Pergamon

Bioorganic & Medicinal Chemistry 10 (2002) 2779–2793

BIOORGANIC & MEDICINAL CHEMISTRY

Synthesis and In Vitro Platelet Aggregation and TP Receptor Binding Studies on Bicyclic 5,8-Ethanooctahydroisoquinolines and 5,8-Ethanotetrahydroisoquinolines

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Received 29 May 2001; accepted 24 January 2002

Abstract—Eighteen novel bicyclic 1-substituted benzyl octahydro- and tetrahydroisoquinolines were synthesized and evaluated for human thromboxane A_2/prostaglandin H_2 (TP) receptor affinity and antagonism of TP receptor-mediated platelet aggregation. In both cases, potency depended more on the presence of methoxy groups on the 1-benzyl moiety than on nitrogen substitution or extent of oxidation of the isoquinoline ring system. The most potent of the bicyclic compounds retained the 5,8-ethanooctahydroisoquinoline ring structure of the parent molecule (1) and required the 3,4,5-trimethoxybenzyl substitution pattern found in the well-characterized tetrahydroisoquinoline antiplatelet agent trimetoquinol. Differences in nitrogen substituent SAR were noted between the mono-methoxylated compounds and the 3,4,5-trimethoxybenzyl derivatives.

Introduction

The pathogenesis of many cardiovascular and thromboembolic diseases can be related to metabolites of arachidonic acid released from plasma membrane phospholipids.[1–5] A bioactive metabolite of particular importance is the potent platelet aggregation inducer and vasoconstrictor thromboxane A_2 (TXA_2). The development of compounds that selectively inhibit the action of TXA_2 at the thromboxane A_2/prostaglandin H_2 (TP) receptor level or by inhibiting its synthesis would be of significant scientific and clinical importance.[6–9] Several potential clinical advantages of TP receptor antagonists over TXA_2 synthase inhibitors have been suggested.[10] Specifically, TP receptor antagonists also block the effects of platelet aggregatory prostaglandin precursors, such as PGH_2. In addition, the TP receptor antagonists do not generate vasoconstricting prostaglandins such as $PGF_{2\alpha}$, and they are therapeutically useful after the in vivo generation of TXA_2. Unfortunately, few potent or selective TP receptor antagonists have been identified, and those that have are primarily derivatives of prostanoic acid.[8,11–13]

Recently cloned,[14] the TP receptor is a 343 amino acid protein coupled to a pertussis toxin-insensitive G_q protein, and has the characteristic helical structure common to members of this receptor family.[15] In addition to the G_q protein, there is evidence that the platelet TP receptor might also associate with a high molecular weight transglutaminase protein known as G_h as well as to a G_{13} protein.[16,17]

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PII: S0968-0896(02)00101-3

Histidine and cysteine residues found in the first extracellular loop of the TP receptor have been hypothesized to be important in binding agonists to the TP receptor, but the specific role of these residues has not been identified.[18,19] Glycoslyation of asparagine residues at positions 4 and 16 of the protein is essential for high affinity binding and specificity, although the addition of sugar to only one of the residues will permit ligand binding.[20] It is believed that two subtypes of the receptor exist, one that regulates aggregatory/secretory responses and another that controls calcium mobilization and shape changes.[5,21] The platelet TP receptor is believed to be distinct from the binding site found in vascular smooth muscle, although the two receptors are expressed by a common gene.[5,22]

Until recently, little was known about TP receptor topography which would facilitate a strictly rational approach to the design of specific antagonists, although a few efforts at rational design have been attempted.[23,24] While the tertiary structure of the protein has not yet been mapped, a 1993 molecular modeling study by Yamamoto et al.[24] yielded a three-dimensional receptor model. In the following year, Jin and Hopfinger[25] identified a spatial pharmacophore for the four recognition sites of the TP receptor which accommodates five known carboxylate-containing TP receptor antagonists, four of which were based on the structure of the endogenous agonist TXA_2. A subsequent four-dimensional QSAR study by Albuquerque et al. provided structural requirements and restrictions for TP receptor antagonists of the 7-oxabicyclo[2.2.1]heptane oxazole structural type.[26] Early TP receptor antagonists suffered from serious pharmacokinetic-based limitations.[27] However, some members of the oxabicycloheptane class of carboxylate-containing antiplatelet agents, such as ifetroban sodium,[28] have demonstrated good TXA_2 antagonist potency with pharmacokinetic profiles consistent with clinical utility. The aforementioned spatial pharmacophore studies should prove useful in the design of new, even more highly potent anionic TP receptor antagonists.

However, not all TP antagonists are modeled after TXA_2.[29] Trimetoquinol (TMQ, Fig. 1), is an amine-containing tetrahydroisoquinoline analogue that exhibits both platelet aggregation inhibitory properties and β-adrenoceptor stimulatory activity.[30–34] A recent QSAR study related binding affinity at both β_2 adrenoceptors and TP receptors to physicochemical parameters of the aromatic substituents of the 1-benzyl moiety.[35] Affinity at both sites was directly related to the lipophilic nature of the *p*-substituent and the molar refraction of one *m*-substituent. The dependence of β_2 receptor affinity on a low or negative resonance parameter value for the second *m*-substitutent indicated the importance of electronic interactions.

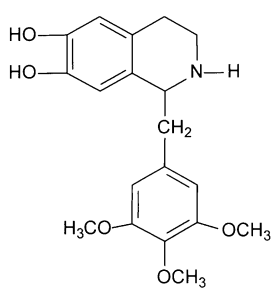


Figure 1. Structure of trimetoquinol (TMQ).

TMQ's antiplatelet and β adrenoceptor agonist activities are stereoselective,[31,36–40] with the *R*(+) isomer exerting the anti-platelet activity through the antagonism of TXA_2 and related endoperoxides at the receptor level.[39,41,42] *S*(−)-TMQ is the most potent β adrenoceptor agonist, but is also capable of selectively inhibiting platelet aggregation induced by non-prostanoids such as phospholipase C, calcium ionophores (e.g., A23187) phorbol esters and low-dose thrombin (in the presence of aspirin).[39,43] In addition to anti-aggregatory action, TMQ also exhibits lipolytic, bronchial relaxant, hypotensive, cardiostimulant and uterine-stimulant activities.[44] Thus, while not selective itself, TMQ can serve as a prototype benzylisoquinoline-based inhibitor of prostaglandin-dependent and independent platelet aggregation, and as a template for the design of selective amine-containing antiplatelet agents.

Two derivatives of bicyclic 1-*p*-methoxybenzyl-5,8-ethanooctahydroisoquinoline (**1** and **2**, Fig. 2) have been synthesized in our laboratory[45] and subsequently shown to non-selectively antagonize platelet aggregation. Tertiary amine **2**, the more potent of the two, exhibited weak TP receptor antagonist action but was up to 50-fold more potent than either TMQ or *N*-methyl-TMQ as an inhibitor of prostaglandin-independent phorbol ester (PMA) and A23187-induced platelet aggregation.[46] These compounds also possess α_2 adrenoceptor antagonist activity.

Compound Design

In order to study the impact of (1) lipophilic and electronic character of the 1-benzyl substituent and (2) steric bulk of the amine substitutent on the extent of TP receptor antagonist action in bicyclic 5,8-ethanooctahydroisoquinoline molecules, compounds **3**–**12** (Fig. 2) were synthesized. The *p*-CH_3 and Cl substituents found in compounds **4**–**9** and **14**–**15** are both more lipophilic than the OCH_3 found on the parent structures **1** and **2**, but differ in their π electron donating/withdrawing character. Their relative TP receptor antagonist potencies would shed light on the relative importance of lipophilicity and electron density distribution within the aromatic arm of the structure. The 3,4,5-trimethoxy substitution pattern found in **10**–**12** and **16** mimics the TMQ structural prototype and tests the hypothesis that this structural arrangement is essential for high TP receptor antagonism in the bicyclic ligands.

The aromatic analogues **13**–**16** (Fig. 2) were byproducts of the Bischler–Napieralski cyclization/reduction step of the synthetic pathway, and were also tested for antiplatelet action. Since it was our hypothesis that the bicyclic antagonists bind to the TP receptor through a

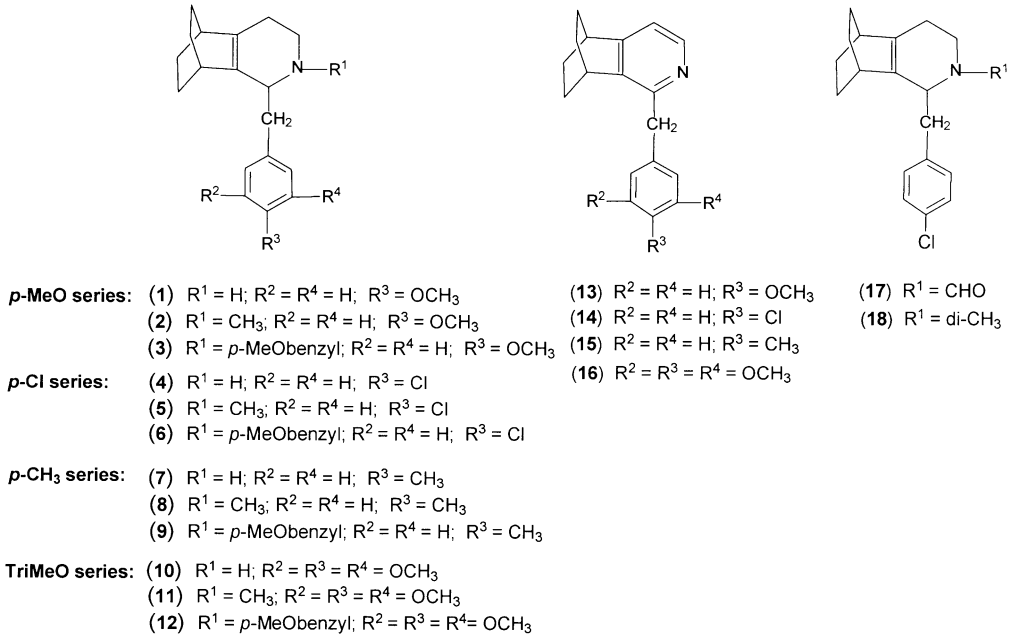


Figure 2. 5,8-Ethanooctahydroisoquinolines and 5,8-ethanotetrahydroisoquinolines synthesized as TP receptor antagonists.

cationic amino nitrogen, non-ionizable **17** and permanently cationic **18** (Fig. 2) were synthesized.

