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Phosphodiesterase inhibitors. Part 3: Design, synthesis and structure–activity relationships of dual PDE3/4-inhibitory fused bicyclic heteroaromatic-dihydropyridazinones with anti-inflammatory and bronchodilatory activity

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ABSTRACT

(−)-6-(7-Methoxy-2-trifluoromethylpyrazolo[1,5-*a*]pyridin-4-yl)-5-methyl-4,5-dihydro-3-(2*H*)-pyridazinone (KCA-1490) is a dual PDE3/4 inhibitor that exhibits potent combined bronchodilatory and anti-inflammatory activity. A survey of potential bicyclic heteroaromatic replacement subunits for the pyrazolo[1,5-*a*]pyridine core of KCA-1490 has identified the 4-methoxy-2-(trifluoromethyl)benzo[*d*]thiazol-7-yl and 8-methoxy-2-(trifluoromethyl)quinolin-5-yl analogues as dual PDE3/4-inhibitory compounds that potently suppress histamine-induced bronchoconstriction and exhibit anti-inflammatory activity in vivo.

1. Introduction

Therapeutic management of asthma and chronic obstructive pulmonary disease (COPD) varies according to the severity of the symptoms, but for many years corticosteroids and β_2-agonists have been the mainstay of treatment regimens.[1] Steroids provide control of underlying inflammatory dysfunction, whilst the bronchodilatory activity of β_2-agonists primarily serves to afford symptomatic relief for airway constriction. In recent years combination treatments comprising an inhaled corticosteroid (ICS) and long-acting β_2-agonist (LABA) have been actively developed, and these allow effective management of the majority of mild-to-moderate asthma.[1] However, long-term use of corticosteroids in high doses can produce several adverse side effects, driving an on-going search for steroid-sparing treatment options.[2] Moreover, the safety of LABAs has also recently come under scrutiny, with the US Food and Drug Administration ordering a series of post-approval clinical trials from 2011 to evaluate their potential involvement in serious adverse outcomes among asthma patients.[3]

One important avenue explored as a new therapeutic option for respiratory disease has focused on the development of inhibitors for the phosphodiesterase (PDE) 4 enzyme family, and this has culminated in the deployment of roflumilast (Fig. 1), which, as the first PDE4-selective inhibitor to gain regulatory approval, has recently been launched for the treatment of severe COPD.[4] The potential utility of PDE4 inhibitors for treatment of respiratory disease derives from their pronounced anti-inflammatory activity, but they also exhibit modest activity for inducing relaxation of airway smooth muscle tissue.[5] However, PDE3 inhibitors are known to be superior to PDE4 inhibitors in mediating relaxation of airway smooth muscle.[6] Thus, either the combination of PDE3 and PDE4 inhibitors or the use of dual PDE3/4 inhibitors may offer enhanced therapeutic potential for asthma and COPD compared to treatment with individual PDE family selective agents alone, and this notion is supported by reports that combined inhibition of PDE3 and PDE4 may function additively or synergistically to induce airway smooth muscle relaxation.[7]

In the search for an alternative approach to the ICS/LABA regimen for asthma and COPD management, we have also evaluated and found promising activity with dual PDE3/4-inhibitory compounds. Thus, we have previously disclosed (−)-6-[7-methoxy-2-(trifluoromethyl)pyrazolo[1,5-*a*]pyridin-4-yl]-5-methyl-4,5-dihydro-3(2*H*)-pyridazinone (KCA-1490, Fig. 1) as a dual PDE3/4 inhibitor with very potent combined bronchodilatory and anti-inflammatory activity and an improved therapeutic window over

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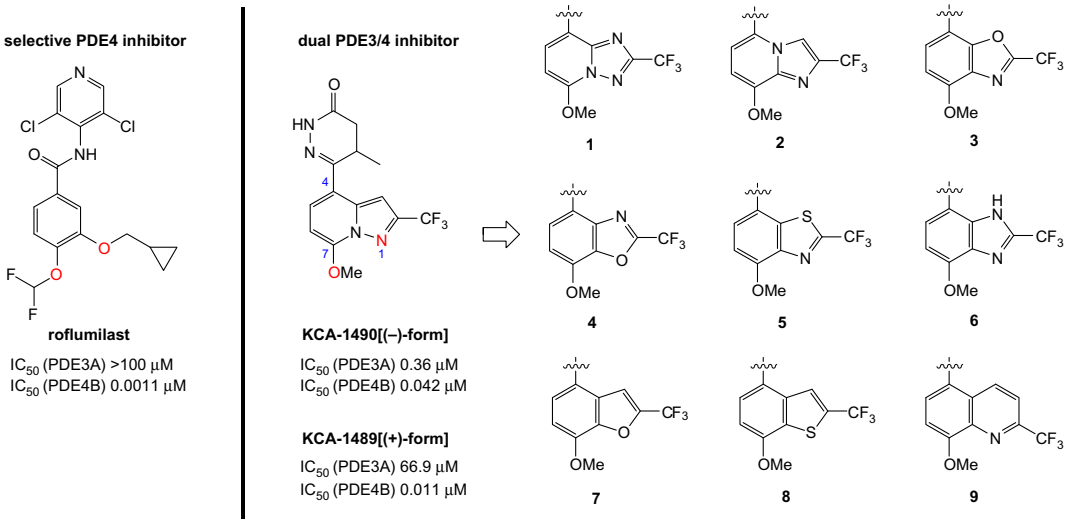


Figure 1. Structures of PDE4-selective inhibitor roflumilast and dual PDE3/4-inhibitory KCA-1490 together with target compounds (**1**–**9**) derived from KCA-1490. Activity data quoted for roflumilast, KCA-1490 and KCA-1489 (the enantiomer of KCA-1490) are derived from enzyme inhibition assays using the core catalytic domains for PDE3A and PDE4B as described previously.[8b]

roflumilast in a number of in vitro and in vivo models used for pharmacological profiling.[8] Our structure–activity relationship studies with compound series related to KCA-1490 revealed that the PDE3-inhibitory activity is heavily dependent on the presence of the 5-methyldihydropyridazinone, a subunit that is strongly represented in established PDE3 inhibitors,[9,10] and we have rationalized this dependence in our previously reported[8b] binding models (summarized here in Fig. 2). The N(1) nitrogen center and the oxygen atom of the 7-methoxypyrazolo[1,5-*a*]pyridine core subunit proved to be critical determinants for potent PDE4 inhibition, and these centers correspond to the catechol ether oxygen atoms of roflumilast (Fig. 1) in the role that they play in engaging the purine-scanning glutamine of the PDE4 catalytic pocket (Fig. 2B).[8b] Evaluation of a series of substituents at the pyrazolopyridine 2-position revealed that a trifluoromethyl group conferred an efficacious balance of PDE3 and PDE4 inhibition and was optimal for activity in our pharmacological models.[8a] Having defined the essential structural features required for dual PDE3/4-inhibitory activity within the confines of our pyrazolo[1,5-*a*]pyridine-based series, there remained considerable scope for structural variation with isosteric replacements for the core bicycle. We therefore set out to evaluate a series of KCA-1490 analogues with alternative fused 5–6 bicyclic heteroaromatics in place of the pyrazolo[1,5-*a*]pyridine (**1**–**8**, Fig. 1) together with the ring expanded analogue, quinoline **9**. The synthesis and activity of these compounds is presented here.

2. Chemistry

Synthesis of triazolopyridine **1** commenced with the formation of *N*-aminopyridinium salt **11** by treatment of parent pyridine **10** with *O*-mesitylenesulfonylhydroxylamine (MSH) (Scheme 1). Construction of the 2-(trifluoromethyl)-[1,2,4]triazolo[1,5-*a*]pyridine bicycle was then accomplished by condensation of salt **11** with

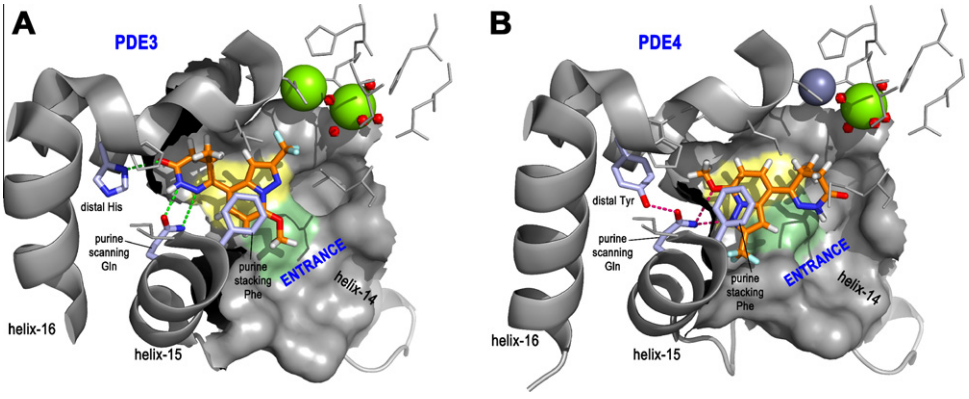
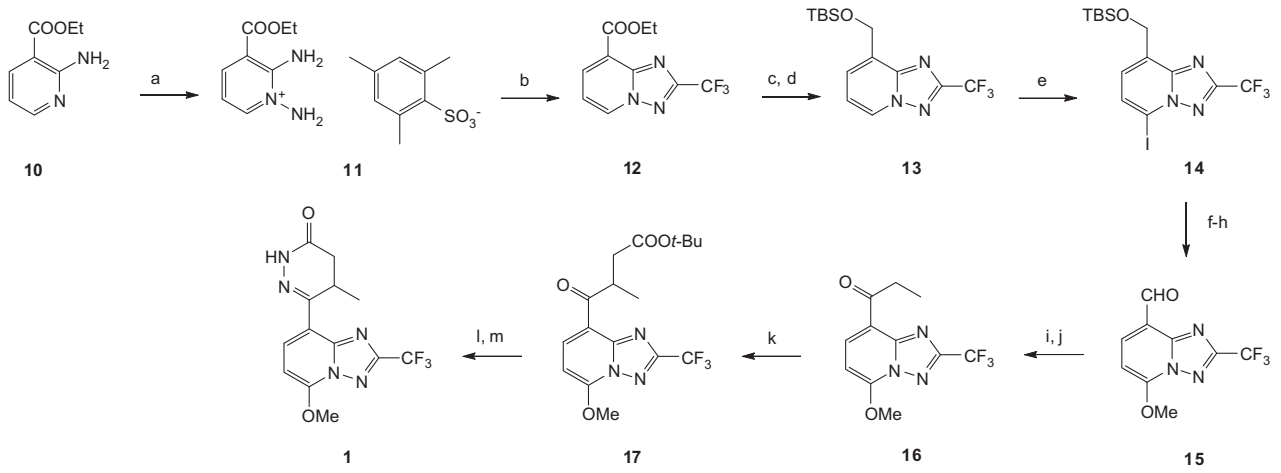


