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Novel Spirotetracyclic Zwitterionic Dual H_1/5-HT_{2A} Receptor Antagonists for the Treatment of Sleep Disorders

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Histamine H_1 and serotonin 5-HT_{2A} receptors mediate two different mechanisms involved in sleep regulation: H_1 antagonists are sleep inducers, while 5-HT_{2A} antagonists are sleep maintainers. Starting from **9′a**, a novel spirotetracyclic compound endowed with good H_1/5-HT_{2A} potency but poor selectivity, very high Cli, and a poor P450 profile, a specific optimization strategy was set up. In particular, we investigated the possibility of introducing appropriate amino acid moieties to optimize the developability profile of the series. Following this zwitterionic approach, we were able to identify several advanced leads (**51**, **65**, and **73**) with potent dual H_1/5-HT_{2A} activity and appropriate developability profiles. These compounds exhibited efficacy as hypnotic agents in a rat telemetric sleep model with minimal effective doses in the range 3−10 mg/kg po.

Introduction

Insomnia, defined as difficulty in initiating and/or maintaining sleep, is one of the most common central nervous system (CNS[a]) disorders. According to the National Sleep Foundation's 2010 Sleep in America Poll, the majority of those surveyed report that they only get a good night's sleep a few nights per week or less, with some ethnic groups doing more poorly than others.[1] The incidence of insomnia in the general population is between 10−30%, and approximately 50% of cases complain of serious daytime consequences, such as inability to concentrate, reduced energy, and memory problems. When this persists for more than 1 month, without an associated mental disorder or physical problem, it is classified as primary insomnia.[2]

Common symptoms of those suffering with a sleep disorder include abnormal sleep behavior and difficulties in one or more of falling asleep, remaining asleep, sleeping for adequate lengths of time, and achieving restorative sleep.[3] Available treatments for sleep disorders include the use of prescription hypnotics, such as benzodiazepines. However, these drugs may be habit-forming, lose their effectiveness after extended use, and metabolize more slowly for certain designated groups, resulting in persisting medicative effects.

Other treatments include over-the-counter antihistamines, e.g., diphenhydramine and dimenhydrinate. These medicines are not designed to be strictly sedative in their activity, and as such, this method of treatment has been associated with a number of adverse side effects, e.g., persistence of the sedating medication after the prescribed time of treatment or the so-called "hangover effect". Many of these side effects result from nonspecific activity in the periphery as well as in the CNS during this period of extended medication.

It has been suggested that brain histamine is involved in the regulation of the sleep−wake cycle, arousal, cognition, and memory primarily through action at the H_1 receptors, producing a reduction of sleep latency in both preclinical[4] and clinical studies.[5] On the other hand, selective blockade of the 5-HT_{2A} receptor has been proven in both preclinical[6] and clinical[7] studies to be efficacious in reducing wake after sleep onset (WASO) and increasing slow wave sleep (SWS) and total sleep time (TST), therefore providing consolidation of sleep. It can be hypothesized that the combination of these two mechanisms may provide an improved hypnotic profile versus the marketed gold standard benzodiazepine-like drugs.[8]

As part of a broad drug discovery strategy aimed at the identification of novel hypnotic agents,[9] we reasoned on the possibility of optimizing (1*S*,3*R*)-5′,11′-dihydrospiro-[cyclopentane-1,10′-dibenzo[*a*,*d*]cyclohepten]-3-yl(dimethyl)-amine (**9′a**, Figure 1), a novel spirotetracyclic compound

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[a] Abbreviations: CNS, central nervous system; hERG, human ether-a-go-go-related gene K^+ channel; PK, pharmacokinetic; P450, cytochrome P450; Cli, intrinsic clearance; fu, fraction unbound; MW, molecular weight; clogD, calculated log *D*; PSA, polar surface area; CHO, Chinese hamster ovary; HEK, human embryonic kidney; Fpo, oral bioavailability; Br:Bl, brain−blood; Clb, blood clearance; Vss, distribution volume; FLIPR, fluorescent imaging plate reader; SWS, slow wave sleep; TST, total sleep time; ECG, electrocardiogram; EMG, electromiogram; CT18, circadian time 18; REM, rapid eye movement; NREM, nonrapid eye movement; RO, receptor occupancy; EWG, electron withdrawing group; RCM, ring closing metathesis; TFA, trifluoroacetic acid; DMP, Dess−Martin periodinane; HPLC, high performance liquid chromatography; NT, not tested.

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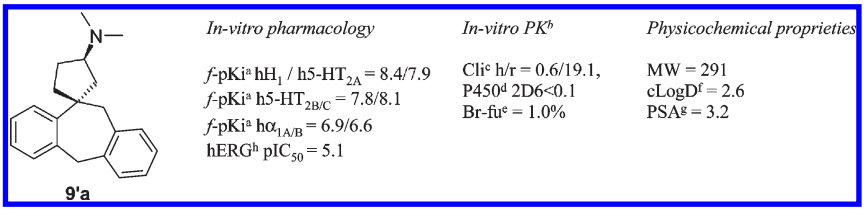


Figure 1. In vitro and physicochemical data of compound **9′a**. Superscript letters indicate the following: (a) functional pK_i measured in FLIPR (fluorescent imaging plate reader) assay;[26] (b) pharmacokinetics; (c) intrinsic clearance in human (h) and rat (r) liver microsomes (mL/min/g liver); (d) CYPEX (see experimental for details); (e) fraction unbound in rat brain; (f) ACD logD 7.4, version 11; (g) Å^2; (h) dofetilide binding. See Experimental Section for details.

endowed with promising dual H_1/5-HT_{2A} in vitro activity.[10] Compound **9′a** represents a low molecular weight lead, although it is characterized by poor selectivity toward human adrenergic α_1 receptors and the human ether-a-go-go-related gene (hERG) potassium channel as well as by suboptimal physiochemical parameters. When preliminary pharmacokinetic data were gathered, a very high rat in vitro intrinsic clearance (Cli), a low free fraction measured in vitro in homogenized brain tissue (Br-fu), and a strong inhibition of the 2D6 isoform of the human CYP450 were also observed. An additional trait of **9′a** is its antagonism of the 5-HT_{2B} and 5-HT_{2C} receptors. While there is some evidence of slight increases in wakefulness and motor activity after blockade of the 5-HT_{2B} receptor,[11] no significant alteration in the percentage distribution of any sleep stage, arousal and light SWS (SWS1), was observed with the inhibition of the 5-HT_{2C} receptor.[12] Therefore, it appears that the presence of 5-HT_{2B} and 5-HT_{2C} activity in **9′a** and in general in our compounds may not represent a potential issue for their hypnotic profile.

It is well-known that the structure of H_1 receptor antagonists can tolerate zwitterion moieties,[13] as they have been intentionally introduced to reduce the sedating properties of centrally acting first generation antihistamines by reducing their brain penetrating capacities. Furthermore, in the second generation H_1 antagonists (e.g., cetirizine and fexofenadine), the introduction of the zwitterion group was responsible for a reduction of the drug−drug interactions and enhanced receptor selectivity profiles with respect to first generation drugs.[14] Despite the fact that zwitterions were initially introduced to limit brain penetration of the first generation antihistamines, scientists at Hypnion, now Lilly, recently demonstrated that zwitterionic structures can penetrate the brain and induce hypnotic activity. In fact, they shaped their sleep program strategy by incorporating zwitterion moieties into known H_1/5-HT_{2A} antagonist scaffolds.[15] This approach led Hypnion to quickly progress several compounds into the clinical phase, one of which, HY-10275 (LY-2624803) whose structure has not yet been disclosed, successfully achieved its proof of concept as a sleep drug.[16]

On the basis of this information, we considered introducing a series of constrained/hindered amino acid derivatives to the top region of this novel template with the aim of optimizing compound **9′a** by eradicating the observed developability issues.

In this article the optimization process of the initial lead **9′a**, which resulted in the identification of compounds **51**, **65**, and **73**, will be described in detail. These compounds demonstrated suitable overall profiles for a hypnotic with in vivo activity in an animal model of sleep disorders.

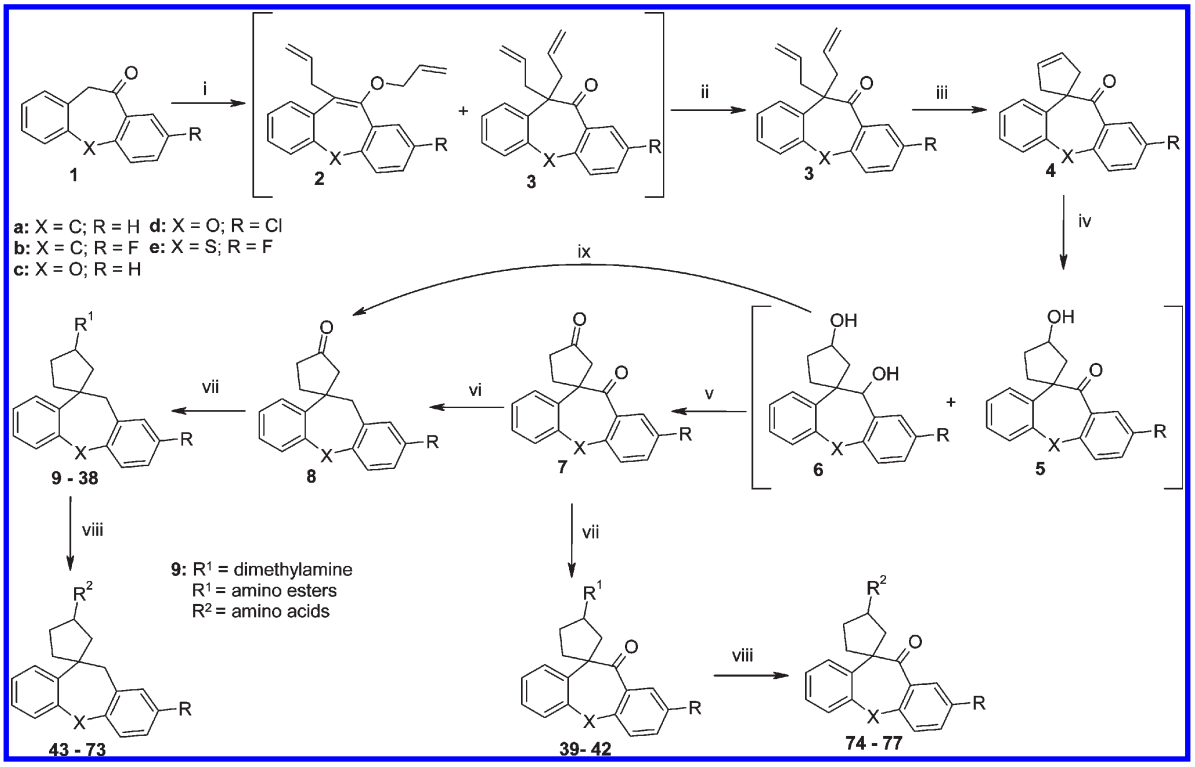
Chemistry

The spirocyclic skeleton of our lead is an unexplored scaffold in organic chemistry; therefore, it was necessary to identify a novel synthetic route to successfully prepare compounds **43**−**73** and **74**−**77** (Scheme 1). The synthesis of compound **9′a** has already been reported in a previous communication,[10] while herein we illustrate an optimized synthesis, which has been adapted for the purpose of introducing substituents on the scaffold (such as R = Cl or F) and different heteroatoms (such as X = O and S) in place of the carbon in the benzhydrylic position in the lower part of the compound. Thus, starting from the appropriately substituted ketone **1**,[17] the first step of the synthesis was a potassium *tert*-butoxide promoted bis-alkylation with allyl bromide.[18] In the case of **1c** and **1d** the reaction led directly to the *C*-diallyl compounds **3c** and **3d**, respectively, whereas a mixture of the corresponding compounds **3** and *C*-allyl/*O*-allyl compound **2** was observed, when starting from **1a**, **1b**, and **1e**. Nevertheless, the mixture of **2** and **3** was readily converted into the single compound **3** by Claisen rearrangement.[19] The diallylation reaction is a crucial step because it permits the construction of the quaternary center. As reported, the intermediates **3** were then converted via ring-closing metathesis (RCM), in good yields, into intermediates **4**, which underwent hydroboration/oxidation to afford a mixture of compounds **5** and **6**, which in turn was oxidize using Dess−Martin periodinane (DMP) to provide **7** in excellent yields. The bis-ketone **7** could be either transformed into the key intermediate **8** via a regioselective hydrogenation reaction or processed through a reductive amination reaction with an amino acid ester and hydrolysis of the ensuing ester to give the final compounds **74**−**77**.

The reduction of the benzylic ketone by hydrogenation is probably the step of the synthesis that would most warrant improvement, as the reaction usually necessitates harsh conditions to proceed, such as a large excess of palladium on carbon, high pressure and lengthy reaction times; nonetheless, the recovery of desired product is good. Many alternative reaction conditions were assessed to reduce this ketone, for instance, a Wolf−Kishner reaction or triethylsilane in trifluoroacetic acid (TFA), but a lack of reactivity or degradation was typically observed. In general, the removal of the benzylic keto group has proven to be difficult from oxygen and carbon-bridged scaffolds (X = O, C) and impossible from sulfur-bridged compounds (X = S) and in some cases when the aromatic ring is substituted (R ≠ H).

Compounds **8** are racemic mixtures that can be resolved by preparative chiral HPLC. For example, intermediates **8a**, **8b**, and **8c** have been resolved and the two enantiomers have been named **8a** (ent 1), **8a** (ent 2), **8b** (ent 1), **8b** (ent 2), and **8c** (ent 1), **8c** (ent 2) depending on their respective retention times in the corresponding HPLC separation. For compounds **8a** (ent 1) and **8a** (ent 2) the absolute stereochemistry was determined by means of ab initio vibrational circular dichroism (VCD) analysis;[10] hence, **8a** (ent 1) was assigned as the (1*R*)-enantiomer and **8a** (**ent 2**) as the (1*S*)-enantiomer.

Scheme 1. General Synthetic Procedure for the Preparation of Compounds **9** and **43**−**77**[a]



[a] (i) *tert*-Butanol, potassium *tert*-butoxide, allyl bromide, 35 °C (starting from **1c** and **1d**, the reaction led directly to **3**); (ii) Claisen rearrangement; (iii) degassed CH_2Cl_2, Grubbs second generation catalyst, room temp; (iv) BH_3 in THF, room temp, H_2O, then NaOH 3 M, H_2O_2, room temp; (v) DMP, dry CH_2Cl_2, room temp; (vi) THF/acetic acid, Pd/C, H_2, room temp; (vii) dimethylamine or appropriate amino esters (R^1; see Tables 1−3), DCE, $NaBH(OAc)_3$, room temp; (viii) KOH, MeOH/H_2O. (ix) To obtain **8b**: (a) H-Cube hydrogenation (30 atm at 60 °C); (b) DMP, dry CH_2Cl_2, room temp.

Henceforth, compound **8a** (ent 1) is named **8a**(*R*) and **8a** (ent 2) is named **8a**(*S*).

