JPP JOURNAL OF Pharmacy and Pharmacology
ROYAL PHARMACEUTICAL SOCIETY
JPP Journal of Pharmacy And Pharmacology
Research Paper

Curcumin analogues inhibit phosphodiesterase-5 and dilate rat pulmonary arteries

Oraya Kruangtip[a], Krongkarn Chootip[b], Prapapan Temkitthawon[a], Kanokwan Changwichit[a], Thipphawan Chuprajob[c], Chatchawan Changtam[d], Apichart Suksamrarn[c], Nantaka Khorana[a], C. Norman Scholfield[a] and Kornkanok Ingkaninan[a]

[a]Department of Pharmaceutical Chemistry and Pharmacognosy, Faculty of Pharmaceutical Sciences and Center of Excellence for Innovation in Chemistry, [b]Department of Physiology, Faculty of Medical Sciences, Naresuan University, Phitsanulok, [c]Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Ramkhamhaeng University, Bangkok, [d]Division of Physical Sciences, Faculty of Science and Technology, Huachiew Chalermprakiet University, Samutprakarn, Thailand

Keywords
curcumin analogues; phosphodiesterase-5; pulmonary artery; pulmonary artery hypertension

Correspondence
Kornkanok Ingkaninan, Faculty of Pharmaceutical Sciences and Center of Excellence for Innovation in Chemistry, Naresuan University, Phitsanulok 65000, Thailand.
E-mail: k_ingkaninan@yahoo.com
Krongkarn Chootip, Department of Physiology, Faculty of Medical Sciences, Naresuan University, Phitsanulok 65000, Thailand.
E-mail: krongkarnc@gmail.com

Abbreviations
Ach, acetylcholine; BSA, bovine serum albumin; cGMP, guanosine cyclic monophosphate; DMSO, dimethylsulphoxide; EC_{40}, 40% of maximal effective concentration; ESMS, electrospray mass spectrometry; IC_{50}, half maximal inhibitory concentration; NMR, nuclear magnetic resonance; PAH, pulmonary artery hypertension; PDE, phosphodiesterase; PE, phenylephrine; QAE, quaternary aminoethyl anion exchanger; sGC, soluble guanylyl cyclase.

Received April 21, 2014
Accepted July 4, 2014

doi: 10.1111/jphp.12302

Abstract

Objectives Phosphodiesterase (PDE)-5 inhibitors are useful as vasodilators for the treatment of pulmonary arterial hypertension. We aimed to study curcumin analogues for PDE5 inhibitory activity and vasorelaxation of rat pulmonary arteries.

Methods Three natural curcuminoids (1–3) and six synthetic analogues (4–9) were tested for PDE5 and PDE6 inhibitory activities using enzymatic radioassay. Their vasorelaxation was measured using freshly isolated segments of rat pulmonary artery and aorta.

Key findings Curcuminoids (1–3) mildly inhibited PDE5 (half maximal inhibitory concentration (IC_{50}) = 18 μM): the metamethoxyl of curcumin was important for PDE5 inhibition. But hydroxyl rearrangements, removing both methoxyls and one ketomethylene, yielded the potent 7 and 9 (IC_{50} = 4 μM) (compared with sildenafil, IC_{50} = 0.03 μM). Only 1, 3 and 4 were PDE5 selective over PDE6. Triazole-carboxylic addition provided water-solubility while preserving potency. All analogues possessed concentration-dependent vasorelaxant activity on pulmonary arteries (40% of maximal effective concentration (EC_{40}) = 29–90 μM, maximum response = 60–90% at 300 μM), while compounds (1–8) were weakly acting in aorta (maximum response <40%). Only demethoxycurcumin (2) and analogues 5, 8, 9 had endothelium-dependent actions. Sildenafil was highly potent (EC_{40} = 0.04 μM) and highly endothelium dependent in pulmonary artery but weak on intact aorta (EC_{40} = 1.8 μM). Activity profiles suggest actions through additional cell pathways for promoting vasorelaxation.

Conclusions Curcumin analogues are potential leads for developing efficacious and selective PDE5 inhibitors and other pathologies of pulmonary hypertension.

Introduction

Idiopathic pulmonary arterial hypertension (PAH) is a relatively rare lung disorder (prevalence ~6 cases/million people[1]) with a poor prognosis. Pulmonary arteries become constricted, thus reducing blood flow to the lungs and increasing pulmonary arterial pressure. This PAH increases the load on the right ventricle, leading to right heart failure and death.[2] Several mechanisms for the disease have been proposed and shown to produce PAH in animal models, but none appears to reflect the human condition. Because of this unclear aetiology, current drug

treatments have focused on symptomatic treatment in the form of vasodilators. Of these, sildenafil, tadalafil, vardenafil and similar drugs have received the most widespread application. These compounds are inhibitors of phosphodiesterase-5 (PDE5), which is found in many cells including the smooth muscle cells of pulmonary vascular tree expressed as PDE5A.[3] This enzyme is upregulated in pulmonary hypoxia and PAH[4] making it an attractive drug target for treatment of these pathologies. The target substrate for PDE5 is the inactivation of guanosine cyclic monophosphate (cGMP) via hydrolysis. Cytosolic cGMP constituatively activates in particular, adenosine triphosphate and big potassium current channels[5] and the resulting increased K-permeability maintains vasorelaxant tone. Thus inhibiting PDE5 favours cGMP accumulation hence promoting vasodilatation.[6,7]

Sildenafil was initially registered as an oral drug for erectile dysfunction, then later approved for PAH treatment[6] and is now licensed for Raynaud's disease.[7] There is a growing list of successful clinical trials with PDE5 inhibitors[8] that may lead to extended licensing by regulatory bodies.[7] Clearly, these inhibitors also cause vasodilatation in other tissues[8] and more especially have retinal-related complications most commonly associated with additional PDE6 blockade.[9] Therefore, the hunt continues for drugs that are specific for diseased targets including the pulmonary arterial circulation and especially erectile dysfunction.

