Bioorganic & Medicinal Chemistry 19 (2011) 2927–2938

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Development and SAR of functionally selective allosteric modulators of $GABA_A$ receptors

Cristobal Alhambra [a], Chris Becker [a], Timothy Blake [a], Amy (Hui-Fang) Chang [a], James R. Damewood Jr. [a], Thalia Daniels [a], Bruce T. Dembofsky [a], David A. Gurley [c], James E. Hall [a], Keith J. Herzog [a], Carey L. Horchler [a], Cyrus J. Ohnmacht [a], Richard Jon Schmiesing [a], Adam Dudley [c], Maria D. Ribadeneira [b], Katherine S. Knappenberger [c], Carla Maciag [c], Mark M. Stein [c], Maninder Chopra [c], Xiaodong F. Liu [c], Edward P. Christian [c], Jeffrey L. Arriza [b], Marc J. Chapdelaine [a,*]

[a] Department of Chemistry, AstraZeneca Pharmaceuticals, Wilmington, DE 19850, USA
[b] Department of Disposition, Metabolism and Pharmacokinetics, AstraZeneca Pharmaceuticals, Wilmington, DE 19850, USA
[c] Department of Neuroscience Biology, AstraZeneca Pharmaceuticals, Wilmington, DE 19850, USA

ARTICLE INFO

Article history:
Received 30 November 2010
Revised 9 March 2011
Accepted 16 March 2011
Available online 29 March 2011

Keywords:
$GABA_A$ receptor
Benzodiazepine
Allosteric modulator
Subtype functional selectivity
Cinnoline
Quinoline
Anxiety

ABSTRACT

Positive modulators at the benzodiazepine site of α2- and α3-containing $GABA_A$ receptors are believed to be anxiolytic. Through oocyte voltage clamp studies, we have discovered two series of compounds that are positive modulators at α2-/α3-containing $GABA_A$ receptors and that show no functional activity at α1-containing $GABA_A$ receptors. We report studies to improve this functional selectivity and ultimately deliver clinical candidates. The functional SAR of cinnolines and quinolines that are positive allosteric modulators of the α2- and α3-containing $GABA_A$ receptors, while simultaneously neutral antagonists at α1-containing $GABA_A$ receptors, is described. Such functionally selective modulators of $GABA_A$ receptors are expected to be useful in the treatment of anxiety and other psychiatric illnesses.

1. Introduction

Quinolines and cinnolines of types **1** and **2** (Table 1) have been reported to be ligands for the benzodiazepine (BZ) binding site of the $GABA_A$ receptors.[1,2] On further exploration, we have identified compounds that are α2/α3 functionally selective over the α1 subtype. SAR studies to enhance this functional selectivity and deliver clinical candidates are described.

1.1. Background

Of the two GABA receptor ion channels, $GABA_A$ and $GABA_C$, $GABA_A$ has received the most detailed characterization because of the variety of chemotypes that elicit a wide range of responses.[3] Among the compounds are BZs that produce alterations in seizures, sedation, motor activity, cognition and level of anxiety.

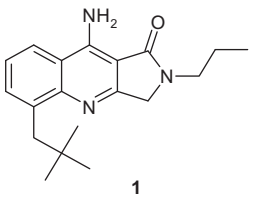
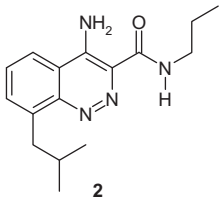
The $GABA_A$ receptor is a ligand-gated chloride ion channel with a pentameric structure composed of a minimum of 16 subunits. Most central nervous system $GABA_A$ receptors contain α, β, and γ-subunits in a 2:2:1 stoichiometry. In particular, α1, α2, α3 and α5 are found in conjunction with β and γ-subunits and provide a BZ binding site at the α,γ-interface. Compounds binding at the BZ site can have three functional effects or three distinct modulatory modes on the chloride current. First, binding at the BZ site may result in agonism or positive allosteric modulation (PAM) and increase the GABA induced chloride current. Second, binding at the BZ site may result in inverse agonism or negative allosteric modulation (NAM) and decrease GABA induced chloride current. Third, binding at the BZ site may produce neutral antagonism (NA) and have no effect on the chloride current. Differing behavioral effects result from these different mechanisms of action at the α2 and α3 subtype receptors; agonists or PAMs are anxiolytic, whereas, inverse agonists or NAMs are anxiogenic and NAs have no apparent physiological effect.[4]

Abbreviations: GABA, γ-aminobutyric acid; CNS, central nervous system; PAM, positive allosteric modulator; α_x-$GABA_AR$, α_x-containing $GABA_A$ receptor; NAM, negative allosteric modulator; NA, neutral antagonist; SAR, structure–activity relationships; BZ, benzodiazepine; DZ, diazepam; Frac, fraction.

* Corresponding author. Tel.: +1 302 478 6341.
E-mail address: marc.chapdelaine@verizon.net (M.J. Chapdelaine).

0968-0896/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2011.03.035

Table 1
Profile of legacy quinoline and cinnoline in current assays

	Desired activity[11b]	 1	 2
A2 pK_i[12]	>8.0	8.0	9.0
A1 Oocyte (Frac DZ at 1 μM)[13]	<0.1	0.44	0.4
A2 Oocyte (Frac DZ at 1 μM)[13]	>0.15	0.27	0.61
A3 Oocyte (Frac DZ at 1 μM)[13]	>0.15	—	0.4
Solubility[14] (μM)	>10	3.4	4.6
Human microsomal clearance[15] (μL/min/mg)	<25	230	120

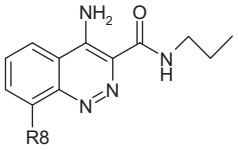
Typically, BZs have near equipotent affinities at α1, α2, α3 and α5 containing $GABA_A$ receptors and display uniform PAM activity at all these subtype receptors. Although many of these BZs are efficacious anxiolytics, they generally profile with a number of undesirable side effects such as ataxia, sedation, potentiation of alcohol, tolerance and dependence upon chronic administration.[4]

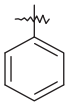
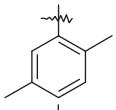
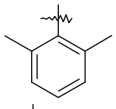
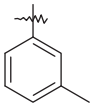
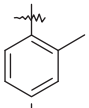
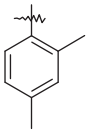
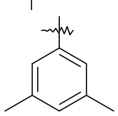
In a series of elegant experiments, it has been demonstrated that the α1 containing subtype of the $GABA_A$ receptor is implicated in the sedation and motor effects typically seen upon administration of classic BZs. On the other hand, α2 and α3 activity is associated with anxiolytic effects.[5] We envisioned that functionally selective ligands would provide improved therapies for anxiety. Compounds with α1 neutral antagonism and α2/α3 agonism should provide a more desirable therapeutic profile—anxiolysis without sedation.[6]

2. Results and discussion

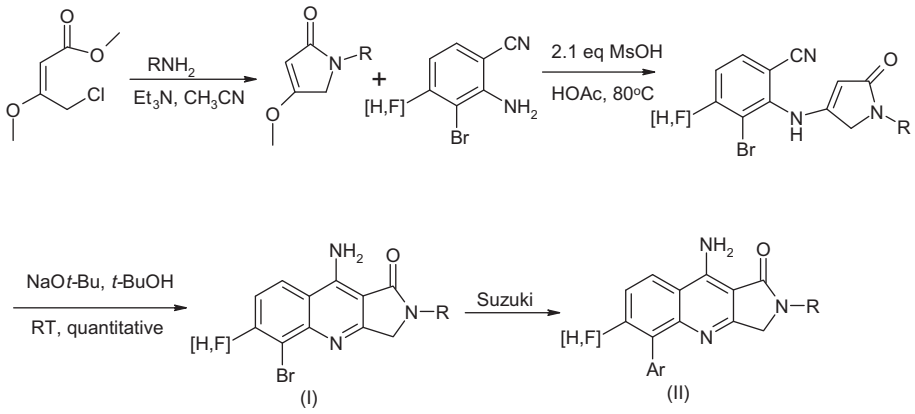
We describe renewed investigation of the quinoline **1** and cinnoline **2** series in this publication and the discovery of $GABA_A$ BZ site ligands with functional selectivity for the α2 and α3 subtypes over the α1 containing subtype.

Table 2
SAR of R^8 phenyl, tolyl and xylyl substitutions



	R^8 structure	A2 pK_i[12]	A1 Oocyte (Frac DZ at 1 μM)[13]	A2 Oocyte (Frac DZ at 1 μM)[13]	A3 Oocyte (Frac DZ at 1 μM)[13]	Sol[14] (μM)	Clint[15] (μL/min/mg)
3		8.18 ± 0.05	−0.2 ± 0.1	0.08 ± 0.01		9.5	76
4		8.26 ± 0.77	0.28 ± 0.04	0.38 ± 0.09		1.4 ± 0.03	25
5		8.07 ± 0.77	−0.26 ± 0.09	0.0 ± 0.01			130
6		8.14 ± 0.3	0.17 ± 0.07	0.23 ± 0.01	0.26 ± 0.05	8 ± 1.4	100
7		8.71 ± 0.08	0.06 ± 0.05	0.03 ± 0.03		26.8 ± 1.04	26
8		8.49 ± 0.11	0.28 ± 0.09	0.11 ± 0.1	0.09 ± 0.06	0.8	25
9		8.05 ± 0.34	0.4 ± 0.14	0.46 ± 0.09	0.17 ± 0.04	0.8 ± 0.24	29

Quinoline Synthesis



R = R^2 as defined in Tables 4, 6 and 7.

Scheme 1. Quinoline synthesis.

