Synthesis and Biological Activity of *N*-Arylpiperazine-Modified Analogues of KN-62, a Potent Antagonist of the Purinergic $P2X_7$ Receptor

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The $P2X_7$ receptor is involved in several processes relevant to inflammation (cytokine release, NO generation, killing of intracellular pathogens, cytotoxicity); thus, it may be an appealing target for pharmacological intervention. The characterization of native and recombinant $P2X_7$ receptor continues to be hindered by the lack of specific and subtype-selective antagonists. However, a tyrosine derivative named KN-62 exhibits selective $P2X_7$ receptor-blocking properties. The present study was designed to evaluate the functional antagonistic properties of a novel series of KN-62-related compounds characterized by the presence of different phenyl-substituted piperazine moieties. Antagonistic activity of KN-62 derivatives was tested on HEK293 cells transduced with the human $P2X_7$ receptor and monocyte-derived human macrophages, a cell type well-known for the high level of expression of this receptor. The biological responses investigated were ATP-dependent Ca^{2+} influx across the plasma membrane, ethidium bromide uptake, and secretion of the cytokine interleukin-1β. KN-62 was characterized by the presence of a phenylpiperazine moiety, and the presence of a one-carbon linker between the piperazine nitrogen and the phenyl ring (compound **61**) increases the activity, while a two-carbon linker (compound **62**) decreases biological activity 10-fold. Also, the nature and the position of substituents on the phenyl ring tethered to the piperazine seemed to exert a fundamental influence on the biological activity. In the series of synthesized compounds, the presence of a fluorine in the para position gives the most potent compound (**63**), while the same atom in the ortho position reduces potency by 3-fold. When the *p*-fluorine was replaced in the same position with other halogens, such as chlorine (compound **64**) or iodine (compound **65**), the activity decreased dramatically. We then tested the activity of the four most potent KN-62 derivatives on ATP-stimulated secretion of IL-1β from monocyte-derived human macrophages, a key cell type in inflammation and innate immunity. Interestingly, compound **68** and **71** caused a complete inhibition of IL-1β release, while with KN-62, **63**, and **85**, there was a small residual cytokine secretion even at concentrations exceeding 100 nM. None of the compounds tested on IL-1β release had any effect on isolated CaMII kinase activity up to 20 μM (not shown).

Introduction

It has been increasingly recognized that extracellular adenosine 5′-triphosphate (ATP) acts as an extracellular messenger by stimulating purinergic P2 receptors, which are considered a promising new target for anti-inflammatory drug development.[1−4] P2 receptors are classified into two families comprising seven subtypes each: G-protein-coupled receptors named P2Y ($P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, and $P2Y_{13}$) and ligand-gated ion channels named P2X ($P2X_{1-7}$).[5−7] Both families are the focus of great attention because of their therapeutic potential. In particular, P2X receptors are under scrutiny as potential targets for the alleviation of pain ($P2X_3$), modulation of endocrine function and fertility ($P2X_4$ and $P2X_7$), treatment of cancer of the reproductive system ($P2X_7$), motor control ($P2X_2$), inflammation and immunity ($P2X_7$), apoptosis ($P2X_1$ and $P2X_7$), and cardiovascular pathology ($P2X_4$).[6,8−10] Unfortunately, characterization of native and recombinant P2 receptor continues to be hindered by the lack of specific and subtype-selective antagonists.

The $P2X_7$ receptor differs strikingly from the other members of the P2X family for its size, 595 amino acids, for the long (242 residues) C-terminal chain, and for its peculiar ability to form a nonselective plasma membrane pore of 3−5 nm size when exposed to sustained stimulation with ATP.[11−13] Opening of the $P2X_7$ pore allows plasma membrane fluxes of ions as well as of hydrophilic molecules with molecular mass up to 900 Da.[11,14−16] The physiological function of $P2X_7$ is as yet unknown; however, its high expression in immune cells, including microglia, macrophages, lymphocytes, and dendritic cells, where it mediates cytotoxic responses, cytochine release, and cell fusion, suggests that it may have an important role in immunomodulation.[17−19] In vivo and in vitro studies show that stimulation of the $P2X_7$ receptor in macrophages and microglial cells is one

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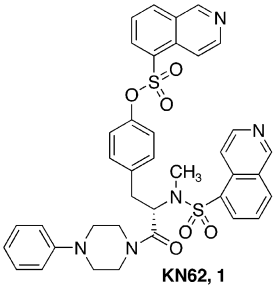
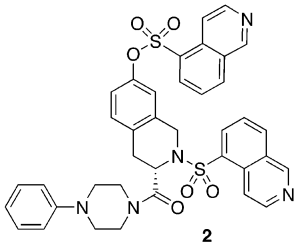
of the most potent stimuli for interleukin-1β (IL-1β) secretion in response to lipopolysaccharide (LPS) stimulation.[20,21] IL-1β is of prime importance in the induction of the immune responses, including facilitating responses to antigens and synthesis of prostaglandins, and in the pathogenesis of local and systemic inflammatory reaction (e.g., septic shock).

Human macrophages have proven to be very useful for the evaluation of $P2X_7$ agonists and antagonists, and among the latter, the KN-62 (1-(*N*,*O*-bis(1,5-isoquinolinesulfonyl)-*N*-methyl-L-tyrosyl)-4-phenylpiperazine) compound (**1**) is, to the best of our knowledge, the most potent antagonist for the human $P2X_7$ receptors with an IC_{50} of 51 nM and complete inhibition at 500 nM.[22−24]



KN62, 1



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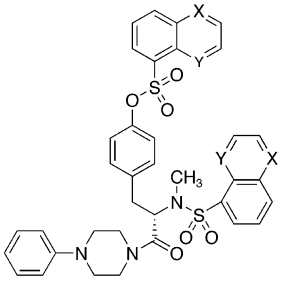
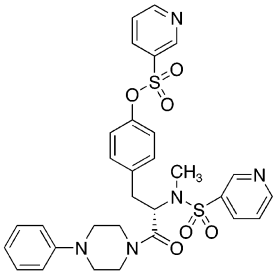


3, X=N, Y=CH
4, X=CH, Y=N
5, X=Y=CH



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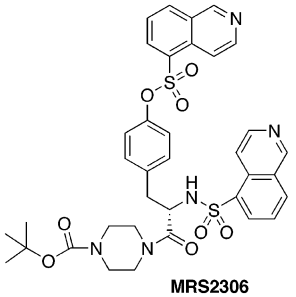


MRS2306

Recently, our group has studied the effect of conformational restriction of KN-62, by the synthesis of conformationally constrained KN-62 analogue, with formula **2**, where the tyrosine backbone was replaced with the 1,2,3,4-tetrahydroisoquinoline (TIC) moiety that can be considered as a "cyclic tyrosine".[25,26] In this way, a six-member ring was introduced to freeze the rotation of the $C_\alpha-C_\beta$ bond of the *N*-methyltyrosine. This ring-constrained *N*-methyltyrosine moiety (TIC) has been inserted in order to restrict conformational freedom and to stabilize the desired bioactive conformation of KN-62 and in an effort to examine the tolerability of the $P2X_7$ receptor to a rigid tyrosine replacement. Unfortunately, this constrained form of KN-62 completely lost the antagonist properties with respect to the parent compound, and this confirms the notion than an extended rather than folded conformation of tyrosine is preferred at the $P2X_7$ receptor.

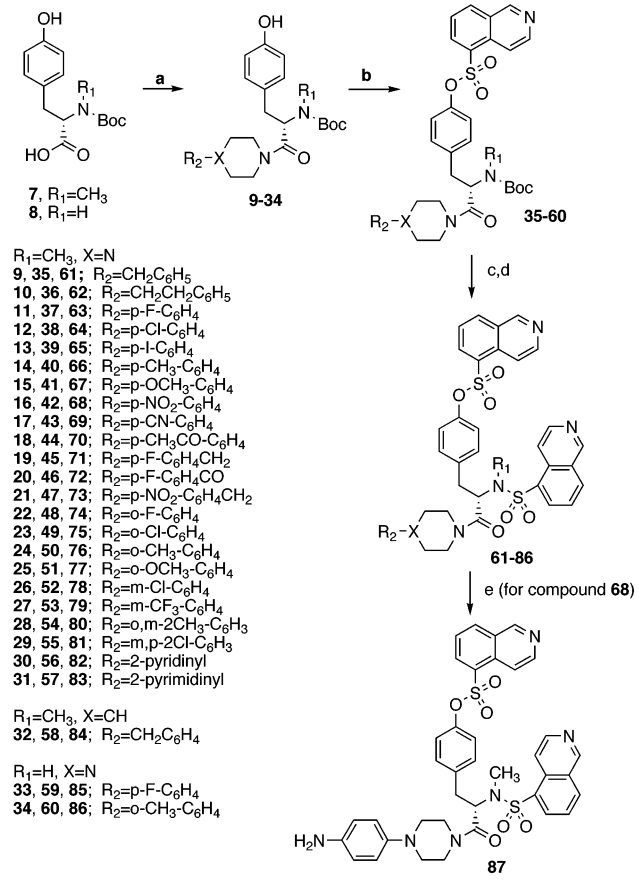
In the structure of KN-62, the importance of the presence of the isoquinoline-5-sulfonyl moieties, linked to the nitrogen and to the hydroxyl group of the *N*-methyltyrosine, for binding capacity to the $P2X_7$ receptor has been studied by the synthesis of a series of KN-62-analogues (compounds **3**−**6**) that possess different arylsulfonyl moieties. In these latter derivatives, the isoquinoline-5-sulfonyl function that characterizes KN-62 has been substituted, with its two isomers corresponding to quinoline-5- and quinoline-8-sulfonyl moieties (compounds **3** and **4**, respectively), with the naphthalene-1-sulfonyl group (compound **5**), and finally with a 3-pyridinesulfonyl moiety (compound **6**) (unpublished results). Unfortunately, neither the compounds **3**−**5** nor the derivative **6** are able to maintain the same antagonist activity as the parent compound KN-62, and this means that the isoquinoline-5-sulfonyl moiety is essential for the activity. In addition, on the lead compound KN-62, the presence of the methyl on the α-nitrogen is not essential for the activity. In fact, its removal is well tolerated without loss of antagonism.[27] In a study in which three positions of KN-62 have been systematically modified corresponding to the α-nitrogen and the hydroxyl function of the tyrosine moiety, along with the phenylpiperazine group, Jacobson et al. have identified a compound named MRS 2306 (1-(*N*,*O*-bis-(1,5-isoquinolinesulfonyl)-*N*-L-tyrosyl)-4-*tert*-butyloxycarbonylpiperazine) that was slightly more potent than the reference compound KN-62.[27]

In the present article, we have focused our attention on the modification of the phenylpiperazine residue. We have performed a systematic structure−activity analysis by examining the effects due to the insertion of different chemical functionalities on the phenyl ring linked to the piperazine nitrogen. These modifications will alter the electronic, steric, and lipophilic/hydrophilic features of the aromatic portion joined to the piperazine, and we hope to obtain compounds more potent than KN-62.

Chemistry

Compounds **61**−**87** were prepared using (L)-*N*-Boc-*N*-methyltyrosine (**7**) and (L)-*N*−-Boc-tyrosine (**8**) as starting materials,[28] following the synthetic sequence highlighted in Scheme 1. These amino-protected precursors **7** and **8** were converted to the activated 1-hydroxy-1,2,3-benzotriazole (HOBt) esters with HOBt and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and were coupled with the appropriate *N*-arylpiperazines[29] to give the amides **9**−**32** and **33**, **34**, respectively, in good yields. Compounds **35**−**58** and **59**, **60** were prepared from the corresponding tyrosylphenol anion of **9**−**32** and **33**, **34**, respectively (generated in situ with sodium hydride), by treatment with a dichloromethane (CH_2Cl_2) solution of 5-isoquinolinesulfonyl chloride.[30,31] The protecting *tert*-butyloxycarbonyl (Boc) group in **35**−**60** was conveniently removed by the use of trifluoroacetic acid (TFA) in a CH_2Cl_2 solution and furnished the corresponding free amine that was then coupled with an excess of 5-isoquinolinesulfonyl chloride, providing the sulfonamides **61**−**86** in acceptable

Scheme 1[a]



[a] Reagents: (a) substituted aryl- or heteroarylpiperazine, HOBt (1.1 equiv), EDC (1.1 equiv), DMF, room temp, 24 h; (b) NaH, isoquinolinesulfonyle chloride, CH_2Cl_2, room temp, 18 h; (c) TFA, CH_2Cl_2, room temp; (d) TEA, isoquinolinesulfonyle chloride, CH_2Cl_2, room temp, 18 h; (e) H_2, 10% Pd/C, MeOH, 4 h.

yields. Compound **87** was obtained by a reductive hydrogenation of **68** in the presence of palladium on charcoal.

The structures of the synthesized compounds **61**−**87** and the yields of the syntheses are presented in Table 1. The range of yields was 10−88%.

Results and Discussion

The antagonist activity of KN-62 derivatives **61**−**87** modified on the arylpiperazine pharmacophore was investigated by the measurements of two ATP-dependent responses: ATP-stimulated Ca^{2+} influx into and ethidium bromide uptake by HEK293 cells transfected with the human $P2X_7$ receptor (HEK293−$P2X_7$). Since HEK293 cells lack endogenous P2X receptors, they are an ideal cell model for the expression of recombinant $P2X_7$ and for antagonist potency studies. Figure 1 shows a typical experiment in which changes in the intracellular Ca^{2+} concentration as a consequence of $P2X_7$ receptor activation were recorded in wild-type and $P2X_7$-transduced HEK293 cells.

The effect of one of the KN-62 derivatives synthesized (see below) is also shown. As expected for a true Ca^{2+} influx, the $P2X_7$-dependent increase in cytoplasmic Ca^{2+} was fully abolished in the absence of extracellular Ca^{2+} (not shown). Results of experiments similar to that shown in Figure 1 performed with a large number of compounds synthesized in our laboratory are summarized in Table 2. Compound **63** was the most active antagonist in this series, and several compounds (i.e., **61**, **66**, **68**, **71**, **73**−**76**, **78**, **79**, **81**, **85**, and **86**) showed an antagonistic activity more potent than KN-62.

