Neurosteroid Analogues. 14. Alternative Ring System Scaffolds: GABA Modulatory and Anesthetic Actions of Cyclopenta[*b*]phenanthrenes and Cyclopenta[*b*]anthracenes

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Although the structural features of binding sites for neuroactive steroids on γ-aminobutryic acid type A ($GABA_A$) receptors are still largely unknown, structure–activity studies have established a pharmacophore for potent enhancement of $GABA_A$ receptor function by neuroactive steroids. This pharmacophore emphasizes the importance of the position and stereochemistry of hydrogen-bonding groups on the steroid. However, the importance of the steroid ring system in mediating hydrophobic interactions with the $GABA_A$ receptor is unclear. We have taken the cyclopenta[*b*]phenanthrene (tetracyclic compounds with a nonlinear ring system different from that of steroids) and cyclopenta[*b*]anthracene (tetracyclic molecules with a linear 6–6–6–5 carbocyclic ring system) ring systems and properly substituted them to satisfy the pharmacophore requirements of the critical hydrogen-bond donor and acceptor groups found in neuroactive steroids. We have found these cyclopenta[*b*]phenanthrene and cyclopenta[*b*]anthracene analogues to have potent activity at the $GABA_A$ receptor, rivaling that of the most potent steroid modulators. Single-channel analysis of electrophysiological data indicates that similarly substituted analogues in the different ring systems affect the kinetic components of macroscopic currents in different ways. Mutations to the hydrogen bonding amino acids at the putative steroid binding site (α1Q241L mutation and α1N407A/Y410F double mutation) produce similar effects on macroscopic current amplitude by the different ring system analogues suggesting that the different kinetic effects are explained by the precise interactions of each analogue with the same binding site(s).

Introduction

Endogenous neurosteroids derived from cholesterol, such as allopregnanolone, are some of the most potent and efficacious allosteric modulators of the γ-aminobutyric acid type A ($GABA_A$)[a] receptor. The physiological effects of neurosteroids are extensive and include regulation of neuronal synaptic function and the sleep cycle.[1,2] At pharmacological doses, these compounds also have anxiolytic, anticonvulsant, and hypnotic properties.[3,4] Previous structure–activity relationship (SAR) studies of neuroactive steroids at $GABA_A$ receptors have established a basic pharmacophore for positive modulation of the $GABA_A$ receptor by neuroactive steroids: a hydrogen-bond-accepting group in a pseudoequatorial configuration at the 17β position and a hydrogen-bond-donating group in the axial 3α configuration (Figure 1).[5–10] Additional SAR studies have examined the effect of adding groups to the tetracyclic steroid system, providing information about sterically available and unavailable regions above and below the steroid framework.[6,11–19] However, there is little information available regarding the importance of the hydrophobic steroid framework for interaction with the $GABA_A$ receptor. Therefore, we have recently focused our attention on understanding its significance through the synthesis and evaluation of neuroactive steroid analogues that have rearranged ring systems yet maintain the critical three-dimensional orientation and distance constraints for the hydrogen-bonding groups.

In a previous study, we synthesized and evaluated a series of tricyclic neuroactive steroid analogues, the benz[*f*]indenes (Figure 1) and found the planar *trans,trans*-benz[*f*]indene analogues to be less active than the steroids they were designed to mimic.[20] We suggested three possible reasons for the reduced activity of these analogues: (1) the flexible hydroxyethyl side chain on benz[*f*]indene C-6 was assuming conformations that were not conducive to interacting with the receptor; (2) the receptor could not accommodate the C-7 and C-8 carbons of the benz[*f*]indenes; or (3) as previous studies had suggested, the C-6/C-7 edge of the steroid B ring provides a critical interaction with the receptor or the lipid surrounding the receptor and the benz[*f*]indenes lack what would be steroid carbon atoms C-4−C-7, thus eliminating this required hydrophobic interaction with the receptor.[17,21–23]

To understand better the behavior of the benz[*f*]indenes, we have synthesized a series of cyclopenta[*b*]phenanthrene analogues (Figure 1 and Chart 1, compounds **1** and **2**). Similar to the benz[*f*]indenes, these analogues have the same linear 6–6–5 carbocyclic framework. However, instead of the flexible hydroxyethyl side chain of the benz[*f*]indenes, the cyclopenta-[*b*]phenanthrenes have a fourth staggered ring that effectively

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[a] Abbreviations: $GABA_A$, γ-aminobutyric acid type A ($GABA_A$); SAR, structure–activity relationship; [^{35}S]-TBPS, [^{35}S]-*tert*-butylbicyclophosphorothionate; LRR, loss of righting reflex; LSR, loss of swimming reflex.

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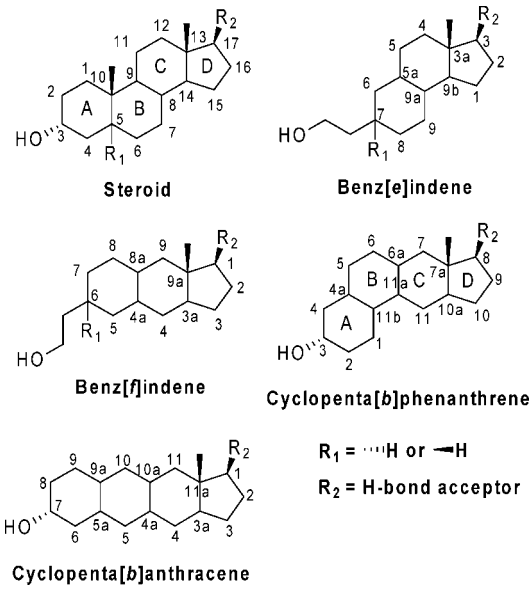
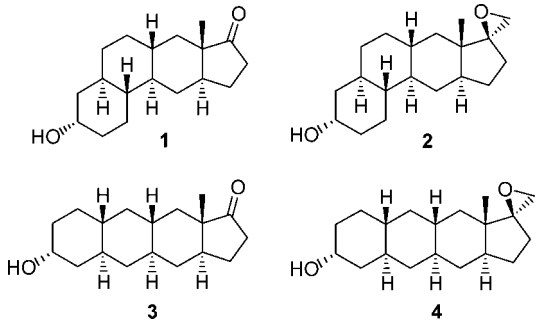


Figure 1. Structure and numbering of the steroid, benz[*e*]indene, benz[*f*]indene, cyclopenta[*b*]phenanthrene, and cyclopenta[*b*]anthracene ring systems.

Chart 1



adds back what would be the steroid A ring and a portion of the steroid B ring (Figure 2A).

In the process of developing chemistry to synthesize the cyclopenta[*b*]phenanthrenes, we also developed chemistry to synthesize the tetracyclic linear cyclopenta[*b*]anthracene analogues (Figure 1 and Chart 1, compounds **3** and **4**).[24] Even though the hydrophobic framework of these analogues is different from that of steroids, when properly aligned, the geometrical relationships between the hydrogen-bonding groups within each molecule can be very similar (Figure 2B). Thus, these cyclopenta[*b*]anthracenes will help further our SAR studies of the steroid hydrophobic framework.

Here, we describe the preparation of the optically pure cyclopenta[*b*]phenanthrene and cyclopenta[*b*]anthracene neuroactive steroid analogues from the previously described enantiomerically pure 3*H*-cyclopenta[*b*]phenanthren-3-one **5** and 7*H*-cyclopent[*b*]anthracen-7-one **8**, respectively.[24] The activities of the cyclopenta[*b*]phenanthrenes and cyclopenta[*b*]anthracenes at $GABA_A$ receptors were evaluated electrophysiologically using rat $\alpha_1\beta_2\gamma_{2L}$ $GABA_A$ receptors expressed in *Xenopus laevis* oocytes and HEK 293 cells. The ability of these compounds to bind to $GABA_A$ receptors was measured by the noncompetitive displacement of [^{35}S]-*tert*-butylbicyclophosphorothionate ([^{35}S]-TBPS) from the picrotoxin binding site on $GABA_A$ receptors in rat brain membranes. Finally, the anesthetic effects of the compounds were determined by measuring the *X. laevis* tadpole loss of righting reflex (LRR) and loss of swimming reflex (LSR).

