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PAPER

Anthranilic acid-based inhibitors of phosphodiesterase: Design, synthesis, and bioactive evaluation†

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Our previous studies identified two 2-benzoylaminobenzoate derivatives **1**, which potently inhibited superoxide ($O_2^{\bullet-}$) generation induced by formyl-L-methionyl-L-leucyl-L-phenylalanine (FMLP) in human neutrophils. In an attempt to improve their activities, a series of anthranilic acid derivatives were synthesized and their anti-inflammatory effects and underlying mechanisms were investigated in human neutrophils. Of these, compounds **17**, **18**, **46**, **49**, and **50** showed the most potent inhibitory effect on FMLP-induced release of $O_2^{\bullet-}$ in human neutrophils with IC_{50} values of 0.20, 0.16, 0.15, 0.06, and 0.29 μM, respectively. SAR analysis showed that the activities of most compounds were dependent on the ester chain length in the A ring. Conversely, a change in the linker between the A and B ring from amide to sulfonamide or *N*-methyl amide, as well as exchanges in the benzene rings (A or B rings) by isosteric replacements were unfavorable. Further studies indicated that inhibition of $O_2^{\bullet-}$ production in human neutrophils by these anthranilic acids was associated with an elevation in cellular cAMP levels through the selective inhibition of phosphodiesterase 4. Compound **49** could be approved as a lead for the development of new drugs in the treatment of neutrophilic inflammatory diseases.

1. Introduction

Inflammatory lung diseases, including asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis, and lung injury are caused by activated neutrophils recruiting and releasing chemoattractants into the airway.[1,2] Although neutrophils play a critical role in the defense of the human body against infections, abnormal activation of neutrophils releases high concentrations of reactive oxygen species (ROS) and elastase, and mediates immunological insults and oxidative damage.[2] The formation of superoxide anion ($O_2^{\bullet-}$), a precursor of other ROS, by NADPH oxidase in human neutrophils is directly or indirectly linked to damage or destruction of surrounding tissues.[3,4] Several experimental studies showed that oxidant stress enhances many pathological processes in various inflammatory diseases and leads to harmful tissue damage.[5] Therefore, it is crucial to prevent neutrophil oxidative burst in inflammatory tissues and organs. To our knowledge, the cyclic nucleotide cAMP is an important second messenger in preventing activation of neutrophils.[6,7] Phosphodiesterases (PDEs) are important in regulating intracellular concentrations of cAMP by catalyzing its hydrolysis and inactivating cAMP. Thus, PDE inhibitors are currently being investigated as anti-inflammatory drugs because of their suppressive effects on neutrophil function.[6,8–10] PDEs have been classified into 11 families (PDE1–PDE11) based on protein sequence, structure, substrate specificity, enzymatic properties, and tissue distribution.[11] Among these, PDE3 and PDE4 were found in neutrophils and were observed to regulate intracellular cAMP levels and modulate the activation of neutrophils.[12,13] However, on the basis of our unpublished data, only PDE4 inhibitors exhibited an inhibitory effect on $O_2^{\bullet-}$ generation in neutrophils. Therefore, we hypothesize that evaluating the inhibitory effect on $O_2^{\bullet-}$ generation in neutrophils is a new way of investigating specific PDE4 inhibitors, and may be the target for a new generation of agents in the treatment of neutrophilic inflammatory disease.

Anthranilic acid derivatives have been recognized to have diverse biological activities,[14–31] in particular, they are enzymatic inhibitors of VEGF receptor kinase,[14] matrix metalloproteinase (MMP),[15] acyl carrier protein synthase (AcpS),[16] methionine aminopeptidase-2,[17,18] phospholipase A_2,[19] human hydroxysteroid dehydrogenase AKR1C1,[20] plasminogen activator inhibitor-1,[21] and cholecystokinin (CCK).[22–24] In addition, they also exhibit anti-cancer, anti-hepatitis C virus (anti-HCV), anti-Alzheimer, anti-inflammatory, anti-platelet, and anti-bacterial activities.[25–35] Of these, the *N*-phenylanthranilic acids, *e.g.* mefenamic acid analogs, have been used as anti-inflammatory agents in therapy.[29–31] Our previous studies identified two 2-benzoylaminobenzoate

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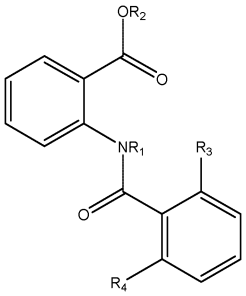
† Electronic supplementary information (ESI) available: HPLC analysis of the target compounds. See DOI: 10.1039/c1ob05714f

‡ These authors contributed equally to this work.

Table 1 Inhibitory effects of 2-benzoylaminobenzoic esters and *N*-methyl 2-benzoylaminobenzoic esters on superoxide anion generation and elastase release by human neutrophils in response to fMLP/CB



	R_1	R_2	R_3/R_4	Anti-inflammation (μM)[a]	
				$O^{2\bullet-}$ generation	NE release
1[b]	H	CH_3	F/H	0.64 ± 0.01	> 20
Sivelestat[b]				> 20	0.050 ± 0.0002
8	H	CH_2CH_3	Cl/H	0.76 ± 0.26	> 20
9	H	CH_2CH_3	Br/H	6.92 ± 2.54	> 20
10	H	$CH_2CH_2CH_3$	F/H	> 20	> 20
11	H	$CH_2CH_2CH_3$	Cl/H	> 20	> 20
12	H	$CH_2CH_2CH_3$	Br/H	> 20	> 20
13	H	$CH_2CH_2CH_2CH_3$	F/H	> 20	> 20
14	H	$CH_2CH_2CH_2CH_3$	Cl/H	> 20	> 20
15	H	$CH_2CH_2CH_2CH_3$	Br/H	> 20	> 20
16	H	CH_3	F/F	0.62 ± 0.07	> 20
17	H	CH_2CH_3	F/F	0.20 ± 0.03	> 20
18	H	$CH_2CH_2CH_3$	F/F	0.16 ± 0.03	> 20
19	CH_3	CH_3	F/F	> 20	> 20
20	CH_3	CH_2CH_3	F/F	> 20	> 20
21	CH_3	$CH_2CH_2CH_3$	F/F	> 20	> 20

[a] Concentration necessary for 50% inhibition (IC_{50}). [b] Compound **1** and sivelestat were used as positive controls.

derivatives, **1**, which potently inhibited $O_2^{\bullet-}$ generation induced by formyl-L-methionyl-L-leucyl-L-phenylalanine (FMLP) in human neutrophils.[32,36] Furthermore, derivative **1** was able to elevate cAMP levels by inhibiting phosphodiesterase, and attenuate hemorrhagic shock-induced lung injury in rats.[36] Accordingly, the leading agent of phosphodiesterase inhibitors, **1**, was modified in an attempt to improve anti-inflammatory activity by increasing the ester chain length, changing the amide to sulfonamide or *N*-methyl amide, as well as extending the space between the two aromatic rings. Furthermore, replacing the aromatic rings with isosteres is also discussed. All synthetic compounds (Tables 1–4) were evaluated for their *in vitro* inhibitory effects on $O_2^{\bullet-}$ generation induced by FMLP in human neutrophils. Herein, we report on the synthesis of anthranilic acid derivatives, as well as their pharmacological data and structure–activity relationships (SARs).

2. Chemistry

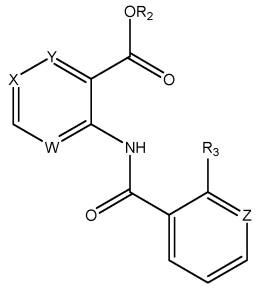
Initially, corresponding anthranilic and *N*-methyl anthranilic esters were synthesized by refluxing anthranilic or *N*-methyl anthranilic acids, respectively, in alcohols/c-H_2SO_4 solutions to yield intermediates, which were subsequently reacted with corresponding substituted benzoyl chlorides to obtain 2-benzoylaminobenzoic esters (**8–18**) and *N*-methyl 2-benzoylaminobenzoic esters (**19–21**) (Table 1 and Scheme 1). To study the isosteres of the aromatic rings, 2-aminopyridine-3-carboxylic acid, 4-aminonicotinic acid, and 3-amino-2-pyrazine carboxylate were esterified and then coupled with the corresponding substituted benzoyl chlorides to afford **22–27** (Table 2 and Scheme 1). Furthermore, the anthranilic esters were transformed to 2-phenylsulfonamidobenzoic esters (**29–37**, Table 3) by reacting with the corresponding benzenesulfonyl chloride (Scheme 2). 2-Phenylacetamidobenzoic esters (**40–51**, Table 4) were prepared by a coupling reaction with 2-benzoylaminobenzoic esters and the corresponding phenylacetic acids in HBTU/DIEA/DCM solution (Scheme 3). In addition, conversion of 2-benzoylaminobenzoic esters to 3-phenylpropanamidobenzoic esters (**52–54**, Table 4, Scheme 3) and 2-cinnamamidobenzoic esters were described using the above protocol (**56** and **58**, Scheme 4). Accordingly, all synthetics (Tables 1–4) were fully characterized using spectroscopic data. The spectroscopic data of the new compounds (**10–15**, **18–26**, **34–37**, **42–48**, **49–54**, and **58**) are shown in the experimental section.

3. Results and discussion

Neutrophil function assay

All synthetics (**8–27**, **29–37**, **40–54**, **56**, and **58**) and positive controls (**1** and sivelestat) were subjected to $O_2^{\bullet-}$ generation and neutrophil elastase release with FMLP as the inducer in neutrophils. Of these, five compounds **17**, **18**, **46**, **49**, and **50** exhibited the most potent and selective inhibitory effects on

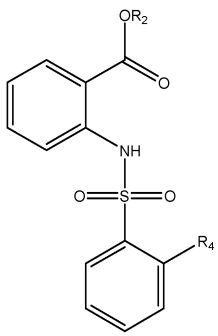
Table 2 Inhibitory effects of the isosteres of 2-benzoylaminobenzoic esters on superoxide anion generation and elastase release by human neutrophils in response to fMLP/CB



	R_2	R_3	W/X/Y/Z	Anti-inflammation (μM)[a]	
				$O^{2\bullet-}$ generation	NE release
1[b]	CH_3	F	CH/CH/CH/CH	0.64 ± 0.01	>20
Sivelestat[b]				>20	0.050 ± 0.0002
8	CH_2CH_3	Cl	CH/CH/CH/CH	0.76 ± 0.26	>20
9	CH_2CH_3	Br	CH/CH/CH/CH	6.92 ± 2.54	>20
22	CH_2CH_3	F	CH/N/CH/CH	1.00 ± 0.20	>20
23	CH_2CH_3	F	N/CH/CH/CH	0.45 ± 0.17	>20
24	CH_2CH_3	Cl	N/CH/CH/CH	12.20 ± 6.97	>20
25	CH_2CH_3	Br	N/CH/CH/CH	11.32 ± 5.92	>20
26	CH_3	F	N/CH/N/CH	>20	>20
27	CH_2CH_3	Cl	CH/CH/CH/N	10.92 ± 2.86	>20

[a] Concentration necessary for 50% inhibition (IC_{50}). [b] Compound **1** and sivelestat were used as positive controls.

Table 3 Inhibitory effects of the isosteres of 2-phenylsulfonamidobenzoic esters on superoxide anion generation and elastase release by human neutrophils in response to fMLP/CB

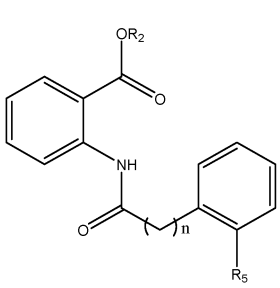


29-37

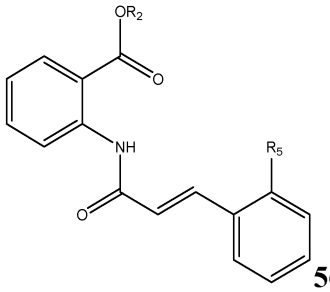
	R_2	R_4	Anti-inflammation (μM)[a]	
			$O^{2\bullet-}$ generation	NE release
1[b]	CH_3	F	0.64 ± 0.01	> 20
Sivelestat[b]			> 20	0.050 ± 0.0002
29	CH_3	F	> 20	> 20
30	CH_3	Cl	> 20	> 20
31	CH_3	Br	> 20	> 20
32	CH_2CH_3	F	6.10 ± 2.14	> 20
33	CH_2CH_3	Cl	2.42 ± 0.15	> 20
34	CH_2CH_3	Br	2.43 ± 0.16	> 20
35	$CH_2CH_2CH_3$	F	2.02 ± 0.56	> 20
36	$CH_2CH_2CH_3$	Cl	1.05 ± 0.40	11.39 ± 2.23
37	$CH_2CH_2CH_3$	Br	0.74 ± 0.0007	> 20

[a] Concentration necessary for 50% inhibition (IC_{50}). [b] Compound **1** and sivelestat were used as positive controls.