The reductive amination reaction that leads to the formation of the second stereogenic center gave good diastereomeric excess; in fact, the amine **9′a** and the amino esters **10**−**38** and **39**−**42** were obtained with a diastereomeric ratio between 70/30 and 90/10. The major diastereoisomer is identified with the prime symbol (′) while the minor with the double prime (″). The separation of the two diastereoisomers was carried out at this stage by using, also in this case, a preparative chiral HPLC because of the difficulty of the separation by conventional silica gel chromatography. The last step of the synthesis was the hydrolysis of the esters **10**−**38** and **39**−**42** to give the final amino acids **43**−**73** and **74**−**77** that were habitually purified by chromatography on a C18 column.

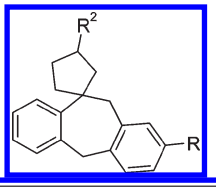
Considering that in the final compounds there are two stereogenic centers and consequentially four stereoisomers, it was imperative to identify the most active isomer in order to simplify the exploration. This analysis was carried out using guvacine as amino ester and both the single known enantiomers of the spiroketone **8a**, **8a**(*R*) and **8a**(*S*) (see Table 1). The amino acid products obtained, **45** and **46**, were tested as a mixture of diastereoisomers, and it was clear that the mixture **46**, obtained from **8a**(*S*) via amino ester **13**, was the most active. The two diastereoisomers of **13** were then separated to give the major **13′** and the minor **13″** isomers. In this case we observed that the major diastereoisomer (**13′**) gave the best compound in terms of a mixed H_1/5-HT_{2A} profile (**47**), while the minor diastereoisomer (**13″**) gave **48**, which is potent at H_1 but inactive at 5-HT_{2A}. Tables 1−3 illustrate the structures of compounds (**43**−**73** and **74**−**77**) prepared according to the procedures described in Scheme 1, along with corresponding data from primary assays. In order to better identify the stereochemistry of the reported compounds, Table 1−3 also contain details of the parent amino esters from which they were derived and the parent spiroketone used in the reductive amination step (Scheme 1, step vii).

Results and Discussion

All the newly prepared compounds were assayed for their agonist and antagonist activities using two kinds of functional assays: a FLIPR (fluorescent imaging plate reader) assay, using stably transfected Chinese hamster ovary (CHO) cells expressing the human H_1 receptor and stably transfected SHSY5Y cells expressing the human 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors; a luminescence (Aequorin) assay using frozen human embryonic kidney (HEK) cells stably expressing the human 5-HT_{2A} receptor. Most of the 5-HT_{2A} data reported in Tables 1−3 were generated using the aequorin assay, and those generated with the FLIPR assay are indicated with a footnote. The well-known ketotifen[20] and MDL-100,907[21] were used as positive controls for H_1 and 5-HT_{2A} receptor antagonists; data obtained were consistent with the corresponding affinities reported in the literature.

Before entering into a detailed discussion of the results obtained, a few general observations can be made. In order to combine the effect on sleep latency of a H_1 antagonist with the effect on sleep maintenance of a 5-HT_{2A} antagonist, we considered, in the first instance, balanced dual H_1/5-HT_{2A} antagonist compounds to achieve comparable receptor occupancy for the two targets. No agonist activity was observed for any of the amino acids considered. High selectivity vs 5-HT_{2B} and 5-HT_{2C} receptors proved elusive; however, this was not

Table 1. Functional Activity (*f*-pK_i[a]) at the Human H_1[b], 5-HT_{2A}[c], 5-HT_{2B},[b] and 5-HT_{2C}[b] Receptors, PSA[d] and clogD[e] for Compounds **43**−**67**[j,k]



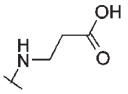
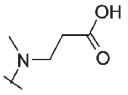
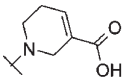
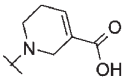
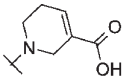
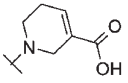
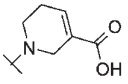
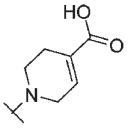
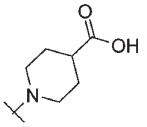
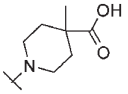
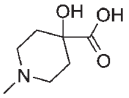
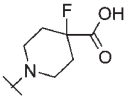
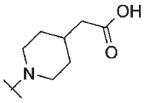
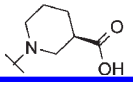
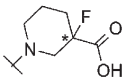
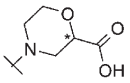
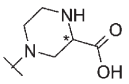
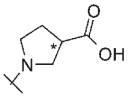
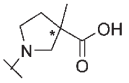
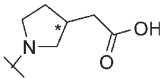
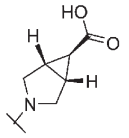
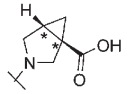
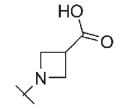
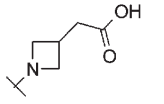
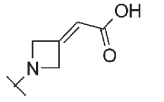
Cmpd	Parent Amino Esters (10-32)	Parent Spiroketone (8)	R^2	R	*f*-pKi[a]				PSA[d]	cLogD[e]
					H_1[b]	5-HT_{2A}[c]	5-HT_{2B}[b]	5-HT_{2C}[b]		
Ketotifen[i]	-	-	-	-	9.8	7.8	7.5	6.7	-	-
MDL-100,907[l]	-	-	-	-	6.0	10.0	5.9	6.5	-	-
9′a	-	8a(*S*)		H	8.4	7.9[b]	7.8	8.1	3	2.5
43	10	8a(*R*)		H	7.6	5.7	<5.7	<5.7	49	2.3
44	11[f]	8a(*S*)		H	8.0	6.8	<5.7	7.0	41	2.2
45	12	8a(*R*)		H	6.8	5.4	<5.7	<5.7	41	2.3
46	13	8a(*S*)		H	7.8	6.4[a]	5.9	6.8	41	2.3
47	13′	8a(*S*)		H	7.6	6.9	<5.7	6.8	41	2.3
48	13″	8a(*S*)		H	7.7	<5.7[b]	<5.7	<5.7	41	2.3
49	14′	8b (ent2)		F	7.4	<6.12	<5.7	6.5	41	2.2
50	15′ isomer 1[h]	8a		H	7.4	7.1	<5.7	6.7	41	2.1
51	16′	8a(*S*)		H	7.3	7.1	6.8	7.2	41	2.0
52	17′ isomer 1[h]	8a		H	7.3	7.4	8.5	7.8	41	2.4
53	18′ isomer 2[h]	8a		H	7.3	7.0	6.6	6.9	61	1.2
54	19′	8a(*S*)		H	7.4	7.3[b]	<5.7	6.0	41	1.7
55	20′ isomer 2[h]	8a		H	7.6	6.2	6.0	<5.6	41	2.0
56	21′	8a(*S*)		H	7.7	7.2[b]	5.9	7.5	41	1.9

Table 1. Continued

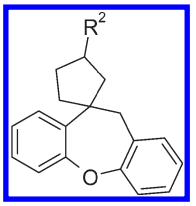
Cmpd	Parent Amino Esters (10-32)	Parent Spiroketone (8)	R^2	R	*f*-pKi[a]				PSA[d]	cLogD[e]
					H_1[b]	5-HT_{2A}[c]	5-HT_{2B}[b]	5-HT_{2C}[b]		
57	22′	8a(*S*)		H	6.1	7.1	<5.7	5.8	41	1.4
58	23′ isomer 2[g]	8a(*S*)		H	7.2	7.0	<5.7	6.5	50	1.0
59	24′ isomer 2[g]	8a(*S*)		H	7.0	6.5	5.9	6.7	53	1.4
60	25′ isomer 2[g]	8a(*S*)		H	7.8	7.7	7.0	7.8	41	1.8
61	26 isomer 2[g]	8a(*S*)		H	7.9	7.9	8.0	7.7	41	2.2
62	27′ isomer 2[g]	8a(*S*)		H	7.5	7.7	6.0	7.7	41	1.6
63	28′ isomer 2[h]	8a		H	7.4	6.2	<5.7	<5.7	41	1.6
64	29′ isomer 2[g]	8a(*S*)		H	7.5	7.5	6.4	7.2	41	1.6
65	30′	8a(*S*)		H	8.2	7.8	7.1	8.2	41	1.6
66	31′ isomer 2[h]	8a		H	6.5	7.3	7.4	<5.7	41	1.2
67	32′ isomer 1[h]	8a		H	6.7	7.3	6.6	6.6	41	0.6

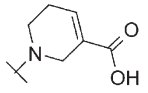
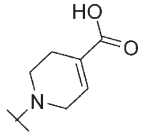
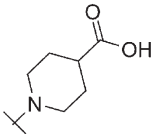
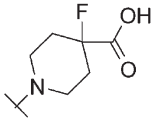
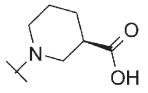
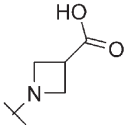
[a] *f*-pK_i = functional pK_i obtained from the FLIPR and aequorin assays. SEM for H_1, 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} data sets is < 0.1 with a minimum of two replicates. [b] FLIPR = fluorescent imaging plate reader. See Experimental Section for assay details. [c] Aequorin assay (intracellular Ca luminescence). See Experimental Section for assay details. [d] Å^2. [e] ACD logD 7.4, version 11. [f] Not isolated. [g] When the reductive amination is performed with a racemic amine (R^1 in Scheme 1) and the chiral ketone **8a**(*S*), the term "isomer 1" is used for the single stereoisomer with the shorter retention time in the conditions of the chiral separation. Conversely the term "isomer 2" is used for the single stereoisomer with the longer retention time in the conditions of the chiral separation. See Experimental Section for details. [h] When the reductive amination is performed with a chiral or achiral amine (R^1 in the Scheme 1) and the racemic ketone **8a**, the term "isomer 1" is used for the single stereoisomer with the shorter retention time in the conditions of the chiral separation. Conversely the term "isomer 2" is used for the single stereoisomer with the longer retention time in the conditions of the chiral separation. See Experimental Section for details. [i] H_1 antagonist positive control. [j] Symbol ∗ represents the following: single unknown enantiomer. [k] Symbols′ and ″ represent the following: The major diastereoisomer after reductive amination reaction was identified with the prime symbol (′) while the minor with the double prime (″). [l] 5-HT_{2A} antagonist positive control.

considered a criterion to preclude compound progression because of the minor role of these two receptors in sleep regulation, as stated previously. With regards to selectivity versus adrenergic α_1 receptors and the hERG potassium channel, which was a concern for our initial hit **9′a** (Figure 1), we never observed significant activity for any of the zwitterionic compounds synthesized and tested in these assays (*f*-pK_i-(h$\alpha_{1A/B}$) < 6 in the FLIPR assay, hERG is discussed in detail below). In terms of activity versus the primary targets we noticed that the zwitterionic modification almost always retained the H_1 activity but reduced the 5-HT_{2A} potency; thus, in light of these preliminary results, it was clear that the challenge for the exploration was to find balanced dual antagonist zwitterionic compounds.

As highlighted above, the objective of this optimization strategy was to identify suitable zwitterionic moieties able to maximize the in vitro affinity versus both the H_1 and 5-HT_{2A} receptors and the selectivity toward undesired biological targets enhancing the druglike characteristics of compound **9′a**. Thus, the *β*-alanine derivative **43**[22] and the *N*-methyl

Table 2. Functional Activity (*f*-pK_i[a]) at the Human H_1,[b] 5-HT_{2A},[c] 5-HT_{2B},[b] and 5-HT_{2C}[b] Receptors, PSA,[d] and clogD[e] for Compounds **68**−**73** (See Table 1 for Footnotes)

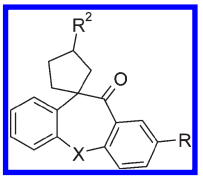


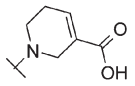
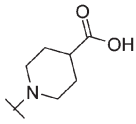
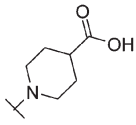
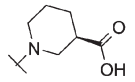
Entry	Parent Amino Esters (33-38)	Parent Spiroketone (8)	R^2	*f*-pKi[a]				PSA[d]	cLogD[e]
				H_1[b]	5-HT_{2A}[c]	5-HT_{2B}[b]	5-HT_{2C}[b]		
68	**33′ isomer 1**[h]	**8c**		7.0	6.4	<5.7	<5.7	50	1.5
69	**34′ isomer 2**[h]	**8c**		7.3	7.6	<5.7	6.0	50	1.4
70	**35**	**8c**		7.1	6.5	5.8	NT	50	1.2
71	**36′ isomer 2**[h]	**8c**		6.8	6.4	<5.7	<5.7	50	1.0
72	**37**	**8c**		6.9	7.0	<5.7	NT	50	1.2
73	**38′**	**8c (ent 2)**		7.3	7.5	6.1	7.5	50	0.8

analogue **44**, characterized by a reduced lipophilicity and increased polar surface area (PSA) with respect to **9′a**, were readily prepared and tested as a mixture of diastereoisomers. Both of them showed good potency at the H_1 receptor (*f*-pK_i = 7.6 and 8.0 for **43** and **44**, respectively), whereas the tertiary amine **44** exhibited higher in vitro activity at the 5-HT_{2A} receptor (*f*-pK_i = 5.7 and 6.8 for **43** and **44**, respectively), although it proved to be less potent than the reference compound **9′a** (*f*-pK_i = 7.9). Compound **43** was found to be more selective than parent compound **9′a**, whereas **44** retained some in vitro activity at the 5-HT_{2C} receptor (*f*-pK_i = 7.0). Notably both compounds were found to be clean in terms of CYP450 inhibition (IC_{50} > 10 μM against all isoforms tested) and affinity for the hERG ion channel (pIC_{50} < 4.2 and pIC_{50} = 4.2 for **43** and **44**, respectively). Further to these promising initial results it was decided to concentrate the exploration on the amino acid moiety with the aim of maximizing the in vitro affinity for the 5-HT_{2A} receptor while maintaining the improved developability properties observed. To this end, a series of modified/constrained β, γ and δ-amino acid derivatives shown in Table 1 was synthesized.

