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Synthesis and pharmacological investigation of azaphthalazinone human histamine H_1 receptor antagonists

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ABSTRACT

5-Aza, 6-aza, 7-aza and 8-aza-phthalazinone, and 5,8-diazaphthalazinone templates were synthesised by stereoselective routes starting from the appropriate pyridine/pyrazine dicarboxylic acids by activation with CDI, reaction with 4-chlorophenyl acetate ester enolate to give a β–ketoester, which was hydrolysed, and decarboxylated. The resulting ketone was condensed with hydrazine to form the azaphthalazinone core. The azaphthalazinone cores were alkylated with *N*-Boc-D-prolinol at *N*-2 by Mitsunobu reaction, de-protected, and then alkylated at the pyrrolidine nitrogen to provide the target H_1 receptor antagonists. All four mono-azaphthalazinone series had higher affinity (pK_i) for the human H_1 receptor than azelastine, but were not as potent as the parent non-aza phthalazinone. The 5,8-diazaphthalazinone was equipotent with azelastine. The least potent series were the 7-azaphthalazinones, whereas the 5-azaphthalazinones were the most lipophilic. The more hydrophilic series were the 8-aza series. Replacement of the *N*-methyl substituent on the pyrrolidine with the *n*-butyl group caused an increase in potency (pA_2) and a corresponding increase in lipophilicity. Introduction of a β-ether oxygen in the *n*-butyl analogues (2-methoxyethyl group) decreased the H_1 pA_2 slightly, and increased the selectivity against hERG. The duration of action in vitro was longer in the 6-azaphthalazinone series. The more potent and selective 6-azaphthalazinone core was used to append an H_3 receptor antagonist fragment, and to convert the series into the long acting single-ligand, dual H_1 H_3 receptor antagonist **44**. The pharmacological profile of **44** was very similar to our intranasal clinical candidate **1**.

1. Introduction

Allergic rhinitis, also known as 'hay fever' affects between 10% and 25% of the world's population and has shown a steady increase in prevalence during the last 40 years.[1] The prevalence of allergic rhinitis may be significantly underestimated because of misdiagnosis, underdiagnosis and failure of patients to seek medical attention. There are two types of allergic rhinitis, seasonal and perennial. The symptoms of seasonal allergic rhinitis include at the early stage nasal itching, irritation and sneezing, and at the late stage rhinorrhoea and nasal congestion. The symptoms of perennial allergic rhinitis are similar, however, nasal congestion may be more pronounced. Either type of allergic rhinitis may also cause other symptoms such as irritation of the throat and/or eyes, epiphora and oedema around the eyes.[2] In addition to the classical symptoms it is now recognised that allergic rhinitis has a significant impact on quality of life, such as social life, sleep disturbance as a result of nasal congestion, which in turn leads to reduced performance at work and school.[3]

Allergic rhinitis and other allergic conditions are associated with the release of histamine from various cell types, but particularly mast cells. The physiological effects of histamine are mediated by four major G-protein-coupled receptors, termed H_1, H_2, H_3, and H_4, which differ in their expression, signal transduction and histamine-binding characteristics.[4] H_1 receptors are widely distributed throughout the CNS and periphery, and play a critical role in regulating inflammatory responses and CNS activity, such as wakefulness. H_1 antagonists, also known as H_1 blockers or antihistamines, are the most commonly used first-line medication for allergic rhinitis.[5,6] First generation H_1 receptor antagonists were introduced 70 years ago and although they were effective, caused sedation attributed to their ability to blockade H_1 receptors in the CNS. Second generation H_1 receptor antagonists were developed with reduced CNS penetration and a corresponding reduction in the sedative side-effects. Oral second generation H_1 receptor antagonists such as cetirizine, desloratadine, fexofenadine, loratadine and levocetirizine (Chart 1)[7] are effective in treating all the

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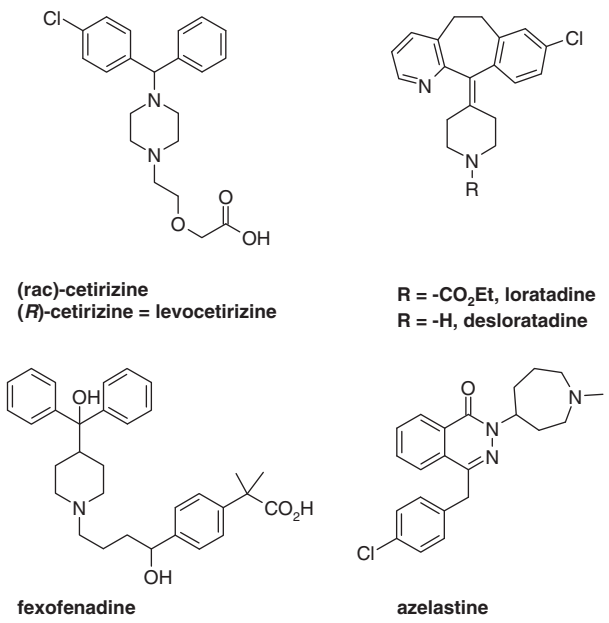


Chart 1. Representative H_1 receptor antagonists used clinically.

symptoms of allergic rhinitis, apart from the nasal congestion. Hence they are often used in combination with α-adrenergic agonist decongestants such as pseudoephedrine. However, the use of α-adrenergic agonists is limited due to their potential to produce hypertension, agitation and insomnia. In addition to the oral antihistamines there are topical treatments available, such as azelastine nasal spray, which has been shown to benefit patients who have not responded adequately to loratadine and fexofenadine, and is significantly more efficacious than cetirizine and levocabastine in patients with seasonal allergic rhinitis.[8,9]

Azelastine (Chart 1), which was originally developed for oral administration, is marketed as a racemic mixture. It is about ten-times more potent than the first-generation H_1 antagonist chlorpheniramine[10] and is oxidatively metabolised to an active metabolite, desmethylazelastine. Adverse effects of azelastine nasal spray include bitter taste (20%), somnolence (11%) and nasal burning (4%).[8,10] Azelastine was resolved and the potency of its enantiomers was reported to be the same as that of the racemic mixture,[11] however, there are no reports associating the adverse effects of azelastine with one of its enantiomers. The expanding role of H_1 antihistamines has recently been reviewed.[12] We have recently published our efforts in optimising phthalazinone-based histamine H_1 receptor antagonists and on the discovery of H_1 and H_3 receptor single-ligand antagonists, culminating in the identification of **1** as a development candidate for the treatment of allergic rhinitis suitable for intranasal administration (Chart 2).[13] Compound **2**, our optimised histamine H_1 receptor antagonist had a measured log*D* of 3.0, which was higher than azelastine (log*D* 2.3), we were therefore interested in analogues of **2** with a lower log*D* in order to improve its selectivity profile. In the present paper we report the synthesis and pharmacological profile of the regioisomeric 5-aza, 6-aza, 7-aza, and 8-aza-phthalazinone analogues of **2**, together with the 5,8-diazaphthalazinone analogue, and the combination of the most potent and selective 6-aza core with an H_3 antagonist fragment to give a novel H_1 H_3 dual antagonist.

2. Chemistry

The preparation of analogues containing a nitrogen atom at the 6- or 7-position of phthalazinone **2** was initially achieved using the non-regiospecific route outlined in Scheme 1, which is analogous to the route used for the preparation of **2**.

This route involved reaction of 3,4-pyridinedicarboxylic anhydride (**3**) with 4-chlorophenylacetic acid (**4**) to give a 7:1 mixture of regioisomeric lactones **5** and **6** in 20% yield. The major isomer's structure was confirmed by NOE and shown to be **5**. Irradiation of the olefinic proton at 7.18 ppm (1H, s) produced a positive NOE to the aromatic doublet at 7.85 ppm (2H, d, *J* 8.5 Hz) and to the pyridine 5-H at 8.10 ppm (1H, dd, *J* 5.5 and 1 Hz) (Fig. 1). The mixture of regioisomeric lactones **5** and **6** was treated with hydrazine and heated in ethanol to 90 °C for 1 h. The resulting regioisomeric mixture of phthalazinones **7** and **8** was separated by column chromatography on silica gel. The major product **7** was obtained in 36% yield, and the minor isomer **8** in 2%. The phthalazinone **7** was alkylated with *N*-Boc-D-prolinol by Mitsunobu coupling to give **9** in 43% yield, and then de-protected by treatment with 4 M hydrogen chloride in 1,4-dioxane to give the secondary amine **10** in 54% yield. The latter compound was converted to the tertiary amine **11** using Eschweiler-Clarke alkylation in 62% yield. In a similar manner the regioisomeric phthalazinone **8** was converted to the tertiary amine **14**, via **12** and **13**. The preparation of phthalazinone **8** as described in Scheme 1 provided sufficient material for initial biological screening, however, compound **8** was obtained as a minor component in a mixture, which required chromatographic separation, and was isolated in very low yield. For this reason an alternative, regioselective route to phthalazinone **8** was investigated, and is outlined in Scheme 2. 3,4-Pyridinedicarboxylic anhydride (**3**) was regioselectively converted to the 4-monomethyl ester **15** according to the method of Joule.[14] The carboxylic acid of **15** was activated by treatment with carbonyl di-imidazole (CDI), treated in situ with sodium hydride, followed by *tert*-butyl 4-chlorophenylacetate to give the β-ketoester **16** in 75%, as a mixture of keto-enol tautomers. The *tert*-butyl ester was cleaved, after treatment with trifluoroacetic acid, and the resulting acid spontaneously decarboxylated, to give the ketone **17** in 73% yield. The synthesis of **8** was completed by reaction of keto-ester **17** with hydrazine in refluxing ethanol and acetic acid to give the required phthalazinone **8**, free of its regioisomer **7**, in 78% yield.

In addition to the *N*-methyl analogues **14** and **2**, the *N*-butyl analogues **18a** and **19a** were prepared by reductive alkylation of the parent secondary amines **13** and **20** with *n*-butyraldehyde and sodium triacetoxyborohydride (Scheme 3). Furthermore the *N*-(2-methoxyethyl) analogues **18b** and **19b** were prepared by

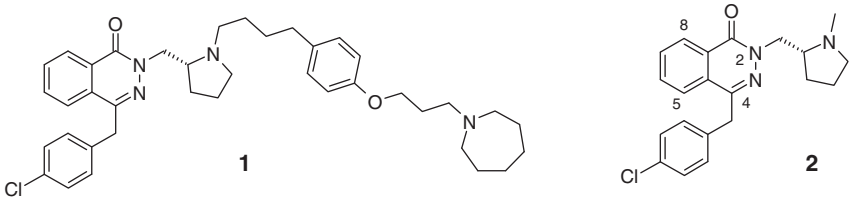
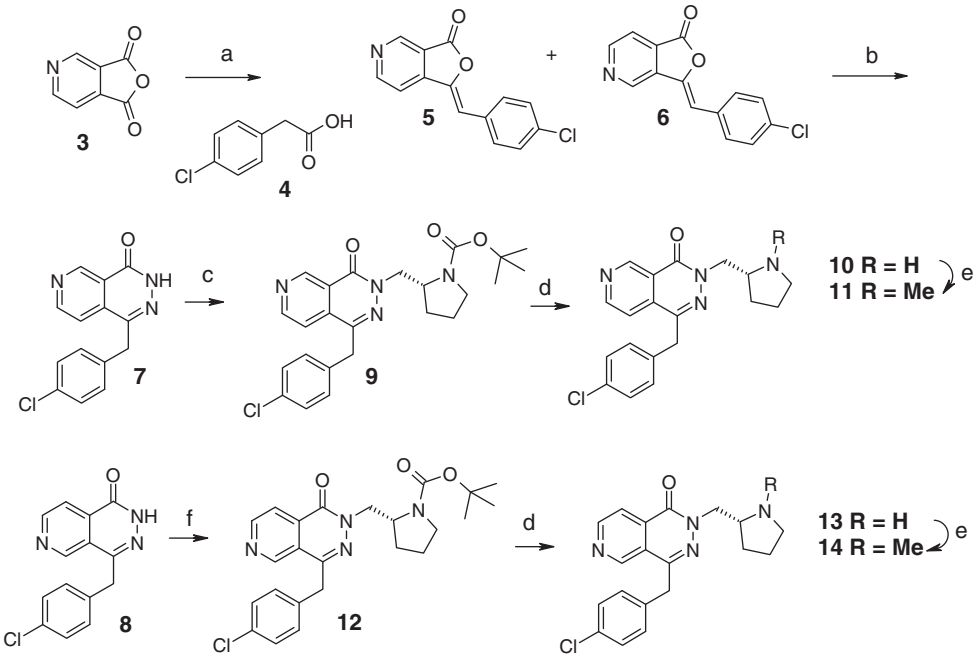


Chart 2. GSK's intranasal histamine H_1H_3 dual receptor antagonist, and phthalazinone H_1 receptor antagonist template.



