Bioorganic & Medicinal Chemistry Letters 19 (2009) 2591–2594

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Pyridopyrimidine based cannabinoid-1 receptor inverse agonists: Synthesis and biological evaluation

John S. Debenham [a,*], Christina B. Madsen-Duggan [a], Junying Wang [a], Xinchun Tong [a], Julie Lao [b], Tung M. Fong [b], Marie-Therese Schaeffer [b], Jing Chen Xiao [b], Cathy C. R.-R. Huang [b], Chun-Pyn Shen [b], D. Sloan Stribling [c], Lauren P. Shearman [c], Alison M. Strack [c], D. Euan MacIntyre [c], Jeffrey J. Hale [a], Thomas F. Walsh [a]

[a] Department of Medicinal Chemistry, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065, USA
[b] Department of Metabolic Disorders, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065, USA
[c] Department of Pharmacology, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065, USA

ARTICLE INFO

Article history:
Received 3 January 2009
Revised 3 March 2009
Accepted 4 March 2009
Available online 9 March 2009

Keywords:
Cannabinoid
CB1
Inverse agonist
Pyridopyrimidine
Obesity

ABSTRACT

The synthesis, SAR and binding affinities are described for cannabinoid-1 receptor (CB1R) specific inverse agonists based on pyridopyrimidine and heterotricyclic scaffolds. Food intake and pharmacokinetic evaluation of several of these compounds indicate that they are effective orally active modulators of CB1R.

The incidence of clinically severe obesity continues to increase globally. In the United States 5% of the population is considered morbidly obese.[1] The estimated cost to the United States for all obesity related issues in 2000 was a staggering $117 billion ($61 billion direct and $56 billion indirect).[2] Obese patients respond well to surgical interventions that modify the gastrointestinal tract limiting food/nutritional intake.[1] Unfortunately, in a study of Medicare beneficiaries undergoing bariatric surgical procedures, one out of 50 patients die within the first 30 days post surgery.[3] Clearly, more conservative approaches for treatment are needed.

Inverse agonists of the cannabinoid-1 receptor (CB1R)[4] have been shown to be effective in reducing food intake with concomitant weight loss in both human[5] and animal[6] studies. In our previous reports,[7] we disclosed the SAR and lead optimization of a screening hit containing a pyridine core arrayed by three hydrophobic domains. This was modified to **1** which effected reductions in body weight (BW) gain and suppressed food intake (FI) in diet induced obese (DIO) rats at 10 mg/kg (48% FI suppression and −8 g BW change vs +7 g vehicle).

Removal of the difluorophenoxy ring of **1** and annulation of a new ring to the core structure ultimately led to naphthyridinone **2**.[8] This compound showed similar binding affinity to **1** but was markedly more brain penetrant in rats (4 h brain/plasma 2.7 vs 0.61). Cannabinoid-1 receptors are primarily located in the CNS[5] therefore **2** had similar in vivo efficacy as **1** at one third of the dose (Fig. 1). Additionally, the acute efficacy of **2** was on par with that of MK-0364/taranabant which has been tested extensively in rodents and humans (Fig. 1). MK-0364 dosed in rats at 3 mg/kg leads to a 49% FI suppression and −10 g BW change versus +10 g vehicle.[9] Since **2** had low clearance in several of our safety assessment species (rats and dogs, but not monkeys),[8] we wanted to further optimize drug candidates by maintaining in vivo efficacy while decreasing half-life. Given that the heterobicyclic core of **2** showed better efficacy at suppressing food intake relative to the monocyclic pyridine core of **1**, we opted to pursue modifications to the naphthyridinone core. Herein we describe the SAR and synthetic efforts utilizing the generalized pyridopyrimidine core **3**.[10]

The construction of the pyrido[2,3-*d*]pyrimidine core system has been reviewed.[11] Since CB1 inverse agonists often contain three hydrophobic domains arrayed around a more polar core[4] we envisioned a hydrophobic domain in the R^1 position. In our SAR efforts, amidines were engaged to position the hydrophobic group. Addition of *t*-butylcarbamidine to the appropriately substituted ethyl 2-chloronicotinate in the presence of DBU at 135 °C afforded the pyridopyrimidinone **5** directly (Scheme 1).

