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Identification of Benzopyran-4-one Derivatives (Isoflavones) as Positive Modulators of $GABA_A$ Receptors

Navnath Gavande,[a] Nasiara Karim,[a] Graham A. R. Johnston,[b] Jane R. Hanrahan,[a] and Mary Chebib*[a]

γ-Aminobutyric acid (GABA) is the most abundant inhibitory neurotransmitter in the mammalian central nervous system (CNS), with 25–50% of synapses being GABA-ergic in nature.[1] GABA influences neurons via three major classes of receptors, which are grouped on the basis of their subunit composition, gating properties, and pharmacological profiles, and termed $GABA_A$, $GABA_B$, and $GABA_C$ ($GABA\rho$) receptors. $GABA_A$ and $GABA_C$ receptors are ligand-gated chloride ion channels, whereas $GABA_B$ receptors are G protein-coupled receptors.[2] The ionotropic $GABA_A$ receptors are transmembrane protein complexes composed of five heteropentameric subunits. To date, 16 human $GABA_A$ receptor subunits have been identified, and they have been classified into α (α_1–α_6), β (β_1–β_3), γ (γ_1–γ_3), δ, ε, π, and θ. Although a wide range of different $GABA_A$ receptor combinations exists in vivo, the $\alpha_1\beta_2\gamma_2$ subunit combination represents the most dominant receptor subtype in the human brain.[2,3]

Enhancement of chloride ion flux at $GABA_A$ receptors by positive modulators is one of the most powerful therapeutic strategies for the treatment of CNS-related disorders, such as generalized anxiety, panic disorders, sleep disturbances, muscle spasms and seizure disorders.[2a,4] Positive modulators of $GABA_A$ receptors, such as benzodiazepines, neuroactive steroids, barbiturates, and loreclezole, have been identified as useful for the treatment of CNS-related disorders. Benzodiazepines are commonly prescribed drugs, but they produce a range of both desirable and unwanted side effects, such as sedation, myorelaxation, rebound anxiety, dependence, tolerance, strong interactions with alcohol, and amnesia.[2a,5] However, based on recent advances in $GABA_A$ receptor pharmacology, subtype-selective agents and modulators that bind to the $GABA_A$ receptor at sites other than the classical benzodiazepine binding site may offer an opportunity to discover novel therapeutic agents with fewer adverse side effects.[5a,6] Thus, the identification of novel templates for positive modulation of $GABA_A$ receptors represent an important objective in drug discovery for the treatment of CNS-related disorders.

Using functional electrophysiological studies on recombinant receptors, we[7] and others[8] recently revealed that flavonoids (flavones/flavanones/flavan-3-ols) are able to interact with binding sites on the $GABA_A$ receptor that are independent of the classical high-affinity, flumazenil-sensitive, benzodiazepine binding site. In contrast, many previous studies demonstrated that a range of naturally occurring and synthetic flavonoids displace radiolabelled benzodiazepines in rat brain tissue binding to the high-affinity benzodiazepine binding site with nanomolar affinity.[9] Thus, the benzopyran-4-one (flavonoid) pharmacophore is emerging as a potential template for the treatment of CNS-related disorders, although the exact mechanism and location of the binding site have not yet been elucidated.

Isoflavones are a subgroup of flavonoids, which differ from flavones in location of the substituted phenyl group (Figure 1). Isoflavones are natural antioxidants and have diverse effects

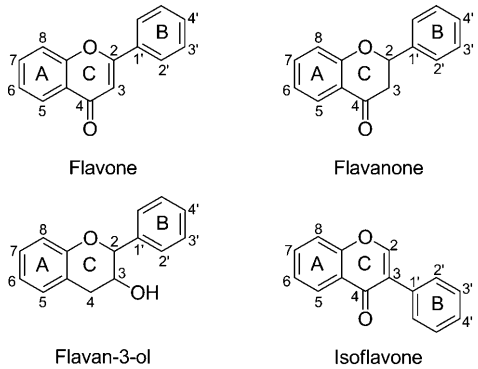

Figure 1. Structures of flavone, flavanone, flavan-3-ol and isoflavone. The numeric positions and ring nomenclature for each scaffold are indicated.

on human health. For example, isoflavones help to prevent osteoporosis by protecting and maintaining strong and healthy bones while reducing bone resorption and improving bone mass.[10] Likewise, isoflavones are known to lower cholesterol levels and assist in preventing the build up of arterial plaques, and they also help to reduce blood pressure by increasing vasodilatation.[11] In addition, they are known to reduce tumour size in various cancers, including prostate and breast cancer,[12] and to help to relieve post-menopausal symptoms.[10] Furthermore, isoflavones are the active ingredients in various marketed products that protect skin from sun damage.

