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Non-peptidic Inhibitors of Human Neutrophil Elastase: The Design and Synthesis of Sulfonanilide-Containing Inhibitors

Katsuhiro Imaki, Takanori Okada, Yoshisuke Nakayama, Yuuki Nagao, Kaoru Kobayashi, Yasuhiro Sakai, Tetsuya Mohri, Takaaki Amino, Hisao Nakai* and Masanori Kawamura

Department of Medicinal Chemistry, Minase Research Institute, Ono Pharmaceutical Co., Ltd, Shimamoto, Mishima, Osaka 618, Japan

Abstract—A novel series of pivaloyloxy benzene derivatives has been identified as potent and selective human neutrophil elastase (HNE) inhibitors. Convergent syntheses were developed in order to identify the inhibitors which are intravenously effective in an animal model. A compound of particular interest is the sulfonanilide-containing analogues. Structure–activity relationships are discussed. Structural requirements for metabolic stabilization are also discussed. Copyright © 1996 Elsevier Science Ltd

Introduction

Human neutrophil elastase (HNE) is a member of the serine proteases produced by human neutrophils. It is released from neutrophils in response to inflammatory stimuli,[1] and has been implicated in the development of various diseases such as emphysema,[2–4] adult respiratory distress syndrome (ARDS),[5,6] cystic fibrosis,[7–9] and rheumatoid arthritis.[10] Under normal conditions, the body protects itself from the potential damaging effects of extracellular HNE with the endogenous α-1-proteinase inhibitor (α_1-PI). However, if the balance between protease and anti-protease is upset due to a decrease in the level of α_1-PI, the excess HNE activity may lead to tissue damage and the development of a disease such as emphysema.[11] The excess HNE produced by this imbalance hydrolyses elastin, the structural protein which gives the lungs their elasticity, and contributes to the development of disease. It has been hypothesized that an appropriate, small molecular weight inhibitor of HNE could restore the imbalance between HNE and α_1-PI in diseases such as emphysema, and would be useful therapeutically in the treatment of such diseases.[12] A variety of structural classes of HNE inhibitors have thus been studied.[13–18]

A series of pivaloyloxybenzene derivatives was discovered to be highly potent in vitro HNE inhibitors, and to possess activity in in vivo models of acute lung injury. Among them ONO-5046 (**49a**) is currently being evaluated in a clinical study of a range of HNE-mediated disorders. A potential drawback of peptidic inhibitors and the reported inhibitors as therapeutic drugs is their low systemic activity, possibly because of their metabolic instability or mechanism-based safety problems.[19] For these reasons, it is considered desirable to design reversible and metabolically stable non-peptidic inhibitors without safety problems. Our approach to these problems is the basis of this paper.

Chemistry

Deoxybenzoin derivatives **4b**, **5**, **6**, **7b**, **8**, **9b** and **10–19** were prepared as shown in Scheme 1. Preparation of derivatives **4b**, **7b** and **9b** was carried out by the Friedel–Crafts *para*-acylation of the substituted benzene derivatives **4a**, **7a** and **9a**, respectively, with the corresponding phenylacetyl chloride in the presence of aluminum chloride. Acidic hydrolysis of **4b** and **7b** provided **5** and **8**, respectively. Acylation of **5** with trifluoroacetic anhydride gave **6**. Demethylation of **9b** with pyridinium hydrochloride gave **10**, which was acylated with acetic anhydride in pyridine to afford **11**. Acylation of **10** with the corresponding acid chloride by the conventional method produced **12–18**. Compound **19** was prepared by treatment of 4-hydroxydeoxybenzoin (**10**) with ethyl isocyanate in the presence of triethylamine.

The treatment of *p*-aminobenzonitrile with benzyl magnesium chloride followed by acidification with sulfuric acid gave **21**, which was converted to **22** with cyanamide (Scheme 2-1). *t*-Butyl *p*-benzoylbenzoate (**24**) was prepared by the condensation of *t*-butyl alcohol and *p*-benzoylbenzoic acid in the presence of DCC (Scheme 2-2).

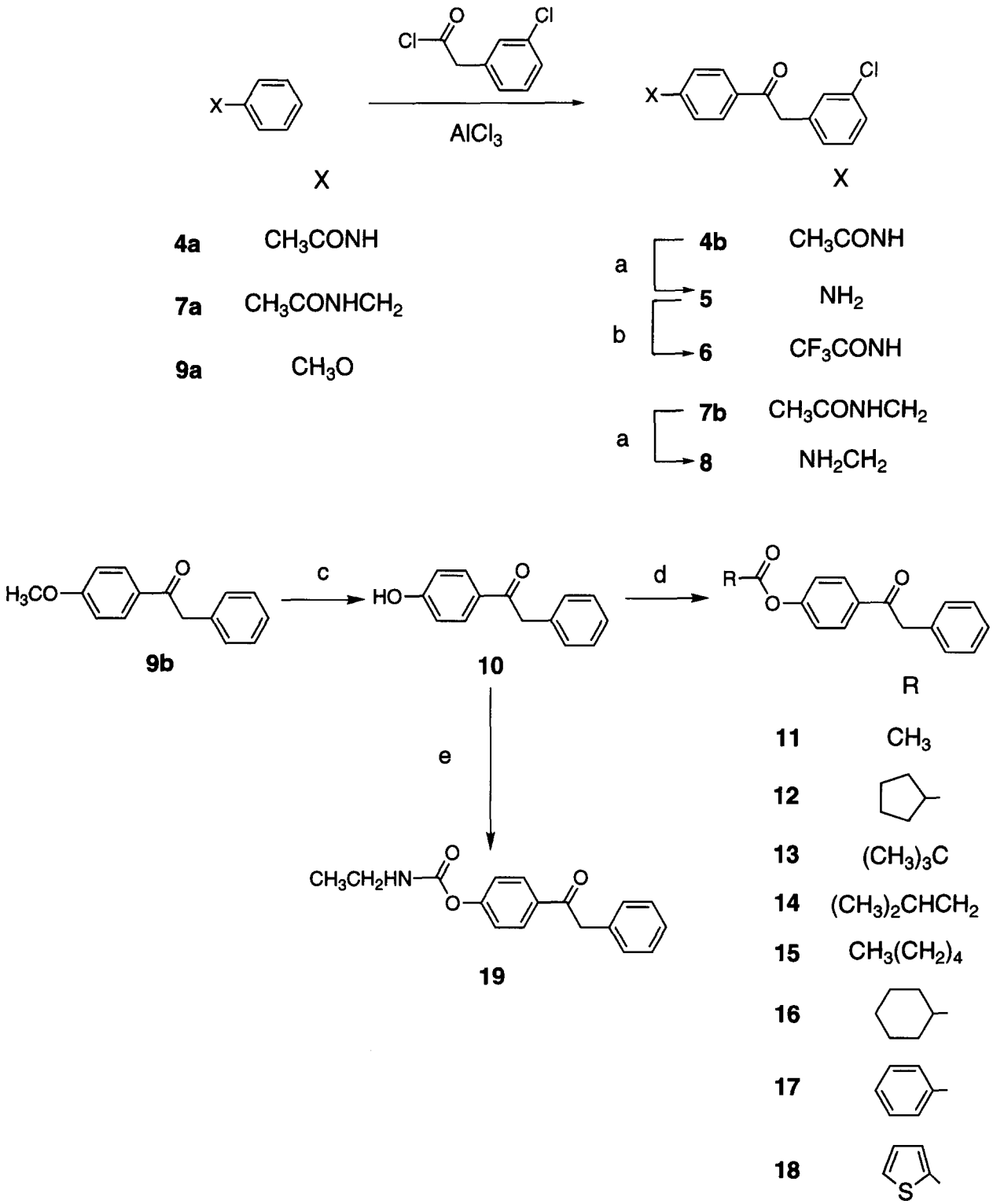
As described in Scheme 3-1, the series of benzophenone derivatives **25a–c** and **26a–c** was prepared by acylation of each phenol with pivaloyl chloride and cyclopentanecarbonyl chloride, respectively.

Scheme 3-2 shows the preparation of the derivatives **27–36** except for the sulfonamide derivatives. Compounds **27–30**, **32**, **34** and **35** were prepared by acylation of the corresponding phenol with pivaloyl chloride. Hydrogenolysis of benzyl ether of **30** gave **31**. Oxidation of **32** with hydrogen peroxide gave **33**. Sequential reactions: condensation of *p*-acetoxybenzoic acid and *p*-methylaniline in the presence of diisopro-

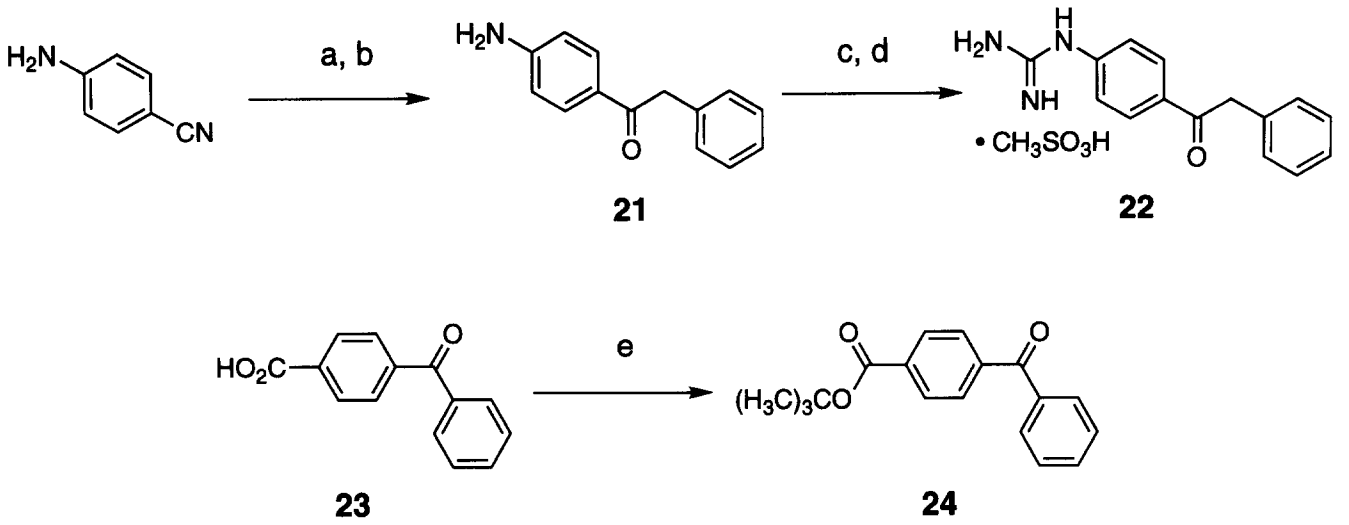
pylcarbodiimide, methanolysis with methanol and potassium carbonate, and then acylation with pivaloyl chloride afforded **36**.

Scheme 4-1 shows the method used to prepare the series of sulfonamides **37–41**. Sequential reactions: sulfonylation of the amines with *p*-methoxybenzenesulfonyl chloride,[20] demethylation,[20] and then acylation of the corresponding *p*-hydroxybenzenesulfonamides with pivaloyl chloride provided **37–41**.

Scheme 4-2 describes the method of preparation of **44–56**, containing more complex sulfonamide moiety in their molecules. Acylation of sodium *p*-hydroxybenz-



Scheme 1. (1) Preparation of **3–9**; (2) preparation of **9–20**. Reagents: [a]conc HCl; [b]$(CF_3CO)_2O$, Py; [c]Py • HCl; [d]RCOCl, Et_3N, CH_2Cl_2; [e]EtNCO, Et_3N, CH_2Cl_2.



Scheme 2. (1) Preparation of **22**; (2) preparation of **24**. Reagents: [a]BzlMgCl, Et_2O; [b]H_2SO_4; [c]NH_2CN; [d]CH_3SO_3H; [e]*t*-BuOH, DCC, DMAP.

enesulfonate with pivaloyl chloride gave **42**, which was treated with thionyl chloride to give a sulfonyl chloride **43**. Sulfonylation of various kinds of amines with **43** provided **44–56**. As described in Scheme 4-3, the *N*-methyl derivative **59** was prepared by the sequential reactions: treatment of **55** with thionyl chloride to give **57**, acylation of benzyl glycinate hydrochloride with **57** followed by debenzylation of **58**.

As shown in Scheme 5, **61–69** were prepared by acylation of *p*-hydroxybenzenesulfonanilide with the corresponding acid chloride.

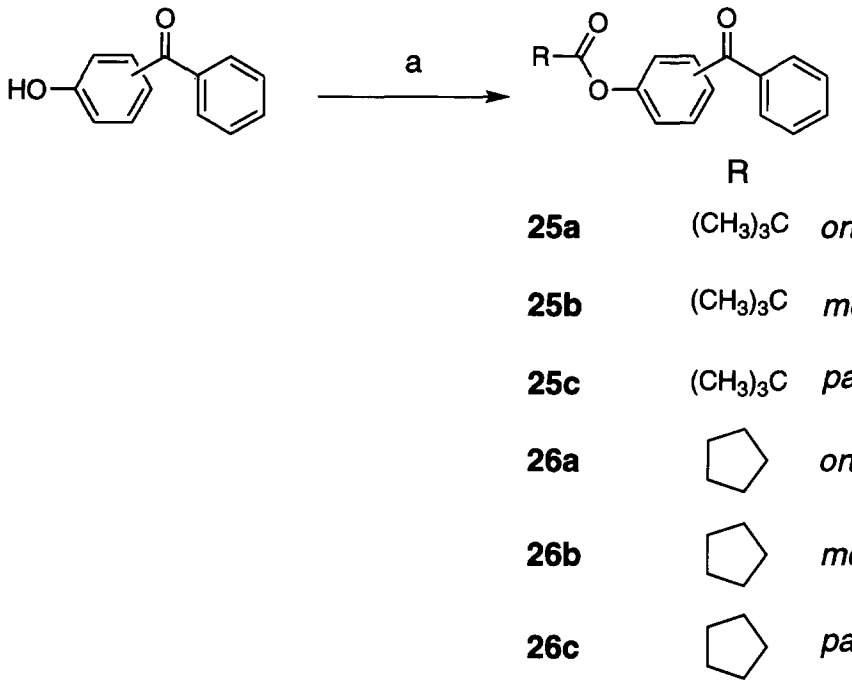
The preparation of **72–74** was accomplished by *N*-sulfonylation of aniline derivatives with 3-methyl-4-pivaloyloxybenzenesulfonyl chloride (**71**) which was obtained by treatment of potassium 3-methyl-4-pivaloyloxybenzenesulfonate (**70**) with thionyl chloride (Scheme 6–1). As shown in Scheme 6–2, **75d** and **76d** were prepared by *N*-sulfonylation of benzyl 2-aminobenzoate with the corresponding benzenesulfonyl chlorides **75b** and **76b** followed by catalytic hydrogenolysis of **75c** and **76c**, respectively.

