Eur. J. Med. Chem. 35 (2000) 463–480

S0223523400001793/REV

Review

Phosphodiesterase 4 inhibitors, structurally unrelated to Rolipram, as promising agents for the treatment of asthma and other pathologies

Vittorio Dal Piaz*, Maria Paola Giovannoni

Dipartimento di Scienze Farmaceutiche, Università di Firenze, Via G. Capponi 9 50121 Florence, Italy

Received 28 February 2000

Abstract – An increase of cyclic adenosine and guanosine monophosphate (cAMP and cGMP) level can be achieved by inhibition of phosphodiesterases (PDEs), which are the enzymes responsible for the conversion of these second messengers into the corresponding 5-monophosphate inactive counterparts. The high heterogeneity in PDE families and in their tissue distribution, as well as their different functional role, make these enzymes very attractive targets for medicinal chemists. The PDE 4 family is particularly abundant in immunocompetent cells, where an increase of cAMP leads to the inhibition of the synthesis and release of pro-inflammatory mediators, cytokines and active oxygen species. Moreover PDE 4 inhibitors are able to reduce bronchial smooth muscle tone in vitro and show bronchodilatory effects in vivo. Thus, the current therapy for asthma, which is based on a combination of β_2 agonists and corticosteroids, could be replaced by treatment with PDE 4 inhibitors. This review mainly covers PDE 4 inhibitors structurally related to xanthines and Nitraquazone, which appear to be very attractive models for the synthesis of novel PDE 4 inhibitors potentially useful for the treatment of asthma, chronic pulmonary obstructive disease and some autoimmune diseases. These compounds could be devoid of the central side-effects (nausea, vomiting, headache) of the archetypal Rolipram, which hampered its development as a drug. The review also highlights the novel structural classes of PDE 4 inhibitors recently reported in the literature. © 2000 Éditions scientifiques et médicales Elsevier SAS

phosphodiesterase 4 inhibitors / chemical classification / asthma / chronic pulmonary obstructive disease / autoimmune diseases

1. Introduction

In the last few years there has been a growing interest for the therapeutic applications of phosphodiesterase 4 inhibitors (PDE 4 inhibitors). There are at least two main reasons for the basis of the rapid development of the chemical, pharmacological and biochemical research in this field.

First, there is a general conviction that the mixed anti-inflammatory and bronchodilatory profile of PDE 4 inhibitors could allow, through the optimization of first generation prototypes, the discovery of new agents able to compete and, perhaps, to replace corticosteroids and β_2 agonists, which represent the basis of the therapeutic management of asthma. Moreover PDE 4 inhibitors may be beneficial in the treatment of chronic obstructive pulmonary disease (COPD), a major respiratory disease for which pharmacological treatment is still inadequate [1].

* Correspondence and reprints: vdp@farmfi.scifarm.unifi.it

Second, new and promising therapeutic applications of PDE 4 inhibitors in certain unmet autoimmune diseases, e.g. rheumatoid arthritis, multiple sclerosis and type 2 diabetes have emerged in recent years [2]. In the meantime the knowledge of the molecular biology of the PDE 4 family has dramatically advanced, allowing the identification of four sub-types, differentially expressed between tissues and cells [3–5]. On this basis, subtype-selective inhibitors have been addressed as potential ways to achieve tissue or cell selectivity, therefore decreasing the side-effects and improving the therapeutic index of first generation PDE 4 inhibitors.

1.1. Why Rolipram-unrelated phosphodiesterase 4 inhibitors?

Several comprehensive reviews on PDE 4 inhibitors have appeared in the literature in recent years [6–10] and a systematic updating has been provided by annual publications by Norman and others [1, 11, 12]. For many reasons the attention of the authors has been mainly

devoted to Rolipram and congeners. In fact Rolipram, since its discovery as a potent and selective PDE 4 inhibitor [13], has represented a useful pharmacological tool for the characterization of this isoenzyme in different tissues, a reference drug in evaluating novel inhibitors, as well as the main template for the synthesis of novel inhibitors. Thus, a plethora of analogues with similar pharmacological and toxicological profiles were synthesized. This fact probably limited the search for different structural classes of inhibitors until it became clear that many of the side-effects displayed by this compound, which hampered its therapeutic development, are connected with its ability to bind with nanomolar affinity to a binding site, which for a long time was considered distinct from the catalytic site of the PDE 4 [14]. From that moment two main directions of research were followed in the field of PDE 4 inhibitors: that of the manipulation of the Rolipram structure, aimed to dissociate catalytic site inhibition and binding site affinity and the search for structurally different inhibitors.

Thus, the number of compounds published or patented as PDE 4 inhibitors, which are more or less closely related to the structure of Rolipram, is much higher with respect to that of the other chemical classes. Nevertheless the few PDE 4 inhibitors therapeutically employed in asthma are still Theophylline and related compounds; moreover, one of the PDE 4 inhibitors more advanced in clinical evaluation, Arofylline [15], belongs to the family of xanthines. Finally the number of patents claiming different chemical classes of PDE 4 inhibitors is increasing in recent years, indicating that pharmaceutical companies are moving from the Rolipram structure, probably in the attempt to avoid the side-effects associated with it. Thus the main focus of this review will be on PDE 4 inhibitors structurally unrelated to Rolipram.

1.2. PDE families

The phosphodiesterases (PDEs) are a superfamily of enzymes which catalyse the hydrolysis of the cyclic nucleotides AMP and GMP to their corresponding inactive 5-monophosphate counterparts. cAMP and cGMP are ubiquitous intracellular second messengers which play a prominent role in the regulation of important cellular functions such as secretion, contraction, metabolism and growth. Thus the elevation of their intracellular levels by PDE inhibition represents a useful strategy for eliciting a variety of pharmacological effects.

At present, at least 11 different families of PDE isoenzymes are known [16, 17]. Some of them are characterized by substrate specificity (cAMP or cGMP), different kinetic properties and different tissue distribution. The molecular cloning of mammalian PDE revealed that each family is populated by one or more distinct gene product (e.g. A, B, C or D for PDE 4). Furthermore alternative mRNA processing allows the production of multiple splice variants [18]. Thus, till now at least 44 distinct human PDE proteins have been identified [16]. For some isoenzymes endogenous regulators such as Ca^{2+} and calmodulin, co-factors, such as Mg^{2+} and possibly Zn^{2+} [12] and synthetic selective inhibitors are known. Thus Milrinone and Siguazodan selectively inhibit PDE 3, Rolipram PDE 4, Zaprinast and Sildenafil PDE 5. In *table I* are reported some features of the seven main families.

1.3. Historic development of PDE inhibitors as drugs

During the 1980s a concerted effort was performed by the pharmaceutical industry to identify selective PDE 3 inhibitors as possible substitutes of cardioactive glycosides in the therapy of cardiac insufficience. In fact, these inhibitors elicit a favourable haemodynamic effect, standing on positive inotropic and vasodilating activity, in both the isolated organ and in whole animals, associated, in some cases, to platelet aggregation inhibition. Unfortunately, long term therapy of patients with this type of drug was demonstrated to be disadvantageous in terms of quality of life versus survival. Thus, today, drugs like Milrinone **1** [22] and a few analogues such as Enoximone **2** [23], Vesnarinone **3** [24] and Pimobendan **4** [25] have only a limited clinical utility in short term treatment of heart failure (*figure 1*).

On the contrary a selective PDE 5 inhibitor, the Sildenafil **5** (Viagra®), endowed with potent peripheral vasodilatory activity, has successfully been launched by Pfizer in many countries for the treatment of male erectile dysfunction [21].

From the beginning of the 1990s growing interest was devoted to identifying selective PDE 4 inhibitors. The type 4 cAMP specific isoenzyme, which was identified in 1985 [26], is particularly abundant in brain and immunocompetent cells; in the eosinophils which are considered the effectors ‘par excellence’ of asthma, this is the only isoform present [9]. Inhibition of PDE 4 in all these cell types produces a marked reduction of inflammatory and immunomodulatory response. Moreover, in vivo PDE 4 inhibitors reduce the tone of bronchial smooth muscle and produce bronchodilation. Taken together these data suggested a possible therapeutic utility of PDE 4 inhibitors as anti-inflammatory and immunomodulatory agents.

Table I. Phosphodiesterase isoenzyme families.

Family	Modulator	Substrate specificity (relative Km)	Location	Inhibitors
PDE 1α	Ca^{2+}/calmodulin stimulated	cAMP > cGMP	airway, vascular smooth muscle, heart, liver, brain, kidney, macrophages	Vinpocetine[a]
PDE 1β	cGMP stimulated	cAMP = cGMP	airway, vascular smooth muscle, heart, liver, brain, kidney, macrophages	Vinpocetine[a]
PDE 2	cGMP stimulated	cAMP < cGMP	airway, smooth muscle, heart, liver, brain, kidney	EHNA[a]
PDE 3	cGMP inhibited	cAMP = cGMP	airway, vascular smooth muscle, heart, liver, platelets, endothelial cells, macrophages, T-lymphocytes	Siguazodan[a] Cilostamide[b] Cilostazol[c]
PDE 4	cAMP specific	cAMP << cGMP	airway, vascular smooth muscle, heart, liver, brain, kidney, endothelial cells, T-lymphocytes, macrophages, neutrophils, monocytes, eosinophils, mast cells	Denbufylline[a] RP73401[a] Nitraquazone[a]
PDE 5	cGMP specific	cAMP >> cGMP	airway, vascular smooth muscle, heart, brain, platelets, macrophages, T-lymphocytes	Zaprinast[a] SKF96231[a] Sildenafil[d]
PDE 6	photoreceptor	cAMP >> cGMP	retina	Zaprinast[a]
PDE 7	cAMP specific		T-lymphocytes	none (Rolipram insensitive)

[a]Reference [9]; [b]reference [19]; [c]reference [20]; [d]reference [21].

2. PDE 4 inhibitors in asthma and COPD [27]

Bronchial asthma is a non-infectious respiratory pathology affecting over 5% of the adult population and perhaps up to 10% of children. In industrialized countries it is rising in prevalence, severity and mortality, despite a substantial increase in prescribed antiasthma treatments [28]. In younger patients the disease frequently increases in severity with time. Acute airway obstruction, bronchial hyper-responsiveness and inflammatory state of the bronchial mucosa with increased levels of inflammatory mediators, are the most evident phenomena which characterize this pathology. Recent studies have highlighted the prominent role of the eosinophils in asthma, suggesting that this pathology could be considered as an eosinophilic bronchitis and therefore an inflammatory disorder [29–33].

Inhaled β_2 receptor agonists and corticosteroids have represented the mainstay of the therapeutic management of asthma for at least 25 years. According to the previous arguments, β_2 adrenoceptor agonists, which inhibit bronchostriction, would provide little more than symptomatic relief, while the anti-inflammatory effect of glucocorticoids may affect disease progression [34, 35]. On the other hand Theophylline, the oldest antiasthma drug, particularly employed in the US and considered for a longtime as only a bronchodilator, also displays a significant anti-inflammatory effect.

