From Hit to Lead. Combining Two Complementary Methods for Focused Library Design. Application to μ Opiate Ligands

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Compound **1** obtained by random screening and displaying a micromolar activity on the μ opiate receptor was chosen as a starting point for optimization. Two complementary concepts of similarity were used for the design of analogues and compared. These are based, respectively, on a computer-aided comparison of pharmacophoric patterns and on topological similarity. The structure−activity relationships are discussed in light of both similarity concepts. Compound **40**, an *N*-methyl-3-(4-oxo-1-phenyl-1,3,8-triazaspiro[4.5]decyl)acetamide derivative, designed by combining the structure−activity relationships enlightened by each method, has a subnanomolar affinity for μ (h) receptor (IC_{50} = 0.9 nM). It is a promising lead, allowing the design of a new series of analogues substituted at the N-3 of the spirocycle moiety.

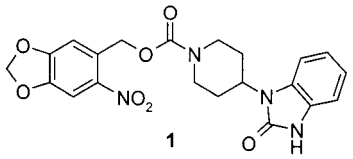
Introduction

In recent years, parallel synthesis[1] and high-throughput screening (HTS),[2] relying in various extents on molecular modeling for library management,[3] description,[4] similarity evaluation,[5] and quantitative structure−activity relationships (SARs),[6] have led to a dramatic increase in throughput in the primary stages of drug discovery.

We report here a generic hit-to-lead strategy, relying on the systematic generation and evaluation of analogues around the parent hit compound. This strategy makes concerted uses of two different analoging methods to cover efficiently the structural space around the hits. The first method is a classical exploratory analoging based on similar topology. The second method relies on (1) algorithms for rapid generation of 3D molecular fingerprints[7] based on multiconformational models of candidate compounds and (2) similarity[8] evaluation tools for the comparison of these 3D fingerprints. It is therefore called the similar pharmacophoric pattern search.

To validate experimentally our hit-to-lead strategy, we have applied it to the well-known μ receptor. Our goal was not primarily to discover new μ ligands but to exemplify our approach in the case of a universally recognized model. To mimic a real situation of drug discovery, a diverse library, of 10560 compounds, fitting the Lipinski rules,[9] obtained by parallel synthesis, was screened at 10 μM on the μ opiate receptor (rat cortex)

Chart 1



1

μ(h) = 1050 nM
κ(h) > 10000 nM
δ(h) > 10000 nM

for binding ability. The hit rate obtained at 10 μM for an inhibition threshold of 50% was 1.7%. Hits were then resynthesized, purified, and tested for affinity against the μ receptor (human) and the two other opiate subtypes (δ, κ). Out of these primary hits, compound **1** (Chart 1) was selected as a starting point for optimization.

Analogues of **1** were designed either by pharmacophoric pattern similarity searching against a virtual library of potentially synthesizable combinatorial products or by exploring various changes of the pharmacophoric pattern, while keeping the topology. Automated parallel synthesis was used for the rapid synthesis of the different analogues. A total of 294 analogues of **1** were synthesized on the 5 μmol scale and screened on the μ (h) receptor at 10 μM. Eventually, 30 compounds were selected for resynthesis and purification on a larger scale, to allow both complete purity and identity assessment and biological evaluation. After this first step, six new analogues displaying combinations of the best structural features evidenced by each analogue design method were synthesized and tested.

Design of the Analogues

To select and synthesize the analogues, two independent methods of analogue design have been used, allowing us to investigate in a systematic way the structural space around **1**.

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(a) Method A consists of exploring the distribution of the *pharmacophoric*[10] elements of the active compound while keeping a similar molecular topology. This method uses modifications of structures such as chain lengthening, substitutions, ring opening or closures, and simplifications to "mutate", "enrich", or "simplify" the distribution of the pharmacophoric groups, also referred to as the pharmacophoric pattern, of the hit. At least one obvious retrosynthetic route of wide chemical scope was available for compound **1** since it was primarily issued from parallel synthesis. Therefore, most of the analogues were generated as combinations of commercially available or purposely synthesized topologically similar but pharmacophorically different analogues of the original building blocks used for the synthesis of **1**.

(b) Method B aims at retrieving analogues that display a similar pharmacophoric pattern, out of a virtual library of all feasible combinations of available building blocks (more than 7000 diversity reagents, in our case). This method does not explicitly take molecular topology into account. Each member of the virtual library was described by an autocorrelogram referred to as an FBPA (fuzzy bipolar pharmacophore autocorrelogram).[7] The 252-dimensional FBPAs are the molecular descriptors at the basis of the similarity metrics used in this work.[8] The FBPA of a molecule M, hereafter denoted as $\Psi_M(a,b,\Delta)$, monitors the numbers of atom pairs within each of the $252 = 21 \times 12$ categories that can be defined in terms of the 21 combinations of six pharmacophoric features $a, b \in$ {hydrophobicity, aromaticity, hydrogen bond acceptor and donor, cation, anion} times 12 considered distance ranges Δ (Å) $\in$ {3−4, 4−5, ..., 14−15}. The associated fuzzy-logic-based dissimilarity score ΔFBP(m,M) between a candidate molecule m and the reference M relies on partial dissimilarity scores $\delta_{a,b}$(m,M) calculated with respect to the atom pair distributions matching every pharmacophore feature pair *a*, *b*:

$$\delta_{a,b}(\mathrm{m,M}) = 1 - \frac{2(\Psi_\mathrm{m} \otimes \Psi_\mathrm{M})_{a,b}}{(\Psi_\mathrm{M} \otimes \Psi_\mathrm{M})_{a,b} + (\Psi_\mathrm{m} \otimes \Psi_\mathrm{m})_{a,b}}$$

where

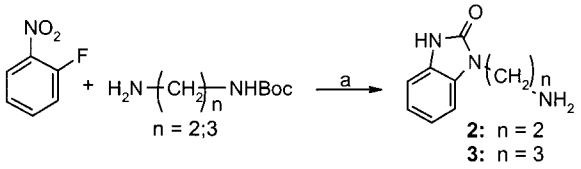
$$(\Psi_\mathrm{m} \otimes \Psi_\mathrm{M})_{a,b} = \sum_{\Delta_1=1}^{N_\mathrm{bin}} \sum_{\Delta_2=1}^{N_\mathrm{bin}} \Psi_\mathrm{m}(a,b,\Delta_1)\, \Psi_\mathrm{M}(a,b,\Delta_2) \times \exp[-\alpha(\Delta_1 - \Delta_2)^2]$$

The global dissimilarity score ΔFBP(m,M) is defined as a weighted average of the pharmacophore pair partial scores, with weighing factors w_k associated with each pharmacophore feature:

$$\Delta\mathrm{FBP(m,M)} = \frac{\sum_{a=1}^{N_\mathrm{f}} \sum_{b=a}^{N_\mathrm{f}} w_a w_b \delta_{a,b}(\mathrm{m,M})}{\sum_{a=1}^{N_\mathrm{f}} \sum_{b=a}^{N_\mathrm{f}} w_a w_b}$$

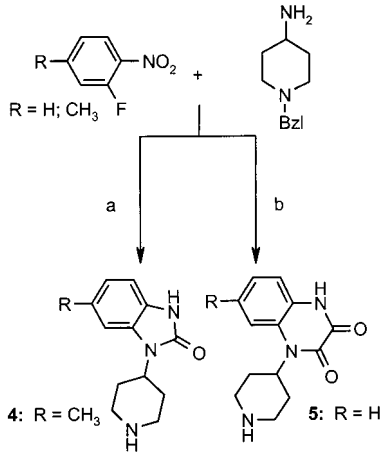
The weighing factors and the fuzziness parameter α were once calibrated to maximize the number of represented biological activity classes within a most diverse subset selected by the dissimilarity metric from a collection of reference ligands of various receptors. Candidates selected from a virtual compound collection and displaying the lowest dissimilarity scores when their FBPAs were compared to that of the reference compound (here, hit **1**) were then, in a second step, superimposed on the reference and reranked according to the more accurate ComPharm similarity score.[8]

Scheme 1[a]



[a] Reagents and Conditions: (a) (i) K_2CO_3, DMF, 70 °C, overnight; (ii) EtOH, Pd−C (10%), $NH_4^+HCOO^-$, 70 °C, overnight; (iii) AcOEt, DIPEA, 0 °C, triphosgene; (iv) TFA/CH_2Cl_2 (50/50).

Scheme 2[a]

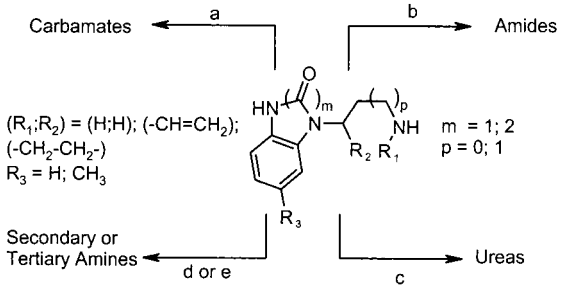


[a] Reagents and Conditions: (a) (i) K_2CO_3, DMF, 70 °C, overnight; (ii) Fe, HCl (concentrated), EtOH, reflux, 3.5 h; (iii) triphosgene, AcOEt, rt, overnight; (iv) Pd−C, HCOOH, MeOH, reflux, overnight; (b) (i) K_2CO_3, DMF, 70 °C, overnight; (ii) Fe, HCl (concentrated), EtOH, reflux, 3.5 h; (iii) oxalic acid, HCl (3 N), reflux, overnight; (iv) Pd−C, HCOOH, MeOH, reflux, overnight.

Chemistry

(a) Library of Exploratory Analogues (Method A, Similar Topology). Compound **1** is a carbamate. Various analogues of the amine moiety were purchased or prepared (Schemes 1 and 2) to determine the effect of ring opening of the piperidine, substitution of one of the hydrogen atoms of the benzimidazolinyl group, and homologation of the 2-oxo-1-benzimidazolinyl group by insertion of another carbonyl moiety. Different alcohols, amines, and carboxylic acids were coupled, using various synthetic methods (Scheme 3), to these amines to generate carbamates, ureas, and amides, respectively. The presence of a basic nitrogen is known to be critical for binding to opiate receptors as well as to many other G-protein-coupled receptors (GPCRs) displaying a conserved aspartate residue in the potential binding cleft.[11] Moreover, this feature has a major impact on the physical−chemical behavior. We thus investigated the synthesis of *N*-(3-phenylpropyl)piperidinyl derivatives as analogues of **1**. Alkylation procedures from aldehyde or halide, which can readily generate a broad structural

Scheme 3[a]



[a] Reagents and conditions: (a) alcohol in DMF, *N,N′*-carbonyldiimidazole (CDI) in THF, 86−98%; (b) carboxylic acid in DMF, CDI in THF, 87−90%; (c) amine in DMF, CDI in THF, 90−92%; (d) aldehyde, $NaHB(OAc)_3$, CH_2Cl_2, 81−88%; (e) halide, DIPEA, DMF, 95−99%.

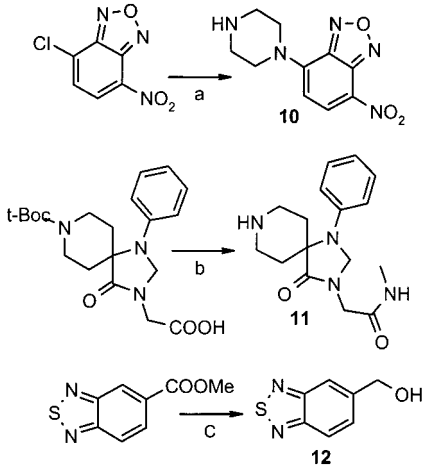
diversity, are the obvious synthetic way to obtain these compounds. However, in our hands, aliphatic aldehydes were not stable in the synthesis conditions, and 3-phenylpropyl halides are poorly reactive. The carbon chain between the basic nitrogen and the phenyl group was thus shortened to use benzyl aldehydes and halides (Scheme 3). These *N*-benzylpiperidinyl analogues are related to the 4-(2-oxo-1-benzimidazolinyl)piperidine derivatives already described as displaying in vivo analgesic properties.[12]

(b) Library of Analogues with Similar Pharmacophoric Patterns (Method B). These compounds could be sorted into two series. The first series consisted of analogues with a topology completely different from that of **1**. Examples are presented in Chart 2 (compounds **6**−**9**). Intermediates **10**−**12** had to be prepared as shown in Scheme 4 to synthesize these analogues. The second and best fitting series consisted of analogues derived from 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one or its substituted derivative **11**.

Analogues displaying a spirocyclic moiety and retrieved by method B could be easily synthesized as described in Scheme 5. Some analogues displaying a good similarity score were derived from alcohol **12**, which had been previously synthesized from the corresponding methyl ester.