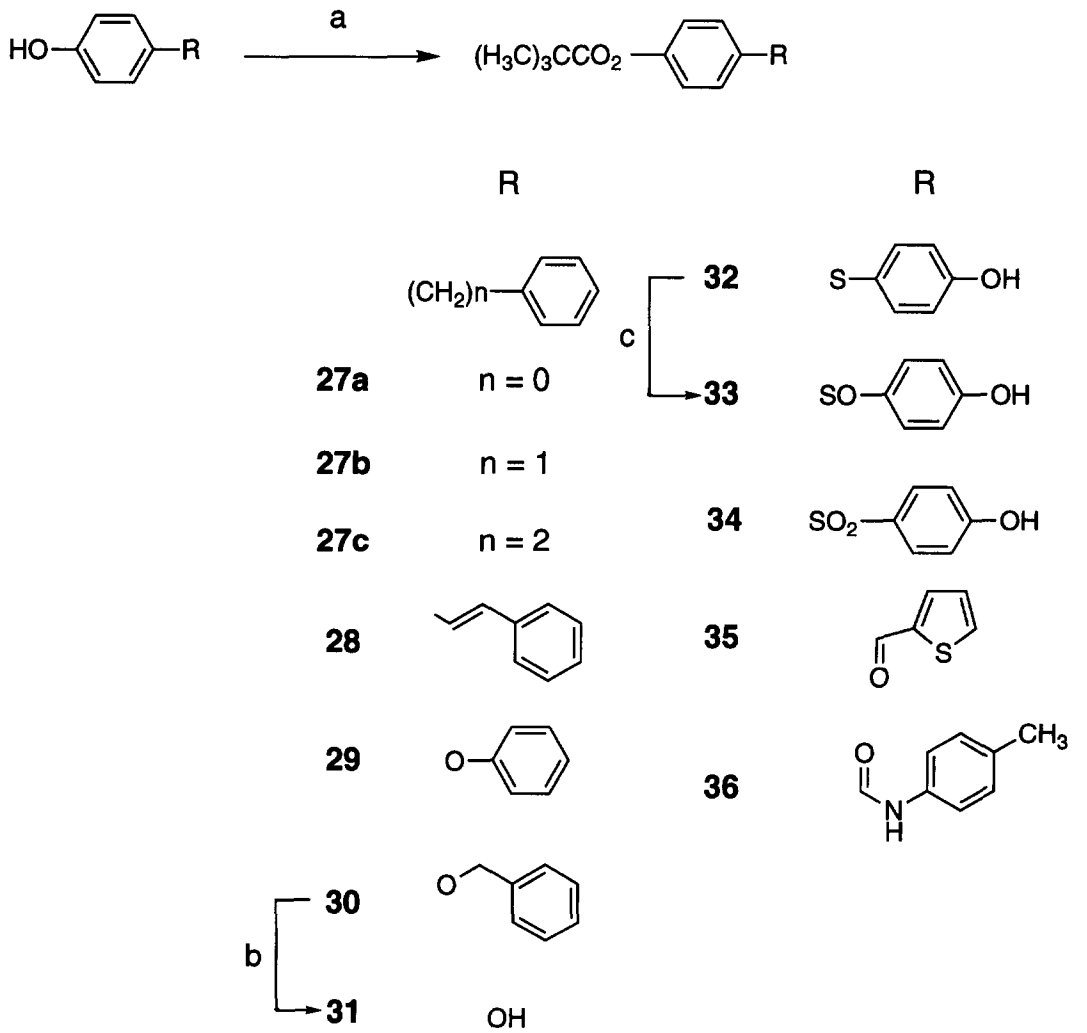
Results and Discussion

The inhibition of neutrophil elastase was studied using a synthetic substrate, Suc-Ala-Pro-Ala-*p*NA. Inhibition was found to be competitive as determined by Lineweaver–Berk plot analysis.[21] The metabolic stability of these inhibitors in guinea-pig plasma was examined using high performance liquid chromatography (HPLC) analysis. Some of the selected compounds (**37**, **48a**, **49a**, **50a** and **50b**) were evaluated in an in vivo study using HNE-induced skin capillary permeability in guinea pig as a newly developed animal model.[21] The intravenous administration of **48a**, **49a**, **50a** and **50b** significantly suppressed the increase of skin capillary permeability induced by HNE at a dose of 10 mg/kg,[21] whereas oral administration of these compounds did not show any significant effect up to 100 mg/kg, because of their presumed metabolic instability in the intestine (shown in Table 8). Only **49a** was weakly active after its oral administration at a higher dose (300 mg/kg).

We screened our compound library for non-peptidic HNE inhibitors and found 3-(4-guanidinobenzoyloxy)-chlorobenzene (**1**), a trypsin inhibitor, which had moderate activity (IC_{50} 8.0 μM) to HNE (Chart 1). Our initial chemical modification was started with the confirmation of indispensable partial structure for the inhibitory activity. The elimination of chloro group from **1** produced **2** with remarkable reduction of the inhibitory activity. As shown in Table 1, chemical modification of **1** to the deoxybenzoin derivative without a guanidino group produced **3** with remarkable reduction of the inhibitory activity (7.3% inhibition at 100 μM). The replacement of the phenyl benzoate moiety of **2** with a deoxybenzoin moiety and removal of the guanidine function produced **9** and **10**, also with remarkable reduction of the inhibitory activity. Deoxybenzoin derivatives **20** and **22** showed 19.4 and 0% inhibition at 100 μM. Thus, both the chloro group and the phenyl benzoate moiety of **1** were found to be necessary for the moderate activity of **1**. The guanidine function was not required for the activity. Moderate to potent inhibitory activity (24.7% inhibition at 100 μM in **18**, IC_{50} 0.066 μM in **13**) was recovered by some of the phenol ester containing derivatives, **11-18**. A urethane derivative, **19**, showed 29% inhibition at 100 μM. Compounds containing aliphatic ester of phenol showed more potent inhibitory activity than the aromatic esters of phenol. Among them, the most potent was the phenol pivalate **13**. The much more potent activity of **13** compared to **24** was estimated to depend partly upon the greater reactivity of its phenol ester moiety compared to the aliphatic *t*-butyl ester moiety of **24** (Tables 1 and 2).

As a result, phenyl pivalate moiety was found to be the most strongly recognized function among the synthe-





Scheme 3. (1) Preparation of **25a–c** and **26a–c**. Reagents: [a]RCOCl, Et_3N, CH_2Cl_2. (2) Preparation of **27–36**. Reagents: [a]$(CH_3)_3CCOCl$, Et_3N, CH_2Cl_2; [b]10% Pd–C, H_2, EtOAc; [c]30% H_2O_2

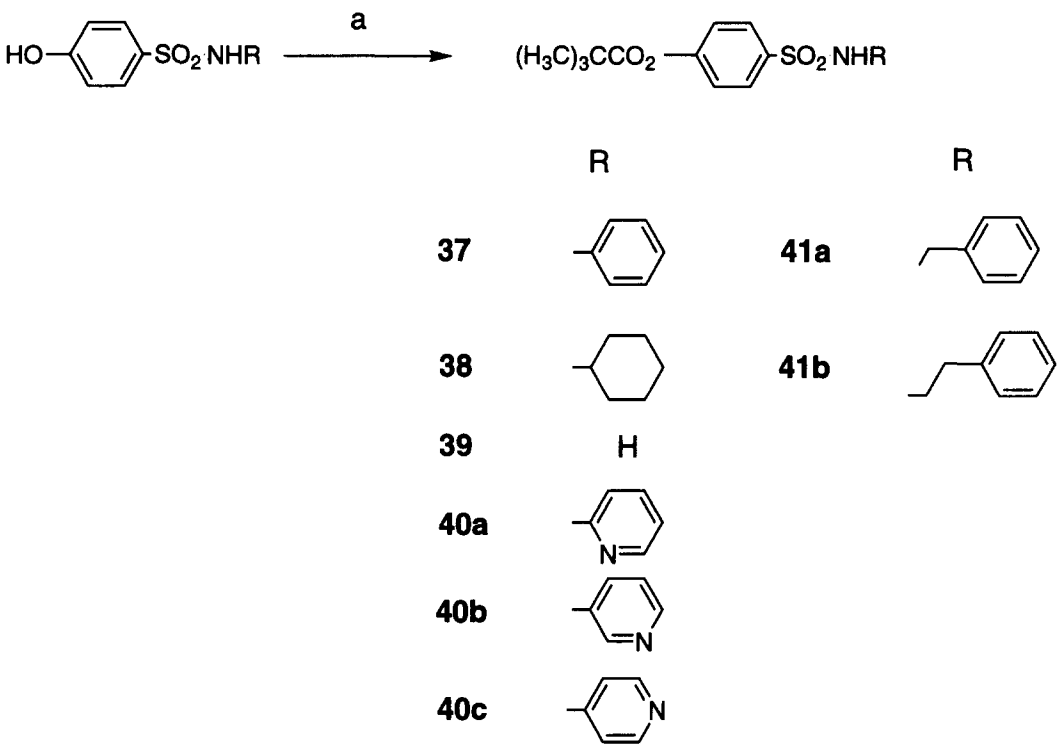
sized derivatives. Compounds **25c** and **26c** were designed to remove the presumed unnecessary methylene moiety of **13** and **12**, respectively.

Based on the results obtained in **25a–c** and **26a–c** (Table 2), *p*-substituted derivatives **25c** and **26c** were thought to show the most potent inhibitory activity among the series because of their less steric hindrance for recognition of the acyloxybenzene moiety. The reverse ester **24** (Table 1), which does not contain phenol ester moiety, was presumed to be inactive because of its intramolecular improper arrangement of the *t*-butyl group and the ester moiety, and/or its lower reactivity than that of **25c**. As such, a series of aliphatic esters of *p*-substituted phenol was discovered to be an attractive entity to the non-peptidic HNE elastase inhibitor.

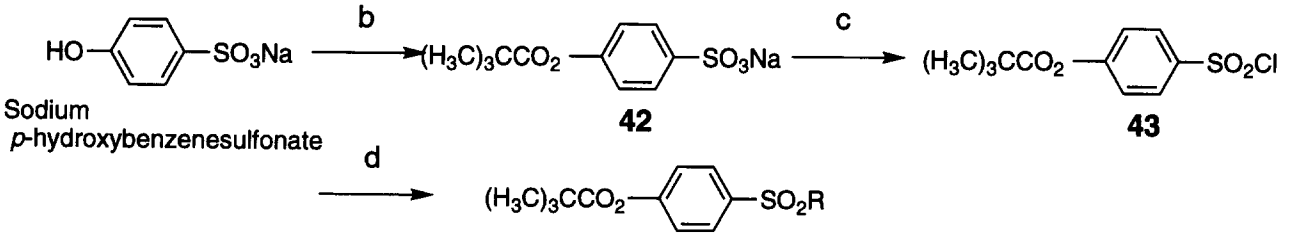
In an attempt to identify an optimized *p*-substituent, chemical modification was made as shown in Tables 2 and 3. Most of the *p*-substituents were tolerated for strong recognition by the enzyme, except for the cinnamoyl of **28**, the hydroxy function of **31** and the sulfonate of **42**. Compounds **27a–c**, **29**, **30**, **32–36**, **37–41** and **43–46** maintained their potent inhibitory activity. Among them, a series of sulfonamides (**37**, **38**, **43**, **44** and **46**) especially showed more potent activity than the other compounds described in Table 3. The sulfonanilide **37** was selected as the best compound for further modification, based on its potent inhibitory activity and the ease of its chemical modification of the aniline nucleus, although aliphatic sulfonamide **44** showed the equivalent activity.

In an attempt to identify an optimized aliphatic acyl moiety, chemical modification of acyl moiety was made maintaining sulfonanilide moiety in the *para*-position as shown in Table 4. An acyl moiety containing a branched chain at the adjacent carbon atom to the acyl carbonyl carbon was necessary to obtain potent inhibitory activity, as illustrated in **37**, **64**, **67** and **68**. The insertion of methylene moiety between the branched carbon and the carbonyl carbon remarkably decreased the inhibitory activity, as illustrated in **65** and **66**. The cyclization of the branched chains of the acyl moiety also decreased the inhibitory activity, as demonstrated in **61–63**. Remarkable bulkiness of an acyl moiety led to the complete loss of its activity up to 20 μM, as shown in **69**, even though it contained a branched carbon atom adjacent to carbonyl carbon. As such, 4-pivaloyloxyphenylsulfonanilide (**37**) was selected as a unique lead compound for further modification.

Regardless of its potent in vitro activity, the compound **37** was not effective on HNE-induced capillary permeability in guinea pig after its intravenous injection, (shown in Table 8). Metabolic instability in the plasma ($T_{1/2}=15$ min) was considered to be the most plausible reason for this systemic ineffectiveness. Three kinds of approaches (Chart 2) were attempted to identify an elastase inhibitor with enough metabolic stability to be active in an in vivo model. Approach (a) was an attempted block of metabolic hydrolysis by introducing a substituent X to the *ortho*-position of the pivaloyloxy group, as shown in general formula I (Chart 2). Approach (b) was also an attempted metabolic stabilization, by reducing affinity to metabolic enzymes

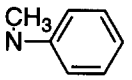


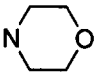
Scheme 4. (1) Preparation of **37–41**; (2) preparation of **42–57**; (3) preparation of **59**. Reagents: [a]$(CH)_3)_3CCOCl$, Et_3N, CH_2Cl_2; [b]$(CH_3)_3CCOCl$, 2 M NaOH, THF; [c]$SOCl_2$, DMF; [d]RNH_2 or R^1R^2NH, Et_3N, CH_2Cl_2; [e]SOCl2; [f]$H_2NCH_2CO_2Bzl$, Et_3N, CH_2Cl_2; [g]10% Pd–C, H_2, EtOAc.



R

44 $NHC(CH_3)_3$

45 

46 

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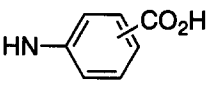
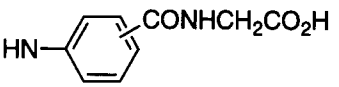


48a *ortho*

48b *meta*

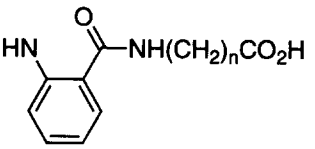
48c *para*



49a *ortho* (ONO-5046)

49b *meta*

49c *para*

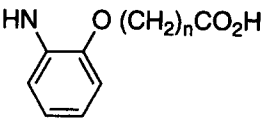


50a n = 2

50b n = 3

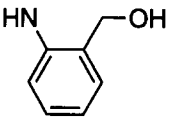
50c n = 4

R



51a n = 2

51b n = 3



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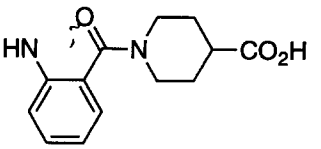

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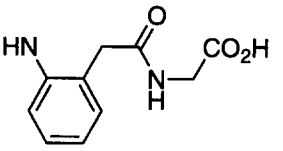

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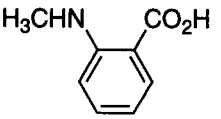

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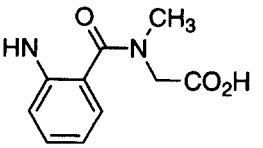

