CHAPTER IV

FINDINGS AND DISCUSSION

Inhibition of PDE5 and PDE6 by curcumin analogs

Three naturally occurring curcumins (1-3) and six synthetic analogues (6-9) were tested using the PDE5 and PDE6 inhibition assays. The highest potency on PDE5 was for 7 and 9 with IC_{50}s of ~4μM. Nevertheless, compounds 7 and 9 were ~100-fold less potent than sildenafil but more effective than the naturally occurring 1-3 (Table 4). 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 4).

Table 4 The inhibitory effects of curcumin and its analogues on PDE5 and PDE6. Values are means ± S.E.M (n=3)

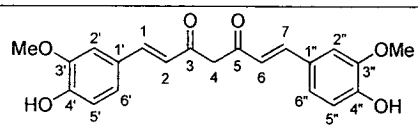
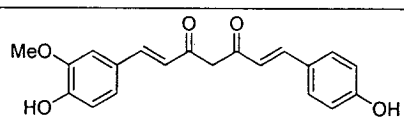
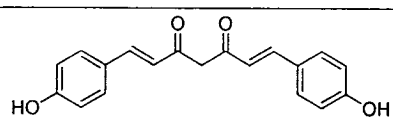
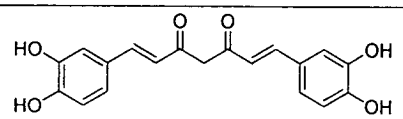
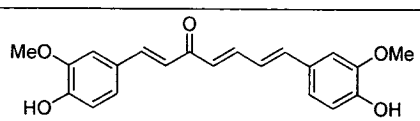
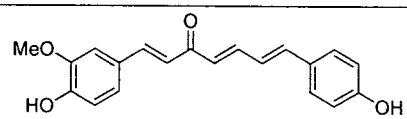
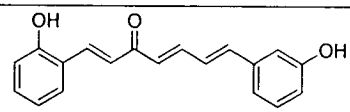
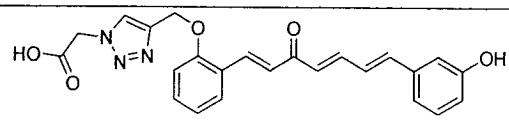
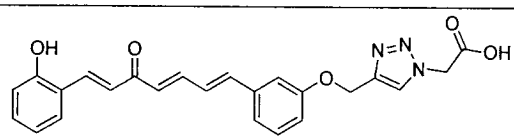
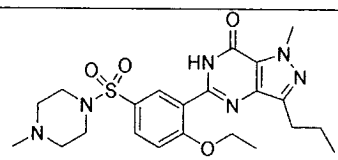
Compounds	Chemical structures	IC_{50} against	
		PDE5 (μM)	PDE6 (μM)
1		18.8 ± 2.1[a]	113.9±20.9[b]
2		50.6 ± 3.3[c]	12.6±2.8[a]
3		94.4 ± 5.2[d]	>500[c]
4		44.5 ± 1.5[c]	>700[d]
5		30.5 ± 5.1[b]	18.1±9.7[a]

Table 4 (cont.)

Compounds	Chemical structures	IC_{50} against	
		PDE5 (μM)	PDE6 (μM)
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

[a-e] Difference within columns (samples not connected by the same letter are statistically diferent at $p<0.05$)

Vasorelaxation of pulmonary artery and aorta by curcuminoids

In isolated pulmonary arteries, the natural curcuminoids (1-3) and synthetic analogs (4-9) demonstrated concentration-dependent vasodilatation (Figure 11). Sildenafil achieved a supramaximal relaxations, 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 achieve 50% of the maximal sildenafil response. Therefore all potencies were expressed as EC_{40}. All of the test analogues showed similar potencies (mean EC_{40} value 58-111 μM) on intact pulmonary arteries (Table 5).

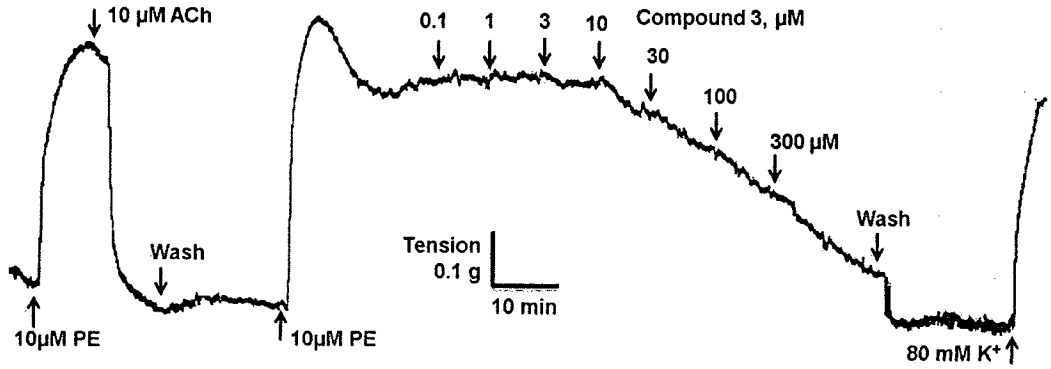


Figure 11 Time-course showing constriction of a endothelium-intact section of pulmonary artery the relaxant effect of compound 3 at 0.1 to 300 µM. PE=phenylephine, ACh=acetylcholine, K= potassium

Teble 5 Vasorelaxant actions of curcumin and its analogues in endothelium-intact and denuded pulmonary arteries. (n=5-6), p-values indicated are for differences between endothelium-intact vs endothelium-denuded. # p<0.0001 for endothelium-intact vs intact aorta

Compounds	EC_{40} (µM)		
	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[#]

Endothelial denudation of pulmonary arteries showed smaller relaxations with compounds 2, 5, 8 and 9 (Table 5)(Figure 12b, e, h, i). Similarly, sildenafil potency was also substantially lower in endothelium denuded pulmonary arteries (Figure 12j). For the remaining compounds 1, 3, 4, 6, and 7 (Figures 1a, c, d, f, g), there was no change in potency with denudation.