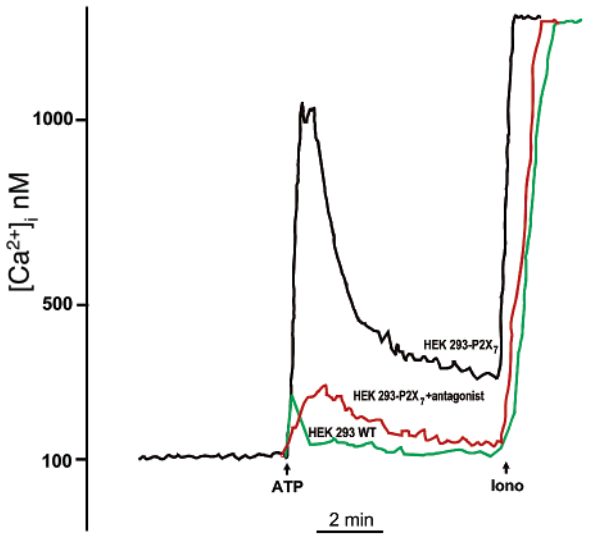


Figure 1. ATP-dependent cytoplasmic Ca^{2+} increases in wild-type and $P2X_7$-transduced HEK293 cells. Cells were resuspended in the fluorometer cuvette in the saline solution described in Materials and Methods at a concentration of 10^6 cells/mL. ATP was 1 mM, and ionomycin was 100 nM. The $P2X_7$ blocker, compound **71**, was added at a concentration of 50 nM 5 min prior to ATP. Black trace, HEK293−$P2X_7$; red trace, HEK293−$P2X_7$ treated with compound **71** prior to ATP; green trace, wild-type HEK293.

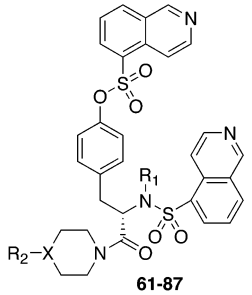
With the aim of investigating the importance of the distance between the piperazine basic nitrogen and the phenyl ring by the insertion of one and two methylene units, compounds **61** and **62** were synthesized and tested. For compound **61**, a methylene spacer slightly improves the activity, which was 2-fold higher than that of the reference compound KN-62, while for derivative **62** (one carbon longer than **61**) the presence of an ethyl spacer was detrimental to the antagonist potency, which was 10- and 30-fold lower than that reported for KN-62 and **61**, respectively. These data suggest that the optimal length of the linker contained one carbon atom, which is presumably important for the relative position of the phenyl ring in the space.

The presence of a basic nitrogen in the piperazine moiety was important for the activity. In fact, its removal by the substitution of the piperazine with a piperidine (compound **84**, the 4-benzylpiperidine analogue of **61**) reduced the activity by 3-fold (IC_{50} = 21.1 vs 65.3 nM for **61** vs **84**, respectively).

Results shown in the Table 1 indicate that the substitution of the phenyl ring with other six-member ring heterocycles with one nitrogen (pyridine, **82**) or two nitrogens (pyrimidine, **83**) results in a 1.5- to 3-fold reduction in activity, respectively.

For the compounds that possess different substituents on the phenyl ring in the para position, they showed variable potencies, where derivative **63**, characterized by the presence of a fluorine, was the most potent compounds of the whole series, being 30-fold more potent than KN-62. The other para-halogenated analogues, such as *p*-chloro (**64**) and *p*-iodo (**65**), showed lower potencies (from 2- to 3-fold) varying with the

Table 1. Physical and Synthetic Data of Novel KN-62 Analogues (**61**−**87**)



61-87

compd	R_1	R_2	X	mp, °C	yield,[a] %	formula[b]	anal.
61	CH_3	$CH_2C_6H_5$	N	92−94	35	$C_{39}H_{37}N_5O_6S_2$	C, H, N
62	CH_3	$CH_2CH_2C_6H_5$	N	98−100	25	$C_{40}H_{39}N_5O_6S_2$	C, H, N
63	CH_3	*p*-F−C_6H_4	N	110−112	38	$C_{38}H_{34}N_5O_6S_2F$	C, H, N
64	CH_3	*p*-Cl−C_6H_4	N	oil	61	$C_{38}H_{34}N_5O_6S_2Cl$	C, H, N
65	CH_3	*p*-I−C_6H_4	N	95−97	44	$C_{38}H_{34}N_5O_6S_2I$	C, H, N
66	CH_3	*p*-CH_3−C_6H_4	N	70−72	58	$C_{39}H_{37}N_5O_6S_2$	C, H, N
67	CH_3	*p*-OCH_3−C_6H_4	N	85−87	47	$C_{39}H_{37}N_5O_7S_2$	C, H, N
68	CH_3	*p*-NO_2−C_6H_4	N	77−79	76	$C_{38}H_{34}N_6O_8S_2$	C, H, N
69	CH_3	*p*-CN−C_6H_4	N	124−127	58	$C_{39}H_{34}N_6O_6S_2$	C, H, N
70	CH_3	*p*-CH_3CO−C_6H_4	N	82−84	43	$C_{40}H_{37}N_5O_7S_2$	C, H, N
71	CH_3	*p*-F−$C_6H_4CH_2$	N	103−105	10	$C_{39}H_{36}N_5O_6S_2F$	C, H, N
72	CH_3	*p*-F−C_6H_4CO	N	124−126	18	$C_{39}H_{34}N_5O_7S_2F$	C, H, N
73	CH_3	*p*-NO_2−$C_6H_4CH_2$	N	145−147	40	$C_{39}H_{36}N_6O_8S_2$	C, H, N
74	CH_3	*o*-F−C_6H_4	N	133−135	76	$C_{38}H_{34}N_5O_6S_2F$	C, H, N
75	CH_3	*o*-Cl−C_6H_4	N	84−86	58	$C_{38}H_{34}N_5O_6S_2Cl$	C, H, N
76	CH_3	*o*-CH_3−C_6H_4	N	84−86	58	$C_{39}H_{37}N_5O_6S_2$	C, H, N
77	CH_3	*o*-OCH_3−C_6H_4	N	85−87	10	$C_{39}H_{37}N_5O_7S_2$	C, H, N
78	CH_3	*m*-Cl−C_6H_4	N	138−140	67	$C_{38}H_{34}N_5O_6S_2Cl$	C, H, N
79	CH_3	*m*-CF_3−C_6H_4	N	81−83	20	$C_{39}H_{34}N_5O_6S_2F_3$	C, H, N
80	CH_3	*o,m*-$2CH_3$−C_6H_3	N	174−176	21	$C_{40}H_{39}N_5O_6S_2$	C, H, N
81	CH_3	*m,p*-2Cl−C_6H_3	N	130−132	64	$C_{38}H_{33}N_5O_6S_2Cl_2$	C, H, N
82	CH_3	2-pyridinyl	N	183−185	48	$C_{37}H_{34}N_6O_6S_2$	C, H, N
83	CH_3	2-pyrimidinyl	N	187−189	32	$C_{36}H_{33}N_7O_6S_2$	C, H, N
84	CH_3	$CH_2C_6H_5$	CH	131−133	35	$C_{40}H_{38}N_4O_6S_2$	C, H, N
85	H	*p*-F−C_6H_4	N	145−147	50	$C_{37}H_{32}N_5O_6S_2F$	C, H, N
86	H	*o*-CH_3−C_6H_4	N	132−134	88	$C_{38}H_{35}N_5O_6S_2$	C, H, N
87	CH_3	*p*-NH_2−C_6H_4	N	80−82	45	$C_{38}H_{36}N_6O_6S_2$	C, H, N

[a] Yield of synthesized compounds after purification by column chromatography. [b] All compounds were analyzed for C, H, N. Analytical results were within 0.4% of theoretical values.

Table 2. Activities of Synthesized Compounds **61**−**87** and KN-62 on the Calcium Influx in Human Monocytes

compd	IC_{50}[a] ± SE (nM)	compd	IC_{50}[a] ± SE (nM)
KN-62 (**1**)	51.1 ± 1.1	**74**	18.5 ± 0.4
61	21.1 ± 0.8	**75**	15.8 ± 0.9
62	600 ± 27	**76**	15.1 ± 1.4
63	1.3 ± 0.1	**77**	84.1 ± 5
64	105 ± 1.1	**78**	14.1 ± 0.3
65	176 ± 3.8	**79**	31.1 ± 1.2
66	13.5 ± 0.2	**80**	1122 ± 25
67	132 ± 4.3	**81**	33.9 ± 0.8
68	5.8 ± 0.6	**82**	170 ± 3.9
69	97.7 ± 2.2	**83**	79.8 ± 0.7
70	70.8 ± 0.8	**84**	65.3 ± 0.8
71	5.8 ± 0.5	**85**	6.0 ± 0.3
72	380 ± 8.8	**86**	28.8 ± 0.3
73	12.3 ± 0.3	**87**	101 ± 1.3

[a] IC_{50} = 50% inhibitory concentration, which represents the mean from dose−response curves of at least three experiments. All experiments were repeated three times.

increasing size of the halogen atom. Starting from compound **64**, placement of chloro in the meta (compound **78**) or ortho (compound **75**) position led to a 6-fold increase of potency. The same derivatives **75** and **78** were also 3-fold more potent than KN-62. In the same compound **64**, the introduction of a second chloro atom in the meta position (compound **81**) led to a 3-fold increase of activity.

For the most potent compound in this series (derivative **63**), placement of fluorine in the meta position (compound **74**) resulted in a 14-fold decrease in potency. The same effect was not observed for derivatives **66** and **76**, which possess methyl groups in the para and ortho positions, respectively, and showed the same potency.

A potency increase was also observed with compounds **66** and **68**, containing nitro and methyl groups in the para position of the phenyl ring, which are 4- and 10-fold more active than KN-62, respectively. Addition of electron-withdrawing groups, such as nitrile (**69**) and acetyl (**70**), and electron-donating groups, such as methoxy (**67**) and amino (**87**), results in a decrease in potency (from 2- to 3-fold). In derivative **67**, when the methoxy group was placed in the ortho position (compound **77**), a 2-fold increase in potency was observed.

These results support the suggestion that the phenylpiperazine moiety interacts with the active site of the receptor and indicate that the compound/receptor interaction can be improved by the presence of adequate chemical groups in the para position of the phenyl ring

For the compound **80**, the dramatic reduction of activity due to the insertion of a second methyl group in the phenyl ring could be a result of the steric bulkiness of the *o,m*-xylyl residue, which may prevent interaction with the receptor.

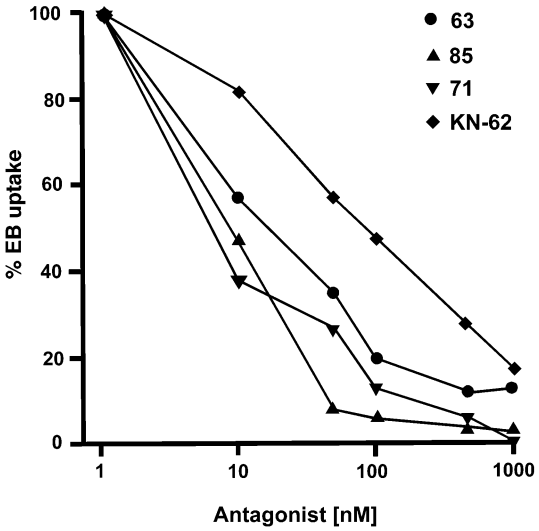


Figure 2. Effect of KN-62 and its derivatives on ethidium bromide uptake. P_2X_7-transduced HEK293 cells were incubated in a fluorometer cuvette in a modified (no Ca^{2+}, 500 mM EGTA added) standard saline solution (see Materials and Methods). The $P2X_7$ antagonists were added 5 min before ATP. Uptake inhibition is expressed as a percentage of maximal ethidium bromide (EB) uptake rate (arbitrary fluorescence units per min) measured in the absence of inhibitor. Experimental points are averages of triplicate determinations.

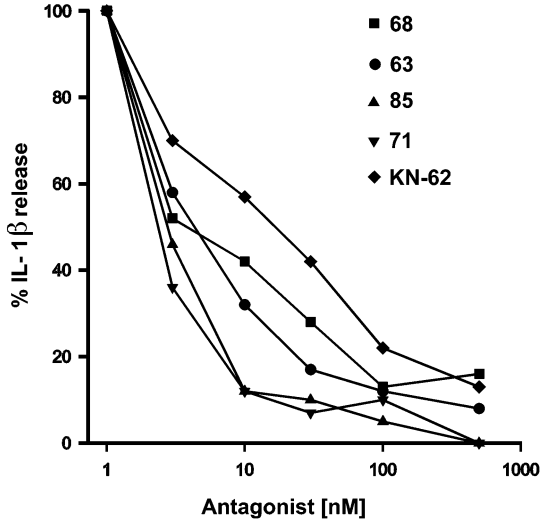


Figure 3. Dose-dependent inhibition of ATP-dependent IL-1β release by KN-62 and its derivatives. Cells were incubated in serum-free RPMI, pretreated with LPS, and stimulated with ATP as described in Materials and Methods. The $P2X_7$ antagonists were added 5 min before ATP. Supernatants were withdrawn 30 min after ATP addition and assayed for IL-1β content. Experimental points are averages of quadruplicate determinations.

The introduction of a methylene spacer separating the piperazine nitrogen and the *p*-fluorinephenyl ring furnished a compound (**71**) that was 4-fold less active than the parent compound **63**. The same effect was observed comparing the activities of **68** and **73**, where the insertion of a methylene unit decreases the potency by 2-fold.

From a comparison of the activities of compounds **63** and **65** with that of **76** and **86**, respectively, a slight change in potency was observed by removal of the methyl on the nitrogen on the α-position of the tyrosine moiety, and this confirms that the presence of this substituent was not fundamental for antagonistic activity.

In conclusion, the structure−activity relationships (SAR) showed that the basicity of the piperazine nitrogen linked to the phenyl-substituted ring was essential for activity (compounds **63** and **71** vs **72**). This is confirmed by replacement of the piperazine moiety with a piperidine (**61** vs **84**), which led to a compound with reduced activity. The most potent $P2X_7$ receptor antagonist identified in this study (compound **63**) contained a *p*-fluorine substituent on the phenylpiperazine moiety. Figure 2 shows the effect of three of the most potent compounds (**63**, **71**, and **85**) on ethidium bromide uptake, a reliable index of activation of the P_2X_7 receptor/pore.

We then tested the activity of the four most potent KN-62 derivatives on ATP-stimulated secretion of IL-1β from monocyte-derived human macrophages, a key cell type in inflammation and native immunity. Figure 3 shows that the KN-62 IC_{50} for IL-1β was lower than that for Ca influx (20 vs 51 nM). Two of the compounds tested, **71** and **85**, had an IC_{50} lower than KN-62 (3.0 and 2.5 nM, respectively).

Interestingly, **71** and **85** caused a complete inhibition of IL-1β release, while with KN-62, **63**, and **68**, there was a small residual cytokine secretion even at concentrations exceeding 100 nM. Incubation of macrophages with the KN-62 derivatives throughout the IL-1β release experiments had no detrimental effects on cell viability, as judged by the lack of morphological alterations (shrinking or swelling), loss of adherence, or release of cytosolic enzymes (not shown), thus indicating that these compounds are good tools for the investigation of inflammatory cell functions in vitro. None of the compounds tested on IL-1β release had any effect on isolated CaMII kinase activity up to 20 μM (not shown).

