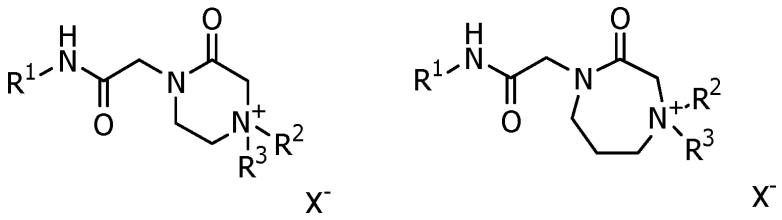
JOURNAL OF combinatorial CHEMISTRY

Article

Synthesis of a Library of 3-Oxopiperazinium and Perhydro-3-oxo-1,4-diazepinium Derivatives and Identification of Bioactive Compounds

Isabel Masip, Clotilde Ferrndiz-Huertas, Carolina Garca-Martnez, Jos A. Ferragut, Antonio Ferrer-Montiel, and Angel Messeguer

J. Comb. Chem., **2004**, 6 (1), 135-141• DOI: 10.1021/cc030002q • Publication Date (Web): 18 November 2003



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

Synthesis of a Library of 3-Oxopiperazinium and Perhydro-3-oxo-1,4-diazepinium Derivatives and Identification of Bioactive Compounds

Isabel Masip,[†] Clotilde Ferrándiz-Huertas,[‡] Carolina García-Martínez,[‡] José A. Ferragut,[‡] Antonio Ferrer-Montiel,[‡] and Angel Messeguer*,[†]

Department of Biological Organic Chemistry, IIQAB, CSIC. J. Girona, 18, 08034 Barcelona, Spain, and Instituto Biología Molecular y Celular, Universidad Miguel Hernández, 03202 Elche, Spain

Received January 4, 2003

The design and synthesis of a library of novel families of 3-oxopiperazinium and perhydro-3-oxo-1,4-diazepinium derivatives is reported. The library was composed of 44 3-oxopiperazinium derivatives (11 of these compounds had a spiranic skeleton) and 22 perhydro-3-oxo-1,4-diazepinium compounds. The synthetic procedure involved a 6-step sequence carried out in solution, along with the use of solid-phase linked scavengers and microwave activation for the rapid removal of the excess of amine reagents. A final cyclization step performed under mild conditions led to the charged heterocyclic moiety. Screening of this library in two biological assays identified active compounds that inhibit the activity of the vanilloid receptor TRPV1 and modulators of the multidrug resistance phenomenon. Thus, this synthetic sequence represents a facile and convenient entry to unprecedented libraries of this sort of tetraalkylammonium derivatives that may be of use for identification of novel scaffolds of diverse biological activity.

Introduction

The advantages in terms of solubility and in some cases of bioavailability induced by the quaternization of amine residues have been employed for optimizing the biological activity of compounds toward selected therapeutic targets. A representative example of this strategy can be the application of tiotropium and its derivatives as bronchodilators in chronic obstructive respiratory diseases.[1] In this same context, N-substituted phenothiazines have been identified as potent anti-leishmania agents.[2] In this case, the quaternization of the tertiary amine fragment was carried out by a final alkylation with methyl iodide (the Menschutkin reaction). On the other hand, a family of transport proteins (SLC22A) containing polyspecific transporters involved in the intestinal absorption and liver or kidney excretion of organic cations has been recently identified.[3,4] Therefore, the search for modulators of these transport proteins could also constitute an attractive goal for potential therapeutic intervention.

In this context, compounds containing 3-oxopiperazinium or perhydro-3-oxo-1,4-diazepinium moieties (Figure 1) are scarce in the literature. Van Heiningen et al. described several cephalosporins bearing piperazinium and 3-oxopiperazinium fragments. Again, these compounds were obtained by a final alkylation of the corresponding amine.[5] The only example in which tetraalkylammonium derivatives were synthesized by a cyclization approach was reported by Fancher et al.[6] In this case, the ammonium derivative was an intermediate in the synthesis of substituted 2-piperazinones as compounds with analgesic activity.

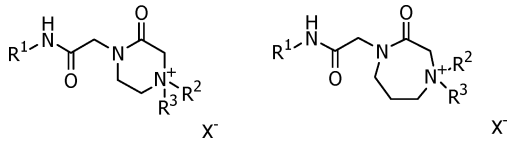


Figure 1. General formula of the library compounds.

The present contribution reports the design and construction of a library of cyclic tetraalkylammonium derivatives of a general formula as illustrated in Figure 1. Screeening of the library identified blockers of the vanilloid receptor TRPV1 and modulators of the multidrug resistance phenomenon. Thus, this library is a source of chemical scaffolds for drug discovery.

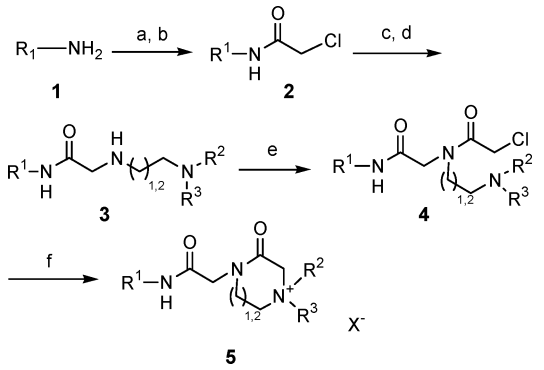
Results and Discussion

Synthetic Strategy and Preliminary Assays. According to our purpose, the main features of this library were (i) the presence of a lipophilic moiety at R^1 by employing a set of primary amines containing an aromatic residue, which could also give rise to the establishment of $\pi-\pi$ or $\pi-$cation interactions with appropriate receptors; (ii) the insertion of the diversity sources R^2 and R^3 by means of aliphatic primary amines containing an additional tertiary amine; this residue would confer the polar character to the molecule, and the nitrogen atom of the tertiary amine would induce the final cyclization step; (iii) the fourth diversity source would be the size of the heterocyclic ring (six- or seven-membered), and its formation would depend on the carbon atom chain

* To whom correspondence should be addressed. Phone: 34 93 400 61 21. Fax: 34 93 204 59 04. E-mail: ampqob@iiqab.csic.es.
[†] CSIC. J. Girona.
[‡] Universidad Miguel Hernández.

