2-Arylindenes and 2-Arylindenones: Molecular Structures and Considerations in the Binding Orientation of Unsymmetrical Nonsteroidal Ligands to the Estrogen Receptor

Gregory M. Anstead,† Scott R. Wilson, and John A. Katzenellenbogen*

Department of Chemistry, University of Illinois, 1209 West California Street, Urbana, Illinois 61801.
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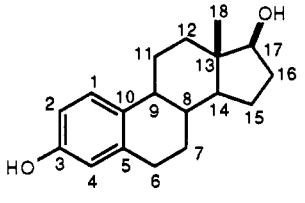
We have studied how 2-arylindene systems, unsymmetrical nonsteroidal estrogens, orient themselves within the binding site of the estrogen receptor, relative to estradiol, by making a comprehensive comparison of the binding affinity of 16 analogues. These analogues are representatives of two major classes, those substituted at C-3 with an ethyl or with a phenyl substituent; within each class there are members that have different patterns of hydroxyl group substitution and C-1 oxo or alkyl substitution. Orientational preferences were inferred from the relative binding affinities and were supplemented by computer graphic molecular overlap studies that utilized crystal structures of selected representative compounds and the known tolerance of the estrogen receptor to substituents on the steroidal ligand estradiol. 2-Arylindenes with a 3-aryl substituent appear to orient with the indene system mimicking the A- and B-rings of estradiol (indene/AB mode). This orientation is supported by the fact that hydroxyl substitution at C-6 in the indene markedly elevates binding relative to hydroxyl substitution at the para position of the 2-phenyl substituent. A C-1 oxo substituent increases binding further, but a C-1 alkyl group has little effect. By contrast, the 2-arylindenes with a C-3 ethyl substituent appear to bind with the pendant C-2 ring, mimicking the A-ring of estradiol (pendant/A mode), as hydroxyl substitution in this ring elevates binding relative to the C-6 hydroxy analogues. C-1 alkyl substitution elevates binding affinity in this series; such a substituent in a C-1 *S* configuration would be projected into the receptor region normally occupied by the high-affinity 7α- or 11β-alkyl estradiols. A C-1 oxo substituent produces only a modest binding enhancement in the C-3 ethyl series. A thermodynamic evaluation of receptor fit suggests that the smaller 3-ethyl-2-arylindenes are more efficient than the 2,3-diarylindenes in the use of the molecular bulk to achieve receptor binding. This analysis of the orientational preference of 2-arylindene nonsteroidal estrogens has important implications in the design of donor/acceptor-substituted 2-arylindenes as fluorescent ligands for the estrogen receptor.

The estrogen receptor (ER) is unique among steroid receptors in being tolerant to large changes in ligand structure.[1] Nonsteroidal estrogens have high binding affinity representatives from many different structural classes: bibenzyls,[2] stilbenes,[3] triarylethylenes[4] and ethanes,[5] phenylindoles,[6] phenylindenes,[7] coumarins,[8] isoflavones,[8] resorcylic acid lactones,[9] and others.[10] The manifold structures of these high-affinity nonsteroidal ER ligands raise questions concerning how these ligands are oriented within the binding site of the ER, as compared to a steroidal ligand such as estradiol (E_2, **1**).[11] Some of



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these nonsteroidal ligands are symmetric, thereby reducing the number of possible ways in which they may be oriented. However, unsymmetrical nonsteroidal ligands pose ambiguities in this regard; it may not be readily apparent which of two nonequivalent phenols may be imitating the crucial C-3 phenolic hydroxyl group of E_2.[12]

Such questions of binding orientation may not be an important issue when the goal of ligand design is simply the acquisition of high binding affinity, but it can be critical in the design of functionalized ligands for the ER. In the course of developing inherently fluorescent ligands for the ER based on the hydroxy-substituted 2-arylindene system,[13] we encountered this problem. The existence of desirable fluorescence characteristics (long emission wavelength, high environmental sensitivity) often requires the incorporation of distinct electron donor and acceptor moieties in the molecule,[14] as illustrated for the 2-arylindene system in Figure 1. Since a 4-hydroxyphenyl group is an effective electron donor, whereas electron acceptors are phenyl groups substituted with nitro, cyano, or acetyl functions, it is important that these substituents by arranged on the 2-phenylindene nucleus in a manner most consistent with high receptor binding affinity. Further-

* Address correspondence to this author at 461 Roger Adams Laboratory, Box 37, Department of Chemistry, University of Illinois, 1209 W. California St., Urbana, IL 61801.

† Present address: Chemistry Department, State University of New York, Stony Brook, NY 11794.

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(14) Weber, G.; Farris, F. J. *Biochemistry* **1979**, *18*, 3075.

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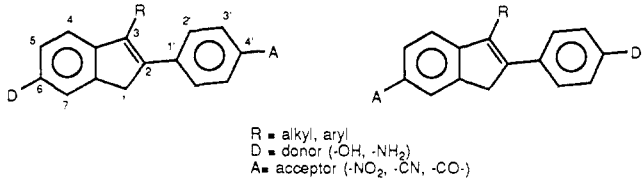


Figure 1. Numbering system and possible structures of donor/acceptor-substituted 2-arylindenes.

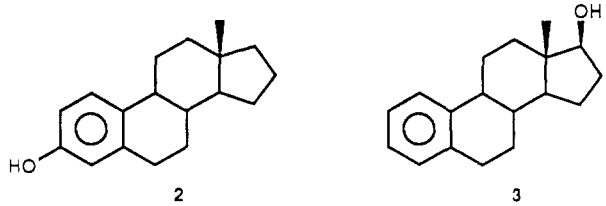
more, elucidation of the structural factors that control the orientation of nonsteroidal estrogens will facilitate the understanding of ER agonism/antagonism at the molecular level.[15]

In this paper, we report the estrogen receptor binding affinities and molecular structures of a series of 2-phenylindenes in which the position of the hydroxyl group and the substituents at C-1 and C-3 are systematically varied. The molecular structures and binding data are used to derive hypothetical orientations of these compounds in the ER binding site.