Experimental Section

Chemical Materials and Methods. General Procedure. All reactions were carried out under an inert atmosphere of dry nitrogen unless otherwise described. Standard hypodermic syringe (glass/metal Luer) techniques were applied for transferring dry solvents. Starting materials were purchased and used without any purification. Reaction courses and product mixtures were routinely monitored by TLC on silica gel (precoated F_{254} Macherey−Nagel plates) and visualized with aqueous $KMnO_4$. Melting points (mp) were determined on a Buchi-Tottoli apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Chemical shifts (δ) are given in ppm upfield from tetramethylsilane. All products reported showed ^{1}H NMR spectra in agreement with the assigned structures. ^{1}H NMR was determined in $CDCl_3$ solution with a Bruker AC200 spectrometer. Microanalytical analyses were conducted by the Mycroanalytical Laboratory of the Chemistry Department of the University of Ferrara. Organic solutions were dried over anhydrous Na_2SO_4. Methanol was distilled from magnesium turnings, dioxan was distilled from calcium hydride, and dry DMF was distilled from calcium chloride and stored over molecular sieves (3 Å). In high-pressure hydrogenation experiments, a Parr shaker on a high-pressure autoclave was used.

General Procedure A for the Synthesis of Compounds 9−34. To a solution of **7a** or **7b** (1 mmol) in dry DMF (5 mL) cooled to 0 °C was added EDC (211 mg, 1.1 mmol, 1.1 equiv), HOBt (1.1 mmol), and a suitable N-substituted piperazine (1.1 mmol).[29] This mixture was stirred for 24 h and then concen-

trated in vacuo. The residue was dissolved in EtOAc (10 mL) and was washed with water (5 mL) and then with brine (5 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue purified by column chromatography using EtOAc/petroleum ether (1:1, v/v) as eluent furnished the derivatives **9**−**34**.

1-[(*S*)-*N*-*tert*-Butyloxycarbonyl-*N*-methyltyrosyl]-4-benzylpiperazine (9). Following general procedure A, this product was obtained as a white solid (yield 98%): mp 104−106 °C, [α] = −63.7, *c* = 0.91% in $CHCl_3$. ^{1}H NMR ($CDCl_3$) *δ*: 1.36 (s, 9H), 2.28 (m, 4H), 2.79 (s, 3H), 2.83 (s, 2H), 2.89 (dd, *J* = 16.7 and 7.3 Hz, 2H), 3.49 (m, 4H), 5.21 (t, *J* = 7.3 Hz, 1H), 5.85 (s, 1H), 6.72 (d, *J* = 8.3 Hz, 2H), 7.05 (d, *J* = 8.3 Hz, 2H), 7.20 (m, 5H).

1-[(*S*)-*N*-*tert*-Butyloxycarbonyl-*N*-methyltyrosyl]-4-phenethylpiperazine (10). Following general procedure A, this product was obtained as a white solid (yield 46%): mp 147−150 °C; [α] = −46.8, *c* = 1.5% in CH_2Cl_2. ^{1}H NMR ($CDCl_3$) *δ*: 1.37 (s, 9H), 2.38 (m, 4H), 2.58 (m, 2H), 2.75 (m, 2H), 2.81 (s, 3H), 2.89 (dd, *J* = 14.1 and 8.3 Hz, 2H), 3.49 (m, 4H), 5.21 (t, *J* = 8.3 Hz, 1H), 5.49 (s, 1H), 6.70 (d, *J* = 8.3 Hz, 2H), 7.00 (d, *J* = 8.3 Hz, 2H), 7.23 (m, 5H).

1-[(*S*)-*N*-*tert*-Butyloxycarbonyl-*N*-methyltyrosyl]-4-(4-fluorophenyl)piperazine (11). Following general procedure A, this product was obtained as a white solid (yield 94%): mp 67−69 °C; [α] = −85.4, *c* = 1.29% in $CHCl_3$. ^{1}H NMR ($CDCl_3$) *δ*: 1.34 (s, 9H), 2.78 (s, 3H), 2.89 (dd, *J* = 16.7 and 7.3 Hz, 2H), 3.07 (m, 4H), 3.50 (m, 4H), 5.21 (t, *J* = 7.3 Hz, 1H), 5.85 (s, 1H), 6.86 (m, 8H).

1-[(*S*)-*N*-*tert*-Butyloxycarbonyl-*N*-methyltyrosyl]-4-(4-chlorophenyl)piperazine (12). Following general procedure A, this product was obtained as a white solid (yield 78%): mp 73−75 °C; [α] = −77, *c* = 0.76% in $CHCl_3$. ^{1}H NMR ($CDCl_3$) *δ*: 1.39 (s, 9H), 1.56 (m, 2H), 2.89 (s, 3H), 2.95 (m, 4H), 3.54 (m, 4H), 5.25 (t, *J* = 7.2 Hz, 1H), 6.78 (m, 3H), 7.02 (d, *J* = 8.4 Hz, 1H), 7.09 (d, *J* = 8.2 Hz, 2H), 7.22 (m, 2H), 9.46 (s, 1H).

1-[(*S*)-*N*-*tert*-Butyloxycarbonyl-*N*-methyltyrosyl]-4-(4-iodophenyl)piperazine (13). Following general procedure A, this product was obtained as a yellow solid (yield 92%): mp 73−75 °C; [α] = −65.4, *c* = 1.02% in $CHCl_3$. ^{1}H NMR ($CDCl_3$) *δ*: 1.38 (s, 9H), 2.82 (s, 3H), 2.93 (dd, *J* = 14.6 and 7.2 Hz, 2H), 3.12 (m, 4H), 3.51 (m, 4H), 5.24 (t, *J* = 7.2 Hz, 1H), 5.82 (s, 1H), 6.61 (d, *J* = 8.8 Hz, 2H), 6.72 (d, *J* = 8.4 Hz, 2H), 7.10 (d, *J* = 8.4 Hz, 2H), 7.52 (d, *J* = 8.8 Hz, 2H).

1-[(*S*)-*N*-*tert*-Butyloxycarbonyl-*N*-methyltyrosyl]-4-(*p*-tolyl)piperazine (14). Following general procedure A, this product was obtained as an oil (yield 93%): [α] = −83.3, *c* = 1.34% in $CHCl_3$. ^{1}H NMR ($CDCl_3$) *δ*: 1.38 (s, 9H), 2.27 (s, 3H), 2.81 (s, 3H), 2.86 (dd, *J* = 14 and 7.6 Hz, 2H), 3.42 (m, 4H), 3.54 (m, 4H), 5.26 (t, *J* = 7 Hz, 1H), 6.80 (m, 4H), 7.06 (m, 4H), 9.5 (s, 1H).

1-[(*S*)-*N*-*tert*-Butyloxycarbonyl-*N*-methyltyrosyl]-4-(4-methoxyphenyl)piperazine (15). Following general procedure A, this product was obtained as a foamy yellow solid (yield 89%): [α] = −63.3, *c* = 1.1% in $CHCl_3$. ^{1}H NMR ($CDCl_3$) *δ*: 1.38 (s, 9H), 2.82 (s, 3H), 2.87 (m, 6H), 3.52 (m, 4H), 3.77 (s, 3H), 5.26 (t, *J* = 7.2 Hz, 1H), 6.71 (d, *J* = 8.4 Hz, 2H), 6.84 (m, 2H), 7.02 (d, *J* = 8.6 Hz, 2H), 7.10 (d, *J* = 8.6 Hz, 2H), 9.46 (s, 1H).

1-[(*S*)-*N*-*tert*-Butyloxycarbonyl-*N*-methyltyrosyl]-4-(4-nitrophenyl)piperazine (16). Following general procedure A, this product was obtained as a yellow solid (yield 88%): mp 211−213 °C; [α] = −66.7, *c* = 1.02% in $CHCl_3$. ^{1}H NMR ($CDCl_3$) *δ*: 1.39 (s, 9H), 2.74 (dd, *J* = 14 and 7.6 Hz, 2H), 2.84 (s, 3H), 3.34 (m, 4H), 3.56 (m, 4H), 5.25 (t, *J* = 7.2 Hz, 1H), 6.71 (d, *J* = 3.4 Hz, 2H), 6.76 (d, *J* = 3.4 Hz, 2H), 7.12 (d, *J* = 8.4 Hz, 2H), 8.12 (d, *J* = 9.2 Hz, 2H), 9.5 (s, 1H).

1-[(*S*)-*N*-*tert*-Butyloxycarbonyl-*N*-methyltyrosyl]-4-(4-cyanophenyl)piperazine (17). Following general procedure A, this product was obtained as an oil (yield 76%): [α] = −49.7, *c* = 1.47% in $CHCl_3$. ^{1}H NMR ($CDCl_3$) *δ*: 1.39 (s, 9H), 1.56 (m, 2H), 2.84 (s, 3H), 3.10 (m, 4H), 3.52 (m, 4H), 5.31 (t, *J* = 7.2 Hz, 1H), 6.73 (d, *J* = 8.6 Hz, 2H), 6.79 (d, *J* = 9.2 Hz, 2H), 7.11 (d, *J* = 8.2 Hz, 2H), 7.48 (d, *J* = 8.8 Hz, 2H), 9.46 (s, 1H).

1-[(*S*)-*N*-*tert*-Butyloxycarbonyl-*N*-methyltyrosyl]-4-(4-acetylphenyl)piperazine (18). Following general procedure A, this product was obtained as a yellow solid (yield 67%): mp 123−125 °C; [α] = −36.2, *c* = 0.5% in $CHCl_3$. ^{1}H NMR ($CDCl_3$) *δ*: 1.34 (s, 9H), 2.53 (s, 3H), 2.84 (s, 3H), 2.93 (dd, *J* = 14.8 and 7.3 Hz, 2H), 3.42 (m, 4H), 3.54 (m, 4H), 5.26 (t, *J* = 7.3 Hz, 1H), 5.91 (s, 1H), 6.77 (dd, *J* = 8.0 and 7.8 Hz, 4H), 7.10 (d, *J* = 8.0 Hz, 2H), 7.87 (d, *J* = 8.0 Hz, 2H).

1-[(*S*)-*N*-*tert*-Butyloxycarbonyl-*N*-methyltyrosyl]-4-(4-fluorobenzyl)piperazine (19). Following general procedure A, this product was obtained as a brown oil (yield 78%): [α] = −62.2, *c* = 0.49% in $CHCl_3$. ^{1}H NMR ($CDCl_3$) *δ*: 1.37 (s, 9H), 2.37 (m, 4H), 2.84 (s, 5H), 2.94 (m, 1H), 3.11 (m, 1H), 3.53 (m, 4H), 4.57 (m, 1H), 5.16 (t, *J* = 7.3 Hz, 1H), 6.78 (d, *J* = 8.1 Hz, 2H), 7.00 (dd, *J* = 8.2 and 8.1 Hz, 4H), 7.21 (d, *J* = 8.2 Hz, 2H).

1-[(*S*)-*N*-*tert*-Butyloxycarbonyl-*N*-methyltyrosyl]-4-(4-fluorobenzoyl)piperazine (20). Following general procedure A, this product was obtained as a white oil (yield 97%): [α] = −10.6, *c* = 1.18% in CH_2Cl_2. ^{1}H NMR ($CDCl_3$) *δ*: 1.38 (s, 9H), 2.85 (s, 3H), 3.00 (m, 2H), 3.50 (m, 8H), 4.57 (m, 1H), 5.16 (m, 1H), 6.73 (d, *J* = 8.3 Hz, 2H), 7.08 (m, 4H), 7.38 (d, *J* = 8.2 Hz, 2H).

1-[(*S*)-*N*-*tert*-Butyloxycarbonyl-*N*-methyltyrosyl]-4-(4-nitrobenzyl)piperazine (21). Following general procedure A, this product was obtained as a brown oil (yield 78%): [α] = −30, *c* = 0.46% in $CHCl_3$. ^{1}H NMR ($CDCl_3$) *δ*: 1.35 (s, 9H), 2.35 (m, 4H), 2.82 (s, 2H), 2.89 (m, 2H), 2.96 (s, 3H), 3.51 (m, 4H), 4.88 (m, 1H), 5.19 (m, 1H), 6.75 (d, *J* = 8.5 Hz, 2H), 7.05 (d, *J* = 8.5 Hz, 2H), 7.46 (d, *J* = 8.3 Hz, 2H), 8.14 (d, *J* = 8.3 Hz, 2H).

1-[(*S*)-*N*-*tert*-Butyloxycarbonyl-*N*-methyltyrosyl]-4-(1-fluorophenyl)piperazine (22). Following general procedure A, this product was obtained as a white solid (yield 78%): mp 73−75 °C; [α] = −77, *c* = 0.76% in $CHCl_3$. ^{1}H NMR ($CDCl_3$) *δ*: 1.38 (s, 9H), 2.84 (s, 3H), 2.94 (m, 4H), 3.11 (dd, *J* = 13.8 and 7.6 Hz, 2H), 3.58 (m, 4H), 5.26 (t, *J* = 7.2 Hz, 1H), 6.76 (m, 3H), 7.01 (m, 5H), 9.46 (s, 1H).

1-[(*S*)-*N*-*tert*-Butyloxycarbonyl-*N*-methyltyrosyl]-4-(2-chlorophenyl)piperazine (23). Following general procedure A, this product was obtained as a white solid (yield 98%): mp 70−72 °C; [α] = −70.3, *c* = 1.15% in $CHCl_3$. ^{1}H NMR ($CDCl_3$) *δ*: 1.38 (s, 9H), 2.84 (s, 3H), 2.91 (m, 6H), 3.71 (m, 4H), 5.25 (t, *J* = 7.2 Hz, 1H), 5.82 (s, 1H), 6.74 (m, 2H), 7.04 (m, 5H), 7.34 (d, *J* = 9.2 Hz, 1H).

1-[(*S*)-*N*-*tert*-Butyloxycarbonyl-*N*-methyltyrosyl]-4-*o*-tolylpiperazine (24). Following general procedure A, this product was obtained as a white solid (yield 95%): mp 80−82 °C; [α] = −66.7, *c* = 1.9% in $CHCl_3$. ^{1}H NMR ($CDCl_3$) *δ*: 1.38 (s, 9H), 2.28 (s, 3H), 2.74 (m, 6H), 2.86 (s, 3H), 3.65 (m, 4H), 5.26 (t, *J* = 7.2 Hz, 1H), 6.01 (s, 1H), 6.74 (m, 2H), 7.10 (m, 6H).

