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Synthesis and SAR study of new phenylimidazole-pyrazolo[1,5-*c*]quinazolines as potent phosphodiesterase 10A inhibitors

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ABSTRACT

A series of phenylimidazole-pyrazolo[1,5-*c*]quinazolines **1a–q** was designed, synthesized and characterised as a novel class of potent phophodiesterase 10A (PDE10A) inhibitors. In this series, 2,9-dimethyl-5-(2-(1-methyl-4-phenyl-1*H*-imidazol-2-yl)ethyl)pyrazolo[1,5-*c*]quinazoline (**1q**) showed the highest affinity for PDE10A enzyme (IC_{50} = 16 nM).

1. Introduction

Phosphodiesterases (PDEs) are a class of key enzymes in cellular signalling pathways. They are bimetallic hydrolases that degrade the intracellular second messengers cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) by catalytic hydrolysis of the 3′–5′ phosphodiester bond, forming the inactive 5′-monophosphate. Thereby, they play a crucial role in regulation of the intracellular levels of these ubiquitous second messengers. PDEs are a super family of enzymes encoded by 21 genes and are subdivided into 11 families (PDE1–PDE11) with over 60 isoforms according to structural and functional properties.[1,2] PDE10A is a dual-substrate (cGMP/cAMP) PDE encoded by a single gene as reported simultaneously by three independent groups in 1999.[3–5] Of the 11 PDE families, PDE10A has the most restricted distribution with mRNA being highly expressed in brain and testes. In the brain, the enzyme is predominantly expressed in the putamen and caudate nucleus by the medium sized spiny neurons of the mammalian striatal complex.[6,7] Based on this unique localisation, drug discovery research has focused extensively on using PDE10A modulators as a novel therapeutic approach for dysfunction in the basal ganglia circuit including Parkinson's disease, Huntington's disease, schizophrenia, addiction and obsessive compulsive disorder.[8–11]

The alkaloid Papaverine (Fig. 1) was reported by Pfizer as the first relatively selective PDE10A inhibitor. Papaverine was shown

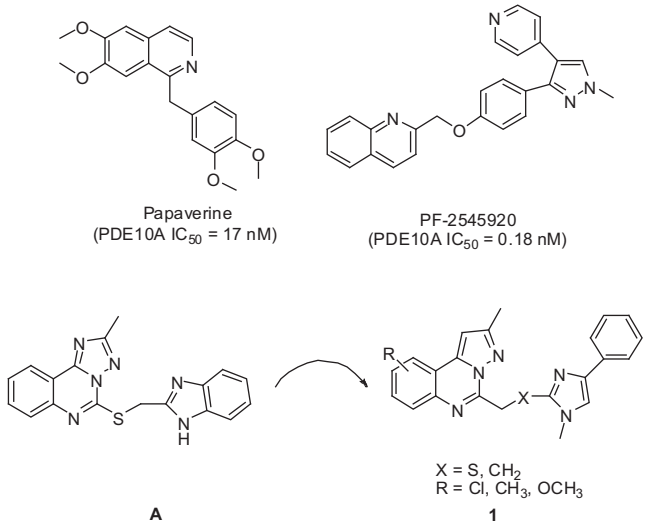

Figure 1.

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to be active in animal models predictive of antipsychotic activity, suggesting the potential use of PDE10A inhibitors for the treatment of schizophrenia.[12]

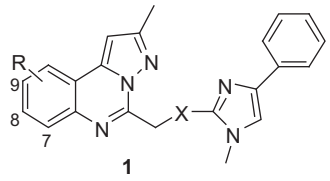
After these findings, a dedicated search for PDE10A selective agents was carried within many laboratories, resulting in the identification of a wide array of chemical entities endowed with high PDE10A affinity.[13–16] Recently, a series of pyrazolyl-phenoxymethyl quinolines were reported by Pfizer as potent PDE10A inhibitors with excellent selectivity versus other PDEs, and within the series, PF-2545920 (Fig. 1) was launched as a clinical candidate for the treatment of schizophrenia.[17]

During a hit-to-lead campaign after a high-throughput screening (HTS), at H. Lundbeck A/S we identified the triazoloquinazoline compound **A** (Fig. 1) as a potent and selective PDE10A inhibitor. An X-ray analysis of the compound in the binding pocket of PDE10A (Fig. 2) revealed that the nitrogen in the one-position of the [1,2,4]triazolo[1,5-*c*]quinazoline was apparently not participating in interactions with the enzyme, and therefore could be replaced by carbon thereby improving the physical chemical properties of the molecules by lowering the polar surface area, which is a critical parameter for obtaining bioavailable compounds with potential for penetrating the blood–brain barrier.[18] Another outcome of the analysis of the X-ray structure was the idea to replace the benzimidazole motif with a 'ring-opened' phenyl imidazole, which resulted in compounds with a basic nitrogen with improved solubility.

As part of our efforts within this framework, we are interested to extend the structure–activity relationship studies (SAR) on PDE10A enzyme by designing a new series of compounds of general formula **1**, derived from **A** by replacing both the triazoloquinazoline system with the pyrazolo[1,5-*c*]quinazoline one, and the benzimidazole motif with the heterobiaryl phenylimidazole fragment.