The results obtained for **43** and **44** suggested that the exploration should be biased toward tertiary amines and, in particular, attention was focused on cyclic amines. Thus, compound **47** emerged with good H_1 activity (*f*-pK_i = 7.6), a satisfactory level of 5-HT_{2A} potency (*f*-pK_i = 6.9), good selectivity versus 5-HT_{2B}, and no selectivity over 5-HT_{2C} (*f*-pK_i = 6.8). Compound **47** was also found to be clean in terms of CYP450 inhibition (IC_{50} > 10 μM against all isoforms tested) and affinity for the hERG ion channel (pIC_{50} < 4.4). The substitution of the tetracyclic scaffold was also investigated, but the scope of the exploration was limited to a specific position, following some indications from the literature,[23] due to synthetic feasibility. Therefore, compound **49** was synthesized and it was found to show high potency on H_1 (*f*-pK_i = 7.4), but unlike the unsubstituted analogue **47**, it was inactive as a 5-HT_{2A} receptor antagonist. The exploration continued on the tetrahydropyridine substructure and the isoguvacine derivative **50** came out with suitable H_1/5-HT_{2A} potencies (*f*-pK_i = 7.4 and *f*-pK_i = 7.1, respectively) and 5-HT_{2B} selectivity. Some isonipecotic acid derivatives were also taken into consideration, and the reference compound for this subclass, compound **51**, maintained a good overall target profile (*f*-pK_i = 7.3/*f*-pK_i = 7.1, on H_1/5-HT_{2A}, respectively) without any selectivity versus 5-HT_{2B} and 5-HT_{2C} as well as resulting to be clean in terms of CYP450 (IC_{50} > 10 μM against all isoforms tested) and hERG ion channel inhibition (pIC_{50} < 4.2). Substitution in the α-position of the acidic moiety of **51** led to

Table 3. Functional Activity (*f*-pK_i[a]) at the Human H_1,[b] 5-HT_{2A},[c] 5-HT_{2B},[b] and 5-HT_{2C}[b] Receptors, PSA,[d] and clogD[e] for Compounds **74**–**77** (See Table 1 for Footnotes)



Cmpd	Parent Amino Esters (39-42)	Parent Spiroketone (8)	R^2	R	X	*f*-pKi[a]				PSA[d]	cLogD[e]
						H_1[b]	5-HT_{2A}[c]	5-HT_{2B}[b]	5-HT_{2C}[b]		
74	**39**	**7a**		H	C	7.1	<5.7	NT	<5.7	58	0.9
75	**40**	**7d**		Cl	O	6.0	6.4	<5.7	NT	67	1.1
76	**41**	**7e**		F	S	6.7	6.9	<5.7	<5.7	58	2.4
77	**42**	**7d**		Cl	O	6.7	<5.7	<5.7	NT	67	1.1

compounds **52**, **53**, and **54** characterized by good H_1/5-HT_{2A} antagonist potencies and increasing selectivity versus 5-HT_{2B} and 5-HT_{2C} passing from methyl to fluorine substitution, suggesting that electron withdrawing groups (EWG) in this position successfully improve selectivity. Compound **54** also shows a clean CYP450 profile (IC_{50} > 10 μM against all isoforms tested) and selectivity over the hERG ion channel (pIC_{50} < 4.4). Homologation of compound **51** was not tolerated for 5-HT_{2A} receptor antagonist activity (compound **55**). Moving to the nipecotic acid derivatives, compound **56** showed potent primary assay activity and 5-HT_{2B} selectivity, while its fluorine substituted analogue, compound **57**, is selective versus both 5-HT_{2B} and 5-HT_{2C} receptors, confirming that an EWG in the α-position enhances selectivity but is poorly active on H_1. The morpholine and piperazine derivatives were also considered and synthesized; both of them are selective over 5-HT_{2B}, but while compound **58** has well balanced target potencies, a slight drop in the 5-HT_{2A} activity was observed for **59**.

A group of pyrrolidines was also studied, and in general, all the compounds synthesized showed increased potency compared to the piperidine derivatives previously described. Compounds **60** and **61** have similar pharmacological profiles, characterized by very high potency on the primary targets and no selectivity at all versus 5-HT_{2B} and 5-HT_{2C} receptors, while the homologated compound **62** retains potency on H_1/5-HT_{2A} with a significantly reduced 5-HT_{2B} activity, displaying a different behavior with respect to the homologated compound **55**. Two [3.1.0] amino acidic fragments were also investigated and the corresponding final compounds **63** and **64** synthesized; they show very interesting profiles with high H_1 antagonist potency. In particular **64** is characterized by a well balanced profile (*f*-pK_i = 7.5/*f*-pK_i = 7.5, on H_1/5-HT_{2A}, respectively), with selectivity versus 5-HT_{2B} (*f*-pK_i = 6.4), the hERG ion channel (pIC_{50} < 4.4), and a clean CYP450 profile (IC_{50} > 10 μM against all isoforms tested).

The analysis of different cyclic secondary amines, which started with piperidine, passing through pyrrolidines and arriving at azetidine derivatives, is now close to completion. Three compounds were prepared belonging to the latter class. The first, compound **65**, is the most active and balanced derivative found (*f*-pK_i = 8.2/*f*-pK_i = 7.8, H_1/5-HT_{2A}, respectively), even if it is not selective versus 5-HT_{2C} (*f*-pK_i = 8.2). Compound **65** also shows a clean CYP450 profile (IC_{50} > 10 μM against all isoforms tested) and selectivity over the hERG ion channel (pIC_{50} < 4.3). The other two azetidine derivatives, **66** and **67**, are characterized by an unbalanced profile versus 5-HT_{2A}, and the former is also characterized by inactivity versus 5-HT_{2C}.

With the aim of increasing PSA and further reducing lipophilicity, we decided to introduce heteroatoms in the scaffold and a number of oxepine derivatives were synthesized (Table 2). Learnings from the previous exploration were transferred to the new oxepine scaffold, and the first two compounds prepared, guvacine derivative **68** and isoguvacine derivative **69**, displayed some very interesting features. In particular, a trend of selectivity versus 5-HT_{2B} and 5-HT_{2C}, driven by the oxepine scaffold, is evident, even if there is also a trend toward reduced potency on the primary targets. In fact **69**, besides compound **54** mentioned above, was the only compound synthesized that combines selectivity (*f*-pK_i ≤ 5.7/*f*-pK_i = 6.0, on 5-HT_{2B}/5-HT_{2C}, respectively) with appropriate potencies on the desired targets (*f*-pK_i = 7.3/*f*-pK_i = 7.6, on H_1/5-HT_{2A}, respectively). Some piperidine derivatives were also considered in this case, and three compounds, **70**, **71**, and **72**, were prepared. Unfortunately, they did not withstand the reduction of potency derived from the oxepine scaffold, showing moderate activities on the target receptors. On the other

Table 4. Summary of the Affinity (pK_i) of Compounds at the Rat Native H_1/5-HT_{2A} Receptors Determined Using Radioligand Binding

	pK_i[a]	
compd	r-H_1[b]	r-5-HT_{2A}[c]
47	8.06	6.93
51	8.33	7.47
54	7.91	6.45
64	8.27	7.16
65	8.25	7.48
69	7.70	6.98
73	7.85	7.47

[a] pK_i values are the mean of two independent experiments performed in duplicate. The difference between the averaged pK_i values was lower than 0.35. [b] [^{3}H]Pyrilamine binding to rat cortical membranes. [c] [^{3}H]-Ketanserin binding to rat cortical membranes.

hand, the azepine derivative **73** was very well tolerated (*f*-pK_i = 7.3/*f*-pK_i = 7.5, on H_1/5-HT_{2A}, respectively) and displayed low activity for the 5-HT_{2B} receptor (*f*-pK_i = 6.1) and no selectivity versus the 5-HT_{2C} receptor (*f*-pK_i = 7.5). Compound **73** showed a clean CYP450 profile (IC_{50} > 10 μM against all isoforms tested) and selectivity over the hERG ion channel (pIC_{50} < 4.3).

Table 3 reports some efforts of structural diversification. Fixing the carbonyl group next to the spiro system, substitution on the phenyl ring on the right-hand side (RHS), and different heteroatoms in the benzhydrylic position in the lower part of the compound (X = C, O, and S) were considered. A comparison between compounds **47**, **68**, and **74**, representative examples from the three scaffolds considered in this exploration, showed how, starting from the carbocyclotetraspiro, then considering the oxepine, and finally moving to the last scaffold described with a carbonyl group, the H_1 activity was basically maintained but the 5-HT_{2A} affinity dropped in the last case. The only compound, in this last subseries, able to maintain a balanced overall target profile, albeit with moderate potency, was **76**, characterized by a fluorine on the RHS phenyl ring and a sulfur on the lower part.

H_1 and 5-HT_{2A} Receptor Binding in Rat Cortex. Competition studies were performed to determine test compound affinity at both H_1 and 5-HT_{2A} receptors in membranes from rat cortex by using [^{3}H]pyrilamine and [^{3}H]ketanserin as radioligands, respectively. Data obtained for the most advanced compounds, **47**, **51**, **54**, **64**, **65**, **69**, and **73**, are reported in Table 4. In general, the pK_i values obtained in binding studies for 5-HT_{2A} were in good agreement with the *f*-pK_i values found in the functional assay in human recombinant cells (Tables 1 and 2), whereas the affinity values for the H_1 receptor were higher than functional potencies. This difference could depend on the species (rat versus human) and/or different conditions of the assays, such as the ionic composition of the incubation media or the incubation time.

Pharmacokinetic Studies. Compounds with favorable in vitro profiles, able to combine H_1 and 5-HT_{2A} potencies and exhibit selectivity versus 5-HT_{2B} and/or 5-HT_{2C}, were progressed through the screening cascade and evaluated in rat pharmacokinetic studies, both in vitro and in vivo (Table 5). All the amino acids considered for progression displayed very low values of intrinsic clearance in rat and human microsomes, low CYP450 inhibitory potencies, thus minimizing the possibility of drug−drug interactions, and increased fraction unbound in brain with respect to the starting lead amine **9′a**. The in vivo pharmacokinetics were investigated in male rat following intravenous (iv) and oral (po) administration, in a cassette dosing experimental design. Brain penetration was determined, in almost all the experiments, after po administration through measurement of the brain to blood AUC_{0-8h} ratio. For **47** brain penetration was measured at 1 h following iv dosing. After iv administration, all the compounds showed low clearance, in agreement with the in vitro results except for **54**. The volume of distribution (Vss) was low for **47** and moderate for the other compounds, and the half-life ($t_{1/2}$) was between 1 and 3 h except for compound **64** where it was longer ($t_{1/2}$ = 5.3 h). After oral administration, all the compounds displayed moderate to high bioavailability (Fpo) with the exception of **54** (Fpo = 22%), and the brain penetration generally ranged from 0.1 to 0.5. Only compounds **64** and **69** showed higher brain penetration of 2.2 and 1.8, respectively. Progression of some compounds was determined on the basis of these results: Compound **54** was stopped because of its inferior in vivo profile, characterized by low bioavailability combined with high blood clearance and also low brain penetration. Compound **64** was stopped as well, although it did not present any major PK issues, as the half-life of 5.3 h did not reflect the hypnotic profile required.

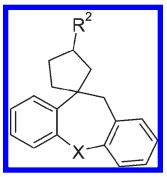
Despite their promising profile, compounds **47** and **69** were put on hold because of a developability concern related to the potential toxicity associated with their tetrahydropyridine substructures.[24]

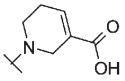
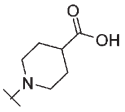
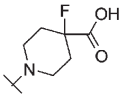
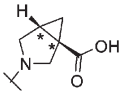
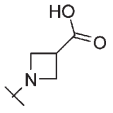
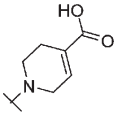
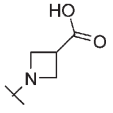
In Vivo Pharmacodynamic Profile. The hypnotic effects of the remaining compounds (**51**, **65**, and **73**) were assessed using telemetric recording of electroencephalogram (EEG) and electromiogram (EMG) in the rat. Simultaneous recording of EEG and EMG allows the accurate derivation of the following sleep parameters: awake, nonrapid eye movement (NREM) sleep, and REM sleep. The test compound's effects were assessed for 5 h immediately after treatment in the active phase, starting at circadian time [CT] 18 (lights off at circadian time 12) of the rat light−dark cycle. CT18 was chosen to allow a maximal window to assess the hypnotic effects of the compound.

The three compounds were tested orally in this paradigm at 1, 3, and 10 mg/kg. All of them showed dose related increases in total sleep time (TST) during the 5 h of observation, reaching a statistically significant effect at 3 mg/kg for compound **65** ($p < 0.01$) and at 10 mg/kg for compounds **51** ($p < 0.01$) and **73** ($p < 0.01$). No effect was observed on the sleep latency (Table 6). An effect of H_1 antagonists on sleep latency has been reported in the literature[4,25] where compounds were tested in a disturbed sleep model; taken together with our results, these data suggest that H_1/5HT_{2A} antagonists may act on sleep onset in disturbed sleep conditions.

Ex Vivo Receptor Occupancy Study. Two compounds, **51** and **65**, were characterized for their receptor occupancy (RO) in rats in order to understand the level of occupancy achieved at both target receptors at doses where **51** and **65** have been shown to produce a hypnotic effect in rats (CT18 model). Three doses were tested (1, 3, and 10 mg/kg, po, 2 h pretreatment). A time point of 2 h after treatment was chosen on the basis of preliminary PK data indicating T_{max} occurred at 2 h for both compounds with plasma exposures C_{max} = 216 ng/mL for compound **51** (at 1 mg/kg po) and C_{max} = 626 ng/mL for compound **65** (at 3 mg/kg po). At this time point the maximal RO values are expected. In the RO study, both compounds showed a linear plasma exposure at T_{max} across the three doses tested (compound **51** showing 119, 303, and 755 ng/mL at 1, 3, and 10 mg/kg, respectively; compound **65** showing 127, 417, and 1217 ng/mL at 1, 3, and 10 mg/kg, respectively).

Table 5. Rat Pharmacokinetic Profile for Compounds



Cmpd	R^2	X	*in-vitro* PK			*in-vivo* PK [d]				
			Cli rat; hum [a] ml/min/g liver	CYP450 [b] IC_{50} (μM)	Br/Bl fu [c] %	CLb ml/min/kg	Vss L/kg	t½ h	Fpo %	Br:Bl
47		CH_2	<0.5; <0.5	All > 10	3.0 / 2.4	2	0.4	2.4	80	0.1
51		CH_2	0.8; <0.5	All > 10	3.1 / 18.2	21	2.9	1.9	65	0.5
54		CH_2	<0.5; <0.5	All > 10	2.0 / 8.4	61	3.8	1.2	22	0.1
64		CH_2	<0.5; <0.5	All > 10	2.3 / 8.0	5	2.4	5.3	62	2.2
65		CH_2	<0.5; <0.5	All > 10	3.2 / 12	9	2.1	3.1	54	0.1
69		O	<0.5; <0.5	All > 10	2.7 / 10.3	16	2.7	3.0	37	1.8
73		O	0.6; <0.5	All > 10	4.0 / 17.1	13	1.9	1.9	100	0.2

[a] Intrinsic clearance in liver microsomes, rat and human. [b] CYPEX assay. [c] fu = fraction unbound in brain−blood rat. [d] In vivo data determined by 0.5 mg/kg iv and 1 mg/kg po administration in rat for compounds **51**, **64**, **73** and by 1 mg/kg iv and 3 mg/kg po administration in rat for compounds **47**, **54**, **65** and **69**. Br:Bl measured by brain to blood AUC_{0-8h} ratio following po dosing for **47** at 1 h following iv dosing.