Structure–activity relationship studies have shown that secondary amine-containing TMQ analogues provide significantly greater TP receptor antagonist action than any tertiary derivative.[44] However, previous studies with **1** and **2** have documented that secondary amine **1** is much less active than its N–CH_3 analogue **2** in inhibiting platelet aggregation stimulated by the stable TP receptor agonist U46619. In fact, **2** was consistently more active than **1** in blocking aggregation in response to mediators of both prostanoid-dependent and -independent aggregation pathways.[46] Given this critical SAR difference it was of interest to investigate whether bulkier nitrogen substituents such as *N*-*p*-methoxybenzyl (among the more potent and platelet-selective of nitrogen substituents in tertiary TMQ-based TP receptor antagonists) also augment the antagonist potency of the bicyclic 5,8-ethanooctahydroisoquinoline structures (**3**, **6**, **9**, and **12**).[47]

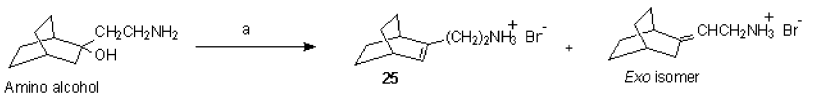
Results

Chemistry

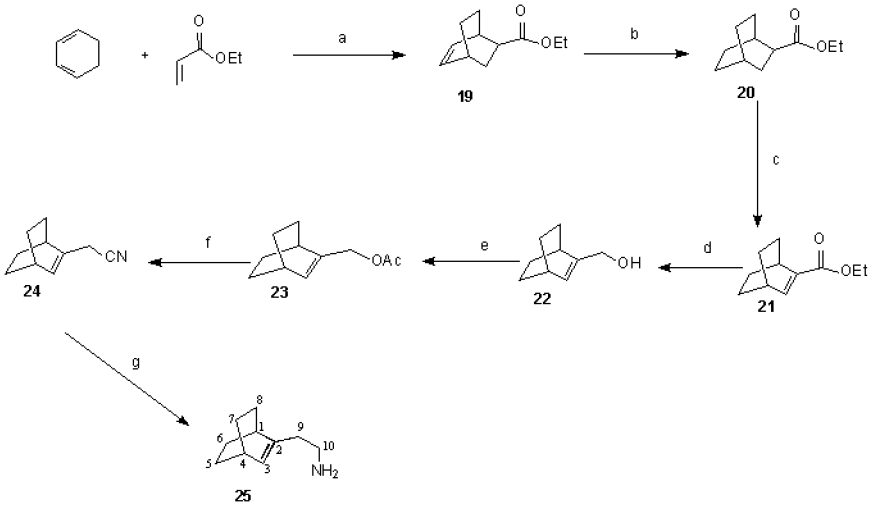
The syntheses of the bicyclic 5,8-ethanooctahydroisoquinolines **1** and **2** have been previously described.[45] A major drawback of the original pathway was the generation of a mixture of olefinic isomers in the synthesis of the critical intermediate 2-(aminoethyl)bicyclo-[2.2.2]oct-2-ene (**25**). Compound **25** and its exocyclic olefinic isomer were produced in equivalent amounts when the precursor amino alcohol was subjected to heat-catalyzed elimination after in situ conversion to the bromide (Scheme 1). These isomeric olefins proved very difficult to separate with conventional purification methods, and the mixture was subjected to acylation and Bischler–Napieralski cyclization to produce target compounds **1** and **2**. In addition, some of the bicyclic octane and octene precursors to the synthesis of the amino alcohol were quite volatile and difficult to isolate without significant product loss. The new route to the desired bicyclic amine **25** described in Scheme 2 avoids these problems, and represents a significant improvement over the original pathway.

The synthesis of key intermediate **25** began with a Diels–Alder reaction with commercially available cyclohexadiene and ethyl acrylate[48] (Scheme 2). An overnight reaction at 140 °C in a sealed tube gave the bicyclic ester **19** in 85% yield. Catalytic reduction of the olefin provided the known[49] saturated analogue **20** which was subjected to lithium diisopropylamide-mediated selenylation with phenylselenium bromide.[50] Oxidative deselenylation with 30% hydrogen peroxide afforded **21**, which had the requisite double bond in desired position.[50] The yield of **21** was routinely above 90%, and the NMR was consistent with that of the known methyl ester.[51] This conjugated ethyl ester was purified by repeated column chromatography and fractional distillation to single peak purity on a capillary gas chromatrograph. However, an impurity that co-eluted and co-distilled with the desired compound prevented the percent carbon observed upon elemental analysis from matching the anticipated value to within the requisite $\pm 0.4\%$. High-resolution electron impact mass spectrometry documented a molecular formula consistent with the given structure.

Conversion of the ester **21** to the corresponding cyanomethyl derivative **24** was achieved by the following sequence of reactions (Scheme 2). Reduction of **21** to the allylic alcohol **22** was accomplished with fresh diisobutylaluminum hydride (Dibal-H) in toluene.[52]



Scheme 1. Synthesis of 2-aminoethylbicyclo[2.2.2]octene isomers. (a) Ph_3PBr_2, *p*-xylene, reflux.



Scheme 2. Synthesis of 2-aminoethylbicyclo[2.2.2]oct-2-ene (**25**): (a) 140 °C, sealed tube, 85%; (b) H_2, Pd, EtOH, rt, 89%; (c) (i) LDA, THF, −78 °C; (ii) PhSeBr, THF, −78 to 0 °C; (iii) 30% H_2O_2,HOAc, 0 °C to rt, 95%; DiBAL-H, CH_2Cl_2, −78 °C, 85%; acetyl chloride, pyridine, CH_2Cl_2, rt, 90%; (d) $Pd(Ph_3P)_4$, CH_3CN, toluene, reflux, 92%; (e) AlH_3 or $LiAlH_4$, THF, 0 °C, 79%.

Despite utilizing conditions that had produced quantitative yields of cyclic allylic alcohols from esters in other systems,[53] the yield of this reaction was variable, particularly upon scale-up. In small scale reactions, yields of 85% were typical. Once isolated and purified, **22** was converted to its corresponding acetate **23** in 90% yield with acetyl chloride and pyridine.[54] The acetate was reacted with trimethylsilyl nitrile in the presence of palladium tetrakistriphenylphosphine in refluxing toluene to provide the nitrile **24** in 92% yield.[55] All of these small bicyclic intermediates suffered the same problem as the conjugated ester **21** with respect to purification. Despite repetitive column chromatography and fractional distillation of the products to single peak purity on the gas chromatograph, high resolution electron impact GC/mass spectrometry was again required to give unequivocable proof of molecular formula. Reduction of nitrile **24** with aluminum hydride generated in situ from lithium aluminum hydride and aluminum chloride[56] gave the pure endocyclic amine **25** in 79% yield. A similar reaction using lithium aluminum hydride alone[57] provided the desired amine in 83% yield.

The condensation of **25** with the appropriately substituted phenylacetyl chlorides **26** (which were generated via reaction of the commercially available acid and oxalyl chloride in dichloromethane) in the presence of dry pyridine provided desired amides **27** in 64–75% yield after chromatographic purification (Scheme 3).[58] The *p*-methoxyphenylacetyl chloride was generated by reacting the commercially available acid with thionyl chloride, as previously described.[45] Bischler–Napieralski cyclization[59,60] of these amides with phosphorus pentoxide, followed by a basic workup and immediate reduction of the intermediate imines with potassium borohydride in methanol, provided a mixture of two major products. These products were purified on a silica gel column and characterized as the desired 5,8-ethano-octahydroisoquinolines (**4**, **7**, and **10**) and aromatic 5,8-ethanotetrahydroisoquinoline (**13**–**16**) byproducts. These octahydro and tetrahydro derivatives were routinely produced in 30–35 and 20–25% yields, respectively. All *N*-*p*-methoxybenzyl analogues (**3**, **6**, **9**, and **12**) were synthesized in moderate to high yield by reacting the secondary amines (**1**, **4**, **7**, and **10**) with *p*-methoxybenzyl chloride in the presence of triethylamine.[61]

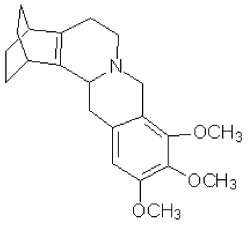


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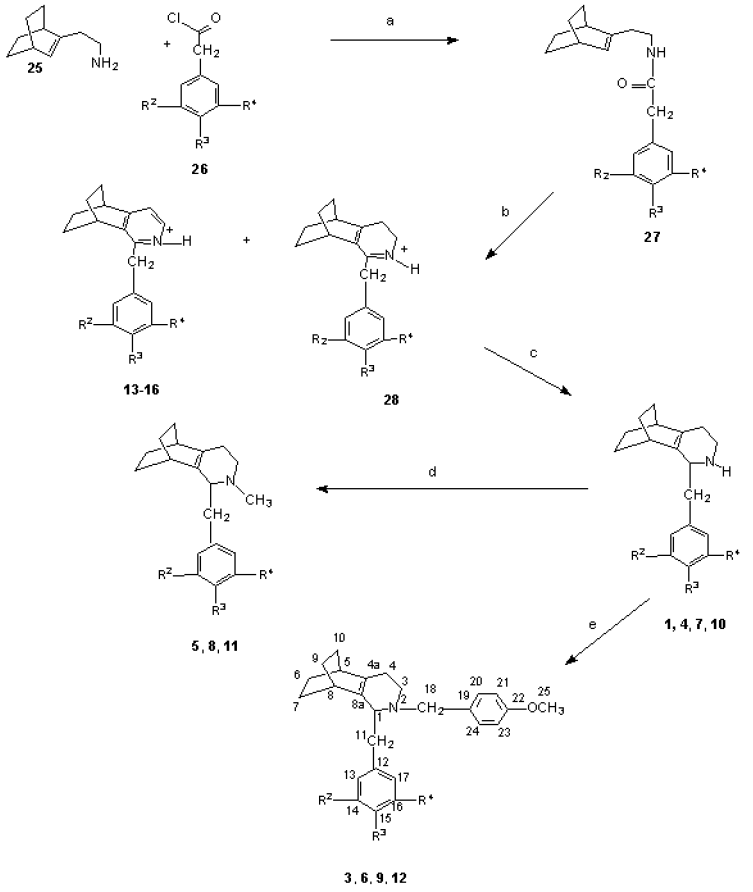
Figure 3. Benzoquinolizine rearrangement product (**29**).

N-Methylation of the *p*-chloro analogue **4** and the *p*-methyl derivative **7** could be accomplished by reaction with 37% formaldehyde/formic acid.[62] Under these same conditions, however, the 3,4,5-trimethoxy congener underwent intramolecular cyclization to produce a benzoquinolizine product **29** (Fig. 3). This product was characterized by ^1H and ^{13}C NMR, and the ^{13}C



Scheme 3. Synthesis of 5,8-ethanooctahydroisoquinolines and 5,8-ethanotetrahydroisoquinolines (**3**–**16**): (a) pyridine, THF, rt, 64–75%; (b) P_2O_5, toluene, reflux, 20–25% of tetrahydro derivatives; (c) KBH_4, NH_4OH, MeOH, rt, 30–35% from **27**; (c) H_2CO, HCOOH or H_2CO, $NaCNBH_3$, CH_3CN, reflux, 25–78%; *p*-MeObenzyl chloride, Et_3N, $CHCl_3$, rt, 65–98%.

resonance values found were in close agreement with a highly similar dibenzo[*a*,*g*]quinolizine alkaloid, tetrahydropalmatine.[63] Methylation of polymethoxylated analogue **10** to provide the desired tertiary amine **11** was accomplished in 75–78% yield by reacting the secondary amine with 37% formaldehyde and sodium cyanoborohydride in acetonitrile at room temperature.[64]

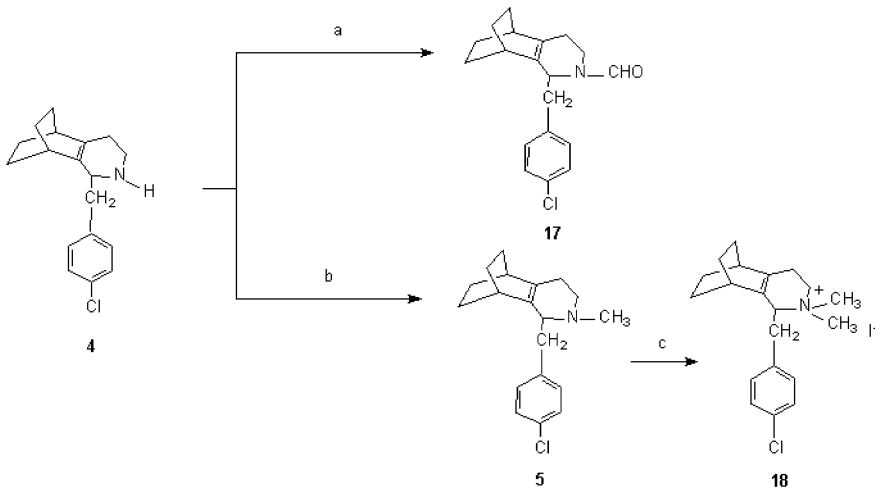
The formamide derivative **17** was synthesized in 61% yield from secondary amine **4** by reaction with ethyl formate (Scheme 4).[65] The NMR spectrum of this compound was complicated by the presence of two isomeric forms. A similar spectroscopic situation was noted in the *N*-formylated analogue of **1**.[45] The quaternary ammonium derivative **18** was synthesized in 76% yield from tertiary amine **5** by reaction with methyl iodide[44] (Scheme 4).