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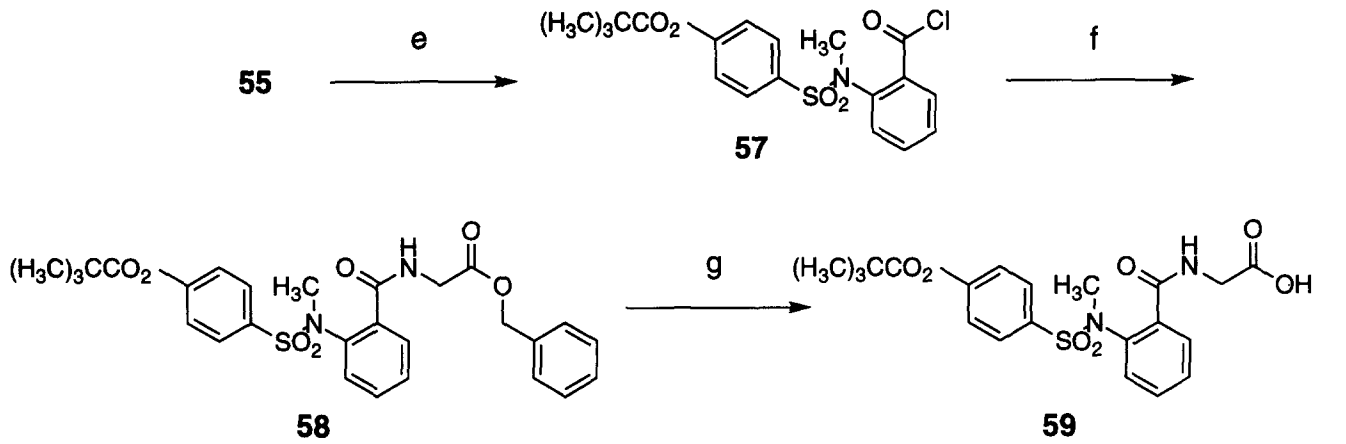
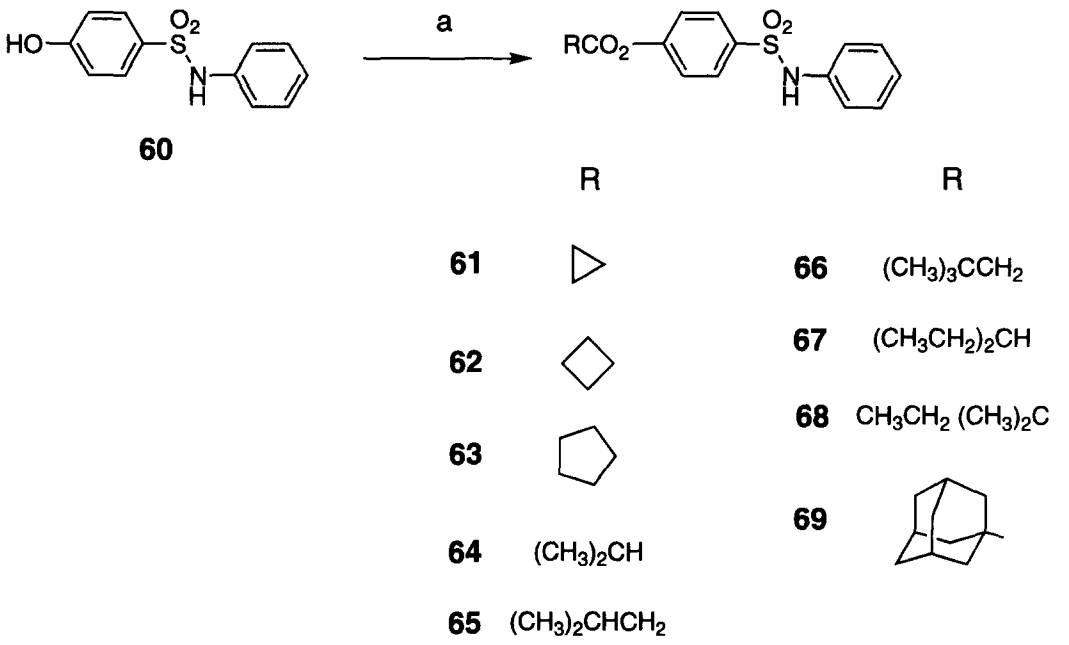
Scheme 4. *continued*



Scheme 4. *continued*

through the chemical modification of the aniline nucleus,[22] as shown in general formula II (Chart 2). The third approach (a+b), was a hybrid of the two approaches, as shown in general formula III (Chart 2). The biological results of approaches (a) and (a+b) are described in Table 5. Each of the modifications caused a significant reduction of in vitro activity and/or the half-life, as illustrated in **72–76**. It was an unexpected result that introduction of the *ortho*-isopropyl group afforded relatively less reduction in in vitro activity for its much greater bulkiness than that of the methyl group, without much effect on their half-lives, as illustrated in **72–74** and **75d**. The introduction of two methyl groups produced **76d**, with remarkable loss of the inhibitory activity. Approach (b) led to the discovery of the desired compounds, as demonstrated in Table 6. The substitution of the *N*-sulfonanilide nucleus with the carboxylic acid group prolonged the half-lives compared to the parent compound **37**, as shown in **48a–c**. In particular, *ortho*-substitution afforded **48a** with both the potent activity and the improved half-life in guinea-pig plasma. The amide formation of **48a–c** with glycine gave **49a–c** more improved half-lives (>60 min). Also in this series, the *ortho*-substituted anilide **49a** provided the most potent in vitro inhibitory activity. Other amides, **50a–c**, produced similar results both in their in vitro activity and their half-lives in the plasma. The replacement of the amide moiety of **50a** and **b** with an ether moiety produced **51a** and **b**, respectively, with a reduction of the half-lives in the plasma. Based on the results obtained in **52–54** (Table 6) and **56** (Table 7), *sec-o*-amide function was strongly suggested to be a requirement for the improved half-lives. The insertion of methylene moiety between the sulfonanilide nucleus and the *o*-amide group provided **54** with remarkable reduction of its half-life in the plasma. The replacement of the *o*-amide function with the hydroxymethyl

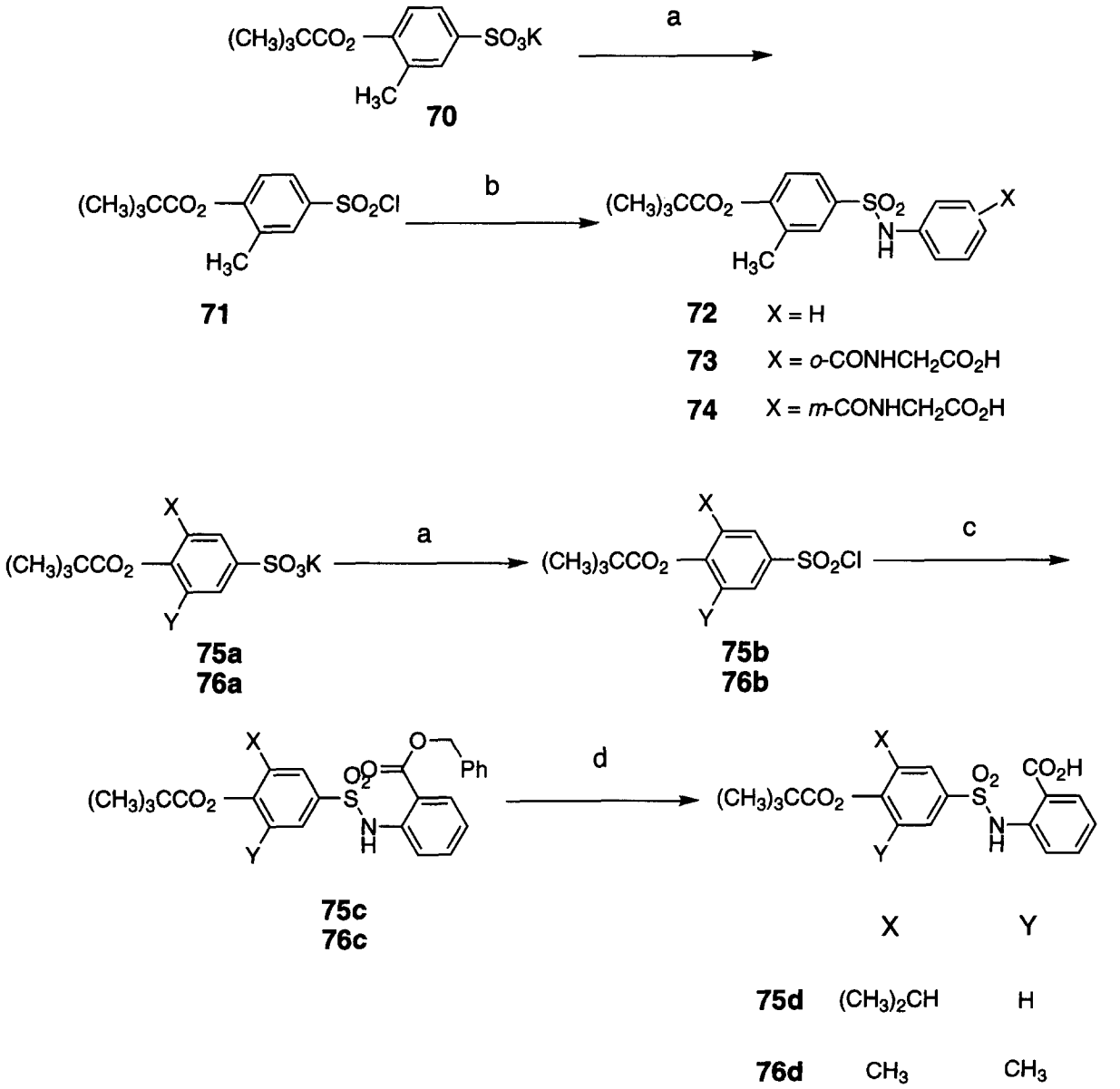


Scheme 5. Preparation of **60–68**. Reagents: [a]RCOCl, Et_3N, CH_2Cl_2.

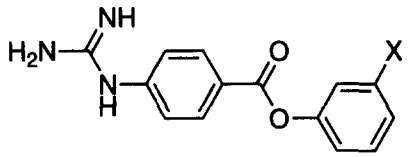
group produced **52**, also with remarkable reduction of its half-life. In any case, *ortho*-substitution of sulfonanilide provided the most potent in vitro inhibitory activity. In order to study the effect of *N*-methylation of the *ortho*-amide or sulfonanilide on both the in vitro inhibitory activity and the metabolic stability in the plasma, compounds **56** and **59** were prepared. Both of the compounds showed less potent activity and less metabolic stability compared to the parent compound **49a**, as shown in Table 7.

Some of the selected compounds, **37**, **48a**, **49a**, **50a** and **b**, were evaluated in an in vivo study using HNE-induced skin capillary permeability in guinea-pig as a newly developed animal model.[21] As shown in Table 8, the intravenous administration of **48a**, **49a**, **50a** and **b** significantly suppressed the increase of skin capillary permeability induced by HNE at a dose of 10 mg/kg,[21] whereas oral administration of these compounds did not show any significant effect up to 100 mg/kg, because of their presumed metabolic instability in the intestine. Only **49a** was weakly active after its oral administration at a higher dose (300 mg/kg).

In summary, we have found a non-peptidic lead **1** during the course of the screening of our compound library in an attempt to develop an HNE elastase inhibitor. Chemical modification of **1** was made to identify a systemically effective elastase inhibitor. The criterion for these pivaloyloxybenzene derivatives to be effective in an in vivo animal model was their adequate metabolic stability in the plasma. Among the synthesized derivatives, the compound **49a** (ONO-5046) was found to be active in the newly developed animal



Scheme 6. (1) Preparation of **72–74**; (2) preparation of **75** and **76**. Reagents: [a]$SOCl_2$, DMF; [b]RNH_2, Py; [c]benzyl *o*-aminobenzoate, Py; [d]H_2, Pd–C, MeOH, EtOAc.



1 X = Cl IC_{50} = 8.0 μM

2 X = H IC_{50} = 150 μM

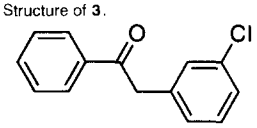
Chart 1. Trypsin inhibitors which showed inhibitory activity against human neutrophil elastase.

Table 1. In vitro activities of deoxybenzoin

Compound	% inhibition of HNE[a] at 100 μM	Compound	% inhibition of HNE[a] at 100 μM
3[d,24]	7.30	**13**	(0.066)[c]
4	37.5	**14**	(6.60)[c]
5	14.8	**15**	43.7
6	NS[b]	**16**	13.6
7	25.2	**17**	6.00
8	17.2	**18**	24.7
9	0.80	**19**	29.1
10	4.60	**20**[e,24]	19.4
11	30.0	**22**	0.00
12	(1.90)[c]	**24**	1.90

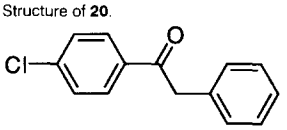
[a]Human neutrophil elastase.
[b]Not soluble.
[c]IC_{50} value against human neutrophil elastase.

d Structure of **3**.



3

e Structure of **20**.



20

Table 2. Effect of substitution on inhibitory activities

Compound	IC_{50} (μM)[a]	Compound	IC_{50} (μM)[a]
25a *ortho*	7.2	**28**	16.0
25b *meta*	0.96	**29**	0.28
25c *para*	0.060	**30**	0.50
26a *ortho*	(42%)[b]	**31**	21.0
26b *meta*	7.0	**32**	0.24
26c *para*	1.0	**33**	0.11
27a	0.47	**34**	0.070
27b	0.15	**35**	0.096
27c	0.43	**36**	0.47

[a]IC_{50} Value against human neutrophil elastase.
[b]% inhibition of HNE at 100 μM.

Table 3. In vitro activities of sulfonamide derivatives

Compound	IC_{50} (μM)[a]	Compound	IC_{50} (μM)[a]
37	0.034	**41a**	0.15
38	0.042	**41b**	0.072
39	0.77	**42**	11.0
40a	0.19	**43**	0.053
40b	0.17	**44**	0.047
40c	0.40	**45**	0.13
		46	0.050

[a]IC_{50} value against human neutrophil elastase.

model[21] after its intravenous administration. The biological profiles of ONO-5046 were also disclosed.[21] IC_{50} values of ONO-5046 on neutrophil elastase derived from human or other animal species were about 130 times higher than that of porcine pancreatic elastase.[21]

Thus, ONO-5046 was discovered to be a highly specific and intravenously effective neutrophil elastase inhibitor, and may be useful to study the roles of neutrophil elastase in vivo. As far as the mechanism-based safety problems are concerned, the exact mechanism of action of ONO-5046 must be clarified. At present, the sequential reversible reactions proposed in trypsin inhibitors[22,23] (acylation of the enzyme, longer duration of the acylated enzyme compared to the substrate-enzyme complex, and then regeneration of the enzyme by hydrolysis) are considered to be the most plausible mechanism of action also in this case, judging from its analogous structural features and the following biological data.

As shown in Figure 1, the IC_{50} value of ONO-5046 was potentiated after 5 min preincubation from 380 to 19 nM. As long as 60 min preincubation was carried out, inhibitory activity remained potent (IC_{50} = 22 nM). Our preliminary experiment using dialysis of the EI-complex restored enzyme activity after 24 h.

As an example, possible mechanisms of recognition of the above-described inhibitors ONO-5046 and **1** by each enzyme are diagrammed in Chart 3. ONO-5046 was estimated to possess hydrophobic interaction between its pivaloyl group and Val 216 of elastase. Dual activity of our lead compound **1** might be explained as illustrated in Chart 3. Compound **1** was thought to have hydrophobic interaction with elastase using its *meta*-substituted chloro group based on its

Table 4. In vitro activities of varieties of 4-acyloxybenzenesulfonanilides

Compound	IC_{50} (μM)[a]	Compound	IC_{50} (μM)[a]
37	0.034	**65**	0.93
61	2.2	**66**	86.0
62	3.3	**67**	0.27
63	0.7	**68**	0.15
64	0.15	**69**	>20.0

[a]IC_{50} value against human neutrophil elastase.

much more potent activity compared to **2**. Compound **1** was considered to show strong interaction with Asp 226 of trypsin using its guanidino group based on the reported examples of the analogous inhibitors.[23] The presumed reversible mechanism of action of ONO-5046 has been proposed as an explanation of the fewer safety problems compared to the reported irreversible elastase inhibitors.[19] A crystallographic analysis of the cocrystallized complex consisting of elastase and ONO-5046 is now under way in our laboratory.

