Bioorganic & Medicinal Chemistry 19 (2011) 7551–7558

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Further structure–activity relationship studies on 8-substituted-3-[2-(diarylmethoxyethylidenyl)]-8-azabicyclo[3.2.1]octane derivatives at monoamine transporters

Shaine A. Cararas [a], Sari Izenwasser [b], Dean Wade [b], Amy Housman [b], Abha Verma [a], Stacey A. Lomenzo [a], Mark L. Trudell [a,*]

[a] Department of Chemistry, University of New Orleans, New Orleans, 2000 Lakeshore Drive, LA 70148, USA
[b] Department of Psychiatry and Behavioral Sciences, University of Miami Miller School of Medicine, Miami, FL 33136, USA

ARTICLE INFO

Article history:
Received 26 August 2011
Revised 5 October 2011
Accepted 10 October 2011
Available online 18 October 2011

Keywords:
GBR 12909
Dopamine transporter
Serotonin transporter
Norepinephrine transporter
Dopamine uptake
Cocaine
Tropane

ABSTRACT

The synthesis and structure–activity relationships of 8-substituted-3-[2-(diarylmethoxyethylidenyl)]-8-azabicyclo[3.2.1]octane derivatives were investigated at the dopamine transporter (DAT), the serotonin transporter (SERT) and norepinephrine transporter (NET). The rigid ethylidenyl-8-azabicyclic[3.2.1]octane skeleton imparted modestly stereoselective binding and uptake inhibition at the DAT. Additional structure–activity studies provided a transporter affinity profile that was reminiscent of the structure–activity of GBR 12909. From these studies, the 8-cyclopropylmethyl group has been identified as a unique moiety that imparts high SERT/DAT selectivity. In this study the 8-cyclopropylmethyl derivative **22e** (DAT K_i of 4.0 nM) was among the most potent compounds of the series at the DAT and was the most DAT selective ligand of the series (SERT/DAT: 1060). Similarly, the 8-chlorobenzyl derivative **22g** (DAT K_i of 3.9 nM) was found to be highly selective for the DAT over the NET (NET/DAT: 1358).

1. Introduction

Despite recent trends showing a slight downward trend in illicit drug use since 1990s, cocaine abuse in any form, either as the hydrochloride salt or as the free base Crack, remains a significant problem among adolescents and adults in the United States. Reports indicate that as recently as 2009, over 1.6 million Americans aged 12 and over were current cocaine users.[1] Although this is a small portion of the total population the societal costs to American citizens are estimated to be in the billions of dollars based on costs related to decreased productivity, crime, and health care.[2] Unfortunately, to date there is no effective medication available for the treatment of cocaine-addicted individuals.

During the past decade tremendous progress has been made towards understanding the role of the dopamine transporter (DAT) in the pharmacological mechanism of cocaine addiction. However, despite these advances, a clinically approved medication for cocaine addiction targeting the DAT has yet to be identified.[3] Some recent studies have suggested that the DAT may not be the sole contributor to the addiction pathology of cocaine and that the serotonin transporter (SERT) is also involved in the brain circuitry of cocaine addiction.[4–7] However, there remains overwhelming evidence that indicates cocaine occupation of binding sites on the DAT contributes significantly to the stimulant, reinforcing effects and abuse liability associated with cocaine use.[8–16] As such, the DAT remains an important target for medication development and selective DAT inhibitors may yet prove to be viable medications for cocaine abuse.[17–19]

Although a plethora of structure–activity studies on a variety of compound classes that exhibit high affinity for the DAT have been completed,[20–26] a highly selective DAT inhibitor that would mirror the selective serotonin reuptake inhibitor (SSRI) antidepressants (e.g., Citalopram and Paroxetine) has yet to be discovered.[27] There are several DAT ligands that exhibit 100-fold selectivity over SERT and norepinephrine transporters (NET) in binding affinity, and there are very few ligands that are DAT selective by three orders of magnitude over SERT.[28–30] However, there exists no DAT ligands that inhibit dopamine, serotonin and norepinephrine uptake with comparable selectivity.[3] This disconnection between DAT binding affinity and dopamine uptake inhibition has been suggested to be one of the problems that may be contributing to the lack of success in advancing a DAT selective agent for clinical development as a cocaine medication.[3]

The aryl-1,4-dialkylpiperazines GBR 12909 (**1**) and GBR 12935 (**2**) have served as useful pharmacological probes for the

* Corresponding author. Tel.: +1 504 280 7337; fax: +1 504 280 6860.
E-mail addresses: MLTCM@jazz.ucc.uno.edu, mtrudell@uno.edu (M.L. Trudell).

0968-0896/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2011.10.028

development of selective dopamine uptake inhibitors.[31] Early studies with GBR 12909 explored the potent and somewhat selective affinity of these ligands for the DAT.[32] This selectivity prompted further investigations with **1** that led to the identification of GBR 12909 as a potential agonist-based substitution therapy for cocaine addiction.[33–35] It exhibited slower onset and longer duration of action than cocaine while possessing much higher affinity for the DAT with a slower dissociation rate. GBR 12909 was also shown to antagonize cocaine-induced elevation of extracellular dopamine levels.[35] The favorable pharmacological profile of **1** led to in vivo studies that demonstrated the ability of GBR 12909 to suppress cocaine self-administration behavior in rhesus monkeys,[36] and exhibit non-stimulant properties in humans.[37] Although the development of GBR 12909 as a cocaine medication was halted after Phase 1 human safety studies identified problematic cardiovascular effects, it has served as an important template for structure activity studies directed toward cocaine medication development.[26]

While studying the structure–activity relationships (SAR) of GBR 12909, a variety of ring systems have been shown to replace the piperazine scaffold and provide compounds with high affinity for the DAT. These include various piperidine (e.g., **3** and **4**) and tropane (e.g., **5** and **6**) scaffolds.[25,26,38,39] These studies have led to the development of a well-defined pharmacophore for high affinity and selectivity.[26] Previous efforts in our laboratories have identified a novel class of GBR-related 8-alkylaryl-3-[2-(diarylmethoxyethylidenyl)]-8-azabicyclo[3.2.1]octane derivatives that exhibit potent and selective affinity for the DAT.[39,40] These preliminary in vitro studies demonstrated GBR-analogues **7**–**12** exhibited GBR-like affinity at the DAT but were up to 300-fold more selective for the DAT over the SERT.[38,39] The potent and highly selective pharmacological profile of these 8-alkylaryl-3-[2-(diarylmethoxyethylidenyl)]-8-azabicyclo[3.2.1]octane derivatives has prompted further investigation of the SAR of this novel class of DAT ligands in search of potent highly selective ligands. It is well documented that N-substitution on 3-phenyltropane-based and benztropine-based DAT inhibitors has little effect on affinity and selectivity, while N-substitution on piperidine analogues of GBR 12909 has been shown to improve DAT selectivity over SERT.[25,41] In lieu of the structural similarities of our lead compound **12** with **1** and the piperidine derivative **3**, our attention has focused on the substituents attached to the nitrogen atom of the tropane ring system to optimize ligand-transporter selectivity. Herein we report the synthesis and biological evaluation at monoamine transporters of a series of novel highly DAT selective 8-substituted-3-[2-(diarylmethoxyethylidenyl)]-8-azabicyclo[3.2.1]octane derivatives (Fig. 1).

2. Results and discussion

2.1. Chemistry

The synthesis of the 8-substituted-3-diarylmethoxyethylidenyl-8-azabicyclo[3.2.1]octane analogues commenced with the protection of the nitrogen atom of 3-tropinone (**13**). Previous studies had revealed that reduction of the basicity of the nitrogen atom significantly enhanced the yield of subsequent reactions on the tropane scaffold, presumably by facilitating the chromatography.[39] This enhancement of the overall yield was worthy of the additional steps involved in the protection/deprotection chemistry. To this end, the carbamate **14** was prepared in 92% on a multigram scale (Scheme 1). The carbamate **14** was subjected to the Masamune–Roush modification of the Horner–Wadsworth–Emmons olefination reaction and afforded the ethylidenyl ester **15** in 87% yield.[42] Reduction of the ester with lithium aluminium hydride furnished the allylic alcohol **16** in 89% yield. The allylic alcohol **16** was then converted into the corresponding benzhydryl ethers **17** or **18** by heating at 145 °C in neat chlorodiphenylmethane or bis(4-fluorophenyl)methylchloride, respectively. This procedure required no workup and after column chromatography of the reaction mixture the ethers **17** (59%) and **18** (66%) were obtained in good yields. The ethers **17** and **18** were converted into the corresponding *N*-secondary amine **19** and **20**, by decarbonylation with hydrazine monohydrate. Concomitant N-alkylation with the appropriate alkylbromide furnished the 8-substituted-3-diarylmethoxyethylidenyl-8-azabicyclo[3.2.1]octane **21** and **22** in overall yields of 30–60% for the two-step process.