2.1. Earlier work with the quinoline and cinnoline series

The earlier investigations of the quinolines and cinnolines, prior to the recognition of the subtypes, primarily had characterized each series through classic membrane binding and in vivo pharmacology. These efforts had identified numerous nanomolar and sub-nanomolar binders in each series. In vivo tests had shown examples in each series to be apparently non-sedating anxiolytics in rodents.[2b] Progression of each series of compounds was halted for similar reasons: low bioavailability attributed to high metabolic clearance and toxicity presumed to result from potential reactive metabolite formation. Nevertheless, evaluation with updated assays determined the quinolines and cinnolines as reasonable starting points for renewed work in search of functionally selective BZ ligands.

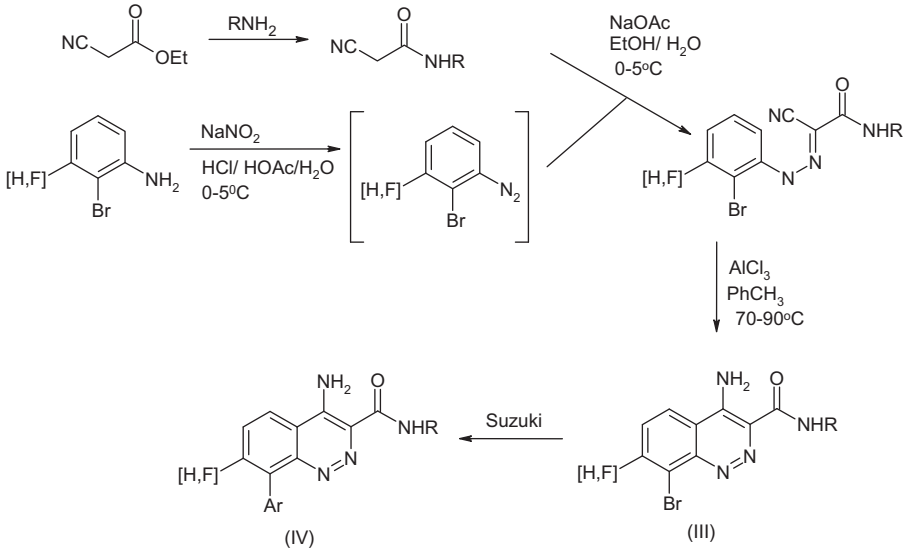
2.2. Challenges facing current effort

Table 1 highlights some of the challenges which needed to be addressed in our current $GABA_A$ effort to enable meeting the desired levels of activity opposite the key in vitro assays. In the instance of the prototypical quinoline **1** and cinnoline **2**, the efficacy at the α1 subtype (expressed relative to diazepam (DZ) efficacy at 1.0 μM concentration) was much greater than desired and human microsomal clearance was an order of magnitude greater than acceptable.

2.2.1. Reevaluation of 8-phenyl cinnolines

Upon evaluation against the updated criteria, the 8-phenyl cinnoline congeners attracted our attention for a number of reasons (Table 2). First, all had maintained high affinity tor the α2

Cinnoline Synthesis



R = R^3 as defined in Tables 2,3,5 and 7.

Scheme 2. Cinnoline synthesis.

binding site—pK_i greater than 8. More intriguingly, when examined through the prism of patch clamp electrophysiology, a range of α1 efficacies were observed relative to the effect of 1.0 μM DZ as measured in the same oocytes. The 8-phenyl compound **3** is an inverse agonist/NAM at α1. The addition and positioning of one and two methyl groups on the 8-phenyl ring dramatically impacts the compounds efficacies. Addition of a single methyl group at the 2-position of the 8-phenyl group, compound **7**, reverses the NAM α1 activity of **3**, making **7** a neutral antagonist at α1. Moving the single methyl group from the 2-position of the 8-phenyl group to the 3-position, **6**, converts the compound to a modest α1 PAM.

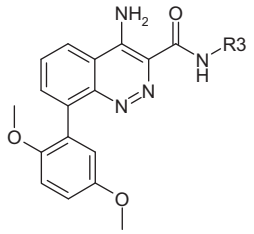
In the case of α2 efficacies, the 2-methyl of **7** had little effect on its α2 efficacy and maintains α2 NA activity like **3**. Moving the single methyl group from the 2-position of the 8-phenyl group to the 3-position, **6**, converts the compound to a modest α2 PAM.

Disubstitution of the 8-phenyl ring with methyl groups produces a range of α1 and α2 efficacies. The 2,5-disubstitution of **4** with methyl groups renders an α1 and α2 PAM. Whereas disposition of the two methyl groups in 2,6-positions of the 8-phenyl ring produces an α1 NAM, **5,** with NA at α2. When methyl groups are displayed at the 2,4-positions of the 8-phenyl ring, **8,** an α1 PAM with weak α2 PAM results. The 3,5-dimethyl derivative, **9**, produced relatively robust α1 and α2 PAMs. Despite the fact that none of the compounds in Table 2 had the profile that we desired, the range of efficacies accessible through the aegis of these methyl groups on the cinnolines encouraged a more in-depth investigation of the role of the substituted-8-aryl and heteroaryl groups on functional activity.[7,8] Correspondingly, substituted-5-aryl and heteroaryls on the quinolines were also evaluated.

2.2.2. Need for improved solubilities

Only four compounds in Table 2 possess aqueous solubilities greater than 1.0 μM. Part of our immediate attention turned to identifying groups bioisosteric to the methyl groups that might improve aqueous solubility and simultaneously add the benefit of the sought functional selectivity.[9] One approach was to investigate the effect of an oxygen spacer placed between the phenyl ring and the methyl group. At a minimum, it was hoped that this would be a means of improving the aqueous solubility of our compounds with an accompanying modest reduction of lipophilicity.[10]

Table 3
SAR of R^3 substitutions of 8-(2,5-dimethoxyphenyl)-cinnoline-carboxamides



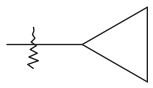
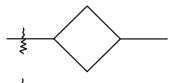
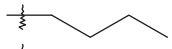
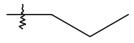
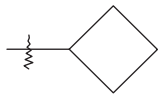
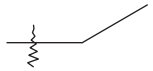
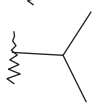
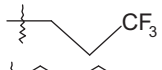
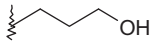
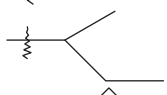
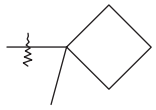
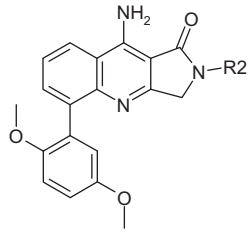
	R^3 structure	A2 pK_i[12]	A1 Oocyte (Frac DZ at 1 μM)[13]	A2 Oocyte (Frac DZ at 1 μM)[13]	A3 Oocyte (Frac DZ at 1 μM)[13]	Sol[14] (μM)	Clint[15] (μL/min/mg)
10		8 ± 0.31	0.14 ± 0.04			8.9	7.4
11		7.86 ± 0.34	0.24 ± 0.02	0.4 ± 0.06		1.8	19
12		7.82 ± 0.34	0.14 ± 0.05	0.33 ± 0.03			44
13		7.7 ± 0.08	0.09 ± 0.06	0.36 ± 0.12	0.34 ± 0.05	3.9 ± 2.5	24
14		7.58 ± 0.25	0.1 ± 0.04	0.28 ± 0.04		4.3 ± 3.9	27
15		7.53 ± 0.16	0.01 ± 0.04	0.23 ± 0.056		8.2	12
16		7.25 ± 0.23	−0.02 ± 0.07	0.2		4.7	12
17		7.04 ± 0.20	0.13 ± 0.04	0.26 ± 0.06		2.8 ± 2.23	35
18		6.96 ± 0.03	0.14 ± 0.05	0.32 ± 0.09		11	8.6
19		6.89 ± 0.02	0.15 ± 0.09	0.33 ± 0.16		2.6	29
20		6.27 ± 0.15				1.2	9.4
21		5.45	0.08 ± 0.05	0.01 ± 0.01		7.9	35

Table 4
SAR of R^2 substitutions of 5-(2,5-dimethoxyphenyl)-quinoline-carboxamides



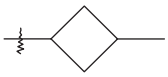
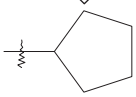
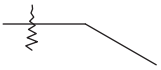
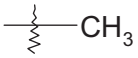
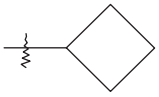
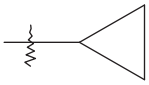
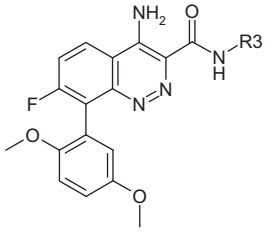
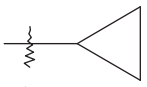
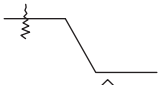
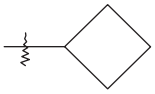
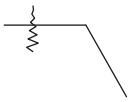
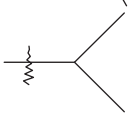
	R^2 structure	A2 pK_i[12]	A1 Oocyte (Frac DZ at 1 μM)[13]	A2 Oocyte (Frac DZ at 1 μM)[13]	A3 Oocyte (Frac DZ at 1 μM)[13]	Sol[14] (μM)	Clint[15] (μL/min/mg)
22		7.84 ± 0.19	0.23 ± 0.05			0.9	97
23		7.89 ± 0.15	0.2 ± 0.12			2.9	63
24		7.22 ± 0.33				0.5	16
25		6.58 ± 0.24				0.8	4.9
26		8.33 ± 0.54	0.07 ± 0.03	0.4 ± 0.05	0.36 ± 0.05	2.3 ± 1.34	47
27		5.73	0.05 ± 0.04	0.05 ± 0.07	0.02 ± 0.02	2.0 ± 0.23	96
28		7.48 ± 0.27	0.06 ± 0.1	0.26 ± 0.06	0.19 ± 0.04	4.9	19

Table 5
SAR of R^3 substitutions of 8-(2,5-dimethoxyphenyl)-7-fluoro-cinnoline-carboxamides



	R^3 structure	A2 pK_i[12]	A1 Oocyte (Frac DZ at 1 μM)[13]	A2 Oocyte (Frac DZ at 1 μM)[13]	A3 Oocyte (Frac DZ at 1 μM)[13]	Sol[14] (μM)	Clint[15] (μL/min/mg)
29		8.94 ± 2.15	0.03 ± 0.02	0.26 ± 0.04	0.22 ± 0.00	8.8 ± 0.31	25
30		8.79 ± 2.13	0.01 ± 0.05	0.23 ± 0.09	0.2 ± 0.04	3.3	53
31		8.45 ± 1.16	−0.01 ± 0.06	0.23 ± 0.03		2.8	22
32		8.24 ± 1.47	−0.06 ± 0.05	0.21 ± 0.03		5.0	4
33		7.25 ± 1.05	0.07 ± 0.03	0.16 ± 0.04		7.8	24

2.3. Synthetic chemistry

Schemes 1 and 2 outline the chemistry used to efficiently obtain the desired quinolines and cinnolines, respectively. Both routes are robust and have allowed access to hundreds of grams of compound from both series.