Table 4 Inhibitory effects of the isosteres of 2-phenylacetamidobenzoic esters and 3-phenylpropanamidobenzoic esters on superoxide anion generation and elastase release by human neutrophils in response to fMLP/CB



40-54



56 and **58**

				Anti-inflammation (μM)[a]	
	R_2	R_5	*n*	$O^{2\bullet-}$ generation	NE release
1[b]	CH_3	F	0	0.64 ± 0.01	> 20
Sivelestat[b]				> 20	0.050 ± 0.0002
40	CH_3	F	1	1.18 ± 0.10	> 20
41	CH_3	Cl	1	1.35 ± 0.01	> 20
42	CH_3	Br	1	3.42 ± 1.04	> 20
43	CH_2CH_3	F	1	0.47 ± 0.33	> 20
44	CH_2CH_3	Cl	1	0.57 ± 0.38	> 20
45	CH_2CH_3	Br	1	0.66 ± 0.11	> 20
46	$CH_2CH_2CH_3$	F	1	0.15 ± 0.03	> 20
47	$CH_2CH_2CH_3$	Cl	1	0.39 ± 0.004	> 20
48	$CH_2CH_2CH_3$	Br	1	0.48 ± 0.21	17.60 ± 7.76
49	$CH_2CH_2CH_2CH_3$	F	1	0.06 ± 0.01	> 20
50	$CH_2CH_2CH_2CH_3$	Cl	1	0.29 ± 0.09	> 20
51	$CH_2CH_2CH_2CH_3$	Br	1	0.67 ± 0.41	> 20
52	CH_2CH_3	F	2	0.95 ± 0.19	> 20
53	CH_2CH_3	Cl	2	1.57 ± 0.42	> 20
54	CH_2CH_3	Br	2	> 20	> 20
56	CH_2CH_3	F	—	> 20	> 20
58	CH_2CH_3	Cl	—	> 20	> 20

[a] Concentration necessary for 50% inhibition (IC_{50}). [b] Compound **1** and sivelestat were used as positive controls.

FMLP-induced $O_2^{\bullet-}$ generation in neutrophils with IC_{50} values of 0.20, 0.16, 0.15, 0.06, and 0.29 μM, respectively.

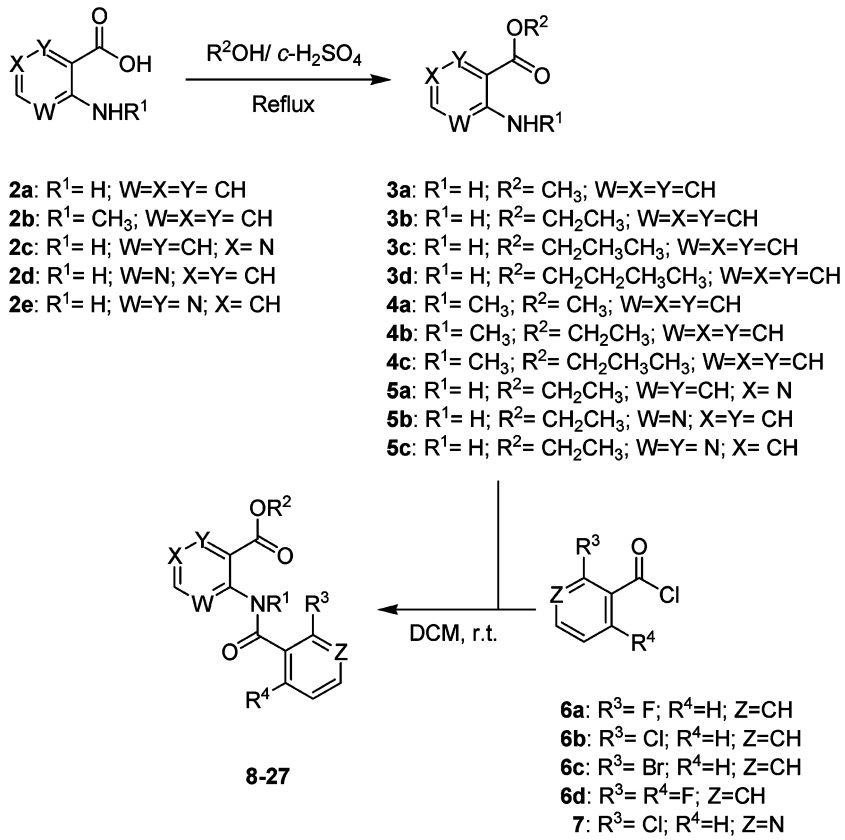
The neutrophil function assay data and SAR analysis showed the enhancement of activity in most synthetics, *e.g.* compounds **16–18** and **29–51**, where the ester chain length in the A ring was increased. A change in the linker between the A and B rings from amide to sulfonamide drastically reduced the inhibitory effect, as seen for **29–34**. The activities of *N*-methyl amide derivatives (**19–21**) vanished due to removal of the intramolecular hydrogen bond, which results in a drastic loss of binding affinity.[37] Furthermore, except for **23**, exchanges in the benzene rings (A or B rings) of **1** by different isosteric replacements (**22–27**) were unfavorable. There are many reports that demonstrate that replacing the CH with an N at each of the aromatic sites leads to improved aqueous solubility, preserves hydrogen bonding, or reduces metabolism.[38] However, the N atom in aromatic rings acts as an electron-withdrawing substitution changing the inductive effect or resonance, and modifies the hydrogen-bonding intensity of the amide between the A and B rings, thus the activities of compounds **22–27** were decreased.

To investigate the bioactive relationship of the spacer between the two aromatic rings, compounds **43–51** were synthesized and they showed an increase in inhibitory effects as the spacer length was increased by one carbon atom, whereas the activities were reversed when the spacer length was increased by two carbon atoms (**52–54**). Moreover, the activities were attenuated by the replacement of a 3-phenylpropanoyl moiety in anthranilic acid (**52–54**) with a cinnamoyl moiety (**56** and **58**).

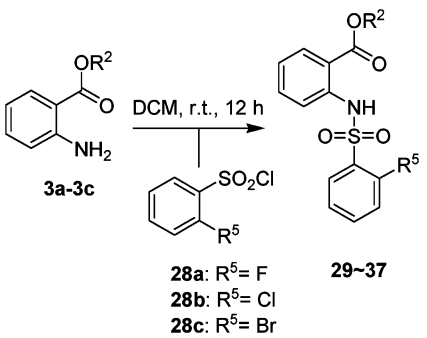
cAMP level determination

Compound **1** was able to enhance cAMP levels and PKA activity by inhibiting phosphodiesterase. To gain further insight into the mechanism of action of active synthetics, cAMP levels in the presence of these active compounds were determined. Neutrophils were incubated with compounds **17**, **18**, **21**, and **49** (1 μM) as described above, and an enzyme immunoassay kit was used to determine cAMP concentrations.[36] The results showed that compound **49** exhibited the most potent inhibitory effects on cAMP degradation, however, **21**, a non-active component for inhibiting $O_2^{\bullet-}$ generation in neutrophils, had no effect on modulating cAMP levels (Fig. 1). These results suggested and confirmed that cAMP is involved in the inhibitory effects on $O_2^{\bullet-}$ generation.

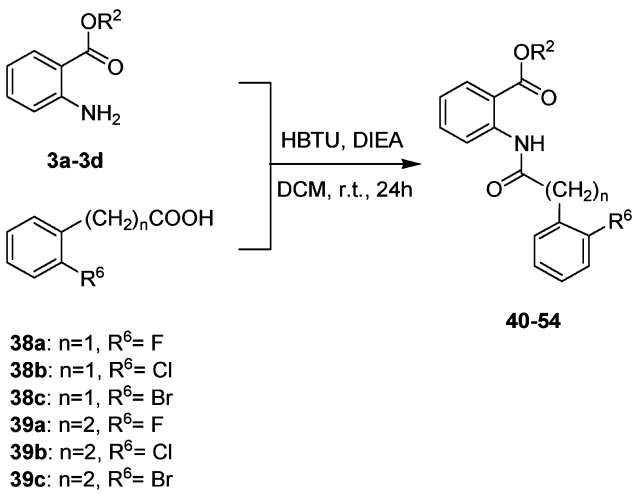
Additionally, in an attempt to examine whether cAMP was involved in the inhibitory effect on $O_2^{\bullet-}$ generation of **49**, a PKA



Scheme 1 The route for the preparation of compounds **8**–**27**.



Scheme 2 The route for the preparation of compounds **29**–**37**.



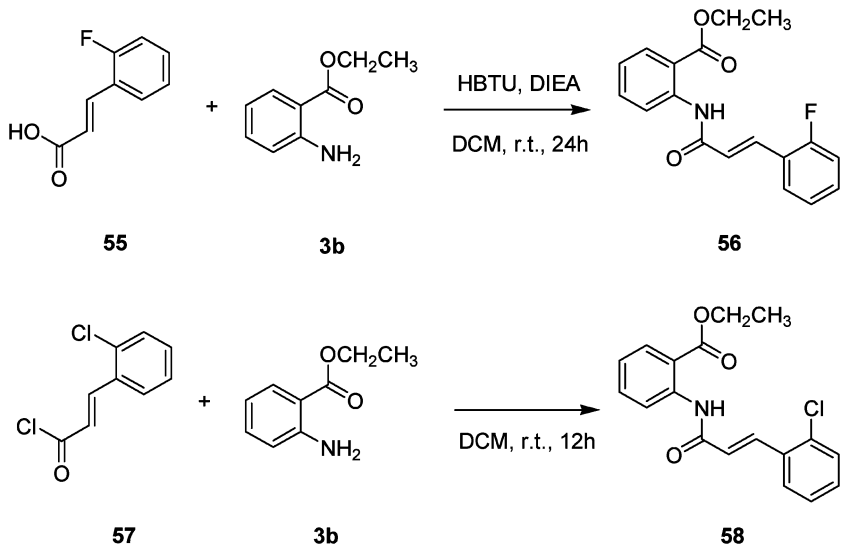
Scheme 3 The route for the preparation of compounds **40**–**54**.

inhibitor, H89 (3 μM), was used to elucidate the mechanism. The result showed negative regulation of FMLP-caused human neutrophil $O_2^{\bullet-}$ generation mediated by **49** was reversed by H89 (Fig. 2). This suggests that cAMP/PKA-dependent pathway mediates the inhibition of FMLP/CB-activated $O_2^{\bullet-}$ production caused by **49**.

Inhibitory effects of compounds on PDEs

Phosphodiesterases, *e.g.* PDE3, PDE4, and PDE5, regulate intracellular cAMP and/or cGMP levels to modulate $O_2^{\bullet-}$ generation in neutrophils. To investigate whether bioactive synthetics regulate cAMP levels by specifically inhibiting subtype phosphodiesterases, the most potent compound (**49**), and three positive controls (Ro20-1724, cilostamide, and zaprinast) were chosen to further evaluate their inhibitory effects on PDE3, PDE4, and PDE5, respectively.[39,40] In the present study, **49** exhibited selective activity on PDE4 (Selective Index; SI = 15.2). Interestingly, compound **49** showed minor inhibitory effects on PDE3 (Table 5), and no effect on PDE5 (the IC_{50} value of zaprinast was 2.5 μM).

cAMP is an important second messenger with a variety of physiological and pathophysiological manifestations. Elevation of intracellular cAMP levels is believed to suppress the activation of neutrophils.[36] Cellular cAMP concentrations are modulated either by synthesis *via* adenylate cyclase or by degradation *via* PDEs.



Scheme 4 The route for the preparation of compounds **56** and **58**.

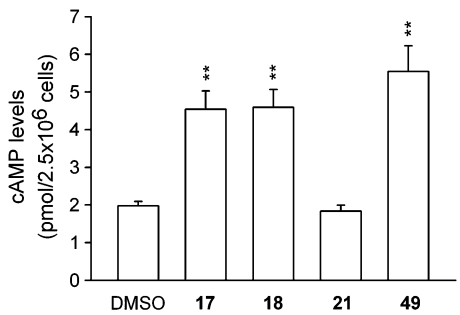


Fig. 1 Effects of compounds **2**, **17**, **18**, **21**, and **49** on cAMP levels. Human neutrophils were incubated with DMSO (control) or compounds **2**, **17**, **18**, **21**, and **49** (1.0 μM) for 5 min before stimulation with or without FMLP (0.1 mM) for another 1 min. cAMP levels were measured by enzyme immunoassay kits. All data are expressed as the mean ± S.E.M. (n = 3). *p < 0.05; **p < 0.01; ***p < 0.001 compared to the control.

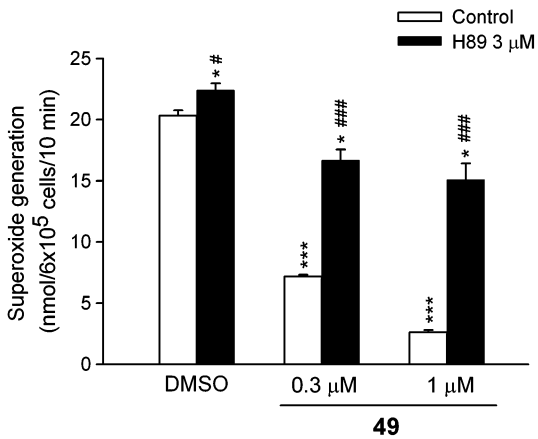


Fig. 2 Effects of the cAMP pathway on **49** caused inhibition of $O_2^{\bullet-}$ release. $O_2^{\bullet-}$ generation was induced by FMLP/CB and measured using SOD-inhibitable cytochrome c reduction. H89 (3 μM). All data are expressed as the mean ± S.E.M. (n = 3). * p < 0.05; ** p < 0.01; *** p < 0.001 compared to the control. # p < 0.01; ## p < 0.01; ### p < 0.001 compared to the corresponding control.

Table 5 Inhibitory effects of **49** on PDE3 and PDE4

	IC_{50} (μM)[a]	
	PDE3	PDE4
49	64.0	4.20
Cilostamide[b]	0.0312	—
Ro20-1724[b]	—	1.58

[a] Concentration necessary for 50% inhibition (IC_{50}). [b] Cilostamide and Ro20-1724 were used as positive controls.

The predominant cAMP-specific PDE in most inflammatory cells belongs to the PDE4 family,[41] and inhibitors of PDE4 are currently being developed clinically as potential anti-inflammatory agents.[42] Human neutrophils contain abundant amounts of PDE4, and the activity of PDE4 plays a significant function in regulating cellular cAMP level. In this study, our results demonstrate that inhibition of $O_2^{\bullet-}$ production in human neutrophils by **49** is associated with an elevation of cellular cAMP concentration through its inhibition of PDE4. **49** was shown to be more effective at inhibiting $O_2^{\bullet-}$ generation than PDE4, indicating that **49** may exhibit an additional cAMP-independent mechanism of action.

