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Azaflavones compared to flavones as ligands to the benzodiazepine binding site of brain $GABA_A$ receptors

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ABSTRACT

A series of azaflavone derivatives and analogues were prepared and evaluated for their affinity to the benzodiazepine binding site of the $GABA_A$ receptor, and compared to their flavone counterparts. Three of the compounds, the azaflavones **9** and **12** as well as the new flavone **13**, were also assayed on $GABA_A$ receptor subtypes ($\alpha_1\beta_3\gamma_{2s}$, $\alpha_2\beta_3\gamma_{2s}$, $\alpha_4\beta_3\gamma_{2s}$ and $\alpha_5\beta_3\gamma_{2s}$), displaying nanomolar affinities as well as selectivity for α1- versus α2- and α3-containing receptors by a factor of between 14 and 26.

GABA (γ-aminobutyric acid) is the major inhibitory neurotransmitter in the central nervous system.[1] The $GABA_A$ receptor is a chloride ion channel complex, consisting of five subunits from eight different classes with multiple isoforms ($\alpha_{1\text{-}6}$, $\beta_{1\text{-}4}$, $\gamma_{1\text{-}4}$, δ, ε, π, θ and $\rho_{1\text{-}3}$).[2,3] The most abundant $GABA_A$ receptor contains α, β, γ subunits in a 2:2:1 stoichiometry, and receptors with different subtype composition are associated with different physiological effects. While α_1-containing receptors are implicated in sedation and anterograde amnesia, α_2- and/or α_3-containing receptors appear to be important for anxiolytic activity.[4,5] The $GABA_A$ receptor has several allosteric modulatory sites, of which the one for benzodiazepines (BZDs) has attracted most attention. A pharmacophore model comprising agonists, inverse agonists, and antagonists for this binding site was proposed in 1995[6] and further developed in a recent study with synthetic flavones,[7,8] resulting in the potent 5′-bromo-2′-hydroxy-6-methylflavone **11** (K_i = 0.9 nM). In Figure 1, **11** is displayed in the pharmacophore model, and it should be noted that flavones only can interact as a hydrogen bond acceptor with H2 of the H2/A3 hydrogen bond donating/accepting site. Aza analogues of flavones, for example, compounds **9** and **12**, could shed light on the difference between a hydrogen bond acceptor and donor by interacting with A3, and thereby provide valuable information for the pharmacophore model. Compounds **16** and **17** were also considered to be of interest, as they, just as the flavones, can interact with H2. In addition, as the introduction of a benzyl group in the position facing the interface region for other types of ligands has improved the potency considerably,[9,10] compounds **12** and **13** were also prepared and included in this study.

The azaflavones **9, 10** and **12** as well as the 2-arylquinolines **16** and **17** were prepared according to the procedure shown in Scheme 1. 2-Arylquinolones **9** and **10** were synthesized in a four-step procedure starting with an acylation of toluidine and *N*-methyltoluidine under Sugasawa conditions to give **3** and **4**.[11] The amides **5** and **6** were generated by reaction with 5-bromo-2-methoxybenzoyl chloride prepared from 5-bromo-2-methoxybenzaldehyde following a previously published protocol.[8]

Cyclization of **5** and **6** in presence of potassium *tert*-butoxide followed by a demethylation with boron tribromide gave quinolones **9** and **10**.[12] Successive treatment of **7** with phosphorus oxychloride, boron tribromide and morpholine gave **16**. In a Suzuki-Miyaura cross-coupling reaction with *B*-benzyl-9-borabicyclo[3.3.1]nonane, the bromo atom of **9, 16** and **11** was substituted by a benzyl group.[13] Condensation of ethyl benzoyl acetate and 4-ethylaniline was carried out neat in the presence of a catalytic amount of $Sc(OTf)_3$ to give β-enamine **19** (see Scheme 2),[14] and subsequent cyclization of the β-enamine in diphenyl ether at 250 °C gave quinolone **20**.

