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Synthesis and biological evaluation of novel pyrimidine derivatives as sub-micromolar affinity ligands of GalR2

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ABSTRACT

GalR1 and GalR2 represent unique pharmacological targets for treatment of seizures and epilepsy. A novel series of 2,4,6-triaminopyrimidine derivatives were synthesized and found to have sub-micromolar affinity for GalR2. Optimization of a series of 2,4,6-triaminopyrimidines led to the discovery of several analogs with IC50 values ranging from 0.3 to 1 μM.

The neuropeptide galanin[1] is widely expressed in the central nervous system (CNS) and peripheral nervous system (PNS) of many different species, including humans.[2–4] Galanin regulates a variety of physiological and pathological processes, including pain, learning, memory, mood, addiction, and food intake.[5] In addition, a critical role for galanin in seizure control has been suggested. Galanin has been shown to exhibit potent anticonvulsant effect in both acute and chronic seizure models in rodents. Intrahippocampal injected galanin strongly and irreversibly attenuated the status epilepticus induced by perforant path stimulation in rats.[6] Mice with null mutation of galanin were more susceptible to develop status epilepticus after perforant path stimulation or systemic kainic acid injection, and exhibited more severe seizures following pentylenetetrazol injection.[7] In contrast, mice that overexpress galanin under a dopamine-β-hydroxylase promoter developed increased resistance to seizure induction in these three models.[7]

The physiological effects of Galanin are known to be mediated by the activation of one or more of the three known G-protein-coupled galanin receptor subtypes designated as GalR1, GalR2 and GalR3. GalR1 and/or GalR2 are particularly relevant to seizures and epilepsy. GalR1 receptor is predominantly coupled to Gi-mediating suppression of forskolin-stimulated cAMP accumulation. In addition to being weakly coupled to Gi, GalR2 is found to couple to Gq, resulting in membrane lipid turnover and inositol phosphate (IP) accumulation.[8] Both GalR1 and GalR2 are G protein-coupled receptors that are expressed at high levels in the hippocampus.[9]

Much effort has been made in recent years toward development of systemically active agonists/ modulators for GalR1 and GalR2 receptors. So far, two nonpeptide, systemically active agonists, Galnon[6] and Galmic[11] (Fig. 1), and a CNS-penetrating peptide galanin analog Gal-B2[10,12] have been synthesized and characterized. While exhibiting micromolar to submicromolar affinities to GalR1 and GalR2, Galnon and Galmic are not selective between the two receptors and thus raise concerns of possible side effects.[13] A newly synthesized peptide analog B-25 has been tested as a galanin analog and shown impressive in vivo efficacy.[10] Peptides typically have short in vivo half lives but the half life of B-25 is greater than 10 h.[10] Moreover, it may be cost-inefficient to produce peptides in sufficient quantities needed for in vivo evaluation. Therefore, there is a continuous need for selective as well as systemically active, nonpeptide, agonist or modulator of GalR1/R2.

As part of our research efforts to discover and develop nonpeptide agonists for the treatment of seizures and epilepsy, we recently reported the discovery of compound CYM2503 (**3**), a peptidomimetic, positive allosteric modulator of GalR2. CYM2503 induces anticonvulsant effects in animal models.[14] However, finding a small-molecule agonist for the GalR2 is particularly challenging. In this report, we describe the discovery of small molecular novel pyrimidine derivatives as sub-micromolar affinity ligands of GalR2.

Screening of our internal compound collection for modulators of the GalR2 receptor resulted in the identification of 4-bromo-N^2,N^6-dicyclohexylpyridine-2,6-diamine (**4**, CYM2024). Although