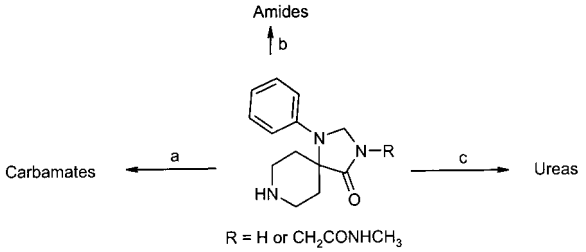
(c) Analytical Control. Each of the 294 analogues synthesized by parallel synthesis, at an "HTS grade", was controlled for purity and identity using LC/MS. In each case, the purity exceeded 80% and the mass spectrum of the major peak was consistent with the expected structure. Further pharmacological characterization was undertaken on fully purified and controlled samples ("SAR grade").

Scheme 4[a]



[a] Reagents and Conditions: (a) (i) DMF, reflux, 1 h; (ii) HCl (3 N), 100 °C, 2 h; (b) (i) THF, CDI, $MeNH_2$·HCl, TEA; (ii) THF, HCl (1 N), Et_2O, H_2SO_4, 65 °C, overnight; (c) (i) LiI, pyridine, reflux, overnight; (ii) ClCOOEt, DIPEA, THF, 0 °C, 1.5 h; (iii) $NaBH_4$, H_2O, 0 °C, 2 h.

Scheme 5[a]



[a] Reagents and conditions: (a) alcohol in DMF, CDI in THF, 85−95%; (b) carboxylic acid in DMF, CDI in THF, 90−94%; (c) amine in DMF, CDI in THF, 90−96%.

Screening

The libraries were tested at 10 μM for binding at the μ human receptor. Crude products displaying a percentage of inhibition above 50% were selected for preliminary IC_{50} determination prior to resynthesis and purification. Eventually, 30 compounds were selected for resynthesis and purification on a larger scale to allow biological characterization on μ (h), κ (h), and δ (h).

Results and Discussion

IC_{50} is defined as the concentration inhibiting 50% of specific binding of 0.5 nM DAMGO, U69593, and DPDPE to μ, κ, and δ receptors, respectively. Binding

Chart 2

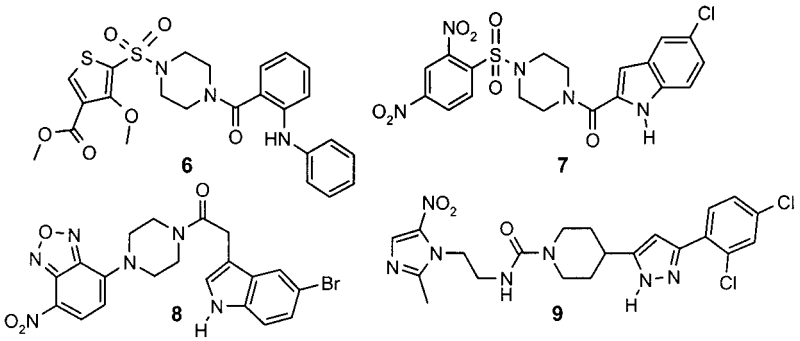
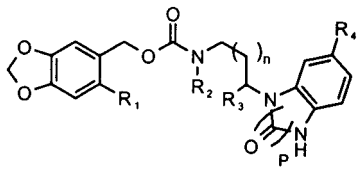
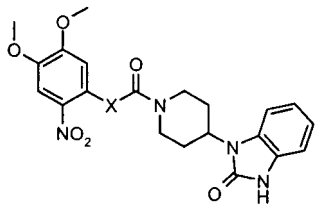


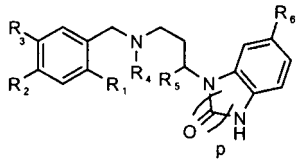
Table 1. Binding Affinities (IC_{50}) on the Opiate Receptors of Exploratory Analogues **13**−**27** of **1** Retrieved by Method A (Similar Topology)



compd no.	R_1	R_2	R_3	R_4	n	p	IC_{50} (nM)[a,b] μ (h)	κ (h)	δ (h)	sol[c] (μM)	log D[d]
1	NO_2	$-CH_2CH_2-$		H	1	1	1050 (1020−1090)	>10000	>10000	<10	f
13	Cl	$-CH_2CH_2-$		H	1	1	147 (142−152)	284 (265−303)	2530 (2507−2554)	<10	4.2
14	Cl	H	H	H	1	1	2480 (1160−3800)	1740 (1429−2051)	>10000	<10	e
15	Cl	H	H	H	0	1	>10000	>10000	>10000	<10	3.2
16	NO_2	$-CH_2CH=$		H	1	1	>10000	6540 (6290−6790)	>10000	f	f
17	Cl	$-CH_2CH_2-$		H	1	2	2590 (2340−2840)	1060 (983−1137)	>10000	<10	1.1
18	Cl	$-CH_2CH_2-$		CH_3	1	1	1235 (1184−1292)	797 (770−825)	5640 (3960−7320)	<10	3.4



compd no.	X	IC_{50} (nM)[a,b] μ (h)	κ (h)	δ (h)	sol[c] (μ M)	log D[d]
19	CH_2	>10000	1320 (1316−1324)	>10000	<10	2.4
20	$-CH=CH-$	960 (959−961)	4030 (3247−4812)	>10000	57.4	3



compd no.	R_1	R_2	R_3	R_4	R_5	R_6	p	IC_{50} (nM)[a,b] μ (h)	κ (h)	δ (h)	sol[c] (μM)	log D[d]
21	Cl	$-OCH_2O-$		$-CH_2CH_2-$		H	1	1.2 (1.1−1.2)	1.7 (1.6−1.8)	37.4 (36.9−37.9)	89.6	3.0
22	Cl	$-OCH_2O-$	H	H		H	1	235 (231−240)	507 (401−612)	3330 (295−03710)	185.7	1.8
23	NO_2	$-OCH_2O-$		$-CH_2CH_2-$		H	1	1.5 (1.4−1.6)	37 (32.4−41.8)	264 (248−279)	<10	2.4
24	NO_2	$-OCH_2O-$		$-CH_2CH=$		H	1	19.3 (17.2−21.3)	311 (286−336)	1040 (1010−1070)	<10	2.9
25	Cl	$-OCH_2O-$		$-CH_2CH_2-$		H	2	6.3 (4.4−8.3)	32 (30−34)	620 (589−651)	46.4	1.3
26	Cl	$-OCH_2O-$		$-CH_2CH_2-$		CH_3	1	2.4 (2.2−2.5)	2.9 (2.7−3.0)	117 (101−133)	<10	2.6
27	Cl	OCH_3	H	$-CH_2CH_2-$		H	1	16 (14.7−17.2)	270 (270−270)	1410 (304−2510)	195.3	2.5

[a] IC_{50} values were obtained from an experiment performed in duplicate from 11 concentrations. Both values are given in parentheses. [b] The radioligands used were [^{3}H]DAMGO (μ (h)), [^{3}H]U69593 (κ (h)), and [^{3}H]DPDPE (δ (h)). [c] The solubility value is determined in PBS, pH 7.4. [d] Partition coefficient between PBS, pH 7.4, and octanol. [e] Determination could not be made since the compound was not detected by MS or UV in the buffer phase (log D likely >4). [f] Not determined.

affinities (IC_{50} values, nM) to the μ, κ, and δ receptors of the selected analogues of **1** are reported in Tables 1−3.

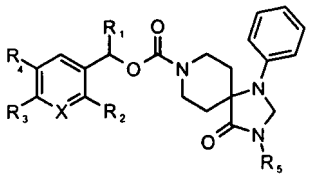
(a) Exploratory Analogues (Method A, Similar Topology). As one might expect,[11] introduction of a basic nitrogen, conjugated with shortening of the chain, enhances binding affinity by 2 or 3 orders of magnitude (**1** vs **23**), while decreasing subtype selectivity. Isosteric replacement of the carbamate in **1** by a cinnamoyl moiety (compound **20**) has poor incidence on the activity (except on κ (h)) but increases solubility. The carbamate link seems not favorable for aqueous solubility.

The open ring analogues **14** and **22** ($IC_{50(\mu)}$ = 2480 nM and $IC_{50(\mu)}$ = 235 nM, respectively) are 10−100 times less active than the corresponding piperidine compounds **13** ($IC_{50(\mu)}$ = 147 nM) and **21** ($IC_{50(\mu)}$ = 1.2 nM). In the meantime, solubility increases and log D decreases. Simultaneous removal of a methylene group and ring opening led to a complete loss of activity (compound **15**). A change in the structural constraint, thanks to a double bond, in the piperidine ring of **1** or **23** led to a 10-fold decrease in activity (**16** or **24**, respectively).

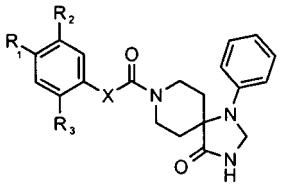
Quinoxalinedione derivatives **17** and **25** were found to be 5−20 times less active than the corresponding benzimidazolones. These analogues displayed the lowest log D of the series.

Affinities of analogues bearing a methyl group at position 6 on the benzimidazolone moiety decrease (**1** and **21** vs **18** and **26**) by only a factor of 2. Interestingly, analogue **16** displays a better affinity for κ (h) than for

Table 2. Binding Affinities (IC_{50}) on the Opiate Receptors of Spirocycle Analogues **28**−**38** of **1** Retrieved by Method B (Similar Pharmacophoric Pattern)



compd no.	R_1	R_2	R_3	R_4	R_5	X	IC_{50} (nM)[a,b] μ (h)	κ (h)	δ (h)	sol[c] (μM)	log D[d]
28	H	NO_2	$-OCH_2O-$		H	CH	>10000	5070 (5000−5140)	>10000	<10	3.1
29	H	Cl	$-OCH_2O-$		$CH_2CONHCH_3$	CH	179 (173−184)	333 (286−380)	1765 (1600−1930)	36.9	3.1
30	H	Cl	$-OCH_2O-$		H	CH	1730 (1700−1759)	2145 (1710−2580)	>10000	<10	2.9
31	H	H	$-OCH_2O-$		H	CH	2285 (2090−2480)	2235 (2080−2390)	>10000	<10	3.7
32	H	H	−thiadiazole−		H	CH	>10000	1480 (1140−1820)	>10000	<10	3.4
33	H	H	H	$-OC_6H_5$	H	CH	>10000	1810 (1510−2100)	>10000	<10	e
34	$-CH_2CH_2-$		H	H	H	CH	1470 (1160−1780)	617 (532−702)	>10000	<10	e
35	H	H	H	H	H	N	>10000	3550 (2350−4750)	>10000	105	2.5



compd no.	R_1	R_2	R_3	X	IC_{50} (nM)[a,b] μ (h)	κ (h)	δ (h)	sol[c] (μM)	log D[d]
36	$-OCH_2O-$		H	$-CH_2NH-$	>10000	2280 (1610−2950)	>10000	28.8	2.8
37	$-OCH_2O-$		NO_2	−CH=CH−	>10000	>10000	>10000	24.3	3.1
38	OCH_3	OCH_3	NO_2	−CH=CH−	>10000	>10000	>10000	34.8	3.0

[a−e] See footnotes of Table 1.

Table 3. Binding Affinities (IC_{50}) on the Opiate Receptors of Analogues **6**−**9** of **1** Retrieved by Method B (Similar Pharmacophoric Pattern)

compd no.	IC_{50} (nM)[a,b] μ (h)	κ (h)	δ (h)	sol[c] (μM)	log D[d]
6	4955 (2740−7170)	7240 (2980−11500)	>10000	<10	4.2
7	2285 (2090−2480)	>10000	>10000	<10	e
8	>10000	1010 (1000−1020)	>10000	<10	3
9	2890 (1170−4610)	>10000	7130 (3160−11100)	46.5	3.4

[a−e] See footnotes of Table 1.

μ (h). Analogue **26**, differing from **21** only by a methyl group, is however much less water soluble.

Interestingly, relative subtle modification of structure (**13**, **1**, and **21** vs **17**, **18**, and **26**) can lead to significant changes in solubility properties.

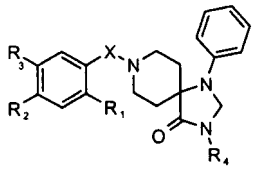
A chlorine atom in the *ortho*-position of the benzyl ring improves both binding and solubility in comparison with the nitro group (**13** and **21** vs **1** and **23**), but selectivity between μ (h) and κ (h) decreases. Suppression of the methylenedioxy group and replacement by a single methoxy substituant (**27** vs **21**) results in a 10-fold decrease of affinity for μ (h) but a better solubility.

The two key features that thus should be taken into consideration for the optimization of compound **1** are the presence of the basic nitrogen, to reach nanomolar activities, and the replacement of the NO_2 group by a chlorine atom, to increase solubility.

(b) Analogues with Similar Pharmacophoric Patterns (Method B). Among others, of micromolar activities, method B produced several analogues where the piperidinylbenzimidazolone is replaced by a phenyltriazaspirodecanone moiety (**28**−**38**). In that series, compound **28** shows no detectable activity, while replacement of the nitro group by a chlorine atom in **30** allows activities similar to that of **1**. *ortho*-Substitution on the benzyl ring allows micromolar activity on μ (h). Interestingly, compound **34**, which bears a 2-indan moiety instead of an *ortho*-subtituted benzyl ring, also displays micromolar activity on μ (h) ($IC_{50(\mu)} = 1470$ nM), and submicromolar activity on κ (h) ($IC_{50(\kappa)} = 617$ nM). In this carbamate series, the only features that increase solubility are the presence of a pyridine ring (**35**) or the presence of an *N*-methylacetamide group on position 3 of the spirodecanone (**29**). Interestingly, the latter substitution was also found to be, in our series, the most beneficial feature for binding. The isosteric replacement of the carbamate link by an amide (compounds **37** and **38**) or a urea (compound **36**) did improve solubility, but had no effect on activity.