* Corresponding author. Tel.: +1 732 594 5497; fax: +1 732 594 2210.
E-mail address: john_debenham@merck.com (J.S. Debenham).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.03.005

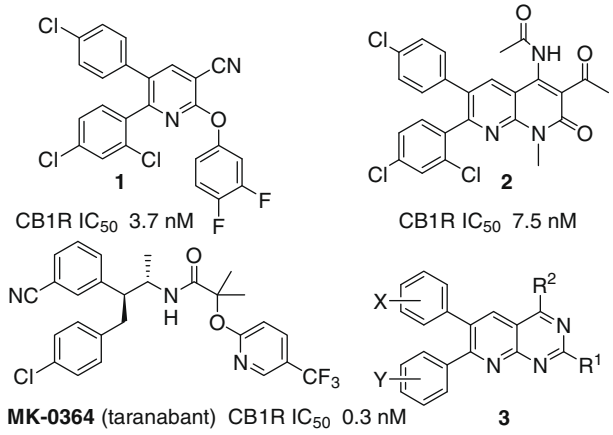
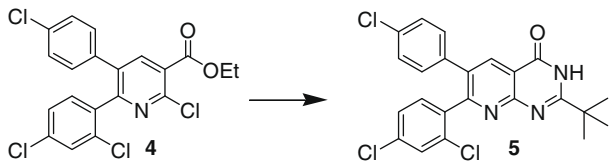


Figure 1. Early Merck leads, MK-0364 and core structure **3**.



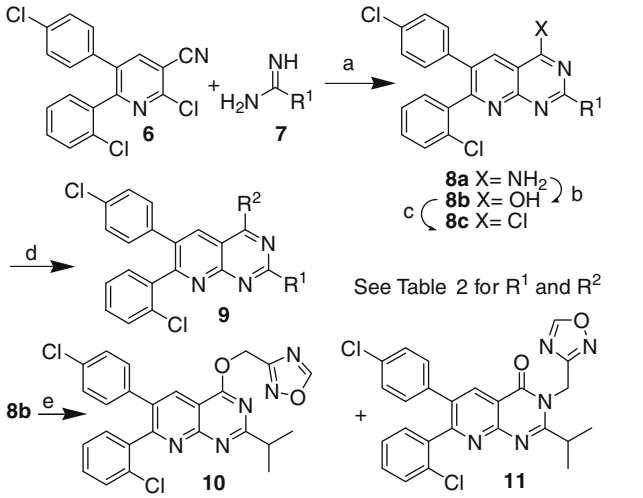
Scheme 1. Reagents and conditions: (a) *tert*-butylcarbamidine hydrochloride, DBU, DMF, 138 °C, 50%.

Alternatively, amidine addition to the 2-chloronicotinonitrile **6** affords the 4-aminopyridopyrimidine analog **8a** which can then be hydrolyzed to the pyridopyrimidinone **8b** with methanesulfonic acid in high yield (Scheme 2). As the 3-ethylcarboxylate group for the starting material in Scheme 1 was prepared from hydrolysis of the 3-cyano group, analogs could be prepared more expeditiously and with greater diversity (both 4-amino and 4-hydroxy variants) utilizing the general route depicted in Scheme 2. After hydrolysis of the amino group, treatment of the resultant pyridopyrimidinone with $POCl_3$ in toluene produced a very reactive 4-chloro derivative **8c** that was ready for nucleophilic displacement or Suzuki cross coupling reactions. Exposure of **8c** to an excess of the necessary amine in THF produced a variety N-substituted analogs in excellent yield. The effects of N- and O-substitution of the 3- and 4-positions, respectively, were also examined. Alkylations were carried out by treating the pyridopyrimidinone **8b** to Cs_2CO_3 and the appropriate alkylation agent as exemplified with the chloromethyl oxadiazole (Scheme 2).