1-[(*S*)-*N*-*tert*-Butyloxycarbonyl-*N*-methyltyrosyl]-4-(2-methoxyphenyl)piperazine (25). Following general procedure A, this product was obtained as a white solid (yield 97%): mp 153−155 °C; [α] = −126.9, *c* = 0.93% in $CHCl_3$. ^{1}H NMR ($CDCl_3$) *δ*: 1.38 (s, 9H), 2.83 (s, 3H), 2.93 (m, 6H), 3.64 (m, 4H), 3.86 (s, 3H), 5.27 (t, *J* = 7.2 Hz, 1H), 5.82 (s, 1H), 6.72 (m, 3H), 6.89 (m, 2H), 7.06 (m, 3H).

1-[(*S*)-*N*-*tert*-Butyloxycarbonyl-*N*-methyltyrosyl]-4-(3-chlorophenyl)piperazine (26). Following general procedure A, this product was obtained as a yellow solid (yield 93%): mp 60−62 °C; [α] = −72, *c* = 0.88% in $CHCl_3$. ^{1}H NMR ($CDCl_3$) *δ*: 1.36 (s, 9H), 2.83 (s, 3H), 2.99 (m, 6H), 3.52 (m, 4H), 5.26 (t, *J* = 7.2 Hz, 1H), 6.76 (m, 4H), 7.12 (m, 4H), 9.46 (s, 1H).

1-[(*S*)-*N*-*tert*-Butyloxycarbonyl-*N*-methyltyrosyl]-4-(3-trifluoromethylphenyl)piperazine (27). Following general procedure A, this product was obtained as a yellow solid (yield 78%): mp 102−104 °C; [α] = −63.4, *c* = 0.8% in $CHCl_3$. ^{1}H NMR ($CDCl_3$) *δ*: 1.39 (s, 9H), 2.83 (s, 3H), 3.04 (m, 6H), 3.56 (m, 4H), 5.25 (t, *J* = 7.2 Hz, 1H), 5.82 (s, 1H), 6.73 (d, *J* = 8.3 Hz, 2H), 7.02 (m, 3H), 7.10 (d, *J* = 8.3 Hz, 2H), 7.35 (m, 1H).

1-[(*S*)-*N*-*tert*-Butyloxycarbonyl-*N*-methyltyrosyl]-4-(2,3-dimethylphenyl)piperazine (28). Following general proce-

dure A, this product was obtained as an oil (yield 91%): [α] = −20.3, *c* = 0.7% in CH_2Cl_2. ^{1}H NMR ($CDCl_3$) *δ*: 1.44 (s, 9H), 2.18 (s, 3H), 2.26 (s, 3H), 2.85 (s, 3H), 2.93 (m, 6H), 3.65 (m, 4H), 5.25 (t, *J* = 7.2 Hz, 1H), 5.82 (s, 1H), 6.76 (m, 3H), 6.94 (m, 1H), 7.06 (m, 3H).

1-[(*S*)-*N*-*tert*-Butyloxycarbonyl-*N*-methyltyrosyl]-4-(3,4-dichlorophenyl)piperazine (29). Following general procedure A, this product was obtained as a white solid (yield 85%): mp 77−78 °C; [α] = −71.4, *c* = 0.42% in $CHCl_3$. ^{1}H NMR ($CDCl_3$) *δ*: 1.39 (s, 9H), 2.84 (s, 3H), 2.99 (m, 6H), 3.56 (m, 4H), 5.25 (t, *J* = 7.2 Hz, 1H), 6.75 (m, 2H), 6.88 (m, 1H), 7.08 (m, 2H), 7.26 (m, 2H), 9.46 (s, 1H).

1-[(*S*)-*N*-*tert*-Butyloxycarbonyl-*N*-methyltyrosyl]-4-pyridin-2-ylpiperazine (30). Following general procedure A, this product was obtained as a white oil (yield 89%): [α] = −57.1, *c* = 0.7% in CH_2Cl_2. ^{1}H NMR ($CDCl_3$) *δ*: 1.39 (s, 9H), 2.83 (s, 3H), 2.97 (d, *J* = 7.6 Hz, 1H), 3.50 (m, 9H), 4.95 (m, 1H), 5.26 (t, *J* = 7.5 Hz, 1H), 6.66 (m, 4H), 7.01 (d, *J* = 8.2 Hz, 1H), 7.08 (d, *J* = 8.4 Hz, 1H), 7.49 (m, 1H), 8.18 (m, 1H).

1-[(*S*)-*N*-*tert*-Butyloxycarbonyl-*N*-methyltyrosyl]-4-pyrimidin-2-ylpiperazine (31). Following general procedure A, this product was obtained as a white solid (yield 91%): mp 109−111 °C; [α] = −54.5, *c* = 0.27% in CH_2Cl_2. ^{1}H NMR ($CDCl_3$) *δ*: 1.38 (s, 9H), 2.83 (s, 3H), 3.02 (m, 2H), 3.53 (m, 5H), 3.88 (m, 3H), 4.92 (m, 1H), 5.26 (t, *J* = 7.5 Hz, 1H), 6.53 (m, 1H), 6.72 (m, 2H), 7.04 (d, *J* = 7.8 Hz, 1H), 7.11 (d, *J* = 7.8 Hz, 1H), 8.32 (m, 1H).

1-[(*S*)-*N*-*tert*-Butyloxycarbonyl-*N*-methyltyrosyl]-4-benzylpiperidine (32). Following general procedure A, this product was obtained as a white solid (yield 97%): mp 70−72 °C; [α] = −61.7, *c* = 0.46% in CH_2Cl_2. ^{1}H NMR ($CDCl_3$) *δ*: 1.39 (s, 9H), 1.62 (m, 5H), 2.50 (m, 3H), 2.86 (dd, *J* = 12.6 and 6.8 Hz, 6H), 3.95 (m, 1H), 4.58 (m, 1H), 5.21 (t, *J* = 6.8 Hz, 1H), 5.85 (s, 1H), 6.72 (d, *J* = 7.5 Hz, 2H), 7.10 (m, 4H), 7.25 (m, 3H).

1-[(*S*)-*N*-*tert*-Butyloxycarbonyltyrosyl]-4-(4-fluorophenyl)piperazine (33). Following general procedure A, this product was obtained as a yellow oil (yield 87%): [α] = +7.0, *c* = 0.43% in CH_2Cl_2. ^{1}H NMR ($CDCl_3$) *δ*: 1.43 (s, 9H), 2.93 (m, 6H), 3.57 (m, 4H), 4.82 (t, *J* = 8.6 Hz, 1H), 5.45(s, 1H), 6.75 (m, 4H), 6.93 (d, *J* = 8.4 Hz, 2H), 7.04 (d, *J* = 8.4 Hz, 2H).

1-[(*S*)-*N*-*tert*-Butyloxycarbonyltyrosyl]-4-*o*-tolylpiperazine (34). Following general procedure A, this product was obtained as a white solid (yield 94%): mp 80−82 °C; [α]=+14.9, *c* = 1.03% in CH_2Cl_2. ^{1}H NMR ($CDCl_3$) *δ*: 1.43 (s, 9H), 2.26 (s, 3H), 2.31 (m, 1H), 2.69 (m, 3H), 2.92 (d, *J* = 6.9 Hz, 2H), 3.29 (m, 1H), 3.46 (m, 1H), 3.69 (m, 2H), 4.85 (dd, *J* = 15.7 and 7.3 Hz, 1H), 5.47 (d, *J* = 8.6 Hz, 1H), 6.20 (s, 1H), 6.74 (d, *J* = 8.4 Hz, 2H), 6.86 (d, *J* = 7.7 Hz, 1H), 7.03 (t, *J* = 8.4 Hz, 3H), 7.15 (t, *J* = 7.4 Hz, 2H).

General Procedure B for the Synthesis of Compounds 35−60. To a suspension of NaH (24 mg of 55−65% oil suspension, 0.6 mmol, 1.2 equiv) in dry THF (5 mL) was added **9**−**34** (0.5 mmol, 1 equiv). After 10 min, the isoquinolinesulfonyl chloride (1 mmol, 2 equiv) dissolved in dry DCM (2 mL) was added. This mixture was stirred for 18 h at room temperature and then concentrated in vacuo. The residue was dissolved in a mixture of EtOAc (10 mL) and saturated aqueous $NaHCO_3$ (5 mL). After the layers were separated, the organic layer was dried (Na_2SO_4) and concentrated in vacuo, and the residue purified by column chromatography using EtOAc/MeOH (9.8:0.2, v/v) as eluent yielded the derivatives **35**−**60**.

1-[(*S*)-*O*-Isoquinolinesulfonyl-*N*-*tert*-butyloxycarbonyl-*N*-methyltyrosyl]-4-benzylpiperazine (35). Following general procedure B, this product was obtained as a yellow oil (yield 90%): [α] = −64.4, *c* = 1.15% in $CHCl_3$. ^{1}H NMR ($CDCl_3$) *δ*: 1.31 (s, 9H), 2.72 (m, 4H), 2.90 (m, 2H), 3.48 (m, 7H), 3.70 (m, 2H), 5.15 (t, *J* = 7 Hz, 1H), 6.76 (t, *J* = 8.6 Hz, 3H), 7.05 (t, *J* = 8.6 Hz, 3H), 7.28 (m, 3H), 7.63 (m, 1H), 8.27 (m, 2H), 8.54 (d, *J* = 6.1 Hz, 1H), 8.79 (d, *J* = 6.1 Hz, 1H), 9.42 (s, 1H).

1-[(*S*)-*O*-Isoquinolinesulfonyl-*N*-*tert*-butyloxycarbonyl-*N*-methyltyrosyl]-4-phenethylpiperazine (36). Following general procedure B, this product was obtained as a white solid (yield 82%): mp 58−60 °C; [α] = −33.8, *c* = 0.73% in CH_2Cl_2. ^{1}H NMR ($CDCl_3$) *δ*: 1.39 (s, 9H), 2.05 (s, 1H), 2.17 (s, 1H), 2.39 (m, 3H), 2.57 (m, 3H), 2.72 (m, 4H), 2.87 (m, 2H), 3.36 (m, 2H), 3.51 (m, 1H), 5.15 (t, *J* = 7 Hz, 1H), 6.77 (m, 2H), 7.03 (d, *J* = 8.3 Hz, 2H), 7.18 (m, 3H), 7.25 (m, 2H), 7.59 (t, *J* = 8.0 Hz, 1H), 8.25 (t, *J* = 5.8 Hz, 2H), 8.53 (d, *J* = 6.2 Hz, 1H), 8.79 (d, *J* = 6.2 Hz, 1H), 9.42 (s, 1H).

1-[(*S*)-*O*-Isoquinolinesulfonyl-*N*-*tert*-butyloxycarbonyl-*N*-methyltyrosyl]-4-(4-fluorophenyl)piperazine (37). Following general procedure B, this product was obtained as a oil (yield 85%): [α] = −54.1, *c* = 1.31% in $CHCl_3$. ^{1}H NMR ($CDCl_3$) *δ*: 1.33 (s, 9H), 2.73 (s, 3H), 2.91 (m, 6H), 3.51 (m, 4H), 5.17 (t, *J* = 7.2 Hz, 1H), 6.77 (t, *J* = 8.6 Hz, 4H), 7.01 (t, *J* = 8.6 Hz, 4H), 7.62 (t, *J* = 7.6 Hz, 1H), 8.26 (t, *J* = 7.6, 2H), 8.52 (d, *J* = 6.1 Hz, 1H), 8.79 (d, *J* = 6.1 Hz, 1H), 9.40 (s, 1H).

1-[(*S*)-*O*-Isoquinolinesulfonyl-*N*-*tert*-butyloxycarbonyl-*N*-methyltyrosyl]-4-(4-chlorophenyl)piperazine (38). Following general procedure B, this product was obtained as a white liquid (yield 86%): [α] = −68.4, *c* = 1% in $CHCl_3$. ^{1}H NMR ($CDCl_3$) *δ*: 1.33 (s, 9H), 1.57 (m, 2H), 2.74 (s, 3H), 3.09 (m, 4H), 3.48 (m, 4H), 5.22 (t, *J* = 7 Hz, 1H), 6.77 (m, 4H), 7.10 (t, *J* = 8.6 Hz, 2H), 7.23 (m, 2H), 7.61 (t, *J* = 8.6 Hz, 1H), 8.25 (dd, *J* = 6.6 and 6.2 Hz, 2H), 8.54 (d, *J* = 6.2 Hz, 1H), 8.80 (d, *J* = 6.2 Hz, 1H), 9.41 (s, 1H).

1-[(*S*)-*O*-Isoquinolinesulfonyl-*N*-*tert*-butyloxycarbonyl-*N*-methyltyrosyl]-4-(4-iodophenyl)piperazine (39). Following general procedure B, this product was obtained as a yellow oil (yield 98%): [α] = −48.8, *c* = 1.22% in $CHCl_3$. ^{1}H NMR ($CDCl_3$) *δ*: 1.34 (s, 9H), 2.73 (s, 3H), 2.98 (m, 6H), 3.51 (m, 4H), 5.21 (t, *J* = 7 Hz, 1H), 6.64 (d, *J* = 8.8, 2H), 6.76 (t, *J* = 8.6, 2H), 7.09 (t, *J* = 8.6, 2H), 7.53 (d, *J* = 8.8, 2H), 7.61 (t, *J* = 7.2 Hz, 1H), 8.25 (t, *J* = 7.2 Hz, 2H), 8.53 (d, *J* = 6.1 Hz, 1H), 8.80 (d, *J* = 6.1 Hz, 1H), 9.41 (s, 1H).

1-[(*S*)-*O*-Isoquinolinesulfonyl-*N*-*tert*-butyloxycarbonyl-*N*-methyltyrosyl]-4-tolylpiperazine (40). Following general procedure B, this product was obtained as a yellow oil (yield 90%): [α] = −10.9, *c* = 1.34% in $CHCl_3$. ^{1}H NMR ($CDCl_3$) *δ*: 1.33 (s, 9H), 2.05 (s, 3H), 2.74 (s, 3H), 2.82 (m, 2H), 3.04 (m, 4H), 3.51 (m, 4H), 5.22 (t, *J* = 7.2 Hz, 1H), 6.79 (t, *J* = 8.4 Hz, 4H), 7.09 (d, *J* = 8.4 Hz, 4H), 7.61 (t, *J* = 7.6 Hz, 1H), 8.26 (m, 2H), 8.53 (d, *J* = 6 Hz, 1H), 8.80 (d, *J* = 6.2 Hz, 1H), 9.41 (s, 1H).

1-[(*S*)-*O*-Isoquinolinesulfonyl-*N*-*tert*-butyloxycarbonyl-*N*-methyltyrosyl]-4-(4-methoxyphenyl)piperazine (41). Following general procedure B, this product was obtained as a yellow oil (yield 82%): [α] = −63.3, *c* = 1.1% in $CHCl_3$. ^{1}H NMR ($CDCl_3$) *δ*: 1.33 (s, 9H), 1.57 (m, 2H), 2.73 (s, 3H), 2.96 (m, 4H), 3.51 (m, 4H), 3.76 (s, 3H), 5.21 (t, *J* = 7 Hz, 1H), 6.82 (m, 4H), 7.10 (t, *J* = 8.6 Hz, 2H), 7.23 (m, 2H), 7.61 (t, *J* = 8.6 Hz, 1H), 8.25 (dd, *J* = 6.6 and 6.2 Hz, 2H), 8.54 (d, *J* = 6.2 Hz, 1H), 8.78 (d, *J* = 6.2 Hz, 1H), 9.42 (s, 1H).