Results and Discussion

Rationale and Experimental Design. The 2-arylindene system has various attributes that make it an ideal lead structure in the development of an integrated fluorescent estrogen. Like other triarylethylene ER ligands, the 2-arylindenes possess a formal *trans*-stilbene chromophore. However, the 2-arylindenes are unique in that the 6/5-ring fusion splays the substituents attached to the double bond apart, permitting a relatively flat disposition of the 2-aryl group, and thereby greatly enhancing the fluorescence quantum yield.[13a] Furthermore, the 2-arylindenes have high-affinity representatives with diverse substituents at C-1 and C-3,[7,13a,c] increasing the possibilities for successful ligand design.

In estradiol, the 3-hydroxyl group is more important for binding than the 17β-OH. Deoxyestradiol (**2**), lacking the 17β-OH, has an RBA of 14%, whereas **3**, without the 3-OH, has an RBA of 1.7%.[16] The presence of at least one phenolic hydroxyl and a lipophilic backbone within certain dimensions may be the only common denominator among the high-affinity nonsteroidal estrogens.



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For the pseudosymmetric 2-arylidene system, it was necessary to know which aryl group imitated the crucial A-ring phenol of estradiol to allow optimization of binding affinity in a system in which only one phenol could be utilized, i.e., a fluorescent donor/acceptor-substituted 2-arylindene. We intended to determine the long-axis orientation of 3-aryl- and 3-ethyl-2-arylindenes by systematically varying the location of a single hydroxyl group, placing it either at the 6-position of the fused aryl ring or at the 4′-position of the 2-aryl ring. A similar approach was used by Pons and co-workers in their study of the binding orientation of triphenylacrylonitriles in the ER

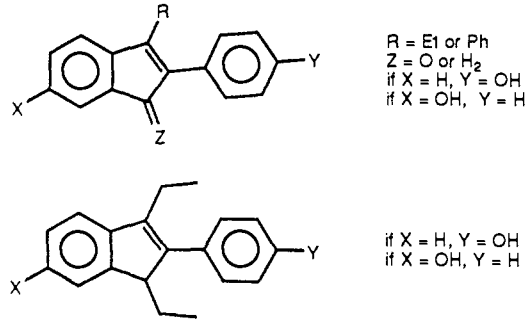
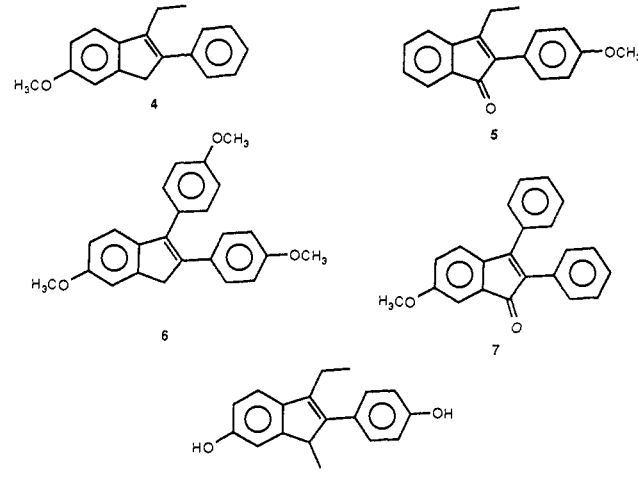


Figure 2. Summary of compounds to probe the orientational preference of the 2-arylindene system.

binding site.[17] Further structural variation was introduced at the C-1 position of the indene [$-CH_2-$ vs $-CO-$ vs $-CH(Et)-$] to probe additional steric and electronic effects on the orientation of the 2-arylidenes in the ER binding site. The compounds under study are summarized in Figure 2. The synthesis of these compounds is described elsewhere.[13a,e]

Molecular Structures. To assess the effects of molecular shape on binding affinity and orientation, crystallographic structure determinations were performed on one member of the two structural classes for which molecular structures have not been reported: the 2-aryl-3-ethylidenes (e.g., **4**) and the 2-aryl-3-ethylindenones (e.g., **5**). Two perspectives on ethylindene **4** and on ethylindenone **5** appear in Figure 3. The crystallographic structures of these molecules were compared with those obtained previously for the related ER ligands **6**, **7**[13a] and **8**.[7]



4 5 6 7 8

Neither indene **4** nor indenone **5** had any intermolecular contacts less than 2.5 Å. The closest intermolecular contact for indene **4** involved the methoxy group, so the observed indene structure, with the possible exception of methoxy rotation, is at or near a local energy minimum.[18] Indenone **5** had one contact which might be construed as a very weak hydrogen bond [H4···ether oxygen, 2.56 (2) Å, C4–H4··· ether oxygen, 174 (2)°, C4···carbonyl oxygen, 3.456 (2) Å; see Figure 1 for numbering scheme]. This intermolecular contact along the *c* axis situated the translationally related C-3 ethyl group terminal carbon atom about 3.9 Å from the closest atom in the C-2 aryl plane, which is longer than

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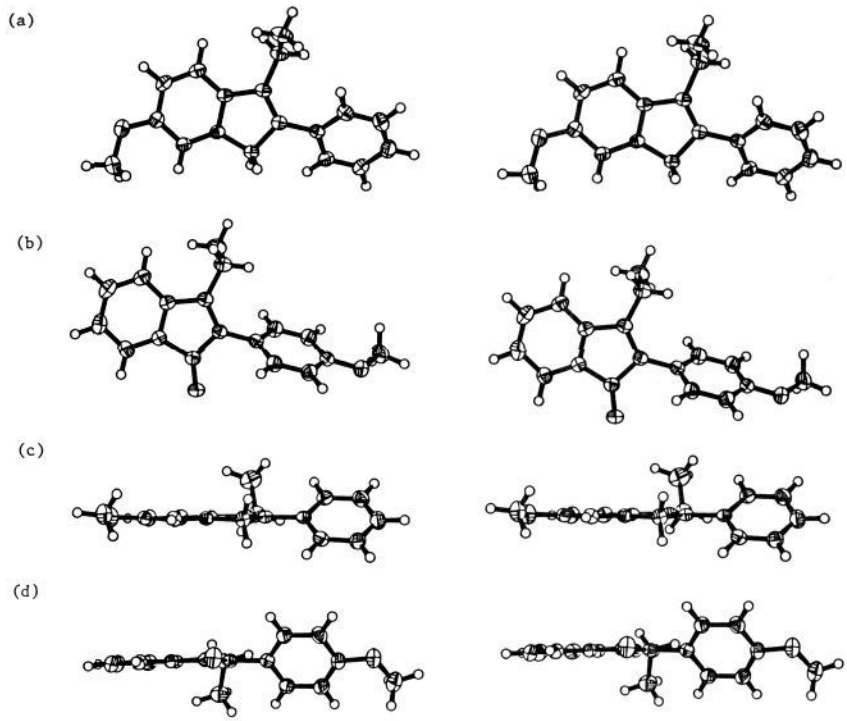

Figure 3. Stereoscopic thermal ellipsoid representations (35% probability): (a and b) 4 and 5 plotted parallel to the best-plane normal, respectively; (c and d) 4 and 5 plotted perpendicular to the best-plane normal, respectively.