Several *Curcuma* species are known to be vasorelaxant including *Curcuma longa*,[10] while curcumin reduces pulmonary arterial pressure.[11] We have shown that extracts of these plants exhibit PDE5 inhibitory activity.[12] This suggests that curcuminoids might provide leads for the development of a new generation of selective PDE5 inhibitors. Therefore, here we aimed to explore the activities of both natural and synthetic curcumin analogues on the inhibition of PDE5 in cell-free assay and on the vasorelaxation of freshly isolated rat pulmonary arteries *in vitro*.

Materials and Methods

Materials

Krebs' solution (mM): (NaCl 122; KCl 5; (N-(2-hydroxyethyl)piperazine-N′-(2-ethane-sulfonic acid)) 10; KH_2PO_4 0.5; NaH_2PO_4 0.5; $MgCl_2$ 1; glucose 11 and $CaCl_2$ 1.8; adjusted to pH 7.3 with 1-N NaOH), acetylcholine (ACh), phenylephrine (PE), ingredients in buffer 1 (100-mM Tris-HCl, pH 7.5; 100-mM imidazole; 15-mM $MgCl_2$; 1.0-mg/ml bovine serum albumin (BSA) and 2.5-mg/ml snake venom from *Crotalus atrox*), ingredients in Buffer 2 (100-mM Tris-HCl, pH 7.5 100-mM imidazole, 15-mM $MgCl_2$, 1.0-mg/ml BSA and 0.5-mg/ml histone) were obtained from Sigma (St Louis, MO, USA). Pentobarbital sodium solution (Nembutal) was obtained from Ceva Sante Animale (Libourne, France). Quaternary aminoethyl anion exchanger (QAE) resin (QAE Sephadex A-25) was purchased from GE Healthcare (Upsala, Sweden). The PDE5 and PDE6 were prepared from rat lung tissues and chicken retinas, respectively.

Preparations of compounds

The natural compounds, (1) curcumin, (2) demethoxycurcumin and (3) bisdemethoxycurcumin, were obtained from the rhizomes of *C. longa*.[13] Compound 4 was synthesised by the demethylation reaction of compound 1 as described previously.[13] Compounds 5–7 were prepared by aldol condensation of substituted cinnamones (10a and 10b) and substituted cinnamaldehydes (11a, 11b and 11c) under a base-catalysed condition, which has recently been reported[14] (Figure 1). Compounds 8 and 9, the acid group-containing analogues of 7, were synthesised by coupling the alkyne analogues 12 and 13 with the azide 14 as shown in Figure 2. Briefly, the cinnamones 10c and 10b were coupled with cinnamaldehydes 11c and 11d by the same method used to synthesise compounds 5–7[14] to yield compounds 12 (32%) and 13 (34%). Each of compounds 12 and 13 was later reacted with 2-azidoacetic acid (14) under click conditions[15] to give compounds 8 and 9 in 69% and 38% yields, respectively. Compound 8: ^{1}H nuclear magnetic resonance (NMR) (400 MHz, acetone-d_6) δ (ppm): 5.36 (s, 2H), 5.38 (s, 2H), 6.62 (d, J = 15.3 Hz, 1H), 6.82 (d, J = 7.8 Hz, 1H), 7.01–7.08 (m, 5H), 7.21 (t, J = 7.8 Hz, 1H), 7.32 (d, J = 7.7 Hz, 1H), 7.36 (d, J = 16.1 Hz, 1H), 7.42 (t, J = 7.7 Hz, 1H), 7.51 (ddd, J = 15.3, 6.6, 3.4 Hz, 1H), 7.73 (d, J = 7.6 Hz, 1H), 7.93 (d, J = 16.1 Hz, 1H), 8.22 (s, 1H). ^{13}C NMR (100 MHz, acetone-d_6) δ (ppm): 51.3, 63.0, 114.0, 114.5, 117.1, 119.7, 122.1, 125.0, 126.3, 126.6, 128.3, 130.1, 130.7, 130.8, 132.4, 138.2, 138.8, 142.0, 143.6, 144.0, 158.6, 158.5, 168.5, 189.2. electrospray mass spectrometry (ESMS) (–ve): m/z 430 $[M-H]^-$. Compound 9: ^{1}H NMR (400 MHz, acetone-d_6) δ (ppm): 5.26 (s, 2H), 5.34 (s, 2H), 6.74 (d, J = 15.2 Hz, 1H), 6.90 (t, J = 7.4 Hz, 1H), 6.98 (d, J = 8.1 Hz, 1H), 7.03 (d, J = 8.1 Hz, 1H), 7.09 (d, J = 15.6 Hz, 1H), 7.17–7.33 (m, 6H), 7.54 (dd, J = 15.1, 10.8 Hz, 1H), 7.69 (d, J = 7.7 Hz, 1H), 8.04 (d, J = 16.1 Hz, 1H), 8.15 (s, 1H). ^{13}C NMR (100 MHz, acetone-d_6) δ (ppm): 51.2, 62.4, 113.8, 116.6, 117.1, 120.8, 121.2, 122.9, 126.0, 126.1, 128.7, 129.4, 130.6, 130.7, 132.4, 138.5, 138.9, 141.5, 143.3, 144.3, 157.7, 159.9, 168.7, 189.2. ESMS (–ve): m/z 430 $[M-H]^-$. The purity of all the test compounds was more than 95% as determined by NMR and thin layer chromatography. The samples were stored at –20°C until use.