All bicyclic octahydro- and tetrahydroisoquinolines were analyzed by ^1H and ^{13}C NMR spectrometry. Characterization and evaluation of the purity of the bicyclic octahydro- and tetrahydroisoquinoline hydrochloride or methanesulfonate salts by melting point was difficult, as the compounds slowly sublimed upon heating. The melting points reported in the Experimental represent the temperature at which a single crystal disappeared.

Pharmacology

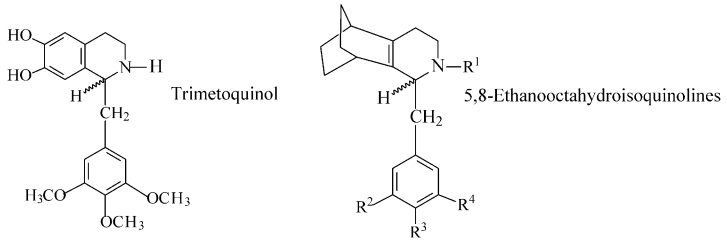
The results of the evaluation of racemic bicyclic compounds **1**–**18** as antagonists of U46619-induced platelet aggregation and as competitive ligands for [^{3}H]SQ29,548-labeled TP receptor sites in human platelets are provided in Table 1.

Preliminary studies documented that none of the bicyclic 5,8-ethanooctahydroisoquinoline molecules caused platelet aggregation in platelet rich plasma. With the exception of **9**, all bicyclic compounds were weak aggregation antagonists with IC_{50} values against U46619-induced platelet aggregation ranging from 5.3 to 550 μM. The most active platelet aggregation inhibitor of the novel bicyclic structures was **10**, which retained the secondary amine and trimethoxybenzyl substitution pattern of the prototypical isoquinoline TP receptor antagonist TMQ. Compound **10** was approximately seven times less active than TMQ in inhibiting thromboxane-induced platelet aggregation, and had about one-third the affinity of TMQ for thromboxane binding sites on platelets. The high significance ($p < 0.0001$) of the relationship between aggregation (pIC_{50}) and displacement of [^{3}H]SQ29,548 binding to TP receptors (pK_I) indicates that the anti-aggregatory activities of these compounds are through blockade of TP receptor sites of human platelets (Fig. 4).



Scheme 4. Synthesis of formylated and quaternary derivatives of a *p*-chloro-5,8-ethanooctahydroisoquinoline: (a) $HCOOC_2H_5$, benzene, 50 °C, 61%; (b) H_2CO, HCOOH, reflux, 40%; (c) MeI, $KHCO_3$, MeOH, rt, 76%.

Table 1. Inhibitory effect of trimetoquinol (TMQ) versus 5,8-ethanooctahydroisoquinolines on U46619-induced aggregation and [^{3}H]SQ29,548 binding to TP-receptors of human platelets[a]



Compound	Platelet aggregation			Platelet binding		
	IC_{50} (μM)	pIC_{50}[b]	P.R.[d]	K_i (μM)	pK_i[c]	P.R.[d]
Trimetoquinol (TMQ)	0.81	6.09±0.09	1.0	0.62	6.21±0.13	1.0
p-OCH_3 series ($R^2=R^4=H$; $R^3=OCH_3$)						
$R^1=H$ (**1**)	177.0	3.45±0.06	0.005	270.1	3.57±0.14	0.002
$R^1=CH_3$ (**2**)	103.1	3.99±0.03	0.008	61.3	4.21±0.08	0.010
R^1=*p*-methoxybenzyl (**3**)	541.2	3.27±0.03	0.002	218.8	3.66±0.13	0.003
Tetrahydro analogue (**13**)	377.3	3.42±0.08	0.002	43.3	4.36±0.06	0.014
p-Cl series ($R^2=R^4=H$; $R^3=Cl$)						
$R^1=H$ (**4**)	128.8	3.89±0.10	0.006	69.7	4.16±0.08	0.009
$R^1=CH_3$ (**5**)	124.0	3.91±0.12	0.010	40.7	4.39±0.74	0.015
R^1=*p*-methoxybenzyl (**6**)	374.4	3.43±0.04	0.007	222.2	3.65±0.20	0.003
R^1=formyl (**17**)	553.8	3.62±0.09	0.001	102.1	3.99±0.05	0.006
R^1=*N*,*N*-dimethyl (**18**)	530.9	3.28±0.08	0.002	95.6	4.02±0.03	0.006
Tetrahydro analogue (**14**)	180.0	3.77±0.10	0.005	64.9	4.17±0.07	0.010
p-CH_3 series ($R^2=R^4=H$; $R^3=CH_3$)						
$R^1=H$ (**7**)	154.9	3.81±0.05	0.005	66.1	4.18±0.09	0.009
$R^1=CH_3$ (**8**)	77.6	4.11±0.19	0.010	37.4	4.43±0.15	0.017
R^1=*p*-methoxybenzyl (**9**)	>800	<3.10	<0.001	48.0	4.32±0.02	0.013
Tetrahydro analogue (**15**)	286.7	3.54±0.08	0.003	95.1	4.02±0.05	0.007
Tri-OCH_3 series ($R^2=R^3=R^4=OCH_3$)						
$R^1=H$ (**10**)	5.3	5.28±0.27	0.15	1.8	5.74±0.05	0.34
$R^1=CH_3$ (**11**)	13.8	4.86±0.05	0.059	8.1	5.09±0.09	0.077
R^1=*p*-methoxybenzyl (**12**)	66.4	4.18±0.08	0.012	6.7	5.17±0.08	0.093
Tetrahydro analogue (**16**)	133.5	3.87±0.06	0.006	9.3	5.03±2.03	0.067

[a]Data represent the mean±SEM of $n=3$–6 experiments. U46619 (0.5–1.5 μM) was used as inducer for aggregation in the presence of 1 mM aspirin incubated for 1 min at 37 °C. [^{3}H]SQ29,548 (1 or 5 nM) was used to label TXA_2 sites; and nonspecific binding was defined by addition of 50 μM of SQ29,548.

[b]$IC_{50}=-\log IC_{50}$, which is the inhibitory concentration reducing half of the maximal response to the inducer.

[c]$pK_i=-\log K_i$, and was determined as $IC_{50}/(1+[\text{ligand}]/K_d)$, where $K_d=3.1$ nM is taken from previous data.[65]

[d]Potency ratio (P.R.)=(IC_{50} or K_i TMQ)/(IC_{50} or K_i) bicycloisoquinolines, where TMQ value is the mean of individual control tested in each experiment. TMQ is the reference compound.

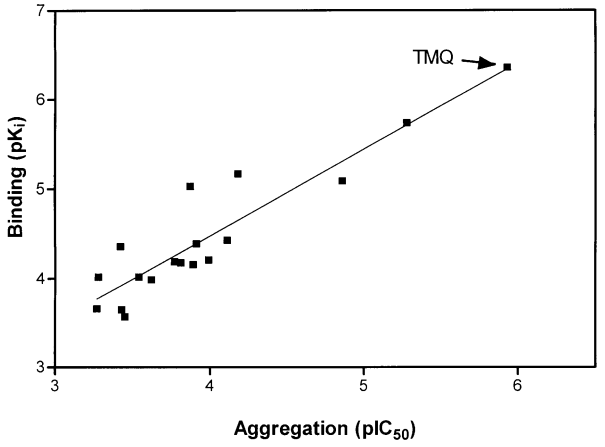


Figure 4. Correlation of anti-aggregatory activities (pIC_{50}) and inhibition of [^{3}H]SQ29548 binding (pK_i) to TP receptors in human platelets by bicyclic octahydro- and tetrahydroisoquinolines ($r = 0.922$, $p < 0.0001$).

Discussion

Analysis of structure–activity relationships for bicyclic 5,8-ethanooctahydroisoquinolines clearly shows that the presence of the TMQ-like trimethoxy substituent pattern on the 1-benzyl group is important for optimal anti-thromboxane activities of aggregation and TP receptor binding. The work of Kumar and Singh suggests that hydrophobicity of the *p*-substituent of TMQ is important to TP receptor binding.[35] Despite the increase in lipophilicity compared to a methoxy group, the lack of potency of all analogues of the *p*-chloro and *p*-methyl series indicates that other physicochemical parameters are operational in this bicyclic series. One possibility is that hydrogen bonding in this molecular area is essential for anti-platelet activity and TP receptor affinity in bicyclic 5,8-ethanooctahydroisoquinolines, although additional analogues with varying steric, electronic and hydrogen-bonding properties would need to be synthesized and tested to confirm or refute the hypothesis. The lack of appreciable activity of the *p*-chloro analogues containing either the *N*,*N*-dimethyl or the *N*-formyl groups precluded assessment of the importance of the cationic nitrogen in binding these compounds to the TP receptor. Studies will continue with the trimethoxy series now that it has been identified as the most potent of the bicyclic receptor ligands.

In general, the nature of the amine substituent (H, CH_3, *p*-methoxybenzyl) or the extent of unsaturation in the nitrogen-containing ring (octahydro versus tetrahydro analogues) did not produce significant changes in the inhibition of platelet aggregation and competitive receptor binding. However, in the most active methoxy-containing structures, the presence of multiple methoxy groups on the aromatic ring appears to change the preference of the receptor for the extent of substitution on the amino nitrogen. In the original *p*-methoxy molecules, tertiary bicyclic ligand **2** was more active than secondary analogue **1** as both an inhibitor of thromboxane-induced aggregation and in displacing the tritiated TP receptor antagonist from its binding site. The relationship is reversed in the 3,4,5-trimethoxy derivatives **10** and **11** and TMQ, indicating that binding to the TP receptor is influenced by the 1-benzyl moiety.

Conclusion

Eighteen novel bicyclic 1-substituted benzyloctahydro- and tetrahydroisoquinolines were synthesized and evaluated for their ability to (1) bind to the human TP receptor and (2) antagonize the activity of a stable TXA_2 analogue at that receptor surface. The activity of the bicyclic ligands was compared to that of the well-known tetrahydroisoquinoline platelet aggregation inhibitor TMQ. The presence of at least one aromatic methoxy group was crucial to the antiplatelet and TP receptor binding activity of the bicyclic ligands. The most potent of these compounds retained the 5,8-ethano-octahydroisoquinoline ring structure of the parent molecule (**1**) and required TMQ's 3,4,5-trimethoxy substitution pattern on the 1-benzyl moiety. An interesting inversion in nitrogen substituent SAR resulted when three methoxy groups (as opposed to one) were present; the bicyclic secondary amine was the most active member of the trimethoxybenzyl family of compounds. Adding bulk (beyond CH_3) to the amino nitrogen of the bicyclic compounds, or oxidizing the nitrogen-containing ring to a pyridine system, does not promote anti-platelet activity or TP receptor affinity. Bicyclic 5,8-ethanooctahydroisoquinolines containing these nitrogen modifications were significantly less active in inhibiting U46619-induced platelet aggregation and [^{3}H]SQ29,548 binding compared to *N*-methyl or secondary amine derivatives. The most active bicyclic ligand (**10**) was significantly less active than TMQ as both an inhibitor of U46619-mediated platelet aggregation and in displacing [^{3}H]SQ29,548 from TP receptors.

Experimental

Chemistry

General. Melting points were determined on a Fisher–Johns melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on either a Varian INOVA or Unity 300 MHz instrument. Chemical shifts are reported in δ units relative to $CHCl_3$ (7.27 ppm). Gas chromatography was conducted on both Hewlett Packard HP 5988A and 5890 Series II chromatographs. High resolution mass spectra were recorded in an EI mode on a Micromass Autospec mass spectrometer at the Nebraska Center for Mass Spectrometry (Lincoln, NE). Microanalyses were performed by either Galbraith Laboratories (Knoxville, TN) or Atlantic Microlabs, Inc. (Norcross, GA). Infrared (IR) spectra were recorded on either a Perkin-Elmer 1600 FT instrument or a Nicolet Avatar 380 FT-IR. R_f values were determined from TLC performed on fluorescent, plastic-backed Selecto Scientific silica gel 60 plates. Column chromatography was carried out with Merck or Fisher silica gel 60 (200–425 mesh). Solvents were dried by distillation after reflux with appropriate drying agents and stored over molecular sieve.

