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Structure-Based Design of Cyclooxygenase-2 Selectivity into Ketoprofen

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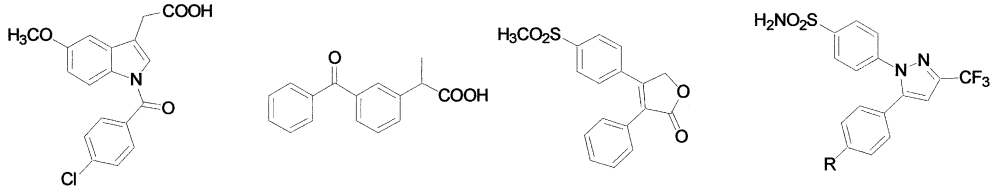
Abstract—We have recently described how to achieve COX-2 selectivity from the non-selective inhibitor indomethacin (**1**) using a combination of a pharmacophore and computer 3-D models based on the known X-ray crystal structures of cyclooxygenases. In the present study we have focused on the design of COX-2 selective analogues of the NSAID ketoprofen (**2**). The design is similarly based on the combined use of the previous pharmacophore together with traditional medicinal chemistry techniques motivated by the comparative modeling of the 3-D structures of **2** docked into the COX active sites. The analysis includes use of the program GRID to detect isoenzyme differences near the active site region and is aimed at suggesting modifications of the basic benzophenone frame of the lead compound **2**. The resulting series of compounds bearing this central framework is exemplified by the potent and selective COX-2 inhibitor **17** (LM-1669).

Introduction

Non-steroidal antiinflammatory drugs (NSAIDs) are useful tools for the treatment of inflammation, pain and fever although they show an undesirable profile of gastric side effects. Because NSAIDs directly target cyclooxygenases (COXs), the discovery of the COX-2 isoform has opened the possibility of developing COX-2 selective inhibitors to act as an effective NSAID with reduced gastric side effects.[1] At present, two COX-2 selective inhibitors have successfully reached the market, Rofecoxib (**3**) and Celecoxib (**4**), (see Scheme 1), inducing a great interest in obtaining isozyme-specific drugs.[2]

To our knowledge, several attempts to derive COX selective inhibitors from the non-selective NSAIDs indomethacin (**1**),[3] ketoprofen (**2**),[4] zomepirac[5] or flurbiprofen[6] have been published. These strategies introduced the desired selectivity by systematic structural modification of the lead NSAIDs. Alternatively, selectivity may be introduced by using the available information on the tricyclic COX-2 selective inhibitors



1 (Indomethacin) **2** (Ketoprofen) **3** (Rofecoxib) **4** (R = CH_3, Celecoxib) **5** (R = Br, SC-558)

Scheme 1.

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structurally related to **3** and **4**. We have recently described the derivation of COX-2 selectivity from the non-selective inhibitor **1**[7] based on a pharmacophore accounting for the activity of the tricyclic COX-2 inhibitors. This methodology allowed us to transfer structural information onto **1** and to identify a small set of novel COX-2 selective inhibitors having the basic *N*-benzyl-indole core.[7]

The structure of the COX-1 and COX-2 isoenzymes, obtained from X-ray crystallography[8] or by homology modeling,[9] are known. Kurumbail et al.[10] have published crystallographic structures of a COX-2 in free form as well as complexed with the COX-2 selective inhibitor SC-558 (**5**), the non-selective inhibitors indomethacin (**1**), and flurbiprofen. In this paper we detail our strategy to obtain selective COX-2 inhibitors based on the modification of the structure of the known potent but non-selective COX inhibitor ketoprofen (**2**). The strategy is intended to obtain selectivity using traditional medicinal chemistry techniques motivated by the comparative modeling of a COX-1 and -2 complexed with **2** together with the available pharmacophore. The latter model contains structural information on the tricyclic COX-2 selective inhibitors having the sulfonyl group believed to play a crucial role on selectivity.

