Bioorganic & Medicinal Chemistry Letters 19 (2009) 5746–5752

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Discovery of the imidazo[1,5-*a*][1,2,4]-triazolo[1,5-*d*][1,4]benzodiazepine scaffold as a novel, potent and selective $GABA_A$ α5 inverse agonist series

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ARTICLE INFO

Article history:
Received 1 July 2009
Revised 30 July 2009
Accepted 31 July 2009
Available online 4 August 2009

Keywords:
$GABA_A$ receptor
Benzodiazepines
Cognition
Imidazole
Triazole
Medicinal chemistry

ABSTRACT

Through iterative design cycles we have discovered a number of novel new classes where the imidazo[1,5-*a*][1,2,4]-triazolo[1,5-*d*][1,4]benzodiazepine was deemed the most promising $GABA_A$ α5 inverse agonist class with potential for cognitive enhancement. This class combines a modest subtype binding selectivity with inverse agonism and has the most favourable molecular properties for further lead optimisation towards a central nervous system (CNS) acting medicine.

Although senile dementia was first recognised by Pythagoras in the seventh century B.C. as a medical condition, and more recently diagnosed pathologically in 1906 by Kraepelin and Alzheimer, still today a significant unmet medical need for the treatment of Alzheimer's disease (and related dementias) exists.[1] Current medicines based on NMDA receptor antagonism (e.g., Memantine)[2] and cholinesterase inhibition (e.g., Donepezil)[3] suffer from well documented modest efficacy in patients and mechanism related side effects for the latter. An alternative pharmacological approach based on selectively targeting the $GABA_A$ α5 receptor subtype offers unique opportunities for the potential treatment of the cognitive deficits in Alzheimer's disease and related dementias.[4]

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian brain and the $GABA_A$ receptor (ligand gated chloride channel) has been, historically, one of the most successful pharmacological targets delivering a large number of clinically differentiated medicines as anti-epileptics, anxiolytics, hypnotics and sedatives which predominantly function via increased receptor activation via the benzodiazepine binding site. Since the discovery of these medicines, innovative molecular biology has revealed that $GABA_A$ receptors comprise of a pentameric assembly of heteromeric subunits in a 2:2:1 stoichiometry with the most common protein assemblies being α1β2γ2, α2β3γ2, α3β3γ2 and α5β3γ2.[5]

Apart from the non-selective antagonist Flumazenil all currently used benzodiazepines are non-selective agonists for these subtypes and these are in clinical use as sedatives, anxiolytics and muscle relaxants. The clinical side effects of these compounds can be explained by interaction with multiple undesired subtypes. Interestingly, clinical studies with β-carbolines, which are non-selective inverse agonists for the $GABA_A$ α1β2γ2, α2β3γ2, α3β3γ2 and α5β3γ2 subtypes, induce the converse pharmacological effects on behaviour including, importantly, enhanced learning and memory. However, due to poor pharmacological profile, these compounds induced convulsions and anxiety in animal and healthy volunteer studies, respectively, so that clinical development was not an option.[6]

Genetic and pharmacological studies have outlined the dominant individual contributions from each $GABA_A$ αxβxγ2 subtype revealing that agonism at $GABA_A$ α1 induces sedation and agonism at $GABA_A$ α2 and/or $GABA_A$ α3 imposes the desired anxiolytic effect

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0960-894X/$ - see front matter © 2009 Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2009.07.153

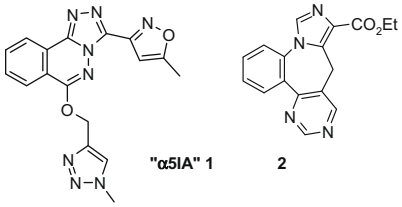


Figure 1. Merck clinical candidate and our selected hit compound.

in rodent models. Conversely, inverse agonism at $GABA_A$ α1 induces pro-convulsant and inverse agonism at $GABA_A$ α2 and/or $GABA_A$ α3 is responsible for an anxiogenic response.