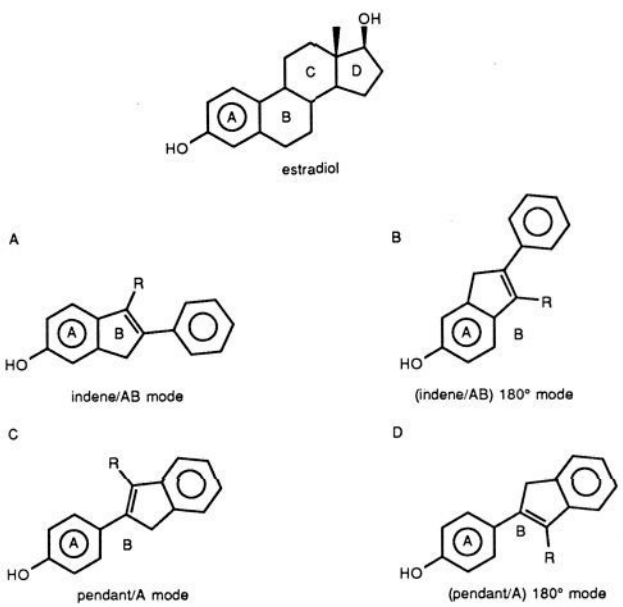

Figure 4. General orientations that 2-arylindenes may adopt in the binding site of the estrogen receptor relative to estradiol. Orientations A and B and orientations C and D are related to each other by a 180° rotation about the C–O bond axis.

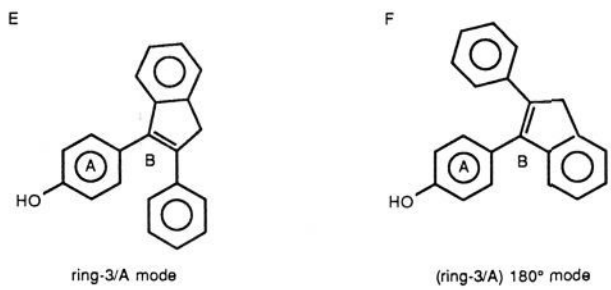

Figure 5. Additional binding orientation modes that may be accessed by 2,3-diarylindenes. Orientations E and F are related to each other by a 180° rotation about the C–O bond axis.

the sum of the van der Waals radii for a methyl group and the half-thickness of an aromatic ring.[19] This weak crystal packing force may have a small influence on the conformation, depending on the broadness of the energy minimum for aryl rotation.[20] Nevertheless, the indenone structure is also probably near a local minimum-energy conformation.[18] The observed conformations of 4 and 5 probably represent global energy minima, because there is only one unique molecule per asymmetric unit[15b] and the intermolecular forces in each case are minimal.[21]

Our primary interest was in the overall molecular shape, which would be dependent on the torsional angles between the three prominant planes (the central double bond with respect to the fused ring system, the C-2 aryl substituent, and the C-3 substituent) within the five molecules. Table I shows a comparison of these angles. Considering the long axis of the molecules, the greatest deviation from planarity is observed in ethylindenone 5, whereas diarylindene 6 is the most planar. The diarylindenone 7, indenestrol A (8), and ethylindene 4 all show a similar dihedral angle between the 2-aryl ring and the indene nucleus. From a comparison of 4 and 8 it can be seen that the C-1 methyl group of indenestrol A contributes negligibley to the torsion of the C-2 substituent.

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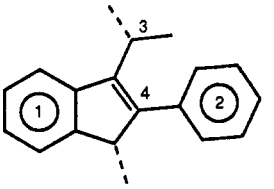
Table I. Normalized Torsional Angles (deg) between Prominent Planes in 2-Arylindenes[a]



torsion angle	ethylindene 4	ethylindenone 5	diarylindene 6[b]	diphenylindenone 7[c]	indenestrol A (8)[d]
1–4	2 (−178)	0 (180)	1 (−179)	5 (+175)	0 (180)
2–4	39 (−141)	53 (+127)	20 (−160)	36 (+144)	36 (−144)
3–4	74 (−106)	70 (+110)	51 (+51)	55 (+125)	80 (−100)
total torsion[e]	115	123	72	96	116

[a] Normalized refers to torsional angles measured without regard to sign (i.e., rotational direction). This convention has been used elsewhere for dihedral angles.[20] The values in parentheses are the measured torsional angles considering the direction of rotation; since these structures were centrosymmetric, only the relative signs per structure are significant. Rotational direction will determine ligand shape and may be a consequence of crystal packing forces; however, global molecular properties such as molecular volume surface area and conjugation will be independent of rotational direction. [b] Reference 13a. [c] For the X-ray crystallographic structures of other 2,3-diarylindenones, see ref 22. [d] Reference 7. [e] Sum of the three prominent normalized torsional angles.

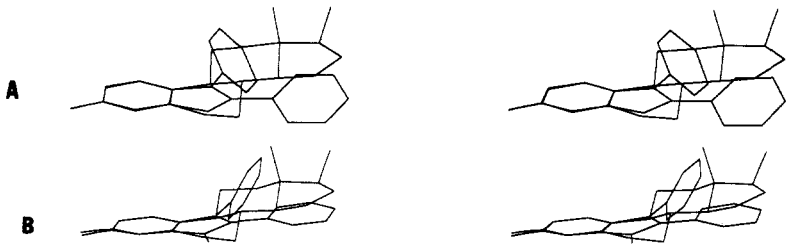


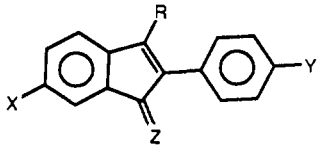
Figure 6. Stereoview of the suggested binding orientation of 2,3-diarylindenes (A) and -indenones (B), relative to estradiol. The structures of the compounds are based on X-ray crystallographic determinations. A rigid fit of the fused aryl rings and the A-ring of the steroid was used.

