CHAPTER I

INTRODUCTION

Rationale for the study

Pulmonary arterial hypertension (PAH) is a rare lung disorder in which the arteries that carry blood from the heart to the lungs become narrowed, thus reducing blood flow through the vessels. As a result, the pressure in pulmonary arteries rises above the normal 15 mm Hg to 25 mm Hg at resting and to more than 50 mm Hg with exercise [1]. This abnormally high pressure is needed to maintain normal pulmonary blood flow and strains the right ventricle of the heart to maintain these elevated pressures. This can lead to the development of right heart failure and death [2]. The symptoms of PAH include pain in the chest, shortness of breath, fatigue and fainting [3]. Although the incidence of the disease is low, 15-50 cases/million people [4], its severity necessitates efficient therapeutic strategies leading to a new efficacious drug. In PAH patients, smaller pulmonary artery with intimal fibrosis and typical increasing of smooth muscle cells are found. High myofibroblast and muscle cell exchange into intima layer lead to pulmonary artery constriction [5].

The pulmonary artery consists of 3 layers i.e., the intima, media and adventitia. The endothelial cell is a main component of intima layer and has an ability to respond to external signals such as hypoxia, shear stress, and inflammation. The endothelial cells then release mediators which diffuse through into the pulmonary vascular smooth muscle layer leads to changes in vascular tone [5]. One such vascular smooth muscle releaxant is nitric oxide (NO) in the lung is a controller of pulmonary perfusion which related with rate of alveolar ventilation. NO is produced in the endothelial cells from arginine via endothelial nitric oxide synthase (eNOS) [6]. Moreover, mediators such as prostaglandin, endothelin (ET-1) and serotonin could affect vascular dilation/constriction. Prostacyclin (PGI_2) causes vascular relaxation. On the other hand, thromboxane A_2 (TXA_2) leads to vascular constriction. In PAH patients, low PGI_2 and high TXA_2 are found [7]. ET-1 causes pulmonary artery constriction and increases smooth muscle cell proliferation (mitogen) and this

contributes to the PAH [8]. Serotonin produced from tryptophan also leads to vasoconstriction [9].

Currently, there are three groups of drugs used for the treatment of PAH i.e., (1) selective pulmonary vasodilators such as PGI_2 analogues, (2) ET-1 receptor antagonists and, (3) phosphodiesterase-5 (PDE5) inhibitors [9].

The examples of PDE5 inhibitors are sildenafil (Revatio® or Viagra®), tadalafil (Cialis®), vardenafil (Levitra®) and similar drugs have received the most wide spread application. These compounds act by inhibiting PDE5, which is highly expressed in pulmonary smooth muscle cells as PDE5A [10]. Inhibition of PDE5 leads to increase of intracellular guanosine 3′, 5′-cyclic monophosphate (cGMP) which activates in particular, ATP (adenosine tri phosphate) and BK (bradykinin) channels [11] and the resulting increased K-permeability results in vasorelaxation as well as longer term anti-proliferative activity [12]. The substrate for PDE5 is cGMP which is normally hydrolysed by PDE5 which releases this vasodilator tone leasing to vascular constriction. Therefore, inactivation of PDE5 will preserve cytosolic cGMP levels and promote vasodilation [13]. Sildenafil was firstly registered as an oral drug for erectile dysfunction, and later in 2005, it was approved by various drug licensing authorities including the United States-FDA for PAH treatment [14]. Clearly, these PDE5 inhibitors also cause vasodilation in other tissues [15] and more especially the retinal photoreceptor transductance disturbance associated with the additional PDE6 blockade [16, 17]. Therefore, the hunt continues for drugs that more specifically target pulmonary arterial smooth muscle.