Results and Discussion

The modeled 3-D structures of the COX isoenzymes complexed with ketoprofen (**2**) were used as the starting point for this study.[4] Comparison of the resulting ketoprofen COX-1 and -2 complexes were performed with the program GRID for the presence of isoenzyme-specific binding sites.[11] Calculations were carried out with the oxygen-containing GRID probes including OH, OC2, O, O:: and O1. The specific water (OH2) and sulfonyl oxygen probes (O=for sulfonamide and OS for sulfone) rendered most significant differences. Particular attention was focused on the cavity near the active site of COX-2 where the selective inhibitors of the tricyclic series position the sulfonyl group.[10] In this region, GRID was able to assess the presence of energetically favourable sulfonyl positions in COX-2, meanwhile, could not predict equivalent sites of interaction in COX-1. The results are shown in Figure 1 with the 5.5 Å spacing between the GRID sulfonyl oxygen spot and the preferred ketoprofen *meta* substitution. The disposition allows sulfonyl-containing substituents extending 4–6 Å. Thus, a number of spacing groups accessible for synthesis were modeled, for example, vinyl, phenyl and so on. Computer limitations excluded systematic modeling of all possible substituents by performing enzyme–ligand

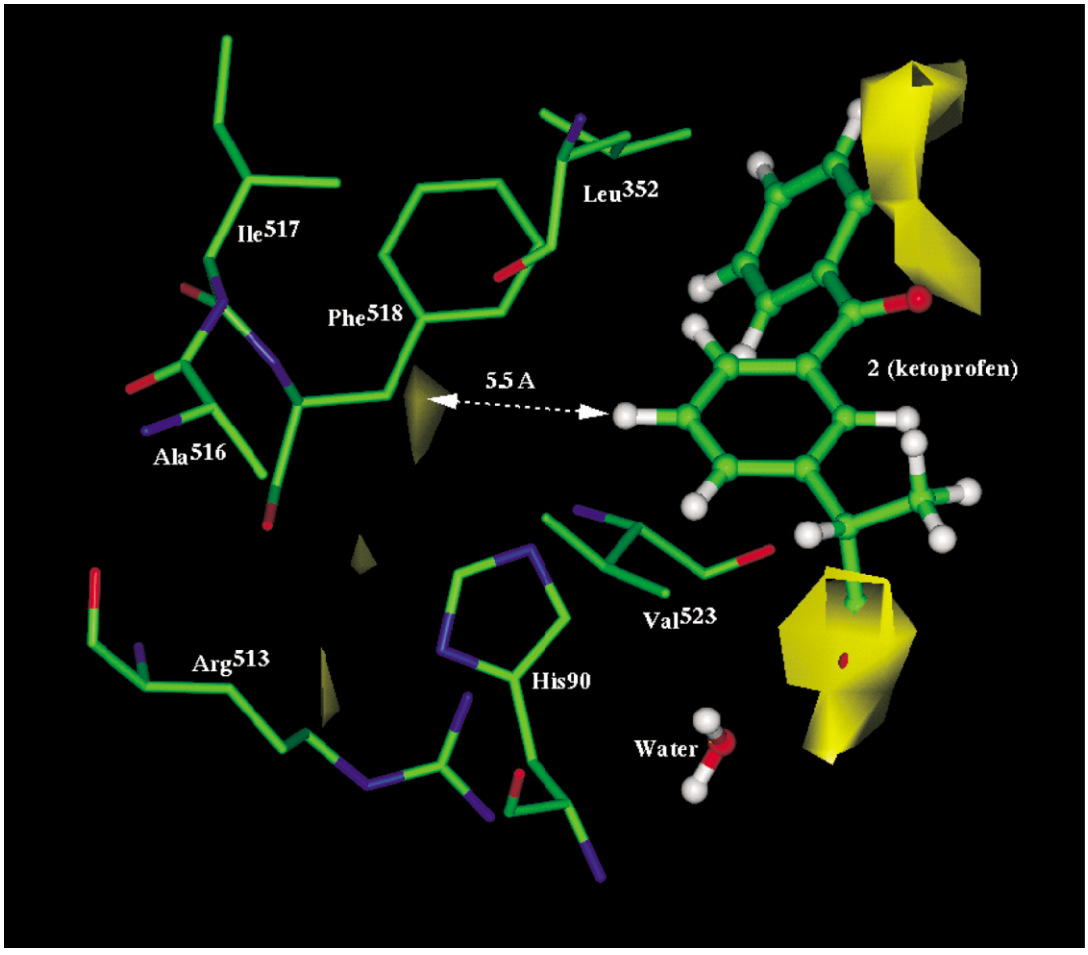


Figure 1. Computer modeled complex of ketoprofen (**2**) docked into the COX-2 active site: Interaction energy maps contoured at −5 kcal/mol (yellow surface) obtained with the GRID standard sulfonamide probe (O=). The ligand and the additional water molecule considered in the complex[4] are shown together with a selected subset of the residues near the GRID spots.

complexes. Moreover, the success on the application of the pharmacophore to the design of COX-2 selective indomethacin analogues prompted us to use this rational approach to the design of new compounds.[7] The model bearing structural information on the tricyclic COX-2 selective inhibitors, for example, having the characteristic sulfonyl group believed to play a crucial role on selectivity, is described in detail in literature[7] and is shown in Figure 2. Moreover, the alignment of **5** and **17** illustrates that the sulfonylvinyl benzophenone skeleton present in **17** may be suitable to obtain selective COX-2 inhibition.