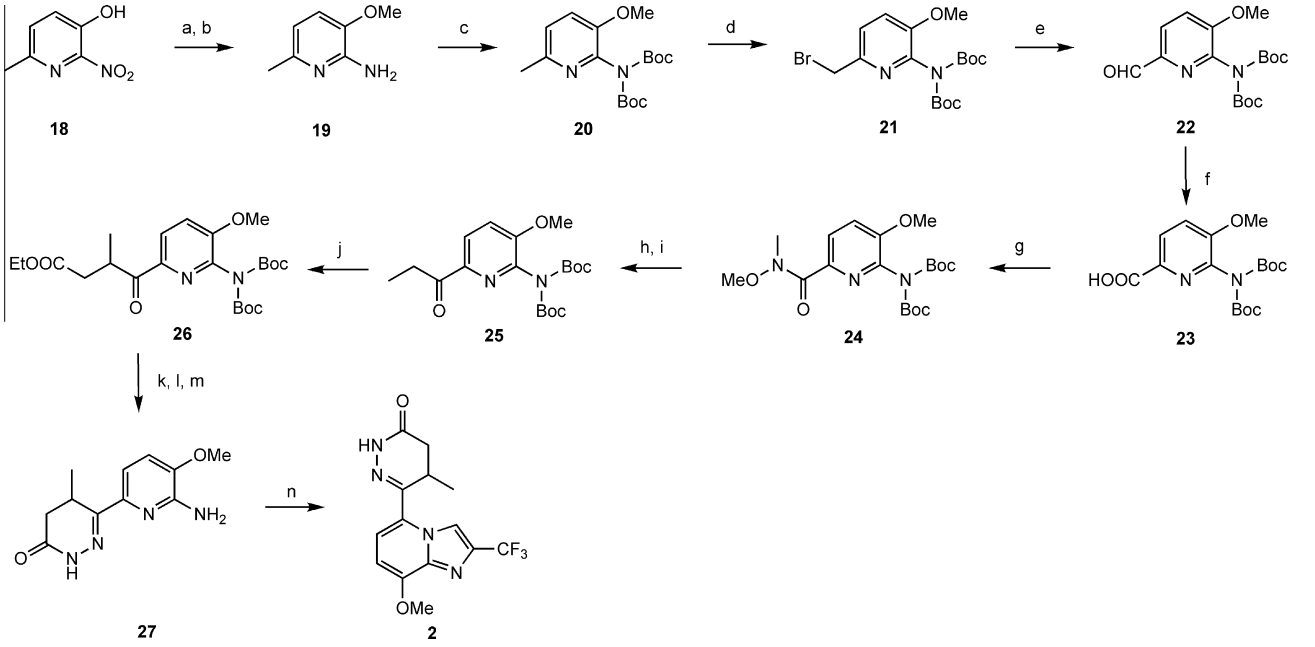
Figure 2. Interaction models for KCA-1490 (orange stick) with PDE3 (panel A) and PDE4 (panel B) based on SAR analysis of our pyrazolopyridine series inhibitors.[8] By analogy to related PDE3 inhibitors,[10] KCA-1490 is predicted to possess the (*R*)-configured 5-methyl-4,5-dihydropyridazinone ring; the antipode (KCA-1489), which has weak PDE3-inhibitory activity, is predicted to be (*S*)-configured. The PDE3 binding model is templated on the co-crystal structure (PDB: 1SO2) of (*R*)-6-(4-{[2-(3-iodobenzyl)-3-oxocyclohex-1-en-1-yl]amino}phenyl)-5-methyl-4,5-dihydropyridazin-3(2*H*)-one with the core catalytic domain from PDE3B.[10b] Residues lining the catalytic pocket of PDE3B are fully conserved in the core catalytic domain of PDE3A (used in the enzyme inhibition assays of the present study). In this binding pose the pyridazinone ring hydrogen bonds (dotted lines) the purine-scanning Gln and distal His residues of the PDE3 catalytic pocket. The PDE4 binding model is templated on the co-crystal structure of zardaverine with the core catalytic domain of PDE4D (PDB: 1XOR, 1MKD).[31] Residues lining the catalytic pocket are fully conserved between PDE4D and PDE4B (used in the assays of the present study). The PDE4 binding model is typical of catechol ether inhibitors and invokes hydrogen bonded engagement of the purine scanning-Gln by the pyrazolopyridine N(1) and methoxy oxygen centers of KCA-1490. Despite the near 180° rotation in the orientation of the inhibitor in the two models, the pyrazolopyridine (corresponding to the heterobicyclic subunits of compounds **1**–**9**) occupies the hydrophobic clamp, a conserved feature in PDE3 and PDE4 formed by Ile (yellow surface) and Phe (green surface) residues on one face of the inhibitor and by the purine-stacking Phe on the other face. The entrance to the catalytic pocket is marked.



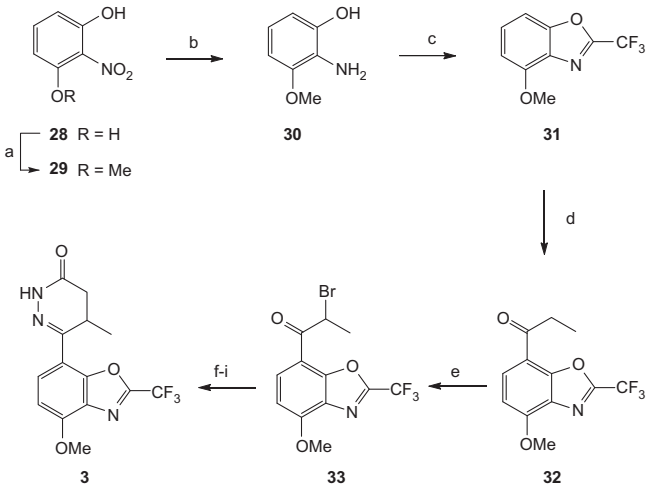
Scheme 1. Reagents and conditions: (a) MSH/CH_2Cl_2, rt (92%); (b) TFAA, Et_3N/MeOH, rt (79%); (c) DIBAL-H/THF, −10 °C; (d) TBSCl, imidazole/DMF, rt (92%, 2 steps); (e) *n*-BuLi/THF, −78 °C then 1,2-diiodoethane, −78 °C (94%); (f) TBAF/THF, rt; (g) MnO_2/$CHCl_3$, 60 °C; (h) NaOMe/MeOH, reflux (35%, 3 steps); (i) EtMgBr/THF, rt; (j) SO_3–pyridine, Et_3N/DMSO, rt (45%, 2 steps); (k) LiHMDS/THF, −78 °C then $BrCH_2COOBu^t$, −78 °C to rt; (l) TFA/CH_2Cl_2, rt; (m) H_2N–NH_2, AcOH/EtOH, reflux (40%, 3 steps).

TFAA to afford **12** in moderate yield. DIBAL-H reduction of the ester followed by TBS protection of the intermediate primary alcohol gave **13**. Iodination at the 5-position of the triazolopyridine, removal of the TBS group and MnO_2 oxidation followed by substitution of the iodide using NaOMe furnished aldehyde **15**. The pyridazinone ring was then constructed by conversion of **15** into ketone **16** through a two-step sequence consisting of Grignard reaction and Parikh–Doering oxidation. Reaction of the enolate derived from **16** with bromoacetate then led to γ-ketoester **17** and thence to target **1** by condensation with hydrazine following cleavage of the *tert*-butyl ester.

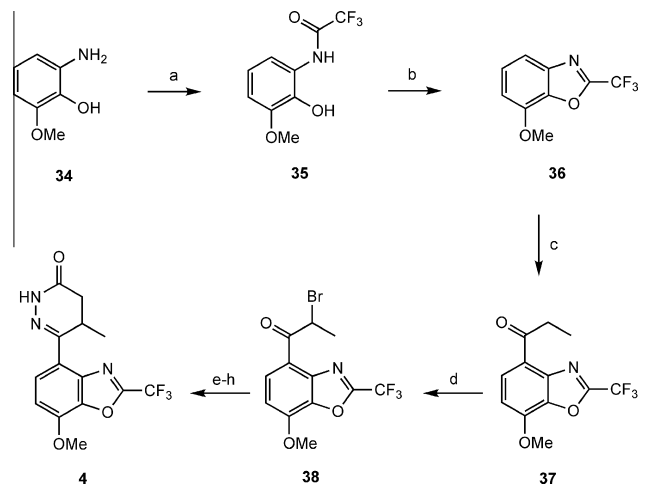
Target imidazopyridine **2** was prepared from pyridine derivative **18** (Scheme 2). Methylation of the phenolic hydroxyl group followed by reduction of the nitro group gave amine **19**. Initially we attempted to complete the imidazo[1,2-*a*]pyridine scaffold from **19** before constructing the dihydropyridazinone ring using a strategy similar to that applied in the synthesis of triazolopyridine **1**. However, in the case of the imidazopyridine, formation of the dihydropyridazinone ring in the final step was unsuccessful and starting material was recovered. This suggested that the reactivity of the carbonyl group at the 5-position of the imidazopyridine was low. We therefore revised the synthetic route to install the pyridazinone ring on pyridine **19** before completing construction of the imidazopyridine ring. Thus, Boc protection of **19** followed by bromination at the benzylic position afforded bromide **21**. Oxidation in two steps gave carboxylic acid **23** via aldehyde **22**. The carboxylic acid was coupled with *N*,*O*-dimethylhydroxylamine to give the Weinreb amide (**24**). Treatment of **24** with EtMgBr resulted in removal of one Boc group as well as formation of the desired ethyl ketone functionality. Reinstallation of the Boc



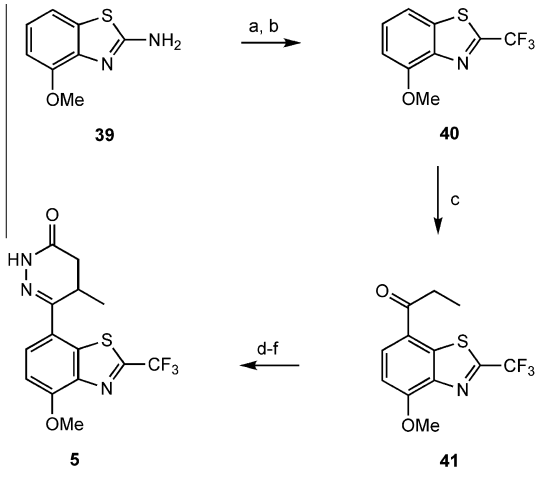
Scheme 2. Reagents and conditions: (a) MeI, K_2CO_3/DMF, rt; (b) H_2 (1 atm), 10% Pd–C/EtOAc, rt (95%, 2 steps); (c) $(Boc)_2O$, Et_3N, DMAP/MeCN, rt (79%); (d) NBS, Bz_2O_2/CCl_4, reflux (69%); (e) NMO, MS4A/MeCN, rt (79%); (f) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene/*t*-BuOH, H_2O, rt (quant.); (g) *N*,*O*-dimethylhydroxylamine, EDCI, HOBt, DIEA/DMF, rt (88%); (h) EtMgBr/THF, −78 °C to rt; (i) $(Boc)_2O$, Et_3N, DMAP/MeCN, rt (85%, 2 steps); (j) LiHMDS/THF, 0 °C then $BrCH_2COOEt$, rt (63%); (k) KOH aq/MeOH, 60 °C; (l) H_2N–NH_2, AcOH/EtOH, reflux; (m) TFA/CH_2Cl_2, rt (89%, 3 steps); (n) 3-bromo-1,1,1-trifluoroacetone/EtOH, 75 °C (19%).



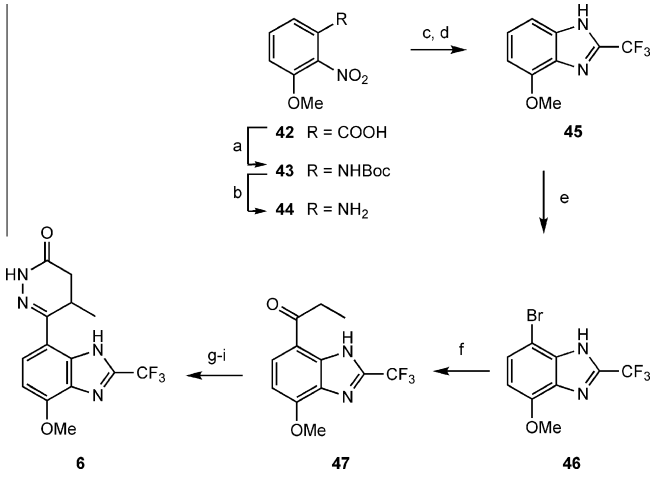
Scheme 3. Reagents and conditions: (a) MeI, K_2CO_3/DMF, rt (32%); (b) H_2 (1 atm), 10% Pd–C/EtOH, rt (98%); (c) TFAA, TsOH/$PhCH_3$, reflux (59%); (d) $AlCl_3$, propionyl chloride/CH_2Cl_2, rt (63%); (e) $CuBr_2$/EtOAc, reflux (61%); (f) di-*t*-butyl malonate, NaH/DMF, rt; (g) TFA/CH_2Cl_2, rt; (h) xylene, 150 °C; (i) $BocNHNH_2$, TsOH/xylene, reflux (31%, 4 steps).



Scheme 4. Reagents and conditions: (a) TFAA/$PhCH_3$, reflux (96%); (b) TsOH/$PhCH_3$, reflux (86%); (c) $TiCl_4$, propionyl chloride/CH_2Cl_2, rt (80%); (d) $CuBr_2$/EtOAc, reflux (94%); (e) di-*t*-butyl malonate, NaH/DMF, rt; (f) TFA/CH_2Cl_2, rt; (g) xylene, 150 °C; (h) $BocNHNH_2$, TsOH/xylene, reflux (45%, 4 steps).



Scheme 5. Reagents and conditions: (a) KOH aq reflux (b) PPSE, TFA, 95 °C (69%, 2 steps); (c) $AlCl_3$, propionyl chloride/$MeNO_2$, rt (quant.); (d) LiHMDS/THF, 0 °C then $BrCH_2COOBu^t$, 0 °C to rt; (e) TFA/CH_2Cl_2, rt; (f) H_2N–NH_2/EtOH, reflux (30%, 3 steps).