Table 6. Sleep Promoting Effects of Compounds **51**, **65**, and **73** Administered Orally at 1, 3, and 10 mg/kg in Rat during Active Phase (Starting CT18)[a]

compd	dose	TST (min)	NREM sleep latency (min)	NREM sleep over 5 h (min)	REM sleep latency (min)	REM sleep over 5 h (min)
51	veh	90.2 ± 10.7	54.2 ± 13.9	80.9 ± 8.5	70.3 ± 12.8	9.3 ± 3.3
	1 mg/kg	94.4 ± 7.4	69.8 ± 20.3	84.7 ± 6.4	83.6 ± 21.0	9.7 ± 1.7
	3 mg/kg	105.2 ± 9.1	51.4 ± 11.5	94.2 ± 8.1	77.9 ± 13.6	11.0 ± 2.1
	10 mg/kg	105.2 ± 9.1*	34.6 ± 6.8	103.7 ± 9.8*	83.8 ± 18.0	12.6 ± 2.0
65	veh	92.1 ± 5.8	39.8 ± 7.1	79.4 ± 5.6	75.3 ± 13.9	12.7 ± 3.2
	1 mg/kg	98.1 ± 10.9	58.1 ± 11.7	87.4 ± 9.3	77.8 ± 23.9	10.7 ± 2.1
	3 mg/kg	134.7 ± 9.8**	51.6 ± 6.0	118.6 ± 9.1**	81.6 ± 9.8	16.1 ± 1.9
	10 mg/kg	145.2 ± 11.5**	33.5 ± 7.2	130.5 ± 10.1**	64.5 ± 8.9	14.6 ± 2.2
73	veh	116.1 ± 7.6	40.4 ± 6.3	105.6 ± 21	57.0 ± 18	10.5 ± 1.3
	1 mg/kg	120.7 ± 7.8	37.8 ± 7.1	107.3 ± 6.6	67.0 ± 12.3	13.3 ± 2.3
	3 mg/kg	138.3 ± 12.9	37.9 ± 7.5	123.2 ± 12	77.7 ± 12.1	15.1 ± 2.7*
	10 mg/kg	155.7 ± 11.6**	31.5 ± 5.7	139.5 ± 10.5**	63.4 ± 8.2	16.2 ± 1.7*

[a] (*) $p < 0.05$ and (**) $p < 0.01$ for each dose versus corresponding vehicle as determined by one-way ANOVA followed by Dunnett's test. Values are expressed as the mean ± SEM; $n = 8$. See Experimental Section for assay details.

[^{3}H]Ketanserin was used as ligand for the 5-HT_{2A} receptor, whereas [^{3}H]pyrilamine was used for the H_1 receptor. Compound **51** showed receptor occupancy levels of 0%, 5.6%, and 18% for the 5-HT_{2A} receptor and 30%, 35%, and 55% for the H_1 receptor. Similarly, compound **65** showed levels of 0.9%, 24%, and 47% for the 5-HT_{2A} receptor and 16%, 45%, and 66% for the H_1 receptor. The two compounds **51** and **65** showed efficacy in the rat sleep model (CT18) at 10 mg/kg po and at 3 mg/kg po, respectively (Table 6), thus suggesting that in order to reach a hypnotic profile, a combination of a low RO

of both H_1 and 5-HT_{2A} receptors is needed; in particular a 5-HT_{2A} receptor occupancy of ~20% is sufficient to observe the in vivo readout.

Conclusions

A new spirotetracyclic class of dual H_1/5-HT_{2A} antagonists, together with the lead optimization process, has been presented. Starting from an unselective and poorly developable lead (**9′a**), a zwitterionic approach successfully delivered high quality leads characterized by low risk of CYP450 inhibition and low affinity vs the hERG potassium channel and adrenergic receptors. On the basis of their favorable overall profile, several leads (**51**, **65**, and **73**), with equivalent in vitro potencies and in vivo profiles, were identified and progressed in the screening cascade toward a pharmacodynamic model of sleep disorders (rat CT18 sleep model), where they exhibited a robust effect, thus confirming a possible application as an alternative therapy to currently available hypnotic drugs with the potential for a reduced side effect burden.

Experimental Section

Biological Test Methods. FLIPR screening assays for H_1, 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} were all run essentially as described in Wigglesworth et al.[26]

5-HT_{2A} Aequorin Assay.[27] Frozen human embryonic kidney (HEK) cells stably expressing the human 5-HT_{2A} serotonin receptor and aequorin apoprotein were thawed and added dropwise to an appropriate volume of warm DMEM media (Gibco Invitrogen 41965-039) containing 10% dialyzed fetal bovine serum (FBS) (Invitrogen, 05-4011DK). Cells were then spun down at 1000 rpm for 5 min at room temperature. The supernatant was poured off and the pellet resuspended in HBSS buffer (Sigma kit H1387) supplemented with HEPES (Sigma H0887), $NaHCO_3$ (Sigma S8761), 0.1% pluronic acid F68 solution (Gibco Invitrogen 24040-032), and 0.1% bovine serum albumin (CalBiochem 126609). A sample was taken and a cell count performed. Cells were diluted down to 2.5×10^6 cells/mL in loading buffer. Coelenterazine [5 μM] (Invitrogen C6780) was added to the cell suspension and the cell vessel wrapped in foil. The cell vessel was put on a windmill rotator (Bibby Stuart) and left overnight at room temperature. Before assay, a sample was taken and a cell count performed. Cells were diluted to an appropriate final density immediately prior to assay. Plates containing compounds (0.5 μL) were placed in a Lumilux, where they were diluted in buffer (20 μL), before additions of cells (20 μL) and a predetermined submaximal concentration of 5-HT (20 μL), while luminescence was monitored. Data were analyzed using area under curve for the entire time course, normalized to in-plate nominal high and low controls, and fitted to a four-parameter logistic equation.

hERG ^{3}H-Dofetilide Binding Assay. hERG activity was measured using ^{3}H-dofetilide binding in a scintillation proximity assay (SPA) format. The activity was measured with a Perkin-Elmer Viewlux imager.

Receptor Binding Assays. The in vitro binding assays of H_1 and 5-HT_{2A} receptors were performed using the rat cerebral cortex. Male Spagrue−Dawley rats (200−250 g) were decapitated, and the cerebral cortex was rapidly removed and homogenized in 20 volumes of ice-cold 50 mM Tris-hydrochloride buffer, pH 7.4. The homogenate was centrifuged at 41000*g* for 15 min at 4 °C. The pellet was resuspended in buffer, incubated at 37 °C for 15 min, and washed twice by the same procedure. The final pellet was resuspended in 4 volumes of ice-cold buffer. The final suspension was distributed into aliquots and stored at −80 °C. Protein content in the membrane preparations was evaluated using the Bradford method (BCA protein assay, PIERCE).

The receptor−ligand binding assays were performed using [ethylene-3*H*]ketanserin hydrochloride (NET-791, 2.22−3.33 TBq/mmol, Perkin-Elmer) for 5-HT_{2A} receptor binding and [pyridinyl-5-3*H*]pyrilamine (TRK608, 1.1 TBq/momol, Amersham) for H_1 receptor binding. Binding experiments were carried out in deep-well 96-well plates in a total volume of 0.5 mL/well containing the radioligand (final concentration of 1 nM [^{3}H]ketanserin or 0.6 nM [^{3}H]pyrilamine), tissue suspension (50 μg/well for 5-HT_{2A} receptor or 150 μg/well for H_1 receptor), various concentrations of test compound, and the appropriate buffer (50 mM Tris-HCl, 10 mM $MgCl_2$, pH7.4, for 5-HT_{2A} receptor or 50 mM HEPES, pH 7.4, for H_1 receptor). The mixture was incubated with shaking at 37 °C for 1 h for 5-HT_{2A} receptor binding or at room temperature for 2 h for H_1 receptor binding.

Samples were rapidly filtered through Whatman GF/B glass-fiber filters presoaked with 0.3% PEI using a cell harvester and then washed 4 times with 4 mL of ice-cold 0.9% NaCl buffer. The filters were dried and counted in a liquid scintillation counter (Tri-Carb 2900TR, Perkin-Elmer). Nonspecific binding was determined by the presence of 10 μM mianserin or cetirizine for 5-HT_{2A} or H_1 receptor, respectively.

Radioligand binding data were analyzed by nonlinear regression analysis using GraphPad Prism 4.0 (GraphPad Software, CA). Curve fitting from competition binding experiments was determined by using a one site competition equation after checking that the Hill slope in the four-parameter logistic equation was not different from 1.0. In this condition, pK_i values of test drugs were calculated according to the Cheng−Prusoff equation from the IC_{50}, and the K_D (equilibrium dissociation constant of the radioligand) was determined at each receptor type (5-HT_{2A}, H_1) through saturation experiments. Results are expressed as mean values of two separate experiments performed in duplicate.

P450 CYPEX Assay. Inhibition (IC_{50}) of human CYP1A2, 2C9, 2C19, 2D6, and 3A4 was determined using Cypex Bactosomes expressing the major human P450s. A range of concentrations (0.1, 0.2, 0.4, 1, 2, 4, and 10 μM) of test compound were prepared in methanol and preincubated at 37 °C for 10 min in 50 mM potassium phosphate buffer (pH 7.4) containing recombinant human CYP450 microsomal protein (0.1 mg/mL; Cypex Limited, Dundee, U.K.) and probe-fluorescent substrate. The final concentration of solvent was between 3% and 4.5% of the final volume. Following preincubation, NADPH regenerating system (7.8 mg of glucose 6-phosphate, 1.7 mg of NADP, and 6 units of glucose 6-phosphate dehydrogenase/mL of 2% (w/v) $NaHCO_3$; 25 μL) was added to each well to start the reaction. Production of fluorescent metabolite was then measured over a 10 min time course using a Spectrafluor Plus plate reader. The rate of metabolite production (AFU/min) was determined at each concentration of compound and converted to a percentage of the mean control rate using Magellan (Tecan software). The inhibition (IC_{50}) of each compound was determined from the slope of the plot using Grafit, version 5 (Erithacus software, U.K.). Miconazole was added as a positive control to each plate. CYP450 isoform substrates used were ethoxyresorufin (ER; 1A2; 0.5 μM), 7-methoxy-4-triflouromethylcoumarin-3-acetic acid (FCA, 2C9, 50 μM), 3-butyryl-7-methoxycoumarin (BMC, 2C19, 10 μM), 4-methylaminomethyl-7-methoxycoumarin (MMC, 2D6, 10 μM), diethoxyfluorescein (DEF, 3A4, 1 μM), and 7-benzyloxyquinoline (7-BQ, 3A4, 25 μM). The test was performed in three replicates.

Intrinsic Clearance (Cli) Assay. Intrinsic clearance (Cli) values were determined in rat and human liver microsomes. Test compounds (0.5 μM) were incubated at 37 °C for 30 min in 50 mM potassium phosphate buffer (pH 7.4) containing 0.5 mg of microsomal protein/mL. The reaction was started by addition of cofactor (NADPH, 8 mg/mL). The final concentration of solvent was 1% of the final volume. At 0, 3, 6, 9, 15, and 30 min, an aliquot (50 μL) was taken, quenched with acetonitrile containing an appropriate internal standard, and analyzed by

HPLC−MS/MS. The intrinsic clearance (Cli) was determined from the first-order elimination constant by nonlinear regression using Grafit, version 5 (Erithacus software, U.K.), corrected for the volume of the incubation and assuming 52.5 mg of microsomal protein/g liver for all species. Values for Cli were expressed as (mL/min)/g liver. The lower limit of quantification of clearance was determined to be when $<15\%$ of the compound had been metabolized by 30 min, and this corresponded to a Cli value of 0.5 (mL/min)/g liver. The upper limit was 50 (mL/min)/g liver.

In Vivo Studies. All experiments were prereviewed and approved by a local animal care committee in accordance with the guidelines of the "Principles of Laboratory Animal Care" (NIH Publication No. 86-23, revised 1985) and with a project license that was obtained according to Italian law (Art. 7, Legislative Decree No. 116, January 27, 1992), which acknowledges European Directive 86/609/EEC on the care and welfare of laboratory animals.

In Vivo Pharmacokinetics. The pharmacokinetics and oral bioavailability of test compounds were determined following iv (bolus) and po (suspension) administration to male Sprague−Dawley rats at 0.5−1 and 1−3 mg free base/kg, respectively. Blood and brain samples were collected at intervals up to 24 h after dosing. Blood and brain samples were subsequently assayed for test compound concentration using a method based on protein precipitation followed by HPLC−MS/MS analysis.

Rat Sleep Study. Male Sprague−Dawley rats (275−300 g, Charles River Italy) were housed singly on a light−dark cycle (15:00−03:00 h light) 1 week prior to surgery. Access to food and water was allowed ad libitum.

In order to collect the biopotential signals, a miniature multichannel telemetric transmitter (TL10M3-F40-EET, Data Sciences Int.) was implanted intraperitoneally into the animals.

To allow recording of cortical electroencephalogram (EEG), two electrodes were fixed permanently, with dental cement, to the skull. They were directly in contact with the dura mater through two drilled holes on the fronto-parietal region. Two electrodes were fixed to the skeletal muscles of the neck for recording electromyogram (EMG).

After recovery from surgery, animals were maintained in their home cage in a temperature controlled environment (21 ± 1 °C) with access to food and water ad libitum. Implanted animals demonstrated a normal behavioral repertoire immediately after recovery from surgery, but to allow normal sleep patterns to be re-established, animals were utilized after 3 weeks. The environmental conditions described above were maintained throughout the sleep studies.

For the duration of the test period freely moving animals remained in their home cages on individual receivers. EEG and EMG signals were recorded continuously using DSI Dataquest A.R.T.

The EEG trace, divided into 10 s epochs, was digitally transformed (FFT transformation) to provide the power spectra of δ, θ, α, and β bands in order to distinguish three different activity patterns in the rat (awake, NREM sleep, and REM sleep). The markers assigned by the automated scoring system (sleep stage, DSI) were transferred to the EEG digital signal and subsequently confirmed by visual examination of the EEG and EMG traces by trained operators, blind to the drug treatment.

Analysis of sleep parameters included latency to NREM sleep (time interval to the first six consecutive NREM sleep epochs after injection), latency to REM sleep (time interval to the first REM sleep epoch after injection), and time spent wake, NREM sleep, REM sleep, and total sleep time (TST).

Drug studies were carried out according to a randomized paired crossover design where, in separate experimental sessions, each animal received control and drug treatments. Compounds were administered orally in a volume of 2 mL/kg in doses of 1, 3, and 10 mg/kg. Animals were treated with compound or respective vehicle 6 h after switch off of the light (circadian time (CT) 18). Recordings were made for the subsequent 5 h test period.

Results were expressed as mean value ± SEM. Statistical analysis was performed by a one-way analysis of variance followed by Dunnett's test.

Ex Vivo Receptor Occupancy by Autoradiography. Sprague−Dawley rats were acutely administered with vehicle (0.5% HPMC po) or test compounds (1, 3, or 10 mg/kg po), and after 2 h they were sacrificed and brains were quickly removed and frozen in precooled isopentane at −20 °C.

H_1 ex Vivo Receptor Occupancy. Coronal brain sections were cut at 14 μm, mounted onto microscope slides, and incubated with 1nM [^{3}H]pyrilamine (TRK608, Amersham) in 50 mM Tris-HCl, pH 7.5, buffer for 10 min at room temperature. Nonspecific binding was determined in the presence of 10 μM pyrilamine. Sections were subsequently washed 4 × 2 min in ice-cold incubation buffer and exposed to imaging plates for 7 days and analyzed with a phosphoimager scanner (Fuji BAS-5000). Receptor occupancy was measured in cerebral cortex.

5-HT_{2A} ex Vivo Receptor Occupancy. Coronal brain sections were cut at 14 μm, mounted onto microscope slides and incubated with 1 nM [^{3}H]ketanserine (NET791, Perkin-Elmer) in 50 mM Tris-HCl + 10 mM $MgCl_2$, pH 7.5, buffer for 10 min at room temperature. Nonspecific binding was determined in the presence of 10 μM mianserin. Sections were subsequently washed 4 × 2 min in ice-cold incubation buffer and exposed to imaging plates for 7 days and analyzed with a phosphoimager scanner (Fuji BAS-5000). Receptor occupancy was measured in cerebral cortex.