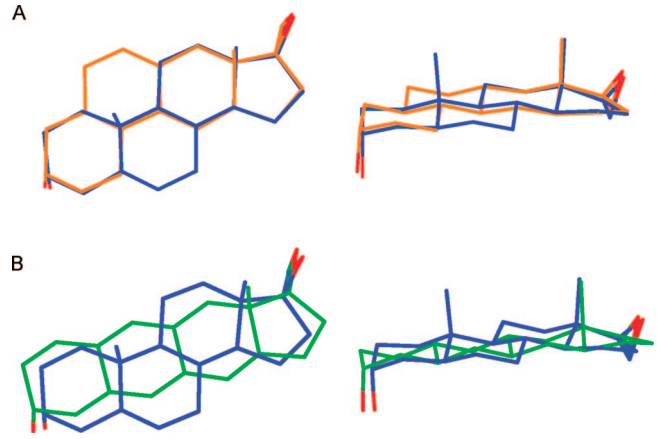


Figure 2. Three-dimensional comparisons of a cyclopenta[*b*]phenanthrene and cyclopenta[*b*]anthracene with a steroid. (A) An overlay (top view, left; edge view, right) of cyclopenta[*b*]phenanthrene **2** (orange) and 5α-reduced steroid **16a** (blue). When compared in this manner, the B-ring of the cyclopenta[*b*]phenanthrene occupies space that would be occupied by a two-carbon bridge between steroid carbons 1 and 11. Additionally, interactions that the bottom portion of the steroid B-ring (carbons C-6 and C-7) would have with the receptor are not present in the cyclopenta[*b*]phenanthrene. (B) An overlay (top view, left; edge view, right) of cyclopenta[*b*]anthracene **4** (green) and 5α-reduced steroid **16a** (blue). As aligned, the distance and orientation between the hydrogen bond donating (OH) and accepting (spiro-epoxide) groups can be maintained. As aligned the hydrophobic space occupied by the tetracyclic cyclopenta[*b*]anthracene rings differs slightly from that of the rings of the 5α-reduced steroid. The ring systems are aligned so that the rings in each molecule are in the same plane.

We have found that analogues having the cyclopenta[*b*]-phenanthrene or cyclopenta[*b*]anthracene system are positive $GABA_A$ receptor modulators that have potent pharmacological activity in the electrophysiological, binding, and tadpole anesthesia assays. In comparative studies with steroids, benz[*e*]indenes, and benz[*f*]indenes, the rank order for positive modulation of $GABA_A$ receptors was steroids = benz[*e*]indenes = cyclopenta[*b*]phenanthrenes = cyclopenta[*b*]anthracenes > benz[*f*]indenes. Based on our studies, we have found alternatives to the steroid ring system that give neuroactive steroid analogues with potent activity and have gained further insight into the portions of the steroid ring system that provide necessary hydrophobic contacts with the receptor.

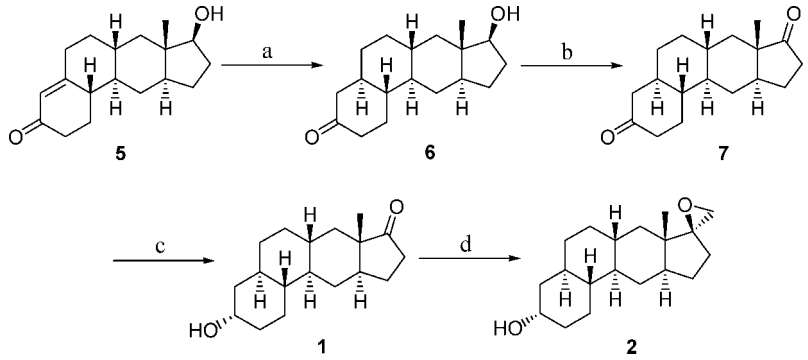
Chemistry

Cyclopenta[*b*]phenanthrene Analogues. Synthesis of the cyclopenta[*b*]phenanthrene analogues began by stereoselectively reducing the previously reported cyclopenta[*b*]phenanthrenone **5**[24] to give the *trans* ring fusion product **6** (65%, Scheme 1). Compound **6** was then oxidized to yield diketone **7** (85%). The C-3 ketone was then selectively reduced with K-Selectride to provide the hydroxy ketone **1**. Compound **1** was then converted to the spiro-epoxide **2** by treatment with $Me_3S^+I^-$ and $KOBu^t$ according to a literature procedure.[25]

Cyclopenta[*b*]anthracene Analogues. Starting from the previously reported cyclopenta[*b*]anthracenone **8**,[24] the cyclopenta[*b*]anthracene analogues were synthesized in a manner identical to that of the cyclopenta[*b*]phenanthrene analogues described above (Scheme 2).

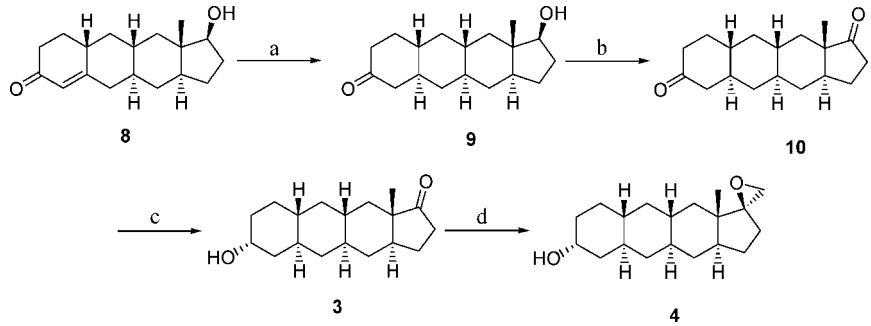
Benz[*f*]indene and Benz[*e*]indene Analogues. Benz[*f*]indene and benz[*e*]indene analogues having a spiro-epoxide on the 5-membered ring were synthesized to compare their activity to that of the corresponding cyclopenta[*b*]phenanthrene and cyclopenta[*b*]anthracene analogues. Starting with either the ben-

Scheme 1[a]



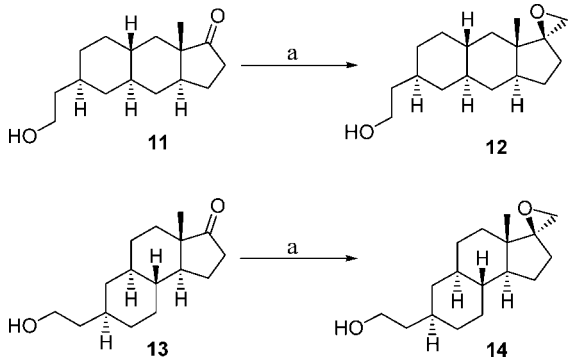
[a] Key: (a) Li/NH_3(liq), THF; (b) PCC, NaOAc, CH_2Cl_2; (c) (1) K-Selectride, THF; (2) NaOH, H_2O_2; (d) TMS^+I^-, $KOBu^t$, DMF.

Scheme 2[a]



[a] Key: (a) Li/NH_3(liq), THF; (b) PCC, NaOAc, CH_2Cl_2; (c) (1) K-Selectride, THF; (2) NaOH, H_2O_2; (d) TMS^+I^-, $KOBu^t$, DMF.

Scheme 3[a]



[a] (a) TMS^+I^-, $KOBu^t$, DMF.

z[*f*]indene hydroxy ketone **11**[20] or the benz[*e*]indene hydroxy ketone **13**,[26] the spiro-epoxide was introduced in one step to yield the benz[*f*]indene spiro-epoxide **12** (60%) or the benz[*e*]indene spiro-epoxide **14** (58%, Scheme 3).[25]

[^{35}S]-TBPS Displacement. TBPS binds to the picrotoxin site on the $GABA_A$ receptor complex. Neuroactive steroids and other $GABA_A$ receptor potentiators are known to displace allosterically TBPS, thus giving an indirect measure of steroid binding to the receptor.[27–29] The results of the binding experiments are summarized in Table 1.