In contrast, most of the test compounds produced weaker actions on aorta compared to intact pulmonary artery except compound **9** which was equally potent on both aorta and pulmonary artery (Figure 12i). Sildenafil was ~40-fold less effective on the aorta than intact pulmonary artery, i.e., only ~5-fold more potent than denuded pulmonary artery (Figure 12j). This could be a consequence of more PDE5 in pulmonary artery but we are unware of any direct comparison of PDE5 in vascular smooth muscle on different arteries.

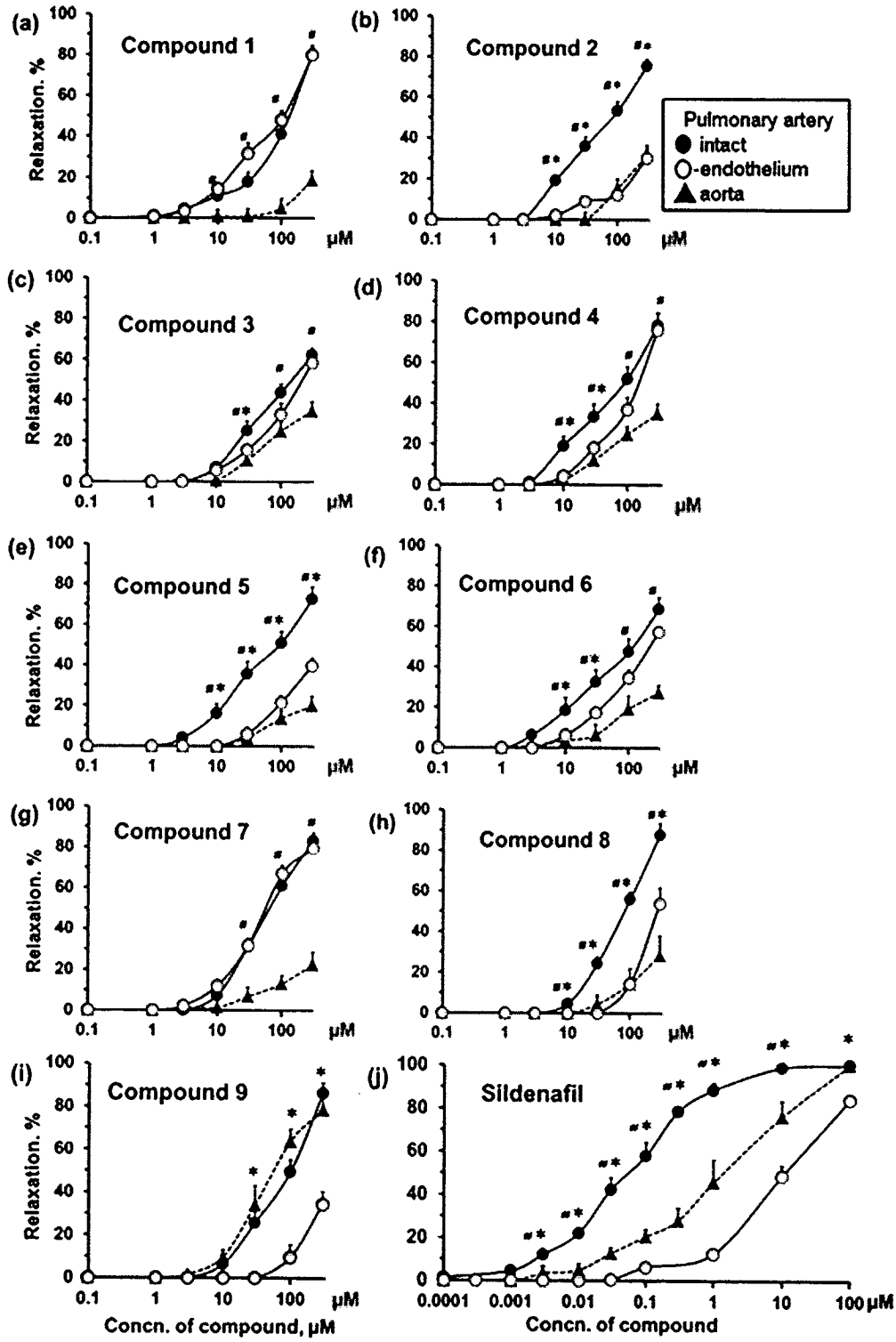


Figure 12 Concentration-vasorelaxation plots for the nine curcumin analogues and sildenafil. Each value is mean + SEM (n=5-6).* $p < 0.05$ compared relaxation of pulmonary arteries with and without endothelium, # $p < 0.05$ comparing endothelium intact pulmonary arteries with aortas