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Il Farmaco 57 (2002) 235–242

IL FARMACO

Review

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Leukotriene modifiers: novel therapeutic opportunities in asthma☆

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Received 15 November 2001; accepted 10 December 2001

Abstract

Cysteinyl leukotrienes (Cys-LT) are powerful proinflammatory autacoids that cause long-lasting bronchoconstriction, plasma leakage, increased mucus production; their biological activity suggests a prominent role in the etiopathology of asthma and several Cys-LT receptor antagonists and synthetase inhibitors have been developed as new antiasthmatic drugs. Zafirlukast was discovered by a mechanism-based approach to drug discovery; early structure–activity relationship analyses of the prototype SRS-A antagonist FPL-55712, lead to the identification of an indole-containing lead compound that was more specific than FPL-55712. Modifications were made on the lipid-like tail, indole backbone and acidic head region of this lead compound, resulting in potent and selective leukotriene receptor antagonists such as ICI-198615 and 204219 (zafirlukast). On the basis of successful results in preclinical asthma models, zafirlukast was recommended for clinical development and became the first leukotriene-modifier to be approved for the treatment of asthma. Leukotriene biosynthesis inhibitors (LSI) also represent a promising approach to the treatment of asthma and may theoretically provide a broader protection than Cys-LT receptor antagonists by inhibition of the synthesis of the two major leukotrienes, the Cys-LT and the chemotactic LTB_4. The LSI BAY X-1005 is the result of a broad chemistry program that identified 15-HETE as an endogenous inhibitor of leukotriene synthesis and REV 5901 as a lead prototypic quinoline-based 5-lipoxygenase (5-LO) inhibitor. Clinical studies demonstrated the effectiveness of BAY X-1005 in experimental conditions such as allergen provocation and cold-air induced asthma. However, no consistent treatment effect in the overall asthma population (mild to moderately severe asthmatics) lead to discontinuation of its development. © 2002 Published by Éditions scientifiques et médicales Elsevier SAS.

Keywords: Leukotriene modifiers; Novel therapeutic opportunity; Asthma

1. Introduction

The leukotrienes are a family of lipid mediators that are derived from arachidonic acid (AA) via the 5-lipoxygenase enzyme [1]. They were first isolated from leukocytes and are characterized by an unusual triene-contaning chemical structure. The cysteinyl-leukotrienes (Cys-LT) are a subclass that contains a peptide moiety attached to the leukotriene backbone. There are three major Cys-LT: LTC_4, LTD_4 and LTE_4. Together they account for the biological activity of slow-reacting substance of anaphylaxis (SRS-A), which was discovered nearly 40 years ago when it was described its ability to produce a slowly developing, long-lasting contraction of isolated guinea pig ileum.

During the 1970s, several investigators became interested in the role of SRS-A in asthma and other allergic diseases because its biological properties appeared relevant to asthma pathophysiology; moreover, inhibitors of other classes of mediators were without significant effect in asthma control. The elucidation of the structure of SRS-A as the Cys-LT in 1979 provided a turning point; it allowed medicinal chemists to plan potential receptor antagonists that were based on the structure of the natural ligand. With the subsequent chemical synthesis of the Cys-LT, sufficient pure compound(s) became available for further studies in asthma, as well as for establishing appropriate animal models in which to characterize newly synthesized compounds. The successful development of a Cys-LT receptor antagonist such as zafirlukast, as well as the identification of other leukotriene modulators, such as

☆ Presented at XXI-SCI-Advanced Course in Medicinal Chemistry, University of Urbino, Urbino, Italy, July 1–5, 2001.

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PII: S0014-827X(02)01209-0

the synthesis inhibitor BAY X-1005, attests to the viability of mechanism-based approach to drug discovery.

2. Cys-LT receptor antagonists: zafirlukast—medicinal chemistry strategy

The structure of the prototype SRS-A antagonist FPL 55712 and subsequently the structure of Cys-LT provided starting points for the chemical design of leukotriene receptor antagonists, contributing important structural information that eventually led to the development of zafirlukast [2].