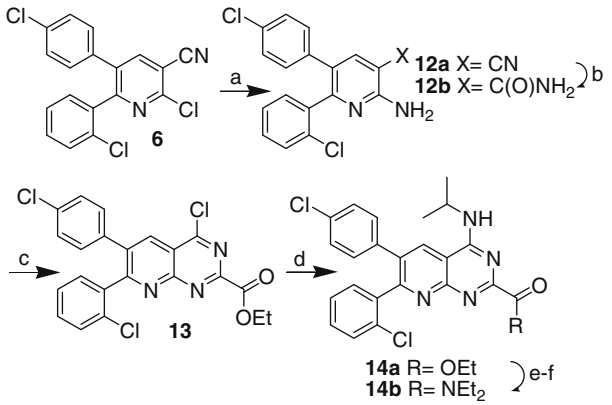
In order to evaluate the effect of amides at the 2-position of the pyridopyrimidine system, **6** was converted to the substituted 2-aminonicotinamide as shown in Scheme 3. Acylation and cyclization with ethyl 2-chloro-2-oxoacetate followed by in situ chlorination with $POCl_3$ provided **13** that was elaborated to the *i*-propyl amino **14a**. Saponification of the ester allowed for amide formation as indicated to afford **14b**.

The chlorine at the 4-position of **8c** provided an opportunity for elaboration of the bicyclic scaffold into a tricyclic core. The fusion of the third ring at this site was readily achieved in two steps (Scheme 4). Exposure of **8c** to excess hydrazine provided hydrazide **15**. This, in turn, was cyclized with either carbonyldiimidazole at room temperature or trimethylorthoformate in refluxing toluene forming the fused triazolone[12] **16** or triazole **17**, respectively (Scheme 4).

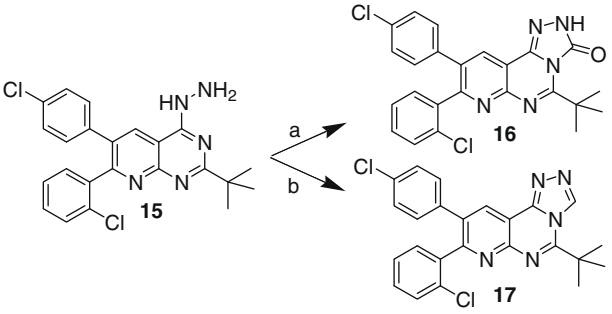
In order to more fully explore the effects of aryl substitution on the newly formed heterotricyclic core, a Suzuki strategy was employed in which triazolone **21** could be reacted with a variety of



Scheme 2. Reagents and conditions: (a) DBU, DMF, 130 °C, 50–66%; (b) $MeSO_3H$, H_2O, 110 °C, 95%; (c) $POCl_3$, toluene, 108 °C, 81%; (d) for amine nucleophiles, excess amine, THF, rt-40 °C or for **35** 4-fluorophenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, DME, H_2O, EtOH, microwave heating 120 °C, 30%; (e) 3-(chloromethyl)-1,2,4-oxadiazole, Cs_2CO_3, DMF, rt, 58% **11**, 6% **10**.

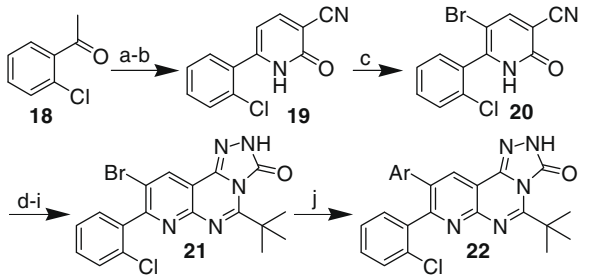


Scheme 3. Reagents and conditions: (a) NH_4OH, dioxane, 100 °C, 95%; (b) H_2SO_4, 100 °C; (c) $ClCOCO_2Et$, toluene, then $POCl_3$, 100 °C, 40%; (d) isopropylamine, THF, rt, 88%; (e) KOH, THF, H_2O, rt; (f) diethylamine, HOBT, EDAC, DIPEA, dimethylacetamide, 40 °C, 22%.