Isoflavones were first linked to $GABA_A$ receptors when three isoflavones were shown to displace [^{3}H]diazepam binding in rat brain membranes.[13] The synthetic coumestan scaffold (6*H*-benzofuro[3,2-*c*]chromen-6-one) showed an inverse agonist effect at the benzodiazepine site of the $GABA_A$ receptor.[14] To date, there are few reports describing the direct inhibitory effects of naturally occurring genistein and daidzein using func-

[a] *N. Gavande, N. Karim, Prof. J. R. Hanrahan, Prof. M. Chebib*
Faculty of Pharmacy, The University of Sydney
A15, Science Road, Sydney, NSW 2006 (Australia)
Fax: (+61) 293-514-391
E-mail: maryc@pharm.usyd.edu.au

[b] *Prof. G. A. R. Johnston*
Adrien Albert Laboratory of Medicinal Chemistry
Department of Pharmacology
The University of Sydney, NSW 2006 (Australia)

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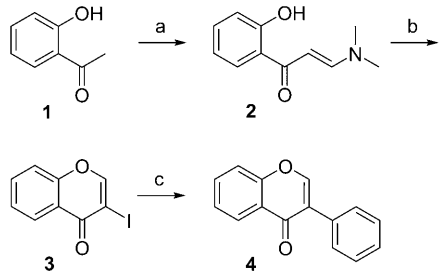
tional electrophysiological studies on recombinant $GABA_A$ receptors.[15] The lack of information on the effects of isoflavones may be due to limited availability of structurally diverse natural and synthetic analogues. Recently, we have discovered synthetic analogues of isoflavones as novel templates for potential dual peroxisome proliferator-activated receptor (PPAR)-α and -γ agonists.[16] During the course of our ongoing research, we envisioned for the first time, that isoflavones exhibit a potent flumazenil-insensitive positive modulation at human recombinant $\alpha_1\beta_2\gamma_{2L}$ receptors expressed in *Xenopus* oocytes. The main goal of this communication is to report the in vitro $GABA_A$ activity of the isoflavones and to find correlations between the structure-driven design paradigm and the pharmacological activity. In order to develop a broader understanding of the structural requirements for $GABA_A$ modulatory activity by isoflavones, we synthesized and biologically evaluated analogues of **4** with the ultimate objective to identify more potent modulators of $GABA_A$ receptors.

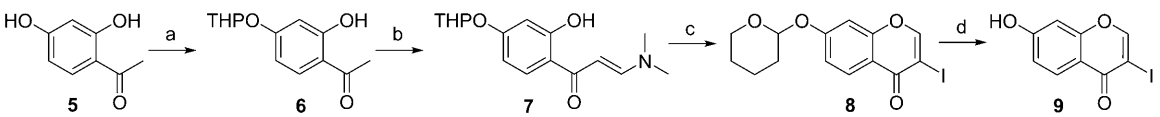
The synthesis of isoflavone **4** is depicted in Scheme 1. Condensation of 2-hydroxyacetophenone with *N*,*N*-dimethylformamide dimethylacetal (DMF-DMA) afforded enaminone **2** under microwave irradiation, enaminone **2** cyclized without further purification directly to 3-iodochromone **3** in the presence of iodine/$CHCl_3$.[17] Suzuki–Miyaura coupling of the 3-iodochromone **3** with phenylboronic acid afforded isoflavone **4**.[16]

The synthesis of isoflavones **10**–**49** was achieved according to previously described procedures (Scheme 2).[16,18] 3-Iodo-7-(tetrahydropyran-2-yloxy)-benzopyran-4-one **8** was considered a key intermediate in the synthesis of the isoflavones **10**–**49**. The synthesis of intermediate **8** was achieved from 2,4-dihydroxy acetophenone through a sequence of reactions: selective protection of the 4-hydroxy group of 2,4-dihydroxy acetophenone by a tetrahydropyran (THP) ether, condensation with DMF-DMA to furnish enaminone **7**, and cyclization using molecular iodine provided intermediate **8**. Compound **9** was prepared by removal of the THP ether using *para*-toluenesulfonic acid (Scheme 2 A). Suzuki–Miyaura coupling of the intermediate **8** with appropriately substituted boronic acids afforded compounds **10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46** and **48** (Scheme 2 B). Subsequent THP

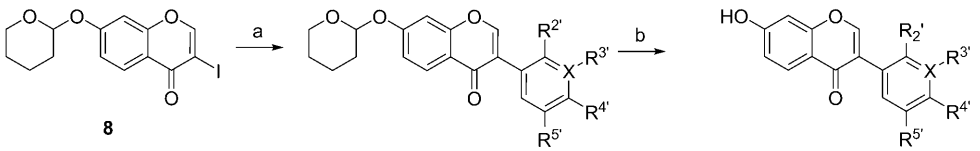


Scheme 1. Synthesis of isoflavone **4**. *Reagents and conditions*: a) $(MeO)_2CHNMe_2$, μwave, 150 °C, 10 min, 90%; b) I_2, pyridine, $CHCl_3$, RT, 12 h, 86%; c) $PhB(OH)_2$, 10% Pd/C, Na_2CO_3, DME/H_2O (1:1), 45 °C, 2 h, 84%.

