

EXPERT OPINION

1. Asthma
2. Current therapies
3. Research focus and future needs
4. Patenting activity
5. Conclusion
6. Expert opinion

informa
healthcare

Small-molecule anti-inflammatory drug compositions for the treatment of asthma: a patent review (2013 – 2014)

Paul Glossop[†], Gavin Whitlock^{*} & Karl Gibson

[†]*Sandexis Medicinal Chemistry Ltd, Kent, UK

Introduction: Asthma is a chronic condition affecting 235 million people worldwide, with prevalence continuing to increase. A significant number of patients have poorly controlled asthma but despite this, a new mechanistic class of small-molecule asthma therapy has not emerged over the past 15 years.

Areas covered: In this article, the authors review the published patent literature from 2013 to 2014 that describes the discovery of novel small-molecule anti-inflammatory agents for the treatment of asthma. This patent analysis was performed using multiple search engines including SciFinder and Free Patents Online.

Expert opinion: This review highlights that significant research is still directed towards the development of novel anti-inflammatory agents for the treatment of asthma. Current standard-of-care therapies are given topically to the lung via an inhaled dose, which the authors believe can offer significant advantages in terms of efficacy and therapeutic index, compared with an oral dose. Several of the patents reviewed disclose preferred compounds and data that suggest an inhaled approach is being specifically pursued. The patents reviewed target a wide range of inflammatory pathways, although none have yet delivered an approved novel medicine for asthma; this gives an indication of both the opportunity and challenge involved in such an endeavor.

Keywords: anti-inflammatory, asthma, bronchodilation, inflammation, inhaled drugs

Expert Opin. Ther. Patents [Early Online]

1. Asthma

Asthma is a chronic disease of the respiratory system, caused by inflammation of the airways leading to bronchial hyperresponsiveness and symptoms such as wheezing, breathlessness, cough and chest tightness [1]. It is thought that the primary risk factors for triggering the disease are genetic and environmental in nature: for example, exposure to a range of allergens such as dust mites, pollen, pets or airborne pollution can lead to an asthma attack or exacerbation [1]. Asthma is a common condition, with ~ 235 million people worldwide living with the disease; asthma is the most common noncommunicable disease among children [1]. Although there is no cure for asthma, symptoms can be well-controlled for many patients with medication that acts either as a preventative therapy or so-called rescue therapy [1]. However, a significant number of patients have asthma that is poorly controlled by existing medicines, resulting in ~ 250,000 deaths per year [2].

Asthma is a complex inflammatory condition that involves activation of a wide range of inflammatory cells such as eosinophils, mast cells, and T helper type 2 (Th2) cells, and the release of pro-inflammatory signaling proteins such as

Article highlights.

- Asthma is a chronic inflammatory condition whose prevalence is continuing to rise.
- Many patients have poorly controlled asthma and consequently, there is still a significant medical need for new safe and effective anti-inflammatory agents to treat this disease.
- A new mechanistic class of small-molecule asthma therapy has not emerged over the past 15 years.
- Many anti-inflammatory approaches to asthma are currently being pursued, in particular, kinase inhibition and chemokine receptor antagonism.
- Inhalation may still be the optimal approach to deliver high levels of sustained lung-targeted efficacy combined with low systemic exposure to provide a large therapeutic index against systemically driven side effects.
- This review highlights that a number of inhaled approaches are specifically being pursued, including inhibition of kinases such as p38 MAPK, phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) and Janus kinase. Inhaled PI3K and p38 mitogen-activated protein kinase (MAPK) inhibitors are currently in clinical development for the treatment of asthma.
- Oral approaches to asthma are also being pursued. For example, chemoattractant receptor-homologous molecule expressed on Th2 cells antagonists (CRTH2) have shown efficacy with no drug-related adverse events during clinical trials in asthmatic patients.

This box summarizes key points contained in the article.

cytokines, chemokines, lipid mediators and growth factors. The bronchoconstriction associated with an allergic asthma exacerbation has two distinct phases: the early airway response (EAR) and the late airway response (LAR) [3]. Different classes of therapy impact on these two phases in distinct ways: for example, long-acting β_2 -adrenoceptor agonists (LABA) are bronchodilators that block the EAR, whereas inhaled corticosteroids (ICS) are anti-inflammatory drugs that modulate the LAR [3]. Consequently, inhaled combination medicines containing both LABA and ICS have emerged as the standard-of-care therapy for chronic asthma.

2. Current therapies

Existing small-molecule asthma therapies are divided into two main categories: i) preventative/maintenance therapy that provides long-term control of symptoms; and ii) so-called rescue therapy that provides rapid bronchodilation and relief of symptoms associated with an acute asthma attack [1]. Although such rescue medications (e.g., salbutamol) are fast-acting, they are also short-acting and therefore not suitable for the control of symptoms. Long-term symptom control is achieved by the once-daily or twice-daily inhalation of LABA or ICS, or their combination products. Widely used inhaled medicines that are administered twice daily include the LABA drugs formoterol and salmeterol, and the ICS drugs budesonide and fluticasone propionate (Figure 1); combination products of these

drug classes include Symbicort® (AstraZeneca) and Advair®/Seretide® (GlaxoSmithKline), respectively, which have been the mainstay of asthma therapy for many years. Once-daily inhaled medicines have recently emerged, including vilanterol (LABA) and fluticasone furoate (ICS) (Figure 1); a combination medicine of these two drugs (Relvar® Ellipta®, GlaxoSmithKline) is approved for the treatment of asthma. In contrast to inhaled medications, there are relatively few orally administered medicines for the treatment of chronic asthma. Examples of oral anti-inflammatory drugs include montelukast, a leukotriene-receptor antagonist that prevents the binding of leukotriene D₄, and zileuton, a 5-lipoxygenase inhibitor that prevents the synthesis of leukotriene D₄ (Figure 1) [4].

3. Research focus and future needs

It is notable that no small-molecule medicine targeting a new mechanism of action has been launched for asthma during the past 15 years, although a number of new biologics have been investigated. For example, the anti-IgE biologic omalizumab is approved for moderate-severe asthma, and a number of anti-cytokine biologics are in late-stage development [5]. Asthma rates are continuing to increase and the estimated global market for asthma medications is predicted to reach \$23 billion per annum by 2023 [6]. This increase in prevalence, coupled with patients who are unresponsive to ICS therapy, indicates that there is still a significant need for new asthma treatments that operate through safe and effective anti-inflammatory pathways. For example, pathways that involve specific pro-inflammatory mediators such as prostaglandin D₂ (PGD₂) and interleukins, and/or pharmacological targets such as spleen tyrosine kinase (Syk), PI3K, PDE4 and chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2). Such targets/pathways have previously been highlighted to have the potential to offer new treatment options for steroid-resistant asthma [7] and are included among the patents of this review. Overall, these research efforts may provide opportunities to stratify the highly heterogeneous population of asthma sufferers into specific subgroups, which may benefit from a more targeted approach to therapy [8].