Memory impairment is a known undesired clinical result of benzodiazepine use and is thought to be due to the agonistic effects at the $GABA_A$ α5 receptor subtype. Therefore, taking into consideration the clinical knowledge from both benzodiazepine and β-carboline pharmacology enough evidence is present to strongly rationalise how selective $GABA_A$ α5 receptor subtype inverse agonists, devoid of convulsant or anxiogenic action, could have a significant clinical impact on the treatment of memory and learning deficits. The additional evidence[7] that the $GABA_A$ α5 receptor subtype is predominant in the hippocampus and the fact that the density and pharmacology of the $GABA_A$ α5 receptor subtype is preserved in post-mortem AD patients is compelling enough a reason to design a medicinal chemistry programme aimed at the discovery of selective $GABA_A$ α5 receptor subtype inverse agonists.

Researchers at Merck have discovered a plethora of functionally selective compound series[8] culminating in an experimental medicine study with 'α5IA' (Fig. 1) which significantly reversed the alcohol induced memory impairment in a double-blind placebo controlled cross-over study in 12 healthy volunteers.[9] This compound was prevented for further clinical use due to dose limits imposed on 'α5IA' due to the potential for precipitation, crystalluria and nephrotoxicity by the main metabolite.[10]

Additional non-selective $GABA_A$ receptor subtype inverse agonists have been studied in the clinics but no information relating to their safety, tolerability or efficacy is currently publicly available.[11]

To the best of our knowledge, very few compounds[12] have been successfully designed that combine some degree of both binding and functional selectivity and this communication, and subsequent ones[13–15] will reveal our successful efforts to identify a number of interesting novel series and compounds with best and first in class pharmacological and pharmacokinetic profiles.

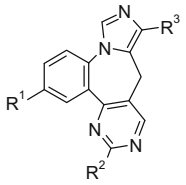
As our source for starting points we decided to perform a high throughput screening (HTS) of our corporate compound library and unsurprisingly we achieved a high hit rate of 'benzodiazepine classes'. We decided to pursue further compound **2** (Fig. 1) (an imidazo [1,5-*a*]pyrimidino[5,4-*d*]benzazepine) due to its interesting pharmacological profile and intellectual property opportunities.

Table 2
Molecular property profile of selected compounds

Compd	Solubility[a] (μg/mL)	Log $D_{7.4}$	Permeation coefficient (10^{-6}cm s^{-1}) (PAMPA)	Cl (mL/min/mg protein)[b] (rat/human)
14	20	2.68	3.6	11/4
20	10	2.68	2.78	–[c]/8

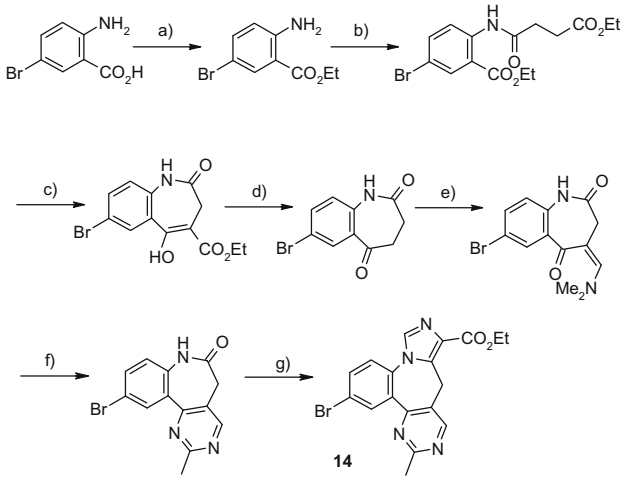
[a] LYSA measurement.
[b] From incubations with liver microsomes.
[c] Not stable in matrix.

Table 1
Binding and efficacy profile of analogues of **2**



Compd	R^1	R^2	R^3	Affinity $GABA_A$ α5β3γ2[a] K_I (nM)	Selectivity[17] K_I (nM) α1β3γ2 / K_I (nM) α5β3γ2	Efficacy $GABA_A$ α5β3γ2[b] (%)
2	H	H	CO_2Et	3.7	21	+11
3	H	Ph	CO_2Et	170.6	6	+11
4	H	PMB	CO_2Et	12.5	16	+59
5	H	nPr	CO_2Et	5.5	12	+19
6	H	Me	CO_2Et	3.8	12	
7	H	iPr	CO_2Et	8.9	11	
8	H	cPr	CO_2Et	7.6	7	
9	F	Me	CO_2Et	4.6	9	
10	F	nPr	CO_2Et	8.0	5	
11	F	iPr	CO_2Et	11.2	7	
12	F	cPr	CO_2Et	7.8	6	
13	Br	H	CO_2Et	20.2	31	−12
14	Br	Me	CO_2Et	12.5	37	−19
15	H	H	H	410.0	>8	
16	H	H	Cl	15.6	8	+15
17	H	Me	Cl	15.6	14	+21
18	Br	H	Cl	136.0	6	−6
19	Br	Me	Cl	61.0	10	−13
20	H—≡	Me	CO_2Et	8.1	70	−29
21	H—≡	Me	Cl	133	>24	−41