The modeling results prompted us to synthesize compounds **6**–**27** having the essential sulfonylvinyl benzophenone frame. Our primary in vitro screening scheme included the inhibition of the PGE_2 generation by LPS-stimulated monocytes isolated from human blood (COX-2)[12] and of the TxB_2 generation in the presence of 1 μM arachidonic acid by platelets isolated from human blood (COX-1).[13] In vitro COX inhibition results are summarized in Table 1. In this set of in vitro tests, compound **17** demonstrated to inhibit the COX-2 activity ($IC_{50} = 0.25$ μM) with moderate effect on COX-1 ($IC_{50} = 18$ μM; see Table 1).

Benzophenones **6**, **7**, **13** and **14**, having the additional sulfonylvinyl substituent, were synthesized and tested against COX-2. Because none of these compounds showed activity, we assumed that, in addition to the pharmacophore minimal requirements, an extra substituent on the benzophenone phenyl ring is needed. Accordingly, compounds with an additional polar hydroxymethyl (**10** and **19**), acetyl (**20**), cyano (**21**), amide (**22**), amine (**23**) or 2-propanoate (**12**) were synthesized together with the compounds **8**, **9**, **11** and **15**–**18**, having an extra alkyl substituent. Among these compounds only the alkyl-substituted 3-sulfonylvinyl benzophenones **16**–**18** were able to significantly inhibit the COX-2 and only **17** inhibited this enzyme selectively (**16** and **18** also presented a significant ability to inhibit COX-1). These results are consistent with the absence of activity described for the compounds of the sulfonyl tricyclic series bearing, simultaneously, a carboxylate.[14] All in all, we hypothesized that the extra substituent should be neither polar nor acidic and, most importantly, that it is an orienting group crucial to retain the pharmacophore geometry. In order to assess the importance of the sulfonyl group for selectivity, the sulfonamide group in **17** was modified for sulfone (**24**), ester (**25**), carboxylate (**26**) and carboxamide (**27**) but none of the variations improved the COX-2 inhibition. Finally, in the human whole blood assay[15] **17** inhibited the COX activity ($IC_{50}^{COX\text{-}2} = 12.0 \pm 0.7$ μM and $IC_{50}^{COX\text{-}1} = 100 \pm 3.3$ μM) with comparable selectivity to the reference compound **3** (0.83 ± 0.13 and 18.3 ± 5.3 μM respectively).