Novel orally active antiasthma drugs displaying both bronchodilatory and anti-inflammatory activity could be very useful substitutes for the above combined therapy with anti-inflammatories and bronchodilators for many reasons:

1. chronic administration of glucocorticoids can have adverse effects, namely in children [36].

2. Systemic side-effects, such as tachycardia, palpitation and headache are observed with inhaled β_2 adrenoceptor agonists, like salbutamol and terbutalin. These adverse reactions may be related to inadvertent swallowing of the inhaled dose, which is absorbed from the gut [37]. Moreover several concerns emerged from studies aimed to clarify the effects of regularly inhaled β_2 agonists on disease control in stable asthmatic subjects. In these patients worsening of the pathology was observed with continuously higher doses or longer-acting inhaled beta-symphatomimetics [38, 39].

3. Side-effects could result from non-selective drug interaction [40].

4. Disease progression could reach a point where airway obstruction significantly reduces inhaled drug delivery.

5. Self-administration of inhaled drugs may be difficult for older people and the disabled.

According to this, bronchial asthma is the major focus of the pharmaceutical industry in the field of respiratory diseases.

As anticipated, the in vitro profile of selective PDE 4 inhibitors is characterized by suppression of the inflammatory and immunomodulatory response, as well as by reduction of bronchial smooth muscle tone. Thus PDE 4

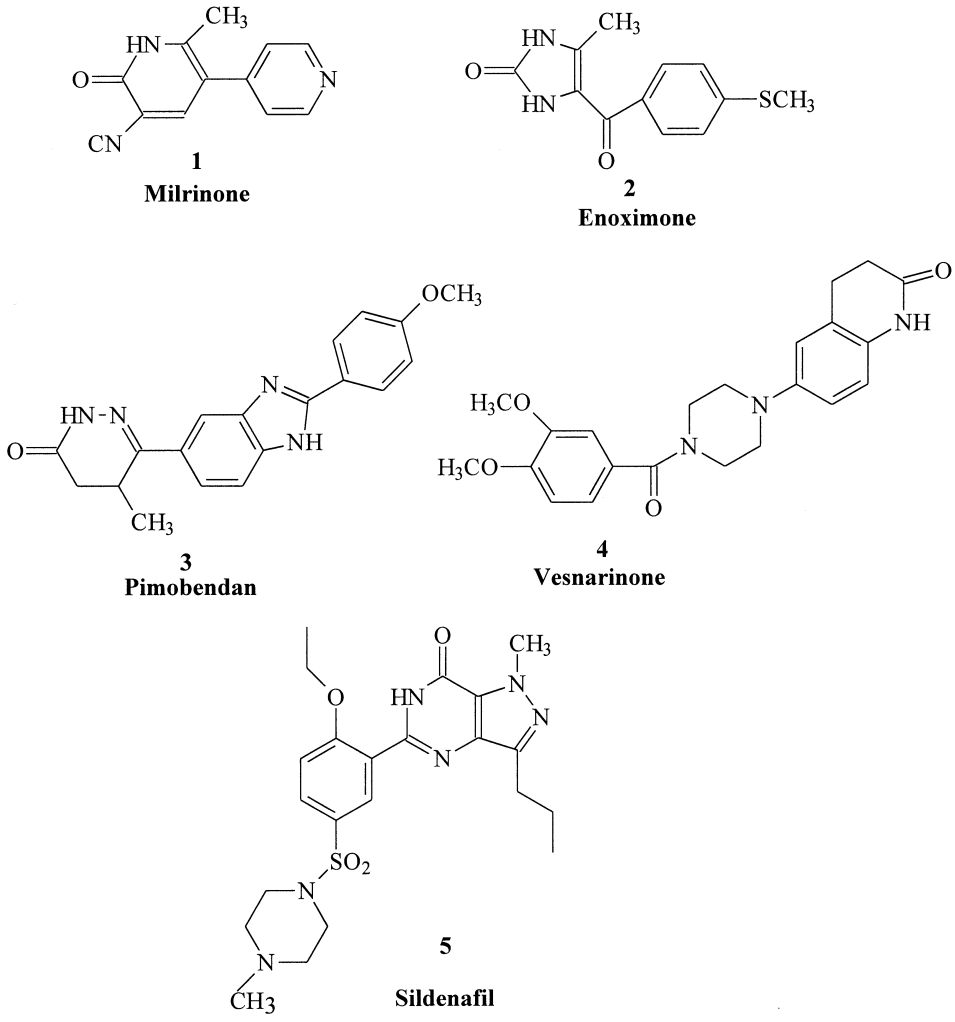


Figure 1. PDE 3 and PDE 5 inhibitors on the market.

inhibitors are capable of down-regulating, in vitro, many of the concerted effects of immunocompetent cells, inflammatory mediators, cytokines and growth factors which are associated with asthma [41–43]. Among these are: inhibition of superoxide generation in monocytes, macrophages, neutrophils and eosinophils, reduction of TNF-α release in monocytes and macrophages and suppression of chemotaxis and phagocytosis. Furthermore, strong evidence exists that these agents are able to reduce the permeability of endothelial cells and the expression of adhesion molecules which are at the basis of most inflammatory processes.

In vivo PDE 4 inhibitors demonstrated bronchodilatory effects and ability to reverse the bronchospasm induced by a variety of agents such histamine, leukotriene D_4 (LTD 4), carbachol or methacholine [10]. In this regard it is interesting to observe that guinea-pig studies demonstrated that PDE 3 inhibitors, as well as mixed PDE 3/PDE 4 inhibitors [44], are also able to reverse the bronchoconstriction induced by the same agonists. In conclusion, the profile of selective PDE 4 inhibitors seems to fulfil the requirements for a unique therapy, hopefully replacing the mixed administration of β_2 agonists and anti-inflammatories, while the typical cardiovascular profile of PDE 3 inhibitors seems to preclude a possible development of these agents, as well as that of the mixed PDE 3/PDE 4 inhibitors as anti-asthma drugs.

Chronic obstructive pulmonary disease (COPD) is a progressive lung disease affecting at least 6% of the population and represents the fourth largest cause of

death in the world [45], being also a major drain of finite health resources. COPD is associated with a chronic inflammation of the airway that involves neutrophils, macrophages and $CD8^+$ T-cells. Smoking cessation programs, bronchodilators and anti-inflammatory drugs demonstrated to be inadequate in managing this pathology. Recent clinical data suggest that PDE 4 inhibitors may be beneficial: thus phase III clinical trials with SB-207499 (Ariflo®), a Rolipram related PDE 4 inhibitor, demonstrated a substantial improvement in pulmonary function in subjects with minimal response to bronchodilator therapy [46]. This suggests that suppression of neutrophil function may be at the basis of the significant patient improvement observed with this PDE 4 inhibitor.

3. Other therapeutic targets of PDE 4 inhibitors

Recently, new and more ambitious therapeutic targets like multiple sclerosis, type II diabetes, rheumatoid arthritis, ulcerative cholitis, atopic dermatitis and other autoimmune diseases emerged for PDE 4 inhibitors. Many of these perspectives are associated with the evidence that PDE 4 selective inhibitors are able to reduce TNF-α production. In fact this cytokine plays a central role in determining several unmet diseases belonging to the autoimmune class.

3.1. Rheumatoid arthritis

In rheumatoid arthritis (RA) a significant increase of TNF-α levels in plasma and synovial fluid was evidenced, suggesting that agents which normalize this cytokine overexpression, subsequent to a deregulated inflammatory response, could be useful in this type of pathology. According to this, TNF-α neutralizing antibodies determined significant clinical improvements in RA patients [47].

PDE 4 inhibitors like Rolipram demonstrated to be able to prevent the onset of the arthritis symptoms, as well as to diminish disease progression if administered after disease development in the rat collagen type II-induced arthritis model [48]. These results were accompanied by the evidence of a strong down-regulation of TNF-α and interferon (IFN) mRNA expression in regional lymph nodes. Thus, at least one pharmaceutical company switched the focus of its research on PDE 4 inhibitors from asthma to rheumatoid arthritis. Promising results were obtained from a pilot clinical study with Piclamilast (RP73401), a potent and selective PDE 4 inhibitor, structurally related to Rolipram, which demonstrated to be able to induce significant symptom relief in patients, associated by a positive trend in reducing serum concentrations of IL-6 and C-reactive protein [49].

Taken together these data seem to confirm the potential of PDE 4 inhibitors as promising drugs in this highly disabling disease.

3.2. Multiple sclerosis

The PDE 4 selective inhibitor Rolipram is effective in suppressing the clinical manifestations of acute autoimmune encephalomyelitis (EAE) in two well-defined experimental models of multiple sclerosis (MS) in rats and monkeys [50–52]. The non-selective Denbufylline can also prevent and ameliorate EAE in rats [53, 54]. It was suggested that these effects are in part due to inhibition of pro-inflammatory cytokine release from autoreactive T lymphocytes and possibly from macrophages.

The discovery of new, more selective, PDE 4 inhibitors devoid of the side-effects of Rolipram could open interesting perspectives for the therapy of this chronic inflammatory disease of the CNS.

3.3. Diabetes

Both Pentoxifylline and Rolipram reduced the severity of insulitis and prevented diabetes in NOD mice, an experimental model of autoimmune diabetes in which interleukin (IL)-12, IFN–γ and TNF-α play an important role. The protection was still efficient after several weeks after withdrawal of the drugs and PDE 4 inhibition seems to be at the basis of the beneficial effects observed in this experimental model of diabetes [55].

3.4. Septic shock

Studies performed in experimental models of endotoxin-induced acute renal failure in rats demonstrated that the selective PDE 4 inhibitor Ro-201724 was effective in reducing renal vascular resistance and glomerular filtration rate impairment, improving survival of the animal treated group [56]. Both selective (Rolipram, Denbufylline) and non-selective pentoxifylline (PTX) PDE 4 inhibitors in experimental models of endotoxin-induced bowel erythrocyte extravasation in rats and in anaesthetized dogs provided evidence that these agents are able to reduce extravasation and blood flow reduction [57]. Moreover a group of 12 patients fulfilling the criteria of septic shock, when given PTX (1 mg/kg/h for 24 h) showed a significant reduction of TNF-α levels, even if IL-6 levels increased, suggesting that PDE 4 inhibitor treatment cannot block the inflammatory overreaction [58]. Taken together these data seem to confirm the therapeutic potential of PDE inhibitors in septic shock.

Further types of pathologies in which increased plasma levels of TNF-α were evidenced, such as inflammatory bowel disease [59] and liver injury [60] were proposed as therapeutic targets of PDE 4 inhibitors.

Moreover PDE 4 selective inhibitors demonstrated effectiveness in inhibiting the decrease in bone mineral density of the femurs from Walker 256/S-bearing rats, suggesting that these agents may be considered as drug candidates for the bone loss disease [61].

3.5. Antileishmania effects

Due to their modulatory activity on cell proliferation and on differentiation processes, PDE inhibitors were also proposed as potential drugs in some parasite induced diseases, e.g. leishmaniasis [62]. This is a pathology affecting millions of people world-wide (annual incidence: 12 million cases), for which available drugs display low efficacy and are characterized by heavy side effects.