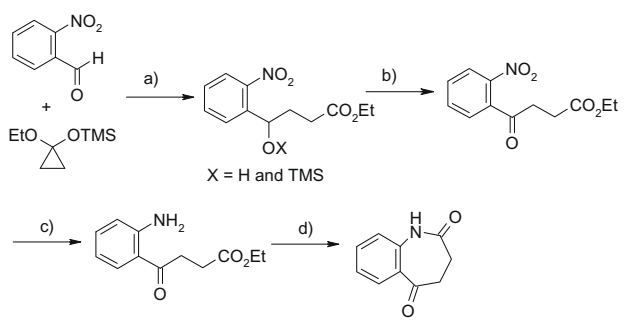
[a] Cloned rat or human receptor subunits were expressed in insect SF9 cells, human HEK293 cells or *Xenopus laevis* oocytes for ^{3}H-Flumazenil $GABA_A$ α_1,α_2,α_3 and α_5 binding or electrophysiology.
[b] Efficacy is determined as the percentage change of a submaximal (EC_{10}) response to GABA.



Scheme 1. Synthesis of **14**. Reagents and conditions: (a) EtOH, HCl (concd), reflux, 13 h, 80%; (b) ethyl succinyl chloride, $CaCO_3$, toluene, reflux, 1 h, 79%; (c) KH, toluene, 70 °C, 2 h, 71%; (d) DMSO, H_2O, 150 °C, 5 h, 65%; (e) $Me_2NCH(OMe)_2$, 120 °C, 1 h, 90%; (f) acetamidine HCl, NaOMe, rt, 4 h, 87%; (g) $POCl_3$, *N*,*N*-dimethyl-*p*-toluidine, LiHMDS, (*E*)-(dimethylaminomethyleneamino)-acetic acid ethyl ester, AcOH, 50%.

Initial SAR exploration revealed that **2** demonstrated very good potency (3.7 nM) at the $GABA_A$ α5 receptor subtype and also high binding selectivity (21-fold) versus the $GABA_A$ α1 receptor subtype (Table 1).[16] However, in functional testing **2** was found to be agonistic at the $GABA_A$ α5 receptor subtype and this trend was shown for compounds **3**–**5**. Introduction of a F-substituent resulted in a reduction of binding selectivity. Pleasingly the introduction of a Br substituent offered the best compromise in potency, binding selectivity[17] and inverse agonism as shown in compound **14**.

We then sought out to identify ester replacements but were surprised to learn that all typical ester isosteres investigated resulted in complete loss of affinity at the $GABA_A$ α5 receptor subtype and additionally any relatively potent examples (~100 nM) were all agonistic in functional response. No substituent (e.g., compound **15**) was not tolerated and, as will be shown in more detail in the subsequent paper, a Cl substituent was found to be a suitable ester replacement where some potency at the $GABA_A$ α5 receptor subtype was retained but it was not able to combine with an inverse agonistic effect within this class. Introduction of a terminal acetylene moiety in compound **20** offered the best compromise in potency, selectivity and inverse agonist effects but replacement of the ester functional group by many groups including a Cl atom proved to be detrimental. Overall compounds **14** and **20** were perhaps the most promising from the imidazo [1,5-*a*]pyrimidino[5,4-*d*]benzazepine series. Characterised by acceptable molecular properties, **14** (Table 2) was selected to be tested in an in vivo passive avoidance test of cognition in rats where activity was demonstrated at 100 mg/kg ip (Supplementary data).



Scheme 2. Alternative route to benzazepinediones. Reagents and conditions: (a) ZnI_2, DCM, rt, 5 min, 77%; (b) PCC, DCM, rt, 20 h, 92%; (c) H_2, Pd/C, EtOAC, rt, 3 h, 80%; (d) NaH, −40 °C to rt, 3 h, 81%.