Chemistry. General Methods. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded either on Varian instruments at 300, 400, or 500 MHz or on Bruker instruments at 300, 400, or 500 MHz. Chemical shifts are reported in ppm (δ) using the residual solvent line as internal standard. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; b, broad. The NMR spectra were recorded at a temperature ranging from 25 to 90 °C. When more than one conformer was detected, the chemical shifts for the most abundant one is reported.

Mass spectra (MS) were run on a 4 II triple quadrupole Agilent MSD 1100 mass spectrometer, operating in ES(+) and ES(−) ionization mode. The usage of this methodology is indicated by "MS".

Optical rotations were measured by using a Jasco DIP-360 digital polarimeter with a path length of 10 cm recorded at the sodium D line.

HPLC−mass spectra were run on an Agilent LC/MSD 1100 mass spectrometer, operating in ES(+) and ES(−) ionization mode coupled with HPLC instrument Agilent 1100 series. LC/MS ES(+) was performed on a Supelcosil ABZ + Plus (33 mm × 4.6 mm, 3 m) (mobile phase, 100% [water + 0.1% formic acid] for 1 min, then from 100% [water + 0.1% formic acid] to 5% [water + 0.1% formic acid] and 95% acetonitrile in 5 min, finally under these conditions for 2 min; T = 40 °C; flow = 1 mL/min. LC/MS ES(−) was performed on a Supelcosil ABZ + Plus (33 mm × 4.6 mm, 3 m) (mobile phase, 100% [water + 0.05% ammonia] for 1 min, then from 100% [water + 0.05% ammonia] to 5% [water + 0.05% ammonia] and 95% acetonitrile in 5 min, finally under these conditions for 2 min; T=40 °C; flow = 1 mL/min. In the mass spectra only one peak in the molecular ion cluster is reported. The usage of this methodology is indicated by "HPLC−MS" in the analytical characterization of the described compounds.

Alternatively mass directed analytical HPLC (Agilent technology HP1100) was carried out using a 19 mm ×100 mm or 30 mm ×100 mm, 5 μm, reversed phase Waters Atlantis column as the stationary phase and a gradient from water + 0.1% formic acid to acetonitrile + 0.1% formic acid as the eluent. The HPLC system was monitored by DAD array detector and an Agilent 110MSD mass spectrometer. The LC elution method

(using Zorbax Eclipse XDB, 4.6 mm × 150 mm, 5 μm C8 column) was the following: 15 min method at 25 °C, mobile phase composed of different $CH_3CN/H_2O-HCOOH$ 0.1% mixtures at a flow rate of 1 mL/min (all solvents were HPLC grade, Fluka).

Alternatively HPLC spectra were performed using a reversed-phase liquid chromatography (ProStar 210/215 PrepStar218) and UV−vis detector (ProStar 325). The LC elution method (using Varian Polaris 5 C-18, 150 mm × 4.6 mm) was the following: 15 min method at 25 °C, mobile phase composed of different $CH_3CN/H_2O-HCOOH$ 0.1% mixtures at a flow rate of 1 mL/min (all solvent were HPLC grade, Fluka).

Alternatively HPLC spectra were performed using a Waters 2690 apparatus at 25 °C using a 3 mm ×100 mm, 3.5 μm, reversed phase X-Terra C-18 column as the stationary phase and a gradient from 5% [water + 0.1% formic acid] to 90% [acetonitrile + 0.1% formic acid] during 19.5 min or 20% [water + 0.1% formic acid] to 95% [acetonitrile + 0.1% formic] during 19 min as the eluent. Flow rate was 0.5 mL/min (all solvents were HPLC grade, Merck). The HPLC system was monitored by DAD array detector at 254 nm and a Micromass Quattromicro mass spectrometer.

Total ion current (TIC) and DAD UV chromatographic traces together with MS and UV spectra associated with the peaks were taken on a UPLC/MS Acquity system equipped with 2996 PDA detector and coupled to a Waters Micromass ZQTM mass spectrometer operating in positive or negative electrospray ionization mode. LC/MS ES(±) was performed using an Acquity UPLC BEH C18 column (50 mm × 21 mm, 1.7 μm particle size) and column temperature of 40 °C (mobile phase A, (water + 0.1% formic acid); mobile phase B, (acetonitrile + 0.075% formic acid); flow rate of 1.0 mL/min; gradient, t = 0 min 3% B, t = 0.05 min 6% B, t = 0.57 min 70% B, t = 1.4 min 99% B, t = 1.45 min 3% B). The usage of this methodology is indicated by "UPLC/MS" in the analytic characterization of the described compounds.

GC−MS (Varian Saturn 2000) was carried out using a Varian Chrompack CP-Sil low bleed/MS 30 m × 0.25 mm, 0.5 μm column as the stationary phase and helium (2 mL/min) as the carrier gas. Injector temperature was 270 °C, and column temperature was increased from 200 to 300 °C at a rate of 10 °C/min and then held at 300 °C for 5 min. Mass detection was performed using chemical ionization (CH_3CN) in the range from 200 m/z to 450 m/z.

For reactions involving microwave irradiation, a Personal Chemistry Emrys optimizer was used.

For hydrogenation reactions, H-Cube continuous-flow hydrogenation reactor occasionally was used.

Flash silica gel chromatography was carried out on silica gel 230−400 mesh (supplied by Merck AG Darmstadt, Germany) or over Varian Mega Be−Si prepacked cartridges or over prepacked Biotage or Isolute Flas silica cartridges. Alternatively chromatographic purifications were performed on columns packed with Merck 60 silica gel, 23−400 mesh, for flash technique. Thin-layer chromatography was carried out using Merck TLC plates Kieselgel 60F-254 and visualized with UV light, 5% phosphomolybdic acid, and aqueous potassium permanganate. SPE-SCX cartridges are ion exchange solid phase extraction columns by supplied by Varian. The eluent used with SPE-SCX cartridges is methanol followed by 2 N ammonia solution in methanol. Oasis HLB extraction cartridges are ion exchange solid phase extraction columns supplied by Waters. The eluent used with HLB cartridges is water followed by methanol.

In a number of preparations, purification was performed using either Biotage manual flash chromatography (Flash+) or automatic flash chromatography (Horizon) systems. All these instruments work with standard Biotage Silica cartridges.

In a number of preparations, purification was performed on a mass-directed autopurification (MDAP) system Fraction Lynx equipped with Waters 2996 PDA detector and coupled with a ZQTM mass spectrometer (Waters) operating in positive and negative electrospray ionization modes ES(+) and ES(−) (mass range 100−1000).

A set of acidic and basic semipreparative gradients have been used.

Method A consisted of the following chromatographic acidic conditions for up to 30 mg of crude: column, 100 mm × 21.2 mm SupelcosilTM ABZ + Plus (5 μm particle size); mobile phase, A [water + 0.1% formic acid]/B [acetonitrile + 0.1% formic acid]; flow rate, 20 mL/min; gradient, 5% B for 1 min, 95% B in 9 min, 100% B in 3.5 min.

Method B consisted of the following chromatographic acidic conditions for up to 100 mg of crude: column, 150 mm × 30 mm XTerra Prep MS C18 (10 μm particle size); mobile phase, A [water + 0.1% formic acid]/B [acetonitrile + 0.1% formic acid]; flow rate, 40 mL/min; gradient, 1% B to 100% B in 7 min lasting for 7.5 min.

Method C consisted of the following chromatographic basic conditions for up to 100 mg of crude: column, 150 mm × 30 mm XTerra Prep MS C18 (10 μm particle size); mobile phase, A [water + 10 mM ammonium carbonate (adjusted to pH 10 with ammonia)]/B [acetonitrile]; flow rate, 40 mL/min; gradient, 10% B for 0.5 min, 95% B in 12.5 min.

General procedures for the preparation of compounds **9** and **43**−**77** are shown in Scheme 1. The purity of the compounds reported in the manuscript was established through HPLC and/or ^{1}H NMR methodologies. All final compounds reported in the manuscript have a purity of >95%.

11,11-Di-2-propen-1-yl-5,11-dihydro-10*H*-dibenzo[*a*,*d*]cyclohepten-10-one (3a). The starting ketone 5,11-dihydro-10*H*-dibenzo[*a*,*d*]cyclohepten-10-one (**1a**, 10 g, 48 mmol, whose preparation has been described in *J. Med. Chem.* **2004**, *47*, 4202−4212) was suspended in *tert*-butanol (100 mL) and heated to 35 °C. Potassium *tert*-butoxide (13.5 g, 120 mmol) and allyl bromide (16.6 mL, 192 mmol) were added at this temperature, and the pink suspension was stirred for 1 h. The suspension was cooled to room temperature and quenched with NH_4Cl (saturated solution). The mixture was partitioned between diethyl ether and water. The organic phase was separated, dried over N_2SO_4, and evaporated in vacuum to give the crude material (13 g) as an orange oil as a mixture of *C*-diallyl (**3a**) and *C*-allyl/*O*-allyl (**2a**), which was progressed to the Claisen rearrangement without any further purification/characterization. The neat mixture (13 g) was heated at 185 °C for 15 min to give the desired product (**3a**, 13 g) that was used in the next step without any further purification. MS (ESI) m/z: 311 $[M + Na]^+$. ^{1}H NMR (400 MHz, chloroform-*d*) δ: 2.75−2.96 (m, 4H), 3.94 (s, 2H), 4.92−5.04 (m, 4H), 5.42−5.55 (m, 2H), 7.10−7.35 (m, 8H).

8-Chloro-11,11-di-2-propen-1-yldibenzo[*b*,*f*]oxepin-10(11*H*)-one (3d). The solution of KO^{t}Bu (2.47 g,22 mmol) in 100 mL of the ^{t}BuOH was stirred for 10 min under a stream of argon at room temperature. Then 8-chlorodibenzo[*b*,*f*]oxepin-10(11*H*)-one (1 g, 4.1 mmol; for preparation see *J. Med. Chem.* **1980**, *23* (5), 494−501) and allyl bromide (9.31 mL,21,8 mmol) were added. The mixture was then heated at 60 °C for 3 h. After the mixture was cooled to room temperature, a saturated solution of $NaHCO_3$ (150 mL) was added in one portion. The mixture was left under magnetic stirring for an additional 15 min. The precipitate was filtered off, and the aqueous phase was then extracted using ethyl acetate (3 × 50 mL). Collected organic layers were washed with saturated $NaHCO_3$ solution, dried with anhydrous Na_2SO_4, and evaporated, giving a crude product (**3d**, 1.8 g) as an oil. GC−MS m/z: 325 $[M + 1]^+$. ^{1}H NMR (400 MHz, chloroform-*d*) δ: 2.85−3.01 (m, 4H), 5.04−5.12 (m, 4H), 5.62−5.76 (m, 2H), 7.14−7.30 (m, 7H).

Spiro[cyclopent-3-ene-1,10′-dibenzo[*a*,*d*]cyclohepten]-11′(5′*H*)-one (4a). To a solution of 11,11-di-2-propen-1-yl-5,11-dihydro-10*H*-dibenzo[*a*,*d*]cyclohepten-10-one (**3a**, 1.00 g, 3.47 mmol) in degassed DCM (1 L) was added Grubbs second generation catalyst (15 mol %, 0.44 g) under argon atmosphere at room

temperature. The reaction mixture was stirred at room temperature overnight. The dark solution was then adsorbed on silica gel (10 equiv wt relative to catalyst) and passed through a pad of silica gel (petroleum ether/diethyl ether 1/1). The filtered solution was stirred with activated charcoal (50 equiv wt relative to product) for 12 h. After the carbon was filtered, the filtrate was concentrated in vacuum and purified by silica gel column chromatography (petroleum ether/diethyl ether = 9/1) to give the title compound (**4a**, 748 mg) as a white solid. MS (ESI) *m/z*: 283 $[M + Na]^+$. ^{1}H NMR (400 MHz, chloroform-*d*) δ: 2.92−2.99 (m, 2H), 3.56−3.63 (m, 2H), 4.36 (m, 2H), 5.71−5.74 (m, 2H), 7.09−7.51 (m, 7H), 7.78−7.88 (m, 1H).

2′-Chloro-11′*H*-spiro[cyclopent-3-ene-1,10′-dibenzo[*b,f*]oxepin]-11′-one (4d). An amount of 3 L of dry DCM was degassed with argon, and 8-chloro-11,11-di-2-propen-1-yldibenzo[*b,f*]oxepin-10(11*H*)-one (**3d**, 6.78 g, 20.8 mmol) and Grubbs second generation catalyst (15.5 mol %, 1.275 g) were added. The solution was stirred under argon atmosphere for 8 h, and then it was passed through a prepacked silica gel column and evaporated. The crude oil obtained (6.5 g) was purified by silica gel column chromatography (*n*-hexane/ethyl acetate = 9:0.2), and the fraction of the product that was obtained gave a precipitate after suspension in hexane to give the title compound (**4d**, 3.5 g). GC−MS *m/z*: 297 $[M + 1]^+$. ^{1}H NMR (400 MHz, chloroform-*d*) δ: 2.92−3.95 (d, 2H), 3.42−3.46 (d, 2H), 5.72 (s, 2H), 7.20−7.35 (m, 6H), 7.45−7.47 (m, 1H).

Mixture of 3-Hydroxyspiro[cyclopentane-1,10′-dibenzo[*a,d*]-cyclohepten]-11′(5′*H*)-one (5a) and 5′,11′-Dihydrospiro[cyclopentane-1,10′-dibenzo[*a,d*]cycloheptene]-3,11′-diol (6a). 1 M borane solution in THF (0.77 mL, 0.77 mmol) was added dropwise to a stirred solution of spiro[cyclopent-3-ene-1,10′-dibenzo-[*a,d*]cyclohepten]-11′(5′*H*)-one (**4a**, 0.200 g, 0.77 mmol) in anhydrous THF (1.6 mL) at room temperature under an N_2 atmosphere, and the mixture was stirred at room temperature for 2.5 h. Water (0.08 mL) was then added dropwise, followed by 3 M sodium hydroxide (0.10 mL). Hydrogen peroxide (0.12 mL, 35%) was then added at a rate to maintain the temperature between 30 and 50 °C, and the reaction mixture was stirred for 16 h at room temperature. Diethyl ether (1.6 mL) was added to the reaction mixture, and the organic phase was washed with brine and water. The organic solvent was evaporated to give a residue which, for analytical purposes, was purified by silica gel column chromatography (5/1 petroleum ether/diethyl ether), affording 3-hydroxyspiro[cyclopentane-1,10′-dibenzo[*a,d*]cyclohepten]-11′(5′*H*)-one (**5a**, 0.091 g) and 5′,11′-dihydrospiro[cyclopentane-1,10′-dibenzo[*a,d*]cycloheptene]-3,11′-diol (**6a**, 0.079 g).

3-Hydroxyspiro[cyclopentane-1,10′-dibenzo[*a,d*]cyclohepten]-11′(5′*H*)-one (5a). MS (ESI) *m/z*: 279 $[M + 1]^+$, 301 $[M + Na]^+$, 261 $[M - H_2O]^+$, 579 $[2M + Na]^+$. ^{1}H NMR (400 MHz, chloroform-*d*) δ: 1.66−1.92 (m, 2H), 2.12−2.21 (m, 1H), 2.32−2.48 (m, 1H), 2.85−2.95 (m, 1H), 3.21−3.30 (m, 1H), 4.35−4.44 (m, 3H), 7.10−7.46 (m, 7H), 7.91−7.95 (m, 1H).