2.3.1. Replacement of methyl groups and variation of 3-N-carboxamide substituents of cinnolines

Replacement of the 2,5-dimethyls of **4** with 2,5-dimethoxy groups yields the comparator compound **13**, Table 3. This replacement of the methyl groups by methoxy groups results in: a modest increase in aqueous solubility to almost four micromolar and a modest diminution in A2 binding, pK_i of 7.7 versus 8.26. Despite the loss of affinity for A2, the two methoxy groups produce a functionally selective compound that is an α2 and α3 PAM with α1 NA. Given the desired functional selectivity of **13**, a survey of 3-N-carboxamide substituents was undertaken while the 8-aryl group remained constant with 2,5-dimethoxy substituents, Table 3.

We have drawn several trends from examination of Table 3. The aqueous solubilities of all the 2,5-dimethoxy cinnoline amides were at minimum equal to or greater than that of parent compound **4**. The nature of the R_3 group can impact the A2 affinity, as witnessed by the 2.5 order of magnitude range of values within the compounds of Table 3. The α1 functional activity appears more sensitive to the nature of the R_3 group than does the α2 functional activity. The α1 functional activity ranges from NAM (**16**), to NA (**13** and **15**) to moderate PAM activity (**11** and **14**). The compounds of Table 3, regardless of their binding affinity, maintain their α2 PAM activity, in all instances but one, **21**.

2.3.2. Replacement of methyl groups and variation of 2-N-lactam substituents of quinolines

A similar but more limited strategy was undertaken with the quinolines. In this instance, the R_5 group was maintained as the 2,5-dimethoxy phenyl and a lesser range of 2-N-sustituents was evaluated, Table 4. Compound **26** emerged from this exercise as a viable candidate with a pK_i of 8.33, α2/α3 PAM (0.4 and 0.36, respectively) and α1 NA (0.07); albeit with clearance values higher than desired as outlined in Table 1. The attractiveness of the functionally selective **28** was lessened by its relatively lower A2 binding affinity when compared to **26**. In general, within the quinoline series, R2 groups other than *N*-cyclobutyl and *N*-cyclopropyl resulted in a loss of binding potency accompanied by undesired increases in α1 PAM activity.

2.4. 7-Fluoro-cinnolines and 6-fluoro-quinolines

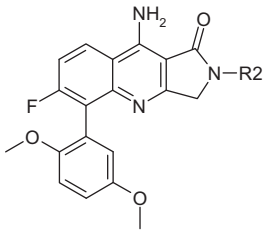
The 7-position of the cinnolines and the 6-position of the quinolines had been identified as sites of probable metabolic oxidation. To address this issue, the 7-fluoro analogues in Table 5 and 6-fluoro analogues in Table 6 were prepared using chemistry presented in Schemes 1 and 2. For the cinnolines, the rank order established for the A2 binding affinity in Table 3 was conserved in the 7-fluoro versions of Table 5. Introduction of the 7-fluoro group on the cinnolines decreased α1 PAM activity, reducing it to the NA range. On the other hand, the α2 PAM activities, while reduced by the addition of the 7-fluoro group to the cinnolines, still remained in the PAM range for all compounds in Table 5. In keeping with literature precedent, the 7-fluoro group had minimal and acceptable impact on the aqueous solubilities of the cinnoline compounds.[10] The effect of the 7-fluoro group on the human microsomal clearance values varied; in several instances the clearance increased, cf. **10** versus **29**, **13** versus **30** and **16** versus **33**. In other instances, the in vitro clearance of 7-fluoro analogues decreased, **14** versus **31** and **15** versus **32**. A rationale for this dichotomy on metabolic susceptibility within the series remains elusive.

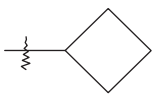
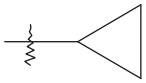
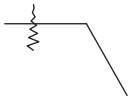
Fewer examples of the 6-fluoro quinolines were pursued, Table 6. A2 binding was modestly improved by introduction of the fluoro-group (compare **26** vs **34**, **28** vs **35** and **24** vs **36**). In those instances where direct comparisons of α1 and α2 efficacies can be made between the non-fluorinated and 6-fluorinated quinolines (**26** vs **34** and **28** vs **35**), it is apparent that the fluoro-substitution has had a minimal effect, if any.

2.5. Alternatives to the 8- and 5-phenyl groups

Alternatives to the 8- and 5-phenyl groups of the cinnoline and quinoline analogues were sought, as were non-methoxy substituents. All possible regioisomers of carbon–carbon linked pyridines,

Table 6
SAR of R^2 substitutions of 5-(2,5-dimethoxyphenyl)-6-fluoro-quinoline-carboxamides



	R^2 structure	A2 pK_i[12]	A1 Oocyte (Frac DZ at 1 μM)[13]	A2 Oocyte (Frac DZ at 1 μM)[13]	A3 Oocyte (Frac DZ at 1 μM)[13]	Sol[14] (μM)	Clint[15] (μL/min/mg)
34		9.0 ± 1.38	0.08 ± 0.04	0.28 ± 0.04		1	58
35		7.86 ± 1.86	0.08 ± 0.06	0.26 ± 0.01	0.26 ± 0.08	1	31
36		7.86 ± 1.44	0.03 ± 0.05	0.16 ± 0.05		0.3	4

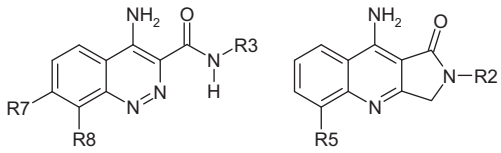
pyrimidines,[16] pyrazines, pyrazoles and pyridazines were evaluated.[7,8] One quinoline, **45**, and nine cinnolines emerged from this effort. Some of the results obtained with these compounds are detailed in Table 7 which highlight the findings: A methoxy group *ortho* to the biphenyl linkage of the cinnolines and quinolines provides the desired in vitro profile (see **13** of Table 3 and Table 7). In the case of the heterocycles, nitrogen at the 3-position relative to the biphenyl linkage also imparts the desired functional activity profile (**38**, **42**, **43**, **44** and **45**). In two cases, a methoxy group *para* to the biphenyl linkage renders the desired activity (**41**, and **42**). A methyl group *meta* to the biphenyl linkage maintained the desired SAR profile in two compounds (**39** and **44**). A fluoro group *ortho* to the biphenyl linkage helped provide the desired profile in one instance, **40**.

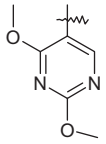
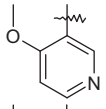
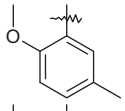
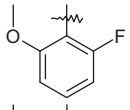
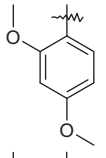
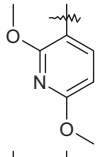
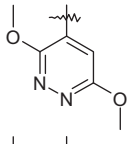
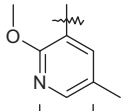
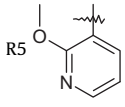
Several other observations were made from our study. There generally appears to be structural similarity of R8 and R5 groups that provide the functional selectivity. In combination with the respective 4- and 9-amino groups of the cinnolines and quinolines that yield the desired activities,[1,2] the results are consistent with the hypothesis that these two series interact with BZ site in similar fashions. Likewise, for almost all compounds where the α3 efficacy was measured, the magnitude of α2 and α3 efficacies closely parallel each other; **9** and **38** are the exceptions.