Besides the spirodecanone template, method B identified several other pharmacophorically similar backbones (**6**−**9**, Table 3) that actually had micromolar IC_{50} values. However, none of these structures were found in a consistent family, rendering the elaboration of the

Table 4. Binding Affinities (IC_{50}) on the Opiate Receptors of Analogues **39**−**44** of **1** Resulting from Combining Methods A and B



compd no.	R_1	R_2	R_3	X	R_4	IC_{50} (nM)[a,b] μ (h)	κ (h)	δ (h)	sol[c] (μM)	log D[d]
39	Cl		$-OCH_2O-$	CH_2	H	2.0 (1.5−2.4)	5.7 (5.7−5.8)	138 (109−167)	20.1	3.0
40	Cl		$-OCH_2O-$	CH_2	$CH_2CONHCH_3$	0.9 (0.9−1.0)	2.1 (2.0−2.3)	11.4 (11.1−11.7)	208. 9	2.9
41	H		$-OCH_2O-$	CH_2	H	16.7 (15.6−17.8)	56.1 (50.1−62.1)	1190 (997−1370)	183. 2	2.2
42	H		−thiadiazole−	CH_2	H	24.9 (24.3−25.4)	104 (86−121)	4120 (3980−4250)	197	2.6
43	H	H	$-OC_6H_5$	CH_2	H	83.5 (83.4−83.7)	101 (96−106)	1840 (1770−1900)	<10	2.9
44	Cl		$-OCH_2O-$	CH_2OCO	CH_2COOH	735 (687−784)	6570 (6490−6650)	>10000	98.1	0.7

[a−d] See footnotes of Table 1.

Table 5. Binding Affinities (IC_{50}) on the Opiate Receptors of Some Reference Ligands

entry	name	IC_{50} (nM)[a,b] μ (h)	κ (h)	δ (h)
1	morphine sulfate	1.0 (0.8−1.2)	217 (191−234)	242 (237−247)
2	codeine monohydrate	520 (507−534)	c	>10000
3	nalbuphine	1.0 (0.8−1.2)	83 (72−94)	353 (349−358)
4	buprenorphine hydrochloride	1.7 (1.1−2.3)	1.2 (1.1−1.3)	0.9 (0.5−1.3)
5	naloxone	2.0 (1.9−2.0)	1.5 (1.2−1.8)	151 (149−153)
6	pethidine	315 (301−329)	2370 (1920−2810)	>10000
7	loperamide hydrochloride	1.5 (1.1−1.8)	155 (126−184)	123 (116−130)
8	fentanyl	1.3 (1.3−1.4)	1580 (991−2180)	431 (362−500)
9	methadone	4.1 (3.8−4.4)	512 (511−513)	1090 (977−1200)

[a,b] See footnotes of Table 1. [c] Could not be determined.

primary SAR difficult. Therefore, at the end of that first optimization cycle, we selected the spirodecanone framework as a basis for a second round of synthesis.

Applying the key features evidenced by method A on the spirodecanone framework, we designed five more analogues (**39**−**43**, Table 4). The syntheses were performed using benzyl aldehydes or benzyl chlorides for *N*-alkylation, except for compound **42**, which was synthesized from alcohol **12** via the corresponding mesylate.

As in the benzimidazolone series, introduction of the basic nitrogen allowed nanomolar activities to be reached and increased aqueous solubility (while log *D* was distributed between 2.5 and 3). Large substituents on the benzyl ring, such as a phenoxy group in **43**, decrease activity by a factor of 40 and have a dramatic impact on solubility. Interestingly, benzothiadiazole seems to be a good bioisoster of the methylenedioxyphenyl group, since analogue **42** has activities, solubility, and log *D* similar to those of analogue **41**. In the case of opiate receptors, benzothiadiazole could be considered as a potential substitute for the methylenedioxyphenyl group that, notwithstanding its occurrence in a number of natural or synthetic therapeutic compounds, is known to potentially inhibit cytochrome P450 and saturate metabolic pathways. Analogue **40** bears a chlorine atom on the benzyl ring and is substituted by an *N*-methylacetamide group on the spiro moiety. It is the most active compound of the series, and it has the best solubility. It is more than 3 orders of magnitude more active than **1**, being within the range of the best reported nonpeptidic ligands (entries 1−9, Table 5). Interestingly, the N-3 substitution on the spirocyclic moiety is quite tolerant since compound **44**, having no basic nitrogen but even bearing a carboxylate group, displayed a submicromolar activity on μ (h). The influence of various substituents at this position on the spirocyclic moiety could be the next point to investigate to optimize selectivity and ADME properties for example. Indeed, the methyl group could be easily replaced to give other amides.

Conclusion

Hit optimization of compound **1** relied on two different analogue design strategies: a computer-aided approach based on a quantitative estimation of the similarity of pharmacophoric patterns in the "structural space", and an open-ended exploratory approach consisting of voluntary changes of the molecular pharmacophores while preserving the overall "skeleton" (topology) of the compounds.

The exploratory approach may introduce dramatic changes in the pharmacophoric patterns of the molecules, with radical effects on the activity. It evidenced here again the beneficial introduction of a basic nitrogen to reach nanomolar activities, on the opiate receptors. The analogues of **1** with similar pharmacophoric patterns included some representatives in which the benzimidazolone moiety has been replaced by a topologically different triazaspirodecanone ring system. Though similar activities for derivatives of these two moieties had already been reported in the serotoninergic and dopaminergic series,[13,14] substitution by a *N*-methylacetamide at N-3 of the spirocyclic moiety has, to our knowledge, never been reported. In our hands, the spirodecanone moiety has proven to be pharmacophorically equivalent to the benzimidazolone, with respect to the opiate receptors, having moreover the advantage of accommodating at the N-3 position a variety of substituents

ranging from hydrogen to carboxylate or *N*-methylcarboxamide. The latter result could be exploited to optimize other properties of the compound such as selectivity or ADME profile, without affecting the binding of the molecule to the μ opiate receptor.

In conclusion, our pharmacophore-oriented analogue design (method B) suggests completely novel molecular skeletons compatible with a primary pharmacophore assumption, and exploratory modifications (method A) can then be applied to refine the assumption. The two methods can therefore be regarded as complementary and should be used in parallel for the elucidation of structure−activity relationships. In the present case, the combination of information provided by each analogue series has allowed us to design chimeric compounds that (1) were more active than the best of the two series, (2) had improved physical−chemical properties, and (3) bear a readily modifiable substituent, opening a promising way to a second step of optimization.

Further biological evaluation of the analogues obtained in these first steps of optimization is now considered. In particular, the use of pharmacological profiling on a large panel of receptors is currently being investigated to study the structure−profile relationships.

Experimental Section

Chemistry. General Information. All commercial reagents and solvents were used without further purification. NMR spectra were recorded on either a Bruker DRX-300 or a Bruker DMX-600 spectrometer. Chemical shifts are in parts per million (ppm). The assignments were made using one-dimensional (1D) ^{1}H and ^{13}C spectra and two-dimensional (2D) HMQC spectra. Mass spectra were determined using a Micromass Platform instrument equipped with an APCI interface. SAR-grade compounds were purified by extraction or preparative HPLC using a Shimadzu LC-10 preparative chromatography system equipped with LC-8A pumps, an FRC-10A fraction collector, an SPD-10A detector, and a Vydac C18 5 μm particle size column, dimensions 250 × 25.4 mm. A gradient starting from 100% H_2O and reaching 100% H_2O/80% CH_3CN within 18 min at a flow rate of 50 mL/min was used. Organic layers obtained after extraction of aqueous solutions were dried over $MgSO_4$ and filtered before evaporation in vacuo. Purity was determined by two different HPLC systems. In the first system, analyses were performed using a C18 TSK-GEL Super ODS 3 μm particle size column, dimensions 50 × 4.6 mm. A gradient starting from 100% H_2O/0.05% TFA and reaching 100% H_2O/80% CH_3CN/0.0425% TFA within 5 min at a flow rate of 2.75 mL/min was used (t_R = retention time). Chromatograms were recorded on an LC/MS instrument using a Micromass Platform mass analyzer coupled to an HP 1100 LC system with a diode array (200−400 nm) detector or using a Micromass ZMD system coupled with a Gilson 307 diode array detector and LC pumps 306. In the second system, analyses were performed on an Adsorbosphere CN 3U from Alltech 3 μm particle size column, dimensions 50 × 4.6 mm, using a Shimadzu instrument equipped with LC-10AS pumps and an SPD-10A dual wavelength (215, 254 nm) detector. A gradient starting from 100% H_2O/0.05% TFA and reaching 100% H_2O/80% CH_3CN/0.0425% TFA within 25 min at a flow-rate of 2.75 mL/min was used (t_R' = retention time). Purification yields were not optimized. Melting points were measured on a Büchi B-450 apparatus and are uncorrected. All the reactions on the 5 μmol scale were performed in 96-well polypropylene plates, Beckmann reference 26 7006. When more material was needed (SAR grade compounds), the reactions were performed 30 or 40 times in the same conditions and reaction products pooled before purification.

General Procedure A for Carbamates (Compounds 1, 13−18, 28−35, and 44). A 42 μL (10.3 μmol, 2.1 equiv) sample of a solution (0.247 M, in THF) of *N*,*N*′-carbonyldiimidazole was added at room temperature to 50 μL (5 μmol, 1 equiv) of a solution (0.1 M, in DMF) of alcohol. After the resulting solution was stirred at room temperature for 2 h, 50 μL (5 μmol, 1 equiv) of a solution (0.1 M, in DMF) of amine was added. The reaction mixture was stirred overnight, evaporated under reduced pressure, and, except for **1**, purified using preparative HPLC. For **1**, the residue was dissolved in AcOEt and purified by acidobasic extractions. The organic layer was dried over $MgSO_4$ and filtered before evaporation.

General Procedure B for Amides (Compounds 8, 19, 20, 37, and 38). A 42 μL (10.3 μmol, 2.1 equiv) sample of a solution (0.247 M, in THF) of *N*,*N*′-carbonyldiimidazole was added at room temperature to 9 μL (5 μmol, 1 equiv) of a solution (0.56 M, in DMF) of acid. After the resulting solution was stirred at room temperature for 2 h, 50 μL (5 μmol, 1 equiv) of a solution (0.1 M, in DMF) of amine was added. The reaction mixture was stirred for 2 h and then evaporated under reduced pressure. The residue was purified by preparative HPLC.

General Procedure C for Ureas (Compounds 9 and 36). A 42 μL (10.3 μmol, 2.1 equiv) sample of a solution (0.247 M, in THF) of *N*,*N*′-carbonyldiimidazole was added at room temperature to 50 μL (5 μmol, 1 equiv) of a solution (0.1 M, in DMF) of the first amine. After the resulting solution was stirred at room temperature for several minutes, 50 μL (5 μmol, 1 equiv) of a solution (0.1 M, in DMF) of the second amine was added. The reaction mixture was stirred overnight and then evaporated under reduced pressure. The residue was purified by preparative HPLC.

General Procedure D for Reductive Amination (Compounds 23 and 24). A mixture of amine (1.5 mmol, 1 equiv), aldehyde (1.5 mmol, 1 equiv), and $NaHB(OAc)_3$ (3.75 mmol, 2.5 equiv) in CH_2Cl_2 was stirred overnight at room temperature and then evaporated under reduced pressure. The residue obtained was triturated in an aqueous solution of Na_2CO_3, washed with H_2O (2 × 10 mL), filtered, and washed with Et_2O/pentane (50/50).

General Procedure E for *N*-Alkylations (Compounds 21, 22, 25−27, 39−41, and 43). A 52 μL (0.3 mmol, 1 equiv) sample of DIPEA and 600 μL of a solution of halide (0.5 M, in DMF) (0.3 mmol, 1 equiv) were added to 3 mL of a solution (0.1 M, in DMF) of amine (0.3 mmol, 1 equiv). The reaction mixture was heated overnight at 70 °C and then evaporated under reduced pressure. The residue obtained was purified by preparative HPLC as described above, except for **22** and **40**, which were purified with HPLC solvents containing 0.5% trifluoroacetic acid.