5′,11′-Dihydrospiro[cyclopentane-1,10′-dibenzo[*a,d*]cycloheptene]-3,11′-diol (6a). MS (ESI) *m/z*: 303 $[M + Na]^+$. ^{1}H NMR (400 MHz, chloroform-*d*) δ: 1.83−2.43 (m, 5H), 2.61−2.71 (m, 1H), 3.81−3.89 (m, 1H), 4.47−4.61 (m, 2H), 5.03 (s, 1H), 7.03−7.61 (m, 8H).

3*H*-Spiro[cyclopentane-1,10′-dibenzo[*a,d*]cycloheptene]-3,11′-(5′*H*)-dione (7a). To a solution of Dess−Martin triacetoxyperiodinane (0.38 g, 0.9 mmol) a mixture of 3-hydroxyspiro-[cyclopentane-1,10′-dibenzo[*a,d*]cyclohepten]-11′(5′*H*)-one and 5′,11′-dihydrospiro[cyclopentane-1,10′-dibenzo[*a,d*]cycloheptene]-3,11′-diol (**5a** and **6a**, 0.100 g, 0.36 mmol) was added in dry DCM (8 mL). The reaction mixture was left at 25 °C for 3.5 h. The mixture was diluted with DCM (10 mL) and washed with NaOH (1 N) and then brine. The organic layer was dried over Na_2SO_4 and after solvent evaporation gave the title compound (**7a**, 92 mg). MS (ESI) *m/z*: 299 $[M + Na]^+$. ^{1}H NMR (400 MHz, chloroform-*d*) δ: 2.26−2.49 (m, 3H), 2.76−2.84 (m, 1H), 3.26−3.46 (m, 2H), 4.32−4.51 (m, 2H), 7.16−7.48 (m, 7H), 7.92−7.96 (m, 1H).

2′-Chloro-3*H*,11′*H*-spiro[cyclopentane-1,10′-dibenzo[*b,f*]oxepin]-3,11′-dione (7d). 2′-Chloro-11′*H*-spiro[cyclopent-3-ene-1,10′-dibenzo[*b,f*]oxepin]-11′-one (**4d**, 2.1 g, 7.08 mmol) was melted in anhydrous THF (100 mL) under argon atmosphere, and 1 M solution of BH_3−THF (8 mL, 8 mmol) was added dropwise. The mixture was left for 4 h under stirring at room temperature. After 4 h, H_2O (20 mL) and 10% NaOH (10 mL) were added followed by 30% H_2O_2 (5 mL). The mixture was stirred overnight and then diluted with H_2O (40 mL) and extracted with diethyl ether. Organic layer was washed with brine, dried, and evaporated, giving a crude product as a mixture of **5d** and **6d** (2 g). The mixture was dissolved in dry DCM (100 mL), and Dess−Martin (triacetoxyperiodinane) (9 g, 21.31 mmol) was added. The mixture was stirred overnight under argon atmosphere. The reaction was worked up by washing three times with NaOH (10% aqueous solution). Water layers were washed with DCM and combined organic layers were dried (Na_2SO_4/$MgSO_4$) and evaporated to give the crude title compound (**7d**, 2.13 g), which was used in the next step without any further purification. HPLC−MS *m/z*: 312.89 $[M + 1]^+$; t_R = 8.44 min. GC−MS *m/z*: 313 $[M + 1]^+$. ^{1}H NMR (400 MHz, chloroform-*d*) δ: 2.18−2.39 (m, 2H), 2.41−2.47 (m, 1H), 2.76−2.79 (d, 1H), 3.07−3.12 (m, 1H), 7.25−7.38 (m, 5H), 7.50−7.52 (m, 1H), 8.01−8.02 (d, 1H).

5′,11′-Dihydro-3*H*-spiro[cyclopentane-1,10′-dibenzo[*a,d*]cyclohepten]-3-one (8a). In a Parr apparatus, 3*H*-spiro[cyclopentane-1,10′-dibenzo[*a,d*]cycloheptene]-3,11′(5′*H*)-dione (**7a**, 2.200 g, 7.96 mmol) was dissolved in THF (40 mL). AcOH (10.00 mL) and wet Pd/C (10% (w/w), 50% w/w of water content) (4.24 g, 3.98 mmol) were added, and the mixture was hydrogenated (0.016 g, 7.96 mmol) under 5 atm pressure for 5 days. During this time three portions of Pd/C (2.5 g, 10% (w/w), 50% w/w of water content) were added. The palladium was then filtered over Celite and the solvent evaporated to afford the title compound (**8a**, 2.2 g) as a racemic mixture. MS (ESI) *m/z*: 285 $[M + Na]^+$. ^{1}H NMR (400 MHz, chloroform-*d*) δ: 2.24−2.32 (m, 2H), 2.51−2.62 (m, 4H), 3.09−3.16 (m, 2H), 4.11−4.21 (m, 2H), 7.04−7.35 (m, 8H).

The racemic mixture of 5′,11′-dihydro-3*H*-spiro[cyclopentane-1,10′-dibenzo[*a,d*]cyclohepten]-3-one (**8a**) was submitted for preparative chiral HPLC (column, Chiralpak IA (25 cm × 2.0 cm), 5 μm; bobile phase, *n*-hexane/(ethanol/methanol 50/50) 96/4% v/v; flow rate, 14 mL/min; UV, 225 nm; 19 mg/inj in CH_2Cl_2/ethanol/methanol/hexane) and gave two enantiomers:

(−)-(1*R*)-5′,11′-Dihydro-3*H*-spiro[cyclopentane-1,10′-dibenzo-[*a,d*]cyclohepten]-3-one (8a(*R*) (ent 1)). $[\alpha]_D^{20}$ −83° (*c* 0.88, $CHCl_3$) (optical rotation was measured on a different batch); retention time = 12.5 min (683 mg).

(+)-(1*S*)-5′,11′-Dihydro-3*H*-spiro[cyclopentane-1,10′-dibenzo-[*a,d*]cyclohepten]-3-one (8a(*S*) (ent 2)). $[\alpha]_D^{20}$ +83° (*c* 0.93, $CHCl_3$) (optical rotation was measured on a different batch); retention time = 14.0 min (655 mg).

3*H*,11′*H*-Spiro[cyclopentane-1,10′-dibenzo[*b,f*]oxepin]-3-one (8c). THF (10 mL) and glacial acetic acid (5 mL) were mixed in a Paar bottle, and 2′-chloro-3*H*,11′*H*-spiro[cyclopentane-1,10′-dibenzo[b, f]oxepin]-3,11′-one (**7d**, 100 mg, 0.319 mmol) and Pd/C (100 mg) were added. The mixture was shaken in the Paar apparatus under H_2 atmosphere (6 bar) at room temperature for 2 days. Then the mixture was filtered through a membrane filter and evaporated. The residue was dissolved in ethyl acetate (10 mL) and washed with $NaHCO_3$ (saturated solution 3 × 15 mL). Organic layer was dried (Na_2SO_4/$MgSO_4$) and evaporated to give an oil that crystallized from MeOH to give the title compound (**8c**, 91.2 mg) as a racemic mixture. GC−MS *m/z*: 265 $[M + 1]^+$. ^{1}H NMR (400 MHz, chloroform-*d*) δ: 2.33−2.49 (m, 4H), 2.62−2.66 (d, 1H), 2.74−2.78 (d, 1H), 3.07−3.18 (m, 2H), 7.02−7.27 (m, 8H).

The racemic mixture of 3*H*,11′*H*-spiro[cyclopentane-1,10′-dibenzo[*b,f*]oxepin]-3-one (**8c**, 550 mg) was submitted for preparative chiral HPLC (column = Chiralcel OJ-H; mobile

phase = *n*-hexane/isopropanol 88/12; flow rate = 14 mL/min; UV, 225 nm; 25 mg/inj) and gave two enantiomers:

3*H*,11′*H*-Spiro[cyclopentane-1,10′-dibenzo[*b*,*f*]oxepin]-3-one (8c (ent 1)). Retention time = 17.1 min (171 mg).

3*H*,11′*H*-Spiro[cyclopentane-1,10′-dibenzo[*b*,*f*]oxepin]-3-one (8c (ent 2)). Retention time = 19.7 min (180 mg).

(1*S*)-*N*,*N*-Dimethyl-5′,11′-dihydrospiro[cyclopentane-1,10′-dibenzo-[*a*,*d*]cyclohepten]-3-amine (9a). Dimethylamine (2.0 M in THF, 0.09 mL, 0.172 mmol, 1.5 equiv) and (+)-(1*S*)-5′,11′-dihydro-3*H*-spiro-[cyclopentane-1,10′-dibenzo[*a*,*d*]cyclohepten]-3-one (**8a**(*S*), 30 mg, 0.115) were mixed in DCE and glacial acetic acid (2 drops). After 20 min at room temperature $NaBH(OAc)_3$ (29 mg, 0.137 mmol, 1.2 equiv) was added and the resulting reaction mixture was stirred at room temperature from 6 h. The workup was done by adding $NaHCO_3$ (saturated solution) and then by extracting with DCM. Products were purified by silica gel column chromatography.

The reaction mixture was quenched with $NaHCO_3$ (saturated aqueous solution) and extracted with dichloromethane. The organic layers were combined, dried (Na_2SO_4), and concentrated in vacuo. The crude product was purified by SCX, affording the title compound (**9**, 21 mg) as a mixture of diastereoisomers (~85/15).

This diastereomeric mixture was submitted for chiral HPLC purification (preparative SFC chromatographic conditions: column, Chiralcel OD-H, 25 cm × 2.0 cm; pressure, 172 bar; flow rate, 22 mL/min; UV detection, CD 220 nm; modifier, ethanol + 0.1% isopropylamine 10%) to give two diastereoisomers:

(1*S*,3*R*)-*N*,*N*-Dimethyl-5′,11′-dihydrospiro[cyclopentane-1,10′-dibenzo[*a*,*d*]cyclohepten]-3-amine (9′a). Retention time = 14.05 min (5.1 mg). ^{1}H NMR (400 MHz, chloroform-*d*) δ: 1.87−2.22 (m, 6H), 2.33 (s, 6H), 2.84 (m, 1H), 3.15 (d, 1H), 3.27 (d, 1H), 4.04 (d, 1H), 4.25 (d, 1H), 7.01−7.32 (m, 8H).

(1*S*,3*S*)-*N*,*N*-Dimethyl-5′,11′-dihydrospiro[cyclopentane-1,10′-dibenzo[*a*,*d*]cyclohepten]-3-amine (9″a). Retention time = 17.39 min (1.9 mg). ^{1}H NMR (400 MHz, chloroform-*d*) δ: 1.85−2.27 (m, 6H), 2.36 (s, 6H), 2.89 (m, 1H), 3.07 (d, 1H), 3.19 (d, 1H), 4.07 (d, 1H), 4.23 (d, 1H), 7.03−7.25 (m, 7H), 7.43−7.49 (m, 1H).

Methyl 1-((1*R*)-5′,11′-Dihydrospiro[cyclopentane-1,10′-dibenzo-[*a*,*d*]cyclohepten]-3-yl)-1,2,5,6-tetrahydro-3-pyridinecarboxylate (12). To a solution of (−)-(1*R*)-5′,11′-dihydro-3*H*-spiro[cyclopentane-1,10′-dibenzo[*a*,*d*]cyclohepten]-3-one (**8a**(*R*), 60 mg, 0.229 mmol) in 1,2-DCE (2 mL) were added methyl 1,2,5,6-tetrahydro-3-pyridinecarboxylate (40.6 mg, 0.229 mmol) and DIPEA (0.044 mL, 0.252 mmol). After the mixture was stirred for 10 min at room temperature, AcOH (2 drops) and $NaBH(OAc)_3$ (72.7 mg, 0.343 mmol) were added. The reaction mixture was stirred at room temperature overnight. The mixture was washed with saturated aqueous $NaHCO_3$ solution and then brine. The layers were separated and the organic layer was evaporated under vacuum to afford a yellow oil, which was dissolved in methanol and purified using a 1 g SCX cartridge (eluting first with MeOH and then 2 M NH_3 in MeOH). Evaporation of the solvent gave the title product (**12**, 55 mg) as a mixture of diastereoisomers as yellow foam. UPLC/MS R_f = 0.63; *m*/*z* (ES) 388.1 $[M + H]^+$. ^{1}H NMR (400 MHz, chloroform-*d*) δ: 6.95−7.37 (m, 9H), 4.15−4.31 (m, 1H), 3.92−4.13 (m, 1H), 3.65−3.85 (m, 3H), 2.99−3.47 (m, 5H), 2.52−2.75 (m, 2H), 1.79−2.51 (m, 8H).

Methyl 1-((1*S*)-5′,11′-Dihydrospiro[cyclopentane-1,10′-dibenzo-[*a*,*d*]cyclohepten]-3-yl)-1,2,5,6-tetrahydro-3-pyridinecarboxylate (13). To a solution of (+)-(1*S*)-5′,11′-dihydro-3*H*-spiro[cyclopentane-1,10′-dibenzo[*a*,*d*]cyclohepten]-3-one, (**8a**(*S*),100 mg, 0.38 mmol) in 1,2-dichloroethane (5 mL) were added methyl 1,2,5,6-tetrahydro-3-pyridinecarboxylate hydrochloride (67.7 mg, 0.381 mmol) and DIPEA (0.073 mL, 0.419 mmol). After the mixture was stirred for 10 min at room temperature, AcOH (0.044 mL, 0.762 mmol) and $NaBH(OAC)_3$ (121 mg, 0.572 mmol) were added. The reaction mixture was stirred at room temperature overnight. Further guvacine methyl esther hydrocloride (0.3 equiv) and $NaBH(OAC)_3$ (0.5 equiv) were added, and the mixture was stirred for an additional 4 h. The mixture was washed with saturated aqueous $NaHCO_3$ solution, then brine, and the organic phase was separated and concentrated under vacuum. The crude product was purified using a SCX cartridge, eluting first with DCM, second with MeOH, and third with NH_3 in MeOH (2 M). After solvent evaporation the desired compound (**13**, 94 mg) was obtained as mixture of diastereoisomers (~85/15). UPLC/MS R_f = 0.66; *m*/*z* (ES) 388.2 $[M + H]^+$.

The diastereomeric mixture of methyl 1-(5′,11′-dihydrospiro-[cyclopentane-1,10′-dibenzo[*a*,*d*]cyclohepten]-3-yl)-1,2,5,6-tetrahydro-3-pyridinecarboxylate (**13**, 94 mg) was submitted for chiral HPLC purification (preparative chromatographic conditions: column = Chiralcel OD-H; mobile phase = *n*-hexane/ethanol 75/25% v/v; flow rate = 0.8 mL/min; DAD = 210−340 nm; CD = 225 nm) to give **13′** and **13″**.

Methyl 1-((1*S*)-5′,11′-Dihydrospiro[cyclopentane-1,10′-dibenzo-[*a*,*d*]cyclohepten]-3-yl)-1,2,5,6-tetrahydro-3-pyridinecarboxylate (13′). Retention time = 8 min (70 mg). ^{1}H NMR (400 MHz, chloroform-*d*) δ: 6.98−7.37 (m, 9H), 4.27 (d, 2H), 4.02 (d, 2H), 3.72−3.79 (m, 3H), 3.36−3.45 (m, 1H), 3.21−3.34 (m, 2H), 3.05−3.19 (m, 2H), 2.55−2.73 (m, 2H), 2.34−2.50 (m, 2H), 2.20−2.33 (m, 2H), 2.08−2.19 (m, 1H), 1.80−2.05 (m, 3H).