2-Ethoxycarbonylbicyclo[2.2.2]oct-5-ene (19). A thick-walled reaction tube was charged with cyclohexadiene (2 g, 25 mmol), ethyl acrylate (2.5 g, 25 mmol), and methylene blue (0.018 g, 0.05 mmol). The tube was sealed and the solution heated at 140 °C for 12–18 h. Distillation (76 °C/<1 mm Hg; lit 106 °C/15 mm Hg[66]) afforded **19** as a clear liquid (3.83 g, 85% yield). ^{1}H NMR ($CDCl_3$) δ 6.3 (t, 1H, C=CH), 6.13 (t, 1H, C=CH), 4.1 (m, O–CH_2), 2.93 (m, 1H, C2-H), 2.58 (m, 2H, C1-H, C4-H), 1.8–1.4 (m, 4H, C3-H_2, C8-H_2), 1.35–1.25 (m, 5H, C7-H_2, CH_3); ^{13}C NMR with DEPT ($CDCl_3$) δ 175.6 (carbonyl carbon), 135.3 (C5), 131.6 (C6), 60.3 (O–CH_2), 43.1 (C2), 32.7 (C1), 30.1 (C3), 29.6 (C4), 25.5, 24.6 (C7, C8), 14.4 (CH_3).

2-Ethoxycarbonylbicyclo[2.2.2]octane (20). A pressure bottle was charged with **19** (2.6 g, 15 mmol), 10% Pd/C (0.75 g), and 100% EtOH (50 mL). The bottle was placed in the Parr hydrogenator and evacuated by aspirator vacuum. The system was then filled with H_2 (50 psi) and allowed to shake for 18 h. The solution was filtered through Celite® and the solvent removed in vacuo to give **20** as a clear liquid (2.4 g, 89% yield). ^{1}H NMR ($CDCl_3$) δ 4.1 (q, 2H, O–CH_2), 2.58 (m, 1H, C2-H), 2.0 (m, 2H, C1-H, C4-H), 1.78–1.4 (m, 13H, C3-H_2, C5-H_2, C6-H_2, C7-H_2, C8-H_2, CH_3), 1.2 (t, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 176 (carbonyl carbon), 60.3 (O–CH_2), 42.1 (C2), 28.4 (C1), 27.6 (C3), 26.5 (C4), 25.4, 25.4, 24.0, 22.1 (C5, C6, C7, C8), 14.5 (CH_3).

2-Ethoxycarbonylbicyclo[2.2.2]oct-2-ene (21). A round-bottom flask was charged with diisopropylamine (11.1 g, 14.8 mL, 0.11 mol) and dry THF (300 mL). The solution was then cooled to −78 °C and to this was added dropwise 1.6 M *n*-butyllithium (75 mL, 0.12 mol). The yellow solution was allowed to stir for 60 min. To this was added **20** (17.3 g, 0.10 mol) dissolved in dry THF (40 mL) and the reaction allowed to stir for 60 min at −78 °C. To this solution was added phenylselenium bromide (25 g, 0.11 mol) dissolved in dry THF (50 mL). The reaction was allowed to warm to 0 °C and allowed to stir at 0 °C for 10 min. H_2O (60 mL) followed by 30% H_2O_2 (32 mL) and acetic acid (12 mL) were added and the reaction allowed to slowly warm to room temperature. The solution was poured into a mixture of 10% $NaHCO_3$ (600 mL) and CH_2Cl_2 (600 mL) and allowed to stir for 15 min. The entire solution was transferred to a separatory funnel and the layers separated. The organic layer was washed with 1 M HCl (300 mL), saturated NaCl (300 mL), and dried over Na_2SO_4. The solution was filtered and the solvent removed in vacuo. Column chromatography (R_f 0.61, 4.5:0.5 petroleum ether/diethyl ether) afforded **21** as a yellow oil (16.3 g, 95% yield). Distillation under vacuum (93–94 °C at <1 mm Hg) afforded **21** as a colorless liquid. ^{1}H NMR ($CDCl_3$) δ 7.3 (d, 1H, C3-H), 4.2 (q, 2H, C9-H_2), 3.2 (s, 1H, C1-H), 2.7 (d, 1H, C4-H), 1.6 (d, 4H, C5-H_2, C7-H_2), 1.2–1.4 (m, 7H, C6-H_2, C8-H_2, C10-H_2); ^{13}C NMR ($CDCl_3$) δ 165.4 (carbonyl carbon), 145.6 (C3), 138.0 (C2), 60.2 (O–CH_2), 31.0 (C1), 29.2 (C4), 25.6, 25.3 (C5, C6, C7, C8), 14.4 (CH_3). Anal. calcd For $C_{11}H_{16}O_2$: C, 73.33; H, 8.89; found: C, 72.37; H, 8.88. HRMS (EI) calcd for $C_{11}H_{16}O_2$ (m^+) 180.1150. Found 180.1149. IR (thin film) cm^{-1} 3052.6 (=CH stretch), 2945.1 (CH stretch), 1711.0 (carbonyl stretch), 1260.3 (C–O stretch), 1081.1 (O–C–C stretch).

2-Hydroxymethylbicyclo[2.2.2]oct-2-ene (22). A round-bottom flask was charged with **21** (16.3 g, 90 mmol) and dry CH_2Cl_2 (200 mL) and cooled to −78 °C. To this was added diisobutylaluminum hydride (180 mL of 1 M in hexanes, 0.18 mol) and the reaction allowed to stir for 2 h. The solution was then allowed to warm to −30 °C and CH_3OH (30 mL) was added. After 20 min, 100 mL of a 50:50 mixture of CH_3OH/H_2O was added and the reaction allowed to warm to room temperature and stirred for 30 min. The solution was filtered through Celite®, transferred to a separatory funnel, and the layers separated. The aqueous layer was then extracted with CH_2Cl_2 (100 mL), the organic layers combined, and dried over Na_2SO_4. The solution was filtered and the solvent removed in vacuo. Column chromatography (R_f 0.22, 4.5:0.5 petroleum ether:ethyl acetate) afforded **22** as a yellow oil (11.0 g, 88% yield). Distillation under vacuum (96–97 °C at <1 mm Hg) afforded **22** as a thick colorless oil in 85% overall yield. ^{1}H NMR ($CDCl_3$) δ 6.1 (d, 1H, C3-H), 4.2 (s, 2H, C9-H_2), 2.5 (m, 2H, C1-H, C4-H), 1.8 (s, 1H, OH), 1.6–1.2 (m, 8H, C5-H_2, C6-H_2, C7-H_2, C8-H_2); ^{13}C NMR ($CDCl_3$) δ 146.2 (C2), 127.7 (C3), 64.6 (C9), 31.6 (C1), 30.0 (C4), 26.5, 26.1 (C5, C6, C7, C8). Anal. calcd for $C_9H_{14}O$: C, 78.26; H, 10.14; found: C, 77.32; H, 10.11. HRMS (EI) calcd for $C_9H_{14}O$ (m^+) 138.1045. Found 138.1042. IR (thin film) cm^{-1} 3316.8 (OH stretch), 2940.0 (CH stretch), 1451.1 (OH bend), 1045.2 (C–O stretch).

2-Acetoxymethylbicyclo[2.2.2]oct-2-ene (23). A round-bottom flask was charged with **22** (5.0 g, 36 mmol), dry pyridine (6.8 g, 7.0 mL, 87 mmol), and dry CH_2Cl_2 (50 mL) and cooled to 0 °C. To this was added acetyl chloride (3.14 g, 2.8 mL, 40 mmol) dissolved in dry CH_2Cl_2 (10 mL). The reaction was allowed to stir at 0 °C for 20 min and at room temperature for 2 h. To this mixture was added H_2O (50 mL) and the solution transferred to a separatory funnel. The organic layer was removed and the aqueous layer extracted with CH_2Cl_2 (25 mL) and the combined organic layers dried over Na_2SO_4. The drying agent was filtered off and the solvent removed in vacuo. Column chromatography (R_f 0.59, 4.5:0.5 petroleum ether:ethyl acetate) afforded **23** as a yellow oil (3.9 g, 60% yield). Alternatively, vacuum distillation (100 °C at <1 mm Hg) afforded clean **23** as a colorless liquid in 90% yield. ^{1}H NMR ($CDCl_3$) δ 6.2 (d, 1H, C3-H), 4.6 (s, 2H, C9-H_2), 2.5 (m, 2H, C1-H, C4-H), 2.1 (s, 3H, CH_3), 1.6–1.2 (m, 8H, C5-H_2, C6-H_2, C7-H_2, C8-H_2); ^{13}C NMR ($CDCl_3$) δ 171.2 (carbonyl carbon), 141.2 (C2), 131.2 (C3), 65.9 (C9), 31.8 (C1), 30.1 (C4), 26.3, 25.9 (C5, C6, C7, C8), 21.2 (CH_3). Anal. calcd For $C_{11}H_{16}O_2$: C, 73.33; H, 8.89; found: C, 71.76; H, 8.83. HRMS (EI) calcd for $C_{11}H_{16}O_2$ (m^+) 180.1150 Found: 180.1145. IR (thin film) cm^{-1} 3032.2 (=CH stretch), 2940.0 (CH stretch), 1741.7 (carbonyl stretch), 1245.0 (C–O stretch), 1024.8 (O–C–C stretch).

2-Cyanomethylbicyclo[2.2.2]oct-2-ene (24). A round-bottom flask was charged with **23** (4.0 g, 23 mmol),

tetrakis(triphenylphosphine)palladium (1.37 g, 1 mmol), and dry toluene (75 mL). To this mixture was added $(CH_3)_3SiCN$ (4.7 g, 6.4 mL, 48 mmol) and the reaction allowed to reflux for 18 h. The reaction was cooled, filtered through Florisil®, and the solvent removed in vacuo. Column chromatography (R_f 0.58, 50:50 petroleum ether/CH_2Cl_2) afforded **24** as a yellow oil (3.0 g, 92% yield). Distillation under vacuum (101 °C at <1 mm Hg) provided **24** as a colorless liquid in 71% yield. ^{1}H NMR ($CDCl_3$) δ 6.25 (d, 1H, C3-H), 3.2 (s, 2H, C9-H_2), 2.6 (m, 1H, C4-H), 2.43 (s, 1H, C1-H), 1.7–1.2 (m, 8H, C5-H_2, C6-H_2, C7-H_2, C8-H_2); ^{13}C NMR with DEPT ($CDCl_3$) δ 134.6 (C2), 131.7 (C3), 117.7 (cyano), 33.9 (C1), 30.5 (C4), 26.3. 25.8 9 (C5, C6, C7, C8), 22.9 (C9). Anal. calcd For $C_{10}H_{13}N$: C, 81.63; H, 8.84; N, 9.52; found: C, 80.68; H, 8.85; N, 9.55. HRMS (EI) calcd for $C_{10}H_{13}N$ (m^+) 147.1048. Found: 147.1044. IR (thin film) cm^{-1} 3042.1 (=CH stretch), 2942.6 (CH stretch), 2249.8 (nitrile stretch).