Procedure F for the Preparation of Compounds 6 and 7. A 25 μL (5 μmol, 1 equiv) sample of a solution (0.2 M, THF) of sulfochloride was added at room temperature to 50 μL (5 μmol, 1 equiv) of a solution (0.1 M, DMF) of *N*-Boc-piperazine. After being stirred for 2 h at room temperature, the reaction mixture was evaporated under reduced pressure, and the residue obtained was dissolved in 100 μL of a solution of TFA/DCM (50/50). The solution was stirred for 1/2 h and then evaporated under reduced pressure. A 42 μL (10.3 μmol, 2.1 equiv) sample of a solution (0.247 M, in THF) of *N*,*N*′-carbonyldiimidazole was added at room temperature to 9 μL (5 μmol, 1 equiv) of a solution (0.56 M, in DMF) of acid. After the resulting solution was stirred at room temperature for 2 h, 50 μL (5 μmol, 1 equiv) of a solution (0.1 M, in DMF) of the deprotected amine was added. The reaction mixture was stirred overnight and then evaporated under reduced pressure. The residue was purified by preparative HPLC.

4-(2-Oxobenzimidazol-1-yl)piperidine-1-carboxylic Acid (6-Nitrobenzo[1,3]dioxol-5-yl)methyl Ester (1) (Procedure A). HPLC: t_R = 2.39 min. ^{1}H NMR (DMSO-d_6, 600 MHz): δ 1.71 (dd, J = 9.9 Hz, J' = 2.1 Hz, 2H), 2.22 (l s, 2H), 3.05−3.07 (m, 2H), 4.15 (d, J = 13.6 Hz, 2H), 4.38−4.39 (m,

1H), 5.36 (s, 2H), 6.26 (s, 2H), 6.97−6.99 (m, 3H), 7.17 (s, 1H), 7.20−7.22 (m, 1H), 7.71 (s, 1H), 10.85 (s, 1H, N*H*CO). MS (APCI+): *m*/*z* 441 [M + H]$^+$.

1-(2-Aminoethyl)-1,3-dihydrobenzimidazol-2-one Trifluoroacetate (2). A mixture of 1-fluoro-2-nitrobenzene (1.72 mL, 16.3 mmol), *tert*-butyl-3-(aminoethyl)carbamate (2.61 mL, 16.3 mmol), and potassium carbonate (3.61 g, 26.1 mmol, 1.6 equiv) in DMF (42 mL) was heated at 70 °C overnight. The reaction mixture was evaporated under reduced pressure. After precipitation of mineral salts with CH_2Cl_2, evaporation of the filtrate afforded 5.2 g of an orange oil. The oil (5.2 g) was dissolved in EtOH (105 mL). Pd−C (10%) (1.96 g, 1.8 mmol) and ammonium formate (4.61 g, 72 mmol, 4 equiv) were then added to the solution. The suspension was heated at 70 °C overnight, filtered on Celite, and rinsed with additional EtOH (2 × 50 mL). Evaporation of the filtrate afforded an oil (2.75 g, 10.95 mmol) which was then dissolved in AcOEt (140 mL) with DIPEA (3.82 mL, 21.8 mmol, 2 equiv). The solution was cooled at 0 °C before addition of triphosgene (1.7 g, 3.61 mmol, 0.33 equiv). After being stirred for 10 min at 0 °C and overnight at room temperature, the reaction mixture was washed with water (3 × 50 mL). The organic layer was dried ($MgSO_4$), filtered, and evaporated under reduced pressure to yield an oil. Flash chromatography on silica gel using a solvent system of CH_2Cl_2/MeOH (97/3) afforded an oil. Recrystallization from CH_3CN afforded 1.1 g of purified residue. The Boc protection was then removed by dissolving the protected amine (1.1 g, 2.99 mmol) in CH_2Cl_2 (7.485 mL), TFA (7.485 mL), and H_2O (0.748 mL) at room temperature. After being stirred for 2 h, the reaction mixture was evaporated under reduced pressure. The resulting oil was triturated with Et_2O to afford 0.946 mg of the white trifluoroacetate salt (20%). HPLC: t_R = 1.18 min. ^{1}H NMR (DMSO-d_6, 600 MHz): δ 3.14−3.16 (m, 2H), 4.06 (t, J = 6.2 Hz, 2H), 7.04−7.07 (m, 3H), 7.19 (dd, J = 5.8 Hz, J' = 1.8 Hz, 1H), 7.90 (br s, 3H, NH_3^+), 10.98 (s, 1H, N*H*CO). MS (APCI+): *m*/*z* 178 [M + H of free base]$^+$.

1-(2-Aminopropyl)-1,3-dihydrobenzimidazol-2-one Trifluoroacetate (3). The title compound was obtained from *tert*-butyl-3-(aminopropyl)carbamate, as described for **2**. Mp: 208−209 °C. HPLC: t_R = 1.40 min. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.93−1.95 (m, 2H), 2.80−2.85 (m, 2H), 3.88 (t, J = 6.6 Hz, 2H), 7.01−7.05 (m, 3H), 7.19 (d, J = 5.5 Hz, 1H), 7.75 (br s, 3H, NH_3^+), 10.95 (s, 1H, N*H*CO). MS (APCI+): *m*/*z* 192 [M + H of free base]$^+$.

(6-Methyl-1-piperidin-4-yl)-1,3-dihydrobenzimidazol-2-one Hydrochloride (4). A mixture of 3-fluoro-4-nitrotoluene (3.26 g, 21 mmol, 1 equiv), 4-amino-1-benzylpiperidine (4 g, 21 mmol, 1 equiv), and K_2CO_3 (4.65 g 33.63 mmol, 1.6 equiv) in DMF (42 mL) was heated at 70 °C overnight, and evaporated under reduced pressure. The red residue was dissolved in CH_2Cl_2 and washed with H_2O (2 × 30 mL). The organic layer was then evaporated under reduced pressure to afford 7.64 g of a red oil. This residue was dissolved in EtOH (150 mL). A mixture of this solution with Fe (7 g, 6 equiv) and concentrated HCl (8.6 mL, 5 equiv) was refluxed for 3.5 h. After cooling, the mixture was filtered on Celite and rinsed with DMF. The filtrate was evaporated under reduced pressure, dissolved in H_2O/AcOEt, and neutralized with Na_2CO_3. The organic layer was washed twice with water, dried ($MgSO_4$), and evaporated under reduced pressure. A mixture of the obtained oil (4.8 g, 16.2 mmol, 1 equiv) in AcOEt (65 mL) with DIPEA (4.61 g, 35,73 mmol, 2.2 equiv) was cooled to 0 °C, and 1.78 g (6 mmol, 0.37 equiv) of triphosgene was added. The mixture was allowed to warm to room temperature and stirred overnight. The solvent was removed under reduced pressure to yield an oil. AcOEt (25 mL) and H_2O (25 mL) were added. The organic layer was evaporated. Recrystallization from CH_3CN (100 mL) provided 2 g (6.2 mmol) of the amine as a brown powder. A solution of the intermediate benzyl-protected amine in MeOH (39 mL) with HCOOH (1.69 mL, 44.8 mmol, 7.2 equiv) and 870 mg of Pd−C (10%) was heated at 60 °C and stirred for 4 h. Evaporation under reduced pressure of the mixture gave a black oil. A solution of this oil in HCl (1 N) (30 mL) and AcOEt (5 mL) was stirred for 30 min at room temperature. The mixture was evaporated under reduced pressure to afford an oil which solidified upon triturating with Et_2O/pentane. The hydrochloride salt was obtained as a beige powder (1.4 g, 25%). HPLC: t_R = 1.48 min. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.77 (d, J = 12 Hz, 2H), 2.29 (s, 3H), 2.57−2.69 (m, 2H), 2.98−3.10 (m, 2H), 3.36 (d, J = 12 Hz, 2H), 4.48 (m, 1H), 6.76 (d, J = 7.84 Hz, 1H), 6.83 (d, J = 7.84 Hz, 1H), 7.33 (s, 1H), 9.15 (m, 2H, H_2N^+(piperidine)), 10.76 (s, 1H, N*H*CO). MS (APCI+): *m*/*z* 232 [M + H of free base]$^+$.

1-Piperidin-4-yl-1,4-dihydroquinoxaline-2,3-dione Hydrochloride (5). A mixture of 2-nitrofluorobenzene (7.46 g, 52.9 mmol), 4-amino-1-benzylpiperidine (10.07 g, 52.9 mmol), and K_2CO_3 (11.54 g, 83.5 mmol) in DMF (100 mL) was heated at 70 °C overnight, and evaporated under reduced pressure. The red residue was dissolved in EtOH (300 mL). A mixture of this solution with Fe (17.4 g, 331 mmol) and concentrated HCl (12 N) (26 mL) was refluxed for 2 h. After cooling, the mixture was evaporated under reduced pressure. The residue was dissolved in HCl (3 N) (130 mL) with oxalic acid (9.36 g, 104 mmol). The mixture was refluxed overnight, cooled, neutralized with a 1 M aqueous solution of Na_2CO_3, and extracted with AcOEt. The organic layer was washed with H_2O (2 × 30 mL), dried ($MgSO_4$), filtered, and evaporated under reduced pressure. The benzyl protection was removed as described for **4** to yield the hydrochloride salt (2.34 g, 8.04 mmol, 15.2%). HPLC: t_R = 1.48 min. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.82 (d, J = 12.9 Hz, 2H), 2.86 (dq, J = 3.6 Hz, J' = 13.2 Hz, 2H), 3.12 (q, J = 13.4 Hz, 2H), 3.34 (d, J = 12 Hz, 2H), 4.82 (m, 1H), 7.13−7.18 (m, 3H), 7.76−7.78 (m, 1H), 9.36 (m, 2H, H_2N^+(piperidine)), 12.0 (s, 1H, N*H*CO). MS (APCI+): *m*/*z* 232 [M + H of free base]$^+$.

4-Methoxy-5-[4-(2-phenylaminobenzoyl)piperazine-1-sulfonyl]thiophene- 3-carboxylic Acid Methyl Ester (6) (Procedure F). HPLC: t_R = 2.82 min and t_R' = 14.75 min. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.00−3.10 (m, 4H), 3.40−3.60 (m, 4H), 3.89 (s, 3H), 3.94 (s, 3H), 6.83 (t, J = 7.3 Hz, 1H), 6.91 (d, J = 7.8 Hz, 2H), 7.06 (t, J = 7.3 Hz, 1H), 7.15 (t, J = 7.7 Hz, 2H), 7.26 (d, J = 8.1 Hz, 1H), 7.31 (dd, J = 1.3 Hz, J' = 7.6 Hz, 1H), 7.40 (dt, J = 1.4 Hz, J' = 8.3 Hz, 1H), 7.82 (s, 1H, PhN*H*Ph), 8.72 (s, 1H). ^{13}C NMR (DMSO-d_6, 300 MHz) δ 41.0, 46.2, 52.8, 63.8, 117.4, 120.1, 120.6, 121.8, 123.8, 126.8, 129.5, 129.7, 131.0, 140.0, 140.5, 144.1, 158.5, 161.4, 168.5. MS (APCI+): *m*/*z* 516 [M + H]$^+$.

(5-Chloro-1*H*-indol-2-yl)[4-(2,4-dinitrobenzenesulfonyl)-piperazin-1-yl]methanone (7) (Procedure F). HPLC: t_R = 2.88 min and t_R' = 15.27 min. MS (APCI+): *m*/*z* 495 [M + H]$^+$. Mixture of two conformers (68/32). (Conformer 1) ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.45−3.48 (m, 4H), 3.93−3.98 (m, 4H), 6.87 (dd, J = 2.0 Hz, J' = 0.6 Hz, 1H), 7.27 (dd, J = 2.1 Hz, J' = 8.8 Hz, 1H), 7.50 (d, J = 8.8 Hz, 1H), 7.74 (d, J = 2.1 Hz, 1H), 8.37 (d, J = 8.7 Hz, 1H), 8.68 (dd, J = 2.3 Hz, J' = 8.7 Hz, 1H), 9.11 (d, J = 2.3 Hz, 1H), 11.87 (d, J = 1.4 Hz, 1H, NH_{indole}). ^{13}C NMR (DMSO-d_6, 300 MHz): δ 45.8, 46.4, 50.4, 104.6, 114.4, 120.8, 121.1, 124.2, 125.0, 127.7, 128.4, 131.5, 133, 134.7, 135.1, 148.5, 150.9, 162.5. (Conformer 2) ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.45−3.48 (m, 4H), 3.93−3.98 (m, 4H), 6.95 (dd, J = 2.0 Hz, J' = 0.6 Hz, 1H), 7.29 (dd, J = 2.1 Hz, J' = 8.8 Hz, 1H), 7.51 (d, J = 8.8 Hz, 1H), 7.76 (d, J = 2.1 Hz, 1H), 8.39 (d, J = 8.7 Hz, 1H), 8.72 (dd, J = 2.3 Hz, J' = 8.7 Hz, 1H), 9.11 (d, J = 2.3 Hz, 1H), 11.93 (d, J = 1.4 Hz, 1H, NH_{indole}). ^{13}C NMR (DMSO-d_6, 300 MHz): δ 45.8, 46.4, 50.4, 104.6, 114.4, 121.7, 123.9, 124.2, 125.0, 128.4, 129.0, 131.5, 134.7, 135.1, 148.9, 150.9, 162.5.

