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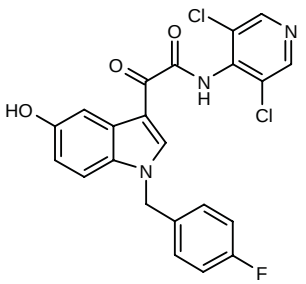
AWD 12-281

Antiasthmatic/Antiinflammatory
Phosphodiesterase 4 Inhibitor

N-(3,5-Dichloropyrid-4-yl)-[1-(4-fluorobenzyl)-5-hydroxy-indol-3-yl]glyoxylic acid amide



$C_{22}H_{14}Cl_2FN_3O_3$
Mol wt: 458.2746
CAS: 247584-20-9
EN: 267691

Abstract

Airway diseases such as bronchial asthma and chronic obstructive pulmonary disease (COPD) are chronic inflammatory diseases whose prevalence is increasing. Current research concerned with developing effective treatments for these conditions have focused on the search for alternatives to the standard corticosteroid antiinflammatory therapy. Selective phosphodiesterase 4 (PDE4) inhibitors have received a considerable amount of attention due to their ability to suppress the functions of several cell types involved in allergic and inflammatory disorders. The selective PDE4 inhibitor AWD 12-281 is the result of a pharmacophore-based synthesis program wherein the optimization process was supported by ligand-based drug design methods. AWD 12-281 was selected for further development for its high affinity and selectivity for the human PDE4 isoenzyme and due to its potent activity and excellent tolerability in models of allergic rhinitis, asthma and COPD, especially after topical treatment.

Synthesis

The synthesis of AWD 12-281 is shown in Scheme 1 (1): The reaction of 5-methoxyindol (I) with 4-fluorobenzyl chloride in DMF gives 5-methoxy-1-(4-fluorobenzyl)indol (II). This compound is acylated with oxalyl chloride, yielding 5-methoxy-[1-(4-fluorobenzyl)indol-3-yl]glyoxylic acid chloride (III), which is transformed with 4-amino-3,5-dichloropyridine into the corresponding amide (IV). The reaction of (IV) with borontribromide in toluene cleaves the methoxy group, yielding AWD 12-281.

Description

M.p. 214.5-6 °C.

Introduction

Several airway diseases such as bronchial asthma and chronic obstructive pulmonary disease (COPD) are chronic inflammatory diseases whose prevalence is increasing. Both are prime diseases to be targeted by phosphodiesterase 4 (PDE4) inhibitors and have been the subject of much research (2-4).

Asthma is a chronic inflammatory disorder of the airways that involves complex interactions among inflammatory cells and the tissues in the airways. Besides activation of mast cells, macrophages and T lymphocytes in

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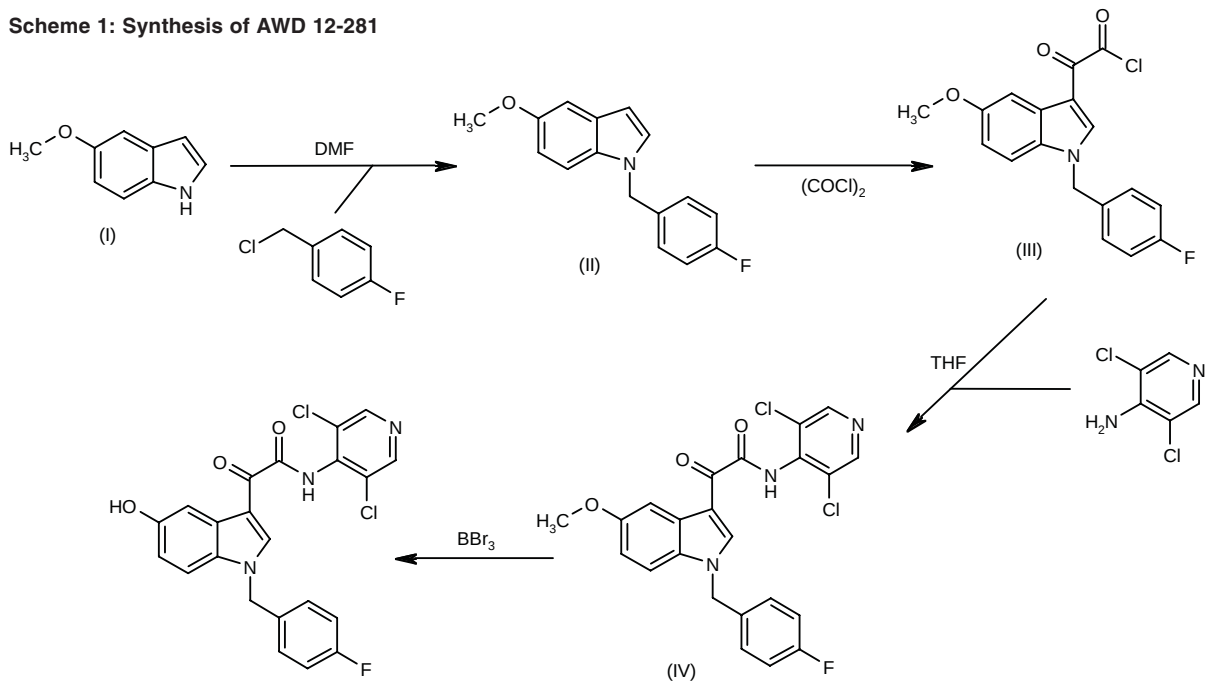
Scheme 1: Synthesis of AWD 12-281



