From Tyrosine to Glycine: Synthesis and Biological Activity of Potent Antagonists of the Purinergic $P2X_7$ Receptor

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The characterization of the native and recombinant $P2X_7$ receptor continues to be hindered by the lack of specific and subtype-selective antagonists with a "druglike" profile. However, a tyrosine derivative named KN-62 exhibits selective $P2X_7$ receptor-blocking properties. As a molecular simplification of KN-62, the present study was designed to evaluate the functional antagonistic properties of a novel series of glycine derivatives characterized by the presence of different phenyl-substituted piperazine moieties. Antagonistic activity of these glycine derivatives was tested on HEK293 cells transfected with the human $P2X_7$ receptor. The most potent $P2X_7$ receptor antagonist identified in this study (compound **4g**) contains an *o*-fluorine substituent on the phenylpiperazine moiety and had an IC_{50} of 12.1 nM. The biological responses investigated were ATP-dependent Ca^{2+} influx across the plasma membrane and ethidium bromide uptake.

Introduction

The $P2X_7$ receptor is an ATP-sensitive, ligand-gated ion channel that, like other members of the P2X family, mediates a nonselective cation conductance when stimulated with an appropriate ligand.[1] Currently, seven different P2X receptor subtypes have been molecularly defined and the $P2X_7$ receptor seems to be the most divergent member of this family in terms of molecular structure, pharmacology, and function.[1] The structural features that more typically differentiate $P2X_7$ from the other members of the P2X subfamily are its size (595 amino acids), its long cytoplasmic carboxyterminal tail (242 residues),[2] and its peculiar ability to form a nonselective plasma membrane pore of 3−5 nm size when exposed to prolonged or repeated agonist stimulation.[3,4] The "large pore" properties of this receptor are usually verified by uptake of fluorescent dyes such as ethidium and YoPro1[a] (4-[(3-methyl-2-(3*H*)-benzoxazolylidene)methyl]-1-[3-(triethylammonio)propyl] diiodide).[1] The mechanisms underlying the process of channel dilation were originally thought to depend on the C-terminus region of the $P2X_7$ receptor.[2] However, emerging evidence indicates that distinct proteins, activated by $P2X_7$ receptors, may play a key role in the dye uptake signal.[5,6] Recent work has identified panx1 as the large pore pathway activated by $P2X_7$ receptors and as an upstream molecule essential for the activation of the inflammatory cascade triggered by activation of this purinergic subtype.[7] Opening of the $P2X_7$ pore allows plasma membrane fluxes of ions as well as of hydrophilic molecules with molecular masses up to 900 Da.[8−10]

Since their discovery in immune cells, $P2X_7$ receptors have been proposed as mediators of inflammation.[11] In particular, $P2X_7$ receptors are found in most immune cells of the periphery and the brain, where their activation leads to multiple downstream events such as cell permeabilization, apoptosis, and/or cytokine release.[12−14] In the case of prolonged stimulation, cell death occurs through necrotic or apoptotic pathways.[8,15,16] Interestingly, $P2X_7$ receptors are rapidly up-regulated and activated after inflammatory insults.[17]

However, despite its selective expression in immunocytes, $P2X_7$ is also present in cells not primarily participating in immunomodulation, such as presynaptic terminals in the central and peripheral nervous system[18] and human epidermal Langerhans cells.[19]

On glial cells, the $P2X_7$ receptor has been shown to mediate release of glutamate, which is known to be involved in the neurotransmission of painful sensory signals.[20] Up-regulation of the $P2X_7$ receptor, most likely on activated microglia, was reported in association with ischemic damage and necrosis by occlusion of rat middle cerebral artery.[21] Recent studies indicate a role of the $P2X_7$ receptor in the generation of superoxide in microglia around β-amyloid plaques in a transgenic model for Alzheimer's disease[22a] and in multiple sclerosis lesions from autopsy brain sections. [22b]

These features make desirable $P2X_7$ antagonists that can be efficiently used in preventing, treating, or ameliorating a variety of pain states (e.g., chronic inflammatory pain and neuropathic pain), inflammatory processes, and neurodegenerative conditions (e.g., stroke and Alzheimer's disease).

Among these, a tyrosine derivative named KN-62 (1-(*N*,*O*-bis(1,5-isoquinolinesulfonyl)-*N*-methyl-L-tyrosyl)-4-phenylpiperazine, compound **1a,** Chart 1) has been considered for several years to be one of the most potent antagonists for the human $P2X_7$ receptors with an IC_{50} of 51 nM.[23−25]

In connection with the attempt to improve the activity and to study the structure−activity relationship of KN-62 inhibition

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[a] Abbreviations: Boc, *tert*-butyloxycarbonyl; Cbz, benzyloxycarbonyl; EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodimide hydrochloride; HEK, human embryonic kidney; HOBt, 1-hydroxybenzotriazole; KN-62, 1-[*N*,*O*-bis-(5-isoquinolinesulfonyl)-*N*-methyl-L-tyrosyl]-4-phenylpiperazine; Pd/C, palladium on activated charcoal; TFA, trifluoroacetic acid; YOPRO-1, quinolinium, 4-[(3-methyl-2-(3*H*)-benzoxazolylidene)methyl]-1-[3-(triethylammonio)propyl] diiodide. These abbreviations and symbols are used in addition to those by the IUPAC-IUB Commission on Biochemical Nomenclature (*J. Biol. Chem.* **1985**, *260*, 14-42).

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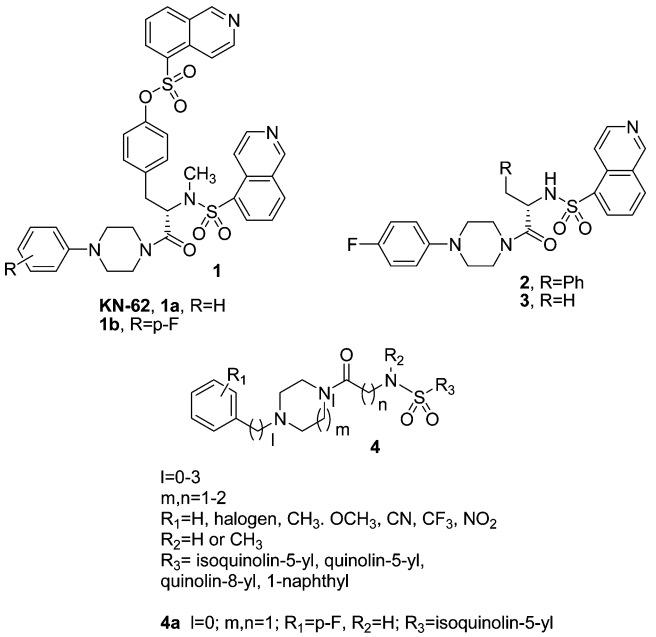
Chart 1. Chemical Structures of L-Tyrosine (**1a** and **1b**), L-Phenylalanine (**2**), L-Alanine (**3**), and Glycine (**4**) Derivatives



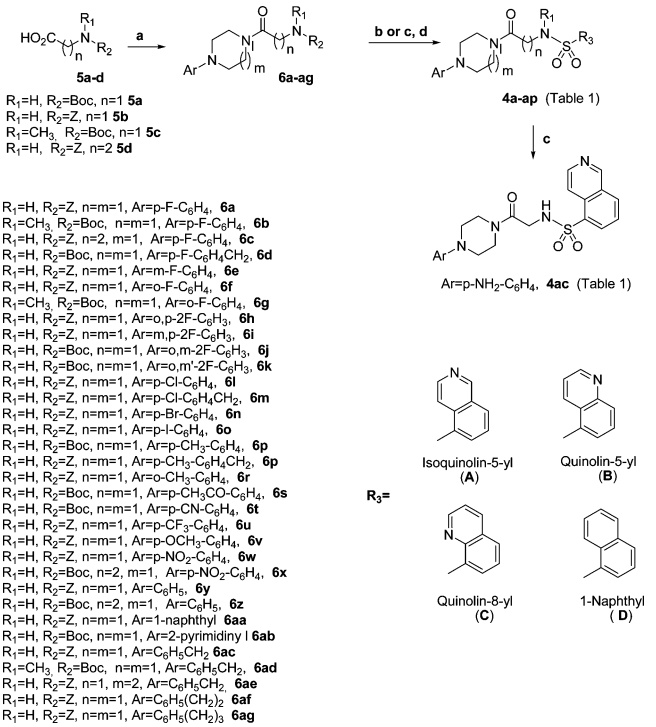
on the $P2X_7$ receptor, our group has prepared a novel series of KN-62-related compounds with general structure **1** and described their functional antagonistic properties, focusing our attention on the systematic modification of the phenylpiperazine residue.[26] In this series, the *p*-fluorophenyl derivative was the most potent compound (**1b**, $IC_{50} = 6.3$ nM), found to be 8-fold more potent than KN-62. The potency of this latter derivative was maintained by removing the methyl group linked to the nitrogen on the α-position of the tyrosine moiety. This was confirmed by the preparation of another series of tyrosine derivatives synthesized by Jacobson's group.[27]

Tyrosine derivatives with general formula **1** do not represent ideal candidates for the discovery of oral drugs because of their high molecular weight (>700 Da), high lipophilicity (clogP > 6), and the presence of metabolically labile sulfonate groups. However, the lack of actvity of the L-phenylalaninylsulfonamido derivative **2** suggests the importance of sulfonate for the $P2X_7$ antagonism. Because of our efforts to synthesize new $P2X_7$ receptor antagonists with druglike properties, by removing the phenyl sulfonate ring to yield the L-alanine derivative **3**, the activity was dramatically reduced. Surprisingly, the replacement of the amino acid L-tyrosine with glycine allowed us to synthesize a new series of compounds with general formula **4**, active at nanomolar concentration and with acceptable molecular weight. In particular, compound **4a** was equipotent with KN-62, although 10-fold less potent than compound **1b**.

Compound **4a** then became the starting point for the synthesis of a series of derivatives with general structure **4**, in which four different positions of the aminoacid glycine were systematically modified in the structure. A large series of compounds were synthesized in order to study the effects of the insertion of electron-donating and electron-withdrawing groups on the phenyl ring linked to the piperazine nitrogen. These modifications served to alter the electronic, steric, and lipo/hydrophilic features of the aromatic portion joined to the piperazine. By synthesis of a small series of sarcosine derivatives (R_2 = Me; **4b**, **4h**, and **4ak**), we were able to evaluated the importance of the presence of a methyl on the α-nitrogen of glycine.

We also evaluated the antagonistic effect of the replacement of the *N*-isoquinoline-5-sulfonyl group in the compound with

Scheme 1[a]



[a] Reagents: (a) arylpiperazine, EDCl, HOBt, DMF, room temp, 18 h; (b) TFA, CH_2Cl_2, room temp; (c) 10% Pd/C, EtOH, or TFA, CH_2Cl_2, room temp; (d) $ArSO_2Cl$, TEA, CH_2Cl_2, room temp.

different arylsulfonyl moieties, corresponding to quinoline-5- and quinoline-8-sulfonyl functions, and with the naphthalene-1-sulfonyl group. Finally, we prepared a small series of homologues of glycine ($n = 2$) corresponding to the β-alanine derivatives **4c**, **4d**, **4ab**, and **4ae**.

Chemistry

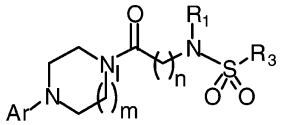
Compounds with general structure **4** were prepared using *N*-Boc-glycine (**5a**), *N*-Cbz-glycine (**5b**), *N*-Boc-sarcosine (**5c**), and *N*-Cbz-β-alanine (**5d**) as starting materials, following the synthetic sequence outlined in Scheme 1. These amino-protected precursors (**5a**−**d**) were converted to activated 1-hydroxy-1,2,3-benzotriazole (HOBt) esters with HOBt and 1-(3-dimethylaminopropyl)-3-ethylcarbodimide hydrochloride (EDC) and coupled with the appropriate commercially available *N*-arylpiperazines[28] to give amides with general formula **6** in good yields. The protecting *tert*-butyloxycarbonyl (Boc) groups were conveniently removed using trifluoroacetic acid (TFA), while the benzyloxycarbonyl functions (Cbz or Z) were cleaved off using a catalytic hydrogenation with 10% palladium on activated charcoal (Pd/C), furnishing the corresponding free amines. These were then coupled with an excess of arylsulfonyl chloride[29] in CH_2Cl_2 in the presence of triethylamine to furnish the respective sulfonamides **4a**−**ap** in acceptable yields. Compound **4ac** was obtained by a reductive hydrogenation of **4aa** in the presence of 10% Pd/C.