4. Patenting activity

In this article, 24 patents published during 2013 and 2014 are reviewed that relate to the discovery of small-molecule drugs as anti-inflammatory agents for the potential treatment of asthma. Owing to the large number of biological targets that can be targeted to prevent or relieve the symptoms of asthma, this review includes small-molecule ligands that bind to a variety of enzymes, receptors or ion channels. However, due to the large number of existing bronchodilator and steroidal therapies already in use, patents relating to the β_2 -adrenoceptor, muscarinic M₃ receptor or glucocorticoid receptor have not been reviewed here [9-11]. Patent activity against

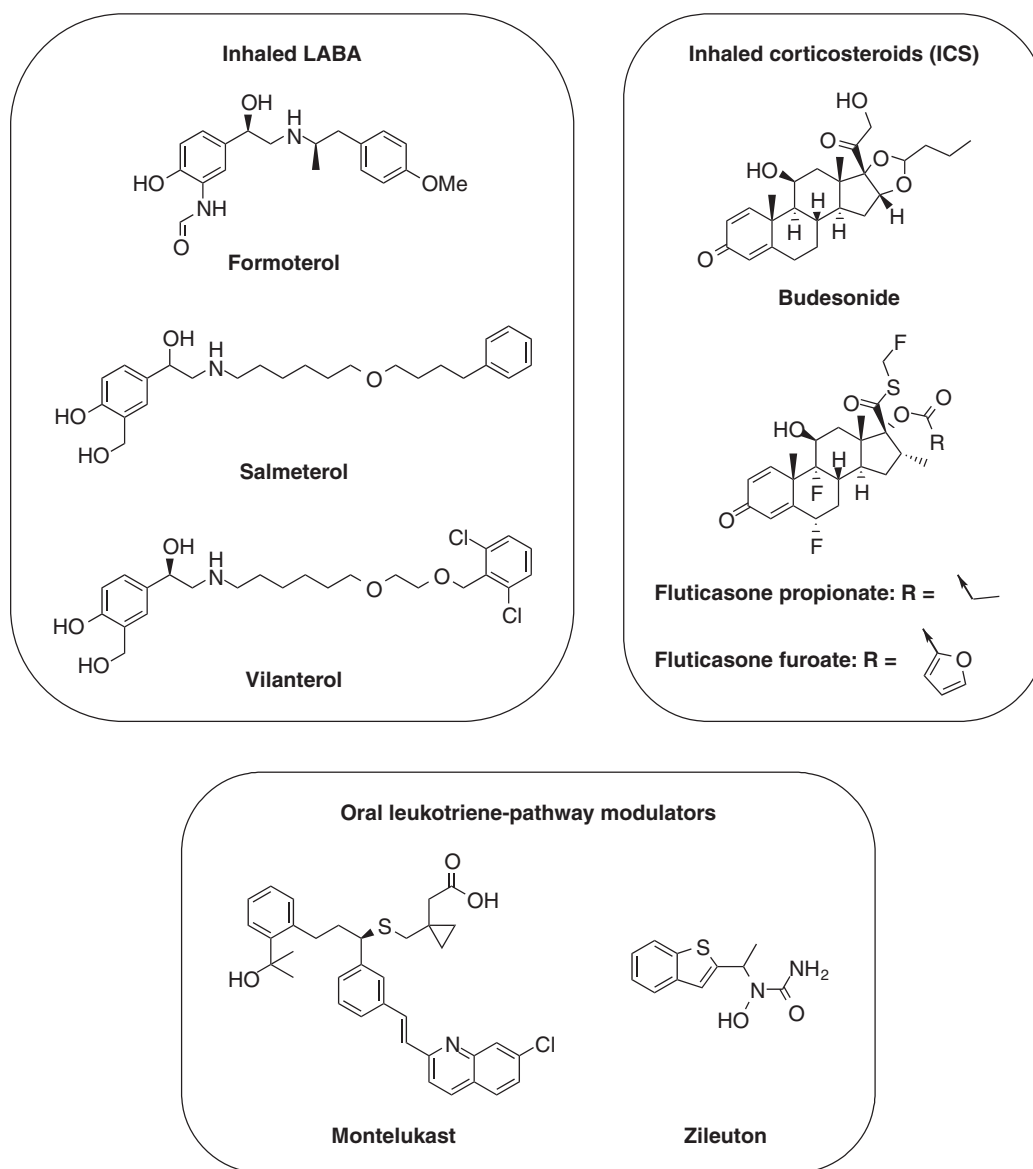


Figure 1. Examples of current medications used for the treatment of asthma.

some of these individual anti-inflammatory targets has been reviewed in previous articles in *Expert Opin. Ther. Patents* [12,13]. The purpose of this review is to focus specifically on patents published in the last 2 years that state asthma as their primary indication or one of their main indications.

The patent review was performed using multiple electronic databases including SciFinder and Free Patents Online. Using these tools, patents were extracted from 2013 to 2014 that contained small-molecule drugs and 'asthma' as a key word; patents relating to biologics such as proteins, vaccines and antibodies are not included in this review. The resulting list of patents was individually assessed to establish their relevance: that is, whether asthma was the primary therapeutic indication or one of the main indications. The resulting

patents that are the subject of this review have been divided into their pharmacological target class: enzymes (including kinases), receptors and ion channels; these sections are further subdivided into specific biological targets within each target class. There is an additional section for patents that contain compounds with activity in phenotypic assays, for which the biological target is unknown.

4.1 Enzymes (including kinases)

4.1.1 Spleen tyrosine kinase

Syk is a non-receptor tyrosine kinase (Tyk) that plays a critical role in regulating the degranulation of mast cells and basophils, and is widely expressed in a variety of inflammatory cells. Syk lies upstream of the signaling cascades which