10.1021/cc030002q CCC: $27.50
Published on Web 11/18/2003

Scheme 1. Solution-Phase Synthesis of 3-Oxopiperazinium and Perhydro-3-oxo-1,4-diazepinium Derivatives.[a]



[a] Reagents and conditions: (a) $ClCOCH_2Cl$, K_2CO_2, CH_2Cl_2, 0 °C, 30 min; (b) Wang aldehyde resin (3.5 equiv/equiv amine), dioxane, microwave irradiation, 40 min; (c) R-NH_2 (3 equiv), K_2CO_2 (3 equiv, dioxane, 90 °C, 3h; (d) Wang aldehyde resin (3 equiv/equiv amine), dioxane, microwave irradiation, 20 min; (e) $ClCOCH_2Cl$ (1 equiv), K_2CO_2 (3 equiv), CH_2Cl_2, 0 °C, 30 min; (f) Al_2O_3, CH_2Cl_2-MeOH (2:1).

intercalated between the nitrogen atoms of the primary and tertiary amine moieties.

Initially, on the basis of our previous experience with the synthesis of a library of 2,5-piperazinediones by using a multiple parallel synthesis approach on solid phase,[7] and on the basis of our recent description of the preparation of a library of peptoids under the positional scanning format,[8] we devised a synthetic sequence involving two acylation steps for the introduction of α-chloroacetyl residues, intercalated by an amination step for introducing the R^2 and R^3 diversity sources, and a final cyclization reaction promoted by the tertiary amino group. However, analysis of this strategy revealed an incompatibility with the use of solid-phase techniques. In fact, the most suitable linkage to the solid support should be through the R^1 diversity source. This restriction would impose the introduction of this diversity after the release of compounds from the resin.

Therefore, a synthetic pathway operating in homogeneous phase combined with the use of solid-phase scavengers for removing the excess of reagents was devised. Preliminary experiments carried out on the amination of 2-chloroacetamide with different primary amines afforded a mixture of products resulting from the reaction of the primary amine at both the 2-methylene and the carbonyl groups of the amide. These results suggested the convenience of avoiding the use of this acetyl building block, except for those bearing already the diversity source R^1. By this procedure, it was expected that the secondary amide formed would not be attacked by the primary amine employed in the amination step. Accordingly, the introduction of the first diversity source R^1 was carried out by reaction of the corresponding primary amine with $ClCH_2COCl$ in the presence of K_2CO_3 in CH_2Cl_2 (Scheme 1). The maximal conversions were achieved by adding 1.5 molar equiv of the amine. Alternative bases to K_2CO_3 did not avoid the need of this amine excess. This observation forced the employment of the scavenger Wang aldehyde HL for removing the unreacted amine.[9,10] The experiments performed with representative amines showed that an excess of 3 to 3.5 equiv of the polymer was sufficient for the complete removal of the amine excess. However, this step demanded in some cases treatments for 24 h at 40−50 °C. We questioned whether the use of microwave activation[11,12] could accelerate these scavenging reactions. Thus, the addition of the Wang aldehyde resin to a dioxane solution of the crude reaction mixture, followed by activation in a domestic microwave oven for 40 min (in case of amines listed in Figure 2) or 20 min (for amines listed in Figure 3), led to the complete removal of the amine excess. This treatment was carried out by 4-min heating periods interspersed by 1-min pauses.

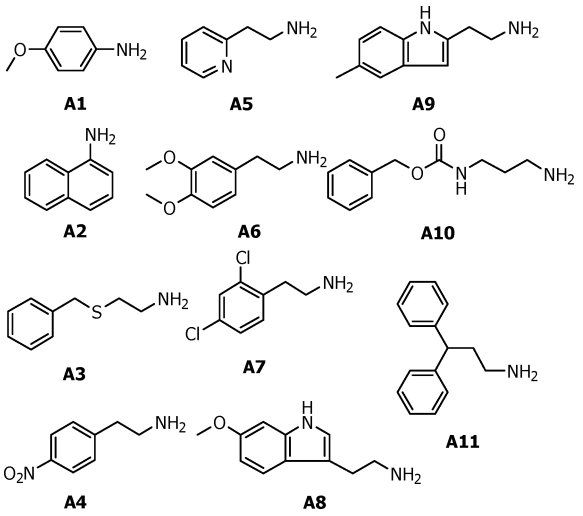


Figure 2. Primary amines introduced as R^1 diversity source in the library synthesis.

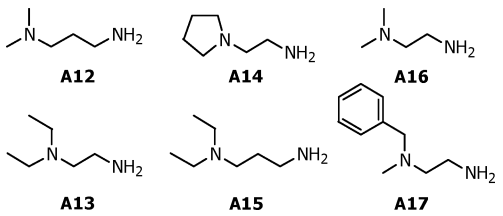


Figure 3. Primary amines introduced as R^2, R^3 diversity source in the library synthesis.

The introduction of the second diversity source, that is, that containing R^2 and R^3, was performed by reaction of the corresponding primary amine with the intermediate chloromethyl derivative **2** to give the N,N′-disubstituted glycinamide **3**. From the different reaction conditions tested, those involving the use of dioxane as solvent in conjunction with 3 molar equiv of the primary amine and K_2CO_3 provided the best results (over 80% yield) at 90 °C for 3 h.

The acylation of the secondary amine moiety of glycinamide **3** using $ClCH_2COCl$ in CH_2Cl_2 under carefully controlled conditions (30 min at 0 °C) led to a crude reaction mixture containing the expected chloroacetyl derivative **4** or the cyclized compound **5**. Notice that the spontaneous cyclization of the chloroacetyl intermediate took place, depending upon the nature of the substituents of the tertiary amine group. To promote the desired cyclization for all the model cases studied, the crude reaction mixture resulting from the treatment with chloroacetyl chloride was stirred in the presence of alumina. By this procedure, the tetraalkylammonium derivatives were systematically obtained in high purities and yields over 80% for this step.

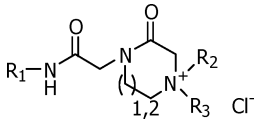
The identification of the cyclic tetraalkylammonium compounds and their differentiation from the chloroacetyl

precursors was carried out by NMR and HPLC/MS. In the ^{13}C NMR spectra, the methylene groups adjacent to the quaternary nitrogen atom exhibited downfield shifts (3−12 ppm) in comparison with the corresponding open intermediate. Likewise, the carbonyl group in β position with respect to the quaternary nitrogen atom (cyclic derivative) was shifted upfield (∼7 ppm) compared to its chemical shift in the acyclic chlorinated precursor. The methylene group adjacent to the quaternary nitrogen atom present in perhydro-3-oxo-1,4-diazepinium derivatives (C-7) appeared at ∼15 ppm upfield in comparison with the same methylene group of the 3-oxopiperazinium derivatives (C-6). Interestingly, the ^{1}H NMR spectra of these compounds showed broad absorptions, indicating a high conformational mobility. The NMR profiles could be satisfactorily resolved by registering the spectra at 60 °C. With respect to the mass spectra, the main difference observed in the cyclic derivatives was the absence of the isotopic chlorine atom profile.

