Novel Azo Derivatives as Prodrugs of 5-Aminosalicylic Acid and Amino Derivatives with Potent Platelet Activating Factor Antagonist Activity

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This paper describes the synthesis of a series of azo compounds able to deliver 5-aminosalicylic acid (5-ASA) and a potent platelet activating factor (PAF) antagonist in a colon-specific manner for the purpose of treating ulcerative colitis. We found it possible to add an amino group on the aromatic moiety of our reported 1-[(1-acyl-4-piperidyl)methyl]-1*H*-2-methylimidazo[4,5-*c*]-pyridine derivatives or on British Biotech compounds BB-882 and BB-823 maintaining a high level of activity as PAF antagonist. A selected compound UR-12715 (**49c**) showed an IC_{50} of 8 nM in the in vitro PAF-induced aggregation assay, and an ID_{50} of 29 μg/kg in the in vivo PAF-induced hypotension test in normotensive rats. Through attachment of **49c** to the 5-ASA via azo functionality we obtained UR-12746 (**70**). Pharmacokinetics experiments with [^{14}C]-**70** allow us to reach the following conclusions, critical in the design of these new prodrugs of 5-ASA. Neither the whole molecule **70** nor the carrier **49c** were absorbed after oral administration of [^{14}C]-**70** in rat as was demonstrated by the absence of plasma levels of radioactivity and the high recovery of it in feces. Effective cleavage of azo bond (84%) by microflora in the colon is achieved. These facts ensure high topical concentrations of 5-ASA and **49c** in the colon. Additionally, **70** exhibited a potent anticolitic effect in the trinitrobenzenesulfonic acid-induced colitis model in the rat. This profile suggests that UR-12746 (**70**) provides an attractive new approach to the treatment of ulcerative colitis.

Inflammatory bowel disease (IBD), which includes Crohn's disease and ulcerative colitis, is a serious disorder of the lower gastrointestinal tract in which tissue damage and inflammation lead to bowel impairment. The more common ulcerative colitis involves inflammation of the lining of the colon, whereas Crohn's disease affects all layers of the intestinal wall. Both disorders are chronic, progressive diseases associated with considerable pain, abdominal cramping, persistent diarrhea, nausea, vomiting, gastrointestinal bleeding, anemia, fever, and weight loss, as well as secondary infections.[1] Although an explanation for the etiopathogenesis has not yet emerged, the inflammatory process itself is well understood. The final common pathway of immune activation in IBD is the local influx of monocytes, macrophages, and polymorphonuclear neutrophils (PMNs). The processes that account for the recruitment of these cells include cytokine generation, complement activation, and eicosanoid biosynthesis. Once the influx of PMNs and macrophages occurs, the production of platelet activating factor (PAF) and leukotrienes, in particular LTB_4, increases, leading to the secondary amplification of the inflammatory response, which produces the clinical manifestations of IBD.[2]

Currently established therapies consist mainly of glucocorticoids and 5-aminosalicylic acid (5-ASA) derivatives. The origin of this second group of compounds was sulfasalazine, which was introduced in the early 1940's and has become the most widely prescribed agent for IBD. Although sulfasalazine has pharmacological activity of its own, its beneficial action in IBD has been shown to be attributable to 5-ASA.[3] When sulfasalazine **1A** is orally ingested, only 12% is absorbed; the rest reaches the colon practically unaltered where it is split by azo reductases of the colonic microflora, releasing **5-ASA**, which is mostly excreted in the feces, and sulfapyridine (**1B**), which is rapidly absorbed (see Figure 1). Thus, although not originally designed as a prodrug, sulfasalazine can be considered as such, given that it ensures colon-specific delivery of 5-ASA and sulfapyridine, which acts merely as a carrier.[4] Despite the benefits of sulfasalazine in the treatment of IBD, its efficacy is limited and side effects and allergic reactions

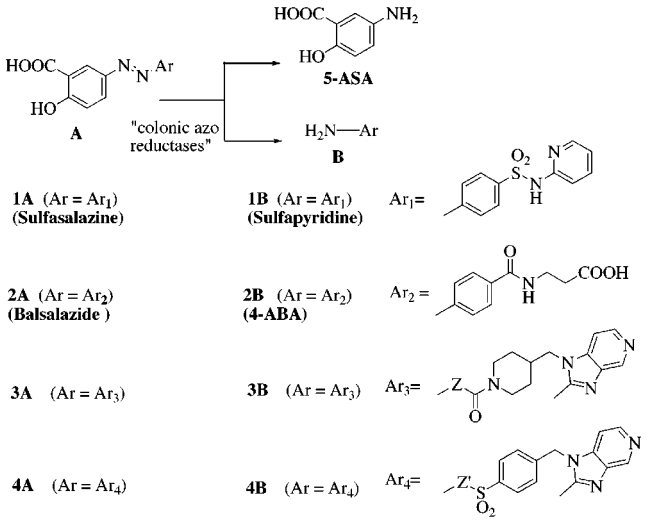

Figure 1. See Schemes 1−3 for identification of Z and Z′.

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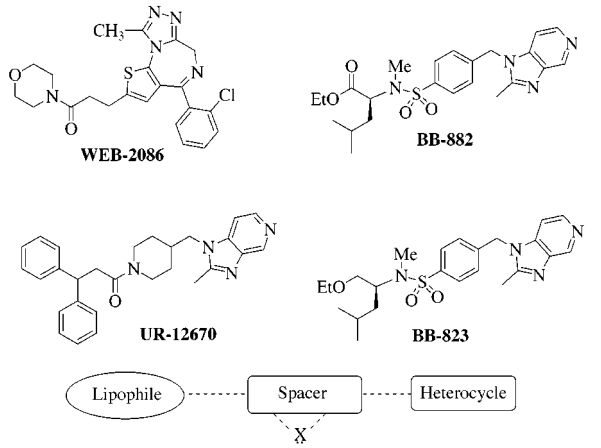


Figure 2. PAF antagonists reference compounds and pharmacophore model.

are common. As the toxic effects of sulfasalazine are attributed to sulfapyridine, efforts have been directed to avoiding or substituting the carrier. Galenic formulations of 5-ASA with an enteric coating (mesalazine) have been developed, and prodrugs based on Figure 1 have been studied. One of them, balsalazide (**2A**), with low systemic absorption and a nontoxic carrier 4-ABA (**2B**), has recently been launched.[5]

We envisioned a new approach to the problem, taking advantage of the azo moiety as the colonic drug delivery system but adding a therapeutic activity to the carrier that, if it is not absorbed in the colon, can act topically with 5-ASA to produce a synergistic effect. We chose to antagonize PAF, a proinflammatory lipid mediator involved in hypersensitivity and inflammatory reactions such as platelet and neutrophil aggregation, increased vascular permeability, and leukocyte adhesion. PAF also stimulates the release of eicosanoids and cytokines. Such direct and indirect actions contribute to the initiation and amplification of inflammatory process, including that of IBD.[6] In fact, the colonic mucosa from patients with ulcerative colitis or Crohn's disease has been shown to produce higher levels of PAF than normal mucosa.[7,8] Moreover, treatment with glucocorticoids effectively reduces PAF levels in colonic mucosa.[9] The objective of the present work was to synthesize aromatic amines **B** with potent PAF antagonist activity and prepare the corresponding azo derivative **A** of 5-ASA. Ideal pharmacokinetic properties of these compounds would comprise negligible systemic absorption of **A**, a high level of azo cleavage and also low absorption of **B** in the colon. These requisites would lead to high, topically active concentrations of both 5-ASA and **B**. Our reasons for taking a topical approach include (a) the expectation of reducing adverse effects, (b) the observation that currently effective therapy with 5-ASA (film-coated tablets, enemas) acts topically, and (c) the assumption that improvement of disease will depend on effective blockade of locally generated high PAF concentrations. This approach differs from previous attempts to use PAF antagonists systematically.[10]

Despite the great diversity observed in the reported PAF antagonists,[11] an important group has shown a lipophilic moiety tethered to an sp^2 nitrogen heterocycle by a spacer containing a hydrogen bond acceptor (see Figure 2). On the basis of our own experience,[12] we considered the lipophilic moiety to be the most flexible one for introducing an aromatic amino group with less distortion of activity. Here we describe the preparation and pharmacological evaluation of new aromatic amines **3B** and **4B**, with potent PAF antagonist activity, by modification of the lipophilic moiety of our reported 1-[(1-acyl-4-piperidyl)methyl]-1*H*-2-methylimidazo[4,5-c]pyridine derivatives and BB-882,[13] respectively. For the most active compounds in both series, we also describe the preparation of the corresponding azo derivatives **3A** and **4A**.

Chemistry

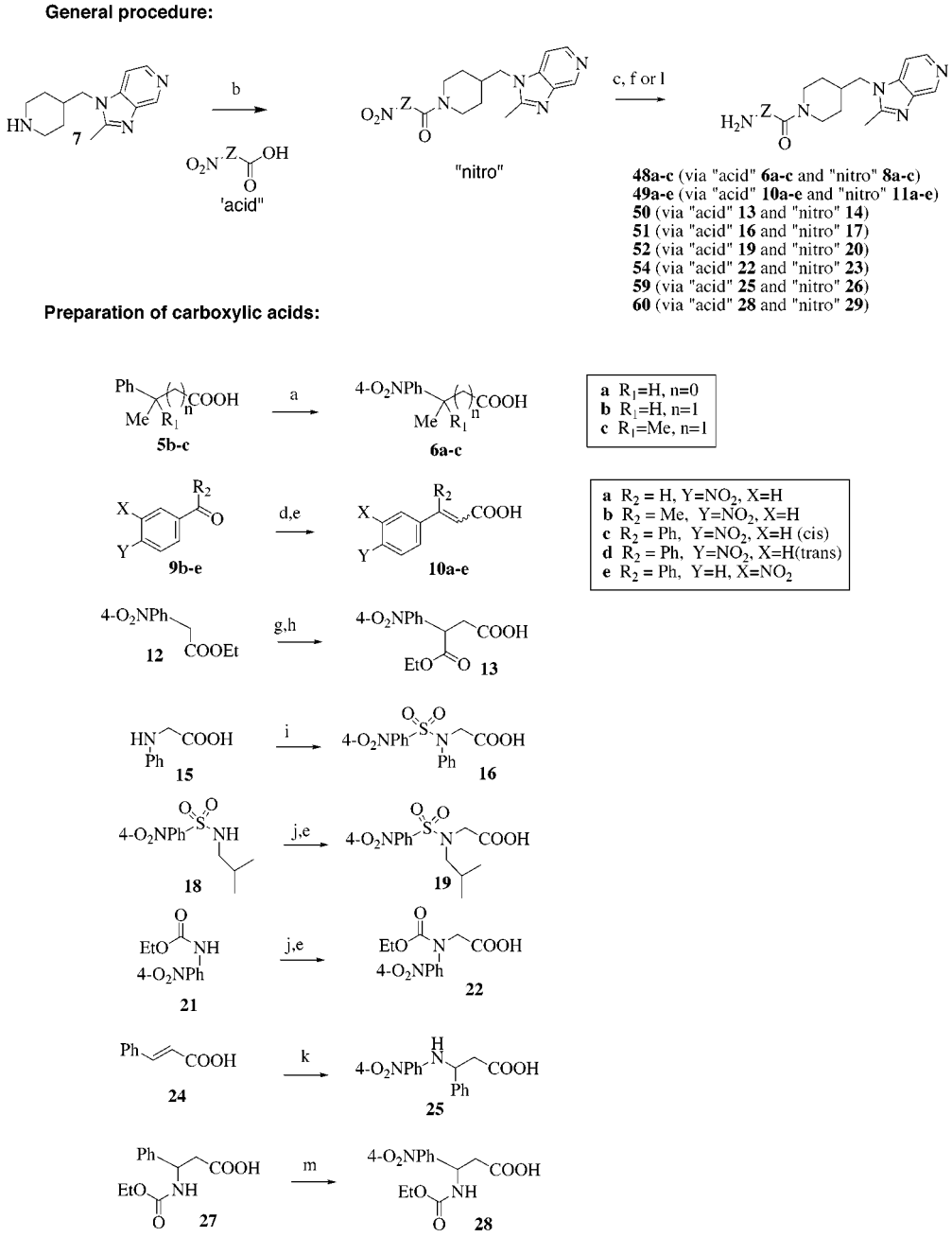
The (piperidylmethyl)imidazopyridines **48**−**52**, **54**, **59**−**60** were prepared by reacting amine **7**[12c] with the appropriate nitroaromatic acid via DCC coupling, followed by reduction of the nitro group by catalytic hydrogenation (Scheme 1). Nitrophenylalkyl acids **6b**,**c** were obtained by nitration of the parent compounds with H_2SO_4/HNO_3.[14] Nitrophenylcinnamic acids **10b**−**e** were prepared from the corresponding commercial nitro aromatic ketones **9b**−**e** by reaction with triethylphosphonoacetate using NaH as the base in refluxing THF followed by basic hydrolysis. Using this method with 4-nitrobenzophenone **9c**, a 2:1 mixture of *Z* and *E* isomers **10c** and **10d** was obtained. *Z* and *E* assignation was confirmed by COSY and NOE experiments. After coupling with **7** and reduction, the *Z* and *E* isomers **49c** and **49d** were separated by column chromatography and evaluated independently. Later, after application of the conditions of the Peterson reaction[15] (Me_3SiCH_2COOEt, LDCA, THF, −78 °C), the ratio of *Z*/*E* was increased to 8:1. In the case of 3-nitrobenzophenone **9e**, a 1:1 mixture of *Z*/*E* was obtained and evaluated without separation.