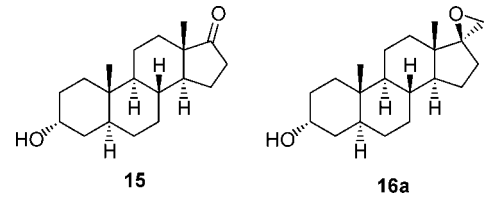
The cyclopenta[*b*]phenanthrene ketone **1** was approximately 3-fold less potent than the analogous 5α-reduced ketosteroid **15** (Chart 2). In contrast, the cyclopenta[*b*]anthracene ketone **3** was equally as potent as ketosteroid **15**. The cyclopenta[*b*]-phenanthrene and cyclopenta[*b*]anthracene spiro-epoxides **2** and **4**, respectively, were potent displacers of [^{35}S]-TBPS, both slightly better than the 5α-reduced spiro-epoxide steroid **16a** (Chart 2). The benz[*e*]indene and benz[*f*]indene spiro-epoxides **14** and **12**, respectively, had equal potency to each other, but a

Table 1. Displacement of [^{35}S]-TBPS by Cyclopenta[*b*]phenanthrenes, Cyclopenta[*b*]anthracenes, Benz[*f*]indenes, Benz[*e*]indenes, and Steroids

compd	IC_{50} (μM)[a]	n_{Hill}
	cyclopenta[*b*]phenanthrenes	
1: [oxo][b]	1.44 ± 0.27	1.02 ± 0.16
2: [epox]	0.04 ± 0	1.15 ± 0.15
	cyclopenta[*b*]anthracenes	
3: [oxo]	0.25 ± 0.07	0.80 ± 0.15
4: [epox]	0.06 ± 0.01	0.93 ± 0.14
	benz[*f*]indenes	
11:[c] [oxo]	5.01 ± 0.8	1.18 ± 0.14
12: [epox]	1.17 ± 0.3	1.48 ± 0.49
	benz[*e*]indenes	
13:[c] [oxo]	4.17 ± 1.07	1.38 ± 0.39
14: [epox]	1.58 ± 0.14	3.17 ± 0.51
	steroids (5α-reduced)	
15:[c] [oxo]	0.41 ± 0.13	0.89 ± 0.2
16a:[d] [epox]	0.11 ± 0.01	1.32 ± 0.16

[a] Results presented are from duplicate experiments performed in triplicate. Error limits are calculated as standard error of the mean. [b] Oxo (or epox) indicates the substituent on the 5-membered ring. [c] Values obtained from ref 20. [d] Values obtained from ref 41.

Chart 2



12-fold reduction in potency relative to the 5α-reduced spiro-epoxide steroid **16a**. The low binding potency of benz[*e*]indene spiro-epoxide **14** was surprising since this compound had potent actions in the other two biological assays (vide infra) and the reason for its low potency as a displacer of TBPS is not apparent.

Table 2. Modulation of Rat $\alpha_1\beta_2\gamma_{2L}$ $GABA_A$ Receptor Function by Cyclopenta[*b*]phenanthrenes, Cyclopenta[*b*]anthracenes, Benz[*f*]indenes, Benz[*e*]indenes, and Steroids

	oocyte electrophysiology[a]			
compd	0.1 μM	1 μM	10 μM	(gating) 10 μM
cyclopenta[*b*]phenanthrenes				
1; [oxo][b]	0.92 ± 0.05	1.32 ± 0.06	6.14 ± 0.34	0.05 ± 0.02
2; [epox]	2.16 ± 0.09	15.7 ± 0.6	29.7 ± 1.3	0.15 ± 0.02
cyclopenta[*b*]anthracenes				
3; [oxo]	1.10 ± 0.26	5.03 ± 1.51	28.4 ± 7.7	0.28 ± 0.09
4; [epox]	1.53 ± 0.13	10.8 ± 1.4	26.3 ± 3.5	0.16 ± 0.05
benz[*f*]indenes				
11;[c] [oxo]	1.00 ± 0.04	1.00 ± 0.06	2.42 ± 0.11	−0.02 ± 0.02
12; [epox]	0.86 ± 0.04	1.54 ± 0.06	5.37 ± 0.22	0.03 ± 0.01
benz[*e*]indenes				
13;[c] [oxo]	0.99 ± 0.11	1.03 ± 0.17	1.05 ± 0.11	0.03 ± 0.07
14; [epox]	1.10 ± 0.08	4.11 ± 0.52	20.0 ± 2	0.04 ± 0.06
steroids (5α-reduced)				
15;[c] [oxo]	0.97 ± 0.02	1.41 ± 0.01	5.44 ± 0.19	0.02 ± 0.01
16a;[d] [epox]	3.11 ± 0.17	21.9 ± 1.3	33.7 ± 2	0.22 ± 0.02

[a] The GABA concentration used for the control response was 2 μM. Each compound was evaluated on at least four different oocytes at the concentrations indicated, and the results reported are the ratio of the currents measured in the presence/absence of added compound. Gating represents direct current gated by 10 μM compound in the absence of GABA, and this current is reported as the ratio of compound only current/2 μM GABA current. Error values are calculated as standard error of the mean ($N \geq 4$). [b] Oxo (or epox) indicates the substituent on the 5-membered ring. [c] Values obtained from ref 20. [d] Values obtained from ref 41.

For the more potent spiro-epoxide series of analogues the general order of binding for the different ring systems was cyclopenta[*b*]phenanthrene = cyclopenta[*b*]anthracene ≥ steroid > benz[*e*]indene = benz[*f*]indene.

Electrophysiology. Initial screening of the functional actions of the compounds at $GABA_A$ receptors was done electrophysiologically using *X. laevis* oocytes expressing rat $\alpha_1\beta_2\gamma_{2L}GABA_A$ receptors (Table 2). The compounds were evaluated at three different concentrations for their ability to enhance currents mediated by 2 μM GABA. Direct gating effects of 10 μM compound in the absence of GABA were also assessed. This method is useful for determining whether compounds are active or inactive, but quantitative comparisons are not meaningful because screening is done on different oocyte preparations (modulator responsiveness varies within and between preparations).

Nevertheless, the results of the screening show the cyclopenta[*b*]phenanthrene and cyclopenta[*b*]anthracene spiro-epoxides **2** and **4**, respectively, to have potentiating and gating actions similar to those of the 5α-reduced spiro-epoxide steroid **16a** (Chart 2). Additionally, at the highest concentration tested (10 μM), it appears that compounds **2** and **4** have similar efficacies. Analogues with a ketone on the 5-membered ring typically have weak activity relative to those analogues having a carbonitrile, acetyl, or spiro-epoxide on the ring. However, the cyclopenta[*b*]anthracene **3** had remarkably high potentiating and direct gating actions at 10 μM.

To determine a rank order of potency, the spiro-epoxide steroids, benz[*e*]indenes, benz[*f*]indenes, cyclopenta[*b*]phenanthrenes, and cyclopenta[*b*]anthracenes were applied at 0.25 μM to the same oocyte, eliminating any differences in modulator responsiveness. The rank order for enhancing GABA-mediated currents at $GABA_A$ receptors was steroids = benz[*e*]indenes = cyclopenta[*b*]phenanthrenes = cyclopenta[*b*]anthracenes > benz[*f*]indenes (Figure 3). This order was slightly different than that for TBPS binding, as relative to the other compounds, the benz[*e*]indene epoxide exhibited higher activity in the functional assay than in the [^{35}S]-TBPS binding assay.

To explore the mechanism of $GABA_A$ receptor potentiation, we tested the effects of mutations to the putative steroid binding site[30] on channel potentiation by steroid **16a**, cyclopenta[*b*]-phenanthrene **2**, and cyclopenta[*b*]anthracene **4**. Wild-type receptors, or receptors containing the α1Q241L mutation or the α1N407A/Y410F double mutation, were transiently expressed in HEK 293 cells and exposed to GABA and 0.1–10 μM **16a**, **2**, or **4**. The GABA concentration in these experiments was held at a concentration corresponding to EC_{25} in the macroscopic dose–response curve (data not shown). The resulting steroid analogue concentration−effect curves are shown in Figure 4. The data indicate that compounds **16a**, **2**, and **4** are roughly equipotent on the wild-type receptor. Potentiation is effectively abolished by the α1Q241L mutation but is minimally affected, at best, by the α1N407A/Y410F double mutation.

In order to examine the kinetic mechanisms of modulation, we conducted single-channel experiments on wild-type receptors exposed to 50 μM GABA and 3 μM compound **16a**, **2**, or **4**. This GABA concentration corresponds to an approximately EC_{40} in the open probability dose–response curve,[31] while the steroid analogue concentrations were based on the macroscopic concentration−effect curves (Figure 4A) and selected to result in maximal potentiation.