Synthesis of the Library. The above preliminary experiments defined the optimal conditions for the six-step synthetic sequence and gave information about the criteria for selecting the different diversity sources. Thus, amines such as 2,6-diethylaniline or 2-(*p*-methoxybenzoyl)ethylamine had to be discarded because of their poor reactivity in the first amination step. The primary amines selected for introducing the first source of diversity R^1 are shown in Figure 2. All amines contained lipophilic fragments as well as residues capable of promoting $\pi-\pi$ or $\pi-$cation interactions with potential receptors; at the same time, the primary amine functionality had diverse substitution at the α- or β-carbon atom. With respect to the primary amines required for the introduction of the R^2, R^3 diversity sources, Figure 3 shows the compounds chosen. With the exception of *N*′-benzyl-*N*′-methyl-1,2-ethylenediamine (**a17**), all of them were commercially available. Four of the amines contained the 1,2-diaminoethyl chain needed to give rise to the 3-oxopiperazinium compounds, whereas two had a 1,3-diaminopropyl chain for generating the perhydro-3-oxo-1,4-diazepinium ring. Likewise, simple radicals were selected for R^2 and R^3 (methyl, ethyl, and benzyl) to ensure that the final cyclization would not be conditioned by steric reasons. In support of this tenet, preliminary experiments showed that the pyrrolidine fragment present in amine **a14** would not constitute an impediment for this cyclization step.

Following the synthetic pathway discussed above, a 66-membered library was constructed by using the multiple parallel synthesis approach in solution. From the general reaction conditions shown in Scheme 1, the only exception was the acylation with amine **a6**, which had to be carried out under careful control of reaction time (15 min) to prevent the formation of overalkylation side-products. The library was composed of 44 3-oxopiperazinium derivatives (11 of these compounds had a spiranic skeleton) and 22 perhydro-3-oxo-1,4-diazepinium compounds. The HPLC analysis of all compounds showed over 80% purity in 75% of the components (Table 1). It should be noted that compounds formed with amine **a16** led to lower purity values when compared to all other amines employed for the introduction of the R^2, R^3 diversity sources. Nonetheless, all compounds were identified by HPLC/MS, and high-resolution mass spectrometry data were obtained for a set of 14 selected examples. In addition, 25% of the tetraalkylammonium derivatives representing the overall set of diversity used were further characterized by ^{1}H and ^{13}C NMR techniques.[13] As reported in Table 1, the overall yield after the six-step sequence was within 40−70% for most of the compounds of the library. Finally, when considered appropriate for characterization and biological evaluation, compounds could be purified by reversed-phase HPLC.

Table 1. Purities and Yields of the Library Compounds[a]



	R^2, R^3					
R^1	a12	a13	a14	a15	a16	a17
a1	$^{81}/_{46}$	$^{82}/_{39}$	$^{100}/_{8}$	$^{96}/_{69}$	$^{60}/_{45}$	$^{65}/_{90}$
a2	$^{85}/_{40}$	$^{92}/_{60}$	$^{79}/_{56}$	$^{73}/_{80}$	$^{73}/_{36}$	$^{73}/_{78}$
a3	$^{84}/_{50}$	$^{75}/_{48}$	$^{82}/_{18}$	$^{97}/_{55}$	$^{66}/_{30}$	$^{63}/_{90}$
a4	$^{78}/_{16}$	$^{80}/_{24}$	$^{68}/_{12}$	$^{92}/_{43}$	$^{71}/_{32}$	$^{71}/_{43}$
a5	$^{88}/_{16}$	$^{75}/_{35}$	$^{90}/_{80}$	$^{100}/_{43}$	$^{85}/_{52}$	$^{78}/_{55}$
a6	$^{84}/_{69}$	$^{81}/_{86}$	$^{100}/_{27}$	$^{97}/_{62}$	$^{70}/_{70}$	$^{66}/_{84}$
a7	$^{93}/_{20}$	$^{100}/_{92}$	$^{81}/_{57}$	$^{88}/_{74}$	$^{70}/_{71}$	$^{70}/_{84}$
a8	$^{82}/_{21}$	$^{86}/_{40}$	$^{81}/_{52}$	$^{89}/_{60}$	$^{57}/_{8}$	$^{65}/_{73}$
a9	$^{93}/_{27}$	$^{84}/_{8}$	$^{78}/_{57}$	$^{94}/_{47}$	$^{67}/_{12}$	$^{69}/_{66}$
a10	$^{68}/_{14}$	$^{88}/_{76}$	$^{84}/_{76}$	$^{80}/_{10}$	$^{60}/_{40}$	$^{85}/_{91}$
a11	$^{81}/_{58}$	$^{100}/_{59}$	$^{94}/_{65}$	$^{91}/_{68}$	$^{89}/_{43}$	$^{92}/_{69}$

[a] Purities, determined by HPLC at 220 nm, are shown in the upper left of each cell, and yields are shown in the lower right.

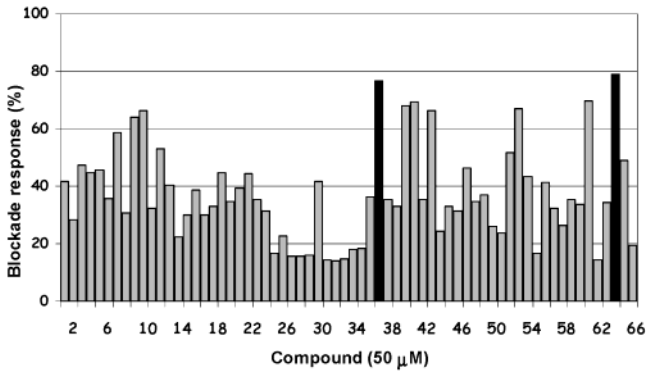


Figure 4. Blockade potency of the library of 3-oxopiperazinium and perhydro-3-oxo-1,4-diazepinium derivatives at 50 μM in the capsaicin-induced channel activity of TRPV1 channel heterogously expressed in *Xeponus* oocytes.