Estrogen Receptor Binding Affinities. The estrogen receptor binding affinities of the orientational probes were determined in a competitive protein binding assay (see Table II). Analogous compounds are included for comparison purposes.

Orientation. One can imagine that there are four general orientations that a 2-arylindene may adopt with respect to estradiol in the estrogen receptor binding site; these are shown schematically in Figure 4. The 2,3-diarylindenes have two additional binding modes, involving the 3-aryl ring as an A-ring mimic (Figure 5).

Binding Orientation of C-3 Aryl-2-phenylindene Systems 9–15. The estrogen receptor binding affinities of the C-3-aryl-substituted 2-arylindene systems **9–15** show a clear pattern indicative of a preferred binding orientation. The very low binding affinity of the C-3 ring hydroxylated indene **11** indicates that the binding modes of Figure 5 cannot be accommodated by the ER. The low affinities of the 2,3-diarylindene **10** and -indenone **13**, in which the phenyl ring attached to C-2 is para-hydroxylated, also suggest that these classes of compounds do not bind to ER in the pendant/A or (pendant/A) 180° modes. By comparison, the fused ring hydroxylated 2,3-diarylindene **9** and -indenone **12** bind relatively well, indicating a preference for the indene/AB mode or (indene/AB) 180° mode. However, the latter is dismissed for the following reasons: (1) overall, it produces poor skeletal overlap with the steroid, and (2) the indene methylene (or indenone carbonyl carbon) is presented near the region of the ER occupied by the C-1 substituent of the steroid, a position of known steric intolerance. The C-1 methyl,[24] hydroxy,[25] chloro,[26] and fluoro[26] analogues of estradiol all have a lower RBA than estradiol. Thus, the indene/AB

Table II. Estrogen Receptor Binding Affinities[a]



compd	R	X	Y	Z	RBA, %[b]
9	C_6H_5	OH	H	H_2	8.9
10	C_6H_5	H	OH	H_2	0.36
11	4-HOC_6H_4	H	H	H_2	0.017
12	C_6H_5	OH	H	O	59
13	C_6H_5	H	OH	O	0.45
14	C_6H_5[c]	H	H	O	0.0095
15	C_6H_5[d]	OH	H	Me, H	12
16	Et	OH	H	H_2	0.58
17	Et	H	OH	H_2	2.3
18	Et[e]	OH	OH	H_2	16
19	Et	OH	H	O	1.2
20	Et	H	OH	O	4.6
21	Et	OH	H	Et, H	2.2
22	Et	H	OH	Et, H	9.3
23	Et[e]	OH	OH	Et, H	79
8	Et[e]	OH	OH	Me, H	81

[a] Determined by competitive radiometric binding assay using rat uterine cytosol as a source of receptor, [^{3}H]estradiol as tracer, and dextran-coated charcoal as adsorbent for the free ligand. For details see ref 23. [b] Binding affinities are expressed relative to that of estradiol = 100% (RBA = relative binding affinity), are the average of duplicate determinations, and are generally reproducible within ±30%. [c] Commercial sample (Aldrich). [d] Reference 2c. [e] Supplied by K. S. Korach.

(24) The RBA of 1-methylestradiol was 15% with rabbit uterine cytosol: Zeelen, F. J.; Bergink, E. W. In *Cytotoxin Estrogens in Hormone Receptive Tumors* (Raus, J., Martens, H., Le-Clercq, G., Eds.) Academic Press: London, 1980; pp 39–48.

(25) The RBA of 1-hydroxyestradiol was 19% using rat uterine cytosol: Carlson, K. E. Unpublished results.

Table III. Orientational Preference Ratios (OPR) for 2-Arylindenes

	site of hydroxylation				
	fused (F)		pendant (P)[d]		
class of compounds	compd no.	RBA	compd no.	RBA	OPR[b]
2,3-diarylindenes	9	8.9	10	0.36	25F[c]
2,3-diarylindenones	12	59	13	0.45	131F[c]
2-aryl-3-ethylindenes	16	0.58	17	2.3	4.0P[c]
2-aryl-3-ethylindenones	19	1.2	20	4.6	3.8P[c]
1,3-diethyl-2-arylindenes	21	2.2	22	9.3	4.2P[c]

[a] Pendant refers to the ring attached to C-2. [b] Orientational preference ratio, i.e., the RBA of the higher affinity compound divided by the RBA of the lower affinity compound. [c] The suffixes F and P refer to fused and pendant, denoting on which ring the hydroxyl gruop produces a higher affinity.

mode is proposed for the binding orientation of 2,3-diarylindenes and -indenones in the ER binding site (see Figure 6).

The very low binding affinity of the nonhydroxylated indenone **14** documents the critical importance of a hydroxyl substituent in this series. The fact that the C-1 methyl analogue **15** has an affinity comparable to the unsubstituted indene **9** indicates that, for this class of compounds, additional aliphatic substitution at C-1 is of little consequence in terms of binding affinity. By contrast, indenone **12** has considerably greater affinity than the corresponding protio- or methyl-substituted systems **9** and **15**, respectively. This may be due to the fact that the C-1 carbonyl group in the indenone may cause a greater increase in the pendant ring twisting than a proton or the C-1 methyl group. This twisting expands the molecular volume and/or surface area of these receptor ligands and thereby increases their binding affinity.[13a,27,28] Also, the C-1 methyl group in **15** may sterically interfere with the receptor-essential volume[29] in the region of the receptor that would be occupied by the C-6 substituents of a steroidal ligand.[13c]