The structures of the synthesized compounds **4a**−**ap** and the yields of the syntheses are presented in Table 1.

Results and Discussion

The potency of derivatives with general formula **4** in the inhibition of ATP-stimulated Ca^{2+} influx and ethidium bromide uptake in HEK293 cells transfected with the human $P2X_7$ receptor (HEK293-$P2X_7$) was investigated. Figure 1 shows typical traces of ATP-dependent calcium increase in HEK 293-

Table 1. Physical and Synthetic Data of Novel Glycine Analogues



compd	Ar	R_1	R_3	*n*	*m*	mp, °C	yield,[a] %	formula[b]	anal.
4a	*p*-F-C_6H_4	H	A	1	1	71−73	62	$C_{21}H_{21}N_4O_3SF$	C, H, N
4b	*p*-F-C_6H_4	Me	A	1	1	58−60	64	$C_{22}H_{23}N_4O_3SF$	C, H, N
4c	*p*-F-C_6H_4	H	A	2	1	73−75	58	$C_{22}H_{23}N_4O_3SF$	C, H, N
4d	*p*-F-C_6H_4	H	D	2	1	63−65	65	$C_{22}H_{22}N_3O_3SF$	C, H, N
4e	*p*-F-$C_6H_4CH_2$	H	A	1	1	50−51	61	$C_{22}H_{23}N_4O_3SF$	C, H, N
4f	*m*-F-C_6H_4	H	A	1	1	68−70	51	$C_{21}H_{21}N_4O_3SF$	C, H, N
4g	*o*-F-C_6H_4	H	A	1	1	67−69	69	$C_{21}H_{21}N_4O_3SF$	C, H, N
4h	*o*-F-C_6H_4	Me	A	1	1	51−52	63	$C_{22}H_{23}N_4O_3SF$	C, H, N
4i	*o*,*p*-2F-C_6H_3	H	A	1	1	186−187	55	$C_{21}H_{20}N_4O_3SF_2$	C, H, N
4j	*m*,*p*-2F-C_6H_3	H	A	1	1	73−75	55	$C_{21}H_{20}N_4O_3SF_2$	C, H, N
4k	*o*,*m*-2F-C_6H_3	H	A	1	1	202−204	57	$C_{21}H_{20}N_4O_3SF_2$	C, H, N
4l	*o*,*m*′-2F-C_6H_3	H	A	1	1	215−217	48	$C_{21}H_{20}N_4O_3SF_2$	C, H, N
4m	*o*-F-C_6H_4	H	B	1	1	62−63	54	$C_{21}H_{21}N_4O_3SF$	C, H, N
4n	*o*-F-C_6H_4	H	C	1	1	52−53	53	$C_{21}H_{21}N_4O_3SF$	C, H, N
4o	*o*-F-C_6H_4	H	D	1	1	68−70	73	$C_{22}H_{22}N_3O_3SF$	C, H, N
4p	*p*-Cl-C_6H_4	H	A	1	1	70−71	58	$C_{21}H_{21}N_4O_3SCl$	C, H, N
4q	*p*-Cl-$C_6H_4CH_2$	H	A	1	1	68−70	54	$C_{22}H_{23}N_4O_3SCl$	C, H, N
4r	*p*-Br-C_6H_4	H	A	1	1	103−15	58	$C_{21}H_{21}N_4O_3SBr$	C, H, N
4s	*p*-I-C_6H_4	H	A	1	1	202−205	52	$C_{21}H_{21}N_4O_3SI$	C, H, N
4t	*p*-CH_3-C_6H_4	H	A	1	1	73−75	58	$C_{22}H_{24}N_4O_3S$	C, H, N
4u	*p*-CH_3-$C_6H_4CH_2$	H	A	1	1	146−148	63	$C_{23}H_{26}N_4O_3S$	C, H, N
4v	*o*-CH_3-C_6H_4	H	A	1	1	153−155	52	$C_{22}H_{24}N_4O_3S$	C, H, N
4w	*p*-CH_3CO-C_6H_4	H	A	1	1	153−155	54	$C_{23}H_{24}N_4O_4S$	C, H, N
4x	*p*-CN-C_6H_4	H	A	1	1	52−53	54	$C_{22}H_{21}N_5O_3S$	C, H, N
4y	*p*-CF_3-C_6H_4	H	A	1	1	88−90	49	$C_{22}H_{21}N_4O_3SF_3$	C, H, N
4z	*p*-OCH_3-C_6H_4	H	A	1	1	76−78	67	$C_{22}H_{24}N_4O_4S$	C, H, N
4aa	*p*-NO_2-C_6H_4	H	A	1	1	170−172	68	$C_{21}H_{21}N_5O_5SF$	C, H, N
4ab	*p*-NO_2-C_6H_4	H	A	2	1	72−73	52	$C_{22}H_{23}N_5O_5S$	C, H, N
4ac	*p*-NH_2-C_6H_4	H	A	1	1	93−95	77	$C_{21}H_{23}N_5O_3S$	C, H, N
4ad	C_6H_5	H	A	1	1	63−65	44	$C_{21}H_{22}N_4O_3S$	C, H, N
4ae	C_6H_5	H	A	2	1	178−180	43	$C_{22}H_{24}N_4O_3S$	C, H, N
4af	1-naphthyl	H	A	1	1	203−205	60	$C_{25}H_{24}N_4O_3S$	C, H, N
4ag	C_6H_5	H	D	1	1	58−60	52	$C_{22}H_{23}N_3O_3S$	C, H, N
4ah	2-pyrimidinyl	H	A	1	1	185−187	52	$C_{19}H_{20}N_6O_3S$	C, H, N
4ai	1-naphthyl	H	D	1	1	86−88	61	$C_{26}H_{25}N_3O_3S$	C, H, N
4aj	$C_6H_5CH_2$	H	A	1	1	202−204	62	$C_{22}H_{24}N_4O_3S$	C, H, N
4ak	$C_6H_5CH_2$	Me	A	1	1	53−55	52	$C_{23}H_{26}N_4O_3S$	C, H, N
4al	$C_6H_5CH_2$	H	A	1	2	58−60	57	$C_{23}H_{26}N_4O_3S$	C, H, N
4am	$C_6H_5CH_2$	H	D	1	1	52−53	54	$C_{23}H_{25}N_3O_3S$	C, H, N
4an	$C_6H_5(CH_2)_2$	H	A	1	1	53−55	46	$C_{23}H_{26}N_4O_3S$	C, H, N
4ao	$C_6H_5(CH_2)_2$	H	D	1	1	50−52	51	$C_{24}H_{27}N_3O_3S$	C, H, N
4ap	$C_6H_5(CH_2)_3$	H	A	1	1	72−74	57	$C_{24}H_{28}N_4O_3S$	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 the theoretical values.

$P2X_7$ cells in the absence and in the presence of **4g** (see below). Results of experiments similar to the one shown in Figure 1, performed with a large number of compounds synthesized in our laboratory, are summarized in Table 2. Compound **4g** was the most active $P2X_7$ receptor antagonist identified in this series, and several compounds (i.e., **4w**, **4aa**, **4aj**, and **4an**) showed an antagonistic activity more potent than KN-62. Figure 2 displays the inhibition curves of the four most potent compounds (**4g**, **4aa**, **4aj**, and **4an**).

Compounds **4g** and **4aa** were also evaluated for selectivity at other P2 receptors ($P2X_1$, $P2X_2$, and $P2X_4$), where they were not found to have significant interactions up to 10 μM.

Initial SAR studies of **4a**−**ap** focused on examining of the effects on $P2X_7$ inhibition induced by changes in the phenylpiperazine portion. As shown in Table 1, the replacement of the fluoro group with a hydrogen (**4ad**) gave an inactive compound. Also, the replacement of the phenyl ring with a pyrimidine (compound **4ah**) decreased the activity dramatically. Some activity was regained with a more bulky 1-naphthyl group (compound **4af**), which was 1.5-fold less potent then KN-62.

The compounds that possessed different substituents at the para position of the phenyl ring showed variable potencies. The derivative **4aa**, characterized by the presence of a nitro group, was the most potent compound of the series, being 3-fold more potent than KN-62. In the series of para-halogenated analogues, only the *p*-fluoro derivative **4a** was active. The increase of the size of the halogen atom from chlorine (**4p**) to bromine (**4r**) and finally to iodine (**4s**) caused a loss of potency. Starting from compound **4a**, the placement of the fluoro group in the meta position (compound **4f**) diminished the activity, while the ortho position substitution (compound **4g**) was beneficial for the activity. This latter compound was 5-fold more potent than KN-62.

Compounds **4a** and **4g** represented the starting point for the synthesis of a large series of derivatives in which we introduced two fluorine atoms in different positions on the phenyl ring. In contrast to the *o*- and *p*-fluorophenyl substitutions, none of the difluoro substituted analogues (**4i**−**l**) yielded encouraging levels of activity. The dramatic reduction of activity due to the insertion of a second fluoro group in the phenyl ring could be attributed

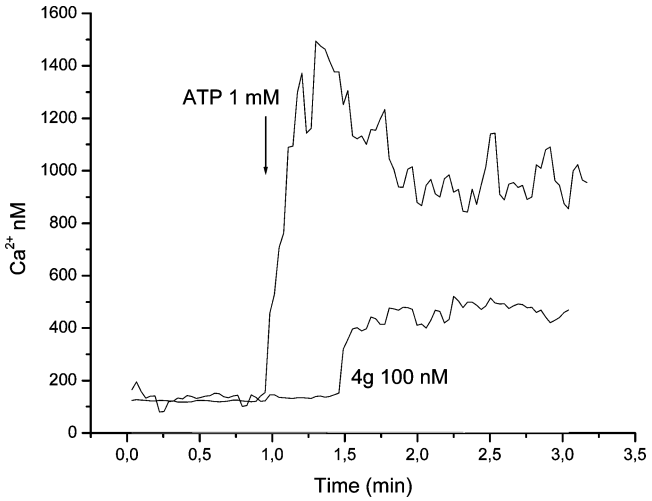


Figure 1. Typical traces that visualize stimulation of cytoplasmic Ca^{2+} increase in $P2X_7$-transfected HEK 293 cells by 1 mM ATP in the absence and in the presence of compound **4g**. Cells were resuspended in the fluorometer cuvette in the saline solution described in Chemical Materials and Methods at a concentration of 10^6 cells/mL. The P2X7 antagonist was added at a concentration of 100 nM 10 min prior to ATP.

Table 2. Activities of Synthesized Compounds with General Formula **6** on the Calcium Influx in HEK 293-$P2X_7$ Cells[a]

compd	$IC_{50} \pm$ SE (nM)	compd	$IC_{50} \pm$ SE (nM)
KN-62	51.1 ± 1.1[26]	**4v**	>1000
4a	60.0 ± 5.2	**4w**	40.2 ± 5.1
4b	>1000	**4x**	>1000
4c	>1000	**4y**	>1000
4d	>1000	**4z**	>1000
4e	>1000	**4aa**	21.1 ± 2.0
4f	>1000	**4ab**	>1000
4g	12.1 ± 3.0	**4ac**	>1000
4h	>1000	**4ad**	>1000
4i	>1000	**4ae**	800 ± 10
4j	>1000	**4af**	90.2 ± 5.1
4k	>1000	**4ag**	>1000
4l	>1000	**4ah**	>1000
4m	>1000	**4ai**	>1000
4n	>1000	**4aj**	35.1 ± 3.0
4o	>1000	**4ak**	100 ± 11
4p	>1000	**4al**	900 ± 7
4q	>1000	**4am**	>1000
4r	>1000	**4an**	40.0 ± 3.1
4s	>1000	**4ao**	>1000
4t	130 ± 10	**4ap**	>1000
4u	>1000		

[a] IC_{50} is the 50% inhibitory concentration representing the mean from dose response curves of at least five experiments. All experiments were repeated five times.

to steric and electronic factors, which may have prevented the interaction of the difluorophenyl residue with the receptor.

Replacing the *p*-fluoro with a *p*-methyl group (compound **4t**) resulted in a 10-fold drop in potency. When the methyl group was moved from the para to the ortho position (compound **4v**), the activity was completely lost.

A potency increase was observed with compounds **4aa** and **4w**, which contain nitro and acetyl groups in the para position of the phenyl ring. These compounds were 3- and 1.5-fold more active than KN-62, respectively. The addition of both electron-withdrawing groups, such as nitrile (**4x**) and trifluoromethyl (**4y**), and electron-donating groups, such as methoxy (**4z**) and amino (**4ac**), was deleterious to the activity. 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 with the presence of chemical groups in the phenyl ring.