Scheme 1. Reagents and conditions: (a) NaOAc, AcOH, 230 °C, 20%; (b) $NH_2NH_2{\cdot}H_2SO_4$, NaOH, EtOH, 90 °C; (c) *N*-Boc-D-prolinol, PPh_3, *i*-PrO_2CNNCO_2Pr-*i*, THF, −15 to 20 °C, 43%; (d) 4 M HCl–dioxane; (e) HCHO, HCO_2H, 100 °C; (f) *N*-Boc-D-prolinol, PPh_3, *tert*-BuO_2CNNCO_2Bu-*tert*, THF, −10 to 20 °C, 100%.

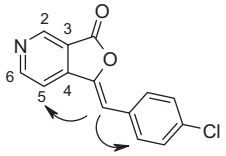
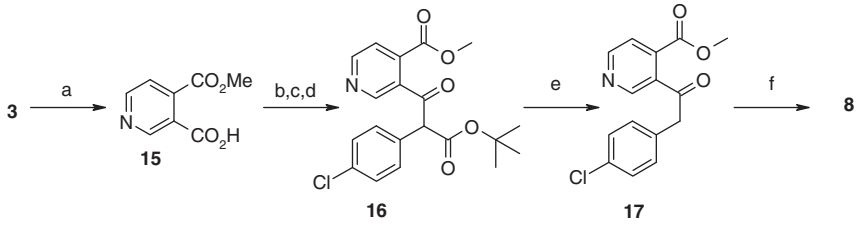


Figure 1. Key NOESY (H ↔ H) confirming the structure of regioisomer **5**.

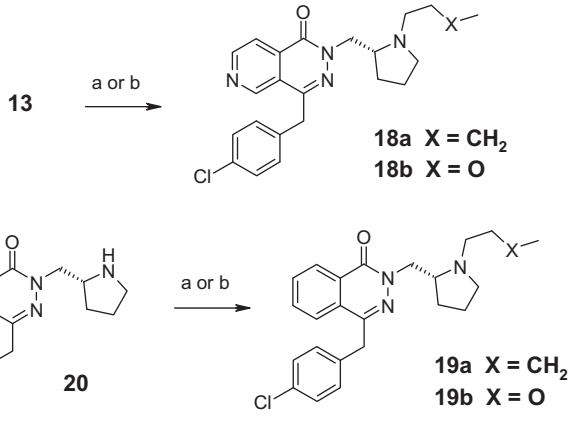
alkylation of **13** and **20** with 2-methoxyethyl bromide in the presence of potassium carbonate in DMF in a microwave oven at 150 °C.

The 5-aza and 8-aza regioisomers were prepared in a similar way to the 6-aza and 7-aza analogues according to Scheme 4. 2,3-Dicarboxylic anhydride (**21**) was converted to the 2-mono-ethyl ester **22**,[15] its structure was confirmed by HMBC correlations (Fig. 2), the free 3-carboxylic acid was activated with CDI and then treated in situ with the enolate of methyl 4-chlorophenyl acetate in DMF to give a mixture of β-keto-ester **23** and the carboxylic acid **24**. Both compounds were obtained as mixtures of keto–enol tautomers containing in addition a small amount of the respective regioisomer arising from addition of the enolate to the ethyl ester instead of the imidazolide. The keto-ester **23** was hydrolysed in aqueous 5 M HCl at 90 °C and spontaneously decarboxylated to give the corresponding ketone **25**. The latter was treated with hydrazine to give phthalazinone **26**. Its structure was confirmed by NOE from the benzylic CH_2 at 4.31 ppm to the 5-*H* of the 8-azaphthalazinone ring at 8.35 ppm, and to the aromatic protons of the chlorophenyl ring at 7.34 ppm (Fig. 3). Phthalazinone **26** was alkylated with *N*-Boc-D-prolinol by Mitsunobu coupling, and then de-protected using TFA to give **27**. Eschweiler-Clarke methylation of **27** gave the methyl analogue **28**, whereas reductive alkylation with butyraldehyde gave the *n*-butyl analogue **29a**. Reaction of **27** with 2-methoxyethyl bromide provided the ether analogue **29b**. The structure of **28** was confirmed by HMBC correlations and shown in Figure 2. The 5-aza analogues were prepared from **24** in a similar manner to give the methyl analogue **32**. The *n*-butyl analogue **33a** was obtained by alkylation of **31** with *n*-butyl bromide, and the ether analogue **33b** from 2-methoxyethyl bromide. The structure of phthalazinone **32** was confirmed by HMBC correlations between 8-*H* of the 5-azaphthalazinone ring to the carbonyl (Fig. 2).

The synthesis of the 5,8-diaza analogues is outlined in Scheme 5. Pyrazinedicarboxylic anhydride (**34**) was treated with methanol to give the mono-methyl ester **35**,[16] and the resulting monocarboxylic acid was activated with CDI and treated with methyl 4-chlorophenylacetate in the presence of sodium hydride to give a 3:1 mixture of the ester **36** and the corresponding carboxylic acid **37**. The mixture of **36** and **37** was hydrolysed and decarboxylated with HCl to give the ketone **38**, which was treated with hydrazine



Scheme 2. Reagents and conditions: (a) NaOMe, MeOH, THF, −70 °C, 45%; (b) CDI, DMF; (c) NaH; (d) *tert*-butyl 4-chlorophenylacetate, 75%; (e) TFA, DCM, 73%; (f) $NH_2NH_2{\cdot}H_2O$, EtOH, AcOH, reflux, 78%.



Scheme 3. Reagents and conditions: (a) *n*-PrCHO, DCM, AcOH, $NaBH(OAc)_3$; (b) 2-$MeOCH_2CH_2Br$, K_2CO_3, DMF, microwave irradiation, 150 °C.

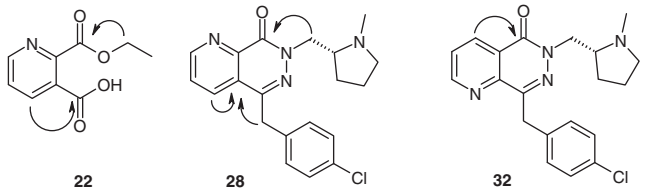
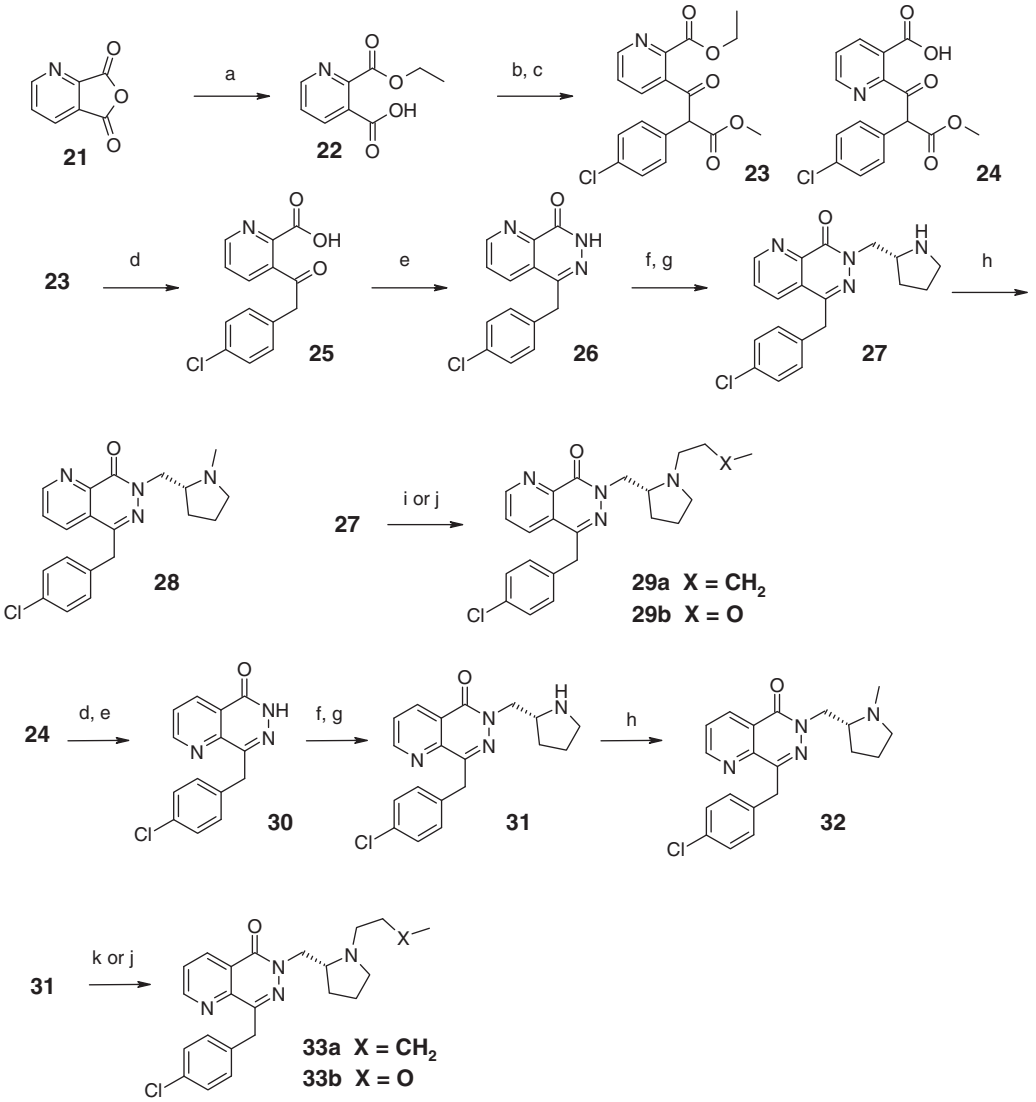


Figure 2. Key HMBC (H → C) correlations confirming the structure of compounds **22**, **28** and **32**.

to provide the phthalazinone **39**. Mitsunobu coupling, followed by TFA cleavage of the resulting *N*-Boc-protected amine **40** gave the secondary amine **41**, which was methylated by the Eschweiler-Clarke procedure to provide the target tertiary amine **42**.

Finally the 6-aza analogue of the dual H_1 H_3 receptor antagonist **1** was prepared by alkylation of **13** with methanesulfonate **43**[13] in acetonitrile in the presence of sodium bicarbonate to give **44** (Scheme 6).



Scheme 4. Reagents and conditions: (a) EtOH, 53%; (b) CDI, DMF, 50 °C; (c) NaH, methyl 4-chlorophenylacetate, 5 °C; (d) 5 M HCl, 90 °C; (e) $NH_2NH_2{\cdot}H_2SO_4$, NaOH, EtOH, 90 °C; (f) *N*-Boc-D-prolinol, PPh_3, *tert*-BuO_2CNNCO_2Bu-*tert*, 20 °C; (g) TFA, DCM, 20 °C; (h) HCHO, HCO_2H, 95 °C; (i) *n*-PrCHO, $NaBH(OAc)_3$, 20 °C; (j) 2-$MeOCH_2CH_2Br$, K_2CO_3, DMF, 150 °C; (k) *n*-BuBr, K_2CO_3, DMF, 100 °C, microwave, 52%.