Although a few selective PDE4 inhibitors have exhibited broad spectrum anti-inflammatory effects, and reached clinical effects in asthma and COPD, the side effects of these inhibitors limit their clinical use.[13,43] Therefore, the development of a new generation of PDE inhibitors combining PDE4 inhibition may be another way forward.[42] The dual PDE3–PDE4 compounds, zardaverine and its related compounds, provide additive or synergistic effects to suppress the activation/function of cells playing a role in inflammatory lung diseases, and provide more bronchodilator and bronchoprotective effects in addition to the beneficial PDE4 effects.[13,41,42] Accordingly, compound **49** showed dual inhibitory effects on PDE3 and PDE4, and no significantly cytotoxic or anti-platelet effects, suggesting that this compound may be approved as a new candidate in the treatment of airway inflammatory diseases.

4. Conclusion

In this study, we synthesized forty-six anthranilic acid derivatives and compared their inhibitory effects on $O_2^{\bullet-}$ generation in neutrophils. The analysis of structure–activity relationships revealed that the ester chain length in the A ring, as well as an amide linker between the A and B rings, played important roles in $O_2^{\bullet-}$ generation. Furthermore, our study demonstrated that **49** inhibited $O_2^{\bullet-}$ generation *via* cAMP-dependent pathways. This compound could be approved as a lead for the development of new agents in the treatment of inflammatory lung diseases.

5. Experimental section

5.1. Materials and methods

All commercial chemicals and solvents are reagent grade and were used without further treatment unless otherwise noted. Reactions were detected by TLC using Merck 60 F254 silica gel aluminum packed plates; spots were recorded under ultraviolet irradiation (254 and 365 nm). The flash column chromatography was performed with silica gel (Silicycle, 70–230 mesh or 230–400 mesh). The purities of synthetics were determined by HPLC using a Jasco PU-1580 intelligent HPLC pump and a Jasco AS 1555-10 intelligent sampler with an Ascentis® C-18 analytical column (Superlcu, 5 μm, 4.6 mm × 250 mm). Detection was conducted at 248 nm in a Jassco UV-1575 UV-Vis detector. Purities of all the tested components were found to be more than 95% unless otherwise stated. The NMR spectra using $CDCl_3$ as solvents were obtained on a Bruker AVANCE-400 MHz FT-NMR spectrometer. Chemical shifts were internally referenced to the solvent signals in TMS. Low-resolution EI-MS were recorded on a Quattro GC-MS spectrometer having a direct inlet system, low-resolution and high-resolution ESI-MS spectra on a Bruker Daltonics APEX II 30e spectrometer.

5.1.1. General procedure for synthesis of 2-benzoylaminobenzoic esters (8–18) and *N*-methyl 2-benzoylaminobenzoic esters (19–21). Compounds **3a–3d** and **4a–4c** were synthesized through refluxing **2a** or **2b**, respectively, in corresponding alcohols (50 ml)/*c*-H_2SO_4 (1.0 ml) for 2 h, the solutions were subsequently neutralized by ammonium water; the resulting mixtures were partitioned between ice water (100 ml) and chloroform; and the organic layers were concentrated. To a mixture solution of **3a–3d** and **4a–4c** (1.0 mmole) in DCM was added the suitable benzoyl chlorides (2.0 mmole). The reaction mixture was stirred at room temperature for 12 h. The solvent was evaporated at reduced pressure. The residue was purified by silica gel column chromatography using a mixture of *n*-hexane–ethyl acetate to afford the products.

*5.1.1.1. Propyl 2-(2-fluorobenzamido)benzoate (**10**).* 51% yield. Colorless oil. ^{1}H NMR ($CDCl_3$) δ 11.89 (1H, d, J = 6.8 Hz, NH), 8.91 (1H, d, J = 8.4 Hz, H-3), 8.07 (1H, dd, J = 8.0, 1.2 Hz, H-6′), 8.05 (1H, dd, J = 8.0, 1.2 Hz, H-6), 7.58 (1H, t, J = 8.4, 1.2 Hz, H-4), 7.48 (1H, td, J = 7.2, 1.2 Hz, H-4′), 7.27 (1H, t, J = 7.6 Hz, H-5′), 7.15 (1H, dd, J = 11.6, 8.4 Hz, H-3′), 7.13 (1H, t, J = 8.0 Hz, H-5), 4.29 (2H, t, J = 6.8 Hz, OC$\underline{H}_2$CH$_2$CH$_3$), 1.79 (2H, qt, J = 7.6, 6.8 Hz, OCH$_2$C$\underline{H}_2$CH$_3$), 1.02 (3H, t, J = 7.6 Hz, OCH$_2$CH$_2$C$\underline{H}_3$). ^{13}C NMR ($CDCl_3$) δ 168.3 (s, -$\underline{C}$OOCH$_2$CH$_2$CH$_3$), 162.7 (s, -CONH-), 160.6 (s, C-2′, $J_{C\text{-}F}$ = 249 Hz), 141.5 (s, C-2), 134.7 (d, C-4), 133.9 (d, C-4′, $J_{C\text{-}F}$ = 9 Hz), 132.1 (d, C-6′), 131.3 (d, C-6), 125.1 (d, C-5′, $J_{C\text{-}F}$ = 3 Hz), 123.4 (d, C-5), 123.2 (s, C-1′, $J_{C\text{-}F}$ = 12 Hz), 121.7 (d, C-3), 117.0 (d, C-3′, $J_{C\text{-}F}$ = 23 Hz), 116.7 (s, C-1), 67.4 (t, -COO$\underline{C}$H$_2$CH$_2$CH$_3$), 22.4 (t, -COOCH$_2\underline{C}$H$_2$CH$_3$), 10.9 (q, -COOCH$_2$CH$_2\underline{C}$H$_3$). ESI-MS (m/z,%): 302 [M + H]$^+$ (100). HRESI-MS m/z 302.1191 [M + H]$^+$ (calcd for $C_{17}H_{17}FNO_3$ 302.1192).

*5.1.1.2. Propyl 2-(2-chlorobenzamido)benzoate (**11**).* 83% yield. Pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 11.59 (1H, br. s, NH), 8.91 (1H, d, J = 8.4 Hz, H-3), 8.07 (1H, dd, J = 8.0, 1.2 Hz, H-6), 7.65 (1H, dd, J = 7.2, 2.0 Hz, H-6′), 7.57 (1H, td, J = 8.4, 1.2 Hz, H-4), 7.42 (1H, dd, J = 8.8, 1.2 Hz, H-3′), 7.34 (2H, m, H-4′, 5′), 7.12 (1H, t, J = 8.0 Hz, H-5), 4.22 (2H, t, J = 6.8 Hz, OC$\underline{H}_2$CH$_2$CH$_3$), 1.74 (2H, qt, J = 7.2, 6.8 Hz, OCH$_2$C$\underline{H}_2$CH$_3$), 0.98 (3H, t, J = 7.2 Hz, OCH$_2$CH$_2$C$\underline{H}_3$). ^{13}C NMR ($CDCl_3$) δ 168.5 (s, -$\underline{C}$OOCH$_2$CH$_2$CH$_3$), 165.8 (s, -CONH-), 141.6 (s, C-2), 136.6 (s, C-1′), 135.0 (d, C-4), 131.8 (d, C-6), 131.6 (s, C-2′), 131.3 (d, C-4′), 131.0 (d, C-3′), 129.6 (d, C-6′), 127.5 (d, C-5′), 123.5 (d, C-5), 120.9 (d, C-3), 116.1 (s, C-1), 67.4 (t, -COO$\underline{C}$H$_2$CH$_2$CH$_3$), 22.3 (t, -COOCH$_2\underline{C}$H$_2$CH$_3$), 10.9 (q, -COOCH$_2$CH$_2\underline{C}$H$_3$). ESI-MS (m/z,%): 318[M + H]$^+$, 320 [M + 2 + H]$^+$. HRESI-MS m/z 318.0899 [M + H]$^+$ (calcd for $C_{17}H_{17}ClNO_3$ 318.0897).

*5.1.1.3. Propyl 2-(2-bromobenzamido)benzoate (**12**).* 63% yield. Brown oil. ^{1}H NMR ($CDCl_3$) δ 11.53 (1H, br. s, NH), 8.91 (1H, d, J = 8.4 Hz, H-3), 8.07 (1H, d, J = 8.0 Hz, H-6), 7.59 (3H, m, H-4, 3′, 6′), 7.37 (1H, t, J = 7.6 Hz, H-5′), 7.27 (1H, t, J = 7.6 Hz, H-4′), 7.12 (1H, t, J = 7.6 Hz, H-5), 4.22 (2H, t, J = 6.8 Hz, OC$\underline{H}_2$CH$_2$CH$_3$), 1.74 (2H, qt, J = 7.6, 6.8 Hz, OCH$_2$C$\underline{H}_2$CH$_3$), 0.98 (3H, t, J = 7.6 Hz, OCH$_2$CH$_2$C$\underline{H}_3$). ^{13}C NMR ($CDCl_3$) δ 168.5 (s, -$\underline{C}$OOCH$_2$CH$_2$CH$_3$), 166.6 (s, -CONH-), 141.6 (s, C-2), 138.8 (s, C-1′), 135.0 (d, C-4), 134.1 (d, C-3′), 131.9 (d, C-6), 131.3 (d, C-4′), 129.3 (d, C-6′), 128.1 (d, C-5′), 123.5 (d, C-5), 120.9 (d, C-3), 120.1 (s, C-2′), 116.1 (s, C-1), 67.4 (t, -COO$\underline{C}$H$_2$CH$_2$CH$_3$), 22.3 (t, -COOCH$_2\underline{C}$H$_2$CH$_3$), 10.9 (q, -COOCH$_2$CH$_2\underline{C}$H$_3$). ESI-MS (m/z,%): 362 [M + H]$^+$, 364 [M + 2 + H]$^+$. HRESI-MS m/z 362.0393 [M + H]$^+$ (calcd for $C_{17}H_{17}BrNO_3$ 362.0392).

*5.1.1.4. Butyl 2-(2-fluorobenzamido)benzoate (**13**).* 48% yield. Colorless oil. ^{1}H NMR ($CDCl_3$) δ 11.90 (1H, br. s, NH), 8.91 (1H, d, J = 8.8 Hz, H-3), 8.06 (2H, m, H-6, 6′), 7.57 (1H, td, J = 8.8, 1.6 Hz, H-4), 7.47 (1H, ddd, J = 11.6, 8.0, 2.0 Hz, H-4′), 7.26 (1H, t, J = 8.0 Hz, H-5′), 7.18 (1H, dd, J = 11.6, 8.0 Hz, H-3′), 7.12 (1H, t, J = 7.6 Hz, H-5), 4.33 (2H, t, J = 6.4 Hz, OC$\underline{H}_2$CH$_2$CH$_2$CH$_3$), 1.74 (2H, tt, J = 7.2, 6.4 Hz, OCH$_2$C$\underline{H}_2$CH$_2$CH$_3$), 1.46 (2H, qt, J = 7.6, 7.2 Hz, OCH$_2$CH$_2$C$\underline{H}_2$CH$_3$), 0.96 (3H, t, J = 7.6 Hz, OCH$_2$CH$_2$CH$_2$C$\underline{H}_3$). ^{13}C NMR ($CDCl_3$) δ 168.3 (s, -$\underline{C}$OOCH$_2$CH$_2$CH$_2$CH$_3$), 162.6 (s, $J_{C\text{-}F}$ = 3 Hz, -CONH-), 160.6 (s, $J_{C\text{-}F}$ = 250 Hz, C-2′), 141.5 (s, C-2), 134.7 (d, C-4), 133.8 (d, $J_{C\text{-}F}$ = 9 Hz, C-4′), 132.0 (d, $J_{C\text{-}F}$ = 2 Hz, C-6′), 131.2 (d, C-6), 125.0 (d, $J_{C\text{-}F}$ = 4 Hz, C-5′), 123.4 (d, C-5), 123.2 (s, $J_{C\text{-}F}$ = 12 Hz, C-1′), 121.8 (d,C-3), 116.8 (d, $J_{C\text{-}F}$ = 23 Hz, C-3′), 116.7 (s, C-1), 65.6 (t, -COO$\underline{C}$H$_2$CH$_2$CH$_2$CH$_3$), 31.0 (t, -COOCH$_2\underline{C}$H$_2$CH$_2$CH$_3$), 19.6 (t, -COOCH$_2$CH$_2\underline{C}$H$_2$CH$_3$), 14.1 (q, -COOCH$_2$CH$_2$CH$_2\underline{C}$H$_3$). ESI-MS (m/z,%): 316 [M + H]$^+$. HRESI-MS m/z 316.1348 [M + H]$^+$ (calcd for $C_{18}H_{19}FNO_3$ 316.1349).

