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3,4-Dihydronaphthalen-1(2*H*)-ones: novel ligands for the benzodiazepine site of α5-containing $GABA_A$ receptors

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Abstract—A series of substituted 3,4-dihydronaphthalen-1(2*H*)-ones with high binding affinity for the benzodiazepine site of $GABA_A$ receptors containing the α5-subunit has been identified. These compounds have consistently higher binding affinity for the $GABA_A$ α5 receptor subtype over the other benzodiazepine-sensitive $GABA_A$ receptor subtypes (α1, α2 and α3). Compounds with a range of efficacies for the benzodiazepine site of α5-containing $GABA_A$ receptors were identified, including the α5 inverse agonist 3,3-dimethyl-8-methylthio-5-(pyridin-2-yl)-3,4-dihydronaphthalen-1(2*H*)-one **22** and the α5 agonist 8-ethylthio-3-methyl-5-(1-oxidopyridin-2-yl)-3,4-dihydronaphthalen-1(2*H*)-one **19**.

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system. $GABA_A$ receptors are GABA-gated chloride ion channels, composed of pentameric assemblies of members of the $GABA_A$ receptor gene family (α1-6, β1-3, γ1-3, δ, ε, θ and π)[1,2] with the majority of $GABA_A$ receptors comprising of α, β and γ-subunits arranged in a 2:2:1 stoichiometry.[3] The binding of GABA to its receptor can be modulated by simultaneous binding of chemical entities to allosteric sites on the ion channel complex. One of the most studied of these allosteric sites is the benzodiazepine (BZ) binding site due to the clinical efficacy of BZ ligands such as diazepam (Valium®), as anxiolytics, anticonvulsants and hypnotics. Based upon their modulatory effects on GABA-induced $GABA_A$ receptor activation, BZ ligands are categorised as either: agonists (which increase the affinity of GABA for the receptor), antagonists (which bind to $GABA_A$ receptors but have no intrinsic efficacy) or inverse agonists (which decrease the affinity of GABA for the receptor), spanning the efficacy spectrum from full agonist through partial agonist, antagonist, partial inverse agonist to full inverse agonist. Currently, all clinically effective BZ ligands are classified as full agonists at the BZ binding site.

$GABA_A$ receptor subtypes, which possess a BZ binding site (and which comprise around 75% of all $GABA_A$ receptor subtypes present in the brain)[4] contain β and γ2-subunits in conjunction with either an α1, α2, α3 or α5-subunit.[4] Receptors containing the α5-subunit account for approximately 5% of the total $GABA_A$ receptor population in the brain, however, in the hippocampus they constitute approximately 20% of all $GABA_A$ receptors.[5] The hippocampus is a region of the brain closely associated with learning and memory processes indicating that there may be a link between $GABA_A$ receptors containing the α5-subunit and these processes.[6,7] We sought to identify selective inverse agonists at the BZ site of $GABA_A$ receptors containing an α5-subunit, as potential cognition enhancers, which may lack the anxiogenic[8] or convulsant[9] (or proconvulsant)[10] side effects observed with nonselective $GABA_A$ full inverse agonists, such as the β-carboline methyl 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM; **1**, Fig. 1).

One of the lead structures on the $GABA_A$ α5 programme, identified using a directed screening approach, was 6,6-dimethyl-3-methylthio-1-(1*H*-pyrazol-5-yl)-6,7-dihydro-2-benzothiophen-4(5*H*)-one **2**, a high affinity $GABA_A$ α5 receptor ligand (K_i: 5.2 nM) with 4–13-fold binding selectivity over the $GABA_A$ α1, α2 and α3 receptor subtypes.[11] Structure–activity relationships in this thiophene series have been reported by Chambers