In this paper we report the synthesis and the biological evaluation of novel phenylimidazole-pyrazolo[1,5-*c*]quinazolines **1a–q** (Table 1), with a methylsulfanyl or an ethylene spacer, bearing different substituents in the phenyl ring of the tricyclic system.

Table 1
Structure and PDE10A potency for compounds **1a–q**



1

Compd	X	R	PDE10A IC_{50}[a] (nM)
A	—	—	12
1a	S	H	48
1b	S	7-Cl	73
1c	S	8-Cl	130
1d	S	9-Cl	110
1e	S	7-OCH_3	83
1f	S	8-OCH_3	100
1g	S	7-CH_3	160
1h	S	9-CH_3	76
1i	CH_2	H	50
1j	CH_2	7-Cl	52
1k	CH_2	8-Cl	50
1l	CH_2	9-Cl	16
1m	CH_2	7-OCH_3	37
1n	CH_2	8-OCH_3	31
1o	CH_2	9-OCH_3	430
1p	CH_2	7-CH_3	78
1q	CH_2	9-CH_3	16

[a] IC_{50} values are means of at least two experiments, and a typical standard deviation was ±30%.

2. Results and discussion

2.1. Chemistry

The synthetic route to the title compounds **1a–q** (Table 1) is outlined in Schemes 1 and 2. The synthesis of the key pyrazolo[1,5-*c*]quinazoline intermediates **4a–i** (Scheme 1) started from 2-methyl-4-quinolinols **2**. These compounds underwent a transposition reaction with hydrazine hydrate to substituted 3-methyl-pyrazolyl-anilines **3a–i** in good to high yields.[19] The pyrazolyl-anilines **3** were converted in high yields into the desired pyrazolo[1,5-*c*]quinazolines **4a–i** in two steps, by reacting them with chloroacetyl chloride, and cyclising the resulting intermediates under acidic conditions. Subsequent nucleophilic substitution of **4a–f,h,i** with 1-methyl-4-phenyl-1,3-dihydro-imidazole-2-thione **5** furnished the desired title compounds **1a–h**.

The phenylimidazole-pyrazolo[1,5-*c*]quinazolines bearing the ethylene spacer **1i–q** (Scheme 2) were synthesized by reduction of the alkene intermediates **8a–i** by hydrogenation with H_2 using transition metal catalyst such as Pd or PtO_2. Intermediates **8a–i** were prepared in satisfactory yields by the Wittig reaction between the phosphonium salts **6** and the aldehyde **7** using NaH as base in DMF. The phosphonium salts were easily obtained by reaction of **4a–i** with triphenylphosphine under standard conditions.

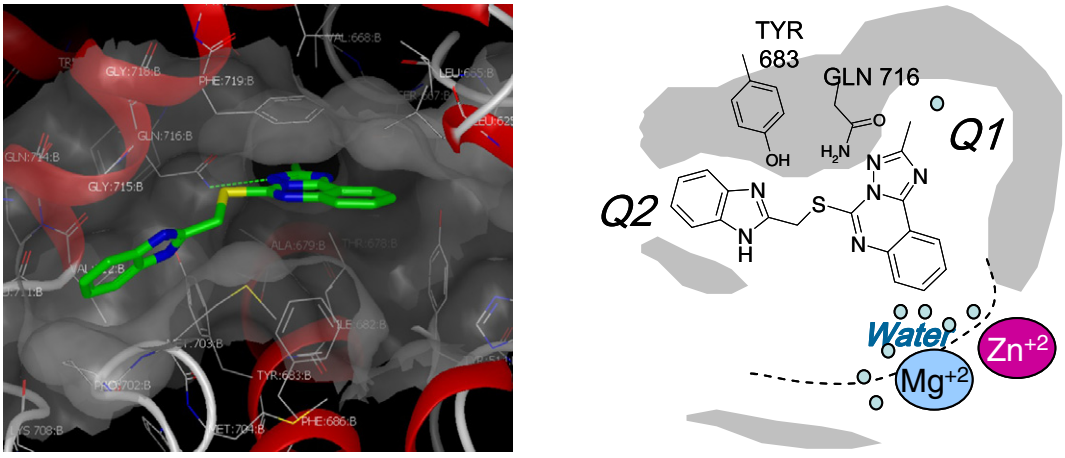
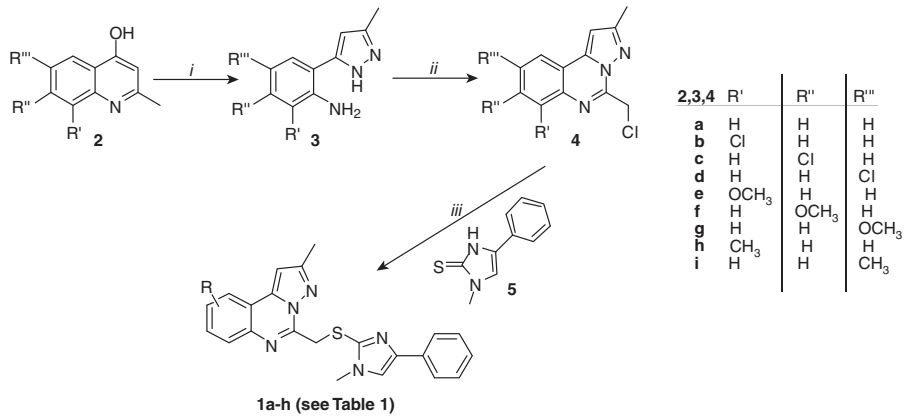
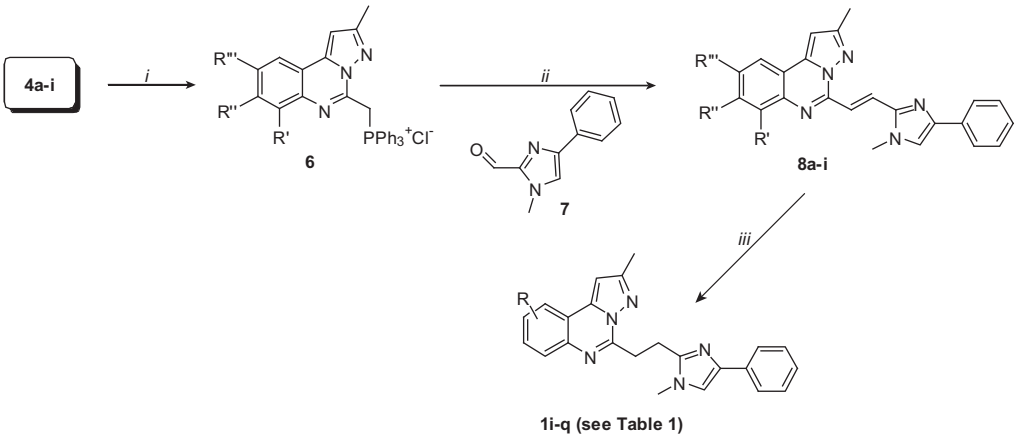