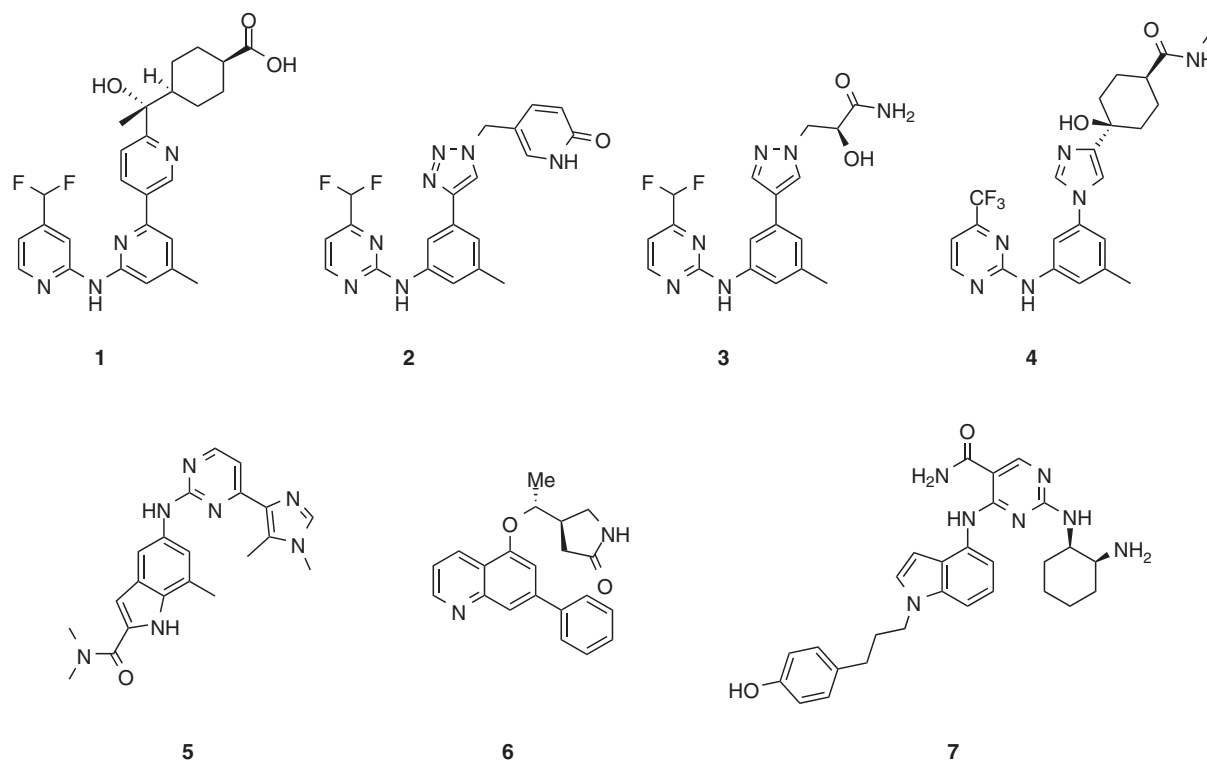


Figure 2. Syk inhibitors, exemplified within recent patent literature (2013 – 2014).

Syk: Spleen tyrosine kinase.

regulate calcium flux and gene transcription and it is essential for the production and secretion of both early and late allergic inflammatory mediators in response to allergen-IgE complexes. As such, Syk inhibition is thought to be a viable strategy for reducing inflammation associated with allergic asthma. The first inhaled Syk inhibitor, R343, was developed by Rigel Pharmaceuticals and Pfizer [14–16]. Phase I clinical studies with an inhaled 12 mg dose of R343 demonstrated a reduction in both EAR and LAR following allergen challenge in mild asthmatics [17]. More recently, R343 completed an 8-week Phase II clinical study for allergic asthma but did not meet its primary endpoint (change in forced expiratory volume in 1 (FEV1) second; a measure of lung function) following an inhaled dose of 1 or 2 mg, although it was relatively safe and well-tolerated [18,19].

A number of patent applications have been published during 2013 – 2014 that claim Syk inhibitors for the treatment of asthma [12]. In particular, Merck Sharp & Dohme Corp. has filed four separate applications [20–23], exemplified by compounds 1 – 4 (Figure 2). Structurally, these compounds all contain a similar framework based on either *N*-(2-pyridyl)-2-aminopyridine or *N*-phenyl-2-aminopyrimidine, which are presumably the hinge-binding units of these kinase inhibitors. Compounds 1 – 4 are mostly distinguished by the substituent on the top right-hand side of the molecules. For example, a patent application published in 2014 exemplifies different

ester prodrugs of carboxylic acid 1 [20]. These prodrugs are reported to have improved aqueous solubility, thus facilitating the production of aqueous formulations for drug administration, including parenteral formulations delivered by injection. The prodrugs are designed to cleave under physiological conditions to release 1 as the active Syk inhibitor, which has an IC_{50} of < 0.5 nM in a homogeneous time-resolved fluorescence (HTRF) assay using recombinant human Syk enzyme. Compound 1 is the only ‘parent’ Syk inhibitor in the application. The other three patent applications containing Syk inhibitors (not prodrugs) were published in 2013 and 2014 [21–23]. These applications exemplify a large number of compounds, including pyridone 2 (IC_{50} = 3.7 nM) [21], and amides 3 (IC_{50} = 2.7 nM) and 4 (IC_{50} = 6.1 nM) [22,23].

Boehringer Ingelheim International GMBH published two patents in 2013 that claim two different series of Syk inhibitors [24,25]. One patent contains inhibitors based on an *N*-(5-indolyl)-2-aminopyrimidine scaffold, such as 5 [24]. The other patent exemplifies a series of quinoline-based inhibitors, for example, 6 [25]. Both of these scaffolds are likely to represent the hinge-binding units of their respective series. Compounds 5 and 6 were tested in a Kinase-Glo[®] luminescent assay using recombinant human Syk enzyme: their IC_{50} values were found to be 7.4 and 0.4 nM, respectively.

Ziarc Pharma Ltd published a patent in 2014 that contains a series of 2-(2-aminocyclohexyl)aminopyrimidine-

5-carboxamides as Syk inhibitors [26]. One of the preferred examples is compound 7, which has an IC_{50} of 1.18 nM in a Kinase-Glo[®] luminescent assay using recombinant human Syk enzyme. The compounds are particularly favored for non-systemic or topical delivery, including inhalation, to provide targeted efficacy and improved safety and toleration through minimal systemic exposure.

4.1.2 p38 mitogen-activated protein kinase

p38 mitogen-activated protein kinase (MAPK) is thought to play a key role in many signaling pathways involved in chronic inflammation present in respiratory diseases such as asthma and chronic obstructive pulmonary disease (COPD) [27]. p38 MAPK is activated by a wide range of pro-inflammatory cytokines that, in turn, release further pro-inflammatory cytokines. Currently, there is one inhaled p38 MAPK inhibitor, PF-03715455 from Pfizer, in clinical development for the treatment of moderate-to-severe asthma [28]. Six patent applications have been published from Respivert Ltd that claim inhaled p38 MAPK inhibitors for the treatment of asthma [13,29-35]. Two of these patents, which published sequentially in 2013, exemplify a series of naphthalene ureas; one of these patents claims 8 (Figure 3) as one of 33 examples [29], whereas the other application is a single-compound patent that claims 8 as the only example [30]. The activity of 8 was determined across a range of *in vitro* assays: p38 MAPK α IC_{50} = 60 nM, determined using a fluorescence resonance energy transfer (FRET) assay; p38 MAPK α K_d = 20 nM, determined using a site-directed competition binding assay conducted by DiscoverRX; cellular IC_{50} = 2.2 – 3.4 nM in human cell assays measuring the lipopolysaccharide (LPS)-induced release of inflammatory mediators such as IL-8 and TNF α . Compound 8 was also found to have a superior anti-inflammatory effect to the ICS fluticasone propionate, when tested for cytokine production in sputum macrophages and bronchial epithelial cells from patients with COPD. Efficacy data from two *in vivo* studies in mice show that 8 provides: dose-dependent and long-lasting inhibition of LPS-induced lung neutrophilia, following intratracheal administration; dose-dependent inhibition of cigarette-smoke-induced lung neutrophilia, following intranasal delivery. Salt forms of 8 are also described, along with post-micronization data for a crystalline solid form of the anhydrous free base [30]; this provides additional evidence that 8 is being developed as an inhaled formulation. Four patents, published in 2014 [31-35], contain urea-based compounds that possess multi-kinase activity against p38MAPK, Syk and Src, for example, indazole 9 (Figure 3) [33]. The activity of 9 was determined across a range of *in vitro* assays: p38 MAPK α IC_{50} = 77 nM, c-Src IC_{50} = 13 nM, Syk IC_{50} = 22 nM, determined using FRET assays; cellular IC_{50} = 2.0 nM in human cell assays measuring the LPS-induced release of IL-8. Efficacy data from *in vivo* studies in mice show that 9 produces dose-dependent inhibition of neutrophilia in the lungs for up to 8 h. The multi-kinase activity

displayed by 9 may offer the potential for increased efficacy via inhibition of multiple signaling pathways (e.g., Syk inhibition [Section 4.1.1]), but there is also a risk that a nonselective profile could introduce unwanted side effects. Compounds 8 and 9 exhibit their long duration of action by virtue of slow dissociation from the p38 MAPK enzyme.

