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Small molecule inhibitors of IgE synthesis

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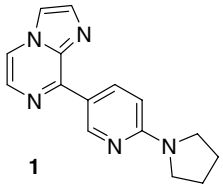
Abstract—A novel series of small molecule inhibitors of IgE synthesis are described. Compounds were optimized for potency, metabolic stability and absence of genetic toxicology.

There is extensive literature evidence supporting immunoglobulin E (IgE) as the key causative factor in allergic disorders. Binding of antigens to specific IgE on mast cells or basophils leads to the cross-linking of IgE receptors, which results in cell activation, degranulation and release of inflammatory mediators such as histamine, leukotrienes and platelet-activating factor (PAF). In particular: (1) levels of IgE are elevated in allergic diseases (asthma, allergic rhinitis) which correlate well with severity of symptoms;[1] (2) genetically engineered mice deficient in IgE or the high affinity IgE receptor have a significantly reduced ability to mount an allergic response;[2] and (3) normal animals can be made allergic by injecting IgE from an allergic animal. More importantly, the key role played by IgE in allergy has been clinically validated by omalizumab, a humanised anti-IgE monoclonal antibody. Omalizumab works by binding to and removing free circulating IgE, and is marketed for the treatment of allergic asthma. Omalizumab has also been demonstrated to be safe and well tolerated. However, high cost may impact on the uptake of this therapy.[3] Nevertheless, Omalizumab has clearly established IgE as a clinically validated target. Thus IgE synthesis inhibitors as oral, disease modifying agents may be seen as a major therapeutic advance. Whilst several classes of small molecule inhibitors of IgE synthesis have been identified,[4] none have yet made it to clinical development; in this paper we wish to report our own efforts to identify such a molecule.

Our initial lead **1** was identified through screening and was shown to be a potent inhibitor of IgE synthesis with an IC_{50} of 55 nM (inhibition of IL4 induced IgE synthesis in human peripheral blood mixed lymphocytes, hPBL);[5] however, this compound was later found to suffer from extensive hepatic and extrahepatic metabolism that rendered it unsuitable for further progression. In particular, the pyrrolidine ring of **1** was found to be metabolically vulnerable. Our initial focus was therefore on the identification of pyrrolidinopyridine replacements with increased metabolic stability.



1

IgE IC_{50} 55 nM / HLM 14min / CYP1A1 < 1min / CYP1A2 < 1min / MW 265 / logD 2.5

Replacement of the pyridyl-pyrrolidine by piperazinyl-pyrimidine gave **2**. Potency increased, as did in vitro human liver microsomal (HLM) stability.[6] However, the compound still showed rapid turnover in isolated CYP1A enzyme.[7] In addition, profiling the compound in our Ames assay indicated that it was a potential mutagen (specifically versus the TA100 strain) both with and without metabolic activation.[8]

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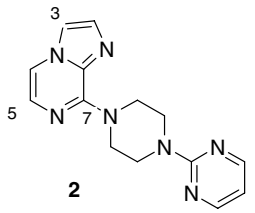
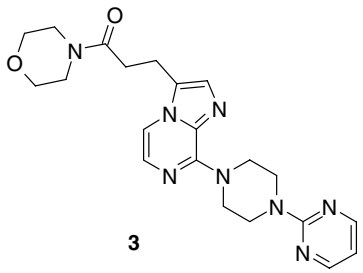
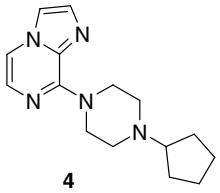


IgE IC_{50} 18 nM / HLM 45min / CYP1A1 <2min / CYP1A2 2min / MW 281 / logD 2.0 / TA100 +ve +/- activation

Since both CYP1A enzymes and TA100 show specificity for flat aromatic substrates,[9] it was proposed that introduction of 3D structure could address both problems simultaneously. Initial attempts to introduce 3D structure focused on the C-3 and C-7 positions of the imidazopyrimidine core primarily as these were the positions with greatest synthetic accessibility. Compounds were indeed identified with reduced CYP1A turnover (e.g., **3** and **4**); however, both compounds were inactive.[10]