Experimental

Chemistry: general

All ^{1}H NMR spectra were obtained using a JEOL FX-90Q or Varian VXR-200s or 500s spectrometer. Mass spectra were obtained on a JEOL JMS-DX-303HF spectrometer. IR spectra were measured on a Perkin Elmer FT-IR 1760X. Melting points were uncorrected. Column chromatography was carried out on silica gel (E. Merck; particle size 0.063–0.02 mm). Thin layer chromatography was performed on silica gel (Merck Art. No. 5715). All solvents were distilled before use.

Preparation of deoxybenzoin derivatives

Deoxybenzoin derivatives **3**, **4b**, **7b** and **9b** were prepared according to the reported method.[24]

3-Chlorodeoxybenzoin (3). R_f 0.66 (25% EtOAc: hexane); ^{1}H NMR ($CDCl_3$): δ 7.95 (2H, d, J=7.0 Hz), 7.60–7.40 (3H, m), 7.30–7.15 (3H, m), 7.10 (1H, m); IR (neat) 3050, 1670, 1590, 1560, 1470 cm^{-1}; MS *m/e* 230 (M^+).

4-Acetylamino-3′-chlorodeoxybenzoin (4b). R_f 0.12 (38% EtOAc:hexane); ^{1}H NMR ($CDCl_3$): δ 7.95 (2H, d, J=8.0 Hz), 7.69 (2H, d, J=8.0 Hz), 7.30–7.10 (3H, m), 4.25 (2H, s), 2.18 (3H, s); IR (KBr) 3300, 1670, 1590, 1520 cm^{-1}; MS *m/e* 287 (M^+), 162.

4-Acetylaminomethyl-3′-chlorodeoxybenzoin (7b). R_f 0.33 (60% EtOAc:hexane); ^{1}H NMR ($CDCl_3$): δ 7.95 (2H, d, J=8.0 Hz), 7.35 (2H, d, J=8.0 Hz), 7.30–7.10 (4H, m), 5.85 (1H, br s), 4.50 (2H, d, J=6.0 Hz), 4.25 (2H, s), 2.18 (3H, s); IR (KBr) 3300, 3100, 1680, 1640, 1600, 1550 cm^{-1}; MS *m/e* 301 (M^+), 176.

4-Methoxydeoxybenzoin (9b). R_f 0.23 (9% EtOAc: hexane); mp 66–68 °C; ^{1}H NMR ($CDCl_3$): δ 7.99 (2H, d, J=9.0 Hz), 7.30–7.20 (5H, m), 6.90 (2H, d, J=9.0

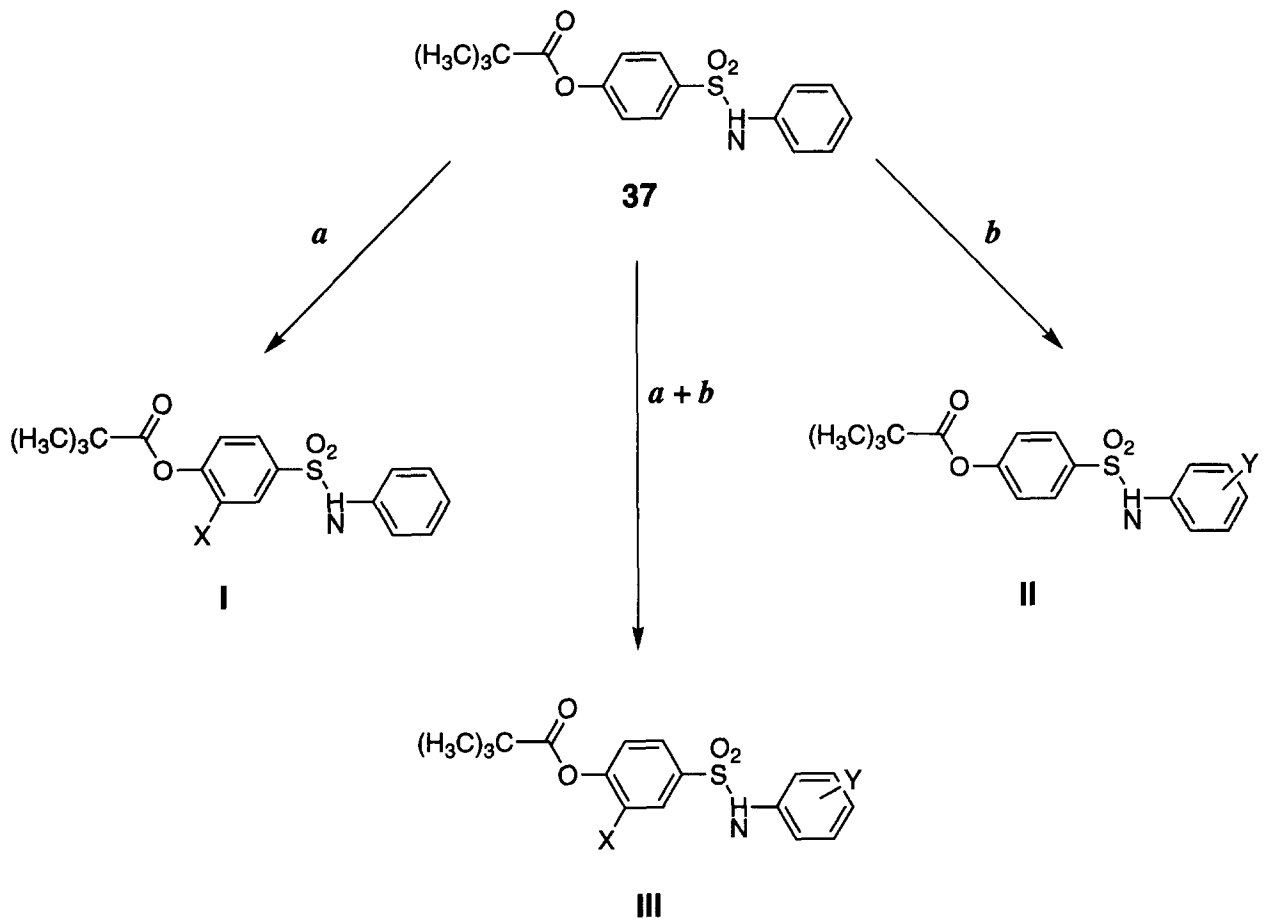


Chart 2. Drug design for metabolic stabilization. (a) Introduction of a substituent X to the *ortho*-positionof the pivaloyloxy group. (b) Introduction of a substituent Y which may reduce affinity to metabolic enzymes to the aniline nucleus.[23] (a+b) Hybridization of a and b.

Table 5. In vitro activities and metabolic stabilities of the pivaloyloxybenzene derivatives prepared by approach (a) (Chart 2)

Compound	IC_{50} (μM)[a]	Half-life in guinea-pig plasma[b] (min)
37	0.034	<15[c]
72	0.21	15–30[d]
73	0.33	>60
74	0.12	15–30[d]
75d	0.092	15–30[d]
76d	100	NT[e]

[a]IC_{50} value against human neutrophil elastase.
[b]See Experimental Section for conditions of HPLC analysis.
[c]The half-life of the compound was estimated to be less than 15 min, because less than 50% of the parent compound was detected by HPLC analysis after 15 min incubation in the plasma.
[d]The half-life of the compound was estimated to be between 15 and 30 min based on HPLC analysis.
[e]Not tested.

Table 6. In vitro activities and metabolic stabilities of the pivaloyloxybenzene derivatives prepared by approach (b) (Chart 2)

Compound	IC_{50} (μM)[a]	Half-life in guinea-pig plasma[b] (min)
37	0.034	<15[c]
48a	0.023	40
48b	0.19	40
48c	0.97	40
49a (ONO-5046)	0.044	>60[d]
49b	0.13	>60[d]
49c	0.74	>60[d]
50a	0.034	>60[d]
50b	0.031	>60[d]
50c	0.023	>60[d]
51a	0.04	<15[c]
51b	0.032	<15[c]
52	0.052	<15[c]
53	0.13	30–60
54	0.061	15–30

[a]IC_{50} value against human neutrophil elastase.
[b]See Experimental Section for conditions of HPLC analysis.
[c]Less than 50% of the parent compound was detected by HPLC analysis after 15 min incubation in the plasma.
[d]The half-life of the compound was estimated to be more than 60 min, because more than 50% of the parent compound was detected by HPLC analysis after 1 h incubation in the plasma.

Table 7. Effect of *N*-methylation on in vitro activities and metabolic stabilities

Compound	IC_{50} (μM)[a]	Half-life in guinea-pig plasma[b] (min)
49a	0.044	>60
56	0.16	40
59	0.12	<15

[a]IC_{50} value against human neutrophil elastase.
[b]See Experimental Section for conditions of HPLC analysis.

Hz), 4.23 (2H, s), 3.86 (3H, s); IR (KBr) 1680, 1600, 1580, 1260, 1180 cm^{-1}; MS *m/e* 226 (M^+), 135.

Preparation of 4-aminophenyl-3′-chlorodeoxybenzoin hydrochloride (5). A mixture of **4** (80 mg, 0.29 mmol) and conc HCl (2 mL) was heated at reflux. After 4 h, the reaction mixture was evapd and the residue washed with Et_2O to give **5** as a solid: R_f 0.40 (38% EtOAc:hexane); ^1H NMR ($CDCl_3$ + CD_3OD): δ 8.10 (2H, d, J=8.0 Hz), 7.45 (2H, d, J=8.0 Hz), 7.40–7.10 (4H, m), 4.30 (2H, s); IR (KBr) 2800, 2550, 1680, 1600, 1570 cm^{-1}; MS *m/e* 245 (M^+).

The following compound **8** was obtained from **7b** according to the procedure described for the preparation of **5** from **4b**.

4-Aminomethyl-3′-chlorodeoxybenzoin hydrochloride (8). R_f 0.15 (9% MeOH:$CHCl_3$); ^1H NMR (CD_3OD): δ 8.10 (2H, d, J=8.0 Hz), 7.58 (2H, d, J=8.0 Hz), 7.40–7.10 (4H, m), 4.36 (2H, s), 4.18 (2H, s); IR (KBr) 3200, 1670, 1590, 1590 cm^{-1}; MS *m/e* 259 (M^+).

Preparation of 4-trifluoroacetylamino-3′-chlorodeoxybenzoin (6). A soln of **8** (30 mg, 0.10 mmol), trifluoroacetic anhydride (0.30 mL) and pyridine (0.50 ml) was stirred at 25 °C. After 0.5 h, the reaction mixture was evapd and the residue washed with hexane to give **6** as a solid: R_f 0.62 (38% EtOAc:hexane); ^1H NMR ($CDCl_3$): δ 8.15 (2H, d, J=8.0 Hz), 7.70 (2H, d, J=8.0 Hz), 7.30–7.10 (4H, m), 4.25 (2H, s); IR (KBr) 3300, 1700, 1670, 1590, 1520 cm^{-1}; MS *m/e* 341 (Ma+), 216.

Preparation of 4-hydroxydeoxybenzoin (10). Compound **9b** was treated with pyridinium hydrogen chloride at 200 °C to give **10** as a solid: R_f 0.21 (25% EtOAc:hexane) ; ^1H NMR ($CDCl_3$): δ 7.95 (2H, d, J=8.0 Hz), 7.40–7.20 (5H, m), 6.85 (2H, d, J=8.0 Hz), 5.94 (1H, br s), 4.24 (2H, s); IR (KBr) 3350, 1660, 1590, 1560 cm^{-1}; MS *m/e* 212 (M^+).

Preparation of 4-acetoxydeoxybenzoin (11). Compound **10** was treated with acetic anhydride and pyridine to give **11** as a solid: R_f 0.42 (25% EtOAc:hexane); ^1H NMR ($CDCl_3$): δ 8.05 (2H, d, J=8.0 Hz), 7.18 (2H, d, J=8.0 Hz), 7.40–7.20 (5H, m), 4.26 (2H, s), 2.32 (3H, s); IR (KBr) 3025, 1750, 1680, 1590, 1500 cm^{-1}; MS *m/e* 254 (M^+).

General method A: preparation of benzyl *p*-cyclopentylcarbonyloxyphenyl ketone (12)

This procedure illustrates the general method for the preparation of **13**–**18**. To a stirred soln of **10** (212 mg, 1 mmol) and triethyamine (0.14 ml, 1 mmol) in CH_2Cl_2 (5 ml) was added cyclopentylcarbonyl chloride (114 mg, 1 mmol) at 0 °C, and the reaction mixture was stirred at 25 °C overnight. Cold water was added to the reaction mixture, and the mixture was extracted with EtOAc. The organic layer was washed with 1 M HCl, brine, dried over $MgSO_4$ and concd. The residue was recrystallized from hexane to give 150 mg (49%) of **12** as a white powder: R_f 0.77 (29% EtOAc:hexane); ^1H

Table 8. Inhibitory effect of the selected compounds on the increase of skin capillary permeability induced by hne in guinea-pigs

Compound	% inhibition			
	i.v. (mg/kg)		p.o. (mg/kg)	
	3	10	100	300
37	NS[a]	NS[a]	NS[a]	NS[a]
48a	NT[b]	41.0 ($p<0.01$)	NT[b]	NS[a]
49a (ONO-5046)	31.7 ($p<0.01$)	51.8 ($p<0.01$)	NS[a]	22.1 ($p<0.01$)
50a	NT[b]	38.0 ($p<0.01$)	NT[b]	NS[a]
50b	NT[b]	34.4 ($p<0.02$)	NT[b]	NS[a]

[a]Not significant.
[b]Not tested.

NMR ($CDCl_3$): δ 8.00 (2H, d, $J=7.9$ Hz), 7.20 (5H, m), 7.12 (2H, d, $J=7.9$ Hz), 4.25 (2H, s), 3.20–2.70 (1H, br), 2.15–1.55 (8H, br); MS *m/e* 308 (M^+).

Benzyl *p*-pivaloyloxyphenyl ketone (13). R_f 0.35 (9% EtOAc:hexane); ^1H NMR ($CDCl_3$): δ 8.00 (2H, d, $J=9.7$ Hz), 7.20 (5H, br), 7.10 (2H, d, $J=9.7$ Hz), 4.20 (2H, s), 1.35 (9H, s); MS *m/e* 296 (M^+).

Benzyl 3-methylbutanoyloxyphenyl ketone (14). R_f 0.77 (29% EtOAc:hexane); ^1H NMR ($CDCl_3$): δ 8.00 (2H, d, $J=9.0$ Hz), 7.20 (5H, br), 7.10 (2H, d, $J=9.0$ Hz), 4.20 (2H, s), 2.44 (2H, d, $J=6.8$ Hz), 2.25 (1H, m), 1.04 (6H, d, $J=7.2$ Hz); MS *m/e* 296 (M^+).

Benzyl *p*-hexanoyloxyphenyl ketone (15). R_f 0.35 (9% EtOAc:hexane); ^1H NMR ($CDCl_3$): δ 7.70 (H, d, $J=9.7$ Hz), 7.00–6.70 (5H, br), 3.90 (2H, s), 2.20 (2H, t, $J=7.2$ Hz), 1.60–0.80 (6H, br), 0.60 (3H, br); MS *m/e* 310 (M^+).