3. Conclusion

Evaluation of the SAR in both the quinolines and cinnolines increased the understanding of the requirements to achieve the desired functional selectivity profile. All compounds in Table 7 produce robust anxiolysis in a punish-responding model that will

Table 7
SAR of R^8 and R^5 aryl and heterocycle substitutions of cinnoline and quinoline-carboxamides



	R3	R7	R8	A2 pK_i[12]	A1 Oocyte (Frac DZ at 1 μM)[13]	A2 Oocyte (Frac DZ at 1 μM)[13]	A3 Oocyte (Frac DZ at 1 μM)[13]	Sol[14] (μM)	Clint[15] (μL/min/mg)
37	*n*-Pr	H		8.13 ± 0.16	0.1 ± 0.04	0.54 ± 0.12	0.42 ± 0.09	1.9	19
38	*n*-Pr	H		8.65 ± 0.55	0.18 ± 0.09	0.29 ± 0.12	0.41 ± 0.14	28.1 ± 5.0	7.7
39	*n*-Pr	H		8.21 ± 0.34	0.13 ± 0.08	0.52 ± 0.15	0.48 ± 0.11	8.3 ± 6.4	31
40	*n*-Pr	H		9.51 ± 0.32	−0.01 ± 0.06	0.19 ± 0.06	0.17 ± 0.03	7.6	46
41	*n*-Pr	F		9.39 ± 0.05	0.06 ± 0.07	0.13 ± 0.09	0.19 ± 0.06	1.4	25
42	*c*-Pr	F		9.42 ± 0.14	0.01 ± 0.05	0.25 ± 0.04	0.19 ± 0.03	2.0 ± 0.79	17
43	*c*-Pr	F		9.25 ± 0.33	−0.01 ± 0.05	0.25 ± 0.07	0.28 ± 0.05	6.2 ± 1.92	20
44	*c*-Pr	H		8.54 ± 0.76	0.03 ± 0.07	0.53 ± 0.08	0.52 ± 0.04	10.8 ± 2.9	18
45	R2 *c*-Bu	R6=H		8.62 ± 0.26	0.07 ± 0.08	0.44 ± 0.08	0.4 ± 0.06	9.4 ± 3.85	35

be detailed in a future publication.[11a] These compounds all possess PAM activity greater than 0.1 at α2 and α3. Compounds **38** and **39** were amongst several that allowed us to determine that in vitro α1 efficacy greater than 0.1 relative to 1.0 μM DZ in oocyte patch clamp generally carried a sedation effect when assessed in an in vivo locomotor activity assay.[11] Ultimately, compounds **13** and **40** were advanced to clinical trials.[17]

4. Experimental

4.1. Chemistry

4.1.1. General

All chemicals and reagents were purchased from Sigma–Aldrich, Fisher Scientific Inc., Strem Chemicals, Matrix Scientific, ACE Synthesis, and Frontier Scientific Inc. All reactions were carried out under a nitrogen atmosphere. Silicycle SilaFlash® cartridges were used for flash chromatography. ^{1}H NMRs were obtained on a Bruker Avance DPX300 NMR, Bruker Avance II 500 NMR, Bruker Avance 700 NMR, and were consistent with the assigned structures. All NMRs were recorded in $CDCl_3$ or DMSO-d_6 unless otherwise specified. High resolution mass spectrometry (HRMS) analyses were determined on an Agilent Technologies 6210 Time-of-Flight LC/MS. All compounds submitted for biological evaluation were greater than 95% pure as determined by ^{1}H NMR and HPLC mass spectroscopy.

4.2. General procedure for quinoline synthesis (see Scheme 1)

4.2.1. Quinolines (II)

9-Amino-6-X-5-(aryl)-2-(alkyl)-2,3-dihydropyrrolo[3,4-*b*]quinolin-1-one (X = H or F).

The corresponding bromide—precursor(I) of Scheme 1 (1 equiv), the corresponding aryl boronic acid reagent (2.2 equiv), tetrakis(triphenylphosphine)palladium(0) (0.05 equiv) and potassium carbonate (3.0 equiv) in DME/ethanol/water (7/2/3) were heated at 90 °C, monitored by LC/MS. The reaction concentration was 0.3 M. Upon completion, the reaction mixture was cooled to room temperature, diluted with chloroform and washed with water. The chloroform layer was dried with magnesium sulfate and evaporated to constant mass. The crude product was loaded onto a silica gel column, and eluted with 0–10% methanol in methylene chloride to give a yellow or tan solid. The solid was further crystalized from ether/methylene chloride, or purified by HPLC to give a white or yellow solid as the desired product in 60–85% yield.

4.2.2. Precursor (I)

9-Amino-4-X-5-bromo-2-(alkyl)-2,3-dihydropyrrolo[3,4-*b*]quinolin-1-one (X = H or F).

A white slurry of 4-X-3-bromo-2-[1-(alkyl)-5-oxo-2,5-dihydro-1*H*-pyrrol-3-ylamino]-benzonitrile (1.0 equiv) in *t*-butanol (0.08–1.00 M) was warmed to 45 °C and treated with sodium *t*-butoxide (1.2 equiv). The resulting green solution was heated at 45 °C for 3 h. The reaction was cooled to room temperature, partitioned between methylene chloride and water and the organic layer was washed with saturated aqueous sodium bicarbonate and the combined aqueous layers were extracted with methylene chloride. The organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated under reduced pressure to give a light tan solid in greater than 80% yield, which was used without further purification.

The intermediate compounds were prepared as follows:

4-X-3-Bromo-2-[1-(alkyl)-5-oxo-2,5-dihydro-1*H*-pyrrol-3-ylamino]-benzonitrile (X = H or F).

2-Amino-3-bromo-4-X-benzonitrile (1.0 equiv) and 1-(alkyl)-4-methoxy-1*H*-pyrrol-2(5*H*)-one (2.0 equiv) were combined in acetic acid (0.5–0.8 M benzonitrile in HOAc) and heated to 80 °C. Methanesulfonic acid (2.5 equiv) was dissolved in acetic acid (10 M) and added dropwise via syringe over 15 min. The reaction was stirred for 1 h at 80 °C and then cooled to rt and placed on a rotary evaporator under high vacuum for 15 min at 55 °C to remove the acetic acid. The resulting oil was dissolved in methylene chloride and slowly added dropwise over 20 min to a solution of saturated aqueous sodium bicarbonate at 0–5 °C, maintaining the pH value above 8. The resultant precipitate from the biphasic system was separated, washed with water twice and then with hexane once, and was dried at 50 °C under high-vacuum to give a tan solid as the title compound in 70–85% yield, which was used without further purification (see Table 8).

4.2.3. 4-Methoxy-1-(alkyl)-1,5-dihydropyrrol-2-one

To a solution of the corresponding alkyl amine (1.2 equiv) in THF (2.0 M) was added simultaneously a solution of (*E*)-4-chloro-3-methoxy-but-2-enoic acid methyl ester (1 equiv) in acetonitrile (1.5 M) and a solution of triethylamine (1 equiv) in acetonitrile (3.6 M) over 30 min at room temperature. After stirring overnight at room temperature or 3 h at 55 °C, the resulting precipitate in the reaction mixture was removed by filtration. The mother liquor was concentrated and purified by flash chromatography on silica gel eluting with a gradient of 20–100% ethyl acetate in hexanes to afford the title compound in greater than 75% yield.

4.2.4. 2-Amino-3-bromobenzonitrile

The title compound was prepared as described in the literature (Campbell, J. B. Jr.; Davenport, T. W.; *Synth. Commun.*, **1989,** 19 (13&14), 2255–2263).

4.2.5. 2-Amino-3-bromo-4-fluoro-benzonitrile

The title compound was prepared as described in the patent literature (Chang, H. F.; Chapdelaine, M.; Dembofsky, B. T.; Herzog, K. J.; Horchler, C.; Schmiesing, R. J. WO2008155572 and US20080318943, 2008).

4.3. General procedure for cinnoline synthesis (see Scheme 2)

4.3.1. Cinnolines (IV)

4-Amino-7-X-8-(aryl)-N-(alkyl)-cinnoline-3-carboxamide (X = H or F).

The corresponding bromide—precursor(III) (1 equiv), the corresponding aryl boronic acid reagent (2.2 equiv), tetrakis(triphenylphosphine)palladium(0) (0.05 equiv) and potassium carbonate (3.0 equiv) in DME/ethanol/water (7/2/3) were heated at 90 °C, monitored by LC/MS. The reaction concentration was 0.3 M. Upon completion, the reaction mixture was cooled to room temperature, diluted with chloroform and washed with water. The chloroform layer was dried with magnesium sulfate, and evaporated to constant mass. The crude product was loaded onto a silica gel column, and eluted with 0–10% methanol in methylene chloride to give a yellow or tan solid. The solid was further crystalized from ether/methylene chloride, or purified by HPLC to give a white, yellow or tan solid as the desired product in 50–85% yield.

4.3.2. Precursor (III)

4-Amino-7-X-8-bromo-N-(alkyl)-cinnoline-3-carboxamide (X = H or F).

To a solution of 2-[(3-X-2-bromophenyl)-hydrazono]-*N*-(alkyl)-2-cyanoacetamide (1.0 equiv) in anhydrous toluene (0.1 M, 1 volume) was added aluminum chloride (3.0 equiv). The reaction was heated with vigorous stirring at 70–90 °C for 1–3 h, cooled, and quenched with Rochelle's salt (saturated aqueous potassium so-

dium tartrate, 0.5 volume). Afterward, the mixture was stirred at room temperature overnight. The aqueous layer was separated, and the organic layer was treated with fresh Rochelle's salt (0.5 volume) while stirring at room temperature for 1 h. After separating the resulting layers, the organic suspension was washed with water and filtered to give give a tan solid. Additionally, the organic filtrate was concentrated, and triturated with ether to give a tan precipitate. The resulting solids were filtered to give a tan solid in 30–70% yield of crude product. The combined tan solid was dried at 50 °C under high-vacuum to give the title compound, which was used without further purification.

The intermediate compounds were prepared as follows:

2-[(3-X-2-Bromophenyl)-hydrazono]-*N*-(alkyl)-2-cyanoacetamide (X = H or F).

Solution A: To a mechanically stirred solution of 3-X-2-bromoaniline (1.0 equiv) in acetic acid (2 M, 1 volume) was added water (0.6 volume) at ambient temperature. The mixture was cooled to 0 °C, and then concentrated aqueous HCl (0.5 volume) added. A precipitate was formed immediately and the suspension was stirred at 0 °C for 20 min. To this suspension was added dropwise a solution of sodium nitrite (1.10 equiv) in water (0.6 volume), maintaining the internal temperature below 5 °C. The resulting clear orange solution was stirred at 0 °C for another 30 min.

Solution B: To a mechanically stirred solution of *N*-(alkyl)-2-cyanoacetamide (1.25 equiv) in ethanol (4.5 volume) was added a solution of sodium acetate (1.60 equiv) in water (120 volume), and chilled to between 0 °C and −5 °C.