Two major problems had to be overcome in the design of leukotriene receptor antagonists that were based on the Cys-LT structure. First, the inherently unstable triene-containing chain had to be replaced with moieties that would add stability to the molecule, and second, the potent agonist actions of the Cys-LT had to be converted into a structure with pure antagonist properties. The results from nuclear magnetic resonance (NMR) [3] and molecular modeling studies led to the use of aryl-containing groups in place of the unstable triene. A series of homo-cinnamyl-containing leukotriene mimics were synthesized; however, these compounds still retained agonist activity. A pure antagonist, with significantly lower potency than the prototype antagonist FPL 55712, was subsequently produced—first by varying the peptidyl chain and then by removing one of the carboxylic acid moieties.

The potency of these early compounds was increased by restricting the molecule into a bioactive conformation. Synthetic work began by incorporating heterocyclic rings, with a focus on indole and indazole ring systems. Substitutions, including addition of a 3-methoxy group to the benzoic acid region, led to a compound with potency comparable to that of FPL 55712; more importantly, its selectivity as a Cys-LT receptor antagonist was fivefold greater [4]. The structure–activity relationships surrounding this lead structure were investigated extensively by focusing on the three regions of the molecule—the lipid-like tail, the indole backbone and the acidic head (Fig. 1) [5].

Increased in vitro potency and selectivity, but more important oral efficacy in animal models, were realized when compounds with branching at the α- or β-carbons in the lipid-like tail were synthesized and evaluated. A carbocyclic moiety was a preferred substitution, and provided a 100-fold increase in potency over the original lead structure. The nature of the acidic head group and the linkage to the heterocycle also influenced antagonist activity.

Replacement of the carboxylic acid head group with phenylsulfonamide produced a further 100-fold increase in potency, with the preferred linkage occurring with a 3-methoxy-phenyl group. The increased potency of these antagonists was accompanied by a longer duration of action in animal models; this enabled studies using a 3-h pretreatment time. At the time of its synthesis compound ICI 198615 was one of the most potent Cys-LT receptor antagonists; however its bioavailability was $< 1\%$ in rats and dogs [6].

Subsequent structure–activity relationship studies were focussed on improving oral bioavailability. Improved in vivo activity was obtained by variations of the indole backbone, with an 'inverted indole' template being a preferred structure [7]. Oral activity was also improved by the addition of *ortho*-substituents to the arylsulfonamide moiety in compounds having an indole backbone [8]. These changes were then incorporated into the inverted indole backbone template to produce zafirlukast (Fig. 1). Zafirlukast retains the potent leukotriene receptor antagonist activity seen in earlier members of this compound series; however, its oral bioavailability is significantly increased to 68% in rats and 67% in dogs.

3. Preclinical evaluation of zafirlukast

Zafirlukast was characterized in various biochemical and isolated tissue studies, which established its potency and selectivity as a Cys-LT antagonist. Zafirlukast antagonized the binding of [^{3}H] LTD_4 to the Cys-LT receptor in guinea pig lung membranes with a K_i of 0.34 nM, making it twofold more potent than LTD_4 and 2000-fold more potent than FPL 55712 [9]. The K_i of zafirlukast was independent of the [^{3}H] LTD_4 concentration, indicating that it is a competitive leukotriene antagonist. When ligand-binding studies were conducted with [^{3}H] LTE_4 or [^{3}H] ICI-198615, the K_i values were 0.23 and 2.6 nM, respectively. Similar K_i values were obtained when the Cys-LT receptors in human lung membranes were evaluated; zafirlukast exhibited a K_i of 1.1 nM against [^{3}H] LTD_4 and 3.7 nM against [^{3}H] ICI-198615. Zafirlukast, at concentrations up to 3 μM, did not compete with [^{3}H] LTC_4 for binding to human lung membranes. The [^{3}H] LTC_4 binding sites in human lung are distinct from the Cys-LT_1 receptor; it remains to be determined if these sites are a Cys-LT_2 receptor subtype.

Zafirlukast antagonized Cys-LT-induced contractions of isolated guinea pig trachea and human bronchi. This agent was a competitive antagonist because it produced parallel rightward shifts of the cumulative leukotriene concentration–response curve without affecting efficacy. The pK_B values of zafirlukast were independent of the antagonist concentration; they ranged from 9.0 to 9.5 against LTD_4 and 9.6–9.7 against LTE_4 on guinea pig trachea and from 8.2 to 8.7 against LTD_4 and 8.3–8.5 against LTE_4 on human airways [9]. Further-

more, the slope of the Schild plot describing the antagonism of LTE_4-induced contractions of guinea pig trachea was not different from unity.