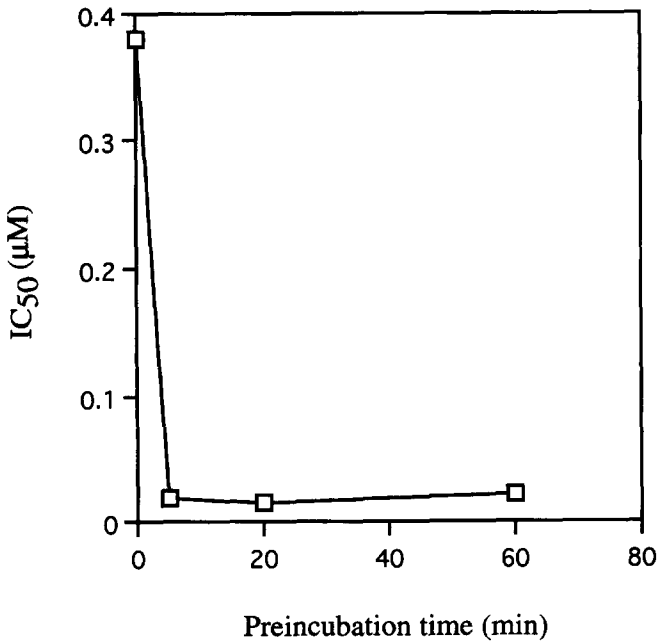


Figure 1. Preincubation experiment of ONO-5046 with elastase. Gradual increase of inhibitory activity (IC_{50} 380 nM after 0 min, 19 nM after 5 min, 15 nM after 20 in and 22 nM after 60 min) was observed after preincubation experiment.

Benzyl *p*-cyclohexylcarbonyloxyphenyl ketone (16). R_f 0.85 (29% EtOAc:hexane); ^1H NMR ($CDCl_3$): δ 8.00 (1H, d, $J=7.2$ Hz), 7.25 (5H, br), 7.10 (2H, d, $J=7.2$ Hz), 4.25 (2H, s), 2.70–2.25 (1H, br); MS *m/e* 322 (M^+).

Benzyl *p*-benzoyloxyphenyl ketone (17). R_f 0.74 (29% EtOAc:hexane); ^1H NMR ($CDCl_3$): δ 8.10 (2H, d, $J=7.2$ Hz), 8.05 (1H, d, $J=7.2$ Hz), 7.65–7.40 (3H, m), 7.29 (2H, d, $J=7.2$ Hz), 7.25 (5H, br), 4.25 (2H, s); MS *m/e* 316 (M^+).

Benzyl *p*-(2-thienylcarbonyl)oxyphenyl ketone (18). R_f 0.66 (29% EtOAc:hexane); ^1H NMR ($CDCl_3$): δ 8.05 (2H, d, $J=10.0$ Hz), 7.96 (1H, d, $J=7.2$ Hz), 7.30 (2H, d, $J=10.0$ Hz), 8.50–7.09 (8H, m), 4.30 (2H, s); MS *m/e* 322 (M^+).

Preparation of benzyl *p*-ethylcarbamoyloxyphenyl ketone (19). To a stirred mixture of **10** (212 mg, 1 mmol) and triethyamine (0.14 mL, 1 mmol) in CH_2Cl_2 (5 ml) was added ethyl isocyanate (71 mg, 1 mmol) at 0 °C. After stirring at 25 °C, the reaction mixture was quenched with cold water and extracted with EtOAc. The organic layer was washed with 1 M HCl and brine, dried over $MgSO_4$ and concd. The residue was recrystallized from hexane to give 260 mg (92%) of **19** as a pale yellow powder: R_f 0.33 (29% EtOAc:hexane); ^1H NMR ($CDCl_3$): δ 7.95 (2H, d, $J=9.0$ Hz), 7.15 (2H, d, $J=9.0$ Hz), 7.20 (5H, m), 4.20 (2H, s), 3.55–3.10 (2H, m), 1.2 (3H, t, $J=7.2$ Hz); MS *m/e* 283 (M^+).

Preparation of 4-guanidinodeoxybenzoin (22). To a stirred soln of 4-cyanoaniline (2.0 g, 17 mmol) in toluene (20 mL) was added 17 ml (17 mmol) of benzyl magnesium chloride (1.0 M in toluene) at 0 °C, and the mixture was heated at reflux overnight. After cooling to room temperature, 50 ml of 25% H_2SO_4 was added. The mixture was heated at reflux for 1 h, cooled to 0 °C and quenched by addition of 2 M NaOH. The mixture was extracted with Et_2O and the organic layer

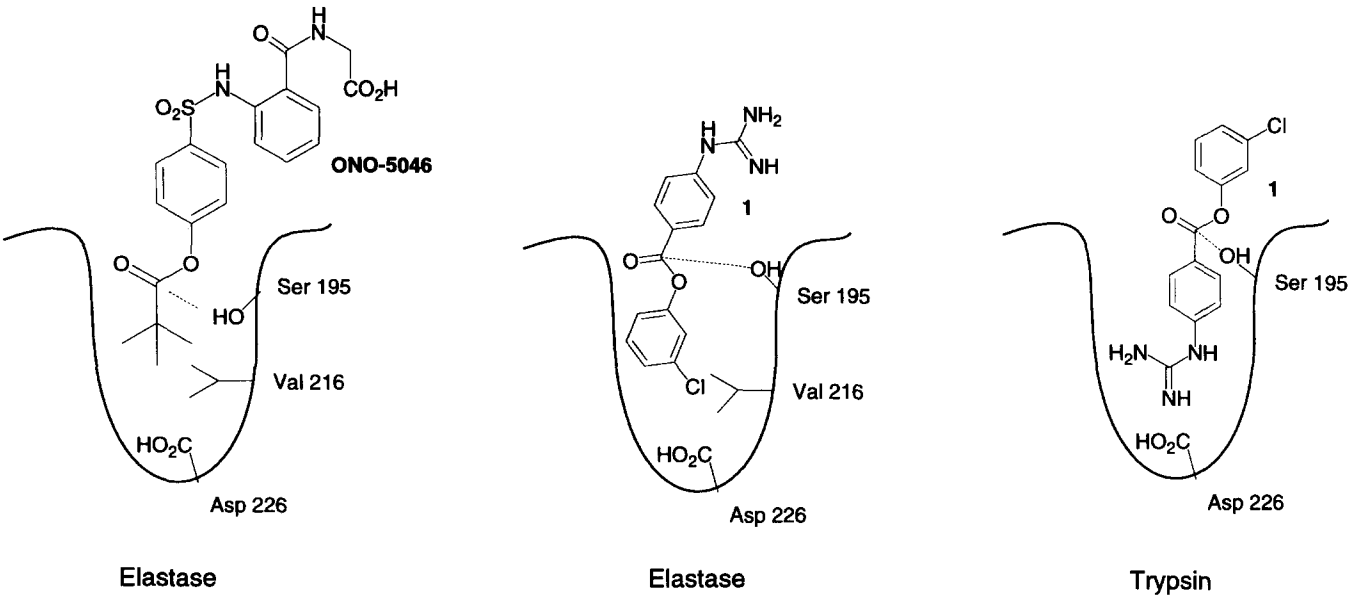


Chart 3. Possible mechanisms of recognition of the inhibitors by the enzymes.

washed with brine, dried over $MgSO_4$ and concd. Recrystallization from EtOAc:hexane gave 350 mg (9.7%) of **21** as a white powder: mp 104–105 °C; R_f 0.26 (CH_2Cl_2); ^1H NMR ($CDCl_3$): δ 7.90 (2H, d, $J=8.0$ Hz), 7.30 (5H, m), 6.65 (2H, d, $J=8.0$ Hz), 4.20 (2H, s); MS *m/e* 211 (M^+).

A mixture of 4-aminodeoxybenzoin (350 mg, 1.66 mmol), NH_2CN (700 mg, 16.6 mmol), water (1 mL), conc HCl (0.5 mL) and EtOH (10 mL) was heated at reflux for 8 h. After concn, the reaction mixture was quenched with aq $NaHCO_3$. The resulting precipitate was collected by filtration, and washed with water and acetone. To the stirred suspension of the product in EtOH (10 mL) was added 0.15 mL CH_3SO_3H, and the resulting precipitate collected by filtration to give 285 mg (49%) of **22** as a solid: mp 169–170 °C; R_f 0.73 (EtOAc:AcOH:H_2O, 3:1:1); ^1H NMR ($CDCl_3$): δ 8.8.11 (2H, d, $J=9.0$ Hz), 7.36 (2H, d, $J=9.0$ Hz), 7.30–7.10 (5H, m), 4.33 (2H, s), 2.70 (3H, s); IR (KBr) 3400, 3100, 1680, 1610, 1570, 1410, 1350, 1200, 1160, 1050 cm^{-1}; MS *m/e* 253 (M^+), 211, 162, 120.

Preparation of *t*-butyl *p*-benzoylbenzoate (24). To a stirred solution of *p*-benzoylbenzoic acid (1.0 g, 4.4 mmol), *t*-butyl alcohol (650 mg, 8.8 mmol) and *p*-dimethylaminopyridine (40 mg, 0.19 mmol) in DMF (20 ml) was added DCC (906 mg, 4.4 mmol) at 0 °C, and stirring was continued at 25 °C overnight. The resulting urea was removed by filtration and the filtrate was concd in vacuo. Purification by column chromatography on silica gel (2% EtOAc:CH_2Cl_2) gave 127 mg (10%) of **24** as a colorless oil: R_f 0.60 (2% EtOAc:CH_2Cl_2) ; ^1H NMR ($CDCl_3$): δ 8.09 (2H, d, $J=7.5$ Hz), 7.81 (2H, d, $J=7.5$ Hz), 7.90–7.50 (2H, m), 7.50–7.40 (3H, m), 1.62(9H, s); MS *m/e* 282 (M^+), 227, 209; IR (neat) 2980, 1715, 1660, 1600, 1440, 1370 cm^{-1}.

General procedure b: preparation of *o*-pivaloyloxybenzophenone (25a)

This procedure illustrates the general method for the preparation of **25b–c** and **26a–c**. To a stirred solution of *o*-hydroxybenzophenone (198 mg, 1 mmol) and triethylamine (0.14 mL, 1 mmol) in CH_2Cl_2 (2 ml) was added pivaloyl chloride (120 mg, 1 mmol) at 0 °C. After stirring at 25 °C for 1 h, the reaction mixture was quenched with cold water and extracted with EtOAc. The organic layer was washed with 1 M HCl and brine, dried over $MgSO_4$ and concd. Purification by column chromatography on silica gel (9% EtOAc:hexane) gave 250 mg (89%) of **25a** as a colorless oil: R_f 0.64 (29% EtOAc:hexane); ^1H NMR ($CDCl_3$): δ 7.80 (2H, dd, $J=7.2$, 1.8 Hz), 7.60–7.00 (7H, m), 1.00 (9H, s); MS *m/e* 282 (M^+).

***m*-Pivaloyloxybenzophenone (25b).** R_f 0.64 (29% EtOAc:hexane); ^1H NMR ($CDCl_3$): δ 7.75 (2H, dd, $J=7.2$ Hz, 1.8 Hz), 7.70–7.20 (7H, m), 1.30 (9H, s); MS *m/e* 282 (M^+).

***p*-Pivaloyloxybenzophenone (25c).** R_f 0.64 (29% EtOAc:hexane); ^1H NMR ($CDCl_3$): δ 7.80 (2H, d, $J=9.0$ Hz), 7.70–7.20 (5H, m), 7.15 (2H, d, $J=9.0$ Hz), 1.35 (9H, s); MS *m/e* 282 (M^+).

***o*-Cyclopentylcarbonyloxybenzophenone (26a).** R_f 0.46 (17% EtOAc:hexane); ^1H NMR ($CDCl_3$): δ 7.80 (2H, d, $J=7.0$ Hz), 7.60–7.20 (7H, m), 2.65 (1H, m), 1.80–1.40 (8H, m); IR (neat) 2960, 2872, 1756, 1668, 1605, 1581, 1481, 1451, 1362, 1317, 1293, 1271, 1202, 1124, 1074 cm^{-1}; MS *m/e* 294 (M^+).

***m*-Cyclopentylcarbonyloxybenzophenone (26b).** R_f 0.70 (29% EtOAc:hexane); ^1H NMR ($CDCl_3$): δ

7.80–7.20 (9H, m), 3.00 (1H, m), 2.20–1.50 (8H, br); MS *m/e* 294 (M^+).

***p*-Cyclopentylcarbonyloxybenzophenone (26c).** R_f 0.70 (29% EtOAc:hexane); ^1H NMR ($CDCl_3$): δ 7.80 (2H, d, $J=9.0$ Hz), 7.80–7.60 (2H, m), 7.60–7.40 (3H, m), 7.10 (2H, d, $J=9.0$ Hz), 3.00 (1H, q, $J=7.2$Hz), 2.20–1.50 (8H, br); MS *m/e* 294 (M^+).

General procedure c: preparation of *p*-pivaloyloxy-*N*-phenylbenzenesulfonamide (37)

This procedure illustrates the general method for the preparation of **38**, **39**, **40a–c** and **41a–b**. To a stirred solution of *p*-hydroxy-*N*-phenylbenzenesulfonamide (1.7 g, 7.0 mmol) which was obtained by demethylation of *p*-methoxy-*N*-phenylbenzenesulfonamide and Et_3N (0.97 ml, 7.0 mmol) in CH_2Cl_2 (10 ml) was added pivaloyl chloride (0.85 mL, 7.0 mmol) at 0 °C. After stirring at 25 °C overnight, the reaction mixture was concd in vacuo and extracted with EtOAc. The organic layer was washed with 1 M HCl, brine, dried over $MgSO_4$ and concd in vacuo. The resulting precipitate was collected by filtration and washed with hexane to give 2.16 g (93%) of **37** as a solid: mp 115–116 °C; R_f 0.30 (29% EtOAc:hexane); ^1H NMR ($CDCl_3$): δ 7.70 (2H, d, $J=9.0$ Hz), 7.30–6.90 (7H, m), 6.50 (1H, br s), 1.30 (9H, s); MS *m/e* 333 (M^+). Anal. calcd for $C_{17}H_{19}NO_4S$: C, 61.61; H, 5.18; N, 4.23. found: C, 61.45; H, 5.09; N, 4.11.

***N*-(4-Pivaloyloxybenzenesulfonyl)cyclohexylamine (38).** R_f 0.82 (29% EtOAc:hexane); ^1H NMR ($CDCl_3$): δ 7.88 (2H, d, $J=9.0$ Hz), 7.18 (2H, d, $J=9.0$ Hz), 4.35 (1H, m), 3.10 (1H, m), 2.00–1.00 (10H, m), 1.40 (9H, m); IR (KBr) 3280, 2940, 1750, 1590, 1480, 1440, 1320, 1210, 1160, 1100 cm^{-1}; MS *m/e* 339 (M^+).

4-Pivaloyloxybenzenesulfonamide (39). Mp 213–214 °C; R_f 0.72 (66% EtOAc:n-hexane); ^1H NMR ($CDCl_3$): δ 7.95 (2H, d, $J=9.0$ Hz), 7.20 (2H, d, $J=9.0$ Hz), 4.75 (2H, br s), 1.35 (9H, s); IR (KBr) 3400, 3280, 1720, 1580, 1480, 1350, 1200, 1160, 1120 cm^{-1}; MS *m/e* 257 (M^+). Anal. calcd for $C_{17}H_{20}N_2O_6S_2$: C, 49.50; H, 4.90; N, 6.79. found: C, 49.73; H, 4.88; N, 6.59.

2-(4-Pivaloyloxybenzenesulfonyl)aminopyridine (40a). R_f 0.64 (9% MeOH/$CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.05 (2H, d, $J=9.0$ Hz), 8.00 (1H, m), 7.80 (1H, m), 7.25 (2H, d, $J=9.0$ Hz), 7.30 (1H, m), 6.90 (1H, t, $J=6.5$ Hz), 1.40 (9H, s); IR (KBr) 3200–2300 (br), 1750, 1630, 1610, 1520, 1490, 1480, 1460, 1380, 1360 cm^{-1}; MS *m/e* 334 (M^+).