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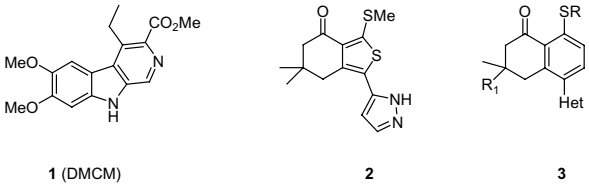
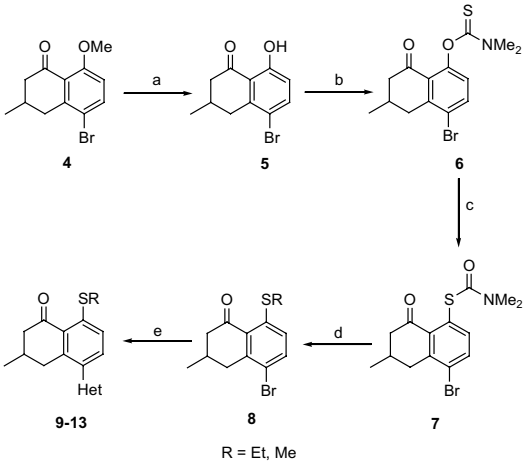
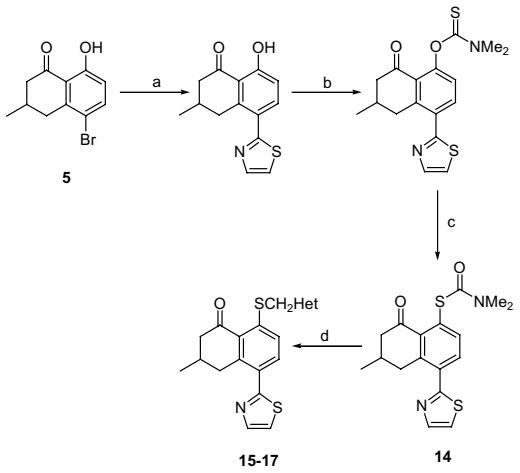


Figure 1.

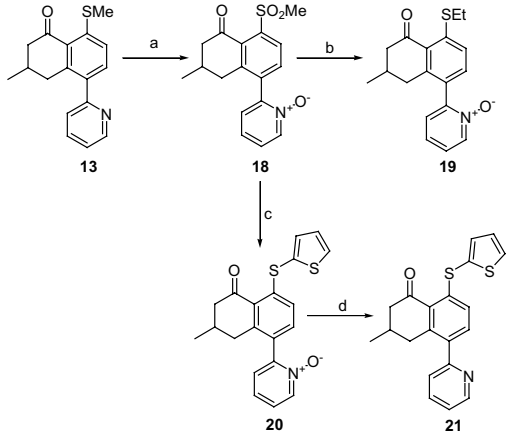
et al.[12] As well as investigating the thiophenes we sought to identify alternative core skeletons based on this novel lead. In particular, the thiophene moiety was replaced with a phenyl ring to give a series of 3,4-dihydronaphthalen-1(2*H*)-ones **3**. This manuscript describes the syntheses of these α-tetralones, and their affinity and efficacy at the BZ site of $GABA_A$ receptors containing an α5-subunit (hereafter referred to as the α5-subtype). To investigate the effect of varying the heterocycle at the C5 position of the tetralone core, the bromophenyl intermediate **8** was utilised as shown in Scheme 1.[13] 5-Bromo-8-methoxy-3-methyl-3,4-dihydronaphthalen-1(2*H*)-one (**4**)[14] was de-methylated using boron tribromide to give the phenol **5**, which was then converted to the *O*-arylthiocarbamate **6** using standard chemistry.[15] The *O*-arylthiocarbamate **6** was then subjected to the thermal Newman–Kwart rearrangement to afford the *S*-arylthiocarbamate **7**, which was readily hydrolysed and subsequently alkylated in situ with ethyl (or methyl) iodide to give bromide **8**. Finally, the Stille cross-coupling procedure was used to introduce the heterocycle. As shown in Scheme 2, the C8-heteroarylmethylthio analogues (**15**,[16] **16** and **17**[17]) were synthesised in an efficient manner from the *S*-arylthiocarbamate **14**, in which a preferred C5-heterocycle such as thiazole was incorporated early in the synthesis. The pyridine-*N*-oxide **19** was obtained from **13** in two steps as shown in Scheme 3. Treatment of **13** with an excess of *m*-CPBA afforded the methanesulfonyl-pyridine-*N*-oxide **18**, which was reacted with sodium