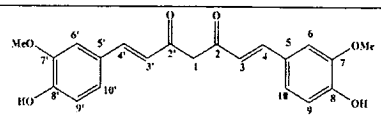
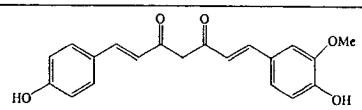
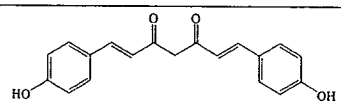
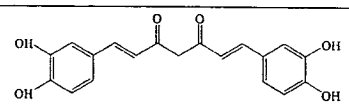
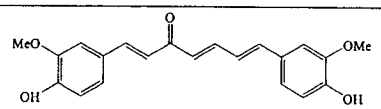
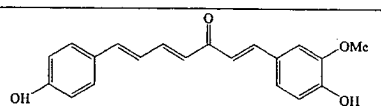
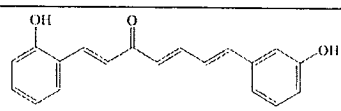
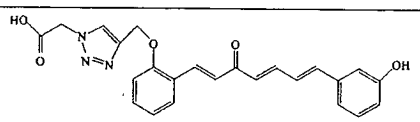
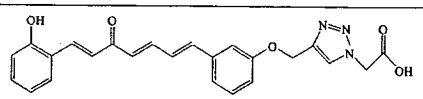
In the previous screening study, our group investigated some Thai medicinal plants for PDE5 inhibitory activity. We found that *Curcuma longa* L. along with some other *Curcuma spp.* showed high PDE5 inhibition [18] (Table 1). In addition, Abusnina and coworkers (2009) reported that curcumin, the main constituent in *C. longa*, showed inhibitory activities on PDE-1-5 with IC_{50}s in the 10-35 μM range [19].

Table 1 Percentage PDE5 inhibition of alcoholic extract of some Curcuma species tested at the final concentration (10 μM)

No.	Scientific Name	%PDE5 inhibition
1	*Curcuma aeruginosa* Roxb.	20.74 ± 1.18
2	*Curcuma longa* L.	84.01 ± 1.29
3	*Curcuma zedoaria* (Berg) Roscoe	88.27 ± 4.12
4	*Curcuma petiolata* Roxb.	81.92 ± 3.53
5	*Curcuma xanthorrhiza* Roxb.	49.57 ± 4.12
6	*Curcuma sp.* (นางคำ)	81.73 ± 4.71
7	*Curcuma sp.* (หัวใหญ่)	41.78 ± 2.35
8	*Curcuma sp.* (เพชรม้า)	19.56 ± 1.76
9	*Curcuma sp.* (ม้าเหลือง)	52.10 ± 3.53
10	*Curcuma sp.* (ม้าขาว)	14.82 ± 2.24
11	*Curcuma sp.* (ม้าห้อ)	27.08 ± 3.29
12	*Curcuma sp.* (มหากำลัง)	18.69 ± 2.00
13	*Curcuma sp.* (หนุมานยกทัพ)	21.88 ± 1.76

This makes curcumin an interesting lead compound for the development of other PDE5 inhibitors with higher activity and specificity. Therefore, curcumin and some of its analogs were tested for their inhibitory effect on PDE5 (Table 2). Thus, synthesised products such as compounds 5, 6 and 7 showed higher activity than the natural products, compounds 1-3. The structural changes and substitution groups are obviously important to PDE5 inhibitory activity.

Table 2 The inhibitory effect of 10 μM curcumin and analogs on PDE5

compounds	structure	% PDE5 inhibition
1		31.39 ± 1.15
2		29.36 ± 3.73
3		12.77 ± 1.01
4		33.76 ± 0.09
5		54.51± 5.96
6		65.37 ± 6.60
7		52.99 ± 5.31
8		33.13 ± 4.73
9		45.82 ± 5.78

Thus, the aim of this study is to investigate the effect of curcumin and these analogs on pulmonary artery in rats. This might lead to the finding of new potential drugs for PAH.

Objectives of the study

To quantitate the vasodilatatory effectiveness of curcumin and its analogs on rat pulmonary artery

To localise the possible tissue targets i.e., endothelium or vascular smooth muscle of curcumin and analogs on vasaodilation effect of rat pulmonary artery

To compare the effect of curcumin and analogs on the responses in rat pulmonary artery and aorta

Scope of the study

The vasodilation effect of curcumin and its analogs will be tested on rat intra-pulmonary artery with and without intact endothelium as well as intact aorta using sildenafil as a positive control.

Hypothesis

Since curcumin and its analogs showed an *in vitro* inhibitory effect on PDE5, this action produces vasorelaxation of the rat pulmonary artery and is mediated through an increased cGMP. If true, then the different natural and synthetic curcumin analogs should display potencies which reflect the cell-free determinations.

Anticipated outcomes

The effects of curcumin and its analogs on rat pulmonary artery, the tissue target and their selectivity will be characterised. The structure activity relationship of the analogs will be discussed in the light of PDE5 sensitivity and different cellular sites of action.