2-(2-Aminoethyl)bicyclo[2.2.2]oct-2-ene (25). A round-bottom flask was charged with $LiAlH_4$ (1.22 g, 32 mmol) and dry THF (35 mL) and cooled to 0 °C. To this mixture was added **24** (2.4 g, 16 mmol) dissolved in dry THF (10 mL) and the reaction allowed to stir for 3 h. The reaction was slowly quenched with H_2O (25 mL) and 0.1 N NaOH (35 mL). The mixture was transferred to a separatory funnel, extracted twice with ethyl ether (75 mL), and the combined organic layers dried over Na_2SO_4. The drying agent was filtered off and the solvent removed in vacuo. Column chromatography (R_f 0.70, 4.0:0.5:0.5 $CHCl_3$/CH_3OH/$N(CH_3CH_2)_3$) afforded **25** as a yellow oil (1.85 g, 79% yield). ^{1}H NMR ($CDCl_3$) δ 5.9 (d, 1H, C3-H), 2.8 (t, 2H, C10-H_2), 2.5 (m, 1H, C4-H), 2.3 (s, 1H, C1-H), 2.2 (t, 2H, C9-H_2), 1.8–1.4 (m, 8H, C5-H_2, C6-H_2, C7-H_2, C8-H_2). As the hydrobromide salt ($CDCl_3$) δ 8.0 (broad s, 3H, NH_3^+), 6.1 (d, 1H, C3-H), 3.2 (m, 2H, C10-H_2), 2.61 (t, 2H, C9-H_2), 2.58 (d, 1H, C4-H), 2.4 (s, 1H, C1-H), 1.6–1.2 (m, remaining aliphatic protons); ^{13}C ($CDCl_3$): δ 140.3 (C2), 130.4 (C3), 38.3 (C10), 33.7 (C1), 32.5 (C9), 30.0 (C4), 26.1, 25.8 (C5, C6, C7, C8).

Substituted phenylacetyl chlorides (26). The acid chlorides that were condensed with amine **25** were synthesized from commercially available acids in the following manner. Under nitrogen, 18.05 mmol substituted phenylacetic acid and 27.08 mmol oxalyl chloride were added to 30 mL dry CH_2Cl_2 containing 1 drop of DMF. The reaction was stirred for 18 h at room temperature prior to evaporating solvent and excess oxalyl chloride. Dry diethyl ether (30 mL) was added to the isolated product until precipitation occurred. The precipitate was filtered, and the filtrate concentrated. Liquid products were purified by vacuum distillation (2 mm Hg). Purified yields approximated 90%. In the case of 3,4,5-trimethoxyphenylacetyl chloride, a solid was isolated when the crude acid chloride was allowed to stand in the cold. This solid acid chloride was purified by recrystallization from CCl_4 and hexane, and isolated in 60–70% purified yield. ***p*-Chlorophenylacetyl chloride**: ^{1}H NMR ($CDCl_3$) δ 7.5 (d, 2H, aromatic), 7.2 (d, 2H, aromatic), 4.18 (s, 2H, CH_2). ***p*-Methylphenylacetyl chloride**: ^{1}H NMR ($CDCl_3$) δ 7.2 (s, 4H, aromatic), 4.20 (s, 2H, CH_2), 2.40 (s, 3H, CH_3). **3,4,5-Trimethoxyphenyl acetyl chloride**: ^{1}H NMR ($CDCl_3$) δ 6.47 (s, 2H, aromatic protons), 4.08 (s, 2H, CH_2), 3.87 (two s, 9H, OCH_3).

[[[(Substituted benzyl)carbonyl]amino]ethyl]bicyclo[2.2.2]oct-2-ene (27). The amides generated from the condensation of amine **25** with the substituted acid chlorides **26** were prepared in the following manner. A solution of 8.28 mmol of **26** and 4.87 mL dry pyridine, under nitrogen, was taken to 0 °C. To this mixture was added, in a dropwise fashion, a solution of 8.28 mmol acid chloride **26** in 35 mL dry THF, and the solution stirred at room temperature for 24 h. The solvent and excess pyridine were removed in vacuo, H_2O (50 mL) and diethyl ether (100 mL) were added to the residue, and the phases were separated. The aqueous phase was extracted twice with diethyl ether, and the combined organic extracts washed sequentially with H_2O, 10% HCl, and dried over anhydrous Mg_2SO_4. The crude products were purified on a silica gel column. A gradient solvent system of 25–66% diethyl ether in petroleum ether was used to purify the *p*-methoxy, *p*-methyl, and *p*-chloro analogues. The trimethoxy analogue was purified by eluting with petroleum ether/diethyl ether (1:1) followed by 2–6% ethyl acetate in ethyl ether. The chromatographed amides were obtained in 64–75% yield and were cyclized directly without further purification. ***p*-Chlorobenzyl derivative**: ^{1}H NMR ($CDCl_3$) δ 7.3 (d, 2H, aromatic, 7.15 (d, 2H, aromatic), 5.75 (d, 1H, C3-H), 5.3 (broad s, 1H, NH), 3.5 (s, 2H, CH_2CO), 3.3 (m, 2H, C10-H_2), 2.5–2.2 (m, 2H, C1-H, C4-H), 2.15 (t, 2H, C9-H_2), 1.6–0.8 (m, 8H, C5-H_2, C6-H_2, C7-H_2, C8-H_2). ***p*-Methylbenzyl derivative**: ^{1}H NMR ($CDCl_3$) δ 6.90–7.20 (m, 4H, aromatic), 5.70–5.65 (d, 1H, C3-H), 5.45–5.35 (broad s, 1H, NH), 3.55 (s, 2H, CH_2CO), 3.35–3.25 (q, 2H, C10-H_2), 2.30 (s, 3H, CH_3), 2.20–2.10 (m, 4H, C1-H, C4-H, C9-H_2), 1.50–0.80 (m, 8H, C5-H_2, C6-H_2, C7-H_2, C8-H_2). **3,4,5-Trimethoxybenzyl derivative**: ^{1}H NMR ($CDCl_3$): δ 6.40 (s, 2H, aromatic), 5.75–5.65 (d, 1H, C3-H), 5.50 (broad s, 1H, NH), 3.80 (two s, 9H, OCH_3), 3.50 (s, 2H, CH_2CO), 3.40–3.20 (q, 2H, C10-H_2), 2.40–2.00 (m, 4H, C1-H, C4-H, C9-H_2), 1.50–0.95 (m, 8H, C5-H_2, C6-H_2, C7-H_2, C8-H_2).

5,8-Ethano-1-(*p*-chlorobenzyl)-1,2,3,4,5,6,7,8-octahydroisoquinoline (4) and 5,8-ethano-1-(*p*-chlorobenzyl)-5,6,7,8-tetrahydroisoquinoline (14). A single necked flask was purged with nitrogen, charged with 1.60 g (5.31 mmol) of the *p*-chlorobenzyl amide (**27**) and 135 mL dry toluene, and heated to 130 °C in an oil bath. In small portions over 90 min, 15.07 g (106.13 mmol) P_2O_5 was added. The reaction mixture was refluxed for 2–3 h, cooled to room temperature, and treated with 3.7 mL concentrated HCl and ice. After brief stirring, the toluene was removed in vacuo and the aqueous layer basified to pH 7–8 with concentrated ammonium hydroxide. The aqueous solution was extracted three times with 120 mL portions of $CHCl_3$, and the combined organic extracts dried over anhydrous Na_2SO_4. The 1.70 g (5.95 mmol) of crude red liquid imine **28** ($R^2 = R^4 = H$, $R^3 = Cl$) which resulted upon solvent

evaporation was dissolved in 20–30 mL MeOH. To this solution was added 483 mg (8.93 mmol) KBH_4 in small portions over 15–20 min, and the reaction was left to stand overnight. The MeOH was then removed in vacuo, and 30 mL H_2O and 100 mL ethyl acetate were added. The organic layer was separated and the aqueous layer extracted twice with 100 mL portions of ethyl acetate. The combined organic extracts were washed with 50 mL brine and dried over anhydrous Na_2SO_4. Concentration of the solvent provided 1.33 g crude liquid product which was purified chromatographically on a silica gel column. Compounds **4** and **14** were successfully separated by eluting sequentially with petroleum ether, ethyl ether, and a 2–5% solution of 2% NH_4OH/MeOH in diethyl ether. A 40% yield (590 mg) of **4** and a 20% yield (310 mg) of **14** were realized. Both products were converted to the hydrochloride salt form in the standard manner and recrystallized from MeOH/diethyl ether. **4**: mp hydrochloride: 192—193 °C. ^{1}H NMR ($CDCl_3$) δ 7.29–7.14 (m, 4H, aromatic protons), 3.55 (dd 1H, C1-H), 3.1–2.9 (m, 2H, C3-H, C11-H), 2.85–2.75 (m, 1H, C3-H), 2.6–2.35 (m, 2H, C5-H, C11-H), 2.25 (broad s, 1H, C8-H), 2.1 (t, 2H, C4-H_2), 1.8–1.6 (broad s, 1H, NH), 1.8–1.2 (m, 8H, remaining protons); ^{13}C NMR ($CDCl_3$) δ 138.48 (C12), 137.33 (C8a), 135.68 (C4a), 131.93 (C15), 130.45 (2C, C13, C17), 128.57 (2C, C14, C16), 56.17 (C1), 40.56 (C3), 38.94 (C11), 34.64 (C5), 31.17 (C8), 27.75 (C4), 27.01, 26.84, 26.07, 25.96 (C6, C7, C9, C10). Anal. calcd for $C_{18}H_{23}NCl_2$: C, 66.67; H, 7.10; N, 4.32; Cl, 21.91; found: C, 66.53; H, 7.03; N, 4.21; Cl, 22.23 (by difference). **14**: mp hydrochloride 175–175.5 °C. ^{1}H NMR ($CDCl_3$) δ 8.4 (d, 1H, C1-H), 7.4–7.0 (m, 4H, C13-H, C14-H, C16-H, C17-H), 6.9 (d, 1H, C4-H), 4.2 (s, 2H, C11-H_2), 3.2 (broad s, 1H, C5a-H), 2.9 (broad s, 1H, C8a-H), 2.0–1.1 (m, 8H, remaining protons); ^{13}C NMR ($CDCl_3$) δ 153.76, 152.90 (C1, C4a), 146.60 (C3), 138.81 (C12), 137.54 (C8a), 131.68 (C15), 129.85 (2C, C13, C17), 128.44 (2C, C14, C16), 117.93 (C4), 40.40 (C11), 33.69 (C5), 29.25 (C8), 25.30, 25.26 (4C, C6, C7, C9, C10). Anal. calcd For $C_{18}H_{19}NCl_2$: C, 67.50; H, 5.94; N, 4.38; found: C, 67.50; H, 5.99; N, 4.30.

5,8-Ethano-1-(*p*-methoxybenzyl)-1,2,3,4,5,6,7,8-octahydroisoquinoline (1) and 5,8-ethano-1-(*p*-methoxybenzyl)-5,6,7,8-tetrahydroisoquinoline (13). Utilizing a similar procedure to that outlined for the synthesis of **14**, 3.50 g (11.70 mmol) of the *p*-methoxybenzyl amide and 33.22 g (233.97 mmol) P_2O_5 were reacted to form the intermediate imine **28** ($R^2 = R^4 = H$, $R^3 = OCH_3$). Reduction of the crude imine with KBH_4, followed by chromatographic purification on a silica gel column with petroleum ether followed by a 5% solution of 5% NH_4OH/MeOH in diethyl ether, provided a 29% yield (960 mg) of **13**, along with a 30% yield (1 g) of **1**. Both products were converted to hydrochloride or methanesulfonate salts and purified by recrystallization. **1**: mp methanesulfonate 132–132.5 °C. ^{1}H NMR ($CDCl_3$) δ 7.15 (d, 2H, Ar–H, $J = 8$ Hz), 6.85 (d, 2H, Ar–H, $J = 8$ Hz), 3.79 (s, 3H, OCH_3), 3.51–3.47 (m, 1H, C1-H), 3.1–2.8 (m, 2H, C3-H, C11-H), 2.85–2.65 (m, 2H, C3-H, NH), 2.52 (m, 1H, C11-H), 2.40 (broad s, 1H, C5-H), 2.3 (broad s, 1H, C8-H), 2.15 (t, 2H, C4-H_2), 1.7–1.0 (m, 8H, remaining protons). This corresponds well with the spectrum obtained on **1** free base synthesized by the original method.[43] ^{13}C NMR ($CDCl_3$) δ 158.02 (C15), 137.34 (C8a), 135.28 (C4a), 131.70 (C12), 130.03 (2C, C13, C17), 113.90 (2C, C14, C16), 56.24 (C1), 55.20 (OCH_3), 40.30 (C3), 38.40 (C11), 34.55 (C5), 31.12 (C8), 27.62 (C4), 26.96, 26.80, 26.07, 25.94 (C6, C7, C9, C10). **13**: mp hydrochloride 146–148 °C. ^{1}H NMR ($CDCl_3$) δ 8.37 (d, 1H, C3-H, $J = 5$ Hz), 7.11 (d, 2H, C14-H, C16-H), 6.98 (d, 1H, C4-H, $J = 5$ Hz), 6.79 (dd, 2H, C13-H, C17-H, $J = 6$ Hz), 4.17 (s, 2H, C11-H_2), 3.75 (s, 3H, OCH_3), 3.24 (broad s, 1H, C8-H), 2.95 (broad s, 1H, C5-H), 1.85–1.1 (m, 8H, remaining protons); ^{13}C NMR ($CDCl_3$) δ 157.65 (C15), 153.68, 153.45 (C1, C4a), 146.31 (C3), 137.32 (C8a), 132.34 (C12), 129.29 (C13, C17), 117.55 (C4), 113.63 (2C, C14, C16), 55.0 (OCH_3), 40.10 (C11), 33.59 (C5), 29.08 (C8), 25.20 (4C, C6, C7, C9, C10). Anal. calcd for $C_{19}H_{22}NOCl \cdot H_2O$: C, 68.37; H, 7.20; N, 4.20; Cl, 10.64; found: C, 68.62; H, 7.32; N, 4.13; Cl, 10.42.