3-(4-Pivaloyloxybenzenesulfonyl)aminopyridine (40b). R_f 0.50 (9% MeOH/$CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.30–8.15 (2H, m), 7.80 (2H, d, $J=8.0$ Hz), 7.65 (1H, m), 7.29 (1H, m), 7.20 (2H, d, $J=8.0$ Hz), 1.35 (9H, s); IR (KBr) 3600–3200 (br), 1750, 1580, 1470, 1400, 1350, 1310, 1260, 1200 cm^{-1}; MS *m/e* 334 (M^+).

4-(4-Pivaloyloxybenzenesulfonyl)aminopyridine (40c). R_f 0.30 (9% MeOH/$CHCl_3$); ^1H NMR (CD_3OD + $CDCl_3$): δ 8.00 (4H, m), 7.20 (2H, d, $J=8.5$ Hz), 7.10 (2H, d, $J=7.0$ Hz), 1.35 (9H, s); IR (KBr) 3000 (br), 1750, 1630, 1590, 1490, 1350 cm^{-1}; MS *m/e* 334 (M^+), 251, 186, 157.

***p*-Pivaloyloxy-*N*-benzylbenzenesulfonamide (41a).** R_f 0.47 (29% EtOAc:hexane); ^1H NMR ($CDCl_3$): δ 7.90 (2H, d, $J=9.0$ Hz), 7.40–7.20 (7H, m), 4.70 (1H, br), 4.20 (2H, d, $J=5.4$ Hz), 1.35 (9H, s); MS *m/e* 347 (M^+).

***p*-Pivaloyloxy-*N*-phenethylbenzenesulfonamide (41b).** R_f 0.41 (29% EtOAc:hexane); ^1H NMR ($CDCl_3$): δ 7.85 (2H, d, $J=9.0$ Hz), 7.40–7.00 (7H, m), 4.35 (1H, br), 3.40–3.10 (2H, m), 2.80 (2H, t, $J=7.2$ Hz), 1.35 (9H, s); MS *m/e* 361 (M^+).

Preparation of *p*-pivaloyloxybenzenesulfonyl chloride (43). To a stirred soln of sodium *p*-hydroxybenzenesulfonate (360 g, 1.55 mol) and NaOH (65 g, 1.55 mol) in H_2O (720 mL) and THF (520 mL) was added pivaloyl chloride (173 mL) at 25 °C. After stirring for 1 h, the reaction mixture was cooled to 5 °C. The resulting solid was filtrated to give 350 g (80%) of sodium *p*-pivaloyloxybenzenesulfonate (**42**): R_f 0.63 ($CHCl_3$:MeOH:AcOH, 10:5:1); ^1H NMR (DMSO-d_6): δ 7.68–7.58 (2H, m), 7.08–6.95 (2H, m), 1.30 (9H, s); IR (KBr) 3490, 2975, 1751, 1594, 1495, 1282 cm^{-1}. Thionyl chloride (107 mL) was added dropwise to DMF (350 mL) at 0 °C. To the stirred DMF (350 mL) was added dropwise thionyl chloride (107 mL, 1.47 mol) at 0 °C. After stirring for 15 min, the resulting mixture was treated with **42** (350 g, 1.25 mol) and then stirred for 30 min at 25 °C. The reaction mixture was poured into cold water and the resulting solid was collected by filtration to give 298 g (86%) of *p*-pivaloyloxybenzenesulfonyl chloride (**43**): R_f 0.84 (29% EtOAc:hexane); ^1H NMR ($CDCl_3$): δ 8.12–8.02 (2H, m), 7.38–7.28 (2H, m), 1.38 (9H, s); IR (KBr) 2982, 1752, 1578, 1482, 1373, 1211 cm^{-1}; MS (EI, m/e) 276 (M^+).

General procedure d: preparation of *N*-(4-pivaloyloxybenzenesulfonyl)*t*-butylamine (44)

This procedure illustrates the general method for the preparation of **45-47**. To a stirred soln of *t*-butylamine (160 mg, 2.2 mmol) and Et_3N (0.5 mL, 3.6 mmol) in CH_2Cl_2 (6 ml) was added *p*-pivaloyloxybenzenesulfonyl chloride (**43**) (500 mg, 1.8 mmol), which was obtained by acylation of sodium *p*-hydroxybenzenesulfonate followed by treatment with thionyl chloride at 0 °C. After stirring at 25 °C overnight, the reaction mixture was concd in vacuo and extracted with EtOAc. The organic layer was washed with 1 M HCl, brine, dried over $MgSO_4$ and concd in vacuo. Recrystallizaion from EtOAc:hexane gave 111 mg (29%) of **44** as a white powder: R_f 0.65 (3% EtOAc:CH_2Cl_2); ^1H NMR ($CDCl_3$): δ 7.95 (2H, d, $J=9.0$ Hz), 7.20 (2H, d, $J=9.0$ Hz), 4.5 (1H, br s), 1.40 (9H, s), 1.25 (9H, s); IR (KBr)

3260, 2980, 1740, 1590, 1480, 1310, 1200 cm^{-1}; MS *m/e* 313 (M^+).

***N*-Methyl-*N*-(4-pivaloyloxybenzenesulfonyl)aniline (45).** R_f 0.61 (3% EtOAc:CH_2Cl_2); ^1H NMR ($CDCl_3$): δ 7.54 (2H, d, J=9.0 Hz), 7.14 (2H, d, J=9.0 Hz), 7.50–6.90 (5H, m), 3.18 (3H, s), 1.36 (9H, s); IR (KBr) 1750, 1590, 1490, 1340, 1210, 1140, 1120 cm^{-1}; MS *m/e* 347 (M^+), 283, 263.

***N*-(4-Pivaloyloxybenzenesulfonyl)morpholine (46).** R_f 0.19 (3% EtOAc:CH_2Cl_2); ^1H NMR ($CDCl_3$): δ 7.85 (2H, d, J=9.0 Hz), 7.30 (2H, d, J=9.0 Hz), 3.90–3.65 (4H, m), 3.20–2.90 (4H, m), 1.40 (9H, s); IR (KBr) 2970, 2860, 1759, 1590, 1480, 1340 cm^{-1}; MS *m/e* 327 (M^+).

***N*-(4-pivaloyloxybenzenesulfonyl)imidazole (47).** R_f 0.52 (3% EtOAc:CH_2Cl_2); ^1H NMR ($CDCl_3$): δ 7.96 (2H, d, J=8.5 Hz), 8.00 (1H, m), 7.28 (2H, d, J=8.5 Hz), 7.28 (1H, m), 7.09 (1H, m), 1.36 (9H, s); MS *m/e* 308 (M^+), 224, 157.

General Procedure E: Preparation of *o*-(*p*-pivaloyloxybenzenesulfonyl-amino)benzoic acid (48a)

This procedure illustrates the general method for the preparation of **48b–c**, **49a–c** and **50a–c**. To a stirred soln of benzyl *o*-aminobenzoate (454 mg, 2.0 mmol) in pyridine (5 mL) was added *p*-pivaloyloxybenzenesulfonyl chloride (552 mg, 2.0 mmol) at 0 °C. After stirring at 25 °C for 1 h, the reaction mixture was concd in vacuo and purified by column chromatography on silica gel (17% EtOAc:hexane), and recrystallization from MeOH gave 580 mg (62%) of benzyl *o*-(*p*-pivaloyloxybenzenesulfonylamino)benzoate as a solid. The mixture of benzyl *o*-(*p*-pivaloyloxybenzenesulfonylamino)benzoate (580 mg, 1.2 mmol) and 10% Pd–C (50 mg) was stirred vigorously under an atmosphere of hydrogen at 25 °C for 1 h. The catalyst was removed by filtration and the filtrate concd in vacuo to give 430 mg (92%) of **48a** as a solid: mp 184–185 °C; R_f 0.37 ($CHCl_3$:MeOH:AcOH = 100:5:1); ^1H NMR ($CDCl_3$): δ 8.20–7.00 (8H, m), 5.50 (1H, br), 1.35 (9H, s); MS *m/e* 377 (M^+). Anal. calcd for $C_{18}H_{19}NO_6S$: C, 57.28; H, 5.08; N, 3.71. found: C, 57.18; H, 4.97; N, 3.78.

***m*-(*p*-Pivaloyloxybenzenesulfonylamino)benzoic acid (48b).** Mp 229–230 °C; R_f 0.31 ($CHCl_3$:MeOH:AcOH, 100:5:1); ^1H NMR ($CDCl_3$): δ 7.85 (2H, d, J=9.0 Hz), 7.80–7.65 (2H, m), 7.50–7.30 (3H, m), 7.20–7.00 (2H, d, J=9.0 Hz); MS *m/e* 377 (M^+). Anal. calcd for $C_{18}H_{19}NO_6S$: C, 57.28; H, 5.08; N, 3.71. found: C, 57.07; H, 4.89; N, 3.70.

***p*-(*p*-Pivaloyloxybenzenesulfonylamino)benzoic acid (48c).** Mp 237–238 °C; R_f 0.56 (50% EtOAc:hexane); ^1H NMR ($CDCl_3$): δ 7.90 (2H, d, J=9.0 Hz), 7.80 (2H, d, J=9.0 Hz), 7.10 (4H, m), 1.35 (9H, s); IR (KBr) 3400, 3300, 2970, 2600, 1750, 1680, 1600, 1340, 1290, 1200, 1160 cm^{-1}; MS *m/e* 377 (M^+). Anal. calcd for $C_{18}H_{19}NO_6S$: C, 57.28; H, 5.08; N, 3.71. found: C, 57.12; H, 4.92; N, 3.65.

***N*-[*o*-(*p*-Pivaloyloxybenzene)sulfonylaminobenzoyl]glycine (49a).** Mp 218–220 °C; R_f 0.40 ($CHCl_3$:MeOH:AcOH, 30:3:1); ^1H NMR ($CDCl_3$): δ 7.80–7.72 (2H, m), 7.70–7.58 (2H, m), 7.45 (1H, m), 7.20–7.10 (3H, m), 3.99 (2H, s), 1.30 (9H, s); IR (KBr) 3432, 2978, 1749, 1722, 1647, 1592, 1523, 1493, 1454, 1408 cm^{-1}; MS *m/e* 434 (M^+), 350, 332, 313, 275. Anal. calcd for $C_{20}H_{22}N_2O_7S$: C, 55.28; H, 5.11; N, 6.45. found: C, 55.35; H, 5.34; N, 6.21.

***N*-[*m*-(*p*-Pivaloyloxybenzene)sulfonylaminobenzoyl]glycine (49b).** Mp 138–139 °C; R_f 0.33 (5% AcOH: EtOAc); ^1H NMR ($CDCl_3$): δ 7.80 (2H, d, J=8.0 Hz), 7.56–7.22 (4H, m), 7.12 (2H, d, J=8.0 Hz), 4.13 (2H, s), 1.32 (9H, s); IR (KBr) 3388, 3177, 2978, 1746, 1729, 1651, 1589, 1540, 1479, 1409, 1244, 1208, 1174, 1161, 1130 cm^{-1}; MS *m/e* 434 (M^+). Anal. calcd for $C_{20}H_{22}N_2O_7S$: C, 55.28; H, 5.11; N, 6.45. found: C, 55.47; H, 5.26; N, 6.35.

***N*-[*p*-(*p*-Pivaloyloxybenzene)sulfonylaminobenzoyl]glycine (49c).** Mp 121–122 °C; R_f 0.26 (5% AcOH: EtOAc); ^1H NMR ($CDCl_3$): δ 7.81 (2H, d, J=8.0 Hz), 7.62 (2H, d, J=8.0 Hz), 7.14 (4H, d, J=8.0 Hz), 4.12 (2H, s), 1.32 (9H, s); IR (KBr) 3411, 3177, 2977, 1732, 1646, 1610, 1546, 1505, 1402, 1346, 1309, 1232, 1207, 1162, 1117 cm^{-1}; MS *m/e* 434 (M^+). Anal. calcd for $C_{20}H_{22}N_2O_7S$: C, 55.28; H, 5.11; N, 6.45. found: C, 55.18; H, 5.03; N, 6.50.

***N*-[*o*-(*p*-Pivaloyloxybenzene)sulfonylaminobenzoyl]-β-alanine (50a).** Mp 134–135 °C R_f 0.46 ($CHCl_3$: MeOH:AcOH = 100:5:1); ^1H NMR ($CDCl_3$): δ 7.70 (2H, br), 7.48–7.20 (3H, m), 7.18–7.00 (3H, m), 6.60–6.50 (1H, m), 3.56 (2H, q, J=6.0 Hz), 2.65 (2H, t, J=6.0 Hz), 1.30 (9H, br s); IR (KBr) 3365, 3230, 1732, 1636, 1604, 1549, 1489, 1401 cm^{-1}; MS *m/e* 448 (M^+), 431, 364, 346, 318. Anal. calcd for $C_{21}H_{24}N_2O_7S$: C, 56.23; H, 5.40; N, 6.25. found: C, 56.11; H, 5.28; N, 6.12.

4-[2-(*p*-Pivaloyloxybenzene)sulfonylaminobenzoyl]aminobutanoic acid (50b). Mp 137–138 °C; R_f 0.36 ($CHCl_3$:MeOH:AcOH, 100:5:1); ^1H NMR ($CDCl_3$): δ 7.70 (2H, d, J=9.0 Hz), 7.70–7.50 (1H, br), 7.40–7.20 (2H, m), 7.10 (1H, d, J=7.2 Hz), 7.00 (2H, d, J=9.0 Hz), 3.30 (2H, t, J=6.8 Hz), 2.40 (2H, t, J=7.2 Hz), 1.90 (2H, q, J=7.2 Hz), 1.35 (9H, s); MS *m/e* 462 (M^+). Anal. calcd for $C_{22}H_{26}N_2O_7S$: C, 57.19; H, 5.68; N, 6.06. found: C, 56.82; H, 5.67; N, 5.83.

6-[*o*-(*p*-Pivaloyloxybenzene)sulfonylaminobenzoyl]aminohexanoic acid (50c). R_f 0.38 ($CHCl_3$:MeOH:AcOH, 100:5:1); ^1H NMR ($CDCl_3$ + CD_3OD): δ 7.70 (2H, d, J=9.0 Hz), 7.65 (1H, br), 7.40–7.20 (2H, m), 7.10 (1H, br), 7.00 (2H, d, J=9.0 Hz), 3.20 (2H, br), 2.30 (2H, br), 1.90–1.20 (6H, br), 1.35 (9H, s); MS *m/e* 490 (M^+).

Preparation of 3-[*o*-(*p*-pivaloyloxybenzene)sulfonylaminophenoxy]propanoic acid (51a). A mixture of *o*-nitrophenol (7.0 g, 50 mmol), β-propiolactone (3.6 g, 50 mmol) and NaOH (2.0 g, 50 mmol) was heated at reflux for 40 min. The reaction mixture was cooled in an ice-water bath and quenched by 1 M HCl. The reaction mixture was extracted with Et_2O, and the organic layer was washed with brine, dried over $MgSO_4$ and concd. The residue was washed with hexane–EtOAc and collected by filtration to give 3.9 g (37%) of 3-(*o*-nitrophenoxy)propionic acid as a solid: R_f 0.30 ($CHCl_3$:MeOH:AcOH = 100:5:1).

A mixture of 3-(*o*-nitrophenoxy)propionic acid (2.11 g, 10 mmol) and 10% Pd-C (200 mg) was stirred vigorously under an atmosphere of hydrogen at 25 °C for 3 h. The catalyst was removed by filtration, and the filtrate concd to give 3-(*o*-aminophenoxy)propionic acid as a crude oil: R_f 0.29 ($CHCl_3$:MeOH:AcOH, 100:5:1).