Alkylation of ethyl 4-nitrophenylacetate **12** with *tert*-butyl bromoacetate followed by *tert*-butyl ester cleavage afforded acid **13**. Reacting 4-nitrophenylsulfonyl chloride with *N*-phenylglycine afforded acid **16**. Alkylation of sulfonamide **18** or carbamate **21** with ethyl bromoacetate, followed by hydrolysis of the ester, gave acids **19** and **22**. Addition of hydrogen bromide to cinnamic acid followed by displacement on bromide yielded 4-nitroanilide derivative **25**. Amino acid derivative **28** was prepared by nitration of **27** with ammonium nitrate in sulfuric acid.

Ureas **31** and **33**, precursors of compounds **55** and **56**, were prepared by reaction of **7** with the isocyanate generated through treatment of the corresponding acids **30** and **32** with diphenyl phosphoryl azide[16] (Scheme 2), whereas urea **35**, precursor of **57**, was obtained by treating **7** with the phenyl carbamate **34**. Coupling 4-aminobenzoic acid with amine derivatives **36**[12c] and **37**, prepared from **7**, yielded compounds **53** and **58**, respectively.

The (aminosulfonylbenzyl)imidazopyridines **61**−**67** were prepared as outlined in Scheme 3. Benzoylation or alkylation of sulfonamides **38a**,**b**[13] afforded, after catalytic hydrogenation, compounds **64**−**66**. Amides **62**−**63** were synthesized by coupling acids **42a**,**b** with 3- or 4-nitrobenzylamines, respectively, via DCC to give **43** and **44** followed by catalytic hydrogenation. Reacting sulfonyl chloride **45** with 4-nitrophenylalanine ethyl ester gave nitro derivative **46**, which was reduced to amine **67**. Sulfonamide **61** was prepared by reacting

Scheme 1[a]



[a] (a) 95−97% H_2SO_4, 100% fuming HNO_3, rt, 0.5 h; (b) **7**, DCC, HOBt, DMF, rt, 24 h; (c) Pd/C 10%, MeOH, H_2, rt, 48 h; (d) $(EtO)_2POCH_2COOEt$, NaH, THF, reflux, 24 h; (e) K_2CO_3, MeOH, H_2O, reflux, 4 h; (f) $SnCl_2 \cdot 2H_2O$, 37% HCl, AcOH, rt, 18 h; (g) $BrCH_2COOBu^t$, NaH, DMF, 60 °C, 24 h; (h) benzene, *p*-TsOH, 100 °C, 30 min; (i) NO_2-$C_6H_4SO_2Cl$, THF, 1 N NaOH, rt, 24 h; (j) $BrCH_2COOEt$, NaH, THF, rt, 24 h; (k) HBr 30% in AcOH, rt, 18 h, then, $NH_2C_6H_4NO_2$, 2-butanone, reflux, 18 h; (l) Zn, $CaCl_2$, EtOH, 50 °C, 45 min; (m) NH_4NO_3, 95−97% H_2SO_4, −15 °C, 1 h.

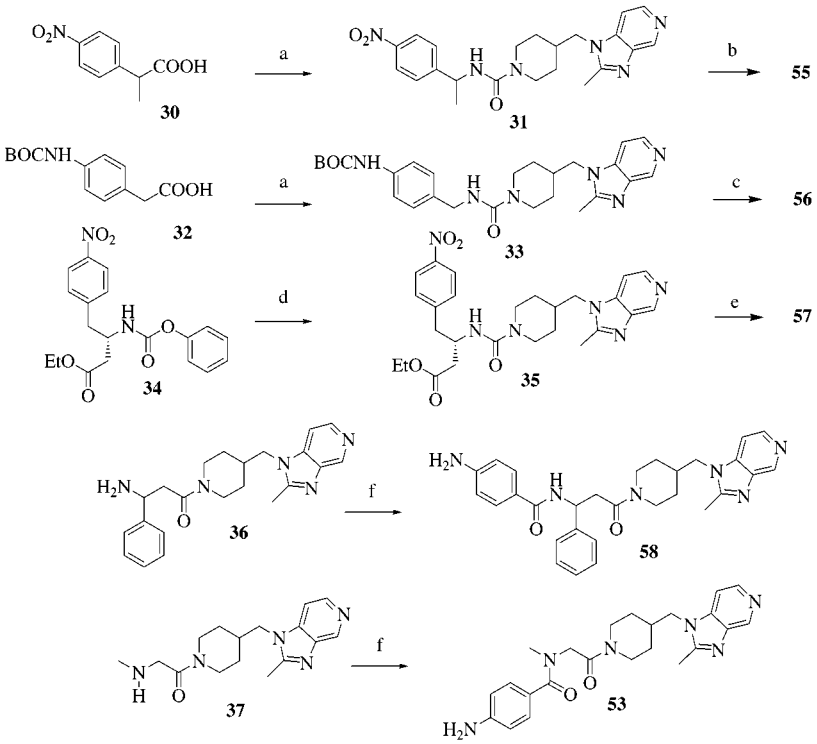
sulfonyl chloride **45** with ethyl 3-(methylamino)benzoate followed by ester hydrolysis, Curtius rearrangement[17] of the formed acid to afford BOC-protected amine **47**, and BOC removal.

Azo derivatives **68**−**74** were all synthesized by diazotization of amines **48c**, **49c**,**d**, **51**, **64a**, and **65a**−**b** with sodium nitrite in 3 M HCl and coupling with salicylic acid as is shown in Scheme 4.

Results and Discussion

The PAF antagonist activities of amines **3B** and **4B** were tested using the in vitro PAF-induced platelet aggregation assay,[18] and the in vivo PAF-induced hypotension test in normotensive rats.[19] In our previous work on 1-[(1-acyl-4-piperidyl)methyl]-1*H*-2-methylimidazo[4,5-*c*]pyridine derivatives, we found some clear trends in the SAR of the acyl radical.[12b,c] The highly active ones usually had in common a phenyl group β to the amidic carbonyl. They were 3-phenylpropanoyl and 3-phenylpropenoyl derivatives or aza analogues such as *N*-phenylglycinyl and benzylaminocarbonyl derivatives. A second substituent, often aromatic, β to the amide was usually necessary. From that work, the 3,3-diphenylpropanoyl derivative UR-12670 was chosen as lead compound (see Table 1). Here, we have prepared a series of compounds **48**−**60** resulting from attachment of an

Scheme 2[a]



[a] (a) $(C_6H_5O)_2PON_3$, benzene, NEt_3, rt, 20 min, 90 °C, 2h, then, **7**, 90 °C, 12 h; (b) $SnCl_2 \cdot 2H_2O$, EtOH, $NaBH_4$; (c) HCl (6.2 N in dioxane), MeOH, rt, 20 h; (d) pyr, **7**, 140 °C, 18 h; (e) Pd/C 10%, MeOH, H_2, rt, 48 h; (f) $NH_2C_6H_4COOH$, DCC, HOBt, DMF, rt, 24 h.

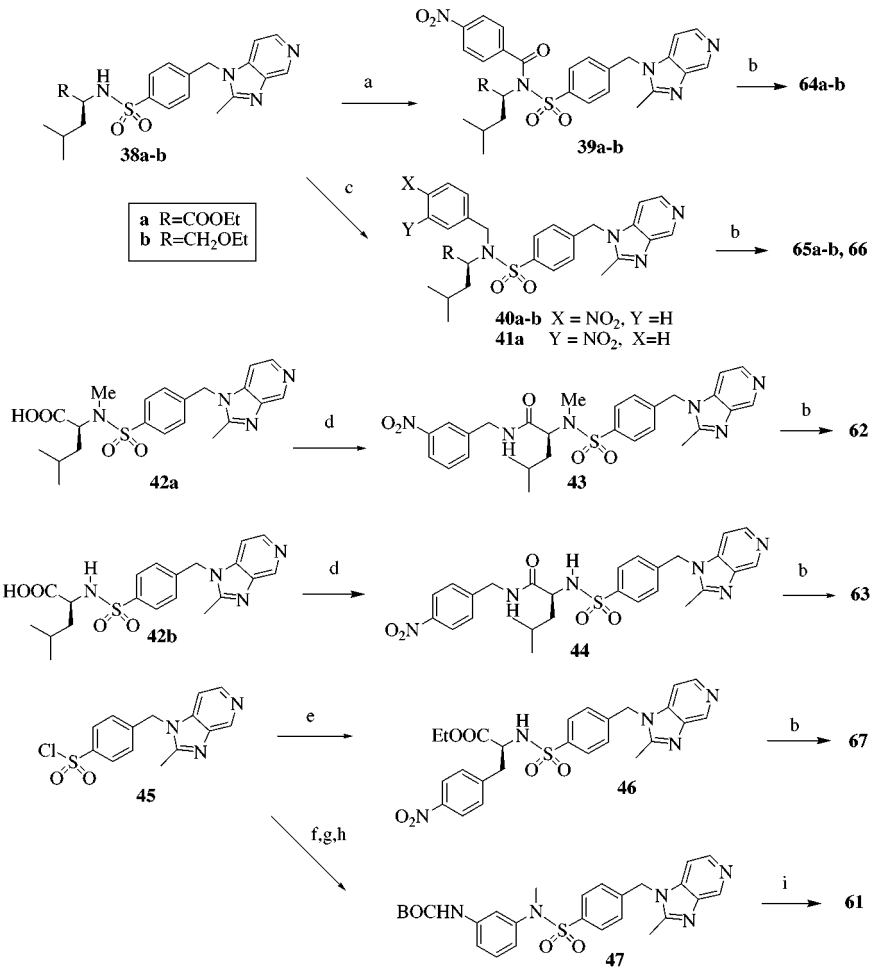
amino group, in position 3 or 4, to one of the aromatic rings of the acyl radical of the aforementioned series. Examining the pharmacological results of the phenylalkyl and phenylalkenyl derivatives **48a**−**c**, **49a**−**e**, and **50** (see Table 1), we observed that the only two compounds that did not follow the mentioned conditions (the shorter compound **48a** and the unsubstituted compound **49a**) were clearly less active. Among the others, dimethyl **48c** and diphenyl **49c** and **49d** were the best in the set of two tests. They were more active than WEB-2086[20] and slightly less active than UR-12670 or BB-882. We judged this activity to be satisfactory taking into account the strong structural restriction that we impose (the presence of an aromatic amino group) and considering that the two above-mentioned reference compounds are among the most active PAF antagonists reported. Among glycinyl derivatives **51**−**54**, only **51** presented acceptable activity. Less tolerant in the presence of the amine were the urea derivatives **55**−**57** and 3-amino-3-phenylpropanoyl derivatives **58**−**60**. Although aniline **59** presented good activity in the aggregation test, it was 10 times less active than, for example, **49c** in the in vivo test.

The second series investigated was that derived from the modification of the BB-882 and BB-823 skeletons.[13] The required aromatic amine was linked to the leucine or leucinol portion in three different ways: **62** and **63** had an amide as an isostere of the ester; in **64a**,**b**, **65a**,**b**, and **66**, the methyl group was replaced with a 4-aminobenzoyl group or a 3- or 4-aminobenzyl group and, in **67**, the isobutyl substituent was replaced with a 4-aminobenzyl group. The pharmacological results gathered in Table 2 suggest that the most flexible position for introducing an additional aromatic ring is the nitrogen of sulfonamide, **65a** being the most potent compound.

Having accomplished our first objective (to have aromatic amines with potent PAF antagonist activity), we then prepared the azo derivatives of the most active compounds of both series (compounds **68**−**74**), which we also tested as PAF antagonists (Table 3). As expected, because of the bulkiness of the radical, the PAF antagonist activity of the azo decreased.