Previous studies have shown that exposure to many potentiating neurosteroids results in specific changes in the intracluster open and closed time properties. In particular, the mean duration and relative frequency of the longest lived open time component are increased, and the prevalence of the activation-related closed time component is decreased.[32,33] As a result, the channel mean open duration is increased, and the mean intracluster closed duration is decreased upon exposure to these steroids, enhancing the channel open probability and, consequently, peak macroscopic current. Previous studies have also found steroid analogues with only a subset of these kinetic effects, suggesting that either different ligand–receptor interactions with the same steroid binding site can modify the type of effect observed in single-channel electrophysiological recordings or, in the case that the kinetic effects are mediated by steroid interactions with separate sites, that some analogues are unable to interact with all three sites.[34,35] The experiments described cannot distinguish between these possibilities.

The major result from the evaluation of the effects of compounds **16a**, **2**, and **4** is that the compounds have specific, nonidentical effects on single-channel currents. Exposure to compound **16a** drastically increases the relative frequency of long openings but is essentially without effect on the duration of long openings and the prevalence of the activation-related closed time component. In contrast, compound **2** affects both the duration and prevalence of long openings but is without effect on the closed times. Finally, compound **4** enhances the relative occurrence of long openings and reduces the prevalence of the activation-related closed times, but is without effect on the duration of long openings. Sample single-channel currents are shown in Figure 5, and the findings are summarized in Table 3.

Tadpole Behavior. With the exception of benz[*e*]indene **13**, ring systems having a ketone on the 5-membered ring induced loss of righting reflex (LRR) with similar potency, within experimental resolution (Table 4). However, cyclopenta[*b*]anthracene **3** was the only compound with a ketone that caused loss of swimming reflex (LSR). The cyclopenta[*b*]phenanthrene and cyclopenta[*b*]anthracene spiro-epoxides **2** and **4**, respectively, were very potent inducers of LRR and LSR. Spiro-epoxide **2** was almost 7-fold better at inducing LRR and 3-fold better at causing LSR than the 5α-reduced steroid spiro-epoxide

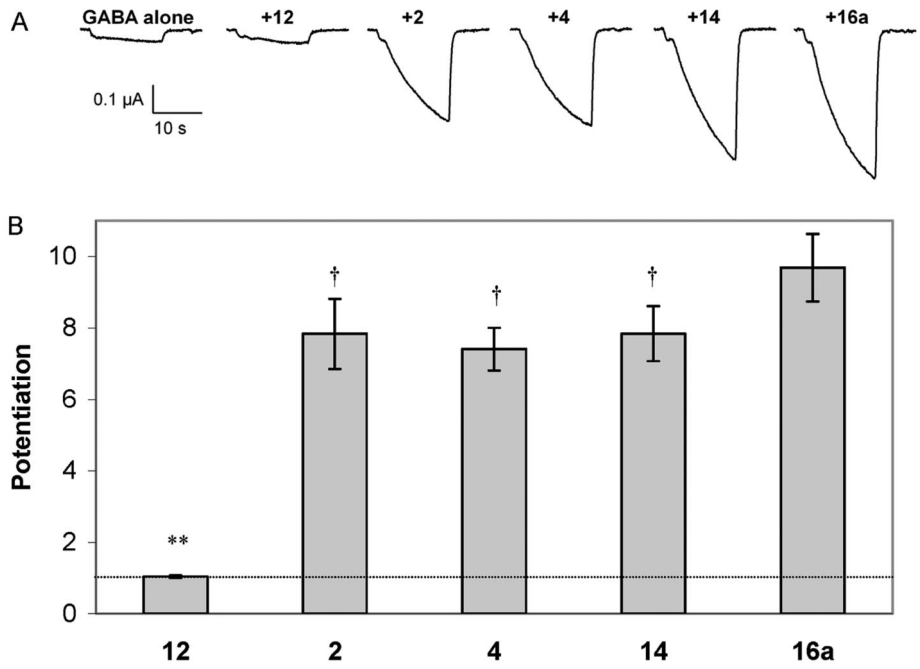


Figure 3. Direct comparison of the ability of spiro-epoxides to modulate $GABA_A$ receptor-mediated chloride currents at 0.25 μM: benz[*f*]indene **12**, cyclopenta[*b*]phenanthrene **2**, cyclopenta[*b*]anthracene **4**, benz[*e*]indene **14**, and steroid **16a**. The GABA concentration used for the control response was 2 μM. The compounds were evaluated on the same oocytes expressing recombinant rat $\alpha_1\beta_2\gamma_{2L}$ receptors. (A) Sample currents from an oocyte clamped to –70 mV and exposed transiently to GABA alone and then GABA plus each of the test compounds. (B) Summary of effects of test compound on GABA responses. The current mediated by GABA alone is set to one as the control value and indicated by the dotted line. Potentiation is calculated as R2/R1, where R2 is the response in the presence of modulators and R1 is the response to GABA alone. Error limits are calculated as standard error of the mean for $n \geq 4$. The significance level applies to comparison to steroid **16a**. $^{**}P < 0.001$. †Not significant.

16a it was designed to mimic. Spiro-epoxide **4** was almost 3-fold better at inducing LRR and approximately 1.5 times better at causing LSR than steroid **16a**.

Discussion

The results of the [^{35}S]-TBPS displacement, channel electrophysiology, and tadpole LRR and LSR experiments indicate that cyclopenta[*b*]phenanthrene and cyclopenta[*b*]anthracene neuroactive steroid analogues have potent activity at $GABA_A$ receptors, rivaling that of the corresponding steroid. Comparing the high activity of the cyclopenta[*b*]phenanthrene system to the low activity of the benz[*f*]indene ring system, it suggests that the space occupied by the C-7 and C-8 carbons of the benz[*f*]indenes (carbons C-5 and C-6 of the cyclopenta[*b*]-phenanthrenes) can be accommodated by the receptor. Additionally, the C-6/C-7 edge of the steroid B ring does not appear to provide a critical hydrophobic interaction with the receptor or the lipid surrounding the receptor, as the potent cyclopenta[*b*]phenanthrene system and the weak benz[*f*]indene system both lack this region.

However, one feature that the potent cyclopenta[*b*]phenanthrenes possess that the weakly modulating benz[*f*]indenes lack is the bottom portion of the steroid A ring, carbons C-4 and C-5 (cyclopenta[*b*]phenanthrene carbons C-1 and C-2). If these carbons are important for interaction with the $GABA_A$ receptor, it is not surprising that benz[*e*]indene analogues have such high activity, given that their flexible hydroxyethyl side chain is able to assume a conformation that closely mimics the bottom portion of the steroid A ring (Figure 1). Conversely, it is not possible for the hydroxyethyl side chain on carbon C-6 of the benz[*f*]indenes to occupy the same space as steroid A ring carbons C-4 and C-5, presumably resulting in lower activity. To the best of our knowledge, this is the first study demonstrating the importance of the steroid carbons C-4 and C-5 for interaction with the $GABA_A$ receptor.

In light of this information, the activity of the cyclopenta-[*b*]anthracene analogues is not unexpected, and the alignment of the cyclopenta[*b*]anthracene and steroid (Figure 2B) is logical. The cyclopenta[*b*]anthracene analogues maintain the critical three-dimensional orientation and distance of the hydrogen-bonding groups, could occupy the space normally filled by carbons C-4 and C-5 of the steroid, and do not have portions of the ring system in areas that have been previously shown to be unavailable sterically.

Based on data from several studies, there is a general consensus that the binding sites for neuroactive steroids are located within the membrane-spanning domains of the $GABA_A$ receptor.[30,33,36] However, there is ongoing debate as to the exact location and number of binding sites. Despite the differences in enantioselectivity that 5α- and 5β-reduced steroids exhibit for $GABA_A$ receptor modulation,[37,38] selective antagonism for the GABAergic actions of 5α-, but not 5β-reduced steroids,[39] and the extremely different three-dimensional shapes of 5α- and 5β-reduced steroids, proposed models for a common binding site for these steroids have been difficult to either prove or disprove.[10,18] Thus, in agreement with the common binding site model, Hosie et al. showed that mutations to the putative steroid binding site diminished channel potentiation by both 5α- and 5β-reduced steroids.[30] Moreover, a recent study demonstrated that mutations to the steroid binding site affected potentiation by both etiocholanolone and its enantiomer, although modulation by the natural steroid was affected more strongly.[35] By contrast, single-channel electrophysiology supports the existence of three distinct sites for neuroactive steroid interaction with the receptor,[34] and similarly, inhibition curves of TBPS binding in the