Scheme 4. Reagents and conditions: (a) CDI, THF, CH_2Cl_2, 89%; (b) triethylorthoformate, NMP, 130 °C, 48%.

boronic acids on the final step of the synthesis, thereby facilitating the rapid assembly of analogs (Scheme 5). The pyridone ring of **19** was prepared by treating chloroacetophenone with dimethylformamide dimethylacetal to yield a vinylogous amide which was reacted with cyanoacetamide under basic conditions to generate

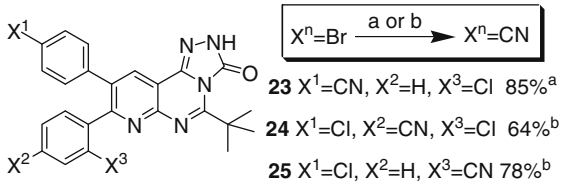


Scheme 5. Reagents and conditions: (a) $(CH_3)_2NCH(OCH_3)_2$, DMF, 90 °C, 89%; (b) 2-cyanoacetamide, DMF, $NaOCH_3$, 95 °C, 51%; (c) NBS, dioxane, MeOH, rt, 88%; (d) $POCl_3$, toluene, 90 °C, 82%; (e) *tert*-butylcarbamidine hydrochloride, DBU, DMA, microwave heating 135 °C, 58%; (f) $MeSO_3H$, H_2O, 110 °C, 92%; (g) $POCl_3$, toluene, 110 °C, 90%; (h) hydrazine hydrate, THF, rt; (i) CDI, CH_2Cl_2, rt, 85%; (j) $ArB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, DME, H_2O, EtOH, microwave heating, 120 °C, 9–37%.

19. Bromination with NBS provided **20** in 88% yield on a 100 g scale. The remaining steps were similar to that described above.

Finally, the effect of replacement of aryl chlorides with cyano groups (Scheme 6) was explored as these groups can be helpful in reducing the log D values, potentially improving physicochemical properties such as solubility. Two palladium catalyzed methods were utilized (Scheme 6). While the substrates were not identical, we found that treatment with $Zn(CN)_2$, $Pd_2(dba)_3$, dppf in slightly aqueous DMF[13] (conditions a) to be more robust and suitable for scale up.

Binding affinities were determined using a standard protocol and all compounds tested were found to be functional inverse agonists of CB1R.[14] In optimization to find an appropriate hydrophobic domain R^1 (Table 1), we found the methyl analogs **26** and **27** least active at 2500 and 1500 nM CB1R, respectively. Not surprisingly,

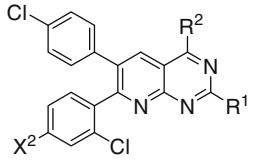


Scheme 6. Reagents and conditions: (a) $Zn(CN)_2$, $Pd_2(dba)_3$, dppf, DMF/H_2O 99:1, 110 °C; (b) $Pd(PPh_3)_4$, 18-crown-6, dioxane, KCN, 90 °C.

increasing the bulk of R^1 from methyl to *i*-propyl and then to *t*-butyl provided the most active compounds, showing over 300-fold improvement to 6–7.4 nM for **30** and **5**, respectively. Further elaboration of R^1 to 4-chlorophenyl did not yield any additional improvement in binding activity. It is of note that there was only a small difference in potency where R^2 was either amino or hydroxy (or its tautomer). The spread was only 1–5-fold, being most pronounced (fivefold) for the *t*-butyl pair **30** and **31** where X^2 was hydrogen.

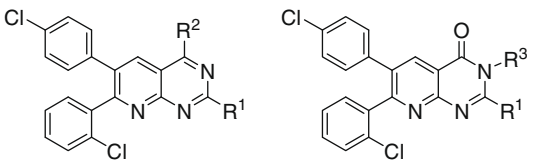
After optimization of R^1, we turned out attention to the further examination of R^2 and substitution of the 3-position nitrogen with R^3 (Table 2). In our examples, the elaboration of R^2 led to highly potent compounds (<10 nM CB1R). The carbon linked 4-fluorophenyl **35** was the most potent at 0.6 nM. The dimethylamino **41**, and hydroxyl fluorinated alkyl **42** were about 12-fold less active at about 9 nM CB1R. Piperazine **36**, diethyl **37**, *t*-butyl **38**, and *i*-propylamino **39** all had similar potencies of 2–4 nM. O-linked derivatives at R^2 were also acceptable with oxadiazole **10** having an IC_{50} of 1 nM. In contrast, the N-linked oxadiazole **11** was 80-fold less ac-

Table 1
Binding affinity of compounds at the human CB1R and CB2R expressed as IC_{50} (nM)