2.2. Biology

All compounds were tested as the oxalate salts. Binding affinities for the dopamine, serotonin and norepinephrine transporters were determined by the ability of the drug to displace the radiolabeled ligands [^{3}H]WIN 35,428, [^{3}H]citalopram, and [^{3}H]nisoxetine, respectively, from the monoamine transporters obtained from rat brain tissue using previously reported assays.[40,43,44] The binding affinities of all compounds listed in Table 1 were initially determined for the DAT. The compounds that exhibited DAT binding affinities with K_i values <100 nM were evaluated at the serotonin and norepinephrine transporters to determine transporter selectivity. Those compounds that exhibited low binding affinity (K_i >100 nM) at the DAT were typically not evaluated further.

2.3. Structure–activity relationships

As expected the 8-alkyl desfluorobenzhydryl analogues **21a**, **21b**, **21c**, **21d** and **21e** were found to exhibit modest affinity for the DAT, similar to the 8-methyl analogue **7** (K_i value of 130 nM) previously reported by Zhang and coworkers.[38] The ethyl analogue **21a** was equipotent with the propyl analogue **21b**. However, the allyl analogue **21c** was more potent than the propyl analogue **21b** while the propargyl analogue **21d** was less potent than either the propyl analogue **21b** or the allyl analogue **21c**. The 8-cyclopropymethyl analogue **21e** (DAT K_i of 169 nM) exhibited weak affinity at the DAT similar to that of the allyl analogue **21c**. Alternatively, the substituted 8-benzyl analogues **21f–h** all exhibited high affinity and were four to seven-fold more potent than the unsubstituted congener **11**. It is noteworthy, that among the desfluorobenzhydryl derivatives **21**, the 8-(4-fluorobenzyl)-congener **21f** exhibited very high selectivity for DAT over both SERT (DAT/SERT = 585) and NET (DAT/NET = 477).

The 4,4′-difluorobenzhydryl analogues **22a–g** proved to be generally more potent than the corresponding desfluoro analogues **21a–g**. This was consistent with the SAR of GBR 12909. The 8-alkyl congeners **22a,b** also exhibited similar DAT affinity to the methyl analogue **8** (K_i of 19 nM).[38] Further, the allyl analogue **22c** (DAT K_i of 21 nM) was twice as potent as the ethyl (**22a**) and the propyl analogues (**22b**). However, it was quite surprising to discover that the 8-cyclopropylmethyl analogue **22e** (DAT K_i of 4.0 nM), was nearly threefold more potent than the other 8-alkyl derivatives and was slightly more potent than GBR 12909. The 8-cyclopropylmethyl analogue **22e** exhibited low nanomolar affinity at the DAT similar to the benzyl derivatives **22f–h** (K_i values of 3–6 nM). However, **22e** was significantly more selective for the DAT over the SERT than 22f–**h**. In fact the greater than 1000-fold SERT/DAT selectivity observed for **22e** is among the highest reported for any DAT ligand.[28–30,44] In addition, **22e** was also selective for DAT over NET (NET/DAT: 625) albeit less than the SERT.

In general, the 8-benzyl analogues **22f–h** exhibited high affinity at DAT and were equipotent with GBR 12909. However, these analogues were consistently more selective for DAT over SERT and NET relative to **1**. It is noteworthy that within the series, SERT affinity was always greater than NET affinity for the 8-benzyl

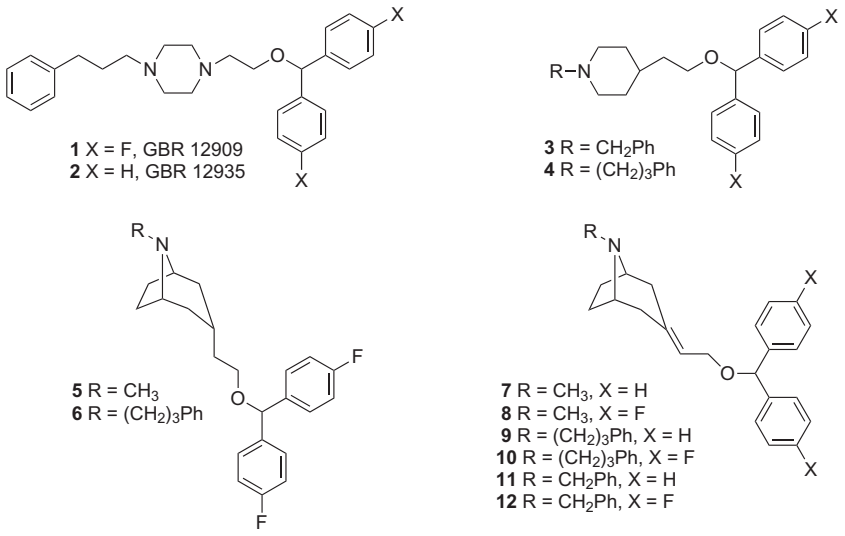
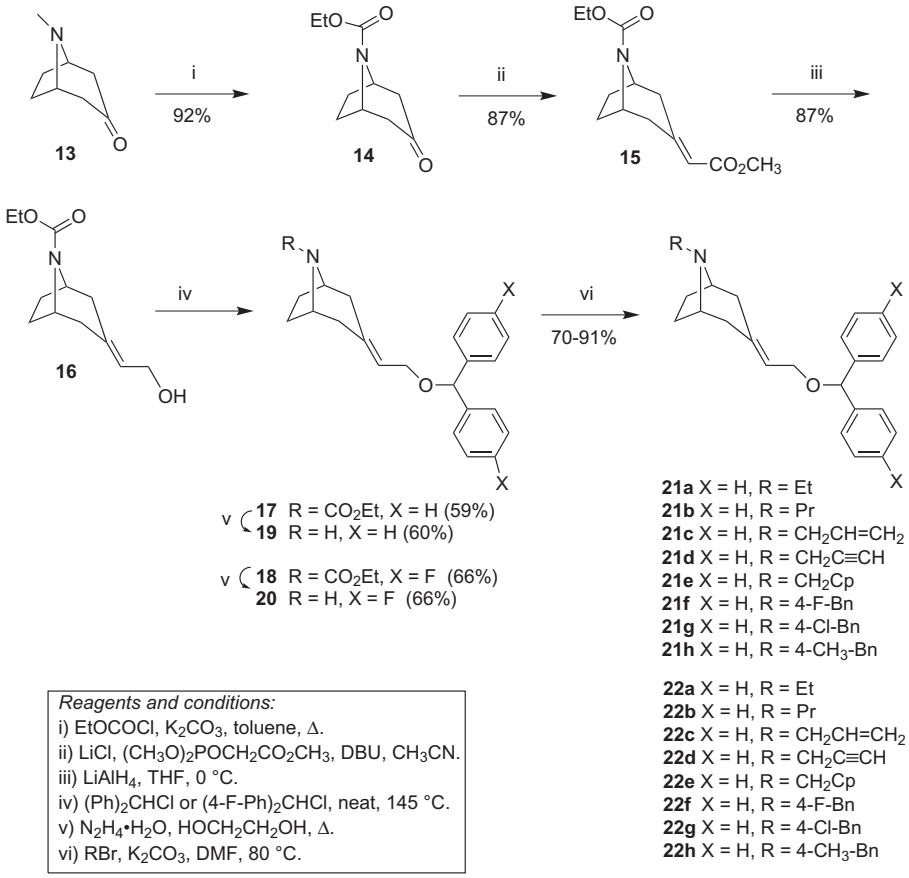


Figure 1. GBR 12909-related dopamine transporter ligands.