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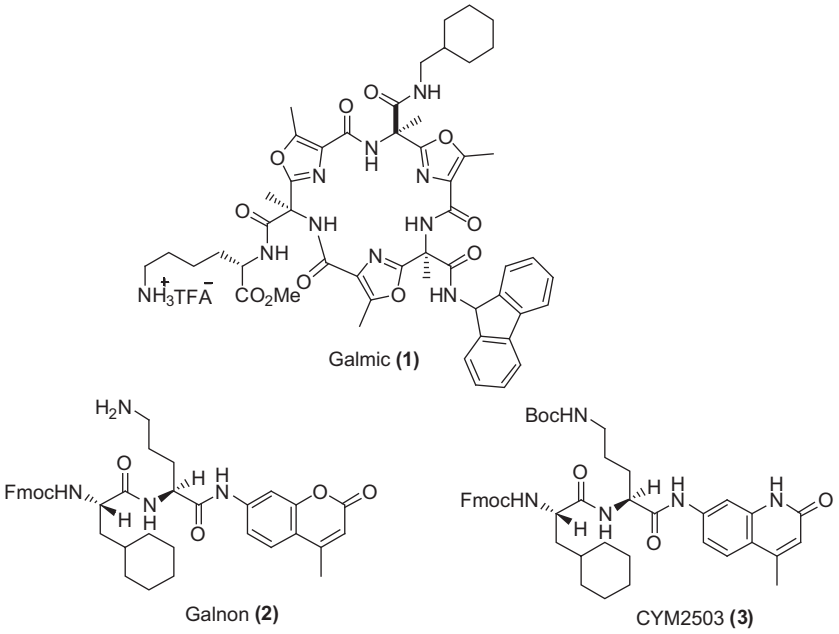
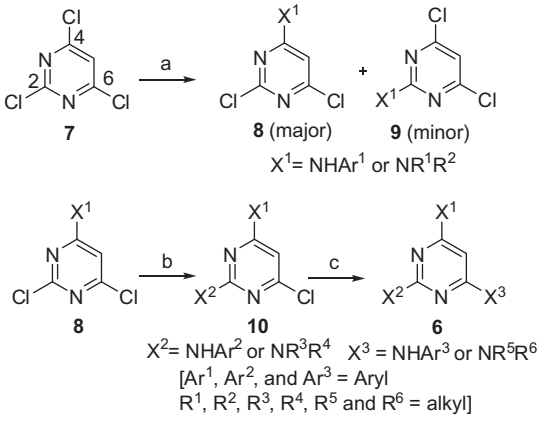


Figure 1. Structures of nonpeptide ligands of the Galanin receptor.

compound **4** has moderate binding affinity to GalR2, we were not able to increase affinity by further chemical modifications with different substitutions on the pyridine core. We then turned our attention to the pyrimidine and triazine system. Specifically, we focused on 2,4,6-triaminopyrimidines and/or triazines, as exemplified by general structures **5** and **6** (Fig. 2).

To develop a structure–activity relationship (SAR) around **5** and **6**, series of pyrimidines and triazines were first synthesized. The triaminopyrimidines were synthesized via routes depicted in Scheme 1. 2,4,6-trichloropyrimidine (**7**) was treated with an appropriate aliphatic amine at 0 °C to give 4-aminopyrimidine **8** in 75–85% yield. The second amino substitution at position 2 was effected by treating compound **8** with an arylamine in the presence of diisopropylethylamine in refluxing *n*-BuOH for 1–4 days to give compound **10**. To shorten the reaction time, compound **8** was treated with an arylamine under microwave conditions at 160 °C to give the diaminopyrimidine **10** in 85–95% yield. Compound **10** was further treated with another arylamine under Buchwald–Hartwig coupling conditions[15] to give triaminopyrimidine derivative **6** in 80–90% yield.

In an alternative method, 2,4,6-trichloropyrimidine (**7**) was first treated with an arylamine in the presence of Na_2CO_3 in refluxing EtOH for 2–4 h to give 4-aminopyrimidine **8** in 85–95% yield.[16] Compound **8** was further treated with another arylamine using diisopropylethylamine followed by final substitution with an aliphatic amine under microwave conditions to afford triaminopyrimidine derivative **6** in 90–95% yield.[17]



Scheme 1. General strategy for the synthesis of triaminopyrimidine derivatives. Reagents and conditions: (a) $R_1R_2NH_2$ (1.0 equiv), Et_3N (1.1 equiv), 0 °C–rt, 30 min, 75–85%; Ar_1NH_2 (2.0 equiv), Na_2CO_3 (2.0 equiv), EtOH, reflux, 2–4 h, 85–95%; (b) Ar_2NH_2 (1.5 equiv), DIPEA (5.0 equiv), *n*-BuOH, reflux, 1–4 days or *n*-BuOH, microwave, 160 °C, 4–7 h, 85–90%; $R_3R_4NH_2$ (1.5 equiv), DIPEA (2.0 equiv), *n*-BuOH, rt, overnight, 90–95%; (c) Ar_3NH_2, $Pd_2(dba)_3$, XPhos, *t*-BuOK, dioxane, 85 °C, overnight, 80-90%; $R_5R_6NH_2$, *n*-BuOH, microwave, 160 °C, 2–5 h, 90–95%.