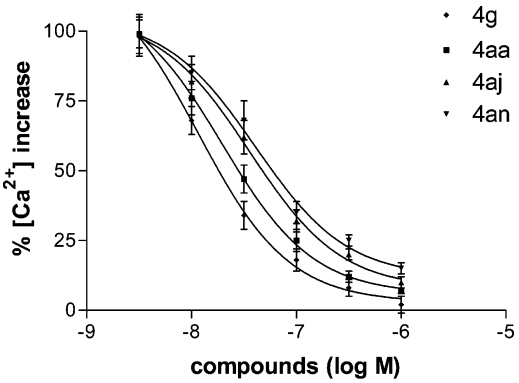


Figure 2. Effect of P2X7 antagonists **4g**, **4aa**, **4aj**, and **4an** on intracellular calcium increase induced by 1 mM ATP. P2X7-transfected HEK 293 cells were incubated in a fluorimeter cuvette with standard saline solution. The P2X7 antagonists were added 10 min before ATP. Experimental points are averages of five determinations.

To investigate the importance of the distance between the piperazine basic nitrogen and the phenyl ring by insertion of one, two, and three methylene units, the corresponding compounds **4aj**, **4an**, and **4ap** were synthesized and tested.

The biological data suggested that the optimal chain length was one or two carbon atoms, which is presumably important for the relative position of the phenyl ring in space. In fact, a methylene or an ethylene spacer (compounds **4aj** and **4an**) improved the activity, making it 1.5-fold higher than that of the reference compound KN-62. However, extending the chain to three carbon atoms (**4ap**) was clearly detrimental to the antagonist potency. Cycle enlargement from piperazine (**4aj**) to homopiperazine (**4al**) led to an almost 30-fold decrease in activity.

A loss of potency was also observed when a substituent such as fluoro, chloro, or methyl (compounds **4e, 4q** and **4u**, respectively) was introduced in the para aryl position of the benzyl group.

By synthesizing three compounds (**4b**, **4h**, and **4ak**), we were able to evaluate the biological effect of the insertion of a N^{α}-methyl group on the glycine skeleton. While N^{α}-methyl analogues **4b** and **4h** were inactive with respect to the parent compounds **4a** and **4g**, compound **4ak** was only 3-fold less active than the corresponding analogue **4aj**.

In the compounds **4b**, **4aa**, and **4ad**, the replacement of the glycine segment with its homologue, corresponding to β-alanine derivatives **4c**, **4ab**, and **4ae**, resulted in inactive compounds.

The $P2X_7$ receptor-binding capacity of the nitrogen of the isoquinoline-5-sulfonyl moiety was studied by replacing this function with a 1-naphthyl group to obtain the corresponding derivatives **4d**, **4o**, **4ag**, **4ai**, **4am**, and **4ao.** Unfortunately, these compounds were not able to maintain the antagonist activity of the parent compounds **4c**, **4g**, **4ad**, **4af**, **4aj**, and **4an**. We found that not only the presence but also the position of the nitrogen atom on the arylsulfonyl moiety is important for the antagonist activity. In fact, in compound **4g** the inhibitory effect was lost when the isoquinoline-5-sulfonyl moiety was replaced with its two isomers corresponding to quinoline-5- and quinoline-8-sulfonyl moieties (derivatives **4m** and **4n**, respectively).

The effect of the four most potent compounds was also assessed on ethidium bromide uptake induced by 1 mM ATP, a typical $P2X_7$ response. Figure 3 shows that the novel

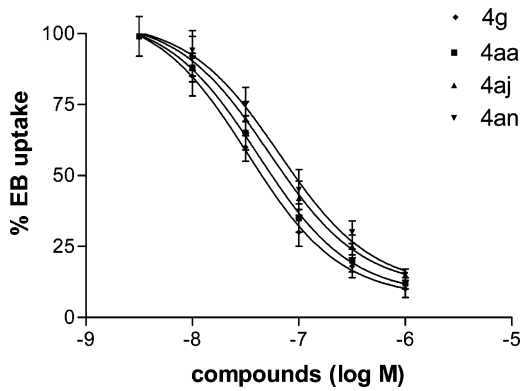


Figure 3. Effect of P2X7 antagonists **4g**, **4aa**, **4aj**, and **4an** on ethidium bromide uptake. $P2X_7$-transfected HEK 293 cells were incubated in a fluorimeter cuvette in the saline solution described in Chemical Materials and Methods. The P2X7 antagonists were added 10 min before ATP. Uptake inhibition is expressed as a percentage of maximal bromide (EB) uptake rate (arbitrary fluorescence units per min) measured in the absence of inhibitor. Experimental points are averages of five determinations.

compounds **4g**, **4aa**, **4aj**, and **4an** were able to reduce the agonist effect with the same order of potency obtained in the calcium studies.

In conclusion, we have discovered a new series of phenylpiperazine derivatives that are potent $P2X_7$ antagonists, in which the original L-tyrosine moiety and the metabolically fragile sulfonate group of KN-62 and its derivatives were replaced with glycine. In the series of phenylpiperazine derivatives, the nature and the position of substituents on the phenyl ring seemed to exert an important influence on the biological activity. In fact, only a few substituents (*p*- and *o*-F, *p*-Me, *p*-$COCH_3$, and *p*-NO_2) were tolerated for the maintenance of antagonism, and the isoquinolinesulfonyl moiety was essential for the activity. Also. a dramatic reduction in potency was observed with the methylation of the nitrogen atom in the glycine moiety. No clear structure−activity relationship can be observed based on data from antagonistic activity, but we have identified several compounds, in particular **4g** and **4aa**, that appear to be more potent than KN-62 and that may be selected for further development.

Experimental Section

Chemical Materials and Methods. General Procedure. All reactions were carried out under in 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. All three were 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 with General Formula 6a−ag. To a solution of *N*-Boc-glycine (**5a**), *N*-Z-glycine (**5b**), Boc-sarcosine (**5c**), and Z-β-alanine (**5d**) (1 mmol) in dry DMF (5 mL) cooled at 0 °C was added EDC (211 mg, 1.1 mmol, 1.1 equiv), HOBt (1.1 mmol). and the suitable N-substituted piperazine (1.1 mmol). This mixture was stirred for 17 h and then concentrated in vacuo. The residue was dissolved in dichloromethane (10 mL), washed with water (5 mL), and then washed with brine (5 mL). The organic layer was dried and concentrated in vacuo. The residue, purified by column chromatography, gave **6a−ag**.

Benzyl Ester of {2-[4-(4-Fluorophenyl)piperazin-1-yl]-2-oxoethyl}carbamic Acid (6a). Eluent for the column chromatography: EtOAc−petroleum ether, 3−7 v/v. The product was obtained as a white solid (yield 76%), mp 118−120 °C. ^{1}H NMR ($CDCl_3$) δ: 3.10 (m, 4H), 3.59 (t, J = 5.2 Hz, 2H), 3.82 (t, J = 5.2 Hz, 2H), 4.07 (d, J = 4.2 Hz, 2H), 5.13 (s, 2H), 5.92 (bs, 1H), 7.00 (m, 4H), 7.37 (m, 5H).

***tert*-Butyl Ester of {2-[4-(4-Fluorophenyl)piperazin-1-yl]-2-oxoethyl}methylcarbamic Acid (6b).** Eluent for the column chromatography: EtOAc−petroleum ether, 4−6 v/v. The product was obtained as a colorless oil (yield 77%). ^{1}H NMR ($CDCl_3$) δ: 1.40 (m, 9H), 2.87 (s, 3H), 3.02 (m, 4H), 3.57 (t, J = 5.0 Hz, 2H), 3.72 (t, J = 5.0 Hz, 2H), 4.04 (s, 2H), 6.90 (m, 2H), 7.39 (m, 1H), 7.72 (m, 1H).

Benzyl Ester of {3-[4-(4-Fluorophenyl)piperazin-1-yl]-3-oxopropyl}carbamic Acid (6c). Eluent for the column chromatography: EtOAc−petroleum ether, 7−3 v/v. The product was obtained as an orange solid, mp 95−97 °C (yield 88%). ^{1}H NMR ($CDCl_3$) δ: 2.51 (t, J = 5.6 Hz, 2H), 3.02 (m, 4H), 3.52 (m, 4H), 3.72 (t, J = 5.6 Hz, 2H), 5.02 (s, 2H), 5.49 (bs, 1H), 6.86 (m, 4H), 7.19 (m, 5H).

***tert*-Butyl Ester of {2-[4-(4-Fluorobenzyl)piperazin-1-yl]-2-oxoethyl}carbamic Acid (6d).** Eluent for the column chromatography: EtOAc−petroleum ether, 9−1 v/v. The product was obtained as a yellow oil (yield 68%). ^{1}H NMR ($CDCl_3$) δ: 1.42 (s, 9H), 2.48 (m, 4H), 3.40 (t, J = 5.2 Hz, 2H), 3.54 (s, 2H), 3.64 (t, J = 5.2 Hz, 2H), 3.93 (d, J = 4.4 Hz, 2H), 5.50 (s, 1H), 6.93 (t, J = 8.6 Hz, 2H), 7.22 (d, J = 8.6 Hz, 1H), 7.26 (d, J = 8.6 Hz, 1H).

Benzyl Ester of {2-[4-(3-Fluorophenyl)piperazin-1-yl]-2-oxoethyl}carbamic Acid (6e). Eluent for the column chromatography: EtOAc−petroleum ether, 4−6 v/v. The product was obtained as a white solid (yield 74%), mp 125−126 °C. ^{1}H NMR ($CDCl_3$) δ: 3.12 (m, 4H), 3.50 (t, J = 4.8 Hz, 2H), 3.73 (t, J = 4.8 Hz, 2H), 4.01 (d, J = 4.2 Hz, 2H), 5.07 (s, 2H), 5.72 (bs, 1H), 6.54 (m, 4H), 7.37 (m, 5H).

Benzyl Ester of {2-[4-(2-Fluorophenyl)piperazin-1-yl]-2-oxoethyl}carbamic Acid (6f). Eluent for the column chromatography: EtOAc−petroleum ether, 4−6 v/v. The product was obtained as a white solid (yield 78%), mp 108−109 °C. ^{1}H NMR ($CDCl_3$) δ: 3.08 (m, 4H), 3.58 (t, J = 5.2 Hz, 2H), 3.82 (t, J = 5.2 Hz, 2H), 4.09 (d, J = 4.4 Hz, 2H), 5.13 (s, 2H), 5.92 (bs, 1H), 6.87 (m, 4H), 7.37 (m, 5H).

***tert*-Butyl Ester of {2-[4-(2-Fluorophenyl)piperazin-1-yl]-2-oxoethyl}methylcarbamic Acid (6g).** Eluent for the column chromatography: EtOAc−petroleum ether, 1−1 v/v. The product was obtained as a colorless oil (yield 65%). ^{1}H NMR ($CDCl_3$) δ: 1.47 (m, 9H), 2.94 (s, 3H), 3.08 (t, J = 5.2 Hz, 4H), 3.62 (t, J = 5.2 Hz, 2H), 3.79 (t, J = 5.2 Hz, 2H), 4.09 (s, 2H), 7.07 (m, 4H).

Benzyl Ester of {2-[4-(2,4-Difluorophenyl)piperazin-1-yl]-2-oxoethyl}carbamic Acid (6h). Eluent for the column chromatography: EtOAc−petroleum ether, 1−1 v/v. The product was obtained as a white solid (yield 71%), mp 112−113 °C. ^{1}H NMR ($CDCl_3$) δ: 3.02 (m, 4H), 3.57 (t, J = 5.0 Hz, 2H), 3.80 (t, J = 5.0 Hz, 2H), 4.08 (d, J = 4.4 Hz, 2H), 5.13 (s, 2H), 5.80 (bs, 1H), 6.83 (m, 3H), 7.33 (m, 5H).

Benzyl Ester of {2-[4-(3,4-Difluorophenyl)piperazin-1-yl]-2-oxoethyl}methylcarbamic Acid (6i). Eluent for the column chromatography: EtOAc−petroleum ether, 6−4 v/v. The product was obtained as a white solid (yield 72%), mp 115−116 °C. ^{1}H NMR ($CDCl_3$) δ: 3.07 (m, 4H), 3.52 (t, J = 5.0 Hz, 2H), 3.72 (t, J = 5.0 Hz, 2H), 4.02 (d, J = 4.4 Hz, 2H), 5.08 (s, 2H), 5.75 (bs,

1H), 6.59 (m, 2H), 7.04 (q, $J = 9.8$ Hz, 1H), 7.33 (m, 3H), 7.50 (m, 1H), 7.85 (m, 1H).