To a stirred solution of 3-(*o*-aminophenoxy)propionic acid in pyridine (20 ml) was added *p*-pivaloyloxyphenylsulfonyl chloride (2.76 g, 10 mmol) at 0 °C. After stirring at 25 °C for 1 h, the reaction mixture was poured into 1 M HCl and the mixture extracted with Et_2O. The organic layer was washed with brine, dried over $MgSO_4$ and concd. The residue was washed with EtOAc–hexane and collected by filtration. Recrystallization from EtOAc–hexane gave 2.19 g (52%) of **51a** as a pale yellow powder: mp 151–152 °C; R_f 0.36 ($CHCl_3$:MeOH:AcOH, 100:5:1); ^1H NMR ($CDCl_3$): δ 7.70 (2H, d, J = 9.0 Hz), 7.70 (1H, br), 7.20–7.00 (5H, m), 6.80 (1H, d, J = 9.0 Hz), 4.00 (2H, t, J = 5.5 Hz), 2.60 (2H, t, J = 5.5 Hz), 1.35 (9H, s); MS *m/e* 421 (M^+). Anal. calcd for $C_{20}H_{23}NO_7S$: C, 56.99; H, 5.51; N, 3.32. found: C, 57.12; H, 5.33; N, 3.15.

4-[*o*-(*p*-Pivaloyloxybenzene)sulfonylaminophenoxy]butanoic acid (51b). Mp 145–146 °C; R_f 0.48 ($CHCl_3$:MeOH:AcOH, 100:5:1); ^1H NMR ($CDCl_3$): δ 7.70 (2H, d, J = 9.0 Hz), 7.45 (1H, dd, J = 9.0, 1.8 Hz), 7.00 (2H, d, J = 9.0 Hz); MS *m/e* 435 (M^+). Anal. calcd for $C_{21}H_{25}NO_7S$: C, 57.91; H, 5.80; N, 3.22. found: C, 57.85; H, 5.76; N, 3.23.

2-Hydroxymethyl-*N*-(*p*-pivaloyloxybenzenesulfonyl)aniline (52). This compound was prepared by the same procedure described in the preparation of **44–47**: R_f 0.76 (66% EtOAc:hexane); ^1H NMR ($CDCl_3$): δ 7.90 (1H, br s), 7.75 (2H, d, J = 9.0 Hz), 7.60–7.00 (6H, m), 4.35 (2H, d, J = 5.0 Hz), 1.35 (9H, s); IR (KBr) 3470, 3050, 2950, 2850, 1750, 1590, 1470, 1320, 1210 cm^{-1}; MS *m/e* 363 (M^+), 345, 279, 261, 238, 196, 122.

***N*-[*o*-(*p*-Pivaloyloxybenzene)sulfonylaminobenzoyl]-4-piperidinecarboxylic acid (53).** R_f 0.50 ($CHCl_3$:MeOH:AcOH, 100:5:1); ^1H NMR ($CDCl_3$): δ 7.70 (2H, d, J = 9.0 Hz), 7.50 (1H, m), 7.30 (1H, m), 7.20–6.90 (4H, m), 3.80 (1H, br), 2.90–2.40 (4H, br), 2.00–1.40 (4H, br), 1.35 (9H, s); MS *m/e* 488 (M^+).

***N*-[*o*-(*p*-Pivaloyloxybenzene)sulfonylaminophenylacetyl]glycine (54).** *Rf* 0.37 (4% AcOH:EtOAc); ^1H NMR ($CDCl_3$): δ 7.77 (2H, d, J = 9.0 Hz), 7.35–7.10 (6H, m), 3.92 (2H, s), 3.40 (2H, s), 1.40 (9H, s); IR (KBr) 1750, 1610, 1530, 1480, 1400 cm^{-1}; MS *m/e* 448 (M^+), 430, 373, 289.

Preparation of *o*-[*N*-methyl-*N*-(*p*-pivaloyloxybenzene)sulfonylamino]benzoic acid (55). To a stirred soln of *N*-methylanthranilic acid (1.51 g, 10 mmol) in pyridine (25 mL) was added *p*-pivaloyloxyphenylsulfonyl chloride (2.76 g, 10 mmol). After stirring at 25 °C for 3 h, the reaction mixture was poured into cold water and the mixture was extracted with EtOAc. The organic layer was washed with 1 M HCl, brine, dried over $MgSO_4$ and concd. Purification by column chromatography on silica gel ($CHCl_3$:THF:AcOH, 100:5:1) gave 540 mg (14%) of **55** as a solid: R_f 0.24 ($CHCl_3$:THF:AcOH, 100:5:1); ^1H NMR ($CDCl_3$): δ 7.95 (1H, dd), 7.68 (2H, dd,), 7.55–7.38 (2H, m), 7.18 (2H, dd), 7.09 (1H, dd), 3.30 (3H, s); MS *m/e* 392 (M^+).

Preparation of *N*-methyl-*N*-[*o*-(*p*-pivaloyloxybenzene)sulfonylaminobenzoyl]glycine (56). To a stirred mixture of sarcosine (1.78 g, 20 mmol) and 2 M NaOH (10 mL) in THF (10 mL) was added *o*-nitrobenzoyl chloride (1.85 g, 10 mmol). After stirring at 25 °C for 1 h, the reaction mixture was poured into 1 M HCl and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$ and concd to give an oily product. A mixture of the oil, 10% Pd–C (100 mg) and 2 M HCl (5 mL) in MeOH (20 mL) was stirred vigorously under an atmosphere of hydrogen at 25 °C for 3 h. The catalyst was removed by filtration and the filtrate concd. The residue was washed with EtOAc-hexane and collected by filtration to give a solid. To a stirred suspension of the solid in pyridine (40 mL) was added *p*-pivaloyloxybenzenesulfonyl chloride (2.76 g, 4.4 mmol). After stirring at 25 °C overnight, the reaction mixture was concd and purification by column chromatography on silica gel ($CHCl_3$:THF:AcOH, 100:5:1) gave 450 mg (23%) of **56** as a solid: mp 95–96 °C; R_f 0.17 ($CHCl_3$:THF:AcOH, 100:5:1); ^1H NMR ($CDCl_3$ + CD_3OD + D_2O): δ 7.80 (2H, d, J = 9.0 Hz), 7.65 (1H, br), 7.10 (2H, d, J = 9.0 Hz), 7.40–7.00 (4H, m), 4.10 (2H, s), 2.70 (3H, s), 1.35 (9H, s); MS *m/e* 449 ($M^+ + 1$).

Preparation of *N*-[*o*-[*N*-methyl-*N*-(*p*-pivaloyloxybenzene)sulfonylamino] benzoyl]glycine (59). A mixture of **55** (540 mg, 1.38 mmol) and thionyl chloride (2 ml) was heated at reflux for 1 h. Thionyl chloride was removed azeotropically with benzene. A solution of the obtained residue in CH_2Cl_2 (3 mL) was added to a stirred mixture of glycine benzyl ester *p*-toluenesulfonate (559 mg, 1.66 mmol) and triethylamine (0.636 mL, 4.56 mmol) in CH_2Cl_2 (5 ml) at 0 °C. After stirring at 25 °C for 1 h, the reaction mixture was poured into 1 M HCl and extracted with CH_2Cl_2. The organic layer was washed with aq $NaHCO_3$, brine, dried over $MgSO_4$ and concd to afford the crude benzyl ester of **58** (676 mg):

R_f 0.80 (9% MeOH:$CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.84–7.64 (4H, m), 7.44–7.19 (9H, m), 6.58 (1H, m), 5.24 (2H, s), 4.30 (2H, br), 3.22 (3H, br s), 1.38 (9H, s); IR (KBr) 2976, 1752, 1667, 1593, 1526, 1482, 1355 cm^{-1}; MS *m/e* 539 (M^+).

A mixture of the crude benzyl ester of **58** (676 mg) and 10% Pd–C (70 mg) in EtOAc (8 ml) was stirred vigorously under an atmosphere of hydrogen at 25 °C for 1 h. The catalyst was removed by filtration, and the filtrate concd to afford 500 mg (81% from **55**) of **59** as a solid: R_f 0.46 ($CHCl_3$:MeOH:AcOH, 30:3:1); ^1H NMR ($CDCl_3$): δ 7.84–7.68 (4H, m), 7.44–7.18 (4H, m), 6.62 (1H, br), 4.24 (2H, d), 3.22 (3H, s), 1.38 (9H, br s); IR (KBr) 3391, 2977, 1756, 1662, 1597, 1535, 1482, 1405 cm^{-1}; MS *m/e* 448 (M^+). Anal. calcd for $C_{21}H_{24}N_2O_7S$: C, 56.23; H, 5.40; N, 6.25. found: C, 55.98; H, 5.53; N, 6.00.

***p*-Pivaloyloxyphenylbenzene (27a)**. R_f 0.54 (9% EtOAc:hexane); ^1H NMR ($CDCl_3$): δ 7.70–7.00 (9H, m), 1.30 (9H, s); MS *m/e* 254 (M^+).

***p*-Pivaloyloxybenzylbenzene (27b)**. R_f 0.62 (20% EtOAc:hexane); ^1H NMR ($CDCl_3$): δ 7.35–7.10 (7H, m), 6.95 (2H, m), 3.95 (2H, br s), 1.35 (9H, s); MS *m/e* 268 (M^+), 184.

***p*-Pivaloyloxy-1,2-diphenylethane (27c)**. R_f 0.66 (9% EtOAc:hexane); ^1H NMR ($CDCl_3$): δ 7.50–6.80 (9H, m), 2.85 (4H, s), 1.35 (9H, s); MS *m/e* 282 (M^+).

***p*-Pivaloyloxystilbene (28)**. R_f 0.58 (9% EtOAc: hexane); ^1H NMR ($CDCl_3$): δ 7.60–7.15 (7H, m), 7.10–6.90 (4H, m), 1.38 (9H, s); MS *m/e* 280 (M^+).

***p*-Pivaloyloxyphenyl phenyl ether (29)**. R_f 0.59 (20% EtOAc:hexane); ^1H NMR ($CDCl_3$): δ 7.40–6.95 (9H, m), 1.35 (9H, s); MS *m/e* 270 (M^+), 187.

***p*-Pivaloyloxyphenyl benzyl ether (30)**. R_f 0.50 (9% EtOAc:hexane); ^1H NMR ($CDCl_3$): δ 7.40 (5H, br s), 7.00 (4H, s), 5.10 (2H, s), 1.35 (9H, s); MS *m/e* 284 (M^+).

***p*-Pivaloyloxyphenol (31)**. R_f 0.46 (29% EtOAc: hexane); ^1H NMR ($CDCl_3$): δ 6.90 (2H, d, J=9.0 Hz), 6.80 (2H, d, J=9.0 Hz), 5.00 (1H, s), 1.35 (9H, s); MS *m/e* 194 (M^+).

4-Hydroxy-4′-pivaloyloxydiphenyl sulfide (32). R_f 0.62 (9% MeOH:$CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.50–6.70 (8H, m), 5.15 (1H, s), 1.30 (9H, s); MS *m/e* 302 (M^+).

4-Hydroxy-4′-pivaloyloxydiphenyl sulfone (34). R_f 0.55 (9% MeOH/$CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.95 (2H, d, J=8.1 Hz), 7.85 (2H, d, J=8.1 Hz), 7.25 (2H, d, J=8.1 Hz), 6.90 (2H, d, J=8.1 Hz), 5.60 (1H, s), 1.35 (9H, s); MS *m/e* 334 (M^+).

4-Pivaloyloxy-1-(2-thienylcarbonyl)benzene (35). R_f 0.62 (29% EtOAc:hexane); ^1H NMR ($CDCl_3$): δ 7.90 (2H, d, J=7.9 Hz), 7.80–7.60 (2H, m), 7.20 (2H, d, J=7.9 Hz), 7.20 (1H, m), 1.35 (9H, s); MS *m/e* 298 (M^+).

4-Methyl-*N*-(*p*-pivaloyloxybenzoyl)aniline (36). R_f 0.46 (3% EtOAc:CH_2Cl_2); ^1H NMR ($CDCl_3$): δ 7.90 (2H, d, J=9.0 Hz), 7.55 (2H, d, J=9.0 Hz), 7.20 (4H, br d, J=9.0 Hz), 2.40 (3H, s), 1.40 (9H, s); IR (KBr) 3300, 2960, 1740, 1640, 1600, 1500 cm^{-1}; MS *m/e* 311 (M^+).

Preparation of 4-hydroxy-4′-pivaloyloxydiphenyl sulfoxide (33). A mixture of 4-hydroxy-4′-pivaloyloxydiphenyl sulfide (1.2 g, 4.0 mmol) and 35% H_2O_2 in AcOH (7 mL) was stirred at 25 °C for 1 h and concd in vacuo. Purification by column chromatography on silica gel (5% MeOH:$CHCl_3$) gave **33** (180 mg, 14%) as a white amorphous product: R_f 0.27 (5% MeOH/$CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.65 (2H, d, J=9.0 Hz), 7.45 (2H, d, J= 9.0 Hz), 7.25 (2H, d, J=9.0 Hz), 6.85 (2H, d, J= 9.0 Hz), 1.35 (9H, s); MS *m/e* 318 (M^+).

General procedure F: preparation of *p*-Cyclopropylcarbonyl-*N*-phenylbenzenesulfonamide (61)

This procedure is the general method for the preparation of **62–69**. To a stirred mixture of *p*-hydroxy-*N*-phenylbenzenesulfonamide (249 mg, 1 mmol) and triethyamine (0.14 mL, 1 mmol) in CH_2Cl_2 (5 ml) was added cyclopropanoyl chloride (0.90 mL, 1 mmol) at 0 °C, and the mixture was stirred at 25 °C overnight. After quenching with cold water, the reaction mixture was extracted with EtOAc. The organic layer was washed with 1 M HCl, brine, dried over $MgSO_4$ and concd in vacuo. Purification by column chromatography on silica gel (2% EtOAc:CH_2Cl_2) afforded 170 mg (54%) of **61** as a white solid: mp 91–92 °C; R_f 0.50 (3% EtOAc/ CH_2Cl_2); ^1H NMR ($CDCl_3$): δ 7.80 (2H, d, J=9.0 Hz), 7.50–6.90 (7H, m), 1.85 (1H, m), 1.30–0.90 (4H, m); MS *m/e* 317 (M^+). Anal. calcd for $C_{16}H_{15}NO_4S$: C, 61.24; H, 5.76; N, 4.20. found: C, 59.87; H, 5.48; N, 4.03.

***p*-Cyclobutylcarbonyloxy-*N*-phenylbenzenesulfonamide (62)**. R_f 0.50 (3% EtOAc/ CH_2Cl_2); ^1H NMR ($CDCl_3$): δ 7.80 (2H, d, J=9.0 Hz), 7.50–6.90 (7H, m), 3.40 (1H, m), 2.60–1.70 (6H, m); MS *m/e* 331 (M^+). Anal. calcd for $C_{17}H_{17}NO_4S$: C, 62.58; H, 5.56; N, 4.06. found: C, 62.39; H, 5.38; N, 3.98.

***p*-Cyclopentylcarbonyloxy-*N*-phenylbenzenesulfonamide (63)**. Mp 69–70 °C; *Rf* 0.44 (29% EtOAc:hexane); ^1H NMR ($CDCl_3$): δ 7.70 (2H, d, J=7.9 Hz), 7.10 (2H, d, J=7.9 Hz), 7.40–6.80 (5H, m), 6.60 (1H, br s), 3.00 (1H, m), 2.20–1.50 (8H, br); MS *m/e* 345 (M^+). Anal. calcd for $C_{18}H_{19}NO_4S$: C, 60.17; H, 5.38; N, 4.39. found: C, 60.00; H, 5.12; N, 4.25.