Binding Orientation of C-3 Ethyl-2-phenylindene Systems 16–23 and 8. The pattern of binding affinity displayed by the C-3-ethyl-substituted 2-phenylindene systems **16–23** and **8** is suggestive of a different binding orientation for these compounds, as compared to the 2,3-diarylindenes. In each of the pairs—**16** and **17**, **19** and **20**, **21** and **22**—the higher binding affinity of the C-2 *p*-hydroxyphenyl partner compared to the 6-hydroxyindene/-one suggests that the pendant/A-ring binding modes (Figure 4 C,D) are preferred. A similar conclusion was reached by Duax et al., on the basis of the overlap of the hydroxy groups and skeletal features of indenestrol A (**8**) with estradiol.[7] The dihydroxy analogues **18** and **23** also bind with greater affinity compared to either of their monohydroxy partners. For C-3 ethylindenes hydroxylated at both the para position of the C-2 ring and at C-6, the (pendant/A) 180° mode produces the best congruence of the indene hydroxyl groups with the C-3 and 17β-hydroxyl groups of estradiol.[7]

In contrast to the C-3 arylindenes, in the C-3 ethyl series, C-1 alkyl groups (methyl or ethyl) cause a substantial increase in the binding affinity, raising the affinity even above that of the corresponding ethylindenones **19** and **20**. This can be rationalized in terms of torsional effects and hydrophobic bonding preferences. Compared to the C-3 arylindenes and -indenones, the C-3 ethylindenes and -indenones have substantially greater pendant group twisting, and thus increased molecular volume and/or surface area (cf. Table I, **4** and **5** vs **6** and **7**), and the C-1 alkyl group on the C-3 ethyl system does not cause further increase in torsion (Table I, **8** vs **4**). However, in both the pendant/A binding orientations, the C-1 alkyl substituent is projected into a region of the receptor that has a strong preference for hydrophobic substituents. In the pendant/A mode, a C-1 ethyl group corresponds to C-7 of a steroidal ligand and its C-7 methyl substituent; in the (pendant/A) 180° mode, to a steroidal 11-ethyl substituent. Occupation of either of these sites is known to enhance receptor binding affinity.[30] Furthermore, it can be predicted that the *S*-enantiomer of the C-1 alkyl-3-ethyl-2-arylindenes would have the higher binding affinity, since in one of the pendant/A orientational moddes, the C-1 alkyl group would then correspond to either the 11β- or 7α-stereochemistries of a substituent of a steroidal ligand, which produce a higher affinity than the other epimers.[30] This prediction has been substantiated by the recent work of Parker et al., who separated the enantiomers of indenestrol A (**8**),[31] and Korach et al., who established their absolute configurations and measured their binding affinities.[32]

By contrast, receptor binding affinity is reduced by polar oxygen substituents at the 7- and 11-sites of a steroid,[33] as would be presented by the ethylindenone systems **19** and **20** when bound in the pendant/A modes. Various orientations of the C-3 ethylindenes are shown in Figure 7.

Orientation Summary. To quantify orientational preference in a pseudosymmetric system, such as the 2-arylindenes, in which one end is functionalized to enhance binding affinity, an orientation preference ratio (OPR) for a pair of compounds can be defined:

$$\text{orientation preference ratio (OPR)} = \frac{\text{RBA of higher affinity compound}}{\text{RBA of lower affinity compound}}$$

with a suffix appended to denote whether the pendant or

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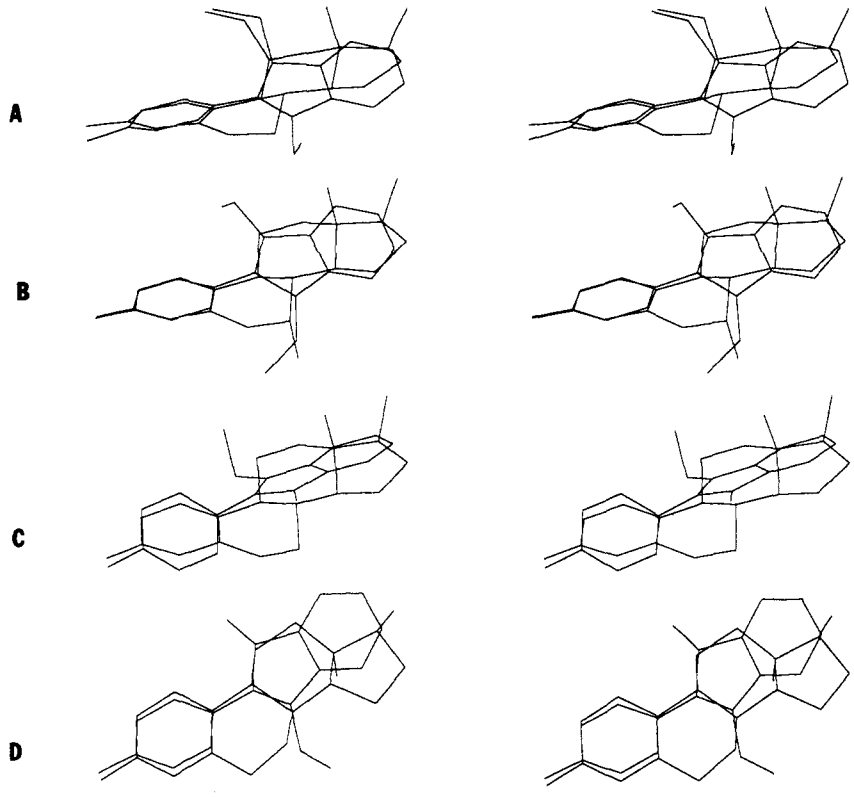

Figure 7. Stereoviews of the binding orientations of C-3 ethyl indenes/-ones relative to steroidal ligands: (A) (*S*)-**22** in (pendant/A) 180° orientation overlapped with 11β-ethylestradiol; (B) (*S*)-**22** in pendant/A mode overlapped with 7α-methylestradiol; (C) ethylidenone **20** in the pendant/A orientation, vs estradiol; (D) ethylindenone **20** in the (pendant/A) 180° orientation, compared to estradiol. The structures of (*S*)-**22**, 11β-ethylestradiol, and 7α-methylestradiol were determined by molecular mechanics. The structures of ethylidenone **20** and estradiol are based on X-ray crystallographic determinations. For fit A, C1, C2, and the pendant ring of the indene were aligned with C11, C9, and the A-ring (C1, C3, C5) of the steroid, respectively. For fit B, C3, C2, and the pendant ring of the indene were aligned with C11, C9, and the A-ring (C1, C3, C5) of the steroid, respectively. For fit C, C3, C2, and the pendant ring of the indene were aligned with C11, C9, and the A-ring (C1, C3, C5, C10) of the steroid, respectively. For fit D, C1, C2, C3, and the pendant ring of the indene were fitted to C11, C9, C8, and the A-ring (C1, C3, C5) of the steroid, respectively. Equal weight was applied in each case.

fused ring hydroxylated compound produces the higher RBA. The results are shown in Table III.