A)



B)



10, **12**, **14**, **16**, **18**, **20**, **22**, **24**, **26**, **28**, **30**, **32**, **34**, **36**, **38**, **40**, **42**, **44**, **46**, **48**

11, **13**, **15**, **17**, **19**, **21**, **23**, **25**, **27**, **29**, **31**, **33**, **35**, **37**, **39**, **41**, **43**, **45**, **47**, **49**

10, **11**: X=C, $R^{2'}$=OCH_3, $R^{3'}$=H, $R^{4'}$=H, $R^{5'}$=H
12, **13**: X=C, $R^{2'}$=H, $R^{3'}$=OCH_3, $R^{4'}$=H, $R^{5'}$=H
14, **15**: X=C, $R^{2'}$=H, $R^{3'}$=H, $R^{4'}$=OCH_3, $R^{5'}$=H
16, **17**: X=C, $R^{2'}$=H, $R^{3'}$=OCH_3, $R^{4'}$=OCH_3, $R^{5'}$=H
18, **19**: X=C, $R^{2'}$=H, $R^{3'}$=OCH_3, $R^{4'}$=H, $R^{5'}$=OCH_3
20, **21**: X=C, $R^{2'}$=H, $R^{3'}$=OCH_3, $R^{4'}$=OCH_3, $R^{5'}$=OCH_3
22, **23**: X=N, $R^{2'}$=OCH_3, $R^{3'}$=none, $R^{4'}$=OCH_3, $R^{5'}$=H
24, **25**: X=C, $R^{2'}$=H, $R^{3'}$=$R^{4'}$=O-CH_2-O, $R^{5'}$=H
26, **27**: X=C, $R^{2'}$=H, $R^{3'}$=$R^{4'}$=O-CH_2-CH_2-O, $R^{5'}$=H
28, **29**: X=C, $R^{2'}$=H, $R^{3'}$=CH_3, $R^{4'}$=OCH_3, $R^{5'}$=CH_3
30, **31**: X=C, $R^{2'}$=H, $R^{3'}$=F, $R^{4'}$=H, $R^{5'}$=H
32, **33**: X=C, $R^{2'}$=H, $R^{3'}$=H, $R^{4'}$=F, $R^{5'}$=H
34, **35**: X=C, $R^{2'}$=F, $R^{3'}$=H, $R^{4'}$=F, $R^{5'}$=H
36, **37**: X=C, $R^{2'}$=H, $R^{3'}$=F, $R^{4'}$=F, $R^{5'}$=F
38, **39**: X=C, $R^{2'}$=OCH_3, $R^{3'}$=F, $R^{4'}$=H, $R^{5'}$=F
40, **41**: X=C, $R^{2'}$=H, $R^{3'}$=H, $R^{4'}$=Cl, $R^{5'}$=H
42, **43**: X=C, $R^{2'}$=H, $R^{3'}$=H, $R^{4'}$=CF_3, $R^{5'}$=H
44, **45**: X=C, $R^{2'}$=H, $R^{3'}$=OCF_3, $R^{4'}$=H, $R^{5'}$=H
46, **47**: X=C, $R^{2'}$=H, $R^{3'}$=NO_2, $R^{4'}$=H, $R^{5'}$=H
48, **49**: X=C, $R^{2'}$=H, $R^{3'}$=OCH_2Ph, $R^{4'}$=H, $R^{5'}$=H

Scheme 2. A) Synthesis of key intermediates.[16,18] *Reagents and conditions*: a) DHP, PPTS, CH_2Cl_2, RT, 4 h; b) $(MeO)_2CHNMe_2$, 95 °C, 3 h; c) I_2, pyridine, $CHCl_3$, RT, 12 h, 92%; d) *p*-TsOH, MeOH/THF (1:1), 60 °C, 1 h, 90%. B) Synthesis of isoflavones **10**–**49** via known procedures from key intermediate **8**.[16,18] *Reagents and conditions*: a) $ArB(OH)_2$, 10% Pd/C, Na_2CO_3, DME/H_2O (1:1), 45 °C, 1–4 h, 74–95%; b) *p*-TsOH, MeOH/THF (1:1), 60 °C, 1–2 h, 76–88%.