4.1.3 Phosphatidylinositol-4,5-bisphosphate 3-kinase

The PI3K pathway regulates the expression and activation of inflammatory mediators, inflammatory cell recruitment and airway remodeling. There are four isoforms of PI3K (α , β , γ and δ), but selective PI3K δ inhibition has been indicated to be a potentially important mechanism to reduce airway inflammation and hyperresponsiveness. Merck Sharp & Dohme Corp. published two patents in 2014 that claim PI3K δ inhibitors for the treatment of asthma [36-38]. Both patents utilize the same purine hinge-binding unit as that found in the endogenous kinase substrate ATP. Examples include 10 and 11 (Figure 3) [36,37], which have IC_{50} < 1.0 nM in a homogeneous time-resolved fluorescence assay using recombinant human PI3K δ enzyme, and > 10-fold selectivity over the PI3K α isoform.

A patent from Gilead Calistoga LLC was published in 2013 that claims a single compound, 12 (Figure 3) [39,40], which is selective for PI3K δ over the other PI3K isoforms: PI3K δ IC_{50} = 0.45 nM, PI3K α IC_{50} = 6,300 nM, PI3K β IC_{50} = 87 nM and PI3K γ IC_{50} = 210 nM, when screened in a SelectScreen kinase-inhibitor assay panel. To analyze PI3K signaling via catalytic subunit isoforms P110 δ and P110 γ , basophil activation was measured in isolated peripheral blood mononuclear cells (PBMC) or whole blood using the Flow2 CAST[®] kit (Bühlmann Laboratories AG). Compound 12 had a basophil PI3K P110 δ EC_{50} = 1.0 nM and PI3K P110 γ EC_{50} = 50 nM, indicating that 12 is selective for PI3K δ both in enzyme and cellular assays. Following oral dosing of 12 (1.52 mg/kg) to rats, all animals in the study group (3/3) were observed to have diarrhea at 8 and 12 h post-dose.

Respivert Ltd published one patent in 2013, covering crystalline forms of a single PI3K inhibitor, 13 (Figure 3) [41,42]. In particular, the crystalline hydrate and anhydrous forms of 13 were assessed for their suitability for inhaled delivery via a dry powder inhaler. Compound 13, also known as RV1729, is currently in Phase I clinical development as a potential new treatment for asthma and COPD [43-45].

4.1.4 Janus kinase

The Janus kinase (Jak) family of kinases (Jak-1, Jak-2, Jak-3 and Tyk-2) play a critical role in inflammatory signaling pathways. They act as hetero- or homodimers in the phosphorylation of Stat (signal transducer and activator of transcription) proteins, which regulate gene transcription of pro-inflammatory mediators. Jak-dependent signaling pathways are believed to play key roles in the activation and recruitment of inflammatory cells in chronic respiratory

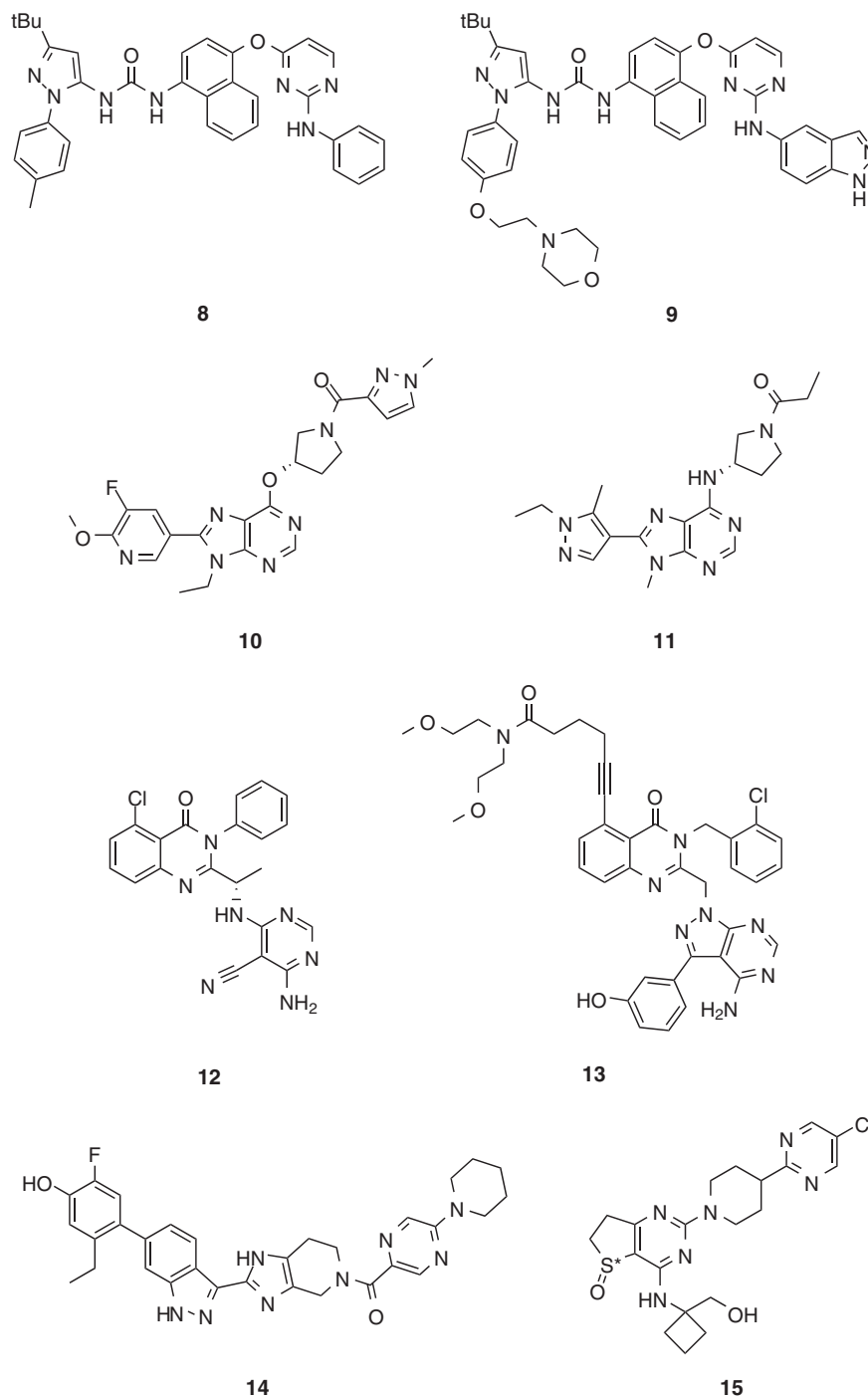


Figure 3. Inhibitors of p38 MAPK, PI3K, Jak and PDE4, exemplified within recent patent literature (2013 – 2014).