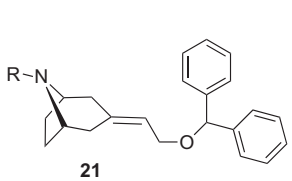


Scheme 1. Synthesis of 8-Substituted-3-[2-(diarylmethoxyethylidenyl)]-8-azabicyclo[3.2.1]octanes.

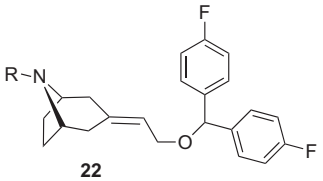
derivatives, while the NET affinity was greater for the 8-alkyl congeners. Among the benzyl derivatives the 4-chlorobenzyl analogue **22g** exhibited the greatest NET/DAT selectivity, with DAT affinity 1358-times more potent than NET. The SERT/DAT selectivity of 168 was relatively high compared to GBR 12909, but significantly less than the 8-cyclopropylmethyl derivative **22e**.

Despite the presence of a tropane scaffold, these analogues **21** and **22** exhibited structure–activity more similar to that described for GBR 12909 derivatives than the 3-phenyltropanes or benztropines. This assessment is based upon the significantly improved DAT affinity and selectivity imparted by substitution of the 8-benzyl substituent. These results are consistent with the SAR of the piperidine derivatives of GBR 12909 and the related pharmacophore.[26,45,46] However, the high affinity of the 8-cyclopropyl methyl analogue **22e** seems to be an anomaly in this system. It is unclear at this time what specific effect the 8-cyclopropylmethyl

Table 1
Inhibition of [^{3}H] WIN 35,428, [^{3}H] Citalopram and [^{3}H] Nisoxetine



21



22

Compd[a]	Code	R	clogP[b]	DAT (K_i, nM)[c]	SERT (K_i, nM)[c]	NET (K_i, nM)[c]	SERT/DAT	NET/DAT
1	GBR 12909		6.24	10.6 ± 1.9[d]	132 ± 0[d]	496 ± 22	13	47
11	SAC-I-37	Bn	6.49	48 ± 5.0	5420 ± 74	1393 ± 147	113	29
12	ALB-IV-41	Bn	6.77	7.9 ± 0.5	2220 ± 740	NT	281	
21a	SAC3-129	Et	5.12	286 ± 85				
22a	SAC3-151	Et	5.41	40 ± 3.0	5958 ± 786	3577 ± 117	149	89
21b	SAC3-111	Pr	5.64	245 ± 20				
22b	SAC3-155	Pr	5.93	41 ± 7.7	5463 ± 318	2847 ± 349	133	69
21c	SAC3-133	$CH_2CH{=}CH_2$	5.49	143 ± 15				
22c	SAC3-149	$CH_2CH{=}CH_2$	5.78	21 ± 2	3461 ± 314	3120 ± 669	167	149
21d	SAC3-157	$CH_2C{\equiv}CH$	4.91	536 ± 49				
22d	SAC3-171	$CH_2C{\equiv}CH$	5.19	NT[e]				
21e	SAC3-159	CH_2Cp	5.54	169 ± 44				
22e	SAC3-169	CH_2Cp	5.83	4.0 ± 2.7	4239 ± 225	2502 ± 157	1060	625
21f	SAC5-165	4-F-Bn	6.63	7.0 ± 1.9	4094 ± 1528	3336 ± 1879	585	477
22f	SAC5-75	4-F-Bn	6.92	4.9 ± 1.3	1145 ± 144	3151 ± 650	234	643
21g	SAC5-161	4-Cl-Bn	7.09	7.6 ± 1.0	544 ± 89	2714 ± 216	72	554
22g	SAC5-77	4-Cl-Bn	7.38	3.9 ± 2.0	656 ± 193	5297 ± 618	168	1358
21h	SAC5-167	4-CH_3-Bn	7.00	13 ± 2.0	746 ± 192	1772 ± 441	57	136
22h	SAC5-137	4-CH_3-Bn	7.29	6.1 ± 1.1	405 ± 98	2750 ± 1267	68	450

[a] All compounds were tested as the oxalate salts. NT:Not tested.
[b] See Ref. 47.
[c] All values are the mean ± SEM of three experiments preformed in triplicate.
[d] IC_{50} values taken from ref. 46.
[e] The compound decomposed in solution and a reliable K_i value could not be obtained.

moiety has on molecular recognition at the monoamine transporters. Relative to the benzyl analogues, **22e** has a diminished capacity for π–π-interactions at the binding site. In addition, with *ClogP* value of 5.83 (Table 1), **22e** is significantly less lipophilic than the 8-benzyl derivatives (e.g., **12**, *ClogP* 6.77).[47] Presumably, the structural variations present around the nitrogen atom in **22e** do not contribute to enhanced DAT affinity but lead to diminished SERT and NET affinity relative to the benzyl analogues **22f–h**.

3. Conclusion

In summary, a series of 8-substituted-3-diarylmethoxyethylidenyl-8-azabicyclo-[3.2.1]octane analogues were synthesized and evaluated for their affinities at dopamine, serotonin and norepinephrine transporters. The rigid ethylidinyl-8-azabicyclic[3.2.1] octane skeleton imparted modestly stereoselective binding and uptake inhibition. Additional structure–activity studies provided a transporter affinity profile that was reminiscent of the SAR of GBR 12909. From these studies, the 8-cyclopropylmethyl group has been identified as a unique moiety that imparts high SERT/DAT selectivity. In this study the 8-cyclopropylmethyl derivative **22e** was among the most potent compounds of the series and is one of the most DAT selective ligands reported to date. Similarly, the 8-chlorobenzyl derivative **22g** was found to be highly selective for the DAT over the NET. Because of the high selectivity of these compounds for the DAT over the other monoamine transporters, **22e** and **22g** offer a unique opportunity to explore the feasibility of the development of a DAT selective cocaine pharmacotherapy. In addition, these highly selective DAT ligands may find additional application toward medication development for other disease states that affect dopminergic pathways (ADHD and Parkinson's disease).[48,49] The pharmacological evaluation of these analogues is currently under investigation and will be reported in due course.

4. Experimental section

4.1. General

All chemicals were purchased from Aldrich Chemical Company and used as received unless otherwise noted. Anhydrous acetonitrile (CH_3CN), chloroform ($CHCl_3$), dichloromethane (CH_2Cl_2), methanol (MeOH), tetrahydrofuran (THF) and toluene, were purchased from Mallinckrodt Baker, Inc. Thin layer chromatography (TLC) 20 × 20 cm glass plates precoated with 250 μm silica gel were purchased from Sorbent Technologies and used to monitor reactions via visualization with short-wave UV light, iodine, potassium permanganate, 2,4-dinitrophenyl hydrazine or Dragondorff's reagent. Chromatography was performed over Sorbent Technologies Silica Gel 60 Å (230–400 mesh). High-pressure hydrogenations were carried out on a Parr apparatus. Proton and carbon NMR were recorded on a Varian-400 MHz nuclear magnetic resonance spectrometer at ambient temperature in deuterated chloroform, acetone or water from Cambridge Isotope Laboratories, Inc. ^{1}H NMR chemical shifts are reported as δ values (ppm) relative to tetramethylsilane. Splitting patterns are designated as: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. ^{13}C NMR chemical shifts are reported as δ values (ppm) relative to chloroform-*d* (77.0 ppm). Optical rotations were measured on Autopol III autopolarimeter at the sodium D line (2 mL sample cell).