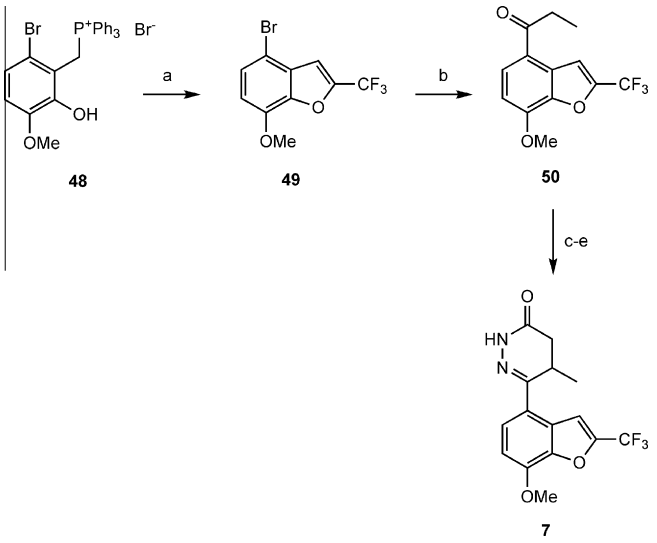


Scheme 6. Reagents and conditions: (a) DPPA, Et_3N/*t*-BuOH, reflux (98%); (b) TFA/CH_2Cl_2, rt (91%); (c) H_2 (1 atm), 10% Pd–C/EtOH, EtOAc, AcOH, rt; (d) TFA, reflux (90%, 2 steps); (e) NBS/$CHCl_3$, rt (63%); (f) *n*-BuLi/THF, −78 °C then *N*,*N*-dimethylpropionamide, −78 °C to rt (46%); (g) LiHMDS/THF, −78 °C to -20 °C then $BrCH_2COOBu^t$, −78 °C to rt; (h) TFA/CH_2Cl_2, rt; (i) H_2N–NH_2/EtOH, reflux (68%, 3 steps).

group was therefore undertaken to obtain the di-Boc derivative (**25**) prior to treatment with base and bromoacetate, affording ketoester **26**. Formation of the dihydropyridazinone ring was then completed by hydrolysis of the ester and condensation with hydrazine. Removal of the Boc protection with TFA gave aminopyridine **27** which was then treated with 3-bromo-1,1,1-trifluoroacetone to furnish target imidazopyridine **2**.

Our synthetic route to the 4-methoxybenzoxazole analogue (**3**) of KCA-1490 is presented in Scheme 3. Monomethylation of resorcinol **28** followed by reduction of the nitro group gave aniline **30**. Construction of the 2-(trifluoromethyl)benzoxazole scaffold was then accomplished by reaction with TFAA under acidic conditions to afford **31**. Friedel–Crafts acylation of **31** afforded 7-propionylbenzoxazole **32**. Application of our standard procedure for formation of the pyridazinone precursor—by treatment of **32** with LiHMDS followed by bromoacetate—generated a complex product mixture with only a trace of the desired γ-ketoester. This suggested that the 4-methoxy-2-(trifluoromethyl)benzoxazole ring was unstable to the alkylation conditions used, and we therefore employed an alternative strategy for preparation of the dihydropyridazinone ring precursor that avoided exposure to the strong base. Thus, bromination of ketone **32** with copper(II) bromide gave α-bromoketone **33**. The latter was converted into a γ-ketoacid dihydropyridazinone precursor through a three-step sequence consisting of nucleophilic substitution with di-*tert*-butyl malonate, deprotection and then decarboxylation. In the final step, our usual conditions for formation of the dihydropyridazinone ring (hydrazine, AcOH/EtOH, reflux) gave only a trace of target **3** due to competing nucleophilic attack of hydrazine at the 2-position of the benzoxazole. Through further investigation we found that the undesired side reaction could be suppressed by treating the keto-acid with $BocNHNH_2$ under acidic conditions, allowing formation of target **3** in moderate yield.[11]

The 7-methoxybenzoxazole target (**4**) was prepared using a similar strategy to that used for construction of the isomeric 4-methoxybenzoxazole analogue (**3**), this time commencing with

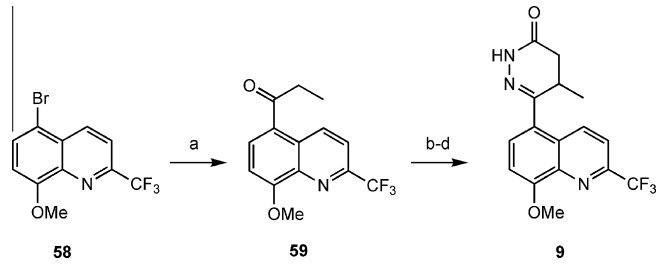


Scheme 7. Reagents and conditions: (a) TFAA, $Et_3N/PhCH_3$, reflux (87%); (b) *n*-BuLi/THF, −78 °C then *N*,*N*-dimethylpropionamide, rt (35%); (c) LiHMDS/THF, −78 °C then $BrCH_2COOBu^t$, −78 °C to rt; (d) TFA/CH_2Cl_2, rt; (e) $H_2N–NH_2$, AcOH/EtOH, reflux (37%, 3 steps).

aniline **34** (Scheme 4). N-acylation of the latter with TFAA followed by cyclization with catalytic TsOH in hot toluene gave 7-methoxy-2-(trifluoromethyl)benzoxazole (**36**) in good yield. Intermediate **36** was then converted into target **4** following a similar six-step sequence to that developed for synthesis of **3** from 4-methoxy-2-(trifluoromethyl)benzoxazole (**31**).

Our synthetic route to the 4-methoxybenzothiazole analogue (**5**) of KCA-1490 is shown in Scheme 5. Hydrolysis of 2-aminobenzothiazole **39**[12] to open the azole ring followed by thiazole reconstruction with TFA in the presence of trimethylsilyl polyphosphate (PPSE)[13] gave the parent 4-methoxy-2-(trifluoromethyl)benzothiazole bicycle (**40**). Friedel–Crafts acylation of **40** with propionyl chloride afforded the key ethyl ketone intermediate (**41**) in quantitative yield. Ketone **41** was then converted into target **5** through a reaction sequence similar to that used for synthesis of **1** from ketone **16** (Scheme 1). Compound **5** was also accessible from intermediate **40** by boronation at C-7 followed by Suzuki cross-coupling with a 6-chloro-5-methylpyridazinone derivative (Supplementary data).

The benzimidazole target (**6**) was synthesized from commercially available benzoic acid **42** (Scheme 6). DPPA-mediated



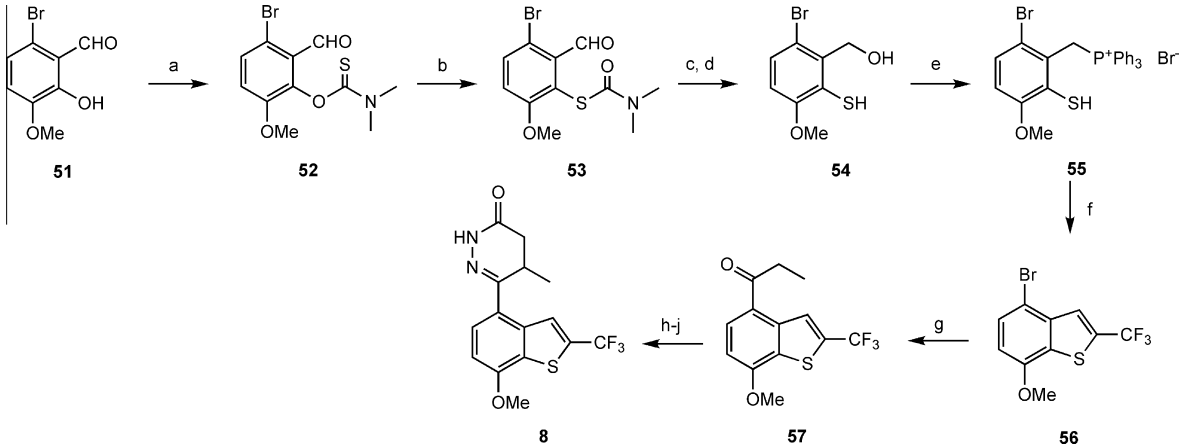
Scheme 9. Reagents and conditions: (a) *n*-BuLi/THF, −78 °C then propionic anhydride, −78 °C (34%); (b) LiHMDS/THF, 0 °C then $BrCH_2COOBu^t$, rt; (c) TFA/CH_2Cl_2, rt; (d) $H_2N–NH_2$, AcOH/EtOH, reflux (43%, 3 steps).

Curtius rearrangement in the presence of *tert*-butanol afforded *N*-Boc aniline **43**;[14] removal of the Boc group, hydrogenation and condensation of the intermediate phenylenediamine with TFA completed construction of the 2-(trifluoromethyl)benzimidazole scaffold in **45**. Bromination at the 7-position of **45** gave bromide **46**. Bromine–lithium exchange and reaction of the resulting benzimidazolyllithium with *N*,*N*-dimethylpropionamide then provided ethyl ketone **47**, which was finally converted into target **6** using the reaction sequence developed for construction of the dihydropyridazinone ring in compound **1**. As with the benzothiazole (**5**), target **6** was also accessible through a Suzuki cross-coupling strategy following bromine–lithium exchange on **46** and boronation (Supplementary data).

The benzofuran target (**7**) was prepared from the known phosphonium salt **48**[15] (Scheme 7). Reaction of the latter with TFAA furnished the benzofuran core structure in **49**. Bromobenzofuran **49** was then converted into **7** in a similar manner to the synthesis of **6** from bromobenzimidazole **46** (Scheme 6).

Synthesis of the benzothiophene analogue (**8**), Scheme 8, was carried out in much the same way as for benzofuran **7** but using the thiophenol equivalent (**55**) of the phenolic phosphonium bromide salt (**48**). Thus, thiocarbamoylation of phenol **51**, thermal rearrangement of the resulting thiocarbamate to **53** and then hydrolysis followed by reduction of the aldehyde afforded benzyl alcohol **54**. The latter was easily converted into the requisite phosphonium salt (**55**) by treatment with triphenylphosphine hydrobromide, and **55** was then transformed into target benzothiophene **8** using the reaction sequence previously applied in the synthesis of benzofuran **7** from phosphonium bromide salt **48**.

Our synthetic route to the 8-methoxyquinoline derivative (**9**) is outlined in Scheme 9. Bromoquinoline **58**[16] was converted into ethyl ketone **59** by bromine–lithium exchange followed by



Scheme 8. Reagents and conditions: (a) dimethylthiocarbamoyl chloride, DABCO/DMF, rt (81%); (b) Ph_2O, 200 °C (57%); (c) NaOH aq/*i*-PrOH, 60 °C; (d) $NaBH_4$/MeOH, rt (quant., 2 steps); (e) Ph_3PHBr/MeCN, reflux; (f) TFAA, $Et_3N/PhCH_3$, reflux (82%, 2 steps); (g) *n*-BuLi/THF, −78 °C then *N*,*N*-dimethylpropionamide, −78 °C to rt (15%); (h) LiHMDS/THF, 0 °C then $BrCH_2COOBu^t$, −78 °C to rt; (i) TFA/CH_2Cl_2, rt; (j) $H_2N–NH_2$, AcOH/EtOH, reflux (72%, 3 steps).

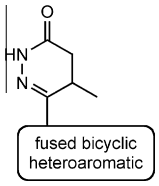
reaction with propionic anhydride. Application of our standard route for installation of the dihydropyridazinone ring then afforded target quinoline **9** in three steps from **59**.

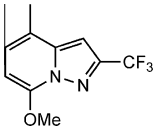
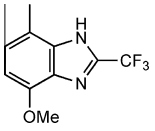
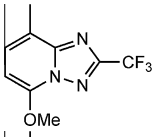
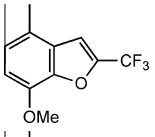
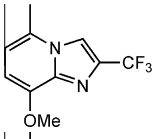
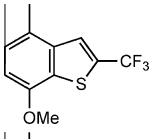
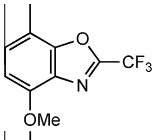
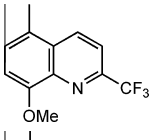
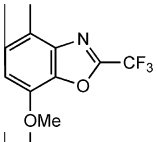
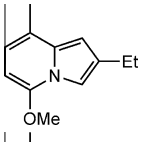
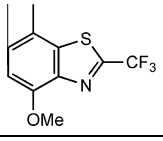
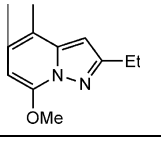
3. Results and discussion

Having previously identified[8a] (−)-6-[7-methoxy-2-(trifluoromethyl)pyrazolo[1,5-*a*]pyridin-4-yl]-5-methyl-4,5-dihydro-3(2*H*)-pyridazinone (KCA-1490) as a dual PDE3/4 inhibitor that exhibited potent combined bronchodilatory and anti-inflammatory activity, we set out to evaluate a range of related compounds (**1**–**9**) in which the pyrazolo[1,5-*a*]pyridine subunit of the parent structure was replaced by an alternative heterobicycle. For the majority of compounds in the target set we chose to replace the pyrazolopyridine by an isosteric 5–6 fused bicycle (**1**–**8**). Quinoline **9** was included in order to provide a structure with an expanded 6–6 fused replacement bicycle, and structurally-related 8-methoxyquinoline carboxamides have also been evaluated as PDE4-selective inhibitors by other groups.[17] For initial assessment the target compounds (**1**–**9**) in the present study were prepared and tested in racemic form, assaying for inhibitory activity against the core catalytic domains from PDE3A and PDE4B using previously reported protocols.[18] Activity data for these compounds and for KCA-1450[8a] (the racemate corresponding to KCA-1490 and its enantiomer KCA-1489) are presented in Table 1.