From the results in Tables II and III, it can be seen that orientation in 2-arylindenes is determined primarily by a single structural feature—the nature of the C-3 substituent, phenyl vs ethyl. Possible polar contributions to binding from a C-1 carbonyl group are not important in determining orientation: the C-3 ethylindenones and C-3 arylindenones have opposite apparent orientational preferences. Aliphatic substitution at C-1 has little effect on orientation preferences: the C-1 methyl-2,3-diarylindene **15** orients differently from the C-1 alkylated ethylindene **22**. Furthermore, the OPR changes little in comparing the three classes of C-3 ethylated compounds. The torsion of substituents with respect to the central double bond is not a determinant of orientation; diphenylindenone **7** has a total torsion approaching that of indenestrol A (**8**) and ethylindene **4**, but the diphenylindenone shows a very strong, opposite orientational preferences.

Thus, the C-3 substituent does appear to exert the most important influence on orientation.[34] In the C-3 phenyl compounds, the C-3 aryl group may be stabilized in the indene/AB orientation by charge-transfer complexation with the receptor. Alternatively, in one of the pendant/A modes, the bulky, protruding C-3 aryl group may interfere with the receptor-essential volume.[29] Conversely, in the pendant/A orientations, the C-3 ethylindenes may tightly occupy the receptor site (vide infra), whereas in the indene/AB mode, there may be steric interference with the ER by the indene C-3 ethyl group in the vicinity of the steroid C-ring.

Thermodynamic Evaluation of Receptor Fit. In the preceding sections, a visual method for receptor fit evaluation was used, with estradiol as the template. Alternatively, the goodness of fit in the receptor binding site can be determined by a thermodynamic approach.[35] In this procedure, the observed free energy of binding of the compound (ΔG_{obsd}), calculated from the RBA, is compared to the sum of empirically derived average binding energies (AVERAGE values, ΔG_{av}) for all the functional groups in the molecule, with additional consideration for the loss of molecular degrees of freedom and overall translational/rotational entropy of the molecule. If $\Delta G_{obsd} > \Delta G_{av}$, the compound matches its receptor well.

The observed free energy of binding[35] is given by

$$\Delta G_{obsd} = RT \ln K_d \quad (1)$$

The dissociation constant K_d can be obtained from the association constant K_a, which can be calculated from the RBA value of the compound, by using the equation of Korenman[36] and a known value of the K_a of estradiol. The RAC, the ratio of association constants, is given by

$$K_a^{competitor}/K_a^{estradiol} = (R)(RBA)/(R + 1 - RBA) \quad (2)$$

where R is the ratio of free to bound [^{3}H]estradiol at half-saturation. The association constant for estradiol, measured under the same conditions as the RBA assay, is 3×10^9 M^{-1}.[37]

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Table IV. Structural Classification and Binding Energies

compd	DOF[a]	no. of functional groups C_{sp^2}	C_{sp^3}	OH	C=O	binding energy, kcal/mol ΔG_{obsd}[b]	ΔG_{av}[c]	diff
9	3	20	1	1	0	10.5	1.2	9.3
10	3	20	1	1	0	8.8	1.2	7.6
11	3	20	1	1	0	7.1	1.2	5.9
12	3	20	0	1	1	11.5	3.8	7.7
13	3	20	0	1	1	7.7	3.8	3.9
14	2	20	0	0	1	6.8	2.0	4.8
15	3	20	2	1	0	10.7	2.0	8.7
16	3	14	3	1	0	9.0	−1.4	10.4
17	3	14	3	1	0	9.8	−1.4	11.2
18	4	14	3	2	0	10.8	0.4	10.4
19	3	14	2	1	1	9.4	1.2	8.2
20	3	14	2	1	1	10.1	1.2	8.9
21	4	14	5	1	0	9.7	−0.5	10.2
22	4	14	5	1	0	10.5	−0.5	11.0
23	5	14	5	2	0	11.7	1.3	10.4
8	4	14	4	2	0	11.7	1.2	10.5
1	2	6	12	2	0	11.8	3.4	8.4
2[d]	1	6	12	1	0	11.7	1.6	10.1

[a] Degrees of freedom. [b] Calculated from eq 1 and 2. [c] Calculated from eq 3. [d] Based on the RBA (74%; rat uterine cytosol) determined in this laboratory by using a commercial sample (Steraloids).

The AVERAGE values were calculated by the equation of Andrews et al.:[35]

$$\Delta G_{av} = -14 - 0.7n_{DOF} + 0.7n_{C_{sp^2}} + 0.8n_{C_{sp^3}} + 2.5n_{OH} + 3.4n_{C=O} \quad (3)$$

where −14 is a standard value for the loss of translational and rotational entropy and n is the number of the indicated functional groups or degress of freedom (DOF) in the molecule. The values of ΔG_{obsd} and ΔG_{av} are summarized in Table IV. Estradiol (**1**) and 17-deoxyestradiol (**2**) are included for comparison.

The results of the thermodynamic analysis show that, despite the relatively low binding affinity of monohydroxyethylidenes **17** and **22**, these compounds fit the ER very well, as evidenced by the large difference between the ΔG_{obsd} and ΔG_{av} values. The compounds are small in molecular size compared to estradiol and the 2,3-diarylindenes, but they utilize this small molecular bulk effectively in binding. This is in accord with the molecular overlays of Figure 7; compared to the 2,3-diarylindenes, the hydrophobic bulk of the ethylindenes is more closely within the steroidal domain, with small portions of the C- and D-rings of the steroid unoccupied by the ethylindene ligand. On the basis of the difference values, the ethylindenes **17** and **22** have a better fit to the ER than estradiol itself. This is consistent with the hypothesis that estradiol binding is dominated by features of the A- and B-rings and least affected by the D-ring.[15]