4.2. 8-Ethoxycarbonyl-8-azabicyclo[3.2.1]octan-3-one (14)

In a dry 500 mL round-bottom flask was added a stir-bar, 3-tropinone (10.0 g, 0.072 mol) and K_2CO_3 (0.050 g). Anhydrous toluene (90 mL) was added followed by 3 equiv of ethylchloroformate (21 mL, 0.22 mol) via syringe, drop wise. The flask was fitted with a condenser, nitrogen bubbler and heated to reflux overnight

with stirring. The mixture was concentrated under vacuum and the brown oil was dissolved in CH_2Cl_2 (100 mL) and washed with H_2O (100 mL). The layers were separated in a separatory funnel and the aqueous layer extracted with CH_2Cl_2 (3 × 50 mL). The organics were combined and dried over Na_2SO_4, filtered and concentrated under vacuum. The residue was purified by column chromatography (SiO_2, EtOAc/hexane, 1:3) to afford **14** (13.1 g, 92%) as a light yellow oil. ^{1}H NMR: δ 4.51 (br s, 2H), 4.16 (q, J = 7.2 Hz, 2H), 2.63 (br s, 2H), 2.31 (dd, J = 17.4, 1.6 Hz, 2H), 2.07–2.03 (m, 2H), 1.65 (d, J = 7.6 Hz, 2H), 1.25 (t, J = 7.2 Hz, 3H). ^{13}C NMRδ 208.3, 154.1, 61.6, 53.2, 49.1, 29.5(2), 28.8(2), 14.9.

4.3. 8-Ethoxycarbonyl-3-methoxycarboethylidenyl-8-azabicyclo[3.2.1]octane (15)

To a dry 500 mL round-bottom flask was added 1.2 equiv (3.4 g, 0.080 mol) LiCl and a stir bar and sealed under a nitrogen balloon. Acetonitrile (180 mL) was added followed by 1.2 equiv of trimethylphosphonoacetate (6.04 mL, 0.037 mol). The mixture was allowed to stir at room temperature for 30 min to dissolve the LiCl. DBU (4.60 mL, 0.031 mol) was added via syringe, drop wise, over 10 min. The protected tropinone **14** (13 g, 0.066 mol) was dissolved in CH_3CN (60 mL) and syringed into the flask. The mixture was all allowed to stir at room temperature overnight under N_2. The mixture was concentrated under reduced pressure and the light brown oil was dissolved in CH_2Cl_2 (100 mL) and washed with H_2O (50 mL). The layers were separated in a separatory funnel and the aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL). The organics were combined and dried over Na_2SO_4, filtered and concentrated under vacuum. The residue was purified by column chromatography (SiO_2, EtOAc/hexane, 1:4) to afford **15** (14.5 g, 87%) as a light yellow oil. ^{1}H NMR: δ 5.79 (s, 1H), 4.39 (br s, 2H), 4.19 (t, J = 6.8 Hz, 2H), 3.69 (s, 3H), 2.69 (br s, 1H), 2.36 (br s, 1H), 2.12 (d, J = 14.4 Hz, 1H), 1.94 (s, 2H), 1.59 (d, J = 7.2 Hz, 2H), 1.28 (t, J = 6.8 Hz, 4H). ^{13}C NMR: δ 165.6, 155.2, 153.2, 118.2, 60.4, 53.6, 53.4, 50.3, 42.1, 35.5, 27.9(2), 14.2. Anal. Calcd for: $C_{13}H_{19}NO_4$: C, 61.64; H, 7.56; N, 5.53. Found: C, 61.46; H, 7.66; N, 5.48.

4.4. 8-Ethoxycarbonyl-3-hydroxymethylethylidenyl-8-azabicyclo[3.2.1]octane (16)

Lithium aluminum hydride (1.2 equiv, 0.87 g, 0.023 mol) and a stir bar were added to a clean, dry 250 mL round-bottom flask and sealed under a nitrogen balloon. THF (35 mL) was added via syringe and the solution brought to 0 °C in an ice-water bath. The ester **15** (3.9 g, 0.015 mol) was dissolved in THF (15 mL) and added drop wise over 20 min via syringe. The mixture was stirred at 0 °C for 1.5 h and quenched by the slow addition of 10% KOH (11 mL). Stirring was continued for an additional hour and the reaction mixture was decanted into a separatory funnel. The white solid was washed with diethyl ether (4 × 30 mL) and the organics added to the seperatory funnel. The organics were washed with phosphate buffer (75 mL) and the buffer solution was extracted with diethyl ether (4 × 30 mL). The organics were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The resulting alcohol was purified by column chromatography (SiO_2, EtOAc/hexane, 1:1) to yield **16** (3.0 g, 89%) as a light yellow oil. ^{1}H NMR: δ 5.58 (m, 1H), 4.32 (s, 1H), 4.16 (m, 1H), 2.53 (d, J = 13.6 Hz, 2H), 2.41(d, J = 14 Hz, 2H), 2.23 (d, J = 14.4 Hz, 2H), 2.03 (d, J = 13.6 Hz, 2H), 1.89 (m, 3H), 1.61 (t, J = 7.6 Hz, 2H), 1.52 (t, J = 7.6 Hz, 3H). ^{13}C NMR: δ 153.7, 134.4, 127.7, 60.7, 57.7, 53.6, 41.6, 34.5(2), 27.8(2), 14.3. Anal. Calcd for: $C_{12}H_{19}NO_3$: C, 63.98; H, 8.50; N, 6.22. Found: C, 63.76; H, 8.61; N, 6.43.

4.5. 3-(2-Diphenylmethoxyethylidenyl)-8-ethoxycarbonyl-8-azabicyclo[3.2.1]-octane (17)

In a dry 10 mL round-bottom flask was added the alcohol **16** (1.1 g, 0.005 mol), diphenylchloromethane (1.5 equiv, 1.3 mL, 0.007 mol) and a stir bar. This was heated neat to 145 °C for 2 h under N_2. The mixture was cooled to room temperature, dissolved in pure ethyl acetate and purified immediately by column chromatography (60% w/w SiO_2, EtOAc/hexane, 1:4) to afford **17** (1.3 g, 66%) as a clear light yellow oil. ^{1}H NMR: δ 7.3–7.22 (m, 10H), 5.63–5.61 (m, 1H), 5.39 (s, 1H), 4.36–4.20 (m, 2H), 4.18–4.10 (m, 2H), 4.04–3.98 (m, 2H), 2.55–2.45 (m, 1H), 2.27–2.07 (m, 1H), 1.95–1.86 (m, 2H), 1.64–1.59 (m, 2H), 1.30–1.21 (m, 3H). ^{13}C NMR: δ 153.6, 142.0 (2), 138.2(4), 128.1(4), 126.7(2), 123.0, 82.3, 64.2, 62.2, 55.1, 54.8, 43.8, 36.9, 29.1, 28.8(2), 14.2. Anal. Calcd for: $C_{25}H_{29}NO_3$: C, 76.70; H, 7.47; N, 3.58. Found: C, 76.54; H, 7.26; N, 4.45.

4.6. 3-[2-Bis(4-fluorophenyl)methoxyethylidenyl]-8-ethoxycarbonyl-8-azabicyclo-[3.2.1]octane (18)

In a dry 10 mL round-bottom flask was added the alcohol **16** (1.4 g, 0.006 mol), bis-(4-fluorophenyl)methylchloride (1.5 equiv, 1.7 mL, 0.009 mol) and a stir bar. This was heated neat to 145 °C for 2 h under N_2. The mixture was cooled to room temperature, dissolved in EtOAc and purified immediately by column chromatography (60% w/w SiO_2, EtOAc/hexane, 1:4) to afford **18** (2.0 g, 75%) as a clear light yellow oil. ^{1}H NMR: δ 7.3–7.26 (m, 4H), 7.03–6.98 (m, 4H), 5.63–5.61 (m, 1H), 5.39 (s, 1H), 4.36–4.20 (m, 2H), 4.18–4.10 (m, 2H), 4.04–3.98 (m, 2H), 2.55–2.45 (m, 1H), 2.27–2.07 (m, 1H), 1.95–1.86 (m, 2H), 1.64–1.59 (m, 2H), 1.30–1.21 (m, 3H). ^{13}C NMR: δ 158.1(2), 153.5, 138.3, 138.1, 134.4(4), 128.5(2), 128.3(2), 123.1, 115.0, 81.2, 69.4, 64.1, 62.0, 55.2, 54.8, 43.8, 36.9, 29.2, 14.3. Anal. Calcd for $C_{25}H_{27}F_2NO_3$: C, 70.24; H, 6.37; N, 3.28. Found: C, 70.55; H, 6.50; N, 3.09.