For the majority of the compounds (**1**–**9**) replacement of the KCA-1450 pyrazolopyridine subunit resulted in activity loss against PDE3—an activity loss that was maximal in the case of the benzimidazole (**6**), where a 10-fold reduction in inhibitory potency was observed. Only two of the compounds exhibited improved PDE3-inhibitory potency over KCA-1450, namely quinoline **9** and benzothiazole **5**, with 2.5-fold and 16-fold enhancements, respectively. In principle the reduced PDE3-inhibitory activity observed for benzimidazole **6** might reflect a conformational bias arising from establishment of an intramolecular hydrogen bond between the imidazole ring and the dihydropyridazinone N(1)-center for the 1*H*-benzo[*d*]imidazol-7-yl tautomer of **6** (shown in Scheme 6). Alternatively an intramolecular hydrogen bond of this type might partially compromise the ligand's interaction with the purine-scanning glutamine because the X-ray

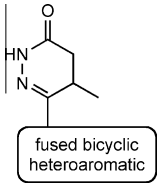
Table 1
SAR survey for optimization of heterobicycle-pyridazinones (in vitro)

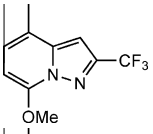
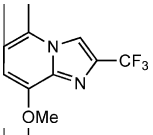
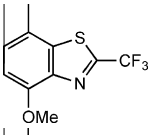
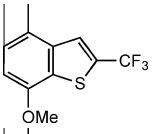
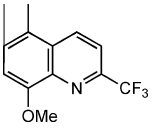


Compound	Fused heterobicycle	Inhibition IC_{50}[a] (μM)		Compound	Fused heterobicycle	Inhibition IC_{50}[a] (μM)	
		PDE3A	PDE4B			PDE3A	PDE4B
KCA-1450[b] (racemic form of KCA-1490)		2.38[c]	0.070[c]	**6**		22.2	0.30
1		10.2	0.43	**7**		19.8	0.38
2		1.45	0.26	**8**		2.85	0.28
3		5.74	1.01	**9**		0.97	0.35
4		6.43	1.52	**60**[b]		20.7	3.49
5		0.15	0.021	**61**[b]		11.3[c]	0.47[c]

[a] Enzyme assays were performed using the core catalytic domains from the PDE3A and PDE4B isoforms according to previously procedures.[18] Data reported are the mean of at least three experiments. Roflumilast was used as a positive control [IC_{50}(PDE4B core cat. domain) 0.0011 μM; IC_{50} (PDE3A core cat. domain) >100 μM].
[b] KCA-1450, **60** and **61** were prepared according to the literature (experimental detail is provided in the Supplementary data).[8a,21]
[c] Pharmacological data have been described in the literature.[8a]

Table 2
SAR survey for optimization of heterobicycle-pyridazinones (in vivo)



Compound	Fused heterobicycle	Inhibitory effect on bronchoconstriction[a]		LPS model[b]
		iv (%)	po (%) (1 mg/kg)	po (1 mg/kg)
KCA-1450 (racemic form of KCA-1490)		97	++	100
2		99	+	62
5		97	++	80
8		17	NT[c]	NT[c]
9		99	++	68

[a] The inhibitory activity of compound on histamine-induced bronchoconstriction in guinea pigs was measured according to an established procedure.[22] iv testing was carried out with compounds dosed at 0.1 mg/kg and is expressed as % inhibition of constriction relative to control. po testing was carried out with compounds at 1 mg/kg dosing; (++) and (+) denote respectively ⩾70% inhibition and 30–69% inhibition of bronchoconstriction relative to control.
[b] Anti-inflammatory activity was assessed in the rat LPS model according to an established procedure.[23]
[c] NT = Not tested.

co-crystal structure of another 5-methyldihydropyridazinone inhibitor bound to PDE3 (PDB: 1SO2)[10b] suggests formation of a loose hydrogen bond between N(1) and the purine-scanning glutamine Nε center (cf. binding model, Fig. 2A). However, relative to KCA-1450, benzofuran **7** exhibits nearly as great a reduction in PDE3-inhibitory activity as benzimidazole **6**. Thus a simple rationalization that links the weaker performance of the benzimidazole as a PDE3 inhibitor to internal hydrogen bonding may not be valid. Interestingly the benzothiophene analogue (**8**) of benzofuran **7** retains essentially all of the PDE3-inhibitory potency of KCA-1450. Taken together with the enhanced activity of quinoline **9** and benzothiazole **5** this may actually indicate that the performance of these compounds is critically affected by the interaction between the inhibitor and the 'hydrophobic clamp'[19] of the PDE catalytic pocket, a conserved feature in PDE3 and PDE4 comprising phenylalanine and isoleucine residues (marked in Fig. 2) that facilitate substrate binding by packing against the two faces of the cyclic nucleotide's purine subunit. Thus, the expanded ring system of the quinoline analogue (**9**) may provide enhanced surface contact between ligand and protein in this region, while the position, size and polarisability of the sulfur center in benzothiazole **5** may potentially allow optimal interaction, thereby accounting for the marked improvement of this compound's in vitro PDE3-inhibitory activity over the parent structure, KCA-1450. An intramolecular non-bonded S···N interaction[20] between the benzothiazole sulfur and dihydropyridazinone N(1) center may also need to be considered in rationalizing the PDE3-inhibitory performance of **5**. In principle, such an interaction might result in controlled conformation and improved fit to the PDE3 catalytic pocket, but the nature of the conformational control would likely be similar to that arising from the possible intramolecular hydrogen bond discussed above for benzimidazole **6**, where the inhibitory potency for PDE3 is 148-fold weaker.

Our earlier SAR data for the pyrazolo[1,5-*a*]pyridine series related to KCA-1450 had strongly implicated a binding mode for the compounds in PDE4 that was distinct from PDE3 (Fig. 2).[8] Key hydrogen bond acceptor roles were invoked for the N(1) center of the pyrazolopyridine subunit and the methoxy group in engaging the side chain amide NH_2 of the purine-scanning glutamine. Thus it is postulated that in PDE4 it is the pyrazolopyridine as opposed to the pyridazinone ring that anchors the inhibitor to this important residue in the catalytic pocket, and it was for this reason that we focused on pyrazolopyridine replacements in our target compound set (**1**–**9**) that conserve a heteroatom at the position corresponding to the pyrazolopyridine N(1) center in KCA-1450. The importance of this heteroatom for the PDE4-inhibitory activity of the compounds is clearly illustrated with the 2-ethyl-5-methoxyindolizine analogue of KCA-1450 (**60**, Table 1), a compound that we

have previously described.[21] Indolizine **60** and KCA-1450 are not fully isosteric, but a direct comparison can be made between **60** and pyrazolopyridine **61** (Table 1),[8a] in which the trifluoromethyl group of KCA-1450 is replaced by ethyl. This reveals a 7.4-fold loss in PDE4-inhibitory activity associated with deletion of the pyrazolopyridine N(1) center; PDE3 inhibition is less severely compromised by this structural change, however, and indolizine **60** is 1.8-fold less active than pyrazolopyridine **61** for inhibition of PDE3 in our assays. Interestingly, with the exception of benzothiazole **5**, all of the pyrazolopyridine replacement analogues in our target set (**1**–**4**, **6**–**9**) show reduced PDE4-inhibitory potency relative to KCA-1450 (at least as measured against the PDE4B core catalytic domain). Compounds **1**, **2**, **6**, **7**, **8** and **9** exhibit remarkably similar levels of PDE4 inhibition with IC_{50}s in the range 0.26–0.43 μM; the two benzoxazoles (**3** and **4**) perform rather less well. It is difficult to rationalize the structure–activity relationships for PDE4 inhibition with this compound set, but the three-fold enhancement in the activity of benzothiazole **5** (relative to KCA-1450) may also reflect improved interactions within the 'hydrophobic clamp' of the PDE4 catalytic pocket as proposed for PDE3 (vide supra). In the context of the heterobicycle-dihydropyridazinone series, benzothiazole may therefore constitute a privileged scaffold for construction of compounds with dual PDE3/4-inhibitory activity.

We have already demonstrated that the parent pyrazolo[1,5-*a*]pyridine structure, KCA-1450, exhibits potent combined bronchodilatory and anti-inflammatory activity, which appears to be linked to the capacity to inhibit both PDE3 and PDE4.[8a] In particular, the (−)-enantiomer (KCA-1490) of this structure, with which the PDE3-inhibitory activity is primarily associated (Fig. 1), strongly outperformed roflumilast in several in vitro and in vivo models used for pharmacological profiling of drugs targeted to respiratory disease.[8a] Based on this experience, four compounds (**2**, **5**, **8** and **9**) were chosen from the target set presented here and evaluated in an initial screen to assess their capacity to suppress histamine-induced bronchoconstriction (0.1 mg/kg, iv in guinea pig)[22] (Table 2). Of these four compounds, imidazopyridine **2**, benzothiazole **5** and quinoline **9** all performed well (affording a 97–99% suppression of bronchoconstriction vs control), whereas the benzothiophene (**8**) had lower activity. Thus, we selected three compounds (**2**, **5** and **9**) for further evaluation against histamine-induced bronchoconstriction (1 mg/kg, po in guinea pig)[22] and in an LPS model used to assess anti-inflammatory activity (1 mg/kg, po in rat).[23] Both the benzothiazole (**5**) and quinoline (**9**) were effective for suppression of histamine-induced bronchoconstriction under po administration. Imidazopyridine **2** was significantly less effective in this regard however. In the LPS model, benzothiazole **5** showed more potent anti-inflammatory activity than **2** or **9**, although both of these compounds exhibited significant levels of activity. However, benzothiazole **5** was rather less effective than KCA-1450, perhaps because of the vulnerability of the benzothiazole scaffold to metabolism[30] under po administration.

4. Conclusions

In this study we have surveyed the potential of several heterobicycles as replacement subunits for the pyrazolo[1,5-*a*]pyridine core of our dual PDE3/4 inhibitor, KCA-1490. Our initial evaluation has been undertaken with the compounds in racemic form, although their PDE3-inhibitory activity is likely associated primarily with only one of the two enantiomers. Thus in our previous work with the parent pyrazolopyridine[8] the (−)-enantiomer (KCA-1490), to which the pharmacological activity is principally linked, was found to possess 185-fold greater potency over its antipode (KCA-1489) for inhibition of PDE3. Inhibitory potency against PDE4 was comparable for the two enantiomers however (see Fig. 1). As we have discussed elsewhere,[8b] the contrasting eudismic ratios for inhibition of PDE3 and PDE4 likely reflect a preference for distinct binding modes in the interaction of the pyrazolopyridine-pyridazinone structures within the catalytic pockets of PDE3 and PDE4 enzymes (Fig. 2). Our observation of a high eudismic ratio for PDE3 inhibition is consistent with the findings of others, where a strong preference for an (*R*)-configured 5-methyldihydropyridazinone inhibitor subunit has been noted.[10a] Of the heterocycles evaluated in the current study, the benzothiazole (in compound **5**) and quinoline (in compound **9**) showed greatest promise in in vitro enzyme inhibition assays undertaken using the core catalytic domains of PDE3A and PDE4B. Both compounds **5** and **9** potently suppress histamine-induced bronchoconstriction in rodents and show promising anti-inflammatory activity in our preliminary screens. Isosteric benzoxazole, triazolopyridine, imidazopyridine, benzofuran, benzimidazole and benzothiophene replacements for the 5–6 fused pyrazolopyridine core of the parent compound showed less promise. The activity of quinoline **9** highlights the potential of 6–6 fused bicyclic heteroaromatic replacements for the pyrazolopyridine subunit of KCA-1490 as well as benzothiazole replacement. Thus, further studies with the enantiomers of benzothiazole **5** and 6–6 fused bicyclic heteroaromatics, such as quinoline derivatives, are now in progress.