*5.1.1.5. Butyl 2-(2-chlorobenzamido)benzoate (**14**).* 55% yield. Colorless oil. ^{1}H NMR ($CDCl_3$) δ 11.58 (1H, br. s, NH), 8.91 (1H, d, J = 8.4 Hz, H-3), 8.06 (1H, dd, J = 8.0, 1.2 Hz, H-6),

7.65 (1H, dd, J = 7.2, 2.0 Hz, H-6′), 7.58 (1H, td, J = 8.4, 1.2 Hz, H-4), 7.44 (1H, dd, J = 7.6, 1.2 Hz, H-3′), 7.38 (1H, td, J = 7.6, 1.2 Hz, H-5′), 7.37 (1H, td, J = 7.6, 2.0 Hz, H-4′), 7.12 (1H, t, J = 8.0 Hz, H-5), 4.28 (2H, t, J = 6.8 Hz, $OCH_2CH_2CH_2CH_3$), 1.71 (2H, tt, J = 7.2, 6.8 Hz, $OCH_2CH_2CH_2CH_3$), 1.43 (2H, qt, J = 7.6, 7.2 Hz, $OCH_2CH_2CH_2CH_3$), 0.94 (3H, q, J = 7.6 Hz, $OCH_2CH_2CH_2CH_3$). ^{13}C NMR ($CDCl_3$) δ 168.5 (s, -$COOCH_2CH_2CH_2CH_3$), 165.8 (s, -CONH-), 141.6 (s, C-2), 136.7 (s, C-1′), 135.0 (d, C-4), 131.8 (s, C-2′), 131.7 (d, C-6), 131.3 (d, C-4′), 131.0 (d, C-3′), 129.6 (d, C-6′), 127.5 (d, C-5′), 123.5 (d, C-5), 121.0 (d, C-3), 116.2 (s, C-1), 65.7 (t, -$COOCH_2CH_2CH_2CH_3$), 30.9 (t, -$COOCH_2CH_2CH_2CH_3$), 19.6 (t, -$COOCH_2CH_2CH_2CH_3$), 14.1 (q, -$COOCH_2CH_2CH_2CH_3$). ESI-MS (m/z,%): 332 $[M + H]^+$, 334 $[M + 2 + H]^+$. HRESI-MS m/z 332.1052 $[M + H]^+$ (calcd for $C_{18}H_{19}ClNO_3$ 332.1053).

*5.1.1.6. Butyl 2-(2-bromobenzamido)benzoate (**15**).* 60% yield. Pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 11.50 (1H, br. s, NH), 8.90 (1H, d, J = 8.0 Hz, H-3), 8.07 (1H, dd, J = 8.0, 2.0 Hz, H-6), 7.60 (3H, m, H-4, 3′, 6′), 7.38 (1H, td, J = 8.0, 0.8 Hz, H-5′), 7.28 (1H, td, J = 8.0, 2.0 Hz, H-4′), 7.13 (1H, t, J = 8.0 Hz, H-5), 4.27 (2H, t, J = 6.8 Hz, $OCH_2CH_2CH_2CH_3$), 1.71 (2H, tt, J = 7.6, 6.8 Hz, $OCH_2CH_2CH_2CH_3$), 1.43 (2H, qt, J = 7.6, 7.2 Hz, $OCH_2CH_2CH_2CH_3$), 0.94 (3H, t, J = 7.2 Hz, $OCH_2CH_2CH_2CH_3$). ^{13}C NMR ($CDCl_3$) δ 168.4 (s, -$COOCH_2CH_2CH_2CH_3$), 166.5 (s, -CONH-), 141.4 (s, C-2), 138.7 (s, C-1′), 134.9 (d, C-4), 134.0 (d, C-3′), 131.7 (d, C-6), 131.2 (d, C-4′), 129.2 (d, C-6′), 127.9 (d, C-5′), 123.4 (d, C-5), 120.8 (d, C-3), 120.0 (s, C-2′), 116.0 (s, C-1), 65.6 (t, -$COOCH_2CH_2CH_2CH_3$), 30.8 (t, -$COOCH_2CH_2CH_2CH_3$), 19.5 (t, -$COOCH_2CH_2CH_2CH_3$), 14.0 (q, -$COOCH_2CH_2CH_2CH_3$). ESI-MS (m/z,%): 376 $[M + H]^+$, 378 $[M + 2 + H]^+$. HRESI-MS m/z 376.0548 $[M + H]^+$ (calcd for $C_{18}H_{19}BrNO_3$ 376.0547).

*5.1.1.7. Propyl 2-(2,6-difluorobenzamido)benzoate (**18**).* 32% yield. Colorless oil. ^{1}H NMR ($CDCl_3$) δ 11.64 (1H, br. s, NH), 8.90 (1H, d, J = 8.0 Hz, H-3), 8.08 (1H, dd, J = 7.6, 1.6 Hz, H-6), 7.60 (1H, td, J = 8.0, 1.2 Hz, H-4), 7.39 (1H, tt, J = 12.4, 8.0 Hz, H-4′), 7.15 (1H, t, J = 7.6 Hz, H-5), 6.99 (2H, t, J = 8.0 Hz, H-3′, 5′), 4.25 (2H, t, J = 6.8 Hz, $OCH_2CH_2CH_3$), 2.77 (2H, qt, J = 7.2, 6.8 Hz, $OCH_2CH_2CH_3$), 1.00 (3H, t, J = 7.2 Hz, $OCH_2CH_2CH_3$). ^{13}C NMR ($CDCl_3$) δ 168.5 (s, -$COOCH_2CH_2CH_3$), 161.6 (s, -CONH-), 159.1 (s, $J_{C\text{-}F}$ = 252, 7 Hz, C-2′, 5′), 141.3 (s, C-2), 135.0 (d, C-4), 132.7 (d, $J_{C\text{-}F}$ = 10 Hz, C-4′), 131.3 (d, C-6), 123.7 (d, C-5), 121.0 (d, C-3), 116.1 (s, C-1), 115.5 (s, $J_{C\text{-}F}$ = 20 Hz, C-1′), 112.5 (d, $J_{C\text{-}F}$ = 25 Hz, C-3′, 5′), 67.4 (t, -$COOCH_2CH_2CH_3$), 22.3 (t, -$COOCH_2CH_2CH_3$), 10.8 (q, -$COOCH_2CH_2CH_3$). ESI-MS (m/z,%): 320 $[M + H]^+$. HRESI-MS m/z 320.1099 $[M + H]^+$ (calcd for $C_{17}H_{16}F_2NO_3$ 320.1098).

*5.1.1.8. Methyl 2-(2,6-difluoro-N-methylbenzamido)benzoate (**19**).* 55% yield. Colorless oil. ^{1}H NMR ($CDCl_3$) δ 7.78 (1H, dd, J = 8.0, 1.2 Hz, H-6), 7.45 (1H, td, J = 8.0, 1.2 Hz, H-4), 7.36 (1H, d, J = 8.0 Hz, H-3), 7.27 (1H, td, J = 8.0, 1.2 Hz, H-5), 7.08 (1H, tt, J = 8.0, 6.8 Hz, H-4′), 6.63 (2H, m, H-3′, 5′), 3.90 (3H, OCH_3), 3.89 (3H, NCH_3). ^{13}C NMR ($CDCl_3$) δ 166.3 (s, -$COOCH_3$), 165.8 (s, -$CONCH_3$), 158.7(s, $J_{C\text{-}F}$ = 250 Hz, C-2′, 6′), 142.1 (s, C-2), 133.4 (d, C-4), 131.9 (d, C-6), 131.4 (d, $J_{C\text{-}F}$ = 10 Hz, C-4′), 129.9 (d, C-5), 29.1 (s, C-1), 129.0 (d, C-3), 125.0 (s, $J_{C\text{-}F}$ = 22 Hz, C-1′), 111.6 (d, $J_{C\text{-}F}$ = 22 Hz, C-3′, 5′), 52.8 (q, -$COOCH_3$), 37.7 (q, -$CONCH_3$). ESI-MS (m/z,%): 306 $[M + H]^+$. HRESI-MS m/z 306.0941 $[M + H]^+$ (calcd for $C_{16}H_{14}F_2NO_3$ 306.0942).

*5.1.1.9. Ethyl 2-(2-fluoro-N-methylbenzamido)benzoate (**20**).* 48% yield. White powder, mp 84–86 °C. ^{1}H NMR ($CDCl_3$) δ 7.81 (1H, d, J = 7.6 Hz, H-6), 7.45 (1H, t, J = 7.6 Hz, H-4, 7.36 (1H, d, J = 7.6 Hz, H-3), 7.29 (1H, t, J = 7.6 Hz, H-5), 7.10 (1H, tt, J = 8.0, 7.6 Hz, H-4′), 6.65 (2H, m, H-3′, 5′), 4.87 (2H, q, J = 6.8 Hz, OCH_2CH_3), 3.48 (3H, s, NCH_3), 1.40 (3H, t, J = 6.8 Hz, OCH_2CH_3). ^{13}C NMR ($CDCl_3$) δ 165.6 (s, -$COOCH_2CH_3$), 161.5 (s, -$CONCH_3$), 142.2 (s, C-2), 133.7 (d, C-4), 133.2 (d, C-6), 131.3 (d, $J_{C\text{-}F}$ = 10 Hz C-4′), 129.9 (d, C-5), 129.7 (s, C-1), 128.9 (d, C-3), 115.2 (s, $J_{C\text{-}F}$ = 23 Hz, C-1′), 111.6 (d, $J_{C\text{-}F}$ = 21 Hz, C-3′, 5′), 62.1 (t, -$COOCH_2CH_3$), 37.9 (q, -$CONCH_3$), 14.5 (q, -$COOCH_2CH_3$). ESI-MS (m/z,%): 320 $[M + H]^+$. HRESI-MS m/z 320.1097 $[M + H]^+$ (calcd for $C_{17}H_{16}F_2NO_3$ 320.1098).

*5.1.1.10. Propyl 2-(2,6-difluoro-N-methylbenzamido)benzoate (**21**).* 52% yield. White powder, mp 72–74 °C. ^{1}H NMR ($CDCl_3$) δ 7.82 (1H, dd, J = 8.0, 1.2 Hz, H-6), 7.45 (1H, td, J = 7.6, 1.2 Hz, H-4), 7.36 (1H, d, J = 7.6 Hz, H-3), 7.29 (1H, t, J = 8.0 Hz, H-5), 7.09 (1H, tt, J = 8.4, 7.6 Hz, H-4′), 6.64 (2H, m, H-3′, 5′), 4.30 (2H, t, J = 6.8 Hz, $OCH_2CH_2CH_3$), 3.52 (3H, s, NCH_3), 1.82 (2H, qt, J = 7.2, 6.8 Hz, $OCH_2CH_2CH_3$), 1.02 (3H, t, J = 7.2 Hz, $OCH_2CH_2CH_3$). ^{13}C NMR ($CDCl_3$) δ 165.5 (s, -$COOCH_2CH_2CH_3$), 161.6 (s, -$CONCH_3$), 158.9 (s, $J_{C\text{-}F}$ = 250 Hz, C-2′, 6′), 142.0 (s, C-2), 133.2 (d, C-4), 131.9 (d, C-6), 131.5 (d, $J_{C\text{-}F}$ = 10 Hz, C-4′), 129.8 (d, C-5), 129.5 (s, C-1), 129.0 (d, C-3), 115.0 (d, $J_{C\text{-}F}$ = 23 Hz, C-1′), 111.7 (d, $J_{C\text{-}F}$ = 21 Hz, C-3′, 5′), 67.7 (t, -$COOCH_2CH_2CH_3$), 37.8 (q, -$CONCH_3$), 22.15 (t, -$COOCH_2CH_2CH_3$), 10.77 (q, -$COOCH_2CH_2CH_3$). ESI-MS (m/z,%): 334 $[M + H]^+$. HRESI-MS m/z 334.1257 $[M + H]^+$ (calcd for $C_{18}H_{18}F_2NO_3$ 334.1255).

*5.1.1.11. Ethyl 2-(2-fluorobenzamido)nicotinate (**22**).* Compound **22** was synthesized in 45% yield from 4-amino-nicotinic acid (**2c**) in similar procedure as described. Pale yellow powder, mp 72–74 °C. ^{1}H NMR ($CDCl_3$) δ 11.55 (1H, d, J = 8.4 Hz, NH), 8.69 (1H, dd, J = 4.8, 1.6 Hz, H-6), 8.36 (1H, dd, J = 7.6, 1.6 Hz, H-4), 8.12 (1H, dd, J = 7.6, 1.2 Hz, H-6′), 7.52 (1H, ddd, J = 10.2, 7.6, 1.2 Hz, H-4′), 7.28 (1H, t, J = 7.6 Hz, H-5′), 7.19 (1H, dd, J = 10.2, 8.4 Hz, H-3′), 7.15 (1H, dd, J = 7.6, 4.8 Hz, H-5), 4.41 (2H, q, J = 6.8 Hz, CH_2CH_3), 1.39 (3H, t, J = 6.8 Hz, CH_2CH_3). ^{13}C NMR ($CDCl_3$) δ 166.7 (s, -$COOCH_2CH_3$), 161.6 (s, -CONH-), 160.1 (s, $J_{C\text{-}F}$ = 249 Hz, C-2′), 153.1 (d, C-6), 152.4 (s, C-2), 140.2 (d, C-4), 134.1 (d, $J_{C\text{-}F}$ = 9 Hz, C-4′), 132.5 (d, C-5′), 125.1 (d, $J_{C\text{-}F}$ = 3 Hz, C-6′), 122.7 (s, $J_{C\text{-}F}$ = 12 Hz, C-1′), 119.3 (d, C-5), 116.6 (d, $J_{C\text{-}F}$ = 24 Hz, C-3′), 113.5 (s, C-3), 62.3 (t, -$COOCH_2CH_3$), 14.4 (q, -$COOCH_2CH_3$). ESI-MS (m/z,%): 289 $[M + H]^+$. HRESI-MS m/z 289.0986 $[M + Na]^+$ (calcd for $C_{15}H_{14}FN_2O_3$ 289.0986).