the airway mucosa, eosinophilic infiltration into the airways plays a key role in the pathogenesis of asthma. Corticosteroid drugs have demonstrated excellent antiinflammatory activity in the current clinical treatment of bronchial asthma. However, their therapeutic effects are also often accompanied by side effects.

Searching for alternative antiinflammatory treatments to corticosteroids, selective PDE4 inhibitors have received a considerable amount of attention because they suppress the functions of several cell types involved in allergic and inflammatory disorders (5, 6).

COPD is a growing public health problem in both developed and developing countries. In fact, expectations are that COPD-related mortality will double in the next 30 years. The main characteristic of COPD is the chronically progressive decrease in lung function resulting from chronic inflammation, airway obstruction and tissue destruction leading to emphysema. The most important risk factor is smoking, but the pathomechanism is not yet clearly understood. However, the airway neutrophilia seems to be critical for the progression of the disease. There is a clear medical need for new agents for this progressive disease for which current treatment is completely unsatisfactory (7).

PDE4 isoenzyme is a key metabolizing enzyme for the degradation of intracellular cAMP in pulmonary inflammatory and immune cells. This enzyme, by preventing the accumulation of intracellular cAMP, promotes bronchoconstriction and airway inflammation (5, 8). Selective inhibition of PDE4 would, therefore, be expected to produce both bronchodilatory and antiinflammatory effects in patients with bronchial asthma or COPD. In addition to the current clinical treatment of bronchial asthma and COPD with corticosteroids, the development of selective PDE4 inhibitors is one of the newer promising concepts in the treatment of airway inflammation (9).

The selective PDE4 inhibitor AWD 12-281 is the result of a pharmacophore-based synthesis program wherein the optimization process was supported by ligand-based drug design methods (10,11), as well as an optimization for topical administration by *in vitro* metabolism studies. AWD 12-281 was selected for further development because of its high affinity and selectivity for the human PDE4 isoenzyme (10), its potent activity in models of allergic rhinitis, asthma and COPD, especially after topical treatment, and its excellent tolerability (12-24).

Pharmacological Actions

AWD 12-281 is a potent (IC_{50} = 9.7 nmol/l) and highly selective inhibitor of the PDE4 isoenzyme, as can be seen in Table I.

PDE isoenzymes are involved in the regulation of cellular signal transduction cascades by the modulation of cyclic nucleotide levels. PDE4 is a major isoenzyme in inflammatory cells. PDE4 inhibitors increase the intracellular cAMP level and hence modulate intracellular functions (*e.g.*, superoxide generation is decreased) and gene transcription (*e.g.*, synthesis and/or release of inflammatory cytokines is decreased), resulting in very potent antiinflammatory effects (9). Based on this mechanism of action, additional effects can be expected in

Table I: Inhibitory activity of different PDE isoforms by AWD 12-281.