Screening of the Library To Find Active Compounds. To determine whether this newly generated diverse library contains biologically active molecules, we screened it to find antagonists of the receptor TRPV1, a neuronal receptor that integrates thermal and chemical stimuli in the peripheral nervous system.[14] TRPV1 is a therapeutic target for the development of new analgesic drugs to treat inflammatory pain.[15,16] The screening assay evaluated the blockade potency of the different compounds on the capsaicin-induced channel activity of TRPV1 heterogously expressed in *Xenopus* oocytes.[17] As illustrated in Figure 4, the library exhibited an average blockade activity at 50 μM of 30%. Notably, compounds **5**{37, 40, 41, 43, 53, 61, and 64} displayed potencies ≥70%, products 37 and 64 being the most active

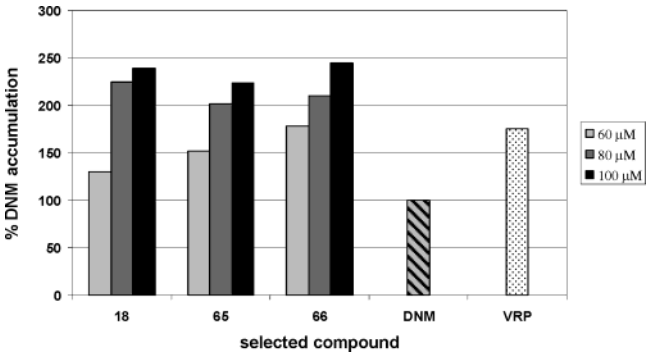


Figure 5. Dose−response results of most active compounds (18, 65, and 66) at 60, 80, and 100 μM on the intracellular daunomycin (DNM) accumulation in the murine laukemic resistant cell line L1210R overexpressing P-glycoprotein. Values for DNM (3 μM) and verapamil (VRP, 5 μM) are also given.

blockers. This blockade potency was selective, as evidenced by the lack of activity on the glutamate receptor NMDA (data not shown). Although these compounds inhibited the TRPV1 activity with significant potency, their blockade efficacy ($IC_{50} \geq 20$ μM) is moderate to low. Nonetheless, these findings provide important information on the structural requirements for TRPV1 channel blockers. For instance, the most active compounds identified so far (37 and 64) contain highly lipophilic residues at R^1 (amines **a7** and **a11**) and the seven-membered tetraalkylamonium ring. Moreover, five out of the seven most active compounds are perhydro-3-oxo-1,4-diazepinium derivatives. Thus, the identified products furnish structural scaffolds that could be used as pillars to design more active and selective TRPV1 blockers.

Similarly, the library was screened to find chemosensitizer agents of the multidrug resistance (MDR) phenotype which renders tumor cells insensitive to antineoplastics, thus representing a serious problem in cancer chemotherapy.[18] MDR phenotype is pleiotropic and frequently associated with the overexpression of efflux pumps, such as P-glycoprotein (P-gp) and the multidrug resistance (associated) protein (MRP) that extrude out antitumoral drugs.[19] To identify molecules that sensitize tumor resistant cells to antineoplastics, we established a screening assay that monitored the intracellular accumulation of the antineoplastic agent daunomycin (DNM) in the murine leukemic resistant cell line L1210R that overexpress the P-glycoprotein.[20] Intracellular drug accumulation was evaluated by flow cytometry taking advantage of the intrinsic DNM fluorescence. The screening of the library identified few compounds that at 100 μM remarkably increased the intracellular accumulation of DNM in L1210R cells, similar to the well-known chemosensitizer verapamil (data not shown). Dose−response relationships of these molecules led to identification of compounds **5**{18, 65, and 66} as the most active chemosensitizers, with an IC_{50} in the range of 20 μM (Figure 5). Notably, these compounds exhibited selectivity toward the P-glycoprotein, as evidenced by the modest sensitizing effect on the human tumor line HL60 that overexpresses the related extrusion pump MRP1. From the structural point of view, the most active chemosensitizers identified are highly lipophilic molecules as a result of the occurrence of aromatic moieties at R^1 (from amines **a3** and **a11**) and at R^2 for two of them (from amine **a17**). In addition, all contain the 3-oxopiperazinium ring. Together, these results indicate the presence of hits in the library that may be used as scaffolds for structure−activity improvement and development of a new generation of useful chemosensitizers.

In conclusion, a procedure has been developed for the synthesis of a library of a novel family of 3-oxopiperazinium and perhydro-3-oxo-1,4-diazepinium derivatives. The six-step sequence was carried out in solution phase using solid-phase scavengers and microwave stimulation for the removal of the excess of amine reagents. The strategy included a final cyclization step under mild conditions for generating the charged heterocyclic moiety. This synthetic sequence represents a facile and convenient entry to new scaffolds and to unprecedented libraries of this sort of tetraalkylammonium derivatives. The screening of this library against two different therapeutic targets, namely, the vanilloid receptor TRPV1 and the MDR phenotype, led to the identification of hit compounds possessing distinctive structural features that will permit the corresponding structure−activity improvements.

Experimental Section

General. All primary amines and solvents were obtained from commercial suppliers and used without further purification. Aldehyde Wang HL resin was obtained from Novabiochem (Switzerland). NMR spectra were recorded on a Varian Unity 300 instrument (300 MHz for ^{1}H and 75 MHz for ^{13}C). Chemical shifts are referenced to D_2O (^{1}H) and acetone-d_6 (^{13}C). HPLC analyses were performed using a Hewlett-Packard HP1100 apparatus equipped with a Kromasil 100 C8 column (15 × 0.46 cm, 5 μm) from Teknokroma (Barcelona, Spain). HPLC/MS analyses were performed with a HP1100 LC/MSD apparatus. High-resolution mass spectra (HRMS-FAB) were carried out at the Mass Spectrometry Service of the University of Córdoba (Spain).

General Procedure for the Preparation of Library Compounds. The library was synthesized with a Carousel Reaction Station from Radleys Discovery Technologies (U.K.). The synthesis involved the following sequential steps:

1. First Amine Coupling. Chloroacetyl chloride (0.2 mmol) was added dropwise to a stirred suspension of the corresponding primary amine (R^1-NH_2, Figure 2) (0.3 mmol, 1.5 equiv) and K_2CO_3 (0.4 mmol) in CH_2Cl_2 (2.5 mL) maintained at 0 °C. The mixture was stirred at this temperature for 30 min, filtered, and evaporated to render a residue which was redissolved in dioxane (0.5 mL) and treated with the 4-benzyloxybenzaldehyde polystyrene resin (Wang aldehyde HL) (0.35 mmol, 3.5 equiv relative to the nucleophile, 2.8 mmol/g). The reaction mixture was placed in a domestic microwave and irradiated (350 W) for 40 min at 4-min intervals. The crude reaction mixture was filtered, and the resin was washed with dioxane (2 mL) and CH_2Cl_2 (3 × 2 mL). The filtrate was evaporated to afford the corresponding N-substituted chloroacetamide **2**.

2. Second Amine Coupling. The residue obtained was dissolved in dioxane (2 mL) and treated with the corresponding primary amine ($R^2R^3NCH_2CH_2NH_2$, Figure 3) (0.6 mmol, 3 equiv) and K_2CO_3 (0.4 mmol). The mixture was stirred at 90 °C for 3 h, filtered, and concentrated to 1 mL.