The specificity of zafirlukast as an antagonist of leukotriene-induced contractions was established against different agonists; zafirlukast did not antagonize adrenergic, histaminergic, serotoninergic, muscarinic or thromboxane (TP1, TP2) receptors or calcium channels [9]. Zafirlukast did antagonize the EP1 receptor, which mediates prostaglandin E_2 (PGE_2)-induced relaxation of guinea pig trachea; however, it was 10 000-fold less potent than it was in the Cys-LT receptor assay.

Zafirlukast was effective in animal models against leukotriene and antigen challenge, whether administered by aerosol, intravenously (i.v.) or orally (p.o.), and whether used to prevent or reverse the leukotriene-induced bronchospasm. Zafirlukast dose-dependently antagonized LTD_4-induced dyspnea in conscious guinea pigs when it was administered 30 min before aerosolized LTD_4 challenge. When administered orally at a dose of 0.5 μmol/kg at varying times before LTD_4 challenge, zafirlukast achieved maximal antagonist activity with a 3-h pretreatment. It also exhibited a long duration of action—with a pharmacodynamic half-life of more than 13 h [9].

Zafirlukast reversed an ongoing LTE_4-induced bronchoconstriction in anesthetized guinea pigs. LTE_4 (3 nmol/kg i.v.) increased pulmonary resistance by 700% and reduced dynamic lung compliance (Cdyn) by 95%.