4-(7-Nitrobenzo[1,2,5]oxadiazol-4-yl)piperazine Hydrochloride (10). A mixture of 4-chloro-7-nitrobenzo[1,2,5]-oxadiazole (4.5 g, 22.5 mmol, 1 equiv), *N*-(*tert*-butyloxycarbonyl)-4-piperidine (5.04 g, 27.1 mmol, 1.2 equiv), and DMF (49.6 mL) was refluxed for 1 h. The reaction mixture was then evaporated under reduced pressure. The residue was then washed with H_2O (3 × 30 mL) and filtered to afford 7.315 g (20.9 mmol) of a yellow powder. A mixture of this product, EtOH (20 mL), and HCl (3 N) (40 mL) was refluxed for 2 h. The mixture was evaporated under reduced pressure, and the residue was

recrystallized from Et_2O/pentane (50/50) to afford 5.75 g of the purple hydrochloride salt (73.1%). Mp: 297−299 °C. HPLC: t_R = 2.04 min. ^{1}H NMR (D_2O−Na_2CO_3, 600 MHz): δ 2.78 (m, 4H), 3.38 (m, 2H), 6.26 (d, J = 9.6 Hz, 1H), 8.6 (d, J = 9.6 Hz, 1H). MS (APCI+): m/z 250 [M + H of free base]$^+$.

2-(5-Bromo-1*H*-indol-3-yl)-1-[4-(7-nitrobenzo[1,2,5]oxadiazol-4-yl)piperazin-1-yl]ethanone (8) was obtained from **10** according to procedure B. HPLC: t_R = 2.54 min. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.70−3.73 (m, 2H), 3.79 (s, 2H), 3.81−3.84 (m, 2H), 4.00−4.10 (m, 4H), 6.54 (d, J = 9.2 Hz, 1H), 7.11 (d, J = 8.6 Hz, J' = 1.9 Hz, 1H), 7.26 (d, J = 8.5 Hz, 1H), 7.29 (s, 1H), 7.71 (d, J = 1.8 Hz, 1H), 8.46 (d, J = 9.2 Hz, 1H), 11.09 (s, 1H, NH_{indole}). ^{13}C NMR (DMSO-d_6, 300 MHz): δ 31.8, 42.0, 45.5, 50.1, 50.4, 104.7, 115, 122.8, 125, 127.1, 137.8. MS (APCI+): m/z 485 [M + H]$^+$.

4-[5-(2,4-Dichlorophenyl)-2*H*-nitroimidazol-1-yl)ethyl]-amide (9) (Procedure C). HPLC: t_R = 2.66 min and t_R' = 13.77 min. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.44−1.56 (m, 2H), 1.93−1.97 (m, 2H), 2.48 (s, 3H), 2.80−2.97 (m, 3H), 3.47 (dt, J = 5.6 Hz, J' = 5.7 Hz, 2H), 3.98−4.02 (m, 2H), 4.41 (t, J = 5.7 Hz, 2H), 6.64 (s, 1H), 6.84 (t, J = 5.4 Hz, 1H, N*H*CON), 7.54 (dd, J = 8.4 Hz, J' = 1.8 Hz, 1H), 7.74 (d, J = 1.7 Hz, 1H), 8.40 (d, J = 8.4 Hz, 1H), 8.11 (s, 1H), 13.0 (s, 1H, NH_{pyrazole}). ^{13}C NMR (DMSO-d_6, 300 MHz): δ 14.5, 31.9, 33.6, 39.9, 43.9, 46.6, 103.0, 128.1, 130.2, 132.0, 132.3, 132.9, 133.8, 139.2, 148.3, 152.2, 157.6. MS (APCI+): m/z 493 [M + H]$^+$.

***N*-Methyl-2-(4-oxo-1-phenyl-1,3,8-triazaspiro[4,5]dec-3-yl)acetamide Hydrochloride (11).** A 3.9 g (10 mmol) sample of 3-[4-oxo-1-phenyl-8-(*tert*-butyloxycarbonyl)-1,3,8-triazaspiro[4,5]decyl]acetic acid was dissolved in a solution of *N*,*N*′-carbonyldiimidazole (0.247 M, in THF) (80 mL). After the reaction mixture was stirred at room temperature for 2 h, 1.35 g of methylamine hydrochloride salt (20 mmol, 2 equiv) and 1 g of triethylamine were added. After being stirred overnight, the reaction mixture was evaporated under reduced pressure. The crude product was dissolved in CH_2Cl_2 and washed with acidic water (pH 2, 3 × 50 mL). The organic layer was evaporated under reduced pressure. HPLC: t_R = 2.44 min. MS (APCI+) m/z 303 ([M + H]$^+$ − Boc). A mixture of the residue, THF (75 mL), HCl (1 N, in Et_2O) (20 mL, 20 mmol, 2 equiv), and H_2SO_4 (560 μL) was stirred overnight at 65 °C. The suspension was filtered and washed with AcOEt (30 mL). The solid was then dissolved in H_2O (50 mL), and Na_2CO_3 was added. The free base was extracted with CH_2Cl_2 (3 × 30 mL), and the organic layer was evaporated under reduced pressure to afford 600 mg (19.8%) of a white powder. HPLC: t_R = 1.47 min. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.63 (d, J = 14 Hz, 2H), 2.53−2.60 (m, 2H), 2.59 (d, $J_{\text{H−NH}}$ = 4.5 Hz, 3H), 3.02 (dd, J = 13 Hz, J' = 4.4 Hz, 2H), 3.22−3.25 (m, 2H), 3.95 (s, 2H), 4.66 (s, 2H), 6.71−6.83 (m, 2H), 6.90 (d, J = 8.2 Hz, 2H), 7.22 (t, J = 7.4 Hz, 2H), 8.00−8.05 (m, 1H, N*H*CO). MS (APCI+): m/z 303 [M + H of free base]$^+$.

Benzo[1,2,5]thiadiazol-5-ylmethanol (12). A mixture of benzo[1,2,5]thiadiazole-5-carboxylic acid methyl ester (3 g, 15.44 mmol) in pyridine (51 mL) and LiI (14.46 g, 108.1 mmol) was refluxed overnight. The solvent was removed under reduced pressure, and the resulting oil was dissolved in H_2O (25 mL). Addition of HCl (3 N) at 0 °C allowed the carboxylic acid intermediate to precipitate. The solid was washed with H_2O (2 × 20 mL) and acetonitrile (2 × 20 mL) and dissolved in THF (65 mL) and DIPEA (2.9 mL, 16.64 mmol). The mixture was cooled to 0 °C, and ClCOOEt (1.54 mL, 16 mmol) was added. After the resulting mixture was stirred for 1.5 h at 0 °C, a solution of $NaBH_4$ (1.36 g, 36.06 mmol) in H_2O (8.5 mL) was added dropwise. The mixture was stirred for 2 h at room temperature and evaporated under reduced pressure. The orange residue was dissolved in AcOEt (40 mL) and washed with a saturated solution of Na_2CO_3 (2 × 30 mL) and brine (1 × 30 mL), separated, dried ($MgSO_4$), and evaporated under reduced pressure to yield 1.45 g (63%) of a yellow powder. Mp: 54.8−55.5 °C. HPLC: t_R = 2.23 min. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 4.74 (d, J = 5.8 Hz, 2H), 5.57 (t, J = 5.7 Hz, 1H, O*H*), 7.69 (dd, J = 1.4 Hz, J' = 9.2 Hz, 1H), 7.98 (s, 1H), 8.07 (d, J = 9.0 Hz, 1H). MS (APCI+): m/z 167 [M + H]$^+$, 208 [M + MeCN]$^+$.

4-(2-Oxo-2,3-dihydrobenzimidazol-1-yl)piperidine-1-carboxylic Acid (6-Chlorobenzo[1,3]dioxol-5-yl)methyl Ester (13) (Procedure A). HPLC: t_R = 2.45 min and t_R' = 14.1 min. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.65 (l d, J = 12 Hz, 2H), 2.17 (ddd, J = 12 Hz, J' = 4.3 Hz, 2H), 2.83−2.90 (l s, 2H), 4.09 (l d, J = 12 Hz, 2H), 4.32 (m, 1H), 5.02 (s, 2H), 6.05 (s, 2H), 6.91−6.93 (m, 3H), 7.04 (s, 1H), 7.09 (s, 1H), 7.10−7.15 (m, 1H), 10.8 (s, 1H, N*H*CO). ^{13}C NMR (DMSO-d_6, 300 MHz): δ 29.4, 41.2, 50.5, 64.8, 103.0, 109.4, 109.8, 110.6, 110.8, 121.2, 121.5, 125.7, 128.0, 129.1, 130.0, 147.4, 148.9, 154.5, 155.0. MS (APCI+): m/z 430 [M + H]$^+$ and 386 [M − 44]$^+$.

[3-(2-Oxo-2,3-dihydrobenzimidazol-1-yl)propyl]carbamic Acid (6-Chlorobenzo[1,3]dioxol-5-yl)methyl Ester (14), Obtained from 3 (Procedure A). HPLC: t_R = 2.42 min and t_R' = 13.40 min. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.69−1.75 (m, 2H), 2.98 (dt, J = 6.2 Hz, J' = 6.7 Hz, 2H), 3.74 (t, J = 7.0 Hz, 2H), 4.92 (s, 2H), 6.03 (s, 2H), 6.93−6.96 (m, 3H), 6.99 (s, 1H), 7.31 (m, 2H), 7.31 (t, J = 5.5 Hz, 1H, N*H*COO), 10.80 (s, 1H, N*H*CON). ^{13}C NMR (DMSO-d_6, 300 MHz): δ 29.5, 39.0, 39.3, 63.9, 103.4, 109.4, 110.8, 111.2, 110.3, 122.2. MS (APCI+): m/z 404 [M + H]$^+$ and 360 [M − 44]$^+$.

[3-(2-Oxo-2,3-dihydrobenzimidazol-1-yl)ethyl]carbamic Acid (6-Chlorobenzo[1,3]dioxol-5-yl)methyl Ester (15), Obtained from 2 (Procedure A). HPLC: t_R = 2.46 min and t_R' = 13.58 min. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.21 (q, $J_{\text{H−NH}}$ = 5.9 Hz, J = 6.1 Hz, 2H), 2.78 (t, J = 6.1 Hz, 2H), 4.88 (s, 2H), 6.04 (s, 2H), 6.90−6.92 (m, 4H), 6.99 (m, 1H), 7.06 (s, 1H), 7.39 (t, $J_{\text{H−NH}}$ = 5.9 Hz, 1H, N*H*COO), 10.7 (s, 1H, N*H*CON). ^{13}C NMR (DMSO-d_6, (300 MHz): δ 40.5, 41.3, 63.6, 102.9, 108.2, 110.4, 110.5, 121.2, 121.5. MS (APCI+): m/z 392 [M + H]$^+$.

4-(2-Oxo-2,3-dihydrobenzimidaol-1-yl)-3,6-dihydro-2*H*-pyridine-1-carboxylic Acid (6-Nitrobenzo[1,3]dioxol-5-yl)methyl ester (16) (Procedure A). HPLC: t_R = 2.43 min. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.45 (s, 2H), 3.64 (l s, 2H), 4.12 (l d, 2H), 5.33 (s, 2H), 5.90 (s, 1H), 6.60 (s, 2H), 6.93−6.98 (m, 4H), 7.03 (m, 1H), 7.16 (s, 1H), 7.68 (s,1H), 10.94 (s, 1H, N*H*CO). ^{13}C NMR (DMSO-d_6, 300 MHz): δ 27.3, 41.1, 43.3, 64.6, 104.6, 105.1, 108.9, 109.6, 110.0, 122.1, 122.8. MS (APCI+): m/z 439 [M + H]$^+$.

4-(2,3-Dioxo-3,4-dihydro-2*H*-quinoxalin-1-yl)piperidine-1-carboxylic Acid (6-chlorobenzo[1,3]dioxol-5-yl)methyl Ester (17), Obtained from 5 and 6-Chloropiperonyl Alcohol (Procedure A). HPLC: t_R = 2.40 min and t_R' = 3.41 min. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.63 (l d, J = 11.2 Hz, 2H), 2.48−2.56 (ddd, J = 12.7 Hz, J' = 4.2 Hz, 2H), 2.83−2.90 (l s, 2H), 4.09 (l d, J = 10 Hz, 2H), 4.68 (m, 1H), 5.05 (s, 2H), 6.09 (s, 2H), 7.04 (s, 1H), 7.09 (s, 1H), 7.15−7.18 (m, 3H), 7.63 (m, 1H),10.7 (s, 1H, N*H*CO). ^{13}C NMR (DMSO-d_6, 300 MHz): δ 27.6, 44.1, 55.3, 64.8, 103.0, 110.8, 110.9, 115.8, 116.9, 123.9, 124.5, 125.8, 127.0, 127.6, 128.0, 147.4, 148.9, 154.9, 156.3. MS (APCI+): m/z 458 [M + H]$^+$ and 414 [M − 44]$^+$.

