapidou, E.; Barrabee, E.; Terracciano, T.; Patel, M. G.; Gullo, V. P.; Burrier, R.; Das, P. R.; Mittleman, S.; Puar, M. S. *Tetrahedron Lett*. **1978**, *387*, 6111.
43. Brewer, A. D.; Davis, R. A. US Patent 4,004,018, 1977.
44. Afonso, C. A. M.; Barros, M. T.; Godinho, L. S.; Maycock, C. D. *Synthesis* **1991,** 575.
45. Caputo, R.; Ferreri, C.; Palumbo, G.; Capozzi, G. *Tetrahedron* **1986,** 2369.
46. Caputo, R.; Ferreri, C.; Palumbo, G. *Synthesis* **1991,** 223.
47. Jeko, J.; Timar, T.; Jaszberenyi, J. C. *J. Org. Chem*. **1991**, *56*, 223.
48. Pless, J. *Angew. Chem. Inter. Ed*. **1973**, *12*, 147.
49. Wang, H.-Y.; Wild, K. D.; Shank, R. P.; Lee, D. H. S. *Neuropeptides* **1999**, *33*, 197.
50. Fisone, G.; Wu, C. F.; Consolo, S.; Nordstrom, O.; Brynne, N.; Bartfai, T.; Melander, T.; Hokfelt, T. *Proc. Natl. Acad. Sci. USA* **1987**, *84*, 7339–7343.
51. Ogren, S.-O.; Pramanik, A. *Neurosci. Lett*. **1991**, *128*, 253.
52. Mastropaolo, J.; Nadi, N. S.; Ostrowski, N. L.; Crawley, J. N. *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 9841.