Figure 2. (a) X-ray structure of compound ligand **A**. (b) Cartoon showing binding interactions and pharmacophore of ligand **A** with the catalytic pocket in the PDE10A enzyme.



Scheme 1. Reagents and conditions: (i) $NH_2NH_2/NH_2NH_2{\cdot}2HCl$, ethylene glycol; (ii) (a) $ClCOCH_2Cl$, K_2CO_3, THF; (b) HCl, dioxane; (iii) DIPEA, CH_3CN.



Scheme 2. Reagents and conditions: (i) PPh_3, CH_3CN; (ii) NaH 60%, DMF; (iii) H_2, C/Pd 10%, THF; or H_2, PtO_2, THF, R = Cl.

2.2. Inhibition of PDE10A activity and SAR study

The ability to inhibit the PDE10A mediated hydrolysis of cAMP was measured using ^{3}H-labelled cAMP in an yttrium silicate SPA beads assay making use of the fact that cAMP has low affinity for yttrium silicate SPA beads, whereas hydrolysed 5′-AMP has high affinity for yttrium silicate SPA beads. The potency in inhibiting PDE10A was calculated and the IC_{50} values are shown in Table 1.

Compared with the starting point compound **A**, the data shows, that it is possible to get almost equipotent PDE10A inhibitors during the drastic chemical changes. That is, reversing the sulfanylmethyl spacer or even replace it with an ethylene spacer while at the same time removing a nitrogen in the 'clamp-scaffold' and ring-opening conversion of the benzimidazole to a phenyl imidazole. For instance this is demonstrated by comparing the starting compound **A** (IC_{50} = 12 nM) with the about four times weaker compounds **1a** (IC_{50} = 48 nM) and compound **1i** (IC_{50} = 50 nM). The sulphur in the spacer is therefore not required for the PDE10A inhibiting activity. The fact that the phenylimidazole-pyrazolo[1,5-*c*]quinazolines preserve the high PDE10A affinity compared with the starting triazoloquinazoline further is consistent with the binding mode depicted from the X-ray, where the nitrogen in the one-position of the [1,2,4]triazolo[1,5-*c*]quinazoline was apparently not participating in interactions with the enzyme, and therefore could be replaced by carbon. The SAR described in Table 1 shows that there is a relatively large tolerability for substituents in the pyrazolo[1,5-*c*]quinazoline ring system. Both electron-donating and electron-accepting substituents are tolerated in the 7-, 8- and 9-positions. It was somewhat surprising to learn, that even the lipophilic substituents like methyl and chloro did not improve the PDE10A affinity in spite of being placed directly in the lipophilic area in the clamp, maybe with the exception of a methyl group in the 9-position combined with an ethylene spacer which gives rise to the most potent compound in this series, namely compound **1q** (IC_{50} = 16 nM).

Having characterised the phenylimidazole-pyrazolo[1,5-*c*]quinazoline as novel chemical entities capable of potent PDE10A inhibition, we next established their selectivity against other PDE enzymes. The selected analogue **1q** and the triazoloquinazoline **A** were found to be highly selective PDE10A inhibitors; the IC_{50} values are higher than 10 μM for the following PDEs: PDE1B, PDE2, PDE3A, PDE4d6, PDE5a, PDE7b, PDE9 and PDE11. Furthermore, profiling of the triazoloquinazoline **A** had shown very limited ability to penetrate the blood–brain barrier with a brain/plasma-ratio (B/P-ratio) in mice of <0.04 in spite of having good bioavailability in the mouse with plasma-levels of 629 ng/ml at a dose of 2 mg/kg (po). Gratifyingly, the evaluation of the compound **1i** showed significant improvement in brain penetration with a B/P-ratio of 0.65. Another critically important point was the low aqueous solubility of the lead compound **A**, which was below detection limit (<0.02 μg/ml). Evaluation of the aqueous solubility of compound **1i** showed with a measured aqueous solubility of 1.8 μg/ml, which is still very low, albeit an improvement in solubility.