4-(6-Methyl-2-oxo-2,3-dihydrobenzimidazol-1-yl)piperidine-1-carboxylic Acid (6-Chlorobenzo[1,3]dioxol-5-yl)-methyl Ester (18), Obtained from 4 (Procedure A). HPLC: t_R = 2.67 min and t_R' = 14.74 min. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.63 (l d, J = 12 Hz, 2H), 2.18 (ddd, J = 12.7 Hz, J' = 4.2 Hz, 2H), 2.26 (s, 3H), 2.83−2.90 (l s, 2H), 4.09 (l d, J = 11.7 Hz, 2H), 4.28 (m, 1H), 5.01 (s, 2H), 6.05 (s, 2H), 6.73 (dd, J = 7.9 Hz, J' = 0.6 Hz, 1H), 6.80 (d, J = 7.9 Hz, 1H), 6.98 (l s, 1H), 7.04 (s, 1H), 7.09 (s, 1H), 10.7 (s, 1H, N*H*CO). ^{13}C NMR (DMSO-d_6, 300 MHz): δ 21.15, 28.6, 43.5, 49.6, 64.2, 103.0, 108.6, 109.1, 109.8, 110.2, 121.3. MS (APCI+): m/z 445 [M + H]$^+$ and 400 [M − 44]$^+$.

1-{1-[2-(6-Nitrobenzo[1,3]dioxol-5-yl)acetyl]piperidin-4-yl}-1,3-dihydrobenzimidazol-2-one (19) (Procedure B). HPLC: t_R = 2.17 min and t_R' = 13.02 min. MS (APCI+): m/z 442 [M + H]$^+$. Mixture of two conformers (52/48). (Conformer 1) ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.67 (l d, J = 12 Hz, 1H), 2.03−2.11 (m, 1H), 2.68 (t, J = 12 Hz, 1H), 3.82 (s, 3H), 3.85 (s, 3H), 4.07 (d, J = 16 Hz), 4.10−4.18 (m, 1H), 4.20 (d, J = 16

Hz, 1H), 6.92−6.95 (m, 3H), 7.06 (s, 1H), 7.24−7.27 (m, 1H), 7.65 (s, 1H), 10.83 (s, 1H, N*H*CON). ^{13}C NMR (DMSO-d_6, 300 MHz): δ 30.0, 39.7, 42.7, 51.2, 57.2, 57.7, 109.1, 110.2, 110.4, 117.0, 122.0. (Conformer 2) ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.76 (l d, J = 12 Hz, 1H), 2.36−2.45 (m, 1H), 3.21−3.28 (m, 1H), 3.82 (s, 3H), 3.85 (s, 3H), 4.07 (d, J = 16 Hz, 1H), 4.20 (d, J = 16 Hz, 1H), 4.41−4.49 (m, 2H), 6.92−6.95 (m, 3H), 7.06 (s, 1H), 7.24−7.27 (m, 1H), 7.65 (s, 1H), 10.83 (s, 1H, N*H*CON). ^{13}C NMR (DMSO-d_6, 300 MHz): δ 30.6, 39.7, 45.8, 51.2, 57.2, 57.7, 109.1, 110.2, 110.4, 117.0, 122.0.

1-{1-[3-(4,5-Dimethoxy-2-nitrophenyl)acryloyl]piperidin-4-yl}-1,3-dihydrobenzimidazol-2-one (20) (Procedure B). HPLC: t_R = 2.36 min and t_R' = 13.84 min. MS (APCI+): m/z 453 [M + H]$^+$. Mixture of two conformers (66/33). (Conformer 1) ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.75−1.78 (m, 2H), 2.29 (l d, J = 9.6 Hz, 1H), 2.77 (t, J = 12 Hz, 1H), 2.92 (t, J = 12 Hz, 1H), 3.25 (t, J = 6.8 Hz, 1H), 3.85 (s, 3H), 3.94 (s, 3H), 6.97−6.99 (m, 3H), 7.23−7.26 (m, 1H), 7.33 (d, J = 15 Hz, 1H), 7.42 (s, 1H), 7.63 (s, 1H), 7.86 (d, J = 15 Hz, 1H), 10.87 (s, 1H, N*H*CON). ^{13}C NMR (DMSO-d_6, 300 MHz): δ 29.8, 42.7, 45.8, 57.2, 57.7, 109.1, 110.2, 110.4, 112.0, 122.0, 123.5, 137.0. (Conformer 2) ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.75−1.78 (m, 2H), 2.30 (l d, J = 9.6 Hz, 1H), 3.85 (s, 3H), 3.94 (s, 3H), 3.97 (d, J = 12 Hz, 1H), 4.40−4.47 (m, 1H), 4.64 (d, J = 13 Hz, 1H), 6.97−6.99 (m, 3H), 7.23−7.26 (m, 1H), 7.42 (s, 1H), 7.63 (s, 1H), 8.19 (d, J = 2 Hz, 1H), 8.55 (d, J = 1 Hz, 1H), 10.83 (s, 1H, N*H*CON). ^{13}C NMR (DMSO-d_6, 300 MHz): δ 30.6, 45.8, 48.0, 57.2, 57.7, 109.1, 110.2, 110.4, 112.0, 122.0, 137.0, 144.8.

1-{1-[(6-Chlorobenzo[1,3]dioxol-5-yl)methyl]piperidin-4-yl}-1,3-dihydrobenzimidazol-2-one (21) (Procedure E). HPLC: t_R = 2.19 min. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.06 (d, J = 9.4 Hz, 2H), 2.13 (t, J = 11.2 Hz, 2H), 2.26−2.38 (m, 2H), 2.90 (d, J = 11.2 Hz, 2H), 3.48 (s, 2H), 4.07−4.17 (m, 1H), 6.03 (s, 2H), 6.91−6.96 (m, 3H), 7.01 (s, 1H), 7.05 (s, 1H), 7.19−7.20 (m, 1H), 10.79 (s, 1H, N*H*CO). ^{13}C NMR (DMSO-d_6, 300 MHz): δ 29.5, 50.9, 53.5, 59.0, 102.7, 109.6, 110.2, 110.8, 121.2, 121.3, 125.5, 129.1, 130.0, 130.1, 147.5, 147.8, 154.5. MS (APCI+): m/z 387 [M + H]$^+$.

1-{3-[[(6-Chlorobenzo[1,3]dioxol-5-yl)methyl]amino]-propyl}-1,3-dihydrobenzimidazol-2-one Trifluoroacetate (22), Obtained from 3 (Procedure E). HPLC: t_R = 2.12 min. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.92−2.02 (m, 2H), 2.95−2.98 (m, 2H), 3.84 (t, J = 6.7 Hz, 2H), 4.09−4.12 (m, 2H), 6.07 (s, 2H), 6.94−6.99 (m, 3H), 7.11−7.14 (m, 3H), 8.85 (s, 2H, NH_2^+), 10.91 (s, 1H, N*H*CO). ^{13}C NMR (DMSO-d_6, 300 MHz): δ 25.7, 37.9, 45.4, 48.0, 103.3, 108.6, 109.8, 110.6, 111.9, 121.4, 121.9, 123.2, 125.6, 126.9, 130.8, 147.6, 149.7, 155.2. MS (APCI+): m/z 360 [M + H of free base]$^+$.

1-{1-[(6-Nitrobenzo[1,3]dioxol-5-yl)methyl]piperidin-4-yl}-1,3-dihydrobenzimidazol-2-one (23) (Procedure D). HPLC: t_R = 1.76 min. ^{1}H NMR (CD$_3$COOD, 300 MHz): δ 2.09 (d, J = 12.3 Hz, 2H), 2.80−2.97 (m, 2H), 3.44 (t, 2H), 3.88 (d, J = 11.7 Hz, 2H), 4.68 (s, 2H), 4.69−4.81 (m, 1H), 6.21 (s, 2H), 7.08−7.12 (m, 2H), 7.17−7.20 (m, 1H), 7.28 (s, 1H), 7.34−7.36 (m, 1H), 7.68 (s, 1H). ^{13}C NMR (CD$_3$COOD, 300 MHz): δ 26.0, 48.1, 52.9, 57.7, 104.7, 106.4, 110.0, 110.8, 113.2, 121.1, 122.1, 122.5, 128.1, 128.7, 144.3, 150.2, 153.0, 155.6. MS (APCI+): m/z 397 [M + H]$^+$.

1-{1-[(6-Nitrobenzo[1,3]dioxol-5-yl)methyl]-1,2,3,6-tetrahydropyridin-4-yl}-1,3-dihydrobenzimidazol-2-one (24) (Procedure D). HPLC: t_R = 1.73 min. ^{1}H NMR (CD$_3$COOD, 300 MHz): δ 3.09 (s, 2H), 3.90 (t, J = 11.4 Hz, 2H), 4.31 (s, 2H), 4.88 (s, 2H), 6.1 (s, 1H), 6.30 (s, 2H), 7.18−7.22 (m, 2H), 7.25−7.36 (m, 2H), 7.42 (s, 1H), 7.77 (s, 1H). ^{13}C NMR (CD$_3$COOD, 300 MHz): δ 23.7, 49.3, 49.8, 56.3, 103.2, 106.1, 109.4, 110.3, 112.8, 119.3, 120.6, 122.0, 122.7, 127.8, 129.3, 130.4, 144.0, 149.7, 152.6, 154.5. MS (APCI+): m/z 395 [M + H]$^+$.

1-{1-[(6-Chlorobenzo[1,3]dioxol-5-yl)methyl]piperidin-4-yl}-1,4-dihydroquinoxaline-2,3-dione (25), Obtained from 5 (Procedure E). HPLC: t_R = 2.09 min. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.85−1.88 (m, 2H), 2.50−2.58 (m, 1H), 2.85−3.01 (m, 2H), 3.23−3.57 (m, 1H), 3.44 (s, 2H), 4.28 (s, 1H), 4.69−4.80 (m, 1H), 6.12 (s, 2H), 7.12−7.30 (m, 5H), 7.59 (s, 1H), 11.98 (s, 1H, N*H*CO). MS (APCI+): m/z 415 [M + H]$^+$.

1-{1-[(6-Chlorobenzo[1,3]dioxol-5-yl)methyl]piperidin-4-yl}-6-methyl-1,3-dihydrobenzoimidazol-2-one (26), Obtained from 4 (Procedure E). HPLC: t_R = 2.34 min. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.58 (d, J = 10.4 Hz, 2H), 2.13 (t, J = 11.2 Hz, 2H), 2.28−2.38 (m, 2H), 2.91 (d, J = 10.2 Hz, 2H), 3.28 (s, 3H), 3.48 (s, 2H), 4.04−4.12 (m, 1H), 6.03 (s, 2H), 6.72 (d, J = 7.9 Hz, 1H), 6.78 (d, J = 7.9 Hz, 1H), 7.01 (s, 1H), 7.02 (s, 1H), 7.05 (s, 1H), 10.65 (s, 1H, N*H*CO). ^{13}C NMR (DMSO-d_6, 300 MHz): δ 22.0, 29.5, 50.9, 53.5, 59.1, 102.7, 109.3, 110.0, 110.2, 110.9, 121.8, 125.5, 126.9, 129.9, 130.3, 140.4, 154.7. MS (APCI+): m/z 400 [M + H]$^+$.

1-[1-(4-Methoxybenzyl)piperidin-4-yl]-1,3-dihydrobenzoimidazol-2-one (27) (Procedure E). HPLC: t_R = 2.10 min. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.58 (d, J = 11.2 Hz, 2H), 2.03 (t, J = 9.6 Hz, 2H), 2.22−2.35 (m, 2H), 2.88 (d, J = 11.2 Hz, 2H), 3.45 (s, 2H), 3.69 (s, 3H), 4.05−4.13 (m, 1H), 6.85 (d, 2H), 6.91−6.96 (m, 3H), 7.15−7.20 (m, 1H), 7.21 (d, 2H), 10.75 (s, 1H, N*H*CO). ^{13}C NMR (DMSO-d_6, 300 MHz): δ 29.5, 51.0, 53.3, 55.8, 62.1, 109.5, 109.6, 114.4, 121.2, 121.3, 125.6, 129.1, 130.0, 130.9, 154.5, 159.1. MS (APCI+): m/z 338 [M + H]$^+$.

4-Oxo-1-phenyl-1,3,8-triazaspiro[4.5]decane-8-carboxylic Acid (6-Nitrobenzo[1,3]dioxol-5-yl)methyl Ester (28) (Procedure A). HPLC: t_R = 2.61 min and t_R' = 13.87 min. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.62 (d, J_{gem} = 14.2 Hz, 2H), 2.35 (m, 2H), 3.46 (m, 2H), 3.91 (dd, 2H), 4.56 (s, 2H), 5.31 (l d, 2H), 6.21 (s, 2H), 6.68 (d, J = 8.25 Hz, 2H), 6.72 (t, J = 7.3 Hz, 1H), 7.13 (s, 1H), 7.16 (dd, J = 7.4 Hz, J = 8.6 Hz, 2H), 7.67 (s, 1H), 8.77 (s, 1H, N*H*CO). ^{13}C NMR (DMSO-d_6, 300 MHz): δ 28.8, 59.1, 64.8, 104.6, 106.5, 109.2, 115.1, 130.3. MS (APCI+): m/z 455 [M + H]$^+$.