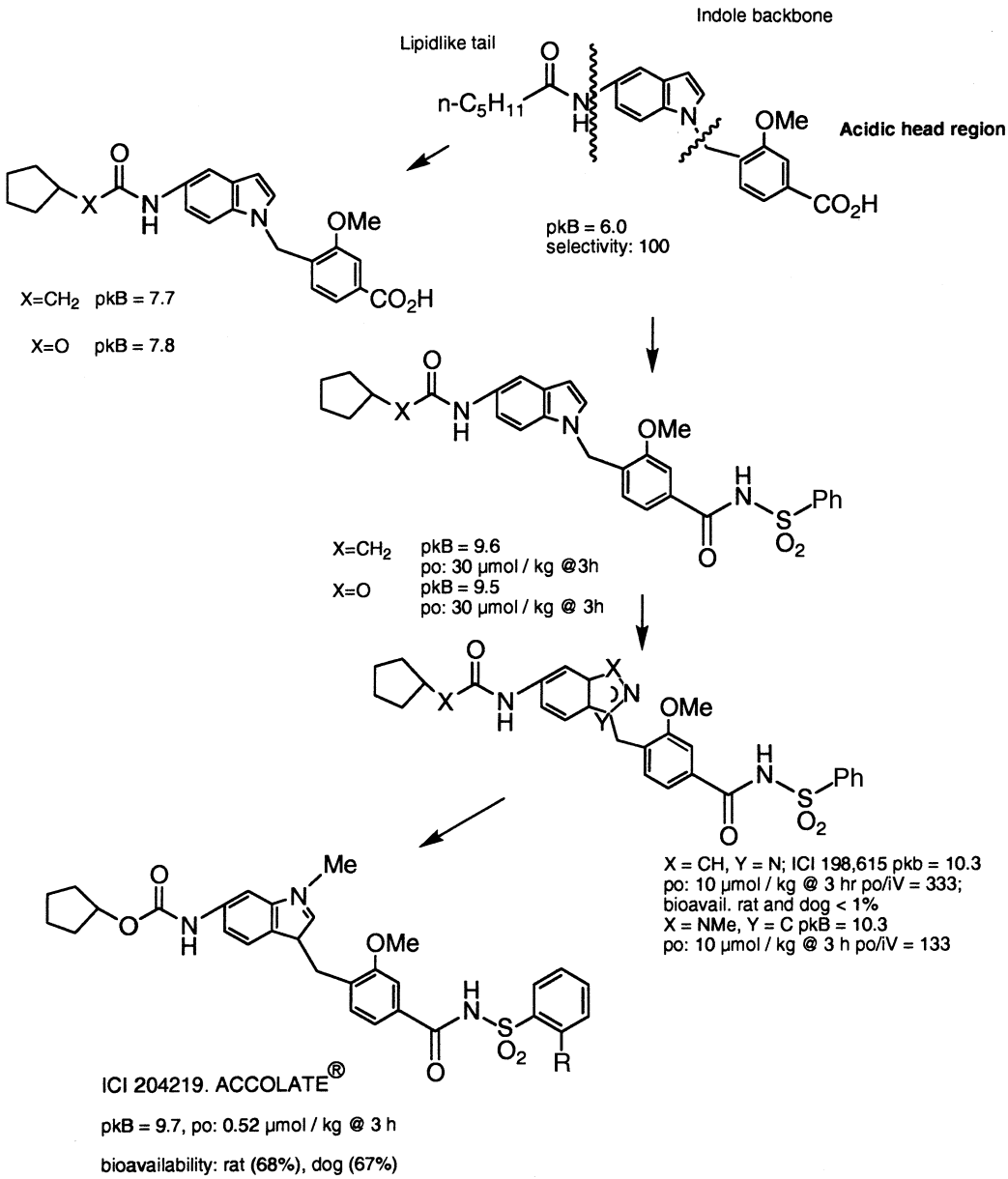

Fig. 1. Structure–activity relationship analyses and synthesis of potent and selective leukotriene receptor antagonist zafirlukast (Accolate®). Adapted from [5].

Zafirlukast (0.3 μmol/kg i.v.) or placebo was administered at the time of peak bronchoconstriction. The time for both pulmonary parameters to return to baseline was significantly shortened by zafirlukast compared with placebo. Qualitatively similar results were obtained when bronchoconstriction was induced with either LTC_4 or LTD_4 [9].

Therefore, in preclinical animal models, zafirlukast prevented and reversed leukotriene and antigen-induced bronchospasm, decreased airway reactivity to metacholine and inhibited airway eosinophilia. This profile of activity made zafirlukast a viable candidate for clinical evaluation [10].

4. Zafirlukast antagonism of LTD_4-induced bronchospasm in humans

Zafirlukast is rapidly and completely absorbed following oral administration of 5-, 10-, 20- or 40-mg tablets. Peak plasma concentrations are achieved by 3 h, and the elimination half-life is 8.7 h. Whereas the pharmacokinetic half-life suggested that three times per day dosing may be needed, pharmacodynamic studies indicates that zafirlukast provides leukotriene antagonist activity for longer periods. A single 40-mg dose of zafirlukast was administered at 2, 12 or 24 h before healthy subjects were challenged with aerosol LTD_4 in a double-blind, placebo-controlled crossover study. After a 2-h pretreatment with zafirlukast, a 117-fold higher LTD_4 dose was required in order to reduce specific airway conductance (SGaw) by 35% than after placebo. Similarly, after the 12- and 24-h pretreatments, the LTD_4 dose that reduced SGaw by 35% was ninefold and fivefold higher with zafirlukast than with placebo.

Thus, antagonist activity was evident at 12 h after oral administration of zafirlukast, which is consistent with a twice-daily dosing regimen.

5. Antiasthmatic efficacy and safety of zafirlukast

Several randomized, double-blind, placebo-controlled, multicenter studies have established the effectiveness and safety of zafirlukast in patients with persistent asthma [11]. Zafirlukast at 20-mg b.i.d. dose produced the most consistent responses; asthma symptom scores were reduced by 27%, morning asthma symptoms by 28%, β_2-agonist use by 31% and nighttime awakenings by 46%. Zafirlukast also improved pulmonary function; Forced expiratory volume in 1 min (FEV_1) was significantly increased (by 11%), whereas placebo had no effect. Efficacy appeared to be related to steady-state trough plasma concentrations of zafirlukast; the improvements in FEV_1 were positively correlated with plasma zafirlukast concentrations, and the reductions in asthma symptoms, including nighttime awakenings and first morning symptoms, were negatively correlated. Zafirlukast is well tolerated by patients with mild-to-moderate asthma; the incidence of adverse events is comparable in the zafirlukast and placebo groups.