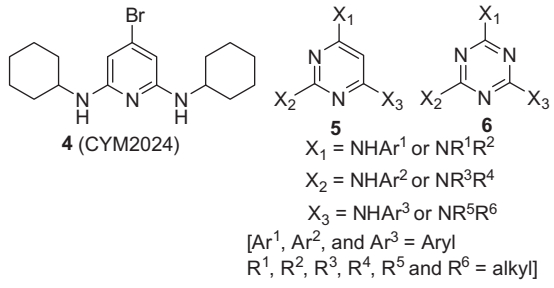
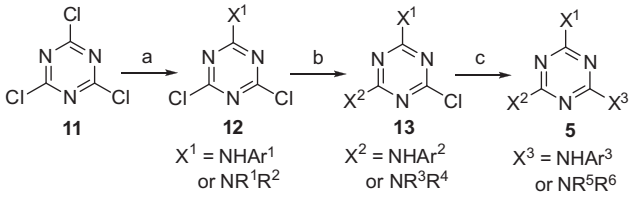
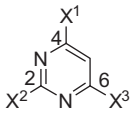


Figure 2. Structures of **4** (CYM2024) and general structures of 2,4,6-triamino pyrimidines and triazines.

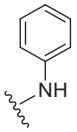
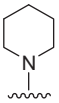
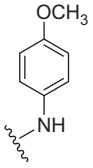
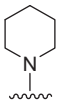
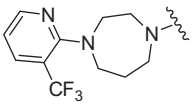


Scheme 2. General strategy for the synthesis of triaminotriazine derivatives. Reagents and conditions: (a) $R_1R_2NH_2$ or Ar_1NH_2, $NaHCO_3$, acetone, H_2O, 0–5 °C, 80–85%; (b) $R_3R_4NH_2$ or Ar_2NH_2, K_2CO_3, THF, rt, 2–4 h, 80–85%; (c) $R_5R_6NH_2$ or Ar_3NH_2, K_2CO_3, dioxane, microwave, 120 °C, 1–4 h, 90–95%.

Table 1
GalR1 and GalR2 binding affinities[a] of compounds **6a**–**6t**



6

Compd[b]	X^1	X^2	X^3	GalR1 IC_{50} (μM)	GalR2 IC_{50} (μM)
6a			2,5-Dimethylaniline	NA	4.57
6b			4-OCH_3-Aniline	8.59	1.09
6c			3- OCF_3-Aniline	34.86	6.62
6d			4- OCF_3-Aniline	NA	NA
6e			4-(OCF_2H)Aniline	NA	3.38
6f			3-(OCF_2H)Aniline	6.9	4.15
6g			Piperonylamine	NA	3.69
6h			3-F,4-OCH_3-Aniline	6.77	8.65
6i		Pyrrolidine	1-Phenylpiperazine	12.8	3.4
6j		Pyrrolidine	3-OCH_3-Aniline	4.6	0.93
6k		Pyrrolidine	4-OCH_3-Aniline	NA	8.04
6l		Cyclohexanamine	4-(OCF_2H)Aniline	27.5	12.5
6m		Cyclohexanamine	3-(OCF_2H)Aniline	5.03	0.77
6n		Cyclohexanamine	4-OCF_3-Aniline	5.93	1.06
6o		Cyclohexanamine	3-OCH_3-Aniline	5.62	1.75
6p		Cyclohexanamine	3-OCF_3-Aniline	6.82	0.55
6q			4-OCH_3-Aniline	3.35	1.92
6r			2,5-Dimethylaniline	3.90	2.08
6s			3-F,4-OCH_3-Aniline	6.32	2.03
6t			3-F,2-CH_3-Aniline	1.30	0.56

[a] Binding affinity was determined using [^{125}I] galanin displacement assay.[19] NA: not active; up to 100 μM.
[b] All compounds were at least 95% pure with the majority being greater than 98% pure by LC–MS[20], ^{1}H- and ^{13}C NMR.