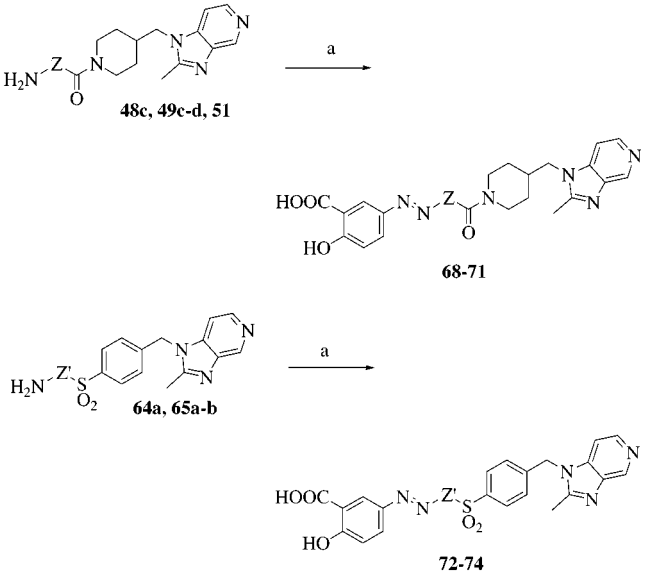
On the basis of the criterion of level of carrier activity and preliminary results of pharmacokinetic studies using nonradioactive compounds administered to rats among others, we chose compound **70** to be labeled with ^{14}C (amidic α carbon) to further investigate the kinetic aspects of the series. When we gave [^{14}C]-**70** orally (50 mg/kg, 50 μCi/kg) to female Sprague−Dawley rats, no plasma levels of radioactivity were detected in the first 48 h post-administration and almost all radioactivity was detected in feces collected during the first 168 h post-administration. At the end of the study, 88.4 ± 9.9% of the total administered radioactivity was recovered in feces whereas only a 0.15 ± 0.06% was recovered in urine. This low urine excretion is in agreement with the absence of radioactivity in plasma, indicating negligible absorption of radioactive material. Feces samples were also analyzed by HPLC with radioactive and UV detection to determine the distribution of **70** and **49c**, in order to know the percentage of azoreduction produced in the gastrointestinal tract. During the first 24 h, 83% of this radioactivity corresponds to [^{14}C]-**49c** and 17% to [^{14}C]-**70**. In the 24 to 48 h period post-administration, all the radioactivity detected in feces corresponded to [^{14}C]-**49c**. The percentage of azoreduction for the accumulated period of 0 to 48 h was 84%. To study the possible absorption of the 5-ASA moiety once it is released from **70** upon cleavage of the azo bond, plasma levels of 5-ASA and its immediate me-

Scheme 3[a]



[a] (a) $NO_2C_6H_4COCl$, NaH, THF, rt, 18 h; (b) Pd/C 10%, MeOH, H_2, rt, 48 h; (c) 3- or 4-$NO_2C_6H_4CH_2OMs$, NaH, THF, rt, 18 h; (d) 3- or 4-$NO_2C_6H_4CH_2NH_2$·HCl, NEt_3, DCC, HOBt, DMF, rt, 24 h; (e) 4-nitrophenylalanine ethyl ester, CH_3CN, NEt_3, rt, 24 h; (f) 3-$MeNHC_6H_4COOEt$, CH_3CN, NEt_3, DMAP, rt, 24 h; (g) KOH, EtOH, H_2O, reflux, 1 h; (h) $(C_6H_5O)_2PON_3$, Bu^tOH, NEt_3, 90 °C, 4 h; (i) HCl (6.2 N in dioxane), MeOH, rt, 2 h.

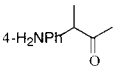
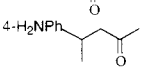
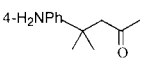
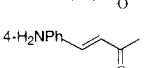
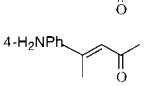
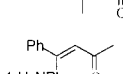
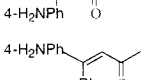
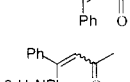
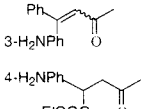
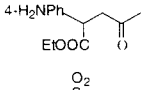
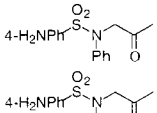
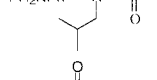
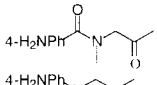
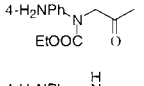
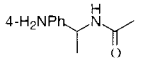
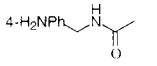
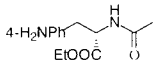
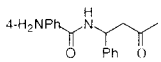
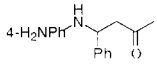
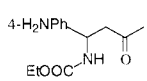
Scheme 4[a]



[a] (a) $NaNO_2$, 3 M HCl, −10 °C, 1 h; then, salicylic acid, 2 N NaOH, 30 min.

tabolite *N*-acetyl-5-ASA were determined in plasma and urine. There were quantifiable plasma levels of 5-ASA and *N*-acetyl-5-ASA only in the samples between 4 and 24 h, in which levels below 0.2 μg/mL were observed. This delayed absorption suggests that, as salicylates can only be absorbed after the azoreduction of **70**, the cleavage of the azo bond must occur in the last portion of the gastrointestinal tract. In urine, only 0.46 ± 0.12% of *N*-acetyl-5-ASA equivalent dose administered was quantified in the first 48 h post-administration. These results met the desired conditions: absence of systemic absorption of the azo derivative and a high level of azo cleavage together with low or absence of absorption of the carrier from the colon. The anticolitic activity profile of **70** was investigated in the trinitrobenzene sulfonic acid model of colitis in rats, showing reduction of colonic damage and inflammatory markers in acute treatments (Table 4). Compound **70** exhibited better activity than sulfasalazine at the same dose, which suggests a role of **49c** in the overall activity. This suggestion is further supported because **70** provides less 5-ASA than sulfasalazine at the same dose expressed in mg/kg. Anticolitic effect of **70** in the TNBS model has been further studied in both acute[21] and chronic[22,23] treatments, showing greater potency than the mere 5-ASA-donor sulfasalazine.[23] Interestingly, it has been demonstrated that the activity of **70** is due to the contribution of both the PAF antagonist carrier **49c** and 5-ASA**.** We administered intracolonically equimolar amounts of **49c**,

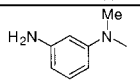
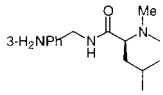
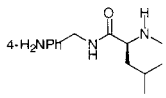
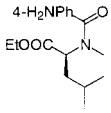
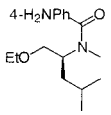
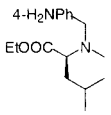
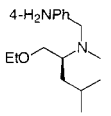
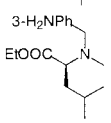
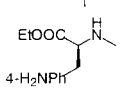
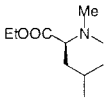
Table 1. PAF Antagonist Activities of Compounds **3B**

comp	R	PAF-induced platelet aggregation IC_{50},[a] μM	PAF-induced hypotension ID_{50},[b] mg/kg iv
48a		0.43 (0.25-0.76)	1.47 (0.85-2.55)
48b		0.011 (0.0078-0.015)	0.24 (0.18-0.31)
48c		0.019 (0.012-0.029)	0.099 (0.072-0.14)
49a		1.20 (0.85-1.65)	1.23 (0.85-1.77)
49b		0.060 (0.045-0.081)	0.17 (0.13-0.22)
49c		0.008 (0.006-0.011)	0.029 (0.017-0.050)
49d		0.005 (0.0038-0.0065)	0.01-0.025
49e		0.19 (0.17-0.22)	0.015 (0.010-0.023)
50		0.074 (0.039-0.141)	0.56 (0.36-0.86)
51		0.075 (0.071-0.078)	0.088 (0.043-0.18)
52		0.61 (0.48-0.78)	0.029 (0.018-0.048)
53		1.27 (0.85-1.90)	–
54		0.25 (0.18-0.35)	–
55		1.60 (1.20-2.15)	–
56		1.55 (1.35-1.75)	2.42 (1.50-3.89)
57		0.032 (0.028-0.036)	1.13 (0.59-2.15)
58		2.20 (1.60-3.05)	0.19 (0.13-0.27)
59		0.029 (0.020-0.045)	0.11 (0.08-0.16)
60		0.27 (0.18-0.40)	0.13 (0.08-0.23)
UR-12670		0.0076 (0.006-0.011)	0.0086 (0.0063-0.012)
WEB-2086		0.091 (0.071-0.12)	0.17 (0.12-0.27)
Lexipafant (BB-882)		0.0036 (0.0021-0.0063)	0.0037 (0.0024-0.0057)

[a] Concentration required to inhibit PAF-induced maximum aggregation by 50%. Parentheses contain 95% confidence limits. [b] Dose required to reduce the lowering of the arterial blood pressure caused by PAF by 50%. Parentheses contain 95% confidence limits.

5-ASA, and **49c** plus 5-ASA. Compound **49c** and 5-ASA cannot be administered separately by oral route, as both compounds are absorbed before reaching the colon. These experiments demonstrated that part of the overall activity (e.g., the decrease in neutrophil infiltration and in LTB_4 mucosa levels) was due to the contribution of topically acting **49c**.[23] Compound **70** (UR-12746) is currently undergoing clinical evaluation in the treatment of ulcerative colitis.

Table 2. PAF Antagonist Activities of Compounds **4B**

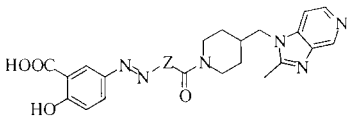
comp	R	PAF-induced platelet aggregation IC_{50},[a] μM	PAF-induced hypotension ID_{50},[b] mg/kg iv
61		0.30 (0.20-0.40)	0.22 (0.14-0.35)
62		0.25 (0.15-0.41)	0.28 (0.19-0.40)
63		0.12 (0.08-0.17)	0.075 (0.057-0.099)
64a		0.030 (0.025-0.038)	0.15 (0.13-0.16)
64b		1.28 (1.23-1.32)	0.30 (0.17-0.55)
65a		0.012 (0.010-0.015)	0.033 (0.018-0.061)
65b		0.016 (0.011-0.023)	0.14 (0.10-0.19)
66		0.017 (0.0091-0.031)	0.043 (0.028-0.017)
67		0.74 (0.54-1.02)	0.19 (0.11-0.33)
Lexipafant (BB-882)		0.0036 (0.0021-0.0063)	0.0037 (0.0024-0.0057)

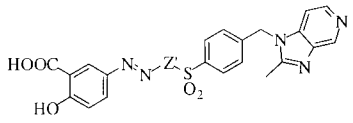
[a],[b] See footnotes to Table 1.

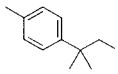
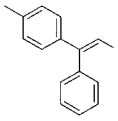
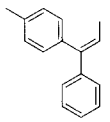
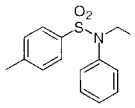
Experimental Section

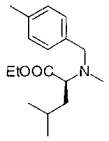
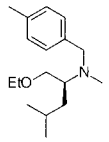
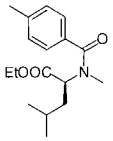
A. Chemistry. Most of the compounds were obtained through purification by column chromatography. After solvent evaporation, solids (probably amorphous) were obtained. Melting points were determined with a Mettler FP 80 central processor melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 983 spectrophotometer. ^{1}H NMR (80 MHz) spectra were recorded on a Brucker AC80 spectrometer, ^{1}H NMR (300 MHz) spectra on a Brucker Avance DPX-300 spectrometer, and the spectras are reported in ppm on the δ scale, from the indicated reference. Mass spectra were measured by electrospray technique (MH^+) on a Waters ZMD mass spectrometer. Combustion analyses were performed with a Carlo Erba 1106 analyzer. Liquid chromatography was performed with a forced flow (flash chromatography) of the indicated solvent system on SDS silica

Table 3. PAF Antagonist Activities of Compounds **3A** and **4A**





Comp.	Z	PAF-induced platelet aggregation IC_{50},[a] μM	PAF-induced hypotension ID_{50},[b] mg/kg iv
68		1.60 (1.20-2.20)	0.11 (0.08-0.13)
69		7.3 (4.3-12.5)	–
70		4.1 (3.3-5.1)	2.81 (1.79-4.40)
71		4.01 (3.61-4.46)	0.19 (0.14-0.36)

Comp.	Z	PAF-induced platelet aggregation IC_{50},[a] μM	PAF-induced hypotension ID_{50},[b] mg/kg iv
72		0.49 (0.31 -0.77)	1.31 (0.90 -1.91)
73		0.40 (0.34-0.46)	2.51 (1.10-5.70)
74		1.47 (1.16-1.87)	0.47 (0.21-1.06)
Sulfasalazine		>200	–
Balsalazide		>200	–

[a,b] See footnotes to Table 1.

Table 4. Effect of UR-12746 (**70**) and Sulfasalazine Treatment (100 mg/kg/day) on Macroscopic and Biochemical Parameters in the Acute Phase of Trinitrobenzenesulfonic Acid (TNBS)-Induced Colitis

group	*n*	damage score[a] (0−10)	colonic wt (mg/cm)	% of animals exhibiting diarrhea	myeloperoxidase (U/g tissue)	LTB_4 (ng/g tissue)
noncolitic	12	0	65.1 ± 4.8	0	62.6 ± 5.6	1.0 ± 0.2
TNBS control group	12	9 (7−10)	167.1 ± 5.0	75	897.3 ± 97.5	6.5 ± 0.5
UR-12746 (**70**)	8	7.5 (6−9)*	149.6 ± 6.7*	25*	445.9 ± 77.6**	4.5 ± 0.6**
sulfasalazine	8	8 (7−9)	154.4 ± 5.7	62.5	683.3 ± 59.6	3.6 ± 0.5**

[a] Score data are expressed as median (range). Colonic weight, myeloperoxidase, and LTB_4 data are expressed as mean ± SEM. *n* is the number of animals per group. *P < 0.05 vs TNBS control group; **P < 0.01 vs TNBS control group.

gel chromagel 60 a C.C. (230−400 mesh). All reactions were performed under anhydrous conditions unless otherwise noted. Solvents for reactions were purchased as "anhydrous" and used as received. All chemical yields are unoptimized and generally represent the result of a single experiment. Some abbreviations have been used: HOBt for 1-hydroxybenzotriazole hydrate, DCC for *N*,*N*′-dicyclohexylcarbodiimide), DPPA for diphenyl phosphoryl azide. The synthesis of (^{14}C)**70** was performed by Amersham Life Science essentially by the same sequence described for **70**, using triethyl phosphono[1−14C]acetate.