5. Experimental

5.1. General

^{1}H NMR spectra were measured with a JEOL JNM-ECA-400 or -ECX-400 (400 MHz) spectrometer. The chemical shifts are expressed in parts per million (δ value) downfield from tetramethylsilane, using tetramethylsilane ($\delta = 0$) and/or residual solvents such as chloroform ($\delta = 7.26$) as an internal standard. Splitting patterns are indicated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br s, broad singlet. Measurements of mass spectra were performed with a JEOL JMS-SX102X mass spectrometer. Data for elemental analyses are within ±0.3% of the theoretical values, and were determined by a Yanaco CHN-corder MT-6. Unless otherwise noted, all the experiments were carried out using anhydrous solvents. Throughout this study, Merck precoated TLC plates (Silica gel 60 F_{254}, 0.25 mm) were used for thin layer chromatographic (TLC) analysis, and all of the spots were visualized using UV light followed by coloring with phosphomolybdic acid or anisaldehyde reagents. Silica gel 60 N (40–50 μm, neutral; Kanto Chemical Co., Inc., Tokyo, Japan) or Chromatorex® NH DM2035 (200–350 mesh; Fuji Silysia Chemical, Ltd., Aichi, Japan) was used for the flash column chromatography. Alternative synthetic routes to **5** and **6** that exploit Suzuki cross-coupling strategy to install the pyridazinone ring on the benzothiazole and benzimidazole core structures are presented in the accompanying Supplementary data. Experimental procedures for synthesis of KCA-1450, **60** and **61** are also detailed in the Supplementary data. A number of the synthetic routes involved N-amination reactions of pyridine substrates using *O*-mesitylenesulfonylhydroxylamine (MSH).[24] **CAUTION**: MSH is explosively unstable as a dry powder and is best handled in CH_2Cl_2 solution.[25]

5.2. Synthetic Chemistry

5.2.1. 1,2-Diamino-3-(ethoxycarbonyl)pyridinium 2,4,6-trimethylbenzenesulfonate (11)

To a stirred solution of ethyl *O*-mesitylenesulfonylacetohydroxamate[24] (28.3 g, 99.3 mmol) in dioxane (40 mL) was added 70% $HClO_4$ (14 mL) at 0 °C. After stirring for 0.5 h at the same temperature, ice-water was added to the reaction mixture and the resulting precipitate collected by filtration. The wet filter cake of MSH

(**CAUTION**) was dissolved in CH_2Cl_2; the organic layer was separated and quickly dried over $MgSO_4$. The resulting solution of MSH was slowly added to a stirred solution of **10** (13.8 g, 82.7 mmol) in CH_2Cl_2 (40 mL) at 0 °C. After stirring for 1 h at room temperature, the reaction mixture was concentrated in vacuo. Diethyl ether was added to the residue and the resulting precipitate was collected by filtration to give **11** (29.0 g, 76.1 mmol, 92%) as a yellow solid. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.34 (3H, t, J = 7.3 Hz), 2.17 (3H, s), 2.50 (6H, s), 4.37 (2H, q, J = 7.3 Hz), 6.74 (2H, s), 6.96 (2H, br s), 7.00 (1H, t, J = 6.7 Hz), 8.41 (1H, dd, J = 6.7, 1.2 Hz), 8.53 (1H, d, J = 6.7 Hz), 8.75 (2H, br s).

5.2.2. Ethyl 2-(trifluoromethyl)-[1,2,4]triazolo[1,5-*a*]pyridine-8-carboxylate (12)

To a stirred solution of **11** (10.0 g, 26.2 mmol) in toluene (75 mL) were added triethylamine (12.5 mL, 89.7 mmol) and TFAA (5.60 mL, 39.3 mmol). After stirring for 13 h under reflux conditions, the mixture was concentrated in vacuo. The residue was dissolved in EtOAc and the resulting mixture was washed with satd $NaHCO_3$ aq followed by brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane–EtOAc = 1.5:1) to give **12** (5.34 g, 20.7 mmol, 79%) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.47 (3H, t, J = 7.3 Hz), 4.54 (2H, q, J = 7.3 Hz), 7.30 (1H, t, J = 7.3 Hz), 8.39 (1H, dd, J = 7.3, 1.2 Hz), 8.81 (1H, dd, J = 7.3, 1.2 Hz).

5.2.3. 8-(((*tert*-Butyldimethylsilyl)oxy)methyl)-2-(trifluoromethyl)-[1,2,4]triazolo[1,5-*a*]pyridine (13)

To a stirred solution of **12** (5.03 g, 19.4 mmol) in THF (150 mL) was slowly added DIBAL-H (0.950 mol/L in *n*-hexane; 40.9 mL, 38.8 mmol) at −10 °C under an argon atmosphere. After stirring for 1 h at the same temperature, the reaction was quenched with 1 mol/L HCl and the resulting mixture was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was dissolved in DMF (100 mL) and imdiazole (3.30 g, 48.5 mmol) and TBSCl (3.51 g, 23.3 mmol) were added at 0 °C. After stirring for 1 h at room temperature, the reaction was quenched with water and the resulting mixture was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane–EtOAc = 30:1) to give **13** (5.90 g, 17.9 mmol, 92% over 2 steps) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.17 (6H, s), 0.99 (9H, s), 5.17 (2H, s), 7.22 (1H, t, J = 6.7 Hz), 7.80 (1H, dd, J = 6.7, 1.2 Hz), 8.53 (1H, dd, J = 6.7, 1.2 Hz).

5.2.4. 8-(((*tert*-Butyldimethylsilyl)oxy)methyl)-5-iodo-2-(trifluoromethyl)-[1,2,4]triazolo[1,5-*a*]pyridine (14)

To a stirred solution of **13** (5.90 g, 17.8 mmol) in THF (120 mL) was slowly added *n*-BuLi (2.71 mol/L in *n*-hexane; 7.23 mL, 19.6 mmol) at −78 °C under an argon atmosphere. After stirring for 0.5 h at the same temperature, 1,2-diiodoethane (5.52 g, 19.6 mmol) was added and the reaction mixture was stirred for 2.5 h at −78 °C. The reaction was quenched with satd $NaHCO_3$ aq and the resulting mixture was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane–EtOAc = 30:1) to give **14** (7.64 g, 16.7 mmol, 94%) as a yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.17 (6H, s), 0.96 (9H, s), 5.14 (2H, d, J = 1.2 Hz), 7.55 (1H, dt, J = 7.9, 1.2 Hz), 7.68 (1H, d, J = 7.9 Hz).

5.2.5. 5-Methoxy-2-(trifluoromethyl)-[1,2,4]triazolo[1,5-*a*]pyridine-8-carbaldehyde (15)

To a stirred solution of **14** (7.64 g, 16.7 mmol) in THF (100 mL) was added TBAF (1.00 mol/L in THF; 33.4 mmol, 33.4 mmol) at 0 °C. After stirring for 1 h at room temperature, the reaction was quenched with water and the resulting mixture was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was dissolved in $CHCl_3$ (150 mL) and MnO_2 (14.5 g, 167 mmol) was added. The reaction mixture was stirred for 5 h at 60 °C and filtered through a pad on Celite®. The filtrate was concentrated in vacuo. The residue was dissolved in MeOH (100 mL) and NaOMe (3.61 g, 66.8 mmol) was added. After stirring for 2 h under reflux conditions, the reaction was quenched with satd NH_4Cl aq and the resulting mixture was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane–EtOAc = 1:1) to give **15** (1.43 g, 5.85 mmol, 35% over 3 steps) as a yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.34 (3H, s), 6.66 (1H, d, J = 7.9 Hz), 8.36 (1H, d, J = 7.9 Hz), 10.59 (1H, s).

5.2.6. 1-(5-Methoxy-2-(trifluoromethyl)-[1,2,4]triazolo[1,5-*a*]pyridin-8-yl)propan-1-one (16)

To a stirred solution of **15** (682 mg, 2.78 mmol) in THF (25 mL) was slowly added EtMgBr (0.970 mol/L in THF; 3.44 mL, 3.34 mmol) at −78 °C under an argon atmosphere. After stirring for 3 h at room temperature, the reaction was quenched with satd $NaHCO_3$ aq and the resulting mixture was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was dissolved in DMSO (9 mL); triethylamine (2.50 mL, 17.9 mmol) and SO_3-pyridine (1.42 g, 8.94 mmol) were then added. After stirring for 1 h at the room temperature, water was added to the reaction mixture and the resulting precipitate was collected by filtration to give **16** (343 mg, 1.25 mmol, 45% over 2 steps) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.27 (3H, t, J = 7.3 Hz), 3.50 (2H, q, J = 7.3 Hz), 4.30 (3H, s), 6.60 (1H, d, J = 8.6 Hz), 8.47 (1H, d, J = 8.6 Hz).

5.2.7. 6-(5-Methoxy-2-(trifluoromethyl)-[1,2,4]triazolo[1,5-*a*]pyridin-8-yl)-5-methyl-4,5-dihydropyridazin-3(2*H*)-one (1)

To a stirred solution of **16** (342 mg, 1.25 mmol) in THF (10 mL) was slowly added LiHMDS (1.00 mol/L in THF; 1.34 mL, 1.34 mmol) at −78 °C under an argon atmosphere. After stirring for 0.5 h at the same temperature, *tert*-butyl bromoacetate (277 μL, 1.88 mmol) was added in one portion and the reaction mixture was stirred for 1 h at room temperature. The reaction was quenched with water and the resulting mixture was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was dissolved in CH_2Cl_2 (15 mL) and treated with TFA (5 mL). The reaction mixture was stirred for 2 h at room temperature and concentrated in vacuo. The residue was dissolved in EtOH (10 mL) and hydrazine monohydrate (182 μL, 3.75 mmol) and AcOH (472 μL, 8.25 mmol) were added. After stirring for 7 h under reflux conditions, the reaction was quenched with water and the resulting mixture was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane–EtOAc = 3:1) to give **1** (166 mg, 0.500 mmol, 40% over 3 steps) as a yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.27 (3H, d, J = 7.3 Hz), 2.54 (1H, dd, J = 17.1, 1.8 Hz), 2.84 (1H, dd, J = 17.1, 6.7 Hz), 4.27 (3H, s), 4.27–4.31 (1H, m), 6.56 (1H, d, J = 8.2 Hz), 8.19 (1H, d, J = 8.2 Hz), 8.58 (1H, br s). HRMS (EI): calcd for $C_{13}H_{12}F_3N_5O_2$ ($[M]^+$) 327.0943, found 327.0943. Anal. calcd for $C_{13}H_{12}F_3N_5O_2$: C, 47.71; H, 3.70; N, 21.40. Found: C, 47.73; H, 3.82; N, 21.10.

5.2.8. 2-Amino-3-methoxy-6-methylpyridine (19)[26]

To a stirred solution of **18** (9.76 g, 63.3 mmol) in DMF (120 mL) were added K_2CO_3 (14.0 g, 101 mmol) and MeI (5.91 mL,

95.0 mmol). After stirring for 2 h at room temperature, the reaction was quenched with water and the resulting mixture was extracted with EtOAc. The organic layer was washed with water followed by brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane–EtOAc = 4:1) to give 3-methoxy-6-methyl-2-nitropyridine (10.1 g) as a white solid.

10% Pd–C (1 g) was added to a solution of 3-methoxy-6-methyl-2-nitropyridine (10.1 g) in EtOAc (300 mL) and the reaction mixture was stirred for 4 h under hydrogen atmosphere (1 atm). The reaction mixture was filtered through a pad of Celite®. The filtrate was concentrated in vacuo to give **19** (8.28 g, 58.9 mmol, 95% over 2 steps) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.33 (3H, s), 3.81 (3H, s), 4.59 (2H, br s), 6.45 (1H, d, J = 7.9 Hz), 6.82 (1H, d, J = 7.9 Hz)

5.2.9. 2-Di(*tert*-butoxycarbonyl)amino-3-methoxy-6-methylpyridine (20)

To a stirred solution of **19** (3.00 g, 21.7 mmol) in MeCN (100 mL) were added $(Boc)_2O$ (28.4 g, 130 mmol), triethylamine (6.05 mL, 43.4 mmol), and DMAP (100 mg, 0.82 mmol). After stirring for 8 h at room temperature, the reaction mixture was concentrated in vacuo. The residue was dissolved in EtOAc and water was added. The organic layer was separated, washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane–EtOAc = 3:1) to give **20** (5.80 g, 17.1 mmol, 79%) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.41 (18H, s), 2.48 (3H, s), 3.81 (3H, s), 7.07 (1H, d, J = 8.6 Hz), 7.14 (1H, d, J = 8.6 Hz).