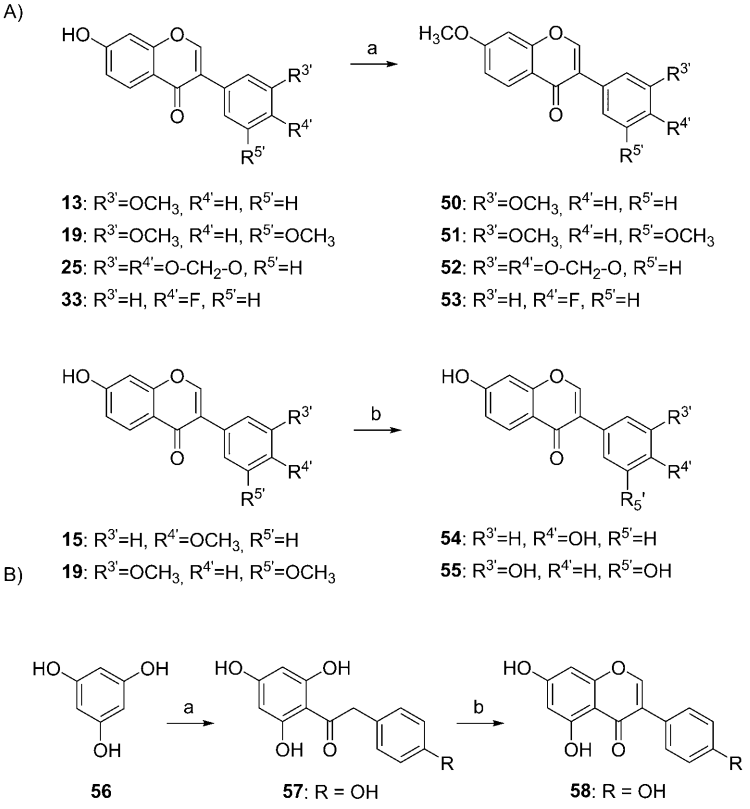
ether cleavage provided the corresponding isoflavones **11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47** and **49** (Scheme 2B).[16]

O-Methylation of 7-hydroxy-benzopyran-4-one derivatives **13**, **19**, **25** and **33** with methyl iodide provided compounds **50**–**53** (Scheme 3A). While, deprotection of the methyl ether groups in compounds **15** and **19** in the presence of boron tribromide afforded compounds **54**–**55** (Scheme 3A).[16] Genistein (**58**) was obtained by Friedel–Crafts acylation of phloroglucinol with the 4-hydroxyphenylacetic acid under microwave irradiation, followed by cyclization of the resultant hydroxyketone **57** using DMF/methanesulfonyl chloride as a carbon atom donor in the presence of boron trifluoride diethyl etherate (Scheme 3B).

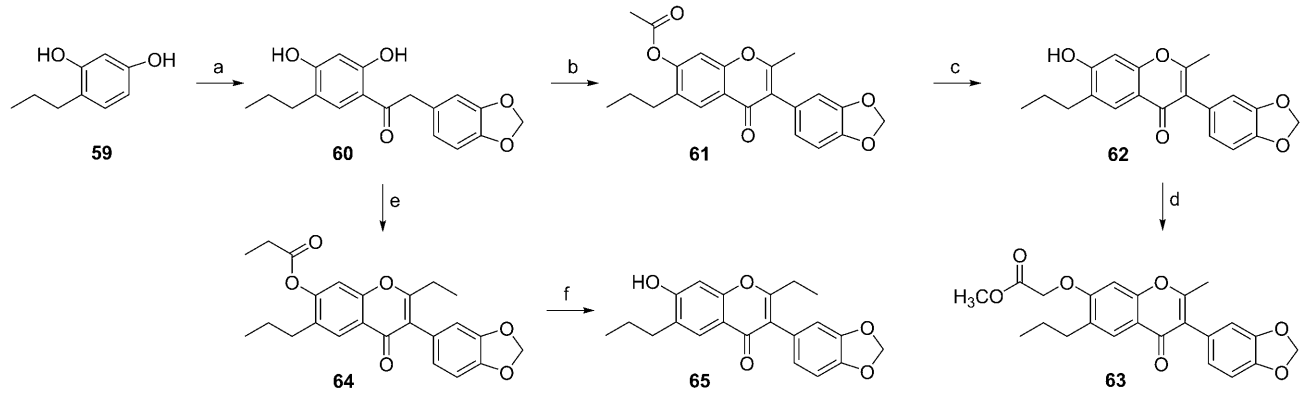
The hydroxyketone **60** obtained by Friedel–Crafts acylation reaction was used to generate compounds **62**–**63** and **65** (Scheme 4). The hydroxyketone **60** was converted to the corresponding isoflavones **61** and **64** via cyclization in the presence of appropriate anhydrides, followed by base hydrolysis, which afforded compounds **62** and **65**.[19] Subsequent alkylation of **62** with methyl bromoacetate in the presence of potassium carbonate in DMF provided **63**.

Modulation of GABA activity on $GABA_A$ receptors by benzopyran-4-one derivatives (isoflavones) was investigated on human recombinant $\alpha_1\beta_2\gamma_{2L}$ $GABA_A$ receptors expressed in *Xenopus* oocytes using the two-electrode voltage clamp method. As shown in Table 1, we examined the effects of varying substituents on the benzopyran-4-one skeleton by determining the potentiation of the GABA EC_5 responses elicited by the co-application of benzopyran-4-one derivatives (30 or 100 μM). GABA EC_5 is the concentration of GABA that elicited a peak current with an amplitude of 5% of the maximal current observed with 1 mM GABA; it was determined for each oocyte and was ~2 to 7 μM. As a result of this initial screening, we identified a number of substituted compounds that positively modulated GABA-induced ion currents with substantially higher potency than unsubstituted isoflavone **4**.