3. Conclusions

In summary, phenylimidazole-pyrazolo[1,5-*c*]quinazoline was developed as a novel clamp-binding heterocycle for binding to

the central pocket of PDE10A based on design considerations from an X-ray analyses of a triazoloquinazoline bound to the catalytic domain of PDE10A. A series of novel and potent PDE10A inhibitors was obtained by combining the tricyclic pyrazolo[1,5-*c*]quinazoline system with a phenylimidazole fragment bound via either a methylsulfanyl or an ethylene spacer from the 2-position.

Particularly, compound **1q**, possessing a methyl group in the 9-position of the pyrazolo[1,5-*c*]quinazoline system and the phenylimidazole bound via the ethylene spacer, resulted the most potent PDE10A inhibitor in this series, with an IC_{50} value of 16 nM.

The selected analogue **1q** was profiled for PDE isoform selectivity and showed very high selectivity for PDE10A. Further work on this interesting class of PDE10A inhibitors will be disclosed in due course.

4. Experimental section

4.1. Chemistry: general procedures

Melting points were obtained on a Köfler melting point apparatus and are uncorrected. NMR spectra were taken on a Varian XL-200 NMR spectrometer with ^1H being observed at 200 MHz. Chemical shifts were reported in δ (ppm) downfield from tetramethylsilane, and coupling constants (*J*) are expressed in hertz. Multiplicities are recorded as s (singlet), br s (broad singlet), d (doublet), t (triplet), dd (doublet of doublets), m (multiplet). Electron ionization mass spectra (70 eV) were recorded with a Hewlett-Packard 5790–5970 MSD gas chromatograph–mass spectrometer. Atmospheric Pressure Ionization Electrospray (API-ES) mass spectra were obtained on an Agilent 1100 series LC/MSD spectrometer. Elemental analyses were performed with a Perkin–Elmer 2400 analyzer, and results were within ±0.40% of the calculated values. Flash chromatography (FC) was performed using Merck silica gel 60 (230–400 mesh ASTM). Organic extracts were dried over anhydrous Na_2SO_4 prior to solvent evaporation. The 2-methyl-4-quinolinols **2** were purchased by BioBlocks. The 1-methyl-4-phenyl-1,3-dihydro-imidazole-2-thione **5** and 1-methyl-4-phenyl-1*H*-imidazole-2-carbaldehyde **7** were purchased by Lundbeck.

4.1.1. General procedure I. Synthesis of 2-(3-methyl-1*H*-pyrazol-5-yl)anilines 3a–i

A mixture of 2-methyl-4-quinolinol **2** (1 equiv, 6.28 mmol), hydrazine dihydrochloride (1 equiv, 6.28 mmol), 99% hydrazine monohydrate (10 equiv, 62.8 mmol) in ethylene glycol (5 ml) was slowly heated to 200 °C and stirred for 5 h at the same temperature. The mixture was then poured onto H_2O (15 ml) and stirred at room temperature for 2 h. The desired compounds were isolated as below indicated.

4.1.1.1. 2-(3-Methyl-1*H*-pyrazol-5-yl)aniline 3a[19]. The resulting precipitate was collected by filtration, washed with H_2O and dried to give the crude cream solid **3a** (80%) which was used without further purification. ^1H NMR ($CDCl_3$) δ 2.22 (s, 3H, CH_3), 5.85 (br s, 2H, NH_2, exch. with D_2O), 6.31 (s, 1H, ArH), 6.74 (m, 2H, ArH), 7.09 (m, 1H, ArH), 7.44 (m, 1H, ArH). MS: *m/z* 173.0 (M^+, base).

4.1.1.2. 2-Chloro-6-(3-methyl-1*H*-pyrazol-5-yl)aniline 3b. The resulting precipitate was collected by filtration, washed with H_2O and dried to give the crude white solid **3b** (90%) which was used without further purification. ^1H NMR ($CDCl_3$) δ 2.34 (s, 3H, CH_3), 5.95 (br s, 2H, NH_2, exch. with D_2O), 6.36 (s, 1H, ArH), 6.65 (t, 1H, *J* = 7.8 Hz, ArH), 7.21 (dd, 1H, J_m = 1.2 Hz, J_o = 8.0 Hz, ArH), 7.39 (dd, 1H, J_m = 1.6 Hz, J_o = 8.0 Hz, ArH), 9.50 (br s, 1H, NH, exch. with D_2O). MS: *m/z* 207.1 (M^+, base).