Determination of phosphodiesterase-5 inhibitory activity

All samples were dissolved in dimethylsulfoxide (DMSO) and diluted with water giving a final DMSO concentration

 © 2014 Royal Pharmaceutical Society,

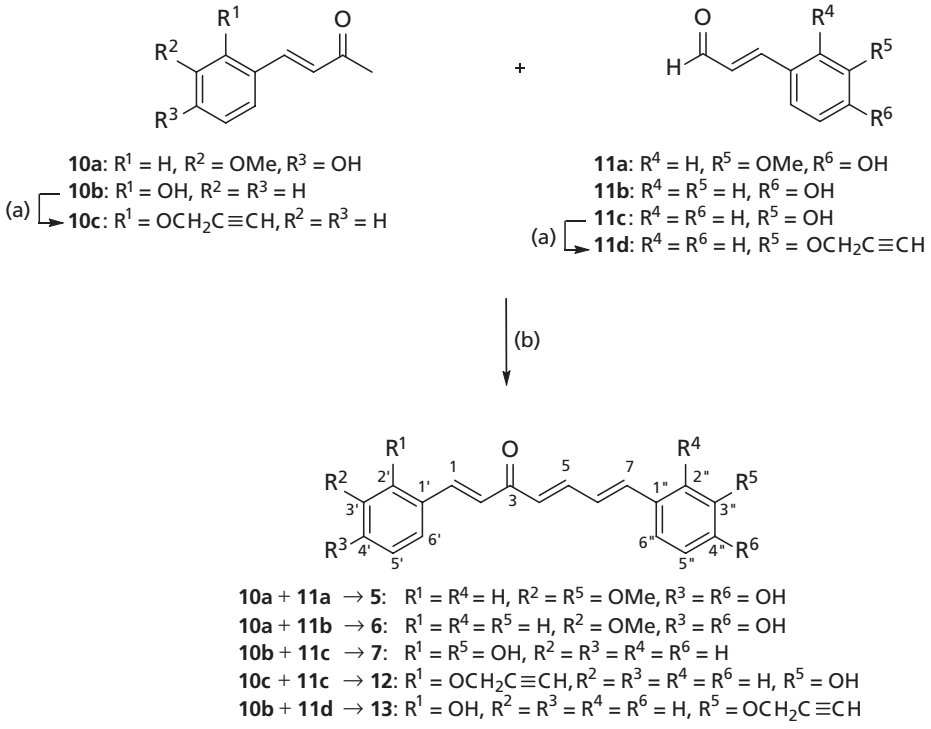


Figure 1 Preparation of compounds 5–7, 12 and 13. Reagents and conditions: (a) propargyl bromide, K_2CO_3, acetone, room temperature. (b) 20% aq. NaOH, EtOH, 0°C– room temperature.

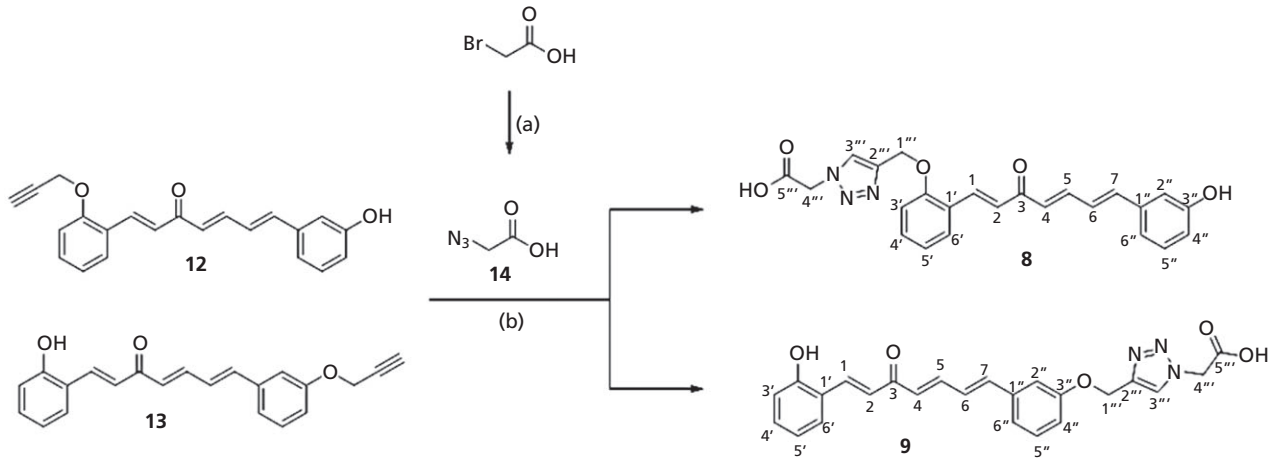


Figure 2 Preparation of compounds 8 and 9. Reagents and conditions: (a) NaN_3, H_2O, room temperature. (b) $CuSO_4$, sodium ascorbate, THF : H_2O (9 : 1).

of 5%. PDE5 was extracted from rat lung tissue as described previously.[16] The PDE5 assay was conducted using the two-step radioactive procedure, which has been modified from Sonnenburg *et al.*, 1998.[17] Twenty microlitres of the following reagents was added to 96-well plates: buffer 1, EGTA, PDE5 solution and test samples or control (5% DMSO in buffer). The reaction was started by adding 20 µl of 5-µM [^{3}H]cGMP (~50 000 cpm) to the reaction mixture and incubated at 30°C for 40 min. Then, 100 µl of 50% QAE resin in water was added to the wells to purify the hydrolysate. The plate was shaken for 10 min and left for 20 min to allow the resin to sediment. The supernatants (100 µl) were transferred to new microplate wells containing 100 µl of fresh 50% QAE resin. The plate was again

shaken and left to permit sedimentation. Then, 100-μl supernatant was mixed and shaken with 200 μl of Microscint (Perkin Elmer, Waltham, MA, USA) 20 for 2 h. The radioactivity was counted by a TopCount NXT (PerkinElmer, Boston, MA, USA), each well for 1 min. The PDE5 activity in the study was standardised to have a hydrolysis activity of 20–25% of the total substrate counts. The calculation of hydrolysis is shown in equation (1). The PDE5 inhibitory activity was calculated using equation (2).