4.7. 3-(2-Diarylmethoxyethylidene)-8-azabicyclo[3.2.1]octane (19 or 20)

In a 250 mL round-bottom flask was added ethylene glycol (88 mL), KOH (4.4 g, 0.079 mol), $NH_2NH_2{\cdot}H_2O$ (0.75 mL, 0.015 mol) and the corresponding protected tropane (**17** or **18**) (0.003 mol). The mixture was heated to reflux for 3 h. The mixture was then cooled to room temperature and poured into water (100 mL) and extracted with Et_2O (6 × 75 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The resulting oil was purified by column chromatography (SiO_2, $CHCl_3$/MeOH/NH_4OH 90:9:1) to afford the nortropane **19** or **20** as a light yellow oil. Compound **19**: Yield (0.62 g, 60%). ^{1}H NMR: δ 7.36–7.22 (m, 10H), 5.51–5.48 (m, 1H), 5.40 (s, 1H), 4.05–3.97 (m, 2H), 3.57–3.54 (m, 2H), 2.42 (d, J = 13.6 Hz, 1H), 2.26 (d, J = 14.0 Hz, 1H) 2.05 (t, J = 14.0 Hz, 2H) 1.84 (br s, 2H), 1.73–1.68 (m, 2H) 1.64–1.59 (m, 1H), 1.51–1.47 (m, 1H). ^{13}C NMR: δ 142.1(2), 138.0(4), 128.1(4), 126.8(2), 122.9, 82.3, 64.1, 55.1, 54.7, 43.8, 36.9, 29.1, 28.8(2). Anal. Calcd for $C_{22}H_{25}NO{\cdot}0.25\ H_2O$: C, 81.75; H, 7.93; N, 4.33. Found: C, 81.57; H, 7.86; N, 4.35. Compound **20**: Yield (577 mg, 66%). ^{1}H NMR: δ 7.36–7.22 (m, 4H), 7.02–6.97 (m, 4H), 5.51–5.48 (m, 1H), 5.40 (s, 1H), 4.05–3.97 (m, 2H), 3.57–3.54 (m, 2H), 2.42 (d, J = 13.6 Hz, 1H), 2.27 (d, J = 14.0 Hz, 1H), 2.05(t, J = 14.0 Hz, 2H), 1.84 (br s, 2H), 1.73–1.68 (m, 2H), 1.64–1.59 (m, 1H), 1.51–1.47 (m, 1H). ^{13}C NMR: δ 158.14(2), 138.3, 138.1, 134.4(4), 128.5(2), 128.3(2), 123.1, 115.0, 81.2, 69.4, 64.1, 55.2, 54.8, 43.8, 36.9, 29.2. Anal. Calcd for $C_{22}H_{23}F_2NO{\cdot}0.50\ H_2O$: C, 72.50; H, 6.63; N, 3.84. Found: C, 72.55; H, 6.50; N, 3.84.

4.8. General procedure of the synthesis of 21 and 22

In a clean, dry 25 mL round-bottom flask was added 600 mg K_2CO_3, the nortropane (**19** or **20**) (2.0 mmol), DMF (15 mL) and the alkyl bromide (2.2 mmol) and a stir bar. The mixture was heated to 80 °C (oil bath) overnight under N_2. The mixture was cooled to room temperature and added to a separatory funnel followed by addition of H_2O (50 mL). The mixture was extracted with diethyl ether (4 × 50 mL) and the combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The oil was purified by column chromatography (SiO_2, EtOAc) and the pure free base was converted into the oxalate salt.

4.9. 3-(2-Diphenylmethoxyethylidenyl)-8-ethyl-8-azabicyclo[3.2.1]octane (21a)

Yellow oil (207 mg, 50%). ^{1}H NMR: δ 7.35–7.22 (m, 10H), 5.20 (t, J = 6.6 Hz, 1H), 5.39 (s, 1H), 4.05–3.94 (m, 2H), 3.34 (d, J = 16 Hz, 2H), 2.64 (d, J = 16 Hz, 1H), 2.55 (q, J = 7 Hz, 2H), 2.28–2.25 (d, J = 13.2 Hz, 1H), 2.13–2.10 (d, J = 14 Hz, 1H), 1.95 (d, J = 14.4 Hz, 1H), 1.87 (s, 2H), 1.56 (t, J = 9.2 Hz, 1H), 1.44 (t, J = 8.8 Hz, 1H), 1.15 (t, J = 7.2 Hz, 3H). Anal. Calcd for $C_{24}H_{29}NO{\cdot}C_2H_2O_4$: C, 71.37; H, 7.14; N, 3.20. Found: C, 71.56; H, 7.39; N, 3.10.

4.10. 3-[2-Bis(4-fluorophenyl)methoxyethylidenyl]-8-ethyl-8-azabicyclo[3.2.1]-octane (22a)

Yellow oil (140 mg, 66%). ^{1}H NMR: δ 7.29–7.26 (m, 4H), 7.03–6.98 (m, 4H), 5.49 (s, 1H), 5.33 (s, 1H), 4.01–3.91 (m, 2H), 3.30 (d, J = 12 Hz, 2H), 2.58–2.47 (m, 3H), 2.19 (d, J = 16 Hz, 1H), 2.07 (d, J = 16 Hz, 1H), 1.91–1.85 (m, 3H), 1.49 (t, J = 7.6 Hz, 1H), 1.38 (t, J = 7.7 Hz, 1H), 1.11 (t, J = 7.6 Hz, 3H). Anal. Calcd for $C_{24}H_{27}F_2NO{\cdot}C_2H_2O_4$: C, 65.95; H, 6.17; N, 2.96. Found: C, 65.10; H, 5.88; N, 2.65.

4.11. 3-(2-Diphenylmethoxyethylidenyl)-8-propyl-8-azabicyclo[3.2.1]octane (21b)

Yellow oil (72 mg, 42%). ^{1}H NMR δ 7.35–7.22 (m, 10H), 5.54 (s, 1H), 5.39 (s, 1H), 4.05–3.94 (m, 2H), 3.38 (br s, 2H), 2.49 (br s, 2H), 2.16 (d, J = 13.2 Hz, 2H), 1.98–1.91 (m, 3H), 1.61 (m, 5H), 0.95 (t, J = 7.4 Hz, 3H). Anal. Calcd for $C_{25}H_{31}NO{\cdot}C_2H_2O_4{\cdot}H_2O$: C, 69.06; H, 7.51; N, 2.98. Found: C, 69.67; H, 7.30; N, 3.01.

4.12. 3-[2-Bis(4-fluorophenyl)methoxyethylidenyl]-8-propyl-8-azabicyclo[3.2.1]-octane (22b)

Yellow oil (64 mg, 68%). ^{1}H NMR: δ 7.29–7.26 (m, 4H), 7.03–6.98 (m, 4H), 5.45 (br s, 1H), 5.35 (s, 1H), 3.98–3.93 (m, 2H), 3.26 (br s, 2H), 2.56 (d, J = 12 Hz, 1H), 2.38 (t, J = 8.0 Hz, 2H), 2.16–2.04 (m, 2H), 1.92–1.84 (m, 3H), 1.56–1.50 (m, 3H), 1.36 (s, 1H), 0.92 (t, J = 7.2 Hz, 3H). Anal. Calcd for $C_{25}H_{29}F_2NO{\cdot}C_2H_2O_4{\cdot}1.5\ H_2O$: C, 63.02; H, 6.66; N, 2.72. Found: C, 62.91; H, 6.89; N, 2.72.

4.13. 8-Allyl-3-(2-diphenylmethoxyethylidenyl)-8-azabicyclo[3.2.1]octane (21c)

Yellow oil (132 mg, 67%). ^{1}H NMR: δ 7.35–7.21 (m, 10H), 5.96 (br s, 1H), 5.52 (br s, 1H), 5.39 (s, 1H), 5.21–5.14 (m, 2H), 4.05–3.93 (m, 2H), 3.30 (d, J = 12 Hz, 2H), 3.11 (d, J = 5.6 Hz, 2H), 2.63 (br s, 1H), 2.24 (br s, 1H), 2.16–2.12 (m, 1H), 1.98–1.95 (m, 1H), 1.86 (m, 2H), 1.60–1.43 (m, 2H). Anal. Calcd for $C_{25}H_{29}NO{\cdot}$-$C_2H_2O_4{\cdot}1.25H_2O$: C, 68.04; H, 7.19; N, 2.94. Found: C, 68.55; H, 6.88; N, 2.78.

