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Benzyloxybenzaldehyde Analogues as Novel Adenylyl Cyclase Activators

Chiung-Yun Chang,[a] Sheng-Chu Kuo,[a] Yi-Lee Lin,[a] Jih-Pyang Wang[b,*] and Li-Jiau Huang[a,*]

[a]*Graduate Institute of Pharmaceutical Chemistry, China Medical College, Taichung 404, Taiwan, ROC*
[b]*Department of Education and Research, Taichung Veterans General Hospital, Taichung 407, Taiwan, ROC*

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Abstract—Several benzyloxybenzaldehyde analogues were prepared and found to have significant inhibitory activity toward neutrophil superoxide formation. Consequently, these compounds were evaluated for cAMP-elevating capability. Among them, benzyloxybenzaldehyde (**7**), exhibiting activity equivalent to forskolin, was determined as an adenylyl cyclase activator since it elevates cAMP levels by activation of adenylyl cyclase but not by inhibition of phosphodiesterase. Having a chemical structure very different from known adenylyl cyclase activators, compound **7** is recommended by us for use as a new lead compound in the future development of adenylyl cyclase activators.

Introduction

Neutrophils play a key role in the host defense against microbial infection through the generation of reactive oxygen species and the release of lysosomal enzymes. However, these protective functions may lead to undesirable tissue damage in the host if prolonged or improperly controlled. This is probably involved in the pathogenesis of many diseases, included emphysema, acute respiratory distress syndrome, atherosclerosis, reperfusion injury, malignancy, and rheumatoid arthritis.[1] Drugs that suppress the neutrophil functions are proposed to ameliorate this tissue damage.

cAMP acts as a ubiquitous intracellular second messenger and is a key player in the signaling pathways in living cells by activating protein kinase and cyclic nucleotide-gated ion channels. Intracellular cAMP levels can be monitored by adenyl cyclase, which synthesizes cAMP, and phosphodiesterase, which hydrolyses cAMP. At least nine different mammalian isoforms of adenylyl cyclase and around 30 forms of enzymes having cAMP phosphodiesterase activity have been identified.[2,3] cAMP-elevating agents decrease the adherence of stimulated neutrophils to endothelium,[4] reduced the release of reactive oxygen species,[5] and decrease the degranulation of lysosomal enzyme.[6] In our continuing search for novel anti-inflammatory agents, we discovered the significant inhibitory effect of compound **7** on fMLP-induced neutrophil superoxide formation. Hence, several of its analogues were prepared and evaluated for their ability to evaluate cellular level of cAMP. This communication deals with the comparative pharmacological evaluation of benzyloxybenzaldehyde analogues (**7**–**11**) in terms of their cAMP-elevating activity.

Results and Discussion

Chemistry

Preparation of 1-substituted benzyloxybenzenes (7–11). As shown in Scheme 1, the starting substituted phenols (**1**–**5**) were subjected to *O*-benzylation by reacting with benzyl chloride in acetone, in the presence of K_2CO_3, to yield the corresponding 1-substituted benzyloxybenzenes (**7**–**11**).[7]

Effect on neutrophil superoxide anion formation. Neutrophils were isolated from pentobarbital-anesthetized rats (Sprague–Dawley, 300–350 g) as previously described.[8] Superoxide anion formation was measured in terms of superoxide dismutase-inhibitable cytochrome *c* reduction.[9] The absorbance change of cytochrome *c* reduction in supernatants was monitored at 550 nm in a microplate

*Corresponding authors. Tel.: +886-4-2205-3366-1007; fax: +886-4-2205-5105; e-mail: ljhuang@mail.cmc.edu.tw. Tel.: +886-4-2359-2525-2043; fax: +886-4-2359-2705; e-mail: w1994@vghtc.vghtc.gov.tw

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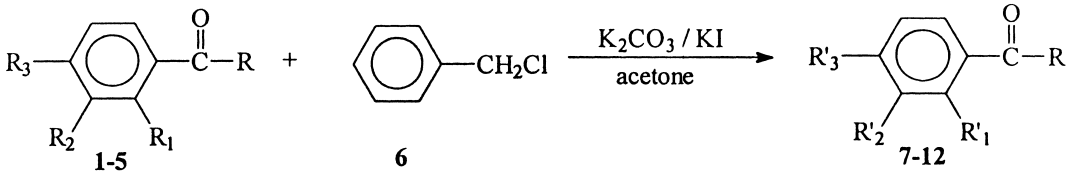
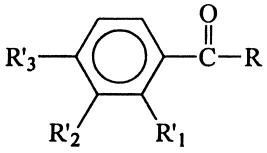