$$\%\text{hydrolysis}_{\text{sample}} = \left(\frac{(\text{CPM}_{\text{sample}} - \text{CPM}_{\text{background}})}{(\text{CPM}_{\text{total count}} - \text{CPM}_{\text{background}})} \right) \times 100 \quad (1)$$

where $\text{CPM}_{\text{sample}}$ is the radioactive count rate of the assay with enzyme and $\text{CPM}_{\text{background}}$ is the count rate without enzyme. $\text{CPM}_{\text{total count}}$ is the count rate of 20 μl of substrate plus 100 ml of buffer 1.

$$\%\text{PDE5 inhibition} = \left[1 - \left(\frac{\%\text{hydrolysis}_{\text{sample}}}{\%\text{hydrolysis}_{\text{control}}} \right) \right] \times 100 \quad (2)$$

where $\%\text{hydrolysis}_{\text{sample}}$ and $\%\text{hydrolysis}_{\text{control}}$ were the enzyme activities of the sample and solvent (1% DMSO) used in the assay, respectively. The IC_{50} values were determined using the test samples at >80% PDE5 inhibition.

Determination of phosphodiesterase-6 inhibitory activity

PDE6 activity was conducted using the procedure previously reported,[14] which has been modified from Huang *et al.*, 1998.[18] Twenty-five millilitres of the following reagents was added to tube: buffer 2, EGTA, PDE6 solution and test samples or control (5% DMSO in buffer). The reaction was started by adding 25 μl of 5-μM [^{3}H]cGMP and incubated at 30°C for 10 min. Then, the reaction was stopped by placing the tube in boiling water for 1 min and cooled for 5 min. The second step of reaction used 25 μl of 2.5-mg/ml snake venom added to the reaction, incubated at 30°C for 5 min. After that, 250 μl of 20-mM Tris-HCl, pH 6.8 (buffer I) was added. The reaction was transferred to a QAE resin column and eluted four times with 500 μl of buffer I. The eluent was mixed with a scintillation cocktail, and the radioactivity was measured using a β-counter. The %hydrolysis of PDE6 was similarly calculated as for PDE5.

Animals

Male Wistar rats (200–250 g) were obtained from the National Laboratory Animal Center, Mahidol University, Nakhornpathom, Thailand. The study was approved by the Animal Ethics Committee, Naresuan University, Phitsanulok, Thailand (NU-AE540416). Animals were housed under standard environmental conditions at 25 ± 2°C, 12-h light and dark cycle, fed with standard rodent diet and tap water in the Center for Animal Research, Naresuan University, Thailand.

Tissue preparation and vascular protocols

Rats were anaesthetised by pentobarbital (65-mg/kg, i.p. injection) and the lungs and aorta were isolated. Intrapulmonary artery was removed from lung and soaked in Krebs' solution to wash off the surrounding loose connective tissue. The vessel was cut into rings 2–3 mm in length and mounted in tissue chambers via a pair of intraluminal wires. The chambers contained Krebs' solution at 37°C and bubbled with air. The rings were incubated for 45–60 min at an optimum tension of 1 g during which the solution was replaced every 15 min. The wires were connected to force transducers to measure isometric tension via a MacLab A/D converter (Chart V5, A.D. Instruments, Castle Hill, NSW, Australia), stored and displayed on personal computer. The arterial contraction and relaxation was tested by sequential application of 10-μM PE and 10-μM ACh. Only vessels showing 80–100% relaxation to ACh were considered as endothelium intact, while in some experiments, the endothelium was predenuded mechanically, and relaxations of <20% were considered as successfully denuded. After washing for 45–60 min, vessels were precontracted by adding 10-μM PE again. When stable contractions were obtained, the samples (containing compounds 1–9) at concentrations of 0.1–100 μM were cumulatively added (Figure 3). The samples were dissolved in DMSO and then diluted with water to obtain the final concentrations of 300, 100, 30, 10, 3, 1, 0.1 μM in 2-ml tissue baths (final solutions contained <0.1% DMSO). Sildenafil was similarly diluted to working concentrations of 0.0001–100 μM.

Statistical analysis

Data were expressed as the mean ± standard error of the mean. Statistical analysis was conducted using Student's *t*-test: unpaired and one-way analysis of variance, followed by Tukey's post-hoc test. *P*-values of < 0.05 were considered significant.