PDE isoform	Preparation	Concentration (nmol/l)	Inhibition (%)
PDE1	Bovine brain enzyme	1000	11.0
PDE2	Human platelets	1000	6.96
PDE3	Human platelets	1000	22.5
PDE4	Human PMNLs	9.7*	50.0
PDE4A	Human recombinant PDE4A	26.3*	50.0
PDE5	Human platelets	1000	19.8
PDE6	Bovine retina	8060	50.0
PDE7	Human recombinant PDE7	1000	4.92
Rolipram binding site	Human PMNLs	105*	50.0

*IC_{50} value in nmol/l

several cell types carrying the PDE4 subtype such as smooth muscle cells and endothelial cells (4, 5). The increased cAMP levels translate into a reduced intracellular calcium release (25). In smooth muscle cells, this leads to a moderate relaxation counteracting the early-phase bronchoconstriction and inhibition of contraction which is important to alleviate bronchial hyperreactivity (6, 8). In endothelial cells, a histamine-induced contraction-like response is alleviated resulting in reduced vascular permeability (9, 17) induced by histamine release in allergic rhinitis (4). In immune system cells, PDE4 inhibition results in a reduced release of cytokines and inflammatory mediators including histamine (5). In addition to these calcium-mediated responses, the rise in cAMP levels results in increased serous secretion of submucosal glands (8, 26). These glands secrete both serous fluid and lysozyme resulting in the digestion and dissolution of mucus plugs. Increased cAMP levels also increase the ciliar activity of lung epithelial cells, resulting in an increased mucociliar clearance of the dissolved and diluted mucus (27).

Because PDE4 inhibitors interfere not only with late-phase inflammation but also increased vascular leakage, bronchoconstriction, bronchial hyperreactivity and mucus secretion, they are potential candidates for the treatment of allergic diseases such as rhinitis, conjunctivitis and bronchial asthma. Furthermore, the broad and potent antiinflammatory activity indicate their therapeutic potential in diseases involving inflammation such as atopic dermatitis, arthritis, psoriasis and inflammatory bowel disease (4). COPD is targeted not only by the anti-inflammatory effect but also by the positive effect on mucus secretion. Based on these known activities, AWD 12-281 was tested in models of mediator release *in vitro* and *in vivo* and in animal models of allergic rhinitis, asthma, COPD, polyarthritis, psoriasis and allergic skin disease.

AWD 12-281 was tested in vitro in models of inflammatory mediator release. AWD 12-281 at concentrations of 0.1-1 μmol/l inhibited the release of inflammatory mediators such as GM-CSF, TNFα and histamine in antigen-stimulated human cells from nasal polyps or human blood, supporting proposed effectiveness in humans. In comparison, the mediator release was reduced by the PDE4 inhibitors rolipram and SB-207499 at the same concentrations and by dexamethasone at lower concentrations (12, 13, 28). AWD 12-281 also demonstrated concentration-dependent inhibition of lipopolysaccharide (LPS)-induced TNF-α release in isolated peripheral blood mononuclear cells (29) and in whole human blood (IC_{50} = 0.8 μmol/l) (28, 30). Treatment with AWD 12-281 (1 μmol/l) resulted in a decrease in histamine- and LPS-mediated increases in intracellular concentrations of Ca^{2+} in human monocytes, demonstrating that PDE4 inhibitors have a potential role in Ca^{2+}-induced signal transduction in human monocytes (29, 31-33). Drugs that are able to inhibit eosinophil functions, especially degranulation, are expected to be very effective antiallergic and antiasthmatic drugs. AWD 12-281 (IC_{50} = 0.55 μmol/l), rolipram (IC_{50} = 0.42 μmol/l) and SB-207499 (IC_{50} = 0.86 μmol/l) were effective inhibitors of complement C5a-induced eosinophil peroxidase release. Interestingly, AWD 12-281, in contrast to rolipram and SB-207499, was able to inhibit eosinophil degranulation (IC_{50} = 16.2 μmol/l) (34).