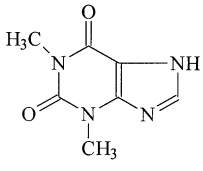
4. The oldest inhibitors

The oldest and most popular compounds displaying PDE 4 inhibitory activity are Theophylline **6**, a weak and non-specific inhibitor and Rolipram **7**, a quite potent (IC_{50} = 0.3 μM) and selective PDE 4 inhibitor (*figure 2*).

4.1. Theophylline

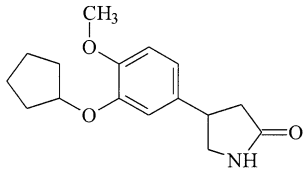
Theophylline began to be generally used in asthma treatment in the 1930s and has been one of the most widely prescribed drugs world-wide. At the present its use is relegated to 3rd-line therapy behind corticosteroids and β_2 selective bronchodilators [63, 64]. The therapeutic utility of Theophylline is closely related to the type of asthma to be managed.

In acute severe asthma, i.v. aminophylline is less effective than inhaled β_2 agonists [65]. Moreover Theophylline is more liable to induce adverse effects. In some cases of asthma deaths toxic concentrations of Theophylline were evidenced [66]. Thus i.v. aminophylline is indicated only for those patients who did not satisfactorily respond to nebulized β agonists.



6

Theophylline



7

Rolipram

Figure 2. Compounds **6** and **7**.

In chronic asthma Theophylline is suggested as an additional bronchodilator when moderate to high doses of inhaled steroids fail to completely control asthma symptoms [67].

Evidence for a sub-population of asthmatics who particularly benefit from Theophylline emerged from a study performed on young patients taking, in addition to oral Theophylline, inhaled steroids, nebulized β_2 agonists, as well as inhaled anticholinergics and cromoglycate. In these cases withdrawal of Theophylline caused a significant deterioration in disease control, which could not be prevented by increased steroids, while it only responded to the re-introduction of Theophylline [68].

The usefulness of Theophylline as an antiasthma drug is due in part to the drug's oral activity and to its ability to control nocturnal asthma symptoms by sustained-release drug preparations. This last property stems from the ability of Theophylline to inhibit the inflammatory response subsequent to the circadian nocturnal troughs of epinephrine and cortisol plasma levels [69].

Common side-effects of Theophylline, such as restlessness and insomnia, which are related to CNS stimulation, generally occur at plasma concentrations higher then 20 mg/L. Higher doses can result in nausea, vomiting, headache and, in extreme cases, life-threatening convulsions and cardiac arrhythmias. Other types of unwanted effects, like increased acid secretion and diuresis may also occur.

Therefore, probably the main problem associated with the use of Theophylline is connected with its narrow therapeutic index. In fact plasma concentrations of 10–15 mg/L are necessary to elicit antiasthma activity; on the other hand blood levels higher than 20 mg/L are able to induce the first side-effects. Further drawbacks are associated with high inter-individual variability of absorption, metabolism and clearance [70] and with the numerous interactions featuring this drug. In this regard recent studies have highlighted a significant reduction in Theophylline clearance due to usual intake of dietary caffeine in subjects consuming 2–7 cups of coffee in one day [71].

Despite its long history and widespread use, the molecular mechanism responsible for the antiasthma effect of Theophylline remains yet to be clarified, due to the myriad of biological effects featuring this compound [72, 73]. Probably several mechanisms like PDE inhibition, adenosine receptor binding and mediator (TNF-α)

antagonism may be operating both for bronchodilatory/ anti-inflammatory activity and for the numerous side-effects.

4.1.1. PDE inhibition

Therapeutically relevant concentrations of Theophylline display small and non-selective inhibition of PDE. Only 5–20% of the total PDE lung expression is inhibited at these plasma levels [74, 75]. Since only PDE 3 and PDE 4 inhibition play a relevant role in determining airway smooth muscle relaxation, it appears improbable that this non-specific and weak inhibition may be responsible for the therapeutic activity of Theophylline. Since from the comparison of PDE inhibition and in vitro airway smooth muscle relaxation [74] emerged an evident parallelism, the more likely hypothesis is that the presence of adenylate cyclase or guanylate cyclase activators may confer a greater functional efficacy to PDE inhibition. Support for this explanation is given by the additional effects of Theophylline and β agonists [76, 77] which elicit bronchodilatation through intracellular cAMP elevation.

4.1.2. Adenosine receptor antagonism

Adenosine has little in vitro effect on airway smooth muscle from healthy subjects, while it causes both in vitro constriction in the same tissue from asthmatics and in vivo bronchoconstriction when inhaled by asthmatics [78, 79]. The latter effect is inhibited by therapeutic levels of Theophylline [80]. The hypothesis that the antiasthma effects could be mediated by adenosine A_1/A_2 antagonism seems to be ruled out by the fact that Theophylline analogues, e.g. Enprofylline, isbufylline and Doxofylline, which lack adenosine receptor antagonism, also display bronchodilatory activity.

More likely, adenosine antagonism may account for some of the CNS, cardiac and gastrointestinal side-effects of Theophylline. These unwanted effects certainly stem, at least in part, from the tendency of Theophylline to indiscriminately inhibit the PDEs in all tissues [81, 82].

4.1.3. Mediator inhibition

This mechanism may account for the antiasthma effect of Theophylline. In fact there is a body of experimental evidence that Theophylline, as well as some analogues, are able to inhibit the hyper-responsiveness of airways induced by TNF-α in animal models [83, 84]. Additional mechanisms which could contribute to the therapeutic activity of Theophylline are catecholamine release and inhibition of calcium flux [85].

4.2. Rolipram

In addition to its sub-micromolar selective PDE 4 inhibitory activity, Rolipram binds with nanomolar affinity to preparations of animal and human brain tissue homogenate. Very recently it was demonstrated that this divergence between enzyme inhibition and binding potency is due to the existence of two different conformers of the PDE 4 isoenzyme [86]. Many Rolipram analogues, as well as PDE 4 inhibitors unrelated to Rolipram, bind with high affinity to the so called Rolipram high affinity binding site (RHABS). Some pharmacological effects of Rolipram, like antidepressant activity and bronchodilatory effects, correlate much better with its affinity for RHABS, while others (inhibition of allergen-induced bronchostriction and inflammatory mediator release), better correlate with enzyme inhibition potency. Side-effects such as nausea, vomiting and headache, which until now hampered the development of many PDE4 inhibitors as drugs, appear at therapeutic doses of potent HARBS ligands. Therefore the best balance of therapeutic benefit and side-effects seems to characterize compounds with high potency as enzyme inhibitors and low affinity for HARBS. This profile features both Rolipram related and unrelated second generation PDE 4 inhibitors.

5. The main chemical classes of PDE 4 inhibitors

Traditionally PDE 4 inhibitors are classified into three main chemical classes:

1. Catechol ethers, in which are grouped a wide variety of flexible molecules of inhibitors structurally related to Rolipram. These agents are characterized by the presence of the substructure **8** in which R_1 is generally a methyl and R_2 a cyclopentyl group; alternatively highly lipophilic groups are present at R_2.
2. Quinazolinediones, in which are classified the PDE 4 inhibitors structurally related to Nitraquazone **9** [87].
3. Xanthines, to which Theophylline **6** belongs.

However, according to the pharmacophoric model proposed by Polimeropoulos et al. [88] for PDE 4 inhibitors, classes 2 and 3 could be referred to the unique general structure **10** in which X = N, CO, CH, Y = bulky alkyl or aryl Z = N, C, and W = N, CH. Inside this class, two subclasses can be distinguished, namely quinazolindiones and xanthines (*figure 3*).

5.1. Quinazolindiones and related compounds

The archetypal quinazolindione moiety of Nitraquazone was extensively manipulated to afford a variety of structure-derived compounds, with Syntex being particu-

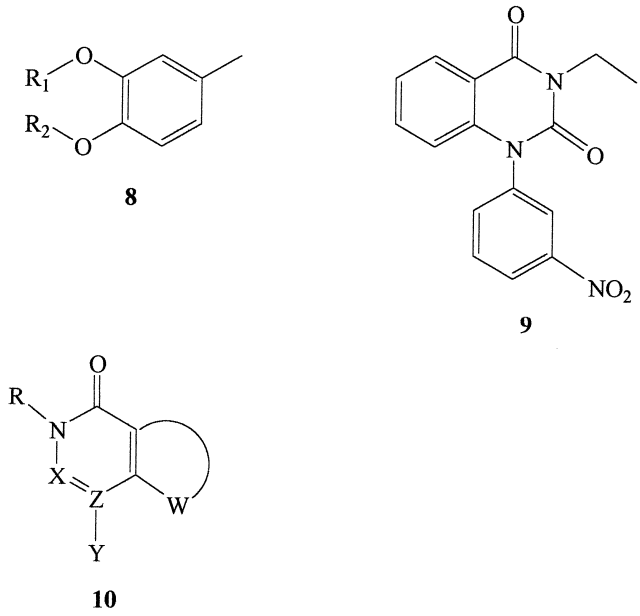


Figure 3. Structures of **8**, **9** and **10**.

larly active in this area. In *figure 4* the conceptual development of some of the most interesting Nitraquazone-derived PDE 4 inhibitors is depicted.

SAR studies demonstrated that the 3′ NO_2 group can be successfully replaced by different non-protic, electron-withdrawing functionalities like Cl, Br, $COOCH_3$.

Benzene–pyridine isosteric replacement, as well as introduction of more space-filling groups at N_3 afforded the Pfizer compound CP 77059 **11** (R_1 = C_6H_5, R_2 = $COOCH_3$) [89] and the Syntex RS 25344 **12** (R_1 = 4-pyridyl, R_2 = NO_2) [90], which proved to be four orders of magnitude more potent with respect to the prototype **9**. Further simplification of the structure, by removing unnecessary functionalities, was associated with further dramatic improvement of activity. Thus the quinoline RS 14203 **13** is one of the most potent PDE 4 inhibitors (IC_{50} = 0.023 nM) [91] (*figure 4*).

The naftiridinones **14** (Sapporo) [92] and **18** [93] arise from a less extended structural simplification of Nitraquazone, whereas compound **15** [94] exhibits the essential substructure of **13**, where the 3′ nitrophenyl group is replaced by a benzotriazole. Compound **16** (Yamanouchi, YM 58977) [95] and **17** (Asta, D-22888) [96] essentially arise from the interchanging of the CO and N-substituted groups with respect to compounds **11**–**12**. In the case of **16** the pyridine nitrogen is also moved in the opposite position, whereas in **17** the phenyl ring appended is also replaced by a fused imidazole system.

The Syntex compounds of type **19** [97], which are nanomolar selective inhibitors, bring together the isosteric benzene–pyridine replacement and the pyrimidone–pyridazinone substitution compared with Nitraquazone. Unfortunately, in the strikingly potent **13**, as well as in the Syntex compounds **19**, the increased potency is accompanied by a concomitant increase in side-effects which limited their development. In the attempt to reduce these adverse reactions, compounds of type **20** [98, 99] were synthesized by our group maintaining the same arylpyridazinone backbone, but replacing the pyridine with a series of five and six membered heterocyclic systems, like pyrrole, pyrazole, 1,2-dihydropyridine and thiophene. These compounds, namely that substituted at pyridazine-2-nitrogen with an ethyl group, compared with Rolipram and Syntex analogues **19**, demonstrated to have weaker PDE 4 inhibitory activity but a significantly better balance between PDE 4 IC_{50} and affinity for HARBS. This property indicates a probable smaller liability to induce emetic effects.