Results

Inhibition of phosphodiesterase-5 and phosphodiesterase-6 by curcumin analogues

Three naturally occurring curcuminoids (1–3) and six synthetic analogues (4–9) were tested using the PDE5 and PDE6 inhibition assays. The highest potency on PDE5 was for compounds 7 and 9 with IC_{50}s of ~4 μM. Nevertheless, compounds 7 and 9 were ~100-fold less potent than sildenafil, but more effective than the naturally occurring

 © 2014 Royal Pharmaceutical Society,

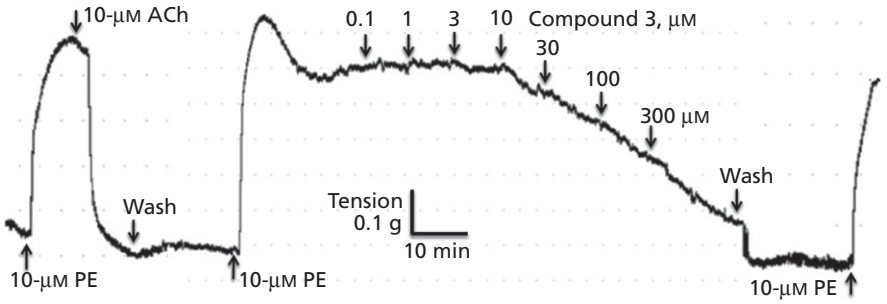


Figure 3 An example time-course showing vasorelaxation of an endothelium-intact pulmonary artery to ACh followed by the relaxant effect of compound 3 at 0.1 to 300 μM. PE, phenylephine, ACh, acetylcholine, K, high potassium solution.

1–3 (Table 1). The other compounds showed IC_{50} values that varied between 10 and 100 μM. For PDE6, the IC_{50}s for compounds 2, 5, 6, 7, 8 and 9 were in the range of 3–20 μM. Only 1, 3 and 4 showed IC_{50}s >100 μM (Table 1).

Vasorelaxation of pulmonary artery and aorta by curcumin analogues

In isolated pulmonary arteries with intact endothelium, the natural curcuminoids (1–3) and synthetic analogues (4–9) demonstrated concentration-dependent vasodilatation (Figures 3 and 4). Sildenafil achieved a supramaximal relaxation, but limited solubility and lower potency prevented us from determining the supramaximal effect for any of the test analogues while some of maximal responses did not even reach 50% of the maximal sildenafil response. Therefore, all potencies were expressed as an EC_{40} (for sildenafil both EC_{50} and EC_{40} were determined). Every test analogue showed similar potencies (mean EC_{40}s were 58–111 μM) on intact pulmonary arteries (Table 2).

Endothelial denudation of pulmonary arteries showed smaller relaxations with compounds 2, 5, 8 and 9 (Table 2) (Figure 4b, 4e, 4h and 4i). Similarly, sildenafil potency was substantially lower in endothelium-denuded pulmonary arteries (Figure 4j). For the remaining compounds 1, 3, 4, 6 and 7 (Figure 4a, 4c, 4d, 4f and 4g), there was no change in potency with denudation.

In contrast, most of the test compounds produced weaker actions on aorta compared with intact pulmonary artery except compound 9, which was equally potent on both aorta and pulmonary artery (Figure 4i). Sildenafil was ~40-fold less effective on the aorta than intact pulmonary artery; that is, only ~5-fold more potent than denuded pulmonary artery (Figure 4j). This could be a consequence of more PDE5 in pulmonary artery but we are unaware of any direct comparison of PDE5 in vascular smooth muscle on different arteries.

Discussion

Structure–activity relationship of curcumin analogues on phosphodiesterases

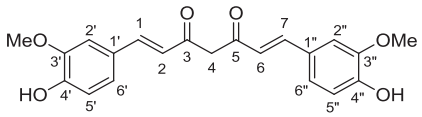
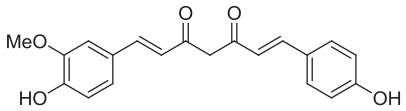
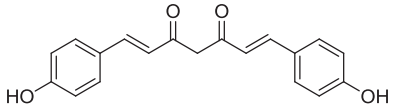
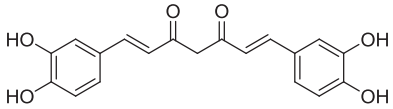
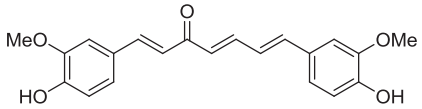
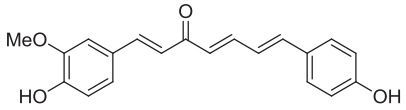
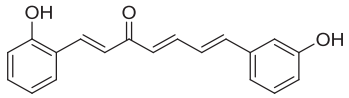
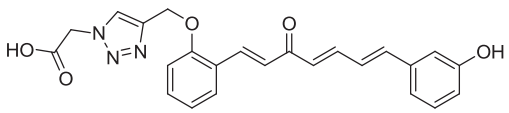
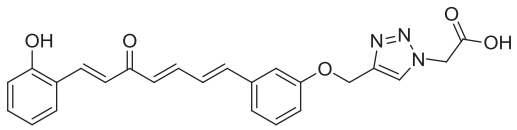
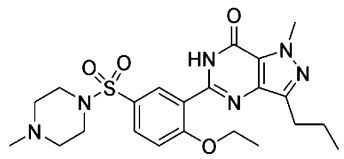
Cell-free experiments showed that all curcumin analogues were moderately active PDE5 inhibitors (Table 1). When the metamethoxyl group was missing from 1, the activities of 2 and 3 were reduced suggesting that this group is important for PDE5 inhibitory activity. Demethylation of 1 gave the more polar analogue 4 resulting in a twofold lower inhibitory activity while removing both methoxyl groups yielded compound 3, which produced a fourfold reduction in activity. It might be possible that these metapositions need these bulky substituents for binding to the active site.[19]

Replacement of the ketomethylene group in 1 giving 5 slightly decreased the inhibitory activity whereas a similar structural modification of 2–6 increased the activity. Taking compound 6 and transposing the two hydroxyl groups from positions 4′ to 2′ and 4″ to 3″ and removing the aromatic methoxyl group yielded the highly potent compound 7.