In a pharmacoeconomic study, patients completed a questionnaire and recorded their responses to therapy and rated their symptoms in a daily diary. Patients treated with zafirlukast had significantly more days without symptoms, lost fewer days from school or work, and required fewer health care contacts than did patients who received placebo [12]. These improved outcomes were evident despite the fact that patients treated with zafirlukast required less of their β-agonist.

A retrospective subgroup analysis was conducted on three 13-week multicenter trials in patients with mild-to-moderate asthma that involved more than 1000 who had received either zafirlukast 20 mg b.i.d. or placebo [13]. Improvements in daytime and nocturnal symptoms, β-agonist usage and pulmonary function were compared for patients who were grouped according to their baseline scores. Zafirlukast provided the greatest percentage improvement in patients who had the greatest degree of nocturnal symptoms or the most compromised pulmonary function at baseline. Such findings suggest that patients with more severe asthma may experience greater benefit than those with less severe disease.

Zafirlukast (Accolate®) has been the first Cys-LT modifier to be approved by the USA-FDA for registration and represents the first new antiasthmatic drug to be developed in the last 20 years. It is now available for antiasthma therapy in Europe.

6. Leukotriene synthesis inhibitors: BAY X-1005—medicinal chemistry strategy

The synthesis of leukotrienes from AA requires the coordinated involvement of 5-lipoxygenase (5-LO) and of a 18-kDa five lipoxygenase activating protein (FLAP) that facilitates substrate transfer to 5-LO by enhancing its activity. The complex interaction of leukotriene synthesis inhibitors (LSIs) directly with FLAP and indirectly with 5-LO is the following.

The 5-LO enzyme residing in the cytoplasm or the nucleoplasm translocates to the nuclear cell membrane upon calcium increase [14]. The calcium dependency of 5-LO translocation explains the extreme effectiveness of the calcium ionophore A23187 to stimulate leukotriene biosynthesis, in addition to the activation of phospholipase A_2 (PLA_2) increasing substrate availability [15]. The exact 5-LO–FLAP interaction sites are still unknown, yet the membrane composition and substrate

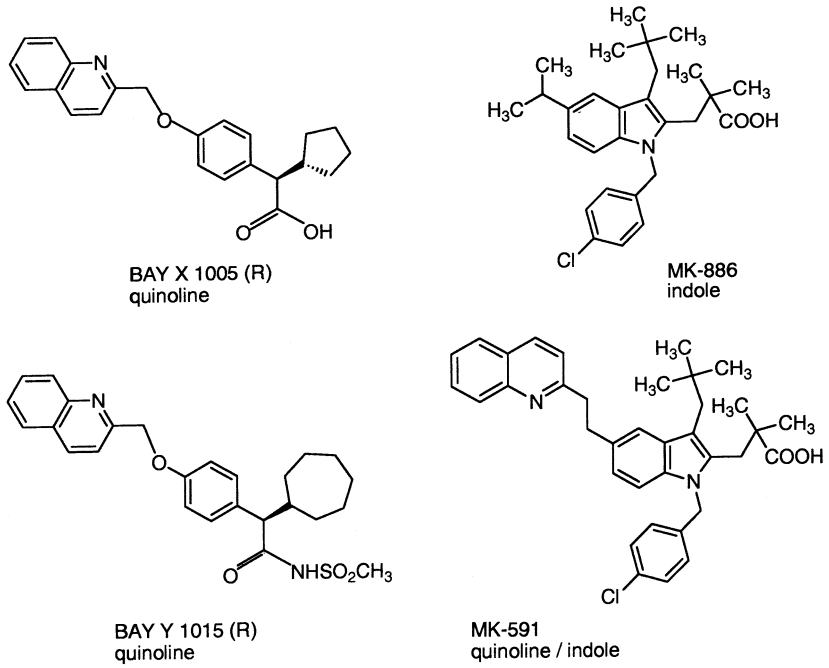

Fig. 2. LSIs; structures of the Bayer and Merck series (see text).

availability at FLAP appear to be important determinants. 5-LO translocation to FLAP determines its activity and substrate specificity. Free fatty acids (FFA) utilization by FLAP and 5-LO comprises endogenous and iso-exogenous substrate. Exogenous substrate is provided by transcellular metabolism. Leukotriene synthesis inhibition by LSIs is achieved by competing with the binding of the 5-LO substrate thereby interfering with AA transfer from FLAP to 5-LO and inhibiting or reversing 5-LO translocation to the nuclear envelope.