4.1.1.3. 5-Chloro-2-(3-methyl-1*H*-pyrazol-5-yl)aniline 3c. The aqueous mixture was extracted with AcOEt, and the combined extracts dried and concentrated. The residue was purified by FC (petroleum ether/AcOEt 1:1) to afford **3c** (40%) as a cream solid. ^1H NMR ($CDCl_3$) δ 2.35 (s, 3H, CH_3), 5.50 (br s, 2H, NH_2, exch. with D_2O), 6.34 (s, 1H, ArH), 6.70 (m, 2H, ArH), 7.37 (d, 1H, *J* = 8.2 Hz, ArH), 9.60 (br s, 1H, NH, exch. with D_2O). MS: *m/z* 207.2 (M^+, base).

4.1.1.4. 4-Chloro-2-(3-methyl-1*H*-pyrazol-5-yl)aniline 3d[20]. The aqueous mixture was extracted with AcOEt, and the combined extracts dried and concentrated. The residue was purified by FC (petroleum ether/AcOEt 1:1) to afford **3d** (74%) as a cream solid. ^1H NMR ($CDCl_3$) δ 2.30 (s, 3H, CH_3), 5.44 (br s, 2H, NH_2, exch. with D_2O), 6.33 (s, 1H, ArH), 6.65 (d, 1H, *J* = 8.6 Hz, ArH), 7.02 (dd, 1H, J_m = 1.6 Hz, J_o = 8.0 Hz, ArH), 7.41 (d, 1H, *J* = 1.6 Hz, ArH), 9.80 (br s, 1H, NH, exch. with D_2O). MS: *m/z* 207.1 (M^+, base).

4.1.1.5. 2-Methoxy-6-(3-methyl-1*H*-pyrazol-5-yl)aniline 3e. The aqueous mixture was extracted with AcOEt, and the combined extracts dried and concentrated. The residue was purified by FC (petroleum ether/AcOEt 1:1) to afford **3e** (47%) as a cream solid. ^1H NMR ($CDCl_3$) δ 2.32 (s, 3H, CH_3), 3.87 (s, 3H, OCH_3), 5.87 (br s, 2H, NH_2, exch. with D_2O), 6.35 (s, 1H, ArH), 6.71 (m, 2H, ArH), 7.11 (d, 1H, *J* = 7.2 Hz, ArH), 12.41 (br s, 1H, NH, exch. with D_2O). MS: *m/z* 203.1 (M^+, base).

4.1.1.6. 5-Methoxy-2-(3-methyl-1*H*-pyrazol-5-yl)aniline 3f. The aqueous mixture was extracted with AcOEt, and the combined extracts dried and concentrated. The residue was purified by FC (petroleum ether/AcOEt 1:1) to afford **3f** (40%) as a cream solid. ^1H NMR ($CDCl_3$) δ 2.31 (s, 3H, CH_3), 3.75 (s, 3H, OCH_3), 5.82 (br s, 2H, NH_2, exch. with D_2O), 6.25 (m, 3H, ArH), 7.25 (d, 1H, *J* = 8.0 Hz, ArH), 11.20 (br s, 1H, NH, exch. with D_2O). MS: *m/z* 203.1 (M^+, base).

4.1.1.7. 4-Methoxy-2-(3-methyl-1*H*-pyrazol-5-yl)aniline 3g. Compound **3g** was obtained following the above general procedure, heating the mixture in a sealed tube at 170 °C for 4 h. The aqueous mixture was extracted with AcOEt, and the combined extracts dried and concentrated. The residue was purified by FC (petroleum ether/AcOEt 1:1) to afford **3g** (53%) as a brown solid. ^1H NMR ($CDCl_3$) δ 2.26 (s, 3H, CH_3), 3.77 (s, 3H, OCH_3), 5.80 (br s, 2H, NH_2, exch. with D_2O), 6.30 (s, 1H, ArH), 6.71 (m, 2H, ArH), 7.01 (d, 1H, *J* = 2.2 Hz, ArH), 11.10 (br s, 1H, NH, exch. with D_2O). MS: *m/z* 203.1 (M^+, base).

4.1.1.8. 2-Methyl-6-(3-methyl-1*H*-pyrazol-5-yl)aniline 3h. The resulting precipitate was collected by filtration, washed with H_2O and dried to give the crude cream solid **3h** (74%) which was used without further purification. ^1H NMR ($CDCl_3$) δ 2.21 (s, 3H, CH_3), 2.33 (s, 3H, CH_3), 5.21 (br s, 2H, NH_2, exch. with D_2O), 6.36 (s, 1H, ArH), 6.69 (t, 1H, *J* = 7.8 Hz, ArH), 7.02 (d, 1H, *J* = 7.6 Hz, ArH), 7.35 (d, 1H, *J* = 7.8 Hz, ArH), 9.80 (br s, 1H, NH, exch. with D_2O). MS: *m/z* 187.1 (M^+, base).

4.1.1.9. 4-Methyl-2-(3-methyl-1*H*-pyrazol-5-yl)aniline 3i. The resulting precipitate was collected by filtration, washed with H_2O and dried to give the crude white solid **3i** (56%) which was used without further purification. ^1H NMR ($CDCl_3$) δ 2.65 (s, 3H, CH_3), 2.33 (s, 3H, CH_3), 5.04 (br s, 2H, NH_2, exch. with D_2O), 6.34 (s, 1H, ArH), 6.66 (d, 1H, *J* = 8.0 Hz, ArH), 6.91 (d, 1H, *J* = 7.8 Hz, ArH), 7.25 (s, 1H, ArH), 10.50 (br s, 1H, NH, exch. with D_2O). MS: *m/z* 187.1 (M^+, base).