Scheme 1. Synthesis of the C5-heterocyclic analogues **9**–**13**. Reagents and conditions: (a) BBr_3, DCM, 0 °C; (b) Me_2NCSCl, DABCO™, DMF; (c) $PhNMe_2$, reflux; (d) KOH, MeOH, reflux then EtI or MeI, 25 °C; (e) Het-$SnBu_3$, 1,4-dioxane, $Pd(PPh_3)_4$, reflux.



Scheme 2. Synthesis of the C8-heteroarylmethylthio derivatives **15**–**17**. Reagents and conditions: (a) 2-tributylstannyl-1,3-thiazole, 1,4-dioxane, $Pd(PPh_3)_4$, reflux; (b) Me_2NCSCl, DABCO™, DMF; (c) $PhNMe_2$, reflux; (d) KOH, MeOH, reflux then $HetCH_2Cl$, 25 °C.

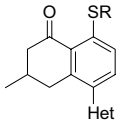


Scheme 3. Synthesis of the pyridine-*N*-oxide **19** and the C8-thienylthio derivative **21**. Reagents and conditions: (a) *m*-CPBA (3.0 equiv), DCM, 1,4-dioxane; (b) NaSEt, THF; (c) thiophene-2-thiol, NaH, THF; (d) PPh_3, 200 °C.

ethanethiolate to afford **19**. Similarly, reaction of **18** with sodium thiophene-2-thiolate produced the thienylthio analogue **20**, which was then reduced with PPh_3 to give the desired pyridine **21**. The C3-*gem*-dimethyl derivative **22** and the C3-unsubstituted tetralone **23** were obtained using similar chemistry to that shown in Scheme 1, starting from 5-bromo-3,3-dimethyl-8-methoxy-3,4-dihydronaphthalen-1(2*H*)-one[13] or 5-bromo-8-methoxy-3,4-dihydronaphthalen-1(2*H*)-one, respectively.[18]

Within the series, compounds that demonstrated good α5 binding affinity[19] were tested in an in vitro efficacy assay using human $GABA_A$ α5 receptors. The efficacy at $GABA_A$ α5 receptors was determined by one of two different methods as described by Chambers et al.[12] The efficacy of those compounds shown in Table 1 were determined using a two-electrode voltage clamp

Table 1. $GABA_A$ receptor subtype affinity of α-tetralones with efficacy measured in *Xenopus* oocytes



Compound	R	Het	K_i (nM) human $GABA_A$ αxβ3γ2 receptors[a]				α5 Selectivity	Oocyte Efficacy[b] α5 (%)
			α5	α1	α2	α3		
DMCM	—	—	2.2 ± 1.0	10 ± 1	13 ± 5	7.5 ± 1.2	3–6	−34 ± 5
CDZ	—	—	368 ± 66	605 ± 136	392 ± 73	471 ± 164	—	+134 ± 13[c]
9	Et		1.9 ± 0.8	25 ± 6	13 ± 7	15 ± 2	7–13	+2 ± 2
10	Et		2.1 (1.8, 2.4)	11 (9, 13)	19 (19, 19)	13 (12, 14)	5–9	+2 ± 1
11	Et		1.0 (1.0, 1.0)	5.4 (4.8, 6.0)	15 (15, 15)	12 (12, 12)	5–15	−8 ± 4
12	Et		2.6 (2.6, 2.6)	37 (35, 39)	38 (37, 39)	16 (12, 20)	6–14	+5 ± 2
13	Me		7.5 (6.3, 8.7)	63 (59, 67)	63 (58, 71)	42 (36, 48)	6–8	−5 ± 2
21			0.5 ± 0.3	2.3 ± 0.5	5.4 ± 0.3	4.5 ± 0.4	5–11	+50 ± 17