1 $R = R_2 = R_3 = H, R_1 = OH$
2 $R = R_1 = R_3 = H, R_2 = OH$
3 $R = R_1 = R_2 = H, R_3 = OH$
4 $R = CH_3, R_1 = OH, R_2 = R_3 = H$
5 $R = NH_2, R_1 = OH, R_2 = R_3 = H$
7 $R = R'_2 = R'_3 = H, R'_1 = OCH_2C_6H_5$
8 $R = R'_1 = R'_3 = H, R'_2 = OCH_2C_6H_5$
9 $R = R'_1 = R'_2 = H, R'_3 = OCH_2C_6H_5$
10 $R = CH_3, R'_1 = OCH_2C_6H_5, R'_2 = R'_3 = H$
11 $R = NH_2, R'_1 = OCH_2C_6H_5, R'_2 = R'_3 = H$

Scheme 1.

Table 1. The inhibitory effect of benzyloxybenzaldehyde analogues on the neutrophil superoxide formation (in vitro)



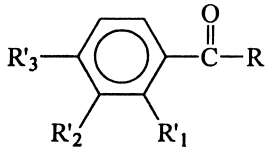
Compounds	R	R'_1	R'_2	R'_3	IC_{50} (μM)[a]
7	H	$OCH_2C_6H_5$	H	H	15.6±4.0
8	H	H	$OCH_2C_6H_5$	H	>30
9	H	H	H	$OCH_2C_6H_5$	14.8±2.3
10	CH_3	$OCH_2C_6H_5$	H	H	12.8±0.9
11	NH_2	$OCH_2C_6H_5$	H	H	19.8±1.0
Trifluoperazine					14.7±0.4

[a]Cells were treated with DMSO (as control) or test compounds. The superoxide anion formation was measured in terms of superoxide dismutase-inhibitable cytochrome *c* reduction. The values are means±SD of 3–5 separate experiments.

reader. The results in this assay were summarized in Table 1 in which compounds **7**, **9**, **10**, and **11** exhibited similar potency of inhibition of neutrophil superoxide anion formation by formyl-methionyl-leucyl-phenylalanine (fMLP). In contrast, the maximal inhibition by compound **8** did not exceed 41%. Since all the above benzyloxybenzaldehyde analogues (**7**–**11**) had a significant effect on neutrophil superoxide formation, we decided to explore the cellular mechanism of inhibition. cAMP is a well known cellular messenger. The increase in cellular cAMP levels is associated with a decrease in the production of superoxide anion by activated neutrophils.[5] We therefore determined the cAMP-elevating activity of benzyloxybenzaldehyde analogues.

cAMP-elevating activity. Neutrophils (2×10^6 cells/0.4 mL) were treated with 30 μM benzyloxybenzaldehyde analogues or forskolin, an adenylyl cyclase activator,[11] for 10 min. Supernatants were acetylated by the addition of 0.025 volumes of triethylamine/acetic anhydride (2:1, v/v). The cAMP contents of the aliquots were assayed using an enzyme immunoassay kit (Amersham). As shown in Table 2, compound **7** and forskolin significantly increased the cellular cAMP levels (about 7-fold). Both **7** and forkolin had a comparable effect on cAMP-elevating activity at various concentrations (Fig. 1). When the benzyloxyl

Table 2. Effect of benzyloxybenzaldehyde analogues on cellular cAMP levels



Compounds	R	R'_1	R'_2	R'_3	cAMP (pmol/2×10^6 cells)[a]	Ratio (relative to forskolin)
Control					0.18±0.05	
7	H	$OCH_2C_6H_5$	H	H	1.30±0.29*	1.10±0.20
8	H	H	$OCH_2C_6H_5$	H	0.56±0.18	0.46±0.07
9	H	H	H	$OCH_2C_6H_5$	0.26±0.11	0.22±0.08
10	CH_3	$OCH_2C_6H_5$	H	H	0.26±0.10	0.22±0.07
11	NH_2	$OCH_2C_6H_5$	H	H	0.29±0.13	0.24±0.10
Forskolin					1.23±0.20*	1.00