4.14. 8-Allyl-3-[2-bis(4-fluorophenyl)methoxyethylidenyl]-8-azabicyclo-[3.2.1]octane (22c)

Yellow oil (158 mg, 66%). ^{1}H NMR: δ 7.29–7.26 (m, 4H), 7.03–6.98 (m, 4H), 5.94 (m, 1H), 5.48 (br s, 1H), 5.34 (s, 1H), 5.16 (m, 2H), 4.01–3.90 (m, 2H), 3.28 (br s, 2H), 3.08 (br s, 2H), 2.56 (br s, 1H), 2.12 (m, 2H), 1.97–1.86 (m, 2H), 1.55–1.43(m, 3H). Anal. Calcd for $C_{25}H_{27}F_2NO{\cdot}C_2H_2O_4{\cdot}H_2O$: C, 64.40; H, 6.21; N, 2.78. Found: C, 64.32; H, 6.02; N, 2.70.

4.15. 3-(2-Diphenylmethoxy-ethylidene)-8-(3-prop-2-ynyl)-8-azabicyclo[3.2.1]octane (21d)

Yellow oil (169 mg, 61%). ^{1}H NMR δ 7.35–7.23 (m, 10H), 5.51 (t, J = 6.4 Hz, 1H), 5.39 (s, 1H), 4.05–3.93 (m, 2H), 3.40 (d, J = 12 Hz, 2H), 3.22 (s, 2H), 2.57 (d, J = 16 Hz, 1H), 2.20–2.13 (m, 2H), 1.98 (d, J = 12 Hz, 1H), 1.85 (br s, 2H) 1.59–1.56 (m, 2H) 1.44 (t, J = 4.4 Hz, 1H). Anal. Calcd for $C_{25}H_{27}NO{\cdot}C_2H_2O_4{\cdot}1.25H_2O$: C 68.98; H 6.60; N 2.98. Found: C 68.67; H 6.36; N 2.93.

4.16. 3-[2-Bis(4-fluorophenyl)methoxyethylidenyl]-8-(3-prop-2-ynyl)-8-azabicyclo-[3.2.1]octane (22d)

Yellow oil (130 mg, 63%). ^{1}H NMR: δ 7.29–7.26 (m, 4H), 7.03–6.98 (m, 4H), 5.50 (t, J = 8 Hz, 1H), 5.34 (s, 1H), 4.01–3.90 (m, 2H), 3.41 (br s, 2H), 3.20 (s, 2H), 2.57 (d, J = 14 Hz, 1H), 2.21–2.12 (m, 2H), 1.88–1.86 (m, 1H), 1.56 (s, 2H), 1.53–1.39 (m, 3H). Anal. Calcd for $C_{25}H_{25}F_2NO{\cdot}C_2H_2O_4{\cdot}H_2O$: C, 64.66; H, 5.83; N, 2.79. Found: C, 64.80; H, 5.50; N, 2.59.

4.17. 3-(2-Diphenylmethoxyethylidenyl)-8-cyclopropylmethyl-8-azabicyclo-[3.2.1]octane (21e)

Yellow oil (227 mg, 45%). ^{1}H NMR: δ 7.36–7.23 (m, 10H), 5.48 (t, J = 6.8 Hz, 1H), 5.39 (s, 1H), 4.05–3.94 (m, 2H), 3.43 (m, 2H), 2.55 (*d*, J = 12 Hz, 1H), 2.33 (d, J = 4 Hz, 2H), 2.20–2.07 (m, 3H), 1.93–1.80 (m, 3H), 1.60–1.37 (m, 1H), 0.94 (m, 1H), 0.52 (m, 2H), 0.12 (m, 2H). Anal. Calcd for $C_{26}H_{31}NO{\cdot}C_2H_2O_4{\cdot}1.25H_2O$: C, 69.12; H, 7.36; N, 2.88. Found: C, 69.00; H, 6.99; N, 2.83.

4.18. 3-[2-Bis(4-fluorophenyl)methoxyethylidenyl]-8-cyclopropylmethyl-8-azabicyclo[3.2.1]octane (22e)

Yellow oil (140 mg, 65%). ^{1}H NMR: δ 7.29–7.26 (m, 4H), 7.03–6.98 (m, 4H), 5.46 (br s, 1H), 5.35 (s, 1H), 4.01–3.90 (m, 2H), 3.42 (m, 2H), 2.56 (br s, 1H), 2.31 (m 2H), 2.17 (m, 1H), 2.08 (d, J = 14.8 Hz, 1H), 1.93 (d, J = 14.4 Hz, 1H), 1.83 (m, 2H), 1.58–143 (m, 2H), 0.51 (d, J = 7.2 Hz, 2H), 0.11 (d, J = 4 Hz, 2H). Anal. Calcd for $C_{26}H_{29}F_2NO{\cdot}C_2H_2O_4{\cdot}H_2O$: C, 64.98; H, 6.43; N, 2.71. Found: C, 64.62; H, 6.17; N, 2.50.

4.19. 3-(2-Diphenylmethoxyethylidenyl)-8-(4-fluorobenzyl)-8-azabicyclo[3.2.1]-octane (21f)

Yellow oil (77 mg, 67%). ^{1}H NMR: δ 7.38–7.21 (m, 12H), 7.04–6.97 (m, 2H), 5.52 (t, J = 6.4 Hz, 1H), 5.39 (s, 1H), 4.04–3.94 (m, 2H), 3.58 (s, 2H), 3.22 (s, 2H), 2.62 (d, J = 13.2 Hz, 1H), 2.25–2.22 (m, 1H), 2.14–2.10 (m, 1H), 1.92 (m, 3H), 1.60–1.53 (m, 2H). Anal. Calcd for $C_{29}H_{30}FNO{\cdot}C_2H_2O_4{\cdot}H_2O$: C, 69.52; H, 6.40; N, 2.62. Found: C, 69.30; H, 6.58; N, 2.44.

4.20. 8-(4-Fluorobenzyl)-3-[2-bis(4-fluorophenyl) methoxyethylidenyl]-8-azabicyclo[3.2.1]octane (22f)

Yellow oil (73 mg, 87%). ^{1}H NMR: δ 7.37–7.25 (m, 6H), 7.06–6.98 (m, 6H), 5.58 (t, J = 6.8 Hz, 1H), 5.35 (s, 1H), 4.01–3.92 (m, 2H), 3.56

(s, 2H), 3.21 (br s, 2H), 2.59 (d, J = 13.6 Hz, 1H), 2.19–1.90 (m, 5H), 1.56 (m, 1H), 1.42 (t, J = 7.6 Hz, 1H). Anal. Calcd for $C_{29}H_{28}F_3NO\cdot C_2H_2O_4$: C, 67.26; H, 5.46; N, 2.53. Found: C, 67.44; H, 5.45; N, 2.56.

4.21. 3-(2-Diphenylmethoxyethylidenyl)-8-(4-chlorobenzyl)-8-azabicyclo[3.2.1]-octane (21g)

Yellow oil (95 mg, 93%). ^{1}H NMR: δ 7.39–7.21 (m, 14H), 5.51 (br s, 1H), 5.38 (s, 1H), 4.01–3.97 (m, 2H), 3.59–3.55 (m, 2H), 3.17 (br s, 2H), 2.56(m, 1H), 2.17–2.09 (m, 2H), 1.89 (m, 3H), 1.58–1.44 (m, 2H). Anal. Calcd for $C_{32}H_{30}ClNO\cdot C_2H_2O_4\cdot H_2O$: C, 67.44; H, 6.21; N, 2.54. Found: C, 67.30; H, 5.98; N, 2.44.