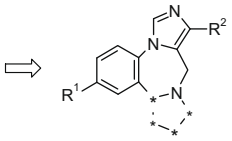


Figure 2. Imidazo benz-'diaza'-series explored.

Since it was not possible to optimally combine both favourable pharmacological and pharmacokinetic profiles within this series we decided to terminate our efforts and begin to explore a number of additional pyrimidine replacements (including pyrimidine isomers, and pyridine and phenyl isomers). All these changes were met with no success, so we terminated these benzazepine-classes.

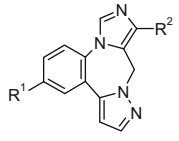
A number of synthetic routes were developed to access the imidazo [1,5-*a*]pyrimidino[5,4-*d*]benzazepines series. The route depicted in Scheme 1[16] was found to be suitable for both small and large scale preparation of derivatives in Table 1. The synthetic strategy relied on an optimised base (KH is far superior to all other bases examined) mediated intramolecular Dieckmann cyclisation to the key benzazepinedione building block. A complimentary route using zinc homoenolate methodology proved to be an efficient alternative reductive cyclisation method (Scheme 2). Transformation of the benzazepinediones to the final products proceeded without event through standard processes.

We then sequentially and thoroughly examined the effect of five-membered rings and designed indoles, indazoles, pyrazoles, imidazoles, triazoles and tetrazole isomers keeping the privileged imidazobenzodiazepine scaffold constant (Fig. 2). The most interesting from the many sub-series examined are shown in Tables 3–8.

Pleasingly, the imidazo[1,5-*a*]pyrazolo[1,5-*d*][1,4]benzodiazepine class delivered very potent $GABA_A$ α5 receptor subtype inverse agonists and in this case ester replacements were even more selective and more efficacious (Table 3). The molecular properties (Table 4) of these classes were also favourable, but since we were seeking higher levels of binding selectivity we decided to study further the effect of additional structural diversity (Tables 5–7).

Imidazo[1,5-*a*]pyrazolo[1,5-*d*][1,4]benzodiazepines were prepared by an efficient sequence of reactions with a key step being a reductive ring cyclisation with subsequent base mediated ring expansion reaction to give the desired pyrazolo[1,5-*d*][1,4]benzo-

Table 3
Binding and efficacy profile of analogues of **22**



Compd	R^1	R^2	Affinity $GABA_A$ α5β3γ2f K_i (nM)	Selectivity[17] K_i (nM) α1β3γ2 / K_i (nM) α5β3γ2	Efficacy $GABA_A$ α5β3γ2[a] (%)
22	H	CO_2Et	0.2	4	−18
23	Cl	CO_2Et	1.4	1	−7
24	H	Me	3.0	14	−26
25	H	Cl	2.0	7	−39
26	H	COcPr	1.0	8	−38
27	Br	Me	49.1	5	−24

[a] See Table 1.

Table 4
Molecular property profile of compound **26** and **27**

	26	**27**
Solubility (μg/mL)[a]	46	9
Log $D_{7.4}$	2.39	2.81
Permeation coefficient (10^{-6}cm s^{-1}) (PAMPA)	3.77	3.27
Cl (mL/min/mg protein)[a] (rat/human)	26/9	8/2
MAB (%)	54/63	78/88

[a] See Table 2.

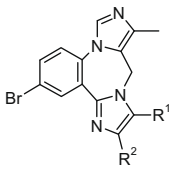
diazepine which was transformed to the final product under standard conditions (Scheme 3).[18]

The diimidazo[1,5-*a*:1′,2′-*d*][1,4]benzodiazepine class allowed us for the first time to achieve very high levels of binding selectivity with concomitant high potency and inverse agonism at the GABA$_A$ α5 receptor subtype but unfortunately this class had the deficiency of relatively high microsomal clearance even with polar substituents such as **33**.

The key building block can be conveniently prepared in three synthetic steps and upon reaction with hydroxylamine gives the seven-membered ring selectively. Selective 'O' 1,4-addition to ethyl propiolate followed by [3,3]-sigmatropic rearrangement and dehydrative cyclisation to the imidazole forms the ester intermediate which could be converted to **33** in a straightforward manner (Scheme 4).