3-(Methylcarbamoylmethyl)-4-oxo-1-phenyl-1,3,8-triazaspiro[4.5]decane-8-carboxylic Acid (6-Chlorobenzo-[1,3]dioxol-5-yl)methyl Ester (29), Obtained from 11 (Procedure A). HPLC: t_R = 2.81 min and t_R' = 14.39 min. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.64 (d, J = 13.6 Hz, 2H), 2.32 (ddd, J = 13.6 Hz, J' = 5.43 Hz, 2H), 2.57 (d, J_{NH-H} = 4.53, 3H), 3.41−3.45 (l s, 2H), 3.88 (l s, 2H), 3.93 (s, 2H), 4.65 (s, 2H), 5.03 (l d, J = 34.4 Hz, 2H), 6.05 (s, 2H), 6.66 (d, J = 8.10 Hz, 2H), 6.74 (t, J = 7.3 Hz, 1H), 7.05 (s, 1H), 7.09 (s, 1H), 7.16 (t, J = 7.2 Hz, 2H), 7.99 (m, 1H, CH$_3$N*H*CO). ^{13}C NMR (DMSO-d_6, 300 MHz): δ 26.8, 30.0, 41.8, 44.5, 65.5, 65.8, 103.7, 111.3, 111.8, 116.1, 119.8, 130.9. MS (APCI+): m/z 515 [M + H]$^+$ and 471 [M − 44]$^+$.

4-Oxo-1-phenyl-1,3,8-triazaspiro[4.5]decane-8-carboxylic Acid (6-Chlorobenzo[1,3]dioxol-5-yl)methyl Ester (30) (Procedure A). HPLC: t_R = 2.74 min and t_R' = 14.59 min. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.59 (d, J = 13.7 Hz, 2H), 2.32 (ddd, J = 13.6 Hz, J' = 5.5 Hz, 2H), 3.42−3.49 (m, 2H), 3.87−3.91 (m, 2H), 4.55 (s, 2H), 4.97 (s, 1H), 5.09 (s, 1H), 6.04 (s, 2H), 6.64 (d, J = 8.0 Hz, 2H), 6.72 (t, J = 6.7 Hz, 1H), 7.04 (s, 1H), 7.09 (s, 1H), 7.14 (t, J = 8.4 Hz, 2H), 8.76 (m, 1H, N*H*CO). ^{13}C NMR (DMSO-d_6, 300 MHz): δ 29.6, 41.9, 60.4, 65.8, 103.7, 111.5, 111.8, 115.7, 119.4, 130.7. MS (APCI+): m/z 445 [M + H]$^+$.

4-Oxo-1-phenyl-1,3,8-triazaspiro[4.5]decane-8-carboxylic Acid Benzo[1,3]dioxol-5-ylmethyl Ester (31) (Procedure A). HPLC: t_R = 2.66 min and t_R' = 13.97 min. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.59 (d, J = 13.8 Hz, 2H), 2.30 (ddd, J = 5.1 Hz, J' = 13.2 Hz, 2H), 3.46 (l s, 2H), 3.90 (dd, J = 4.1 Hz, J' = 12.7 Hz, 2H), 4.55 (s, 2H), 4.98 (ld, J = 7.5 Hz), 5.96 (s, 2H), 6.63 (d, J = 8.0 Hz, 2H), 6.71 (t, J = 7.3 Hz, 1H), 6.8−6.85 (m, 2H), 6.91 (d, J = 0.6 Hz, 1H), 7.13 (t, J = 7.4 Hz, 2H), 8.75 (s, 1H, N*H*CO). ^{13}C NMR (DMSO-d_6, 300 MHz): δ 29.3, 41.6, 59.7, 59.7, 67.6, 102.6, 109.6, 110.0, 115.9, 119.4, 123.1, 130.8. MS (APCI+): m/z 410 [M + H]$^+$ and 366 [M − 44]$^+$.

4-Oxo-1-phenyl-1,3,8-triazaspiro[4.5]decane-8-carboxylic Acid Benzo[1,2,5]thiadiazol-5-ylmethyl Ester (32), Obtained from 12 (Procedure A). HPLC: t_R = 2.64 min and t_R' = 12.07 min. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.63 (d, J = 13.6 Hz, 2H), 2.34 (ddd, J = 13.6 Hz, J' = 5.5 Hz, 2H), 3.5 (l s, 2H), 3.97 (l s, 2H), 4.55 (s, 2H), 5.31 (l d, J = 9.6 Hz,

2H), 6.64 (d, $J = 8.0$ Hz, 2H), 6.67 (t, $J = 7.3$ Hz, 1H), 7.07 (t, $J = 7.6$ Hz, 2H), 7.7 (dd, $J = 9.0$ Hz, $J' = 1.4$ Hz, 1H), 8.02 (s, 1H), 8.07 (d, $J = 9.0$ Hz, 1H), 8.77 (s, 1H, N*H*CO). ^{13}C NMR (DMSO-d_6, 300 MHz): δ 29.8, 42.0, 60.2, 67.3, 115.8, 119.5, 120.4, 122.8, 130.6, 131.4. MS (APCI+): m/z 424 $[M + H]^+$.

4-Oxo-1-phenyl-1,3,8-triazaspiro[4.5]decane-8-carboxylic Acid 3-Phenoxybenzyl Ester (33) (Procedure A). HPLC: $t_R = 3.38$ min. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.59 (d, $J = 14$ Hz, 2H), 2.24−2.35 (m, 2H), 3.4−3.6 (l s, 2H), 3.90 (dd, $J = 12.3$ Hz, $J' = 3.6$ Hz, 2H), 4.55 (s, 2H), 5.08 (l d, $J = 10.8$ Hz, 2H), 6.64 (d, $J = 8.4$ Hz, 2H), 6.70 (t, $J = 7.3$ Hz, 1H), 6.91 (m, 3H), 6.96 (d, $J = 0.9$ Hz, 1H), 7.06−7.12 (m, 2H), 7.13 (t, $J = 8.3$ Hz, 2H), 7.30−7.37 (m, 3H), 8.76 (s, 1H, CON*H*). ^{13}C NMR (DMSO-d_6, 300 MHz): δ 29.8, 42.1, 60.5, 67.3, 115.7, 118.4, 119.0, 119.4, 120.5, 123.5, 125.3, 130.5, 132.7. MS (APCI+): m/z 458 $[M + H]^+$.

4-Oxo-1-phenyl-1,3,8-triazaspiro[4.5]decane-8-Carboxylic Acid Indan-1-yl Ester (34) (Procedure A). HPLC: $t_R = 3.09$ min. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.57 (l s, 2H), 1.96−2.02 (m, 1H), 2.34−2.40 (l s, 2H), 2.44−2.47 (m, 1H), 2.81−2.86 (m, 1H), 2.92−2.97 (m, 1H), 3.41−3.45 (m, 2H), 3.85−3.87 (m, 2H), 4.54 (s, 2H), 6.04 (ls, 1H), 6.65 (d, $J = 8.1$ Hz, 2H), 6.72 (t, $J = 7.2$ Hz, 1H), 7.15−7.19 (m, 3H), 7.26 (d, $J = 3.8$ Hz, 2H), 7.35 (d, $J = 7.3$ Hz, 1H), 8.75 (s, 1H, N*H*CO). ^{13}C NMR (DMSO-d_6, 300 MHz): δ 29.5, 30.9, 41.8, 60.5, 80, 115.7, 119.7, 126.5, 128.2, 130.4, 130.8. MS (APCI+): m/z 392 $[M + H]^+$.

4-Oxo-1-phenyl-1,3,8-triazaspiro[4.5]decane-8-carboxylic Acid Pyridin-3-ylmethyl Ester (35) (Procedure A). HPLC: $t_R = 1.87$ min. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.60 (d, $J = 13.8$ Hz, 2H), 2.31 (ddd, $J = 13.5$ Hz, $J' = 5.5$ Hz, 2H), 3.48 (l s, 2H), 3.92 (dd, $J = 13.1$ Hz, $J' = 4.9$ Hz, 2H), 4.55 (s, 2H), 5.13 (l d, $J = 9.9$ Hz, 2H), 6.63 (d, $J = 8.0$ Hz, 2H), 6.72 (t, $J = 7.3$ Hz, 1H), 7.15 (t, $J = 7.4$ Hz, 2H), 7.37 (ddd, $J = 4.9$ Hz, $J' = 7.9$ Hz, $J'' = 0.7$ Hz, 1H), 7.77 (dt, $J = 7.9$ Hz, $J' = 1.8$ Hz, 1H), 8.51 (dd, $J = 4.8$ Hz, $J = 1.6$ Hz, 1H), 8.58 (d, $J = 1.7$ Hz, 1H), 8.76 (s, 1H, N*H*CO). ^{13}C NMR (DMSO-d_6, 300 MHz): δ 29.8, 41.7, 60.2, 65.7, 115.7, 119.5, 125.1, 130.8, 137.4, 150.5, 150.7. MS (APCI+): m/z 367 $[M + H]^+$.

4-Oxo-1-phenyl-1,3,8-triazaspiro[4.5]decane-8-carboxylic Acid (Benzo[1,3]dioxol-5-ylmethyl)amide (36) (Procedure C). HPLC: $t_R = 2.23$ min and $t_R' = 12.29$ min. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.49 (d, $J = 13.5$ Hz, 2H), 2.35 (ddd, $J = 5.2$ Hz, $J' = 13.5$ Hz, 2H), 3.37 (dt, $J = 2.8$ Hz, $J' = 12.9$ Hz, 2H), 4.25 (dd, $J = 3.3$ Hz, $J' = 12.3$ Hz, 2H), 4.15 (d, $J_{NH-H} = 5.7$ Hz, 2H), 4.54 (s, 1H), 5.93 (s, 2H), 6.59 (d, $J = 8.1$ Hz, 2H), 6.68 (t, $J = 7.3$ Hz, 1H), 6.72 (dd, $J = 7.8$ Hz, $J' = 1.6$ Hz, 1H), 6.78 (s, 1H), 6.81 (dd, $J = 1.5$ Hz, $J' = 4.8$ Hz, 1H), 7.08 (t, $J = 7.5$ Hz, 2H), 7.12 (t, $J = 5.8$ Hz, 1H, OCON*H*), 8.71 (s, 1H, N*H*CO). ^{13}C NMR (DMSO-d_6, 300 MHz): δ 29.2, 42.1, 43.4, 60.2, 102.3, 109.4, 109.6, 115.4, 119.2, 121.8, 130.8. MS (APCI+): m/z 409 $[M + H]^+$.

8-[3-(6-Nitrobenzo[1,3]dioxol-5-yl)acryloyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (37) (Procedure B). HPLC: $t_R = 2.67$ min and $t_R' = 12.44$ min. MS (APCI+): m/z 451 $[M + H]^+$. Mixture of two conformers (50/50). (Conformer 1) ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.68 (d, $J = 14.5$ Hz, 2H), 2.30−2.40 (m, 2H), 3.29−3.39 (m, 1H), 4.24 (d, $J = 11.1$ Hz, 2H), 4.57 (s, 2H), 6.22 (s, 2H), 6.67 (d, $J = 7.9$ Hz, 2H), 6.70 (t, $J = 7.3$ Hz, 1H), 7.16 (t, $J = 7.5$ Hz, 2H), 7.27 (d, $J = 15.1$ Hz, 1H), 7.60 (s, 1H), 7.61 (s, 1H), 7.71 (d, $J = 15.1$ Hz, 1H), 8.79 (s, 1H, N*H*CO). ^{13}C NMR (DMSO-d_6, 300 MHz): δ 29.8, 40.3, 60.2, 105.2, 106.4, 108.7, 115.9, 119.6, 123.9, 130.9, 137.4. (Conformer 2) ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.68 (d, $J = 14.5$ Hz, 2H), 2.30−2.40 (m, 2H), 3.75 (t, 1H), 4.37 (d, $J = 11.1$ Hz, 2H), 4.57 (s, 2H), 6.22 (s, 2H), 6.67 (d, $J = 7.9$ Hz, 2H), 6.70 (t, $J = 7.3$ Hz, 1H), 7.16 (t, $J = 7.5$ Hz, 2H), 7.27 (d, $J = 15.1$ Hz, 1H), 7.60 (s, 1H), 7.61 (s, 1H), 7.71 (d, $J = 15.1$ Hz, 1H), 8.79 (s, 1H, N*H*CO). ^{13}C NMR (DMSO-d_6, 300 MHz): δ 30.1, 42.4, 60.2, 105.2, 106.4, 108.7, 115.9, 119.6, 123.9, 130.9, 137.4.