***tert*-Butyl Ester of {2-[4-(2,3-Difluorophenyl)piperazin-1-yl]-2-oxoethyl}methylcarbamic Acid (6j).** Eluent for the column chromatography: EtOAc−petroleum ether, 6−4 v/v. The product was obtained as an oil (yield 96%). ^{1}H NMR ($CDCl_3$) δ: 1.45 (s, 9H), 3.08 (m, 4H), 3.56 (t, $J = 5.2$ Hz, 2H), 3.80 (t, $J = 5.2$ Hz, 2H), 4.02 (d, $J = 4.4$ Hz, 2H), 5.62 (bs, 1H), 6.72 (m, 1H), 6.84 (m, 1H), 7.02 (m, 1H).

***tert*-Butyl Ester of {2-[4-(2,5-Difluorophenyl)piperazin-1-yl]-2-oxoethyl}methylcarbamic Acid (6k).** Eluent for the column chromatography: EtOAc−petroleum ether, 7−3 v/v. The product was obtained as a white solid (yield 87%). ^{1}H NMR ($CDCl_3$) δ: 1.45 (s, 9H), 3.08 (m, 4H), 3.56 (t, $J = 5.2$ Hz, 2H), 3.78 (t, $J = 5.2$ Hz, 2H), 4.00 (d, $J = 4.4$ Hz, 2H), 5.62 (bs, 1H), 6.61 (m, 2H), 7.02 (m, 1H).

Benzyl Ester of {2-[4-(4-Chlorophenyl)piperazin-1-yl]-2-oxoethyl}carbamic Acid (6l). Eluent for the column chromatography: EtOAc−petroleum ether, 7−3 v/v. The product was obtained as a white solid (yield 74%), mp 145−146 °C. ^{1}H NMR ($CDCl_3$) δ: 3.16 (m, 4H), 3.62 (t, $J = 5.2$ Hz, 2H), 3.84 (t, $J = 5.2$ Hz, 2H), 4.07 (d, $J = 4.4$ Hz, 2H), 5.13 (s, 2H), 5.77 (bs, 1H), 6.93 (d, $J = 9.0$ Hz, 2H), 7.26 (m, 5H), 7.38 (d, $J = 9.0$ Hz, 2H).

Benzyl Ester of {2-[4-(4-Chlorobenzyl)piperazin-1-yl]-2-oxoethyl}carbamic Acid (6m). Eluent for the column chromatography: EtOAc. The product was obtained as a white solid (yield 68%), mp 93−95 °C. ^{1}H NMR ($CDCl_3$) δ: 2.39 (m, 4H), 3.34 (t, $J = 5.0$ Hz, 2H), 3.544 (s, 2H), 3.59 (t, $J = 5.0$ Hz, 2H), 3.95 (d, $J = 4.0$ Hz, 2H), 5.06 (s, 2H), 5.74 (bs, 1H), 7.27 (m, 9H).

***tert*-Butyl Ester of {2-[4-(4-Bromophenyl)piperazin-1-yl]-2-oxoethyl}carbamic Acid (6n).** Eluent for the column chromatography: EtOAc−petroleum ether, 4−6 v/v. The product was obtained as a white solid (yield 65%), mp 110−111 °C. ^{1}H NMR ($CDCl_3$) δ: 1.45 (m, 9H), 314 (m, 4H), 3.58 (t, $J = 5.0$ Hz, 2H), 3.82 (t, $J = 5.0$ Hz, 2H), 4.01 (d, $J = 4.4$ Hz, 2H), 5.51 (bs, 1H), 6.83 (d, $J = 8.6$ Hz, 2H), 7.38 (d, $J = 8.6$ Hz, 2H),.

***tert*-Butyl Ester of {2-[4-(4-Iodophenyl)piperazin-1-yl]-2-oxoethyl}carbamic Acid (6o).** Eluent for the column chromatography: EtOAc−petroleum ether, 4−6 v/v. The product was obtained as a white solid (yield 78%), mp 110−112 °C. ^{1}H NMR ($CDCl_3$) δ: 1.38 (m, 9H), 3.09 (m, 4H), 3.48 (t, $J = 5.4$ Hz, 2H), 3.74 (t, $J = 5.4$ Hz, 2H), 3.93 (d, $J = 4.4$ Hz, 2H), 5.43 (bs, 1H), 6.63 (d, $J = 7.4$ Hz, 2H), 7.49 (d, $J = 7.4$ Hz, 2H).

***tert*-Butyl Ester of [2-Oxo-2-(4-*p*-tolylpiperazin-1-yl)ethyl]carbamic Acid (6p).** Eluent for the column chromatography: EtOAc−petroleum ether, 1−1 v/v. The product was obtained as a white solid (yield 69%), mp 118−120 °C. ^{1}H NMR ($CDCl_3$) δ: 1.36 (s, 9H), 2.18 (s, 3H), 3.03 (m, 4H), 3.45 (t, $J = 4.8$ Hz, 2H), 3.69 (t, $J = 4.8$ Hz, 2H), 3.90 (d, $J = 4.4$ Hz, 2H), 5.42 (s, 1H), 6.75 (d, $J = 7.8$ Hz, 2H), 6.98 (d, $J = 7.8$ Hz, 2H).

Benzyl Ester of {2-[4-(4-Methylbenzyl)piperazin-1-yl]-2-oxoethyl}carbamic Acid (6q). Eluent for the column chromatography: EtOAc−petroleum ether, 7−3 v/v. The product was obtained as a colorless oil (yield 53%). ^{1}H NMR ($CDCl_3$) δ: 2.27 (s, 3H), 2.36 (m, 4H), 3.31 (t, $J = 4.8$ Hz, 2H), 3.42 (s, 2H), 3.56 (t, $J = 4.8$ Hz, 2H), 3.93 (d, $J = 4.2$ Hz, 2H), 5.05 (s, 2H), 5.73 (bs, 1H), 7.08 (d, $J = 8.2$ Hz, 2H), 7.12 (d, $J = 8.2$ Hz, 2H), 7.30 (m, 5H).

Benzyl Ester of [2-Oxo-2-(4-*o*-tolylpiperazin-1-yl)ethyl]carbamic Acid (6r). Eluent for the column chromatography: EtOAc−petroleum ether, 1−1 v/v. The product was obtained as a white solid (yield 73%), mp 134−136 °C. ^{1}H NMR ($CDCl_3$) δ: 2.33 (s, 3H), 2.92 (m, 4H), 3.55 (t, $J = 4.8$ Hz, 2H), 3.79 (t, $J = 4.8$ Hz, 2H), 4.09 (d, $J = 4.4$ Hz, 2H), 5.42 (s, 2H), 5.83 (bs, 1H), 6.98 (d, $J = 7.8$ Hz, 1H), 7.03 (d, $J = 7.8$ Hz, 1H), 7.14 (t, $J = 7.4$ Hz, 1H), 7.37 (m, 7H).

***tert*-Butyl Ester of {2-[4-(4-Acetylphenyl)piperazin-1-yl]-2-oxoethyl}carbamic Acid (6s).** Eluent for the column chromatography: EtOAc−petroleum ether, 6−4 v/v. The product was obtained as a colorless oil (yield 76%). ^{1}H NMR ($CDCl_3$) δ: 1.45 (s, 9H), 2.53 (s, 3H), 3.39 (m, 4H), 3.57 (t, $J = 5.4$ Hz, 2H), 3.80 (t, $J = 5.4$ Hz, 2H), 4.01 (d, $J = 3.8$ Hz, 2H), 5.44 (bs, 1H), 6.90 (d, $J = 9.0$ Hz, 2H), 7.90 (d, $J = 9.0$ Hz, 2H).

***tert*-Butyl Ester of {2-[4-(4-Cyanophenyl)piperazin-1-yl]-2-oxoethyl}carbamic Acid (6t).** Eluent for the column chromatography: EtOAc−petroleum ether, 6−4 v/v. The product was obtained as a white solid (yield 78%), mp 110−112 °C. ^{1}H NMR ($CDCl_3$) δ: 1.45 (s, 9H), 3.36 (m, 4H), 3.55 (t, $J = 5.6$ Hz, 2H), 3.76 (t, $J = 5.6$ Hz, 2H), 4.00 (d, $J = 4.4$ Hz, 2H), 5.62 (s, 1H), 6.85 (d, $J = 9.0$ Hz, 2H), 7.51 (d, $J = 9.0$ Hz, 2H).

Benzyl Ester of {2-[4-(4-Trifluoromethylphenyl)piperazin-1-yl]-2-oxoethyl}carbamic Acid (6u). Eluent for the column chromatography: EtOAc−petroleum ether, 4−6 v/v. The product was obtained as a white solid (yield 87%), mp 155−157 °C. ^{1}H NMR ($CDCl_3$) δ: 3.27 (m, 4H), 3.59 (t, $J = 4.8$ Hz, 2H), 3.80 (t, $J = 4.8$ Hz, 2H), 4.07 (d, $J = 3.8$ Hz, 2H), 5.13 (s, 2H), 5.83 (bs, 1H), 6.94 (d, $J = 8.6$ Hz, 2H), 7.37 (m, 5H), 7.51 (d, $J = 8.6$ Hz, 2H).

***tert*-Butyl Ester of {2-[4-(4-Methoxyphenyl)piperazin-1-yl]-2-oxoethyl}carbamic Acid (6v).** Eluent for the column chromatography: EtOAc−petroleum ether, 6−4 v/v. The product was obtained as a pink solid (yield 72%), mp 82−84 °C. ^{1}H NMR ($CDCl_3$) δ: 1.35 (s, 9H), 2.97 (m, 4H), 3.45 (t, $J = 5.2$ Hz, 2H), 3.63 (t, $J = 5.2$ Hz, 2H), 3.68 (s, 3H), 3.92 (d, $J = 4.2$ Hz, 2H), 5.42 (s, 1H), 6.77 (m, 4H).

***tert*-Butyl Ester of {2-[4-(4-Nitrophenyl)piperazin-1-yl]-2-oxoethyl}carbamic Acid (6w).** Eluent for the column chromatography: EtOAc−petroleum ether, 3−7 v/v. The product was obtained as a yellow solid (yield 75%), mp 125−127 °C. ^{1}H NMR ($CDCl_3$) δ: 1.45 (s, 9H), 3.48 (m, 4H), 3.59 (t, $J = 5.4$ Hz, 2H), 3.82 (t, $J = 5.4$ Hz, 2H), 4.03 (d, $J = 4.2$ Hz, 2H), 5.50 (s, 1H), 6.84 (d, $J = 7.4$ Hz, 2H), 8.17 (d, $J = 7.4$ Hz, 2H).

***tert*-Butyl Ester of {3-[4-(4-Nitrophenyl)piperazin-1-yl]-3-oxopropyl}carbamic Acid (6x).** Eluent for the column chromatography: EtOAc−petroleum ether, 4−6 v/v. The product was obtained as a yellow solid (yield 95%), mp 160−162 °C. ^{1}H NMR ($CDCl_3$) δ: 1.42 (s, 9H), 2.56 (t, $J = 5.6$ Hz, 2H), 3.46 (m, 6H), 3.62 (t, $J = 5.6$ Hz, 2H), 3.78 (t, $J = 5.6$ Hz, 2H), 5.32 (s, 1H), 6.82 (d, $J = 9.4$ Hz, 2H), 8.12 (d, $J = 9.4$ Hz, 2H).

Benzyl Ester of [2-(4-Phenylpiperazin-1-yl)-2-oxoethyl]carbamic Acid (6y). Eluent for the column chromatography: EtOAc−petroleum ether, 4−6 v/v. The product was obtained as a white solid (yield 71%), mp 121−123 °C. ^{1}H NMR ($CDCl_3$) δ: 3.20 (m, 4H), 3.63 (m, 2H), 3.84 (m, 2H), 4.09 (d, $J = 4.4$ Hz, 2H), 5.13 (s, 2H), 5.94 (bs, 1H), 6.87 (m, 3H), 7.37 (m, 7H).

Benzyl Ester of [3-Oxo-3-(4-phenylpiperazin-1-yl)propyl]carbamic Acid (6z). Eluent for the column chromatography: EtOAc−petroleum ether, 1−1 v/v. The product was obtained as a pink solid (yield 82%), mp 94−96 °C. ^{1}H NMR ($CDCl_3$) δ: 2.05 (m, 2H), 2.56 (m, 2H), 3.16 (m, 4H), 3.52 (m, 2H), 3.74 (m, 2H), 5.07 (s, 2H), 5.62 (bs, 1H), 6.89 (m, 3H), 7.33 (m, 7H).