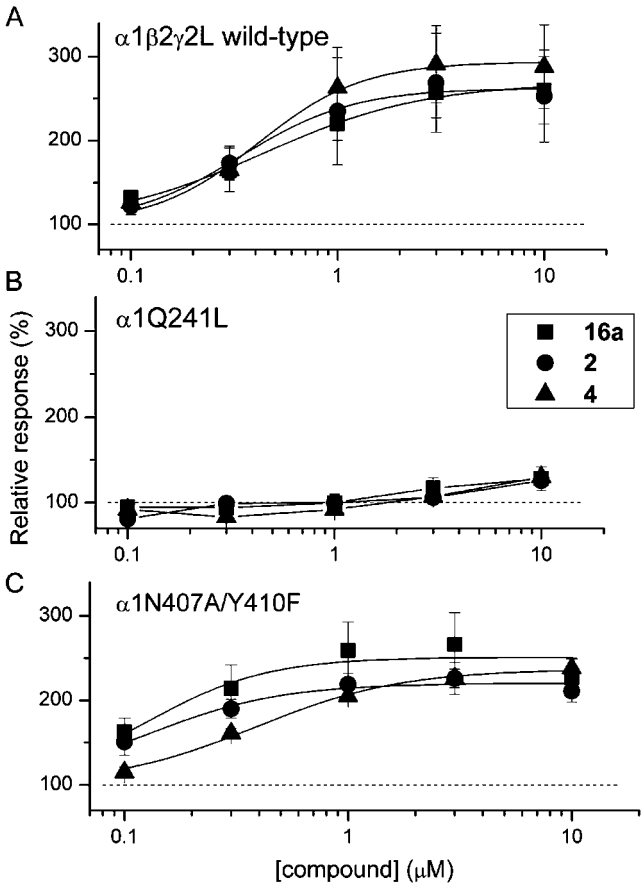


Figure 4. α1Q241L mutation but not the α 1N407A/Y410F double mutation significantly reduces the ability of steroid **16a** (squares), cyclopenta[*b*]phenanthrene **2** (circles), and cyclopenta[*b*]anthracene **4** (triangles) to potentiate the $\alpha1\beta2\gamma2$L $GABA_A$ receptors. Steroid analogue concentration–response curves for the $\alpha1\beta2\gamma2$L wild-type receptor (A), the α1Q241L mutant receptor (B), and the α1N407A/Y410F double mutant receptor (C). The receptors were exposed to a GABA concentration corresponding to an EC_{25} in the presence of 0.1–10 μM compound. The GABA concentrations were 5 μM (wild-type), 20 μM (α1Q241L), or 10 μM (α1N407A/Y410F). The data points show mean ± SEM from 3 to 8 cells. In A and C, the curves were fitted to response = offset + (maximal response − offset) $[\text{steroid}]^n/([\text{steroid}]^n + EC_{50}{}^n)$. The best-fit parameters for the wild-type receptor were as follows. For steroid **16a**, maximal response = 269 ± 8%, EC_{50} = 0.44 ± 0.07 μM, n = 1.1 ± 0.2. For cyclopenta[*b*]phenanthrene **2**, maximal response = 262 ± 9%, EC_{50} = 0.34 ± 0.06 μM, n = 1.6 ± 0.4. For cyclopenta[*b*]anthracene **4**, maximal response = 294 ± 9%, EC_{50} = 0.41 ± 0.05 μM, n = 1.7 ± 0.3. The best-fit parameters for the α1N407A/Y410F double-mutant receptor were as follows. For steroid **16a**, maximal response = 251 ± 16%, EC_{50} = 0.13 ± 0.05 μM, n = 1.6 ± 1.1. For cyclopenta[*b*]phenanthrene **2**, maximal response = 220 ± 7%, EC_{50} = 0.13 ± 0.03 μM, n = 1.4 ± 0.5. For cyclopenta[*b*]anthracene **4**, maximal response = 237 ± 5%, EC_{50} = 0.39 ± 0.05 μM, n = 1.3 ± 0.2. The offset was constrained at 100%. No fitting was attempted for the data from the α1Q241L mutant receptor.

presence of steroids suggest the presence of at least two interaction sites for some steroids.[7]

Our data are in agreement with the notion that interactions with the classic neurosteroid binding site(s) mediate the actions of compounds **16a**, **2**, and **4**. Potentiation by the compounds was abolished by the α1Q241L mutation. Mutations to this site strongly affect potentiation by neuroactive steroids[30,35] as well as the three-ring benz[*e*]indene steroid analogues[34] but not potentiation by barbiturates or benzodiazepines.[30] Interestingly, we found that the α1N407A/Y410F double mutation had minimal effects, at best, on channel potentiation by compounds **16a**, **2**, and **4**. Residues α1N407 and α1Y410 have been proposed to act as the hydrogen-bond donor groups interacting with the hydrogen-bond acceptor group at position C-17 on the D ring of the steroid molecule, and these mutations drastically reduce potentiation by several steroids containing a 20-keto group.[30] Thus, the lack of effect of the α1N407A/Y410F double mutation on potentiation by compounds **16a**, **2**, and **4** is puzzling. However, it is not unprecedented; a previous study also showed that the effects of this double mutation are dependent on the structure of the steroid modulator.[34] In that study, an 18-norsteroid (compound B285) with a 17β-CN group retained activity in receptors having an α1N407A/Y410F mutation (numbered as α1N408A/Y411F in the cited study). Taken together, the results of both studies suggest that residues α1N407 and α1Y410 are not required as hydrogen-bond donating amino acids in order for some compounds to exert potent actions at the neurosteroid binding site(s) on $GABA_A$ receptors. Additional studies will be required to elaborate further the role of residues α1N407 and α1Y410 in modulation of $GABA_A$ receptors by endogenous neurosteroids and neurosteroid analogues.

Conclusion

In this study, we have prepared and examined cyclopenta[*b*]-phenanthrene and cyclopenta[*b*]anthracene analogues of neuroactive steroids to further our understanding of the role of the steroid backbone in the actions of neuroactive steroids at $GABA_A$ receptors. The cyclopenta[*b*]phenanthrene and cyclopenta[*b*]anthracene analogues exhibited potent activity, comparable to their analogous benz[*e*]indenes and neuroactive steroids. Due to the high activity of the cyclopenta[*b*]phenanthrenes, compared to the benz[*f*]indene analogues, we have concluded that new hydrophobic contacts provided by what would be a two carbon bridge between steroid carbons 1 and 11 are allowed, hydrophobic contacts provided by a portion of the steroid B ring are not necessary, and conversely, hydrophobic contacts normally provided by the steroid A ring are critical for interaction with the $GABA_A$ receptor. Electrophysiological studies with $GABA_A$ receptors containing mutations to a putative steroid binding site suggest that these analogues are acting at the same receptor site as neuroactive steroids. Finally, the essentially undiminished actions of the series of spiro-epoxide compounds on $GABA_A$ receptors with the α1N407A/Y410F mutation raise questions regarding the role of amino acids α1N407 and α1Y410 as interacting hydrogen-bonding residues at the putative steroid binding site.

Experimental Section

General Methods. Solvents were either used as purchased or dried and purified by standard methodology. Flash chromatography was performed using silica gel (32−63 μm) purchased from Scientific Adsorbents (Atlanta, GA). Melting points were determined on a Kofler micro hot stage and are uncorrected. FT-IR spectra were recorded as films on a NaCl plate (unless stated otherwise). NMR spectra were recorded in $CDCl_3$ (unless stated otherwise) at ambient temperature operating at 300 MHz (^{1}H) or 75 MHz (^{13}C). Elemental analyses were performed by M-H-W Laboratories (Phoenix, AZ).