The affinity for the benzodiazepine binding site of the $GABA_A$ receptor was determined by displacement of ^{3}H-flumazenil in rat cortical tissue as previously described.[7] The results are shown in Table 1. The subtype selectivity of some of the most potent compounds was tested by assaying their ability to displace ^{3}H-flumazenil in membranes from HEK293 cells expressing rat $\alpha_1\beta_3\gamma_2$, $\alpha_2\beta_3\gamma_2$, $\alpha_3\beta_3\gamma_2$ and $\alpha_5\beta_3\gamma_2$ $GABA_A$ receptor subtypes (Table 2).

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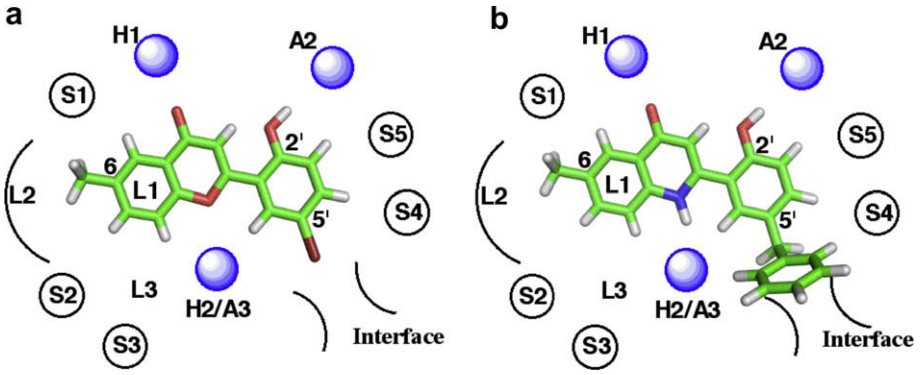
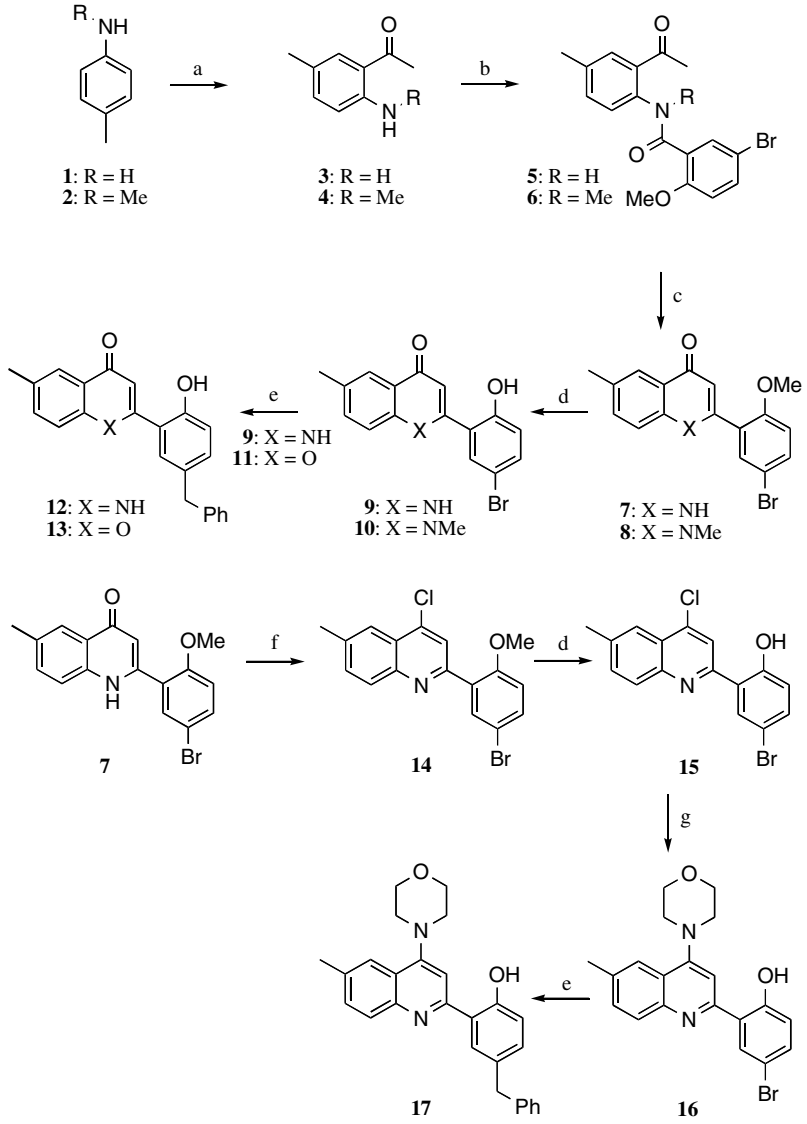
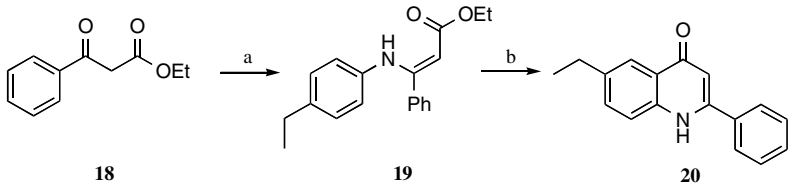