Finally, the nicotinamide ethers **21** can be regarded as the open models of Nitraquazone obtained by separation of the pyrimidindione moiety of Nitraquazone analogues **11** and **12**. This type of compound, firstly reported by Vinick et al. [100] in 1991, more recently aroused renewed interest since some terms display 100-fold selectivity for inhibition of the PDE 4D isoform in comparison with the other PDE 4 isoforms. The interest for these PDE 4 inhibitors was also increased by their reduced affinity for HARBS.

Further PDE 4 inhibitors like **22**, based on the same pyrido[2,3-d]pyrazinone backbone, displaying a 20 nM IC_{50}, were recently disclosed by Fujisawa [101, 102]. These agents bear, in position 4, a phenyl group with more and more larger side-chains appended (*figure 5*).

Novel classes of PDE 4 inhibitors like **23** and **24**, based on the same quinoline, quinolone and naftiridone template featuring **14**, **15** and **16**, but bearing, as the original structural element the presence of carboxamido or sulfonamido groups were disclosed by Chiroscience in 1997 [103, 104]. For these compounds IC_{50}s are not reported.

In conclusion, the best pharmacophoric model for the most potent Nitraquazone-like inhibitors seems to be based on an almost flat heteroaromatic area, generally formed by a 6–6 condensed system. One (hetero)aromatic or a cycloalkyl system A is connected to the flat portion through a methylene spacer; another, bearing an electron-withdrawing substituent in 3′, is directly appended (**25**). Both carbonyl dipoles of Nitraquazone are not essential features and can be replaced by =N– and =CH– (compounds **13** and **22**). The 3′ substituted aromatic system can be replaced by a fused five membered nitrogen

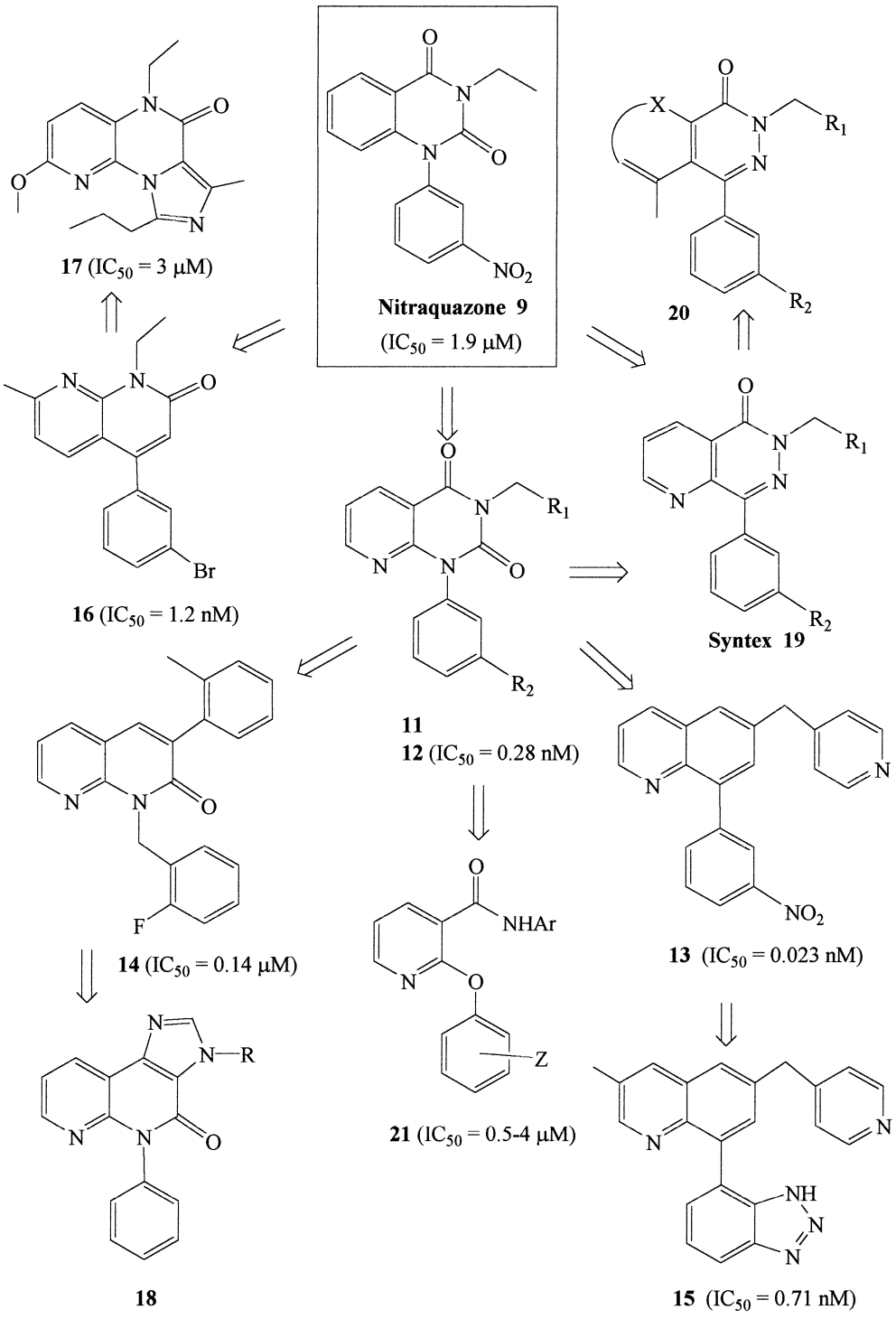


Figure 4. Conceptual development of some of the most interesting nitraquazone-derived PDE 4 inhibitors.

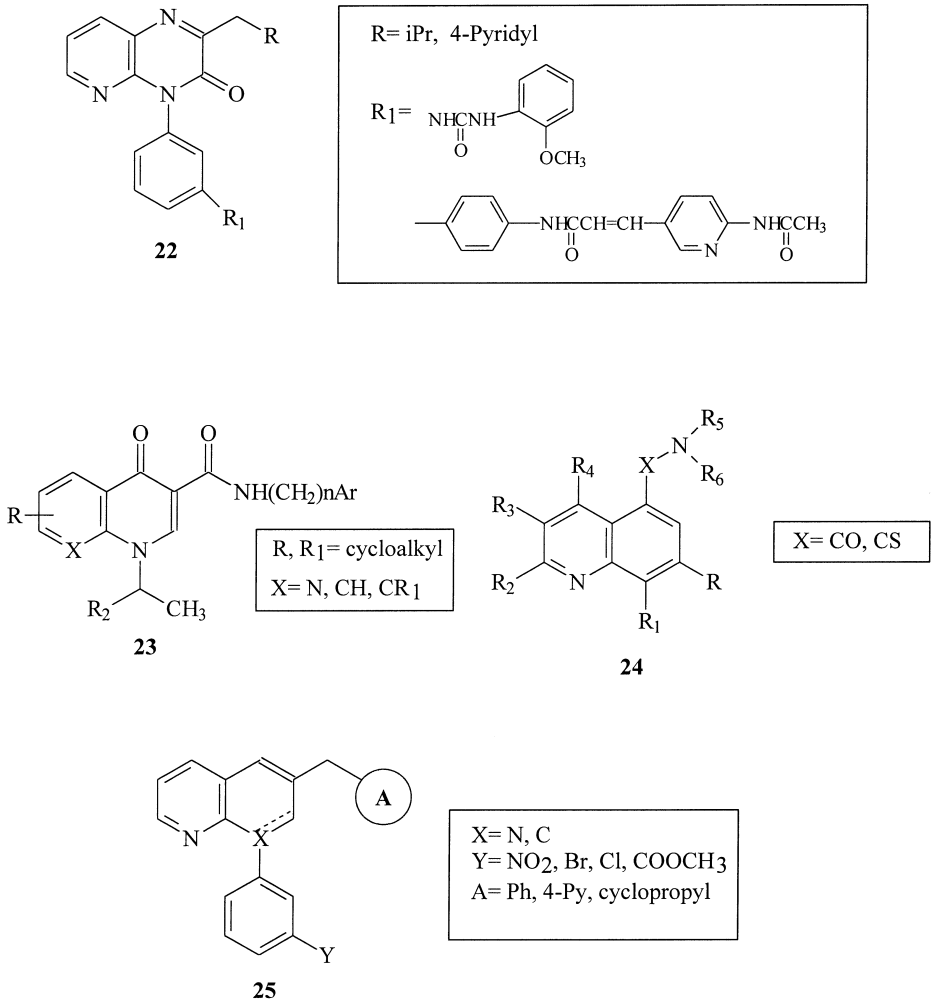


Figure 5. Compounds **22**–**25**.

heterocycle (compounds **15** and **17**) or by space-filling lipophilic groups containing amide or urea functions (compounds **22**). Carboxamido or sulfonamido groups can be inserted instead of the carbonyl dipole in position 4 of Nitraquazone (compounds **24**).

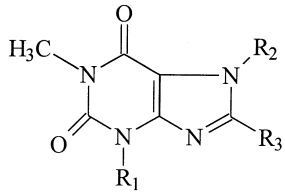
The next challenge in this class of PDE 4 inhibitors will be to combine nanomolar inhibitory potency with strongly reduced affinity for RHABS in order to improve the therapeutic index.

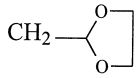
5.2. Xanthines and related compounds

In the attempt to improve the therapeutic index of Theophylline, a variety of synthetic xanthines have been studied (*tables II* and *III*). A relevant contribution in studying SARs in this class was made by a Japanese group directed by Miyamoto [105–109], who recently reported an interesting series of heterocyclic-fused xanthines [110]. Among the therapeutically useful synthetic xanthines, Dyphylline, was approved for asthma therapy and has appeared in the US market; Bamifylline and Doxofylline were marketed in Europe [111]. Enprofylline was extensively investigated, but its development was discontinued [112, 113]. Verofylline was clinically evaluated, but did not reach the market [114]. IBMX (isobutyl methylxanthine) is a relatively weak, non-selective PDE 4 inhibitor [115, 116].

Denbufylline is a selective PDE 4 inhibitor showing low adenosine receptor affinity whose development was discontinued due to poor pharmacokinetics [117]. Generally these synthetic xanthines therapeutically used as

Table II. 1-Methyl xanthines.

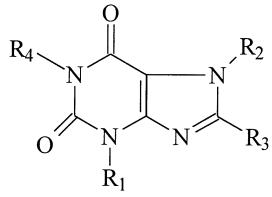


	R_1	R_2	R_3
Teophylline	CH_3	H	H
IBMX	$CH_2CH(CH_3)_2$	H	H
Isbufylline	CH_3	$CH_2CH(CH_3)_2$	H
Doxofylline	CH_3		H
Dyphylline	CH_3	$CH_2CH(OH)CH_2OH$	H
Verofylline	$CH_2CH(CH_3)C_2H_5$	H	CH_3
Bamifylline	CH_3	$(CH_2)_2N(C_2H_5)(CH_2)_2OH$	$CH_2C_6H_5$

antiasthma drugs exhibit low or moderate PDE 4 inhibitory activity (*table IV*).