Jak: Janus kinase; MAPK: Mitogen-activated protein kinase; PDE4: Phosphodiesterase-4; PI3K: Phosphatidylinositol-4,5-bisphosphate 3-kinase.

diseases such as asthma and COPD. In 2013, Pfizer Ltd published a patent containing a series of indazole-based Jak inhibitors for the treatment of asthma [46]. In total, 65 compounds are exemplified; 14 is the most preferred compound (Figure 3). The activity of 14 was extensively determined in a range of *in vitro* assays: Jak-1 and Jak-3 enzyme: IC_{50} = 5.4 and

0.5 nM, respectively, determined using the appropriate Jak enzyme in a Caliper assay; whole-cell IC_{50} = 140 nM, in a Jak-1/3 reporter-gene assay in activated B-cells; cellular Jak-1 and Jak-2 IC_{50} = 75 and 176 nM, respectively, determined using PathHunter[®] assay technology (developed by DiscoverRX); functional Jak IC_{50} = 70 and 45 nM in stimulated

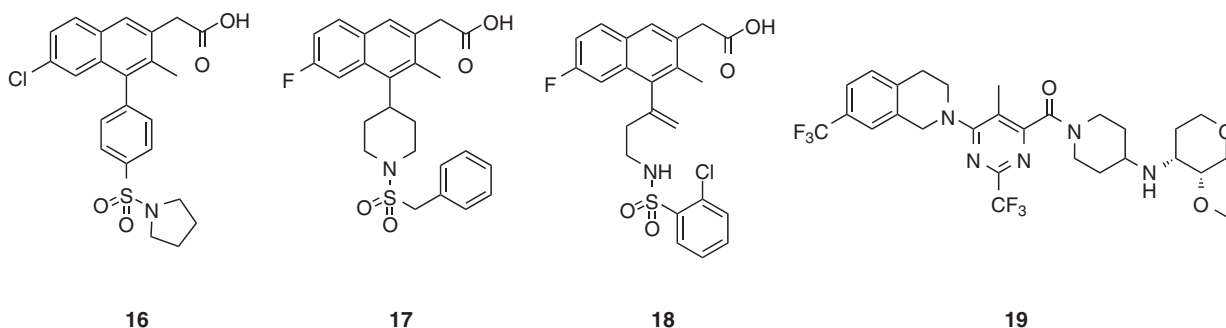


Figure 4. Agonists/antagonists of CRTH2 and CCR2, exemplified within recent patent literature (2013 – 2014).

CRTH2: Chemoattractant receptor-homologous molecule expressed on Th2 cells.

human PBMC, measuring IFN- γ and tyrosine-phosphorylated Stat5 (pYStat5), respectively. The *in vitro* duration of action of 14 in PBMC was > 8.8 h, suggesting that compounds of this patent application provide a long-lasting anti-inflammatory effect. The patent mentions that there is “a need to provide new Jak inhibitors... particularly compounds which can be administered by inhalation”, suggesting that compounds such as 14 are perhaps intended to be delivered by inhalation. As the *in vitro* data suggest that compound 14 inhibits Jak-1, Jak-2 and Jak-3 isoforms (i.e., a pan-Jak inhibitor), inhaled administration would certainly help to maximize efficacy and minimize potential side-effects (e.g., neutropenia) related to systemically mediated Jak-2 inhibition.

4.1.5 Phosphodiesterase-4 (PDE4)

The PDE4 enzyme subfamily contains four isoforms (PDE4A–D), of which PDE4A, PDE4B and PDE4D are expressed on inflammatory cells such as eosinophils, lymphocytes, macrophages and neutrophils [47]. Selective PDE4 inhibitors have recently been developed with the aim of improving the TI over non-selective PDE inhibitors such as theophylline. Clinical studies with orally administered selective PDE4 inhibitors have shown efficacy against relevant asthma endpoints such as inhibition of allergen challenge, exercise-induced bronchoconstriction and improvements in lung function [47]. However, the tolerability of these orally administered drugs is still limited by on-target side effects such as gastrointestinal symptoms [47], which are a well-known issue with PDE4 inhibitors. It has been suggested that the use of inhaled formulations or the development of an oral subtype-selective PDE4B inhibitor may be required to provide efficacy without these side effects [47]. GSK has advanced an inhaled PDE4 inhibitor, GSK256066, to Phase II clinical studies in patients with mild asthma: GSK256066 showed reduction in both the EAR and LAR following allergen challenge [48]. Boehringer Ingelheim International GMBH published a patent in 2013 that claims piperidino-dihydrothienopyrimidine sulfoxides as PDE4 inhibitors [49]. This application follows their publication between 2006 and 2009 of three related patents [50–52].

Compounds of the present invention contain a different substitution pattern and are reported to have increased potency against PDE4B and reduced potential for gastrointestinal side effects. The patent claims two specific compounds [49], one of which is the chiral sulfoxide 15 (Figure 3) that has been synthesized in quantities exceeding 100 g. Crystalline solid forms of 15 are also exemplified with characterization data. Compound 15 has an *in vitro* PDE4B IC₅₀ of 7.2 nM in a scintillation proximity assay (no data reported for the other PDE4 isoforms) and has shown potent anti-inflammatory activity in an *in vivo* model of LPS-induced lung inflammation in rats; following oral administration by gavage, 15 demonstrated an ED₅₀ of 0.1 mg/kg. Gastrointestinal studies in conscious rats demonstrated that oral administration of 15 had no effect on gastric emptying or gastrointestinal transit at doses up to 30-fold the efficacious ED₅₀.

4.2 Receptors

4.2.1 Chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2)

Activated mast cells release PGD₂, which binds to the PGD₂ receptor 1 (DP1) and the CRTH2 receptor (also known as the PGD₂ receptor 2 [DP2]). This activation of Chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2) by PGD₂ results in chemotaxis of eosinophils, basophils and Th2 cells. Therefore, it is believed that CRTH2 antagonists should inhibit recruitment and activation of those cell types in subjects with asthma, with a consequent reduction in airway inflammation and hyperreactivity. Indeed, a number of CRTH2 antagonists have been progressed to clinical studies. For example, OC000459 has been shown to reduce airway inflammation and improve lung function in moderate, persistent asthma at an oral dose of 25 mg once-daily for 12 weeks [53].

Three patents were published in 2013 from F. Hoffman-La Roche AG [54–56]. These applications claim substituted naphthylacetic acids as antagonists or partial agonists of CRTH2 that may be useful in treating asthma. Sulfonamides 16 – 18 (Figure 4) represent one example from each patent [54–56], although it should be noted that 18 is one of only two



Figure 5. Agonists/antagonists of GABA, TRPA1 and Na_v, exemplified within recent patent literature (2013 – 2014).

examples from its patent [56]. The cellular potencies of 16 – 18 were found to be 8, 8 and 4.6 nM, respectively, when evaluated in human Th2 cells, measuring inhibition of induced IL-13 production, detected by ELISA.

4.2.2 Chemokine receptor 2

Chemokines are cytokines released by a range of cell types, which then recruit inflammatory cells such as mast cells, basophils, eosinophils and neutrophils to the site of inflammation. Chemokine receptors such as chemokine (C-C) receptor 2 (CCR2) have been implicated as important mediators of inflammatory processes; for example, CCR2 is highly expressed on monocytes and is a critical regulator of monocyte trafficking. Therefore, a selective CCR2 antagonist may provide a therapeutic benefit in the treatment of asthma by reducing movement of monocytes to a site of inflammation. To date, no CCR2 antagonists have been assessed in clinical studies with asthmatic patients. Boehringer Ingelheim International GMBH published a patent in 2013 claiming selective CCR2 antagonists that are particularly useful for the treatment of asthma [57]. Forty-six examples are included, all of which are based on a central pyrimidine amide. Compound **19** (Figure 4) has a CCR2 K_i of 3 nM in a binding assay using membranes prepared from human acute monocytic leukemia (THP-1) cells. Compounds of the application show selectivity over CCR5; for example, **19** has a K_i of 265 nM in a binding assay using membranes prepared from CHO cells expressing human CCR5.