[a]Cells were treated with DMSO (as control), 30 μM benzyloxybenzaldehyde analogues or forskolin. The cAMP contents were assayed using an enzyme immunoassay kit. The values are means±SD of 4–6 separate experiments.
*$P<0.01$, compared with the control value.

group of compound **7** was moved from the *ortho* to *meta* position (**8**), the cAMP-elevating activity reduced by half. In addition, the shift of the same benzyloxy group to the *para* position (**9**) or change of the –CHO group of **7** to $-COCH_3$ (**10**) or $-CONH_2$ (**11**) resulted in nearly abolished cAMP-elevating activity. These results indicate that the inhibition of superoxide anion formation by **7**, but not other analogues, appears attributable to elevation of cellular cAMP. The mechanisms mediating the inhibition of neutrophil superoxide formation by **8**–**11** require further research.

As intracellular levels of cAMP are regulated by the rate of cAMP production by adenylyl cyclase and the rate of cAMP degradation by phosphodiesterase, we next examined the effect of **7** on phosphodiesterase activity. Phosphodiesterase isoenzymes have been classified into seven distinct families,[12] which differ in their affinities and specificity for cyclic nucleotides. Of these, type 3, 4, and 7 isoenzymes appear to be most important for the regulation of cAMP in different cell types. Type 4 is the predominant isoenzyme of cAMP phosphodiesterase in neutrophils.[10] Neutrophil cytosolic fraction (as

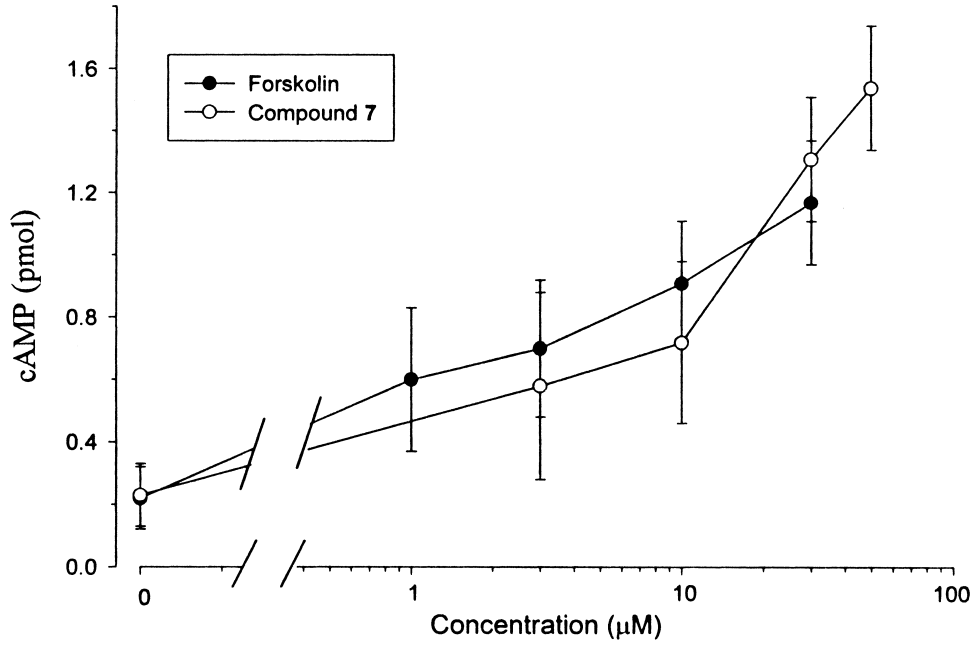


Figure 1. The concentration dependence of the increase of cellular cAMP levels by compound **7**. Cell were treated with DMSO (as control), the indicated concentrations of **7** or forskolin for 10 min. The cAMP contents were assayed using an enzyme immunoassay kit. Values are means ± SD of 3–5 separate experiments.