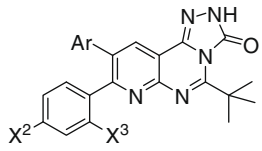
Compound	R^1	R^2	X^2	CB1R:CB2R
26	Me	OH	H	2500:>2000
27	Me	NH_2	H	1500:1100
28	*i*-Pr	OH	H	130:7300
29	*i*-Pr	NH_2	H	190:>2000
30	*t*-Bu	OH	H	7.4:8600
5	*t*-Bu	OH	Cl	6.0:5500
31	*t*-Bu	NH_2	H	40:4900
32	*t*-Bu	NH_2	Cl	11:5200
33	4-Cl-Ph	OH	H	59:>2000
34	4-Cl-Ph	NH_2	H	16:>2000

Table 2
Binding affinity of compounds at the human CB1R and CB2R expressed as IC_{50} (nM)



Compound	R^2	R^3	R^1	CB1R: CB2R
35	4-F–Ph	—	*t*-Bu	0.63:9833
36	–NPiperazine	—	*t*-Bu	1.9:1800
37	$-NEt_2$	—	*t*-Bu	2.2:2700
38	–NH*t*-Bu	—	*t*-Bu	2.7:>2000
39	–NH*i*-Pr	—	*t*-Bu	4.1:>2000
14b	–NH*i*-Pr	—	$CONEt_2$	150:12,000
40	–OMe	—	*t*-Bu	7.5:8400
41	$-NMe_2$	—	*t*-Bu	8.5:1800
42	$-NHCH_2CF_2CH_2OH$	—	*t*-Bu	9.5:>2000
10	–OCH$_2$(1,2,4-oxadiazol-3yl)	—	*i*-Pr	1.1:6300
11	—	–CH$_2$(1,2,4-oxadiazol-3yl)	*i*-Pr	82: 9800

Table 3
Binding affinity of compounds at the human CB1R and CB2R expressed as IC_{50} (nM)



Compound	Ar	X^2	X^3	CB1R:CB2R
16	4-Cl-Ph	H	Cl	1.9:3800
43	3-Cl-Ph	H	Cl	34:2700
23	4-CN-Ph	H	Cl	5.9:18,000
24	4-Cl-Ph	CN	Cl	1.0:19,000
25	4-Cl-Ph	H	CN	3.2:2400
44	4-MeO-Ph	H	Cl	3.0:891
45	4-CF_3-Ph	H	Cl	1.1:9200
46	4-Ac-Ph	H	Cl	10:5800
47	4-NMe_2-Ph	H	Cl	28:770
48	4-CN-3-Pyr	H	Cl	100:18,000

Table 4
Rat overnight (18 h) body weight change (g) and food intake[a]

Compound	ΔBW vehicle (control)	ΔBW compound	Food intake suppression (%)
5	+5	+1	21
42	+4	−4	39
16	+5	−9	50
23	+5	−5	36
25	+6	−2	25
2	+12	−4	68

[a] BW = body weight. All rats were dosed at 3 mg/kg. All p values were <0.05.

Table 5
Pharmacokinetic profiles (Monkey = Rhesus macaque)

Compound (animal)	F (%)	$T_{1/2}$ (h)	Clp (mL/min/kg)	Rat brain/plasma 0.25 h, 4 h
16 (rat)	33	5.6	12	0.14, 0.59
23 (rat)	53	3.5	4.0	NA
23 (dog)	2.1	7.4	24	NA
23 (monkey)	9.7	9.9	5.3	NA
2 (rat)	93	>8	12	0.9, 2.7

tive. Increasing the hydrophilicity of R^1 by installation of diethylamide **14b** showed a 37-fold drop in binding affinity relative to **39** demonstrating the preference for more hydrophobic groups at this position.

Annulation of the triazolone ring between the 3- and 4-positions was also very well tolerated in the binding assay (Table 3). 4-Substituted phenyl groups were critical for good activity at CB1R: 4-chloro **16**, 4-OMe **44**, 4-CF_3 **45** were all very attractive at 1–3 nM CB1R. When the 4-chloro was moved to the 3-position (**43**), an 18-fold drop in potency to 34 nM was observed. Exchange of chloro with the cyano group resulted in little difference in binding affinity for the cyano analogs **23**, **24**, and **25**. 4-Acetyl **46** at 10 nM was also reasonably well tolerated compared to 4-chloro **16**. As the aryl group (Ar) became substantially more polar we saw a 17-fold drop in activity to 100 nM CB1R for 4-cyano-pyridine **48**. Of note was the des-oxy variant **17** (Scheme 4), where loss of the oxygen only resulted in a fourfold drop in activity (8.0 nM CB1R:3400 CB2R).