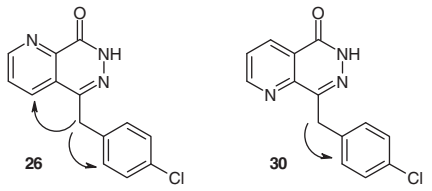
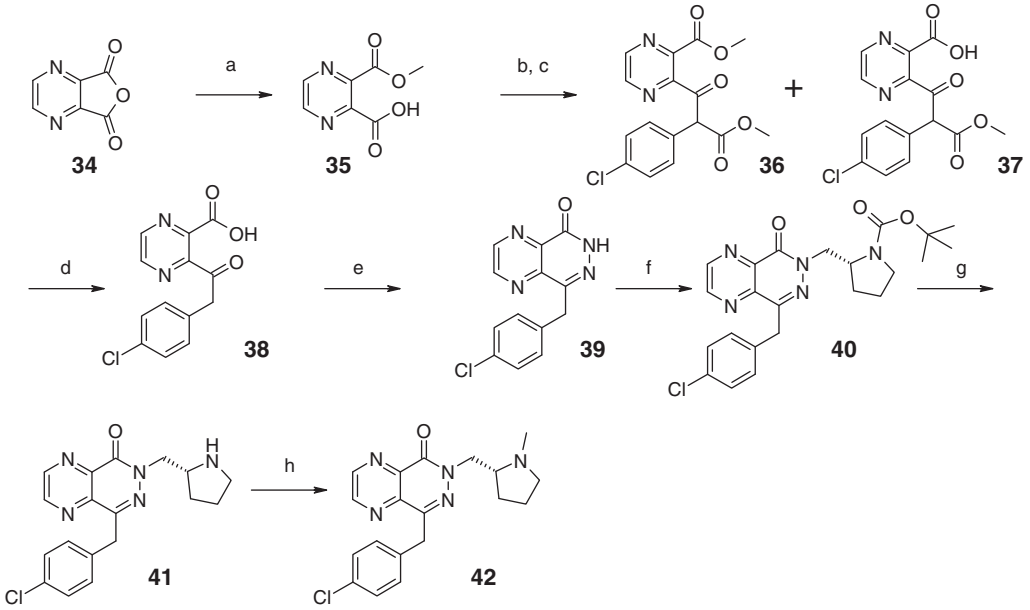


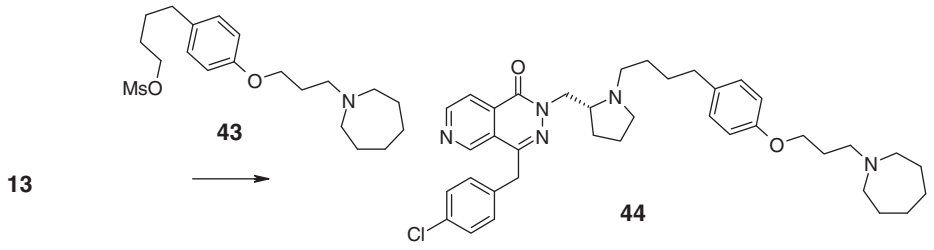
Figure 3. Key NOESY (H ↔ H) confirming structures of phthalazinones **26** and **30**.

3. Results and discussion

The H_1 receptor affinity of compounds was evaluated in vitro using recombinant human histamine H_1 receptor in intact CHO cells by means of plate-based calcium imaging. Inhibition of agonist-induced cellular calcium mobilisation was monitored using the calcium sensing dye Fluo-4AM in a FLIPR instrument.[17] The compounds were also evaluated in membranes from CHO cells transfected with cloned human histamine H_3 receptors for their ability to reduce histamine stimulated GTP-γ-S binding as determined by Scintillation Proximity detection. The adrenergic α_{1A} and α_{1B} receptor affinity of the test compounds was assessed in intact Ratl fibroblast cells by means of plate-based calcium imaging. The hERG activity was measured in ^{3}H-dofetilide radioligand binding assay, and the data from all the above screens are summarised in Table 1. Azelastine and the phthalazinone **2** were used as reference compounds with affinities (pK_i) for H_1 of 8.9 and 9.8, respectively. The 5,8-diaza analogue **42** was equipotent with azelastine (pK_i 8.9), but more hydrophilic ($\log D_{7.4}$ 1.7, azelastine $\log D_{7.4}$ 2.3). Furthermore **42** was more selective than azelastine over the human H_3 receptor, and the adrenergic receptors α_{1A} and α_{1B}. Agonists of the latter receptors act on smooth muscle causing constriction. The 5-, 6-, 7- and 8-aza analogues **32**, **14**, **11** and **28**, respectively were more potent than azelastine, and less lipophilic than the parent phthalazinone analogue **2**. Analogues **14**, **28** and **32** had similar H_1 receptor antagonist activity and were more potent than **11**, however, all four *N*-methyl analogues were less potent than **2**. Similar findings were observed with the *N*-butyl analogues of the azaphthalazinones, the 6-aza analogue **18a** being slightly more potent than the other *N*-butyl analogues. The $\log D$ of the azaphthalazinones varied with the position of the nitrogen atom in the aromatic ring, the more lipophilic being the 5-aza analogue **32** ($\log D_{7.4}$ 2.7), whereas the 8-aza analogue **28** was the least lipophilic ($\log D_{7.4}$ 1.7). The *N*-butyl analogues displayed a similar trend in $\log D$, the 5-aza analogue **33a** having the highest value (3.4) and the 8-aza analogue **29a** the lowest (2.8). All of the above azaphthalazinone analogues were more selective than azelastine towards the H_3 receptor. Azaphthalazinones were slightly less selective than **2** towards the H_3 receptor, however, they were still between 2.7 and 3.2 log units more selective for H_1 over H_3 receptor, and at least 10-fold more selective against other receptors, such as the adrenergic α_{1A} and α_{1B} receptors. Azelastine and **2** exhibited high affinity for the hERG channel in the defetilide binding assay (7.0 and 6.7, respectively). In the *N*-methyl series the



Scheme 5. Reagents and conditions: (a) MeOH, 20 °C, 75%; (b) CDI, DMF, 61 °C; (c) NaH, methyl 4-chlorophenylacetate, 5 °C, 99%; (d) 5 M HCl, H_2O, dioxane, 100 °C; (e) $NH_2NH_2{\cdot}H_2O$, NaOH, 95 °C, 99%; (f) *N*-Boc-D-prolinol, *tert*-BuO_2CNNCO_2Bu-*tert*, PPh_3, THF, 20 °C; (g) TFA, DCM, 20 °C; (h) HCHO, HCO_2H, 95 °C, 47%.



Scheme 6. Reagents and conditions: $NaHCO_3$, MeCN, 80 °C, 6 days, 19%.

Table 1
Antagonist affinity[a] of target compounds at the human H_1 receptor (determined by fluorescence imaging plate reader), human H_3 receptor (determined by a functional GTPγ[S]-assay), affinity at the human α_{1A} and α_{1B} receptors (determined in intact fibroblast cells by means of plate-based calcium imaging), measured log*D* at pH 7.4, and dofetilide hERG binding affinity.

Compound	H_1 pK_i (n)	H_3 pK_i (n)	α_{1A} pK_i (n)	α_{1B} pK_i (n)	Log$D_{7.4}$	hERG pIC_{50}
Azelastine	8.92 ± 0.02 (364)	6.83 ± 0.05 (56)	7.3 ± 0.0 (145)	7.3 ± 0.0 (97)	2.3	7.0
2	9.79 ± 0.08 (10)	6.36 ± 0.02 (6)	7.5 ± 0.3 (14)	8.0 ± 0.3 (12)	3.0	6.7
19a	9.04 ± 0.05 (4)	6.36 ± 0.00 (4)	7.8 ± 0.1 (4)	7.8 ± 0.1 (4)	3.1	7.8
19b	9.29 ± 0.07 (6)	6.4 ± 0.1 (6)	7.7 ± 0.3 (3)	7.9 ± 0.1 (3)	3.3	6.6
11	9.16 ± 0.07 (6)	6.4 ± 0.1 (6)	7.7 ± 0.1 (4)	6.9 ± 0.2 (4)	2.2	5.9
14	9.46 ± 0.08 (13)	6.4 ± 0.0 (11)	7.9 ± 0.2 (9)	7.6 ± 0.2 (8)	1.9	5.6
18a	9.40 ± 0.09 (16)	6.2 ± 0.0 (12)	8.2 ±0.1 (8)	8.1 ± 0.1 (8)	3.2	7.3
18b	9.60 ± 0.05 (8)	6.2 ± 0.0 (7)	7.9 ± 0.1 (5)	8.0 ± 0.1 (3)	2.7	5.9
28	9.45 ± 0.06 (6)	6.6 ± 0.1 (6)	6.2 ± 0.0 (3)	6.8 ± 0.2 (3)	1.7	5.6
29a	9.2 ± 0.1 (4)	6.2 ± 0.0 (4)	7.0 ± 0.3 (2)	7.0 ± 0.3 (2)	2.8	7.1
29b	9.0 ± 0.1 (18)	6.2 ± 0.0 (6)	6.5 ± 0.1 (3)	6.5 ± 0.2 (3)	2.1	5.3
32	9.5 ± 0.1 (6)	6.5 ± 0.1 (5)	6.7 ± 0.1 (3)	7.3 ± 0.1 (4)	2.7	6.8
33a	9.2 ± 0.1 (15)	6.2 ± 0.1 (13)	7.3 ± 0.1 (8)	7.7 ± 0.1 (9)	3.4	8.4
33b	9.27 ± 0.08 (4)	6.2 ± 0.0 (4)	6.8 ± 0.1 (2)	7.4 ± 0.1 (2)	2.9	7.2
42	8.89 ± 0.06 (4)	6.2 ± 0.0 (4)	6.4 ± 0.0 (2)	6.6 ± 0.2 (2)	1.7	5.9
44	7.76 ± 0.05 (9)	9.25 ± 0.04 (7)	7.5 ± 0.2 (3)	7.4 ± 0.1 (3)	—	6.8
1	8.0 ± 0.1 (36)	9.6 ± 0.0 (33)	7.4 ± 0.0 (17)	7.5 ± 0.1 (14)	3.2	7.3

[a] Shows mean ± SEM (where applicable) of estimated functional pK_i for n <3 the SEM is the SD. n = number of experiments

regioisomers with the lowest hERG affinity were the 6-azaphthalazinone **14** and 8-aza analogue **28**. A similar trend was observed in the *N*-butyl series, where the 5-azaphthalazinone **33a** had the highest affinity for hERG, whereas the 6-aza (**18a**) and 8-azaphthalazinone (**29a**) had the lowest hERG affinity. One way of reducing further hERG liability is to reduce the basicity of the amino group by introducing a β-oxygen. Compounds **18b**, **19b**, **29b** and **33b** were found to have significantly reduced hERG affinity by between 1.2 and 1.9 log units. The series with the lowest hERG liability were again the 6-aza and 8-aza regioisomers **18b** and **29b**.