The lack of GABA enhancement by compound **9** and the magnitude of the activity enhancement for compounds **8, 62, 63** and **65** suggest that substitution at the A-, B- and C-rings plays a major role in determining the modulatory activity. The substitution pattern of the isoflavone B-ring was also found to be important for activity. Introduction of alkoxyl/organofluorine substituents at the 2′, 3′, 4′, or 5′ po-



Scheme 3. A) Synthesis of compounds **50**–**55**. *Reagents and conditions*: a) MeI, K_2CO_3, DMF, RT, 4 h, 84–90%; b) BBr_3, CH_2Cl_2, 0 °C→RT, 8 h, 57–65%. B) Synthesis of genistein **58**. *Reagents and conditions*: a) 4-hydroxyphenylacetic acid, $BF_3{\cdot}Et_2O$, ionic liquid ([bmim]-[BF_4]), μwave, 100 °C, 10 min, 64%; b) $BF_3{\cdot}Et_2O$, DMF, $MeSO_2Cl$, 100 °C, 3 h, 70%.



Scheme 4. *Reagents and conditions*: a) 3,4-(methylenedioxy)phenylacetic acid, $BF_3{\cdot}Et_2O$, ionic liquid ([bmim][BF_4]), μwave, 100 °C, 10 min, 66%; b) Ac_2O, Et_3N, 120–130 °C, 12 h, 68%; c) NaOH, reflux, 2 h, 76%; d) $BrCH_2COOCH_3$, K_2CO_3, DMF, 90 °C, 8 h, 85%; e) $(EtCO)_2O$, Et_3N, 120–130 °C, 15 h, 70%; f) NaOH, reflux, 2 h, 72%.

Table 1. Modulation of $\alpha_1\beta_2\gamma_{2L}$GABA$_A$ receptors by benzopyran-4-one derivatives (isoflavones).[a] Structures are given in Schemes 1–4 and full chemical names can be found in the Supporting Information.

Compd	Modulation of GABA EC_5[b] [%]			Compd	Modulation of GABA EC_5[b] [%]		
	30 μM[c]	100 μM[c]	Solubility[e]		30 μM[c]	100 μM[c]	Solubility[e]
4	21±8	59±12	Sol	**36**	NA	ND	PSol[g]
8	207±79	324±106	Sol (PSol)[f]	**37**	9±5	40±5	Sol
9	NA	NA	Sol	**39**	74±33	223±12	Sol
10	32±11	54±7	Sol[g]	**40**	NA	11±3	Sol[g]
11	44±15	94±23	Sol	**41**	18±10	29±7	Sol
12	47±8	71±12	Sol[g]	**42**	9±2	12±5	Sol[g]
13	97±47	166±74	Sol (Sol)[f]	**43**	22±4	48±9	Sol
15	19±13	40±26	Sol	**44**	31±10	ND	PSol
17	23±12	59±8	Sol[g]	**45**	247±54	328±66	Sol (PSol)[f]
19	125±29	242±31	Sol	**46**	NA	21±5	Sol[g]
20	45±18	98±14%	Sol[g]	**47**	15±7	25±2	Sol
21	−2±1	−10±4 (−69±13)[d]	Sol	**48**	8±5	ND	PSol
22	82±21	150±7	Sol[g]	**49**	24±11	ND	PSol
23	53±5	92±10	Sol	**50**	143±75	220±56	Sol (Sol)[f]
25	NA	12±2	Sol	**51**	26±14	102±15	Sol
27	NA	−14±5 (−26±8)[d]	Sol	**52**	6±4	19±5	Sol[g]
29	208±43	267±65	Sol (PSol)[f]	**53**	21±8	35±10	Sol[g]
30	NA	12±8	Sol[g]	**54**	−3±1	−9±2	Sol
31	189±62	252±84	Sol (PSol)[f]	**55**	−9±5	−33±7 (−61±12)[d]	Sol
32	NA	13±2	Sol[g]	**58**	−6±2	−14±3	Sol
33	7±4	19±5	Sol	**62**	352±67	574±113	Sol (PSol)[f]
34	22±17	58±10	Sol[g]	**63**	588±117	726±161	Sol (PSol)[f]
35	123±54	218±77	Sol (PSol)[f]	**65**	884±176	1057±193	Sol (PSol)[f]