4.3 Ion channels

4.3.1 GABA receptor

The GABA_A receptor is a ligand-gated ion channel that has been shown to be expressed on pulmonary epithelial cells and, therefore, may influence smooth muscle contractility.

However, other data suggest that GABA_A receptor levels are elevated in mice that have been challenged with ovalbumin, suggesting that GABA_A receptor activation is associated with the induction of asthma symptoms [58]. Despite the uncertainties associated with this target, one patent was published in 2014 by UWM Research Foundation, Inc., claiming GABA_A agonists useful in the control of airway hyperresponsiveness and inflammation in asthma [59]. Ten compounds based on a benzodiazepine scaffold are exemplified, of which two are stated to possess the desired selectivity bias toward the $\alpha 4$ subunit of the GABA_A receptor; one of these compounds is **20** (Figure 5), which has been extensively evaluated in a range of biological assays. *In vitro* studies in tracheal rings from wild-type mice showed that **20** was able to inhibit acetylcholine-induced smooth muscle contraction; this level of inhibition was not observed in mice deficient in the GABA_A $\alpha 4$ subunit. Compounds of the application are also claimed to have reduced benzodiazepine-like CNS activity.

4.3.2 Transient receptor potential cation channel, subfamily A, member 1

Transient receptor potential cation channel, subfamily A, member 1 (TRPA1) is a non-selective cation channel found in sensory neurons. TRPA1 is activated by a wide range of noxious substances (including some known to be present in asthma-triggering allergens) and by endogenous reactive substances produced during inflammation. Therefore, the blockade of TRPA1 may provide a method for reducing the inflammation associated with chronic asthma. Cubist Pharmaceuticals, Inc. published a patent in 2014 describing a series of tetrahydropurinyl α -methyl amide derivatives that inhibit TRPA1 [60]. Compound 21 (Figure 5) is the most preferred example and has a human TRPA1 $IC_{50} < 25$ nM.



It is believed that the high levels of potency observed with this series are driven by the chiral methyl group between the tetrahydropuriny and amide groups. *In vivo* studies with oral doses of 21 (3 and 10 mg/kg) in a sheep model of allergic asthma showed inhibition of the EAR (51 and 72%, respectively), the LAR (78 and 79%, respectively) and airway hyperresponsiveness (100% at both doses). Single-dose toxicity studies with oral and intravenous formulations showed that 21 was well tolerated up to doses of 300 mg/kg.

The mucosal surfaces in lung that cover the bronchi and bronchioles provide innate defense mechanisms to protect the body from external pathogens and particles. The major defense mechanism is the mucocilliary escalator, which cleanses the surfaces of the lungs with liquid/mucus to carry debris from the lung to the pharynx, where it is swallowed. Many respiratory diseases result in mucosal surfaces that are perfused with insufficient protective liquid, as a result of an imbalance between epithelial fluid secretion and absorption. Both these processes are thought to be controlled by salt transport: for example, too much absorption is thought to be a result of overactive Na^+ transport. Therefore, one approach for the potential treatment of respiratory diseases such as asthma, and particularly cystic fibrosis (CF), is to develop compounds that block the voltage-gated sodium (Na_v) channel (found on the epithelial surface) to decrease the absorption of epithelial fluid; such compounds are often referred to as epithelial sodium channel inhibitors/modulators [61]. Consequently, this should increase the efficiency of mucocilliary clearance to reduce infection/inflammation and improve lung function. In 2013, Parion Sciences, Inc. published a patent claiming a single compound for the treatment of respiratory diseases including asthma, COPD and cystic fibrosis [62]. The preferred single enantiomer 22 (Figure 5) is stated to promote hydration of mucosal surfaces (and hence clear mucus from the lung) via blockade of the Na_v channel; in human bronchial epithelial cells, 22 has an IC_{50} of 2.4 nM. *In vivo* studies in sheep with aerosolized doses of 22 (16 $\mu\text{g}/\text{kg}$) showed an increase in mucocilliary clearance

Allergic diseases such as asthma are associated with a Th2-biased immune response to allergens, which are associated with elevated levels of IgE and downstream effects on mast cells. A compound that stimulates Toll-like receptors (TLR) has the potential to rebalance the Th2- and Th1-mediated responses towards Th1 and reduce the Th2-type inflammatory response known to occur in asthma. In 2014, three patent applications were published sequentially by GlaxoSmithKline LLC that claim pyrrolo[3,2]pyrimidine-4-amines as inducers of human IFN α , possibly via TLR [63-65]. These applications exemplify a small number of compounds that include piperazine 23 and piperidines 24 and 25 (Figure 6) [63-65]. Compounds were tested in an *in vitro* assay evaluating induction of IFN- α and TNF- α using fresh human whole blood. IFN- α pEC₅₀ values for 23 – 25 were found to be ≥ 5.8 , ≥ 6.4 and ≥ 5.3 , respectively. TNF- α pEC₅₀ values for 23 – 25 were found to be ≤ 5.3 , ≥ 5.0 and ≤ 5.3 , respectively, suggesting that these compounds show some selectivity for the induction of IFN- α over TNF- α .

This review of small-molecule anti-inflammatory drug compositions for the treatment of asthma, published in the patent literature between 2013 and 2014, has highlighted that research is being performed against a wide range of pro-inflammatory targets and pathways. Combined with the number of patents published, this indicates that biotechnology and pharmaceutical companies, and the healthcare sector in general, remain engaged in the search for novel

anti-inflammatory therapies for this chronic condition. Potent modulators of different target classes appear to have potential utility in attenuating the inflammatory response: that is, kinase/enzyme inhibitors, receptor agonists/antagonists and ion-channel blockers are all exemplified in the patents reviewed. In particular, there are a few patents that include information suggesting that compounds of those inventions have been developed for inhaled delivery, for example, preferred compounds such as p38 MAPK inhibitor 8, PI3K inhibitor 13, Jak inhibitor 14 and Na_v blocker 22. However, the majority of patents reviewed do not specify a preferred administration method, although there are a number of interesting/preferred compounds, including PI3K inhibitor 12, PDE4 inhibitor 15, GABA_A agonist 20 and TRPA1 antagonist 21. Prodrug forms of the Syk inhibitor 1 are unlikely to be intended for inhaled administration. Apart from PI3K inhibitor 13 (Phase I) [43,44], it is too early to say whether any of these drug molecules or therapeutic approaches has entered clinical trials. However, the wide-ranging nature of their pharmacology and chemical matter provides promising opportunities for the discovery of a new class of drugs for the treatment of asthma.