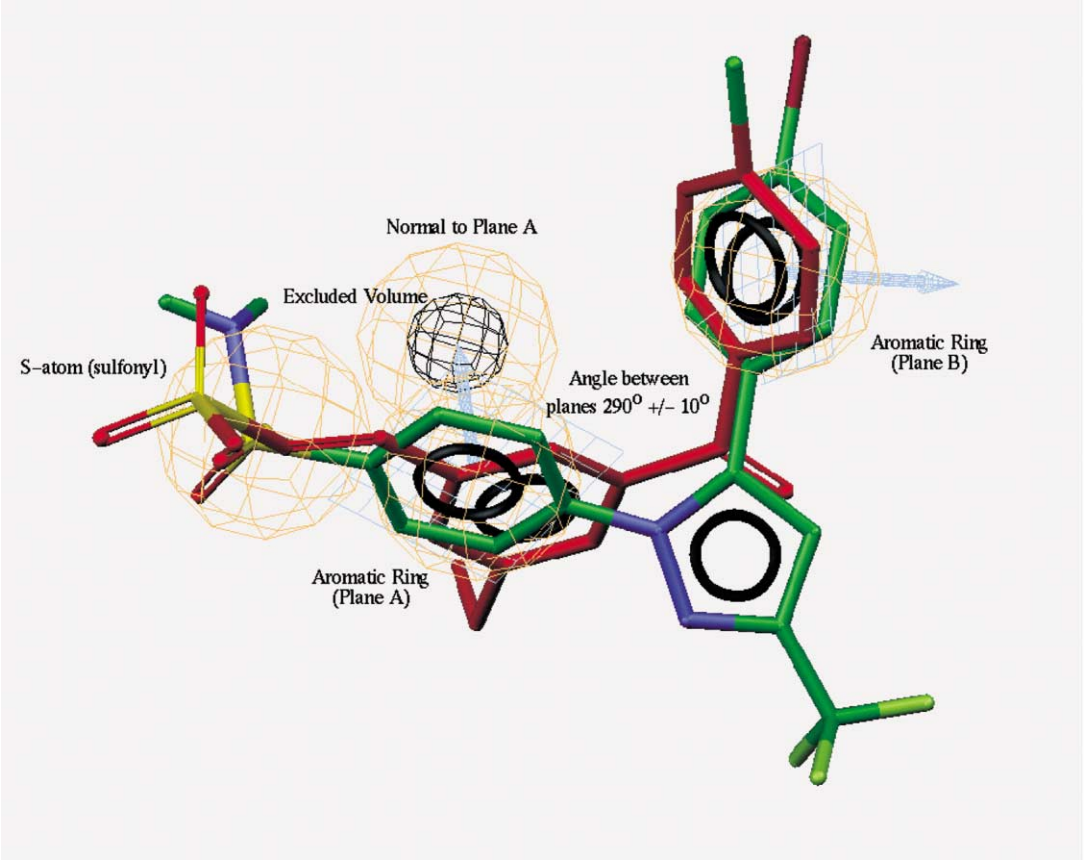
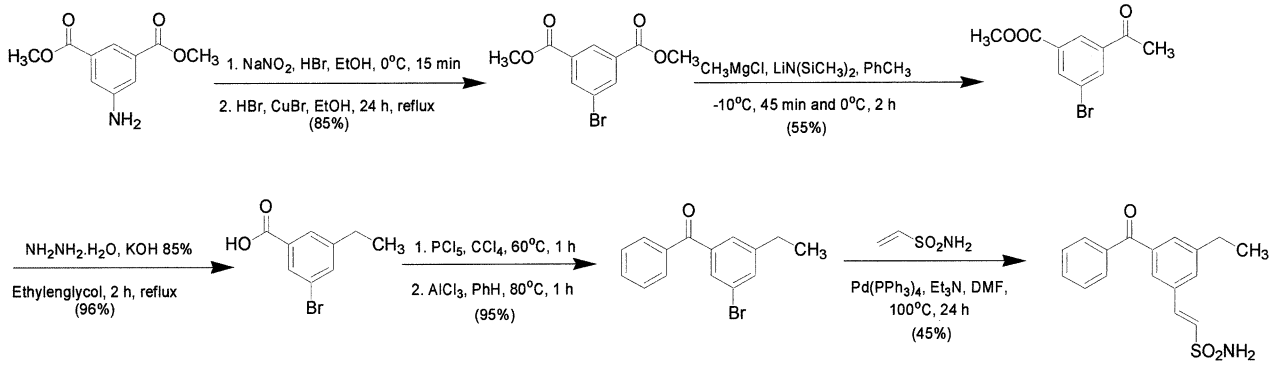