(4a*R*,6a*R*,7*S*,8*S*,10a*S*,11a*R*,11b*R*)-Hexadecahydro-8-hydroxy-7a-methyl-3*H*-cyclopenta[*b*]phenanthren-3-one (6). A three-neck flask fitted with a gas condenser and dropping funnel was cooled to −78 °C, and ammonia (75 mL) was condensed. Lithium (55 mg, 8.0 mmol) was added, and the resulting blue solution was stirred for 0.5 h. To this was added a solution of compound **5** (275 mg, 1.0 mmol) in dry THF (20 mL). After 1 h of stirring, saturated

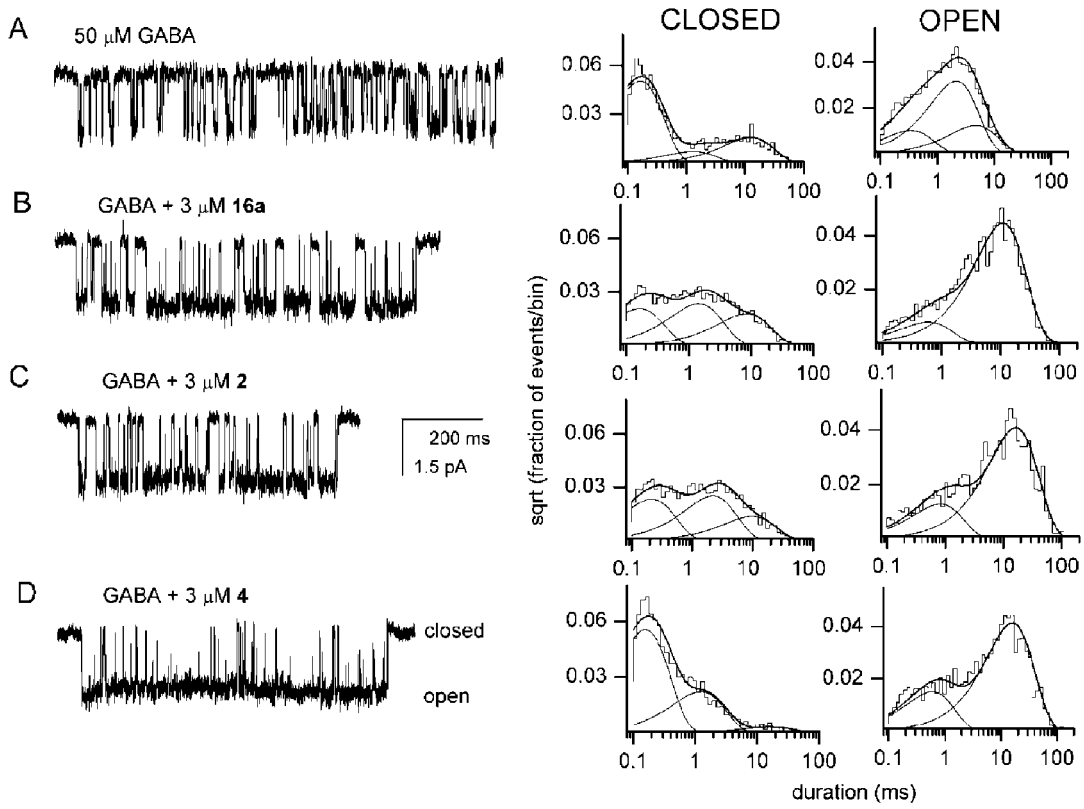


Figure 5. Exposure to compounds **16a**, **2**, and **4** modifies wild-type $\alpha 1\beta 2\gamma 2$L receptor open and closed time properties. Sample single-channel currents, and the respective open and closed time histograms in the presence of 50 μM GABA (A), GABA + 3 μM compound **16a** (B), GABA + 3 μM compound **2** (C), or GABA + 3 μM compound **4** (D). Channel openings are downward deflections. In the presence of GABA alone, the open times were 0.36 ms (18%), 4.7 ms (56%), and 7.0 ms (26%), and the closed times were 0.38 ms (21%), 2.5 ms (32%), and 6.4 ms (47%). In the presence of GABA + compound **16a**, the open times were 0.56 ms (15%) and 10.0 ms (81%), and the closed times were 0.15 ms (34%), 1.3 ms (44%), and 7.9 ms (29%). In the presence of GABA + compound **2**, the open times were 0.76 ms (24%) and 14.9 ms (76%), and the closed times were 0.20 ms (38%), 2.0 ms (41%), and 9.1 ms (21%). In the presence of GABA + compound **4**, the open times were 0.54 ms (28%) and 14.3 ms (72%), and the closed times were 0.15 ms (68%), 1.1 ms (27%), and 15.2 ms (4%). Note that in control recordings, the open time histograms were best-fitted to the sum of three exponentials, but in the presence of compounds **16a**, **2**, or **4**, the open time histograms contained only two components. The summary of averaged data from all patches is given in Table 3.

Table 3. Summary of Single-Channel Kinetic Analysis[a]

compound	OT long (ms)	fraction OT long	fraction CT3	*N*
control	7.6 ± 3.0	0.19 ± 0.13	0.29 ± 0.02	8
3 μM **16a**	10.3 ± 1.8[†]	0.81 ± 0.03**	0.22 ± 0.09[†]	3
3 μM **2**	16.5 ± 5.6*	0.76 ± 0.05**	0.26 ± 0.09[†]	3
3 μM **4**	12.5 ± 3.4[†]	0.67 ± 0.05**	0.04 ± 0.03**	3

[a] The wild-type receptor was activated by 50 μM GABA (control) and exposed additionally to 3 μM steroid **16a**, cyclopenta[*b*]phenanthrene **2**, or cyclopenta[*b*]anthracene **4**. The open time histograms were best-fitted to three (control) or two components (compounds **16a**, **2**, and **4**), and the mean durations and prevalence of the longest-lived component are shown (OT long and fraction OT long). The intracluster closed time histograms contained three components, and the prevalence of the longest lived component (Fraction CT3) is shown. The number of patches (*N*) is given next to the kinetic parameters. Previous work has shown that many potentiating neuroactive steroids and analogues selectively affect these kinetic parameters.[32,34] The control data (GABA alone) are from ref 33. Statistical analysis was carried out using ANOVA with Bonferroni correction (Systat 7.0; Systat Software, Inc., Point Richmond, CA). The significance level applies to comparison to control condition. *$P < 0.05$. **$P < 0.001$. [†]Not significant. Note that when the receptors were exposed to any of the steroid analogues, we were unable to resolve all three open time components that are present in control recordings. The longer lived component in the presence of the analogues is likely a product of merged intermediate and long openings. We would like to point out that when the fraction of long openings determined in the presence of any of the analogues was compared to the sum of the fractions of the intermediate and long openings determined under control conditions, none of the comparisons was statistically significant.

NH_4Cl was added, and the reaction mixture was allowed to come to room temperature overnight. The reaction was extracted with CH_2Cl_2, the combined organic fractions were washed with brine and dried, and the solvent was evaporated. Column chromatography (20% EtOAc in hexanes) gave compound **6** (180 mg, 65%) as a white solid: mp 125–127 °C; $[\alpha]^{25}_D = -50.1$ ($c = 0.99$, $CHCl_3$); ^{1}H NMR δ 3.66 (1H, t, $J = 8.2$ Hz), 2.39–2.23 (4H, m), 2.12–2.00 (2H, m), 1.78–1.46 (6H, m), 1.44–1.10 (9H, m), 1.06–0.80 (3H, m), 0.75 (3H, s); ^{13}C NMR δ 211.7, 81.7, 48.5, 47.9, 46.0, 45.0, 44.6, 43.8, 43.1, 41.2, 38.0, 34.3, 33.8, 30.3, 30.1, 29.2, 25.4, 11.2; IR ν_{max}3422, 2908, 1713, 1049, 732 cm^{-1}; HRMS (EI) *m*/*z* calcd for $C_{18}H_{28}O_2$ 276.2089, found 276.2093.

(4a*R*,6a*R*,7a*S*,10a*S*,11a*R*,11b*R*)-Dodecahydro-7a-methyl-1*H*-cyclopenta[*b*]phenanthrene-3,8(2*H*,4*H*)-dione (7). Compound **6** (160 mg, 0.579 mmol) was dissolved in CH_2Cl_2. To this was added NaOAc (143 mg, 1.74 mmol), followed by PCC (250 mg, 1.2 mmol). The reaction was stirred under N_2 at room temperature for 3 h. It was then filtered through a short stack of silica gel and the solvent removed in vacuo to yield a pale yellow oil. Column chromatography (15% EtOAc in hexanes) gave diketone **7** (135 mg, 85%) as a white solid: mp 130–131 °C; $[\alpha]^{25}_D$ +13.7 (c = 0.96, $CHCl_3$); ^{1}H NMR δ 2.46–2.25 (5H, m), 2.13–2.04 (2H, m), 2.03–1.85 (2H, m), 1.73–1.54 (5H, m), 1.45–0.96 (9H, m), 0.88 (3H, s); ^{13}C NMR δ 220.3, 211.3, 48.4, 48.0, 47.8, 45.9, 45.6, 43.7, 41.1, 39.1, 37.8, 35.6, 34.2, 33.6, 30.1, 28.9, 24.0, 13.5; IR ν_{max} 2916, 1737, 1715, 1187, 1046 cm^{-1}; HRMS (EI) *m*/*z* calcd for $C_{18}H_{26}O_2$ 274.1933, found 274.1939.