Many of the compounds described herein were very active at CB1R and were highly selective against CB2R (100–19,000-fold). Several compounds were evaluated in vivo to determine their anorexigenic effects in DIO rats (Table 4). All compounds were dosed orally at 3 mg/kg and rats were monitored for 18 h to determine changes in body weight (BW) and ability to suppress food intake (FI) relative to vehicle control.[8] Both bicyclic **5** and **42** were active in suppressing food intake (21–39%, respectively) and reducing overnight BW relative to control. Tricyclic **16** was 3–5-fold more potent than **5** and **42** at CB1R and showed the strongest response with a 50% suppression of FI. Tricyclic **23** and **25** were also effective at acute suppression of overnight FI (36–25%, respectively).

Pharmacokinetic properties for select compounds are shown in Table 5. The potent in vivo action of previously reported **2** on the modulation of feeding behavior is the result of extended drug exposure over time, as reflected by its long half-life, and its favorable brain/plasma ratio. Tricyclic **16** shows a shorter half-life, less oral bioavailability and less brain penetration, yet only afforded a small decrease in response. Cyano analog **23** has the shortest half-life in the rat. In higher species, **23** also had a much shorter half-life than **2** along with an unfortunate drop in bioavailability.

In summary, we have shown that both the heterobicyclic pyrido[2,3-*d*]pyrimidine and heterotricyclic pyrido[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidin-3(2*H*)-one core structures (typified by **42** and **16**) can lead to potent and specific CB1R inverse agonists that are effective in modulating feeding behavior to suppress both FI and BW gain. Importantly, tricyclic **16** achieved similar in vivo efficacy to **2** at 3 mg/kg oral dosing, but with a reduced half-life. Further SAR studies and pharmacological evaluation of related heterobicyclic systems will be reported in due course.