5.2.10. 6-Bromomethyl-2-di(*tert*-butoxycarbonyl)amino-3-methoxypyridine (21)

To a stirred solution of **20** (6.34 g, 18.7 mmol) in CCl_4 (50 mL) were added NBS (3.67 g, 20.6 mmol) and benzoyl peroxide (20.0 mg, 82.5 μmol) and the reaction mixture was stirred for 4 h under reflux conditions. The resulting precipitate was filtered off and the filtrate was concentrated in vacuo. The resulting solid was recrystallized from EtOAc–*n*-hexane to give **21** (6.33 g, 12.9 mmol, 69%) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.40 (18H, s), 3.86 (3H, s), 4.53 (2H, s), 7.21 (1H, d, J = 8.6 Hz), 7.37 (1H, d, J = 8.6 Hz).

5.2.11. 6-Di(*tert*-butoxycarbonyl)amino-5-methoxypyridine-2-carbaldehyde (22)

To a stirred suspension of NMO (3.55 g, 30.3 mmol) and 4 Å molecular sieves (powder, 5 g) in MeCN (80 mL) was added a solution of **21** (6.33 g, 15.2 mmol) in MeCN (20 mL). After stirring for 4 h at room temperature, the reaction mixture was filtered through a pad of silica gel. The filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane–EtOAc = 3:1) to give **22** (5.80 g, 17.1 mmol, 79%) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.42 (18H, s), 3.96 (3H, s), 7.35 (1H, d, J = 8.5 Hz), 8.00 (1H, d, J = 8.5 Hz), 9.94 (1H, s).

5.2.12. 6-Di(*tert*-butoxycarbonyl)amino-5-methoxypyridine-2-carboxylic acid (23)

$NaClO_2$ (80%; 2.48 g, 27.4 mmol), $NaH_2PO_4{\cdot}2H_2O$ (1.22 g, 7.83 mmol), and 2-methyl-2-butene (3.70 mL, 35.2 mmol) were added to a solution of **22** (2.76 g, 7.83 mmol) in *tert*-butanol (80 mL) and water (20 mL). After stirring for 4 h at room temperature, the reaction mixture was acidified with 0.5 mol/L HCl and extracted with EtOAc. The organic layer was washed with water followed by brine, dried over Na_2SO_4, and concentrated in vacuo to give **23** (2.95 g, 7.83 mmol, quant.) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.41 (18H, s), 3.97 (3H, s), 7.42 (1H, d, J = 8.6 Hz), 8.22 (1H, d, J = 8.6 Hz), 10.24 (1H, br s).

5.2.13. 6-Di(*tert*-butoxycarbonyl)amino-*N*,5-dimethoxy-*N*-methylpicolinamide (24)

To a stirred solution of **23** (1.62 g, 4.40 mmol) in DMF (50 mL) were added EDCI·HCl (1.27 g, 6.60 mmol), *N*,*O*-dimethylhydroxylamine hydrochloride (644 mg, 6.60 mmol), $HOBt{\cdot}H_2O$ (1.01 g, 6.60 mmol), and DIEA (3.45 mL, 19.8 mmol) and the reaction mixture was stirred for 2 h at room temperature. The reaction was then quenched with water and the resulting mixture was extracted with EtOAc. The organic layer was washed with water followed by brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane–EtOAc = 1:1 to EtOAc) to give **24** (1.60 g, 3.87 g, 88%) as a colorless amorphous powder. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.40 (18H, s), 3.42 (3H, br s), 3.81 (3H, s), 3.90 (3H, s), 7.29 (1H, d, J = 8.6 Hz), 7.82 (1H, br s).

5.2.14. 1-(6-Di(*tert*-butoxycarbonyl)amino-5-methoxypyridin-2-yl)propan-1-one (25)

To a stirred solution of **24** (1.60 g, 3.89 mmol) in THF (25 mL) was slowly added EtMgBr (0.970 mol/L in THF; 12.0 mL, 11.7 mmol) at −78 °C under an argon atmosphere and the reaction mixture was allowed to gradually warm to room temperature over 3 h. The reaction was quenched with satd NH_4Cl aq and the resulting mixture was extracted with EtOAc. The organic layer was washed with water followed by brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was dissolved in MeCN (50 mL), and treated with $(Boc)_2O$ (3.39 g, 15.6 mmol), triethylamine (1.08 mL, 7.78 mmol) and DMAP (20 mg, 0.164 mmol). After stirring for 2 h at room temperature, the reaction mixture was concentrated in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane–EtOAc = 3:1 to EtOAc) to give **25** (1.26 g, 3.31 mmol, 85% over 2 steps) as a pale yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.19 (3H, t, J = 7.3 Hz), 1.41 (18H, s), 3.14 (2H, q, J = 7.3 Hz), 3.92 (3H, s), 7.29 (1H, d, J = 8.6 Hz), 8.06 (1H, d, J = 8.6 Hz).

5.2.15. Ethyl 4-(6-di(*tert*-butoxycarbonyl)amino-5-methoxypyridin-2-yl)-3-methyl-4-oxobutanoate (26)

To a stirred solution of **25** (108 mg, 0.284 mmol) in THF (5 mL) was slowly added LiHMDS (1.00 mol/L in THF; 0.312 mL, 0.312 mmol) at 0 °C under an argon atmosphere. After stirring for 0.5 h at the same temperature, ethyl bromoacetate (39.4 mL, 0.36 mmol) was added at −78 °C and the reaction mixture was allowed to gradually warm to room temperature over 3 h. The reaction was quenched with satd NH_4Cl aq and the resulting mixture was extracted with EtOAc. The organic layer was washed with water followed by brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane–EtOAc = 3:1 to EtOAc) to give **26** (84.0 mg, 179 mmol, 63%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.20 (3H, t, J = 7.3 Hz), 1.23 (3H, d, J = 7.3 Hz), 1.40 (18H, s), 2.48 (1H, dd, J = 16.5, 6.1 Hz), 2.89 (1H, dd, J = 16.5, 8.6 Hz), 3.93 (3H, s), 4.03–4.16 (2H, m), 4.25–4.37 (1H, m), 7.30 (1H, d, J = 8.6 Hz), 8.07 (1H, d, J = 8.6 Hz).

5.2.16. 6-(6-amino-5-methoxypyridin-2-yl)-5-methyl-4,5-dihydropyridazin-3(2*H*)-one (27)

To a stirred solution of **26** (733 mg, 1.57 mmol) in MeOH (30 mL) was added 4 mol/L KOH aq (1.57 mL, 6.29 mmol) and the reaction mixture was stirred for 4 h at 60 °C. The reaction mixture was concentrated in vacuo, acidified with 1 mol/L HCl and extracted with EtOAc. The organic layer was washed with water followed by brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was dissolved in EtOH (50 mL) and treated with hydrazine monohydrate (0.230 mL, 4.71 mmol). After stirring for 3 h under reflux conditions, the reaction mixture was concentrated in vacuo,

the residue redissolved in EtOAc and the solution was filtered through a pad of silica gel. The filtrate was concentrated in vacuo. The residue was dissolved in CH_2Cl_2 (15 mL), and treated with TFA (15 mL). After stirring for 12 h at room temperature, the reaction mixture was concentrated in vacuo and neutralized with satd $NaHCO_3$ aq. The resulting precipitate was collected by filtration to give **27** (328 mg, 1.40 mmol, 89% over 3 steps) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.21 (3H, d, J = 7.3 Hz), 2.43 (1H, d, J = 16.5 Hz), 2.69 (1H, dd, J = 16.5, 6.7 Hz), 3.61–3.73 (1H, m), 3.88 (3H, s), 5.04 (2H, br s), 6.93 (1H, d, J = 7.9 Hz), 7.21 (1H, d, J = 7.9 Hz), 9.17 (1H, br s).

5.2.17. 5-Methyl-6-(2-(trifluoromethyl)imidazo[1,2-*a*]pyridin-5-yl)-4,5-dihydropyridazin-3(2*H*)-one (2)

To a stirred solution of **27** (328 mg, 1.40 mmol) in EtOH (50 mL) was added 3-bromo-1,1,1-trifluoroacetone (535 mg, 2.80 mmol) and the reaction mixture was stirred for 5 h at 70 °C. Additional 3-bromo-1,1,1-trifluoroacetone (535 mg, 2.80 mmol) was added and the reaction mixture was stirred for a further 72 h at 70 °C. The reaction mixture was then concentrated in vacuo; $CHCl_3$ and 1 mol/L HCl were added, and the organic layer was separated. The organic extract was washed with satd $NaHCO_3$ aq followed by brine, dried over Na_2SO_4, and concentrated in vacuo. The resulting solid was recrystallized from $CHCl_3$-*i*-Pr_2O to give **2** (87.1 mg, 266 μmol, 19%) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.35 (3H, d, J = 7.3 Hz), 2.58 (1H, dd, J = 17.1, 1.2 Hz), 2.80 (1H, dd, J = 17.1, 6.7 Hz), 3.37–3.48 (1H, m), 4.11 (3H, s), 6.66 (1H, d, J = 7.9 Hz), 7.26 (1H, d, J = 7.9 Hz), 8.77 (1H, br s), 9.34 (1H, s). LRMS (EI): 326 ([M]$^+$). Anal. calcd for $C_{14}H_{13}F_3N_4O_2$: C, 51.54; H, 4.02; N, 17.17. Found: C, 51.34; H, 3.95; N, 17.04.

5.2.18. 3-Methoxy-2-nitrophenol (29)[27]

To a stirred solution of **28** (25.0 g, 161 mmol) in DMF (500 mL) were added K_2CO_3 (6.80 g, 49.2 mmol) and MeI (11.0 mL, 177 mmol). After stirring for 7 h at room temperature, the reaction mixture was concentrated in vacuo and diluted with water. The resulting mixture was washed with EtOAc; the aqueous layer was acidified with 1 mol/L HCl and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane–EtOAc = 2:1) to give **29** (8.78 g, 51.9 mmol, 32%) as a yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.95 (3H, s), 6.54 (1H, dd, J = 8.6, 1.2 Hz), 6.71 (1H, dd, J = 8.6, 1.2 Hz), 7.40 (1H, t, J = 8.6 Hz), 10.22 (1H, s).

5.2.19. 2-Amino-3-methoxyphenol (30)[28]

To a stirred solution of **29** (8.78 g, 51.9 mmol) in EtOH (250 mL) was added 10% Pd–C (1 g) and the reaction mixture was stirred for 2.5 h at room temperature under a hydrogen atmosphere (1 atm). The reaction mixture was filtered through a pad of Celite®. The filtrate was concentrated in vacuo to give **30** (7.09 g, 51.0 mmol, 98%) as a yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.85 (3H, s), 6.46 (1H, d, J = 7.9 Hz), 6.48 (1H, d, J = 7.9 Hz), 7.40 (1H, t, J = 7.9 Hz), 10.22 (1H, s).

5.2.20. 4-Methoxy-2-(trifluoromethyl)benzo[*d*]oxazole (31)

To a stirred solution of **30** (2.00 g, 14.4 mmol) in toluene (53 mL) was added TFAA (2.20 mL, 15.6 mmol). After stirring for 5 h under reflux conditions, *p*-toluenesulfonic acid monohydrate (274 mg, 1.44 mmol) was added and the reaction mixture was stirred for 2 h under reflux conditions with a Dean-Stark trap. The mixture was then cooled to room temperature, quenched with satd $NaHCO_3$ aq and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane–EtOAc = 4:1) to give **31** (1.84 g, 8.47 mmol, 59%) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.08 (3H, s), 6.90 (1H, d, J = 7.9 Hz), 7.25 (1H, d, J = 8.6 Hz), 7.46 (1H, t, J = 8.6, 7.9 Hz). LRMS (EI): 217 ([M]$^+$).

5.2.21. 1-(4-Methoxy-2-(trifluoromethyl)benzo[*d*]oxazol-7-yl)propan-1-one (32)

To a stirred suspension of $AlCl_3$ (2.21 g, 16.6 mmol) in CH_2Cl_2 (55 mL) were added propionyl chloride (1.45 mL, 16.7 mmol) and **31** (1.20 g, 4.39 mmol). After stirring for 3 days at room temperature, 5% HCl was added and the resulting mixture was extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane–EtOAc = 4:1) to give **32** (955 mg, 3.50 mmol, 63%) as a pale yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.29 (3H, t, J = 7.3 Hz), 3.17 (2H, q, J = 7.3 Hz), 4.15 (3H, s), 6.98 (1H, d, J = 8.6 Hz), 8.17 (1H, d, J = 8.6 Hz). LRMS (EI): 273 ([M]$^+$).