(3*S*,4a*R*,6a*R*,7a*S*,10a*S*,11a*R*,11b*R*)-Hexadecahydro-3-hydroxy-7a-methyl-8*H*-cyclopenta[*b*]phenanthren-8-one (1). Compound **7** (130 mg, 0.47 mmol) was dissolved in THF (15 mL). The reaction was cooled to −78 °C, and K-Selectride (1.0 M solution in THF, 0.95 mL, 0.95 mmol) was added. The reaction was monitored by

Table 4. Effects of Cyclopenta[*b*]phenanthrenes, Cyclopenta[*b*]anthracenes, Benz[*f*]indenes, Benz[*e*]indenes and Steroids on Tadpole Righting and Swimming Responses

compd	tadpole LRR[a] ED_{50} (μM)	tadpole LRR (nHill)	tadpole LSR[b] ED_{50} (μM)	tadpole LSR (nHill)
		cyclopenta[*b*]phenanthrenes		
1; [oxo] [c]	3.17 ± 0.24	−3.24 ± 1.48	none[d]	
2; [epox]	0.20 ± 0.01	−1.74 ± 0.19	0.87 ± 0	−20.4 ± 0.4
		cyclopenta[*b*]anthracenes		
3; [oxo]	1.86 ± 0.89	−1.34 ± 0.57	5.48 ± 0.12	−33.3 ± 0.1
4; [epox]	0.48 ± 0.01	−2.02 ± 0.27	1.73 ± 0.04	−36.4 ± 0.1
		benz[*f*]indenes		
11;[e] [oxo]	4.01 ± 0.14	−7.37 ± 0.9	none	
12; [epox]	3.46 ± 0	−20.7 ± 0	none	
		benz[*e*]indenes		
13;[e] [oxo]	16.4 ± 0.5	−4.79 ± 0.44	none	
14; [epox]	3.17 ± 0.02	−15.3 ± 1.3	5.48 ± 0.17	−33.3 ± 0.1
		steroids (5α-reduced)		
15;[e] [oxo]	3.38 ± 0.9	−2.83 ± 2.7	none	
16a;[f] [epox]	1.35 ± 0.01	−3.69 ± 0.08	2.76 ± 0.01	−21.1 ± 0.7

[a] LRR = loss of righting reflex. Error limits are calculated as standard error of the mean ($N = 10$ animals at each of five or more different concentrations). [b] LSR = loss of swimming reflex. Error limits are calculated as standard error of the mean ($N = 10$ animals at each of five or more different concentrations). [c] Oxo (or epox) indicates the substituent on the 5-membered ring. [d] None is defined as no response at 10 μM. [e] Values obtained from ref 20. [f] Values obtained from ref 41.

TLC (50% EtOAc in hexanes) and complete after 1.5 h. The reaction was stopped by addition of 10% NaOH (5 mL), followed by 30% H_2O_2 (5 mL), and stirring was continued for 0.5 h at room temperature. The mixture was then extracted with EtOAc (3 × 15 mL), and the organic layers were combined. The organic layer was washed with brine (2 × 10 mL), dried, and filtered and the solvent removed. Chromatography (20% EtOAc in hexanes) gave 114 mg (87%) of product **1** as a white solid: mp 152–154 °C; $[\alpha]^{25}_D$ = +38.5 ($c = 1.0$, $CHCl_3$); ^{1}H NMR δ 4.11–4.08 (1H, m), 2.45–2.36 (1H, m), 2.13–2.00 (1H, m), 1.91–1.77 (3H, m), 1.74–1.35 (10H, m), 1.26–0.94 (7H, m), 0.85 (3H, s), 0.82–0.69 (2H, m); ^{13}C NMR δ 221.0, 66.3, 48.4, 47.8, 47.1, 45.7, 40.4, 39.4, 38.1, 36.1, 35.7, 34.0, 33.9, 32.8, 28.4, 24.1, 23.3, 13.6; IR ν_{max} 3440, 2917, 1733, 1013, 732 cm^{-1}. Anal. ($C_{18}H_{28}O_2$) C, H.

(3*S*,4a*R*,6a*R*,7a*S*,8*S*,10a*S*,11a*R*,11b*R*)-Hexadecahydro-7a-methylspiro[8*H*-cyclopenta[*b*]phenanthrene-8,2′-oxiran]-3-ol (2). Compound **1** (70 mg, 0.25 mmol) was dissolved in DMF (5 mL). To this trimethylsulfonium iodide (71 mg, 0.35 mmol) and potassium *tert*-butoxide (39 mg. 0.35 mmol) were added. The reaction was stirred at room temperature for 2 h. At this time, the reaction was transferred to a separatory funnel, and CH_2Cl_2 was added. The organic layer was washed with water (2 × 5 mL) and brine (2 × 10 mL), dried, and filtered and the solvent removed. Chromatography (5% EtOAc in hexanes to 25% EtOAc in hexanes) gave product **2** (46 mg, 63%) as a white solid: mp 164–166 °C; $[\alpha]^{25}_D = -20.2$ ($c = 0.58$, $CHCl_3$); ^{1}H NMR δ 4.10–4.08 (1H, m), 2.91 (1H, d, $J = 5.1$ Hz), 2.61 (1H, d, $J = 5.1$ Hz), 1.97–1.60 (7H, m), 1.56–1.34 (7H, m), 1.26–0.93 (8H, m), 0.87 (3H, s), 0.85–0.68 (2H, m); ^{13}C NMR δ 70.5, 66.5, 53.6, 48.3, 47.2, 47.0, 42.1, 40.5, 40.2, 37.9, 36.2, 34.3, 34.0, 32.9, 29.0, 28.9, 25.8, 23.4, 14.4; IR ν_{max} 3434, 2914, 1444, 1378, 1050 cm^{-1}. Anal. ($C_{19}H_{30}O_2$) C, H.

(1*S*,3a*S*,4a*R*,5a*S*,9a*R*,10a*R*,11a*S*)-Hexadecahydro-1-hydroxy-11a-methyl-7*H*-cyclopenta[*b*]anthracen-7-one (9). A three-neck flask fitted with a gas condenser and dropping funnel was cooled to −78 °C, and ammonia (75 mL) was condensed. Lithium (65 mg, 10 equiv) was added, and the resulting blue solution was stirred for 0.5 h. To this was added a solution of compound **8** (0.25 g, 0.93 mmol) in dry THF (25 mL). After 1 h of stirring, saturated NH_4Cl was added, and the reaction mixture was allowed to come to room temperature overnight. The reaction was extracted with ether, and the combined organic fractions were washed with brine, dried and the solvent evaporated. Column chromatography (30% EtOAc in hexanes) gave compound **9** (0.18 g, 70%) as a white solid: mp 185–187 °C (acetone−hexanes); $[\alpha]^{25}_D$ +55.0 ($c = 1.2$, $CHCl_3$); ^{1}H NMR δ 3.65 (1H, dd, $J = 7.8$, 8.7 Hz), 2.34–2.25 (3H, m), 2.08–1.78 (4H, m), 1.71–1.22 (12H, m), 1.08–0.86 (3H, m), 0.81–0.76 (2H, m), 0.73 (3H, s); ^{13}C NMR δ 211.7, 81.6, 48.3, 45.0, 44.1, 43.5, 43.4, 43.3, 41.8, 41.4, 41.1, 40.2, 38.1, 33.1, 32.4, 30.3, 25.3, 11.2; IR ν_{max}3419, 2908, 1713, 1445, 1044 cm^{-1} Anal. ($C_{18}H_{28}O_2$) C, H.