[a] Determined electrophysiologically in *Xenopus laevis* oocytes expressing $\alpha_1\beta_2\gamma_{2L}$GABA$_A$ receptors as previously described.[7] Data are the mean ±SEM of modulation in 3–5 different oocytes. [b] Percent modulation of the chloride ion flux triggered by GABA EC_5 dose (5 μM) and test compound. [c] Concentration of test compound. NA: no activity was observed at the tested concentration. ND: not determined as compound was only partially soluble at 100 μM. [d] Values in parentheses refer to percent modulation of the chloride ion flux triggered by GABA EC_{50} dose (30 μM) and compound (300 μM). [e] Compound solubility at 100 μM in extracellular buffer (ND96) with 0.6% DMSO, pH 7.4 at room temperature (25 °C) and is classified as soluble (Sol) or partially soluble (PSol). [f] Description in parentheses refer to compound solubility at 300 μM in extracellular buffer (ND96) with 0.6% DMSO, pH 7.4 at room temperature (25 °C). [g] This compound was only partially or not soluble in 100 mM pure DMSO (stock solution) but was soluble in extracellular buffer (ND96) with 0.6% DMSO, pH 7.4 at room temperature (25 °C).

sitions of the isoflavone B-ring showed that the presence of a hydrogen-bond acceptor on the 2′ or 3′ positions results in compounds with significantly higher modulation, with substituent position and hydrogen-bond acceptor capability determining the extent of activity. 3′-Methoxy-substituted isoflavone **13** exhibited higher positive modulation (97% enhancement) compared with the 2′- (**11**) and 4′-substituted isoflavones (**15**) (44% and 19% enhancement, respectively) at 30 μM. Without exception, the other 3′-substituted isoflavones were also found to be the more active when compared with the corresponding 2′- and 4′-substituted isoflavones. The 3′-trifluoromethoxyl-substituted isoflavone **45** (EC_{50} = 14.7 μM; Table 2 and Figure 2B) displayed an even more efficacious positive modulatory effect than 3′-methoxy-7-hydroxyisoflavone **13**

Table 2. Potency and efficacy of isoflavones as positive modulators of recombinant $\alpha_1\beta_2\gamma_{2L}$GABA$_A$ receptors expressed in *Xenopus* oocytes.[a]

Compd	EC_{50} [μM] (95% CI)	E_{Max} [%]	n_H	C log *P*[b]	TPSA [Å^2][c]	Predicted log BB[d]
8	27.3±3.6 (17.38–43.01)	349±58	1.58±0.3	3.03	44.76	−0.06
13	44.7±5.4 (39.30–55.61)	197±31	1.07±0.1	2.64	55.76	−0.28
29	16.3±3.1 (12.17–22.06)	255±43	1.96±0.5	3.64	55.76	−0.13
31	19.5±4.5 (12.54–31.17)	262±36	1.35±0.3	2.85	46.53	−0.11
35	83.9±6.1 (68.74–123.12)	416±64	1.01±0.3	2.99	46.53	−0.09
45	14.7±4.3 (10.20–22.25)	300±55	1.60±0.4	3.75	55.76	−0.11
50	36.3±7.2 (29.73–53.11)	266±43	0.98±0.2	3.02	44.76	−0.06
62	15.4±2.5 (8.16–28.73)	554±71	1.21±0.5	4.38	64.99	−0.15
63	12.5±5.3 (7.70–20.16)	688±76	1.44±0.2	4.96	80.29	−0.29
65	9.3±2.8 (5.99–14.61)	1079±153	1.20±0.3	4.91	64.99	−0.07

[a] Data are the mean ±SEM of modulation in 3–5 different oocytes. [b] C log *P* calculated using ChemBioDraw Ultra 12.0 (CambridgeSoft). [c] Topological polar surface area (TPSA) calculated using ChemBioDraw Ultra 12.0 (CambridgeSoft). [d] Predicted log BB calculated using the formula: log BB = (−0.0148·TPSA) + (0.152·C log *P*) + 0.139; predicted log BB > 0.0 is predicative of a concentration in the brain greater than the concentration in the blood, while compounds with log BB > −0.3 are considered capable of crossing the BBB. The experimental protocol is detailed in the Supporting Information. Abbreviations: maximal current response or maximum effect (E_{Max}), Hill coefficient (n_H), blood—brain barrier (BBB).