*5.1.1.12. Ethyl 4-(2-fluorobenzamido)nicotinate (**23**).* Compound **23** was synthesized in 49% yield from 2-aminonicotinic acid (**2d**) in a procedure similar to **22**. White powder, mp 74–76 °C. ^{1}H NMR ($CDCl_3$) δ 12.00 (1H, d, J = 8.0 Hz, NH), 9.21 (1H, s, H-2), 8.81 (1H, d, J = 6.0 Hz, H-6), 8.66 (1H, d, J = 6.0 Hz, H-5), 8.07 (1H, td, J = 8.4, 1.6 Hz, H-6′), 7.54 (1H, ddd, J = 13.2, 8.4, 1.6 Hz, H-4′), 7.30 (1H, t, J = 8.4 Hz, H-5′), 7.22 (1H, dd, J = 11.2, 8.4 Hz, H-3′), 4.46 (2H, q, J = 6.8 Hz, OCH_2CH_3), 1.44 (3H, t, J = 6.8 Hz, OCH_2CH_3). ^{13}C NMR ($CDCl_3$) δ 167.5 (s, -$COOCH_2CH_3$), 163.2 (s, -CONH-),160.2 (s, $J_{C\text{-}F}$ = 250 Hz, C-2′), 155.0 (d, C-2), 152.9 (d, C-6), 147.7 (s, C-4), 134.6 (d, $J_{C\text{-}F}$ = 9 Hz, C-6′), 132.3 (d, C-5′), 125.4 (d, $J_{C\text{-}F}$ = 3 Hz, C-4′), 122.1 (d, $J_{C\text{-}F}$ = 12 Hz, C-3′), 116.9 (s, $J_{C\text{-}F}$ = 23 Hz, C-1′), 114.7 (d, C-5), 112.0 (s, C-3), 62.2 (t, -$COOCH_2CH_3$), 14.5 (q, -$COOCH_2CH_3$). ESI-MS

(*m*/*z*,%): 289 $[M + H]^+$. HRESI-MS *m*/*z* 289.0989 $[M + H]^+$ (calcd for $C_{15}H_{14}FN_2O_3$ 289.0988).

*5.1.1.13. Ethyl 2-(2-chlorobenzamido)nicotinate (**24**).* Compound **24** was synthesized in 58% yield from 2-aminonicotinic acid (**2d**) in a procedure similar to **8**. Pale yellow powder, mp 108–110 °C. ^{1}H NMR ($CDCl_3$) δ 11.25 (1H, br. s, NH), 8.63 (1H, dd, *J* = 4.8, 1.6 Hz, H-6), 8.35 (1H, dd, *J* = 8.0, 2.0 Hz, H-4), 7.67 (1H, dd, *J* = 8.0, 2.0 Hz, H-3 ′), 7.43 (1H, td, *J* = 8.0, 2.0 Hz, H-5 ′), 7.38 (1H, dd, *J* = 8.0, 2.0 Hz, H-6 ′), 7.35 (1H, td, *J* = 8.0, 2.0 Hz, H-4 ′), 7.13 (1H, dd, *J* = 8.0, 4.8 Hz, H-5), 4.33 (1H, q, *J* = 6.8 Hz, $OC\underline{H}_2CH_3$), 1.38 (1H, t, *J* = 6.8 Hz, $OC\underline{H}_2CH_3$). ^{13}C NMR ($CDCl_3$) δ 166.3 (s, -$\underline{C}OOCH_2CH_3$), 164.3 (s, -CONH-), 152.7 (d, C-6), 151.8 (s, C-2), 139.6 (s, C-1′), 135.9 (d, C-4), 131.2 (s, C-2′), 130.7 (d, C-4′), 130.1 (d, C-3′), 129.2 (d, C-6′), 126.8 (d, C-5′), 118.6 (d, C-5), 112.1 (s, C-3), 61.8 (t, -$COO\underline{C}H_2CH_3$), 13.8 (q, -$COOCH_2\underline{C}H_3$). ESI-MS (*m*/*z*,%): 305 $[M + H]^+$, 307 $[M + 2 + H]^+$. HRESI-MS *m*/*z* 305.0691 $[M + H]^+$ (calcd for $C_{15}H_{14}ClN_2O_3$ 305.0693).

*5.1.1.14. Ethyl 2-(2-bromobenzamido)nicotinate (**25**).* Compound **25** was synthesized in 62% yield from 2-aminonicotinic acid (**3d**) in a procedure similar to **9**. Pale yellow powder, mp 98–100 °C. ^{1}H NMR ($CDCl_3$) δ 11.21 (1H, br. s, NH), 8.61 (1H, dd, *J* = 4.8, 2.0 Hz, H-6), 8.34 (1H, dd, *J* = 8.0, 2.0 Hz, H-4), 7.61 (2H, dd, *J* = 8.0, 0.8 Hz, H-3′, 6′), 7.39 (1H, td, *J* = 8.0, 0.8 Hz, H-5′), 7.30 (1H, td, *J* = 8.0, 0.8 Hz, H-4′), 7.13 (1H, dd, *J* = 8.0, 4.8 Hz, H-5), 4.37 (2H, q, *J* = 6.4 Hz, $OC\underline{H}_2CH_3$), 1.37 (3H, t, *J* = 6.4 Hz, $OCH_2C\underline{H}_3$). ^{13}C NMR ($CDCl_3$) δ 166.9 (s, -$\underline{C}OOCH_2CH_3$), 165.7 (s, -CONH-), 153.2 (d, C-6), 152.3 (s, C-2), 140.3 (d, C-4), 138.7 (s, C-1′), 133.9 (d, C-3′), 131.8 (d, C-4′), 129.4 (d, C-6′), 128.0 (d, C-5′), 119.7 (d, C-5), 119.3 (s, C-2′), 112.8 (s, C-3), 62.4 (t, -$COO\underline{C}H_2CH_3$), 14.48 (q, -$COOCH_2\underline{C}H_3$). ESI-MS (*m*/*z*,%): 349 $[M + H]^+$, 357 $[M + 2 + H]^+$. HRESI-MS *m*/*z* 349.0185 $[M + H]^+$ (calcd for $C_{15}H_{14}BrN_2O_3$ 349.0188).

*5.1.1.15. Methyl 3-(2-fluorobenzamido)pyrazine-2-carboxylate (**26**).* Compound **26** was synthesized in 14% yield from 3-aminopyrazine-2-carboxylic acid (**3e**) in a procedure similar to described. White powder, mp 123–125 °C. ^{1}H NMR ($CDCl_3$) δ 11.58 (1H, d, *J* = 9.6 Hz, NH), 8.70 (1H, d, *J* = 2.0 Hz, H-6), 8.47 (1H, d, *J* = 2.0 Hz, H-5), 8.16 (1H, t, *J* = 7.6 Hz, H-6′), 7.57 (1H, ddd, *J* = 7.6, 5.2, 2.0 Hz, H-4′), 7.33 (1H, t, *J* = 7.6 Hz, H-5′), 7.23 (1H, dd, *J* = 11.6, 7.6 Hz, H-3′), 4.08 (3H, s, OCH_3). ^{13}C NMR ($CDCl_3$) δ 166.5 (s, -$\underline{C}OOCH_3$), 161.6 (s, -CONH-), 159.6 (s, $J_{C\text{-}F}$ = 248 Hz, C-2′), 149.8 (s, C-3), 147.0 (d, C-6), 139.2 (d, C-5), 134.8 (d, $J_{C\text{-}F}$ = 9 Hz, C-6′), 132.8 (d, C-5′), 130.4 (s, C-2), 125.4 (d, C-4′), 121.8 (d, $J_{C\text{-}F}$ = 11 Hz, C-3′), 116.8 (s, $J_{C\text{-}F}$ = 23 Hz, C-1′), 54.0 (q, -$COO\underline{C}H_3$). ESI-MS (*m*/*z*,%): 276 $[M + H]^+$. HRESI-MS *m*/*z* 276.0786 $[M + H]^+$ (calcd for $C_{13}H_{11}FN_3O_3$ 276.0784).

5.1.2 General procedure for synthesis of 2-phenylsulfonamidobenzoic esters (29–37). Compounds **3a–3c** were synthesized in a procedure similar to described. To a mixture solution of **3a–3c** (1.0 mmole) in DCM (20.0 ml) was added the corresponding benzenesulfonyl chloride (**28a–28c**, 1.5 mmole). The reaction mixture was stirred at room temperature for 12 h. The solvent was evaporated at reduced pressure. The residue was purified by silica gel column chromatography using a mixture of *n*-hexane–acetone to afford the products.

*5.1.2.1. Ethyl 2-(2-bromophenylsulfonamido)benzoate (**34**).* 23% yield. Pale yellow powder, mp 88–90 °C. ^{1}H NMR ($CDCl_3$) δ 11.33 (1H, br. s, NH), 8.26 (1H, dd, *J* = 7.6, 1.2 Hz, H-3), 7.96 (1H, dd, *J* = 8.0, 1.6 Hz, H-6), 7.63 (1H, d, *J* = 8.4 Hz, H-3′), 7.49 (1H, d, *J* = 8.4 Hz, H-6′), 7.44 (1H, td, *J* = 7.2, 1.2 Hz, H-4), 7.35 (2H, m, H-4′, 5′), 6.97 (1H, t, *J* = 7.6 Hz, H-5), 4.38 (2H, q, *J* = 7.2 Hz, $OC\underline{H}_2CH_3$), 1.39 (3H, t, *J* = 7.2 Hz, $OCH_2C\underline{H}_3$). ^{13}C NMR ($CDCl_3$) δ 168.2 (s, -$\underline{C}OOCH_2CH_3$), 140.2 (s, C-2), 138.8 (s, C-1′), 135.9 (d, C-4), 134.7 (d, C-3′), 134.5 (d, C-4′), 132.7 (d, C-6), 131.8 (d, C-6′), 128.0 (d, C-5′), 122.7 (s, C-1), 120.7 (s, C-2′), 117.2 (d, C-5), 115.9 (d, C-3), 62.1 (t, -$COO\underline{C}H_2CH_3$), 14.6 (q, -$COOCH_2\underline{C}H_3$). ESI-MS (*m*/*z*,%): 406 $[M + Na]^+$, 408 $[M + 2 + Na]^+$. HRESI-MS *m*/*z* 405.9725 $[M + Na]^+$ (calcd for $C_{15}H_{14}BrNO_4SNa$ 405.9728).

*5.1.2.2. Propyl 2-(2-fluorophenylsulfonamido)benzoate (**35**).* 29% yield. Pale yellow powder, mp 79–81 °C. ^{1}H NMR ($CDCl_3$) δ 11.13 (1H, br. s, NH), 7.98 (2H, m, H-6, 6′), 7.63 (1H, d, *J* = 8.4 Hz, H-3), 7.54 (1H, m, H-4′), 7.40 (1H, td, *J* = 8.4, 1.2 Hz, H-4), 7.27 (1H, t, *J* = 7.6 Hz, H-5′), 7.11 (1H, t, *J* = 9.6 Hz, H-3′), 7.03 (1H, t, *J* = 7.6 Hz, H-5), 4.30 (2H, t, *J* = 6.8 Hz, $OC\underline{H}_2CH_2CH_3$), 1.79 (2H, qt, *J* = 7.2, 6.8 Hz, $OCH_2C\underline{H}_2CH_3$), 1.02 (3H, t, *J* = 7.2 Hz, $OCH_2CH_2C\underline{H}_3$). ^{13}C NMR ($CDCl_3$) δ 168.2 (s, -$\underline{C}OOCH_2CH_2CH_3$), 159.3 (s, C-2′, $J_{C\text{-}F}$ = 255 Hz), 140.3 (s, C-2), 136.1 (d, C-4′, $J_{C\text{-}F}$ = 9 Hz), 134.8 (d, C-4), 131.7 (d, C-6′), 131.3 (d, C-6), 127.5 (s, C-1′, $J_{C\text{-}F}$ = 14 Hz), 124.8 (d, C-5′, $J_{C\text{-}F}$ = 4 Hz), 123.2 (d, C-5), 118.1 (d, C-3), 117.6 (d, C-3′, $J_{C\text{-}F}$ = 21 Hz), 116.2 (s, C-1), 67.7 (t, -$COO\underline{C}H_2CH_2CH_3$), 22.3 (t, -$COOCH_2\underline{C}H_2CH_3$), 10.9(q, t, -$COOCH_2CH_2\underline{C}H_3$). ESI-MS (*m*/*z*,%): 360 $[M + Na]^+$. HRESI-MS *m*/*z* 360.0682 $[M + Na]^+$ (calcd for $C_{16}H_{16}FNO_4SNa$ 360.0684).

*5.1.2.3. Propyl 2-(2-chlorophenylsulfonamido)benzoate (**36**).* 39% yield. Yellow powder, mp 83–85 °C. ^{1}H NMR ($CDCl_3$) δ 11.33 (1H, br. s, NH), 8.22 (1H, dd, *J* = 7.6, 1.6 Hz, H-3), 7.98 (1H, dd, *J* = 8.0, 1.6 Hz, H-6), 7.54 (1H, d, *J* = 8.4 Hz, H-6′), 7.41 (4H, m, H-4, 3′, 4′, 5′), 7.00 (1H, t, *J* = 8.0 Hz, H-5), 4.30 (2H, t, *J* = 6.8 Hz $OC\underline{H}_2CH_2CH_3$), 1.80 (2H, qt, *J* = 7.2, 6.8 Hz, $OCH_2C\underline{H}_2CH_3$), 1.03 (3H, t, *J* = 7.2 Hz $OCH_2CH_2CH_3$). ^{13}C NMR ($CDCl_3$) δ 168.2 (s, -$COOCH_2CH_2CH_3$), 140.23 (s, C-2), 136.9 (s, C-1′), 134.8 (d, C-2′), 134.7 (d, C-6), 132.5 (d, C-4′), 134.4 (d, C-3′), 132.4 (d, C-4), 131.8 (d, C-6′), 127.4 (d, C-5′), 122.9 (d, C-5), 117.2 (d, C-3), 115.8 (s, C-1), 67.6 (d, -$COO\underline{C}H_2CH_2CH_3$), 22.3 (d, -$COOCH_2\underline{C}H_2CH_3$), 10.9 (q, -$COOCH_2CH_2\underline{C}H_3$). ESI-MS (*m*/*z*,%): 376 $[M + Na]^+$, 378 $[M + 2 + Na]^+$. HRESI-MS *m*/*z* 376.0386 $[M + Na]^+$ (calcd for $C_{16}H_{16}ClNO_4S$ Na 376.0384).