5.2.22. 2-Bromo-1-(4-methoxy-2-(trifluoromethyl) benzo[*d*]oxazol-7-yl)propan-1-one (33)

To a stirred solution of **32** (92.0 mg, 0.337 mmol) in EtOAc (4.2 mL) was added $CuBr_2$ (150 mg, 0.672 mmol). After stirring for 2 h under reflux conditions, the reaction mixture was filtered through a pad of Celite®. The filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane–EtOAc = 3:1) to give **33** (72.3 mg, 205 μmol, 61%) as a pale yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.97 (3H, d, J =6.7 Hz), 4.18 (3H, s), 5.45 (1H, q, J = 6.7 Hz), 7.03 (1H, d, J = 8.6 Hz), 8.25 (1H, d, J = 8.6 Hz). LRMS (EI): 351 ([M]$^+$).

5.2.23. 6-(4-Methoxy-2-(trifluoromethyl)benzo[*d*]oxazol-7-yl)-5-methyl-4,5-dihydropyridazin-3(2*H*)-one (3)

To a stirred solution of di-*tert*-butyl malonate (856 mg, 3.96 mmol) in DMF (15 mL) was added 60% NaH (198 mg, 4.95 mmol) at 0 °C under an argon atmosphere. After stirring for 0.5 h at room temperature, a solution of **33** (541 mg, 1.54 mmol) in DMF (15 mL) was slowly added at 0 °C. The reaction mixture was then stirred for 1 h at room temperature and quenched with satd NH_4Cl aq The resulting mixture was extracted with EtOAc. The organic layer was washed with water followed by brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane–EtOAc = 3:1) to give the intermediate di-*tert* butyl ester which was subsequently dissolved in CH_2Cl_2 (10 mL) and treated with TFA (5 mL). After stirring for 1 h at room temperature, the reaction mixture was concentrated in vacuo. The residue was then dissolved in xylene (20 mL) and the resulting solution was stirred for 2 h at 150 °C. *p*-Toluenesulfonic acid monohydrate (296 mg, 1.56 mmol) and *tert*-butyl carbazate (618 mg, 4.68 mmol) were then added. After stirring for 3 h under reflux conditions with a Dean-Stark trap. The reaction mixture was concentrated in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane–acetone = 3:2) to give **3** (157 mg, 0.480 mmol, 31% over 4 steps) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.32 (3H, d, J = 7.3 Hz), 2.55 (1H, dd, J = 17.1, 1.2 Hz), 2.80 (1H, dd, J = 17.1, 6.7 Hz), 3.51–3.59 (1H, m), 4.12 (3H, s), 6.96 (1H, d, J = 9.2 Hz), 7.86 (1H, d, J = 9.2 Hz), 8.65 (1H, s). LRMS (FAB): 328 ([M+H]$^+$). Anal. calcd for $C_{14}H_{12}F_3N_3O$: C, 51.38; H, 3.70; N, 12.84. Found: C, 51.08; H, 3.69; N, 12.68.

5.2.24. 2,2,2-Trifluoro-*N*-(2-hydroxy-3-methoxyphenyl) acetamide (35)

To a stirred solution of **34** (1.92 g, 13.8 mmol) in toluene (50 mL) was added TFAA (2.10 mL, 15.2 mmol). After stirring for 12 h under reflux conditions, water was added and the resulting mixture was extracted with EtOAc. The organic layer was washed with water followed by brine, dried over Na_2SO_4, and concentrated

in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane–EtOAc = 3:1) to give **35** (3.11 g, 13.3 mmol, 96%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.92 (3H, s), 5.78 (1H, s), 6.76 (1H, dd, J = 8.8, 1.2 Hz), 6.91 (1H, t, J = 8.8 Hz), 7.87 (1H, dd, J = 8.8, 1.2 Hz), 8.35 (1H, br s).

5.2.25. 7-Methoxy-2-(trifluoromethyl)benzo[*d*]oxazole (36)

To a stirred solution of **35** (2.60 g, 11.1 mmol) in toluene (120 mL) was added *p*-toluenesulfonic acid monohydrate (2.10 g, 11.1 mmol). After stirring for 16 h under reflux conditions with a Dean-Stark trap. The reaction mixture was cooled to room temperature, poured into satd $NaHCO_3$ aq and extracted with EtOAc. The organic layer was washed with water followed by brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane–EtOAc = 4:1) to give **36** (2.06 g, 9.55 mmol, 86%) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.06 (3H, s), 7.02 (1H, dd, J = 8.0, 1.2 Hz), 7.39 (1H, t, J = 8.0 Hz), 7.46 (1H, dd, J = 8.0, 1.2 Hz).

5.2.26. 1-(7-Methoxy-2-(trifluoromethyl)benzo[*d*]oxazol-4-yl)propan-1-one (37)

To a stirred solution of $TiCl_4$ (1.00 mol/L in CH_2Cl_2; 2.90 mL, 2.90 mmol) were added propionyl chloride (0.254 mL, 2.91 mmol) and a solution of **36** (420 mg, 1.93 mmol) in CH_2Cl_2 (5 mL). After stirring for 2 days at room temperature, 5% HCl was added and the resulting mixture was extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane–EtOAc = 5:1) to give **37** (422 mg, 1.55 mmol, 80%) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.26 (3H, t, J = 7.3 Hz), 3.39 (2H, q, J = 7.3 Hz), 4.11 (3H, s), 7.08 (1H, d, J = 8.6 Hz), 8.13 (1H, d, J = 8.6 Hz). LRMS (EI): 273 ($[M]^+$).

5.2.27. 2-Bromo-1-(7-methoxy-2-(trifluoromethyl)benzo[*d*]oxazol-4-yl)propan-1-one (38)

Compound **38** was prepared from **37** by the method described for the preparation of compound **33** from **32**. Yield: 94% (white solid). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.95 (3H, d, J = 6.7 Hz), 4.13 (3H, s), 6.20 (1H, q, J = 6.7 Hz), 7.12 (1H, d, J = 8.6 Hz), 8.22 (1H, d, J = 8.6 Hz). LRMS (EI): 351 ($[M]^+$).

5.2.28. 6-(7-Methoxy-2-(trifluoromethyl)benzo[*d*]oxazol-4-yl)-5-methyl-4,5-dihydropyridazin-3(2*H*)-one (4)

Compound **4** was prepared from **38** by the method described for the preparation of compound **3** from **33**. Yield: 45% over 4 steps (white solid). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.27 (3H, d, J = 7.3 Hz), 2.53 (1H, dd, J = 17.1, 1.2 Hz), 2.82 (1H, dd, J = 17.1, 7.3 Hz), 4.06–4.16 (1H, m), 4.09 (3H, s), 7.06 (1H, d, J = 8.6 Hz), 7.91 (1H, d, J = 8.6 Hz), 8.65 (1H, s). LRMS (EI): 327 ($[M]^+$). Anal. calcd for $C_{14}H_{12}F_3N_3O$: C, 51.38; H, 3.70; N, 12.84. Found: C, 51.54; H, 3.65; N, 12.88.

5.2.29. 4-Methoxy-2-(trifluoromethyl)benzo[*d*]thiazole (40)

A mixture of **39** (50.0 g, 277 mmol) in 1 mol/L KOH aq (275 mL, 275 mmol) was stirred for 25 h under reflux conditions under an argon atmosphere. Ice water was added and the mixture was washed with CH_2Cl_2. The aqueous layer was acidified with conc. HCl (to approximately pH 3) and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and concentrated in vacuo to give 2-amino-3-methoxybenzenethiol (36.3 g). To a mixture of 2-amino-3-methoxybenzenethiol (36.3 g) in TFA (50 mL) was added trimethylsilyl polyphosphate (50 mL). After stirring for 2 h at 95 °C, the reaction mixture was concentrated in vacuo; water was added, and the resulting mixture was extracted with EtOAc. The organic layer was washed with satd $NaHCO_3$ aq, water followed by brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane–EtOAc = 30:1) to give **40** (44.7 g, 192 mmol, 69% over 2 steps) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.08 (3H, s), 6.92 (1H, d, J = 8.6 Hz), 7.44–7.76 (2H, m).

5.2.30. 1-(4-Methoxy-2-(trifluoromethyl)benzo[*d*]thiazol-7-yl)propan-1-one (41)

To a stirred solution of $AlCl_3$ (18.9 g, 142 mmol) in nitromethane (100 mL) was added propionyl chloride (14.2 mL, 163 mmol) at 0 °C followed by a solution of **40** (11.0 g, 47.2 mmol) in nitromethane (10 mL) at room temperature. After stirring for 11 h at room temperature, the reaction mixture was poured into ice. The resulting precipitate was collected by filtration, washed with water, and dried in vacuo. The solid was triturated with *n*-hexane and filtered to give **41** (13.7 g, 47.2 mmol, quant.) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.31 (3H, t, J = 7.3 Hz), 3.14 (2H, q, J = 7.3 Hz), 4.18 (3H, s), 7.08 (1H, d, J = 8.6 Hz), 8.20 (1H, d, J = 8.6 Hz).

5.2.31. 6-(4-Methoxy-2-(trifluoromethyl)benzo[*d*]thiazol-7-yl)-5-methyl-4,5-dihydropyridazin-3(2*H*)-one (5)

Compound **5** was prepared from **41** by the method described for the preparation of compound **1** from **16**. Yield: 30% over 3 steps (white solid). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.34 (3H, d, J = 7.3 Hz), 2.59 (1H, d, J = 17.1 Hz), 2.81 (1H, dd, J = 17.1, 6.7 Hz), 3.55–3.58 (1H, m), 4.16 (3H, s), 7.09 (1H, d, J = 8.6 Hz), 7.76 (1H, d, J = 8.6 Hz), 8.68 (1H, s). HRMS (EI): calcd for $C_{14}H_{12}F_3N_3O_2S$ ($[M]^+$) 343.0602, found 343.0594.

5.2.32. *tert*-Butyl 3-methoxy-2-nitrophenylcarbamate (43)

To a stirred solution of **42** (10.0 g, 50.7 mmol) in *tert*-butanol (50 mL) were added DPPA (11.5 mL, 53.4 mmol) and triethylamine (7.40 mL, 53.1 mmol). After stirring for 10 h under reflux conditions, the reaction mixture was concentrated in vacuo. The residue was dissolved in EtOAc; the resulting mixture was washed with satd $NaHCO_3$ aq followed by brine, dried over Na_2SO_4, and concentrated in vacuo. The resulting precipitate was triturated with *n*-hexane and filtered to give **43** (13.3 g, 49.6 mmol, 98%) as a yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.50 (9H, s), 3.90 (3H, s), 6.71 (1H, dd, J = 8.6, 1.2 Hz), 7.39 (1H, t, J = 8.6 Hz), 7.55 (1H, br s), 7.77 (1H, dd, J = 8.6, 1.2 Hz).

5.2.33. 3-Methoxy-2-nitroaniline (44)

To a stirred solution of **43** (13.3 g, 49.6 mmol) in CH_2Cl_2 (100 mL) was added TFA (20 mL) and the reaction mixture was stirred for 4 h at room temperature. The mixture was then concentrated in vacuo to a residue that was dissolved in EtOAc. The resulting solution was poured into satd $NaHCO_3$ aq and the organic layer was separated, washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The resulting precipitate was triturated with *n*-hexane and filtered to give **44** (7.55 g, 44.9 mmol, 91%) as a yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.88 (3H, s), 6.31 (1H, dd, J = 8.6, 1.2 Hz), 7.36 (1H, dd, J = 8.6, 1.2 Hz), 7.16 (1H, t, J = 8.6 Hz).

5.2.34. 4-Methoxy-2-(trifluoromethyl)-1*H*-benzo[*d*]imidazole (45)

To a stirred solution of **44** (7.75 g, 44.9 mmol) in EtOAc (100 mL), EtOH (100 mL) with a few drops of AcOH was added 10% Pd–C (775 mg). After stirring for 11 h at room temperature under a hydrogen atmosphere (1 atm), the reaction mixture was filtered through a pad of Celite®. The filtrate was concentrated in vacuo to give the intermediate diamine (6.49 g) which was then dissolved in TFA (75 mL) initially at 0 °C. The resulting mixture was subsequently stirred for 4 h under reflux conditions before concentration to a residue in vacuo. The residue was dissolved in EtOAc and the resulting solution was poured into satd $NaHCO_3$

aq The organic layer was separated, washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The resulting powder was triturated with *n*-hexane and filtered to give **45** (8.69 g, 40.2 mmol, 90% over 2 steps) as a brown solid. ^{1}H NMR (CD_3OD, 400 MHz): δ 4.01 (3H, s), 6.89 (1H, d, *J* = 8.0 Hz), 7.24 (1H, d, *J* = 8.0 Hz), 7.32 (1H, t, *J* = 8.0 Hz).