In addition to the assays reported above, a more precise, lower throughput, modified version of the human H_1 FLIPR assay was run, which provided apparent pA_2 values. Antagonist pA_2 values were determined by generating histamine concentration–response curves either in the absence or presence of a single concentration of antagonist (100 nM) at 30 min incubation. The histamine concentration–response curves were analysed using a four-parameter logistic equation to determine the mid-point (EC_{50}) of the curve. Antagonist pA_2 values were calculated using the equation $pA_2 = \log(DR - 1) - \log[B]$ where DR, the dose ratio, is the EC_{50} in the presence of antagonist divided by the EC_{50} for the control curve, and [B] is the molar concentration of the antagonist tested. The data for azaphthalazinones are summarised in Table 2 and compared to azelastine (pA_2 9.7), and to the phthalazinones **2** and **19a** (pA_2 10.0 and 10.4, respectively. Duration of action in vitro was determined in the FLIPR assay by incubation of adherent CHO cells with antagonist for 30 min, followed by washing, and then by repeat histamine challenges at intervals of 90 and 270 min at 37 °C. Agonist dose ratios were converted to receptor occupancies, which were plotted against time. A measure of duration was obtained from the gradient of the percent receptor occupancy versus time plot. Results were statistically analysed and related to azelastine in the same assay, and expressed as slower, no-difference or faster wash-out than azelastine, with slower wash-out equating to longer duration of action. The 5- and 6-azaphthalazinone analogues were more potent than the 7- and 8-aza analogues. Furthermore the 5-azaphthalazinone **33a** and 6-aza **14** exhibited duration similar to that of azelastine, and were longer acting than the parent phthalazinone **2**. The 5-azaphthalazinone analogues were more lipophilic and had the highest liability for the hERG channel and were therefore rejected. In contrast the 6-aza series was the most potent, selective, and longer acting, and was chosen for appending the H_3 fragment used in the dual

Table 2
Antagonist pA_2 affinity at the human H_1 receptor, determined by fluorescence imaging plate reader, and in vitro duration

Compound	pA_2 ± SEM[a]	*n*	Wash-out
Azelastine	9.7 ± 0.1	19	Reference
2	10.0 ± 0.1	4	Faster
19a	10.4 ± 0.3	7	—
11	8.6 ± 0.4	2	—
14	9.5 ± 0.1	7	No difference
18a	10.0 ± 0.1	10	No difference
18b	9.6 ± 0.1	12	No difference
28	9.3 ± 0.1	8	—
29a	9.5 ± 0.3	2	—
29b	9.1 ± 0.1	6	Faster
32	9.5 ± 0.1	6	—
33	9.8 ± 0.1	8	No difference
42	8.4 ± 0.1	3	—
44	9.0 ± 0.1	17	Slower
1	9.1 ± 0.1	11	Slower

[a] All pA_2 values taken from curve shifts generated at 30 min incubation time and with 100 nM antagonist. Table 2 shows mean pA_2 ± SEM for n <3 the SEM is the SD. n = number of experiments.

pharmacology single-ligand antagonist **1** to convert the series into a dual H_1 H_3 receptor antagonist.[13] Compound **44** had a similar H_1 potency to **1** (Tables 1 and 2) and only slightly reduced pK_i for the H_3 receptor. The selectivity of **44** against the adrenergic α_{1A} and α_{1B} receptors was similar to **1,** however, the selectivity of **44** over hERG was better than **1**. Furthermore both **1** and **44** had longer duration of action than azelastine. Appendage of the H_3 fragment to other H_1 antagonists, such as **18b**, would be expected to provide additional single-ligand dual H_1 H_3 antagonists as potential back-up compounds to **1**. Further to our report that **1** was selected for further development,[13] the compound progressed to phase I and II safety and efficacy studies in seasonal allergic rhinitis. Single-dose intranasal suspensions of the napadisylate salt of **1** (0.22, 1.10 mg) failed to demonstrate clinically significant attenuation of symptoms of allergic rhinitis induced in an environmental allergen challenge chamber.[19] Three-day repeat dosing of a 1 mg solution of the dihydrochloride salt of **1** demonstrated a statistically significant attenuation of nasal symptoms, but this was less than cetirizine, which was used as a reference. Furthermore the dihydrochloride salt of **1** was poorly tolerated by 87% of the subjects who reported nasal discomfort. Since combined H_1 H_3 antagonism did not demonstrate sufficient differentiation from H_1 antagonism

in reducing nasal congestion, no further work was carried out in these series.

4. Conclusion

The regioselective synthesis and pharmacological profile of 5-aza, 6-aza, 7-aza, and 8-aza-phthalazinone analogues of 4-[(4-chlorophenyl)methyl]-2-{[(2*R*)-1-methyl-2-pyrrolidinyl]methyl}-1(2*H*)-phthalazinone, the optimised (*R*-enantiomer) histamine H_1 receptor antagonist **2**, together with the 5,8-diazaphthalazinone analogue are reported. All four mono-azaphthalazinone series had higher affinity for the human H_1 receptor than azelastine, but were not as potent as the parent phthalazinone antagonist **2**. The 5,8-diazaphthalazinone was equipotent with azelastine. The most potent H_1 antagonists were found to be the 5- and 6-aza series. Introduction of nitrogen in the phthalazinone core brought down the lipophilicity of the analogues with the most hydrophilic series being the 6- and 8-aza series, which were also more selective over hERG. Introduction of an ether β-oxygen brought the hERG affinity of the 6-aza and 8-aza analogues further down, especially so for the 8-aza series. The 6-azaphthalazinone core, which was found to be the more potent and selective template, was used to append an H_3 receptor antagonist fragment and to provide the long acting single-ligand dual H_1 H_3 receptor antagonist **44**. The profile of **44** was very similar to our intranasal development candidate **1** (GSK1004723), which unfortunately failed to demonstrate sufficient differentiation of H_3 over and above H_1 antagonism in reducing nasal congestion, and hence this series was not progressed.[13,18,19]

5. Experimental

5.1. Chemistry

Organic solutions were dried over anhydrous Na_2SO_4 or $MgSO_4$. TLC was performed on Merck 0.25 mm Kieselgel 60 F_{254} plates. Products were visualised under UV light and/or by staining with aqueous $KMnO_4$ solution. LCMS analysis was conducted on a Supelcosil LCABZ+PLUS column (3.3 cm × 4.6 mm id) eluting with 0.1% formic acid and 0.01 M ammonium acetate in water (solvent A), and 0.05% formic acid and 5% water in acetonitrile (solvent B), using the following elution gradient 0.0–0.7 min 0% B, 0.7–4.2 min 100% B, 4.2–5.3 min 0% B, 5.3–5.5 min 0% B at a flow rate of 3 mL min^{-1}. The mass spectra were recorded on a Fisons VG Platform spectrometer using electrospray positive and negative mode (ES+ve and ES−ve). Column chromatography was performed on Flashmaster II. The Flashmaster II is an automated multi-user flash chromatography system, available from Argonaut Technologies Ltd, which utilizes disposable, normal phase, SPE cartridges (2–100 g). Mass-directed auto-preparative HPLC (MDAP) was conducted on a Waters FractionLynx system comprising of a Waters 600 pump with extended pump heads, Waters 2700 auto-sampler, Waters 996 diode array and Gilson 202 fraction collector on a 10 cm × 2.54 cm id ABZ+ column, eluting with 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B), using an appropriate elution gradient over 15 min at a flow rate of 20 mL min^{-1} and detecting at 200–320 nm at room temperature. Mass spectra were recorded on Micromass ZMD mass spectrometer using electro spray positive and negative mode, alternate scans. The software used was *MassLynx* 3.5 with *OpenLynx* and *FractionLynx* options. ^{1}H NMR spectra were recorded at 400 MHz. The chemical shifts are expressed in ppm relative to tetramethylsilane. High resolution positive ion mass spectra were acquired on a Micromass Q-Tof 2 hybrid quadrupole time-of-flight mass spectrometer. The purity of all compounds screened in the biological assays was examined by LCMS analysis and was found to be ⩾95%, unless otherwise specified.

5.1.1. 11-[(4-Chlorophenyl)methyl]pyrido[3,4-*d*]pyridazin-4(3*H*)-one (7) and 4-[(4-chlorophenyl)methyl]pyrido[3,4-*d*]pyridazin-1(2*H*)-one (8)

A mixture of solid 4-chlorophenylacetic acid (20.9 g, 122 mmol) and 3,4-pyridinedicarboxylic anhydride (**3**) (18.3 g, 122 mmol) was treated with sodium acetate (400 mg, 4.8 mmol) and acetic acid (7 mL, 122 mmol). The mixture was heated to 230 °C in a Dean-Stark apparatus for 3.75 h. The mixture was allowed to cool to room temperature, and then partially purified by filtration on silica (250 g) eluting with ethyl acetate–cyclohexane, followed by ethyl acetate to give a 7:1 mixture of (1*Z*)-1-[(4-chlorophenyl)methylidene]furo[3,4-*c*]pyridin-3(1*H*)-one (**5**) and (3*Z*)-3-[(4-chlorophenyl)methylidene]furo[3,4-*c*]pyridin-1(3*H*)-one (**6**) (6.26 g, 20%): LCMS rt = 3.32 min, 89%, ES+ve *m/z* 258/260 $(M+H)^+$; ^{1}H NMR δ (DMSO-d_6) major isomer 9.21 (1H, s, pyridine 2-H), 8.98 (1H, d, *J* 5 Hz, pyridine 6-H), 8.10 (1H, d, *J* 5 Hz, pyridine 5-H), 7.85 (2H, d, *J* 8.5 Hz, aromatic 2-H and 6-H), 7.58 (2H, d, *J* 8.5 Hz, aromatic 3-H and 5-H), 7.18 (1H, s, olefinic). On irradiation of the olefinic proton at 7.18 a NOE was observed to the two aromatic protons doublet at 7.85 and to the 5-H doublet at 8.10, confirming that the major isomer was (1*Z*)-1-[(4-chlorophenyl)methylidene]furo[3,4-*c*]pyridin-3(1*H*)-one (**5**). The 7:1 mixture of regioisomers (5.87 g, 22.8 mmol) was dissolved in ethanol (50 mL) and treated with hydrazine sulfate (3.56 g, 27.3 mmol) and 1 M sodium hydroxide (54 mL). The mixture was heated to 90 °C for 1 h, allowed to cool to room temperature, and diluted with water (250 mL). The precipitated solid was collected by filtration, washed with water (50 mL), dissolved in acetone, and re-evaporated to give a red solid (4.46 g, 72%). A portion (3.2 g) was purified by chromatography on silica (750 g) eluting with a gradient of 10–100% ethyl acetate–dichloromethane, followed by 10% methanol–dichloromethane to give the minor isomer 4-[(4-chlorophenyl)methyl]pyrido[3,4-*d*]pyridazin-1(2*H*)-one (**8**) (100 mg, 2%): LCMS rt = 2.71 min, 90%, ES+ve *m/z* 272/274 $(M+H)^+$; ^{1}H NMR δ (DMSO-d_6) 12.90 (1H, s), 9.33 (1H, s), 8.95 (1H, d, *J* 5 Hz), 8.08 (1H, d, *J* 5 Hz), 7.37 (4H, s), 4.40 (2H, s), (contains 4% of the major isomer), and 1-[(4-chlorophenyl)methyl]pyrido[3,4-*d*]pyridazin-4(3*H*)-one (**7**) (2.22 g, 36%): LCMS rt = 2.73 min, 99%, ES+ve *m/z* 272/274 $(M+H)^+$; ^{1}H NMR δ (DMSO-d_6) 12.92 (1H, s), 9.45 (1H, s), 8.99 (1H, d, *J* 5.5 Hz), 7.81 (1H, d, *J* 5.5 Hz), 7.35 (4H, s), 4.30 (2H, s) (contains 6% of the minor isomer).