The 2,4,6-triaminotriazine series was prepared by the consecutive nucleophilic substitution of cyanuric chloride (**11**) with different nucleophiles as shown in Scheme 2. Commercially available cyanuric chloride was first treated with an aliphatic or aromatic amine at 0 °C to yield the monoaminotriazine **12** in 80–85% yield. The second chloride of compound **12** was substituted by various amines at room temperature to provide diaminotriazine **13** in 80–85% yield. Compound **13** was further treated with an aliphatic or aromatic amine under microwave conditions at 120 °C to give triaminotriazine **5** in 90–95% yield. All intermediates and final compounds provided satisfactory ^{1}H, ^{13}C NMR, LCMS, and mass spectral data.[18]

With the initial libraries of pyrimidines and triazines in hand, we then evaluated their affinities to GalR1 and GalR2. All compounds were first screened at 1 and 10 μM in duplicate for affinity to GalR1 and GalR2. The affinity screening was performed twice for each receptor subtype. Compounds that exhibited greater than 50% binding inhibition for GalR2 or 60% binding inhibition for GalR1 at either 1 or 10 μM concentration were progressed to full curve binding assay for the corresponding receptor subtype.

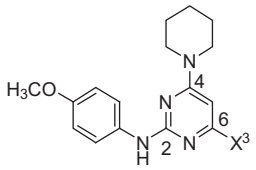
Unexpectedly, none of the triazine series exhibited significant binding affinities to GalR1 and GalR2. Fortunately, we were able to produce meaningful SAR for the 2,4,6-triaminopyrimidines (~250) that were synthesized and screened for binding affinity. Based on these results, we made additional optimization efforts on substitution patterns at the pyrimidine nucleus to improve the binding affinity to GalR1 and GalR2. The SAR of a focused library of 2,4,6-triaminopyrimidines is shown in Tables 1–3.

When the 2- and 4-position of pyrimidine were substituted, respectively, with aniline and piperidine, the resulting triaminopyrimidines (**6a**–**6h**) were nonselective between GalR1 and GalR2 with moderate binding affinity. When the 4-position was substituted with *p*-OCH_3-aniline and the 2- and 6- positions of the pyrimidine were modified with other aromatic/aliphatic amines, the best binding affinity of this series was obtained for compounds **6j**, **6m**, **6n** and **6p**. However, this series of compounds did not exhibit selectivity between GalR1 and GalR2. Interestingly, when 2- and 4-positions of pyrimidine were substituted with piperidine and 1-(3-(trifluoromethyl)pyridin-2-yl)-1,4-diazepane, the resulting triaminopyrimidines were showed even higher binding affinity with nonselectivity between GalR1 and GalR2 (Table 1).