Solution A was poured into solution B, maintaining the internal temperature below 0 °C. An orange precipitate was formed gradually. The mixture was stirred below 0 °C overnight, and then the orange precipitate was collected by filtration, washed with water (100 mL × 3), and dried at 50 °C under high vacuum to remove water. An orange solid was obtained in greater than 95% yield, which was the '*E*' isomer, and used for the next step without further purification.

4.3.3. *N*-(Alkyl)-2-cyanoacetamide

To ethyl-2-cyanoacetate (1.0 equiv) was added alkylamine (2–3 equiv) at room temperature. After stirring overnight at room temperature or 3 h at 50 °C, the reaction mixture was concentrated to give a wax-like solid. The solid was triturated with stirring in hexane/ether(3/1) for 10 min. The solid was filtered, washed with hexane/ether (3/1) once, and dried under vacuum to give the title compound in near quantitative yield, which was used in the next step without further purification.

4.3.4. Alternative approach for precursor (III)

4-Amino-7-X-8-bromo-N-(alkyl)-cinnoline-3-carboxamide (X = H or F).

To the 4-amino-7-X-8-bromo-cinnoline-3-carboxylic acid sulfate (1 equiv) in dimethylformamide (DMF) at 0 °C was added in one portion carbonyldiimidazole (1.6 equiv). The mixture was stirred at 0 °C for 15 min, the ice bath was removed and stirring continued at room temperature for 2 h. To this turbid mixture at 0 °C was added in one portion a preformed solution of the alkyl amine (1.1 equiv) and *N*,*N*-diisopropylethylamine (DIEA) (1.1 equiv) in DMF. The reaction concentration was 0.3 M. The pale yellow clear solution was allowed to warm to room temperature, and monitored by LC/MS. Upon completion, the volatiles were removed in vacuo to give a crude product residue which was subjected to a silica gel column eluting with a 0–50% ethyl acetate in methylene chloride gradient to give a solid as the title product in 80–85% yield, which was used without further purification.

The intermediate compounds were prepared as follows.

4-Amino-7-X-8-bromo-cinnoline-3-carboxic acid sulfate(X = H or F).

4-Amino-7-X-8-bromo-*N*-propyl-cinnoline-3-carboxamide was suspended in 80% concentrated sulfuric acid solution (just enough to cover the starting material). The reaction was refluxed at 100 °C overnight. The reaction mixture was cooled to 0 °C, and the resulting tan precipitate was isolated by filtration, washed with acetonitrile, and dried at 50 °C under high vacuum to remove water. The title compound, isolated in 80% yield, was used without further purification.

4.4. The analytical data of the cinnolines and quinolines

4.5. Biological assays

4.5.1. GABAA2 binding assay

$GABA_A\alpha2$ Binding Assay: Compounds were incubated with membranes from Sf9 cells expressing the α2, β3, γ2 subunits (Paragon Bioservices, 10 μg/well) in assay buffer (50 mM TRIS-Citrate, 200 mM NaCl, pH 7.8) and [^{3}H]-Flunitrazepam (Cat #NET 567) was added to a concentration of 2 nM. Total assay volume was 250 μl. Non-specific binding was determined using 10 μM flumazenil. Plates were shaken gently for 1 h at rt. The reaction was terminated by filtration with Whatman GF/B filters using a Brandell MT-24 cell harvester and washed (3×) with cold assay buffer. Radioactivity was determined on a Packard Topcount NXT. K_i values were reported as the mean of three independent assays.

4.5.2. GABAA oocyte assays

Evaluation of $GABA_A\alpha_{1-3}$ subunit positive modulatory activity by two-electrode voltage clamp: Ovarian lobes of Xenopus laevis frogs were purchased from NASCO (Fort Atkinson, WI) and were torn open carefully in OR2 solution (82 NaCl, 2.5 HEPES, 1.5 NaH_2PO_4, 1 $MgCl_2$, 0.1 EDTA, in mM, pH 7.4). The oocytes were defolliculated by incubating twice in a total of 25 mL of OR2 solution containing 0.2% collagenase IA (Sigma C5894) for a total of 60 min on a test tube rocker rocking at 0.5 Hz. After several washes with OR2, oocytes were stored in 0.5× Leibovitz's L-15 medium (Sigma L1518) containing 0.1 mg/mL gentamicin (Sigma G1914), 100 units/mL of penicillin, and 0.1 mg/mL of streptomycin (Sigma P4333). Stage V or VI oocytes were selected and injected with cRNA on the same or following day. Each oocyte was injected with 20–40 nL of capped and linearized cRNA containing 0.2–2 ng of α, β, or γ subunits of the human $GABA_A$ receptor genes. The cRNA ratio of α:β:γ were 1:1:10, 1:1:2, 1:1:10, and 1:1:5 by weight for (α1)2(β2)2γ2, (α2)2(β3)2γ2, (α3)2(β3)2γ2, and (α5)2(β3)2γ2, respectively. Oocytes were used for two-electrode voltage clamp (TEVC) experiments within 2–10 days of injection.

TEVC recording was carried out using OpusXpress 6000A (Molecular Devices, Sunnyvale, CA), which allows simultaneous recording from 8 oocytes. Oocytes were perfused with ND96 (96 NaCl, 2 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 5 HEPES, in mM, pH 7.5) at a flow rate of 2 ml/min. Oocytes were impaled with 2 micro-glass electrodes filled with 3 M KCl. Tip resistances of 0.5-2 MΩ and 0.5–10 MΩ were used for the current and voltage electrodes, respectively. Membrane potential was held at −60 mV. Oocytes with leak current above 50 nA at the holding potential were discarded.

To determine EC_{10} concentration of GABA for each oocyte, a series of 30 s-pulses with increasing concentrations of GABA was applied to oocytes at 4.5 min-intervals. To test a modulator, oocytes were pre-treated with the compound for 100 s before they were co-treated with GABA at EC_{10} for 30 s. A set of 3 pulses of GABA alone at EC_{10} prior to modulator testing was conducted and the average of the three readings was used to establish the baseline GABA response. For concentration–response curves, increasing

Table 8
Analytical data of cinnoline and quinoline-carboxamides (**1**–**45**)