Methyl 1-((1*S*)-5′,11′-Dihydrospiro[cyclopentane-1,10′-dibenzo-[*a*,*d*]cyclohepten]-3-yl)-1,2,5,6-tetrahydro-3-pyridinecarboxylate (13″). Retention time = 10 min (11 mg). ^{1}H NMR (400 MHz, chloroform-*d*) δ: 7.39−7.52 (m, 1H), 7.09−7.27 (m, 6H), 6.99−7.09 (m, 2H), 4.24 (d, 1H), 4.06 (d, 1H), 3.75 (s, 3H), 3.01−3.40 (m, 5H), 2.54−2.74 (m, 2H), 2.25−2.46 (m, 2H), 1.87−2.19 (m, 4H).

Ethyl 1-((1*S*)-5′,11′-Dihydrospiro[cyclopentane-1,10′-dibenzo-[*a*,*d*]cyclohepten]-3-yl)-4-piperidinecarboxylate (16). To a solution of (+)-(1*S*)-5′,11′-dihydro-3*H*-spiro[cyclopentane-1,10′-dibenzo[*a*,*d*]cyclohepten]-3-one (**8a**(*S*), 74 mg, 0.27 mmol) and ethyl isonipecotate (0.082 mL, 0.534 mmol) in 1,2-dichloroethane (4 mL) was added AcOH (2 drops). The reaction mixture was stirred at room temperature for 1 h, and then $NaBH(OAC)_3$ (85 mg, 0.400 mmol) was added. The resulting mixture was stirred overnight. The mixture was diluted with DCM and washed with a saturated solution of $NaHCO_3$, brine. The phases were separated, and the organic layer was concentrated under vacuum. The crude mixture was purified by passing through a 12 g Si-redisep cartridge (eluting with cHex/EtOAc, from 100:0 to 80:20) to afford the title compound (**16**, 82 mg) as mixture of two diastereoisomers. UPLC/MS R_f = 0.63; *m*/*z* (ES) 404.2 $[M + H]^+$.

This diastereomeric mixture of ethyl 1-(5′,11′-dihydrospiro-[cyclopentane-1,10′-dibenzo[*a*,*d*]cyclohepten]-3-yl)-4-piperidinecarboxylate (**16**) was submitted for chiral chromatography purification (preparative chromatographic conditions: column = Chiralcel OD-H; mobile phase = *n*-hexane/isopropanol 85/15% v/v; flow rate = 1 mL/min; DAD = 210−340 nm; CD = 225 nm) to give **16′**.

Ethyl 1-((1*S*)-5′,11′-Dihydrospiro[cyclopentane-1,10′-dibenzo-[*a*,*d*]cyclohepten]-3-yl)-4-piperidinecarboxylate (16′). Only the major diastereoisomer was isolated: retention time = 7 min (60 mg). ^{1}H NMR (400 MHz, chloroform-*d*) δ: 6.84−7.48 (m, 8H), 3.86−4.40 (m, 3H), 2.74−3.44 (m, 4H), 1.62−2.48 (m, 15H), 1.16−1.39 (m, 3H).

Ethyl 1-((1*S*)-5′,11′-Dihydrospiro[cyclopentane-1,10′-dibenzo-[*a*,*d*]cyclohepten]-3-yl)-4-fluoro-4-piperidinecarboxylate (19). To a solution of (+)-(1*S*)-5′,11′-dihydro-3*H*-spiro[cyclopentane-1,10′-dibenzo[*a*,*d*]cyclohepten]-3-one (**8a**(*S*), 58 mg, 0.221 mmol) and ethyl 4-fluoro-4-piperidinecarboxylate (prepared as described in WO/2002/032893[28]) (58.1 mg, 0.332 mmol) in 1,2-dichloroethane (3 mL) was added a drop of AcOH. The reaction mixture was stirred at room temperature for 0.5 h, and then $NaBH(OAC)_3$ (70.3 mg, 0.332 mmol) was added. The resulting reaction mixture was stirred for 3 h, quenched with $NaHCO_3$ (saturated aqueous solution), and extracted with dichloromethane. The organic layers were combined, dried (Na_2SO_4), and concentrated in vacuo.

The crude product was purified by flash chromatography on silica gel (20 g), eluting with a gradient of dichloromethane/methanol 99.5/0.5 to 99/1 to afford the title compound (**19**, 30 mg) as a mixture of diastereoisomers (~10/90).

This diastereomeric mixture was submitted for chiral HPLC purification (preparative chromatographic conditions: column = Chiralcel OJ-H; mobile phase = *n*-hexane/EtOH 85/15% v/v; flow rate = 0.8 mL/min; DAD = 210−340 nm; CD = 225 nm) to give **19′**.

Ethyl 1-((1*S*)-5′,11′-Dihydrospiro[cyclopentane-1,10′-dibenzo-[*a*,*d*]cyclohepten]-3-yl)-4-fluoro-4-piperidinecarboxylate (19′). Only the major diastereoisomer was isolated: retention time = 21.9 min (21 mg). UPLC/MS R_f = 0.70; *m*/*z* (ES) 422.2 $[M + H]^+$. ^{1}H NMR (400 MHz, chloroform-*d*) δ: 7.31−7.32 (m, 1H), 7.14−7.21 (m, 6H), 7.00−7.08 (m, 1H), 4.20−4.35 (m, 3H), 4.02 (d, 1H), 3.31 (d, 1H), 3.12 (d, 1H), 2.97−3.08 (m, 2H), 2.74−2.90 (m, 1H), 1.78−2.49 (m, 12H), 1.34 (t, 3H).

Ethyl 3-((1*S*)-5′,11′-Dihydrospiro[cyclopentane-1,10′-dibenzo-[*a*,*d*]cyclohepten]-3-yl)-3-azabicyclo[3.1.0]hexane-1-carboxylate (29). To a solution of (+)-(1*S*)-5′,11′-dihydro-3*H*-spiro[cyclopentane-1,10′-dibenzo[*a*,*d*]cyclohepten]-3-one (**8a**(*S*), 70 mg, 0.267 mmol) and ethyl 3-azabicyclo[3.1.0]hexane-1-carboxylate (83 mg, 0.534 mmol, for preparation see WO/2007/055093[29]) in 1,2-dichloroethane (5 mL) under nitrogen was added AcOH (2 drops). The reaction mixture was stirred at room temperature for 1 h 30 min, and then $NaBH(OAC)_3$ (85 mg, 0.400 mmol) was added. The resulting mixture was stirred overnight. The mixture was diluted with DCM. The organic phase was then washed with saturated solution of $NaHCO_3$, then brine and concentrated under vacuum. The crude product was purified through Si-redisep cartridge (12 g), eluting with cHex/EtOAc (from 100:0 to 80:20) to afford the title compound (**29**, 82 mg) as a mixture of two diastereoisomeric racemates. UPLC/MS R_f = 0.67; *m*/*z* (ES) 402.2 $[M + H]^+$.

This mixture was submitted for chiral HPLC separation (preparative chromatographic conditions: column = Chiralcel OJ-H; mobile phase = *n*-hexane/ethanol/isopropanol 96/2/2% v/v′; flow rate = 1 mL/min; DAD = 210−340 nm; CD = 225 nm) to obtain **29″** isomer 1 and **29″** isomer 2; minor isomers were not characterized.

Ethyl 3-((1*S*)-5′,11′-Dihydrospiro[cyclopentane-1,10′-dibenzo-[*a*,*d*]cyclohepten]-3-yl)-3-azabicyclo[3.1.0]hexane-1-carboxylate (29′ Isomer 1). Retention time = 13.62 min (28 mg). ^{1}H NMR (400 MHz, chloroform-*d*) δ: 7.29−7.34 (m, 1 H), 7.08−7.24 (m, 6 H), 7.00−7.07 (m, 1 H), 4.22 (d, 1 H), 4.14 (q, 2 H), 4.04 (d, 1 H), 3.21−3.29 (m, 1 H), 3.08−3.16 (m, 3 H), 2.96−3.06 (m, 1 H), 2.68 (d, 1 H), 2.41−2.51 (m, 1 H), 1.77−2.15 (m, 7 H), 1.44−1.51 (m, 1 H), 1.29−1.37 (m, 1 H), 1.25 (t, 3 H).

Ethyl 3-((1*S*)-5′,11′-Dihydrospiro[cyclopentane-1,10′-dibenzo-[*a*,*d*]cyclohepten]-3-yl)-3-azabicyclo[3.1.0]hexane-1-carboxylate (29′ Isomer 2). Retention time = 17.76 min (26 mg). ^{1}H NMR (400 MHz, chloroform-*d*) δ: 7.26−7.32 (m, 1 H), 7.08−7.25 (m, 6 H), 6.98−7.07 (m, 1 H), 4.12−4.26 (m, 3 H), 4.05 (d, 1 H), 3.21−3.29 (m, 2 H), 3.14 (d, 1 H), 2.97−3.07 (m, 2 H), 2.78 (d, 1 H), 2.33−2.42 (m, 1 H), 1.80−2.13 (m, 7 H), 1.43−1.54 (m, 1 H), 1.33−1.39 (m, 1 H), 1.28 (t, 3 H).

Methyl 1-((1*S*)-5′,11′-Dihydrospiro[cyclopentane-1,10′-dibenzo-[*a*,*d*]cyclohepten]-3-yl)-3-azetidinecarboxylate (30). A mixture of (+)-(1*S*)-5′,11′-dihydro-3*H*-spiro[cyclopentane-1,10′-dibenzo[*a*,*d*]-cyclohepten]-3-one (**8a**(*S*), 5.45 g, 20.77 mmol) and methyl 3-azetidinecarboxylate hydrochloride (3.78 g, 24.9 mmol) in dry DCM (25 mL) was stirred for 15 min. $NaBH(OAC)_3$ (8.81 g, 41.5 mmol) was then added and the reaction mixture stirred overnight, quenched with $NaHCO_3$ (saturated aqueous solution), and extracted with dichloromethane. The organic layers were combined, dried (Na_2SO_4), and concentrated in vacuo. The crude product was purified by flash chromatography on silica gel, eluting with a gradient of MeOH in dichloromethane (from 0% to 3%) to afford the title compound (**30**, 6.7 g) as a mixture of two diastereoisomers. UPLC/MS R_f = 0.61; *m*/*z* (ES) 362.11 $[M + H]^+$.

The diastereomeric mixture (**30**, 4.5 g) was submitted for chiral HPLC purification (semipreparative SFC conditions: column = Chiralcel OD-H; modifier = ethanol 15%; flow rate = 2.5 mL/min; pressure = 120 bar; temperature = 38 °C; detector = 220 nm) to give two single diastereoisomers, but only the major distereoisomer was characterized:

Methyl 1-((1*S*)-5′,11′-Dihydrospiro[cyclopentane-1,10′-dibenzo-[*a*,*d*]cyclohepten]-3-yl)-3-azetidinecarboxylate (30′). Retention time = 4.9 min (2.62 g). ^{1}H NMR (400 MHz, chloroform-*d*) δ: 7.30−7.02 (m, 8 H), 4.13 (q, 2 H), 3.75 (s, 3 H), 3.63−3.57 (m, 2 H), 3.39−3.08 (m, 6 H), 2.15−1.65 (m, 6 H).

Methyl 1-(11′*H*-Spiro[cyclopentane-1,10′-dibenzo[*b*,*f*]oxepin]-3-yl)-1,2,3,6-tetrahydro-4-pyridinecarboxylate (34). To 3*H*,11′*H*-spiro[cyclopentane-1,10′-dibenzo[*b*,*f*]oxepin]-3-one (**8c**,100 mg, 0.378 mmol) in DCE (5 mL) were added methyl 1,2,3,6-tetrahydro-4-pyridinecarboxylate (81 mg, 0.574 mmol) HCl salt and DIPEA (0.066 mL, 0.378 mmol). After the mixture was stirred for 10 min, sodium triacetoxyborohydride (120 mg, 0.567 mmol) and acetic acid (2 drops) were added. The mixture was left stirring overnight. The reaction was quenched with $NaHCO_3$. The organic layer was separated and the water phase extracted with DCM. The combined organic layers were dried over Na_2SO_4 and the solvent was evaporated to give the title compound (**34**, 150 mg) as a mixture of two diastereoisomeric racemates. *m*/*z* (ES): 390.2 $[M + H]^+$.

This mixture was submitted for chiral HPLC separation (preparative chromatographic conditions: column = Chiralcel OJ-H; mobile phase = *n*-hexane/(ethanol + 0.1% isopropylamine) 93/7% v/v; flow rate = 14 mL/min; DAD = 225 nm) to give **34′** isomer 1 and **34′** isomer 2 (the minor isomers were not characterized).

Methyl 1-(11′*H*-Spiro[cyclopentane-1,10′-dibenzo[*b*,*f*]oxepin]-3-yl)-1,2,3,6-tetrahydro-4-pyridinecarboxylate (34′, Isomer 1). Retention time = 17.23 min (28 mg). ^{1}H NMR (400 MHz, chloroform-*d*) δ: 7.26−7.32 (m, 1 H), 7.00−7.25 (m, 7 H), 6.87−6.94 (m, 1 H), 3.76 (s, 3 H), 3.05−3.32 (m, 4 H), 2.85−2.99 (m, 1 H), 2.59−2.71 (m, 2 H), 2.38−2.52 (m, 3 H), 2.14 (d, 5 H).

Methyl 1-(11′*H*-Spiro[cyclopentane-1,10′-dibenzo[*b*,*f*]oxepin]-3-yl)-1,2,3,6-tetrahydro-4-pyridinecarboxylate (34′, Isomer 2). Retention time = 26.42 min (27 mg). ^{1}H NMR (400 MHz, chloroform-*d*) δ: 7.27−7.33 (m, 1 H), 7.01−7.24 (m, 7 H), 6.87−6.95 (m, 1 H), 3.76 (s, 3 H), 3.06−3.31 (m, 4 H), 2.83−2.99 (m, 1 H), 2.60−2.70 (m, 2 H), 2.38−2.51 (m, 3 H), 2.14 (s, 5 H).

Methyl 1-(11′*H*-Spiro[cyclopentane-1,10′-dibenzo[*b*,*f*]oxepin]-3-yl)-3-azetidinecarboxylate (38). 3*H*,11′*H*-Spiro[cyclopentane-1,10′-dibenzo[b,f]oxepin]-3-one (**8c** (ent 2), 70 mg, 0.265 mmol) and methyl 3-azetidinecarboxylate hydrochloride (48.2 mg, 0.318 mmol) in acetonitrile (4 mL) were stirred under nitrogen to give a colorless solution. After the mixture was stirred for 30 min at room temperature, $NaBH(OAC)_3$ (84 mg, 0.397 mmol) was added. The mixture was stirred overnight. Water was added. The solution was concentrated under reduced pressure and the aqueous layer extracted with DCM. The phases were separated on a hydrophobic frit, and the combined organic solvent was evaporated. The product was purified using a NH_2 5 g column, eluting with EtOAc/cyclohexane 1:9 to give the title compound (**38**, 85 mg) as a mixture of two diastereoisomers. UPLC/MS R_f = 0.65; *m*/*z* (ES) 364.06 $[M + H]^+$.