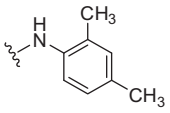
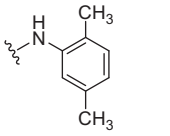
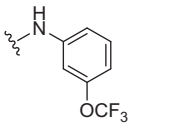
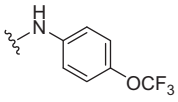
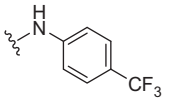
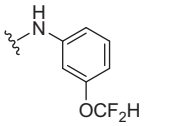
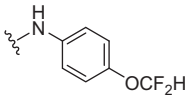
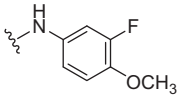
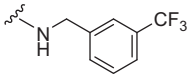
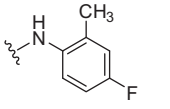
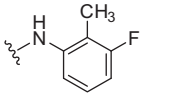
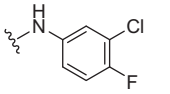
After many modifications on the pyrimidine core, we found that *p*-OCH_3-aniline at 2-position and piperidine at 4-position were necessary for selective binding affinity for GalR2. We then maintained the two optimal substituents at 2- and 4- positions and varied the substitutions at 6-position (structure **6A**). Most of the compounds from this series show selective binding affinity for GalR2 with IC50s ranging from 0.33 to 1.0 μM. Small structural changes of the substituents on the aromatic ring at 6-position of pyrimidine core influenced the binding affinities of these compounds. Compounds with 4-OCF_3 and 3-OCF_3 anilines **6w** (CYM2229) and **6x** (CYM2235) have same binding affinities. In a similar manner, compound **6v** with 2,5-dimethyl substituted aniline has more binding affinity (twofold) than its regio-isomer 2,4-dimethyl derivative (**6u**). Though, dimethyl anilines showed reasonable selective binding affinities against GalR2, it is worthwhile to evaluate the binding affinity of the mono-methyl substituent anilines. We then prepared all 3 possible regio-isomers (*o*-, *m*- and *p*-) of mono-methyl aniline derivatives, but none of the derivatives showed binding affinity for both GalR1 and GalR2. In the most of the cases, 2-methyl-fluoro anilines at the 6-position of pyrimidine core shows selective and good binding affinity for GalR2 [**6dd** and **6ee** (CYM2248)]. Surprisingly, compound with 4-OCF_2H substituted aniline (**6aa**) has no binding affinity, where as compound with 3-OCF_2H substituent **6z** (CYM223) has good binding affinity with IC_{50} = 0.54 μM (Table 2). Anilines with electron withdrawing groups (NO_2, CN) at the 6-position did not show significant binding affinity for either receptors (not listed in the table).

Next SAR studies (Table 3) at the 4-position were performed while maintaining *p*-OCH_3-aniline at 2-position and 3-F-4-OCH_3

Table 2
GalR1 and GalR2 binding affinities[a] of compound series **6A** (**6u–6ff**)

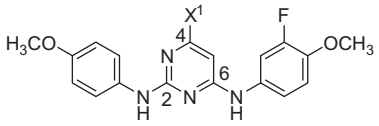


6A

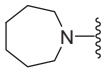
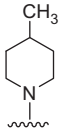
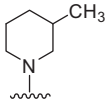
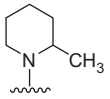
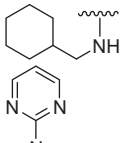
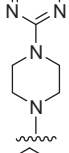
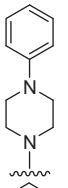
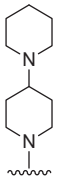
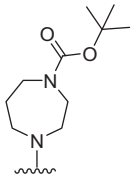
Compd[b]	X^3	GalR1 IC_{50} (μM)	GalR2 IC_{50} (μM)
6u		NA	1.18
6v		NA	0.44
6w		NA	0.38
6x		NA	0.33
6y		NA	0.78
6z		NA	0.54
6aa		NA	NA
6bb		NA	0.43
6cc		NA	1.93
6dd		NA	0.98
6ee		NA	0.38
6ff		NA	4.18

[a] Binding affinity was determined using [^{125}I] galanin displacement assay.[19] NA: not active; up to 100 μM.
[b] All compounds were at least 95% pure with the majority being greater than 98% pure by LC–MS[20], ^{1}H- and ^{13}C NMR.

Table 3
GalR1 and GalR2 binding affinities[a] of compound series **6B** (**6gg–6qq**)



6B

Compd[b]	X^1	GalR1 IC_{50} (μM)	GalR2 IC_{50} (μM)
6gg		NA	NA
6hh		5.48	0.77
6ii		NA	2.85
6jj		NA	NA
6kk		6.78	1.34
6ll		NA	NA
6mm		3.84	NA
6nn		3.84	NA
6oo		6.82	24.85
6pp		16.01	NA
6qq		2.82	9.07

[a] Binding affinity was determined using [^{125}I] galanin displacement assay.[19] NA: not active; up to 100 μM.
[b] All compounds were at least 95% pure with the majority being greater than 98% pure by LCMS[20], ^{1}H- and ^{13}C NMR.