Benzyl Ester of [2-(4-Phenylpiperazin-1-yl)-2-oxoethyl]carbamic Acid (6aa). Eluent for the column chromatography: EtOAc−petroleum ether, 4−6 v/v. The product was obtained as a white solid (yield 71%), mp 121−123 °C. ^{1}H NMR ($CDCl_3$) δ: 3.20 (m, 4H), 3.63 (m, 2H), 3.84 (m, 2H), 4.09 (d, $J = 4.4$ Hz, 2H), 5.13 (s, 2H), 5.94 (bs, 1H), 6.87 (m, 3H), 7.37 (m, 7H).

Benzyl Ester of [2-Oxo-2-(4-pyrimidin-2-yl-piperazin-1-yl)-ethyl]carbamic Acid (6ab). Eluent for the column chromatography: EtOAc−petroleum ether, 7−3 v/v. The product was obtained as a white solid (yield 95%), mp 103−105 °C. ^{1}H NMR ($CDCl_3$) δ: 3.45 (t, $J = 4.8$ Hz, 2H), 3.73 (t, $J = 4.8$ Hz, 2H), 3.86 (m, 4H), 4.00 (d, $J = 4.4$ Hz, 2H), 5.07 (s, 2H), 5.75 (bs, 1H), 6.55 (t, $J = 4.8$ Hz, 1H), 7.29 (m, 5H), 8.33 (d, $J = 5.0$ Hz, 2H),.

Benzyl Ester of [2-(4-Benzylpiperazin-1-yl)-2-oxoethyl]carbamic Acid (6ac). Eluent for the column chromatography: EtOAc−petroleum ether, 8−2 v/v. The product was obtained as a colorless oil (yield 78%). ^{1}H NMR ($CDCl_3$) δ: 2.34 (m, 2H), 3.58 (m, 2H), 3.62 (t, $J = 4.8$ Hz, 2H), 3.76 (t, $J = 4.8$ Hz, 2H), 3.84 (s, 2H), 4.06 (d, $J = 4.2$ Hz, 2H), 5.12 (s, 2H), 5.80 (bs, 1H), 7.35 (m, 10H).

***tert*-Butyl Ester of [2-(4-Benzylpiperazin-1-yl)-2-oxoethyl]-methylcarbamic (6ad).** Eluent for the column chromatography:

EtOAc. The product was obtained as a colorless oil (yield 68%). ^{1}H NMR ($CDCl_3$) δ: 1.44 (s, 9H), 2.43 (m, 4H), 2.90 (s, 3H), 3.50 (m, 2H), 3.54 (m, 2H), 3.62 (m, 2H), 4.03 (s, 2H), 7.31 (m, 5H).

Benzyl Ester of [2-(4-Benzyl[1,4]diazepan-1-yl)-2-oxoethyl]-carbamic Acid (6ae). Eluent for the column chromatography: EtOAc−MeOH, 9.5−0.5 v/v. The product was obtained as a colorless oil (yield 62%). ^{1}H NMR ($CDCl_3$) δ: 1.84 (m, 2H), 2.59 (m, 4H), 3.40 (t, J = 6.0 Hz, 2H), 3.56 (s, 2H), 3.60 (t, J = 6.0 Hz, 2H), 3.94 (t, J = 4.4 Hz, 2H), 5.06 (s, 2H), 5.80 (bs, 1H), 7.26 (m, 10H).

Benzyl Ester of [2-Oxo-2-(4-phenethylpiperazin-1-yl)ethyl]-carbamic Acid (6af). Eluent for the column chromatography: EtOAc. The product was obtained as a colorless oil (yield 60%). ^{1}H NMR ($CDCl_3$) δ: 2.54 (m, 4H), 2.64 (m, 2H), 2.82 (m, 2H), 3.44 (m, 2H), 3.70 (m, 2H), 4.02 (s, 2H), 5.12 (s, 2H), 5.90 (bs, 1H), 7.21 (m, 3H), 7.31 (m, 4H), 7.36 (m, 3H).

Benzyl Ester of {2-Oxo-2-[4-(3-phenylpropyl)piperazin-1-yl]-ethyl}carbamic Acid (6ag). Eluent for the column chromatography: EtOAc−MeOH, 9−1 v/v. The product was obtained as a colorless oil (yield 79%). ^{1}H NMR ($CDCl_3$) δ: 2.60 (m, 4H), 3.25 (d, J = 6.2 Hz, 2H), 3.45 (d, J = 4.6 Hz, 2H), 3.70 (t, J = 4.6 Hz, 2H), 4.02 (d, J = 4.2 Hz, 2H), 5.11 (s, 2H), 5.80 (bs, 1H), 6.20 (m, 1H), 6.54 (d, J = 15.8 Hz, 1H), 7.33 (m, 10H).

General Procedure for Removing the Boc Protecting Group. The Boc derivative (1.5 mmol) was 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 and concentrated in vacuo. The residue obtained was used for the next reaction without any purification.

General Procedure for Removing the Benzyloxycarbonyl (Z) Protecting Group. A solution of Z-derivative (1 mmol) in 10 mL of a EtOH was hydrogenated over 75 mg of 10% Pd/C at 50 psi for 3 h. The catalyst was removed by filtration and the filtrate was concentrated under reduced pressure, giving a residue that was used without purification for the next step.

General Procedure for the Synthesis of Compounds 4a−ap. To a solution of the appropriate amine (0.5 mmol) in dry DCM (5 mL) was added at 0 °C Et_3N (70 μL, 0.5 mmol, 1 equiv) and the corresponding arylsulfonyl chloride (1 mmol, 2 equiv). The reaction mixture was allowed to slowly warm up to room temperature and then stirred for 12 h. After the end of this time, a saturated aqueous $NaHCO_3$ solution (2 mL) was added to the reaction mixture. After the layers were separated, the organic layer was dried and concentrated in vacuo. The residue, after purification for column chromatography, furnished the appropriate product.

Isoquinolinesulfon-5-yl-{2-[4-(4-fluorophenyl)piperazin-1-yl]-2-oxoethyl}amide (4a). Eluent for the column chromatography: EtOAc. The product was obtained as a white solid. ^{1}H NMR ($CDCl_3$) δ: 2.98 (m, 4H), 3.39 (t, J = 4.8 Hz, 2H), 3.65 (t, J = 4.8 Hz, 2H), 3.82 (d, J = 4.0 Hz, 2H), 6.07 (bs, 1H), 6.82 (m, 2H), 6.96 (m, 2H), 7.69 (t, J = 8.0 Hz, 1H), 8.20 (d, J = 8.4 Hz, 1H), 8.43 (m, 2H), 8.72 (d, J = 6.0 Hz, 1H), 9.35 (s, 1H). Anal. ($C_{21}H_{21}N_4O_3SF$) C, H, N.

Isoquinolinesulfon-5-yl-{2-[4-(4-fluorophenyl)piperazin-1-yl]-2-oxoethyl}methylamide (4b). Eluent for the column chromatography: EtOAc−MeOH, 9.5−0.5 v/v. The product was obtained as a white solid. ^{1}H NMR ($CDCl_3$) δ: 2.87 (s, 3H), 2.97 (t, J = 4.6 Hz, 2H), 3.05 (t, J = 4.6 Hz, 2H), 3.61 (m, 4H), 4.10 (s, 2H), 6.84 (m, 4H), 7.65 (t, J = 7.8 Hz, 1H), 8.15 (d, J = 8.0 Hz, 1H), 8.40 (d, J = 7.6, 2H), 8.45 (d, J = 7.6, 2H), 8.61 (d, J = 6.2 Hz, 1H), 9.28 (s, 1H). Anal. ($C_{22}H_{23}N_4O_3SF$) C, H, N.

Isoquinolinesulfon-5-yl-{3-[4-(4-fluorophenyl)piperazin-1-yl]-3-oxopropyl}amide (4c). Eluent for the column chromatography: EtOAc−MeOH, 9.75−0.25 v/v. The product was obtained as a white solid. ^{1}H NMR ($CDCl_3$) δ: 2.53 (t, J = 5.6 Hz, 2H), 3.04 (m, 4H), 3.12 (m, 2H), 3.44 (m, 2H), 3.72 (t, J = 5.6 Hz, 2H), 6.06 (bs, 1H), 6.92 (m, 4H), 7.70 (t, J = 7.4 Hz, 1H), 8.22 (d, J = 8.4 Hz, 1H), 8.42 (d, J = 7.6, 1H), 8.49 (d, J = 7.6, 1H), 8.71 (d, J = 6.4 Hz, 1H), 9.36 (s, 1H). Anal. ($C_{22}H_{23}N_4O_3SF$) C, H, N.

Naphthalenesulfon-1-yl-{2-[4-(4-fluorophenyl)piperazin-1-yl]-2-oxoethyl}amide (4d). Eluent for the column chromatography: EtOAc−petroleum ether, 3−7 v/v. The product was obtained as a white solid. ^{1}H NMR ($CDCl_3$) δ: 2.99 (m, 4H), 3.39 (t, J = 4.4 Hz, 2H), 3.65 (t, J = 4.42 Hz, 2H), 3.79 (d, J = 4.4 Hz, 2H), 6.01 (bs, 1H), 6.86 (t, J = 8.8 Hz, 2H), 6.99 (t, J = 8.8 Hz, 2H), 7.54 (t, J = 8.8 Hz, 1H), 7.62 (t, J = 8.8 Hz, 1H), 7.69 (t, J = 8.8 Hz, 1H), 7.93 (d, J = 8.8 Hz, 1H), 8.07 (d, J = 8.8 Hz, 1H), 8.26 (d, J = 8.8 Hz, 1H), 8.71 (d, J = 8.0 Hz, 1H). Anal. ($C_{22}H_{22}N_3O_3SF$) C, H, N.

Isoquinolinesulfon-5-yl-{2-[4-(4-fluorobenzyl)piperazin-1-yl]-2-oxoethyl}amide (4e). Eluent for the column chromatography: EtOAc. The product was obtained as a white solid. ^{1}H NMR ($CDCl_3$) δ: 2.32 (m, 4H), 3.24 (t, J = 4.6 Hz, 2H), 3.45 (s, 2H), 3.49 (t, J = 4.6 Hz, 2H), 3.75 (s, 2H), 6.05 (s, 1H), 6.98 (t, J = 8.6 Hz, 2H), 7.19 (d, J = 8.6 Hz, 1H), 7.26 (d, J = 8.6 Hz, 1H), 7.68 (t, J = 8.0 Hz, 1H), 8.20 (d, J = 8.0 Hz, 1H), 8.43 (d, J = 7.4 Hz, 2H), 8.72 (d, J = 6.0 Hz, 1H), 9.35 (s, 1H). Anal. ($C_{22}H_{23}N_4O_3SF$) C, H, N.

Isoquinolinesulfon-5-yl-{2-[4-(3-fluorophenyl)piperazin-1-yl]-2-oxoethyl}amide (4f). Eluent for the column chromatography: EtOAc−MeOH, 9.8−0.2 v/v. The product was obtained as a white solid. ^{1}H NMR ($CDCl_3$) δ: 3.01 (m, 4H), 3.34 (t, J = 5.0 Hz, 2H), 3.58 (t, J = 5.0 Hz, 2H), 3.75 (d, J = 4.2 Hz, 2H), 6.03 (bs, 1H), 6.44 (m, 2H), 7.07 (t, J = 8.0 Hz, 1H), 7.19 (s, 1H), 7.63 (t, J = 7.8 Hz, 1H), 8.14 (d, J = 8.2 Hz, 1H), 8.38 (d, J = 6.0 Hz, 2H), 8.67 (d, J = 6.2 Hz, 1H), 9.29 (s, 1H). Anal. ($C_{21}H_{21}N_4O_3SF$) C, H, N.

Isoquinolinesulfon-5-yl-{2-[4-(2-fluorophenyl)piperazin-1-yl]-2-oxoethyl}amide (4g). Eluent for the column chromatography: EtOAc−petroleum ether, 8−2 v/v. The product was obtained as a white solid. ^{1}H NMR ($CDCl_3$) δ: 2.97 (t, J = 5.2 Hz, 2H), 3.02 (t, J = 5.2 Hz, 2H), 3.51 (t, J = 5.2 Hz, 2H), 3.64 (t, J = 5.2 Hz, 2H), 3.90 (d, J = 4.0 Hz, 2H), 6.15 (bs, 1H), 6.89 (t, J = 7.6 Hz, 1H), 7.02 (m, 3H), 7.57 (m, 2H), 7.65 (t, J = 7.2 Hz, 1H), 8.08 (d, J = 7.2 Hz, 1H), 8.43 (d, J = 7.2 Hz, 1H), 9.12 (s, 1H). Anal. ($C_{21}H_{21}N_4O_3SF$) C, H, N.