Arofylline (LAS 31025) (*table III*) is one of the most interesting xanthine derivatives and is in phase III clinical trials for oral asthma therapy [124]. The chemical novelty of this compound stands on the presence of the 4-chlorophenyl fragment at N 3. Arofylline is a weak (IC_{50} = 5.5 μM), but selective, inhibitor (selectivity versus PDE 3 > 50). Compared with Theophylline, Arofylline is many fold more potent as a PDE 4 inhibitor, showing affinity for adenosine A_1 and A_2 receptors in the same order of magnitude. Compared with the more potent PDE 4 inhibitor Rolipram (IC_{50} = 0.30 μM), Arofylline is 25–30-fold less emetic in conscious dogs. Unlike Theophylline, Arofylline lacks stimulant central effects, as measured by spontaneous motor activity in mice, and it is devoid of effects on the cardiac function. In a double blind, parallel, randomized clinical trial in 232 patients

Table III. Further xanthines.



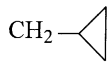
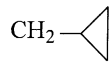
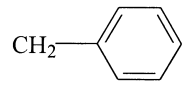
	R_1	R_2	R_3	R_4
Pentoxifylline	CH_3	CH_3	H	$(CH_2)_4COCH_3$
Enprofylline	$n.C_3H_7$	H	H	H
Cipamfylline (BRL 61063)		H	NH_2	
Denbufylline (BRL 30892)	$n.C_4H_9$	CH_2COCH_3	H	$n.C_4H_9$
Arofylline	$p.ClC_6H_4$	H	H	C_2H_5
Chiroscience 245412	$m.OCH_3C_6H_4$	H	H	

Table IV. PDE 4 inhibitory activity and adenosine affinity of some xanthines.

Xanthine	Cell/tissue	PDE 4 IC_{50} (μM)	Adenosine A_1 IC_{50} (μM)	Adenosine A_2 IC_{50} (μM)	Reference
Theophylline	human bronchus	150	20	29	[118]
	human eosinophils	290			[119]
IBMX	dog trachea	9	3.2		[116]
	human eosinophils	14			
ICI-63197	guinea-pig lung	4.6			[120]
Penthoxyfylline	human bronchus	45			[118]
Denbufylline	rat brain	0.8	20	46	[117]
Isbufylline	rat lung	1 400	85		[121]
	rat brain	1 900			[121]
BRL-61063		1.9			[122]
Arofylline	guinea-pig heart	5.3	3.7	7.2	[123]

with moderate asthma, Arofylline (slow release, 90 mg/day) demonstrated significant improvement of the pulmonary function, as well as a safe profile [125].

PDE inhibitors structurally related to the Arofylline-like compound 245412 (*table III*) were reported by a Chiroscience group. A close analogue (2-thienylmethyl and o.tolil instead of benzyl and m.methoxyphenyl, respectively) showed an improved ratio for PDE 4 inhibitory activity versus affinity for HARBS. This compound, dosed orally at 10 mg/kg in guinea-pigs, showed an excellent inhibition of eosinophilia produced by a range of mediators. No emesis or CNS effects were observed in ferrets at the same dose level [126].

Isbufylline, (*table II*) 7-isobutyltheophylline [127], is a very weak PDE inhibitor (IC_{50} in the millimolar range), which shows low affinity for adenosine receptors and is devoid of significant CNS and cardiovascular effects in animal models [128]. The compound is able to reduce eosinophil recruitment into guinea-pig airways sensitized by antigen inhalation. Double-blind controlled clinical trials showed efficacy in a limited number of asthmatic subjects accompanied by safety and good tolerance at dose levels ranging from 20–320 mg [121].

The emorheological agent Pentoxifylline (*table III*) is an alkylated xanthine therapeutically useful for the treatment of peripheral vascular and cerebrovascular diseases, as well as of a number of other pathologies caused by defective regional microcirculation [129]. Pentoxifylline is a weak PDE 4 inhibitor, but significantly inhibits TNF-α synthesis [130]. Pentoxifylline acts primarily by increasing red blood cell deformation by reducing blood viscosity, platelet aggregation and thrombus formation.

Cipamfylline (BRL60063) was the prototype of a series of potent PDE 4 inhibitors synthesized by Smith-Kline Beecham. The unwanted affinity for HARBS and adenosine receptors featuring the parent drug were strongly reduced by specific structural modifications [131].

Among the xanthine analogues (*figure 6*) Ibudilast emerged as a moderately potent (IC_{50} = 0.8 μM) non-specific PDE 4 inhibitor [132] which was marketed in Japan as an orally active antiasthma drug [133]. Again, PDE 4 inhibition could account only in part for its therapeutic activity.

Euroceltique disclosed compounds **26a**–**b** as members of a series of purines, isoguanines and dithioxanthines which inhibit PDE 4 from bovine tracheal smooth muscle with 30 nM potency [134]. Compound **26b** reduced bronchial hyper-responsiveness to histamine in guinea-pigs at 0.5 mg/kg/day s.c.

SCA 40 is a potent antibronchospastic in guinea-pigs [135], which is also able to suppress spontaneous tone on human isolated bronchus. In animal and human bronchus precontracted with different spasmogens, SCA 40 showed pD_2 values within the range 6.85–8.61. The presence of a bromine on position 6 and an amino(alkylamino) group on position 8 plays a critical role in the activity. Inhibitory activity on PDE 4 from bovine trachea was in the micromolar range, but antibronchospastic effects could also be due to its more potent effect on PDE 3. Chemical manipulation of this lead afforded selective PDE 4 inhibitors.

ICI63197 is a weak (IC_{50} = 4.6 μM) selective inhibitor of PDE 4 from guinea-pig lung [136] showing some similarity with the xanthine skeleton.

Among hybrid structures between the xanthine system and Rolipram (*figure 7*), V11294A **27** is one of the most interesting. This compound, which is a quite potent (IC_{50} = 200–300 nM, human lung) and selective PDE 4 inhibitor developed by Napp [137], is now in phase II

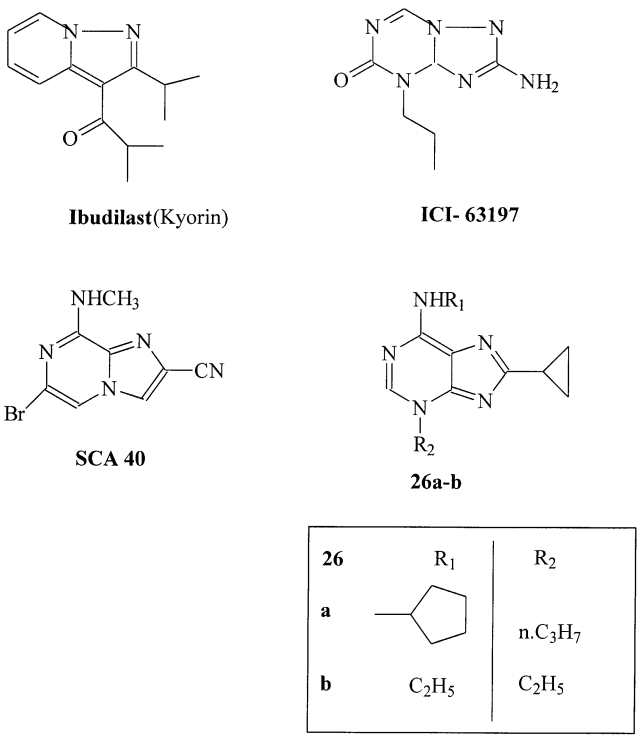


Figure 6. Xanthine analogues.

clinical trials for asthma. This well absorbed, non-emetic compound at the oral dose of 300 mg is able to inhibit PDE 4 in human volunteers for 24 h [138].

Compound RPR 132703 **28** (Rhone Poulenc) whose structural similarity with Rolipram is less evident, being a 1,4-phenolic diether, contains the 3,5-disubstituted pyridine fragment of Piclamilast, a potent Rolipram analogue, which progressed into phase II and was recently discontinued [11]. Compound **28** was reported to be a potent orally active inhibitor of LPS-induced TNF-α release in an allergic rat model [139].

6. Novel structural classes of PDE 4 inhibitors

PDE 4 inhibitors based on the benzofuran template were firstly reported by a Glaxo group [140]. The rationale at the basis of the choice of this completely novel structure was the replacement of the 3-cyclopentyloxy-4-methoxy sub-unit **29** of Rolipram with the conformationally constrained 7-methoxy benzofuran fragment **30** (*figure 8*).

Several patents provided more insights on the structural requirements for the activity by disclosing potent PDE 4 inhibitors based on the same benzofuran back-

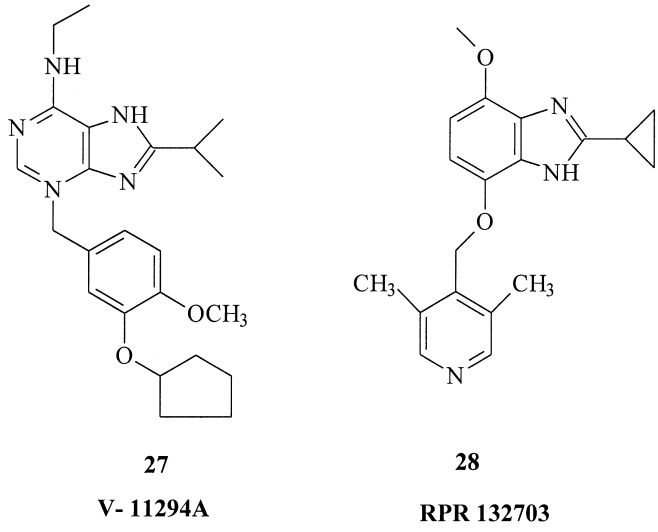


Figure 7. Hybrids of xanthine and Rolipram.

bone. Representative compounds are **31** and **33** (Bayer) [141, 142] and **32** and **34** (Chiroscience) [143, 144]. Compound **31** at 1 μM concentration increased by 400% cAMP concentration in human neutrophils stimulated by fMLP (N-formylmethionyl leucyl phenyl alanine) and at the dose of 1 mg/kg i.v. reduced skin oedema induced by the same agent (*figure 9*).

Very recently a Rhone Poulenc Rorer group reported on compounds of general structure **36** which showed PDE 4 inhibitory activity in the nanomolar range [145].

Many 7-methoxy spirobenzofurans with potent PDE 4 inhibitory activity (–log IC_{50} = 7.98) displaying bronchodilatory and anti-inflammatory activity, which are exemplified by **38** were disclosed by Byk Gulden [146]. Compounds **35** and **37** were synthesized in Kyowa laboratories [147, 148].