Figure 1. Binding mode of (a) the high affinity flavonoid 5′-bromo-2′-hydroxy-6-methyl flavone (**11**) and (b) the 2-aryl-quinolone **12**, in the pharmacophore model discussed in the text. H1 is a H-bond donor site, A2 is a H-bond acceptor site, H2/A3 is a site that both accepts and donates H-bonds, L1, L2 and L3 represents lipophilic pockets while S1, S2, S3, S4 and S5 denote regions of steric repulsive ligand–receptor interaction (or receptor essential volume).

In general, the azaflavones appear to be less potent than the flavones. The affinities of **9**, **12** and **20** are 24, 3 and 7 times lower than that of their corresponding flavone analogues **11**,[8] **13** and **21**.[8] It has been postulated that BZDR ligands require the ability to



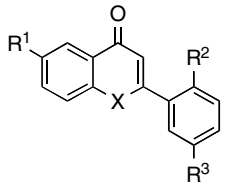
Scheme 1. Reagents and conditions: (a) $AlCl_3$, BCl_3, MeCN, toluene, reflux, 2 h, then HCl (1 M), 80 °C, 30 min (yield 48% for **3** and 30% for **4**); (b) 5-bromo-2-methylbenzoyl chloride, Et_3N, THF, rt, 18 h (yield 82% for **5** and 84% for **6**); (c) KO^{t}Bu, ^{t}BuOH, 70 °C, 16 h (yield 86% for **7** and 100% for **8**); (d) BBr_3, CH_2Cl_2, 40 °C, 8 h (yield 79% for **9**, 95% for **10** and 89% for **15**); (e) K_3PO_4, $Pd(OAc)_2$, S-Phos, *B*-Bn-9-BBN, DMF, 60 °C, 16 h (yield 67% for **12**, 57% for **13** and 85% for **17**); (f) $POCl_3$, 90 °C, 1 h (yield 95%); (g) DIPEA, morpholine, DMF, 90 °C, 1 h (yield 81%).



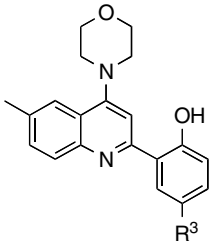
18 19 20

Scheme 2. Reagents and conditions: (a) $Sc(OTf)_3$, 4-ethylaniline (yield 77%); (b) diphenyl ether, reflux, 30 min (yield 63%).