Isoquinolinesulfon-5-yl-{2-[4-(2-fluorophenyl)piperazin-1-yl]-2-oxoethyl}methylamide (4h). Eluent for the column chromatography: EtOAc−MeOH, 9.5−0.5 v/v. The product was obtained as a white solid. ^{1}H NMR ($CDCl_3$) δ: 2.88 (s, 3H), 2.93 (t, J = 4.8 Hz, 2H), 3.04 (t, J = 4.8 Hz, 2H), 3.63 (m, 4H), 4.12 (s, 2H), 6.99 (m, 4H), 7.66 (t, J = 7.8 Hz, 1H), 8.15 (d, J = 8.4 Hz, 1H), 8.43 (m, 2H), 8.61 (d, J = 6.2 Hz, 1H), 9.28 (s, 1H). Anal. ($C_{22}H_{23}N_4O_3SF$) C, H, N.

Isoquinolinesulfon-5-yl-{2-[4-(2,4-difluorophenyl)piperazin-1-yl]-2-oxoethyl}amide (4i). Eluent for the column chromatography: EtOAc. The product was obtained as a white solid. ^{1}H NMR ($CDCl_3$) δ: 2.87 (m, 4H), 3.33 (t, J = 5.4 Hz, 2H), 3.74 (t, J = 5.4 Hz, 2H), 3.77 (d, J = 3.8 Hz, 2H), 6.01 (bs, 1H), 6.76 (m, 3H), 7.64 (t, J = 7.6 Hz, 1H), 8.14 (d, J = 8.4 Hz, 1H), 8.39 (t, J = 7.6 Hz, 2H), 8.65 (d, J = 6.0 Hz, 1H), 9.30 (s, 1H). Anal. ($C_{21}H_{20}N_4O_3SF_2$) C, H, N.

Isoquinolinesulfon-5-yl-{2-[4-(3,4-difluorophenyl)piperazin-1-yl]-2-oxoethyl}amide (4j). Eluent for the column chromatography: EtOAc−MeOH, 9.8−0.2 v/v. The product was obtained as a white solid. ^{1}H NMR ($CDCl_3$) δ: 2.95 (m, 4H), 3.33 (t, J = 5.0 Hz, 2H), 3.58 (t, J = 5.0 Hz, 2H), 3.77 (d, J = 3.8 Hz, 2H), 5.96 (bs, 1H), 6.60 (m, 1H), 6.90 (q, J = 9.0 Hz, 1H), 7.00 (s, 1H), 7.64 (t, J = 7.4 Hz, 1H), 8.17 (d, J = 8.0 Hz, 1H), 8.42 (t, J = 6.8 Hz, 2H), 8.66 (d, J = 6.4 Hz, 1H), 9.32 (s, 1H). Anal. ($C_{21}H_{20}N_4O_3SF_2$) C, H, N.

Isoquinolinesulfon-5-yl-{2-[4-(2,3-difluorophenyl)piperazin-1-yl]-2-oxoethyl}amide (4k). Eluent for the column chromatography: EtOAc. The product was obtained as a white solid. ^{1}H NMR ($CDCl_3$) δ: 3.02 (m, 4H), 3.41 (t, J = 5.2 Hz, 2H), 3.68 (t, J = 5.2 Hz, 2H), 3.84 (d, J = 4.0 Hz, 2H), 6.05 (bs, 1H), 6.65 (t, J = 6.8 Hz, 1H), 6.82 (q, J = 8.6 Hz, 1H), 6.96 (m, 1H), 7.74 (t, J =

7.6 Hz, 1H), 8.26 (d, J = 7.6 Hz, 1H), 8.50 (d, J = 7.6 Hz, 1H), 8.58 (m, 1H), 8.72 (m, 1H), 9.44 (s, 1H). Anal. ($C_{21}H_{20}N_4O_3SF_2$) C, H, N.

Isoquinolinesulfon-5-yl-{2-[4-(2,5-difluorophenyl)piperazin-1-yl]-2-oxoethyl}amide (4l). Eluent for the column chromatography: EtOAc. The product was obtained as a white solid. ^{1}H NMR ($CDCl_3$) δ: 2.90 (m, 4H), 3.43 (t, J = 5.4 Hz, 2H), 3.61 (t, J = 5.4 Hz, 2H), 3.76 (d, J = 4.0 Hz, 2H), 5.97 (bs, 1H), 6.49 (m, 1H), 6.96 (m, 2H), 7.67 (t, J = 7.8 Hz, 1H), 8.18 (d, J = 8.2 Hz, 1H), 8.38 (d, J = 7.6 Hz, 1H), 8.48 (d, J = 7.6 Hz, 1H), 8.68 (d, J = 6.0 Hz,1H), 9.33 (s, 1H). Anal. ($C_{21}H_{20}N_4O_3SF_2$) C, H, N.

Quinolinesulfon-5-yl-{2-[4-(2-fluorophenyl)piperazin-1-yl]-2-oxoethyl}amide (4m). Eluent for the column chromatography: EtOAc−petroleum ether, 6−4 v/v. The product was obtained as a white solid. ^{1}H NMR ($CDCl_3$) δ: 2.96 (t, J = 5.2 Hz, 2H), 3.04 (t, J = 5.2 Hz, 2H), 3.50 (t, J = 5.2 Hz, 2H), 3.68 (t, J = 5.2 Hz, 2H), 3.90 (d, J = 4.0 Hz, 2H), 6.89 (t, J = 7.6 Hz, 1H), 7.02 (m, 3H), 7.20 (bs, 1H), 7.58 (dd, J = 4.0 and 8.4 Hz, 1H), 7.65 (t, J = 8.4 Hz, 1H), 8.08 (d, J = 8.0 Hz, 1H), 8.26 (d, J = 8.4 Hz, 1H), 8.43 (d, J = 7.2 Hz, 1H), 9.12 (m, 1H). Anal. ($C_{21}H_{21}N_4O_3SF$) C, H, N.

Quinolinesulfon-8-yl-{2-[4-(2-fluorophenyl)piperazin-1-yl]-2-oxoethyl}amide (4n). Eluent for the column chromatography: EtOAc−petroleum ether, 6−4 v/v. The product was obtained as a white solid. ^{1}H NMR ($CDCl_3$) δ: 2.95 (t, J = 5.2 Hz, 2H), 3.01 (t, J = 5.2 Hz, 2H), 3.48 (t, J = 5.2 Hz, 2H), 3.63 (t, J = 5.2 Hz, 2H), 3.90 (d, J = 4.0 Hz, 2H), 6.89 (t, J = 8.0 Hz, 1H), 7.02 (m, 2H), 7.20 (t, J = 7.8 Hz, 1H), 7.26 (d, J = 7.8 Hz, 1H), 7.55 (t, J = 8.4 Hz, 1H), 7.64 (t, J = 8.0 Hz, 1H), 8.06 (d, J = 8.4 Hz, 1H), 8.26 (d, J = 8.0 Hz, 1H), 8.423 (d, J = 7.2 Hz, 1H), 9.12 (m, 1H). Anal. ($C_{21}H_{21}N_4O_3SF$) C, H, N.

Naphthalenesulfon-1-yl-{2-[4-(2-fluorophenyl)piperazin-1-yl]-2-oxoethyl}amide (4o). Eluent for the column chromatography: EtOAc−petroleum ether, 4−6 v/v. The product was obtained as a white solid. ^{1}H NMR ($CDCl_3$) δ: 2.96 (m, 4H), 3.38 (t, J = 5.2 Hz, 2H), 3.65 (t, J = 5.2 Hz, 2H), 3.79 (d, J = 4.0 Hz, 2H), 6.01 (t, J = 4.0 Hz, 1H), 6.86 (t, J = 8.4 Hz, 1H), 7.02 (m, 3H), 7.54 (t, J = 8.0 Hz, 1H), 7.62 (t, J = 8.0 Hz, 1H), 7.72 (t, J = 8.0 Hz, 1H), 7.92 (d, J = 8.0 Hz, 1H), 8.07 (d, J = 8.0 Hz, 1H), 8.26 (d, J = 8.0 Hz, 1H), 8.70 (d, J = 8.0 Hz, 1H). Anal. ($C_{22}H_{22}N_3O_3SF$) C, H, N.

Isoquinolinesulfon-5-yl-{2-[4-(4-chlorophenyl)piperazin-1-yl]-2-oxoethyl}amide (4p). Eluent for the column chromatography: EtOAc−MeOH, 9.9−0.1 v/v. The product was obtained as a white solid. ^{1}H NMR ($CDCl_3$) δ: 3.09 (m, 4H), 3.42 (t, J = 5.2 Hz, 2H), 3.64 (t, J = 5.2 Hz, 2H), 3.82 (d, J = 4.0 Hz, 2H), 6.07 (bs, 1H), 6.89 (d, J = 6.2 Hz, 2H), 7.24 (d, J = 6.2 Hz, 2H), 7.69 (t, J = 8.0 Hz, 1H), 8.22 (d, J = 8.0 Hz, 1H), 8.45 (m, 2H), 8.72 (d, J = 6.0 Hz, 1H), 9.36 (s, 1H). Anal. ($C_{21}H_{21}N_4O_3SCl$) C, H, N.

Isoquinolinesulfon-5-yl-{2-[4-(4-chlorobenzyl)piperazin-1-yl]-2-oxoethyl}amide (4q). Eluent for the column chromatography: EtOAc−MeOH, 9.5−0.5 v/v. The product was obtained as a white solid. ^{1}H NMR ($CDCl_3$) δ: 2.27 (m, 4H), 3.16 (t, J = 4.6 Hz, 2H), 3.37 (s, 2H), 3.42 (t, J = 4.6 Hz, 2H), 3.68 (d, J = 4.0 Hz, 2H), 6.00 (s, 1H), 7.19 (m, 4H), 7.62 (t, J = 7.8 Hz, 1H), 8.14 (d, J = 8.2 Hz, 1H), 8.37 (d, J = 8.2 Hz, 2H), 8.64 (d, J = 6.0 Hz, 1H), 9.29 (s, 1H). Anal. ($C_{22}H_{23}N_4O_3SCl$) C, H, N.

Isoquinolinesulfon-5-yl-{2-[4-(4-bromophenyl)piperazin-1-yl]-2-oxoethyl}amide (4r). Eluent for the column chromatography: EtOAc. The product was obtained as a yellow solid. ^{1}H NMR ($CDCl_3$) δ: 3.04 (m, 4H), 3.11 (t, J = 5.0 Hz, 2H), 3.34 (t, J = 5.0 Hz, 2H), 3.91 (d, J = 4.0 Hz, 2H), 6.07 (bs, 1H), 6.88 (d, J = 8.6 Hz, 2H), 7.37 (d, J = 8.6 Hz, 2H), 7.80 (t, J = 7.6 Hz, 1H), 8.32 (d, J = 7.60 Hz, 1H), 8.48 (m, 1H), 8.51 (d, J = 6.0 Hz, 1H), 8.66 (d, J = 6.0 Hz, 1H), 9.47 (s, 1H). Anal. ($C_{21}H_{21}N_4O_3SBr$) C, H, N.

Isoquinolinesulfon-5-yl-{2-[4-(4-iodophenyl)piperazin-1-yl]-2-oxoethyl}amide (4s). Eluent for the column chromatography: EtOAc−petroleum ether, 9−1 v/v. The product was obtained as a brown solid. ^{1}H NMR (DMSO-d_6) δ: 2.99 (m, 4H), 3.40 (m, 4H), 3.80 (d, J = 5.4 Hz, 2H), 6.64 (d, J = 8.8 Hz, 2H), 7.44 (d, J = 8.8 Hz, 2H), 7.72 (t, J = 7.8 Hz, 1H), 8.00 (t, J = 5.4 Hz, 1H), 8.22 (d, J = 8.4 Hz, 1H), 8.32 (d, J = 8.4 Hz, 1H), 8.46 (d, J = 6.2 Hz, 1H), 8.58 (d, J = 6.2 Hz, 1H), 9.32 (s, 1H). Anal. ($C_{21}H_{21}N_4O_3SI$) C, H, N.