The high-affinity 2,3-diarylindene **9** and -indenone **12** produce lower difference values, indicative of a somewhat poorer fit with the ER binding site as compared to the ethylindenes **17** and **22**. This is consistent with the molecular superposition of Figure 6, in which substantial portions of the indene and indenone lie outside of the steroidal envelope and thus may not contribute effectively to binding. Nevertheless, the relatively high difference values obtained for both classes of compounds supports the assumption that these compounds bind to the ER in a low-energy conformation (i.e., similar to the X-ray crystallographic structure);[21] binding in a strained geometry would reduce ΔG_{obsd} and thus the difference value.[35,38]

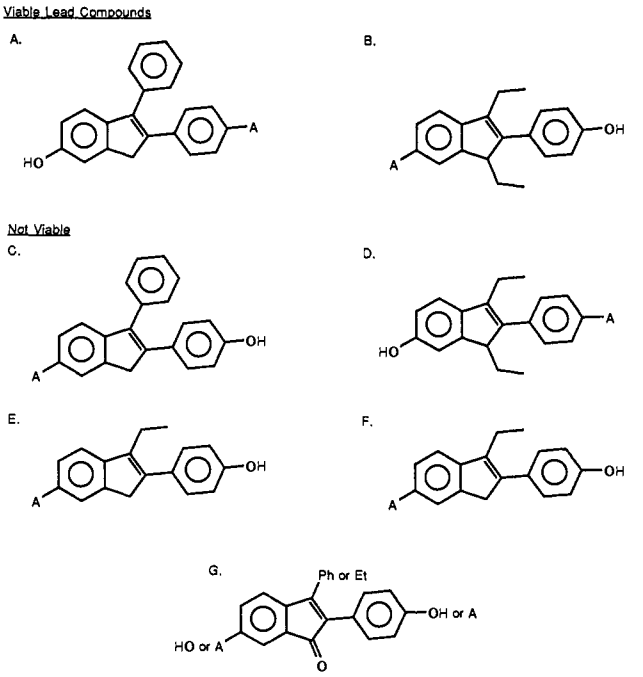

Figure 8. Accepted and rejected lead compounds in integrated fluorescent estrogen design (A = acceptor group). Several compounds of type A have been described elsewhere.[13c]

Orientation in the Design of Integrated Fluorescent Estrogens. The evaluation of the receptor binding affinities of various monohydroxylated 2-arylindenes facilitates the development of donor/acceptor-substituted integrated fluorescent estrogens by indicating the optimum position of the donor group in the arylindene structural manifold. The indenes can be divided into two categories on the basis of the results reported herein: (1) the higher affinity monohydroxy compounds **9** and **22**, which may be successful lead compounds in the design of donor/acceptor-substituted ER ligands, and (2) the low-affinity monohydroxy compounds **10**, **16**, **17**, and **21**, which are not

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Table V. Crystal Data for 4 and 5

formula	$C_{18}H_{18}O$	$C_{18}H_{16}O_2$
crystal system	trigonal	triclinic
space group	$R\bar{3}$	$P\bar{1}$
a, Å	14.248 (4)	9.519 (2)
b, Å	a	9.868 (2)
c, Å	35.583 (4)	7.988 (3)
α, deg	90	90.39 (2)
β, deg	90	109.16 (2)
γ, deg	120	99.24 (2)
V, Å^3	6256 (3)	698.1 (7)
Z	18	2
density calcd, g/cm^3	1.196	1.257
crystallizing solvent	chloroform	hexane–ethyl acetate
crystal habit	tabular (colorless)	prismatic (orange)
crystal dimensions, mm	0.2 × 0.6 × 0.8	0.2 × 0.3 × 0.5
μ cm^{-1}	0.67	0.75
transmission factor range	0.989–0.959 (numerical)	not applied
extinction	[5.1 (6)] × 10^{-8}	not applied
2θ limit, deg (octants)	53.0 ($+h \pm k + l$)	53.0 ($\pm h \pm k - l$)
intensities (unique, R_i)	4847 (2874, 0.018)	3162 (2873, 0.015)
intensities > $2.58\sigma(I)$	1653	1782
R (all intensities)	0.042 (0.087)	0.040 (0.076)
R_w [for $w = 1/\sigma^2(F_0) + pF_0^2$]	0.049 (p = 0.016)	0.044 (p = 0.020)
max density in ΔF map, e/Å^3	0.16	0.13

viable candidates for future fluorescent ligand development. The indenones are rejected as potential fluorescent ligands because the carbonyl group induces intersystem crossing, decreasing the fluorescent quantum yield.[13a] These results are summarized in Figure 8.

Conclusions. The detection of variable orientational modes of small ligands binding to maromolecules is a formidable task unless the binary complex can be crystallized.[39] Unfortunately, an ER–ligand complex has not yet been crystallized. Thus, we have adopted indirect methods for orientation determination based on the overlap of the skeletal and hydrogen-bonding features of ER ligands with those of the natural ligand, E_2.

Our results indicate that 2,3-diarylindenes-indenones bind with the fused aryl ring imitating the steroidal A-ring, and the cyclopentyl unit occupies essentially the same region as the B-ring. By contrast, the 2-aryl-3-ethyl analogues appear to adopt an alternate binding mode in which the pendant 2-phenyl group mimics the A-ring of estradiol. These conclusions are drawn from an analysis of the effect of hydroxyl substitution on binding affinity[40] and considerations of the distribution of substituents within the receptor when the indene system is superimposed upon estradiol and its analogues.

These findings are of importance in designing high-affinity nonsteroidal ligands for the estrogen receptor based on the 2-arylindene skeleton and are being used in our development of inherently fluorescent ligands for the estrogen receptor.

Experimental Section

Calculations and Molecular Graphics. Molecular mechanics calculations were performed with the MAXIMIN option of the SYBYL Molecular Modeling System (Version 3.4, Tripos Associates, St. Louis, MO). The initial geometries of 11β-ethylestradiol and 7α-methylestradiol were based on the X-ray crystallographic structure of estradiol hemihydrate,[41] as was the structure of estradiol used in the molecular superpositions. The initial geometry of (*S*)-**22** was based on the X-ray crystallographic structure of indenestrol A.[7] In the energy minimization of (*S*)-**22**, only the ethyl group at C-1 was allowed to rotate; the rest of the molecule was treated as an aggregate. Molecular superpositions were performed with the SYBYL system, using the FIT command.

Biochemical Methods. Complete experimental details for the relative binding affinity determinations can be found in ref 23. A synopsis of this method is given in the footnotes of Table II.