Table 1
K_i values of flavone analogues tested on ^{3}H-flumazenil binding in vitro to rat cortical membranes



7, **9**, **10**, **11**, **12**, **13**, **20**, **21**, **22**



16, **17**

Compound	R^1	R^2	R^3	X	K_i value[a] (nM)
7	$-CH_3$	–OMe	–Br	NH	730 ± 130
9	$-CH_3$	–OH	–Br	NH	22 ± 4
10	$-CH_3$	–OH	–Br	NMe	1100 ± 310
11[8]	$-CH_3$	–OH	–Br	O	0.9 ± 0.2
12	$-CH_3$	–OH	$-CH_2C_6H_5$	NH	2.0 ± 0.3
13	$-CH_3$	–OH	$-CH_2C_6H_5$	O	0.6 ± 0.3
16	—	—	–Br	—	840 ± 55
17	—	—	$-CH_2C_6H_5$	—	400 ± 68
20	$-CH_2CH_3$	–H	–H	NH	1200 ± 260
21[8]	$-CH_2CH_3$	–H	–H	O	180 ± 40
22[8]	$-CH_3$	–OMe	–Br	O	>1500

[a] Each K_i value is mean ± SD of three determinations.

Table 2
The affinity of selected flavone analogues tested on ^{3}H-flumazenil binding to $\alpha_1\beta_3\gamma_{2s}$, $\alpha_2\beta_3\gamma_{2s}$, $\alpha_3\beta_3\gamma_{2s}$ and $\alpha_5\beta_3\gamma_{2s}$ $GABA_A$ receptor subtypes

Compound	K_i α_1[a] (nM)	K_i α_2[a] (nM)	K_i α_3[a] (nM)	K_i α_5[a] (nM)
9	39 ± 8	120 ± 34	99 ± 20	nd
12	1.2 ± 0.4	18 ± 2.9	31 ± 7.8	7.3 ± 0.8
13	0.80 ± 0.25	19 ± 9	11 ± 6	2.4 ± 1.1

[a] Each K_i value is mean ± SD of three determinations. nd, not determined.

adopt a planar or close to planar arrangement of the ring systems for an efficient binding,[6] and the 2′-hydroxyl group constitutes a sterical hindrance for the adoption of a coplanar conformation among all the potent analogues tested in this study. The energy difference between the coplanar conformation and the lowest energy conformation, with a twisted conformation, was calculated to 13 kJ/mol for flavone **13**, implying that 2′-hydroxyl substituted flavones are unlikely to adopt a planar conformation upon binding. Conformational analyses were performed by Macromodel (version 9.5),[15] and force field calculation were undertaken using MMFFs in gas phase.[16] Instead, the biologically active conformation is probably somewhat twisted. For the azaflavones, the additional sterical hindrance between the N–H and the aryl group makes a planar conformation even less probable (35.7 kJ/mol difference between planar and most stable conformation for **12**, and 23 kJ/mol for compound **20**). The N-methylated **10** is consequently considerably less potent. A reasonable interpretation of the SAR data is that the H2/A3 hydrogen acceptor/donor does not occupy the exactly same space or bind ligands with the same binding angle. In particular, the H2 interacting ligands could be somewhat tilted compared to the A3 interacting ligands. This would imply that a SAR study cannot in a straight forward fashion be translated between the H2 and A3 interactive benzodiazepine analogues. However, it is interesting to note that this general trend is not followed by **7**, which is more potent than its flavone analogue **22**,[8] indicating that a hydrogen bond from the ligand to A3 is as important as one from H2 to the ligand in compounds of comparable planarity. An interesting observation is that the shift from 5′-bromo- to 5′-benzyl substitution does not significantly affect the affinity in the flavone series (**11** and **13**), whereas a 10-fold increase in affinity is observed in the azaflavone series (**9** and **12**). For the two arylquinolines **16** and **17**, it is obvious that the potency is considerably lower compared to the corresponding flavones and azaflavones. In other types of BZDR ligands it has been shown that the interaction of ring systems similar to the morpholine group with H1 is acceptable,[17] excluding steric interactions as the cause for the low affinity. Instead, the strong hydrogen bond between the 2′-hydroxyl group and the quinoline nitrogen (considerably stronger than that present in the 2′-hydroxylflavones, −22 kJ/mol for **16** compared to 1.4 kJ/mol for **11**) will hamper compounds **16** and **17** from adopting the active conformation.