3-Methyl-3-(4-nitrophenyl)butanoic Acid (6c). To the 3-methyl-3-phenylbutyronitrile (21 g, 0.13 mol) were added slowly H_2O (125 mL) and 95−97% H_2SO_4 (100 mL), and the mixture was refluxed for 48 h. Next, H_2O was added, and the resulting solution was extracted with $CHCl_3$. The organic phase was washed with 2 N NaOH, and the aqueous phase was acidified with 5 N HCl and extracted with $CHCl_3$. The combined organic extracts were dried and concentrated to afford 3-methyl-3-phenylbutanoic acid **5c** (20.7 g, 90%). ^{1}H NMR (80 MHz, $CDCl_3$) δ (TMS): 10.8 (m, 1H), 7.29 (s, 5H), 2.61 (s, 2H), 1.43 (s, 6H). To cooled (0 °C) 95−97% H_2SO_4 (18.5 mL) was added **5c** (10 g, 56 mmol). Next, a cooled solution of 100% fuming HNO_3 (3 mL) in 95−97% H_2SO_4 (6.2 mL) was added dropwise, and the reaction mixture was stirred at 0 °C for 30 min and then at room temperature for 30 min more. The mixture was poured into ice and the resulting solution was allowed to stand in the refrigerator overnight. The precipitate was filtered, washed with H_2O and dried, to afford a crude product that was purified by chromatography on silica gel ($CHCl_3$:MeOH, 3%) to give **6c** (5.9 g, 47%). ^{1}H NMR (80 MHz, $CDCl_3$) δ (TMS): 8.16 (d, J = 6.5 Hz, 2H), 7.55 (d, J = 6.5 Hz, 2H), 3.5 (m, 1H), 2.70 (s, 2H), 1.50 (s, 6H).

3-(4-Nitrophenyl)butanoic Acid (6b). The title compound was obtained by nitration of 3-phenylbutanoic acid under the same conditions described for **6c** (21%). ^{1}H NMR (80 MHz, $CDCl_3$) δ (TMS): 8.12 (d, J = 8.7 Hz, 2H), 7.50 (d, J = 8.7 Hz, 2H), 3.31 (m, 1H), 2.61 (d, J = 7.4 Hz, 2H), 1.32 (d, J = 7.0 Hz, 3H).

1-[[1-[3-Methyl-3-(4-nitrophenyl)butanoyl]-4-piperidyl]-methyl]-1*H*-2-methylimidazo[4,5-*c*]pyridine (8c). General Procedure A for DCC Coupling. To a mixture of **7**[12c] (6 g, 26 mmol), **6c** (5.8 g, 26 mmol), and HOBt (3.64 g) in DMF (180 mL), was added DCC (5.28 g, 26 mmol) at 0 °C and under a nitrogen atmosphere, and the reaction mixture was stirred at room temperature for 18 h. The white insoluble material was filtered off, and the solvents were removed in vacuo. The residue was taken up in chloroform and washed with saturated $NaHCO_3$ solution, water, and brine, dried, and concentrated. The residue (8.3 g) was purified by chromatography on silica gel ($CHCl_3$:MeOH, 10%) to afford **8c** (3.21 g, 28%). ^{1}H NMR

(80 MHz, $CDCl_3$) δ (TMS): 8.96 (s, 1H), 8.37 (d, J = 5.5 Hz, 1H), 8.11 (d, J = 8.4 Hz, 2H), 7.51 (d, J = 8.4 Hz, 2H), 7.18 (d, J = 5.5 Hz, 1H), 4.55 (m, 1H), 3.95 (d, J = 7.1 Hz, 2H), 3.83 (m, 1H), 2.59 (s, 3H), 3−0.5 (complex signal, 9H), 1.50 (s, 6H).

1-[[1-[3-(4-Aminophenyl)-3-methylbutanoyl]-4-piperidyl]-methyl]-1*H*-2-methylimidazo[4,5-*c*]pyridine (48c). General Procedure B for Hydrogenation of Nitro Derivatives. A solution of **8c** (2.27 g, 5.2 mmol) in MeOH (100 mL) was hydrogenated at atmospheric pressure in the presence of 10% Pd/C (0.25 g) for 18 h. The catalyst was filtered off, and the solvent was removed to afford 1.26 g of a crude that was purified by chromatography on silica gel ($CHCl_3$:MeOH, 10%) to yield **48c** (1.1 g, 52%). ^{1}H NMR (80 MHz, $CDCl_3$) δ (TMS): 8.96 (s, 1H), 8.38 (d, J = 5.5 Hz, 1H), 7.18 (m, 3H), 6.62 (d, J = 5.5 Hz, 2H), 4.65 (m, 1H), 3.86 (d, J = 7.1 Hz, 2H), 3.63 (m, 2H), 3.50 (m, 1H), 2.59 (s, 3H), 3−0.5 (complex signal, 9H), 1.25 (s, 6H). MS *m/e* 406 (MH^+). mp: 172−173 °C. Anal. ($C_{24}H_{31}N_5O\cdot0.75H_2O$) C, H, N.

The following examples were prepared essentially as described above:

1-[[1-[2-(4-Aminophenyl)propionyl]-4-piperidyl]methyl]-1*H*-2-methylimidazo[4,5-*c*]pyridine (48a): (34%) ^{1}H NMR (80 MHz, $CDCl_3$) δ (TMS): 8.96 (s, 1H), 8.38 (d, J = 5.5 Hz, 1H), 7.05 (m, 1H), 6.99 (d, J = 9.5 Hz, 2H), 6.61 (d, J = 9.5 Hz, 2H), 4.74 (m, 1H), 3.77 (m, 4H), 2.53 (s, 3H), 3−0.5 (complex signal, 9H), 1.37 (d, J = 6.9 Hz, 3H). MS *m/e* 378 (MH^+). mp: 91−95 °C. Anal. ($C_{22}H_{27}N_5O\cdot0.5H_2O$) C, H, N.

1-[[1-[3-(4-Aminophenyl)butanoyl]-4-piperidyl]methyl]-1*H*-2-methylimidazo[4,5-*c*]pyridine (48b): (35%) ^{1}H NMR (80 MHz, $CDCl_3$+CD_3OD) δ (TMS): 8.88 (s, 1H), 8.33 (d, J = 5.5 Hz, 1H), 7.30 (d, J = 5.5 Hz, 1H), 7.02 (d, J = 9.0 Hz, 2H), 6.67 (d, J = 9.0 Hz, 2H), 4.64 (m, 1H), 3.93 (m, 3H), 3.80 (m, 2H), 3.20 (m, 1H), 2.63 (s, 3H), 3−0.5 (complex signal, 9H), 1.31 (d, J = 6.9 Hz, 3H). MS *m/e* 392 (MH^+). mp: 116−117 °C. Anal. ($C_{23}H_{29}N_5O\cdot1.5H_2O$) C, H, N.

***E*-1-[[1-[3-(4-Aminophenyl)propenoyl]-4-piperidyl]-methyl]-1*H*-2-methylimidazo[4,5-*c*]pyridine (49a):** (71%) ^{1}H NMR (80 MHz, $CDCl_3$) δ (TMS): 8.99 (s, 1H), 8.40 (d, J = 5.5 Hz, 1H), 7.62 (d, J = 15.3 Hz, 1H), 7.32 (d, J = 8.4 Hz, 2H), 7.20 (d, J = 5.5 Hz, 1H), 6.64 (d, J = 15.3 Hz, 1H), 6.63 (d, J = 8.3 Hz, 2H), 4.40 (m, 1H), 4.00 (d, J = 7.3 Hz, 2H), 3.94 (m, 1H), 2.80 (m, 2H), 2.64 (s, 3H), 2.4−1.2 (m, 7H). MS *m/e* 376 (MH^+). mp: 115−120 °C. Anal. ($C_{22}H_{25}N_5O\cdot0.5H_2O$) C, H, N.

***E*-1-[[1-[3-(4-Aminophenyl)-2-butenoyl]-4-piperidyl]-methyl]-1*H*-2-methylimidazo[4,5-*c*]pyridine (49b):** (22%) ^{1}H NMR (80 MHz, $CDCl_3$) δ (TMS): 8.98 (s, 1H), 8.38 (d, J = 5.5 Hz, 1H), 7.27 (m, 3H), 6.60 (d, J = 8.5 Hz, 2H), 6.17 (s, 1H), 4.67 (m, 1H), 3.90 (d, J = 7.2 Hz, 2H), 3.81 (m, 1H), 2.63 (s, 3H), 2.23 (s, 3H), 3.1−0.5 (complex signal, 9H). MS *m/e* 390 (MH^+). mp: 106−110 °C. Anal.($C_{23}H_{27}N_5O\cdot0.5H_2O$) C, H, N.

***Z*- and *E*-3-(4-Nitrophenyl)-3-phenylpropenoic Acid (10c and 10d).** To a cooled (0 °C) suspension of 50% NaH (24.66 g, 0.51 mol) in THF (375 mL) was added dropwise triethyl phosphonoacetate (88.2 mL, 44 mmol). The mixture was stirred for 45 min and 4-nitrobenzophenone (102 g, 0.45 mmol) in THF (525 mL) was added. The resulting mixture was refluxed for 18 h under an argon atmosphere and then allowed to cool and partitioned between H_2O and EtOAc. The organic phase was dried and concentrated to a residue (115 g). This crude material was dissolved in MeOH (600 mL), a solution of K_2CO_3 (87.2 g) in H_2O (288 mL) was added, and the resulting mixture was refluxed for 4 h. MeOH was removed under reduced pressure, water was added, and the solution extracted with hexane. The aqueous solution was then brought up to acid pH with 5 N HCl and extracted with $CHCl_3$. Evaporation of the solvent afforded a brown solid (102 g, 85%) as a mixture of the *Z* isomer **10c** and *E* isomer **10d** in a 6:4 ratio. ^{1}H NMR (80 MHz, $CDCl_3$) δ (TMS): 8.20 (m, 2H), 7.33 (m, 7H), 6.70 (m,1H), 6.44 (s, 0.6 H), 6.38 (s, 0.4 H).

Pure **10c** was also stereoselectively prepared by the following alternative method: to a cooled (−78 °C) solution of 1.6 M *n*-BuLi in hexane (60 mL, 96 mmol) in 200 mL of dry THF was added dropwise dicyclohexylamine (19.2 mL), and the resulting solution was stirred at −78 °C for 30 min. Next, ethyl trimethylsilyl acetate (17.46 mL, 95 mmol) was added dropwise, and after 30 min of stirring 4-nitrobenzophenone (20.7 g, 91 mmol) in THF (250 mL) was added. The resulting mixture was stirred for 30 min at −78 °C and then allowed to warm to room temperature. The solvent was removed, and the residue was partitioned between 0.5 N HCl and EtOAc. The insoluble material was filtered off and discarded, and the organic phase was separated, dried, and evaporated to give 35 g of a crude product. HPLC analysis (Licrospher 100CN column, eluent: MeOH-phosphate buffer pH = 6.8, 40:60, UV detection at λ = 220, flux of 1 mL/min) showed an 8:1 *Z*/*E* isomer ratio together with 10% of starting ketone. This crude product was dissolved in 153 mL of MeOH, a solution of K_2CO_3 (26.5 g) in water (74 mL) was added, and the resulting mixture was refluxed for 18 h. MeOH was removed, and the residue was acidified with 5 N HCl and extracted with $CHCl_3$. The organic solution was dried and concentrated to afford 30 g of a crude product. This was recrystallized from EtOH (100 mL) to give **10c** (16 g, 66% for the two steps) (containing only 1% of *E* isomer, as shown by HPLC analysis using a Licrospher RP-18 column, MeOH−phosphate buffer pH = 3 60:40, UV detection at λ = 220 nm, flux 1 mL/min). ^{1}H NMR (80 MHz, $CDCl_3$) δ (TMS): 8.23 (d, J = 8.0 Hz, 2H), 7.33 (m, 7H), 6.70 (m,1H), 6.44 (s, 1H).