1-[(*S*)-*O*-Isoquinolinesulfonyl-*N*-*tert*-butyloxycarbonyl-*N*-methyltyrosyl]-4-(4-nitrophenyl)piperazine (42). Following general procedure B, this product was obtained as a yellow solid (yield 79%): mp 46−47 °C; [α] = −58.71, *c* = 1.01% in $CHCl_3$. ^{1}H NMR ($CDCl_3$) *δ*: 1.34 (s, 9H), 2.83 (dd, *J* = 14 and 6.4 Hz, 2H), 2.95 (s, 3H), 3.44 (m, 4H), 3.56 (m, 4H), 5.19 (t, *J* = 7.2 Hz, 1H), 6.79 (dd, *J* = 7.2 and 3.6 Hz, 4H), 7.10 (d, *J* = 8.6 Hz, 2H), 7.61 (t, *J* = 7.8 Hz, 1H), 8.11 (d, *J* = 7.4 Hz, 2H), 8.25 (t, *J* = 8.8 Hz, 2H), 8.51 (d, *J* = 6.2 Hz, 1H), 8.80 (d, *J* = 6.2 Hz, 1H), 9.51 (s, 1H).

1-[(*S*)-*O*-Isoquinolinesulfonyl-*N*-*tert*-butyloxycarbonyl-*N*-methyltyrosyl]-4-(4-cyanophenyl)piperazine (43). Following general procedure B, this product was obtained as a yellow oil (yield 97%): [α] = +4.21, *c* = 1.4% in $CHCl_3$. ^{1}H NMR ($CDCl_3$) *δ*: 1.34 (s, 9H), 2.74 (s, 3H), 3.09 (m, 6H), 3.49 (m, 4H), 5.22 (t, *J* = 7 Hz, 1H), 6.79 (t, *J* = 8.6 Hz, 4H), 7.11 (t, *J* = 8.4 Hz, 3H), 7.51 (d, *J* = 8.6 Hz, 1H), 7.63 (t, *J* = 7.6 Hz, 1H), 8.26 (dd, *J* = 6.8 and 4.4 Hz, 2H), 8.51 (d, *J* = 6 Hz, 1H), 8.80 (d, *J* = 6 Hz, 1H), 9.41 (s, 1H).

1-[(*S*)-*O*-Isoquinolinesulfonyl-*N*-*tert*-butyloxycarbonyl-*N*-methyltyrosyl]-4-(4-acetylphenyl)piperazine (44). Following general procedure B, this product was obtained as an

oil (yield 98%): [α] = −51.82, *c* = 1.2% in $CHCl_3$. ^1H NMR ($CDCl_3$) *δ*: 1.35 (s, 9H), 2.53 (s, 3H), 2.74 (s, 3H), 3.49 (m, 10H), 5.19 (t, *J* = 7.2 Hz, 1H), 6.77 (t, *J* = 8.6, 2H), 6.84 (t, *J* = 8.9 Hz, 2H), 7.10 (t, *J* = 8.6 Hz, 2H), 7.62 (t, 1H), 7.89 (d, *J* = 8.9 Hz, 2H), 8.28 (d, *J* = 6.2 Hz, 2H), 8.51 (d, *J* = 6.2 Hz, 1H), 8.80 (d, *J* = 6.2 Hz, 1H), 9.42 (s, 1H).

1-[(*S*)-*O*-Isoquinolinesulfonyl-*N*-*tert*-butyloxycarbonyl-*N*-methyltyrosyl]-4-(4-fluorobenzyl)piperazine (45). Following general procedure B, this product was obtained as a brown oil (yield 77%): [α] = −38.9, *c* = 0.66% in $CHCl_3$. ^1H NMR ($CDCl_3$) *δ*: 1.32 (s, 9H), 2.72 (s, 3H), 2.90 (m, 3H), 3.50 (m, 7H), 3.73 (m, 2H), 5.15 (t, *J* = 7 Hz, 1H), 6.78 (t, *J* = 8.6 Hz, 2H), 7.03 (t, *J* = 8.6 Hz, 4H), 7.25 (t, *J* = 6.6 Hz, 2H), 7.64 (m, 1H), 8.28 (d, *J* = 7.8 Hz, 2H), 8.54 (d, *J* = 6.1 Hz, 2H), 8.81 (d, *J* = 6.2 Hz, 1H), 9.42 (s, 1H).

1-[(*S*)-*O*-Isoquinolinesulfonyl-*N*-*tert*-butyloxycarbonyl-*N*-methyltyrosyl]-4-(4-fluorobenzoyl)piperazine (46). Following general procedure B, this product was obtained as a white oil (yield 97%): [α] = −27.4, *c* = 0.74% in CH_2Cl_2. ^1H NMR ($CDCl_3$) *δ*: 1.35 (s, 9H), 1.62 (m, 5H), 2.50 (m, 3H), 2.72 (s, 3H), 2.90 (m, 3H), 3.90 (m, 1H), 4.50 (m, 1H), 5.12 (t, *J* = 6.2 Hz, 1H), 6.76 (d, *J* = 8.4 Hz, 2H), 7.08 (d, *J* = 8.6 Hz, 4H), 7.23 (m, 3H), 7.67 (m, 1H), 8.27 (d, *J* = 6.0 Hz, 2H), 8.54 (d, *J* = 6.1 Hz, 1H), 8.80 (d, *J* = 6.0 Hz, 1H), 9.42 (s, 1H).

1-[(*S*)-*O*-Isoquinolinesulfonyl-*N*-*tert*-butyloxycarbonyl-*N*-methyltyrosyl]-4-(4-nitrobenzyl)piperazine (47). Following general procedure B, this product was obtained as a yellow oil (yield 54%): [α] = −44.5, *c* = 0.44% in CH_3OH. ^1H NMR ($CDCl_3$) *δ*: 1.30 (s, 9H), 2.73 (m, 4H), 2.90 (m, 2H), 3.50 (m, 7H), 3.70 (m, 2H), 5.15 (t, *J* = 7 Hz, 1H), 6.79 (t, *J* = 8.3 Hz, 2H), 7.06 (t, *J* = 8.5 Hz, 2H), 7.49 (d, *J* = 8.3 Hz, 2H), 7.65 (t, *J* = 7.7 Hz, 1H), 8.18 (d, *J* = 8.3 Hz, 2H), 8.29 (d, *J* = 7.7 Hz, 2H), 8.54 (d, *J* = 6.0 Hz, 1H), 8.80 (d, *J* = 6.1 Hz, 1H), 9.42 (s, 1H).

1-[(*S*)-*O*-Isoquinolinesulfonyl-*N*-*tert*-butyloxycarbonyl-*N*-methyltyrosyl]-4-(1-fluorophenyl)piperazine (48). Following general procedure B, this product was obtained as a yellow oil (yield 98%): [α] = −44.23, *c* = 1.04% in $CHCl_3$. ^1H NMR ($CDCl_3$) *δ*: 1.34 (s, 9H), 2.75 (s, 3H), 2.92 (m, 6H), 3.52 (m, 4H), 5.22 (t, *J* = 7 Hz, 1H), 6.79 (m, 5H), 7.14 (m, 4H), 7.62 (t, *J* = 7.6 Hz, 1H), 8.26 (t, *J* = 7.2 Hz, 1H), 8.53 (t, *J* = 6 Hz, 1H), 8.82 (d, *J* = 6 Hz, 1H), 9.41 (s, 1H).

[(*S*)-*O*-Isoquinolinesulfonyl-*N*-*tert*-butyloxycarbonyl-*N*-methyltyrosyl-4-(2-chlorophenyl)piperazine (49). Following general procedure B, this product was obtained as an oil (yield 98%): [α] = −40.6, *c* = 1.23% in $CHCl_3$. ^1H NMR ($CDCl_3$) *δ*: 1.34 (s, 9H), 2.76 (s, 3H), 2.88 (m, 6H), 3.62 (m, 4H), 5.19 (t, *J* = 7 Hz, 1H), 6.79 (t, *J* = 8.6, 2H), 7.05 (dd, *J* = 6.2 and 8.6, 4H), 7.22 (d, *J* = 6.2 Hz, 1H), 7.37 (d, *J* = 7.9 Hz, 1H), 7.64 (t, 1H), 8.28 (t, *J* = 7, 2H), 8.57 (d, *J* = 6.1 Hz, 1H), 8.80 (d, *J* = 6.1 Hz, 1H), 9.44 (s, 1H).

1-[(*S*)-*O*-Isoquinolinesulfonyl-*N*-*tert*-butyloxycarbonyl-*N*-methyltyrosyl]-4-*o*-tolylpiperazine (50). Following general procedure B, this product was obtained as a yellow oil (yield 72%): [α] = −47.0, *c* = 1.27% in $CHCl_3$. ^1H NMR ($CDCl_3$) *δ*: 1.38 (s, 9H), 2.28 (s, 3H), 2.74 (m, 6H), 2.86 (s, 3H), 3.65 (m, 4H), 5.26 (t, *J* = 7.2 Hz, 1H), 6.81 (dd, *J* = 9.0 and 8.4 Hz, 3H), 7.02 (dd, *J* = 8.9 and 8.5 Hz, 3H), 7.18 (t, *J* = 7.4 Hz, 2H), 7.72 (t, *J* = 7.7 Hz, 1H), 8.27 (dd, *J* = 8.4 and 6.1 Hz, 2H), 8.54 (d, *J* = 6 Hz, 1H), 8.82 (d, *J* = 6 Hz, 1H), 9.43 (s, 1H).

1-[(*S*)-*O*-Isoquinolinesulfonyl-*N*-*tert*-butyloxycarbonyl-*N*-methyltyrosyl]-4-(2-methoxyphenyl)piperazine (51). Following general procedure B, this product was obtained as a yellow oil (yield 64%): [α] = −61.0, *c* = 1.54% in $CHCl_3$. ^1H NMR ($CDCl_3$) *δ*: 1.34 (s, 9H), 2.75 (s, 3H), 2.91 (m, 6H), 3.51 (m, 4H), 3.87 (s, 3H), 5.20 (t, *J* = 7 Hz, 1H), 6.76 (d, *J* = 8.5, 2H), 6.83 (t, *J* = 6.6, 3H), 7.06 (d, *J* = 8.5, 3H), 7.61 (t, *J* = 6.6 Hz, 1H), 8.27 (m, 2H), 8.54 (d, *J* = 6.0 Hz, 1H), 8.81 (d, *J* = 6.0 Hz, 1H), 9.42 (s, 1H).

1-[(*S*)-*O*-Isoquinolinesulfonyl-*N*-*tert*-butyloxycarbonyl-*N*-methyltyrosyl]-4-(3-chlorophenyl)piperazine (52). Following general procedure B, this product was obtained as a yellow oil (yield 98%): mp 79−81 °C; [α] = −42.2, *c* = 0.62% in $CHCl_3$. ^1H NMR ($CDCl_3$) *δ*: 1.35 (s, 9H), 2.73 (s, 3H), 2.92 (m, 6H), 3.51 (m, 4H), 5.21 (t, *J* = 7 Hz, 1H), 6.79 (m, 5H), 7.14 (m, 4H), 7.61 (t, *J* = 7.2 Hz, 1H), 8.26 (t, *J* = 7.2 Hz, 1H), 8.53 (d, *J* = 6.2 Hz, 1H), 8.80 (d, *J* = 4.2 Hz, 1H), 9.41 (s, 1H).

1-[(*S*)-*O*-Isoquinolinesulfonyl-*N*-*tert*-butyloxycarbonyl-*N*-methyltyrosyl]-4-(3-trifluoromethylphenyl)piperazine (53). Following general procedure B, this product was obtained as a white solid (yield 75%): mp 74−76 °C; [α] = −48.9, *c* = 0.76% in $CHCl_3$. ^1H NMR ($CDCl_3$) *δ*: 1.35 (s, 9H), 2.74 (s, 3H), 3.01 (m, 6H), 3.65 (m, 4H), 5.20 (t, *J* = 7 Hz, 1H), 6.79 (t, *J* = 8.6 Hz, 2H), 7.08 (t, *J* = 8.6 Hz, 5H), 7.37 (t, *J* = 7.8 Hz, 1H), 7.66 (t, *J* = 7.8 Hz, 1H), 8.29 (dd, *J* = 8.1 and 4.6 Hz, 2H), 8.58 (d, *J* = 6.1 Hz, 1H), 8.81 (d, *J* = 6.1 Hz, 1H), 9.44 (s, 1H).

1-[(*S*)-*O*-Isoquinolinesulfonyl-*N*-*tert*-butyloxycarbonyl-*N*-methyltyrosyl]-4-(2,3-dimethylphenyl)piperazine (54). Following general procedure B, this product was obtained as a yellow oil (yield 44%): [α] = −70, *c* = 0.2% in CH_2Cl_2. ^1H NMR ($CDCl_3$) *δ*: 1.39 (s, 9H), 2.21 (s, 3H), 2.27 (s, 3H), 2.76 (s, 3H), 2.82 (m, 6H), 3.58 (m, 4H), 5.20 (t, *J* = 7 Hz, 1H), 6.80 (t, *J* = 8.3 Hz, 3H), 6.95 (m, 1H), 7.08 (t, *J* = 8.4 Hz, 3H), 7.66 (m, 1H), 8.26 (t, *J* = 8.4, 2H), 8.55 (d, *J* = 6.1 Hz, 1H), 8.81 (d, *J* = 6.1 Hz, 1H), 9.41 (s, 1H).

1-[(*S*)-*O*-Isoquinolinesulfonyl-*N*-*tert*-butyloxycarbonyl-*N*-methyltyrosyl−4-(3,4-dichlorophenyl)piperazine (55). Following general procedure B, this product was obtained as a yellow solid (yield 52%): mp 85−87 °C; [α] = +12, *c* = 0.5% in $CHCl_3$. ^1H NMR ($CDCl_3$) *δ*: 1.34 (s, 9H), 2.73 (s, 3H), 2.92 (m, 6H), 3.51 (m, 4H), 5.19 (t, *J* = 7.0 Hz, 1H), 6.74 (m, 2H), 6.90 (s, 1H), 7.10 (d, *J* = 7.4 Hz, 1H), 7.32 (d, *J* = 7.4 Hz, 1H), 7.63 (t, *J* = 7.6 Hz, 1H), 8.26 (dd, *J* = 7 and 3.6 Hz, 1H), 8.54 (t, *J* = 6 Hz, 1H), 8.81 (d, *J* = 6 Hz, 1H), 9.42 (s, 1H).