*5.1.2.4. Propyl 2-(2-bromophenylsulfonamido)benzoate (**37**).* 21% yield. Yellow powder, mp 101–103 °C. ^{1}H NMR ($CDCl_3$) δ 11.37 (1H, br. s, NH), 8.28 (1H, dd, *J* = 8.0, 1.6 Hz, H-3), 7.79 (1H, dd, *J* = 8.0, 1.2 Hz, H-6), 7.64 (1H, d, *J* = 7.2 Hz, H-3′), 7.51 (1H, d, *J* = 8.8 Hz, H-6′), 7.46 (1H, t, *J* = 7.2 Hz, H-4), 7.36 (1H, t, *J* = 7.2 Hz, H-4′, 5′), 6.99 (1H, t, *J* = 7.6 Hz, H-5), 4.30 (1H, t, *J* = 6.8 Hz, $OC\underline{H}_2CH_2CH_3$), 1.80 (2H, qt, *J* = 7.2, 6.8 Hz, $OCH_2C\underline{H}_2CH_3$), 1.08 (3H, t, *J* = 7.2 Hz, $OCH_2CH_2C\underline{H}_3$). ^{13}C NMR ($CDCl_3$) δ 168.2 (s, -CO), 140.2 (s, C-2), 138.7 (s, C-1′), 135.9 (d, C-4), 134.8 (d, C-3′), 134.6 (d, C-4), 132.7 (d, C-6), 131.8 (d, C-6′), 128.0 (d, C-5′), 122.7 (s, C-1), 120.7 (s, C-2′), 117.1 (d, C-5), 115.8 (d, C-3), 67.6 (t, -$COO\underline{C}H_2CH_2CH_3$), 22.4 (t, -$COOCH_2\underline{C}H_2CH_3$), 10.9 (q, -$COOCH_2CH_2\underline{C}H_3$). ESI-MS (*m*/*z*,%): 420 $[M + Na]^+$, 422 $[M + 2 + Na]^+$. HRESI-MS *m*/*z* 419.9880 $[M + Na]^+$ (calcd for $C_{16}H_{16}BrNO_3SNa$ 419.9880).

5.1.3. General procedure for synthesis of 2-phenylacetamidobenzoic esters (40–51) and 3-phenylpropanamidobenzoic esters (52–54). Compounds **3a–3d** were synthesized in a procedure similar to described. To a solution of **3a–3d** (1.0 mmole) with the corresponding phenylacetic acids (**38a–38c**) or phenylpropionic acids (**39a–39c**) (each 1.5 mmole) in CH_2Cl_2 (20 mL), respectively, were added successively coupling agents HBTU (1.5 mmol) and DIEA (3.0 mmol). The reaction mixture was stirred at room temperature for 24 h. The solvent was evaporated at reduced pressure. The residue was purified by silica gel column chromatography using a mixture of *n*-hexane–acetone to afford the products.

*5.1.3.1. Methyl 2-(2-(2-bromophenyl)acetamido)benzoate (**42**).* 24% yield. Pale yellow powder, Mp: 88–90 °C. ^{1}H NMR ($CDCl_3$) δ 11.03 (1H, br. s, NH), 8.73 (1H, d, J = 8.4 Hz, H-3), 7.98 (1H, dd, J = 8.0, 1.2 Hz, H-6), 7.61 (1H, d, J = 8.0 Hz, H-6′), 7.51 (1H, td, J = 8.4, 1.2 Hz, H-4), 7.43 (1H, dd, J = 7.2, 1.2 Hz, H-5′), 7.35 (1H, t, J = 7.2 Hz, H-4′), 7.19 (1H, td, J = 8.0, 1.2 Hz, H-3′), 7.06 (1H, t, J = 8.0 Hz, H-5), 3.94 (2H, s, Ar-C$\underline{H}_2$-NCO), 3.84 (3H, s, OC$\underline{H}_3$). ^{13}C NMR ($CDCl_3$) δ 169.1 (s, -$\underline{C}$OOCH$_3$), 168.7(s, -CONH-), 141.6(s, C-2), 134.9(s, C-1′), 134.7 (d, C-4), 133.5 (d, C-3′), 131.2 (d, C-4′), 129.5 (d, C-6′), 128.3 (d, C-5′), 125.8 (d, C-5), 123.1 (d, C-3), 120.9 (s, C-2′), 115.7 (s, C-1), 52.6 (q, -COO$\underline{C}$H$_3$), 46.12 (t, $\underline{C}$H$_2$). ESI-MS (*m*/*z*,%): 370 [M + Na]$^+$, 372 [M + 2 + Na]$^+$. HRESI-MS *m*/*z* 370.0055 [M + Na]$^+$ (calcd for $C_{16}H_{14}BrNO_3Na$ 370.0057).

*5.1.3.2. Ethyl 2-(2-(2-fluorophenyl)acetamido)benzoate (**43**).* 25% yield. Yellow oil. ^{1}H NMR ($CDCl_3$) δ 11.18 (1H, br. s, NH), 8.71 (1H, d, J = 8.0 Hz, H-3), 8.01 (1H, dd, J = 8.0, 1.6 Hz, H-6), 7.51 (1H, td, J = 8.0, 1.6 Hz, H-6′), 7.39 (1H, dd, J = 8.0, 1.6 Hz, H-4), 7.29 (1H, m, H-4′), 7.15 (1H, t, J = 8.0 Hz, H-5′), 7.10 (1H, t, J = 9.6 Hz, H-3′), 7.06 (1H, t, J = 8.0 Hz, H-5), 4.36 (2H, q, J = 7.2 Hz, OC$\underline{H}_2$CH$_3$), 3.84 (2H, s, Ar-C$\underline{H}_2$-NCO), 1.40 (3H, t, J = 7.2 Hz, OCH$_2$C$\underline{H}_3$). ^{13}C NMR ($CDCl_3$) δ 169.3 (s, -$\underline{C}$OOCH$_2$CH$_3$), 168.4 (s, -CONH-), 162.8 (s, C-2′, $J_{C\text{-}F}$ = 245 Hz), 141.78 (s, C-2), 134.8 (d, C-4), 132.2 (d, C-4′, $J_{C\text{-}F}$ = 4 Hz), 131.1 (d, C-6), 129.7 (d, C-6′, $J_{C\text{-}F}$ = 8 Hz), 124,8 (d, C-5′, $J_{C\text{-}F}$ = 4 Hz), 123.0 (d, C-5), 122.1 (s, C-1′, $J_{C\text{-}F}$ = 16 Hz), 120.8 (d, C-3), 116.0 (s, C-1), 115.9 (d, C-3′, $J_{C\text{-}F}$ = 4 Hz), 61.7 (t, -COO$\underline{C}$H$_2$CH$_3$), 39.1 (t, CH$_2$, J = 3 Hz), 14.6 (q, -COOCH$_2$$\underline{C}H_3$). ESI-MS (*m*/*z*,%): 324 [M + Na]$^+$. HRESI-MS *m*/*z* 324.1012 [M + Na]$^+$ (calcd for $C_{17}H_{16}FNO_3Na$ 324.1010).

*5.1.3.3. Ethyl 2-(2-(2-chlorophenyl)acetamido)benzoate (**44**).* 22% yield. pale yellow powder, mp 59–61 °C. ^{1}H NMR ($CDCl_3$) δ 11.12 (1H, br. s, NH), 8.72 (1H, d, J = 8.0 Hz, H-3), 8.03 (1H, dd, J = 8.0, 1.2 Hz, H-6), 7.51 (1H, td, J = 8.0, 1.2 Hz, H-4), 7.42 (2H, m, H-3′, 6′), 7.28 (2H, m, H-4′, 5′), 7.07 (1H, t, J = 8.0 Hz, H-5), 4.31 (2H, q, J = 6.8 Hz, OC$\underline{H}_2$CH$_3$), 3.92 (2H, s, Ar-C$\underline{H}_2$-NCO), 1.37 (3H, t, J = 6.8 Hz, OCH$_2$C$\underline{H}_3$). ^{13}C NMR ($CDCl_3$) δ 169.2 (s, -$\underline{C}$OOCH$_2$CH$_3$), 168.4 (s, -CONH-), 141.7 (s, C-2), 135.2 (s, C-1′), 134.8 (s, C-4), 133.0 (d, C-6), 132.3 (d, C-4′), 131.1 (s, C-2′), 130.1 (d, C-3′), 129.3 (d, C-6′), 127.6 (d, C-5′), 123.0 (d, C-5), 120.9 (d, C-3), 116.0 (s, C-1), 61.7 (t, -COO$\underline{C}$H$_2$CH$_3$), 43.6 (t, CH$_2$), 14.6 (q, -COOCH$_2$$\underline{C}H_3$). ESI-MS (*m*/*z*,%): 340 [M + Na]$^+$, 342 [M + 2 + Na]$^+$. HRESI-MS *m*/*z* 340.0716 [M + Na]$^+$ (calcd for $C_{17}H_{16}ClNO_3Na$ 340.0713).

*5.1.3.4. Ethyl 2-(2-(2-bromophenyl)acetamido)benzoate (**45**).* 24% yield. pale yellow powder, mp 83–85 °C. ^{1}H NMR ($CDCl_3$) δ 11.13 (1H, br. s, NH), 8.75 (1H, d, J = 7.6 Hz, H-3), 8.02 (1H, dd, J = 7.6, 1.2 Hz, H-6), 7.62 (1H, d, J = 8.0 Hz, H-3′), 7.53 (1H, td, J = 7.6, 1.2 Hz, H-4), 7.45 (1H, dd, J = 8.0, 1.6 Hz, H-6′), 7.36 (1H, t, J = 8.0 Hz, H-5′), 7.20 (1H, td, J = 8.0, 1.6 Hz, H-4′), 7.08 (1H, t, J = 7.6 Hz, H-5), 4.32 (2H, q, J = 7.2 Hz OC$\underline{H}_2$CH$_3$), 3.96 (2H, s, Ar-C$\underline{H}_2$-NCO), 1.39 (3H, t, J = 7.2 Hz, OCH$_2$C$\underline{H}_3$). ^{13}C NMR ($CDCl_3$) δ 169.0 (s, -$\underline{C}$OOCH$_2$CH$_3$), 168.3 (s, -CONH-), 141.7 (s, C-2), 134.8 (s, C-1′), 133.4 (s, C-4), 132.4 (s, C-3′), 131.1 (s, C-4′), 129.5 (s, C-6′), 128.2 (s, C-5′), 125.8 (s, C-5), 123.0 (s, C-3), 123.9 (s, C-2), 116.0 (s, C-1), 61.7 (s, -COO$\underline{C}$H$_2$CH$_3$), 46.1 (s, CH$_2$), 14.6 (s, -COOCH$_2$$\underline{C}H_3$). ESI-MS (*m*/*z*,%): 384 [M + Na]$^+$, 386 [M + 2 + Na]$^+$. HRESI-MS *m*/*z* 384.0211 [M + Na]$^+$ (calcd for $C_{17}H_{16}BrNO_3Na$ 384.0214).

*5.1.3.5. Propyl 2-(2-(2-fluorophenyl)acetamido)benzoate (**46**).* 38% yield. Pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 11.23 (1H, br. s, NH), 8.74 (1H, d, J = 8.8 Hz, H-3), 8.03 (1H, dd, J = 8.0, 1.6 Hz, H-6), 7.53 (1H, td, J = 8.8, 1.6 Hz, H-4), 7.42 (1H, td, J = 8.0, 1.2 Hz, H-6′), 7.31 (1H, m, H-4′), 7.17 (1H, td, J = 8.0, 1.2 Hz, H-5′), 7.12 (1H, dd, J = 8.8, 1.2 Hz, H-3′), 7.08 (1H, t, J = 8.0 Hz, H-5), 4.26 (2H, t, J = 6.8 Hz, OC$\underline{H}_2$CH$_2$CH$_3$), 3.84 (2H, s, Ar-C$\underline{H}_2$-NCO), 1.80 (2H, qt, J = 7.6, 6.8 Hz, OCH$_2$C$\underline{H}_2$CH$_3$), 1.05 (3H, t, J = 7.6 Hz, OCH$_2$CH$_2$C$\underline{H}_3$). ^{13}C NMR ($CDCl_3$) δ 169.3 (s, -COOCH$_2$CH$_2$CH$_3$), 168.5 (s, -CONH-), 162.8 (s, C-2′, $J_{C\text{-}F}$ = 244 Hz), 141.8 (s, C-2), 134.8 (d, C-4), 132.2 (d, C-4′, $J_{C\text{-}F}$ = 4 Hz), 131.1 (d, C-6), 129.7 (d, C-6′, $J_{C\text{-}F}$ = 8 Hz), 124.9 (d, C-5′, $J_{C\text{-}F}$ = 4 Hz), 123.0 (d, C-5), 122.2 (d, C-1′, $J_{C\text{-}F}$ = 15 Hz), 120.8 (d, C-3), 116.1 (d, C-3′, $J_{C\text{-}F}$ = 16 Hz), 115.9 (s, C-1), 67.3 (t, -COO$\underline{C}$H$_2$CH$_2$CH$_3$), 39.1 (t, $J_{C\text{-}F}$ = 2 Hz, CH$_2$), 22.4 (t, -COOCH$_2$$\underline{C}H_2CH_3$), 10.9 (q, -COOCH$_2CH_2$$\underline{C}H_3$). ESI-MS (*m*/*z*,%): 338 [M + Na]$^+$. HRESI-MS *m*/*z* 338.1168 [M + Na]$^+$ (calcd for $C_{18}H_{18}FNO_3Na$ 338.1165).