5.2.35. 7-Bromo-4-methoxy-2-(trifluoromethyl)-1*H*-benzo[*d*]imidazole (46)

To a stirred solution of **45** (5.54 g, 25.6 mmol) in $CHCl_3$ (130 mL) was added NBS (5.02 g, 28.2 mmol). After stirring for 2 h at room temperature, the reaction was quenched with satd $NaHCO_3$ aq and the organic layer was separated. The organic extract was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane–EtOAc = 4:1) to give **46** (4.72 g, 16.0 mmol, 63%) as a brown solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.00 (3H, s), 6.71 (1H, d, *J* = 8.6 Hz), 7.46 (1H, d, *J* = 8.6 Hz), 10.1 (1H, br s).

5.2.36. 1-(4-Methoxy-2-(trifluoromethyl)-1*H*-benzo[*d*]imidazol-7-yl)propan-1-one (47)

To a stirred solution of **46** (800 mg, 2.71 mmol) in THF (20 mL) was slowly added *n*-BuLi (1.58 mol/L in *n*-hexane; 3.90 mL, 6.16 mmol) at −78 °C under an argon atmosphere. After stirring for 1 h at the same temperature, *N*,*N*-dimethylpropionamide (0.890 mL, 8.10 mmol) was added in one portion at −78 °C and the reaction mixture was allowed to gradually warm to room temperature over 3 h. The reaction was quenched with satd NH_4Cl aq and the resulting mixture was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$) to give **47** (342 mg, 1.26 mmol, 46%) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.29 (3H, t, *J* = 7.3 Hz), 3.08 (2H, q, *J* = 7.3 Hz), 4.15 (3H, s), 6.80 (1H, d, *J* = 8.6 Hz), 7.96 (1H, d, *J* = 8.6 Hz), 11.40 (1H, br s). LRMS (EI): 272 ([M]$^+$).

5.2.37. 6-(4-Methoxy-2-(trifluoromethyl)-1*H*-benzo[*d*]imidazol-7-yl)-5-methyl-4,5-dihydropyridazin-3(2*H*)-one (6)

Compound **6** was prepared from **47** by the method described for the preparation of compound **1** from **16**. Yield: 68% over 3 steps (pale yellow solid). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.33 (3H, d, *J* = 7.3 Hz), 2.57 (1H, d, *J* = 17.1 Hz), 2.79 (1H, dd, *J* = 17.1, 6.7 Hz), 3.50–3.57 (1H, m), 4.11 (3H, s), 6.81 (1H, d, *J* = 8.6 Hz), 7.55 (1H, d, *J* = 8.6 Hz), 8.79 (1H, br s), 11.30 (1H, br s). HRMS (EI): calcd for $C_{14}H_{13}F_3N_4O_2$ ([M]$^+$) 326.0991, found 326.0961.

5.2.38. 4-Bromo-7-methoxy-2-(trifluoromethyl)benzofuran (49)

To a stirred suspension of **48**[15,29] (2.20 g, 3.94 mmol) in toluene (20 mL) were added TFAA (1.62 mL, 11.5 mmol) and triethylamine (1.64 mL, 11.8 mmol). After stirring for 5 h under reflux conditions, the reaction was quenched with water and the resulting mixture was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane–EtOAc = 10:1) to give **49** (1.01 g, 3.42 mmol, 87%) as a pale yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.01 (3H, s), 6.82 (1H, d, *J* = 8.6 Hz), 7.20–7.21 (1H, m), 7.38 (1H, d, *J* = 8.6 Hz).

5.2.39. 1-(7-Methoxy-2-(trifluoromethyl)benzofuran-4-yl)propan-1-one (50)

Compound **50** was prepared from **49** by the method described for the preparation of compound **47** from **46**. Yield: 35% (white solid). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.26 (3H, t, *J* = 7.3 Hz), 3.05 (2H, q, *J* = 7.3 Hz), 4.10 (3H, s), 6.93 (1H, d, *J* = 8.6 Hz), 7.90 (1H, d, *J* = 8.6 Hz), 8.01 (1H, q, *J* = 1.2 Hz).

5.2.40. 6-(7-Methoxy-2-(trifluoromethyl)benzofuran-4-yl)-5-methyl-4,5-dihydropyridazin-3(2*H*)-one (7)

Compound **7** was prepared from **50** by the method described for the preparation of compound **1** from **16**. Yield: 37% over 3 steps (white solid). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.30 (3H, d, *J* = 7.3 Hz), 2.52 (1H, dd, *J* = 16.8, 0.6 Hz), 2.77 (1H, dd, *J* = 16.8, 6.7 Hz), 3.40–3.48 (1H, m), 4.07 (3H, s), 6.93 (1H, d, *J* = 8.3 Hz), 7.45 (1H, d, *J* = 8.3 Hz), 7.89–7.90 (1H, m), 8.56 (1H, s). HRMS (EI): calcd for $C_{15}H_{13}F_3N_2O_3$ ([M]$^+$) 326.0878, found 326.0886. Anal. calcd for $C_{15}H_{13}F_3N_2O_3$: C, 55.22; H, 4.02; N, 8.59. Found: C, 55.13; H, 4.02; N, 8.42.

5.2.41. *O*-(3-Bromo-2-formyl-6-methoxyphenyl) dimethylcarbamothioate (52)

To a stirred solution of **51** (231 mg, 1.00 mmol) in DMF (4 mL) were added DABCO (224 mg, 2.00 mmol) and dimethylthiocarbamoyl chloride (247 mg, 2.00 mmol). After stirring for 12 h at room temperature, the reaction mixture was concentrated in vacuo. EtOAc and water were added, and the organic layer was separated. The organic extract was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The resulting solid was triturated with diisopropyl ether and filtered to give **52** (258 mg, 0.810 mmol, 81%) as a pale yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.40 (3H, s), 3.45 (3H, s), 3.86 (3H, s), 7.05 (1H, d, *J* = 8.6 Hz), 7.51 (1H, d, *J* = 8.6 Hz), 10.20 (1H, s). LRMS (EI): 317 ([M]$^+$).

5.2.42. *S*-3-Bromo-2-formyl-6-methoxyphenyl dimethylcarbamothioate (53)

A solution of **52** (5.78 g, 18.2 mmol) in diphenyl ether (57 mL) was stirred for 0.5 h at 200 °C. The reaction mixture was cooled to room temperature and purified by silica gel column chromatography (*n*-hexane–EtOAc = 1:1) to give **53** (3.28 g, 10.4 mmol, 57%) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.00 (3H, br s), 3.16 (3H, br s), 3.89 (3H, s), 6.97 (1H, d, *J* = 9.2 Hz), 7.64 (1H, d, *J* = 9.2 Hz), 10.25 (1H, s). LRMS (EI): 317 ([M]$^+$).

5.2.43. (6-Bromo-2-mercapto-3-methoxyphenyl)methanol (54)

To a stirred solution of **53** (2.44 g, 7.67 mmol) in *i*-PrOH (60 mL) was added 1 mol/L NaOH aq (15.3 mL, 15.3 mmol). After stirring for 0.5 h at 60 °C, the reaction mixture was concentrated in vacuo. The residue was acidified with 1 mol/L HCl and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was dissolved in MeOH (60 mL) and treated with $NaBH_4$ (580 mg, 15.3 mmol) at 0 °C. After stirring for 0.5 h at room temperature, the reaction was quenched with 1 mol/L HCl and the resulting mixture was concentrated in vacuo. The residue was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated in vacuo to give **54** (1.95 g, 7.67 mmol, quant. over 2 steps) as a light purple oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.99 (1H, br s), 3.90 (3H, s), 4.46 (1H, s), 4.93 (2H, s), 6.71 (1H, d, *J* = 8.6 Hz), 7.33 (1H, d, *J* = 8.6 Hz). LRMS (EI): 248 ([M]$^+$).

5.2.44. 4-Bromo-7-methoxy-2-(trifluoromethyl) benzo[*b*]thiophene (56)

To a stirred solution of **54** (1.95 g, 7.83 mmol) in MeCN (15 mL) was added triphenylphosphine hydrobromide (2.90 g, 8.45 mmol) and the reaction mixture was stirred for 17 h under reflux conditions. Solvent was concentrated in vacuo and EtOAc was added. The resulting powder was filtered to give phosphonium salt **55** (4.39 g) as a white solid. To a stirred suspension of **55** (4.39 g) in toluene (60 mL) were added triethylamine (3.17 mL, 22.7 mmol) and TFAA (1.18 mL, 8.43 mmol). After stirring for 3 h under reflux conditions, water was added and the resulting mixture was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by

silica gel column chromatography (*n*-hexane–EtOAc = 10:1) to give **56** (2.00 g, 6.42 mmol, 82% in 2 steps) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.00 (3H, s), 6.75 (1H, d, *J* = 8.6 Hz), 7.53 (1H, d, *J* = 8.6 Hz), 7.79 (1H, q, *J* = 1.2 Hz). LRMS (EI): 310 ($[M]^+$).

5.2.45. 1-(7-Methoxy-2-(trifluoromethyl)benzo[*b*]thiophen-4-yl)propan-1-one (57)

Compound **57** was prepared from **56** by the method described for the preparation of compound **47** from **46**. Yield: 15% (white solid). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.27 (3H, t, *J* = 7.3 Hz), 3.07 (2H, q, *J* = 7.3 Hz), 4.09 (3H, s), 6.89 (1H, d, *J* = 8.6 Hz), 8.05 (1H, d, *J* = 8.6 Hz), 8.80 (1H, q, *J* = 1.2 Hz). LRMS (EI): 288 ($[M]^+$).

5.2.46. 6-(7-Methoxy-2-(trifluoromethyl)benzo[*b*]thiophen-4-yl)-5-methyl-4,5-dihydropyridazin-3(2*H*)-one (8)

Compound **8** was prepared from **57** by the method described for the preparation of compound **1** from **16**. Yield: 72% over 3 steps (white solid). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.30 (3H, d, *J* = 7.3 Hz), 2.53 (1H, dd, *J* = 17.1, 1.8 Hz), 2.80 (1H, dd, *J* = 17.1, 6.7 Hz), 3.37–3.47 (1H, m), 4.06 (3H, s), 6.90 (1H, d, *J* = 8.6 Hz), 7.58 (1H, d, *J* = 8.6 Hz), 8.54 (1H, q, *J* = 1.2 Hz), 8.62 (1H, s). LRMS (EI): 342 ($[M]^+$). Anal. calcd for $C_{15}H_{13}F_3N_2O_2S$: C, 52.62; H, 3.83; N, 8.18. Found: C, 52.46; H, 3.76; N, 8.12.

5.2.47. 1-(8-Methoxy-2-trifluoromethylquinolin-5-yl)propan-1-one (59)

To a stirred solution of **58**[16] (3.86 g, 12.6 mmol) in THF (100 mL) was slowly added *n*-BuLi (2.71 mol/L in *n*-hexane, 5.20 mL, 14.1 mmol) at −78 °C under an argon atmosphere. After stirring for 1 h at the same temperature, propionic anhydride (3.50 mL, 27.2 mmol) was added. After stirring for 2.5 h at −78 °C, the reaction was quenched with satd NH_4Cl aq and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane–EtOAc = 5:1) to give **59** (1.21 g, 4.27 mmol, 34%) as a yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.29 (3H, t, *J* = 7.3 Hz), 3.11 (2H, q, *J* = 7.3 Hz), 4.18 (3H, s), 7.12 (1H, d, *J* = 8.6 Hz), 7.88 (1H, d, *J* = 8.6 Hz), 8.23 (1H, d, *J* = 8.6 Hz), 9.65 (1H, d, *J* = 8.6 Hz).

5.2.48. 6-(8-Methoxy-2-trifluoromethylquinolin-5-yl)-5-methyl-4,5-dihydropyridazin-3(2*H*)-one (9)

Compound **9** was prepared from **59** by the method described for the preparation of compound **1** from **16**. Yield: 43% over 3 steps (yellow solid). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.24 (3H, t, *J* = 7.3 Hz), 2.58 (1H, dd, *J* = 16.8, 3.4 Hz), 2.90 (1H, dd, *J* = 16.8, 6.7 Hz), 3.29–3.32 (1H, m), 4.15 (3H, s), 7.16 (1H, d, *J* = 8.3 Hz), 7.70 (1H, d, *J* = 8.3 Hz), 7.82 (1H, d, *J* = 9.2 Hz), 8.65 (1H, br s), 8.97 (1H, d, *J* = 9.2 Hz). HRMS (EI): calcd for $C_{16}H_{14}F_3N_3O_2$ ($[M]^+$) 337.1038, found 337.1041. Anal. calcd for $C_{16}H_{14}F_3N_3O_2$: C, 56.97; H, 4.18; N, 12.46. Found: C, 56.67; H, 4.13; N, 12.36.

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Supplementary data

Supplementary data (alternative synthetic routes for preparation of **5** and **6**; synthetic routes to **60**, **61** and KCA-1450) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2012.01.033.

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