The diastereomeric mixture (**38**) was submitted for chiral HPLC purification (semipreparative chiral SFC conditions: column = Chiralpak AD-H; modifier = ethanol + 0.1% isopropylamine, 10%; flow rate = 52 mL/min; pressure = 120 bar; temperature = 38 °C; DAD = 220 nm) to give **38′** (only the major diastereoisomer was characterized).

Methyl 1-(11′*H*-Spiro[cyclopentane-1,10′-dibenzo[*b*,*f*]oxepin]-3-yl)-3-azetidinecarboxylate (38′). Retention time = 7.0 min (19 mg). ^{1}H NMR (400 MHz, chloroform-*d*) δ: 7.29−7.00 (m, 8 H), 3.73 (s, 3 H), 3.58−2.94 (m, 8 H), 2.21−1.58 (m, 6 H).

1-((1*R*)-5′,11′-Dihydrospiro[cyclopentane-1,10′-dibenzo[*a*,*d*]-cyclohepten]-3-yl)-1,2,5,6-tetrahydro-3-pyridinecarboxylic Acid Hydrochloride Salt (45). To a solution of methyl 1-((1*R*)-5′,11′-dihydrospiro[cyclopentane-1,10′-dibenzo[*a*,*d*]cyclohepten]-3-yl)-1,2,5,6-tetrahydro-3-pyridinecarboxylate (**12**, 55 mg, 0.142 mmol) in methanol/water 3:1 (2.4 mL) was added LiOH (16.99 mg, 0.710 mmol), and the reaction mixture was left to stir for 4 h. Further LiOH (17 mg, 0.71 mmol) was added, and the mixture was left to stir overnight. The organic solvent was evaporated under vacuum and the aqueous phase washed with DCM. The reaction mixture was slowly acidified with 3 N HCl, checking the pH of the solution. A white precipitate formed at pH 1. The solid was filtered and dried under vacuum. The solid was triturated from EtOAc to give the title compound (**45**, 41 mg). UPLC/MS R_f = 0.62; *m*/*z* (ES) 374.3 $[M + H]^+$. ^{1}H NMR (400 MHz, chloroform-*d*) δ: 6.95−7.37 (m, 9H), 4.15−4.31 (m, 1H), 3.92−4.13 (m, 1H), 3.65−3.85 (m, 3H), 2.99−3.47 (m, 5H), 2.52−2.75 (m, 2H), 1.79−2.51 (m, 8H).

Methyl 1-((1*S*)-5′,11′-Dihydrospiro[cyclopentane-1,10′-dibenzo-[*a*,*d*]cyclohepten]-3-yl)-1,2,5,6-tetrahydro-3-pyridinecarboxylate (46). Methyl 1-((1*S*)-5′,11′-dihydrospiro[cyclopentane-1,10′-dibenzo[*a*,*d*]-cyclohepten]-3-yl)-1,2,5,6-tetrahydro-3-pyridinecarboxylate (**13**, 66 mg, 0.17 mmol) was dissolved in methanol (1.8 mL)/water (0.6 mL), and LiOH (20.39 mg, 0.852 mmol) was added. The reaction mixture was left under stirring overnight. Methanol was evaporated under vacuum and the aqueous phase washed with DCM. The aqueous phase was acidified with 3 N HCl, checking the pH of the solution (until pH 1), but only a few milligrams of the desired compound precipitated. The compound was retained by the organic phase (DCM). Solvent was removed to give the title compound (**46**, 25 mg) as free base. UPLC/MS R_f = 0.63; *m*/*z* (ES) 374.17 $[M + H]^+$. Data reported for the major diastereoisomer: ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.30−7.40 (m, 1H), 7.09−7.22 (m, 5H), 6.96−7.07 (m, 2H), 3.97−4.24 (m, 2H), 3.59−3.85 (m, 2H), 3.44−3.56 (m, 1H), 3.14−3.37 (m, 2H), 2.92−3.09 (m, 2H), 1.84−2.75 (m, 5H).

1-((1*S*)-5′,11′-Dihydrospiro[cyclopentane-1,10′-dibenzo[*a*,*d*]-cyclohepten]-3-yl)-1,2,5,6-tetrahydro-3-pyridinecarboxylic Acid (47). To a solution of methyl 1-((1*S*)-5′,11′-dihydrospiro-[cyclopentane-1,10′-dibenzo[*a*,*d*]cyclohepten]-3-yl)-1,2,5,6-tetrahydro-3-pyridinecarboxylate (**13′**, 70 mg, 0.181 mmol) in methanol (6 mL) and water (1 mL) was added LiOH (21.63 mg, 0.903 mmol). The reaction mixture was left to stir at room temperature overnight. Further LiOH (1.2 equiv) and water (0.5 mL) were added, and the resulting mixture was stirred at 40 °C for 6 h. The methanol was then evaporated under vacuum, and the reaction mixture was acidified with 3 N HCl (until pH ∼ 1). This solution was purified using an HLB cartridge (5 g) by eluting first with water and then MeOH to give the title compound (**47**, 56 mg). UPLC/MS R_f = 0.61; *m*/*z* (ES) 374.2 $[M + H]^+$. ^{1}H NMR (400 MHz, chloroform-*d*) δ: 7.30−7.40 (m, 1H), 7.09−7.22 (m, 5H), 6.96−7.07 (m, 2H), 3.97−4.24 (m, 2H), 3.59−3.85 (m, 2H), 3.44−3.56 (m, 1H), 3.14−3.37 (m, 2H), 2.92−3.09 (m, 2H), 1.84−2.75 (m, 5H).

1-((1*S*)-5′,11′-Dihydrospiro[cyclopentane-1,10′-dibenzo[*a*,*d*]-cyclohepten]-3-yl)-1,2,5,6-tetrahydro-3-pyridinecarboxylic Acid (48). To a solution of methyl 1-((1*S*)-5′,11′-dihydrospiro-[cyclopentane-1,10′-dibenzo[*a*,*d*]cyclohepten]-3-yl)-1,2,5,6-tetrahydro-3-pyridinecarboxylate (**13″**, 11 mg, 0.028 mmol) in methanol (1.5 mL) and water (0.15 mL) was added LiOH (3.40 mg, 0.142 mmol). The reaction mixture was left to stir overnight at room temperature and then at 40 °C for 4 h. The methanol was evaporated under vacuum, and the reaction mixture was acidified with 3 N HCl, checking the pH of the solution (until pH 1). This solution was purified using an HLB cartridge (2 g) by eluting first with water and then MeOH to give the title compound (**48**, 5.8 mg). UPLC/MS R_f = 0.61; *m*/*z* (ES) 374.2 $[M + H]^+$. ^{1}H NMR (400 MHz, chloroform-*d*) δ: 7.47−7.73 (m, 1 H), 6.86−7.33 (m, 8 H), 3.98−4.28 (m, 2 H), 3.60−3.96 (m, 3 H), 2.85−3.36 (m, 4 H), 1.86−2.74 (m, 7 H), 1.10−1.39 (m, 2 H).

(−)-1-((1*S*)-5′,11′-Dihydrospiro[cyclopentane-1,10′-dibenzo-[*a*,*d*]cyclohepten]-3-yl)-4-piperidinecarboxylic Acid (51). LiOH (17.80 mg, 0.743 mmol) was added to a solution of ethyl 1-((1*S*)-5′,11′-dihydrospiro[cyclopentane-1,10′-dibenzo[*a*,*d*]-cyclohepten]-3-yl)-4-piperidinecarboxylate (**16′**, 60 mg, 0.149 mmol) in ethanol (5 mL) and water (1 mL). The reaction mixture was left to stir at 70 °C for 4 h, overnight at room temperature, and then at 70 °C for further for 3 h. The ethanol was evaporated under vacuum, and the mixture was acidified with 3 N HCl (until pH 1 was reached). The solution was purified using an HLB cartridge (6 g) by eluting first with water and then MeOH to afford the title compound (**51**, 55 mg). $[\alpha]_D^{20}$ −18.6° (*c* 0.63, MeOH) (optical rotation was measured on a different batch and for the HCl salt). UPLC/MS R_f = 0.62; *m*/*z* (ES) 376.2 $[M + H]^+$. ^{1}H NMR (400 MHz, chloroform-*d*) δ: 7.30−7.44 (m, 1 H), 6.99−7.23 (m, 7 H), 3.95−4.25 (m, 2 H), 3.14−3.71 (m, 6 H), 1.80−2.70 (m, 12 H).

1-((1*S*)-5′,11′-Dihydrospiro[cyclopentane-1,10′-dibenzo[*a*,*d*]-cyclohepten]-3-yl)-4-fluoro-4-piperidinecarboxylic Acid (54). To a solution of ethyl 1-((1*S*)-5′,11′-dihydrospiro[cyclopentane-1,10′-dibenzo[*a*,*d*]cyclohepten]-3-yl)-4-fluoro-4-piperidinecarboxylate (**19′**, 21 mg, 0.050 mmol) in THF (1.5 mL) and water (0.5 mL) was added LiOH (4.77 mg, 0.199 mmol), and the mixture was heated under reflux for 4 h. The solvent was concentrated at reduced pressure, and the residue was dissolved in water and then neutralized with HCl (1 M). This mixture was purified by C18 column (10 g) to give the title compound (**54**, 14 mg) as a white solid. UPLC/MS R_f = 0.64; *m*/*z* (ES) 394.2 $[M + H]^+$. ^{1}H NMR (500 MHz, chloroform-*d*) δ: 7.00−7.40 (m, 8 H), 4.15 (d, 1 H), 4.06 (d, 1 H), 3.71−3.85 (m, 1 H), 3.51−3.70 (m, 1 H), 3.29−3.44 (m, 1 H), 3.31 (d, 1 H), 3.17 (d, 1 H), 2.84−3.03 (m, 2 H), 2.49−2.83 (m, 4 H), 2.11−2.36 (m, 5 H), 1.79−2.00 (m, 1 H).

3-((1*S*)-5′,11′-Dihydrospiro[cyclopentane-1,10′-dibenzo[*a*,*d*]-cyclohepten]-3-yl)-3-azabicyclo[3.1.0]hexane-1-carboxylic Acid (64). To a mixture of ethyl 3-((1*S*)-5′,11′-dihydrospiro[cyclopentane-1,10′-dibenzo[*a*,*d*]cyclohepten]-3-yl)-3-azabicyclo[3.1.0]-hexane-1-carboxylate (**29′** isomer 2, 26 mg, 0.065 mmol) in ethanol (5 mL) and water (1 mL) was added KOH (14.53 mg, 0.259 mmol). The resulting mixture was heated at 100 °C in a microwave reactor (Personal Chemistry Emrys optimizer) for 30 min. The solvent was then evaporated under vacuum and the mixture acidified with 3 N HCl (until pH 1 was reached). The solution was purified with a HLB cartridge (6 g), eluting first with water and then MeOH to afford the title compound (**64**, 18.8 mg). UPLC/MS R_f = 0.63; *m*/*z* (ES) 374.2 $[M + H]^+$. ^{1}H NMR (400 MHz, chloroform-*d*) δ: 7.40−7.57 (m, 1 H), 7.08−7.25 (m, 5 H), 6.97−7.08 (m, 2 H), 4.02−4.19 (m, 2 H), 3.88−4.02 (m, 1 H), 3.52−3.84 (m, 3 H), 3.21−3.37 (m, 2 H), 3.05−3.18 (m, 1 H), 2.53−2.72 (m, 1 H), 2.34−2.53 (m, 1 H), 2.03−2.28 (m, 4 H), 1.73−1.97 (m, 2 H), 1.58−1.71 (m, 1 H).

1-((1*S*)-5′,11′-Dihydrospiro[cyclopentane-1,10′-dibenzo[*a*,*d*]-cyclohepten]-3-yl)-3-azetidinecarboxylic Acid Formate Salt (65). In a 50 mL round-bottomed flask was dissolved methyl 1-((1*S*)-5′,11′-dihydrospiro[cyclopentane-1,10′-dibenzo[*a*,*d*]cyclohepten]-3-yl)-3-azetidinecarboxylate (**30′**, 66 mg, 0.183 mmol) in methanol (5 mL) and water (2.5 mL) to give a colorless solution. KOH (41.0 mg, 0.730 mmol) was added, and the mixture was stirred at room temperature overnight. MS monitoring showed that the reaction was complete. Solvent was removed and the residue taken up with HCl 1 M and passed through an HLB 6 g column (water and MeOH to elute). Then a second purification was done by Fraction Lynx acid method to give the title compound as a yellow solid (**65**, 57.9 mg). UPLC/MS t_R = 0.58; *m*/*z* (ES) 348.08 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.33−6.94 (m, 8 H), 4.17−3.99 (m, 2 H), 3.48−3.37 (m, 2 H), 3.26−3.15 (m, 5 H), 3.14−3.06 (m, 1 H), 2.01−1.90 (m, 2 H), 1.85−1.61 (m, 4 H).

1-(11′*H*-Spiro[cyclopentane-1,10′-dibenzo[*b*,*f*]oxepin]-3-yl)-1,2,3,6-tetrahydro-4-pyridinecarboxylic Acid (69). To a solution

of methyl 1-(11′*H*-spiro[cyclopentane-1,10′-dibenzo[*b*,*f*]oxepin]-3-yl)-1,2,3,6-tetrahydro-4-pyridinecarboxylate (**34′** isomer 2, 27 mg, 0.069 mmol) in methanol (2 mL) were added water (1 mL) and lithium hydroxide (1.660 mg, 0.069 mmol). The mixture was heated at 45 °C for 4 h. The MeOH was evaporated and the water phase acidified with HCl (2 N in water) until pH < 1. The suspension was purified by C18 cartridge (5 g) by using water and then MeOH as eluent to obtain, after solvent evaporation, a cream solid (17 mg). The product was further purified by Fraction Lynx HPLC to give the title compound as a white solid (**69**, 8.2 mg). *m*/*z* (ES): 376.1 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.32−7.39 (m, 1 H), 7.04−7.30 (m, 7 H), 6.74−6.84 (m, 1 H), 3.40−2.20 (m, 15 H).

1-(11′*H*-Spiro[cyclopentane-1,10′-dibenzo[*b*,*f*]oxepin]-3-yl)-3-azetidinecarboxylic Acid Formic Acid Salt (73). To a colorless solution of methyl 1-(11′*H*-spiro[cyclopentane-1,10′-dibenzo-[*b*,*f*]oxepin]-3-yl)-3-azetidinecarboxylate (**38′**, 19 mg, 0.052 mmol) in methanol (2 mL) and water (1 mL) was added KOH (11.73 mg, 0.209 mmol), and the mixture was stirred at room temperature overnight. UPLC/MS monitor showed that the reaction was complete. The solvent was removed and the product was purified by Fraction Lynx acid method to give the title compound as white solid (**73**, 20 mg). UPLC/MS $R_f = 0.57$; *m*/*z* (ES) 350.04 $[M + H]^+$. ^{1}H NMR (400 MHz, chloroform-*d*) δ: 8.40 (bs, 1H), 7.22−6.90 (m, 8 H), 4.34−2.95 (m, 8 H), 2.75−1.84 (m, 6 H).

Supporting Information Available: Experimental procedures and supporting data for compounds **43**, **44**, **49**, **50**, **52**, **53**, **55**−**63**, **66**−**68**, and **70**−**72**. This material is available free of charge via the Internet at http://pubs.acs.org.

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