[a] Inhibition of [^{3}H]Ro 15-1788 binding to recombinant human $GABA_A$ receptor subtypes. K_i values are the mean ± SEM of at least three independent determinations. Where no SEM value is given the value is the mean of two independent determinations and the values of each determination are given in parentheses.

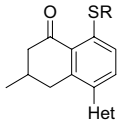
[b] Efficacy is determined as the percentage modulation of the submaximal (EC_{20}) response to GABA. Values given are the mean ± SEM of at least three individual cells from the α5β3γ2 human receptor subtype transiently expressed in *Xenopus laevis* oocytes.

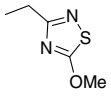
[c] Value is the mean ± SEM of four individual cells using CDZ = 10 μM.

recording from *Xenopus laevis* oocytes, transiently expressing the $GABA_A$ α5β3γ2 receptor subtype.[20,21] The efficacy is measured as the percentage modulation of the submaximal (EC_{20}) response to GABA using a drug concentration equivalent to 100× the K_i value, which would be anticipated to give a maximal response. The efficacy values shown in Tables 2 and 3 were determined using a whole cell patch clamp recording[22] from mouse fibroblast L(tk$^-$) cells, stably expressing the human $GABA_A$ α5β3γ2 receptor.[19] The efficacy is measured as the percentage maximum modulation of the current relative to a submaximal (EC_{20}) GABA response. Positive values represent a potentiation of the GABA-induced current (agonism) whereas negative values represent an attenuation (inverse agonism). All compounds were compared relative to the full inverse agonist DMCM[23] and the full agonist chlordiazepoxide (CDZ).

Table 1 shows the binding and efficacy data for a selected number of analogues in which the heterocycle is varied at C5. As is clearly indicated, various five- and six-membered heterocycles can be tolerated at this position with α5 binding selectivity in the range of 5–15-fold. Measurement of the efficacy in *X. laevis* oocytes of the ethylthio derivatives **9**–**12**, showed that all four compounds have low intrinsic efficacy (−8% to +5%) and, given that the full agonist CDZ and the full inverse agonist DMCM have efficacies of +134% and −34%, respectively, tetralones **9**–**12** are regarded as *antagonists* at the α5-subtype. Replacement of the ethylthio group in **9** with methylthio (**13**; K_i(α5): 7.5 nM) has a slightly detrimental effect on α5 affinity but no significant effect on efficacy. However, introducing a thienylthio group at the C8-position afforded the subnanomolar α5-subtype ligand **21** (K_i: 0.5 nM), which is a *partial agonist* (oocyte efficacy: +50%) at the BZ site on the $GABA_A$ α5 receptor. Table 2 shows the binding and efficacy data for a selected number of C8-heteroarylmethylthio derivatives (**15**–**17**) and the C5-pyridine-*N*-oxide **19**. It should be noted that for these analogues due to the availability of the stably transfected cloned cell line the $GABA_A$ α5 efficacy was recorded using L(tk$^-$) cells.[24] The heteroarylmethylthio derivatives **15**–**17** retain high binding affinity at α5-containing receptors (K_i: 1.0–3.2 nM) and for the two triazole analogues (**16** and **17**) a modest increase in α5-subtype binding selectivity was obtained, compared to the ethylthio analogue **11**. Whilst all three heteroarylmethylthio derivatives have very similar α5 binding affinity, their in vitro efficacy is significantly

Table 2. $GABA_A$ receptor subtype affinity of α-tetralones with efficacy measured in L(tk$^-$) cells