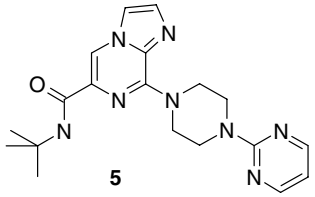


IgE IC_{50} >10 μM / CYP1A1 >120min / CYP1A2 >120min / MW 422



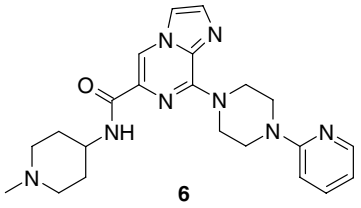
IgE IC_{50} >10 μM / HLM >120min / CYP1A2 >120min / MW 271

A breakthrough came with the identification of **5** where 3D structure could be incorporated at the C-5 position via an amide linker without significant loss of potency. This compound was also shown to be negative in the Ames assay. At the time of synthesis the predicted log*D* for **5** was <2, however, the measured value of 3.7 was considerably higher than expected and was thought to be, at least in part, the reason for the high turnover in both HLM and CYP1A1 observed for this compound.

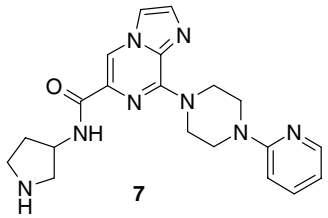


IgE IC_{50} 39 nM / HLM 3min / CYP1A1 13min / MW 381 / logD 3.7 / TA100–ve +/- activation

With the acid precursor **12** in hand a range of more polar amides were prepared. Incorporation of a basic centre in the amide at C-5 and replacement of the pyrimidine ring with a pyridine ring resulted in identification of compounds such as **6** and **7**, which show reduced log*D* and concomitant increased metabolic stability in both HLM and CYP1A. A number of close analogues were prepared, maintaining c log $P < 2.5$.

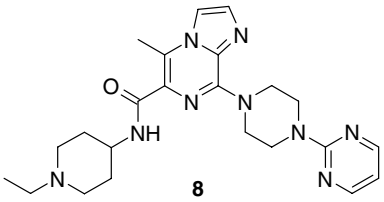


IgE IC_{50} 37 nM / HLM >120min / CYP1A1 45min / CYP1A2 95min / MW 421 / clogP 2.2



IgE IC_{50} 118 nM / HLM >120min / CYP1A2 >120min / MW 392 / clogP 1.7

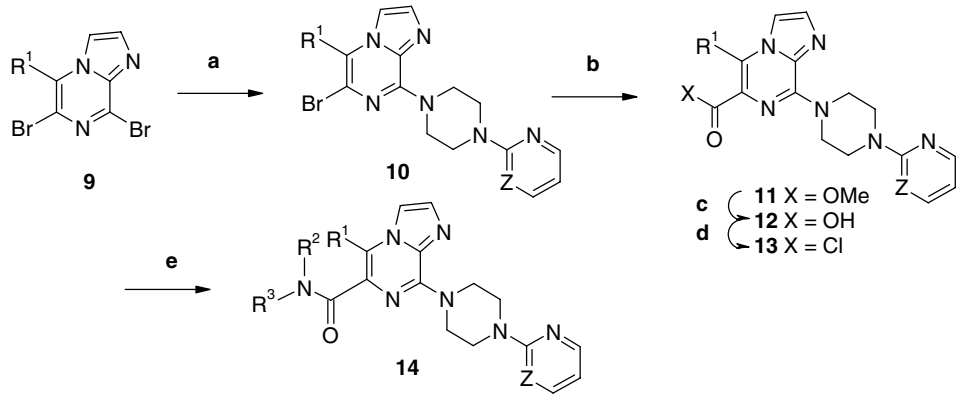
Introduction of a C-4 methyl in conjunction with an *N*-ethyl substituent on the piperidine gave compound **8**,[11] which showed excellent levels of potency and metabolic stability and thus met our initial goals in this programme.



IgE IC_{50} 49 nM / HLM > 120min / CYP1A1 > 120min / Caco - 2 37/25; MW 450 / logD 1.2

The imidazopyrazine amides **14** were prepared by reaction of suitably substituted piperazines with dibromo-imidazopyrazines **9**,[12] which resulted in selective substitution at C-7. Palladium-mediated carbonylation in the presence of methanol gave the required ester **11**, which was hydrolysed and converted to the acid chloride **13** under standard conditions. Reaction of the acid chloride with amines afforded the desired products **14** (Scheme 1).

In summary, we have described the optimization of a series of small molecule inhibitors of IgE synthesis,[13] focusing on potency, in vitro metabolism (particularly by CYP1A1 and CYP1A2) and absence of genetic toxicity.