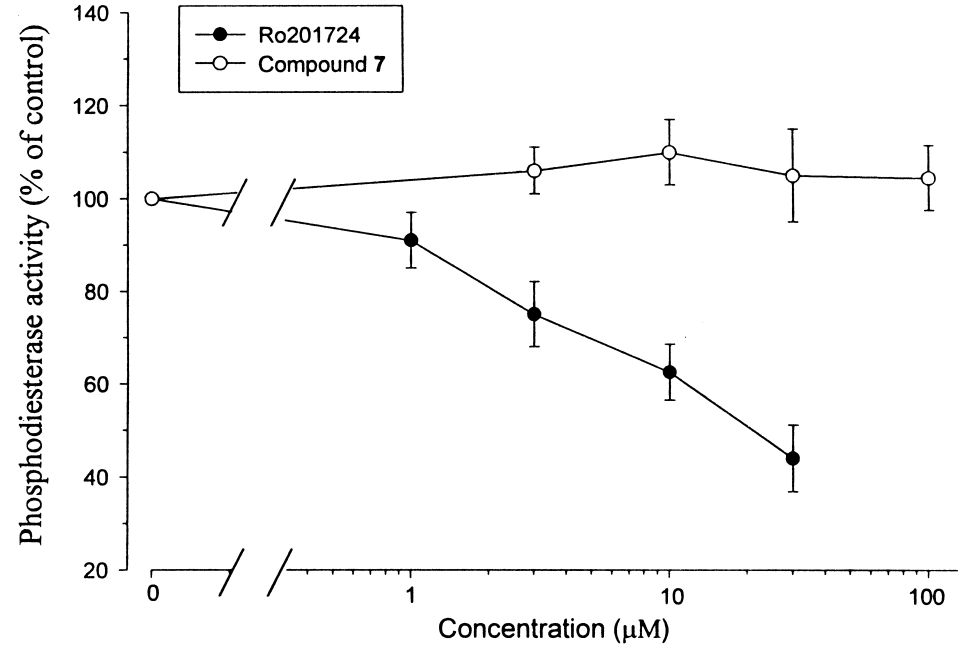


Figure 2. Effect of compound **7** on phosphodiesterase activity. Neutrophil cytosolic fractions were incubated with DMSO (as control), the indicated concentrations of **7** or Ro201724 in the presence of [^{3}H]cAMP for 30 min at 37 °C. Phosphodiesterase activities are expressed as percent of the control value (44.8 ± 6.8 pmol/min). Values are means ± SD of 4–5 separate experiments.

phosphodiesterase enzyme source) was incubated with 1 μM cAMP (0.05 μCi [^{3}H]cAMP) (Amersham) and various concentrations of **7** and Ro201724, a type 4 phosphodiesterase inhibitor,[13] for 30 min at 37 °C. Then the formed AMP was converted to nucleotide by incubation with *Crotalus atrox* snake venom (1 mg/mL in 0.2 M Tris–HCl, pH 8.0).[10] Nucleoside product was separated through AG 1-X8 resin (formate) (Bio-Rad) and radioactivity was detected in a liquid scintillation counter. As shown in Fig. 2, only Ro201724 suppressed the phosphodiesterase activity. Compound **7** had no effect on cAMP degradation. These results indicate that compound **7** elevates cAMP levels through activation of adenylyl cyclase but does not inhibit phosphodiesterase.

There are many indirect adenylyl cyclase activators which cause accumulation of cellular cAMP through the influence on adenylyl cyclase-coupled receptors or signaling pathways. Forskolin, the only known direct adenylyl cyclase activator, stimulates adenylyl cyclase by interaction with the catalytic subunit of the enzyme.[11] As an adenylyl cyclase activator, compound **7** has a chemical structure unlike forskolin and the known indirect activators. Therefore, we recommend the use of compound **7** as a new lead compound in the future development of adenylyl cyclase activators. Currently, its mechanism of action is under investigation and will be reported separately.

References and Notes

1. Babior, B. M. *Am. J. Med.* **2000**, *109*, 33.
2. Sunahara, R. K.; Dessauer, C. W.; Gilman, A. G. *Annu. Rev. Pharmacol. Toxicol.* **1996**, *36*, 461.
3. Houslay, M. D.; Milligan, G. *Trends Biochem. Sci.* **1997**, *22*, 217.
4. Cronstein, B. N.; Levin, R. I.; Philips, M.; Hirschhorn, R.; Abramson, S. B.; Weissmann, G. *J. Immunol.* **1992**, *148*, 2201.
5. Cronstein, B. N.; Rosenstein, E. D.; Kramer, S. B.; Weissmann, G.; Hirschhorn, R. *J. Immunol.* **1985**, *135*, 1366.
6. Richter, J. *J. Leukoc. Biol.* **1992**, *51*, 270.