*5.1.3.6. Propyl 2-(2-(2-chlorophenyl)acetamido)benzoate (**47**).* 40% yield. pale yellow powder, mp 101–103 °C. ^{1}H NMR ($CDCl_3$) δ 11.16 (1H, br. s, NH), 8.75 (1H, d, J = 8.0 Hz, H-3), 8.02 (1H, dd, J = 8.0, 1.2 Hz, H-6, H-6), 7.53 (1H, td, J = 8.0, 1.2 Hz, H-4), 7.44 (2H, m, H-3′, 6′), 7.29 (2H, m, H-4′, 5′), 7.08 (1H, t, J = 8.0 Hz, H-5), 4.32 (2H, t, J = 6.8 Hz, OC$\underline{H}_2$CH$_2$CH$_3$), 3.94 (2H, s, Ar-C$\underline{H}_2$-NCO), 1.78 (2H, qt, J = 7.2, 6.8 Hz, OCH$_2$C$\underline{H}_2$CH$_3$), 1.04 (3H, t, J = 7.2 Hz, OCH$_2$CH$_2$C$\underline{H}_3$). ^{13}C NMR ($CDCl_3$) δ 169.2 (s, -$\underline{C}$OOCH$_2$CH$_2$CH$_3$), 168.4 (s, -CONH-), 141.7 (s, C-2), 135.2 (s, C-1′), 134.8 (d,C-4), 133.0 (s, C-2′), 132.3 (d, C-6), 131.0 (d, C-4′), 130.1(d, C-3′), 129.3 (d, C-6′), 127.6 (d, C-5′), 123.0 (d, C-5), 120.9 (d, C-3), 116.0 (s, C-1), 67.2 (t, -COO$\underline{C}$H$_2$CH$_2$CH$_3$), 43.6 (t, CH$_2$), 22.3 (t, -COOCH$_2$$\underline{C}H_2CH_3$), 10.9 (q, -COOCH$_2CH_2$$\underline{C}H_3$). ESI-MS (*m*/*z*,%): 354 [M + Na]$^+$, 356 [M + 2 + Na]$^+$. HRESI-MS *m*/*z* 354.0873 [M + Na]$^+$ (calcd for $C_{18}H_{18}ClNO_3Na$ 354.0870).

*5.1.3.7. Propyl 2-(2-(2-bromophenyl)acetamido)benzoate (**48**).* 40% yield. Pale yellow needles, mp 57–59 °C. ^{1}H NMR ($CDCl_3$) δ 11.14 (1H, br. s, NH), 8.75 (1H, d, J = 8.0 Hz, H-3), 8.03 (1H, dd, J = 8.0, 1.2 Hz, H-6), 7.63 (1H, d, J = 8.0 Hz, H-6′), 7.54 (1H, td, J = 8.4, 1.2 Hz, H-4), 7.45 (1H, dd, J = 7.6, 1.2 Hz, H-3′), 7.36 (1H, td, J = 8.4, 1.2 Hz, H-5′), 7.20 (1H, td, J = 7.6, 1.2 Hz,H-4′), 7.09 (1H, t, J = 7.6 Hz, H-5), 4.23 (2H, t, J = 6.8 Hz, OC$\underline{H}_2$CH$_2$CH$_3$), 3.96 (2H, s, Ar-C$\underline{H}_2$-NCO), 1.80 (2H, qt, J = 7.2, 6.8 Hz, OCH$_2$C$\underline{H}_2$CH$_3$), 1.05 (3H, t, J = 7.2 Hz, OCH$_2$CH$_2$C$\underline{H}_3$). ^{13}C NMR ($CDCl_3$) δ 169.1 (s, -$\underline{C}$OOCH$_2$CH$_2$CH$_3$), 168.4 (s, -CONH-), 141.7 (s, C-2), 134.8 (d, C-4), 134.8 (s, C-1′), 133.4 (d, C-3′), 132.4 (d, C-6), 131.1 (d, C-4′), 129.5 (d, C-6′), 128.2 (d, C-5′), 125.8 (d, C-5), 123.0 (d, C-3), 120.1 (s, C-2′), 116.0 (s, C-1),

67.2 (t, -COOCH$_2$CH$_2$CH$_3$), 46.1 (t, -COOCH$_2$CH$_2$CH$_3$), 22.4 (t, CH$_2$), 10.9 (q, -COOCH$_2$CH$_2$CH$_3$). ESI-MS (*m/z*,%): 398 [M + Na]$^+$, 400 [M + 2 + Na]$^+$. HRESI-MS *m/z* 398.0368 [M + Na]$^+$ (calcd for $C_{18}H_{18}BrNO_3Na$ 398.0369).

*5.1.3.8. Butyl 2-(2-(2-fluorophenyl)acetamido)benzoate (**49**).* 62% yield. Pale yellow powder, mp 48–50 °C. ^{1}H NMR ($CDCl_3$) δ 11.20 (1H, br. s, NH), 8.72 (1H, d, J = 8.4 Hz, H-3), 7.95 (1H, d, J = 7.6 Hz, H-6), 7.42 (1H, t, J = 8.4 Hz, H-4), 7.36 (1H, t, J = 7.6 Hz, H-6′), 7.24 (1H, ddd, J = 7.2, 6.4, 1.6 Hz, H-4′), 7.10 (1H, t, J = 7.6 Hz, H-5′), 7.07 (1H, t, J = 9.2 Hz, H-3′), 7.00 (1H, t, J = 7.6 Hz, H-5), 4.23 (2H, t, J = 6.8 Hz, OCH$_2$CH$_2$CH$_2$CH$_3$), 3.78 (2H, s, Ar-CH$_2$-NCO), 2.67 (2H, q, J = 8.0, 6.8 Hz, OCH$_2$CH$_2$CH$_2$CH$_3$), 1.42 (2H, qt, J = 8.0, 7.2 Hz, OCH$_2$CH$_2$CH$_2$CH$_3$), 0.95 (3H, t, J = 7.2 Hz, OCH$_2$CH$_2$CH$_2$CH$_3$). ^{13}C NMR ($CDCl_3$) δ 169.1 (s, -CONH-), 168.4 (s, -COOCH$_2$CH$_2$CH$_2$CH$_3$), 161.6 (s, C-2′, $J_{C\text{-}F}$ = 245 Hz), 141.1 (s, C-2), 134.0 (d, C-4), 131.5 (d, C-6′, $J_{C\text{-}F}$ = 3 Hz), 130.3 (d, C-6), 128.9 (s, C-4′, $J_{C\text{-}F}$ = 8 Hz), 124.0 (d, C-5′, $J_{C\text{-}F}$ = 3 Hz), 122.2 (d, C-5), 121.4 (d, C-1′, $J_{C\text{-}F}$ = 16 Hz), 120.0 (d, C-3), 115.3 (d, C-3′), 115.0 (d, C-1), 65.5 (t, -COOCH$_2$CH$_2$CH$_2$CH$_3$), 40.0 (t, CH$_2$, J = 2 Hz), 31.0 (t, -COOCH$_2$CH$_2$CH$_2$CH$_3$), 19.6 (t, -COOCH$_2$CH$_2$CH$_2$CH$_3$), 14.1 (q, -COOCH$_2$CH$_2$CH$_2$CH$_3$). ESI-MS (*m/z*,%): 330 [M + H]$^+$. HRESI-MS *m/z* 330.1504 [M + H]$^+$ (calcd for $C_{19}H_{21}FNO_3$ 330.1505).

*5.1.3.9. Butyl 2-(2-(2-chlorophenyl)acetamido)benzoate (**50**).* 61% yield. pale yellow powder, mp 49–51 °C. ^{1}H NMR ($CDCl_3$) δ 11.14 (1H, br. s, NH), 8.74 (1H, d, J = 8.0 Hz, H-3), 7.94 (1H, d, J = 8.0 Hz, H-6), 7.43 (1H, td, J = 8.0 Hz, 1.2 Hz, H-4), 7.38 (1H, d, J = 7.6 Hz, H-6′), 7.36 (1H, dd, J = 8.0, 1.2 Hz, H-3′), 7.23 (1H, td, J = 7.6, 1.6 Hz, H-5′), 7.69 (1H, td, J = 7.6, 2.0 Hz, H-4′), 6.99 (1H, t, J = 8.0 Hz, H-5), 4.20 (2H, t, J = 6.8 Hz, OCH$_2$CH$_2$CH$_2$CH$_3$), 3.89 (2H, s, Ar-CH$_2$-NCO), 1.66 (2H, tt, J = 7.2, 6.8 Hz, OCH$_2$CH$_2$CH$_2$CH$_3$), 1.41 (2H, qt, J = 7.2, 6.8 Hz, OCH$_2$CH$_2$CH$_2$CH$_3$), 0.95 (3H, t, J = 6.8 Hz, OCH$_2$CH$_2$CH$_2$CH$_3$). ^{13}C NMR ($CDCl_3$) δ 169.0 (s, -CONH-), 168.3 (s, -COOCH$_2$CH$_2$CH$_2$CH$_3$), 141.8 (s, C-2), 135.1 (d, C-4), 134.7 (s, C-1′), 133.0 (s, C-2′), 132.3 (d, C-6), 131.0 (d, C-4′), 130.0 (d, C-3′), 129.2 (d, C-6′), 127.6 (d, C-5′), 122.9 (d, C-5), 120.7 (d, C-3), 115.8 (s, C-1), 65.4 (t, -COOCH$_2$CH$_2$CH$_2$CH$_3$), 43.5 (t, CH$_3$), 31.0 (t, -COOCH$_2$CH$_2$CH$_2$CH$_3$), 19.6 (t, -COOCH$_2$CH$_2$CH$_2$CH$_3$), 14.1 (q, -COOCH$_2$CH$_2$CH$_2$CH$_3$). ESI-MS (*m/z*,%): 346 [M + H]$^+$, 348 [M + 2 + H]$^+$. HRESI-MS *m/z* 346.1207 [M + Na]$^+$ (calcd for $C_{19}H_{21}ClNO_3$ 346.1209).

*5.1.3.10. Butyl 2-(2-(2-bromophenyl)acetamido)benzoate (**51**).* 74% yield. yellow powder, mp 41–43 °C. ^{1}H NMR ($CDCl_3$) δ 11.12 (1H, br. s, NH), 8.74 (1H, d, J = 8.0 Hz, H-3), 7.94 (1H, d, J = 8.0 Hz, H-6), 7.54 (1H, d, J = 8.0 HzH-3′), 7.44 (1H, t, J = 7.6 Hz, H-4), 7.39 (1H, d, J = 8.0 Hz, H-6′), 7.28 (1H, t, J = 7.6 Hz, H-5′), 7.11 (1H, t, J = 7.6 Hz, H-4′), 7.00 (1H, t, J = 7.6 Hz, H-5), 4.20 (2H, t, J = 6.8 Hz, OCHCH$_2$CH$_2$CH$_3$), 3.90 (2H, s, Ar-CH$_2$-NCO), 1.67 (2H, tt, J = 7.6, 6.8 Hz, OCHCH$_2$CH$_2$CH$_3$), 1.42 (2H, qt, J = 7.6, 6.8 Hz, OCHCH$_2$CH$_2$CH$_3$), 0.94 (3H, t, J = 6.8 Hz, OCHCH$_2$CH$_2$CH$_3$). ^{13}C NMR ($CDCl_3$) δ 168.9 (s, -CONH-), 168.3 (s, -COOCH$_2$CH$_2$CH$_2$CH$_3$), 141.8 (s, C-2), 134.8 (d, C-4), 134.7 (d, C-1′), 133.3 (d, C-3′), 132.4 (d, C-6), 131.0 (d, C-4′), 129.5 (d, C-6′), 128.2 (d, C-5′), 125.7 (d, C-5), 122.9 (d, C-3), 120.7 (s, C-2′), 115.9 (s, C-1), 65.4 (t, -COOCH$_2$CH$_2$CH$_2$CH$_3$), 46.0 (t, CH$_2$), 31.0 (t, -COOCH$_2$CH$_2$CH$_2$CH$_3$), 19.6 (t, -COOCH$_2$CH$_2$CH$_2$CH$_3$), 14.2 (q, -COOCH$_2$CH$_2$CH$_2$CH$_3$). ESI-MS (*m/z*,%): 390 [M + H]$^+$, 392 [M + 2 + H]$^+$. HRESI-MS *m/z* 390.0706 [M + H]$^+$ (calcd for $C_{19}H_{21}ClNO_3$ 390.0705).

*5.1.3.11. Ethyl 2-(3-(2-fluorophenyl)propanamido)benzoate (**52**).* 39% yield. Pale yellow powder, mp 58–60 °C. ^{1}H NMR ($CDCl_3$) δ 11.12 (1H, br. s, NH), 8.71 (1H, d, J = 8.0 Hz, H-3), 8.02 (1H, dd, J = 8.0, 1.2 Hz, H-6), 7.52 (1H, td, J = 8.8, 1.6 Hz, H-6′), 7.26 (1H, td, J = 8.0, 1.2 Hz, H-4), 7.17 (1H, m, H-4′), 7.03 (3H, m, H-5, 3′, 5′), 4.35 (2H, q, J = 6.8 Hz, OCH$_2$CH$_3$), 3.10 (2H, t, J = 8.0 Hz, CH$_2$CH$_2$), 2.76 (2H, t, J = 8.0 Hz, CH$_2$CH$_2$), 1.40 (3H, t, J = 6.8 Hz, OCH$_2$CH$_3$). ^{13}C NMR ($CDCl_3$) δ 171.2 (s, -COOCH$_2$CH$_3$), 168.6 (s, -CONH-), 161.6 (s, C-2′, $J_{C\text{-}F}$ = 244 Hz), 141.9 (s, C-2), 134.9 (s, C-4), 131.2 (d, C-6′, $J_{C\text{-}F}$ = 6 Hz), 131.1 (d, C-6), 128.4 (d, C-4′, $J_{C\text{-}F}$ = 8 Hz), 127.9 (s, C-1′, $J_{C\text{-}F}$ = 15 Hz), 124.5 (d, C-5′, $J_{C\text{-}F}$ = 4 Hz), 122.7 (d, C-5), 120.8 (d, C-3), 115.8 (s, C-3′, $J_{C\text{-}F}$ = 22 Hz), 115.6 (d, C-1), 61.8 (t, -COOCH$_2$CH$_3$), 38.9 (t, CH$_2$CH$_2$), 25.3 (t, CH$_2$CH$_2$, J = 3 Hz), 14.6 (q, -COOCH$_2$CH$_3$). ESI-MS (*m/z*,%): 316 [M + H]$^+$. HRESI-MS *m/z* 316.1346 [M + H]$^+$ (calcd for $C_{18}H_{19}FNO_3$ 316.1349).