5,8-Ethano-1-(*p*-methylbenzyl)-1,2,3,4,5,6,7,8-octahydroisoquinoline (7) and 5,8-ethano-1-(*p*-methylbenzyl)-5,6,7,8-tetrahydroisoquinoline (15). Utilizing a similar procedure to that outlined for the synthesis of **4** and **13**, 1.35 g (4.75 mmol) of the *p*-methylbenzyl amide and 13.49 g (95 mmol) P_2O_5 were reacted to form the intermediate imine **28** ($R^2 = R^4 = H$, $R^3 = CH_3$). Reduction of the crude imine with KBH_4, followed by chromatographic purification on a silica gel column with petroleum ether followed by (1) diethyl ether, (2) a 2–5% solution of 2% NH_4OH/MeOH in diethyl ether, and (3) a 4–5% solution of 5% NH_4OH/MeOH in diethyl ether as eluant provided a 31% yield (390 mg) of **7**, along with a 30% yield (380 mg) of **15**. Both products were converted to hydrochloride salts and purified by recrystallization from MeOH/diethyl ether. **7**: mp hydrochloride 179–180 °C. ^{1}H NMR ($CDCl_3$) assignments verified by COZY: δ 7.15 (s, 4H, aromatic protons), 3.6 (dd, 1H, C1-H), 3.15–3.0 (m, 2H, C3-H, C11-H), 2.87 (s, 1H, NH), 2.86–2.78 (m, 1H, C3-H), 2.66–2.58 (m, 1H, C11-H), 2.47 (broad s, 1H, C5-H), 2.37 (s, 3H, Ar–CH_3), 2.3 (broad s, 1H, C8-H), 2.24–2.18 (t, 2H, C4-H_2), 1.62–1.25 (m, 8H, remaining protons); ^{13}C NMR ($CDCl_3$) δ 137.37 (C15), 135.17 (C12), 136.57 (C8a), 135.47 (C4a), 129.09, 128.91 (4C, C13, C14, C16, C17), 56.11 (C1), 40.23 (C3), 38.77 (C11), 34.48 (C5), 31.08 (C8), 27.59 (C4), 26.89, 26.75, 26.02, 25.90 (C6, C7, C9, C10), 20.89 (Ar-CH_3). IR (Thin film) cm^{-1}: 3100 (NH stretch), 2932 (CH stretch), 1108 (CH in plane bend), 767.3 (CH out of plane bend). Anal. calcd for $C_{19}H_{26}NCl$: C, 75.12; H, 8.57; N, 4.61; Cl, 11.70; found: C, 75.08; H, 8.76; N, 4.45; Cl, 11.22; Karl Fischer water = 1.52%. **15**: mp hydrochloride 198–199 °C. ^{1}H NMR ($CDCl_3$) δ 8.35 (d, 1H, C3-H, $J = 5$ Hz), 7.05 (m, 4H, C13-H, C14-H, C16-H, C17-H), 6.85 (d, 1H, C4-H, $J = 5$ Hz), 4.2 (s, 2H, C11-H_2), 3.25 (broad s, 1H, C5-H), 2.9 (broad s, 1H, C8-H), 2.2 (s, 3H, Ar–CH_3), 1.75–1.0 (m, 8H, remaining protons); ^{13}C NMR ($CDCl_3$) δ 153.54, 153.39 (C1, C4a), 146.29 (C3). 137.32, 137.10 (C15, C8a), 135.08 (C12), 128.86, 128.23 (C13, C14, C16, C17), 117.52 (C4), 40.54 (C11), 33.56 (C5), 29.06 (C8), 25.17 (4C, C6, C7, C9, C10), 20.81

(Ar–CH_3). Anal. calcd for $C_{19}H_{22}NCl$: C, 76.13; H, 7.35; N, 4.67; Cl, 11.85; found: C, 76.25; H, 7.43; N, 4.57; Cl, 11.59; Karl Fischer water = 0.62%.

5,8-Ethano-1-(3,4,5-trimethoxybenzyl)-1,2,3,4,5,6,7,8-octahydroisoquinoline (10) and 5,8-ethano-1-(3,4,5-trimethoxybenzyl)-5,6,7,8-tetrahydroisoquinoline (16). Utilizing a similar procedure to that outlined for the synthesis of **4** and **13**, 3.15 g (8.77 mmol) of the 3,4,5-trimethoxybenzyl amide and 24.90 g (175.38 mmol) P_2O_5 were reacted to form the intermediate imine **28** ($R^2 = R^3 = R^4 = OCH_3$). Reduction of the crude imine with KBH_4, followed by chromatographic purification on a silica gel column with diethyl ether followed by an 8–10% solution of 5% NH_4OH/MeOH in diethyl ether as eluant provided a 19% yield (580 mg) of **10**, along with a 17% yield (500 mg) of **16**. Both products were converted to methanesulfonate salts and purified by recrystallization. **10**: mp methanesulfonate 177–178 °C ^1H NMR ($CDCl_3$) δ 6.45 (s, 2H, aromatic protons), 3.85 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 3.55 (m, 1H, C1-H), 3.0 (m, 2H, C11-H, C3-H), 2.75 (m, 1H, C3-H), 2.6 (broad s, 1H, NH), 2.55–2.4 (m, 2H, C11-H, C8-H), 2.35–2.0 (m, 3H, C5-H, C4-H_2), 1.65–1.2 (m, 8H, remaining protons); ^{13}C NMR ($CDCl_3$) δ 153.13 (2C, C14, C16), 136.85 (C8a), 136.0 (C12), 135.62 (C4a), 135.20 (C15), 105.83 (2C, C13, C17), 60.77 (OCH_3), 56.02 (3C, C1,OCH_3), 40.76 (C3), 39.82 (C11), 34.53 (C5), 30.90 (C8), 27.43 (C4), 26.95, 26.78, 25.95, 25.87 (C6, C7, C9, C10). Anal. calcd for $C_{22}H_{33}NSO_6$: C, 60.14; H, 7.52; N, 3.19; found: C, 60.04; H, 7.77; N, 3.11. **16**: mp methanesulfonate 175–176 °C. ^1H NMR ($CDCl_3$) δ 8.35 (d, 1H, C3-H, $J = 5$ Hz), 7.0 (d, 1H, C4-H, $J = 5$ Hz), 6.4 (s, 2H, C13-H, C17-H), 4.2 (s, 2H, C11-H_2), 3.7 (s, 9H, OCH_3), 3.3 (broad s, 1H, C5-H), 2.95 (broad s, 1H, C8-H), 1.8–1.25 (m, 8H, remaining protons); ^{13}C NMR ($CDCl_3$) δ 154.23 (C1); 153.40 (3C, C4a, C14, C16), 146.55 (C3), 137.88 (C8a), 136.85 (C12), 136.07 (C15), 118.07 (C4), 106.24 (2C, C13, C17), 60.97 (OCH_3), 56.37 (2C, OCH_3), 41.30 (C11), 34.08 (C5), 29.64 (C8), 25.64, 25.55 (4C, C6, C7, C9, C10). Anal. calcd for $C_{22}H_{29}NSO_6$: C, 60.69; H, 6.67; N, 3.22; found: C, 60.70; H, 6.94; N, 3.18.

5,8-Ethano-1-(*p*-chlorobenzyl)-2-methyl-1,2,3,4,5,6,7,8-octahydroisoquinoline (5). A mixture of 200 mg (0.70 mmol) **4**, 116.89 mg (2.54 mmol) 98% HCO_2H, and 63 mg (2.10 mmol) 37% H_2CO was stirred for 10 min at room temperature and then refluxed 24 h in an oil bath at 110 °C. The volatile materials were removed in vacuo and the pH adjusted to 8 with a saturated $NaHCO_3$ solution. The emulsion was extracted three times with 50 mL portions of CH_2Cl_2 and dried over anhydrous Na_2SO_4. The crude product was purified by recrystallization of the hydrochloride salt from MeOH/diethyl ether to give 80 mg (40% yield) of crystalline product. Mp hydrochloride 205–206 °C. ^1H NMR ($CDCl_3$) δ 7.4–7.0 (s, 4H, aromatic protons), 3.4–3.5 (m, 1H, C1-H), 3.1–2.95 (m, 2H, C3-H, C11-H), 2.85–2.75 (m, 1H, C3-H), 2.5–2.65 (m, 1H, C11-H), 2.30 (s, 3H, NCH_3), 2.2 (broad s, 2H, C5-H, C4-H), 2.1 (broad s, 1H, C8-H), 2.1–1.9 (m, 1H, C4-H), 1.6–1.1 (m, 8H, remaining protons); ^{13}C NMR ($CDCl_3$) δ 139.66 (C12), 136.56 (C8a), 134.52 (C4a), 131.34 (C15), 130.32 (2C, C13, C17), 128.11 (2C, C14, C16), 63.75 (C1), 46.74 (C3), 42.33 (N–CH_3), 37.68 (C11), 34.43 (C5), 32.27 (C8), 26.80, 26.34 (2C), 25.94, (C6, C7, C9, C10), 23.59 (C4). Anal. calcd for $C_{19}H_{27}Cl_2NO{\cdot}0.5H_2O$: C, 65.71; H, 7.78; N, 4.03; Cl, 20.46; found: C, 65.31; H, 8.05; N, 4.03; Cl, 20.44.

5,8-Ethano-2-methyl-1-(*p*-methylbenzyl)-1,2,3,4,5,6,7,8-octahydroisoquinoline (8). Utilizing a similar procedure to that outlined for the synthesis of **5**, 190 mg (0.71 mmol) of **7**, 130.69 mg (2.84 mmol) 88% HCO_2H, and 63.9 mg (2.31 mmol) 37% H_2CO were reacted to form 170 mg crude **8** which was purified as the hydrochloride salt by recrystallization from MeOH/diethyl ether. A 25% yield (50 mg) of pure **8** hydrochloride was obtained. Mp hydrochloride 189–190 °C. ^1H NMR ($CDCl_3$) δ 7.0–7.2 (m, 4H, aromatic protons), 3.75 (m, 1H, C1-H), 3.55–3.4 (m, 2H, C3-H, C11-H), 3.15 (t, 1H, C3-H), 2.9–2.75 (dd, 1H, C11-H), 2.3 (s, 3H; NCH_3), 2.25 (s, 3H, Ar–CH_3), 2.2 (broad s, 2H, C5-H, C4-H), 2.05 (broad s, 2H, C8-H, C4-H), 1.5–1.1 (m, 8H, remaining protons); ^{13}C NMR ($CDCl_3$) δ 137.81 (C15), 136.86 (C8a), 135.10 (C12), 133.97 (C4a), 128.82, 128.78 (4C, C13, C14, C16, C17), 63.90 (C1), 46.71 (C3), 42.24 (N–CH_3), 38.01 (C11), 34.37 (C5), 32.26 (C8); 26.76, 26.31, 25.90 (2C) (C6, C7, C9, C10), 23.77 (C4), 21.00 (ArCH_3). Anal. calcd for $C_{20}H_{30}NCl{\cdot}0.5H_2O$: C, 73.50; H, 9.19; N, 4.29; Cl, 10.87; found: C, 73.38; H, 9.24; N, 4.20; Cl, 10.84.