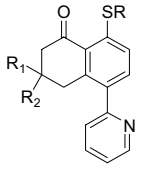
Compound	R	Het	K_i (nM) human $GABA_A$ αxβ3γ2 receptors[a]				α5 Selectivity	L(tk$^-$) Efficacy[b] α5 (%)
			α5	α1	α2	α3		
DMCM	—	—	2.2 ± 1.0	10 ± 1	13 ± 5	7.5 ± 1.2	3–6	−57 ± 1
CDZ	—	—	368 ± 66	605 ± 136	392 ± 73	471 ± 164	—	+99 ± 3[c]
15			3.2 ± 0.1	40 ± 1	34 ± 5	35 ± 7	10–13	+57 ± 7
16			1.0 ± 0.2	38 ± 4	20 ± 5	16 ± 3	16–38	+29 ± 3
17			2.6 (2.5, 2.7)	92 (87, 97)	57 (52, 62)	36 (30, 42)	14–35	+6 ± 4
19	Et		25 ± 6	>300	271 ± 58	286 ± 27	>11	+80 ± 9

[a] Inhibition of [^{3}H]Ro 15-1788 binding to recombinant human $GABA_A$ receptor subtypes. K_i values are the mean ± SEM of at least three independent determinations. Where no SEM value is given the value is the mean of two independent determinations and the values of each determination are given in parentheses.

[b] Efficacy is determined as the percentage maximum modulation of the current relative to a submaximal (EC_{20}) GABA response. Values are the mean maximum modulation ± SEM from at least three individual fitted concentration–response curves produced in the α5β3γ2 human receptor subtype stably expressed in mouse fibroblast L(tk$^-$) cells.

[c] Value is the mean ± SEM from 27 cells produced using CDZ = 3 μM.

Table 3. $GABA_A$ receptor subtype affinity and efficacy of C3 modified α-tetralones



Compound	R	R_1	R_2	K_i (nM) human $GABA_A$ αxβ3γ2 receptors[a]				α5 Selectivity	L(tk$^-$) Efficacy[b] α5 (%)
				α5	α1	α2	α3		
DMCM	—	—	—	2.2 ± 1.0	10 ± 1	13 ± 5	7.5 ± 1.2	3–6	−57 ± 1
CDZ	—	—	—	368 ± 66	605 ± 136	392 ± 73	471 ± 164	—	+99 ± 3[c]
13	Me	H	Me	7.5 (6.3, 8.7)	63 (59, 67)	63 (58, 71)	42 (36, 48)	6–8	+2 ± 1
22	Me	Me	Me	19 ± 7	229 ± 16	60 ± 10	59 ± 6	3–12	−32 ± 3
23	Et	H	H	103 ± 24	>300	>300	>300	>3	—

[a] Inhibition of [^{3}H]Ro 15-1788 binding to recombinant human $GABA_A$ receptor subtypes. K_i values are the mean ± SEM of at least three independent determinations. Where no SEM value is given the value is the mean of two independent determinations and the values of each determination are given in parentheses.

[b] Efficacy is determined as the percentage maximum modulation of the current relative to a submaximal (EC_{20}) GABA response. Values are the mean maximum modulation ± SEM from at least three individual fitted concentration–response curves produced in the α5β3γ2 human receptor subtype stably expressed in mouse fibroblast L(tk$^-$) cells.