Water solubility was a major challenge for curcumin analogues (1–7) and solubility was improved by adding a triazole carboxylic group to the 2′ hydroxyl group in 7 to give 8 but this compromised PDE5 potency. In contrast, a similar substitution on the opposite 3″-hydroxyl group of 7 yielding 9 preserved the inhibitory activity as well as offering superior water solubility.

The inhibitory activity on PDE6 is another concern because it disrupts the cGMP signalling pathway used in retinal transduction and this is avoided in the highly selective PDE5 inhibitor, tadalafil.[19] The inhibition by these compounds on PDE6 suggests that 1, 3 and 4 had weak actions compared with the corresponding actions on PDE5 and accords with the 10-fold selectivity of sildenafil.[20] These results suggest that further modification of curcuminoid analogues could achieve the required specificity and high activity needed to realise clinical usefulness.

Table 1 The inhibitory effects of curcumin and its analogues on PDE5 and PDE6. Values are means ± standard error of the mean ($n = 3$)

Compounds	Chemical structures	IC_{50} against	
		PDE5 (μM)	PDE6 (μM)
1		18.8 ± 2.1[a]	113.9 ± 20.9[b]
2		50.6 ± 3.3[c]	12.6 ± 2.8[a]
3		94.4 ± 5.2[d]	>500[c]
4		44.5 ± 1.5[c]	>700[d]
5		30.5 ± 5.1[b]	18.1 ± 9.7[a]
6		27.6 ± 5.7[b]	7.6 ± 1.8[a]
7		4.4 ± 1.6[a]	4.0 ± 2.1[a]
8		17.1 ± 2.0[a]	5.1 ± 2.0[a]
9		3.9 ± 0.6[a]	2.8 ± 2.3[a]
Sildenafil		0.03 ± 0.01[e]	ND

PDE, phosphodiesterase. [a–e]Difference within columns (samples not connected by the same letter are statistically diferent at $P < 0.05$). PDE, phosphodiesterase.

Vasorelaxant effects of curcumin analogues

In these experiments, sildenafil potency on intact pulmonary arteries expressed as EC_{40} was 0.04 μM (0.074 ± 0.016 μM calculated as EC_{50}), which accords with previous work in rat pulmonary artery[21] and aorta.[22] Furthermore, the potency of sildenafil here was similar to the cell-free action on PDE5 protein. Endothelial removal caused a dramatic decrease in sildenafil potency (200-fold less) both here and in previous work on aorta.[20] This

© 2014 Royal Pharmaceutical Society,

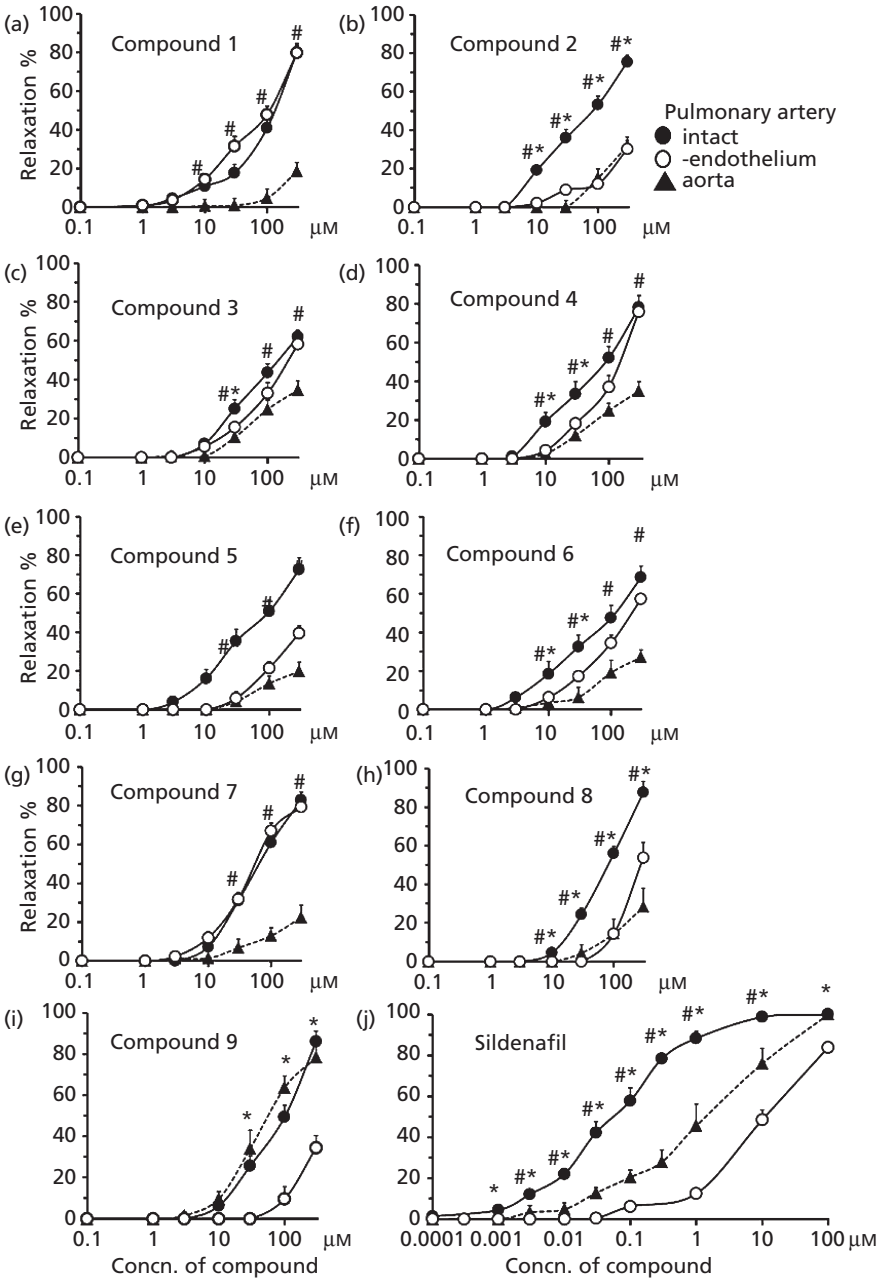


Figure 4 Concentration–vasorelaxation plots for the nine curcumin analogues and sildenafil. Each value is mean ± standard error of the mean (n = 5–6). *P < 0.05 compared relaxation of pulmonary arteries with and without endothelium, #P < 0.05 comparing endothelium-intact pulmonary arteries with aortas.