Scheme 1. R_1 = H, Me; Z = N, C. Reagents and conditions: (a) 1-(2-pyrimidinyl)piperazine, NEt_3, EtOH, 78 °C, 3 h; (b) NEt_3, [1,1′-bis(diphenylphosphino)ferrocene] dichloropalladium(II), 100 psi CO, MeOH, 100 °C, 18 h; (c) 1 N LiOH, THF, rt, 4 h; (d) $(COCl)_2$, DMF, CH_2Cl_2, 2 h; (e) R^2R^3NH, NEt_3, CH_2Cl_2, rt, 18 h.

Acknowledgments

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References and notes

1. Sears, M. R.; Burrows, B.; Flannery, E. M.; Herbison, G. P.; Hewitt, C. J.; Holdaway, M. D. *N. Eng. J. Med.* **1991**, *325*, 1067; Burrows, B.; Martinez, F. D.; Halonen, M.; Barbee, R. A.; Cline, M. G. *N. Eng. J. Med.* **1989**, *320*, 271; Barbee, R. A.; Halonen, M. *Chest* **1991**, *99*, 20; Lane, S. J.; Kemeny, D. M. *Clin. Exp. Allergy* **1994**, *24*, 1001.
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4. Representative examples include: Berger, M.; Albrecht, B.; Berces, A.; Ettmayer, P.; Neruda, W.; Woisetschlaeger, M. *J. Med. Chem.* **2001**, *44*, 3031; Hasegawa, M.; Takenouchi, K.; Takahashi, K.; Takeuchi, T.; Komoriya, K.; Uejima, Y.; Kamimura, T. *J. Med. Chem.* **1997**, *40*, 395.
5. Compound potency (IgE IC_{50}) was determined in isolated peripheral blood lymphocytes (PBL). The cells were incubated with various concentrations of each compound in the presence of interleukin-4 (IL-4) for 12 days. At the end of the incubation period, supernatants were recovered and IgE level was determined using an IgE ELISA. IC_{50} values were calculated and data normalized to an internal standard to take into account donor to donor variability. The cytotoxicity of compounds was also evaluated and shown to be unrelated to efficacy. For selected compounds, compound concentration was evaluated during the course of the experiment and was shown to remain unchanged.
6. Compounds (1 μM) were incubated at 37 °C with human liver microsomes (0.5 μM) for 60 min. Concentration of compound was measured over the time course of the experiment and used to determine a disappearance half-life.
7. (a) Compounds (1 μM) were incubated at 37 °C with recombinant CYP1A1 or CYP1A2 (0.15 μM) for 60 min. Concentration of compound was measured over the time course of the experiment and used to determine a disappearance half-life; CYP1A1 is predominantly expressed in extra-hepatic tissues. See: (b) Smith, G.; Stubbins, M. J.; Harries, L. W.; Wolf, C. R. *Xenobiotica* **1998**, *28*, 1129.
8. Ames, B. N.; McCann, J.; Yamasaki, E. *Mutat. Res.* **1975**, *31*, 347; Ames, D. N.; Maron, D. M. *Mutat. Res.* **1983**, *113*, 173.
9. For CYP1A enzymes see, for example: (a) Lewis, D. F. V. *Current Med. Chem.* **2003**, *10*, 1955; (b) Benigni, R. *Chem. Rev.* **2005**, *105*, 1767.
10. It is likely that the reduced lipophilicity of these compounds also contributes to their increased metabolic stability.
11. ^{1}H NMR (400 MHz, CD_3OD) δ 1.39 (3H, t), 2.04–2.15 (2H, m), 2.24–2.27 (2H, m), 2.99 (3H, s), 3.09–3.15 (2H, m), 3.22 (2H, q), 3.66–3.69 (2H, m), 4.14–4.25 (4H, m), 7.04 (1H, t), 8.00 (1H, d), 8.24 (1H, d), 8.65 (1H, d), N.B. 1H obscured by solvent (NHC*H*) LRMS (electrospray): 460 $[M+H]^+$ Microanalysis: Found C, 52.23; H, 6.84; N, 22.59. $C_{24}H_{33}N_9O{\cdot}2HCl{\cdot}0.9H_2O$ requires C, 52.15; H, 6.71; N, 22.81%.
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13. Despite extensive experimentation we were unable to ascertain the specific mechanism of action for these compounds.