[c] Value is the mean ± SEM from 27 cells produced using CDZ = 3 μM.

different; whereas the 1,2,3-triazole **17** is an *antagonist* at the α5-subtype (L(tk$^-$) efficacy: +6%) the N-methylated-1,2,4-triazole **16** and the 5-methoxy-1,2,4-thiadiazole **15** are both *partial agonists* (L(tk$^-$) efficacy: +29% and +57%, respectively). N-Oxidation of **9** to produce **19** (K_i(α5): 25 nM) results in an order of magnitude decrease in α5 affinity and a large increase in efficacy, to afford what is essentially a *full agonist* (L(tk$^-$) efficacy: +80%) at the α5-subtype. Table 3 shows the effect of methyl substitution at the C3-position. Comparison of the C3-methyl and C3-unsubstituted derivatives **9** (K_i(α5): 1.9 nM) and **23** (K_i(α5): 103 nM), respectively,

clearly demonstrates that a C3-substituent is crucial for high α5-subtype binding affinity, with the unsubstituted analogue exhibiting 50-fold lower α5 affinity. The introduction of a second methyl substituent at C3 does not improve affinity (cf. thiomethyl analogues **13** (K_i(α5): 7.5 nM) and **22** (K_i(α5): 19 nM) but it does have a profound effect on the $GABA_A$ α5 efficacy—converting an antagonist (**13**, L(tk$^-$) efficacy: +2%) into an *inverse agonist* (**22**, L(tk$^-$) efficacy: −32%). Throughout the course of the programme a variety of C3-*gem*-dimethyl derivatives were synthesised but they had unacceptably low α5-subtype affinity (i.e., $K_i > 10$ nM) and, as a consequence, were not pursued any further. It should be noted that for all those compounds that possess a single C3 methyl group the data given in the tables are for the racemic mixture. However, the pyridyl derivative **9** has been resolved into its individual enantiomers using chiral HPLC. The data obtained for these enantiomers showed that whilst there was an order of magnitude difference in their α5-subtype binding affinity (K_i values 0.5 and 10 nM) there was no significant efficacy difference (L(tk$^-$) efficacy: +6% and +18%).

From the selected analogues shown in the tables it is clear that relatively minor structural changes at different positions on the tetralone core could produce a significant effect on $GABA_A$ α5 efficacy. However, it was found that these structure–efficacy observations were not consistent across the series, and as a consequence a robust structure–efficacy relationship was not established.

In summary, a series of 3,4-dihydronaphthalen-1(2*H*)-ones has been identified as a novel class of ligands at the BZ site of $GABA_A$ receptors, with higher binding affinity for receptors containing the α5-subunit compared to the α1, α2 and α3-containing subtypes. Within the series compounds with a range of efficacies were identified, from the α5-subtype agonist **19** through partial agonists and antagonists to the α5-subtype inverse agonist **22**.