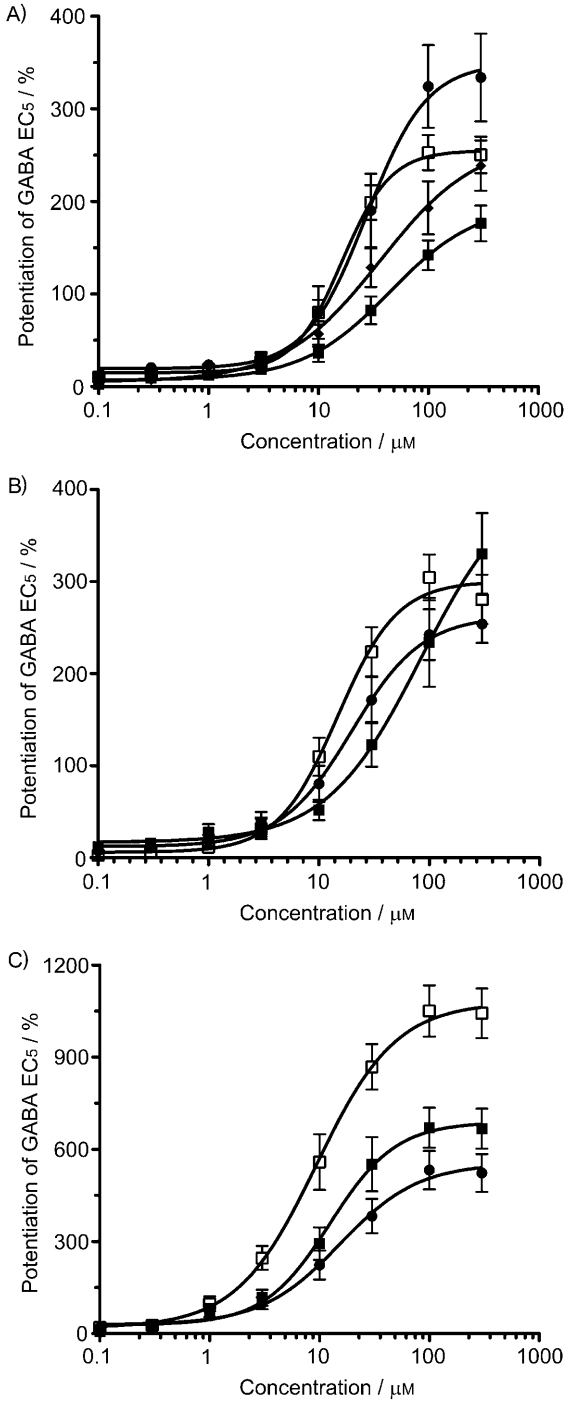


Figure 2. Concentration–response curves for the potentiation of GABA EC_5-induced ion currents by A) isoflavone **8** (●), **13** (■), **29** (□) and **50** (◆); B) isoflavone **31** (●), **35** (■) and **45** (□); C) isoflavone **62** (●), **63** (■) and **65** (□) at human $\alpha_1\beta_2\gamma_{2L}$ $GABA_A$ receptors expressed in *Xenopus* oocytes. Data are expressed as the mean ± SEM of modulation in 3–5 different oocytes.

(EC_{50} = 44.7 μM; Table 2 and Figure 2A) and 3′-fluoro-7-hydroxyisoflavone **31** (EC_{50} = 19.5 μM; Table 2 and Figure 2B), confirming that substituent position and hydrogen-bond acceptor capability determine activity.

Lower enhancement was observed for compounds **20, 36, 37**, and **51**, indicating that multiple substituents (three or more) on the B-ring result in increased steric bulk, which reduces GABA activity enhancement. With the exception of 3′,5′-dimethyl-4′-methoxy-substituted isoflavone **29**, which displayed a significant increase in activity (208% modulation at 30 μM, EC_{50} = 16.3 μM) compared with other poly-substituted isoflavones. In addition, replacement of the 3′-methoxy group (**13**) with a benzyloxy moiety (**49**) resulted in a fourfold decrease in GABA activity enhancement at 30 μM concentration. This indicates that there are no large lipophilic pockets in the binding site surrounding the isoflavone B-ring. Further modifications on the B-ring are underway to understand the complete pharmacophore of these compounds.

In contrast to the positive modulation associated with the B-ring methoxy substituent, hydroxy-substituted isoflavones (**54**, **55** and **58**) displayed negative modulation of GABA-induced ion currents. Dunne, Huang et al. also reported a similar inhibitory activity for compound **54** and **58**.[15] Interestingly, trimethoxyphenyl and 1,4-benzodioxane substitution at C-3 (**21** and **27**, respectively) also exhibited negative modulation of GABA-induced ion currents at lower (5 μM), as well as higher (30 μM) concentrations of GABA (Table 1).

To further explore the structure–activity relationships, we examined the role of substituents in each ring. Hydroxy (7-OH) or alkyl ether (7-OTHP or 7-OMe) substitution at C-7-on the A-ring is well tolerated, as most isoflavones with this substitution pattern demonstrated a positive modulatory effect at $GABA_A$ receptors. 7-Methoxy analogues **50**, **52** and **53** exhibited increased GABA enhancement activity compared with the corresponding 7-hydroxy analogues **13**, **25** and **33**. Interestingly, the 7-OTHP-substituted compound (**8**), in which the phenyl ring at the C-3 position is replaced with an iodo group, also displayed enhancement of GABA-induced ion currents with an EC_{50} value of 27.3 μM (Table 2 and Figure 2A). This result suggests that the presence of a hydrogen-bond acceptor at C-7 may elicit a more favorable interaction with the $GABA_A$ receptor binding site.