Further PDE 4 inhibitors belonging to miscellaneous chemical classes are depicted in *figure 10*. The represen-

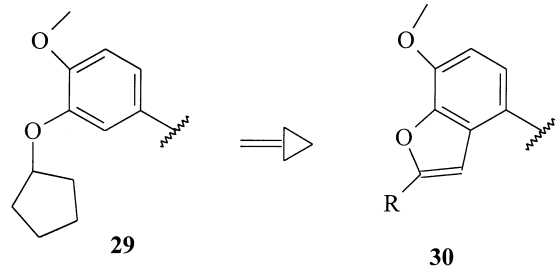


Figure 8. Structures of **29** and **30**.

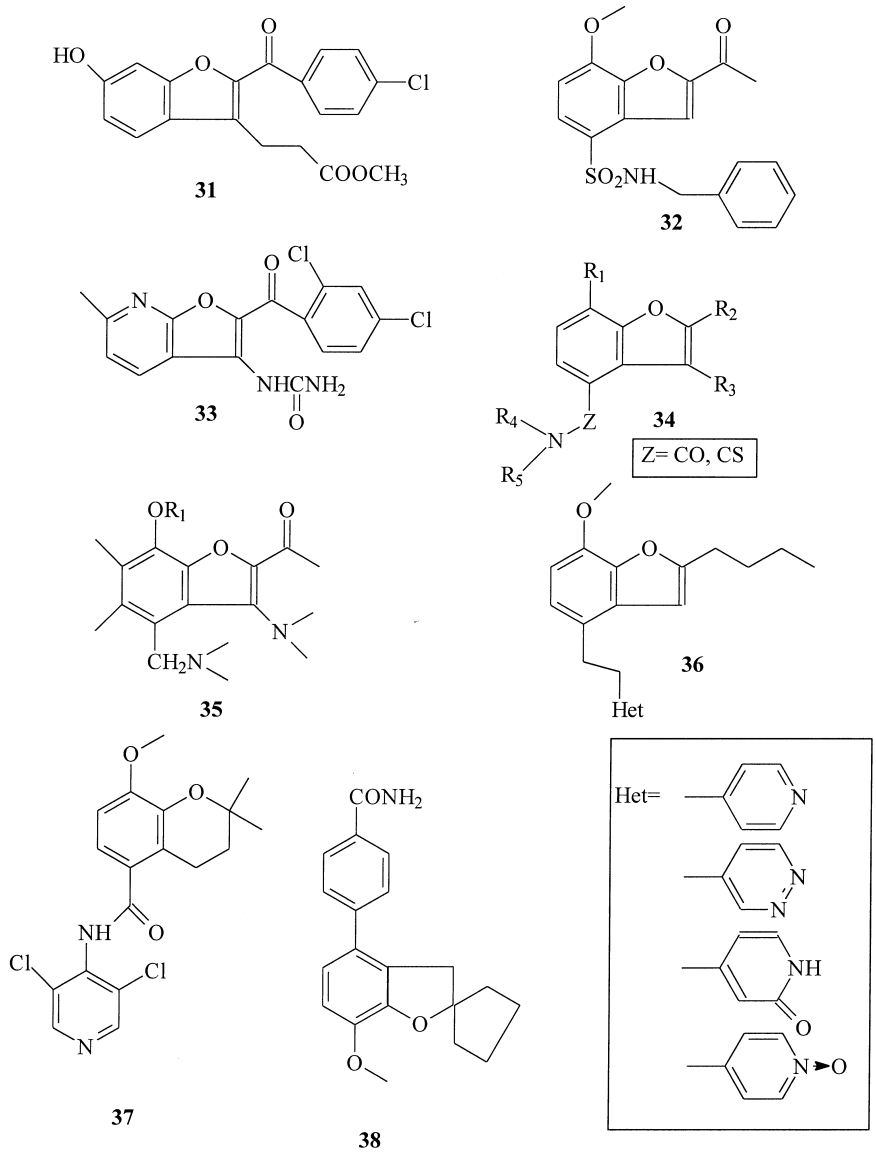


Figure 9. Benzofurans, benzopyrans and related compounds.

tative indazole **39** [149] and the pyrazolo[3,4-c]pyridine **40** were synthesized in the Pfizer laboratories [150].

The benzodiazepine derivatives **41a–b** were reported by a Parke Davis group [151]. In this series, SAR studies demonstrated the critical role played by the 5-membered fused ring, being both the non-cyclic benzodiazepine and the 6-membered analogue devoid of activity. Compound CI-1018 (R = CH_3, Het = 4-pyridyl) was a moderately potent PDE 4 inhibitor from U937 cell line (IC_{50} = 1.1 μM), also displaying good selectivity versus other PDE isoenzymes. CI-1018 proved to be a long lasting and potent inhibitor in antigene-induced pulmonary eosinophilia in rats without emetic effect at the therapeutic dose (Phase I). It is interesting to observe that diazepam itself proved to be a moderately potent PDE 4 inhibitor [152].

2,4,6-Trisubstituted triazines **42** represent a structurally novel class of PDE 4 inhibitors (IC_{50} = 150 nM) endowed with potent bronchodilatory effect, synthesized at UCB Pharma [153].

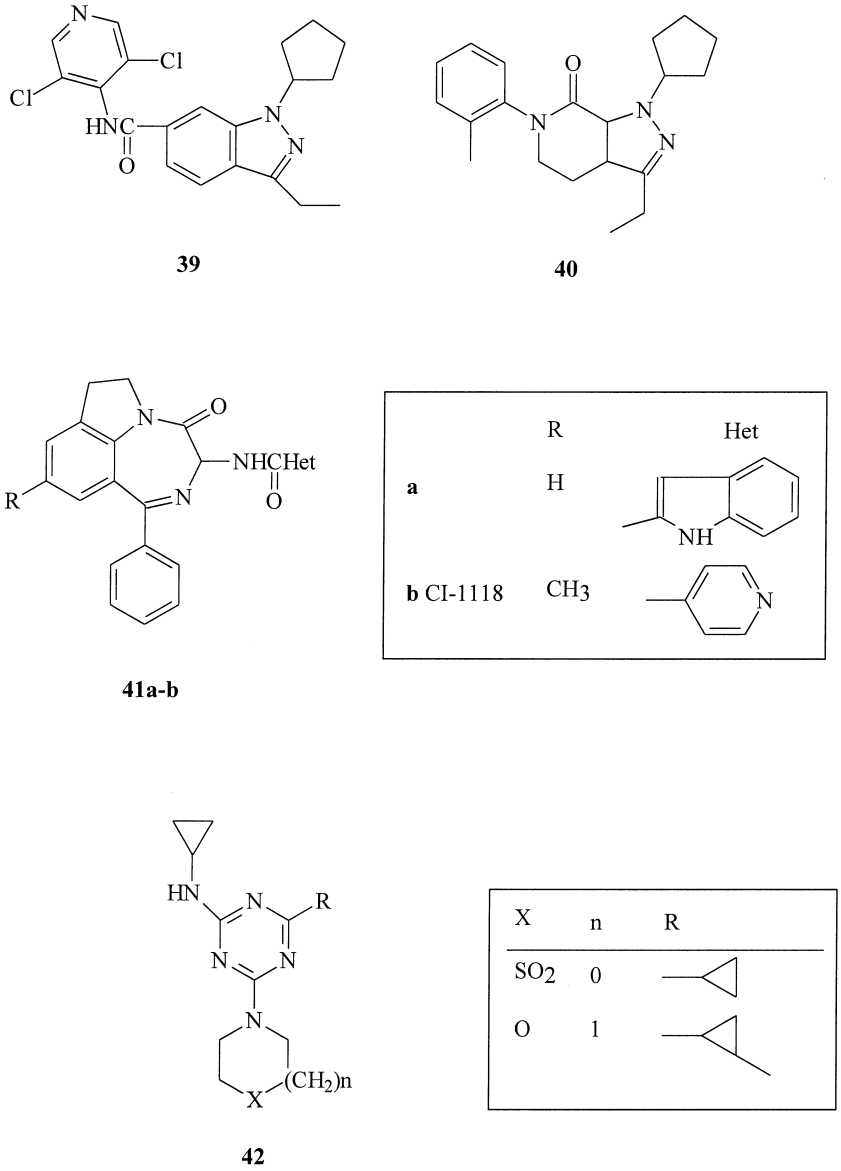


Figure 10. Miscellaneous chemical classes of PDE 4 inhibitors.

7. Conclusions

In recent years there has been a spectacular evolution of the knowledge on the biochemical aspects of the target (PDE 4) and on the pharmacological and toxicological profile of its inhibitors. Four subtypes were identified and their tissue/cell distribution and function clarified. Interaction with PDE 4C, which is heavily expressed in neuronal tissue, seems to shift the activity toward CNS, while PDE 4D, which is the predominant subtype in inflammatory cells, probably mediates anti-inflammatory and immunomodulatory effects. Moreover it is generally accepted that high affinity for HARBS is associated with more propensity to induce side effects. Thus, combining in the same molecule high potency at the catalytic site and reduced affinity for HARBS with selectivity for PDE 4D, may afford the ideal pharmacological and toxicological profile for drugs with clinical efficacy in asthma, COPD and some autoimmune diseases. Indeed SB 207499 (Ariflo®), which seems to be the PDE 4 inhibitor closer to the market for COPD [1] shows 10-fold selectivity for the

subtype 4D and preferentially binds the catalytic site versus HARBS.

On the other hand, therapeutically useful antiasthma drugs, like Theophylline and related compounds, generally display low or moderate potency at the catalytic site. This holds for Arofylline, which is one of the PDE 4 inhibitors most advanced in clinical evaluation as an antiasthma drug. These data seem to suggest that for clinical efficacy in asthma, high potency at the catalytic site of PDE 4 inhibitors is not an essential requirement. Conversely a range of incompletely understood effects, featuring Theophylline and congeners, would be more appropriate. The discontinued development observed in recent years of several very potent PDE 4 inhibitors, due to their lack of efficacy [11], seems to support this hypothesis. Therefore the Theophylline scaffold may be very useful for designing new antiasthma drugs in which its good pharmacokinetic profile could be coupled with reduced cardiovascular and CNS side effects, which presently limit the therapeutic utility of this old, but not obsolete drug.

The significant degree of optimism of the analysts on the clinical useful of PDE 4 inhibitors in COPD, asthma, RA and in some dermatological diseases opens very interesting perspectives for the researchers working in this area of medicinal chemistry.

Acknowledgements

Prof. Daniela Barlocco of Milano University is gratefully acknowledged for helpful discussion.

References

[1] Norman P., Exp. Opin. Ther. Patents 9 (1999) 1101–1117.

[2] Burnouf C., Pruniaux M.P., Szilagyi C.M., in: Bristol J.A. (Ed.), Annual Reports in Medicinal Chemistry, Academic Press, San Diego, 1998, pp. 91–125.

[3] Bolger G., Michaeli T., Martins T., Souness J.E., Rao S., Cell. Signal. 9 (1997) 2–13.

[4] Engels P., Fichtel K., Lubbert H., FEBS Lett. 350 (1994) 291–295.

[5] Conti M., Swinnen J.V., Tsicalas K.E., Jin S.L.C., Adv. Second Messenger Phosphoprotein Res. 25 (1992) 87–99.

[6] Palacios J.M., Beleta J., Segarra V., Il Farmaco 50 (1995) 819–827.

[7] Cavalla D., Frith R., Curr. Med. Chem. 2 (1995) 561–572.