aniline at 6-position (structure **6B**). When piperidine was replaced with lower membered pyrrolidine (**6gg**), higher membered azepane (**6hh**) or substituted piperidine (**6jj**, **6kk**, **6ll** and **6pp**) resulted in a loss of selectivity and/or binding affinity for GalR2. SAR around the position-2 underlined the unique role of piperidine

in binding. The replacement of carbon at 4th position of piperidine with O, S, SO_2, NH, substituted (alkyl or aryl) NH, or removal of cyclic constraint were in fact not tolerated and caused significant loss of GalR2 binding affinity. Replacement of *p*-OCH_3-aniline at 2-position with *p*-OC_2H_5, *p*-isopropoxy or *p*-Cl aniline also caused loss of affinity. In fact, while keeping piperidine at the 4-position and interchanging of 3-F-4-OCH_3 and *p*-OCH_3-aniline from 6-position to 2-position also caused significant loss of GalR2 binding affinity (not listed in the table). Among all pyrimidine derivatives, compounds **6mm** and **6nn** shows selective binding affinity for GalR1 with IC50 3.8 μM each.

In summary, small molecular novel 2,4,6-triaminopyrimidine derivatives were designed, synthesized, and subsequently tested for affinities for GalR1 and GalR2. Eight compounds of the series of structure **6A**, where *p*-OCH_3-aniline at the 4-position and piperidine at the 2-position of the pyrimidine core exhibited the highest affinity for GalR2 receptor with IC_{50} ranging from 0.33 to 1.0 μM. Further results from this lab regarding the activity of these and similar compounds will be presented in due course.