4.1.2. General procedure II. Synthesis of 5-(chloromethyl)-2-methylpyrazolo[1,5-*c*]quinazolines 4a–i

To a cooled suspension (0 °C) of 2-(3-methyl-1*H*-pyrazol-5-yl)aniline **3** (1 equiv, 1.44 mmol), K_2CO_3 (10 equiv, 14.40 mmol) in THF (5 ml), was dropwise added a solution of chloroacetyl chloride (2 equiv, 2.88 mmol) in Et_2O (1 ml), and the whole stirred for 1 h at room temperature. The mixture was slowly added of H_2O and extracted with CH_2Cl_2; the combined organic layers dried and concentrated. The residue was solubilised in dioxane (15 ml) with 0.2 N HCl (0.1 ml) and refluxed for 1 h. The solution was concentrated and the residue purified by FC or used without further purification.

4.1.2.1. 5-(Chloromethyl)-2-methylpyrazolo[1,5-*c*]quinazoline 4a. The crude cream solid (82%) was used without further purification. ^{1}H NMR ($CDCl_3$) δ 2.57 (s, 3H, CH_3), 5.13 (s, 2H, CH_2), 6.81 (s, 1H, ArH), 7.53–7.67 (m, 2H, ArH), 7.92–7.98 (m, 2H, ArH). MS: *m/z* 231.0 (M^+, base).

4.1.2.2. 7-Chloro-5-(chloromethyl)-2-methylpyrazolo[1,5-*c*]quinazoline 4b. The residue was purified by FC (petroleum ether/AcOEt 8.5:1.5) to afford **4b** (80%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 2.59 (s, 3H, CH_3), 5.17 (s, 2H, CH_2), 6.84 (s, 1H, ArH), 7.48 (t, 1H, *J* = 8.0 Hz, ArH), 7.72 (d, 1H, *J* = 8.0 Hz, ArH), 7.88 (d, 1H, *J* = 8.0 Hz, ArH). MS: *m/z* 265.0 (M^+, base).

4.1.2.3. 8-Chloro-5-(chloromethyl)-2-methylpyrazolo[1,5-*c*]quinazoline 4c. The residue was purified by FC (petroleum ether/AcOEt 8.5:1.5) to afford **4c** (92%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 2.57 (s, 3H, CH_3), 5.11 (s, 2H, CH_2), 6.80 (s, 1H, ArH), 7.52 (d, 1H, *J* = 8.0 Hz, ArH), 7.87 (d, 1H, *J* = 8.8 Hz, ArH), 7.94 (s, 1H, ArH). MS: *m/z* 265.0 (M^+, base).

4.1.2.4. 9-Chloro-5-(chloromethyl)-2-methylpyrazolo[1,5-*c*]quinazoline 4d. The residue was purified by FC (petroleum ether/AcOEt 8.5:1.5) to afford **4d** (84%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 2.56 (s, 3H, CH_3), 5.10 (s, 2H, CH_2), 6.78 (s, 1H, ArH), 7.56 (d, 1H, *J* = 7.6 Hz, ArH), 7.88 (m, 2H, ArH). MS: *m/z* 265.0 (M^+, base).

4.1.2.5. 5-(Chloromethyl)-7-methoxy-2-methylpyrazolo[1,5-*c*]quinazoline 4e. The residue was purified by FC (petroleum ether/AcOEt 1:1) to afford **4e** (70%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 2.57 (s, 3H, CH_3), 4.08 (s, 3H, OCH_3), 5.18 (s, 2H, CH_2), 6.80 (s, 1H, ArH), 7.08 (m, 1H, ArH), 7.54 (m, 2H, ArH). MS: *m/z* 261.1 (M^+, base).

4.1.2.6. 5-(Chloromethyl)-8-methoxy-2-methylpyrazolo[1,5-*c*]quinazoline 4f. The residue was purified by FC (petroleum ether/AcOEt 7:3) to afford **4f** (65%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 2.54 (s, 3H, CH_3), 3.92 (s, 3H, OCH_3), 5.11 (s, 2H, CH_2), 6.66 (s, 1H, ArH), 7.17 (d, 1H, *J* = 8.6 Hz, ArH), 7.35 (s, 1H, ArH), 7.83 (d, 1H, *J* = 8.8 Hz, ArH). MS: *m/z* 261.1 (M^+, base).

4.1.2.7. 5-(Chloromethyl)-9-methoxy-2-methylpyrazolo[1,5-*c*]quinazoline 4g. The residue was purified by FC (petroleum ether/AcOEt 7:3) to afford **4g** (83%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 2.56 (s, 3H, CH_3), 3.94 (s, 3H, OCH_3), 5.11 (s, 2H, CH_2), 6.75 (s, 1H, ArH), 7.25 (m, 2H, ArH), 7.84 (d, 1H, *J* = 8.8 Hz, ArH). MS: *m/z* 261.1 (M^+, base).