References and notes

1. Barnard, E. A.; Skolnick, P.; Olsen, R. W.; Mohler, H.; Sieghart, W.; Biggio, G.; Braestrup, C.; Bateson, A. N.; Langer, S. Z. *Pharmacol. Rev.* **1999**, *50*, 291–313.
2. Bonnert, T. P.; McKernan, R. M.; Farrar, S.; Le Bourdelles, B.; Heavens, R. P.; Smith, D. W.; Hewson, L.; Rigby, M. R.; Sirinathsinghji, D. J. S.; Brown, N.; Wafford, K. A.; Whiting, P. J. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 9891–9896.
3. Farrar, S. J.; Whiting, P. J.; Bonnert, T. P.; McKernan, R. M. *J. Biol. Chem.* **1999**, *274*, 10100–10104.
4. McKernan, R. M.; Whiting, P. J. *Trends Neurosci.* **1996**, *19*, 139–143.
5. Sur, C.; Quirk, K.; Dewar, D.; Atack, J. R.; McKernan, R. M. *Mol. Pharmacol.* **1998**, *54*, 928–933.
6. Collinson, N.; Kuenzi, F. M.; Jarolimek, W.; Maubach, K. A.; Cothliff, R.; Sur, C.; Smith, A.; Otu, F. M.; Howell, O.; Atack, J. R.; McKernan, R. M.; Seabrook, G. R.; Dawson, G. R.; Whiting, P. J.; Rosahl, T. W. *J. Neurosci.* **2002**, *22*, 5572–5580.
7. Crestani, F.; Keist, R.; Fritschy, J.-M.; Benke, D.; Vogt, K.; Prut, L.; Bluthmann, H.; Mohler, H.; Rudolph, U. *Proc. Natl. Am. Sci.* **2002**, *99*, 8980–8985.
8. Dorow, R.; Horowski, R.; Paschelke, G.; Amin, M.; Braestrup, C. *Lancet* **1983**, *2*, 98–99.
9. Petersen, E. N. *Eur. J. Pharmacol.* **1983**, *94*, 117–124.
10. Little, H. J.; Nutt, D. J.; Taylor, S. C. *Br. J. Pharmacol.* **1984**, *83*, 951–958.
11. Chambers, M. S.; Atack, J. R.; Bromidge, F. A.; Broughton, H. B.; Cook, S.; Dawson, G. R.; Hobbs, S. C.; Maubach, K. A.; Reeve, A. J.; Seabrook, G. R.; Wafford, K.; MacLeod, A. M. *J. Med. Chem.* **2002**, *45*, 1176–1179.
12. Chambers, M. S.; Atack, J. R.; Broughton, H. B.; Collinson, N.; Cook, S.; Dawson, G. R.; Hobbs, S. C.; Marshall, G.; Maubach, K. A.; Pillai, G. V.; Reeve, A. J.; MacLeod, A. M. *J. Med. Chem.* **2003**, *46*, 2227–2240.
13. Bryant, H. J.; Chambers, M. S.; Hobbs, S. C. U.S. Patent 6,156,761, 2000.
14. Nishiyama, T.; Kameoka, H. *Chem. Express* **1991**, *6*, 109–113.
15. Sebok, P.; Timar, T.; Eszenyi, T.; Patonay, T. *Synthesis* **1994**, 837–840.
16. For the synthesis of **15**, 5-chloro-3-chloromethyl-1,2,4-thiadiazole was the alkylating agent used in step (d) of Scheme 2. During the reaction, methoxide displaced the chloride on the thiadiazole.
17. Compound **17** was synthesised from the SEM-protected triazole analogue using 5-chloromethyl-1-{[2-(trimethylsilyl)ethoxy]methyl}-1*H*-1,2,3-triazole as the alkylating agent in step (d) of Scheme 2. The SEM group was removed by reaction with 2 N HCl in ethanol at reflux.
18. Chatterjee, A.; Hazra, B. G. *Tetrahedron* **1980**, *36*, 2513–2519.
19. Hadingham, K. L.; Wingrove, P.; Le Bourdelles, B.; Palmer, K. J.; Ragan, C. I.; Whiting, P. J. *Mol. Pharmacol.* **1993**, *43*, 970–975.
20. Casula, M. A.; Bromidge, F. A.; Pillai, G. V.; Wingrove, P. B.; Martin, K.; Maubach, K.; Seabrook, G. R.; Whiting, P. J.; Hadingham, K. L. *J. Neurochem.* **2001**, *77*, 445–451.
21. Hadingham, K. L.; Garret, E. M.; Wafford, K. A.; Bain, C.; Heavens, R. P.; Sirinathsinghji, D. J. S.; Whiting, P. J. *Mol. Pharmacol.* **1996**, *49*, 253–259.
22. Horne, A. L.; Hadingham, K. L.; Macaulay, A. J.; Whiting, P.; Kemp, J. A. *Br. J. Pharmacol.* **1992**, *107*, 732–737.
23. Lui, R.; Zhang, P.; McKernan, R. M.; Wafford, K.; Cook, J. M. *Med. Chem. Res.* **1995**, *5*, 700–709.
24. Since efficacy recordings using L(tk$^-$) cells provided full concentration–response curves this method of efficacy measurement was preferred to that obtained using oocytes. It should be noted that obtaining concentration–response curves in oocytes is a problematic process, due to the rapid de-sensitisation of the $GABA_A$ receptor at relatively high concentrations.