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17. *General synthesis of 2,4,6-triaminopyrimidine compounds: Step-1: 4-amino-2,6-dichloro pyrimidine:* 2,4,6-trichloro pyrimidine (1.0 mmol) in ethanol (5 mL) was treated with an aromatic amine (1.1 mmol) in the presence of Na_2CO_3 (1.1 mmol) at rt. The mixture was stirred at reflux for 2–4 h until completion of the reaction. The reaction progress was followed by TLC. After completion of the reaction, an equal volume of water was added with cooling. The resulting white precipitate was filtered, washed with water, and dried in vacuum over night to yield 4-substituted 2,6-dichloro pyrimidine. In case of no precipitation, ethanol was removed by rota vap., and the residue was dissolved in CH_2Cl_2. The organic layer was washed twice with water, brine, dried (Na_2SO_4), filtered, and concentrated. The resulting crude was purified by column chromatography to afford the 4-amino-2,6-dichloro pyrimidines in 85–95% yield. *Step-2*: *2,4-diamino-6-chloropyrimidine*: 4-amino-2,6-dichloro pyrimidine (1.0 mmol) prepared from the above procedure was treated with another aliphatic amine or aromatic amine (2.0 mmol) in the presence of DIEPA (5.0 mmol) in *n*-BuOH (5 mL) at rt. For an aliphatic amine the reaction mixture was stirred at rt for overnight. For an aromatic amine the reaction mixture was refluxed for 24–72 h or placed in microwave (150 °C, 2–7 h) until completion of the reaction. The reaction progress was followed by TLC. After completion of the reaction, solvents were removed by rota vap., and the residue was dissolved in CH_2Cl_2. The organic layer was washed twice with water, brine, dried (Na_2SO_4), filtered, and concentrated. The resulting crude was purified by column chromatography (EtOAc/hexane) to afford the 2,4-diammino-6-chloropyrimidines in 85–90% yield. *Step-3: 2,4,6-triaminopyrimidine*: 2,4-diamino-6-chloropyrimidine (1.0 mmol) prepared from the above procedure was treated with another suitable aliphatic amine or aromatic amine (3.0 mmol). For an aliphatic amine, 2,4-diamino-6-chloropyrimidine (1.0 mmol) was treated with aliphatic amine (3.0 mmol) and DIPEA (5.0 mmol) in *n*-BuOH (5 mL) and placed in microwave (150 °C) for 3–7 h. After the completion of the reaction (monitored by TLC), solvents were removed and the residue was dissolved in EtOAc. The organic layer was washed twice with water, brine, dried (Na_2SO_4), filtered, and concentrated. The resulting crude was purified by column chromatography to afford the 2,4,6-triaminopyrimidines 90–95% yield. For aromatic amine; 2,4-diamino-6-chloropyrimidine (1.0 equiv) was dissolved in dioxane under argon and to that were added $Pd_2(dba)_3$ (10 mol %), Xantphos (10 mol %), aromatic amine (1.2 mmol), *t*-BuOK (1.2 mmol). The resulting solution was degassed with argon for 5 min and heated to 85 °C for overnight. The reaction mixture was filtered through a pad of celite, washed with CH_2Cl_2 (2 × 10 mL) and the resulting filtrate was concentrated. The resulting crude was purified by flash column chromatography to yield 2,4,6-triaminopyrimidines in 90–95% yield.
18. Spectral data for selected compounds:*N^2-(4-methoxyphenyl)-6-(piperidin-1-yl)-N^4-(4-(trifluoromethoxy)phene-yl)pyrimidine-2,4-diamine[**6x** (CYM2235)]:* ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 (d, *J* = 8.2 Hz, 2H), 7.31 (d, *J* = 8.1 Hz, 2H), 7.14 (d, *J* = 8.3 Hz, 2H), 6.83 (d, *J* = 8.5 Hz, 2H), 6.61 (br s, 1H), 5.44 (s, 1H), 3.78 (s, 3H), 3.51 (br s, 4H), 1.60 (br s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 164.01, 161.71, 159.87, 154.92, 144.44, 138.79, 133.70, 122.29, 121.99, 121.48, 113.99, 75.90, 55.64, 45.56, 25.66, 24.88. ESI-MS (*m*/*z*): calcd for $C_{23}H_{24}F_3N_5O_2$ 459.46, found 460.5 $[M+H]^+$. LC–MS >98%, t_R = 3.25 min, *m*/*z* 460 $[M+H]^+$.
*N^4-(3-fluoro-2-methylphenyl)-N^2-(4-methoxyphenyl)-6-(piperidin-1-yl)pyrimidine-2,4-diamine [**6ee** (CYM2248)]:* ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 (d, *J* = 8.2 Hz, 2H), 7.07 (dt, *J* = 14.7, 7.7 Hz, 2H), 6.88 (br s, 1H), 6.76 (dd, *J* = 16.2, 8.3 Hz, 3H), 6.40 (br s, 1H), 5.13 (s, 1H), 3.69 (s, 3H), 3.38 (br s, 4H), 2.05 (s, 3H), 1.48 (br s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 164.03, 163.45, 162.55, 160.22, 159.78, 154.71, 139.58 (d, *J* = 6.3 Hz), 133.87, 126.78 (d, *J* = 10.1 Hz), 121.27, 120.27 (d, *J* = 3.0 Hz), 119.98 (d, *J* = 17.5 Hz), 113.96, 111.65 (d, *J* = 5.1 Hz), 75.33, 55.62, 45.51, 25.63, 24.86, 9.74 (d, *J* = 5.1 Hz). ESI-MS (*m*/*z*): calcd for $C_{23}H_{26}FN_5O$ 407.48, found 408.56 $[M+H]^+$. LC–MS >98%, t_R = 3.28 min, *m*/*z* 408 $[M+H]^+$.
*N^2-(4-methoxyphenyl)-6-(piperidin-1-yl)-N^4-(3-(trifluoromethoxy)phenyl)pyrimidine-2,4-diamine **6w** [(CYM2229)]:* ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 (d, *J* = 8.3 Hz, 2H), 7.24 (s, 1H), 7.18 (d, *J* = 7.0 Hz, 1H), 7.05 (d, *J* = 8.0 Hz, 1H), 6.77 (d, *J* = 7.2 Hz, 3H), 6.61 (br s, 1H), 5.43 (s, 1H), 3.71 (s, 3H), 3.44 (br s, 4 H), 1.52 (br s, 6H), 1.18 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 16 3.95, 161.28, 159.90, 155.02, 149.80, 141.67, 133.58, 130.19, 121.63, 118.85, 114.74, 114.04, 113.34, 100.18, 76.30, 55.63, 45.57, 25.64, 24.89. ESI-MS (*m*/*z*): calcd. for $C_{23}H_{24}F_3N_5O_2$ 459.46, found 469.56 $[M+H]^+$. LC–MS >98%, t_R = 2.07 min, *m*/*z* 470 $[M+H]^+$.
*N^4-(3-fluoro-4-methoxyphenyl)-N^2-(4-methoxyphenyl)-6-(piperidin-1-yl)pyrimidine-2,4-diamine [**6bb** (CYM2233)]:* ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 (d, *J* = 8.0 Hz, 2H), 7.07 (d, *J* = 12.8 Hz, 1H), 6.88 (s, 1H), 6.85–6.67 (m, 4H), 5.57 (s, 1H), 5.26 (s, 1H), 3.77 (s, 3H), 3.68 (s, 3H), 3.39 (br s, 4H), 1.49 (br s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 163.98, 162.39, 159.86, 154.74, 153.96, 150.71, 144.08 (d), 133.83, 133.36 (d), 121.31, 118.10, 113.96, 111.56, 111.28, 75.19, 56.71, 55.61, 45.50, 25.61, 24.85. ESI-MS (*m*/*z*): calcd for $C_{23}H_{26}FN_5O_2$ 423.48, found 424.56 $[M+H]^+$. LC–MS >98%, t_R = 1.94 min, *m*/*z* 425 $[M+H]^+$.
*N^4-(3-(difluoromethoxy)phenyl)-N^2-(4-methoxyphenyl)-6-(piperidin-1-yl)pyrimidine-2,4-diamine. [**6z** (CYM2231)]:* ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 (d, *J* = 8.8 Hz, 2H), 7.24–7.10 (m, 2H), 6.96 (d, *J* = 8.0 Hz, 1H), 6.88 (s, 1H), 6.75 (d, *J* = 8.8 Hz, 2H), 6.66 (d, *J* = 9.0 Hz, 1H), 6.39 (s, 1H), 5.43 (s, 1H), 3.70 (s, 3H), 3.43 (br s, 4H), 1.51 (br s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 163.93, 161.38, 159.86, 154.92, 151.88, 141.72, 133.65, 130.18, 121.57, 117.51, 116.03, 113.98, 113.10, 111.84, 76.31, 55.60, 45.54, 25.62, 24.87. ESI-MS (*m*/*z*): calcd for $C_{23}H_{25}F_2N_5O_2$ 441.47, found 442.56 $[M+H]^+$. LC–MS >98%, t_R = 2.12 min, *m*/*z* 443 $[M+H]^+$.
19. In the full curve binding, compounds were tested in concentrations between 10 nM and 100 μM, and a galanin control was always included in each plate. An IC_{50} is calculated if the lowest portion of the nonlinear regression 1 site competition curve is below 35% of the total binding. Ligand competition binding of ^{125}I porcine galanin to the membrane preparations was performed