This solution was treated with the Wang aldehyde HL resin (1.2 mmol, 3 equiv relative to the nucleophile, 2.8 mmol/g) for 20 min in the microwave oven (4-min intervals). The resin was filtered and washed with dioxane (2 mL) and CH_2Cl_2 (3 × 2 mL). The filtrate was evaporated to obtain the glycinamide **3**.

3. Second Acylation. Chloroacetyl chloride (0.2 mmol) was added to a stirred suspension of glycinamide **3** and K_2CO_3 (0.4 mmol) in CH_2Cl_2 (2.5 mL) at 0 °C, and the mixture was stirred for 30 min, filtered, and evaporated to dryness.

4. Cyclization. The former residue was redissolved in a 2:1 mixture of CH_2Cl_2/MeOH (2 mL), and the solution was treated with basic aluminum oxide for 1 h at 20 °C. The crude reaction mixture was filtered, and the solvent was removed to give the corresponding cyclic tetraalkylammonium compounds **5** as colorless solid or yellowish oil.

4-[(4-Methoxyphenylcarbamoyl)-methyl]-1,1-dimethyl-perhydro-3-oxo-1,4-diazepinium Chloride (5a). ^{1}H NMR (D_2O, acetone-d_6): δ 7.24 (d, J = 8.5 Hz, 2H), 6.80 (d, J = 8.5 Hz, 2H), 4.33 (bs, 2H), 4.23 (s, 2H), 3.69 (4H), 3.60 (s, 3H), 3.20 (s, 6H), 2.25 (2H). ^{13}C NMR: 167.6 (CO), 165.5 (CO), 156.5 (C), 122.9 (2 × CH), 114.7 (2 × CH), 67.7 (CH_2), 66.9 (CH_2), 55.6 (CH_3), 53.2 (CH_2), 49.3 (CH_2), 23.8 (CH_2). HRMS calcd for $C_{16}H_{24}N_3O_3^+$: 306.1818. Found: 306.1807.

1-Benzyl-4-[(4-methoxyphenylcarbamoyl)-methyl]-1-methyl-3-oxopiperazinium Chloride (5b). ^{1}H NMR (D_2O, acetone-d_6): δ 7.68 (bs, 5H), 7.50 (d, J = 9 Hz, 2H), 7.0 (d, J = 9 Hz, 2H), 4.80 (2H), 4.48 (s, 2H), 4.03 (bs, 2H), 3.89 (bs, 2H), 3.85 (s, 3H), 3.38 (s, 3H). ^{13}C NMR: 166.9 (CO), 163.5 (C), 162.3 (CO), 156.6 (C), 133.4 (2 × CH), 131.6 (CH), 129.7 (2 × CH), 125.9 (C), 122.9 (2 × CH), 114.6 (2 × CH), 69.0 (CH_2), 59.8 (CH_2), 55.8 (CH_3), 55.4 (CH_2), 49.6 (CH_2), 48.0 (CH_3), 43.0 (CH_2). HRMS calcd for $C_{21}H_{26}N_3O_3^+$: 368.1974. Found: 368.1971.

1,1-Diethyl-4-[(1-naphthyl)carbamoylmethyl]-3-oxopiperazinium Chloride (5c). ^{1}H NMR (D_2O, acetone-d_6): δ 7.74 (d, J = 8.5 Hz, 1H), 7.70 (d, J = 8.5 Hz, 1H), 7.62 (t, J = 4.5 Hz, 1H), 7.35 (bt, J = 7.2 Hz, 2H), 7.30 (bs, 1H), 7.29 (bs, 1H), 4.29 (s, 2H), 3.60 (bs, 4H), 3.30 (h, J = 7.2 Hz, 4H), 1.10 (t, J = 7.2 Hz, 6H). ^{13}C NMR: 168.8 (CO), 162.7 (CO), 134.3 (C), 131.7 (C), 128.8 (C), 128.7 (CH), 127.7 (CH), 127.2 (CH), 126.9 (CH), 126.0 (CH), 123.9 (CH), 122.5 (CH), 55.2 (2 × CH_2), 52.6 (CH_2), 49.7 (CH_2), 42.8 (CH_2), 6.9 (2 × CH_3).

4-[[2-(Benzylsulfanyl)ethylcarbamoyl]-methyl]-1,1-dimethyl-perhydro-3-oxo-1,4-diazepinium Chloride (5d). ^{1}H NMR, (D_2O, acetone-d_6): δ 7.3−7.1 (5H), 4.06 (bs, 2H), 3.62 (sa, 4H), 3.25 (t, J = 6 Hz, 2H), 3.2 (s, 3H), 3.15 (2H), 2.49 (t, J = 6.5 Hz, 2H), 2.2 (2H). ^{13}C NMR: 169.6 (CO), 165.5 (CO), 135.6 (C), 129.2 (2 × CH), 129.0 (2 × CH), 127.6 (CH), 62.5 (CH_2), 52.5 (CH_2), 49.1 (CH_2), 38.8 (CH_2), 35.5 (CH_2), 29.9 (CH_2), 23.8 (CH_2), 3.2 (2 × CH_3). HRMS calcd for $C_{18}H_{28}N_3O_2S^+$: 350.1902. Found: 350.1903.

1-Benzyl-4-[[2-(benzylsulfanyl)ethylcarbamoyl]-methyl]-1-methyl-3-oxopiperazinium Chloride (5e). ^{1}H NMR, (D_2O, acetone-d_6): δ 7.7−7.5 (5H), 7.5−7.4 (ca, 5H), 5.0 (2H), 4.37 (s, 2H), 4.10 (4H), 3.90 (s, 2H), 3.56 (t, J = 7 Hz, 2H), 3.45 (s, 3H), 2.77 (t, J = 7 Hz, 2H). ^{13}C NMR: 168.7 (CO), 162.2 (CO), 133.5 (2 × CH), 131.7 (CH), 129.9 (2 × CH), 129.2 (2 × CH), 128.9 (2 × CH), 127.5 (CH), 69.1 (CH_2), 60.2 (CH_2), 55.5 (CH_2), 49.1 (CH_2), 48.3 (CH_3), 42.9 (CH_2), 38.9 (CH_2), 35.6 (CH_2), 30.7 (CH_2). HRMS calcd for $C_{23}H_{30}N_3O_2S^+$: 412.2059. Found: 412.2053.