4.1.2.8. 5-(Chloromethyl)-2,7-dimethylpyrazolo[1,5-*c*]quinazoline 4h. The residue was purified by FC (petroleum ether/AcOEt 8.5:1.5) to afford **4h** (76%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 2.57 (s, 3H, CH_3), 2.74 (s, 3H, CH_3), 5.13 (s, 2H, CH_2), 6.78 (s, 1H, ArH), 7.45 (m, 2H, ArH), 7.80 (d, 1H, *J* = 7.2 Hz, ArH). MS: *m/z* 245.2 (M^+, base).

4.1.2.9. 5-(Chloromethyl)-2,9-dimethylpyrazolo[1,5-*c*]quinazoline 4i. The residue was purified by FC (petroleum ether/AcOEt 8.5:1.5) to afford **4i** (58%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 2.54 (s, 3H, CH_3), 2.56 (s, 3H, CH_3), 5.11 (s, 2H, CH_2), 6.77 (s, 1H, ArH), 7.45 (d, 1H, *J* = 8.6 Hz, ArH), 7.74 (s, 1H, ArH), 7.82 (d, 1H, *J* = 8.6 Hz, ArH). MS: *m/z* 245.2 (M^+, base).

4.1.3. General procedure III. Synthesis of 2-methyl-5-((1-methyl-4-phenyl-1*H*-imidazol-2-ylthio)methyl)pyrazolo[1,5-*c*]quinazolines 1a–h

A mixture of 5-(chloromethyl)-2-methylpyrazolo[1,5-*c*]quinazoline **4** (1 equiv, 0.76 mmol), 1-methyl-4-phenyl-1,3-dihydro-imidazole-2-thione **5** (1 equiv, 0.76 mmol), *N*-ethyldiisopropylamine (DIPEA) (3 equiv, 2.28 mmol) in dry CH_3CN (13 ml) was heated at 80 °C for 3 h. The solution was added of H_2O and extracted with CH_2Cl_2; the combined organic layers dried and concentrated. Analytically pure product was isolated after purification by FC (petroleum ether/AcOEt 1:1, 2:8, AcOEt) and trituration with an appropriate solvent as below indicated.

4.1.3.1. 2-Methyl-5-((1-methyl-4-phenyl-1*H*-imidazol-2-ylthio)-methyl)pyrazolo[1,5-*c*]quinazoline 1a. Yield 57%. Mp 190–192 °C (AcOEt/*i*Pr_2O). ^{1}H NMR ($CDCl_3$) δ 2.49 (s, 3H, CH_3), 3.46 (s, 3H, NCH_3), 4.78 (s, 2H, CH_2), 6.76 (s, 1H, ArH), 7.19 (s, 1H, ArH), 7.21–7.37 (m, 3H, ArH), 7.49–7.96 (m, 6H, ArH). API-ES *m/z*: 386.1 (MH^+). Anal. ($C_{22}H_{19}N_5S$) C, H, N.

4.1.3.2. 7-Chloro-2-methyl-5-((1-methyl-4-phenyl-1*H*-imidazol-2-ylthio)methyl)pyrazolo[1,5-*c*]quinazoline 1b. Yield 68%. Mp 180 °C (AcOEt/*i*Pr_2O). ^{1}H NMR ($CDCl_3$) δ 2.50 (s, 3H, CH_3), 3.57 (s, 3H, NCH_3), 4.82 (s, 2H, CH_2), 6.77 (s, 1H, ArH), 7.20 (s, 1H, ArH), 7.22–7.43 (m, 5H, ArH), 7.62–7.66 (m, 2H, ArH), 7.82 (d, 1H, *J* = 7.8 Hz, ArH). API-ES *m/z*: 420.1 (MH^+). Anal. ($C_{22}H_{18}ClN_5S$) C, H, N.

4.1.3.3. 8-Chloro-2-methyl-5-((1-methyl-4-phenyl-1*H*-imidazol-2-ylthio)methyl)pyrazolo[1,5-*c*]quinazoline 1c. Yield 52%. Mp 155–156 °C (AcOEt/*i*Pr_2O). ^{1}H NMR ($CDCl_3$) δ 2.48 (s, 3H, CH_3), 3.50 (s, 3H, NCH_3), 4.76 (s, 2H, CH_2), 6.73 (s, 1H, ArH), 7.20 (s, 1H, ArH), 7.24–7.43 (m, 5H, ArH), 7.64–7.86 (m, 3H, ArH). API-ES *m/z*: 420.1 (MH^+). Anal. ($C_{22}H_{18}ClN_5S$) C, H, N.

4.1.3.4. 9-Chloro-2-methyl-5-((1-methyl-4-phenyl-1*H*-imidazol-2-ylthio)methyl)pyrazolo[1,5-*c*]quinazoline 1d. Yield 60%. Mp 184–185 °C (AcOEt/*i*Pr_2O). ^{1}H NMR ($CDCl_3$) δ 2.49 (s, 3H, CH_3), 3.49 (s, 3H, NCH_3), 4.76 (s, 2H, CH_2), 6.74 (s, 1H, ArH), 7.19 (s, 1H, ArH), 7.21–7.35 (m, 3H, ArH), 7.52 (m, 1H, ArH), 7.63–7.74 (m, 3H, ArH), 7.88 (s, 1H, ArH). API-ES *m/z*: 420.1 (MH^+). Anal. ($C_{22}H_{18}ClN_5S$) C, H, N.