Isoquinolinesulfon-5-yl-[2-oxo-2-(4-*p*-tolylpiperazin-1-yl)ethyl]amide (4t). Eluent for the column chromatography: EtOAc−petroleum ether, 7−3 v/v. The product was obtained as a white solid. ^{1}H NMR ($CDCl_3$) δ: 2.25 (s, 3H), 3.01 (m, 4H), 3.38 (t, J = 5.2 Hz, 2H), 3.64 (t, J = 5.2 Hz, 2H), 3.83 (d, J = 4.0 Hz, 2H), 6.17 (bs, 1H), 6.76 (d, J = 7.8 Hz, 2H), 7.06 (d, J = 7.8 Hz, 2H), 7.67 (t, J = 7.8 Hz, 1H), 8.21 (d, J = 8.0 Hz, 1H), 8.44 (d, J = 7.8 Hz, 2H), 8.70 (d, J = 6.0 Hz, 1H), 9.34 (s, 1H). Anal. ($C_{22}H_{24}N_4O_3S$) C, H, N.

Isoquinolinesulfon-5-yl-{2-[4-(4-methylbenzyl)piperazin-1-yl]-2-oxoethyl}amide (4u). Eluent for the column chromatography: EtOAc−MeOH, 9.5−0.5 v/v. The product was obtained as a white solid. ^{1}H NMR ($CDCl_3$) δ: 2.32 (s, 3H), 2.42 (m, 4H), 3.19 (t, J = 4.8 Hz, 2H), 3.46 (t, J = 4.8 Hz, 2H), 3.48 (s, 2H), 3.74 (d, J = 3.6 Hz, 2H), 6.10 (s, 1H), 7.12 (m, 4H), 7.67 (t, J = 7.8 Hz, 1H), 8.19 (d, J = 8.2 Hz, 1H), 8.42 (d, J = 8.2 Hz, 2H), 8.70 (d, J = 6.2 Hz, 1H), 9.34 (s, 1H). Anal. ($C_{23}H_{26}N_4O_3S$) C, H, N.

Isoquinolinesulfon-5-yl-[2-oxo-2-(4-*o*-tolylpiperazin-1-yl)ethyl]amide (4v). Eluent for the column chromatography: EtOAc−petroleum ether, 7−3 v/v. The product was obtained as a yellow solid. ^{1}H NMR ($CDCl_3$) δ: 2.17 (s, 3H), 2.84 (m, 4H), 3.40 (t, J = 5.2 Hz, 2H), 3.62 (t, J = 5.2 Hz, 2H), 3.88 (d, J = 4.2 Hz, 2H), 6.20 (bs, 1H), 6.84 (d, J = 7.8 Hz, 1H), 6.96 (d, J = 7.8 Hz, 1H), 7.22(m, 2H), 7.67 (t, J = 7.6 Hz, 1H), 8.22 (d, J = 7.8 Hz, 1H), 8.46 (d, J = 7.8 Hz, 2H), 8.70 (d, J = 6.0 Hz, 1H), 9.52 (s, 1H). Anal. ($C_{22}H_{24}N_4O_3S$) C, H, N.

Isoquinolinesulfon-5-yl-{2-[4-(4-acetylphenyl)piperazin-1-yl]-2-oxoethyl}amide (4w). Eluent for the column chromatography: EtOAc. The product was obtained as a white solid. ^{1}H NMR ($CDCl_3$) δ: 2.34 (s, 3H), 3.12 (m, 4H), 3.39 (t, J = 5.4 Hz, 2H), 3.72 (t, J = 5.4 Hz, 2H), 3.90 (d, J = 4.2 Hz, 2H), 6.09 (bs, 1H), 6.76 (d, J = 8.6 Hz, 2H), 7.64 (d, J = 8.6 Hz, 2H), 7.67 (t, J = 7.8 Hz, 1H), 8.21 (d, J = 7.8 Hz, 1H), 8.42 (d, J = 5.8 Hz, 2H), 8.67 (d, J = 5.8 Hz, 1H), 9.25 (s, 1H). Anal. ($C_{23}H_{24}N_4O_4S$) C, H, N.

Isoquinolinesulfon-5-yl-{2-[4-(4-cyanophenyl)piperazin-1-yl]-2-oxoethyl}amide (4x). Eluent for the column chromatography: EtOAc−petroleum ether, 6−4 v/v. The product was obtained as a white solid. ^{1}H NMR ($CDCl_3$) δ: 3.27 (m, 4H), 3.43 (t, J = 5.2 Hz, 2H), 3.66 (t, J = 5.2 Hz, 2H), 3.82 (d, J = 4.2 Hz, 2H), 6.08 (bs, 1H), 6.80 (d, J = 9.0 Hz, 2H), 7.49 (d, J = 9.0 Hz, 2H), 7.68 (t, J = 7.8 Hz, 1H), 8.21 (d, J = 8.4 Hz, 1H), 8.40 (d, J = 5.2 Hz, 1H), 8.44 (d, J = 5.2 Hz, 1H), 8.71 (d, J = 5.2 Hz, 1H), 9.34 (s, 1H). Anal. ($C_{22}H_{21}N_5O_3S$) C, H, N.

Isoquinolinesulfon-5-yl-{2-[4-(4-trifluoromethylphenyl)piperazin-1-yl]-2-oxoethyl}amide (4y). Eluent for the column chromatography: EtOAc. The product was obtained as a white solid. ^{1}H NMR ($CDCl_3$) δ: 3.17 (m, 4H), 3.39 (t, J = 4.6 Hz, 2H), 3.62 (t, J = 4.6 Hz, 2H), 3.76 (d, J = 4.4 Hz, 2H), 5.93 (bs, 1H), 6.81 (d, J = 8.6 Hz, 2H), 7.42 (d, J = 8.6 Hz, 2H), 7.64 (t, J = 8.2 Hz, 1H), 8.15 (d, J = 8.2 Hz, 1H), 8.37 (t, J = 6.8 Hz, 1H), 8.42 (t, J = 6.8 Hz, 1H), 8.66 (d, J = 6.0 Hz, 1H), 9.31 (s, 1H). Anal. ($C_{22}H_{21}N_4O_3SF_3$) C, H, N.

Isoquinolinesulfon-5-yl-{2-[4-(4-methoxyphenyl)piperazin-1-yl]-2-oxoethyl}amide (4z). Eluent for the column chromatography: EtOAc−petroleum ether, 8−2 v/v. The product was obtained as a white solid. ^{1}H NMR ($CDCl_3$) δ: 3.95 (m, 4H), 3.38 (t, J = 5.2 Hz, 2H), 3.63 (t, J = 5.2 Hz, 2H), 3.75 (s, 3H), 3.82 (d, J = 4.2 Hz, 2H), 6.09 (bs, 1H), 6.83 (m, 4H), 7.68 (t, J = 8.0 Hz, 1H), 8.21 (d, J = 8.2 Hz, 1H), 8.40 (d, J = 5.6 Hz, 1H), 8.47 (d, J = 5.6 Hz, 1H), 8.72 (d, J = 6.2 Hz, 1H), 9.35 (s, 1H). Anal. ($C_{22}H_{24}N_4O_4S$) C, H, N.

Isoquinolinesulfon-5-yl-{2-[4-(4-nitrophenyl)piperazin-1-yl]-2-oxoethyl}amide (4aa). Eluent for the column chromatography: EtOAc−MeOH, 9.5−0.5 v/v. The product was obtained as a yellow solid. ^{1}H NMR ($CDCl_3$) δ: 3.39 (m, 6H), 3.69 (t, J = 5.2 Hz, 2H), 3.83 (d, J = 4.4 Hz, 2H), 5.96 (d, J = 4.4 Hz, 1H), 6.78 (d, J = 7.4 Hz, 2H), 7.69 (t, J = 7.4 Hz, 1H), 8.11 (d, J = 7.4 Hz, 2H),

8.22 (d, J = 8.2 Hz, 1H), 8.42 (m, 2H), 8.73 (d, J = 6.2 Hz, 1H), 9.36 (s, 1H). Anal. ($C_{21}H_{21}N_5O_5SF$) C, H, N.

Isoquinolinesulfon-5-yl-{3-[4-(4-nitrophenyl)piperazin-1-yl]-3-oxopropyl}amide (4ab). Eluent for the column chromatography: EtOAc. The product was obtained as a yellow solid. ^{1}H NMR ($CDCl_3$) δ: 2.57 (t, J = 5.4 Hz, 2H), 3.27 (q, J = 5.2 Hz, 2H), 3.40 (m, 4H), 3.49 (t, J = 5.0 Hz, 2H), 3.72 (d, J = 5.0 Hz, 2H), 5.96 (d, J = 4.4 Hz,, 1H), 6.80 (d, J = 9.2 Hz, 2H), 7.70 (t, J = 7.8 Hz, 1H), 8.18 (m, 3H), 8.45 (t, J = 8.6 Hz, 2H), 8.73 (d, J = 6.2 Hz, 1H), 9.40 (s, 1H). Anal. ($C_{22}H_{23}N_5O_5S$) C, H, N.

Isoquinolinesulfon-5-yl-{2-[4-(4-aminophenyl)piperazin-1-yl]-2-oxoethyl}amide (4ac). 4ac was recrystallized from ethyl ether. The product was obtained as a yellow solid. ^{1}H NMR ($CDCl_3$) δ: 2.88 (m, 4H), 3.04 (m, 2H), 3.36 (t, J = 5.2 Hz, 2H), 3.62 (t, J = 5.2 Hz, 2H), 3.80 (d, J = 4.4 Hz, 2H), 6.02 (bs, 1H), 6.57 (d, J = 8.2 Hz, 2H), 6.76 (t, J = 8.2 Hz, 1H), 7.68 (t, J = 8.2 Hz, 1H), 8.18 (d, J = 7.4 Hz, 2H), 8.44 (t, J = 6.2 Hz, 2H), 8.72 (d, J = 5.8 Hz, 1H), 9.34 (s, 1H). Anal. ($C_{21}H_{23}N_5O_3S$) C, H, N.

Isoquinolinesulfon-5-yl-[2-(4-phenylpiperazin-1-yl)-2-oxoethyl]amide (4ad). Eluent for the column chromatography: EtOAc−MeOH, 9.8−0.2 v/v. The product was obtained as a white solid. ^{1}H NMR ($CDCl_3$) δ: 3.05 (m, 4H), 3.42 (t, J = 4.8 Hz, 2H), 3.63 (t, J = 4.8 Hz, 2H), 3.82 (d, J = 3.6 Hz, 2H), 6.11 (bs, 1H), 6.88 (m, 3H), 7.24 (m, 2H), 7.67 (t, J = 7.8 Hz, 1H), 8.20 (d, J = 8.2 Hz, 1H), 8.43 (m, 2H), 8.69 (d, J = 8.2 Hz, 1H), 9.34 (d, J = 8.0 Hz, 1H). Anal. ($C_{21}H_{22}N_4O_3S$) C, H, N.

Isoquinolinesulfon-5-yl-[3-oxo-3-(4-phenylpiperazin-1-yl)propyl]amide (4ae). Eluent for the column chromatography: EtOAc−MeOH, 9.75−0.25 v/v. The product was obtained as a white solid. ^{1}H NMR ($CDCl_3$) δ: 2.52 (t, J = 5.8 Hz, 2H), 3.09 (m, 4H), 3.26 (m, 2H), 3.49 (t, J = 4.6 Hz, 2H), 3.69 (t, J = 4.6 Hz, 2H), 6.02 (bs, 1H), 6.94 (m, 3H), 7.29 (t, J = 7.8 Hz, 2H), 7.72 (t, J = 7.8 Hz, 1H), 8.24 (d, J = 8.2 Hz, 1H), 8.46 (m, 2H), 8.74 (d, J = 6.2 Hz, 1H), 9.36 (m, 1H). Anal. ($C_{22}H_{24}N_4O_3S$) C, H, N.

Isoquinolinesulfon-5-yl-[2-(4-naphthalen-1-yl-piperazin-1-yl)-2-oxoethyl]amide (4af). Eluent for the column chromatography: EtOAc−petroleum ether, 8−2 v/v. The product was obtained as a white solid. ^{1}H NMR ($CDCl_3$) δ: 3.04 (m, 4H), 3.48 (m, 4H), 3.87 (d, J = 3.8 Hz, 2H), 6.08 (bs, 1H), 6.99 (d, J = 6.8 Hz, 1H), 7.48 (m, 2H), 7.56 (t, J = 8.2 Hz, 1H), 7.74 (t, J = 8.2 Hz, 1H), 7.82 (m, 3H), 8.20 (d, J = 8.2 Hz, 1H), 8.44 (t, J = 7.0 Hz, 2H), 8.73 (d, J = 6.2 Hz, 1H), 9.36 (s, 1H). Anal. ($C_{25}H_{24}N_4O_3S$) C, H, N.