References and notes

1. Buchwald, H.; Avidor, Y.; Braunwald, E.; Jensen, M. D.; Pories, W.; Fahrbach, K.; Schoelles, K. *J. Am. Med. Assoc.* **2004**, *292*, 1724.
2. U.S. Department of Health and Human Services. The Surgeon General's call to action to prevent and decrease overweight and obesity. [Rockville, MD]: U.S. Department of Health and Human Services, Public Health Service, Office of the Surgeon General; [2001]. Available from: US GPO, Washington.
3. The rates of 30-day, 90-day and 1 year mortality were 2.0%, 2.8%, and 4.6%, respectively: Flum, D. R.; Salem, L.; Dellinger, E. P.; Cheadle, A.; Chan, L. *J. Am. Med. Assoc.* **2005**, *294*, 1903.
4. For a leading review of reported compounds see: Jagerovic, N.; Fernandez-Fernandes, C.; Goya, P. *Curr. Top. Med. Chem.* **2008**, *8*, 205.
5. Després, J.-P.; Golay, A.; Sjöström, L. *New Engl. J. Med.* **2005**, *353*, 2121.
6. Cota, D.; Marsicano, G.; Tschöp, M.; Grübler, Y.; Flachskamm, C.; Schubert, M.; Auer, D.; Yassouridis, A.; Thöne-Reinecke, C.; Ortmann, S.; Tomassoni, F.; Cervino, C.; Nisoli, E.; Linthorst, A. C. E.; Pasquali, R.; Lutz, B.; Stalla, G. K.; Pagotto, U. *J. Clin. Invest.* **2003**, *112*, 423.
7. (a) Meurer, L. C.; Finke, P. E.; Mills, S. G.; Walsh, T. F.; Toupence, R. B.; Debenham, J. S.; Wang, J.; Tong, X.; Fong, T. M.; Lao, J.; Schaeffer, M.-T.; Chen, J.; Shen, C.-P.; Stribling, D. S.; Shearman, L. P.; Strack, A. M.; MacIntyre, D. E.; Van der Ploeg, L. H. T.; Goulet, M. T. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 645. and 1755; (b) Madsen-Duggan, C.; Debenham, J. S.; Walsh, T. F.; Toupence, R. B.; Huang, S. X.; Wang, J.; Tong, X.; Lao, J.; Fong, T. M.; Schaeffer, M.-T.; Xiao, J. C.; Shen, C.-P.; Stribling, D. S.; Shearman, L. P.; Strack, A. M.; Van der Ploeg, L. H. T.; Goulet, M. T. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2031.
8. Debenham, J. S.; Madsen-Duggan, C. B.; Walsh, T. F.; Wang, J.; Tong, X.; Doss, G. A.; Lao, J.; Fong, T. M.; Schaeffer, M.-T.; Xiao, J. C.; Huang, C. R.-R. C.; Shen, C.-P.; Feng, Y.; Marsh, D. J.; Stribling, D. S.; Shearman, L. P.; Strack, A. M.; MacIntyre, D. E.; Van der Ploeg, L. H. T.; Goulet, M. T. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 681.
9. Fong, T. M.; Guan, X.-M.; Marsh, D. J.; Shen, C.-P.; Stribling, D. S.; Rosko, K. M.; Lao, J.; Yu, H.; Feng, Y.; Xiao, J. C.; Van der Ploeg, L. H. T.; Goulet, M. T.; Hagmann, W. K.; Lin, L. S.; Lanza, T. J., Jr.; Jewell, J. P.; Liu, P.; Shah, S. K.; Qi, H.; Tong, X.; Wang, J.; Xu, S. S.; Francis, B.; Strack, A. M.; MacIntyre, D. E.; Shearman, L. P. *J. Pharm. Exp. Ther.* **2007**, *321*, 1013.
10. All yields provided are for unoptimized reactions.
11. Sako, M.. In *Science of Synthesis*; Yamamoto, Y., Ed.; Thieme, 2004; Vol. 16, pp 1155–1267.
12. Unoptimized procedure for **16**: *5-tert-Butyl-8-(2-chlorophenyl)-9-(4-chlorophenyl)pyrido[3,2-e][1,2,4]triazolo[4,3-c]pyrimidin-3(2H)-one*. To **8c** where R^1 = *t*-Bu (252 mg, 0.569 mmol) in THF (7 mL) was added hydrazine hydrate (0.7 mL, 14.4 mmol). The reaction aged at rt for about 25 min and was concentrated. The residue was diluted with EtOAc, washed with brine, dried (Na_2SO_4), filtered and concentrated to afford **15** which was used without further purification. To **15** (assumed 0.569 mmol) in CH_2Cl_2 (10 mL) was added 1,1′-carbonyldiimidazole (420 mg, 2.59 mmol) and the reaction aged about 18 h at rt. The reaction was diluted with EtOAc, washed with brine and concentrated. The residue was purified via flash chromatography, on silica gel, gradient eluted with 0–10% EtOAc in CH_2Cl_2 affording **16** (235 mg, 89%). HPLC/MS: 464.0 (M+1), 466.0 (M+3); R_t = 3.75 min (Waters C18 XTerra 3.5 μm 3.0 × 50 mm column with gradient 10:90–100 v/v CH_3CN/H_2O + v 0.05% TFA over 3.75 min then hold at 100 CH_3CN + v 0.05% TFA for 1.75 min; flow rate 1.0 mL/min, UV wavelength 254 nm). ^{1}H NMR (500 MHz, $CDCl_3$): δ 10.27 (s, 1 H); 8.47 (s, 1 H); 7.45–7.42 (m, 1 H); 7.33–7.24 (m, 3 H); 7.22 (d, J = 8.35 Hz, 2 H); 7.13 (d, J = 8.35 Hz, 2 H); 1.68 (s, 9 H).
13. Maligres, P.; Waters, M. S.; Fleitz, F.; Askin, D. *Tetrahedron Lett.* **1999**, *40*, 8193.
14. The reported binding results were an average of 2–8 independent determinations (each done in replicate) which were normally within 50% of the average value. For both the binding and functional assay conditions, see: Felder, C. C.; Joyce, K. E.; Briley, E. M.; Mansouri, J.; Mackie, K.; Blond, O.; Lai, Y.; Ma, A. L.; Mitchell, R. L. *Mol. Pharmacol.* **1995**, *48*, 443.