Figure 2. Pharmacophore with **5** (SC-558, green) and **17** (red) fitted. The pharmacophore contains a sulfonyl S-atom, an aromatic ring plane A with a fixed position of the normal to the plane, an additional aromatic ring plane B and an excluded volume. The chemical features are drawn as brown globes except for the additional globe accounting for the position orthogonal to the aromatic ring plane A and the excluded volume (black globe).

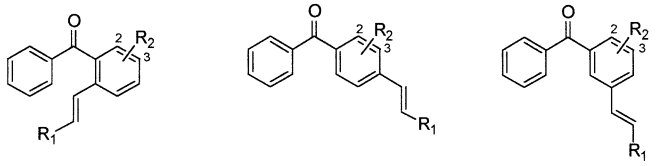


Scheme 2.

Synthesis

The compounds in Table 1, exemplified by the synthesis of **17** shown in Scheme 2, were prepared as previously described in the literature.[16,17] In summary, **17** was obtained as follows: the amino group in 5-amino-isophthalic acid dimethyl ester was converted into the corresponding bromo derivative by treatment with $NaNO_2$/HBr followed by HBr/CuBr. One of the carboxylate groups of the formed 5-bromo-isophthalic acid dimethyl ester was then methylated [CH_3MgCl, $LiN(SiCH_3)$] to produce the acetyl that was reduced with $NH_2NH_2.H_2O$/KOH to form the 3-bromo-5-ethylbenzoic acid. Friedel–Craft reaction of the corresponding acyl chloride (PCl_5/CCl_4, 60 °C) with benzene ($AlCl_3$, 80°C) produced the 5-amino-isophthalic acid dimethyl ester that was reacted with ethenesulfonic acid amide to give the desired product **17** in 20% yield over the 5 steps (see Scheme 2).

Table 1. In vitro COX inhibition results in human blood monocytes (COX-2) and platelets (COX-1)



Compd	Subst.[a]	R_1	R_2	COX-2,[a,b] IC_{50} (μM)	COX-1,[b,c] IC_{50} (μM)
6	A	SO_2NH_2	H	43% @ 20 μM	39% @ 10 μM
7	B	SO_2CH_3	H	6% @ 20 μM	14% @ 10 μM
8	B	SO_2NH_2	3-CH_3	14% @ 20 μM	0% @ 10 μM
9	B	SO_2NH_2	3-CH_2CH_3	5.6	6.3
10	B	SO_2NH_2	3-CH_2OH	8.4	25% @ 10 μM
11	B	SO_2NH_2	2-CH_3	41% @ 20 μM	0% @ 10 μM
12	C	SO_2CH_3	3-$CH(CH_3)COOH$	9.7	n.t.
13	C	SO_2CH_3	H	Inact. @ 500 μM	n.t.
14	C	SO_2NH_2	H	37% @ 20 μM	0% @ 10 μM
15	C	SO_2NH_2	2-CH_3	19	49% @ 10 μM
16	C	SO_2NH_2	2-CH_2CH_3	0.77	0.20
17 (LM-1669)	C	SO_2NH_2	3-CH_2CH_3	0.20	18
18	C	SO_2NH_2	3-$CH=C(CH_3)_2$	0.98	3.6
19	C	SO_2NH_2	3-CH_2OH	9.7	5.4
20	C	SO_2NH_2	3-$COCH_3$	20	6.4
21	C	SO_2NH_2	3-CN	5.6	1.7
22	C	SO_2NH_2	3-$CON(CH_3)_2$	31% @ 20 μM	n.t.
23	C	SO_2NH_2	3-$CH_2NH(CH_2CH_3)_2$	7.1	n.t.
24	C	SO_2CH_3	3-CH_2CH_3	40% @ 20 μM	16% @ 10 μM
25	C	$CO_2CH_2CH_3$	3-CH_2CH_3	5.0	n.t.
26	C	CO_2H	3-CH_2CH_3	2.6	n.t.
27	C	$CONH_2$	3-CH_2CH_3	1.8	0.074
2 (ketoprofen)[d]				0.026	0.0020
3 (rofecoxib)				0.28	0% @ 10 μM
4 (celecoxib)				0.11	1.88