(3a*S*,4a*R*,5a*S*,9a*R*,10a*R*,11a*S*)-Tetradecahydro-11a-methyl-1*H*-cyclopenta[*b*]anthracene-1,7(4*H*)-dione (10). Compound **9** (175 mg, 0.63 mmol) was dissolved in CH_2Cl_2. To this was added NaOAc (310 mg, 3.8 mmol), followed by PCC (540 mg, 2.5 mmol). The reaction was stirred under N_2 at room temperature for 2 h. It was then filtered through a short stack of silica gel and the solvent removed in vacuo to yield a yellow solid. Column chromatography (30% EtOAc in hexanes) gave diketone **10** (158 mg, 91%) as a white solid: mp 194–195 °C (acetone−hexanes); $[\alpha]^{25}_D$ +104 ($c = 0.99$, $CHCl_3$); ^{1}H NMR δ 2.45–2.26 (4H, m), 2.11–1.82 (4H, m), 1.71–1.54 (6H, m), 1.40–1.16 (5H, m), 1.01–0.92 (3H, m), 0.86 (3H, s), 0.81–0.77 (1H, m); ^{13}C NMR δ 220.4, 211.2, 48.2, 45.6, 43.4, 43.2, 41.7, 41.3, 40.8, 39.8, 38.7, 37.8, 35.6, 33.0, 32.0, 23.8, 13.6; IR ν_{max} 2908, 1730, 1707, 1180, 1040 cm^{-1} Anal. ($C_{18}H_{26}O_2$) C, H.

(3a*S*,4a*R*,5a*S*,7*R*,9a*R*,10a*R*,11a*S*)-Hexadecahydro-7-hydroxy-11a-methyl-1*H*-cyclopenta[*b*]anthracen-1-one (3). Compound **10** (150 mg, 0.55 mmol) was dissolved in THF (15 mL). The reaction was cooled to −78 °C, and K-Selectride (1.0 M solution in THF, 1.1 mL, 1.1 mmol) was added. The reaction was monitored by TLC (50% EtOAc in hexanes) and complete after 1.5 h. The reaction was stopped by addition of 10% NaOH (6 mL), followed by 30% H_2O_2 (6 mL), and stirring was continued for 0.5 h at room temperature. The mixture was then extracted with EtOAc (3 × 15 mL), and the organic layers were combined. The organic layer was washed with brine (2 × 10 mL), dried, and filtered and the solvent removed. Chromatography (30% EtOAc in hexanes) gave product **3** (125 mg, 82%) as a white solid: mp 220–221 °C (acetone−hexanes); $[\alpha]^{25}_D$ +63.8 ($c = 0.35$, $CHCl_3$); ^{1}H NMR δ 4.11–4.09 (1H, m), 2.43–2.36 (1H, m), 2.10–2.03 (1H, m), 1.87–1.31 (13H, m), 1.29–1.11 (3H, m), 1.03–0.87 (3H, m), 0.86 (3H, s), 0.84–0.75 (3H, m); ^{13}C NMR δ 220.9, 66.8, 48.3, 45.8, 44.3, 43.2, 41.0, 40.7, 39.9, 38.9, 38.2, 36.4, 35.7, 32.9, 32.4, 27.1, 23.9, 13.7; IR ν_{max} 3219, 2916, 1733, 1013, 978 cm^{-1}. Anal. ($C_{18}H_{28}O_2$) C, H.

(1*S*,3a*S*,4a*R*,5a*S*,7*R*,9a*R*,10a*R*,11a*S*)-Hexadecahydro-11a-methyl-spiro[1*H*-cyclopenta[*b*]anthracene-1,2′-oxiran]-7-ol (4). Compound **3** (59 mg, 0.21 mmol) was dissolved in DMF (2 mL). To this were added trimethylsulfonium iodide (71 mg, 0.35 mmol) and potassium *tert*-butoxide (39 mg. 0.35 mmol). The reaction was stirred at room temperature for 2 h, and then water was added to precipitate a white solid. This solid was filtered, washing with more water. Chromatography (10% EtOAc in hexanes to 20% EtOAc in hexanes) gave product **4** (38 mg, 61%) as a white solid: mp 151–153 °C; $[\alpha]^{25}_D$ +8.6 ($c = 0.5$, $CHCl_3$); ^{1}H NMR δ 4.11–4.09 (1H, m), 2.90 (1H, d, $J = 5.1$ Hz), 2.60 (1H, d, $J = 5.1$ Hz), 1.99–1.91 (1H, m), 1.84–1.60 (4H, m), 1.58–1.35 (9H, m), 1.32–0.91 (7H, m), 0.87 (3H, s), 0.85–0.70 (3H, m); ^{13}C NMR δ 70.5, 66.8, 53.6, 47.0,

44.3, 43.2, 41.5, 41.4, 40.8, 40.7, 40.0, 38.1, 36.5, 32.9, 32.8, 28.9, 27.2, 25.6, 14.5; IR ν_{max} 3376, 2903, 1444, 1371, 1059 cm^{-1}. Anal. ($C_{19}H_{30}O_2$) C, H.

(1*S*,3a*S*,4a*R*,6*S*,8a*R*,9a*S*)-Dodecahydro-9a-methyl-spiro[1*H*-benz[*f*]indene-1,2′-oxirane]-6-ethanol (12). Compound **11** (59 mg, 0.21 mmol) was dissolved in DMF (2 mL). To this were added trimethylsulfonium iodide (71 mg, 0.35 mmol) and potassium *tert*-butoxide (39 mg, 0.35 mmol). The reaction was stirred at room temperature for 2 h. At this time, the reaction was transferred to a separatory funnel, and CH_2Cl_2 was added. The organic layer was washed with water (2 × 5 mL) and brine (2 × 10 mL), dried, and filtered and the solvent removed. Chromatography (5% EtOAc in hexanes to 25% EtOAc in hexanes) gave product **12** (37 mg, 60%) as a white solid: mp 74–76 °C; $[\alpha]^{25}_D$ + 1.78 (c = 6.8, $CHCl_3$); ^{1}H NMR δ 3.70 (2H, m), 2.90 (1H, d, J = 5.1 Hz), 2.60 (1H, d, J = 5.1 Hz), 2.02–1.92 (1H, m), 1.82–1.27 (12H, m), 1.26–1.22 (2H, m), 1.16–1.03 (2H, m), 0.99–0.92 (2H, m), 0.88 (3H, s), 0.83–0.69 (2H, m); ^{13}C NMR δ 70.5, 60.9, 53.5, 47.1, 44.1, 41.6, 40.8, 40.5, 40.3, 38.2, 34.6, 34.0, 33.4, 33.2, 29.0, 25.6, 14.5; IR ν_{max} 3401, 2911, 1446, 1376, 1047 cm^{-1}. Anal. ($C_{17}H_{28}O_2$) C, H.

(3*S*,3a*S*,5a*R*,7*R*,9a*R*,9b*S*)-Dodecahydro-3a-methyl-spiro[3*H*-benz[*e*]indene-3,2′-oxirane]-7-ethanol (14). Compound **13** (30 mg, 0.11 mmol) was dissolved in DMF (2 mL). To this were added trimethylsulfonium iodide (36 mg, 0.18 mmol) and potassium *tert*-butoxide (20 mg, 0.18 mmol). The reaction was stirred at room temperature for 2 h. At this time, the reaction was transferred to a separatory funnel, and CH_2Cl_2 was added. The organic layer was washed with water (2 × 5 mL) and brine (2 × 10 mL), dried, and filtered and the solvent removed. Chromatography (5% EtOAc in hexanes to 25% EtOAc in hexanes) gave product **14** (18 mg, 58%) as an oil: $[\alpha]^{25}_D$ −3.58 (c = 0.53, $CHCl_3$); ^{1}H NMR δ 3.71 (2H, m), 2.92 (1H, d, J = 5.1 Hz), 2.61 (1H, d, J = 5.1 Hz), 2.02–1.94 (1H, m), 1.84–1.57 (5H, m), 1.49–0.93 (13H, m), 0.89 (3H, s), 0.81–0.70 (2H, m); ^{13}C NMR δ 70.6, 60.8, 53.6, 51.9, 43.9, 41.5, 40.8, 40.3, 39.8, 34.4, 33.9, 33.1, 30.4, 29.2, 29.1, 23.2, 14.4; IR ν_{max} 3400, 2918, 1444, 1376, 1054 cm^{-1}; HRMS (EI) *m/z* calcd for $C_{17}H_{28}O_2$ 264.2089, found 264.2076.

[^{35}S]-TBPS Binding Methods. The methods used were as described previously.[40]

***Xenopus* Oocyte Electrophysiological Methods.** The methods used were as described previously.[40]

HEK Cell Electrophysiological Methods. Receptor expression and whole-cell and single-channel recordings were carried out as described previously.[32,34]

Tadpole Behavioral Methods. The methods used were as described previously.[40]

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Supporting Information Available: Elemental analyses results for target compounds **1**, **2**, **3**, **4**, and **12**, ^{1}H and ^{13}C NMR spectra for compound **14**, and X-ray crystallographic data and projection views of compound **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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