1-[(*S*)-*O*-Isoquinolinesulfonyl-*N*-*tert*-butyloxycarbonyl-*N*-methyltyrosyl]-4-pyridin-2-ylpiperazine (56). Following general procedure B, this product was obtained as a white solid (yield 90%): mp 56−58 °C; [α] = −59.3, *c* = 0.43% in CH_2Cl_2. ^1H NMR ($CDCl_3$) *δ*: 1.35 (s, 9H), 2.75 (s, 3H), 3.00 (m, 1H), 3.45 (m, 8H), 3.85 (m, 1H), 5.19 (t, *J* = 7 Hz, 1H), 6.69 (t, *J* = 8.4 Hz, 4H), 7.07 (t, *J* = 8.4 Hz, 2H), 7.51 (t, *J* = 6.6 Hz, 1H), 7.62 (m, 1H), 8.23 (t, *J* = 7.1 Hz, 3H), 8.53 (d, *J* = 6.1 Hz, 1H), 8.89 (d, *J* = 6.2 Hz, 1H), 9.41 (s, 1H).

1-[(*S*)-*O*-Isoquinolinesulfonyl-*N*-*tert*-butyloxycarbonyl-*N*-methyltyrosyl]-4-pyrimidin-2-ylpiperazine (57). Following general procedure B, this product was obtained as a white solid (yield 80%): mp 104−106 °C; [α] = −48.6, *c* = 0.37% in CH_2Cl_2. ^1H NMR ($CDCl_3$) *δ*: 1.35 (s, 9H), 2.76 (s, 3H), 3.00 (m, 2H), 3.53 (m, 5H), 3.85 (m, 3H), 5.19 (t, *J* = 7 Hz, 1H), 6.54 (m, 1H), 6.78 (t, *J* = 8.4 Hz, 2H), 7.08 (t, *J* = 8.4 Hz, 2H), 7.63 (m, 1H), 8.29 (m, 4H), 8.53 (d, *J* = 6.1 Hz, 1H), 8.81 (d, *J* = 6.2 Hz, 1H), 9.42 (s, 1H).

1-[(*S*)-*O*-Isoquinolinesulfonyl-*N*-*tert*-butyloxycarbonyl-*N*-methyltyrosyl]-4-benzylpiperidine (58). Following general procedure B, this product was obtained as a white solid (yield 90%): mp 100−102 °C; [α] = −39.3, *c* = 0.4% in CH_2Cl_2. ^1H NMR ($CDCl_3$) *δ*: 1.31 (s, 9H), 2.72 (m, 4H), 2.90 (m, 2H), 3.48 (m, 7H), 3.70 (m, 2H), 5.15 (t, *J* = 7 Hz, 1H), 6.76 (t, *J* = 8.6 Hz, 3H), 7.05 (d, *J* = 8.6 Hz, 3H), 7.28 (m, 3H), 7.63 (m, 1H), 8.27 (d, *J* = 7.8 Hz, 2H), 8.54 (d, *J* = 6.1 Hz, 1H), 8.79 (d, *J* = 6.1 Hz, 1H), 9.42 (s, 1H).

1-[(*S*)-*O*-Isoquinolinesulfonyl-*N*-*tert*-butyloxycarbonyltyrosyl]-4-(4-fluorophenyl)piperazine (59). Following general procedure B, this product was obtained as a white solid (yield 79%): mp 87−89 °C; [α] = −1.6, *c* = 0.63% in CH_2Cl_2. ^1H NMR ($CDCl_3$) *δ*: 1.39 (s, 9H), 2.65 (m, 1H), 2.92 (m, 6H), 3.43 (m, 2H), 3.78 (m, 1H), 4.76 (m, 1H), 5.33 (d, 1H), 6.82 (m, 4H), 7.01 (m, 4H), 7.54 (t, *J* = 7.6 Hz, 1H), 8.22 (dd, *J* = 7.5 and 8.2 Hz, 2H), 8.53 (d, *J* = 6.1 Hz, 1H), 8.80 (d, *J* = 6.1 Hz, 1H), 9.41 (s, 1H).

1-[(*S*)-*O*-Isoquinolinesulfonyl-*N*-*tert*-butyloxycarbonyltyrosyl]-4-*o*-tolylpiperazine (60). Following general procedure B, this product was obtained as a white solid (yield 73%): mp 73−75 °C; [α] = +22.5, *c* = 1.3% in CH_2Cl_2. ^1H NMR ($CDCl_3$) *δ*: 1.39 (s, 9H), 2.27 (s, 3H), 2.42 (m, 1H), 2.75 (m, 3H), 2.91 (d, *J* = 6.7 Hz, 2H), 3.15 (m, 1H), 3.51 (m, 3H), 4.80

(m, 1H), 5.37 (d, J = 8.5 Hz, 1H), 6.80 (d, J = 8.5 Hz, 2H), 6.90 (d, J = 7.8 Hz, 1H), 7.05 (t, J = 8.4 Hz, 3H), 7.18 (t, J = 7.3 Hz, 2H), 7.54 (t, J = 7.8, 1H), 8.22 (dd, J = 8.2 and 7.3 Hz, 2H), 8.54 (d, J = 6.2 Hz, 1H), 8.81 (d, J = 6 Hz, 1H), 9.41 (s, 1H).

General Procedure for Removal of the Boc Protecting Group from Compounds 35−60. The esters **35−60** (1.5 mmol) **were** stirred at room temperature in a mixture of TFA/CH_2Cl_2 (1:1, 5 mL) for 3 h. The volatiles were removed in vacuo, and the residue was diluted with 5% aqueous $NaHCO_3$ (5 mL). The aqueous mixture was extracted with CH_2Cl_2 (3 × 5 mL), and the combined organic extracts were dried (Na_2SO_4) and concentrated in vacuo. The residue obtained was used for the next reaction without any purification.

General Procedure C for the Synthesis of Compounds 61−87. To a stirred solution of the appropriate free amine (0.5 mmol) in dry DCM (5 mL) were added Et_3N (70 μL, 0.5 mmol, 1 equiv) and then dropwise isoquinolinesulfonyl chloride (1 mmol, 2 equiv) dissolved in DCM (3 mL), under cooling with ice. The reaction mixture obtained was slowly warmed to room temperature and stirred for 18 h. After this time, the mixture was diluted with DCM (5 mL) and washed with saturated aqueous $NaHCO_3$ (2 mL), water (5 mL), and brine (5 mL). After the layers were separated, the organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue obtained, subjected to purification by column chromatography using a stepwise gradient of methanol (0.2−0.5%) in methylene chloride, furnished the appropriate products **61−87**.

1-[(*S*)-*N*,*O*-Bis(isoquinolinesulfonyl)-*N*-methyltyrosyl]-4-benzylpiperazine (61). Following general procedure C, this product was obtained as a yellow solid: [α] = −33.8, c = 0.95% in $CHCl_3$. ^{1}H NMR ($CDCl_3$) δ: 1.95 (m, 1H), 2.16 (m, 1H), 2.25 (t, J = 4.7 Hz, 2H), 2.49 (dd, J = 12.4 and 4.6 Hz, 1H), 3.04 (m, 4H), 3.19 (dd, J = 10.3 Hz, 2H), 3.40 (m, 4H), 5.06 (dd, J = 10.3 and 4.5 Hz, 1H), 6.76 (d, J = 8.6 Hz, 2H), 6.95 (d, J = 8.6 Hz, 2H), 7.27 (m, 5H), 7.59 (t, J = 7.8 Hz, 1H), 7.68 (t, J = 7.6 Hz, 1H), 8.25 (dd, J = 8.2 and 7.7 Hz, 4H), 8.39 (d, J = 6.1 Hz, 1H), 8.56 (d, J = 6.2 Hz, 1H), 8.66 (d, J = 6.2 Hz, 1H), 8.83 (d, J = 6.1 Hz, 1H), 9.35 (s, 1H), 9.43 (s, 1H). Anal. ($C_{39}H_{37}N_5O_6S_2$) C, H, N.

1-[(*S*)-*N*,*O*-Bis(isoquinolinesulfonyl)-*N*-methyltyrosyl]-4-phenethylpiperazine (62). Following general procedure C, this product was obtained as a yellow solid: [α] = +33.2, c = 0.84% in CH_2Cl_2. ^{1}H NMR ($CDCl_3$) δ: 1.94 (m, 6H), 2.47 (m, 2H), 2.73 (m, 5H), 3.04 (m, 4H), 4.31 (m, 1H), 6.12 (m, 1H), 6.63 (d, J = 8.5 Hz, 2H), 6.86 (d, J = 8.5 Hz, 2H), 7.22 (m, 5H), 7.62 (t, J = 7.8 Hz, 2H), 8.23 (m, 5H), 8.51 (d, J = 6 Hz, 1H), 8.68 (d, J = 6.2 Hz, 1H), 8.80 (d, J = 6.2 Hz, 1H), 9.32 (s, 1H), 9.41 (s, 1H). Anal. ($C_{40}H_{39}N_5O_6S_2$) C, H, N.

1-[(*S*)-*N*,*O*-Bis(isoquinolinesulfonyl)-*N*-methyltyrosyl]-4-(4-fluorophenyl)piperazine (63). Following general procedure C, this product was obtained as a yellow solid: [α] = −56.6, c = 0.45% in $CHCl_3$. ^{1}H NMR ($CDCl_3$) δ: 2.42 (dd, J = 12.6 and 6.2 Hz, 1H), 2.63 (m, 1H), 2.89 (m, 2H), 3.03 (s, 3H), 3.25 (dd, J = 12.6 Hz, 2H) 3.55 (m, 4H), 5.12 (dd, J = 12 and 6.2 Hz, 1H), 6.80 (m, 4H), 6.99 (t, J = 8.5 Hz, 4H), 7.55 (t, J = 7.9 Hz, 1H), 7.71 (t, J = 7.9 Hz, 1H), 8.25 (d, J = 8 Hz, 4H), 8.42 (d, J = 6.3 Hz, 1H), 8.52 (d, J = 6.3 Hz, 1H), 8.68 (d, J = 6.3 Hz, 1H), 8.81 (d, J = 6.3 Hz, 1H), 9.36 (s, 1H), 9.41 (s, 1H). Anal. ($C_{38}H_{34}N_5O_6S_2F$) C, H, N.

1-[(*S*)-*N*,*O*-Bis(isoquinolinesulfonyl)-*N*-methyltyrosyl]-4-(4-chlorophenyl)piperazine (64). Following general procedure C, this product was obtained as a yellow oil (yield 61%): [α] = −43.4, c = 0.89% in $CHCl_3$. ^{1}H NMR ($CDCl_3$) δ: 2.44 (dd, J = 12.6 and 6.2 Hz, 2H), 2.92 (m, 4H), 3.00 (s, 3H), 3.57 (m, 4H), 5.11 (dd, J = 6.6 and 6.2 Hz, 1H), 6.76 (d, J = 8.6 Hz, 4H), 6.95 (d, J = 8.6 Hz, 2H), 7.23 (d, J = 6.6 Hz, 1H), 7.54 (d, J = 8 Hz, 1H), 7.70 (t, J = 7.8 Hz, 1H), 8.23 (m, 4H), 8.41 (d, J = 6 Hz, 1H), 8.49 (d, J = 6 Hz, 1H), 8.67 (d, J = 6.2 Hz, 1H), 8.80 (d, J = 6 Hz, 1H), 9.35 (s, 1H), 9.41 (s, 1H). Anal. ($C_{38}H_{34}N_5O_6S_2Cl$) C, H, N.

1-[(*S*)-*N*,*O*-Bis(isoquinolinesulfonyl)-*N*-methyltyrosyl]-4-(4-iodophenyl)piperazine (65). Following general procedure C, this product was obtained as a yellow solid: [α] = −46.0, c = 0.73% in $CHCl_3$. ^{1}H NMR ($CDCl_3$) δ: 2.42 (dd, J = 12.6 and 6.2 Hz, 1H), 2.63 (m, 1H), 2.99 (m, 5H), 3.22 (m, 2H), 3.55(m, 4H), 5.11 (dd, J = 13.6 and 6 Hz, 1H), 6.63 (d, J = 8.9 Hz, 2H), 6.77 (d, J = 8.4 Hz, 2H), 6.96 (d, J = 8.4 Hz, 2H), 7.54 (dd, J = 8.8 and 7.8 Hz, 3H), 7.71 (t, J = 7.8 Hz, 1H), 8.24 (d, J = 7.6 Hz, 4H), 8.42 (d, J = 6.3 Hz, 1H), 8.50 (d, J = 6.1 Hz, 1H), 8.68 (d, J = 6.1 Hz, 1H), 8.81 (d, J = 6.1 Hz, 1H), 9.36 (s, 1H), 9.41 (s, 1H). Anal. ($C_{38}H_{34}N_5O_6S_2I$) C, H, N.

1-[(*S*)-*N*,*O*-Bis(isoquinolinesulfonyl)-*N*-methyltyrosyl]-4-*p*-tolylpiperazine (66). Following the general procedure, this product was obtained as a foamy yellow solid: [α] = −41.5, c = 0.45% in $CHCl_3$. ^{1}H NMR ($CDCl_3$) δ: 2.28 (s, 3H), 2.89 (m, 2H), 3.04 (s, 3H), 3.21 (m, 4H), 3.49 (m, 4H), 5.14 (dd, J = 12 and 6.2 Hz, 1H), 6.77 (dd, J = 6.2 and 3 Hz, 2H), 6.96 (d, J = 8.6 Hz, 2H), 7.09 (d, J = 8.4 Hz, 2H), 7.49 (t, J = 7.8 Hz, 1H), 7.70 (t, J = 7.8 Hz, 1H), 8.13 (d, J = 7.4 Hz, 2H), 8.17 (t, J = 8.8 Hz, 2H), 8.24 (t, J = 7.8 Hz, 1H), 8.31 (d, J = 7.2 Hz, 1H), 8.41 (d, J = 6 Hz, 1H), 8.51 (d, J = 6.2 Hz, 1H), 8.68 (d, J = 6.2 Hz, 1H), 8.81 (d, J = 6 Hz, 1H), 9.35 (s, 1H), 9.40 (s, 1H). Anal. ($C_{39}H_{37}N_5O_6S_2$) C, H, N.