7. Experimental details for synthesis: **2-Benzyloxybenzaldehyde** (**7**). 2-Hydroxybenzaldehyde (12.2 g, 100 mmol) was dissolved in acetone (150 mL). Benzyl chloride (15 mL, 130 mmol), anhydrous K_2CO_3 (20 g) and anhydrous KI (26 g) were added. The mixture was stirred under reflux (50–60) for 6 h, and then evaporated in vacuo. Water (100 mL) was added and the mixture was extracted with $CHCl_3$. The separated organic layer was dried with $MgSO_4$, and then evaporated in vacuo. Finally the residue was eluted through a silica gel column with *n*-hexane/EtOAc (4:1) to give pure compound **7** (18.4 g, 87%) as pale orange prism crystals. Mp 48–49 °C; MS (EI, 70 eV): *m*/*z* 212 (M^+); found C, 79.17; H, 5.54%. $C_{14}H_{12}O_2$ requires C, 79.25; H, 5.66%; UV, λ_{max} (log ε): 215.4 (4.46), 253.6 (4.00), 318.2 (3.89); IR (KBr): 1685 (C=O); ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.20 (2H, s, –OCH_2–), 7.01—7.09 (2H, m, H-3, 5), 7.35–7.48 (5H, m, H-2′, 3′, 4′, 5′, 6′), 7.54 (1H, ddd, *J* = 8.1, 7.7, 1.8 Hz, H-4), 7.87 (1H, dd, *J* = 8.0, 2.0 Hz, H-6), 10.58 (1H, s, –CHO); ^{13}C NMR (50 MHz, $CDCl_3$): δ 70.24 (–OCH_2–), 112.82 (C-3), 120.80 (C-5), 124.96 (C-1), 127.08 (C-2′, 6′), 128.06 (C-4′), 128.23 (C-6), 128.52 (C-3′, 5′), 135.70 (C-4), 135.86 (C-1′), 160.84 (C-2), 189.54 (–CHO). **3-Benzyloxybenzaldehyde** (**8**). 3-Hydroxybenzaldehyde (12.2 g, 100 mmol) was treated in the same manner as described for compound **7**. The residue was eluted through a silica gel column with *n*-hexane/EtOAc (9:1) to give pure compound **8** (19.5 g, 92%) as white prism crystals. Mp 57–58 °C; MS (EI, 70 eV): *m*/*z* 212 (M^+); found C, 79.20; H, 5.59%. $C_{14}H_{12}O_2$ requires C, 79.25; H, 5.66%; UV, λ_{max} (log ε): 215.0 (4.57), 251.0 (4.46), 311.0 (3.92); IR (KBr): 1685 (C=O); ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.13 (2H, s, –OCH_2–), 7.23–7.32 (1H, m, H-4), 7.34–7.49 (8H, m, H-2′, 3′, 4′, 5′, 6′, 2, 5, 6), 9.98 (1H, s, –CHO); ^{13}C NMR (50 MHz, $CDCl_3$): δ 0.01 (–OCH_2–), 113.07 (C-2), 121.97 (C-4), 123.45 (C-6), 127.32 (C-2′, 6′), 127.99 (C-4′), 128.46 (C-3′, 5′), 129.90 (C-5), 136.09 (C-1′), 137.61 (C-1), 159.10 (C-3), 191.85 (–CHO). **4-Benzyloxybenzaldehyde** (**9**). 4-Hydroxybenzaldehyde (12.2 g, 100 mmol) was treated in the same manner as described for compound **7**. The residue was eluted through a silica gel column with *n*-hexane/EtOAc (9:1) to give pure compound **9** (19.5 g, 92%) as white needles. Mp 67–68 °C; MS (EI, 70 eV): *m*/*z* 212 (M^+); found C, 79.13; H, 5.56%. $C_{14}H_{12}O_2$ requires C, 79.25, H, 5.66%; UV, λ_{max} (log ε): 214.0 (4.60), 252.0 (4.23), 315.0 (3.91); IR (KBr): 1685 (C=O); ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.16 (2H, s, –OCH_2–), 7.09 (2H, d, *J* = 8.8 Hz, H-3, 5), 7.35–7.47 (5H, m, H-2′, 3′, 4′, 5′, 6′), 7.85 (2H, d, *J* = 8.8 Hz, H-2, 6), 9.89 (1H, s, –CHO); ^{13}C NMR (50 MHz, $CDCl_3$): δ 70.05 (–OCH_2–), 114.93 (C-3, 5), 127.26 (C-2′, 6′), 128.11 (C-4′), 128.51 (C-3′, 5′), 129.91 (C-1), 131.78 (C-2, 6), 135.73 (C-1′), 163.52 (C-4), 190.57 (–CHO). **2-Benzyloxyacetophenone** (**10**). 