4-[[2-(Benzylsulfanyl)ethylcarbamoyl]-methyl]-1,1-diethyl-perhydro-3-oxo-1,4-diazepinium Chloride (5f). ^{1}H NMR, (D_2O, acetone-d_6): δ 7.2−7.0 (5H), 4.23 (bs, 2H), 4.07 (s, 2H), 3.63 (s, 2H), 3.58 (t, J = 6 Hz, 4H), 3.44 (m, J = 7 Hz, 4H), 3.26 (t, J = 6.5 Hz, 2H), 2.46 (t, J = 6.5 Hz, 2H), 2.2 (2H), 1.25 (t, J = 7 Hz, 6H). ^{13}C NMR: 169.6 (CO), 165.6 (CO), 138.6 (C), 129.2 (2 × CH), 129.0 (2 × CH), 127.5 (CH), 63.2 (2 × CH_2), 62.5 (CH_2), 52.5 (CH_2), 49.2 (CH_2), 38.7 (CH_2), 35.3 (CH_2), 30.0 (CH_2), 23.0 (CH_2), 7.3 (2 × CH_3). HRMS calcd for $C_{20}H_{32}N_3O_2S^+$: 378.2215. Found: 378.2210.

1,1-Diethyl-4-{[2-(4-nitrophenyl)-ethylcarbamoyl]-methyl}-perhydro-3-oxo-1,4-diazepinium Chloride (5 g). ^{1}H NMR (D_2O, acetone-d_6): δ 8.25 (d, J = 8.5 Hz, 2H), 7.65 (d, J = 8.5 Hz, 2H), 4.50 (bs, 2H), 4.31 (s, 2H), 3.88 (bs, 4H), 3.73 (m, J = 7 Hz, 4H), 3.63 (t, J = 7 Hz, 2H), 3.08 (t, J = 7 Hz, 2H), 2.45 (2H), 1.55 (t, J = 7 Hz, 6H). ^{13}C NMR: 169.3 (CO), 165.3 (CO), 148.2 (C), 146.2 (C), 130.6 (2 × CH), 123.8 (2 × CH), 63.4 (2 × CH_2), 62.7 (CH_2), 52.6 (CH_2), 49.2 (CH_2), 40.5 (CH_2), 35.2 (CH_2), 23.2 (CH_2), 7.5 (2 × CH_3). HRMS calcd for $C_{19}H_{29}N_4O_4^+$: 377.2189. Found: 377.2179.

4-[(2-(2-Pyridyl)-ethylcarbamoyl)-methyl]-3-oxopiperazinium-1-spiro-1′-pyrrolidinium Chloride (5h). ^{1}H NMR (D_2O, acetone-d_6): δ 8.65 (d, J = 4.5 Hz, 1H), 8.30 (s, NH), 7.98 (t, J = 7.5 Hz, 1H), 7.55 (d, J = 7.5 Hz, 2H), 7.50 (t, J = 4.5 Hz, 1H), 4.45 (s, 2H), 4.37 (s, 2H), 4.00 (bs, 6H), 3.80 (t, J = 7 Hz, 2H), 3.46 (2H), 3.18 (t, J = 7 Hz, 2H), 2.50 (bs, 4H). ^{13}C NMR: 168.6 (CO), 162.5 (CO), 158.8 (C), 148.9 (CH), 138.1 (CH), 124.3 (CH), 122.6 (CH), 64.4 (2 × CH_2), 60.5 (CH_2), 54.6 (CH_2), 49.2 (CH_2), 43.7 (CH_2), 39.6 (CH_2), 37.1 (CH_2), 21.6 (2 × CH_2). HRMS calcd for $C_{17}H_{26}N_4O_2^+$: 318.2056. Found: 318.2067.

4-{[2-(3,4-Dimethoxyphenyl)-ethylcarbamoyl]-methyl}-3-oxopiperazinium-1-spiro-1′-pyrrolidinium Chloride (5i). ^{1}H NMR (D_2O, acetone-d_6): δ 7.60−6.50 (3H), 3.92 (s, 2H), 3.79 (s, 2H), 3.66 (t, J = 6 Hz, 2H), 3.6−3.5 (12H), 3.23 (t, J = 7 Hz, 2H), 2.53 (t, J = 7 Hz, 2H), 2.08 (bs, 4H). ^{13}C NMR: 168.7 (CO), 162.7 (CO), 148.3 (C), 146.9 (C), 132.5 (C), 121.7 (CH), 112.6 (CH), 111.9 (CH), 64.2 (2 × CH_2), 55.8 (2 × CH_3O), 55.8 (CH_2), 55.7 (CH_2), 49.1 (CH_2), 43.5 (CH_2), 41.0 (CH_2), 34.5 (CH_2), 21.5 (2 × CH_2). HRMS calcd for $C_{20}H_{30}N_3O_4^+$: 376.2236. Found: 376.2236.

4-{[2-(2,4-Dichlorophenyl)-ethylcarbamoyl]-methyl}-1,1-diethyl-3-oxopiperazinium Chloride (5j). ^{1}H NMR (D_2O, acetone-d_6): δ 7.29 (s, 1H), 7.12 (bs, 2H), 3.97 (s, 2H), 3.69 (t, J = 6 Hz, 2H), 3.49 (t, J = 6 Hz, 2H), 3.43 (m, J = 6.5 Hz, 4H), 3.36 (t, J = 6.5 Hz, 2H), 2.76 (t, J = 6.5 Hz, 2H), 1.23 (t, J = 6.5 Hz, 6H). ^{13}C NMR: 168.7 (CO), 162.6 (CO), 135.7 (C), 134.8 (C), 132.7 (CH), 129.2 (CH), 127.5 (CH), 55.3 (2 × CH_2), 52.5 (CH_2), 48.9 (CH_2), 42.5 (CH_2), 39.1 (CH_2), 32.5 (CH_2), 6.9 (2 × CH_3).

1-Benzyl-4-{[2-(2,4-dichlorophenyl)-ethylcarbamoyl]-methyl}-1-methyl-3-oxopiperazinium Chloride (5k). ^{1}H

NMR (D_2O, acetone-d_6): δ 7.51 (bs, 5H), 7.18−7.13 (3H), 4.7 (2H), 4.16 (s, 2H), 4.12 (s, 2H), 3.86 (bs, 2H), 3.74 (bs, 2H), 3.37 (bs, 2H), 3.17 (s, 3H), 2.80 (bs, 2H). ^{13}C NMR: 168.6 (CO), 162.2 (CO), 135.8 (C), 134.7 (C), 133.4 (2 × CH), 132.6 (CH), 131.6 (CH), 129.8 (2 × CH), 128.9 (CH), 127.5 (CH), 126.0 (C), 68.7 (CH_2), 60.0 (CH_2), 55.3 (CH_2), 49.1 (CH_2), 48.3 (CH_3), 42.8 (CH_2), 39.0 (CH_2), 32.5 (CH_2). HRMS calcd for $C_{22}H_{26}N_3O_2Cl_2^+$: 434.1402. Found: 434.1396.