5.1.2. 1-[(4-Chlorophenyl)methyl]-3-[(2*R*)-2-pyrrolidinylmethyl]pyrido[3,4-*d*]pyridazin-4(3*H*)-one (10)

A solution of triphenylphosphine (1.57 g, 6.0 mmol) in anhydrous THF (10 mL) was cooled to −20 °C and di-isopropyl azodicarboxylate (1.0 mL, 5.1 mmol) was added. The resulting suspension was stirred at −15 °C for ten minutes and then treated with a suspension of *N*-BOC-D-prolinol (636 mg, 3.16 mmol) and 1-[(4-chlorophenyl)methyl]pyrido[3,4-*d*]pyridazin-4(3*H*)-one (**7**) (592 mg, 2.17 mmol) in THF (12 mL). The mixture was allowed to warm to 20 °C and stirred for 18 h. The reaction mixture was treated with methanol (10 mL) and solvent was removed under reduced pressure. The residue was purified by chromatography on a silica cartridge (100 g) on Flashmaster eluting with a gradient of 0–100% ethyl acetate–cyclohexane over 60 min to give the BOC-protected product **9** (625 mg, 43%): MS ES+ve *m/z* 455/457 $(M+H)^+$. The product (625 mg, 1.37 mmol) was dissolved in dioxane (5 mL), treated with 4 M hydrogen chloride in dioxane (10 mL) and stirred at 20 °C for 1 h. The suspension was concentrated under reduced pressure; the residue was dissolved in methanol and applied to a methanol pre-conditioned SCX-2 ion-exchange cartridge. The cartridge was washed with methanol and then eluted with 10%

aqueous ammonia in methanol. The ammoniacal fraction was concentrated under reduced pressure and the residue was purified by chromatography on a silica cartridge (100 g) on Flashmaster eluting with a gradient of 0–30% [(methanol containing 1% triethylamine) – dichloromethane] over 60 min to give **10** (413 mg, 54%): LCMS rt = 2.26 min, 92%, ES+ve *m/z* 355/357 $(M+H)^+$; ^1H NMR δ (DMSO-d_6) 9.50 (1H, s), 9.00 (1H, d, *J* 5.5 Hz), 7.81 (1H, d, *J* 5.5 Hz), 7.44 (2H, d, *J* 8 Hz), 7.36 (2H, d, *J* 8 Hz), 4.45 (2H, m), 4.36 (2H, m), 3.87 (1H, m), 3.31–3.20 (2H, m), 3.18–3.08 (1H, m), 2.18–2.06 (1H, m), 2.03–1.84 (2H, m), 1.81–1.69 (1H, m).

5.1.3. 1-[(4-Chlorophenyl)methyl]-3-{[(2*R*)-1-methyl-2-pyrrolidinyl]methyl}pyrido[3,4-*d*]pyridazin-4(3*H*)-one (11)

A solution of **10** (145 mg, 0.4 mmol) in formic acid (1.85 mL) was treated with formaline solution (37% w/v, 3.38 mL) and the mixture was heated to 100 °C for 4 h. The reaction mixture was cooled to room temperature and partitioned between dichloromethane (20 mL) and 2 M hydrochloric acid (20 mL). The phases were separated and the aqueous was washed with dichloromethane (20 mL). The aqueous phase was basified with 2 M sodium hydroxide (40 mL) and extracted with dichloromethane (20 mL). All the organic extracts were combined and concentrated under reduced pressure. The residue was purified by chromatography on a silica cartridge (100 g) on Flashmaster eluting with a gradient of 0–15% [(methanol containing 1% triethylamine)–dichloromethane] over 60 min to give **11** (92 mg, 62%) as a white solid: LCMS rt = 2.24 min, 94%, ES+ve *m/z* 369/371 $(M+H)^+$; ^1H NMR (CD_3OD) 9.54 (1H, s), 8.90 (1H, d, *J* 5.5 Hz), 7.78 (1H, d, *J* 5.5 Hz), 7.36–7.26 (4H, m), 4.43 (1H, dd, *J* 13, 4 Hz), 4.34 (2H, s), 4.16 (1H, dd, *J* 13, 8 Hz), 3.11–3.03 (1H, m), 2.81–2.72 (1H, m), 2.42 (3H, s), 2.34–2.23 (1H, m), 1.97–1.84 (1H, m), 1.85–1.68 (3H, m).

5.1.4. 4-[(Methyloxy)carbonyl]-3-pyridinecarboxylic acid (15)[14]

A suspension of 3,4-pyridinedicarboxylic anhydride (**3**) (26.7 g, 180 mmol) in dry tetrahydrofuran (250 mL) was treated at −70 °C under nitrogen with a suspension of sodium methoxide (11.2 g, 2.01 mol) in dry methanol (50 mL). The reaction mixture was allowed to warm to room temperature and stirred for 18 h. The solvents were removed under reduced pressure and the residue was dissolved in water (350 mL). This solution was acidified to pH 2 using concentrated hydrochloric acid. The resultant solid was collected by filtration, washed with water, and dried in vacuo at 45 °C to give **15** (14.6 g, 45%) as a white solid: LCMS rt = 0.98 min, ES+ve *m/z* 182 $(M+H)^+$; ^1H NMR δ (DMSO-d_6) 13.80 (1H, br), 9.02 (1H, s), 8.87 (1H, d, *J* 5 Hz), 7.63 (1H, d, *J* 5 Hz), 3.84 (3H, s).

5.1.5. Methyl 3-{2-(4-chlorophenyl)-3-[(1,1-dimethylethyl)oxy]-3-oxopropanoyl}-4-pyridinecarboxylate (16)

To a solution of **15** (1.81 g, 10 mmol) in dry dimethylformamide (90 mL) under nitrogen was added carbonyl diimidazole (1.7 g, 10.5 mmol). The reaction mixture was heated at 50 °C for 90 min and then cooled to −5 °C in an ice-salt bath. To this was added *tert*-butyl 4-chlorophenylacetate (2.38 g, 10.5 mmol), followed by portion wise addition of sodium hydride (60% dispersion in mineral oil, 1.4 g, 35 mmol) over 15 min. The reaction mixture was stirred at −5 °C for 10 min and then warmed to room temperature. After 2 h the reaction mixture was poured into a saturated solution of ammonium chloride (100 mL), and extracted with ethyl acetate (3 × 100 mL). The combined organic solutions were washed with water (2 × 100 mL) and brine (2 × 100 mL). The organic phase was dried ($MgSO_4$) and the solvent removed in vacuo. The residue was dissolved in dichloromethane (5 mL) and applied to a silica cartridge (100 g). This was eluted using a gradient of 0–50% ethyl acetate in cyclohexane over 60 min. The required fractions were evaporated in vacuo to give **16** (2.94 g, 75%, mixture of keto- and enol-forms) as a pale brown oil: LCMS rt = 3.41 min, 46% and 3.63 min, 48%, ES+ve *m/z* 390/392 $(M+H)^+$; ^1H NMR δ ($CDCl_3$) 13.47 (0.5H, s), 8.81 (0.5H, d, *J* 4.5 Hz), 8.71 (0.5H, s), 8.59 (0.5H, d, *J* 5 Hz), 8.29 (0.5H, s), 7.71 (0.5H, d, *J* 5 Hz), 7.59 (0.5H, d, *J* 5 Hz), 7.35 (2H, s), 7.27 (2H, s), 7.08 (1H, d, *J* 8 Hz), 7.00 (1H, d, *J* 8 Hz), 5.17 (0.5H, s), 3.96 (1.5H, s), 3.91 (1.5H, s), 1.50 (4.5H, s), 1.44 (4.5H, s).

5.1.6. Methyl 3-[(4-chlorophenyl)acetyl]-4-pyridinecarboxylate (17)

A solution of **16** (2.94 g, 7.5 mmol) in dry dichloromethane (12 mL) was treated with trifluoroacetic acid (5 mL) and the mixture was stirred at room temperature under nitrogen for 20 h. The solvent was removed under reduced pressure and the residue was dissolved in dichloromethane (5 mL). This was applied to a silica cartridge (100 g) and eluted with a gradient of 0–100% ethyl acetate in cyclohexane over 60 min. The required fractions were combined and evaporated under reduced pressure to give **17** (1.59 g, 73%) as a pale orange oil: MS ES+ve *m/z* 290/292 $(M+H)^+$; ^1H NMR δ ($CDCl_3$) 8.82 (1H, d, *J* 5 Hz), 8.66 (1H, s), 7.72 (1H, d, *J* 5 Hz), 7.32 (2H, d, *J* 8 Hz), 7.21 (2H, d, *J* 8 Hz), 4.15 (2H, s), 3.96 (3H, s)

5.1.7. 4-[(4-Chlorophenyl)methyl]pyrido[3,4-*d*]pyridazin-1(2*H*)-one (8)

A solution of **17** (1.59 g, 5.5 mmol) in ethanol (60 mL) was treated with hydrazine hydrate (0.3 mL, 6 mmol) and a few drops of acetic acid. The reaction mixture was heated at reflux for 3 h. The reaction mixture was allowed to cool, the solid was collected by filtration, washed with ethanol (10 mL), and dried in vacuo to give **8** (1.17 g, 78%) as a white solid: LCMS rt = 2.73 min, 98%, ES+ve *m/z* 272/274 $(M+H)^+$; ^1H NMR δ (DMSO-d_6) 12.90 (1H, s), 9.33 (1H, s), 8.95 (1H, d, *J* 5 Hz), 8.08 (1H, d, *J* 5 Hz), 7.37 (4H, s), 4.40 (2H, s).

5.1.8. 4-[(4-Chlorophenyl)methyl]-2-[(2*R*)-2-pyrrolidinylmethyl]pyrido[3,4-*d*]pyridazin-1(2*H*)-one (13)

A solution of triphenylphosphine (10.42 g, 40 mmol) in anhydrous tetrahydrofuran (80 mL) was treated with a solution of di-*tert*-butyl azodicarboxylate (8.38 g, 36 mmol) in anhydrous tetrahydrofuran (60 mL) at −10 °C. The solution was allowed to warm to 15 °C and then cooled to 0–5 °C. To the slight suspension was added a suspension of **8** (5.98 g, 22 mmol) and *N*-Boc-D-prolinol (5.14 g, 25.6 mmol) in anhydrous tetrahydrofuran (100 mL). The suspension was allowed to warm to ambient temperature and stirred for 23 h. The solvent was removed in vacuo to leave an oil (30 g): LCMS rt = 3.48 min, ES+ve *m/z* 455/457. The crude product (30 g) was dissolved in 1,4-dioxane (80 mL), treated with 4 M hydrogen chloride in 1,4-dioxane (80 mL, 320 mmol), and stirred at ambient temperature for 5 h. The solvent was removed under reduced pressure and the residue was partitioned between 1 M aqueous hydrochloric acid (400 mL) and ethyl acetate (200 mL). The phases were separated and the aqueous phase washed with ethyl acetate (200 mL). The combined organic extracts were washed with 1 M aqueous hydrochloric acid (200 mL). The combined aqueous extracts were basified to pH 10 using 2 M aqueous sodium hydroxide (350 mL) and the resulting suspension extracted with ethyl acetate (2 × 400 mL, 1 × 200 mL). The combined organic extracts were concentrated under reduced pressure to give **13** (7.83 g, 100%): LCMS rt = 2.15 min, 99%, ES+ve *m/z* 355/357 $(M+H)^+$; ^1H NMR δ (CD_3OD) 9.25 (1H, s), 8.91 (1H, d, *J* 5 Hz), 8.20 (1H, d, *J* 5 Hz), 7.38–7.28 (4H, m), 4.43 (2H, s), 4.33–4.21 (2H, m), 3.63–3.52 (1H, m), 3.04–2.94 (1H, m), 2.84–2.75 (1H, m), 1.98–1.72 (3H, m), 1.62–1.49 (1H, m).