confirms that the vascular smooth muscle relaxation was largely mediated through the endothelium-releasing vasodilator factors even though sildenafil is acting on vascular smooth muscle.[22]

All the curcuminoids were vasorelaxant using endothelium-intact pulmonary artery and all had similar potencies (Table 2). Compounds 2, 5, 8, 9 and possibly 4 had actions that indicate that the endothelium was necessary. But, these four compounds had similar potencies that did not reflect those variations seen in the cell-free assays on either PDE5 of PDE6. Thus for compounds 2, 5, 8 and 9, there was clearly endothelium dependency, but the poor potency correlation with the cell-free studies does not clearly indicate that they are acting on PDE5.

Table 2 Vasorelaxant actions of curcumin and its analogues on rat endothelium-intact and denuded pulmonary arteries and aorta. (n = 5–6), P-values listed are for differences between endothelium-intact versus endothelium-denuded pulmonary arteries.

	EC_{40} (μM)		
Compounds	Pulmonary artery intact	Pulmonary artery denuded	Aorta-intact
1	109 ± 23	93 ± 30 (P = 0.82)	>300 ± 0[#]
2	58 ± 10	>300 ± 0 (P < 0.0001)	>300 ± 0[#]
3	111 ± 29	184 ± 31 (P = 0.14)	>300 ± 0[#]
4	58 ± 20	121 ± 20 (P = 0.058)	>300 ± 0[#]
5	76 ± 24	>300 ± 0 (P < 0.0001)	>300 ± 0[#]
6	98 ± 28	147 ± 21 (P = 0.17)	>300 ± 0[#]
7	59 ± 8	52 ± 6 (P = 0.74)	>300 ± 0[#]
8	59 ± 8	208 ± 17 (P < 0.0001)	>300 ± 0[#]
9	71 ± 15	>300 ± 0 (P < 0.0001)	46 ± 11
Sildenafil	0.042 ± 0.009	8.4 ± 0.8 (P < 0.0001)	1.8 ± 1.0[#]

[#]P < 0.0001 for endothelium-intact pulmonary arteries versus intact aorta.

Compounds 1 and 7 were unaffected by endothelial denudation suggesting that they acted directly on vascular smooth muscle through a mechanism probably not directly on PDE5. These might include actions on soluble guanylyl cyclase[23] on β-receptors, or cytosolic Ca^{2+} handling.[24]

There are two important differences between the cell-free and vascular relaxation studies, which might affect potency of our compounds: (1) the compounds have to gain access to the cell interior, and numerous bioavailability studies have shown that the membrane permeability of at least curcumin itself is very poor;[25] (2) a vast number of cellular effects for curcuminoids have been described.[26]

However, the very high concentrations needed to have any effect on the aorta suggest that these compounds have some selectivity for the pulmonary artery. This alone indicates that the compounds may form the basis for the development of drugs that selectively target the pulmonary circulation. Finally, the multiple cellular actions of curcuminoids[26–28] may be an asset in the treatment of PAH where there are multiple pathologies including inflammation, PDE5 upregulation, ionchannelopathies, vasoconstriction, endothelial dysfunction and vascular hyperplasia.

Conclusions

Curcumin analogues showed PDE5 inhibitory activity with varying potencies and some showed selectivity for PDE5 over PDE6. There were clear endothelium-dependent vasorelaxant effects to which the pulmonary artery was more sensitive compared with the aorta. These results suggest that these curcuminoids could underpin the further development of highly selective and potent compounds, which could discriminate the pulmonary arterial circulation by targeting several coincident pathologies of PAH including PDE5 upregulation.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

The financial supports from Naresuan University, the Center of Excellence for Innovation in Chemistry (PERCH-CIC), Office of the Higher Education Commission, Ministry of Education, Thailand and The Thailand Research Fund are acknowledged.

References

1. Ling Y *et al*. Changing demographics, epidemiology, and survival of incident pulmonary arterial hypertension: results from the pulmonary hypertension registry of the United Kingdom and Ireland. *Am J Respir Crit Care Med* 2012; 186: 790–796. doi: 10.1164/rccm.201203-0383OC.
2. Barst RJ *et al*. Diagnosis and differential assessment of pulmonary arterial hypertension. *J Am Coll Cardiol* 2004; 43(Suppl. 12 S): 40S–47S. doi: 10.1016/j.jacc.2004.02.032.
3. Sebkhi A *et al*. Phosphodiesterase type 5 as a target for the treatment of hypoxia-induced pulmonary hypertension. *Circulation* 2003; 107: 3230–3235. doi: 10.1161/01.CIR.0000074226.20466.B1.
4. Scipioni A *et al*. Immunohistochemical localisation of PDE5 in rat lung during pre- and postnatal development. *J Biomed Biotechnol* 2009; 2009: 1–7. doi: 10.1155/2009/932961.
5. Schoeffter P *et al*. Role of cyclic AMP- and cyclic GMP-phosphodiesterases in the control of cyclic nucleotide levels and smooth muscle tone in rat isolated aorta. A study with selective inhibitors. *Biochem Pharmacol* 1987; 36: 3965–3972.
6. Galiè N *et al*. Guidelines on diagnosis and treatment of pulmonary arterial hypertension The Task Force on Diagnosis and Treatment of Pulmonary Arterial Hypertension of the European Society of Cardiology. *Eur Heart J* 2004; 25: 2243–2278. doi: 10.1016/j.ehj.2004.09.014.
7. Chrysant SG. Effectiveness and safety of phosphodiesterase 5 inhibitors in patients with cardiovascular disease