4.22. 8-(4-Chlorobenzyl)-3-[2-bis(4-fluorophenyl) methoxyethylidene]-8-azabicyclo-[3.2.1]octane (22g)

Yellow oil (56 mg, 74%). ^{1}H NMR: δ 7.35–7.23 (m, 8H), 7.04–6.97 (m, 4H), 5.48 (t, J = 6.8 Hz, 1H), 5.35 (s, 1H), 4.01–3.91 (m, 2H), 3.56 (s, 2H), 3.19 (br s, 2H), 2.60–2.56 (d, J = 14.4 Hz, 1H), 2.20 (d, J = 14 Hz, 1H), 2.10 (d, J = 14 Hz, 1H), 1.96–1.86 (m, 3H), 1.56 (m, 1H), 1.42 (t, J = 8.8 Hz, 1H). Anal. Calcd for $C_{29}H_{28}F_2ClNO\cdot C_2H_2O_4\cdot H_2O$: C, 63.32; H, 5.49; N, 2.38. Found: C, 63.70; H, 5.26; N, 2.63.

4.23. 3-(2-Diphenylmethoxyethylidenyl)-8-(4-methylbenzyl)-8-azabicyclo[3.2.1]-octane (21h)

Yellow oil (74 mg, 60%). ^{1}H NMR: δ 7.39–7.11 (m, 14H), 5.49 (t, J = 6.4 Hz, 1H), 5.39 (s, 1H), 4.04–3.97 (m, 2H), 3.56 (s, 2H), 3.21 (br s, 2H), 2.59 (d, J = 13.6 Hz, 1H), 2.33 (s, 3H), 2.23–2.19 (m, 1H), 2.11–2.08 (m, 1H), 1.93 (m, 3H), 1.57–1.43 (m, 2H). Anal. Calcd for $C_{30}H_{33}NO\cdot C_2H_2O_4\cdot H_2O$: C, 72.29; H, 7.01; N, 2.63. Found: C, 72.30; H 6.98; N 2.44.

4.24. 3-[2-Bis(4-fluorophenyl)methoxyethylidenyl-8-(4-methylbenzyl)-8-azabicyclo-[3.2.1]octane (22h)

Yellow oil (153 mg, 85%). ^{1}H NMR: δ 7.21–7.16 (m, 4H), 7.04 (d, J = 8.0 Hz, 4H), 6.94–6.90 (m, 4H), 5.39 (t, J = 6.8 Hz, 1H), 5.27 (s, 1H), 3.90–3.86 (m, 2H), 3.49 (s, 2H) 3.16–3.13 (m, 2H), 2.54–2.50 (d, J = 16 Hz, 1H), 2.26 (s, 3H), 2.14 (d, J = 14 Hz, 1H), 2.01 (d, J = 14 Hz, 1H), 1.86–1.83 (m, 3H), 1.46 (t, J = 8 Hz, 1H), 1.33 (t, J = 8 Hz, 1H). Anal. Calcd for $C_{30}H_{31}F_2NO\cdot C_2H_2O_4\cdot H_2O$: C, 66.65; H, 6.29; N, 2.42. Found: C, 66.30; H 5.98; N 2.44.

4.25. [^{3}H]WIN 35,428 binding assay

Male Sprague–Dawley rats (200–250 g, Taconic, Germantown, NY) were decapitated and their brains removed to an ice-cooled dish for dissection of the caudate-putamen. The tissue was homogenized in 30 volumes ice-cold modified Krebs-HEPES buffer (15 mM HEPES, 127 mM NaCl, 5 mM KCl, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 1.3 mM NaH_2PO_4, 10 mM glucose, pH adjusted to 7.4) using a Teflon/glass homogenizer and centrifuged at 20,000g for 10 min at 4 °C. The resulting pellet was then washed two more times by resuspension in ice-cold buffer and centrifugation at 20,000g for 10 min at 4 °C. Fresh homogenates were used in all experiments. Binding assays were conducted in modified Krebs-HEPES buffer on ice, essentially as previously described.[40] The total volume in each tube was 0.5 mL and the final concentration of membrane after all additions was approximately 0.3% (w/v) corresponding to 150–300 μg of protein/sample. Increasing concentrations of the drug being tested were added to triplicate samples of membrane suspension. Five minutes later, [^{3}H]WIN 35,428 (final concentration 1.5 nM) was added and the incubation was continued for 1 h on ice. The incubation was terminated by the addition of 3 mL of ice-cold buffer and rapid filtration through Whatman GF/B glass fiber filter paper (presoaked in 0.1% BSA in water to reduce non-specific binding) using a Brandel Cell Harvester (Gaithersburg, MD). After filtration, the filters were washed with three additional 3 mL washes and transferred to scintillation vials. Absolute ethanol (0.5 mL) and Beckman Ready Value Scintillation Cocktail (2.75 mL) were added to the vials which were counted the next day at an efficiency of about 36%. Under these assay conditions, an average experiment yielded approximately 6000 dpm total binding per sample and approximately 250 dpm non-specific binding. Nonspecific binding was defined as binding in the presence of 100 μM cocaine. K_i values were derived from 14 point competition assays using increasing concentrations of unlabeled compounds (0.05 nM to 100 μM) against 1.5 nM [^{3}H]WIN 35,428. Data were analyzed with GraphPad Prism software (San Diego, California).

4.26. [^{3}H]Citalopram binding assay

Brains from male Sprague-Dawley rats weighing 200–225 g (Taconic Labs) were removed, midbrain dissected and rapidly frozen. Membranes were prepared by homogenizing tissues in 25 volumes (w/v) of 50 mM Tris containing 120 mM NaCl and 5 mM KCl, (pH 7.4 at 25 °C), using a Brinkman Polytron (setting 6 for 20 s) and centrifuged at 20,000g for 10 min at 4 °C. The resulting pellet was resuspended in buffer, recentrifuged and resuspended in buffer to a concentration of 7.5 mg/ml. Ligand binding experiments were conducted in assay tubes containing 4.0 ml buffer for 60 min at room temperature, essentially as previously described.[39] Each tube contained 1.4 nM [^{3}H]citalopram (GE Healthcare) and 1.5 mg midbrain tissue (original wet weight). Nonspecific binding was determined using 1 μM citalopram. Incubations were terminated by rapid filtration through Whatman GF/B filters, presoaked in 0.3% polyethylenimine, using a Brandel R48 filtering manifold (Brandel Instruments Gaithersburg, Maryland). The filters were washed twice with 5 ml cold buffer and transferred to scintillation vials. Beckman Ready Safe (3.0 ml) was added and the vials were counted the next day using a Beckman 6000 liquid scintillation counter (Beckman Coulter Instruments, Fullerton, California). Data were analyzed by using GraphPad Prism software (San Diego, California).

4.27. [^{3}H]Nisoxetine binding assay

Frontal cortex of male Sprague-Dawley rats was removed and frozen. Membranes were prepared by homogenizing tissues in 50 mM Tris (120 mM NaCl, 5 mM KCl; pH 7.4 at 25 °C) and centrifuging (50,000g for 10 min at 4 °C. The resulting pellet was then washed and centrifuged two more times. The final pellet was resuspended to a concentration of 80 mg/ml (original wet weight). Assays were conducted in the above Tris buffer, essentially as previously described.[40] Volume totaled 0.5 mL with tissue concentration of 8 mg/tube. [^{3}H]Nisoxetine (specific activity 80 Ci/mmol; final concn 0.5 nM, New England Nuclear, Boston, MA) was added and the incubation continued for 1 h on ice. Incubations were terminated by rapid filtration through Whatman GF/B filters, presoaked in 0.05% polyethylenimine (PEI). Nonspecific binding was defined using 1 μM desipramine. For these assays, an initial screen was conducted to assess displacement of nisoxetine at a concentration of 1 μM of the unknown compound. If there was greater than 50% displacement of nisoxetine, a K_i value was determined in subsequent studies.

Acknowledgments

This research was supported by the National Institute on Drug Abuse, (MLT, DA11528 and DA023916) and by a Ruth L. Kirschstein National Research Service Award (SAC, DA14155).