Known PDE4 inhibitors like rolipram have the disadvantage of being emetogenic (4, 7). This was associated by others to a binding site different from the PDE4 inhibitory binding site (named the rolipram binding site). PDE4 inhibitors with emetogenic potential do not discriminate well between the catalytic (PDE4) and the rolipram binding site (5). The low affinity of AWD 12-281 to the rolipram binding site indicates an excellent profile making side effects such as emesis unlikely (14-16). This was supported by *in vivo* studies comparing antiinflammatory activity and emetogenic potential in the same species after oral and i.p. administration (16). The good tolerability is further improved when topical administration is used. Indeed, AWD 12-281 is optimized for topical administration (17, 19, 20, 22). The compound has very low oral bioavailability (< 3%) and is exceptionally suited for the topical treatment of allergic rhinitis, bronchial asthma and COPD.

Currently two topical formulations, nasal spray and dry powder for inhalation, have been developed. Nasal spray is indicated for the treatment of allergic rhinitis. The symptoms of allergic rhinitis, *i.e.* watery rhinorrhea, nasal congestion and itching can be related to increased vascular permeability, eosinophilic inflammation and

inflammatory mediator release. The allergen-induced watery rhinorrhea can be induced in different animal models of allergic rhinitis. Topically administered AWD 12-281 inhibited allergen-induced increase in microvascular permeability and rhinorrhea, with a rapid onset of action. In actively sensitized Brown Norway rats, the allergan-induced increase in microvascular permeability was inhibited with an IC_{50} of 58 nmol/l (17). In actively sensitized domestic pigs AWD 12-281 markedly inhibited the allergen-induced rhinorrhea with a calculated ID_{50} of 96 μg/nostril following intranasal administration (14). The effects were dose-dependent and comparable to those of azelastine (100 μg/nostril, 58% inhibition) and beclomethasone (50 and 100 μg/nostril, 47 and 60% inhibition, respectively). Eosinophilic inflammation resulting in nasal congestion cannot be evaluated in established models of allergic rhinits. However, in several experiments, the potent antiinflammatory effects of AWD 12-281 were shown after topical and systemic administration.

The late-phase airway reaction to antigen in sensitized guinea pigs or Brown Norway rats is characterized by a large accumulation of eosinophils in bronchoalveolar lavage (BAL) fluid 24 h (guinea pigs) or 48 h (rats) after allergen challenge. The ability of AWD 12-281 to inhibit the accumulation of eosinophils was used as an indicator of its antiinflammatory efficacy in experimental asthma models and also to evaluate some of the pharmacodynamic properties of the compound. AWD 12-281 inhibited the accumulation of eosinophils in BAL fluid when administered prophylactically in small doses intratracheally as a dry powder (ID_{50} = 7 μg/kg in rats and 63 μg/kg in guinea pigs). The efficacy of AWD 12-281 was similar to that observed with beclomethasone at an equivalent dose by topical administration (17, 19, 20). Consistent with the pharmacokinetic data, AWD 12-281 was less potent when administered orally and required large doses (30 mg/kg). These data also indicate that AWD 12-281, administered as dry powder inhalation, may be useful in the treatment of late-phase eosinophilia in bronchial asthma.

The bronchodilatory activity and effects on bronchial hyperreactivity of AWD 12-281 were addressed in both isolated human bronchus *in vitro* and in animal models *in vivo*. AWD 12-281 at concentrations up to 1 μmol/l had a weak relaxant effect on the spontaneous tone of isolated human airways. In passively sensitized human airways, AWD 12-281 protected against allergen-induced contractions. In contrast to rolipram and RPR-73401, AWD 12-281 (10 μmol/l) completely suppressed the contractile response to a submaximal allergen concentration, being the first selective PDE4 inhibitor to exhibit this activity (24). The potential bronchodilatory activity was also assesed in animal models *in vivo*. AWD 12-281 (at doses of 1-3 mg/kg intrapulmonary) was also shown to reduce early-phase bronchoconstriction in sensitized guinea pigs (15) and to potentiate the bronchodilatory effect of β_2-mimetic drugs like salbutamol. In addition, allergen-induced bronchial hyperreactivity in response to methacholine was investigated in actively sensitized BP-2 mice. The repeated administration of AWD 12-281 (10-60 mg/kg i.p. for 5 doses) alleviated bronchial hyperreactivity in a dose dependent manner, supporting the future use of AWD 12-281 in bronchial asthma (12).