6. Expert opinion

The critical factor for the development of novel anti-inflammatory agents for the treatment of asthma will be the ability to demonstrate that efficacy can be achieved with a sufficiently high TI to allow chronic dosing in patients. In particular, the pediatric asthmatic population will require dosing regimens that provide efficacy combined with exquisite drug safety. This profile will be important for new agents to be deemed as viable therapies. Existing oral anti-inflammatory agents approved for the treatment of non-asthmatic indications include the oral PDE4 inhibitor roflumilast. Although a number of clinical studies have demonstrated that roflumilast is effective at improving asthma symptoms and FEV1 second [66], roflumilast has only been approved for the treatment of severe chronic obstructive pulmonary disease [67]. Roflumilast is associated with a number of adverse events such as diarrhea, weight loss, nausea, headache and an increase in psychiatric adverse events. New anti-inflammatory agents for asthma will need to demonstrate a large TI over side effects driven by both on-target and off-target activity. We believe that the optimal way to deliver this profile of sustained efficacy with high TI is to administer the drug by the inhalation, an approach that has successfully discovered the existing respiratory portfolio of bronchodilator and ICS drugs. The clear advantage of pursuing this strategy is that it is able to provide compounds with high levels of sustained lung-targeted efficacy (high potency and long

duration-of-action) and low systemic exposure (rapid metabolic clearance) to provide a large TI against systemically driven off-target, and even on-target, side effects. Compounds that possess this profile are the result of focused research projects that specifically set out to implement and deliver this concept. Therefore, the application of these principles is at the very forefront of medicinal chemistry design, and biological and pharmacokinetic evaluation; an approach that is often referred to as 'inhalation by design'. Consequently, from the patents reviewed, we believe that specific inhaled drug compositions (e.g., p38 MAPK inhibitor 8, PI3K inhibitor 13 and Jak inhibitor 14) provide the greatest chance of becoming a new asthma drug – providing, of course, that their mechanism of action proves to be safe and efficacious against this respiratory disease. However, there is no doubt that inhaled drug discovery and development remains a long, complicated and expensive process. Therefore, partnering opportunities between smaller biotechnology companies and pharmaceutical companies with an established pedigree in inhaled delivery is likely to be critical for the discovery of new drugs that enter clinical trials and ultimately reach the market for asthmatic patients.

New oral drugs for the treatment of asthma will need to meet the same exacting criteria as those for inhaled drugs: that is, high efficacy and large TI. However, as oral drugs deliver their efficacy through high levels of systemic exposure, in order to be both effective and safe, the primary, on-target activity will need to be associated with non-significant side effects. This added level of complexity will preclude the pursuit of some targets (e.g., Jak inhibition) as an oral approach to asthma. From the reviewed patents that may constitute oral approaches to asthma, CRTH2 antagonists (e.g., 16 – 18) appear to be the most promising, as multiple clinical agents have shown evidence of efficacy following chronic dosing to asthmatic patients. For example, OC000459 has been shown to improve FEV1 second in eosinophilic asthma patients with no drug-related adverse events [53].

Declaration of interest

The authors have performed consultancy work in the area of inhaled drug discovery and have an ongoing consultancy agreement with Ziarco Pharma Ltd. P Glossop is a named inventor on one of the patents (WO2014045029) reviewed in this article. G Whitlock is a named inventor on two of the patents (WO2013014567 and WO2014045029) reviewed in this article. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Bibliography

- WHO. Asthma. World Health Organ Fact Sheet 307. 2013. Available from: <http://www.who.int/mediacentre/factsheets/fs307/en/>
- WHO. Global surveillance, prevention and control of chronic respiratory diseases: a comprehensive approach. 2007. Available from: <http://books.google.com/books?hl=en&lr=&id=gdj5iU5FrXEC&pgis=1>
- Grammer LC, Greenberger PA. Patterson's allergic diseases. 7th edition. Lippincott, Williams & Wilkins, UK; 2009
- Montuschi P. Leukotrienes, antileukotrienes and asthma. *Mini Rev Med Chem* 2008;8:647-56
- Walsh GM. An update on biologic-based therapy in asthma. *Immunotherapy* 2013;5:1255-64
- PR Newswire. Asthma Treatment Market (Drug) Worth \$23 Billion by 2023. Available from: <http://www.prnewswire.com/news-releases/asthma-treatment-market-drug-worth-23-billion-by-2023-284128681.html>
- Barnes PJ. Severe asthma: advances in current management and future therapy. *J Allergy Clin Immunol* 2012;129:48-59
- Montuschi P. Toward a personalized pharmacotherapy of respiratory diseases. *Front Pharmacol* 2010;1:131
- Cazzola M, Page CP, Rogliani P, Matera MG. β_2 -agonist therapy in lung disease. *Am J Respir Crit Care Med* 2013;187:690-6
- Prat M, Gavalda A, Fonquerna S, Miralpeix M. Inhaled muscarinic antagonists for respiratory diseases: a review of patents and current developments (2006-2010). *Expert Opin Ther Pat* 2011;21:1543-73
- Norman P. Boehringer Ingelheim's selective glucocorticoid receptor agonist development candidate: evaluation of WO2010141331, WO2010141332 and WO2010141333. *Expert Opin Ther Pat* 2011;21:1137-42
- Norman P. Spleen tyrosine kinase inhibitors: a review of the patent literature 2010-2013. *Expert Opin Ther Pat* 2014;24:573-95
- Bühler S, Laufer SA. p38 MAPK inhibitors: a patent review (2012-2013). *Expert Opin Ther Pat* 2014;24:535-54
- Singh R, Masuda ES, Payan DG. Discovery and development of spleen tyrosine kinase (SYK) inhibitors. *J Med Chem* 2012;55:3614-43
- Pfizer Ltd. Xinafoate salt of N4-(2,2-difluoro-4H-benzo-[1,4]-oxazin-3-one)-6-yl]-5-fluoro-N2-[3-(methylaminocarbonylmethyleneoxy)phenyl]-2,4-pyrimidinediamine. WO031011; 2009
- Rigel Pharmaceuticals, Inc. Method and device for administering xinafoate salt of N4-(2,2-difluoro-4H-benzo-[1,4]-oxazin-3-one)-6-yl]-5-fluoro-N2-[3-(methylaminocarbonylmethyleneoxy)phenyl]-2,4-pyrimidinediamine. WO059631; 2013
- Allan R, Jones I, Bartley M, et al. A randomised, placebo controlled trial of the effect of an inhaled syk inhibitor on allergen induced airways responses in mild asthma [abstract no A2774]. American Thoracic Society International Conference Meetings Abstracts; 2012
- Rigel Pharmaceuticals, Inc. A Safety and Efficacy Study of Inhaled R940343 in Patients With Mild to Moderate Asthma. Available from: <https://clinicaltrials.gov/ct2/show/record/NCT01591044?term=Rigel+R343&rank=1>
- Rigel Pharmaceuticals, Inc. Rigel's R343 Did Not Meet Primary Endpoint in Asthma Study. Available from: <http://ir.rigel.com/phoenix.zhtml?c=120936&p=irol-newsArticle&ID=1849671&highlight=>
- Merck Sharp & Dohme Corp. Prodrug bipyrilidylaminopyridines as syk inhibitors. WO074421; 2014
- Merck Sharp & Dohme Corp. Triazolyl derivatives as syk inhibitors. WO048065; 2014
- Merck Sharp & Dohme Corp. Pyrazolyl derivatives as syk inhibitors. WO192125; 2013
- Merck Sharp & Dohme Corp. Imidazolyl analogs as syk inhibitors. WO192128; 2013
- Boehringer Ingelheim International GmbH. Amino-indolyl-substituted imidazolyl-pyrimidines and their use as medicaments. WO156608; 2013
- Boehringer Ingelheim International GmbH. Substituted quinolines and their use as medicaments. WO014060; 2013
- Ziarco Pharma Ltd. 2-(2-aminocyclohexyl)amino-pyrimidine-5-carboxamides as spleen tyrosine kinase (syk) inhibitors. WO045029; 2014
- Chung KF. p38 mitogen-activated protein kinase pathways in asthma and COPD. *Chest* 2011;139:1470-9
- Pfizer. An Evaluation Of PF-03715455 In Moderate To Severe Uncontrolled Asthma. Available from: <https://clinicaltrials.gov/ct2/show/NCT02219048?term=NCT02219048&rank=1>
- Respivert Ltd. L-pyrazolyl-3-(4-((2-anilinopyrimidin-4-yl)oxy)naphthalen-1-yl) ureas as P38 MAP kinase inhibitors. WO050756; 2013
- Respivert Ltd. 1-pyrazolyl-3-(4-((2-anilinopyrimidin-4-yl)oxy) naphthalen-1-yl) ureas as P38 MAP kinase inhibitors. WO050757; 2013
- Respivert Ltd. Kinase inhibitors. WO033446; 2014
- Respivert Ltd. Diaryl urea derivatives as P38 MAP kinase inhibitors. WO033447; 2014
- Respivert Ltd. Pyrazole derivatives as P38 MAP inhibitors. WO033448; 2014
- Respivert Ltd. Kinase inhibitors. WO033449; 2014
- Norman P. Respivert's multikinase inhibitors, an evaluation of WO2014033446 to WO2014033449. *Expert Opin Ther Pat* 2014;24:1397-407
- Merck Sharp & Dohme Corp. Purine inhibitors of human phosphatidylinositol 3-kinase delta. WO075392; 2014
- Merck Sharp & Dohme Corp. Purine inhibitors of human phosphatidylinositol 3-kinase delta. WO075393; 2014
- Norman P. Evaluation of WO2014075392 and WO2014075393, Merck's first PI3K δ inhibitor filings. *Expert Opin Ther Pat* 2014;24:1277-82
- Gilead Calistoga LLC. Compositions and methods of treating a disease with (S)-4-amino-6-((1-(5-chloro-4-oxo-3-phenyl-3,4-dihydroquinazolin-2-yl)ethyl)amino)pyrimidine-5-carbonitrile. WO116562; 2013
- Norman P. Evaluation of WO2013116562A, an orally active PI3K δ inhibitor for the treatment of