[a]PGE_2 generation by LPS-stimulated monocytes isolated from human blood.
[b]Dose–response curves were analysed by non-linear regression using the Hill equation as implemented in the software Prism.[18] The values shown correspond either to the IC_{50} or to the % inhibition produced by tested compounds at the selected doses. Values are the mean of at least two independent determinations.
[c]TxB_2 generation in the presence of 1 μM arachidonic acid by platelets isolated from human blood.
[d]Activity was measured with the *S*-(+)-enantiomer of ketoprofen.

Conclusions

This work describes the development of novel COX-2 selective inhibitors starting from a non-selective inhibitor ketoprofen (**2**) to obtain the potent and selective inhibitor **17**. Traditional medicinal chemistry techniques have been employed driven by the combined use of a pharmacophore together with the comparative modeling of COX-1 and 2 using GRID to detect isoenzyme differences. The optimal activity has been obtained with the 3-sulfonylvinyl benzophenone frame with an additional non-polar substituent required for selectivity, for example *meta*-ethyl. **17** (LM-1669) has shown to selectively inhibit COX-2 in human isolated cell and whole blood assays.

References and Notes

1. Smith, W. L.; Garavito, R. M.; DeWitt, D. L. *J. Biol. Chem*. **1996**, *271*, 33157.
2. Ehrich, E.; Dallob, A.; van Hecken, A.; Depre, M.; DeLepeleire, I.; Brideau, C.; Hilliard, D.; Tanaka, W.; Mukhopadhyay, S.; Seibold, J.; DeSchepper, P.; Gertz, B. *Arthr. Rheum*. **1996**, *39*, s81 (abstr. 328). Penning, T. D.; Talley, J. J.; Bertenshaw, S. R.; Carter, J. S.; Collins, P. W.; Docter, S.; Graneto, M. J.; Lee, L. F.; Malecha, J. W.; Miyashiro, J. M.; Rogers, R. S.; Rogier, D. J.; Yu, S. S.; Anderson, G. D.; Burton, E. G.; Cogburn, J. N.; Gregory, S. A.; Koboldt, C. M.; Perkins, W. E.; Seibert, K.; Veenhuizen, A. W.; Zhang, Y. Y.; Isakson, P. C. *J. Med. Chem*. **1997**, *40*, 1347.
3. Black, W. C.; Bayly, C.; Belley, M.; Chan, C. C.; Charleson, S.; Denis, D.; Gauthier, J. Y.; Gordon, R.; Guay, D.; Kargman, S.; Lau, C. K.; Leblanc, Y.; Mancini, J.; Ouellet, M.; Percival, D.; Roy, P.; Skorey, K.; Tagari, P.; Vickers, P.; Wong, E.; Xu, L.; Prasit, P. *Bioorg. Med. Chem. Lett*. **1996**, *6*, 725.
4. Palomer, A.; Pérez, J. J.; Navea, S.; Llorens, O.; Pascual, J.; García, M. L.; Mauleón, D. M. *J. Med. Chem*. **2000**, *43*, 2280.