Compound	NMR	HRMS (ESI, MH^+, *m/z*)
1	^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 0.93 (s, 9H) 1.00 (t, *J* = 7.3 Hz, 3H) 1.72 (sxt, *J* = 7.4 Hz, 2H) 3.23 (s, 2H) 3.57 (t, *J* = 7.2 Hz, 2H) 4.39 (s, 2H) 6.29 (br s, 2H) 7.38 (t, *J* = 7.6 Hz, 1H) 7.54 (d, *J* = 6.7 Hz, 1H) 7.69 (d, *J* = 8.2 Hz, 1H)	312.2070
2	^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.97 (d, *J* = 6.6 Hz, 6H) 1.03 (t, *J* = 7.4 Hz, 3H) 1.69 (sxt, *J* = 7.3 Hz, 2H) 2.25 (dt, *J* = 13.5, 6.8 Hz, 1H) 3.28 (d, *J* = 7.3 Hz, 2H) 3.42–3.54 (m, 2H) 7.54–7.63 (m, 2H) 7.66–7.75 (m, 1H) 8.59 (br s, 1H)	287.1867
3	^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 1.00 (t, *J* = 7.5 Hz, 3H) 1.61–1.72 (m, 2H) 3.46 (q, *J* = 6.8 Hz, 2H) 7.39–7.46 (m, 1H) 7.50 (t, *J* = 7.5 Hz, 2H) 7.66–7.72 (m, 2H) 7.73 (d, *J* = 8.2 Hz, 1H) 7.78–7.83 (m, 1H) 7.83–7.89 (m, 1H) 8.58 (br s, 1H)	307.1556
4	^{1}H NMR (300 MHz, $CDCl_3$) δ 8.55 (br, 1H), 7.87 (m, 1H), 7.75–7.64 (m, 2H), 7.23–7.07 (m, 3H), 3.44 (apparent quartet, *J* = 7.0 Hz, 2H), 2.35 (s, 3H), 2.01 (s, 3H), 1.64 (apparent sextet, *J* = 7.0 Hz, 2H), 0.99 (t, *J* = 7.4 Hz, 3H)	335.1865
5	^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 0.99 (t, *J* = 7.3 Hz, 3H) 1.64 (sxt, *J* = 7.2 Hz, 2H) 1.92 (s, 6H) 3.44 (q, *J* = 6.7 Hz, 2H) 7.15 (d, *J* = 7.3 Hz, 2H) 7.23 (t, *J* = 7.6 Hz, 1H) 7.61 (d, *J* = 7.0 Hz, 1H) 7.74 (t, *J* = 7.6 Hz, 1H) 7.89 (d, *J* = 8.6 Hz, 1H) 8.54 (br s, 1H)	335.1865
6	^{1}H NMR (300 MHz, $CDCl_3$) δ 8.58 (t, *J* = 5.0 Hz, 1H), 7.85 (dd, *J* = 8.3, 1.4 Hz, 1H), 7.79 (dd, *J* = 7.3, 1.4 Hz, 1H), 7.71 (dd, *J* = 8.1, 7.3 Hz, 1H), 7.50 (s, 1H), 7.48 (s, 2H), 7.39 (t, *J* = 7.9 Hz, 1H), 7.23 (s, 1H), 3.46 (q, *J* = 6.7 Hz, 2H), 2.44 (s, 3H), 1.67 (sextet, *J* = 7.3 Hz, 2H), 1.00 (t, *J* = 7.4 Hz, 3H)	321.1710
7	^{1}H NMR (700 MHz, DMSO-d_6) δ 8.86 (br s, 1H), 8.62 (d, *J* = 7.91 Hz, 1H), 7.86 (t, *J* = 7.70 Hz, 1H), 7.79 (d, *J* = 7.14 Hz, 1H), 7.40 (t, *J* = 7.46 Hz, 1H), 7.36 (d, *J* = 7.34 Hz, 1H), 7.31 (t, *J* = 7.42 Hz, 1H), 7.23 (d, *J* = 7.42 Hz, 1H), 3.30 (q, *J* = 6.74 Hz, 2H), 1.99 (s, 3H), 1.57 (sextet, *J* = 7.29 Hz, 2H), 0.89 (t, *J* = 7.46 Hz, 3H)	321.1709
8	^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 0.99 (t, *J* = 7.5 Hz, 3H) 1.64 (sxt, *J* = 7.2 Hz, 2H) 2.03 (s, 3H) 2.39 (s, 3H) 3.44 (m, *J* = 6.1 Hz, 2H) 7.10 (d, *J* = 7.6 Hz, 1H) 7.13–7.21 (m, 2H) 7.64–7.77 (m, 2H) 7.86 (d, *J* = 8.2 Hz, 1H) 8.55 (br s, 1H)	335.1867
9	^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.00 (t, *J* = 7.4 Hz, 3H) 1.59–1.77 (m, 2H) 2.34–2.45 (m, 6H) 3.46 (q, *J* = 6.8 Hz, 2H) 7.06 (s, 1H) 7.26 (s, 2H) 7.66–7.74 (m, 1H) 7.74–7.79 (m, 1H) 7.80–7.87 (m, 1H) 8.58 (br s, 1H)	335.1868
10	^{1}H NMR (300 MHz, $CDCl_3$) δ 8.56 (br m, 1H), 7.85 (a dd, *J* = 2.2, 7.6 Hz, 1H), 7.73 (m, 2H), 6.95 (m, 2H), 6.91 (m, 1H), 3.77 (s, 3H), 3.63 (s, 3H), 2.96 (m, 1H), 0.88 (m, 2H), 0.65 (m, 2H)	365.1607
11	^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 9.08–9.23 (m, 1H) 8.39 (dd, *J* = 8.24, 1.83 Hz, 1H) 7.66–7.79 (m, 2H) 7.00–7.08 (m, 1H) 6.92–7.00 (m, 1H) 6.80–6.89 (m, 1H) 4.23–4.70 (m, 1H) 3.73 (s, 3H) 3.55 (s, 3H) 2.27–2.46 (m, 3H) 1.88–2.09 (m, 1H) 1.69–1.83 (m, 1H) 1.00–1.21 (m, 3H)	393.1935
12	^{1}H NMR 300 MHz, $CDCl_3$) δ 8.54 (t, *J* = 4.7 Hz, 1H), 7.86 (dd, *J* = 7.6, 2.2 Hz, 1H), 6.96 (t, *J* = 3.0 Hz, 1H), 6.96 (s, 1H), 6.93 (d, *J* = 1.9 Hz, 1H), 7.76–7.68 (m, 2H), 3.79 (s, 3H), 3.64 (s, 3H), 3.48 (q, *J* = 6.6 Hz, 2H), 1.61 (quintet, *J* = 7.2 Hz, 2H), 1.43 (sextet, *J* = 7.3 Hz, 2H), 0.94 (t, *J* = 7.3 Hz, 3H)	381.1922
13	^{1}H NMR (300 MHz, $CDCl_3$) δ 8.59 (br, 1H), 7.89 (dd, *J* = 7.8 Hz, *J*′ = 1.9 Hz, 1H), 7.65–7.77 (m, 2H), 6.85–7.20 (m, 3H), 3.79 (s, 3H), 3.64 (s, 3H), 3.35–3.55 (m, overlapped with H_2O), 1.64 (m, *J* = 7.3 Hz, 2H), 0.99 (t, *J* = 7.4 Hz, 3H)	367.1765
14	^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.60–1.75 (m, 2H) 2.10–2.29 (m, 4H) 3.55 (s, 3H) 3.69–3.77 (m, 3H) 4.43–4.57 (m, 1H) 6.86 (d, *J* = 3.1 Hz, 1H) 6.97 (dd, *J* = 8.9, 3.1 Hz, 1H) 7.03 (d, *J* = 9.2 Hz, 1H) 7.68–7.72 (m, 1H) 7.72–7.77 (m, 1H) 8.39 (dd, *J* = 8.1, 1.4 Hz, 1H) 9.20 (d, *J* = 8.2 Hz, 1H)	379.1763
15	^{1}H NMR (300 MHz, $CDCl_3$) δ 8.51 (br t, *J* = 5.2 Hz, 1H), 7.86 (dd, *J* = 7.5, 2.1 Hz, 1H), 7.76–7.68 (m, 2H), 6.97–6.91 (m, 3H), 3.79 (s, 3H), 3.64 (s, 3H), 3.52 (dq, *J* = 5.7, 7.3 Hz, 2H), 1.26 (t, *J* = 7.3 Hz, 3H)	353.1607
16	^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.76 (d, *J* = 8.24 Hz, 1H) 8.39 (dd, *J* = 8.24, 1.53 Hz, 1H) 7.66–7.79 (m, 2H) 7.00–7.06 (m, 1H) 6.93–6.99 (m, 1H) 6.85 (d, *J* = 3.05 Hz, 1H) 4.11–4.23 (m, 1H) 3.73 (s, 3H) 3.55 (s, 3H) 1.22 (d, *J* = 6.71 Hz, 6H)	367.1763
17	^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 2.47 (qt, *J* = 10.7, 6.9 Hz, 2H) 3.64 (s, 3H) 3.75 (q, *J* = 6.8 Hz, 2H) 3.79 (s, 3H) 6.89–6.98 (m, 3H) 7.64–7.81 (m, 2H) 7.83–7.90 (m, 1H) 8.71 (br s, 1H)	421.1481
18	^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.71 (quin, *J* = 6.5 Hz, 2H) 3.37–3.46 (m, 2H) 3.50 (q, *J* = 6.0 Hz, 2H) 3.55 (s, 3H) 3.68–3.78 (m, 3H) 4.50 (t, *J* = 5.2 Hz, 1H) 6.86 (d, *J* = 3.1 Hz, 1H) 6.97 (dd, *J* = 8.9, 3.1 Hz, 1H) 7.03 (d, *J* = 9.0 Hz, 1H) 7.68–7.72 (m, 1H) 7.72–7.77 (m, 1H) 8.39 (d, *J* = 8.1 Hz, 1H) 9.10 (t, *J* = 5.8 Hz, 1H)	383.2
19	^{1}H NMR (500 MHz, DMSO-d_6) δ 9.07 (d, *J* = 4.6 Hz, 1H), 8.39 (d, *J* = 8.2 Hz, 1H), 7.76–7.70 (m, 2H), 7.03 (d, *J* = 9.1 Hz, 1H), 6.97 (dd, *J* = 8.9, 3.0 Hz, 1H), 6.87 (d, *J* = 3.2 Hz, 1H), 3.73 (s, 3H), 3.55 (s, 3H), 2.86 (d, *J* = 4.8 Hz, 3H)	339.1714
20	^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.71 (d, *J* = 8.85 Hz, 1H) 8.40 (dd, *J* = 8.39, 1.68 Hz, 1H) 7.68–7.77 (m, 2H) 7.00–7.05 (m, 1H) 6.94–6.99 (m, 1H) 6.85 (d, *J* = 3.05 Hz, 1H) 3.94–4.05 (m, 1H) 3.73 (s, 3H) 3.55 (s, 3H) 1.49–1.66 (m, 2H) 1.19 (d, *J* = 6.41 Hz, 3H) 0.88 (t, *J* = 7.48 Hz, 3H)	381.1921
21	^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.83 (s, 1H) 8.40 (dd, *J* = 8.39, 1.68 Hz, 1H) 7.65–7.78 (m, 2H), 7.00–7.06 (m, 1H) 6.93–6.99 (m, 1H) 6.85 (d, *J* = 3.05 Hz, 1H) 3.73 (s, 3H) 3.55 (s, 3H) 2.38–2.47 (m, 2H) 1.95–2.10 (m, 2H) 1.77–1.89 (m, 2H) 1.53 (s, 3H)	393.1924
22	^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.31 (dd, *J* = 8.4, 1.4 Hz, 1H) 7.64 (br s, 1H) 7.53 (dd, *J* = 7.0, 1.2 Hz, 1H) 7.44 (dd, *J* = 8.1, 7.2 Hz, 1H) 7.00 (d, *J* = 8.9 Hz, 1H) 6.90 (dd, *J* = 9.0, 3.2 Hz, 1H) 6.75 (d, *J* = 3.1 Hz, 1H) 4.37 (s, 2H) 3.71 (s, 3H) 3.56 (s, 3H) 1.95 (s, 2H) 1.44 (s, 6H) 0.80 (t, *J* = 7.3 Hz, 3H)	404.1968
23	^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.31 (d, *J* = 8.5 Hz, 1H) 7.52–7.56 (m, 1H) 7.46 (d, *J* = 7.2 Hz, 1H) 7.01 (d, *J* = 9.2 Hz, 1H) 6.91 (dd, *J* = 8.9, 3.1 Hz, 1H) 6.76 (d, *J* = 3.1 Hz, 1H) 4.56 (quin, *J* = 7.7 Hz, 1H) 4.28 (s, 2H) 3.71 (s, 3H) 3.57 (s, 3H) 1.83 (d, *J* = 6.4 Hz, 2H) 1.52–1.76 (m, 6H)	404.1965
24	^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.25 (t, *J* = 7.3 Hz, 3H) 3.53–3.72 (m, 5H) 3.80 (s, 3H) 4.32 (s, 2H) 6.36 (br s, 2H) 6.87–7.02 (m, 3H) 7.50 (dd, *J* = 8.3, 7.2 Hz, 1H) 7.68 (dd, *J* = 7.2, 1.5 Hz, 1H) 7.84 (dd, *J* = 8.3, 1.5 Hz, 1H)	364.1659
25	^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 3.15 (s, 3H) 3.66 (s, 3H) 3.80 (s, 3H) 4.32 (s, 2H) 6.35 (br s, 2H) 6.85–7.02 (m, 3H) 7.50 (dd, *J* = 8.3, 7.2 Hz, 1H) 7.68 (dd, *J* = 7.0, 1.3 Hz, 1H) 7.84 (dd, *J* = 8.3, 1.1 Hz, 1H)	350.1498
26	^{1}H NMR (500 MHz, DMSO-d_6) δ 8.32 (d, *J* = 8.2 Hz, 1H), 7.58 (br s, 2H), 7.55 (dd, *J* = 7.2, 1.2 Hz, 1H), 7.46 (dd, *J* = 8.3, 7.1 Hz, 1H), 7.02 (d, *J* = 9.0 Hz, 1H), 6.91 (dd, *J* = 9.0, 3.1 Hz, 1H), 6.78 (d, *J* = 3.1 Hz, 1H), 4.72 (q, *J* = 8.6 Hz, 1H), 4.40 (s, 2H), 3.72 (s, 3H), 3.56 (s, 3H), 2.31 m, 2H), 2.11m 2H), 1.68 (m, 2H)	390.1808
27	^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.27–8.35 (m, 1H) 7.55 (dd, *J* = 7.2, 1.4 Hz, 1H) 7.42–7.49 (m, 1H) 7.01 (d, *J* = 8.8 Hz, 1H) 6.91 (dd, *J* = 9.0, 3.2 Hz, 1H) 6.77 (d, *J* = 3.1 Hz, 1H) 4.82–4.96 (m, 0.5H) 4.50–4.61 (m, 0.5H) 4.42 (s, 1H) 4.38 (s, 1H) 3.68–3.77 (m, 3H) 3.54–3.60 (m, 3H) 2.38–2.51 (m, 0.5H) 2.24–2.33 (m, 2H) 1.99–2.12 (m, 0.5H) 1.76–1.95 (m, 2H) 1.18 (d, *J* = 7.0 Hz, 1.5H) 1.07 (d, *J* = 6.4 Hz, 1.5H)	406.2124
28	^{1}H NMR (500 MHz, DMSO-d_6) δ 8.31 (dd, *J* = 8.3, 1.0 Hz, 1H), 7.60 (br s, 1H), 7.54 (dd, *J* = 7.1, 1.3 Hz, 1H), 7.46 (dd, *J* = 8.4, 7.4 Hz, 1H), 7.00 (d, *J* = 9.1 Hz, 1H), 6.90 (dd, *J* = 8.7, 3.0 Hz, 1H), 6.76	376.1654