and hypertension. *Curr Hypertens Rep* 2013; 15: 475–483. doi: 10.1007/s11906-013-0377-9.
8. Kukreja RC *et al*. Emerging new uses of phosphodiesterase-5 inhibitors in cardiovascular diseases. *Exp Clin Cardiol* 2011; 16: e30–e35.
9. Kerr NM, Danesh-Meyer HV. Phosphodiesterase inhibitors and the eye. *Clin Experiment Ophthalmol* 2009; 37: 514–523. doi: 10.1111/j.1442–9071.2009.02070.x.
10. Adaramoye OA *et al*. Hypotensive and endothelium-independent vasorelaxant effects of methanolic extract from *Curcuma longa* L. in rats. *J Ethnopharmacol* 2009; 124: 457–462. doi: 10.1016/j.jep.2009.05.021.
11. Lin Q *et al*. Effect of curcumin on pulmonary hypertension and wall collagen of pulmonary arterioles of chronic hypoxic hypercapnic rats. *Chin J Appl Physiol* 2006; 22: 257–261.
12. Temkitthawon P. *Investigation of phosphodiesterase-5 inhibitory activity from Thai medicinal plants*. Naresuan University, Thailand: PhD thesis, 2012.
13. Changtam C *et al*. Curcuminoid analogues with potent activity against *Trypanosoma* and *Leishmania* species. *Eur J Med Chem* 2010; 45: 941–956.
14. Chuprajob T *et al*. Synthesis, cytotoxicity against human oral cancer KB cells and structure–activity relationship studies of trienone analogues of curcuminoids. *Bioorg Med Chem Lett* 2014; 24: 2839–2844.
15. Kategaonkar AH *et al*. Synthesis and biological evaluation of new 2-chloro-3-((4-phenyl-1*H*-1,2,3-triazol-1-yl)methyl)quinoline derivatives via click chemistry approach. *Eur J Med Chem* 2010; 45: 3142–3146.
16. Temkitthawon P *et al*. *Kaempferia parviflora*, a plant used in traditional medicine to enhance sexual performance contains large amounts of low affinity PDE5 inhibitors. *J Ethnopharmacol* 2011; 137: 1437–1441. doi: 10.1016/j.jep.2011.08.025.
17. Sonnenburg WK *et al*. Identification, quantitation, and cellular localization of PDE1 calmodulin-stimulated cyclic nucleotide phosphodiesterases. *Methods* 1998; 14: 3–19. doi: 10.1006/meth.1997.0561.
18. Huang D *et al*. Molecular determinants of cGMP binding to chicken cone photoreceptor phosphodiesterase. *J Biol Chem* 2004; 279: 48143–48151. doi: 10.1074/jbc.M404338200.
19. Cahill KB *et al*. Identification of amino acid residues responsible for the selectivity of tadalafil binding to two closely related phosphodiesterases, PDE5 and PDE6. *J Biol Chem* 2012; 287: 41406–41416. doi: 10.1074/jbc.M112.389189.
20. Teixeira CE *et al*. Differential effects of the phosphodiesterase type 5 inhibitors sildenafil, vardenafil, and tadalafil in rat aorta. *J Pharmacol Exp Ther* 2006; 316: 654–661. doi: 10.1124/jpet.105.092544.
21. Pauvert O *et al*. Effect of sildenafil on cyclic nucleotide phosphodiesterase activity, vascular tone and calcium signaling in rat pulmonary artery. *Br J Pharmacol* 2003; 139: 513–522. doi: 10.1038/sj.bjp.0705277.
22. Mochida H *et al*. Sildenafil and T-1032, phosphodiesterase type 5 inhibitors, showed a different vasorelaxant property in the isolated rat aorta. *Eur J Pharmacol* 2002; 440: 45–52. doi: 10.1016/S0014-2999(02)01339-0.
23. Dash JR, Parija SC. Spasmolytic effect of curcumin on goat ruminal artery is endothelium independent and by activation of sGC. *Res Vet Sci* 2013; 95: 588–593. doi: 10.1016/j.rvsc.2013.04.029.
24. Xu P-H *et al*. The relaxant effect of curcumin on porcine coronary arterial ring segments. *Vascul Pharmacol* 2007; 47: 25–30. doi: 10.1016/j.vph.2007.03.003.
25. Wahlang B *et al*. Identification of permeability-related hurdles in oral delivery of curcumin using the Caco-2 cell model. *Eur J Pharm Biopharm* 2011; 77: 275–282. doi: 10.1016/j.ejpb.2010.12.006.
26. Shishodia S. Molecular mechanisms of curcumin action: gene expression. *Biofactors* 2013; 39: 37–55. doi: 10.1002/biof.1041.
27. Metzler M *et al*. Curcumin uptake and metabolism. *Bio Factors* 2013; 39: 14–20. doi: 10.1002/biof.1042.
28. Bronte E *et al*. Role of curcumin in idiopathic pulmonary arterial hypertension treatment: A new therapeutic possibility. *Med Hypotheses* 2013; 81: 923–926. doi: 10.1016/j.mehy.2013.08.016.