Pure **10d** was also stereoselectively prepared by the following alternative method: A mixture of ethyl *trans*-cinnamate (4.4 g, 27 mmol), 4-bromonitrobenzene (6 g, 29.7 mol), triphenylphosphine (0.26 g), tributylamine (8 mL), and palladium acetate (57 mg) in CH_3CN (20 mL) was heated under argon atmposphere at reflux for 2 days. The cooled mixture was partitioned between 0.5 N NaOH and $CHCl_3$, and the organic phase was separated, dried, and concentrated. The residue was purified by chromatography on silica gel (hexane:EtOAc, 20%), and hydrolyzed with K_2CO_3 (as described above for **10c**) to afford 2.4 g (31%) as a white solid. ^{1}H NMR (80 MHz, $CDCl_3$) δ (TMS): 8.18 (d, J = 8.0 Hz, 2H), 7.33 (m, 8H), 6.39 (s, 1H).

***Z*- and *E*-1-[[1-[3-(4-Aminophenyl)-3-phenylpropenoyl]-4-piperidyl]methyl]-1*H*-2-methylimidazo[4,5-*c*]pyridine (49c and 49d).** Following general procedures A and B and starting from a mixture of **10c** and **10d**, a mixture of isomers was obtained which were separated by chromatography on silica gel ($CHCl_3$:MeOH, 10%) to afford a slower eluting isomer, **49c** (54%), and a faster eluting isomer, **49d** (22%). **49c**: ^{1}H NMR (80 MHz, $CDCl_3$) δ (TMS): 8.95 (s, 1H), 8.36 (d, J = 5.5 Hz, 1H), 7.29 (s, 5H), 7.07 (m, 3H), 6.65 (d, J = 6.5 Hz, 2H), 6.07 (s, 1H), 4.70 (m, 1H), 3.82 (m, 3H), 2.57 (s, 3H), 2.8−0.5 (complex signal, 9H). MS *m/e* 452 (MH^+). mp: 121−135 °C. Anal. ($C_{28}H_{29}N_5O\cdot0.75H_2O$) C, H, N. **49d**: ^{1}H NMR (80 MHz, $CDCl_3$) δ (TMS): 8.96 (s, 1H), 8.38 (d, J = 5.5 Hz, 1H), 7.30 (s, 5H), 7.05 (m, 3H), 6.60 (d, J = 6.5 Hz, 2H), 6.17 (s, 1H), 4.60 (m, 1H), 3.81 (m, 3H), 2.55 (s, 3H), 2.8−0.5 (complex signal, 9H). MS *m/e* 452 (MH^+). mp: 223−224 °C. Anal. ($C_{28}H_{29}N_5O\cdot0.5H_2O$) C, H, N.

Alternatively, **49c** was obtained by coupling of **7** with **10c** to give **11c**, following general procedure A, followed by reduction with $SnCl_2$ performed as follows. To a solution of $SnCl_2\cdot2H_2O$ (80.7 g, 0.357 mol) in 37% HCl (121 mL), cooled in an ice bath, was added dropwise a solution of **11c** (57.4 g, 0.119 mol) in AcOH (201 mL). The resulting viscous solution was stirred for 18 h and poured slowly on cooled 5 N NaOH (500 mL). The solid precipitate was filtered, washed with water, suspended in a mixture of $CHCl_3$:MeOH 5% (750 mL), heated at 50 °C, and filtered again. The filtrate was dried over Na_2SO_4, and after elimination of solvents almost to dryness, heated AcOEt (600 mL) was added and the white precipitate was formed, separated, and dried to give **49c** (44 g, 82%).

3-Ethoxycarbonyl-3-(4-nitrophenyl)propionic acid (13). To a 0 °C cooled suspension of 50% NaH (1.2 g, 26 mmol) in DMF (25 mL) was added dropwise a solution of ethyl 4-nitrophenylacetate (5 g, 23.9 mol) in DMF (25 mL), and the mixture was stirred 1 h at room temperature. Then, *tert*-butyl bromoacetate (3.52 mL, 23.9 mol) was added, and the mixture was heated at 60 °C for 24 h. After addition of water (1 mL), solvents were removed, and the residue was partitioned

between water and EtOAc. The organic phase was dried and concentrated to a residue. This was purified by chromatography on silica gel (hexane:EtOAc 1:1), to give 6.5 g (77%) of *tert*-butyl ester of **13**. This ester (2 g, 6.1 mmol) was hydrolyzed by treatment with *p*-toluenesulfonic acid (0.2 g, 1.1 mmol) in benzene (10 mL) at reflux for 30 min. After elimination of the solvent and purification of the residue by chromatography on silica gel (hexane:EtOAc, 50%), **13** was obtained (0.64 g, 40%). ^{1}H NMR (80 MHz, $CDCl_3$) δ (TMS): 9.61 (s, 1H), 8.19 (d, J = 8.4 Hz, 2H), 7.49 (d, J = 8.4 Hz, 2H), 4.16 (q, J = 7.3 Hz, 2H), 4.14 (m, 1H), 3.30 (dd, J = 17.5 Hz, J = 9.0 Hz, 1H), 2.76 (dd, J = 17.3 Hz, J = 6.1 Hz, 1H), 1.20 (t, J = 7.3 Hz, 3H).

1-[[1-[3-(4-Aminophenyl)-3-ethoxycarbonylpropionyl]-4-piperidyl]methyl]-1*H*-2-methylimidazo[4,5-*c*]pyridine (50). The title compound was prepared from **13** following general procedures A and B (16%, 2 steps). ^{1}H NMR (80 MHz, $CDCl_3$) δ (TMS): 8.98 (s, 1H), 8.41 (d, J = 5.5 Hz, 1H), 7.26 (m, 1H), 7.07 (d, J = 9.5 Hz, 2H), 6.62 (d, J = 9.5 Hz, 2H), 4.65 (m, 1H), 3.98 (m, 6H), 2.62 (s, 3H), 3.3−1 (complex signal, 11H), 1.19 (t, J = 6.5 Hz, 3H). MS *m/e* 450 (MH^+). mp: 204−205 °C. Anal. ($C_{25}H_{31}N_5O_3\cdot H_2O$) C, H, N.

[*N*-(4-Nitrophenyl)sulfonyl-*N*-phenylamino]acetic Acid (16). To a solution of *N*-phenylglycine (4.1 g, 30 mmol) in a mixture of THF (35 mL), water (35 mL), and 1 N NaOH (35 mL), cooled in an ice bath, was added dropwise a solution of 4-nitrobenzenesulfonyl chloride (6.5 g, 29 mmol) in THF (20 mL). The resulting mixture was stirred for 52 h and then extracted with ether; the aqueous phase was acidifed with 2 N HCl and extracted with $CHCl_3$. The organic solution was dried and concentrated to afford **16** (6 g, 59%). ^{1}H NMR (80 MHz, $CDCl_3$) δ (TMS): 8.30 (d, J = 8.4 Hz, 2H), 7.85 (d, J = 8.4 Hz, 2H), 7.30 (m, 5H), 4.44 (s, 2H).

1-[[[[*N*-(4-Aminophenylsulfonyl)-*N*-phenylamino]acetyl-4-piperidyl]methyl]-1*H*-2-methylimidazo[4,5-*c*]pyridine (51). The title compound was prepared from **16** following general procedures A and B (18%, 2 steps). ^{1}H NMR (80 MHz, $CDCl_3$) δ (TMS): 8.79 (s, 1H), 8.38 (d, J = 5.5 Hz, 1H), 7.45 (d, J = 8.8 Hz, 2H), 7.26 (m, 6H), 6.94 (d, J = 8.8 Hz, 2H), 4.36 (m, 1H), 4.35 (s, 2H), 4.00 (d, J = 7.2 Hz, 2H), 3.98 (m, 1H), 2.63 (s, 3H), 3.3−1.0 (complex signal, 9H). mp: 147−157 °C. Anal. ($C_{27}H_{30}N_6O_3S\cdot 2H_2O$) C, H, N, S.

***N*-Ethoxycarbonyl-*N*-(4-nitrophenyl)aminoacetic Acid (22).** *N*-(Ethoxycarbonyl)-4-nitroaniline (1.7 g, 8 mmol) was dissolved in THF (5 mL) and was then added dropwise to a cooled (0 °C) suspension of 50% NaH (0.48 g, 10 mmol) in dry THF (10 mL). The mixture was stirred at room temperature for 30 min, and then ethyl bromoacetate (0.89 mL, 8 mmol) was added. The reaction mixture was stirred at room temperature for 48 h and refluxed for 24 h. The residue was taken up in $CHCl_3$ and phosphate buffer and extracted with $CHCl_3$ (2×). Evaporation of the solvent gave the ethyl *N*-Ethoxycarbonyl-*N*-(4-nitrophenyl)aminoacetate (1.54 g). This product was dissolved in MeOH (35 mL), a solution of K_2CO_3 (1.33 g) in H_2O (18 mL) was added, and the mixture was refluxed for 3 h. Volatiles were removed, and the resulting solution was extracted with hexane. The aqueous phase was made acid and extracted with $CHCl_3$. The organic extracts were dried and concentrated to afford 0.7 g (50%) of **22**. ^{1}H NMR (80 MHz, $CDCl_3$) δ (TMS): 8.21 (d, J = 6.5 Hz, 2H), 7.50 (d, J = 6.5 Hz, 2H), 5.89 (broad s, 1H), 4.45 (s, 2H), 4.16 (q, J = 7.1 Hz, 2H), 1.26 (t, J = 7.1 Hz, 3H).

1-[[1-[[*N*-(4-Aminophenylsulfonyl)-*N*-isobutylamino]acetyl]-4-piperidyl]methyl]-1*H*-2-methylimidazo[4,5-*c*]pyridine (52). Prepared as described for **54** using **19** (7%, 2 steps).^{1}H NMR (80 MHz, $CDCl_3$) δ (TMS): 8.96 (s, 1H), 8.39 (d, J = 5.5 Hz, 1H), 7.51 (d, J = 8.8 Hz, 2H), 7.20 (d, J = 5.5 Hz, 1H), 6.63 (d, J = 8.8 Hz, 2H), 4.50 (m, 3H), 3.89 (m, 5H), 3.00 (m, 2H), 2.62 (s, 3H), 3.1−1.2 (m, 8H), 0.84 (d, J = 6.5 Hz, 6H). MS *m/e* 499 (MH^+). mp: 112−116 °C. Anal. ($C_{25}H_{34}N_6O_3S\cdot 1.5H_2O$) C, H, N, S.

1-[[1-[[*N*-(4-Aminobenzoyl)-*N*-methylamino]acetyl]-4-piperidyl]methyl]-1*H*-2-methylimidazo[4,5-*c*]pyridine (53). Preparation from **37** following general procedure A gave **53** (32%). ^{1}H NMR (80 MHz, $CDCl_3$) δ (TMS): 8.97 (s, 1H), 8.38 (d, J = 5.5 Hz, 1H), 7.25 (m, 3H), 6.61 (d, J = 9.0 Hz, 2H), 4.64 (m, 1H), 4.01 (m, 2H), 3.98 (d, J = 7.1 Hz, 2H), 3.10 (s, 3H), 2.62 (s, 3H), 3.3−1 (complex signal, 10H). MS *m/e* 421 (MH^+). mp: 120−124 °C. Anal. ($C_{23}H_{28}N_6O_2\cdot 1.25H_2O$) C, H, N.

1-[[1-[[*N*-(4-Aminophenyl)-*N*-ethoxycarbonylamino]-acetyl]-4-piperidyl]methyl]-1*H*-2-methylimidazo[4,5-*c*]-pyridine (54). The title compound was prepared from **22** following general procedures A and B (32%, 2 steps). ^{1}H NMR (80 MHz, $CDCl_3$) δ (TMS): 8.98 (s, 1H), 8.40 (d, J = 5.5 Hz, 1H), 7.20 (d, J = 5.5 Hz, 1H), 7.10 (d, J = 7.05 Hz, 2H), 6.60 (d, J = 7.05 Hz, 2H), 4.65 (m, 1H), 4.30 (m 2H), 4.10 (m, 4H), 3.80 (m, 3H), 2.62 (s, 3H), 3.1−1.3 (m, 7H), 1.17 (t, J = 6.9 Hz, 3H). MS *m/e* 451 (MH^+). mp: 137−138 °C. Anal. ($C_{24}H_{30}N_6O_3\cdot H_2O$) C, H, N.

1-[[1-[[1-(4-Aminophenyl)ethylamino]carbonyl]-4-piperidyl]methyl]-1*H*-2-methylimidazo[4,5-*c*]pyridine (55). A solution of **31** (prepared as described for **33**, starting from **30**) (0.8 g, 1.89 mmol) and $SnCl_2\cdot 2H_2O$ (2.128 g, 9.4 mmol) in EtOH (25 mL) was heated at 60 °C, and then a solution of $NaBH_4$ (0.035 g, 0.94 mmol) in EtOH (15 mL) was added dropwise. The reaction mixture was heated at 60 °C for 1 h and was then cooled to 10 °C, made basic, and extracted with $CHCl_3$. The organic phase was dried and concentrated to a residue which was chromatographed on silica gel ($CHCl_3$:MeOH:NH_3, 60:20:0.2) to afford **55** (36 mg, 5%). ^{1}H NMR (80 MHz, $CDCl_3$) δ (TMS): 8.96 (s, 1H), 8.36 (d, J = 5.5 Hz, 1H), 7.18 (d, J = 5.5 Hz, 1H), 7.11 (d, J = 8.3 Hz, 2H), 6.62 (d, J = 8.3 Hz, 2H), 4.90 (quint, J = 6.7 Hz, 1H), 3.96 (d, J = 6.4 Hz, 1H), 3.96 (m, 4H), 2.64 (m, 2H), 2.61 (s, 3H), 2.10 (m, 1H), 1.43 (d, J = 6.6 Hz, 3H), 1.26 (m, 6H). MS *m/e* 393 (MH^+). mp: 122−128 °C. Anal. ($C_{22}H_{28}N_6O\cdot 1.5H_2O$) C, H, N.