Naphthalenesulfon-1-yl-[2-(4-phenylpiperazin-1-yl)-2-oxoethyl]amide (4ag). Eluent for the column chromatography: EtOAc−petroleum ether, 4−6 v/v. The product was obtained as a white solid. ^{1}H NMR ($CDCl_3$) δ: 3.07 (m, 4H), 3.38 (t, J = 4.8 Hz, 2H), 3.66 (t, J = 4.8 Hz, 2H), 3.80 (d, J = 4.4 Hz, 2H), 5.97 (bs, 1H), 6.88 (m, 3H), 7.27 (m, 2H), 7.54 (t, J = 8.0 Hz, 1H), 7.63 (t, J = 8.0 Hz, 1H), 7.70 (t, J = 8.0 Hz, 1H), 7.95 (d, J = 8.0 Hz, 1H), 8.08 (d, J = 8.0 Hz, 1H), 8.25 (d, J = 8.0 Hz, 1H), 8.71 (d, J = 8.0 Hz, 1H). Anal. ($C_{22}H_{23}N_3O_3S$) C, H, N.

Isoquinolinesulfon-5-yl-[2-oxo-2-(4-pyrimidin-2-ylpiperazin-1-yl)ethyl]amide (4ah). Eluent for the column chromatography: EtOAc−MeOH, 9−1. The product was obtained as a yellow solid. ^{1}H NMR ($CDCl_3$) δ: 3.30 (t, J = 4.8 Hz, 2H), 3.57 (t, J = 4.8 Hz, 2H), 3.82 (m, 4H), 3.84 (d, J = 4.0 Hz, 2H), 6.06 (t, J = 4.4 Hz, 1H), 6.55 (t, J = 4.6 Hz, 1H), 7.71 (t, J = 8.2 Hz, 1H), 8.22 (d, J = 8.4 Hz, 1H), 8.30 (d, J = 5.0 Hz, 2H), 8.45 (t, J = 5.6 Hz, 1H), 8.51 (d, J = 5.6 Hz, 1H), 8.72 (d, J = 4.4 Hz, 1H), 9.36 (s, 1H). Anal. ($C_{19}H_{20}N_6O_3S$) C, H, N.

Naphthalenesulfon-1-yl-[2-(4-naphthalen-1-ylpiperazin-1-yl)-2-oxoethyl]amide (4ai). Eluent for the column chromatography: EtOAc−petroleum ether, 4−6 v/v. The product was obtained as a white solid. ^{1}H NMR ($CDCl_3$) δ: 3.14 (m, 4H), 3.49 (m, 2H), 3.83 (m, 2H), 4.11 (m, 2H), 6.04 (bs, 1H), 7.00 (d, J = 8.8 Hz, 1H), 7.12 (d, J = 8.8 Hz, 1H), 7.38 (m, 3H), 7.57 (m, 2H), 7.72 (t, J = 8.8 Hz, 1H), 7.94 (d, J = 8.8 Hz, 1H), 8.08 (m, 2H), 8.12 (d J = 8.8 Hz, 1H), 8.29 (d, J = 8.8 Hz, 1H), 8.74 (d, J = 8.4 Hz, 1H). Anal. ($C_{26}H_{25}N_3O_3S$) C, H, N.

Isoquinolinesulfon-5-yl-[2-(4-benzylpiperazin-1-yl)-2-oxoethyl]amide (4aj). Eluent for the column chromatography: EtOAc−MeOH, 9−1 v/v. The product was obtained as a yellow solid. ^{1}H NMR ($CDCl_3$) δ: 2.30 (m, 4H), 3.18 (t, J = 4.8 Hz, 2H), 3.50 (m, 4H), 3.70 (s, 2H), 5.99 (bs, 1H), 7.02 (m, 5H), 7.64 (t, J = 7.8 Hz, 1H), 8.12 (d, J = 7.8 Hz, 1H), 8.40 (d, J = 7.8 Hz, 1H), 8.44 (d, J = 7.8 Hz, 1H), 8.68 (d, J = 7.8 Hz, 1H), 9.30 (s, 1H). Anal. ($C_{22}H_{24}N_4O_3S$) C, H, N.

Isoquinolinesulfon-5-yl-[2-(4-benzylpiperazin-1-yl)-2-oxoethyl]methylamide (4ak). Eluent for the column chromatography: EtOAc−MeOH, 9−1 v/v. The product was obtained as a white solid. ^{1}H NMR ($CDCl_3$) δ: 2.39 (t, J = 5.2 Hz, 2H), 2.46 (t, J = 5.2 Hz, 2H), 2.92 (s, 3H), 3.50 (m, 6H), 4.12 (s, 2H), 7.32 (m, 5H), 7.69 (t, J = 7.8 Hz, 1H), 8.20 (d, J = 8.0 Hz, 1H), 8.48 (d, J = 6.2 Hz, 2H), 8.67 (d, J = 6.2 Hz, 1H), 9.32 (s, 1H). Anal. ($C_{23}H_{26}N_4O_3S$) C, H, N.

Isoquinolinesulfon-5-yl-[2-(4-benzyl[1,4]diazepan-1-yl)-2-oxoethyl]amide (4al). Eluent for the column chromatography: EtOAc−MeOH, 8−2 v/v. The product was obtained as a white solid. ^{1}H NMR ($CDCl_3$) δ: 1.75 (m, 2H), 2.52 (m, 4H), 3.24 (t, J = 7.0 Hz, 2H), 3.54 (m, 4H), 3.75 (d, J = 6.0 Hz, 2H), 6.09 (bs, 1H), 7.25 (m, 5H), 7.64 (t, J = 7.6 Hz, 1H), 8.17 (d, J = 7.6 Hz, 1H), 8.42 (d, J = 7.6 Hz, 1H), 8.44 (d, J = 7.6 Hz, 1H), 8.68 (d, J = 7.6 Hz, 1H), 9.33 (s, 1H). Anal. ($C_{23}H_{26}N_4O_3S$) C, H, N.

Naphthalenesulfon-1-yl-[2-(4-benzylpiperazin-1-yl)-2-oxoethyl]amide (4am). Eluent for the column chromatography: EtOAc−petroleum ether, 6−4 v/v. The product was obtained as a white solid. ^{1}H NMR ($CDCl_3$) δ: 2.32 (m, 4H), 3.20 (t, J = 5.2 Hz, 2H), 3.48 (m, 4H), 3.73 (s, 2H), 5.99 (bs, 1H), 7.29 (m, 5H), 7.52 (t, J = 8.4 Hz, 1H), 7.62 (t, J = 8.4 Hz, 1H), 7.69 (t, J = 8.4 Hz, 1H), 7.94 (t, J = 8.4 Hz, 1H), 8.07 (t, J = 8.4 Hz, 1H), 8.24 (t, J = 8.4 Hz, 1H), 8.70 (t, J = 8.4 Hz, 1H). Anal. ($C_{23}H_{25}N_3O_3S$) C, H, N.

Isoquinolinesulfon-5-yl-[2-oxo-2-(4-phenethylpiperazin-1-yl)-ethyl]amide (4an). Eluent for the column chromatography: EtOAc−MeOH, 9−1 v/v. The product was obtained as a white solid. ^{1}H NMR ($CDCl_3$) δ: 2.41 (m, 4H), 2.58 (t, J = 5.0 Hz, 2H), 2.71 (t, J = 5.0 Hz, 2H), 3.26 (t, J = 4.8 Hz, 2H), 3.76 (t, J = 4.8 Hz, 2H), 3.77 (d, J = 4.4 Hz, 2H), 6.1 (bs, 1H), 7.21 (m, 5H), 7.67 (t, J = 7.8 Hz, 1H), 8.19 (d, J = 8.2 Hz, 1H), 8.42 (d, J = 6.2 Hz, 2H), 8.69 (d, J = 6.2 Hz, 1H), 9.34 (s, 1H). Anal. ($C_{23}H_{26}N_4O_3S$) C, H, N.

Naphthalenesulfon-1-yl-[2-oxo-2-(4-phenethylpiperazin-1-yl)-ethyl]amide (4ao). Eluent for the column chromatography: EtOAc−petroleum ether, 8−2 v/v. The product was obtained as a white solid. ^{1}H NMR ($CDCl_3$) δ: 2.42 (m, 4H), 2.56 (t, J = 8.4 Hz, 2H), 2.73 (t, J = 8.4 Hz, 2H), 3.24 (t, J = 4.4 Hz, 2H), 3.53 (s, 2H), 3.73 (d, J = 3.6 Hz, 2H), 5.98 (bs, 1H), 7.18 (m, 3H), 7.26 (m, 2H), 7.53 (t, J = 8.0 Hz, 1H), 7.61 (t, J = 8.0 Hz, 1H), 7.68 (t, J = 8.0 Hz, 1H), 7.94 (t, J = 8.0 Hz, 1H), 8.07 (t, J = 8.0 Hz, 1H), 8.23 (t, J = 8.0 Hz, 1H), 8.69 (t, J = 8.0 Hz, 1H). Anal. ($C_{24}H_{27}N_3O_3S$) C, H, N.

Isoquinolinesulfon-5-yl-{2-oxo-2-[4-(3-phenylpropyl)piperazin-1-yl]amide (4ap). Eluent for the column chromatography: EtOAc−MeOH, 9−1 v/v. The product was obtained as a white solid. ^{1}H NMR ($CDCl_3$) δ: 1.76 (m, 2H), 2.32 (m, 6H), 2.57 (t, J = 7.6 Hz, 2H), 3.22 (t, J = 5.2 Hz, 2H), 3.49 (t, J = 5.2 Hz, 2H), 3.75 (d, J = 3.4 Hz, 2H), 6.06 (bs, 1H), 7.19 (m, 5H), 7.67 (t, J = 7.8 Hz, 1H), 8.19 (d, J = 8.4 Hz, 1H), 8.39 (d, J = 6.0 Hz, 1H), 8.42 (d, J = 7.8 Hz, 1H), 8.71 (d, J = 6.2 Hz, 1H), 9.34 (s, 1H). Anal. ($C_{24}H_{28}N_4O_3S$) C, H, N.

Biological Materials and Methods. Cell Cultures. HEK293 cells were cultured in a 1:1 DMEM/F-12 medium (Lonza, Verviers, Belgium) containing 15% heat inactivated FCS (Lonza, Verviers, Belgium), 100 U/mL penicillin, and 100 μg/mL streptomycin. HEK 293-P2X$_7$ cells were kindly provided by Prof. A. Surprenant from Department of Biomedical Science, University of Sheffield, U.K. Stable clones were cultured in the same medium containing G418 sulfate (Geneticin) (Sigma Aldrich, Milan, Italy) at a concentration of 0.2 mg/mL.

Ca^{2+} Measurements. Changes in $[Ca^{2+}]_i$ were measured with the fluorescent indicator Fura-2/AM (Calbiochem Inalco, Milan, Italy) as previously described.[30] Briefly, HEK 293-P2X$_7$ cells were loaded with 4 μM Fura-2/AM in a saline solution (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 $CaCl_2$, 20 mM HEPES, pH 7.4) for 30 min at 37 °C in the presence of 250 μM sulfinpyrazone. Cells were then centrifuged at 1000*g* for 10 min to remove the extracellular dye and resuspended in saline solution. Ca^{2+} traces were obtained by using a LS50, Perkin-Elmer (Norwalk, CT) spectrofluorimeter at an excitation wavelength of 340 and 380 nm and emission wavelength of 505 nm. Measurements were performed in samples that were thermostatically controlled (37 °C) and continuously stirred in cuvettes. After a stable baseline had been established, ATP was added and emitted light recorded. All experiments were performed five times.

Ethidium Uptake. ATP-dependent increase in plasma membrane permeability was measured using the extracellular fluorescent tracer ethidium bromide (Sigma Aldrich, Milan, Italy) as previously described.[26] Cells were incubated in a thermostat-controlled fluorometer cuvette (37 °C) in a modified Ca^{2+}-free saline solution containing EGTA for 20 min in the dark at a concentration of 10^6 cells/mL in the presence of 20 μM ethidium bromide and stimulated with 1 mM ATP. Cell suspension was incubated with synthesized antagonists in the range 5−1000 nM for 10 min at 37 °C before fluorimetric analysis in a LS50, Perkin-Elmer (Norwalk, CT) spectrofluorimeter at 37 °C. Fluorescence changes were monitored at the wavelength pair 360 and 580 nm. All experiments were repeated five times.

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Supporting Information Available: Synthesis and spectroscopic data for compounds **2** and **3**; elemental analysis results of compounds **4a**−**ap**. This material is available free of charge via the Internet at http://pubs.acs.org.

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