8-[3-(4,5-Dimethoxy-2-nitrophenyl)acryloyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (38) (Procedure B). HPLC: $t_R = 2.67$ min and $t_R' = 12.86$ min. MS (APCI+): m/z 467 $[M + H]^+$. Mixture of two conformers (50/50). (Conformer 1) ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.60 (m, 2H), 2.40−2.45 (m, 2H), 3.30−3.35 (m, 1H), 3.84 (s, 3H), 3.93 (s, 3H), 4.24 (d, 2H, $J = 11.1$ Hz), 4.58 (s, 2H), 6.68 (d, $J = 8.1$ Hz, 2H), 6.71 (t, $J = 7.4$ Hz, 1H), 7.17 (t, $J = 7.5$ Hz, 2H), 7.27 (d, $J = 15.2$ Hz, 1H), 7.36 (s, 1H), 7.58 (s, 1H), 7.82 (d, $J = 15.2$ Hz, 1H), 8.79 (s, 1H, N*H*CO). ^{13}C NMR (DMSO-d_6, 300 MHz): δ 29.8, 40.4, 57.6, 58.3, 60.2, 109.4, 112.1, 116.0, 119.8, 123.9, 130.8, 138.1. (Conformer 2) ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.60 (m, 2H), 2.40−2.45 (m, 2H), 3.75−3.80 (m, 1H), 3.84 (s, 3H), 3.93 (s, 3H), 4.37 (d, 2H, $J = 11.1$ Hz), 4.58 (s, 2H), 6.68 (d, $J = 8.1$ Hz, 2H), 6.71 (t, $J = 7.4$ Hz, 1H), 7.17 (t, $J = 7.5$ Hz, 2H), 7.27 (d, $J = 15.2$ Hz, 1H), 7.36 (s, 1H), 7.58 (s, 1H), 7.82 (d, $J = 15.2$ Hz, 1H), 8.79 (s, 1H, N*H*CO). ^{13}C NMR (DMSO-d_6, 300 MHz): δ 31.1, 43.5, 57.6, 58.3, 60.2, 109.4, 112.1, 116.0, 119.8, 123.9, 130.8, 138.1.

8-[(6-Chlorobenzo[1,3]dioxol-5-yl)methyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (39) (Procedure E). HPLC: $t_R = 2.26$ min. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.56 (d, $J = 13$ Hz, 2H), 2.48−2.56 (m, 2H), 2.72−2.88 (m, 4H), 3.51 (s, 2H), 4.56 (s, 2H), 6.06 (s, 2H), 6.75 (t, $J = 7.3$ Hz, 1H), 6.86 (d, $J = 8.2$ Hz, 2H), 7.04 (s, 1H), 7.07 (s, 1H), 7.24 (t, $J = 7.3$ Hz, 2H), 8.64 (s, 1H, N*H*CO). ^{13}C NMR (DMSO-d_6, 300 MHz): δ 28.8, 49.6, 58.5, 58.8, 59.1, 102.3, 109.8, 110.4, 114.7, 118.1, 125.1, 129.5, 143.7, 147.0, 176.6. MS (APCI+): m/z 400 $[M + H]^+$.

2-{8-[(6-Chlorobenzo[1,3]dioxol-5-yl)methyl]-4-oxo-1-phenyl-1,3,8-triazaspiro[4.5]dec-3-yl}-*N*-methylacetamide Trifluoroacetate (40), Obtained from 11 (Procedure E). HPLC: $t_R = 2.26$ min. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.90 (d, $J = 14.4$ Hz, 2H), 2.57 (d, $J_{NH-H} = 4.5$ Hz, 3H), 2.69−2.80 (m, 2H), 3.46 (d, $J = 10.0$ Hz, 2H), 3.64−3.71 (m, 2H), 3.95 (s, 2H), 4.36 (s, 2H), 4.65 (s, 2H), 6.12 (s, 2H), 6.79 (t, $J = 7.2$ Hz, 1H), 6.88 (d, $J = 8.4$ Hz, 2H), 7.22 (t, $J = 7.7$ Hz, 2H), 7.23 (s, 1H), 7.26 (s, 1H), 8.03 (d, $J_{NH-H} = 4.5$ Hz, 1H, N*H*CO). ^{13}C NMR (DMSO-d_6, 300 MHz): δ 26.4, 27.2, 43.9, 49.3, 57.4, 58.4, 64.7, 103.6, 110.9, 113.3, 115.5, 119.6, 125.6, 130.0, 143.2, 143.2, 150.4, 167.7, 173.6. MS (APCI+): m/z 471 $[M + H$ of free base$]^+$.

8-(Benzo[1,3]dioxol-5-ylmethyl)-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (41) (Procedure E). HPLC: $t_R = 2.19$ min. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.51 (d, $J = 13.4$ Hz, 2H), 2.42−2.52 (m, 2H), 2.65−2.68 (d, $J = 7.8$ Hz, 4H), 3.38 (s, 2H), 4.52 (s, 2H), 5.94 (s, 2H), 6.68−6.75 (m, 2H), 6.71−6.82 (m, 3H), 6.87 (d, $J = 1.4$ Hz, 1H), 7.21 (t, $J = 7.4$ Hz, 2H), 8.59 (s, 1H, N*H*CO). ^{13}C NMR (DMSO-d_6, 300 MHz): δ 29.3, 49.9, 59.0, 59.5, 62.7, 101.6, 108.7, 109.8, 115.0, 118.4, 122.7, 129.9, 133.0, 144.2, 146.9, 148.0, 177.1. MS (APCI+): m/z 366 $[M + H]^+$.

8-(Benzo[1,2,5]thiadiazol-5-ylmethyl)-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one Trifluoroacetate (42). A 500 μL sample of a solution (1.2 M, in DCM) of $MeSO_2Cl$ (2 equiv) was added dropwise to 188 μL of a solution (1.6 M, in DCM) of benzo[1,2,5]thiadiazol-5-ylmethanol (**12**) (1 equiv) and DIPEA (1 equiv) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 30 min. The reaction mixture was washed with water (2 × 1 mL), HCl (10%) (2 × 1 mL), and a saturated solution of $NaHCO_3$ (2 × 1 mL). The organic layer was evaporated under reduced pressure. The residue obtained was dissolved in 143 μL of THF. A 150 μL sample of a solution (2 M, in THF) of 1-phenyl-1,3,8-triazaspiro-[4.5]decan-4-one was added to this solution. The reaction mixture was stirred for 1 h at room temperature and evaporated under reduced pressure. The residue was purified by preparative HPLC with H_2O/TFA (99.95/0.05) and H_2O/CH_3-CN/TFA (19.96/80/0.04), to give 11.6 mg of the trifluoroacetate salt (7.8%). HPLC: $t_R = 2.16$ min. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.91 (d, $J = 14.5$ Hz, 2H), 2.72−2.81 (m, 2H), 3.51 (d, $J = 10.8$ Hz, 2H), 3.73−3.84 (m, 2H), 4.61 (s, 2H), 4.64 (s, 2H), 6.80 (t, $J = 7.3$ Hz, 1H), 6.90 (d, $J = 8.1$ Hz, 2H), 7.20 (t, $J = 8.3$ Hz, 2H), 7.90 (dd, $J = 9.0$ Hz, $J' = 1.5$ Hz, 1H), 8.20 (d, $J = 9.0$ Hz, 1H), 8.41 (s, 1H), 9.04 (s, 1H, N*H*CO). ^{13}C NMR (DMSO-d_6, 300 MHz): δ 27.0, 49.6, 57.4, 59.7, 115.5, 119.3,

122.5, 125.8, 130.7, 132.7, 133.0, 143.6, 154.8, 175.6. MS (APCI+): *m/z* 366 [M + H of free base]$^+$.

8-(3-Phenoxybenzyl)-1-phenyl-1,3,8-triazaspiro[4.5]-decan-4-one (43) (Procedure E). HPLC: t_R = 2.61 min. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.56 (d, J = 13.2 Hz, 2H), 2.45−2.50 (m, 2H), 2.71−2.73 (d, J = 7.8 Hz, 4H), 3.55 (s, 2H), 4.56 (s, 2H), 6.76 (t, J = 7.3 Hz, 1H), 6.82 (d, J = 8.2 Hz, 2H), 6.88−6.92 (m, 1H), 7.01−7.04 (m, 2H), 7.08−7.17 (m, 2H), 7.21 (T, J = 7.5 Hz, 2H), 7.35−7.48 (m, 4H), 8.65 (s, 1H, N*H*CO). ^{13}C NMR (DMSO-d_6, 300 MHz): δ 29.1, 50.0, 58.8, 59.5, 62.3, 115.0, 117.8, 119.1, 119.5, 119.7, 124.4, 125.5, 128.8, 130.7, 130.9, 144.1, 157.4, 157.7, 177.0. MS (APCI+): *m/z* 414 [M + H]$^+$.

3-(Carboxymethyl)-4-oxo-1-phenyl-1,3,8-triazaspiro-[4.5]decane-8-carboxylic Acid (6-Chlorobenzo[1,3]dioxol-5-yl)methyl Ester (44). A suspension of 3-[4-oxo-1-phenyl-8-(*tert*-butyloxycarbonyl)-1,3,8-triazaspiro[4.5]decyl]acetic acid (3.9 g, 10 mmol), THF (30 mL), HCl (1 N, in Et_2O) (20 mL, 20 mmol, 2 equiv), and H_2SO_4 (560 μL) was stirred overnight at 65 °C. The reaction mixture was then evaporated under reduced pressure. The residue was triturated with AcOEt (50 mL) and Et_2O (2 × 50 mL) to afford 3.87 g (100%) of a gray powder of 3-(4-oxo-1-phenyl-1,3,8-triazaspiro[4.5]decyl)acetic acid as its sulfuric acid salt. The product was used without further purification. HPLC: t_R = 1.45 min. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.77 (d, J = 14.3 Hz, 2H), 2.71 (dt, J = 4.8 Hz, J' = 14.2 Hz, 2H), 3.29−3.31 (m, 2H), 3.47−3.55 (m, 2H), 4.10 (s, 2H), 4.70 (s, 2H), 6.81 (t, J = 7.3 Hz, 1H), 6.95 (d, J = 8.2 Hz, 2H), 7.25 (t, J = 7.6 Hz, 2H), 8.75 (br s, 2H, NH_2^+). MS (APCI+): *m/z* 290 [M + H of free base]$^+$. The title compound was then prepared from this intermediate according to procedure A. HPLC: t_R = 2.82 min and t_R' = 14.04 min. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.60 (d, J = 13.6 Hz, 2H), 2.32 (m, 2H), 3.35 (l s, 2H), 3.90 (dd, 2H), 4.07 (s, 2H), 4.66 (s, 2H), 5.03 (l d, J = 18 Hz, 2H), 6.04 (s, 2H), 6.67 (d, J = 8.15 Hz, 2H), 6.76 (t, J = 7.3 Hz, 1H), 7.05 (s, 1H), 7.09 (s, 1H), 7.17 (t, J = 7.6 Hz, 2H), 9.04 (s, 1H, COO*H*). ^{13}C NMR (DMSO-d_6, 300 MHz): δ 30, 41.8, 43.6, 65.0, 65.7, 104.1, 111.3, 111.7, 116.2, 120, 130.7. MS (APCI+): *m/z* 502 [M + H]$^+$ and 458 [M − 44]$^+$.

Molecular Modeling. All computations were performed on desktop Silicon Graphics workstations using a proprietary software integrated into Cerius^2 environment thanks to the software developer kit (SDK) from Molecular Simulations Inc. (San Diego, CA).

Receptor Binding Assays. The affinities for the different receptors were determined using conventional in vitro receptor binding methodology. All incubations of the radioligand with membranes, and the studied compounds or the nonspecific product, were terminated by adding cold buffer followed by rapid filtration using a cell harvester from Packard. The bound radioactivity was counted using a Topcount from Packard, thanks to a scintillation liquid (Formula 989 or Microscint from Packard). Reference compounds were assayed in duplicate, using at least eight concentrations. All IC_{50} calculations were done using nonlinear regression. Primary screening of the 10560 compounds was done on membranes from rat cerebral cortex.[15] Compounds were tested at 10 μM in duplicate against [^{3}H]DAMGO (1 nM). Naloxone (1 μM) was used to determine nonspecific binding. Screening of the analogues (1 μM in duplicate) and determination of IC_{50} values (eight concentrations in duplicate) were done on human recombinant receptors, expressed in mammalian cells.[16] The radioligand and nonspecific ligand were, respectively, [^{3}H]DAMGO (0.5 nM) and naloxone (10 μM) for μ (h), [^{3}H]U69593 (0.5 nM) and U50488 (10 μM) for κ (h), and [^{3}H]DPDPE (0.5 nM) and naltrexone (10 μM) for δ (h).

Solubility Determination. A 10 mM solution of the compound in DMSO was diluted to 0.2 mM in PBS (pH 7.4) or to 0.2 mM in methanol. The solubility was obtained by comparison of the HPLC peaks areas.[17]

log *D* Determination. log *D* is the partition coefficient of the compounds between 1-octanol and PBS (pH 7.4). It was evaluated by LC/MS.[18]

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Supporting Information Available: NMR spectra, mass spectra, and HPLC chromatograms for all the compounds described in the Experimental Section. This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) In this paper, the word pharmacophore is used in a sense differing from the definition given by IUPAC (Wermuth, C.-G.; Ganellin, C. R.; Lindberg, P.; Mitscher, L. A. Glossary of terms used in Medicinal Chemistry (IUPAC recommendations 1997). *Annu. Rep. Med. Chem.* **1998**, *33*, 385−395). Pharmacophore refers here to the ensemble of all steric and electronic features of a given molecule, which could potentially ensure interactions with a biological target structure.

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