1-[(*S*)-*N*,*O*-Bis(isoquinolinesulfonyl)-*N*-methyltyrosyl]-4-(4-methoxyphenyl)piperazine (67). Following general procedure C, this product was obtained as a white solid: [α] = −48.4, c = 0.94% in $CHCl_3$. ^{1}H NMR ($CDCl_3$) δ: 2.48 (dd, J = 12 and 4.4 Hz, 2H), 2.83 (m, 4H), 3.16 (s, 3H), 3.52 (m, 4H), 3.77 (s, 3H), 5.11 (dd, J = 6.6 and 6.2 Hz, 1H), 6.76 (m, 6H), 6.96 (d, J = 8.4 Hz, 2H), 7.51 (d, J = 8 Hz, 1H), 7.69 (t, J = 8 Hz, 1H), 8.23 (m, 4H), 8.41 (d, J = 6.2 Hz, 1H), 8.51 (d, J = 6.2 Hz, 1H), 8.67 (d, J = 6.2 Hz, 1H), 8.80 (d, J = 6.2 Hz, 1H), 9.35 (s, 1H), 9.40 (s, 1H). Anal. ($C_{39}H_{37}N_5O_7S_2$) C, H, N.

1-[(*S*)-*N*,*O*-Bis(isoquinolinesulfonyl)-*N*-methyltyrosyl]-4-(4-nitrophenyl)piperazine (68). Following general procedure C, this product was obtained as a yellow solid: [α] = −86, c = 1.02% in $CHCl_3$. ^{1}H NMR ($CDCl_3$) δ: 2.34 (dd, J = 14 and 6.2 Hz, 2H), 3.01 (s, 3H), 3.28 (m, 4H), 3.64 (m, 4H), 5.19 (dd, J = 13.8 and 6.2 Hz, 1H), 6.78 (d, J = 5.2 Hz, 2H), 6.81 (d, J = 5.6 Hz, 2H), 6.98 (d, J = 8.6 Hz, 2H), 7.59 (t, J = 7.8 Hz, 1H), 7.73 (t, J = 7.8 Hz, 1H), 8.13 (d, J = 7.4 Hz, 2H), 8.17 (d, J = 7.4 Hz, 2H), 8.20 (t, J = 8.8 Hz, 1H), 8.23 (t, J = 8.8 Hz, 1H), 8.44 (d, J = 6.2 Hz, 1H), 8.50 (d, J = 6.2 Hz, 1H), 8.68 (d, J = 6.2 Hz, 1H), 8.80 (d, J = 6 Hz, 1H), 9.37 (s, 1H), 9.42 (s, 1H). Anal. ($C_{38}H_{34}N_6O_8S_2$) C, H, N.

1-[(*S*)-*N*,*O*-Bis(isoquinolinesulfonyl)-*N*-methyltyrosyl]-4-(4-cyanophenyl)piperazine (69). Following general procedure C, this product was obtained as a yellow solid: [α] = −67.2, c = 0.75% in $CHCl_3$. ^{1}H NMR ($CDCl_3$) δ: 2.17 (dd, J = 12.6 and 6.2 Hz, 2H), 3.00 (s, 3H), 3.17 (m, 4H), 3.69 (m, 4H), 5.11 (dd, J = 6.6 and 6 Hz, 1H), 6.80 (m, 4H), 6.97 (d, J = 6.8 Hz, 2H), 7.52 (d, J = 8.6 Hz, 1H), 7.58 (t, J = 7.6 Hz, 1H), 7.72 (t, J = 7.8 Hz, 1H), 8.30 (m, 4H), 8.41 (d, J = 6 Hz, 1H), 8.49 (d, J = 6 Hz, 1H), 8.67 (d, J = 6.2 Hz, 1H), 8.81 (d, J = 6 Hz, 1H), 9.37 (s, 1H), 9.42 (s, 1H). Anal. ($C_{39}H_{34}N_6O_6S_2$) C, H, N.

1-[(*S*)-*N*,*O*-Bis(isoquinolinesulfonyl)-*N*-methyltyrosyl]-4-(4-acetylphenyl)piperazine (70). Following general procedure C, this product was obtained as a yellow solid: [α] = −56.9, c = 1.12% in $CHCl_3$. ^{1}H NMR ($CDCl_3$) δ: 2.42 (dd, J = 12.6 and 6.2 Hz, 1H), 2.53 (s, 3H), 2.90 (m, 1H), 3.02 (s, 3H), 3.21 (m, 5H), 3.63 (m, 3H), 5.14 (dd, J = 12 and 6.2 Hz, 1H), 6.79 (t, J = 8.5, 4H), 6.95 (d, J = 8.5 Hz, 2H), 7.52 (t, J = 7.8 Hz, 1H), 7.71 (t, J = 7.8 Hz, 1H), 7.89 (d, J = 8.9 Hz, 2H), 8.24 (d, J = 7.4 Hz, 4H), 8.41 (d, J = 6.1 Hz, 1H), 8.49 (d, J = 6.2 Hz, 1H), 8.68 (d, J = 6.2 Hz, 1H), 8.79 (d, J = 6.1 Hz, 1H), 9.35 (s, 1H), 9.39 (s, 1H). Anal. ($C_{40}H_{37}N_5O_7S_2$) C, H, N.

1-[(*S*)-*N*,*O*-Bis(isoquinolinesulfonyl)-*N*-methyltyrosyl]-4-(4-fluorobenzyl)piperazine (71). Following general procedure C, this product was obtained as a yellow solid: [α] = −21.5, c = 0.72% in $CHCl_3$. ^{1}H NMR ($CDCl_3$) δ: 1.95 (m, 1H), 2.24 (m, 3H), 2.49 (dd, J = 12.4 and 4.6 Hz, 1H), 3.04 (m, 4H), 3.14 (m, 2H), 3.38 (m, 4H), 5.06 (dd, J = 10.3 and 4.5 Hz, 1H), 6.78 (d, J = 8.4 Hz, 2H), 6.99 (d, J = 8.4 Hz, 4H), 7.22 (m, 2H), 7.66 (t, J = 7.8 Hz, 2H), 8.25 (m, 4H), 8.39 (d, J = 6.2 Hz, 1H), 8.56 (d, J = 6 Hz, 1H), 8.66 (d, J = 6.3 Hz, 1H), 8.83

(d, J = 6.2 Hz, 1H), 9.36 (s, 1H), 9.44 (s, 1H). Anal. ($C_{39}H_{36}N_5O_6S_2F$) C, H, N.

1-[(*S*)-*N*,*O*-Bis(isoquinolinesulfonyl)-*N*-methyltyrosyl]-4-(4-fluorobenzoyl)piperazine (72). Following general procedure C, this product was obtained as a yellow solid: [α] = −37.8, c = 0.82% in CH_2Cl_2. ^{1}H NMR ($CDCl_3$) δ: 2.49 (m, 1H), 2.79 (m, 1H), 3.03 (s, 3H), 3.28 (m, 6H), 3.57 (m, 2H), 5.07 (dd, J = 10.9 and 4.3 Hz, 1H), 6.79 (d, J = 8.4 Hz, 2H), 6.97 (d, J = 8.5 Hz, 2H), 7.12 (t, J = 8.6 Hz, 2H), 7.44 (m, 2H), 7.72 (t, J = 7.9 Hz, 2H), 8.31 (m, 5H), 8.54 (d, J = 5.9 Hz, 1H), 8.68 (d, J = 6 Hz, 1H), 8.84 (d, J = 6 Hz, 1H), 9.38 (s, 1H), 9.45 (s, 1H). Anal. ($C_{39}H_{34}N_5O_7S_2F$) C, H, N.

1-[(*S*)-*N*,*O*-Bis(isoquinolinesulfonyl)-*N*-methyltyrosyl]-4-(4-nitrobenzyl)piperazine (73). Following general procedure C, this product was obtained as a yellow solid: [α] = −28.4, c = 0.37% in $CHCl_3$. ^{1}H NMR ($CDCl_3$) δ: 1.95 (m, 1H), 2.27 (m, 3H), 2.49 (dd, J = 12.4 and 4.6 Hz, 1H), 2.02 (m, 4H), 3.21 (dd, J = 10.3 Hz, 2H), 3.49 (m, 4H), 5.12 (dd, J = 10.3 and 4.5 Hz, 1H), 6.82 (d, J = 8.4 Hz, 2H), 6.97 (d, J = 8.6 Hz, 2H), 7.48 (d, J = 8.4 Hz, 2H), 7.68 (d, J = 8.3 Hz, 2H), 8.18 (d, J = 8.6 Hz, 3H), 8.30 (d, J = 7.5 Hz, 3H), 8.40 (d, J = 6 Hz, 1H), 8.55 (d, J = 6.2 Hz, 1H), 8.67 (d, J = 6 Hz, 1H), 8.83 (d, J = 6.3 Hz, 1H), 9.37 (s, 1H), 9.45 (s, 1H). Anal. ($C_{39}H_{36}N_6O_8S_2$) C, H, N.

1-[(*S*)-*N*,*O*-Bis(isoquinolinesulfonyl)-*N*-methyltyrosyl]-4-(1-fluorophenyl)piperazine (74). Following general procedure C, this product was obtained as a yellow solid: [α] = −58.4, c = 0.44% in $CHCl_3$. ^{1}H NMR ($CDCl_3$) δ: 2.49 (m, 2H), 3.06 (s, 3H), 3.22 (m, 4H), 3.63 (m, 4H), 5.11 (dd, J = 13.6 and 6 Hz, 1H), 6.79 (m, 4H), 7.00 (m, 4H), 7.57 (t, J = 8.2 Hz, 1H), 7.70 (t, J = 7.8 Hz, 1H), 8.25 (m, 4H), 8.41 (d, J = 6.2 Hz, 1H), 8.52 (d, J = 6.2 Hz, 1H), 8.67 (d, J = 6.2 Hz, 1H), 8.81 (d, J = 6.2 Hz, 1H), 9.36 (s, 1H), 9.42 (s, 1H). Anal. ($C_{38}H_{34}N_5O_6S_2F$) C, H, N.

1-[(*S*)-*N*,*O*-Bis(isoquinolinesulfonyl)-*N*-methyltyrosyl]-4-(2-chlorophenyl)piperazine (75). Following general procedure C, this product was obtained as a yellow solid: [α] = −39.2, c = 0.92% in $CHCl_3$. ^{1}H NMR ($CDCl_3$) δ: 2.48 (dd, J = 12.4 and 4.6 Hz, 2H), 2.83 (m, 2H), 3.07 (s, 3H), 3.23 (t, J = 10.4 Hz, 2H), 3.58 (m, 4H), 5.12 (dd, J = 10.3 and 4.5 Hz, 1H), 6.77 (d, J = 8.6 Hz, 2H), 6.97 (d, J = 8.6 Hz, 4H), 7.23 (d, J = 7.8 Hz, 1H), 7.38 (d, J = 8.8 Hz, 1H), 7.58 (t, J = 7.8 Hz, 1H), 7.71 (t, J = 7.7 Hz, 1H), 8.25 (dd, J = 8.3 and 7.4 Hz, 4H), 8.42 (d, J = 6.2 Hz, 1H), 8.54 (d, J = 6 Hz, 1H), 8.68 (d, J = 5.9 Hz, 1H), 8.82 (d, J = 6.2 Hz, 1H), 9.37 (s, 1H), 9.43 (s, 1H). Anal. ($C_{38}H_{34}N_5O_6S_2Cl$) C, H, N.

1-[(*S*)-*N*,*O*-Bis(isoquinolinesulfonyl)-*N*-methyltyrosyl]-4-*o*-tolylpiperazine (76). Following general procedure C, this product was obtained as a yellow solid: [α] = −39.2, c = 0.92% in $CHCl_3$. ^{1}H NMR ($CDCl_3$) δ: 2.28 (s, 3H), 2.35 (m, 1H), 2.49 (dd, J = 12.4 and 4.6 Hz, 1H), 2.68 (m, 2H), 3.06 (s, 3H), 3.30 (m, 2H), 3.58 (m, 4H), 5.12 (dd, J = 10.3 and 4.5 Hz, 1H), 6.81 (t, J = 9.0 and 8.4 Hz, 3H), 7.02 (dd, J = 8.9 and 8.5 Hz, 3H), 7.18 (t, J = 7.4 Hz, 2H), 7.56 (t, J = 7.7 Hz, 1H), 7.72 (t, J = 7.7 Hz, 1H), 8.27 (dt, J = 8.4 and 6.1 Hz, 4H), 8.42 (d, J = 6.2 Hz, 1H), 8.54 (d, J = 6 Hz, 1H), 8.68 (d, J = 6.2 Hz, 1H), 8.82 (d, J = 6 Hz, 1H), 9.37 (s, 1H), 9.43 (s, 1H). Anal. ($C_{39}H_{37}N_5O_6S_2$) C, H, N.

1-[(*S*)-*N*,*O*-Bis(isoquinolinesulfonyl)-*N*-methyltyrosy]-4-(2-methoxyphenyl)piperazine (77). Following general procedure C, this product was obtained as a yellow solid: [α] = −29.8, c = 0.45% in $CHCl_3$. ^{1}H NMR ($CDCl_3$) δ: 2.51 (dd, J = 12.6 and 6.2 Hz, 1H), 2.62 (m, 1H), 2.87 (m, 2H), 3.07 (s, 3H), 3.22 (m, 2H), 3.56 (m, 4H), 3.87 (s, 3H), 5.15 (dd, J = 13.6 and 6 Hz, 1H), 6.76 (d, J = 8.6 Hz, 2H), 6.97 (d, J = 7.9 Hz, 4H), 7.30 (m, 1H), 7.63 (m, 3H), 8.11 (d, J = 8.1 Hz, 1H), 8.27 (d, J = 7.3 Hz, 3H), 8.44 (m, 1H), 8.54 (d, J = 6 Hz, 1H), 8.75 (m, 2H), 9.40 (m, 2H). Anal. ($C_{39}H_{37}N_5O_7S_2$) C, H, N.

1-[(*S*)-*N*,*O*-Bis(isoquinolinesulfonyl)-*N*-methyltyrosyl]-4-(3-chlorophenyl)piperazine (78). Following general procedure C, this product was obtained as a yellow solid: [α] = −58.8, c = 0.6% in $CHCl_3$. ^{1}H NMR ($CDCl_3$) δ: 2.42 (m, 2H), 2.99 (s, 3H), 3.22 (m, 4H), 3.59 (m, 4H), 5.12 (dd, J = 12 and 6.2 Hz, 1H), 6.79 (m, 4H), 6.89 (m, 3H), 7.21 (t, J = 8 Hz, 1H), 7.54 (t, J = 8 Hz, 1H), 7.72 (t, J = 8 Hz, 1H), 8.26 (m, 4H), 8.42 (d, J = 6.2 Hz, 1H), 8.51 (d, J = 6.2 Hz, 1H), 8.68 (d, J = 6.2 Hz, 1H), 8.81 (d, J = 6.2 Hz, 1H), 9.37 (s, 1H), 9.42 (s, 1H). Anal. ($C_{38}H_{34}N_5O_6S_2Cl$) C, H, N.