5. Luong, C.; Miller, A.; Barnett, J.; Chow, J.; Ramesha, C.; Browner, M. F. *Nat. Str. Biol*. **1996**, *3*, 927.
6. Bayly, C. I.; Black, C.; Leger, S.; Ouimet, N.; Ouellet, M.; Percival, M. D. *Bioorg. Med. Chem. Lett*. **1999**, *9*, 307.
7. Palomer, A.; Cabré, F.; Pascual, J.; Campos, J.; Trujillo, M. A.; Entrena, A.; Gallo, M. A.; García, M. L.; Mauleón, D.; Espinosa, A. *J. Med. Chem*. **2002**, in press.
8. Picot, D.; Loll, P. J.; Garavito, R. M. *Nature* **1994**, *367*, 243. McKeever, B. M.; Pandya, S.; Percival, M. D.; Ouellet, M.; Bayly, C.; O'Neill, G. P.; Bastien, L.; Kennedy, B. P.; Adam, M.; Cromlish, W.; Roy, P.; Black, W. C.; Guay, D.; LeBlanc, Y. *10th Conf. Prost. Related Cpds*. **1996**, *55*, 20 (abstr. 63).
9. Filizola, M.; Pérez, J. J.; Palomer, A.; Mauleón, D. *J. Mol. Graph*. **1997**, *15*, 290.
10. Kurumbail, R. G.; Stevens, A. M.; Gierse, J. K.; McDonald, J. J.; Stegeman, R. A.; Pak, J. Y.; Gildehaus, D.; Miyashiro, J. M.; Penning, T. D.; Seibert, K.; Isakson, P. C.; Stallings, W. C. *Nature* **1996**, *384*, 644.
11. GRID version 17.0. MDL, Oxford, 1998. Goodford, P. J. A. *J. Med. Chem*. **1985**, *28*, 849. Boobbyer, D. N. A.; Goodford, P. J.; McWhinnie, P. M.; Wade, C. R. *J. Med. Chem*. **1993**, *36*, 148.
12. Carabaza, A.; Cabré, F.; Rotllan, E.; Gómez, M.; Gutiérrez, M.; García, M. L.; Mauleón, D. *J. Clin. Pharmacol*. **1996**, *36*, 505.
13. Riendeau, D.; Charleson, S.; Cromlish, W.; Mancini, J. A.; Wong, E.; Guay, J. *Can. J. Phys. Pharmacol*. **1997**, *75*, 1088.
14. Reitz, D. B.; Isakson, P. C. *Curr. Pharm. Des* **1995**, *1*, 211.
15. Brideau, C.; Kargman, S.; Liu, S.; Dallob, A. L.; Ehrich, E. W.; Rodger, I. W.; Chan, C. C. *Inflammation. Res*. **1996**, *45*, 68.
16. Mauleón, D.; Garcia, M. L.; Palomer, A.; Pascual, J. Spanish Patent Application 9,902,287, 1999.
17. Analytical data for the selected active compound: 2-(3-Benzoyl-5-ethyl-phenyl)-ethenesulfonic acid amide (**17**, LM-1669). Mp 123–126 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.24 (3H, t, CH_3), 2.20 (2H, broad, NH), 2.59 (2H, d, CH_2), 7.29 (1H, d, ethyl–H), 7.35 (1H, d, Ar–H), 7.36 (2H, dd, Ar–H), 7.44 (1H, s, Ar–H), 7.45 (1H, dd, Ar–H), 7.55 (1H, ddd, ethyl–H), 7.56 (1H, d, Ar–H), 7.70 (2H, dd, Ar–H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 187.0, 139.8, 137.8, 137.6, 136.0, 134.5, 132.2, 130.1, 130.6, 129.3, 128.7, 128.4, 128.2, 125.0, 112.0, 29.0, 16.1.
18. GraphPad PRISM Version 2.0. GraphPad Software: San Diego, CA, 1998.