[8] Lombardo L.J., Curr. Pharm. Des. 1 (1995) 255–268.

[9] Palfreyman M.N., Souness J.E., in: Ellis G.P., Luscombe D.K. (Eds.), Progress in Medicinal Chemistry, Elsevier, Amsterdam, 1996, pp. 1–52.

[10] Raeburn D., Karlsson J.A., J. Pharm. Pharmacol. 49 (1997) 19–24.

[11] Norman P., Exp. Opin. Ther. Patents 8 (1998) 771–784.

[12] Karlsson J.A., Aldous D., Exp. Opin. Ther. Patents 7 (1997) 989–1003.

[13] Schneider H.H., Schmiechen R., Brezinski M., Seidler J., Eur. J. Pharmacol. 127 (1986) 105–115.

[14] Barnette M.S., Christensen S.B., Essayan D.M., Grous M., Prabhakar U., Rush J.A., Kagey-Sobota A., Torphy T.J., J. Pharmacol. Exp. Ther. 284 (1998) 420–426.

[15] Norman P., Current Opinion in Anti-inflammatory & Immunomodulatory Investigational Drugs 1 (1999) 48–50.

[16] William Harvey Research Conferences: “PDE inhibitors: drugs with an expanding range of therapeutic uses”. Nice, December 1–3, 1999.

[17] Beavo J.A., Physiol. Rev. 75 (1995) 725–748.

[18] Torphy T.J., Am. Respir. Crit. Care Med. 157 (1998) 351–370.

[19] Nishi T., Tabusa F., Tanaka T., Ueda H., Shimizu T., Kanabe T., Kimura Y., Nakagawa K., Chem. Pharm. Bull. 31 (1983) 852–860.

[20] Watanabe K., Nakase H., Kimura K., Arzneim.-Forsch. 36 (1986) 1022–1024.

[21] Terrett N.K., Bell A.S., Brown D., Ellis P., Bioorg. Med. Chem. Lett. 6 (1996) 1819–1824.

[22] Alousi A.A., Canter J.M., Montenaro M.J., Fort D.J., Ferrari R.A., J. Cardiovasc. Pharmacol. 5 (1983) 792–803.

[23] Vernon M.W., Heel M.W., Brodgen R.N., Drugs 42 (1991) 997–1017.

[24] Feldman A.M., Bristow M.R., Parmley W.W., Carson P.E., Pepine C., Gilbert E.M. et al., N. Engl. J. Med. 329 (1993) 149–155.

[25] Van Meel J.C.A., Arzneim.-Forsch. 35 (1985) 284–288.

[26] Nemoz G., Prigent A.F., Moueqqit M., Fougier S., Macovschi O., Pacheco H., Biochem. Pharmacol. 34 (1985) 2997–3000.

[27] Barnett M.S., in: Jucker F. (Ed.), Progress in Drug Research Vol. 53, Birkhauser Verlag, Basel, 1999, pp. 1193–1229.

[28] Weinberger S.E., N. Engl. J. Med. 328 (1993) 1389–1397.

[29] Whelan C.J., Drugs Today 32 (1996) 295–311.

[30] Jeffery P.K., Am. Rev. Respir. Dis. 143 (1991) 1152–1158.

[31] Laitinen L.A., Laitinen A., Haahtela T., Am. Rev. Respir. Dis. 147 (1993) 697–704.

[32] Bradley B.L., Azzawi M., Jacobson M.J., Allergy Clin. Immunol. 88 (1991) 661–674.

[33] Bousquet J., Chanez P., Vignola A.M., Lacoste J.Y., Michel F.B., Crit. Care Med. 150 (1994) S33–S38.

[34] McFadden Jr. E.R., Gilbert I.A., N. Engl. J. Med. 327 (1992) 1928–1937.

[35] Barnes P.J., Eur. Respir. J. 5 (1992) 11226–11236.

[36] Lipworth B.J., Pharmacol. Ther. 58 (1993) 173–209.

[37] Martindale, The complete drug reference, 32nd Edition, Pharmaceutical Press, London, 1999, pp. 758–761.

[38] Sears M.R., Taylor D.R., Print C.G., Lake D.C., Li Q., Flannery E.M. et al., Lancet 336 (1990) 1391–1396.

[39] Barnes P.J., Chung K.F., Trends Pharmacol. Sci. 13 (1992) 20–23.

[40] Torphy T.J., Livi J.P., Balcarek White J.R., Chilton F.H., Undem B.J., in: Strada S.J., Hidaka H. (Eds.), Advances in Second Messenger and Phosphoprotein Research, 25, Raven Press, NY, 1992, p. 289.

[41] Barnes P.J., Rodger I.W., Holgata S.T., Asthma - Basic Mechanism and Clinical Management, Academic Press, 1989.

[42] Kay A.B., Eosinophils, Allergy and Asthma, Blackwell Scientific Publications, 1990.

[43] Holgata S.T., The role of Inflammatory Process in Airway Hyper-responsiveness, Blackwell Scientific Publications, 1989.

[44] Shahid M., Nicholson C.D., Naunyn-Schimiedeberger’s Arch. Pharmacol. 342 (1990) 698–705.

[45] Norman P., Drugs News Persp. 11 (1998) 431–437.

[46] Norman P., Financial Times Pharmaceuticals Management Reports (1999).

[47] Elliot M.J., Maini R.N., Feldmann M., Kalden J.R., Antoni C., Smolen J.S. et al., Lancet 344 (1994) 1105–1110.

[48] Nyman U., Mussener A., Larsson E., Lorentzen J., Clin. Exp. Immunol. 108 (1997) 415–419.

[49] Souness J.E., Foster M., Drugs 1 (1998) 541–553.

[50] Sommer N., Loschmann P.A., Northoff G.H., Weller M., Steinbrecher A., Steinbach J.P. et al., Nat. Med. 1 (1995) 244–248.

[51] Jung S., Zielasek J., Kellner G., Donhauser T., Toyca K., Hartung H.P., J. Neuroimmunol. 68 (1996) 1–11.

[52] Genain C.P., Roberts T., Davis R.L., Nguyen M.H., Uccelli A., Faulds D. et al., Proc. Natl. Acad. Sci. USA 92 (1995) 3601–3605.

[53] Ekholm D., Hemmer B., Gao G., Vergelli M., Martin R., Manganiello V., J. Immunol. 159 (1997) 1520–1529.

[54] Rott O., Cash E., Fleischer B., Eur. J. Immunol. 23 (1993) 1745–1751.

[55] Liang L., Beshay E., Prud'homme J.I., Diabetes 47 (1998) 570–575.

[56] Carcillo J.A., Herzer W.A., Mi Z., Thomas M.J., Jackson E.K., J. Pharmacol. Exp. Ther. 279 (1996) 1197–1204.

[57] Cardelus I., Gras J., Jauregui J., Llenas J., Palacios J.M., Eur. J. Pharmacol. 299 (1996) 153–159.

[58] Staudinger T., Presterl E., Graninger W., Locker G.J., Knapp S., Laczika K. et al., Intensive Care Med. 22 (1996) 888–893.

[59] Reimund J.M., Dumont D., Muller C.D., Kenney J.S., Kedinger M., Baumann R., Piondron P., Duclos B., Gut 40 (1997) 475–480.

[60] Gantner F., Kusters S., Wendel A., Hatzlmann A., Schudt C., Tiegs G.J., Pharmacol. Exp. Ther. 280 (1997) 53–60.

[61] Miyamoto K., Waki Y., Horita T., Kasugai S., Ohya K., Biochem. Pharmacol. 54 (1997) 613–617.

[62] Rascon A., Acta Cientifica Venezolana 48 (1997) 145–153.

[63] Pauwels R., Life Sci. 52 (1993) 2171–2179.

[64] Frew A.J., Holgate S.T., Drugs 46 (1993) 847–862.

[65] Bowler S.D., Mitchell C.A., Armstrong J.G., Eur. Respir. Dis. 70 (1987) 280–283.

[66] Eason J., Makowe H.L., J. Respir. Med. 83 (1989) 219–226.

[67] Barnes P.J., Pauwels R.A., Eur. Respir. J. 7 (1994) 579–591.

[68] Brenner M., Berkowitz R., Marshall N., Strunk R.C., Clin. Allergy 18 (1988) 143–150.

[69] Barnes P.J., Am. J. Med. 85 (Suppl. 1B) (1988) 64–70.

[70] Persson C.G.A., Andersson K.E., Kjellin G., Life Sci. 38 (1986) 1057–1072.

[71] Sato J., Nakata H., Owada E., Kikuta T., Umetsu M., Ito K., Eur. J. Clin. Pharmacol. 44 (1993) 295–298.

[72] Persson C.G.A., Pauwels R.A., in: Page C.P., Barnes P.J. (Eds.), Handbook of Experimental Pharmacology, Springer Verlag, Berlin, 1991, pp. 207–225.

[73] Barnes P.J., Mode of action of Theophylline: a multiplicity of actions, Intern. Congr. Symp. Series 126 (1988) 39–45.

[74] Bergstrand H., Eur. Respir. Dis. 61 (Suppl. 109) (1980) 37–44.

[75] Polson J.B., Kazanowsky J.J., Goldman A.L., Szentivany A., Clin Exp. Pharmacol. Physiol. 5 (1978) 535–539.

[76] Handslip P.D.J., Dart A.M., Davies B.T.I., Thorax 36 (1981) 741–744.

[77] Jenne J.W., Chest 92 (1981) 7S–14S.

[78] Cusley M.J., Tattersfield A.E., Holgate S.T., Am. Rev. Respir. Dis. 129 (1984) 380–384.

[79] Bjork T., Gustafson L.E., Dahlen S.E., Am. Rev. Respir. Dis. 145 (1992) 1087–1091.

[80] Mann J.S., Holgate S.T., Brit. J. Clin. Pharmacol. 19 (1985) 85–92.

[81] Church M.K., Featherstone R.L., Cushley M.J., Mann J.S., Holgate S.T., J. Allergy Clin. Immunol. 78 (1986) 670–675.

[82] Howell R.E., Muehsam W.T., Kinnier W.J., Life Sci. 46 (1990) 563–568.

[83] Kips J.C., Tavernier J.H., Pelemam R.A., Joos G.F., Pawels R.A., Int. Arch. Allergy Appl. Immunol. 99 (1992) 478–481.

[84] Lilly C.M., Sandhu J.S., Ishizaki A., Harada H., Yonemaru M., Larrick J.W. et al., Am. Rev. Respir. Dis. 139 (1989) 1361–1368.

[85] Barnes P.J., Pauwels R.A., Eur. Respir. J. 7 (1994) 579–591.

[86] Souness J.E., Rao S., Cell. Signal. 9 (1997) 227–238.

[87] Glaser T., Traber J., Agents Action 15 (1984) 341–348.

[88] Polymeropoulos E.E., Hofgen N., Quant. Struct.-Act. Relat. 16 (1997) 231–234.

[89] Lowe III J.A., Archer R.L., Chapin D.S., Cheng J.B., Helwey D., Johonson J.C. et al., J. Med. Chem. 34 (1991) 624–628.