References and notes

1. Substance Abuse and Mental Health Services Administration. *Results from the 2009 National Survey on Drug Use and Health: Volume I. Summary of National Findings* (Office of Applied Studies, NSDUH Series H-38A, HHS Publication No. SMA 10-4586Findings), Rockville, MD, 2010.
2. Cartwright, W. S. *Pharmaeconomics* **2000**, 405.
3. Acri, J. In *Dopamine Transporters: Chemistry, Biology and Pharmacology*; Trudell, M. L., Izenwasser, S., Eds.; John Wiley & Sons: New York, 2008; p 391.
4. Liu, S.; Cunningham, K. A. *Drug Alcohol Depend.* **2006**, *81*, 275.
5. Filip, M.; Bubar, M. J.; Cunningham, K. A. *Psychopharmacology* **2006**, *183*, 482.
6. Howell, L. L.; Carroll, F. I.; Votaw, J. R.; Goodman, M. M.; Kimmel, H. L. *J. Pharmacol. Exp. Ther.* **2007**, *320*, 757.
7. Jin, C.; Navarro, H. A.; Carroll, F. I. *J. Med. Chem.* **2008**, *51*, 8048.
8. Ritz, M. C.; Lamb, R. J.; Goldberg, S. R.; Kuhar, M. J. *Science* **1987**, *237*, 1219.
9. Ritz, M. C.; Kuhar, M. J. *J. Pharmacol. Exp. Ther.* **1989**, *248*, 1010.
10. Bergman, J.; Madras, B. K.; Johnson, S. E.; Spealman, R. D. *J. Pharmacol. Exp. Ther.* **1989**, *251*, 150.
11. Kuhar, M. J.; Ritz, M. C.; Boja, J. W. *Trends Neurosci.* **1991**, *14*, 299.
12. Volkow, N. D.; Wang, G.-J.; Fischman, M. W.; Foltin, R. W.; Fowler, J. S.; Abumrad, N. N.; Vitkun, S.; Logan, J.; Gatley, S. J.; Pappas, N.; Hitzemann, R.; Shea, C. E. *Nature* **1997**, *386*, 827.
13. Carroll, F. I.; Howell, L. L.; Kuhar, M. J. *J. Med. Chem.* **1999**, *42*, 2721.
14. Howell, L. L.; Wilcox, K. M. *J. Pharmacol. Exp. Ther.* **2001**, *298*, 1.
15. Volkow, N. D.; Fowler, J. S.; Wang, G.-J. *Behav. Pharmacol.* **2002**, *13*, 355.
16. Pierce, R. C.; Kumaresan, V. *Neurosci. Biobehav. Rev.* **2006**, *30*, 215.
17. Carroll, F. I. *J. Med. Chem.* **2003**, *46*, 1775.
18. Dutta, A. K.; Zhang, S.; Kolhatkar, R.; Reith, M. E. A. *Eur. J. Pharmacol.* **2003**, *479*, 93.
19. Grabowski, J.; Shearer, J.; Merrill, J.; Negus, S. S. *Addict. Behav.* **2004**, *29*, 1439.
20. Runyon, S. P.; Carroll, F. I. *Curr. Top. Med. Chem.* **2006**, *6*, 1825.
21. Runyon, S. P.; Carroll, F. I. In *Dopamine Transporters, Chemistry, Biology and Pharmacology*; Trudell, M. L., Izenwasser, S., Eds.; Wiley: New York, 2008; pp 125–169.
22. Newman, A. H.; Agoston, G. E. *Curr. Med. Chem.* **1998**, *5*, 301.
23. Newman, A. H.; Kulkarni, S. S. *Med. Res. Rev.* **2002**, *22*, 1.
24. Newman, A. H.; Katz, J. L. In *Dopamine Transporters, Chemistry, Biology and Pharmacology*; Trudell, M. L., Izenwasser, S., Eds.; Wiley: New York, 2008; pp 171–209.
25. Kharkar, P. S.; Dutta, A. K.; Reith, M. A. E. In *Dopamine Transporters, Chemistry, Biology and Pharmacology*; Trudell, M. L., Izenwasser, S., Eds.; Wiley: New York, 2008; pp 233–264.
26. Prisinzano, T.; Rice, K. C. In *Dopamine Transporters, Chemistry, Biology and Pharmacology*; Trudell, M. L., Izenwasser, S., Eds.; Wiley: New York, 2008; pp 211–231.
27. Moltzen, E. K.; Bang-Andersen, B. *Curr. Top. Med. Chem.* **2006**, *6*, 1801.
28. Ghorai, S.; Cook, C.; Davis, M.; Venkataraman, S.; George, C.; Beardsley, P.; Reith, M. E. A.; Dutta, A. *J. Med. Chem.* **2003**, *46*, 1220.
29. Rothman, R. B.; Lewis, B.; Dersch, C.; Xu, H.; Radesca, L.; de Costa, B. R.; Rice, K. C.; Kilburn, R. B.; Akunne, H. C.; Pert, A. *Synapse* **1993**, *14*, 34.
30. Hsin, L. W.; Dersch, C. M.; Baumann, M. H.; Stafford, D.; Glowa, J. R.; Rothman, R. B.; Rice, K. C. *J. Med. Chem.* **2002**, *45*, 1321.
31. van der Zee, P.; Koger, H. S.; Gootjes, J.; Hespe, W. *Eur. J. Med. Chem.* **1980**, *15*, 263.
32. Andersen, P. H. *Eur. J. Pharmacol.* **1989**, *166*, 493.
33. Rothman, R. B.; Glowa, J. R. *Mol. Neurobiol.* **1995**, *11*, 1–9.
34. Prisinzano, T.; Rice, K. C.; Bauman, M. H.; Rothman, R. B. *Curr. Med. Chem. CNS Agents* **2004**, *4*, 47.
35. Rothman, R. B.; Mele, A.; Reid, A. A.; Akunne, H. C.; Greig, N.; Thurkauf, A.; de Costa, B. R.; Rice, K. C.; Pert, A. *Pharmacol. Biochem. Behav.* **1991**, *40*, 387.
36. Glowa, J. R.; Fantegrossi, W. E.; Lewis, D. B.; Matecka, D.; Rice, K. C.; Rothman, R. B. *J. Med. Chem.* **1996**, *39*, 4689.
37. Sogaard, U.; Michalow, J.; Butler, B.; Lund Laursen, A.; Ingersen, S. H.; Skrumsager, B. K.; Rafaelsen, O. J. *Int. Clin. Psychopharm.* **1990**, *5*, 237.
38. Zhang, Y.; Joseph, D. B.; Bowen, W. D.; Flippen-Anderson, J. L.; Dersch, C. M.; Rothman, R. B.; Jacobson, A. E.; Rice, K. C. *J. Med. Chem.* **2001**, *44*, 3937.
39. Bradley, A. L.; Izenwasser, S.; Wade, D.; Cararas, S. A.; Trudell, M. L. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 629.
40. Zhang, S.; Izenwasser, S.; Wade, D.; Xu, L.; Trudell, M. L. *Bioorg. Med. Chem.* **2006**, *14*, 7943.
41. Rothman, R. B.; Baumann, M. H.; Prisinzano, T. E.; Newman, A. H. *Biochem. Pharmacol.* **2008**, *75*, 2.
42. Blanchette, M. A.; Choy, W.; Davis, J. T.; Essenfeld, A. P.; Masamune, S.; Roush, W. R.; Sakai, T. *Tetrahedron Lett.* **1984**, *25*, 2183.
43. Lomenzo, S. A.; Rhoden, J.; Izenwasser, S.; Wade, D.; Kopajtic, T.; Katz, J. L.; Trudell, M. L. *J. Med. Chem.* **2005**, *48*, 1336.
44. Rhoden, J.; Bouvet, M.; Izenwasser, S.; Wade, D.; Lomenzo, S. A.; Trudell, M. L. *Bioorg. Med. Chem.* **2005**, *13*, 5623.
45. Dutta, A. K.; Davis, M. C.; Fei, X. S.; Beardsley, P. M.; Cook, C. D.; Reith, M. E. *J. Med. Chem.* **2002**, *45*, 654.
46. Dutta, A. K.; Coffey, L. L.; Reith, M. E. *J. Med. Chem.* **1997**, *40*, 35.
47. *ClogP* values were calculated using software from Collaborative Drug Discovery, Inc. www.collaborativedrug.com.
48. Glase, S. A.; Dooley, D. J. In *Annual Reports in Medicinal Chemistry*; Doherty, A. M., Ed.; Academic Press: San Diego, CA:, 2004; Vol. 39, pp 1–12.
49. Johnson, T. H.; Brotchie, J. M. *Curr. Opin. Investig. Drug* **2004**, *4*, 720.