We next turned our attention to a novel class of imidazo[1,5-*a*][1,2,4]triazolo[4,3-*d*][1,4]benzodiazepines where we were able to develop an extensive data set from more than 180 derivatives (selected examples shown in Table 6).[19] As expected small substituents at R^1 resulted in very potent GABA$_A$ α5 receptor subtype binding and increasing lipophilicity or polarity resulted in increased binding selectivity versus the GABA$_A$ α1,2,3 receptor subtypes (for brevity only GABA$_A$ α1 shown but similar selectivity is

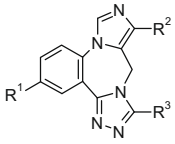
Table 5
Binding and efficacy profile of analogues of **28**



Compd	R^1	R^2	Affinity GABA$_A$ α5β3γ2f K_I (nM)	Selectivity[17] K_I (nM) α1β3γ2 / K_I (nM) α5β3γ2	Efficacy GABA$_A$ α5β3γ2 (%)[a]
28	H	H	6.6	35	−35
29	H	Me	119.0	27	−8
30	Me	H	11.7	75	−19
31	H	CO_2Et	5.4	43	−34
32	H	CH_2OH	8.1	101	−15
33	H	CH_2N–pyrolidinone	1.6	118	−25

[a] See Table 1.

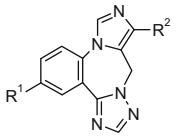
Table 6
Binding and efficacy profile of analogues of **34**



Compd	R^1	R^2	R^3	Affinity GABA$_A$ α5β3γ2f K_I (nM)	Selectivity[17] K_I (nM) α1β3γ2 / K_I (nM) α5β3γ2	Efficacy GABA$_A$ α5β3γ2[a] (%)
34	H	CO_2Et	H	0.8	8	
35	F	CO_2Et	H	1.7	7	
36	Cl	CO_2Et	H	1.2	29	−2
37	Br	CO_2Et	H	0.6	65	−5
38	Me	CO_2Et	H	2.1	43	+20
39	MeO	CO_2Et	H	1.4	26	−12
40	HO	CO_2Et	H	0.6	28	−13
41	H—≡	CO_2Et	H	2.3	59	−19
42	H—≡	Cl	H	33.6	94	−24
43	NC	Cl	H	25.3	11	−22
44	Br	Cl	H	27.4	33	−5
45	Br	I	H	7.8	32	−10
46	Br	Me	H	150.0	18	−20
47	Br	H—≡	H	7.8	60	0
48	Br	COiPr	H	55.5	38	−11
49	MeO	CO_2Et	Me	14.5	33	+55
50	MeO	CO_2Et	Ph	53.8	59	−31
51	MeO	CO_2Et	Bn	22.9	89	+75
52	Br	CO_2Et	Bn	19.1	47	+40

[a] see Table 1

Table 7
Binding and efficacy profile of analogues of **53**



Compd	R1	R2	Affinity $GABA_A$ α5β3γ2f K_I (nM)	Selectivity: K_I (nM) αxβ3γ2			Efficacy $GABA_A$ α5β3γ2 (%)[a]
				K_I (nM) α5β3γ2			
				vs α1	vs α2	vs α3	
				1	1	1	−16
54	Cl	CO_2Et	0.2	5	15	6	−35
55	Br	CO_2Et	0.3	6	13	6	−25
56	Me	CO_2Et	0.5	7	7	6	−22
57	cPr	CO_2Et	0.6	3	1	1	−56
58	OMe	CO_2Et	0.2	5	9	5	−39
59	OCF_3	CO_2Et	0.8	7	16	19	−39
60	H—≡	CO_2Et	0.6	18	7	11	−51
61	iPrCOHN	CO_2Et	6.4	2	11	6	−29
62	H	CO_2Et	0.2	2	2	2	
63	H	H	6.4	16	0	0	−37
64	H	Et	0.5	15	16	19	−52

[a] See Table 1.