1,1-Diethyl-4-{[2-(6-methoxy-1*H*-indol-3-yl)-ethylcarbamoyl]-methyl}-3-oxopiperazinium Chloride (5l). ^{1}H NMR (D_2O, acetone-d_6): δ 7.40 (d, J = 8.5 Hz, 1H), 7.23 (s, 1H), 7.19 (s, 1H), 6.87 (d, J = 8.5 Hz, 1H), 4.19 (s, 4H, H-2), 3.91 (s, 3H), 3.84 (t, J = 6.5 Hz, 2H), 3.59 (8H), 2.97 (t, J = 6.5 Hz, 2H), 2.22 (bs, 1H, NH), 1.43 (t, J = 6.5 Hz, 6H). ^{13}C NMR: 168.6 (CO), 162.4 (CO), 153.4 (C), 132.2 (C), 128.0 (C), 124.4 (CH), 112.8 (CH), 112.0 (C), 111.7 (CH), 101.3 (CH), 59.3 (CH_2), 56.3 (CH_3), 55.5 (2 × CH_2), 52.7 (CH_2), 49.1 (CH_2), 42.3 (CH_2), 40.4 (CH_2), 24.8 (CH_2), 7.1 (2 × CH_3). HRMS calcd for $C_{21}H_{31}N_4O_3^+$: 387.2396. Found: 387.2400.

4-[(3-(Benzyloxycarbonylamino)propylcarbamoyl)-methyl]-3-oxopiperazinium-1-spiro-1′-pyrrolidinium Chloride (5m). ^{1}H NMR (D_2O, acetone-d_6): δ 7.2−7.0 (bs, 5H), 4.84 (s, 2H), 3.96 (s, 2H), 3.67 (t, J = 6 Hz, 2H), 3.60 (t, J = 6 Hz, 2H), 3.52 (t, J = 7 Hz, 4H), 3.02 (t, J = 6.5 Hz, 2H), 2.93 (t, J = 6.5 Hz, 2H), 2.07 (bt, 4H), 1.45 (m, J = 6.5 Hz, 2H). ^{13}C NMR: 168.9 (CO), 162.8 (CO), 158.4 (CO), 136.8 (C), 129.0 (2 × CH), 128.56 (CH), 127.8 (CH), 66.8 (CH_2), 64.2 (2 × CH_2), 55.0 (CH_2), 49.3 (CH_2), 43.7 (CH_2), 38.1 (CH_2), 36.9 (CH_2), 28.8 (CH_2), 21.5 (2 × CH_2). HRMS calcd for $C_{21}H_{31}N_4O_4^+$: 403.2345. Found: 403.2329.

1-Benzyl-4-[(3-(benzyloxycarbonylamino)propylcarbamoyl)-methyl]-1-methyl-3-oxopiperazinium Chloride (5n). ^{1}H NMR (D_2O, acetone-d_6): δ 7.6 (bs, 5H), 7.3 (bs, 5H), 5.09 (s, 2H), 4.80 (2H), 4.28 (s, 2H), 3.97 (bs, 2H), 3.88 (bs, 2H), 3.31 (bs, 2H), 3.28 (s, 3H), 3.22 (t, J = 7 Hz, 2H), 1.78 (m, J = 7 Hz, 2H). ^{13}C NMR: 168.8 (CO), 162.2 (CO), 158.1 (CO), 136.8 (C), 133.3 (2 × CH), 131.6 (CH), 129.8 (CH), 128.9 (2 × CH), 128.4 (CH), 127.8 (CH), 125.9 (C), 66.8 (CH_2), 66.7 (CH_2), 60.0 (CH_2), 55.3 (CH_2), 49.2 (CH_2), 48.2 (CH_3), 42.8 (CH_2), 38.3 (CH_2), 37.0 (CH_2), 29.3 (CH_2).

4-[(3,3-Diphenyl-propylcarbamoyl)-methyl]-1,1-diethyl-perhydro-3-oxo-1,4-diazepinium Chloride (o). ^{1}H NMR (D_2O, acetone-d_6): δ 7.46 (8H), 7.35 (2H), 4.5 (s, 2H), 4.31 (s, 2H), 4.18 (t, J = 7 Hz, 1H), 3.87 (4H), 3.72 (m, J = 7 Hz, 4H), 3.34 (t, J = 7 Hz, 2H), 2.50 (m, J = 7 Hz, 2H), 2.46 (m, 2H), 1.56 (t, J = 7 Hz, 6H). ^{13}C NMR: 169.0 (CO), 165.5 (CO), 144.8 (C), 128.9 (4 × CH), 127.9 (2 × CH), 126.7 (4 × CH), 63.2 (2 × CH_2), 62.4 (CH_2), 52.5 (CH_2), 49.1 (CH_2), 48.8 (CH), 38.5 (CH_2), 34.3 (CH_2), 22.9 (CH_2), 7.3 (2 × CH_3). HRMS calcd for $C_{26}H_{36}N_3O_2^+$: 422.2807. Found: 422.2811.

4-[(3,3-Diphenyl-propylcarbamoyl)-methyl]-1,1-dimethyl-3-oxopiperazinium Chloride (p). ^{1}H NMR (D_2O, acetone-d_6): δ 7.4−7.2 (8H), 7.2 (2H), 4.15 (s, 2H), 4.04 (t, J = 7.5 Hz, 2H), 3.96 (t, J = 6 Hz, 1H), 3.85 (t, J = 6 Hz, 2H), 3.43 (s, 6H, 2 × CH_3), 3.23 (t, J = 7.5 Hz, 2H), 2.33 (m, 2H). ^{13}C NMR: 168.5 (CO), 162.3 (CO), 144.9 (C), 128.9 (4 × CH), 127.9 (2 × CH), 126.7 (4 × CH), 57.6 (CH_2), 52.7 (2 × CH_3), 49.1 (CH_2), 48.9 (CH), 43.0 (CH_2), 38.6 (CH_2), 34.2 (CH_2). HRMS calcd for $C_{23}H_{30}N_3O_2^+$: 380.2338. Found: 380.2328.