1-[[1-[3-[*N*-(4-Aminobenzoyl)amino]-3-phenylpropionyl]-4-piperidyl]methyl]-1*H*-2-methylimidazo[4,5-*c*]pyridine (58). Preparation from **36**[12c] following general procedure A gave **58** as a white solid (75%). ^{1}H NMR (80 MHz, $CDCl_3$) δ (TMS): 8.97 (s, 1H), 8.45 (m, 1H), 8.38 (d, J = 5.5 Hz, 1H), 7.72 (d, J = 8.3 Hz, 2H), 7.34 (m, 6H), 6.65 (d, J = 8.3 Hz, 2H), 5.50 (m, 1H), 4.64 (m, 1H), 3.80 (m, 5H), 2.57 (s, 3H), 3−0.5 (complex signal, 9H). MS *m/e* 497 (MH^+). mp: 132−142 °C. Anal. ($C_{29}H_{32}N_6O_2\cdot H_2O$) C, H, N.

3-[(4-Nitrophenyl)amino]-3-phenylpropionic Acid (25). A mixture of *trans*-cinnamic acid (2 g, 13 mmol) and HBr (30% solution in AcOH, 40 mL) was stirred at room temperature for 18 h and then evaporated to dryness. The resulting solid was taken up in 2-butanone (100 mL), and *p*-nitroaniline (5 g, 36 mmol) was added. The reaction mixture was refluxed for 18 h, allowed to cool, and partitioned between $CHCl_3$ and 1 N HCl. The organic phase was dried and concentrated to a residue. This was purified by chromatography on silica gel (hexane:EtOAc, 50%) to afford **25** as a yellow solid (0.78 g, 21%). ^{1}H NMR (80 MHz, $CDCl_3$) δ (TMS) 7.99 (d, J = 9.2 Hz, 2H), 7.33 (m, 5H), 6.56 (d, J = 9.2 Hz, 2H), 4.92 (t, J = 6.3 Hz, 1H), 3.44 (m, 2H), 2.82 (d, J = 6.5 Hz, 2H).

1-[[1-[3-[(4-Aminophenyl)amino]-3-phenylpropionyl]-4-piperidyl]methyl]-1*H*-2-methylimidazo[4,5-*c*]pyridine (59). To a solution of **26** (prepared following general procedure A, using **25**) (226 mg, 0.4 mmol) in EtOH (5 mL) and H_2O (0.6 mL) was added a solution of $CaCl_2$ (33.6 mg) in H_2O (0.26 mL) and powdered zinc (0.58 g). The resulting mixture was heated at 50 °C for 45 min and filtered through Celite, and the filtrate was concentrated. The residue was purified by chromatography on silica gel ($CHCl_3$:MeOH 10%) to afford **59** as a white solid (0.17 g, 91%). ^{1}H NMR (80 MHz, $CDCl_3$) δ (TMS): 8.93 (s, 1H), 8.34 (d, J = 5.5 Hz, 1H), 7.31 (m, 6H), 6.44 (broad s, 4H), 4.62 (m, 2H), 3.80 (d, J = 7.0 Hz, 2H), 3.52 (m, 4H), 2.55 (s, 3H), 3.0−0.5 (complex signal, 9H). A solution of the title compound in $CHCl_3$ was treated with a solution of HCl (4 N in Et_2O), to afford the hydrochloride of **59**. mp: 189−195 °C. Anal. ($C_{28}H_{32}N_6O.4HCl\cdot 2H_2O$) C, H, N.

3-[*N*-(Ethoxycarbonyl)amino]-3-(4-nitrophenyl)propionic Acid (28). To a −16 °C cooled solution of ammonium nitrate (0.86 g, 10 mmol) in 95−97% H_2SO_4 (9 mL) was added slowly **27** (2 g, 8 mmol). The resulting mixture was stirred 1

h at −16 °C, poured onto ice, and extracted with $CHCl_3$. The organic phase was dried and concentrated to give 1.8 g (82%) of **28**. ^{1}H NMR (80 MHz, $CDCl_3$) δ (TMS): 9.66 (s, 1H), 8.17 (d, J = 6.5 Hz, 2H), 7.50 (d, J = 6.5 Hz, 2H), 6.2 (m, 1H), 5.22 (q, J = 7.5 Hz, 1H), 4.11 (q, J = 7.1 Hz, 2H), 2.91 (d, J = 6.2 Hz, 2H), 1.20 (t, J = 7.1 Hz, 3H).

1-[[1-[3-(4-Aminophenyl)-3-[*N*-(ethoxycarbonyl)amino]-propionyl]-4-piperidyl]methyl]-1*H*-2-methylimidazo[4,5-*c*]pyridine (60). The title compound was prepared from **28** following general procedures A and B (33%, 2 steps). ^{1}H NMR (80 MHz, $CDCl_3$) δ (TMS): 8.94 (s, 1H), 8.35 (d, J = 5.5 Hz, 1H), 7.21 (d, J = 5.5 Hz, 1H), 7.07 (d, J = 9.5 Hz, 2H), 6.63 (m, 3H), 4.95 (m, 1H), 4.60 (m, 1H), 4.06 (q, J = 7.2 Hz, 2H), 3.88 (m, 3H), 2.59 (s, 3H), 3.6−0.5 (complex signal, 11H), 1.19 (t, J = 7.2 Hz, 3H). mp: 113−116 °C. Anal. ($C_{25}H_{32}N_6O_3\cdot 0.5H_2O$) C, H, N.

1-[[1-[(4-Aminophenylmethylamino)carbonyl]-4-piperidyl]methyl]-1*H*-2-methylimidazo[4,5-*c*]pyridine (56). DPPA (1.6 mL, 7.6 mmol) was added to a solution of **32** (1.9 g, 7.6 mmol) and NEt_3 (0.85 mL) in benzene (40 mL) and stirred for 20 min at room temperature and for 2 h at 90 °C. Then, **7** (1.2 g, 5 mmol) was added, and the resulting mixture was stirred for 12 h at 90 °C. After cooling, the mixture was partitioned between 1 N NaOH and AcOEt. The organic phase was dried and concentrated to a residue which was chromatographed on silica gel ($CHCl_3$:MeOH 9:1) to afford 2.3 g (96%) of **33**. ^{1}H NMR (80 MHz, $CDCl_3$) δ (TMS): 8.96 (s, 1H), 8.36 (d, J = 5.5 Hz, 1H), 7.28 (m, 5H), 6.90 (s, 1H), 5.05 (m, 1H), 4.31 (d, J = 5.2 Hz, 2H), 4.03 (m, 2H), 3.96 (d, J = 7.1 Hz, 2H), 2.62 (m, 2H), 2.60 (s, 3H), 2.1−1.2 (m, 5H), 1.50 (s, 9H). mp: 125−130 °C. Anal. ($C_{26}H_{34}N_6O_3\cdot 0.5H_2O$) C, H, N.

A solution of **33** (1 g, 20 mmol) in MeOH (30 mL) was treated with a solution of HCl (6.2 N in dioxane) (1.4 mL) for 20 h. After concentration, the residue was taken up in 2 N NaOH and extracted with $CHCl_3$ to give 0.55 g (70%) of **56** as a white solid. ^{1}H NMR (80 MHz, $CDCl_3$) δ (TMS): 8.92 (s, 1H), 8.34 (d, J = 5.5 Hz, 1H), 7.19 (d, J = 5.5 Hz, 1H), 7.05 (d, J = 8.2 Hz, 2H), 6.58 (d, J = 8.2 Hz, 2H), 5.05 (m, 1H), 4.26 (d, J = 5.2 Hz, 2H), 4.08 (m, 2H), 3.96 (d, J = 7.1 Hz, 2H), 3.40 (m, 2H), 2.60 (m, 2H), 2.59 (s, 3H), 2.1−1.2 (m, 5H);. MS *m/e* 379 (MH^+). mp: 104−109 °C. Anal. ($C_{21}H_{26}N_6O\cdot H_2O$) C, H, N.

(*S*)-1-[[1-[[*N*-[2-(4-Aminophenyl)-1-ethoxycarbonylethyl]-amino]carbonyl]-4-piperidyl]methyl]-1*H*-2-methylimidazo[4,5-*c*]pyridine (57) A solution of **34** (0.84 g, 2.4 mmol) and **7** (0.6 g, 2.4 mmol) in pyridine was heated to reflux for 18 h. After concentration, the residue was partitioned between 0.5 N NaOH and $CHCl_3$. The organic phase was dried and concentrated to a residue which was purified by chromatography on silica gel ($CHCl_3$:MeOH, 5%) to afford **35** as a white solid (64%). This was hydrogenated following procedure B to give **57**. ^{1}H NMR (80 MHz, $CDCl_3$) δ (TMS): 8.96 (s, 1H), 8.36 (d, J = 5.5 Hz, 1H), 7.20 (d, J = 5.5 Hz, 1H), 6.88 (d, J = 9.2 Hz, 2H), 6.57 (d, J = 9.2 Hz, 2H), 4.97 (d, J = 8.1 Hz, 1H), 4.65 (q, J = 8.12 Hz, 1H), 4.18 (q, J = 6.5 Hz, 2H), 4.00 (m, 1H), 3.98 (d, J = 7.3 Hz, 2H), 2.98 (d, J = 5.8 Hz, 2H), 2.80 (m, 5H), 2.61 (s, 3H), 2.1−1.4 (complex signal, 5H), 1.25 (t, J = 6.5 Hz, 3H). MS *m/e* 465 (MH^+). mp: 87−96 °C. Anal. ($C_{25}H_{32}N_6O_3\cdot H_2O$) C, H, N.

***N*-[(*S*)-1-[(3-Aminobenzyl)aminocarbonyl]-3-methylbutyl]-*N*-methyl-4-(1*H*-2-methylimidazo[4,5-*c*]pyridylmethyl)phenylsulfonamide (62).** Starting from **42a** and 3-nitrobenzylamine and following general procedure A and B gave **62** (42%) ^{1}H NMR (80 MHz, CD_3OD) δ (TMS): 8.83 (s, 1H), 8.28 (d, J = 5.5 Hz, 1H), 7.72 (d, J = 8.2 Hz, 2H), 7.46 (d, J = 5.5 Hz, 1H), 7.20 (d, J = 8.2 Hz, 2H), 6.93 (m, 1H), 6.48 (m, 3H), 5.59 (s, 2H), 4.75 (s, 3H), 4.47 (m, 1H), 3.94 (m, 2H), 2.90 (s, 3H), 2.60 (s, 3H), 1.35 (m, 3H), 0.88 (d, J = 5.7 Hz, 6H). MS *m/e* 535 (MH^+). mp: 100−104 °C. Anal. ($C_{28}H_{34}N_6O_3S\cdot 0.25H_2O$) C, H, N, S.

***N*-[(*S*)-1-[(4-Aminobenzyl)aminocarbonyl]-3-methylbutyl]-4-(1*H*-2-methylimidazo[4,5-*c*]pyridylmethyl)phenylsulfonamide (63).** Preparation from **42b** and 4-nitrobenzylamine following general procedure A and B gave **63** (44%). ^{1}H NMR (80 MHz, $CDCl_3$ + CD_3OD) δ (TMS): 8.92 (s, 1H), 8.28 (d, J = 5.5 Hz, 1H), 7.80 (d, J = 8.2 Hz, 2H), 7.33 (d, J = 8.2 Hz, 2H), 7.19 (m, 3H), 6.89 (d, J = 8.3 Hz, 2H), 6.59 (d, J = 8.3 Hz, 2H), 5.44 (s, 2H), 3.98 (m, 2H), 3.70 (m, 3H), 2.59 (s, 3H), 1.35 (m, 3H), 0.83 (d, J = 5.7 Hz, 3H), 0.70 (d, J = 5.7 Hz, 3H). MS *m/e* 521 (MH^+). mp: 249−250 °C. Anal. ($C_{27}H_{32}N_6O_3S\cdot 1.5H_2O$) C, H, N, S.