X-ray Crystallography. Crystals of **4** were grown by slow evaporation from chloroform at −30 °C. Crystals of **5** were obtained by rotary evaporation of a hexane–ethyl acetate solution. Diffraction data were measured at room temperature using an Enraf–Nonius diffractometer equipped with monochromated Mo radiation [λ(Kα) = 0.71073 Å). Final cell dimensions were obtained by a least-squares fit to the automatically centered settings for 25 reflections ($2\theta > 20°$). Three reference reflections monitored during each experiment showed no significant variation. Intensity data were corrected for Lorentz polarization effects. Crystal data for both compounds are listed in Table V. Space group assignments for both crystals were suggested by cell geometry and average values of the normalized structure factors; choices were confirmed by successful refinement.

Both structures were solved by direct methods (MULTAN[42] for **4** and SHELX[43] for **5**); correct positions for all non-hydrogen atoms were deduced from E maps. For both compounds, difference Fourier electron density maps revealed positions for all hydrogen atoms, and the final least-squares refinement cycle (SHELX) included independent parameters for all positions, anisotropic thermal coefficients for all non-hydrogen atoms, and isotropic thermal parameters for hydrogen atoms. For compound **4**, hydrogen thermal parameters were constrained to a single variable and refinement of an empirical isotropic extinction parameter[44] compensated for a skewed variance in the agreement between observed and calculated structure factors with respect to structure factor amplitude. For both experiments, the final difference Fourier map had no significant features. Atomic scattering factors, mass attenuation coefficients, and anomalous dispersion corrections were taken from ref 45.

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Supplementary Material Available: Atomic numbering schemes for 4 and 5 and tables of atomic coordinates, thermal parameters, bond distances, and bond angles (15 pages). Ordering information is given on any current masthead page.

4-(Phosphonoalkyl)- and 4-(Phosphonoalkenyl)-2-piperidinecarboxylic Acids: Synthesis, Activity at *N*-Methyl-D-aspartic Acid Receptors, and Anticonvulsant Activity

Alan J. Hutchison,* Michael Williams, Christoff Angst, Reynalda de Jesus, Louis Blanchard, Robert H. Jackson, E. Jay Wilusz, Deborah E. Murphy, Patrick S. Bernard, Josef Schneider, Thomas Campbell, Wayne Guida, and Matthew A. Sills

Research Department, Pharmaceuticals Division, CIBA-GEIGY Corporation, 556 Morris Avenue, Summit, New Jersey 07901.
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A series of 4-(phosphonoalkyl)- and 4-(phosphonoalkenyl)-2-piperidinecarboxylic acids were synthesized, and their biological activity was assessed as competitive ligands for the NMDA receptor, both in vitro by using a receptor binding assay ([^{3}H]CGS 19755 binding) and in vivo by using an NMDA seizure model in mice. The analogues were also evaluated in [^{3}H]AMPA and [^{3}H]kainate binding to assess their affinity for non-NMDA excitatory amino acid receptor subtypes. A number of these analogues show potent and selective NMDA antagonistic activity both in vitro and in vivo. Most notable are 4-(phosphonomethyl)-2-piperidinecarboxylic acid (**1a**) (CGS 19755) and the phosphonopropenyl analogue **1i**, both of which show anticonvulsant activity in the 1–2 mg/kg ip range. With the aid of computer-assisted modeling, a putative bioactive conformation for AP-5 is hypothesized from the SAR data presented and a preliminary model for the antagonist-preferring state of the NMDA receptor is presented.

Amino acids have an intimate role in neutrotransmission processes in the mammalian CNS.[1] γ-Aminobutyric acid (GABA) and its analogues have inhibitory actions mediated via two distinct receptor subtypes termed GABA-A and GABA-B. The excitatory amino acids, aspartate and glutamate, mediate their actions via at least three classes of receptors which are generally represented by the prototypical agonists *N*-methyl-D-aspartatic acid (NMDA), quisqualic acid (QUIS), and kainic acid (KA).[2] Of these the NMDA receptor has been the most studied. Excess activity at this receptor has deleterious effects on CNS function. Antagonists of the NMDA receptor could thus have potential utility in a number of CNS disorders, most notably in the treatment of epilepsy and the neuronal damage resulting from cerebral ischemia.[3] The present paper describes the development of potent and selective ligands for the NMDA receptor subtype.

At the initiation of these studies the most potent competitive NMDA antagonists known were 2-amino-5-phosphonopentanoic acid (AP-5) and 2-amino-7-phosphonoheptanoic acid (AP-7), discovered by Watkins[4] (see Figure 1). We sought to enhance the biological activity of these templates by the classical medicinal chemistry strategy of conformational restriction. Although both AP-5 and AP-7 are extremely flexible molecules possessing many energetically accessible conformers, we made the initial assumption that the fully extended (all-anti) conformer was the bioactive one for AP-5 as shown in Figure 1. Furthermore, it was known that *cis*-piperidine-2,3-dicarboxylic acid (*cis*-PDA) was a reasonably potent NMDA partial agonist,[5] suggesting that the piperidine-2-carboxylic acid moiety could fit within the exclusion volume of the NMDA receptor site. The superimposition of the all-anti AP-5 conformer and *cis*-PDA led to the synthesis of **1a** (CGS 19755), which was initially identified in a functional assay involving acetylcholine release and subsequently characterized with a binding assay using [^{3}H]CPP [[3-(±)-(2-carboxypiperazin-4-yl)prop-1-yl]phosphonic acid] as the ligand.[6] As a result **1a** was found out to be a potent and selective competitive NMDA antagonist[7] that is an effective anticonvulsant[8] and antiischemic agent[9] which is presently undergoing extensive biological and toxicological evaluation.

This paper is concerned with the synthesis and SAR of a number of 4-(phosphonoalkyl)-2-piperidinecarboxylic acid analogues of **1a** as both receptor ligands and anticonvulsants. The present structure–activity data were derived by using [^{3}H]CGS 19755 (**1a**) as ligand because its higher affinity permits the use of a filtration methodology to isolate bound radioactivity, in contrast to CPP which requires the use of the more time-consuming centrifugation methodology.[10] Affinity of the analogues at quisqualate

* Present address: Nurogen Corp., 35 NE Industrial Rd, Branford, CT 06405.

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