7. Chemistry of the leukotriene synthesis inhibitors BAY X-1005 and BAY Y-1015

15-Hydroxyeicosatetraenoic acid (15-HETE) was identified as an endogenous inhibitor of 5-LO and served as a lead for the prototypic quinoline-based 5-LO inhibitor REV 5901 [16], a weak inhibitor of LTB_4 synthesis in human PMNLs (IC_{50} 3.2 μM) devoid of oral activity in the AA-induced mouse ear inflammation test (30). A broad chemistry program was started to improve potency and oral activity. The quinolylmethoxyphenyl core structure turned out to be essential for in vitro LTB_4 synthesis inhibition; however, structural variations in the side-chain of the phenyl residue finally led to the potent and orally active LSI BAY X-1005 (Fig. 2) [17]. Introduction of an acidic carboxyl group was the breakthrough in achieving oral activity. Repositioning of the side-chain from *meta* to *para* position improved potency, and the cyclopentyl residue instead of a linear alkyl residue contributed to good oral activity.

Further improvement of the in vitro potency of this type of compound in whole cells is easily achieved by further increasing the membrane affinity (lipophilicity) [18]. However, a higher partition coefficient (liposome–water system) results in a higher plasma albumin binding and thus—as a rule—does not improve activity in human whole blood. Plasma albumin binding in this class of compounds depends also on the acidity of the functional group in the side-chain. A decrease in acidity correlates with lower plasma protein binding, but usually compromises pharmacokinetics. A well-balanced degree of protein binding thus seemed to be critical to achieving further improved compounds.

BAY Y-1015 (Figs. 2 and 3) is the result of such an optimization program, where partition coefficients and plasma protein binding were used as additional structure–activity relationship parameters. In comparison to BAY X-1005, BAY Y-1015 demonstrates slightly reduced plasma protein binding but clearly improved activity in human PMNLs and human whole blood [19].

BAY X-1005 is the (*R*)-(−)-enantiomer, with higher activity in human whole blood. A very economical stereoselective synthesis for this enantiomer was developed (Fig. 3). The starting material [4-(2-quinolinylmethoxy) phenyl] acetic acid (I) is esterified with D-menthol and toluene sulfonic acid in boiling toluene. Following alkylation with cyclopentylbromide and excess potassium *tert*-butylate in DMF leads to a diastereomeric mixture (II). Under the reaction con-

ditions the diastereomer (III) precipitates, whereas the remaining soluble diastereomer undergoes continuous epimerization. After crystallization, a yield of $>85\%$ of the desired diastereomer (III) results with a diastereomeric excess (de) $>99\%$. Acidic cleavage with sulfuric acid yields the (*R*)-(−)-enantiomer BAY X-1005 [20].

BAY Y-1015 is the enantiomer with the same absolute conformation (R) and can be prepared in a similar way.

8. Experimental pharmacology of the LSI BAY X-1005 and BAY Y-1015: in vitro effects

BAY X-1005 inhibits the synthesis of the 5-LO products LTB_4, LTC_4 and 5-HETE at a mean effective concentration of about 0.2–0.03 μM, irrespective of the cell type (neutrophil or eosinophil) or the stimulus used (20). There is no direct effect of LSI on 5-LO.

The potency for leukotriene synthesis inhibition reflects the binding affinity of BAY X-1005 to FLAP as determined by the K_d (0.165 μml). BAY X-1005 has no effect on the release of the main 5-LO substrate AA and the release of platelet activating factor (PAF), demonstrating that this LSI has no effect on PLA_2. The 5-LO specificity was shown with human whole blood activated by using the calcium ionophore A-23187 [21]. There was no effect on the constitutively expressed cyclooxygenase. BAY X-1005 was purposely designed as a new class of LSIs free of oxygen radical-quenching properties, which was confirmed in vitro [21].