***N*-(4-Nitrobenzoyl)-*N*-[4-(1*H*-2-methylimidazo[4,5-*c*]pyridylmethyl)-phenylsulfonyl]-L-leucine Ethyl Ester (39a).** To a solution of **38a** (2.4 g, 5.4 mmol), obtained as described in WO 92/03423, in dry THF (80 mL) was added 50% NaH (0.23 g), and the resulting mixture was heated at 50 °C for 1.5 h and then it was allowed to cool to room temperature; 4-nitrobenzoyl chloride (1.2 g, 6.4 mmol) in THF (20 mL) was added, and the mixture was stirred for 52 h. The solvent was removed, and the residue was partitioned between 0.5 N NaOH and $CHCl_3$. The organic phase was separated, dried, and concentrated to afford 2.8 g of a crude product, which was purified by column chromatography ($CHCl_3$:MeOH, 10%) to afford **39a** (1.9 g, 65%). ^{1}H NMR (80 MHz, $CDCl_3$) δ (TMS): 9.02 (s, 1H), 8.40 (d, J = 5.5 Hz, 1H), 8.11 (d, J = 8.8 Hz, 2H), 7.87 (d, J = 8.8 Hz, 2H), 7.53 (d, J = 8.8 Hz, 2H), 7.11 (d, J = 8.8 Hz, 2H), 7.10 (d, J = 5.5 Hz, 1H), 5.38 (s, 2H), 4.85 (t, J = 6.7 Hz, 1H), 4.28 (q, J = 7.1 Hz, 2H), 2.58 (s, 3H), 2.11 (t, J = 6.7 Hz, 2H), 1.78 (quint, J = 6.7 Hz, 1H), 1.30 (t, J = 7.1 Hz, 3H), 0.94 (d, J = 7.3 Hz, 3H), 0.86 (d, J = 7.3 Hz, 3H). mp: 68−71 °C. Anal. ($C_{29}H_{31}N_5O_7S$) C, H, N, S.

***N*-(4-Aminobenzoyl)-*N*-[4-(1*H*-2-methylimidazo[4,5-*c*]-pyridylmethyl)-phenylsulfonyl]-L-leucine Ethyl Ester (64a).** A solution of **39a** (1.91 g, 0.0032 mol) in MeOH (100 mL) was hydrogenated at atmospheric pressure in the presence of 10% Pd/C (0.3 g) for 18 h. The catalyst was filtered off, and the solvent was removed to afford 1.62 g of a crude product, which was purified by column chormatography ($CHCl_3$:MeOH:NH_3, 60:4:0.2) to yield 0.6 g (33%) of a white solid. ^{1}H NMR (80 MHz, $CDCl_3$) δ (TMS): 9.00 (s, 1H), 8.35 (d, J = 5.5 Hz, 1H), 7.98 (d, J = 8.8 Hz, 2H), 7.32 (d, J = 8.8 Hz, 2H), 7.10 (m, 3H), 6.40 (d, J = 8.8 Hz, 2H), 5.33 (s, 2H), 4.95 (dd, J = 8.0 Hz, J = 4.5 Hz, 1H), 4.20 (q, J = 7.1 Hz, 2H), 2.54 (s, 3H), 2.0 (m, 5H), 1.32 (t, J = 7.1 Hz, 3H), 0.86 (d, J = 7.3 Hz, 3H), 0.74 (d, J = 7.3 Hz, 3H). MS *m/e* 564 (MH^+). mp: 91−94 °C. Anal. ($C_{29}H_{33}N_5O_5S\cdot 0.25H_2O$) C, H, N, S.

***N*-(4-Aminobenzoyl)-*N*-[(*S*)-1-isobutyl-2-ethoxyethyl]-4-(1*H*-2-methylimidazo[4,5-*c*]pyridylmethyl)phenylsulfonamide (64b).** Prepared as described for **64a**, starting from **38b**, **64b** was obtained as a white solid (46%). ^{1}H NMR (80 MHz, $CDCl_3$) δ (TMS): 9.03 (s, 1H), 8.38 (d, J = 5.5 Hz, 1H), 7.92 (d, J = 8.2 Hz, 2H), 7.48 (d, J = 8.2 Hz, 2H), 7.07 (m, 3H), 6.48 (d, J = 8.3 Hz, 2H), 5.36 (s, 2H), 4.40 (m, 1H), 4.00 (m, 2H), 3.50 (m, 4H), 2.57 (s, 3H), 1.56 (m, 3H), 1.08 (t, J = 8.9 Hz, 3H), 0.78 (m, 6H). MS *m/e* 550 (MH^+). mp: 89−92 °C. Anal. ($C_{29}H_{35}N_5O_4S\cdot 0.5H_2O$) C, H, N, S.

***N*-(4-Nitrobenzyl)-*N*-[4-(1*H*-2-methylimidazo[4,5-*c*]pyridylmethyl)-phenylsulfonyl]-L-leucine Ethyl Ester (40a).** To a solution of **38a** (3 g, 6.7 mmol) in dry THF (30 mL), cooled in an ice bath, was added 50% NaH (0.28 g), and the resulting mixture was stirred at 0 °C for 0.5 h. Next, a solution of 4-nitrobenzyl mesylate (2.3 g, 9.9 mmol) in THF (20 mL) was added, and the reaction mixture was stirred at room temperature for 18 h. The solvent was removed, and the residue was partitioned between 0.5 N NaOH and $CHCl_3$. The organic phase was separated, dried, and concentrated to afford 6 g of a crude product. This was purified by column chromatography ($CHCl_3$:MeOH:NH_3, 60:2:0.2) to give 2.12 g (55%) of a yellow solid. ^{1}H NMR (80 MHz, $CDCl_3$) δ (TMS): 9.03 (s, 1H), 8.48 (d, J = 5.5 Hz, 1H), 8.15 (d, J = 8.7 Hz, 2H), 7.75 (d, J = 8.7 Hz, 2H), 7.57 (d, J = 8.7 Hz, 2H), 7.17 (m, 3H), 5.40 (s, 2H), 4.80 (d, J = 17 Hz, 1H), 4.51 (d, J = 17 Hz, 1H), 4.50 (m, 1H), 3.79 (m, 2H), 2.60 (s, 3H), 1.43 (m, 3H), 1.00 (t, J = 7.1 Hz, 3H), 0.87 (d, J = 5.6 Hz, 3H), 0.58 (d, J = 5.6 Hz, 3H). mp: 60−65 °C. Anal. ($C_{29}H_{33}N_5O_6S\cdot 0.5H_2O$).

***N*-(4-Aminobenzyl)-*N*-[4-(1*H*-2-methylimidazo[4,5-*c*]pyridylmethyl)-phenylsulfonyl]-L-leucine Ethyl Ester (65a).** Prepared as described for **64a** using **40a**, **65a** was obtained as a white solid (48%). ^{1}H NMR (80 MHz, $CDCl_3$) δ (TMS):

9.01 (s, 1H), 8.36 (d, J = 5.5 Hz, 1H), 7.69 (d, J = 8.7 Hz, 2H), 7.12 (m, 5H), 6.49 (d, J = 8.7 Hz, 2H), 5.36 (s, 2H), 4.58 (m, 1H), 4.50 (d, J = 15.7 Hz, 1H), 4.18 (d, J = 15.7 Hz, 1H), 3.80 (m, 2H), 3.60 (m, 2H), 2.58 (s, 3H), 1.47 (m, 3H), 1.00 (t, J = 7.1 Hz, 3H), 0.81 (d, J = 5.6 Hz, 3H), 0.60 (d, J = 5.6 Hz, 3H);. MS *m/e* 550 (MH^+). mp: 69−73 °C. Anal. ($C_{29}H_{35}N_5O_4S$) C, H, N, S.

***N*-(4-Nitrobenzyl)-*N*-[(*S*)-1-isobutyl-2-ethoxyethyl]-4-(1*H*-2-methylimidazo[4,5-*c*]pyridylmethyl)phenylsulfonamide (40b).** Prepared as described for **40a**, using **38b** (obtained as described in WO 92/03422), **40b** was obtained as a yellow oil (48%). ^{1}H NMR (80 MHz, $CDCl_3$) δ (TMS): 9.03 (s, 1H), 8.38 (d, J = 5.5 Hz, 1H), 8.10 (d, J = 8.7 Hz, 2H), 7.75 (d, J = 8.7 Hz, 2H), 7.52 (d, J = 8.7 Hz, 2H), 7.10 (m, 3H), 5.38 (s, 2H), 4.46 (s, 2H), 4.07 (quint, J = 6.2 Hz, 1H), 3.29 (m, 4H), 2.59 (s, 3H), 1.45 (m, 1H), 1.05 (m, 2H), 0.78 (m, 9H).

***N*-(4-Aminobenzyl)-*N*-[(*S*)-1-isobutyl-2-ethoxyethyl]-4-(1*H*-2-methylimidazo[4,5-*c*]pyridylmethyl)phenylsulfonamide (65b).** Prepared as described for **64a**, using **40b**, **65b** was obtained as a white solid (46%). ^{1}H NMR (80 MHz, $CDCl_3$) δ (TMS): 9.04 (s, 1H), 8.37 (d, J = 5.5 Hz, 1H), 7.75 (d, J = 8.7 Hz, 2H), 7.10 (m, 5H), 6.47 (d, J = 8.7 Hz, 2H), 5.35 (s, 2H), 4.22 (dd, J = 16, 19 Hz, 2H), 4.05 (m, 1H), 3.16 (m, 6H), 2.59 (s, 3H), 1.45 (m, 1H), 1.10 (m, 2H), 0.90 (t, J = 7.0 Hz, 3H), 0.77 (d, J = 6.1 Hz, 3H), 0.69 (d, J = 6.1 Hz, 3H). MS *m/e* 536 (MH^+). mp: 67−72 °C. Anal. ($C_{29}H_{37}N_5O_3S\cdot0.5H_2O$) C, H, N, S.

***N*-[4-(1*H*-2-methylimidazo[4,5-*c*]pyridylmethyl)-phenylsulfonyl]-L-(4-aminophenyl)alanine Ethyl Ester (67).** To a solution of 4-nitrophenylalanine ethyl ester (0.43 g, 1.8 mmol) in acetonitrile (6 mL) and Et_3N (0.8 mL) was added **45** hydrochloride salt (1.5 mmol) suspended in acetonitrile (4 mL). The resulting mixture was stirred for 2 h, poured on 0.5 N NaOH (15 mL), and extracted with $CHCl_3$. The organic phase was separated, dried, and concentrated to afford 0.9 g of a crude product. This was purified by column chromatography ($CHCl_3$:MeOH:NH_3, 60:4:0.2) to give 0.16 g of **46** (22%) as a yellow solid. ^{1}H NMR (80 MHz, $CDCl_3$) δ (TMS): 8.89 (s, 1H), 8.28 (d, J = 5.5 Hz, 1H), 8.02 (d, J = 8.2 Hz, 2H), 7.62 (d, J = 8.2 Hz, 2H), 7.34 (m, 2H), 7.28 (d, J = 8.3 Hz, 2H), 7.04(d, J = 8.2 Hz, 2H), 5.38 (s, 2H), 3.95 (m, 3H), 3.06 (m, 2H), 2.55 (s, 3H), 1.04 (t, J = 8.9 Hz, 3H). After catalytic hydrogenation following procedure B and purification by column chromatography ($CHCl_3$:MeOH:NH_3, 60:10:0.2), **67** was obtained (21%). MS *m/e* 494 (MH^+). mp: 92−95 °C. Anal. ($C_{25}H_{27}N_5O_4S.0.5CHCl_3\cdot0.5H_2O$) C, H, N, S.

***Z*-2-Hydroxy-5-[[4-[3-[4-[(2-methyl-1*H*-imidazo[4,5-*c*]-pyridin-1-yl)methyl]-1-piperidinyl]-3-oxo-1-phenyl-1-propenyl]phenyl]azo]benzoic Acid (70). General Procedure C.** In a 2 L flask was placed **49c** (64.8 g, 0.143 mol). The flask was then cooled in an ice bath, and a solution of fuming HCl (67.5 mL) in water (664 mL) was added. Keeping the flask in the ice bath, a solution of $NaNO_2$ (10.9 g, 0.159 mol) in water (96 mL) was added dropwise, and the mixture was stirred for 45 min. In a beaker was placed a solution of salicylic acid (19.04 g, 0.137 mol) in 2 N NaOH (408 mL) and water (92 mL). To this solution, cooled to 18 °C, the 0 °C cold diazonium salt was added dropwise via cannula. The color of the solution gradually changed to deep red-orange, and toward the end of the reaction an abundant orange-yellow solid precipitated. Toward the end of the addition of the diazonium salt solution, pH was controlled so that the solution remained basic until the end of the addition, when it was stirred for 30 min. EtOH (550 mL) was added, and after cooling in an ice bath, the pH was brought to 3 with 5 N HCl. The resulting mixture was heated to reflux, the solid formed was separated, washed with water, suspended in MeOH (500 mL), and heated. The solid was separated, suspended again in MeOH, and filtered. After drying, **70** was obtained as an orange solid (60.9 g, 71%). ^{1}H NMR (300 MHz, CD_3OD + D_2O + NaOD) δ (CD_3OD): 8.72 (s, 1H), 8.22 (m, 1H), 8.03 (s, 1H), 7.70 (m, 3H), 7.26 (m, 8H), 6.60 (dd, J = 9.0 Hz, J = 3.5 Hz, 1H), 6.25 (s, 1H), 4.88 (solvent), 4.44 (d, J = 12.2 Hz, 1H), 3.78 (m, 2H), 3.63 (m, 1H), 2.72 (m, J = 12.3 Hz, 1H), 2.50 (m, J = 12.3 Hz, 1H), 2.45 (s, 3H), 1.92 (m, 1H), 1.50 (d, J = 13.1 Hz, 1H), 0.93 (m, 2H), −0.20 (q, J = 11.9 Hz, 1H); (KBr): 3417, 3068, 2490, 1640, 1616, 1593, 1462, 1317, 842 cm^{-1}. MS *m/e* 601 (MH^+). mp: >300 °C. Anal. ($C_{35}H_{32}N_6O_4\cdot0.5H_2O$) C, H, N.