Table 8
Molecular property profile of selected compounds

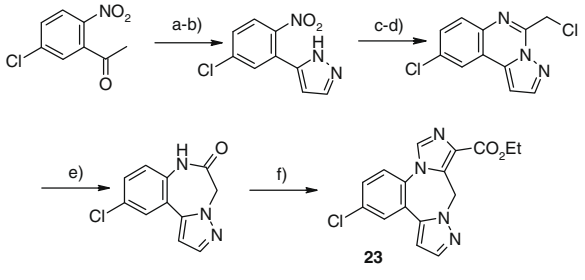
Compd	Solubility[a] (μg/mL)	Log $D_{7.4}$	Permeation coefficient (10^{-6}cm s^{-1}) (PAMPA)	Cl (mL/min/mg protein)[a] (rat/human)
54	15	1.83	5.59	19/50
63	231	0.73	2.87	4/8
64	100	1.35	6.79	3/0

[a] See Table 2.

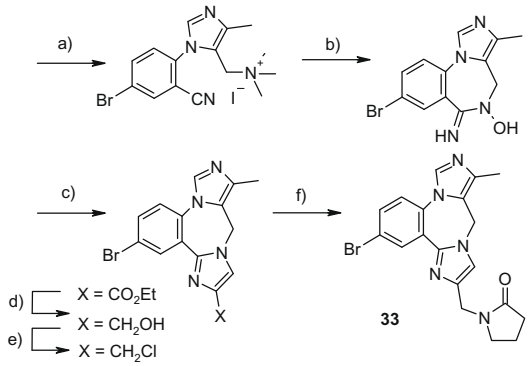
normally achieved for all subtypes within this class). The introduction of the terminal acetylene moiety **41** again proved to give a balanced profile but replacement of the ester to typical isosteres or amides resulted in functional agonism at the $GABA_A$ α5 receptor subtype for all R^1 substituents. Pleasingly, we were able to replace the ester with a Cl substituent but this was met with a 15-fold drop in potency although the selectivity versus the $GABA_A$ α1 receptor subtype was enhanced. This trend was also observed with our preferred Br substituent as R^1. Examination of the opportunities for the R^3 substituent variation revealed that all substituents except phenyl resulted in an agonistic functional response at the $GABA_A$ α5 receptor subtype. Attempts to introduce F and/or OMe substituents onto the phenyl ring (not shown in Table 6) were met with reduced affinity (>300 nM) and pyridine isomer replacements were also not tolerated. As a result of the relatively low solubility, high lipophilicity of this class coupled to the fact that it was not possible to identify single compounds which met our overall target profile we abandoned this class even though **39** was tested in an in vivo passive avoidance test of cognition in rats where activity was demonstrated at 3 mg/kg ip (Supplementary data).

Our preferred route to imidazo[1,5-*a*][1,2,4]triazolo[4,3-*d*][1,4]benzodiazepines is outlined in Scheme 5 where the efficient condensation of 1H-benzo[*d*][1,3]oxazine-2,4-diones with N-protected glycine derivatives proceeds efficiently and is convenient for large scale preparation. This allowed imidazole formation to proceed first followed by acid (cat. TfOH in TFA) deprotection of the preferred 2,4-dimethoxybenzyl protecting group to proceed smoothly. Amide activation via formation and isolation of the imidoyl chloride followed by cyclisation to the 1,2,4-triazole with formylhydrazine completed an overall efficient reaction sequence (Scheme 5).[19]

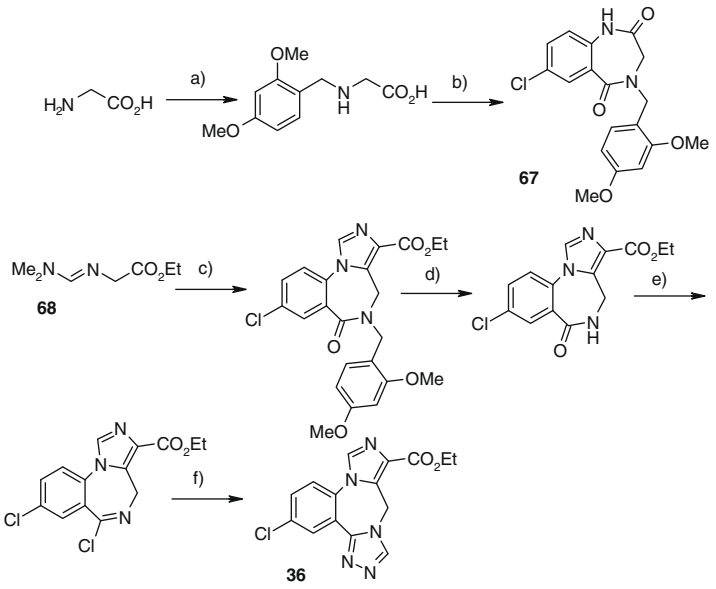
From the possible triazole isomers the imidazo[1,5-*a*][1,2,4]-triazolo[1,5-*d*][1,4]benzodiazepine class proved to have overall the most favoured balanced profile and Table 7 details the initial development of this class.[20] Initially we were excited to observe that all derivatives had excellent potency at the $GABA_A$ α5 receptor subtype and concomitant good levels of inverse agonism—nearly all full inverse agonists. In this class we were able to successfully