	(d, J = 3.0 Hz, 1H), 4.21 (s, 2H), 3.72 (s, 3H), 3.55 (s, 3H), 2.89 (septet, J = 3.7 Hz, 1H), 0.84–0.73 (m, 4H)	
29	^{1}H NMR (500 MHz, DMSO-d_6) δ 8.99 (d, J = 4.8 Hz, 1H), 8.51 (a dd, J = 5.5, 9.3 Hz, 1H), 7.72 (t, J = 9.0 Hz, 1H), 7.06 (a d, J = 9.0 Hz, 1H), 7.00 (m, 1H), 6.86 (a d, J = 3.0 Hz, 1H), 3.72 (s, 3H), 3.58 (s, 3H), 2.93 (m, 1H), 0.68 (m, 4H)	383.1516
30	^{1}H NMR (300 MHz, $CDCl_3$) δ 8.50 (t, J = 4.7 Hz, 1H), 7.89 (dd, J = 9.3, 5.1 Hz, 1H), 7.52 (t, J = 8.8 Hz, 1H), 6.98 (m, 2H), 6.91 (dd, J = 2.4, 0.9 Hz, 1H), 3.79 (s, 3H), 3.67 (s, 3H), 3.44 (q, J = 6.7 Hz, 2H), 1.64 (sextet, J = 7.3 Hz, 2H), 0.99 (t, J = 7.4 Hz, 3H)	385.1668
31	^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.58–1.76 (m, 2H) 2.03–2.30 (m, 4H) 3.58 (s, 3H) 3.73 (s, 3H) 4.49 (sxt, J = 8.1 Hz, 1H) 6.87 (d, J = 2.6 Hz, 1H) 6.96–7.04 (m, 1H) 7.04–7.12 (m, 1H) 7.72 (t, J = 9.0 Hz, 1H) 8.51 (dd, J = 9.0, 5.7 Hz, 1H) 9.20 (d, J = 8.3 Hz, 1H)	397.1668
32	^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.25 (t, J = 7.2 Hz, 3H) 3.41–3.59 (m, 2H) 3.67 (s, 3H) 3.76–3.83 (m, 3H) 6.91 (dd, J = 2.3, 1.1 Hz, 1H) 6.94–7.05 (m, 2H) 7.52 (dd, J = 9.0, 8.3 Hz, 1H) 7.89 (dd, J = 9.2, 5.1 Hz, 1H) 8.46 (br s, 1H)	371.1512
33	^{1}H NMR (500 MHz, *DMSO*-d_6) δ ppm 9.11 (br s, 1H) 8.75 (d, J = 8.24 Hz, 1H) 8.51 (dd, J = 9.31, 5.65 Hz, 1H) 8.31 (br s, 1H) 7.72 (dd, J = 9.00, 9.00 Hz, 1H) 7.07 (d, J = 10.00 Hz, 1H) 7.01 (dd, J = 9.00, 4.00 Hz, 1H) 6.86 (d, J = 3.05 Hz, 1H) 4.10–4.23 (m, 1H) 3.73 (s, 3H) 3.58 (s, 3H) 1.21 (d, J = 6.41 Hz, 6H)	385.1670
34	^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.83 (dd, J = 9.2, 5.8 Hz, 1H) 7.29 (d, J = 8.5 Hz, 1H) 6.93–7.02 (m, 2H) 6.87 (d, J = 2.6 Hz, 1H) 6.35 (br s, 2H) 4.80–4.96 (m, 1H) 4.38 (s, 2H) 3.80 (s, 3H) 3.68 (s, 3H) 2.16–2.32 (m, 4H) 1.70–1.84 (m, 2H).	408.1719
35	^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 (dd, J = 9.7, 5.8 Hz, 1H), 7.31 (t, J = 8.8 Hz, 1H), 7.01–6.93 (m, 2H), 6.86 (d, J = 2.1 Hz, 1H), 6.37 (s, 2H), 4.24 (s, 2H), 3.79 (s, 3H), 3.67 (s, 3H), 2.92–2.83 (m, 1H), 0.93–0.79 (m, 4H)	394.1562
36	^{1}H NMR (300 MHz, DMSO-d_6) δ 8.42 (dd, J = 9.3, 5.8 Hz, 1H), 7.67 (s, 2H), 7.42 (t, J = 8.9 Hz, 1H), 7.04 (d, J = 8.9 Hz, 1H), 6.95 (dd, J = 8.9, 3.5 Hz, 1H), 6.74 (d, J = 3.0 Hz, 1H), 4.30 (s, 2H), 3.72 (s, 3H), 3.58 (s, 3H), 3.48 (q, J = 7.2 Hz, 2H), 1.14 (t, J = 7.3 Hz, 3H)	382.1564
37	^{1}H NMR (300 MHz, $CDCl_3$) δ 8.52 (br m, 1H), 8.33 (s, 1H), 7.91 (dd, J = 7.7, 2.0 Hz, 1H), 7.70–7.77 (m, 2H), 4.06 (s, 3H), 3.93 (s, 3H), 3.46 (apparent q, J = 6.5 Hz, 2H), 1.67(apparent sextet, J = 7.2 Hz, 2H), 1.00 (t, J = 7.4 Hz, 3H)	369.1669
38	^{1}H NMR (300 MHz, $CDCl_3$) δ 8.58 (d, J = 5.8 Hz, 1H), 8.53 (br, 1H), 5.11 (m, 6H), 8.46 (s, 1H), 7.94 (t, J = 4.9 Hz, 1H), 7.74 (d, J = 4.8 Hz, 2H), 6.96 (d, J = 5.8 Hz, 1H), 3.78 (s, 3H), 3.45 (q, J = 6.7 Hz, 2H), 1.66 (m, overlapped with H_2O), 1.00 (t, J = 7.4 Hz, 3H)	338.1609
39	^{1}H NMR (300 MHz, $CDCl_3$) δ 8.56 (br, 1H), 7.88–7.82 (m, 1H), 7.76–7.65 (m, 2H), 7.22–7.12 (m, 2H), 6.93 (m, 1H), 3.67 (s, 3H), 3.45 (apparent q, J = 7.0 Hz, 2H), 2.34 (s, 3H), 1.65 (apparent sextet, J = 7.0 Hz, 2H), 0.99 (t, J = 7.0 Hz, 3H)	351.1817
40	^{1}H NMR (300 MHz, $CDCl_3$) δ 8.54 (br, 1H), 7.93 (m, 1H), 7.78–7.69 (m, 2H), 7.42–7.31 (m, 1H), 6.89–6.80 (m, 2H), 3.70 (s, 3H), 3.44 (apparent quartet, J = 7.0 Hz, 2H), 1.64 (apparent sextet, J = 7.0 Hz, 2H), 0.99 (t, J = 7.0 Hz, 3H)	355.1556
41	^{1}H NMR (500 MHz, $CDCl_3$) δ 8.51 (br s, 1H), 7.86 (dd, J = 9.4, 5.2 Hz, 1H), 7.50 (t, J = 8.8 Hz, 1H), 7.27 (d, J = 9.2, 1H), 6.66 (dd, J = 8.2, 2.3 Hz, 1H), 6.63 (d, J = 2.3 Hz, 1H), 3.87 (s, 3H), 3.71 (s, 3H), 3.44 (q, J = 6.7 Hz, 2H), 1.64 (sextet, J = 7.3 Hz, 2H), 0.99 (t, J = 7.4 Hz, 3H)	385.1669
42	^{1}H NMR (500 MHz, *DMSO*-d_6) δ 9.02 (d, J = 4.8 Hz, 1H), 8.51 (m, 1H), 7.73 (t, J = 9.1 Hz, 1H), 7.66 (d, J = 8.0 Hz, 1H), 6.51 (d, J = 8.0 Hz, 1H), 3.94 (s, 3H), 3.78 (s, 3H), 2.93 (m, 1H), 0.70 (m, 4H)	384.1467
43	^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 0.62–0.66 (m, 2H) 0.86–0.90 (m, 2H) 2.93–2.99 (m, 1H) 3.97 (s, 3H) 4.11 (s, 3H) 7.05 (s, 1H) 7.54 (dd, J = 9.2, 8.4 Hz, 1H) 8.00 (dd, J = 9.3, 5.1 Hz, 1H) 8.43 (br s, 1H)	385.1420
44	^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.60–0.66 (m, 2H) 0.84–0.91 (m, 2H) 2.32 (s, 3H) 2.91–2.99 (m, 1H) 3.84 (s, 3H) 7.48 (d, J = 2.4 Hz, 1H) 7.52 (dd, J = 9.1, 8.5 Hz, 1H) 7.90 (dd, J = 9.2, 5.2 Hz, 1H) 8.09 (d, J = 1.6 Hz, 1H) 8.48 (br s, 1H)	350.1613
45	^{1}H NMR (300 MHz, $CDCl_3$) δ 8.23 (dd, J = 5.0, 1.7 Hz, 1H), 7.86 (dd, J = 8.5, 1.3 Hz, 1H), 7.69 (dd, J = 7.0, 1.5 Hz, 1H), 7.66 (dd, J = 7.2, 2.1 Hz, 1H), 7.48 (t, J = 7.6 Hz, 1H), 7.00 (dd, J = 7.2, 5.1 Hz, 1H), 6.45 (br s, 2H), 4.89 (quintet, J = 8.7 Hz, 1H), 4.38 (s, 2H), 3.87 (s, 3H), 2.25 (q, J = 7.7 Hz, 4H), 1.76 (quintet, J = 8.1 Hz, 2H)	361.1659