5.1.9. 4-[(4-Chlorophenyl)methyl]-2-{[(2*R*)-1-methyl-2-pyrrolidinyl]methyl}pyrido[3,4-*d*]pyridazin-1(2*H*)-one (14)

A solution of **13** (39 mg, 0.11 mmol) in formic acid (0.5 mL) was treated with 37% formaldehyde solution in water and methanol (0.91 mL, 11 mmol) and the mixture was heated to 100 °C for 4.5 h. The reaction mixture was partitioned between DCM (10 mL) and 2 M HCl solution (10 mL). The aqueous phase was further extracted with DCM (10 mL), and then basified with 2 M NaOH solution and extracted with DCM (2 × 10 mL). All the organic extracts were combined and concentrated under reduced pressure. The residue was purified by column chromatography on a silica cartridge (20 g) on Flashmaster eluting with 0–50% MeOH in DCM over 30 min. Appropriate fractions were combined and evaporated under reduced pressure to give **14** (26 mg, 64%): LCMS rt = 2.25 min, 98%, ES+ve *m/z* 369/371 $(M+H)^+$; ^{1}H NMR (CD_3OD) 9.25 (1H, s), 8.91 (1H, d, *J* 5 Hz), 8.23–8.17 (1H, m), 7.37–7.26 (4H, m), 4.43 (1H, dd, *J* 13, 4 Hz), 4.43 (2H, s), 4.17 (1H, dd, *J* 13, 8 Hz), 3.07 (1H, ddd, *J* 9, 6, 3 Hz), 2.82–2.73 (1H, m), 2.43 (3H, s), 2.36–2.24 (1H, m), 1.97–1.86 (1H, m), 1.85–1.71 (3H, m).

5.1.10. 2-{[(2*R*)-1-Butyl-2-pyrrolidinyl]methyl}-4-[(4-chlorophenyl)methyl]pyrido[3,4-*d*]pyridazin-1(2*H*)-one formic acid salt (18a)

A solution of **13** (35 mg, 0.1 mmol) in DCM (0.9 mL) and acetic acid (0.1 mL) was treated with *n*-butyraldehyde (16 mg, 0.22 mmol) and the mixture was stirred for 30 min before it was treated with triacetoxyborohydride (45 mg, 0.21 mmol). The reaction mixture was stirred overnight at room temperature and then was acidified with 2 M hydrochloric acid, neutralised with sodium bicarbonate solution, and extracted with DCM. The organic solution was dried (hydrophobic frit) and evaporated under reduced pressure. The residue was dissolved in methanol and purified by MDAP to give **18a** (29 mg, 63%): MS ES+ve *m/z* 411/413 $(M+H)^+$; ^{1}H NMR δ (CD_3OD) 9.30 (1H, s), 8.96 (1H, d, *J* 5 Hz), 8.44 (1H, s), 8.23 (1H, d, *J* 5 Hz), 7.38–7.29 (4H, m), 4.63–4.50 (2H, m), 4.45 (2H, s), 3.75 (1H, s), 3.68–3.56 (1H, m), 3.45–3.33 (1H, m), 3.12–3.01 (1H, m), 3.10–2.89 (1H, m), 2.28–2.15 (1H, m), 2.10–2.02 (1H, m), 2.02–1.89 (2H, m), 1.69 (2H, quint, *J* 7.8 Hz), 1.46–1.32 (2H, m), 0.96 (3H, t, *J* 7.4 Hz).

5.1.11. 4-[(4-Chlorophenyl)methyl]-2-({(2*R*)-1-[2-(methyloxy)ethyl]-2-pyrrolidinyl}methyl)pyrido[3,4-*d*]pyridazin-1(2*H*)-one (18b)

A suspension of **13** (28 mg, 0.08 mmol) and potassium carbonate (22 mg, 0.16 mmol) in DMF (1 mL) was treated with 2-methoxyethyl bromide (22 mg, 0.16 mmol) and the mixture was heated to 150 °C for 15 min in a microwave oven. The reaction mixture was filtered and the filtrate was loaded onto a SCX-2 ion-exchange column (10 g) eluting with MeOH and then with a 10% aqueous ammonia solution in MeOH. The ammoniacal fractions were combined and concentrated under nitrogen in a blow-down unit. The residue was purified by MDAP to give **18b** (8.1 mg, 25%) as a gum: LCMS rt = 2.35 min, 87%, ES+ve *m/z* 413/415 $(M+H)^+$; ^{1}H NMR δ (CD_3OD) 9.25 (1H, s), 8.91 (1H, d, *J* 5 Hz), 8.20 (1H, d, *J* 5 Hz), 7.38–7.28 (4H, m), 4.43 (2H, s), 4.35 (1H, dd, *J* 13, 4 Hz), 4.12 (1H, dd, *J* 13, 8 Hz), 3.57–3.46 (2H, m), 3.30 (3H, s), 3.21–3.13 (1H, m), 3.09–2.98 (2H, m), 2.62–2.54 (1H, m), 2.39–2.30 (1H, m), 1.90–1.73 (4H, m).

5.1.12. 2-{[(2*R*)-1-Butyl-2-pyrrolidinyl]methyl}-4-[(4-chlorophenyl)methyl]-1(2*H*)-phthalazinone (19a)

Was prepared in a similar way to **18a** (57.6 mg, 70%): LCMS rt = 2.60 min, 100%, ES+ve *m/z* 410/412 $(M+H)^+$; ^{1}H NMR δ (CD_3OD) 8.46–8.38 (1H, m), 7.99 (1H, br d, *J* 7.5 Hz), 7.89 (2H, s), 7.36–7.26 (4H, m), 4.69 (1H, dd, *J* 14, 5 Hz), 4.60 (1H, dd, *J* 14, 5 Hz), 4.45–4.32 (2H, m), 3.95 (1H, d, *J* 5 Hz), 3.76–3.65 (1H, m), 3.55–3.41 (1H, m), 3.28–3.19 (1H, m), 3.15–3.04 (1H, m), 2.31 (1H, q, *J* 8 Hz), 2.20–2.07 (1H, m), 2.06–1.92 (2H, m), 1.72 (2H, quint, *J* 7.5 Hz), 1.52–1.36 (2H, m), 0.96 (3H, t, *J* 7.5 Hz).

5.1.13. 4-[(4-Chlorophenyl)methyl]-2-({(2*R*)-1-[2-(methyloxy)ethyl]-2-pyrrolidinyl}methyl)-1(2*H*)-phthalazinone trifluoroacetate salt (19b)

Was prepared in a similar way to **18b**: MS ES+ve *m/z* 412/414 $(M+H)^+$; ^{1}H NMR δ (CD_3OD) 8.41 (1H, dd, *J* 7.5, 2 Hz), 7.99 (1H, dd, *J* 7.5, 2 Hz), 7.93–7.84 (2H, m), 7.34–7.28 (4H, m), 4.62 (2H, d, *J* 5 Hz), 4.39 (2H, s), 4.09–4.00 (1H, m), 3.78–3.65 (4H, m), 3.34 (3H, s), 3.39–3.32 (1H, m), 3.30–3.25 (1H, m), 2.36 2.26 (1H, m), 2.18–1.95 (3H, m).

5.1.14. 2-[(Ethyloxy)carbonyl]-3-pyridinecarboxylic acid (22)

A suspension of 2,3-pyridinedicarboxylic anhydride (**21**) (19.3 g, 0.13 mol) in EtOH (100 mL) was heated to reflux for 2 h. The clear solution was allowed to cool to 20 °C overnight. The reaction mixture was concentrated under reduced pressure and the residual solid was triturated in ether to give **22** (13.36 g, 53%) as a white crystalline solid: LCMS rt = 1.53 min, 99%, ES+ve *m/z* 196 $(M+H)^+$; ^{1}H NMR δ (DMSO-d_6) 13.72 (1H, br s, CO_2H), 8.77 (1H, dd, *J* 5, 1.5 Hz, 6-H), 8.29 (1H, dd, *J* 8, 1.5 Hz, 4-H), 7.67 (1H, dd, *J* 8, 5 Hz, 5-H), 4.33 (2H, q, *J* 7 Hz, –OCH_2CH$_3$), 1.31 (3H, t, *J* 7 Hz, –OCH$_2$CH_3); ^{13}C NMR δ (DMSO-d_6) 166.2 (CO_2Et), 165.9 (CO_2H), 151.8 (6-C), 151.45 (2-C), 137.84 (4-C), 125.50 (3-C), 125.06 (5-C), 61.26 (OCH_2), 13.66 (OCH$_2$CH_3). HMBC correlations: from 4.33 (q) to 166.2 (CO_2Et); from 8.29 (4-H) to 165.9 (CO_2H).

5.1.15. Ethyl 3-[2-(4-chlorophenyl)-3-(methyloxy)-3-oxopropanoyl]-2-pyridinecarboxylate (23) and 2-[2-(4-chlorophenyl)-3-(methyloxy)-3-oxopropanoyl]-3-pyridinecarboxylic acid (24)

A solution of **22** (1.95 g, 10 mmol) in DMF (10 mL) was treated with carbonyl diimidazole (1.70 g, 10.5 mmol) portionwise under nitrogen at 50 °C. Gas evolution occurs as soon as CDI was added, and the mixture was heated overnight at 53 °C. LCMS indicated completion of carboxylic acid activation (rt = 0.69 min, 83%, ES+ve *m/z* 246 $(M+H)^+$ for the imidazolide). The reaction mixture was cooled in ice-salt and treated with methyl 4-chlorophenylacetate (2.03 g, 11 mmol) under nitrogen, followed by sodium hydride (60% oil dispersion; 1.4 g, 35 mmol). As soon as the first portion of NaH was added the colour of the reaction mixture changed from colourless to red. The mixture was stirred at −10 °C for 2 h, and then allowed to warm to 5 °C and stirred for a further 2 h. The mixture was carefully quenched with 2 M HCl solution (18 mL, 26 mmol) and extracted with ethyl acetate. The organic solution was washed with brine, dried ($MgSO_4$), and evaporated under reduced pressure to give an oil (2.59 g). This was dissolved in dichloromethane and purified by column chromatography on a silica (100 g) cartridge on Flashmaster eluting with a gradient of 0–100% ethyl acetate–cyclohexane over 60 min. Fractions eluting with rt between 32 min and 45 min were combined and evaporated to give a yellow oil: a mixture containing ethyl 3-[2-(4-chlorophenyl)-3-(methyloxy)-3-oxopropanoyl]-2-pyridinecarboxylate (**23**) as the major component (1.2 g, 33%). LCMS rt = 3.10 min, 33%, and rt = 3.21 min, 53%, ES+ve *m/z* 360/362 $(M+H)^+$; ^{1}H NMR δ ($CDCl_3$) 13.2 (1H, s), 8.77 (1H, dd, minor), 8.59 (1H, dd, major), 7.66 (1H, dd, minor), 7.48 (1H, dd, minor), 7.34 (m), 7.22 (1H, dd, major), 7.12 (2H, d, *J* 8 Hz), 7.05 (2H, d, *J* 8 Hz), 5.28 (1H, s), 4.48 and 4.46 (2H, 2 overlapping q, *J* 7 Hz), 4.01 (m), 3.80 (3H, s, major), 3.72 (3H, s, minor), 1.45 (3H, m).

The highly coloured aqueous phase and brine washings were combined and evaporated under reduced pressure. The resulting brown solid was washed with ethyl acetate–methanol (4:1, 100 mL) and the filtrate was evaporated under reduced pressure

to give a mixture containing crude 2-[2-(4-chlorophenyl)-3-(methyloxy)-3-oxopropanoyl]-3-pyridinecarboxylic acid (**24**) as a brown oil (1.3 g): LCMS rt = 0.89 min, 33%, ES+ve *m/z* 334/336 $(M+H)^+$.