1-Benzyl-4-[(3,3-diphenyl-propylcarbamoyl)-methyl]-1-methyl-3-oxopiperazinium (q). ^{1}H NMR (D_2O, acetone-d_6): δ 7.7−7.6 (5H), 7.4−7.2 (10H), 4.80 (2H), 4.26 (s, 2H), 4.19 (bs, 2H), 4.01 (t, J = 7.5 Hz, 1H), 3.92 (2H), 3.87 (2H), 3.28 (s, 3H), 3.22 (t, J = 7.5 Hz, 2H), 2.33 (m, 2H). ^{13}C NMR: 168.5 (CO), 162.2 (CO), 144.9 (C), 133.3 (2 × CH), 131.6 (CH), 129.8 (2 × CH), 129.0 (4 × CH), 127.9 (4 × CH), 126.7 (2 × CH), 125.9 (C), 68.9 (CH_2), 60.0 (CH_2), 55.3 (CH_2), 49.1 (CH_2), 48.8 (CH), 48.3 (CH_3), 42.9 (CH_2), 38.5 (CH_2), 34.2 (CH_2). HRMS calcd for $C_{29}H_{34}N_3O_2^+$: 456.2651. Found: 456.2648.

Recombinant Rat TRPV1 Channels Expression in *Xenopus* Oocytes and Channel Blockade. All the procedures have been described in detail elsewhere.[17] Whole-cell currents from rat TRPV1-injected oocytes were recorded in Mg^{2+}-Ringer's solution (in mM: 10 Hepes pH 7.4, 115 NaCl, 2.8 KCl, 0.1 $BaCl_2$, 2.0 $MgCl_2$) with a two-microelectrode voltage-clamp amplifier at 20 °C. TRPV1 channels were activated by application of 10 μM capsaicin in the absence or presence of increasing concentrations of individual compounds at a holding potential (V_h) of −80 mV.

Daunomycin Accumulation Assays. Daunomycin-resistant murine leukemia L1210R and human promyelocytic HL60R cells, overexpressing P-glycoprotein (P-gp) and the multidrug resistance-assciated protein (MRP1), respectively, were washed once with HBS buffer, and the pellet was resuspended in HBS at 1 × 10^6/mL per sample. Thereafter, cells were incubated with 3 μM daunomycin (control) and 5 μM verapamil (as reference of chemosensitizing effect) for 1 h at 37 °C. After incubation, intracellular daunomycin accumulation was determined by flow cytometry.[20]

Acknowledgment. This work was supported by grants from Fundació La Marató de TV3, from CICYT (SAF98-0059 to A.M. and SAF2000-0142 to A.F.M.), from FIS (01/0038-01, -02, and -04) to A.M., J.A.F., and A.F.M., respectively), from GVA (to A.F.M.), and from FEDER (2FD97-1760-C03-03).

Supporting Information Available. ^{1}H and ^{13}C NMR spectra of selected title compounds (six pages). This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Disse, B.; Speck, G.; Rominger, K. L.; Witek, T. J.; Hammer, R. *Life Sci.* **1999**, *64*, 457−464.
(2) Khan, M. O. F.; Austin, S. E.; Chan, C.; Yin, H.; Marks, D.; Vaghjiani, S. N.; Kendrich, H.; Yardley, V.; Croft, S. L.; Douglas, K. T. *J. Med. Chem.* **2000**, *43*, 3148−3156.
(3) Groothuis, G. M. M.; Meijer, D. K. F. *J. Hepatol.* **1996**, *24*, 3−28.
(4) Van Montfoort, J. E.; Müller, M.; Groothuis, G. M. M.; Meijer, D. K. F.; Koepsell, H.; Meier, P. J. *J. Pharmacol. Exp. Ther.* **2001**, *298*, 110−115.
(5) Van Heyningen, E.; Brown, C. N. *J. Med. Chem.* **1965**, *8*, 174−181.
(6) Fancher, O. E.; Hayao, S.; Strucker, W. G. *J. Med. Chem.* **1964**, *7*, 154−158.

(7) Carbonell, T.; Masip, I.; Sánchez-Baeza, F.; Delgado, M.; Araya, E.; Llorens, O.; Corcho, F.; Pérez, J. J.; Pérez-Payá, E.; Messeguer, A. *Mol. Diversity* **2000**, *5*, 131−143.
(8) Humet, M.; Carbonell, T.; Masip, I.; Sánchez-Baeza, F.; Mora, P.; Cantón, E.; Gobernado, M.; Abad, C.; Pérez-Payá, E.; Messeguer, A. A *J. Comb. Chem.* **2003**, *5*, 597−605.
(9) Booth, R. J.; Hodges, J. C. *Acc. Chem. Res.* **1999**, *32*, 18−26.
(10) Hodges, J. C. *Synlett* **1999**, *1*, 152−158.
(11) Stadler, A.; Kappe, C. O. *J. Comb. Chem.* **2001**, *3*, 624−630.
(12) Lidström, P.; Tierney, J.; Wathey, B.; Westman, J. *Tetrahedron* **2001**, *57*, 9225−9283.
(13) In some six- and seven-membered ring derivatives, the C-2 methylene group was detected neither by ^{1}H nor by ^{13}C NMR. This fact could be due to a dynamic exchange process derived from the influence of the positive charge on the solvent molecules ($D_2O-CD_3COCD_3$ mixtures), which would cause a partially ordered distribution of these molecules around the salt. Under these conditions, the CH_2 absorption would appear as a set of peaks of low intensity that would be hidden in the background noise. In some examples, a better definition of this absorption was obtained by registering the ^{13}C NMR spectra at higher temperatures.
(14) Caterina, M. J.; Julius, D. *Annu. Rev. Neurosci.* **2001**, *24*, 487−517.
(15) Zygmunt, P. M.; Peterson, J.; Andersson, D. A.; Chuang, H.-H.; Sørgård, M.; Di Marzo, V.; Julius, D.; Högestätt, E. D. *Nature* **1999**, *400*, 452−457.
(16) Williams, M.; Kowaluk, E. A.; Arneric, S. P. *J. Med. Chem.* **1999**, *42*, 1481−1500.
(17) García-Martínez, C.; Humet, M.; Planells-Cases, R.; Sanchez-Baeza, F.; Carbonell, T.; Pérez-Payá, E.; Belmonte, C.; De Felipe, C.; Messeguer, A.; Ferrer-Montiel, A. V. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 2374−2379.
(18) Ambudkar, S. V.; Dey, S.; Hrycyna, C. A.; Ramachandra, M.; Pastan, I.; Gottesman, M. M. *Annu. Rev. Pharmacol. Toxicol.* **1999**, *39*, 361−398.
(19) Borst, P.; Oude Elferink, R. *Annu. Rev. Biochem.* **2002**, *71*, 537.
(20) Soto, F.; Planells-Cases, R.; Canaves, J. M.; Ferrer-Montiel, A. V.; Aleu, J.; Gamarro, F.; Castanys, S.; Gonzalez-Ros, J. M.; Ferragut, J. A. *Eur. J. Cancer* **1993**, *29*, 2144−2150.

CC030002Q