1-[(*S*)-*N*,*O*-Bis(isoquinolinesulfonyl)-*N*-methyltyrosyl]-4-(3-trifluoromethylphenyl)piperazine (79). Following general procedure C, this product was obtained as a yellow solid: [α] = −61.5, c = 0.4% in $CHCl_3$. ^{1}H NMR ($CDCl_3$) δ: 2.48 (dd, J = 12 and 4.4 Hz, 1H), 2.75 (m, 1H), 2.93 (m, 5H), 3.30 (m, 2H), 3.62 (m, 4H), 5.11 (dd, J = 6.6 and 6.2 Hz, 1H), 6.78 (d, J = 8.6 Hz, 2H), 6.99 (d, J = 8.6 Hz, 4H), 7.15 (d, J = 7.8 Hz, 1H), 7.40 (t, J = 8.8 Hz, 1H), 7.55 (t, J = 7.8 Hz, 1H), 7.72 (t, J = 7.7 Hz, 1H), 8.23 (m, 4H), 8.43 (d, J = 6 Hz, 1H), 8.51 (d, J = 6.3 Hz, 1H), 8.68 (d, J = 6.4 Hz 1H), 8.80 (d, J = 6 Hz 1H), 9.36 (s, 1H), 9.41 (s, 1H). Anal. ($C_{39}H_{34}N_5O_6S_2F_3$) C, H, N.

1-[(*S*)-*N*,*O*-Bis(isoquinolinesulfonyl)-*N*-methyltyrosyl]-4-(*o*,*m*-xylyl)piperazine (80). Following general procedure C, this product was obtained as a brown solid: [α] = −17.5, c = 1.91% in CH_2Cl_2. ^{1}H NMR ($CDCl_3$) δ: 2.19 (s, 3H), 2.27 (s, 3H), 2.49 (dd, J = 12.4 and 4.6 Hz, 1H), 2.68 (m, 3H), 3.06 (s, 3H), 3.25 (m, 2H), 3.58 (m, 4H), 5.12 (dd, J = 10.3 and 4.5 Hz, 1H), 6.76 (t, J = 8.7 Hz, 2H), 6.84 (d, J = 7.2 Hz, 1H), 7.03 (t, J = 8.7 Hz, 2H), 7.12 (t, J = 8.7 Hz, 2H), 7.56 (t, J = 7.7 Hz, 1H), 7.71 (t, J = 7.7 Hz, 1H), 8.26 (m, 4H), 8.44 (d, J = 6.1 Hz, 1H), 8.54 (d, J = 6.2 Hz, 1H), 8.68 (d, J = 6.2 Hz, 1H), 8.82 (d, J = 6 Hz, 1H), 9.36 (s, 1H), 9.42 (s, 1H). Anal. ($C_{40}H_{39}N_5O_6S_2$) C, H, N.

1-[(*S*)-*N*,*O*-Bis(isoquinolinesulfonyl)-*N*-methyltyrosy]l-4-(3,4-dichlorophenyl)piperazine (81). Following general procedure C, this product was obtained as a yellow solid: [α] = −73.4, c = 0.44% in $CHCl_3$. ^{1}H NMR ($CDCl_3$) δ: 2.96 (m, 2H), 3.02 (s, 3H), 3.41 (m, 4H), 3.63 (m, 4H), 5.16 (dd, J = 13.6 and 6 Hz, 1H), 6.80 (m, 4H), 6.94 (t, J = 8.4 Hz, 3H), 7.57 (t, J = 8 Hz, 1H), 7.72 (t, J = 8 Hz, 1H), 8.22 (m, 4H), 8.44 (d, J = 6 Hz, 1H), 8.52 (d, J = 6 Hz, 1H), 8.66 (d, J = 6 Hz, 1H), 8.81 (d, J = 6 Hz, 1H), 9.37 (s, 1H), 9.42 (s, 1H). Anal. ($C_{38}H_{33}N_5O_6S_2Cl_2$) C, H, N.

1-[(*S*)-*N*,*O*-Bis(isoquinolinesulfonyl)-*N*-methyltyrosyl]-4-pyridin-2-ylpiperazine (82). Following general procedure C, this product was obtained as a yellow solid: [α] = −55.8, c = 0.41% in CH_2Cl_2. ^{1}H NMR ($CDCl_3$) δ: 2.46 (dd, J = 12.7 and 4.3 Hz, 1H), 2.91 (m, 1H), 3.05 (s, 3H), 3.21 (t, J = 10.6 Hz, 3H), 3.42 (m, 3H), 5.09 (dd, J = 10.4 and 4.4 Hz, 1H), 6.60 (d, J = 8.6 Hz, 1H), 6.70 (d, J = 8.4 Hz, 3H), 6.96 (d, J = 8.4 Hz, 2H), 7.53 (m, 2H), 7.70 (t, J = 7.8 Hz, 1H), 8.12 (d, J = 7.3 Hz, 1H), 8.22 (m, 3H), 8.30 (d, J = 7.3 Hz, 1H), 8.41 (d, J = 6.2 Hz, 1H), 8.51 (d, J = 6.2 Hz, 1H), 8.67 (d, J = 6.3 Hz, 1H), 8.80 (d, J = 6.1 Hz, 1H), 9.35 (s, 1H), 9.40 (s, 1H). Anal. ($C_{37}H_{34}N_6O_6S_2$) C, H, N.

1-[(*S*)-*N*,*O*-Bis(isoquinolinesulfonyl)-*N*-methyltyrosyl]-4-pyrimidin-2-ylpiperazine (83). Following general procedure C, this product was obtained as a yellow solid: [α] = −54.6, c = 0.76% in CH_2Cl_2. ^{1}H NMR ($CDCl_3$) δ: 2.46 (dd, J = 12.7 and 4.3 Hz, 1H), 3.00 (m, 1H), 3.06 (s, 3H), 3.41 (m, 6H), 3.80 (m, 2H), 5.09 (dd, J = 10.4 and 4.4 Hz, 1H), 6.56 (t, J = 8.6 Hz, 1H), 6.74 (d, J = 8.5 Hz, 2H), 6.96 (d, J = 8.5 Hz, 2H), 7.57 (t, J = 7.8 Hz, 1H), 7.70 (t, J = 7.8 Hz, 1H), 8.21 (t, J = 7.6 Hz, 4H), 8.33 (m, 2H), 8.40 (d, J = 6.3 Hz, 1H), 8.52 (d, J = 6.1 Hz, 1H), 8.67 (d, J = 6 Hz, 1H), 8.82 (d, J = 6 Hz, 1H), 9.35 (s, 1H), 9.41 (s, 1H). Anal. ($C_{36}H_{33}N_7O_6S_2$) C, H, N.

1-[(*S*)-*N*,*O*-Bis(isoquinolinesulfonyl)-*N*-methyltyrosyl]-4-benzylpiperidine (84). Following general procedure C, this product was obtained as a yellow solid: [α] = −24, c = 0.42% in CH_2Cl_2. ^{1}H NMR ($CDCl_3$) δ: 1.50 (m, 2H), 2.39 (m, 6H), 2.70 (m, 1H), 3.04 (s, 3H), 3.25 (m, 2H), 3.80 (m, 1H), 4.40 (m, 1H), 5.10 (m, 1H), 6.73 (m, 1H), 6.88 (d, J = 8.6 Hz, 2H), 7.05 (m, 3H), 7.24 (m, 3H), 7.68 (m, 2H), 8.26 (m, 4H), 8.41 (d, J = 6.1 Hz, 1H), 8.56 (d, J = 6.2 Hz, 1H), 8.68 (d, J = 6.2 Hz, 1H), 8.82 (d, J = 6 Hz, 1H), 9.35 (s, 1H), 9.43 (s, 1H). Anal. ($C_{40}H_{38}N_4O_6S_2$) C, H, N.

1-[(*S*)-*N*,*O*-Bis(isoquinolinesulfonyl)tyrosyl]-4-(4-fluorophenyl)piperazine (85). Following general procedure C, this product was obtained as a yellow solid: [α] = +47.7, c =

0.62% in CH_2Cl_2. ^1H NMR ($CDCl_3$) δ: 2.22 (m, 2H), 2.50 (m, 4H), 3.06 (m, 3H), 3.25 (m, 1H), 4.35 (m, 1H), 5.99 (d, 1H), 6.65 (d, J = 8.4 Hz, 2H), 6.78 (m, 2H), 6.88 (d, J = 8.5 Hz, 2H), 6.99 (t, J = 8.5 Hz, 2H), 7.58 (m, 2H), 8.16 (t, J = 8 Hz, 2H), 8.27 (t, J = 6.5 Hz, 3H), 8.51 (d, J = 6.3 Hz, 1H), 8.70 (d, J = 6.3 Hz, 1H), 8.82 (d, J = 6 Hz, 1H), 9.30 (s, 1H), 9.41 (s, 1H). Anal. ($C_{37}H_{32}N_5O_6S_2F$) C, H, N.

1-[(*S*)-*N*,*O*-Bis(isoquinolinesulfonyl)tyrosyl]-4-*o*-tolylpiperazine (86). Following general procedure C, this product was obtained as a yellow solid: [α] = +80.3, c = 1% in CH_2Cl_2. ^1H NMR ($CDCl_3$) δ: 2.24 (s, 3H), 2.36 (m, 3H), 2.54 (m, 1H), 2.80 (d, J = 7.2 Hz, 2H), 2.93 (m, 1H), 3.06 (m, 1H), 3.25 (m, 2H), 4.35 (t, J = 7.5 Hz, 1H), 6.09 (d, J = 9.1 Hz, 1H), 6.4 (d, J = 8.3 Hz, 2H), 6.85 (dd, J = 8.6 and 7.8 Hz, 3H), 7.03 (t, J = 7.4 Hz, 1H), 7.18 (m, 2H), 7.55 (t, J = 7.9 Hz, 1H), 7.63 (t, J = 7.7 Hz, 1H), 8.24 (d, J = 7.5 Hz, 5H), 8.52 (d, J = 6 Hz, 1H), 8.69 (d, J = 6.2 Hz, 1H), 8.82 (d, J = 6 Hz, 2H), 9.33 (s, 1H), 9.41 (s, 1H). Anal. ($C_{38}H_{35}N_5O_6S_2$) C, H, N.

1-[(*S*)-*N*,*O*-Bis(isoquinolinesulfonyl)-*N*-methyltyrosyl]-4-(4-aminophenyl)piperazine (87). Following general procedure C, this product was obtained as a yellow solid: [α] = −48.4, c = 0.90% in $CHCl_3$. ^1H NMR ($CDCl_3$) δ: 2.48 (m, 2H), 2.83 (m, 4H), 3.16 (s, 3H), 3.52 (m, 4H), 4.00 (m, 2H), 5.11 (dd, J = 6.6 and 6.2 Hz, 1H), 6.28 (d, J = 8.3 Hz, 2H), 6.34 (d, J = 8.3 Hz, 2H), 6.76 (d, J = 8.4 Hz, 2H), 6.96 (d, J = 8.4 Hz, 2H), 7.51 (d, J = 8 Hz, 1H), 7.69 (t, J = 8 Hz, 1H), 8.23 (m, 4H), 8.41 (d, J = 6.2 Hz, 1H), 8.51 (d, J = 6.2 Hz, 1H), 8.67 (d, J = 6.2 Hz, 1H), 8.80 (d, J = 6.2 Hz, 1H), 9.35 (s, 1H), 9.40 (s, 1H). Anal. ($C_{38}H_{36}N_6O_6S_2$) C, H, N.

Biological Materials and Methods. General Procedures. Cell Cultures. Human monocytes were isolated from buffy coats by gradient on a Ficoll (Ficoll-Paque, Research Grade, Amersham Pharmacia Biotech AB, Cologno Monzese, Italy) and by adherence on plastic Petri dishes as described by Colotta et al.[32] After isolation, cells were kept in culture for 5 days in RPMI 1640 medium containing 2 mM glutamine, 5% human serum, 100 U/mlL penicillin, and 100 mg/mL streptomicyn. Experiments were performed in saline solution containing 125 mM NaCl, 5 mM KCl, 1 mM $MgSO_4$, 1 mM Na_2PO_4, 5.5 mM glucose, 5 mM $NaHCO_3$, 1 mM $CaCl_2$, and 20 mM HEPES (pH 7.4). HEK293 cells were cultured in DME/F-12, 1:1, medium (Sigma, St. Louis, MO) containing 15% heat-inactivated FCS (Life Technologies, Paisley, Scotland), 100 U/mL penicillin, and 100 μg/mL streptomycin. Stable clones were cultured in the same medium containing G418 sulfate (Geneticin) (Calbiochem, La Jolla, CA) at 0.2 mg/mL. Visualization of transfected cells was performed in a saline solution (standard saline solution) containing 125 mM NaCl, 5 mM KCl, 1 mM $MgSO_4$, 1 mM Na_2HPO_4, 5.5 mM glucose, 5 mM $NaHCO_3$, 1 mM $CaCl_2$, and 20 mM HEPES (pH 7.4).

Changes in Plasma Membrane Permeability. ATP-dependent increases in plasma membrane permeability were measured with the extracellular fluorescent tracer ethidium bromide (Molecular Probes, Inc., Eugene, OR). For ethidium bromide uptake, cells were incubated in a thermostat-controlled fluorometer cuvette (37 °C) for 20 min in the dark at a concentration of 10^6 cells/mL in the presence of 20 μM ethidium bromide and challenged with 1 mM ATP. The cell suspension was incubated with KN62 or with the synthesized compounds (10−1000 nM) for 5 min at 37 °C before fluorometric analysis in a stirred cuvette at 37 °C in a fluorometer (model LS50, Perkin-Elmer Ltd., Beaconsfield, U.K.) equipped with magnetic stirring and temperature control. Fluorescence changes were monitored at the wavelength pair 360/580 nm. Cells were also analyzed with an inverted fluorescence microscope (Olympus IMT-2, Olympus Optical Co. Ltd., Tokyo, Japan) for qualitative assesment of ethidium bromide uptake. All experiments were repeated three times.

Ca^{2+} Measurements. Changes in Ca^{2+} were measured with the fluorescent indicator fura-2/AM (Molecular Probes, Inc., Eugene, OR) as described previously.[32] Briefly, cells were loaded with 4 mM of fura-2/AM and incubated in a thermostat-controlled (37 °C) and magnetically stirred fluorometer cuvette (model LS50, Perkin-Elmer Ltd., Beaconsfield, U.K.). Intracellular Ca^{2+} concentration was determined with the 340/380 excitation ratio at an emission wavelength of 500 nM. All experiments were repeated three times.

Cytokine Release. IL-1β release was measured in macrophage monolayers primed for 2 h with bacterial endotoxin (lipopolysaccharide, LPS) at 10 μg/mL and was stimulated with 3 mM ATP for 30 min. Inhibitors, when used, were added 5 min prior to ATP. Supernatants were centrifuged for 5 min at 900*g* to remove floating cells and were assayed for IL-1β content by ELISA (R&D Systems, Minneapolis, MN).

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