[90] Wilhelm R.S., Chin R.L., Devens B.H., Alvarez R., Int. Pat Appl. WO 93 19068; Chem. Abst. 120 (1994) 164213.

[91] Wilhelm R.S., Fatherlee P.R., Chin R.L., Int. Pat. Appl. WO 94 22852; Chem. Abstr. 122 (1995) 55901.

[92] Matsuura A., Ashizawa N., Agawura R., Kumonana T., Hase T., Shimizu C., Kinihara T., Kobayashi F., Biol. Pharm. Bull. 17 (1994) 498–503.

[93] Suzuki F., Kurada T., Kawanita T., Manabe H., Kitamura S., Ohmori K. et al., J. Med. Chem. 35 (1992) 4866–4874.

[94] Luond R., Pombo-Villar E., Int. Pat. Appl. WO 97 18208; Chem. Abstr. 127 (1997) 50650.

[95] Hisamichi H., Takayama K., Iwata M., Kubata H., Kawano N., Aoki M., Isomura Y., 215th ACS National Meeting, Dallas 215 (1998) MEDI 49.

[96] Szelenyi S.K., Poppe H., Dent G., Magnussen H., Rabe K.F., Am. J. Resp. Crit. Care Med. 156 (1997) A624.

[97] Wilhelm R., Loe B., Alvarez R., Devens B., Fong A., P-32, 8th RSC-SCI Medicinal Chemistry Symposium, Cambridge, UK, 10-13 September, 1995.

[98] Dal Piaz V., Giovannoni M.P., Castellana C., Palacios J.M., Beleta J., Doménech T., Segarra V., J. Med. Chem. 40 (1997) 1417–1421.

[99] Dal Piaz V., Giovannoni M.P., Castellana C., Palacios J.M., Beleta J., Doménech T., Segarra V., Eur. J. Med. Chem. 33 (1998) 789–797.

[100] Vinick F.J., Saccomano N.A., Koe B.K., Nielsen J.A., Williams I.H., Thadeio P.F. et al., J. Med. Chem. 34 (1991) 86–89.

[101] Shimazaki N., Watanabe S., Sawada A., Hemmi K., UK Pat Appl. GB-2291422 (1996); Chem Abstr. 125 (1996) 10857.

[102] Shimazaki N., Sawada A., Watanabe S., Int. Pat. Appl. WO 97 24355; Chem. Abstr. 127 (1997) 149156.

[103] Beasley S.C., Cooper N., Gowers L., Gregory J.P., Haughan A.F., Hellewell P.G. et al., Bioorg. Med. Chem. Lett. 8 (1998) 2629–2634.

[104] Dyke H.J., Montana J.G., Lowe C., Kendall H.J., Hannah J., Sabin V.M., Int. Pat. Appl. WO 97 44036 (1998); Chem. Abstr. 128 (1999) 48146.

[105] Miyamoto K., Kurita M., Ohmae S., Sakai R., Sanae F., Takagi K., Eur. J. Pharmacol. 267 (1994) 317–322.

[106] Miyamoto K., Yamamoto Y., Kurita M., Sakai R., Konno K., Sanae F. et al., J. Med. Chem. 36 (1993) 1380–1386.

[107] Sakai R., Conno K., Yamamoto Y., Sanae F., Takagi T., Hasegawa T. et al., J. Med. Chem. 35 (1992) 4039–4044.

[108] Miyamoto K., Sakai R., Yamamoto Y., Konno K., Sanae F., Hasegawa T., Takagi K., J. Pharm. Pharmacol. 44 (1992) 888–892.

[109] Takagi K., Hasegawa T., Kuzuya T., Ogawa K., Watanabe T., Satake K. et al., Jpn. J. Pharmacol. 46 (1988) 373–378.

[110] Sawanishi H., Suzuki H., Yamamoto S., Waki Y., Kasugai S., Ohya K. et al., J. Med. Chem. 40 (1997) 3248–3253.

[111] Connor D.T., Unangst P.C., in: Wolff M.E. (Ed.), Burger's Medicinal Chemistry and Drug Discovery, 5th Edition, John Wiley and Sons Inc., NY, 1996, pp. 535–538.

[112] Persson C.G.A., Kjellin G., Acta Pharmacol. Toxicol. 49 (1981) 313–316.

[113] Lunell E., Svedmyr N., Andersson K.E., Persson C.G.A., Eur. J. Clin. Pharmacol. 22 (1982) 395–402.

[114] Grassi C., Casali L., Rampulla C., Rossi A., Int. J. Clin. Pharmacol. Ther. Toxicol. 18 (1980) 518–522.

[115] Li T., Volpp K.T., Applebury M.L., Proc. Natl. Acad. Sci. USA 87 (1990) 293–297.

[116] Shahid M., van Amsterdam R.G.M., de Boer J., den Berge R.E., Nicholson C.D., Zaagsma J., Br. J. Pharmacol. 104 (1991) 471–477.

[117] Nicholson C.D., Jackman S.A., Wilke R., Br. J. Pharmacol. 97 (1989) 889–897.

[118] Cortjio J., Bou J., Beleta J., Cardelus I., Llenas J., Morcillo E., Gristwood R.W., Br. J. Pharmacol. 108 (1993) 562–568.

[119] Hatzelmann A., Tenor H., Schudt C., Br. J. Pharmacol. 114 (1995) 821–831.

[120] Lowe Iii J.A., Cheng J.B., Drugs Future 17 (1992) 799–807.

[121] Manzini S., Meini S., Giachetti A., Beani L., Borea P.A., Antonelli T., Ballati L., Bacciarelli C., Arzneim.-Forsch. 40 (1990) 1205–1213.

[122] Buckle D.R., Arch J.R.S., Connolly B.J., Fenwick A.E., Fostre K.A., Murray K. et al., J. Med. Chem. 37 (1994) 476–485.

[123] Beleta J., Bou J., Miralpeix M., Domenech T., Berga P., Gristwood R.W., Palacios J.M., 3rd International Conference on Cyclic Nucleotide Phosphodiesterase: from Genes to Therapies, July 18–21, 1996, Glasgow, UK.

[124] Norman P., Current Opinion in Anti-inflammatory & Immunomodulatory Investigational Drugs 1 (1999) 48–50.

[125] Lauria X., Ferrer P., ALA/ATS (1997) International Conference, San Francisco, USA, May 16–21.

[126] Montana J.G., Cooper N., Dyke H.L., Gowers L., Gregory J.P., Hellewell P.G. et al., Bioorg. Med. Chem. Lett. 8 (1998) 2925–2930.

[127] Agostini O., Bacciarelli C., Bonacchi G., Fedi M., Manzini S., US Pat. 5, 314, 890 (1994); Chem. Abstr. 116 (1992) 151442.

[128] Manzini S., Fedi M., Bonacchi G., Agostino O., Drugs Future 15 (1990) 228–229.

[129] Ward P.A., Clissold S.P., Drugs 34 (1987) 50–97.

[130] Strieter R.M., Remick D.G., Ward P.A., Spengler R.N., Lynch Iii J.P., Larrick J., Kunken S.L., Biochem. Biophys. Res. Comm. 155 (1988) 1230–1235.

[131] Buckle D.R., Arch J.R.S., Connoly B.J., Fenwick A.E., Foster K.A., Murray K.J. et al., J. Med. Chem. 37 (1994) 476–485.

[132] Souness J.E., Scott L.C., Tonikinson A., Giembycz M.A., Raeburn D., Br. J. Pharmacol. 108 (1993) 71.

[133] Kawasaki A., Hoshino K., Osaki R., Mizushima Y., Yano S., J. Asthma 29 (1992) 245–252.

[134] Cavalla D., Hofer P., Gehrige A., Wintergest P., Int. Pat. Appl. WO 95 00516 (1995); Chem. Abstr. 122 (1995) 239459.

[135] Vitse O., Laurent F., Pocock T.M., Benezech V., Zanik L., Elliot K.R.F. et al., Bioorg. Med. Chem. Lett. 7 (1999) 1059–1065.

[136] Davies G.E., J. Pharm. Pharmacol. 25 (1973) 681–689.

[137] Cavalla D., Gale D.D., Drug News Perspect. 10 (1997) 470–476.

[138] Gale D.D., Landells L.J., Spina D., Am. Resp. Crit. Care. Med. 159 (1999) A611.

[139] Aldous D., Bower S., Escott K.J., Geiger L.E., Iqbal S., Mariucci M. et al., 15th EFMC Int. Symp. Med. Chem. (1997) September 6–10, Edimburgh, Abstr. P337.

[140] Stafford J.A., Feldman P.L., Marron B.E., Schoenen F.J., Valvano N.L., Unwalla R.J. et al., Bioorg. Med. Chem. Lett. 4 (1994) 1855–1859.

[141] Fisher R., Braunlich G., Mohrs K.H., Hanko R., Butler-Ransohoff J.E., Es-Sayed M. et al., Eur. Pat. Appl. EP-623607 (1994); Chem. Abstr. 122 (1995) 160466.

[142] Braunlich G., Schneider S., Es-Sayed M., Henning R., Sperzel M., Fisher R. et al., Int. Pat. Appl. WO 9802440; Chem. Abstr. 128 (1998) 140689.

[143] Dyke H., Kendall H.J., Lowe C., Montana J.G., Int. Pat. Appl. WO 97 20833; Chem. Abstr. 127 (1997) 108836.

[144] Dyke H., Lowe C., Montana J.G., Kendall H.J., Sabin V.J., Int. Pat Appl. WO 97 44337; Chem. Abstr. 128 (1999) 48134.

[145] McGarry D.G., Regan J.R., Voltz F.A., Hulme C., Moriarty K.J., Djurich S.W. et al., Bioorg. Med. Chem. Lett. 7 (1999) 1131–1139.

[146] Amschler H., Beer T., Gutterer B., Flockerzi D., Martin T., Tibaut U. et al., Int. Pat. Appl. WO 96 36625, Chem. Abstr. 126 (1997) 74737.

[147] Oshima E., Matsusaki T., Iida K., Ichimura M., Int. Pat Appl. WO 98 22452; Chem. Abstr. 129 (1999) 16048.

[148] Nakazato N., Oshima E., Kawakita T., Yanagawa K., Ichimura M., Manbe H., Sato S., Kakai Tokyo Koho JP 10147585 A2; Chem. Abstr. 129 (1999) 5487.

[149] Marfat A., Int. Pat Appl. WO 98 09961; Chem. Abstr. 128 (1999) 217368.

[150] Duplantier A.J., Andresen C.J., Cheng J.B., Cohan V.L., Decker C., Di Capua F.M. et al., J. Med. Chem. 41 (1998) 2268–2277.

[151] Pascal Y., Andrianjara C.R., Auclair E., Avenel N., Bertin B., Calvet A. et al., Bioorg. Med. Chem. Lett. 10 (2000) 35–38.

[152] Collado M.C., Beleta J., Martinez E., Miralpeix M., Domenech T., Palacios J.M., Hernandez J., Brit. J. Pharmacol. 123 (1998) 1047–1054.

[153] Leroux F., van Keulen B.J., Daliers J., Pommery N., Hènichart J.P., Bioorg. Med. Chem. Lett. 7 (1999) 509–516.