5,8-Ethano-2-methyl-1-(3,4,5-trimethoxybenzyl)-1,2,3,4,-5,6,7,8-octahydroisoquinoline (11). Utilizing a similar procedure to that outlined for the synthesis of **5**, 62 mg (0.18 mmol) **10**, 25.9 mg (0.86 mmol) 37% HCO_2H and 18 mg (0.29 mmol $NaCNBH_3$ in 0.54 mL CH_3CN provided 50 mg (78% yield) of crude **11**, which was converted to the hydrochloride salt and recrystallized from MeOH/diethyl ether in the standard manner. Mp hydrochloride 145–146 °C. ^1H NMR ($CDCl_3$) δ 6.5 (s, 2H, aromatic protons), 3.9 (s, 6H, OCH_3), 3.85 (s, 3H, OCH_3), 3.2–3.1 (m, 1H, C1-H), 3.05–2.85 (m, 2H, C3-H, C11-H), 2.85–2.7 (t, 1H, C3-H), 2.7–2.55 (m, 1H, C11-H), 2.35 (s, 3H, NCH_3), 2.2 (broad s, 1H, C5-H), 2.1 (broad s, 1H, C8-H), 1.85 (m, 1H, C4-H), 1.6–1.1 (m, 8H, remaining protons); ^{13}C NMR ($CDCl_3$) δ 152.76 (2C C14, C16), 136.80, 136.70 (C8a, C15), 135.96 (C12), 134.30 (C4a), 105.76, 105.56 (C13, C17), 63.44 (C1), 60.77 (OCH_3), 55.94 (2C, OCH_3), 46.88 (C3), 42.32 (NCH_3), 38.62 (C11), 34.38 (C5), 32.17 (C8), 26.74, 26.33, 26.29, 25.91 (C6, C7, C9, C10), 23.66 (C4). Anal. calcd for $C_{22}H_{32}NO_3Cl{\cdot}1.33H_2O$: C, 63.23; H, 8.30; N, 3.35; found: C, 63.43; H, 8.30; N, 3.10.

5,8-Ethano-1,2-di-(*p*-methoxybenzyl)-1,2,3,4,5,6,7,8-octahydroisoquinoline (3). $CHCl_3$ (0.73 mL) was added to 100 mg (0.35 mmol) **1**, and to this solution was added 75 mg (0.70 mmol) Et_3N and 54.81 mg (0.35 mmol) *p*-methoxybenzyl chloride. The reaction was stirred at room temperature for 20 h prior to dilution with 50 mL CH_2Cl_2 and 20 mL H_2O. The layers were separated and the aqueous layer extracted twice with 50 mL portions

of CH_2Cl_2. The combined organic extracts were washed with 20 mL each of H_2O and brine, and dried over anhydrous Na_2SO_4. Purification was accomplished through silica gel chromatography utilizing a 2% solution of 2% NH_4OH/MeOH in diethyl ether as eluant. A 78% yield (110 mg) of purified **3** was obtained. The hydrochloride salt was formed in the standard manner and recrystallized from MeOH/diethyl ether. Mp hydrochloride 90–91 °C. ^{1}H NMR ($CDCl_3$) δ 7.04 (d, 2H, C20-H, C24-H, J = 9 Hz); 6.92 (d, 2H, C21-H, C23-H, J = 9 Hz), 6.77 (d, 2H, C13-H, C17-H, J = 9 Hz), 6.68 (d, 2H, C14-H, C16-H, J = 8 Hz), 3.80 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.54–3.42 (dd, 3H, C18-H_2, C1-H, J = 13 Hz), 3.22–3.21 (m, 1H, C3-H), 2.99 (t, 1H, C11-H), 2.75–2.69 (m, 2H, C3-H, C11-H), 2.25 (broad s, 1H, C5-H), 2.15 (broad s, 1H, C5-H), 1.85–1.75 (m, 2H, C4-H_2), 1.65–1.1 (m, 8H, remaining protons); ^{13}C NMR ($CDCl_3$) δ 158.14 (C22), 157.61 (C15), 137.15 (C8a), 134.14 (C4a), 133.19 (C12), 132.05 (C19), 130.19, 129.67 (4C, C13, C17, C20, C24), 113.19 (4C, C14, C16, C21, C23), 61.13 (C1), 56.52 (C18), 55.23, 55.15 (OCH_3), 43.25 (C3), 38.45 (C11), 34.46 (C5), 32.53 (C8), 26.78 (C11), 26.40 (2C), 26.21 (4C, C6, C7, C9, C10), 21.94 (C4). Anal. calcd for $C_{27}H_{34}ClNO{\cdot}0.75H_2O$: C, 71.52; H, 7.95; N, 3.09; Cl, 7.84; found: C, 71.52; H, 7.94; N, 2.96; Cl, 7.86.

5,8-Ethano-1-(*p*-chlorobenzyl)-2-(*p*-methoxybenzyl)-1,2,3,4,5,6,7,8-octahydroisoquinoline (6). Utilizing a similar procedure to that outlined for the synthesis of **3**, 150 mg (0.53 mmol) **4** in 1 mL $CHCl_3$ was reacted with 107.66 mg (1.06 mmol) Et_3N and 82.22 mg (0.53 mmol) *p*-methoxybenzyl chloride to produce 210 mg (98% yield) of **6** which was purified as the hydrochloride salt by recrystallization from MeOH/diethyl ether. Mp 152–153 °C. ^{1}H NMR ($CDCl_3$) δ 7.15 (d, 2H, C14-H, C16-H, J = 8 Hz); 7.0 (d, 2H, C13-H, C17-H, J = 8 Hz), 6.8 (d, 2H, C20-H, C24-H, J = 8 Hz), 6.6 (d, 2H, C21-H, C23-H, J = 8 Hz), 3.8 (s, 3H, OCH_3), 3.65–3.35 (m, 3H, C1-H, C18-H_2), 3.3–3.1 (m, 1H, C3-H), 3.0–2.85 (t, 1H, C11-H), 2.8–2.5 (m, 2H, C3-H, C11-H), 2.25 (broad s, 1H, C5-H), 2.15 (broad s, 1H, C8-H), 1.9–1.7 (m, 2H, C4-H_2), 1.4–1.1 (m, 8H, remaining protons); ^{13}C NMR ($CDCl_3$) δ 158.23 (C22), 139.52 (C12), 136.72 (C8a), 134.60 (C4a), 131.79 (C19), 131.14 (C15), 130.73 (2C, C13, C17), 129.71 (2C, C20, C24), 127.74 (2C, C14, C16), 113.20 (2C, C21, C23), 60.66 (C1), 56.49 (C18), 55.20 (OCH_3), 43.45 (C3), 38.56 (C11), 34.50 (C5), 32.55 (C8), 26.80, 26.48, 26.42, 26.24 (4C, C6, C7, C9, C10), 21.80 (C4). Anal. calcd for $C_{26}H_{31}NOCl_2{\cdot}0.5H_2O$: C, 68.86; H, 7.28; N, 3.09; Cl, 15.67; found: C, 69.11; H, 7.34; N, 3.11, Cl, 15.86.

5,8-Ethano-2-(*p*-methoxybenzyl)-1-(*p*-methybenzyl)-1,2,-3,4,5,6,7,8-octahydroisoquinoline (9). Utilizing a similar procedure to that outlined for the synthesis of **3**, 60 mg (0.22 mmol) **7**, 45.21 mg (0.45 mmol) Et_3N, and 37.05 mg (0.22 mmol) *p*-methoxybenzyl chloride in 0.44 mL $CHCl_3$ were reacted to produce 60 mg (70% yield) of **9**, which was purified as the hydrochloride salt by recrystallization from MeOH/diethyl ether. Mp hydrochloride 164–165 °C. ^{1}H NMR ($CDCl_3$) δ 7.0 (s, 4H, C13-H, C14-H, C16-H, C17-H), 6.9 (d, 2H, C20-H, C24-H, J = 8 Hz), 6.6 (d, 2H, C21-H, C23-H, J = 8 Hz), 3.8 (s, 3H, OCH_3), 3.5 (m, 3H, C1-H, C18-H_2), 3.2 (m, 1H, C3-H), 3.0 (t, 1H, C11-H), 2.7 (m, 3H, C3-H, C11-H), 2.35 (s, 3H, Ar-CH_3), 2.3 (broad s, 1H, C5-H), 2.15 (broad s, 1H, C8-H), 1.85–1.65 (m. 2H, C4-H_2), 1.6–1.15 (m, 8H, remaining protons); ^{13}C NMR ($CDCl_3$) δ 158.47 (C22), 138.17 (C15), 137.48 (C8a), 135.06 (C12), 134.41 (C4a), 132.25 (C19), 130.02 (2C, C20, C24), 129.51 (2C, C13, C17), 128.76 (2C, C14, C16), 113.5 (2C, C21, C23), 61.47 (C1), 56.81 (C18), 55.44 (OCH_3), 43.49 (C3), 39.27 (C11), 34.77 (C5), 32.82 (C8), 27.09, 26.74 (2C), 26.51 (4C, C6, C7, C9, C10), 22.26 (C4), 21.33 ($ArCH_3$). Anal. calcd for $C_{27}H_{34}NOCl$: C, 76.50; H, 7.08; N, 3.30; Cl, 8.38; found: C, 76.10; H, 8.46; N, 3.01; Cl, 8.30; Karl Fischer water < 1.19%.

5,8-Ethano-2-(*p*-methoxybenzyl)-1-(3,4,5-trimethoxybenzyl)-1,2,3,4,5,6,7,8-octahydroisoquinoline (12). Utilizing a similar procedure to that outlined for the synthesis of **3**, 82 mg (0.24 mmol) **10**, 59.21 mg (0.48 mmol) Et_3N, and 45.22 (0.24 mmol) *p*-methoxybenzyl chloride in 0.56 mL $CHCl_3$ gave crude **12**. The compound was purified by silica gel chromatography utilizing diethyl ether/petroleum ether (1:1), diethyl ethyl, and 5% MeOH in $CHCl_3$ as eluant. A 65% yield (72 mg) of purified **12** was obtained. The compound was converted to the methanesulfonate salt in the standard manner and recrystallized from MeOH/diethyl ether. Mp methanesulfonate 150–151 °C. ^{1}H NMR ($CDCl_3$) δ 6.95 (d, 2H, C20-H, C24-H, J = 8 Hz), 6.70 (d, 2H, C21-H, C23-H, J = 8 Hz), 6.3 (s, 2H, C13-H, C17-H), 3.6–3.35 (m, 3H, C1-H, C18-H_2), 3.3–3.1 (m, 1H, C3-H), 3.05–2.95 (m, 1H, C11-H), 2.85–2.6 (m, 2H, C3-H, C11-H), 2.2 (broad s, 1H, C5-H), 2.1 (broad s, 1H, C8-H), 1.9–1.4 (m, 2H, C4-H_2), 1.65–1.15 (m, 8H, remaining protons); ^{13}C NMR ($CDCl_3$) δ 158.17 (C22), 152.50 (2C, C14, C16), 136.88, 136.76 (C15, C8a), 136.0 (C12), 134.32 (C4a), 131.88 (C19), 129.76 (2C, C20, C24), 113.08 (2C, C21, C23), 106.10 (2C, C13, C17), 60.80, 60.58 (C1, OCH_3), 56.47 (C18), 55.83, 55.08 (2C, OCH_3), 43.53 (C3), 39.61 (C11), 34.42 (C5), 32.44 (C8), 26.78, 26.44, 26.38, 26.17 (4C, C6, C7, C9, C10), 21.95 (C4). Anal. calcd for $C_{30}H_{40}NSO_7{\cdot}0.75H_2O$: C, 71.52; H, 7.95; N, 3.09; S, 7.84; found: C, 71.52; H, 7.94; N, 2.96; S, 7.86.