In vitro data on BAY X-1005 bridging to the target indication allergic asthma were generated using isolated guinea pig trachea (Schultz-Dale test: IC_{50} 0.36 μM) and human lung tissue determining Cys-LT synthesis inhibition (IC_{50} 0.18 μM) and functional smooth muscle contraction (IC_{50} 0.27 μM) [22].

9. In vivo effects

Unequivocal evidence for the antiinflammatory properties of BAY X-1005 (2 × 25 and 50 mg/kg p.o.) and BAY Y-1015 came from studies using an acute zymosan-induced murine arthritis model superimposed on chronic collagen-induced arthritis [23]. A murine colitis model also indicated significant antiinflammatory effects of BAY Y-1015 at 8 mg/kg p.o. b.i.d.

Cardioprotective effects were demonstrated in a 3-day rabbit coronary artery legation experiment on a variety of parameters such as mortality and electrocardiogram normalization after early infusion of a total dose of 40 mg/kg of BAY X-1005 [24].

Significant animal data regarding allergic asthma were generated in the guinea-pig employing the ovalbumin-sensitized animal (Konzett–Rossler test). It is of interest to note that the data on the rat whole blood ex vivo test after oral application of BAY X-1005 match those of the guinea-pig in the Konzett–Rossler test. In the *Ascaris suum*-antigen-sensitized sheep, BAY X-1005 inhibits the early and late allergic reaction both after oral dosing or following aerosol exposure.

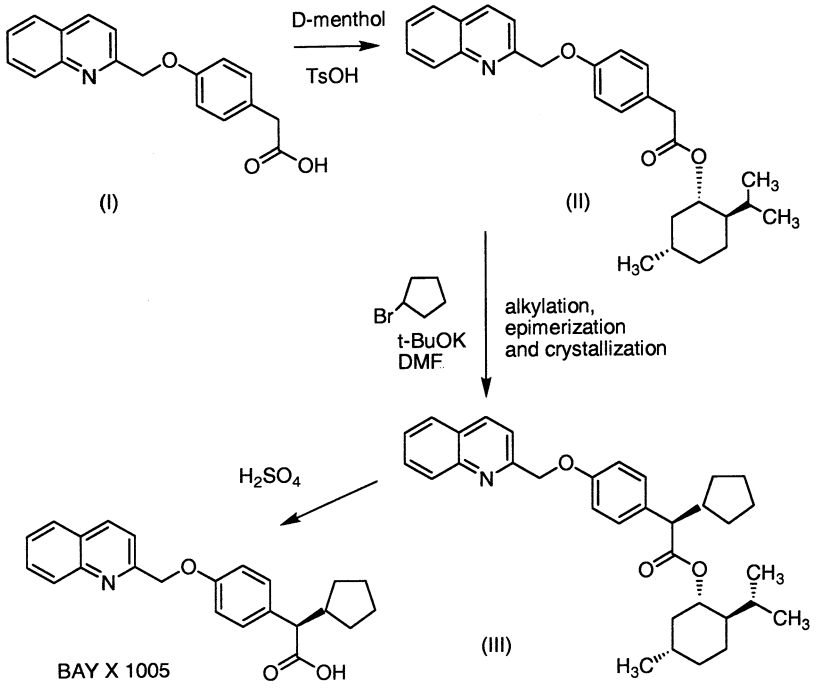

Fig. 3. Stereoselective synthesis of BAY X-1005 (see text).

10. Safety aspects of BAY X-1005/BAY Y-1015

BAY X-1005 proved to be extremely safe. Preclinical pharmacology showed no side effects. Toxicology indicated no limitations with regard to safety for starting clinical studies in volunteers. Clinical phase I studies up to doses of 750 mg equiv. to C_{max} levels of about 7 mg/l (fasted) and 13.7 mg/l after breakfast resulted in virtually no adverse effects [25]. Clinical phase II studies in allergic asthmatic patients again did not demonstrate any significant side effects.

Only BAY Y-1015 demonstrated enzyme-inducing properties at higher doses in humans, which owing to the liability of drug interaction resulted in the termination of further clinical development. Enzyme induction has also been observed with the already marketed direct 5-LO inhibitor zileuton.