In contrast to bronchial asthma, the predominant cells in lung lavage obtained from patients suffering from COPD are neutrophils. Therefore, drugs which inhibit neutrophil inflammation may be of therapeutic interest. Neutrophilia was induced in the lungs of rats, ferrets and pigs by exposure to a lipopolysaccharide (LPS) aerosol. At 6 h (ferrets, rats) or 4-6 h (pigs) after LPS, at the time point of maximal influx in neutrophils, lungs were lavaged. The inhibitory effect of AWD 12-281 on the accumulation of neutrophils was used as an indicator of the drug's efficacy in experimental inflammation and also to evaluate some of the pharmacodynamic properties of the compound. AWD 12-281 potently inhibited LPS-induced lung neutrophilia in BAL fluid and was most effective when administered in small doses intratracheally as a dry powder, with ID_{50} values of 0.02 and 10 μg/kg in rats and ferrets, respectively (16, 18, 20, 21). In domestic pigs, inhaled AWD 12-281 (2 and 4 mg/pig) inhibited LPS-induced lung neutrophilia by 61 and 86% after 4 h and by 21 and 65% after 6 h, respectively. AWD 12-281 was about 5-10 times less effective than beclomethasone (0.4 mg/pig, inhaled). Dexamethasone (0.28 mg/kg i.v.) totally suppressed airways neutrophilia (21, 22).

To characterize effects on airways mucus secretion, the PDE4 inhibitors were tested on tracheal phenol red secretion in mice. The compounds were given 1 h prior to the i.p. application of phenol red. AWD 12-281, SB-207499 and roflumilast (10 mg/kg i.p.) strongly enhanced phenol red secretion into the tracheal lumen by 100, 57 and 48%, respectively. AWD 12-281 enhanced the tracheal phenol red output with an ED_{50} value of 1.79 mg/kg i.p. These findings indicate that AWD 12-281 would have a positive effect in COPD and asthma in man, where it would potentiate normal mucus secretion from submucosal glands (21, 23).

AWD 12-281 has a low emetic potential even when administered intravenously or intrapulmonary (14). No negative findings were found in pharmacological and toxicological safety studies. Following intrapulmonary administration of even high doses of AWD 12-281, no damage of airways mucosa was observed.

Based on the results of these *in vitro* and *in vivo* experiments, AWD 12-281, administered as a nasal spray, may be very effective in the treatment of allergic rhinitis including nasal congestion. The drug's low oral bioavailability permits using the inhalation route of administration for clinical development which minimizes side effects associated with orally available PDE4 inhibitors while increasing the local drug concentration. A powder inhalation form for the treatment of bronchial asthma and COPD is currently being evaluated in toxicology studies. Based on the potent antiinflammatory activity of AWD 12-281, other diseases involving chronic inflammation are potential future indications. Early data indicate that AWD 12-281 has beneficial effects in models of conjunctivitis, atopic dermatitis (35), psoriasis and polyarthritis.

Pharmacokinetics

According to the first preliminary pharmacokinetic studies in rats, the bioavailibity of AWD 12-281 is extremely low (~ 3%). Furthermore, AWD 12-281 can apparently be absorbed by the lungs.

Toxicology

Acute toxicity studies were performed in rats and mice using both oral and intraperitoneal administration. In all four studies, no or only minor symptoms of toxicity occurred. No lethality was noted at doses up to 2150 mg/kg body weight which was the highest dose tested.

Results of a 4-week study of repeated oral administration in rats and dogs demonstrated no effects on the health of animals. The no adverse effect doses in rats and dogs were ~ 1000 mg/kg and ~ 215 mg/kg, respectively.

The toxicological profile of AWD 12-281 was evaluated after repeated dose (4 weeks) intranasal administration in rats and dogs using preformulations intended for use in man. The studies were completed without signs of local or systemic toxicity. Dry powder inhalation formulations of the drug are currently being evaluated in toxicological studies.

AWD 12-281 was well tolerated in animals and was free of any mutagenic and genotoxic effects.

Clinical Studies

Ongoing phase II clinical studies are investigating the use of AWD 12-281, as a nasal suspension, for the treatment of allergic rhinitis. Additional studies are under way using inhaled forms of AWD 12-281 for the management of adult and pediatric patients with bronchial asthma and COPD.

Source

AWD GmbH/elbion (DE).

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