5,8-Ethano-2-formyl-1-(*p*-chlorobenzyl)-1,2,3,4,5,6,7,8-octahydroisoquinoline (17). To a solution of 60 mg (0.21 mmol) **4** in 2.6 mL benzene was added 0.60 mL (544 mg, 7.35 mmol) ethyl formate, and the reaction mixture heated to 50 °C for 24 h. The reaction was cooled and the excess reactant and solvent removed in vacuo. Diethyl ether (50 mL) was added to the residue, the solution washed sequentially with 20 mL H_2O, 20 mL 10% HCl, and 20 mL H_2O, and dried over anhydrous Na_2SO_4. When concentrated, 40 mg (61% yield) of crude **17** was obtained. The compound was purified via silica gel chromatography utilizing petroleum ether/diethyl ether (1:1) as eluant. A 45% purified yield (30 mg) was obtained. Mp 120–121 °C. ^{1}H NMR ($CDCl_3$) δ (two isomers) 7.95, 7.5 (s, 1H, CHO), 7.3–6.8 (m, 8H, aromatic protons), 4.95 (t, 1H, C1-H), 4.5 (dd, 1H, C1-H), 3.9 (broad d, 1H, C3-H), 3.5 (dd, 1H, C3-

H), 3.1–2.4 (m, 6H, C11-H_2, C3-H_2), 2.9 (t, 2H, C4-H_2), 2.45 (broad s, 2H, C5-H_2), 2.35–2.2 (broad s and m, 4H, C8-H_2, C4-H_2), 1.7–1.1 (m, 16H, remaining protons); ^{13}C NMR ($CDCl_3$) selected δ (two isomers) 161.28, 161.22 (CHO), 136.34, 136.24 (C12), 135.22, 135.04 (C8a), 134.84 (C4a), 132.68 (C15), 130.73, 130.56 (C13, C17), 128.87, 128.27 (C14, C16), 58.42 (C1), 51.16 (C3), 40.63 (C11), 34.34, 33.80 (C5), 32.14, 31.91 (C8), 27.68 (C4). Anal. calcd for $C_{19}H_{22}NOCl$: C, 72.26; H, 6.97; N, 4.43; found: C, 72.39; H, 7.22; N, 4.08. Karl Fischer water = <3.19%.

5,8-Ethano-1-(*p*-chlorobenzyl)-2,2-dimethyl-1,2,3,4,5,6,7,8-octahydroisoquinoline iodide (18). To a solution of 60 mg (0.20 mmol) **5** in 4 mL MeOH was added 0.2 mL MeI and 200 mg $KHCO_3$. The mixture was stirred at room temperature for 24 h and the excess solvent and CH_3I removed in vacuo. After filtration and concentration, 67 mg (76% yield) crude **18** was obtained. Purification was by recrystallization from MeOH/diethyl ether. Mp 198–199 °C. ^{1}H NMR ($CDCl_3$) δ 7.50 (d, 2H, aromatic protons, J = 8 Hz), 7.29 (dd, 2H, aromatic protons), 4.66 (t, 1H, C1-H, J = 7 Hz), 3.85 (broad m, 2H, C3-H, C11-H), 3.6–3.45 (m, 4H, NCH_3, C3-H), 3.35 (s, 3H, NCH_3), 2.9–3.1 (m, 1H, C11-H), 2.7–2.5 (m, 2H, C4-H_2), 2.45 (broad s, 1H, C5-H), 1.85 (broad s, 1H, C8-H), 1.6–1.0 (m, 8H, remaining protons); ^{13}C NMR ($CDCl_3$) δ 134.55 (C12), 133.58, 133.29 (C8a, C4a), 132.37 (C15), 131.11 (2C, C13, C17), 128.94 (2C, C14, C16), 71.68 (C1), 55.71 (C3), 52.23 (NCH_3), 50.64 (NCH_3), 36.23 (C_{11}), 33.56, 33.30 (C8, C5), 26.59, 25.58, 24.92 (2C) (C6, C7, C9, C10), 23.22 (C4). Anal. calcd for $C_{20}H_{27}NClI$: C, 53.90; H, 6.06; N, 3.15; found: C, 53.93; H, 5.94; N, 3.04; Karl Fischer water <1.44%.

1,4-Ethano-1,3,5,8,13,13a-hexahydro-9,10-dimethoxy-6*H*-dibenzo[*a*,*g*]quinolizine (29). A mixture of 90 mg (0.26 mmol) **10**, 45.46 mg (0.95 mmol) H_2CO_2 and 170.86 mg (0.76 mmol) 37% H_2CO was refluxed for 24 h. Volatile materials were removed in vacuo, the pH adjusted to 8 with saturated $NaHCO_3$ and the resulting emulsion extraced with CH_2Cl_2. After drying over Na_2SO_4, the solvent was evaporated to give 30 mg (0.0845 mmol) **29**. The product was purified via silica gel column chromatography and sequentially eluted with the following: petroleum ether, 1:1 petroleum ether/diethyl ether, diethylether and 5% MeOH in $CHCl_3$. ^{1}H NMR ($CDCl_3$) δ 6.45 (s, 2H, C12-H_2), 4.1–3.9 (2s, 2H, C8-H_2), 3.85–3.75 (3s, 9H, OCH_3), 3.45–3.3 (2s, 1H, C14-H), 3.15–3.0 (m, 3H, C13-H, C6-H_2), 2.75–2.6 (m, 1H, C13-H), 2.6 (s, 1H, C5-H), 2.55–2.45 (m, 1H, C4-H), 2.35 (broad s, 1H, C1-H), 2.1–1.95 (broad d, 1H, C5-H), 1.7–1.2 (m, 8H, C2-H_2, C2′-H_2, C3-H_2, C3′-H_2); ^{13}C NMR ($CDCl_3$) δ 151.7 (C9), 149.7 (C11), 139.8 (C10), 135.7 (C4a), 14.6 (C14a), 130.3 (C12a), 120.9 (C8a), 107.2 (C12), 60.8, 60.4 (C9-OCH_3, C11-OCH_3), 58.2 (C10-OCH_3), 55.8 (C14), 53.1 (C8), 51.6 (C6), 34.3, 33.9 (C1, C4), 29.8 (C5), 27.3, 26.8, 25.9, 25.8 (C2, C2′, C3, C3′).

Platelet aggregation

Platelet preparation. Human blood (55–110 mL) was drawn from medial cubital vein of donors, and mixed with 0.1 M sodium citrate (9:1 = blood/sodium citrate). Platelet-rich plasma (PRP) was prepared by centrifugation of the blood sample at 200*g* (Sorvall RT 6000, DuPont Company) for 15 min at room temperature, and the supernatant transferred as PRP. An aliquot of PRP (1 mL) was further centrifuged at 2000*g* for 2 min in an Eppendorf microcentrifuge (5415C, Brinkmann Instruments Inc., Germany), and the supernatant was utilized as platelet-poor plasma (PPP). Aliquots (900 μL) of PPP were added to 180 μL phosphate buffer (10 mM sodium phosphate, 0.9% NaCl, pH 7.4) to make diluted PPP. Diluted PPP (450 μL) was used to set the 100% transmittance for experiments.

Protocol. Preliminary studies were conducted to determine whether each compound was an inducer of platelet aggregation. Antagonist activities were determined by incubating varying concentrations of each compound with PRP for 1 min prior to addition of U46619. Monophasic platelet aggregation by U46619, a TXA_2 agonist, was measured in aspirin-treated human platelets. Cuvettes contained a final volume 420 μL of appropriate amount of phosphate buffer (10 mM), aspirin (1 mM), PRP (350 μL) and U46619 (0.5–1.5 μM) with a magnetic teflon stir bar spinning at 1000 rpm at 37 °C for 4 min.

Measurements. Aggregometers (Chrono-Log Corp., Model 560-VS and Model 660, Havertown, PA, USA) were interfaced with personal computers to analyze changes in aggregation. Changes in turbidity of the samples in the aggregometer were acquired, monitored and stored using the computer program (AGGRO/LINK, 1993). As platelets aggregate, the turbidity of PRP decreases with a corresponding increase in light transmittance. Aggregation responses for each experiment were measured and expressed as percentage of the maximal light transmittance (T_{max}) in the presence of inducer.

Data analysis. Values of IC_{50} and pIC_{50} for each antagonist on aggregation were calculated using the GraphPad Prism computer program.[67] Data represent the mean ± SEM of n = 3–6 experiments.

Platelet binding

Isolation of washed platelets. PRP was used in preparing washed platelets as described previously.[68] PRP and PGE_1 (1 μM final) were gently mixed and spun at 1000*g* (Clay-Adams, Inc.) for 4 min. The sediment was collected, resuspended in 50 mM Tris–saline (7 g/L Trizma HCl, 0.67 g/L Trizma base and 6.53 g/L NaCl, pH 7.4) containing 5 mM EGTA and 1 μM PGE_1, and recentrifuged at 1000*g* for 4 min. Platelets were resuspended in Tris–saline in the presence of 1 μM PGE_1 and centrifuged again. Platelet concentration was determined with a hemocytometer and adjusted so the final suspension contained 1×10^9 intact platelets/mL in Tris–saline.

Measurement of competitive equilibrium binding. The specific binding of [^{3}H]SQ29,548 (1 or 5 nM final, specific activity 46 Ci/mmol, DuPont Company, Wilmington,

DE, USA) to human platelets (2×10^8 platelets/mL final) was conducted in a final volume of 0.5 mL at room temperature. Nonspecifically bound ligand was defined by 50 μM [^{3}H]SQ29,548, and specific binding was determined as the difference between total binding and nonspecific binding. Specific binding varied between 94.5 and 98.1% of total binding. Inhibition of specifically bound radioligand by bicyclic octahydroisoquinoline analogues was determined using various concentrations of the analogues after a 30 min incubation. Samples were rapidly filtered by vacuum through Whatman GF/C fiber filters on a Brandel cell harvester (Model M 12-RI), and washed three times (6 mL each) with ice-cold 50 mM Tris–saline buffer (pH 7.4). The filter disks were transferred to plastic vials containing 10 mL of scintillation cocktail (Ultima Gold™XR, Packard Instrument Company), and vortexed. [^{3}H] was measured in a liquid scintillation spectrometer (LKB Wallac Liquid Scintillation, Model 1219 Rackbeta).

Data analysis. Percent specific binding was calculated as (total binding−non-specific binding)/total binding× 100%. Individual inhibitory concentrations reducing half of the maximal responses (IC_{50} values) were determined using GraphPad Prism. K_I values were obtained using $IC_{50}/(1+[\text{ligand}]/K_d)$, where K_d (3.1 nM) was taken from previously published work.[69] Potency ratio (P.R.) values were calculated as (IC_{50} or K_I TMQ)/(IC_{50} or K_I) bicyclohydroisoquinolines, where values for TMQ were used as reference standard for these studies. Linear regression for correlation of aggregation (pIC_{50}) and binding (pK_I) was plotted and analyzed. Data represent the mean ± SEM of $n=3$–6 experiments.

Acknowledgements

The authors gratefully acknowledge financial support for this project from the Nebraska Department of Health. The Nebraska Center for Mass Spectrometry, which performed the high resolution analysis of selected bicyclic molecules, is also acknowledged.

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