Additionally, in the case of compound **62** (352% modulation at 30 μM), introduction of a propyl group at C-6 (A-ring) and a methyl group at C-2 (C-ring) gave rise to a significant increase in activity compared with the corresponding parent compound **25** (inactive at 30 μM). More notably, replacement of the C-2 methyl group (**62**) with an ethyl group (**65**) resulted in even greater enhancement of the $GABA_A$ EC_5 response (884% modulation at 30 μM, EC_{50} = 9.3 μM). Interestingly, in the case of compound **63**, conversion of a hydroxy group to a methyl acetate in position C-7 resulted in a 1.5-fold increase in enhancement compared with compound **62**. Compounds **62**, **63** and **65** (EC_{50} = 15.4, 12.5 and 9.3 μM, respectively; Table 2 and Figure 2C) are the most efficacious positive modulators at $\alpha_1\beta_2\gamma_{2L}$ $GABA_A$ receptors. This implies that there are large lipophilic pockets in the binding site surrounding the isoflavone A- and C-rings.

Most notably, the modulatory action of isoflavones (**8, 13, 29, 31, 35, 45, 50, 62, 63** and **65**) on GABA-induced ion currents were unaffected by the benzodiazepine antagonist flu-

mazenil (10 μM) at $\alpha_1\beta_2\gamma_{2L}$ $GABA_A$ receptors (see Supporting Information for details). This suggests that these compounds do not bind to the classical high-affinity benzodiazepine binding site, which is sensitive to flumazenil and located at the α–γ subunits interface.[20] Figure 3 shows the potentiation of the GABA EC_5 (5 μM) by isoflavone **65** (30 μM) at $\alpha_1\beta_2\gamma_{2L}$ $GABA_A$ receptors; this response is insensitive to flumazenil. Furthermore, the physicochemical properties of isoflavones are clearly in

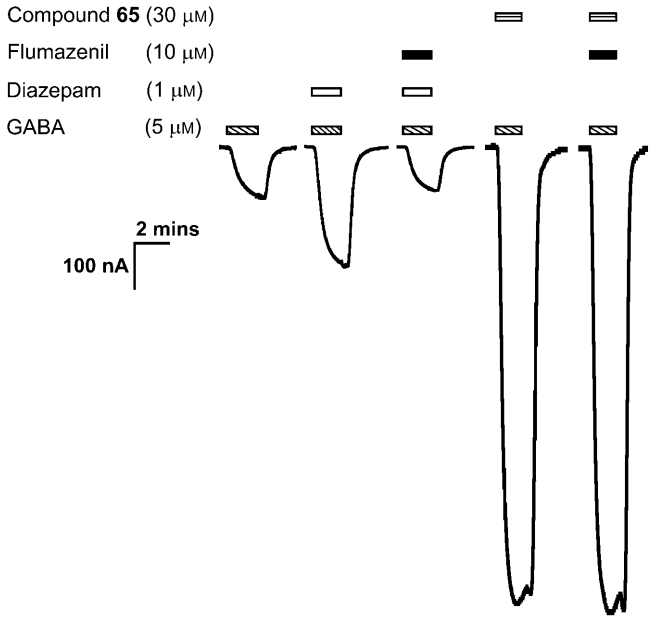


Figure 3. Sample current trace (nA vs min) from individual oocytes showing potentiation of the GABA EC_5 (5 μM) response (▨) by 30 μM isoflavone **65** (▭) at human recombinant $\alpha_1\beta_2\gamma_{2L}$ $GABA_A$ receptors which is not inhibited by 10 μM benzodiazepine antagonist flumazenil (■) while potentiation of GABA EC_5 response (▨) by 1 μM diazepam (▭) is inhibited by 10 μM flumazenil (■).

alignment with the "rules of thumb" for blood–brain barrier (BBB) permeation, which have emerged from studies of brain permeation data (Table 2).[21] These physicochemical properties are comparable to those of neuroactive drugs for the treatment of CNS-related disorders, emphasizing drug-like properties of isoflavones (see Supporting Information for details).

In conclusion, a series of isoflavone derivatives were synthesized and their modulatory effect evaluated on the $\alpha_1\beta_2\gamma_{2L}$ $GABA_A$ receptors. This is the first demonstration of isoflavones as positive modulators of $GABA_A$ receptors. Based on these studies, it is clear that substitution of the A-, B- and C-rings plays an important role in determining $GABA_A$ modulation activity. Flumazenil-insensitive modulation by the isoflavones suggests that these compounds might not bind to the benzodiazepine binding site, and as such may not possess the unwanted side effects associated with classical benzodiazepine binding site active ligands. Systematic structure-driven design led to the discovery of compounds **29, 45, 62**, **63** and **65** as the most efficacious positive modulators at $\alpha_1\beta_2\gamma_{2L}$ $GABA_A$ receptors. Isoflavone **65** was the most potent (EC_{50} = 9.3 μM) and efficacious (E_{max} = 1079%) of these derivatives. Further modification of compound **29, 31, 45, 62**, **63** and **65** could lead to even more potent and perhaps subtype-specific modulators of $GABA_A$ receptors, which might be attractive drug candidates for CNS-related disorders, possessing fewer side effects than classical benzodiazepine-related drugs. Additional structure–activity studies are underway to understand the interactions of these compounds with $GABA_A$ receptors in order to characterize their subtype-specific modulation and pharmacological profile.

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