in a volume of 150 μL in a 96-well plate. Cell membranes were diluted in Hepes buffer containing 25 mM Hepes, pH 7.4, 10 mM $MgCl_2$, 1 mM $CaCl_2$, 0.5% BSA, and 1× protease inhibitor. The ^{125}I porcine galanin was diluted in Tris buffer (50 mM Tris–HCl, pH 7.4, 14 mM $MgCl_2$, 2.45% BSA). Compounds and rat galanin were diluted in 20% DMSO. Eighty microliters cell membrane (2.5 μg for GalR1 and 5 μg for GalR2 binding), 55 μL ^{125}I porcine galanin, and 15 μL compound/galanin preparations were combined and incubations were carried out at room temperature for 1 h. The ^{125}I porcine galanin was used at 0.1 nM and 0.15 nM for GalR1 and GalR2 binding, respectively. The reactions were terminated by rapid vacuum filtration through glass fiber filters (Packard Bioscience), which had been pretreated with 0.3% polyethylenimine. After three washes with cold PBS solution (pH 7.4) containing 0.01% (vol/vol) Triton X-100, the filter was counted with Cobra II auto-γ-counting systems (Packard Bioscience). All data were analyzed by nonlinear regression (Prism; GraphPad).

20. All compounds were characterized by LC–MS, and gave satisfactory results in agreement with the proposed structure. Purity was determined by a C18 reverse phase HPLC column [PHENOMENEX–LUNA (50 × 4.60 mm, 3 μ)] in 10–90% CH_3CN/H_2O containing 0.02% AcOH. Flow rate 1 mL/min; (5 min gradient) and monitored by a UV detector operating at 254 nm. Data collection was in the positive ion mode. LC–MS M+H signals were consistent with expected molecular weight for all reported products.