- asthma. Expert Opin Ther Pat 2014;24:603-7
41. Respivert Ltd. Crystalline PI3 kinase inhibitors. WO136076; 2013
42. Norman P. Evaluation of WO2013136076: two crystalline forms of the phosphatidylinositol 3-kinase- δ inhibitor RV-1729. Expert Opin Ther Pat 2014;24:471-5
43. Respivert Ltd. A Study to Investigate the Safety, Tolerability and Pharmacokinetics of Single and Repeat Doses of RV1729. Available from: <https://clinicaltrials.gov/ct2/show/NCT01813084?term=RV1729&rank=2>
44. Respivert Ltd. A Study to Investigate the Safety, Tolerability and Pharmacokinetics of Single and Repeat Doses of RV1729 for up to 28 Days. Available from: <https://clinicaltrials.gov/ct2/show?term=RV1729&rank=1>
45. A Study to Investigate Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Repeat Doses of RV1729 for 28 Days in Patients With COPD. Available from: <https://clinicaltrials.gov/ct2/show/NCT02140346?term=RV1729&rank=3>
46. Pfizer Ltd. Indazoles. WO014567; 2013
47. Lu S, Liu N, Dass SB, et al. Randomized, placebo-controlled study of a selective PDE4 inhibitor in the treatment of asthma. Respir Med 2009;103:342-7
48. Singh D, Petavy F, Macdonald AJ, et al. The inhaled phosphodiesterase 4 inhibitor GSK256066 reduces allergen challenge responses in asthma. Respir Res 2010;11:26
49. Boehringer Ingelheim International GmbH. Novel piperidino-dihydrothienopyrimidine sulfoxides and their use for treating COPD and asthma. WO026797; 2013
50. Boehringer Ingelheim International GmbH. Substituted piperidino-dihydrothienopyrimidines. WO050248; 2009
51. Boehringer Ingelheim International GmbH. Dihydrothienopyrimidine for treating inflammatory disorders. WO118793; 2007
52. Boehringer Ingelheim International GmbH. Dihydrothienopyrimidines for the treatment of inflammatory diseases. WO111549; 2006
53. Pettipher R, Hunter MG, Perkins CM, et al. Heightened response of eosinophilic asthmatic patients to the CRTH2 antagonist OC000459. Allergy 2014;69:1223-32
54. F. Hoffmann-LA Roche AG. Substituted naphthylacetic acids. WO127841; 2013
55. F. Hoffmann-LA Roche AG. Piperidinyl naphthylacetic acids. WO087544; 2013
56. F. Hoffmann-LA Roche AG. Alkenyl naphthylacetic acids for treating respiratory disorders. WO068322; 2013
57. Boehringer Ingelheim International GmbH. Novel and selective CCR2 antagonists. WO010839; 2013
58. Xiang Y-Y, Wang S, Liu M, et al. A GABAergic system in airway epithelium is essential for mucus overproduction in asthma. Nat Med 2007;13:862-7
59. UWM Research Foundation, Inc. Novel GABA_A agonists and methods of using to control airway hyperresponsiveness and inflammation in asthma. WO047413; 2014
60. Cubist Pharmaceuticals, Inc. Inhibiting the transient receptor potential A1 ion channel. WO113671; 2014
61. Schoenberger M, Althaus M. Novel small molecule epithelial sodium channel inhibitors as potential therapeutics in cystic fibrosis - a patent evaluation. Expert Opin Ther Pat 2013;23:1383-9
62. Parion Sciences, Inc. 3,5-diamino-6-chloro-N-(N-(4-(2-(hexyl (2,3,4,5,6-pentahydroxyhexyl)amino)ethoxy)phenyl)butyl)carbamidoyl)pyrazine-2-carboxamide. WO003386; 2013
63. Glaxosmithkline LLC. Novel compounds. WO081643; 2014
64. Glaxosmithkline LLC. Novel compounds. WO081644; 2014
65. Glaxosmithkline LLC. Novel compounds. WO081645; 2014
66. Bateman ED, Izquierdo JL, Harnest U, et al. Efficacy and safety of roflumilast in the treatment of asthma. Ann Allergy Asthma Immunol 2006;96:679-86
67. Baye J. Roflumilast (daliresp): a novel phosphodiesterase-4 inhibitor for the treatment of severe chronic obstructive pulmonary disease. P T 2012;37:149-61

Affiliation

Paul Glossop^{†1} BSc CChem FRSC,
Gavin Whitlock^{†2} PhD CChem FRSC &
Karl Gibson² PhD CChem FRSC
^{†,*}Authors for correspondence
¹Senior Medicinal Chemist,
Sandexis Medicinal Chemistry Ltd, Innovation
House, Discovery Park, Ramsgate Road,
Sandwich, Kent, CT13 9ND, UK
Tel: +44 0 1304 892369;
E-mail: paul.glossop@sandexis.co.uk
²Partner and Co-founder,
Sandexis Medicinal Chemistry Ltd, Innovation
House, Discovery Park, Ramsgate Road,
Sandwich, Kent, CT13 9ND, UK
Tel: +44 0 1304 892369;
Email: gavin.whitlock@sandexis.co.uk