5.1.16. 5-[(4-Chlorophenyl)methyl]pyrido[2,3-*d*]pyridazin-8(7*H*)-one (26)

The crude **23** (1.2 g, 3.3 mmol) was suspended in 5 M aqueous hydrochloric acid (15 mL, 75 mmol) and heated to 90 °C with vigorous stirring overnight. LCMS rt = 0.85 min, 60%, ES+ve *m/z* 276/278 $(M+H)^+$ for 3-[(4-chlorophenyl)acetyl]-2-pyridinecarboxylic acid and 1.23 min, 27%, ES+ve *m/z* 258/260 $(M+H)^+$ for the corresponding lactone. Hydrazine sulfate (430 mg, 3 mmol) was added to the reaction mixture, followed by sodium hydroxide (40 mL) and the mixture was heated to 90 °C for 1 h. Additional hydrazine sulfate (260 mg, 2 mmol) and ethanol (10 mL) were added and heated to 90 °C for 2.5 h. The mixture was allowed to cool to room temperature and the precipitated solid was collected by filtration, dried in vacuo at 60 °C to give a 5:1 mixture of 8-azaphthazinone (**26**) and 5-azaphthalazinone (**30**) (300 mg, 33%) as a white solid: LCMS rt = 0.87 min, 79%, and rt = 1.01 min, 15%, ES+ve *m/z* 272/274 $(M+H)^+$; ^{1}H NMR δ (DMSO-d_6) major 12.83 (1H, s, N*H*), 9.03 (1H, dd, *J* 4.5, 1.5 Hz, 7-H), 8.35 (1H, dd, *J* 8.5, 1.5 Hz, 5-H), 7.86 (1H, dd, *J* 8.5, 4.5 Hz, 6-H), 7.34 (4H, m, Ar), 4.31 (2H, s, ArCH_2–), NOE was observed from singlet at 4.31 to multiplet at 7.34 and dd at 8.35; minor 12.83 (1H, s, N*H*), 9.14 (1H, dd, *J* 4.5, 1.5 Hz, 6-H), 8.59 (1H, dd, *J* 8.5, 1.5 Hz, 8-H), 7.90 (1H, dd, overlaps with major isomer's 7.87 dd), 7.34 (4H, m, Ar), 4.36 (2H, s, ArCH_2-), NOE was observed from singlet at 4.36 to m at 7.34; LCHRMS rt = 10.77 min, found 272.0596. $C_{14}H_{11}ClN_3O$ requires 272.0591 and rt = 13.01 min, found 272.0602. $C_{14}H_{11}ClN_3O$ requires 272.0591. The filtrate was concentrated to half-volume under reduced pressure whereupon a solid precipitated. The solid was collected by filtration to give additional quantity of **26** (255 mg, 28%) LCMS rt = 0.87 min 76%, and rt = 1.01 min, 6%, ES+ve *m/z* 272/274 $(M+H)^+$; ^{1}H NMR δ (DMSO-d_6) 12.83 (1H, s), 9.03 (1H, dd, *J* 4.5, 1 Hz), 8.35 (1H, dd, *J* 8, 1 Hz), 7.86 (1H, dd, *J* 8, 4.5 Hz), 7.37−7.31 (4H, m), 4.31 (2H, s).

5.1.17. 5-[(4-Chlorophenyl)methyl]-7-[(2*R*)-2-pyrrolidinylmethyl]pyrido[2,3-*d*]pyridazin-8(7*H*)-one (27)

The solution of 8-azaphthalazinone (**26**) (538 mg, 2 mmol), *N*-Boc-D-prolinol (478 mg, 2.38 mmol), triphenylphosphine (1.05 g, 4 mmol) in THF (12 mL) was treated with di-*tert*-butyl azodicarboxylate (920 mg, 4 mmol) at room temperature under nitrogen. The mixture was stirred for 1.5 h and then concentrated under reduced pressure. The residue was purified by chromatography on a silica cartridge (100 g) on Flashmaster eluting with a gradient of 0−25% methanol-dichloromethane over 20 min. Appropriate fractions were combined and evaporated under reduced pressure to give *N*-BOC-**27** (1.055 g). LCMS rt = 1.23 min, 85%, ES+ve *m/z* 455/457 $(M+H)^+$. This product was dissolved in dichloromethane (15 mL) and treated with TFA (10 mL). The mixture was stirred for 20 min and then concentrated under reduced pressure. The residue was dissolved in methanol and applied to a methanol pre-conditioned SCX-2 ion-exchange cartridge (50 g). The cartridge was washed with methanol and then eluted with 10% aqueous ammonia in methanol. The ammoniacal fractions were combined and evaporated under reduced pressure to give 5-[(4-chlorophenyl)methyl]-7-[(2*R*)-2-pyrrolidinylmethyl]pyrido[2,3-*d*]pyridazin-8(7*H*)-one (**27**) (560 mg, 79%): LCMS rt = 0.75 min, 95%, ES+ve *m/z* 355/357 $(M+H)^+$; ^{1}H NMR δ (CD_3OD) 9.01 (1H, dd, *J* 4, 1 Hz), 8.34 (1H, dd, *J* 8, 1 Hz), 7.82 (1H, dd, *J* 8, 4 Hz), 7.30 (4H, m), 4.36 (2H, s), 4.35–4.30 (2H, m), 3.66–3.58 (1H, m), 3.06–2.99 (1H, m), 2.86–2.79 (1H, m), 2.00–1.75 (3H, m), 1.65–1.55 (1H, m).

5.1.18. 5-[(4-Chlorophenyl)methyl]-7-{[(2*R*)-1-methyl-2-pyrrolidinyl]methyl}pyrido[2,3-*d*]pyridazin-8(7*H*)-one formate salt (28)

Was prepared by Eschweiler-Clarke methylation in a similar way to **14:** LCMS rt = 2.19 min, 98%, ES+ve *m/z* 369/371 $(M+H)^+$; ^{1}H NMR δ (CD_3OD) 9.04 (1H, dd, *J* 4.5, 1 Hz, 7-H), 8.46 (1H, s, *H*CO_2H), 8.40 (1H, dd, *J* 8.3, 1 Hz, 5-H), 7.86 (1H, dd, *J* 8.3, 4.5 Hz, 6-H), 7.35–7.28 (4H, m, Ar), 4.60 (1H, dd, *J* 14, 5 Hz, NCH_2CH), 4.53 (1H, dd, *J* 14, 5.8 Hz, NCH_2CH), 4.38 (2H, s, CH_2Ar), 3.61–3.45 (2H, m, C*H*NMe and one of MeNCH_2), 3.00–2.88 (1H, m, one of MeNCH_2), 2.84 (3H, s, -NCH_3), 2.28–2.14 (1H, m, one of CHCH_2CH$_2$), 2.08–1.89 (3H, m, one of CHCH_2CH$_2$ and CHCH$_2$CH_2).

5.1.19. 7-{[(2*R*)-1-Butyl-2-pyrrolidinyl]methyl}-5-[(4-chlorophenyl)methyl]pyrido[2,3-*d*]pyridazin-8(7*H*)-one trifluoroacetic acid salt (29a)

Was obtained in a similar way to **18a**: LCMS rt = 2.47 min, 97%, ES+ve *m/z* 411/413 $(M+H)^+$; ^{1}H NMR δ (CD_3OD) 9.08 (1H, dd, *J* 4.3, 1.0 Hz), 8.43 (1H, dd, *J* 8.4, 1.4 Hz), 7.89 (1H, dd, *J* 8.4, 4.5 Hz), 7.36–7.28 (4H, m), 4.73 (1H, dd, *J* 14.5, 5 Hz), 4.63 (1H, dd, *J* 14.5, 5 Hz), 4.40 (2H, s), 4.01–3.91 (1H, m), 3.78–3.70 (1H, m), 3.63–3.51 (1H, m), 3.28–3.20 (1H, m), 3.14–3.05 (1H, m), 2.39–2.28 (1H, m), 2.21–2.07 (1H, m), 2.06–1.95 (2H, m), 1.79–1.68 (2H, m), 1.48–1.36 (2H, m), 0.97 (3H, t, *J* 7.4 Hz).

5.1.20. 5-[(4-Chlorophenyl)methyl]-7-({(2*R*)-1-[2-(methyloxy)ethyl]-2-pyrrolidinyl}methyl)pyrido[2,3-*d*]pyridazin-8(7*H*)-one formate salt (29b)

Was prepared in a similar way to **18b** MS ES+ve *m/z* 413/415 $(M+H)^+$; ^{1}H NMR δ (CD_3OD) 9.07 (1H, br d, *J* 4.5 Hz), 8.42 (1H, br d, *J* 8 Hz), 8.28 (1H, br s), 7.88 (1H, dd, *J* 8, 4.5 Hz), 7.36–7.29 (4H, m), 4.68–4.56 (2H, m), 4.40 (2H, s), 4.02–3.94 (1H, m), 3.77–3.60 (4H, m), 3.36 (3H, s), 3.35–3.28 (2H, obscured by CHD_2OD), 3.28–3.19 (1H, m), 2.34–2.24 (1H, m), 2.16–1.95 (3H, m).

5.1.21. 8-[(4-Chlorophenyl)methyl]pyrido[2,3-*d*]pyridazin-5(6*H*)-one (30)

Was prepared in a procedure similar to that for **26** from crude 2-[2-(4-chlorophenyl)-3-(methyloxy)-3-oxopropanoyl]-3-pyridinecarboxylic acid **24** (1.3 g) to give a 4:1 mixture of 5- and 8-azaphthalazinone (**30**:**26**): LCMS rt = 0.87 min, 20% and rt = 1.01 min, 80%, ES+ve *m/z* 272/274 $(M+H)^+$; ^{1}H NMR δ (DMSO-d_6) 12.8 (1H, s), 9.15 (1H, dd, *J* 5, 2 Hz), 8.60 (1H, dd, *J* 8, 2 Hz), 7.85 (1H, dd, *J* 8, 5 Hz), 7.32 (4H, m), 4.36 (2H, s).

5.1.22. 8-[(4-Chlorophenyl)methyl]-6-[(2*R*)-2-pyrrolidinylmethyl]pyrido[2,3-*d*]pyridazin-5(6*H*)-one (31)

MS ES+ve *m/z* 355/357 $(M+H)^+$; ^{1}H NMR δ ($CDCl_3$) 9.06 (1H, dd, *J* 4.5, 1.5 Hz), 8.67 (1H, br d, *J* 8 Hz), 7.65 (1H, dd, *J* 8, 4.5 Hz), 7.37 (2H, d, *J* 8 Hz), 7.25 (2H, d, *J* 8 Hz), 4.43 (2H, s), 4.38–4.30 (2H, m), 3.84–3.73 (1H, m), 3.11–2.95 (2H, m), 1.90–1.75 (3H, m), 1.63–1.52 (1H, m).

5.1.23. 8-[(4-Chlorophenyl)methyl]-6-{[(2*R*)-1-methyl-2-pyrrolidinyl]methyl}pyrido[2,3-*d*]pyridazin-5(6*H*)-one (32)

Was prepared by Eschweiler-Clarke methylation in a similar way to **14:** LCMS rt = 2.40 min, 98%, ES+ve *m/z* 369/371 $(M+H)^+$; ^{1}H NMR δ (CD_3OD) 9.12 (1H, dd, *J* 4.5, 1.5 Hz, 6-H), 8.67 (1H, dd, *J* 8.2, 1.5 Hz, 8-H), 7.81 (1H, dd, *J* 8.2, 4.5 Hz, 7-H), 7.37 (2H, d, *J* 8.3 Hz, aromatic 2-H and 6-H), 7.25 (2H, d, *J* 8.3 Hz, aromatic 3-H and 5-H), 4.47 (1H, dd, *J* 13.5, 4.5 Hz, one of NCH_2), 4.45 (2H, s, ArCH_2), 4.31 (1H, dd, *J* 13.5, 7.0 Hz, one of NCH_2CH), 3.28–3.21 (1H, m, one of MeNCH_2), 3.15–3.04 (1H, m, one of NCH$_2$C*H*), 2.59 (3H, s, NCH_3), 2.58–2.53 (1H, m, one of MeNCH_2), 2.06–1.96 (1H, m, one of CHCH_2CH$_2$), 1.91–1.79 (3H, m, one of CHCH_2CH$_2$ and

$CHCH_2CH_2$); ^{13}C NMR δ (101 MHz, CD_3OD) 161.6, 156.8, 150.3, 146.6, 138.5, 136.3, 133.5, 132.1, 129.5, 127.9, 125.4, 67.1, 58.2, 53.7, 41.2, 37.1, 29.8, 22.9.