*5.1.3.12. Ethyl 2-(3-(2-chlorophenyl)propanamido)benzoate (**53**).* 37% yield. White powder, mp 48–50 °C. ^{1}H NMR ($CDCl_3$) δ 11.13 (1H, br. s, NH), 8.72 (1H, d, J = 8.4 Hz, H-3), 8.06 (1H, dd, J = 8.0, 1.2 Hz, H-6), 7.51 (1H, td, J = 8.4, 1.2 Hz, H-4), 7.38 (1H, dd, J = 7.2, 1.2 Hz, H-3′), 7.29 (1H, dd, J = 7.2, 1.6 Hz, H-6′), 7.15 (1H, td, J = 7.2, 1.2 Hz, H-5′), 7.12 (1H, td, J = 7.2, 1.6 Hz, H-4′), 7.04 (1H, t, J = 8.0 Hz, H-5), 4.39 (2H, q, J = 7.2 Hz, OCH$_2$CH$_3$), 3.18 (2H, t, J = 8.0 Hz, CH$_2$CH$_2$), 2.76 (2H, t, J = 8.0 Hz, CH$_2$CH$_2$), 1.38 (3H, t, J = 7.2 Hz, OCH$_2$CH$_3$). ^{13}C NMR ($CDCl_3$) δ 171.2 (s, -COOCH$_2$CH$_3$), 168.6 (s, -CONH-), 141.9 (s, C-2), 138.6 (s, C-4), 134.9 (s, C-1′), 134.4 (d, C-6), 131.2 (s, C-2′), 131.0 (d, C-4′), 130.0 (d, C-3′), 128.2 (d, C-6′), 127.3 (d, C-5′), 122.7 (d, C-5), 120.7 (d, C-3), 115.5 (s, C-1), 61.8 (t, -COOCH$_2$CH$_3$), 38.5 (t, CH$_2$CH$_2$), 29.7 (t, CH$_2$CH$_2$), 14.6 (q, -COOCH$_2$CH$_3$). ESI-MS (*m/z*,%): 332 [M + H]$^+$, 334[M + 2 + H]$^+$. HRESI-MS *m/z* 332.1054 [M + H]$^+$ (calcd for $C_{18}H_{19}ClNO_3$ 332.1053).

*5.1.3.13. Ethyl 2-(3-(2-bromophenyl)propanamido)benzoate (**54**).* 40% yield. White powder, mp 49–51 °C. ^{1}H NMR ($CDCl_3$) δ 11.13 (1H, br. s, NH), 8.72 (1H, d, J = 7.6 Hz, H-3), 8.00 (1H, d, J = 7.6 Hz, H-6), 7.51 (1H, d, J = 8.0 Hz, H-3′), 7.50 (1H, d, J = 7.6 Hz, H-6′), 7.29 (1H, d, J = 7.6 Hz, H-5′), 7.20 (1H, t, J = 8.0 Hz, H-4′), 7.04 (2H, t, J = 7.6 Hz, H-4, 5), 4.34 (2H, q, J = 7.2 Hz, OCH$_2$CH$_3$), 3.18 (2H, t, J = 8.0 Hz, CH$_2$CH$_2$), 2.76 (2H, t, J = 8.0 Hz, CH$_2$CH$_2$), 1.38 (3H, t, J = 7.2 Hz, OCH$_2$CH$_3$). ^{13}C NMR ($CDCl_3$) δ 171.1 (s, -COOCH$_2$CH$_3$), 168.6 (s, -CONH-), 141.9 (s, C-2), 140.4 (s, C-1′), 134.9 (d, C-4), 133.3 (d, C-3′), 131.2 (d, C-6), 131.0 (d, C-4′), 128.4 (d, C-6′), 128.0 (d, C-5′), 124.8 (d, C-5), 122.8 (d, C-3), 120.7 (s, C-2′), 115.5 (s, C-1), 61.8 (t, -COOCH$_2$CH$_3$), 38.6 (t, CH$_2$CH$_2$), 32.3 (t, CH$_2$CH$_2$), 14.6 (q, -COOCH$_2$CH$_3$). ESI-MS (*m/z*,%): 376 [M + H]$^+$, 378 [M + 2 + H]$^+$. HRESI-MS *m/z* 376.0546 [M + H]$^+$ (calcd for $C_{18}H_{19}BrNO_3$ 376.0548).

5.1.4. (E)-Ethyl 2-(3-(2-fluorophenyl)acrylamido)benzoate (56). Compound **56** was synthesized in 5% yield from 2-fluorocinnamic acid (**55**, 1.5 mmole) in a procedure similar to **54**. White powder, mp 108–110 °C. ^{1}H NMR ($CDCl_3$) δ 11.43 (1H, br. s, NH), 8.87 (1H, d, J = 8.8 Hz, H-3), 8.08 (1H, dd, J = 8.0, 1.2 Hz, H-6), 7.88 (1H, d, J = 16.0 Hz, H-α), 7.59 (2H, m, H-4, 6′), 7.35 (1H, ddd, J = 8.0, 5.8, 1.6 Hz, H-4′), 7.53 (1H, t, J = 8.0 Hz, H-5′), 7.13 (1H, dd, J = 10.0, 8.0 Hz, H-3′), 7.11 (1H, t,

J = 8.0 Hz, H-5), 6.74 (1H, d, J = 16.0 Hz, H-β), 4.41 (2H, q, J = 7.2 Hz, OC$\underline{H}_2$CH$_3$), 1.44 (3H, t, J = 7.2 Hz, OCH$_2$C$\underline{H}_3$). ^{13}C NMR ($CDCl_3$) δ 168.9 (s, -$\underline{C}$OOCH$_2$CH$_3$), 164.7 (s, -CONH-), 161.8 (s, $J_{C\text{-}F}$ = 253 Hz, C-2′), 142.3 (s, C-2), 135.4 (d, C-α), 135.0 (d, C-4), 131.7 (d, $J_{C\text{-}F}$ = 9 Hz, C-4′), 131.3 (d, C-6), 129.8 (d, C-5′), 125.1 (d, $J_{C\text{-}F}$ = 6 Hz, C-3′), 124.8 (d, $J_{C\text{-}F}$ = 3 Hz, C-6′), 123.2 (s, $J_{C\text{-}F}$ = 11 Hz, C-1′), 123.0 (d, C-5), 121.0 (d, C-3), 116.6 (d, C-β), 115.7 (s, C-1), 61.9 (t, -COO$\underline{C}$H$_2$CH$_3$), 14.6 (q, -COOCH$_2\underline{C}$H$_3$). ESI-MS (m/z,%): 314 [M + H]$^+$. HRESI-MS m/z 314.1194 [M + H]$^+$ (calcd for $C_{18}H_{17}FNO_3$ 314.1192).

5.2. Biological assays

5.2.1. Preparation of human neutrophils[36]. Blood was taken from healthy human donors (20–32 years old) by venipuncture, using a protocol approved by the institutional review board at Chang Gung Memorial Hospital. Neutrophils were isolated with a standard method of dextran sedimentation prior to centrifugation in a Ficoll Hypaque gradient and hypotonic lysis of erythrocytes.[36] Purified neutrophils that contained >98% viable cells, as determined by the trypan blue exclusion method, were resuspended in calcium (Ca^{2+})-free HBSS buffer at pH 7.4, and were maintained at 4 °C before use.

5.2.2. Measurement of $O_2^{\bullet-}$ generation[36]. The assay of $O_2^{\bullet-}$ generation was based on the SOD-inhibitable reduction of ferricytochrome *c*.[36] In brief, after supplementation with 0.5 mg ml^{-1} ferricytochrome *c* and 1 mM Ca^{2+}, neutrophils were equilibrated at 37 °C for 2 min and incubated with drugs for 5 min. Cells were activated with 100 nM FMLP for 10 min. When FMLP was used as a stimulant, CB (1 μg ml^{-1}) was incubated for 3 min before activation by the peptide (FMLP/CB). Changes in absorbance with the reduction of ferricytochrome *c* at 550 nm were continuously monitored in a double-beam, six-cell positioner spectrophotometer with constant stirring (Hitachi U-3010, Tokyo, Japan). Calculations were based on the differences in the reactions with and without SOD (100 U ml^{-1}) divided by the extinction coefficient for the reduction of ferricytochrome *c* (ε = 21.1/mM/10 mm).

5.2.3. Measurement of elastase release. Degranulation of azurophilic granules was determined by elastase release as described previously.[36] Experiments were performed using MeO-Suc-Ala-Ala-Pro-Val-*p*-nitroanilide as the elastase substrate. Briefly, after supplementation with MeO-Suc-Ala-Ala-Pro-Val-*p*-nitroanilide (100 μM), neutrophils (5×10^5 ml^{-1}) were equilibrated at 37 °C for 2 min and incubated with drugs for 5 min. Cells were activated by100 nM FMLP and 0.5 μg ml^{-1} CB, and changes in absorbance at 405 nm were continuously monitored to assay elastase release. The results are expressed as the percent of the initial rate of elastase release in the FMLP/CB-activated, drug-free control system.

5.2.4. Determination of cAMP concentrations[36,43]. cAMP levels were assayed using an enzyme immunoassay kit (Amersham Biosciences, Buckinghamshire, UK). Human neutrophils were incubated with drugs for the indicated time before stimulation with or without FMLP for another 1 min, and the reaction was terminated by adding 0.5% dodecyltrimethylammonium bromide. Samples were then centrifuged at 3000 × *g* for 5 min at 4 °C. The supernatants were used as a source for the cAMP samples. The assay was performed according to the manufacturer's instructions.

5.2.5. Phosphodiesterase assay[39,40]. PDE activity was modified and determined as the method.[39,40] Washed human platelets were used for both PDE3 and PDE5 analyses and human U937 cells for PDE4. Purified protein containing PDE3, 4 or 5 enzyme and concentration of each test compounds were resuspended in 50 mM Tris–HCl containing 5 mM $MgCl_2$ (pH 7.5). Subsequently, the enzyme (11.5 mg mL^{-1}, 10 μL) was incubated with Tris–HCl (80 μL) and 10 μM cyclic GMP or cyclic AMP substrate (final concentration 1.01 μM containing 0.01 μM [^{3}H]-cyclic GMP or [^{3}H]-cyclic AMP) was added. After 15 min at 25 °C, the samples were heated to 100 °C for 2 min. The reaction was terminated by boiling for 2 min and the resulting AMP was converted to adenosine by the addition of 10 mg mL^{-1} (10 μL) snake venom nucleotidase and further incubation at 25 °C for 20 min. An AGI-X2 resin (200 ml) was added to bind all unconverted cyclic GMP or cyclic AMP. After centrifugation, the supernatant was removed for determination of radiolabelled guanosine or adenosine by a liquid scintillation counter. The test compounds were dissolved separately in DMSO and tested at concentrations of 300, 100, 30, 10, 3, 1, and 0.3 μM, respectively.

5.2.6. Statistical analysis. Results are expressed as the mean ± S.E.M. Data were analysed using the GraphPad Prism software (GraphPad Software, San Diego, CA). Statistical analysis was performed using Student's *t*-test or one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. A value of $p < 0.05$ was considered statistically significant.

5.2.7. Cytotoxic assay. Compounds were tested against HepG2 cells using the MTT method as described before.[44] A total of 1×10^5 HepG2 cells were seeded in 24-well plates for 24 h and made quiescent by incubating in DMEM containing 0.2% FBS for 24 h before use and cells were incubated with control and **49** (100 μM) followed by incubating for another 24 h. For the growth rate determination, isopropanol solution mixed with tetrazolium salt was added to the wells and incubated for an additional 4 h at 37 °C.[45] The optical density of the dissolved material was measured spectrophotometrically at 570 nm, and assays were performed in triplicate.

5.2.8. Preparation of washed human platelets. Human blood anticoagulated with acid citrate dextrose (ACD) was obtained from healthy human volunteers who had not taken any drugs within the last two weeks. The platelet suspension was then prepared according to the washing procedure previously described.[32,33] Platelets were finally suspended in Tyrode's solution containing Ca^{2+} (2 mM), glucose (11.1 mM) and bovine serum albumin (3.5 mg ml^{-1}) at a concentration of 3×10^8 platelets ml^{-1}.

5.2.9. Measurement of platelet aggregation. Platelet aggregation was measured turbidimetrically with a light-transmission aggregometer (Chrono-Log Co., USA). The platelet suspension was incubated with dimethyl sulfoxide (DMSO, vehicle) or test compounds (10 μM) at 37 °C for 3 min under a stirring condition (1200 rpm) prior to the addition of thrombin as inducer. The extent of platelet aggregation was measured as the maximal increase of light transmission within 5 min after the treatment of thrombin.[32,33]

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