11. Clinical evaluation of BAY X-1005 in patients with allergic asthma

BAY X-1005 was investigated in a comprehensive phase I and phase II clinical program in about 1000 subjects patients, using dosages from 50 to 750 mg in single dose and 50–500 mg b.i.d. in multiple-dose studies. Duration of treatment was up to 6 weeks for 500 mg b.i.d. and up to 1 year for 250 mg b.i.d.

11.1. Phase I

BAY X-1005 was rapidly absorbed. In the investigated dose range (see above), the area under the time–response curve (AUC) and C_{max} increased dose dependently; $t_{1/2}$ was 4–8 h. Multiple-dose administration did not reveal a marked accumulation. No clinically relevant interactions with theophylline, antacids or histamine H_2-antagonists occurred [26]. BAY X-1005 was subjectively and objectively well tolerated. No major adverse events occurred during the entire clinical program. The safety profile of BAY X-1005 was excellent.

11.2. Phase II: efficacy profile of BAY X-1005

BAY X-1005 was assessed in a limited phase IIa clinical program evaluating BAY X-1005 effects versus placebo in two antigen challenge tests, a cold air provocation test and a bronchomotor tone study.

An allergen provocation test in ten patients with mild asthma was performed to investigate the effects of single-dose 750-mg BAY X-1005 versus placebo. The mean maximal fall was 7.1% in the BAY X-1005 group and 21% in the placebo group ($P < 0.001$). As a parameter of Cys-LT synthesis, urinary LTE_4 was measured and found to be significantly reduced (76%) within the first 2 h after allergen challenge [27].

The protective effects against inhaled allergen challenge were subsequently confirmed and extended in a study involving eight atopic asthmatics. The effects of 500 mg b.i.d. BAY X-1005 for 3 and a half days were compared with placebo on the allergen-induced early response (EAR, $>15\%$ fall in FEV_1 0–3 h after allergen inhalation) and the late response (LAR, $>15\%$ fall in FEV_1 3–7 h after allergen inhalation). Treatment with BAY X-1005 attenuated the magnitude of both the allergen-induced EAR and LAR. The mean maximal fall of FEV, during the EAR was 26.6% during placebo treatment and 11.4% during treatment with BAY X-1005 with a mean protection of 57.1%. The mean maximal fall in FEV, during the LAR was 19.8% during placebo treatment and 10.7 during BAY X-1005 treatment with a mean protection by BAY X-1005 of 46.0 AUC 0–3 h was also reduced after treatment with BAY X-1005 by 86.5% compared with placebo and the AUC 3–7 h by 59.6%.

The results mirror those of the leukotriene antagonists Accolate and MK-571, but are superior to the direct 5-LO inhibitor zileuton [28].

The results of these studies prompted further clinical efficacy trials. The phase II clinical program comprised a pilot efficacy trial and two comprehensive dose-ranging studies.

Statistically significant and clinically relevant therapeutic effects of the LSI BAY X-1005 were observed in patients with moderate to severe asthma, but not in mild asthmatic patients. This led unexpectedly to termination of the project.

12. Conclusions

Cys-LT receptor antagonists represent the first new drugs introduced in asthma therapy in the past 20 years and they can be used in persistent asthma irrespective of its degree of severity. Leukotriene modifiers can improve lung function and decrease the requirement of β_2-adrenergic agonists, resulting in significant symptom control, especially at night. This new class of compounds is characterized by an excellent patient compliance which is achieved by oral treatment and in patients with mild persistent asthma they may represent first-line therapy. In subjects with moderate-to-severe chronic persistent asthma leukotriene modifiers therapy can be coupled to inhaled corticosteroids to achieve an optimal control of asthma. Cys-LT receptor antagonists are powerful, competitive inhibitors of the Cys-LT_1 receptor, with pA_2 values in the nM range, and they are remarkably safe.

Cys-LT synthesis inhibitors have potentially broader actions than receptor antagonists since they also inhibit formation of the chemotactic factor LTB_4 as well as of the 5-LO-derived hydroxyeicosatetraenoic acids.

However Cys-LT synthesis inhibitors so far experienced do not seem to achieve a complete (or almost complete, above 90%) and long lasting (at least 24 h) blockade of mediator formation which is considered a prerequisite for optimal clinical efficacy.

In addition to providing orally available, safe and effective therapy, these new drugs are powerful pharmacological tools that are now teaching much about the pathobiology of asthma.

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