Subtype affinity testing was performed with compounds **9, 12** and **13** on recombinant $\alpha_1\beta_3\gamma_{2s}$, $\alpha_2\beta_3\gamma_{2s}$, $\alpha_4\beta_3\gamma_{2s}$ and $\alpha_5\beta_3\gamma_{2s}$ receptor subtypes (Table 2). All compound investigated in this study display selectivity for $\alpha_1\beta_3\gamma_{2s}$ over the other receptor subtypes. Interestingly, the substitution of a bromo to a benzyl group in the 6-position of the aryl quinolone (**9**–**12**) resulted in 5 times higher α_2/α_1 K_i ratio and 8 times higher α_3/α_1 K_i ratio as well as an increased affinity, making the highly subtype selective derivatives **12** and **13** valuable for the development of a subtype specific pharmacophore model.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.09.092.

References and notes

1. Sieghart, W. *Pharmacology* **2006**, *54*, 231.
2. Chebib, M.; Johnston, G. A. R. *J. Med. Chem.* **2000**, *43*, 1427.
3. Johnston, G. A. R. *Curr. Pharm. Des.* **2005**, *11*, 1867.
4. Rudolph, U.; Crestani, F.; Benke, D.; Brunig, I.; Benson, J. A.; Fritschy, J. M.; Martin, J. R.; Bluethmann, H.; Mohler, H. *Nature* **1999**, *401*, 796.
5. Rudolph, U.; Crestani, F.; Möhler, H. *Trends Pharmacol. Sci.* **2001**, *22*, 188.
6. Zhang, W.; Koehler, K.; Zhang, P.; Cook, J. *Drug Des. Discov.* **1995**, *12*, 193.

7. Dekermendijan, K.; Kahnberg, P.; Witt, M.; Sterner, O.; Nielsen, M.; Liljefors, T. *J. Med. Chem.* **1999**, *42*, 4343.
8. Kahnberg, P.; Lager, E.; Rosenberg, C.; Schougaard, J.; Camet, L.; Sterner, O.; Østergaard Nielsen, E.; Nielsen, M.; Liljefors, T. *J. Med. Chem.* **2002**, *45*, 4188.
9. Albaugh, P.; Marshall, L.; Gregory, J.; White, G.; Hutchison, A.; Ross, P.; Gallagher, D.; Tallman, J.; Crago, M.; Cassella, J. *J. Med. Chem.* **2002**, *45*, 5043.
10. Lager, E.; Andersson, P.; Nilsson, J.; Pettersson, I.; Østergaard Nielsen, E.; Nielsen, M.; Sterner, O.; Liljefors, T. *J. Med. Chem.* **2006**, *49*, 2526.
11. Prasad, K.; Lee, G. T.; Chaudhary, A.; Girgis, M. J.; Streemke, J. W.; Repic, O. *Org. Process Res. Dev.* **2003**, *7*, 723.
12. Li, L.; Wang, H.-K.; Kuo, S.-C.; Wu, T.-S.; Lednicer, D.; Lin, C. M.; Hamel, E.; Lee, K.-H. *J. Med. Chem.* **1994**, *37*, 1126.
13. Flaherty, A.; Trunkfield, A.; Barton, W. *Org. Lett.* **2005**, *7*, 4975.
14. Yadav, J. S.; Kumar, V. N.; Rao, R. S.; Priyadarshini, A. D.; Rao, P. P.; Reddy, B. V. S.; Nagaiah, K. *J. Mol. Catal. A: Chem.* **2006**, *256*, 234.
15. Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caulfield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440.
16. Halgren, T. A. *J. Comput. Chem.* **1999**, *29*, 720.
17. Andersson, K. E.; Lundt, B. F.; Jorgensen, A. S.; Braestrup, C. *Eur. J. Med. Chem.* **1996**, *31*, 417.
18. (Given in Supplementary Material) Anwer, B.; Sged, S. A.; Tanveer, A. F. *Tetrahedron Lett.* **1976**, *36*, 3217.