***p*-Isobutyryloxy-*N*-phenylbenzenesulfonamide (64)**. Mp 75–76 °C; R_f 0.30 (29% EtOAc:hexane); ^1H NMR ($CDCl_3$): δ 7.70 (2H, d, J=9.0 Hz), 7.40–6.90 (7H, m), 6.45 (1H, br), 2.80 (1H, m), 1.30 (6H, d, J=7.2 Hz); MS *m/e* 319 (M^+). Anal. calcd for $C_{16}H_{17}NO_4S$: C,

61.24; H, 5.76; N, 4.20. found: C, 61.49; H, 5.45; N, 4.18.

p-Isovaleryloxy-N-phenylbenzenesulfonamide (65). R_f 0.52 (29% EtOAc:hexane); ^{1}H NMR ($CDCl_3$): δ 7.70 (2H, d, J=9.0 Hz), 7.30–6.80 (7H, m), 6.50 (1H, br s), 2.40 (2H, d, J=5.4 Hz), 2.15 (1H, m), 1.00 (6H, d, J=7.2 Hz); MS *m/e* 333 (M^+). Anal. calcd for $C_{17}H_{19}NO_4S$: C, 60.55; H, 4.77; N, 4.41. found: C, 60.89; H, 4.55; N, 4.30.

p-(3,3-Dimethylbutanoyloxy)-N-phenylbenzenesulfonamide (66). Mp 97–98 °C, R_f 0.46 (29% EtOAc:hexane); ^{1}H NMR ($CDCl_3$): δ 7.70 (2H, d, J=9.0 Hz), 7.40–6.90 (7H, m), 2.40 (2H, s), 1.10 (9H, s); MS *m/e* 347 (M^+). Anal. calcd for $C_{18}H_{21}NO_4S$: C, 62.22; H, 6.10; N, 4.03. found: C, 62.32; H, 6.20; N, 3.76.

p-(2-Ethylbutanoyloxy)-N-phenylbenzenesulfonamide (67). Mp 91 °C; R_f 0.52 (29% EtOAc:hexane); ^{1}H NMR ($CDCl_3$): δ 7.70 (2H, d, J=9.0 Hz), 7.40–6.80 (7H, m), 6.50 (1H, br s), 2.45 (1H, m), 1.90–1.50 (4H, m), 1.10–0.90 (6H, t, J=7.2 Hz); MS *m/e* 347 (M^+). Anal. calcd for $C_{18}H_{21}NO_4S$: C, 62.22; H, 6.10; N, 4.03. found: C, 62.10; H, 5.91; N, 3.92.

p-(2,2-Dimethylbutanoyloxy)-N-phenylbenzenesulfonamide (68). Mp 81–82 °C; R_f 0.47 (29% EtOAc:hexane); ^{1}H NMR ($CDCl_3$): δ 7.75 (2H, d, J=9.0 Hz), 7.40–6.80 (7H, m), 6.50 (1H, br s), 1.90–1.50 (2H, m), 1.30 (6H, s), 0.90 (3H, t, J=7.2 Hz); MS *m/e* 347 (M^+). Anal. calcd for $C_{18}H_{21}NO_4S$: C, 62.22; H, 6.10; N, 4.03. found: C, 62.53; H, 5.98; N, 3.94.

p-(1-Adamantylcarbonyloxy)-N-phenylbenzenesulfonamide (69). Mp 180–181 °C; R_f 0.46 (29% EtOAc:hexane); ^{1}H NMR ($CDCl_3$): δ 7.70 (2H, d, J=9.0 Hz), 7.40–6.90 (5H, m), 6.40 (1H, br s), 2.00 (9H, br s), 1,70 (6H, br s); MS *m/e* 411 (M^+). Anal. calcd for $C_{23}H_{25}NO_4S \cdot 1/2H_2O$: C, 65.68; H, 6.24; N, 3.33. found: C, 66.04; H, 5.90; N, 3.32.

General procedure G: preparation of 3-methyl-4-pivaloyloxy-N-phenylbenzenesulfonamide (72)

This procedure illustrates the general method for preparation of **73** and **74**. A mixture of *o*-cresol and 93% H_2SO_4 was stirred at 100 °C for 1.5 h. After quenching with 35% KOH, the reaction mixture was concd in vacuo. The obtained solid was collected by filtration and washed with MeOH to afford 1.2 g (27%) of potassium 4-hydroxy-3-methylbenzenesulfonate as a solid. To a stirred mixture of potassium 4-hydroxy-3-methylbenzenesulfonate (1.2 g, 5.3 mmol) in 2 M NaOH (2.6 mL, 5.2 mmol) and THF (1.7 mL) was added pivaloyl chloride (720 mg, 6 mmol) at 25 °C, and the reaction mixture was stirred at 25 °C for 20 min. After evapn, the resulting solid was collected by filtration and washed with cold water to afford 350 mg (22%) of potassium 3-methyl-4-pivaloyloxybenzenesulfonate as a solid. To a stirred soln of potassium 3-methyl-4-pivaloyloxybenzenesulfonate (350 mg, 1.2 mmol) in DMF (4 mL) was added thionyl chloride (0.26 mL, 3.6 mmol) at 0 °C. After stirring at 25 °C for 30 min, the reaction mixture was poured into cold water and extracted with hexane:EtOAc (1:1). The organic layer was washed with brine, dried over $MgSO_4$ and concd to give 270 mg (78%) of 3-methyl-4-pivaloyloxybenzenesulfonyl chloride (**71**). To a stirred soln of aniline (46 mg, 0.50 mmol) in pyridine (1 mL) was added 3-methyl-4-pivaloyloxybenzenesulfonyl chloride (116 mg, 0.40 mmol) at 0 °C. After stirring at 25 °C for 1 h, the reaction mixture was quenched with 1 M HCl and extracted with hexane:EtOAc (1:1). The organic layer was washed with brine, dried over $MgSO_4$ and concd in vacuo. The resulting precipitate was collected by filtration and washed with MeOH to give 108 mg (78%) of **72** as a pale red solid: *Rf* 0.44 (29% EtOAc:hexane); ^{1}H NMR ($CDCl_3$): δ 7.70–7.50 (2H, m), 7.40–7.00 (6H, m), 6.45 (1H, br s), 2.20 (3H, s), 1.35 (9H, s); MS *m/e* 347 (M^+).

N-[o-(3-Methyl-4-pivaloyloxybenzene)sulfonylaminobenzoyl]glycine (73). R_f 0.41 ($CHCl_3$:MeOH:AcOH, 30:3:1); ^{1}H NMR ($CDCl_3$) δ 7.70 (1H, d, J=6.5 Hz), 7.60–7.40 (3H, m), 7.30–7.10 (2H, m), 6.95 (1H, d, J=7.2 Hz), 6.50 (1H, m), 4.00 (2H, m), 2.10 (3H, s), 1.35 (9H, s); IR (KBr), 2970, 1740, 1630, 1600, 1520, 1480, 1390, 1330, 1260, 1230, 1150, 1090 cm^{-1}; MS *m/e* 448 (M^+), 364, 3436, 289, 194.

N-[m-(3-Methyl-4-pivaloyloxybenzene)sulfonylaminobenzoyl]glycine (74). R_f 0.26 ($CHCl_3$:MeOH:AcOH, 30:3:1); ^{1}H NMR ($CDCl_3$ + CD_3OD): δ 7.60–6.90 (7H, m), 4.10 (2H, s), 2.15 (3H, s), 1.35 (9H, s); MS *m/e* 448 (M^+).

Preparation of N-(3-isopropyl-4-pivaloyloxybenzene) sulfonylanthranilic acid (75d). A mixture of *o*-isopropylphenol (2.72 g, 20 mmol) and 93% H_2SO_4 (2.11 ml, 20 mmol) was heated for 1 h. To the stirred reaction mixture was added a soln of KOH (1.12 g, 20 mmol) in H_2O (2 mL) at ambient temperature. The reaction mixture was concd in vacuo and the residue washed with MeOH to afford 3.92 g (77%) of potassium 4-hydroxy-3-isopropylbenzensulfonate (**75a**) as a solid. To a mixture of **75a** (3.92 g, 15 mmol) and 2 M NaOH (7.5 mL, 15 mmol) in THF (3 ml) was added pivaloyl chloride (1.8 g, 15 mmol) at 0 °C, and the mixture was stirred at 25 °C for 20 min. After concentrating in vacuo, the obtained residue was washed with MeOH by filtration. The filtrate obtained by removal of insoluble substance was evapd. To the residue were added DMF (30 mL) and thionyl chloride (3.25 mL, 45 mmol) at 0 °C. The mixture was stirred at 25 °C for 1 h and then poured into cold water. The reaction mixture was extracted with ether:hexane (2:1), and the organic layer was washed with brine, dried over $MgSO_4$ and concd in vacuo to give the crude product of 3- isopropyl 4-pivaloyloxy benzenesulfonyl chloride (**75b**) (4.77 g). To a stirred soln of benzyl *o*-aminobenzoate (227 mg, 1 mmol) in pyridine (3 ml) was added crude **75b** (318 mg, ca 1 mmol) at 0 °C, and the mixture was stirred at

25 °C for 1 h. The reaction mixture was concd in vacuo and purified by column chromatography on silica gel (9% EtOAc:hexane) to afford 260 mg (51%) of benzyl *N*-(3-isopropyl-4-pivaloyloxy-benzene)sulfonylanthranilate (**75c**) as a solid: R_f 0.23 (9% EtOAc:hexane). A mixture of **75c** (260 mg) and 10% Pd–C (20 mg) in 4 mL of 50% MeOH:EtOAc was stirred vigorously under an atmosphere of hydrogen at 25 °C for 20 min. After removal of the catalyst by filtration, the filtrate was concd in vacuo to give 190 mg (100%) of **75d** as a solid: R_f 0.32 ($CHCl_3$:MeOH:AcOH, 100:5:1); ^{1}H NMR ($CDCl_3$): δ 8.00 (1H, d, J=9.0 Hz), 7.90–7.50 (4H, m), 7.25 (1H, d, J=9.0 Hz), 7.10 (1H, d, J=9.0 Hz), 3.00 (1H, br), 1.35 (9H, s), 1.15 (6H, d, J = 7.2 Hz); MS *m/e* 419 (M^+).

Preparation of *N*-(3,5-dimethyl-4-pivaloyloxybenzenesulfonyl)anthranilic acid (76d). To a stirred mixture of benzyl *o*-aminobenzoate (372 mg, 1.6 mmol) and pyridine (0.5 mL, 6.2 mmol) in CH_2Cl_2 (5 mL) was added 500 mg (1.6 mmol) of 3,5-dimethyl-4-pivaloyloxybenzenesulfonyl chloride (**76b**) at 0 °C. After stirring at 25 °C overnight, the reaction mixture was concd in vacuo and extracted with EtOAc. The organic layer was washed with 1 M HCl, brine, dried over $MgSO_4$ and concd in vacuo. The resulting precipitate was collected by filtration and washed with Et_2O to give 600 mg (80%) of benzyl *N*-(3,5-dimethyl-4-pivaloyloxybenzenesulfonyl)anthranilate (**76c**) as a solid. A mixture of **76c** (600 mg, 1.2 mmol) and 10% Pd–C (300 mg) in 60 ml of AcOH:EtOAc:THF (2:3:1) was stirred vigorously under an atmosphere of hydrogen at 25 °C for 3 h. The reaction mixture was filtrated, and the filtrate was concd in vacuo to give 340 mg (69%) of **76d** as a solid: R_f 0.28 (EtOAc:hexane:AcOH, 20:40:1); ^{1}H NMR ($CDCl_3$): δ 7.95 (1H, br d, J = 8.0 Hz), 7.70–7.30 (4H, m), 7.10 (1H, br t, J=8.0 Hz), 2.10 (6H, s), 1.40 (9H, s); IR (KBr) 3000 (br), 1750, 1660, 1580, 1480, 1420, 1340, 1250, 1150, 1100 cm^{-1}; MS *m/e* 405 (M^+).

Determination of enzyme inhibitory activity

The enzyme activity was determined by a synthetic chromogenic substrate, suc-Ala-Pro-Ala-*p*NA. The substrate (0.1 mM) was incubated with the enzyme (1×10^{-3} U) and the inhibitor in Tris–HCl buffer (0.1 M, pH 8.0) containing 0.2 M sodium chloride in a final volume of 1.0 ml. After incubation at 37 °C for 30 min, the absorbance at 405 nm was spectrophotometrically measured and percent inhibition of the compound was calculated by the following equation.

$$\text{Inhibition } (\%) = \{1 - \Delta OD(\text{test-blank})/\Delta OD(\text{control-blank})\} \times 100.$$

The enzyme inhibitory activity was expressed as a concentration required for 50% inhibition.

Preincubation experiment of ONO-5046 with elastase

A mixture of ONO-5046 in DMSO (0.01 mL) and 0.8 unit/mL human polymorphonuclear elastase (0.05 ml) was incubated in 0.2 mM HEPES buffer (pH 8.0, 0.5 mL), 2.5 M NaCl (0.2 mL) 1% polyethyleneglycol 6000 (0.1 mL) and distilled water (0.13 mL) at 37 °C for an indicated period (0, 5, 20, 60 min). A soln of 5 mM MeO-Suc-Ala-Ala-Pro-Val-pNA in DMSO (0.01 ml) was added to the above mixture and incubated at 37 °C for 5 min. The reaction was terminated by 50% acetic acid (0.1 mL) and the released *p*-nitroanilide (pNA) was measured spectrophotometrically at 405 nm. Percent inhibition of ONO-5046 was calculated by the following equation.

$$\text{Inhibition } (\%) = \{1 - \Delta OD(\text{test-blank})/\Delta OD(\text{control-blank})\} \times 100$$

The enzyme inhibitory activity was expressed as a concentration required for 50% inhibition.

Evaluation of metabolic stability[22] in guinea pig plasma

Compounds were dissolved in acetonitrile to a final concentration of 6 mg/mL. To the plasma (0.5 mL) was added the soln of the compound (5 μL) and the mixture was incubated at 37 °C (n=3). Aliquots of 0.1 mL each were taken at regular intervals and quenched by the addition of 3.5 mL of acetonitrile containing n-propyl *p*-hydroxybenzoate as an internal standard (IS). After centrifugation at 3000 rpm for 10 min, the supernatant was concd in vacuo. The residue was dissolved in 0.1 ml of the mobile phase, and 10–20 μl of this soln was loaded onto the HPLC column. HPLC was carried out using a Uvidec 100 III (JASCO) apparatus equipped with an ERC-ODS-1161 column at 240 nm. The mobile phase was 0.02 M KH_2PO_4:acetonitrile (3:2), and the flow rate was 1.0 mL/min.

Evaluation of in vivo activity in guinea pig. Capillary permeability

Male Hartley strain guinea pigs (n=3) weighing 250–350 g were injected with human neutrophil elastase (HNE; 20×10^{-3} U in 100 μl PBS/site) in the clipped-back skin. Compounds were administered intravenously 30 s before HNE injection. Immediately after HNE injection, 1.7 mL/kg of Evans Blue solution (2% w/v) was injected intravenously. Thirty minutes after the Evans blue injection, the guinea pigs were killed by bleeding via the carotic aorta, and the blue spots in the skin were cut off and solubilized by 1 mL of 1 M KOH at 37 °C overnight. The absorbance of acetone:0.6 N phosphate soln (13:5 v/v)-extracted dye was measured with a spectrophotometer at 620 nm (the index for permeability).

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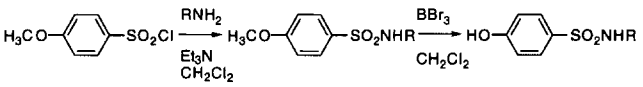

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