2-Hydroxyacetophenone (13.6 g, 100 mmol) was treated in the same manner as described for compound **7**. The residue was eluted through a silica gel column with $CHCl_3$/*n*-hexane (1:2) to give pure compound **10** (15.8 g, 70%) as colorless prism crystals. Mp 41–42 °C; MS (EI, 70 eV): *m*/*z* 226 (M^+); found C, 79.58; H, 6.11%. $C_{15}H_{14}O_2$ requires C, 79.65, H, 6.19%; UV, λ_{max} (log ε): 211.0 (4.57), 246.0 (4.10), 305.0 (3.80); IR (KBr): 1665 (C=O); ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.63 (3H, s, –CH_3), 5.17 (2H, s, –OCH_2–), 7.02–7.06 (2H, m, H-3, 5), 7.39–7.50 (6H, m, H-2′, 3′, 4′, 5′, 6′, 4), 7.78 (1H, dd, *J* = 8.0, 2.0 Hz, H-6); ^{13}C NMR (50 MHz, $CDCl_3$): δ 31.98 (–CH_3), 70.40 (–OCH_2–), 112.58 (C-3), 120.66 (C-5), 127.38 (C-2′, 6′), 128.05 (C-4′), 128.42 (C-1), 128.51 (C-3′, 5′), 130.27 (C-6), 133.47 (C-4), 135.98 (C-1′), 157.82 (C-2), 199.76 (–$COCH_3$). **2-Benzyloxybenzamide** (**11**). 2-Hydroxybenzamide (13.7 g, 100 mmol) was treated in the same manner as described for compound **7**. The residue was eluted through a silica gel column with $CHCl_3$ to give pure compound **11** (21.6 g, 95%) as white needles. Mp 114–115 °C; MS (EI, 70 eV): *m*/*z* 227 (M^+); found C, 73.85; H, 5.65; N, 6.14%. $C_{14}H_{13}NO_2$ requires C, 74.00; H, 5.73; N, 6.17%; UV, λ_{max} (log ε): 210.0 (4.57), 225.0 (4.36), 290.0 (3.87); IR (KBr): 1647 (C=O), 3406 (NH_2); ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.19 (2H, s, –OCH_2–), 6.08 (1H, br, N-Ha), 7.05–7.14 (2H, m, H-3, 5), 7.38–7.52 (6H, m, H-2′, 3′, 4′, 5′, 6′, 4), 7.75 (1H, br, N-Hb), 8.25 (1H, dd, *J* = 7.8, 1.9 Hz, H-6); ^{13}C NMR (50 MHz, $CDCl_3$): δ 71.06 (–OCH_2–), 112.45 (C-3), 120.92 (C-1), 121.29 (C-5), 127.64 (C-2′, 6′), 128.49 (C-4′), 128.75 (C-3′, 5′), 132.46 (C-6), 133.12 (C-4), 135.34 (C-1′), 156.90 (C-2), 166.78 (–$CONH_2$).

8. Wang, J. P.; Raung, S. L.; Kuo, Y. H.; Teng, C. M. *Eur. J. Pharmacol.* **1995**, *288*, 341.
9. Markert, M.; Andrews, P. C.; Babior, B. M. *Meth. Enzymol.* **1984**, *105*, 358.
10. Wright, C. D.; Kuipers, P. J.; Kobylarz-Singer, D.; Devall, L. J.; Klinkefus, B. A.; Weishaar, R. E. *Biochem. Pharmacol.* **1990**, *40*, 699.
11. Seamon, K. B.; Daly, J. W. *Adv. Cyclic Nucleotide Protein Phosph. Res.* **1986**, *20*, 1.
12. Beavo, J. A.; Conti, M.; Heaslip, R. *J. Mol. Pharmacol.* **1994**, *46*, 39.
13. Reeves, M. L.; Leigh, B. K.; England, P. J. *Biochem. J.* **1987**, *241*, 535.