2-Hydroxy-5-[[4-[3-[4-[(2-methyl-1*H*-imidazo[4,5-*c*]pyridin-1-yl)methyl]-1-piperidinyl]-3-oxo-1,1-dimethyl-1-propyl]phenyl]azo]benzoic Acid (68). The title compound was prepared from **48c** following general procedure C. After bringing the pH to 3, extraction with $CHCl_3$, and removal of the solvent afforded a crude product which was purified by chromatography on silica gel ($CHCl_3$:MeOH 20%) to give an orange solid (55%). ^{1}H NMR (80 MHz, $CDCl_3$ + CD_3OD) δ (TMS): 8.88 (s, 1H), 8.48 (m, 2H), 7.95 (m, 3H), 7.48 (m, 4H), 7.00 (d, J = 8.9 Hz, 1H), 4.59 (m, 1H), 4.08 (m, 1H), 3.82 (d, J = 7.1 Hz, 2H), 3.55 (m, 1H), 2.57 (s, 3H), 3−0.5 (complex signal, 9H), 1.61 (s, J = 3H), 1.56 (s, 3H). MS *m/e* 555 (MH^+). mp: 185−190 °C. Anal.($C_{31}H_{34}N_6O_4\cdot H_2O$) C, H, N.

***E*-2-Hydroxy-5-[[4-[3-[4-[(2-methyl-1*H*-imidazo[4,5-*c*]-pyridin-1-yl)methyl]-1-piperidinyl]-3-oxo-1-phenyl-1-propenyl]phenyl]azo]benzoic Acid (69).** The title compound was prepared from **49d** following general procedure C. After bringing the pH to 3, extraction with $CHCl_3$ and removal of the solvent afforded a crude product which was purified by chromatography on silica gel ($CHCl_3$:MeOH 20%) to give an orange solid (44%). ^{1}H NMR (80 MHz, $CDCl_3$+CD_3OD) δ (TMS): 8.93 (s, 1H), 8.45 (m, 2H), 7.92 (m, 3H), 7.40 (m, 8H), 7.30 (d, J = 8.9 Hz, 1H), 6.39 (s,1H), 4.65 (m, 1H), 4.28 (m, 1H), 3.94 (m, 3H), 2.61 (s, 3H), 3−0.5 (complex signal, 6H). MS *m/e* 601 (MH^+). mp: 228−232 °C. Anal. ($C_{35}H_{32}N_6O_4.0.75CHCl_3$) C, H, N.

2-Hydroxy-5-[[4-[2-[4-[(2-methyl-1*H*-imidazo[4,5-*c*]pyridin-1-yl)methyl]-1-piperidinyl]-2-oxo-1-ethyl-*N*-phenylaminosulfonyl]phenyl]azo]benzoic Acid (71). The title compound was prepared from **51** following general procedure C. After bringing the pH to 3, the solid formed was separated and dissolved in DMF to be purified by chromatography on silica gel ($CHCl_3$:MeOH 20%) to give an orange solid (25%). ^{1}H NMR (80 MHz, DMSO-d_6) δ (TMS): 9.06 (s, 1H), 8.33 (m, 2H), 7.82 (m, 7H), 7.26 (m, 5H), 6.80 (d, J = 8.9 Hz, 1H), 4.14 (m, 5H), 2.88 (s, 3H), 3−1 (complex signal, 9H). MS *m/e* 668 (MH^+). mp: 195−200 °C. Anal. ($C_{34}H_{33}N_7O_6S\cdot3H_2O$) C, H, N, S.

***N*-[4-(4-Hydroxy-3-carboxyphenylazo)benzoyl]-*N*-[4-(1*H*-2-methylimidazo[4,5-*c*]pyridylmethyl)phenylsulfonyl]-L-leucine Ethyl Ester (74).** The title compound was prepared from **64a** following general procedure C. After bringing the pH to 3, the solid formed was separated and dissolved in DMF to be purified by chromatography on silica gel ($CHCl_3$:MeOH 20%) to give an orange solid (30%). ^{1}H NMR (80 MHz, DMSO-d_6) δ (TMS): 9.10 (s, 1H), 8.34 (m, 2H), 7.74 (m, 5H), 7.35 (m, 4H), 6.91 (d, J = 8.7 Hz, 2H), 5.73 (s, 2H), 5.60 (m, 2H), 5.02 (m, 1H), 4.19 (q, J = 7.08 Hz, 2H), 2.59 (s, 3H), 1.91 (m, 3H), 1.21 (t, J = 7.0 Hz, 3H), 0.89 (t, J = 6.1 Hz, 3H), 0.82 (d, J = 6.1 Hz, 3H);. MS *m/e* 713 (MH^+). mp: 173−180 °C. Anal. ($C_{36}H_{36}N_6O_8S\cdot1.5H_2O$) C, H, N, S.

***N*-[4-(4-Hydroxy-3-carboxyphenylazo)benzyl]-*N*-[4-(1*H*-2-methylimidazo[4,5-*c*]pyridylmethyl)phenylsulfonyl]-L-leucine Ethyl Ester (72).** The title compound was prepared from **65a** following general procedure C. After bringing the pH to 3, extraction with $CHCl_3$ and removal of the solvent afforded a crude product which was purified by chromatography on silica gel ($CHCl_3$:MeOH 20%) to give an orange solid (18%). ^{1}H NMR (80 MHz, $CDCl_3$+CD_3OD) δ (TMS): 8.97 (s, 1H), 8.53 (s, 1H), 8.37 (m, 1H), 8.00 (d, J = 9.0 Hz, 1H), 7.75 (m, 4H), 7.18 (m, 6H), 5.43 (s, 2H), 4.58 (dd, J = 10.7, 13 Hz, 2H), 4.52 (m, 1H), 4.06 (m, 2H), 3.89 (q, J = 7.08 Hz, 2H), 2.60 (s, 3H), 1.57 (m, 3H), 1.08 (t, J = 7.0 Hz, 3H), 0.95 (t, J = 5.1 Hz, 3H), 0.64 (d, J = 5.1 Hz, 3H). MS *m/e* 699 (MH^+). mp: 177−176 °C. Anal. ($C_{36}H_{38}N_6O_7S\cdot0.5CHCl_3$) C, H, N, S.

***N*-[4-(4-Hydroxy-3-carboxyphenylazo)benzyl]-*N*-[(*S*)-1-isobutyl-2-ethoxyethyl]-4-(1*H*-2-methylimidazo[4,5-*c*]pyridylmethyl)phenylsulfonamide (73)**. The title compound was prepared from **65b** following general procedure C. After bringing the pH to 3, extraction with $CHCl_3$ and removal of

the solvent afforded a crude product which was purified by chromatography on silica gel ($CHCl_3$:MeOH 20%) to give an orange solid (35%). ^{1}H NMR (80 MHz, $CDCl_3$ + CD_3OD) δ (TMS): 8.95 (s, 1H), 8.51 (s, 1H), 8.37 (m, 1H), 8.00 (d, J = 9.0 Hz, 1H), 7.66 (m, 4H), 7.33 (m, 3H), 7.00 (d, J = 8.5 Hz, 3H), 5.35 (s, 2H), 4.42 (s, 2H), 4.22 (m, 1H), 3.33 (m, 5H), 2.57 (s, 3H), 1.55 (m, 1H), 1.28 (m, 3H), 0.95 (t, J = 7.1 Hz, 3H), 0.85 (t, J = 6.0 Hz, 3H), 0.77 (d, J = 6.0 Hz, 3H). MS *m/e* 685 (MH^+). mp: 170−173 °C. Anal. ($C_{36}H_{40}N_6O_6S\cdot H_2O$) C, H, N, S.

B. Biological Methods: Inhibition of Platelet Aggregation in Vitro. Platelet-aggregation studies were performed by the method of Born.[18] Blood was collected in 3.16% sodium citrate (1 volume per 9 volumes of blood) by cardiac puncture from male New Zealand rabbits (2−2.5 kg body weight). Platelet rich plasma (PRP) was prepared by centrifuging the blood at 250 g for 10 min at 4 °C. The PRP was diluted with platelet-poor plasma obtained by further centrifuging at 3000*g* for 10 min. The platelet number was adjusted to 3.5×10^5 cells/mm^3. Platelet aggregation was induced by C18-PAF (1.5×10^{-8} M) and measured with a dual-channel aggregometer Chrono-log 560. Activity was expressed as the IC_{50} value, i.e., the concentration required to inhibit platelet aggregatory response (measured as maximum aggregation) by 50%. The values shown in the tables were calculated by linear regression from a single experimental curve with no less than four data points, each point being the mean of the percentage inhibition at a given concentration obtained from one to three independent experiments. Only points in the range of 15−85% inhibition were used for IC_{50} calculation.

Inhibition of PAF-Induced Hypotension in Normotensive Rats.[19] Male Sprague−Dawley rats, weighing 180 to 220 g, were anesthetized with sodium pentobarbital (50 mg/kg ip). Blood pressure was recorded from the left carotid artery using a Statham pressure transducer coupled to a Beckman R611 recorder. Right and left femoral veins were catheterized to inject the test compound and PAF (0.5 μg/kg). Test compounds were administered by intravenous injection (1 mL/kg, dissolved in saline) 3 min before PAF injection. Blood pressure was monitored, and percentage inhibition of PAF-induced hypotension with respect to controls was calculated. The results were expressed as ID_{50} values, i.e., the dose of test compound required to inhibit hypotension by 50%, or as percentage inhibition at a given dose of test compound. The ID_{50} values were calculated by linear regression from a single experimental curve with no less than four points, each point being the mean of the percentage inhibition at a given dose obtained from two or more independent experiments. Only points in the range of 15−85% inhibition were used for ID_{50} calculation.

Trinitrobenzenesulfonic Acid (TNBS)-Induced Colitis in the Rat.[23] Female Wistar rats (180−220 g) were fasted overnight. Under anesthesia, animals were given 30 mg of TNBS dissolved in 0.25 mL of 50% ethanol (v/v) by means of a Teflon cannula inserted 8 cm through the anus. Rats from the noncolitic group received 0.25 mL of phosphate buffered saline. Compounds were given orally in 1% (w/v) methylcellulose suspension for 5 days before colitis induction, as well as 24 h thereafter. Animals were sacrificed 48 h after colitis induction, and the entire colon was removed. The colon was longitudinally opened and scored for macroscopically visible damage on a 0−10 scale by two observers unaware of the treatment, according to the criterion described by Bell.[24] Myeloperoxidase activity and LTB_4 production were measured as described in Galvez et al.[23] Differences among means were tested for statistical significance using one-way analysis of variance and post hoc least significance tests. Nonparametric data (score) are expressed as median (range) and were analyzed with the Mann−Whitney U test. Differences among proportions (diarrhea) were analyzed with the χ^2 test. Statistical significance was set at $P < 0.05$.

Oral Administration of (^{14}C)70. Pharmacokinetics. An oral solution of (^{14}C)**70** (50 mg/kg and 50 μCi/kg) was given to 31 female Sprague−Dawley rats (stock solution: 9.9 mg of **70** was dissolved in 90 μL of 1 N NaOH added to 580 μL of water; afterward a 40 μL (250 μCi/mL) of (^{14}C)**70** and 290 μL of 0.01 N HCl were added). Blood samples were drawn into vials containing EDTA at specified times until 48 h postdosing. After collection, all blood samples were immediately centrifuged at 3000*g* for 10 min at 4 °C. Plasma was removed and stored frozen at −20 °C until analysis. Urine and feces samples were collected at specified times until 168 h postdosing. Feces samples were weighed and homogenized with 2 mL of water per gram, and urine sample volumes were measured before being stored frozen until analysis.

Radioactivity of each sample was determined with a liquid scintillation counter. The matrix concentration of (^{14}C)**70** and (^{14}C)**49c** were determined using an HPLC method involving gradient elution and both UV and radioactive detection. Plasma samples were deproteinized by adding MeOH. (^{14}C)-**70** and (^{14}C)**49c** were extracted from urine and feces with MeOH. The quantification limit of (^{14}C)**70** in plasma samples was 200 ng/mL. The matrix concentrations of 5-ASA and *N*-acetyl-5-ASA were determined using an HPLC method involving isocatric elution and fluorescence detection. Plasma samples were deproteinized by adding acetonitrile; urine samples were diluted with mobile phase and feces samples extracted with MeOH. The quantification limit of 5-ASA and *N*-acetyl-5-ASA in plasma samples was 5 ng/mL and in urine samples was 50 ng/mL.

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