5.1.24. 6-{[(2*R*)-1-Butyl-2-pyrrolidinyl]methyl}-8-[(4-chlorophenyl)methyl]pyrido[2,3-*d*]pyridazin-5(6*H*)-one (33a)

A solution of **31** (19 mg, 0.054 mmol) in DMF (0.5 mL) was treated with potassium carbonate (18 mg, 0.13 mmol) and butyl bromide (0.009 mL, 0.08 mmol) and the mixture was heated for 15 min at 100 °C in a microwave oven. A further portion of butyl bromide (0.009 mL, 0.08 mmol) was added and the mixture was heated for a further 15 min at 100 °C. The reaction mixture was applied to a methanol pre-conditioned SCX-2 ion-exchange column (10 g) and washed with methanol, followed by elution with 10% aqueous ammonia in methanol. The ammoniacal fractions were concentrated under reduced pressure and the residue was purified by MDAP to give **33a** (11.6 mg, 52%): LCMS rt = 2.61 min, 98%, ES+ve *m/z* 411/413 $(M+H)^+$; ^1H NMR δ (CD_3OD) 9.15 (1H, dd, *J* 4.5, 1.8 Hz), 8.70 (1H, dd, *J* 8.0, 1.5 Hz), 7.83 (1H, dd, *J* 8.0, 4.5 Hz), 7.37 (2H, d, *J* 8.3 Hz), 7.25 (2H, d, *J* 8.5 Hz), 4.53 (2H, d, *J* 5.3 Hz), 4.50–4.42 (2H, m), 3.74–3.65 (1H, m), 3.61–3.53 (1H, m), 3.37–3.33 (1H, m), 3.04–2.96 (1H, m), 2.94–2.85 (1H, m), 2.22–2.12 (1H, m), 2.08–1.88 (3H, m), 1.69–1.60 (2H, m), 1.42–1.31 (2H, m), 0.93 (3H, t, *J* 7.4 Hz).

5.1.25. 8-[(4-Chlorophenyl)methyl]-6-({(2*R*)-1-[2-(methyloxy)ethyl]-2-pyrrolidinyl}methyl)pyrido[2,3-*d*]pyridazin-5(6*H*)-one formate salt (33b)

Was prepared in a similar way to **18b**: MS ES+ve *m/z* 413/415 $(M+H)^+$; ^1H NMR δ (CD_3OD) 9.14 (1H, dd, *J* 4.5, 2 Hz), 8.69 (1H, dd, *J* 8, 2 Hz), 8.48 (1H, br s), 7.82 (1H, dd, *J* 8, 4.5 Hz), 7.37 (2H, d, *J* 8 Hz), 7.25 (2H, d, *J* 8 Hz), 4.51 (1H, dd, *J* 14, 5 Hz), 4.46 (2H, s), 4.39 (1H, dd, *J* 14, 7 Hz), 3.70–3.55 (3H, m), 3.54–3.46 (1H, m), 3.45–3.37 (1H, m), 3.33 (3H, s), 3.08–3.00 (1H, m), 2.97–2.84 (1H, m), 2.15–2.04 (1H, m), 2.01–1.85 (3H, m).

5.1.26. 13-[(Methyloxy)carbonyl]-2-pyrazinecarboxylic acid (35)[16]

2,3-Pyrazinedicarboxylic anhydride (**34**) (15.3 g, 102 mmol) was dissolved in methanol (100 mL) and the solution was stood at room temperature for 3 days. The solvent was removed under reduced pressure and the residue was crystallised from ethyl acetate to give **35** (13.85 g, 75%): MS ES+ve *m/z* 183 $(M+H)^+$; ^1H NMR δ (DMSO-d_6) 8.91 (1H, d, *J* 2 Hz), 8.90 (1H, d, *J* 2 Hz), 3.90 (3H, s).

5.1.27. Methyl 3-[2-(4-chlorophenyl)-3-(methyloxy)-3-oxopropanoyl]-2-pyrazinecarboxylate (36)

A solution of **35** (1.89 g, 10.4 mmol) in DMF (10 mL) was treated portionwise with CDI (1.8 g, 11 mmol) and heated to 61 °C under nitrogen. A further portion of CDI (0.2 g, 1.2 mmol) was added after 2.5 h, the mixture was heated for 2 h, and then allowed to cool to room temperature overnight. Methyl 4-chlorophenylacetate (2.2 g, 12 mmol) was added to the mixture, followed by DMF (2 mL) and the mixture was cooled in an ice-salt bath. Sodium hydride (60% oil dispersion; 1.4 g, 35 mmol) was added portionwise and the mixture was stirred for 1.5 h. Saturated aqueous ammonium chloride solution (50 mL) was added to the reaction mixture and the product was extracted with ethyl acetate (25 mL × 3). The organic extracts were washed with aqueous NH_4Cl, 2 M HCl, water, and twice with brine, dried ($MgSO_4$) and evaporated to give a 3:1 mixture of methyl 3-[2-(4-chlorophenyl)-3-(methyloxy)-3-oxopropanoyl]-2-pyrazinecarboxylate (**36**) and 3-[2-(4-chlorophenyl)-3-(methyloxy)-3-oxopropanoyl]-2-pyrazinecarboxylic acid (**37**) (3.62 g) LCMS rt = 0.79 min, 22%, ES+ve *m/z* 335/337 $(M+H)^+$ for acid, and rt = 1.10 min, 78%, ES+ve *m/z* 349/351 $(M+H)^+$ ester.

5.1.28. 8-[(4-Chlorophenyl)methyl]pyrazino[2,3-*d*]pyridazin-5(6*H*)-one (39)

The mixture from the previous experiment (3.62 g) was suspended in 5 M HCl (20 mL) and heated to 100 °C for 20 min. The ester did not dissolve by this time, so 4 M HCl in dioxane (8 mL) was added and heating was continued for a further 3.5 h. LCMS rt = 0.77 min, 52%, ES+ve *m/z* 277/279 $(M+H)^+$ for keto-acid **38**, then allowed to cool to room temperature and treated with sodium hydroxide (5.28 g, 132 mmol) cautiously. Hydrazine monohydrate (0.49 mL, 10 mmol) was added to the mixture and heated to 95 °C for 20 min. More hydrazine monohydrate (0.2 mL, 4 mmol) and sodium hydroxide (80 mg, 2 mmol) were added and heating was continued for a further 1.5 h. The mixture was concentrated under reduced pressure, the residue was extracted with ethyl acetate, and the filtrate evaporated to a gum. The gum was triturated in ether to give **39** as a glass (2.8 g): LCMS rt = 0.91 min, 72%, ES+ve *m/z* 273/275 $(M+H)^+$; ^1H NMR δ (DMSO-d_6) 13.0 (1H, br), 9.18 (1H, d, *J* 2 Hz), 9.12 (1H, d, *J* 2 Hz), 7.32 (4H, s), 4.35 (2H, s).

5.1.29. 8-[(4-Chlorophenyl)methyl]-6-[(2*R*)-2-pyrrolidinylmethyl]pyrazino[2,3-*d*]pyridazin-5(6*H*)-one (41)

Was obtained by a procedure similar to that described for **13**: LCMS rt = 0.78 min, 97%, ES+ve *m/z* 356/358 $(M+H)^+$; ^1H NMR δ (CD_3OD) 9.15 (1H, d, *J* 2 Hz), 9.07 (1H, d, *J* 2 Hz), 7.38 (2H, d, *J* 8 Hz), 4.25 (2H, d, *J* 8 Hz), 4.50–4.40 (4H, m), 3.89–3.82 (1H, m), 3.28–3.22 (1H, m), 2.20–1.72 (3H, m).

5.1.30. 8-[(4-Chlorophenyl)methyl]-6-{[(2*R*)-1-methyl-2-pyrrolidinyl]methyl}pyrazino[2,3-*d*]pyridazin-5(6*H*)-one (42)

LCMS rt = 2.27 min, 92%, ES+ve *m/z* 370/372 $(M+H)^+$; ^1H NMR δ (CD_3OD) 9.17 (1H, d, *J* 2 Hz), 9.08 (1H, d, *J* 2 Hz), 8.37 (1H, br s),7.37 (2H, d, *J* 8.3 Hz), 7.27 (2H, d, *J* 8 Hz), 4.68–4.57 (2H, m), 4.52–4.42 (2H, m), 3.77–3.68 (1H, m), 3.65–3.57 (1H, m), 3.14–3.05 (1H, m), 2.92 (3H, s), 2.32–2.23 (1H, m), 2.12–1.97 (3H, m).

5.1.31. 4-[(4-Chlorophenyl)methyl]-2-({(2*R*)-1-[4-(4-{[3-(hexahydro-1*H*-azepin-1-yl)propyl]oxy}phenyl)butyl]-2-pyrrolidinyl}methyl)pyrido[3,4-*d*]pyridazin-1(2*H*)-one (44)

A mixture of **13** (54 mg, 0.15 mmol), 4-(4-{[3-(hexahydro-1*H*-azepin-1-yl)propyl]oxy}phenyl)butyl methanesulfonate (**43**)[13] (78 mg, 0.20 mmol) and sodium bicarbonate (28 mg, 0.33 mmol) in MeCN (3 mL) was heated at 80 °C with stirring for 6 days under a nitrogen atmosphere. The cooled reaction mixture was filtered, and the filtrate was concentrated in vacuo. The residue was partitioned between water and DCM, and the organic phase separated using a hydrophobic frit. The aqueous phase was diluted with brine and further extracted with DCM (×6). The combined organic extracts were concentrated in vacuo and the residue was purified by MDAP. The relevant fractions were concentrated, to give two batches of material, each containing a different impurity. Each of these batches was further separately purified by chromatography on silica [2 g, eluted with 2–4% (10% aqueous ammonia in MeOH)−DCM]. Concentration of the appropriate fractions from these two purifications gave **44** (18 mg, 19%): LCMS rt = 2.37 min, 100%, ES+ve *m/z* 643/645 $(M+H)^+$ and 322/323 $(M/2+H)^+$; ^1H NMR δ (DMSO-d_6) 9.23 (1H, s), 8.89 (1H, d, *J* 5.5 Hz), 8.17 (1H, d, *J* 5 Hz), 7.33–7.27 (4H, m), 7.00 (2H, d, *J* 8 Hz), 6.76 (2H, d, *J* 8 Hz), 4.37 (2H, s), 4.30 (1H, dd, *J* 13, 4.5 Hz), 4.11 (1H, dd, *J* 13, 8 Hz), 3.94 (2H, t, *J* 6 Hz), 3.14–3.07 (1H, m), 3.01−2.94 (1H, m), 2.85–2.75 (1H, m), 2.73–2.65 (6H, m), 2.48 (2H, t, *J* 6.5 Hz), 2.35–2.20 (4H, m), 1.97–1.89 (2H, m), 1.88–1.58 (12H, m), 1.57–1.45 (4H, m); ^1H NMR δ (CD_3OD) 9.23 (1H, s), 8.89 (1H, d, *J* 5.3 Hz), 8.17 (1H, d, *J* 5.5 Hz), 7.33–7.26 (4H, m), 7.00 (2 H, d, *J* 8.5 Hz), 6.77 (2H, d, *J* 8.5 Hz), 4.38 (2H, s), 4.31 (1H, dd, *J* 13.0, 4.5 Hz), 4.11 (1H, dd, *J* 13.0, 8.0 Hz), 3.94 (2H, t, *J* 6.1 Hz), 3.15–3.07 (1H, m), 2.98 (1 H, dd, *J* 7.8, 4.8 Hz), 2.84–2.76 (1H, m), 2.74–2.65 (6

H, m), 2.48 (2 H, t, *J* 6.3 Hz), 2.35–2.20 (2H, m), 1.98–1.89 (2H, m), 1.89–1.44 (16 H, m).5.9 Biological assays have been reported previously[18,20]

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