Scheme 3. Synthesis of **23**. Reagents and conditions: (a) $Me_2NCH(OMe)_2$, toluene, 100 °C, 3 h, 84%; (b) hydrazine, EtOH, rt, 16 h, 87%; (c) H_2, Pd/C, EtOAC, rt, 3 h, 82%; (d) chloroacetyl chloride, pyridine, THF, 5–10 °C, 1 h then HCl. Dioxane, 90 °C, 2 h, 69%; (e) NaOH, dioxane, rt, 2 h, 80%; (f) $POCl_3$, *N*,*N*-dimethyl-*p*-toluidine, 1,2-dichloroethane, 85 °C, then add KOBu-*t*, ethyl isocyanoacetate, NMP, −20 °C to rt, 30 min, 67%.



Scheme 4. Synthesis of **33**. Reagents and conditions: (a) see Ref. X; (b) hydroxylamine HCl, triethylamine, DMF, 3 h, 120 °C, 68%; (c) ethyl propiolate, K_2CO_3, EtOH, 80 °C, 5 h, 35%; (d) $LiAlH_4$, THF, rt, 1 h, 54%; (e) $SOCl_2$, rt, on, 92%; (f) NaH, pyrolidinone, DMF, rt, 3 h, 32%.



Scheme 5. Synthesis of **36**. Reagents and conditions: (a) 2,4-dimethoxybenzaldehyde, NaOH, MeOH then H_2, Pd/C, MeOH, rt, 24 h, 85%; (b) 6-chloro-1H-benzo[*d*][1,3]oxazine-2,4-dione, xylene, 140 °C, 2 h, 70%; (c) **67**, $POCl_3$, *N*,*N*-dimethyl-*p*-toluidine, toluene, reflux, 2.5 h then **68**, LiHMDS, THF, −70 °C to rt, 1 h then AcOH, H_2O, −30 °C to reflux, 30 min., 70%; (d) cat. TfOH, TFA, rt, 2 h, 85%; (e) $POCl_3$, *N*,*N*-dimethyl-*p*-toluidine, chlorobenzene, reflux, 2.5 h, 87%; (f) formylhydrazine, Hünigs base, chlorobenzene, reflux, 1 h, 80%.

replace the ester substituent by H or ethyl and maintain full inverse agonism although with low and modest levels of binding selectivity, respectively. Additional successful ester replacements and the synthetic routes to imidazo[1,5-*a*][1,2,4]-triazolo[1,5-*d*][1,4]benzodiazepines will be discussed in more detail.[13]

Overall the class showed favourable molecular properties (Table 8) for a CNS lead series and we therefore decided to focus our lead optimisation efforts on the imidazo[1,5-*a*][1,2,4]-triazolo[1,5-*d*][1,4]benzodiazepine class and the subsequent papers will describe in detail our successful endeavours to identify further potent and binding selective $GABA_A$ α5 receptor subtype inverse agonists from this class.

In summary we have identified through iteratively designed screening cycles a number of novel binding and selective inverse agonist series for the $GABA_A$ α5 receptor subtype with the imidazo[1,5-*a*][1,2,4]-triazolo[1,5-*d*][1,4]benzodiazepine class being the most preferred for further lead optimisation.

Acknowledgements

Special thanks to Brigitte Algeyer, Béatrice David, Cécile Guiziani, Rachel Haab, Marie-Laurence Harle-Yge, Maria Karg, Marie Claire Pflimlin, Pascal Pflimlim, Regina Wolf for technical excellence in their work.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.07.153.

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