concentrations of the modulator were applied on the same oocyte at 5 min-intervals.

Modulation was calculated according to the amplitude change of GABA current caused by the modulator, as follows:

[(current evoked by modulator
+ GABA/current evoked by GABA alone)]

The current amplitude was measured from baseline to peak using Clampfit (Molecular Devices, Sunnyvale, CA). Concentration–response curves with variable slopes were plotted and fitted with Prism GraphPad using non-linear regression with no fitting constraints imposed (GraphPad Software, Inc. San Diego, CA).

4.6. Description of the dried-DMSO solubility method

A volume of 160 μL compound as 5 mM DMSO stock solution in a 96 well Multitier plate was obtained from AstraZeneca central liquid dispensary. A volume of 160 μL compound dispensed as 5 mM stock solution from the Astrazeneca central liquid storage was dispensed into a Multitier plate. The plate was placed on a Tecan automation platform where 60 μL of the stock solution is diluted to 1 mM in DMSO, then serially diluted using 40%ACN/60%H_2O to make 50 μM, 5 μM, and 1 μM plates which were used as LC (50 μM) and LC/MS/MS (1 μM) calibration standards for compound quantitation and LC/MSMS method development (5 μM). The initial plate containing the remaining 100 μL solution was then placed in GeneVac where DMSO was removed at 40 °C under full vacuum for 4 h. After drying, StirStix were added to the plate using a 96-well dispenser. The plate was then returned to the Tecan workstation where 800 μL of pH 7.4, 0.1 M sodium phosphate buffer was dispensed. The plate was capped and placed on an Eppendorf ThermomixerR where the solution was mixed at 750 RPM and thermostated at 25 °C for 24 h. After mixing, the plate was centrifuged at 3000 RPM for 30 min to separate the bulk of remaining insolubles from the solution. Using the Tecan, the midpoint solution was transferred to two analytical plates for parallel LC (340 μL) and LC/MS/MS quantitation (160 μL).

4.7. Determination of metabolic stability of compounds

Microsomal metabolic stability was assessed by incubation of test compound with human microsomes. Compound (1 μM final concentration) was incubated with 0.5 mg/ml microsomes in 100 mM potassium phosphate buffer containing 1 mM NADPH. Compounds were incubated for 25 min at 37 °C. Samples were removed at 0, 5, 10, 15, 20 and 25 min, stopping the reaction by adding to an equal volume of acetonitrile/methanol (1:1, v/v) containing 0.1% formic acid. Unmetabolized parent was quantitated by LC/MS/MS in MRM mode. Intrinsic clearance was estimated using the following equation: Clint = $V_{max}/K_m = K_e$/PMS where K_e is the elimination rate constant and PMS is the final protein concentration in mg/ml.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.03.035.

References and notes

1. Campbell, J. B.; Warawa, E. J. European Patent Application 87303949.9, 1987.
2. (a) Patel, J. B.; Meiners, B. A.; Salama, A. I.; Malick, J. B.; Resch, J. F.; U'Pritchard, D. C.; Giles, R. E.; Hesp, B.; Goldberg, M. E. *Prog. Clin. Biol. Res.* **1990**, *361* (Curr. Future Trends Anticonvulsant, Anxiety, Stroke Ther.), 483.; (b) Resch, J. F. Ger. (East) Patent DD 249011, 1987.; (c) Resch, J. F. European Patent Application 89300865.6, 1989.
3. Sieghart, W.; Fuchs, K.; Tretter, V.; Ebert, V.; Jechlinger, M.; Hoger, H.; Adamiker, D. *Neurochem. Int.* **1999**, *34*, 379.
4. (a) Korpi, E. R.; Matilla, M. J.; Wisden, W.; Luddens, H. *Ann. Med.* **1997**, *29*, 275; (b) Atack, J. R. *Curr. Drug Targets CNS Neurol. Disord.* **2003**, *2*, 213.
5. (a) McKernan, R. M.; Rosahl, T. W.; Reynolds, D. S.; Sur, C.; Wafford, K. A.; Atack, J. R.; Farrar, S.; Myers, J.; Cook, G.; Ferris, P.; Garrett, L.; Bristow, L.; Marshall, G.; Maccaulay, A.; Brown, N.; Moore, K. W.; Carling, R. W.; Street, L. J.; Castro, J. L.; Ragan, C. I.; Dawson, G. R.; Whiting, P. J. *Nat. Neurosci.* **2000**, *3*, 587; (b) Low, K.; Crestani, F.; Keist, R.; Benke, D.; Brunig, I.; Benson, J. A.; Fritschy, J.-M.; Rulicke, T.; Bluethman, H.; Mohler, H.; Rudolph, U. *Science* **2000**, *290*, 131.
6. (a) Atack, J. R. *Expert Opin. Invest. Drugs* **2005**, *14*, 601; For a brief review of preclinical and clinical data supporting the potential of a selective α2/α3 $GABA_A$ modulator such as TPA023 and other selective modulators to be anxiolytic see: (b) Atack, J. R. *Adv. Pharmacol.* **2009**, *57*, 137. and references cited therein.
7. Chapdelaine, M. J.; Ohnmacht, C. J.; Becker, C.; Chang, H.-F.; Dembofsky, B. T. US Patent 2007/0142328 A1.; (b) Alhambra, C.; Chang, H.-F.; Chapdelaine, M.; Herzog, K. J.; Schmiesing, R. J. WO Patent 2011/021979 A1.
8. Chang, H.-F.; Chapdelaine, M. J.; Dembofsky, B. T.; Herzog, K. J.; Horchler, C.; Schmiesing, R. J. WO Patent 2008/155572 A2.
9. For general reviews on bioisosteres in medicinal chemistry see: (a) Thornber, C. W. *Chem. Soc. Rev.* **1979**, *8*, 563; (b) Olesen, P. H. *Curr. Opin. Drug Disc. Dev.* **2001**, *4*, 471; (c) Patani, G. A.; LaVoie, E. J. *Chem. Rev.* **1996**, *96*, 3147.
10. For a discussion of matched pair analysis and the effect of a structural change on physical properties see: Leach, A. G.; Jones, H. D.; Cosgrove, D. A.; Kenny, P. W.; Ruston, L.; MacFaul, P.; Wood, J. M.; Colclough, N.; Law, B. *J. Med. Chem.* **2006**, *49*, 6672.
11. (a) Christian, E. P.; Snyder, D. H.; Song, W.; Gurley, D. A.; Quirk, M. C.; Smolka, J.; McLaughlin, J.; Maier, D. L.; Ding, M. Gharahdaghi, F.; Liu, X. F.; Chopra, M.; Ribadeneria, M.; Chapdelaine, M. J.; Dudley, A.; Arriza, J.; Maciag, C.; Doherty, J. J. *Neuropsychopharmacology*, submitted for publication.; (b) The desired values in Table 1 were used as a first filter for ranking compounds for further in vitro characterization; they evolved as the project progressed.
12. Where standard deviations are included, the results reflect a minimum of three replicates. See Section 4.3.1 for a description of the assay.
13. Where standard deviations are included, the results reflect a minimum of three replicates. See Section 4.3.2 for a description of the assay.
14. The results reflect a minimun of three replicates. See Section 4.3.3 for a description of the assay.
15. The results reflect a minimun of three replicates. See Section 4.3.4 for a description of the assay.
16. For further discussion of the unique properties imparted by incorporation of pyrimidines onto the cinnolines and quinolines see 'developing dual functional allosteric modulators of $GABA_A$ receptors'" Liu, X. F.; Chang, H.-F.; Schmiesing, R. J.; Wesolowski, S. S.; Knappenberger, K. S.; Arriza, J. L.; Chapdelaine, M. J. *Bioorg. Med. Chem.* **2010**, *18*, 8374.
17. (a) Lappalainen, Jaakko, personal communication; (b) www.clinicaltrials.gov.