4.1.3.5. 7-Methoxy-2-methyl-5-((1-methyl-4-phenyl-1*H*-imidazol-2-ylthio)methyl)pyrazolo[1,5-*c*]quinazoline 1e. Yield 74%. Mp 197–198 °C (AcOEt). ^{1}H NMR ($CDCl_3$) δ 2.44 (s, 3H, CH_3), 3.49 (s, 3H, NCH_3), 3.97 (s, 3H, OCH_3), 4.84 (s, 2H, CH_2), 6.72 (s, 1H, ArH), 7.02 (m, 1H, ArH), 7.17 (s, 1H, ArH), 7.19–7.69 (m, 7H, ArH). API-ES *m/z*: 416.1 (MH^+). Anal. ($C_{23}H_{21}N_5OS$) C, H, N.

4.1.3.6. 8-Methoxy-2-methyl-5-((1-methyl-4-phenyl-1*H*-imidazol-2-ylthio)methyl)pyrazolo[1,5-*c*]quinazoline 1f. Yield 68%. Mp 174–175 °C (AcOEt). ^{1}H NMR ($CDCl_3$) δ 2.46 (s, 3H, CH_3), 3.44 (s, 3H, NCH_3), 3.87 (s, 3H, OCH_3), 4.75 (s, 2H, CH_2), 6.62 (s, 1H, ArH), 7.10–7.36 (m, 7H, ArH), 7.68 (d, 1H, *J* = 8.0 Hz, ArH), 7.81 (d, 1H, *J* = 8.8 Hz, ArH). API-ES *m/z*: 416.1 (MH^+). Anal. ($C_{23}H_{21}N_5OS$) C, H, N.

4.1.3.7. 2,7-Dimethyl-5-((1-methyl-4-phenyl-1*H*-imidazol-2-ylthio)methyl)pyrazolo[1,5-*c*]quinazoline 1g. Yield 71%. Mp 189–190 °C (AcOEt/*i*Pr_2O). ^{1}H NMR ($CDCl_3$) δ 2.51 (s, 3H, CH_3),

2.55 (s, 3H, CH_3), 3.38 (s, 3H, NCH_3), 4.78 (s, 2H, CH_2), 6.74 (s, 1H, ArH), 7.17 (s, 1H, ArH), 7.22–7.41 (m, 5H, ArH), 7.69–7.74 (m, 3H, ArH). API-ES *m/z*: 400.1 (MH^+). Anal. ($C_{23}H_{21}N_5S$) C, H, N.

4.1.3.8. 2,9-Dimethyl-5-((1-methyl-4-phenyl-1*H*-imidazol-2-yl-thio)methyl)pyrazolo[1,5-*c*]quinazoline 1h. Yield 70%. Mp 155 °C (AcOEt/iPr$_2$O). ^{1}H NMR ($CDCl_3$) δ 2.49 (s, 3H, CH_3), 2.52 (s, 3H, CH_3), 3.43 (s, 3H, NCH_3), 4.76 (s, 2H, CH_2), 6.72 (s, 1H, ArH), 7.18 (s, 1H, ArH), 7.21–7.41 (m, 5H, ArH), 7.66–7.71 (m, 3H, ArH). API-ES *m/z*: 400.1 (MH^+). Anal. ($C_{23}H_{21}N_5S$) C, H, N.

4.1.4. General procedure IV. Synthesis of (*E*)-2-methyl-5-(2-(1-methyl-4-phenyl-1*H*-imidazol-2-yl)vinyl)pyrazolo[1,5-*c*]quinazolines 8a–i

A mixture of 5-(chloromethyl)-2-methylpyrazolo[1,5-*c*]quinazoline **4** (1 equiv, 0.95 mmol) and triphenylphosphine (1 equiv, 0.95 mmol) in CH_3CN (4 ml) was heated at 80 °C for 5 h. The solution was concentrated and the residue triturated with THF, filtered and dried to give the desired ((2-methylpyrazolo[1,5-*c*]quinazolin-5-yl)methyl)triphenylphosphonium chloride **6** in almost quantitative yields as white solid, which was used without characterisation.

To a suspension of 60% NaH in mineral oil (1.1 equiv, 0.88 mmol) in DMF (4 ml) cooled to −5 °C, the phosphonium salt **6** (1 equiv, 0.76 mmol) was portionwise added and the resulting yellow solution stirred for 30′ at the same temperature. 1-Methyl-4-phenyl-1*H*-imidazole-2-carbaldehyde **7** (1 equiv, 0.76 mmol) was portionwise added and the resulting mixture stirred at room temperature for 1.5 h. The whole was poured onto H_2O, extracted with CH_2Cl_2 and the combined organic layers dried and concentrated. The residue was purified by FC (petroleum ether/AcOEt 6:4, 1:1, AcOEt) to give the pure product as a yellow solid.

4.1.4.1. (*E*)-2-Methyl-5-(2-(1-methyl-4-phenyl-1*H*-imidazol-2-yl)-vinyl)pyrazolo[1,5-*c*]quinazoline 8a. Yield 60%. ^{1}H NMR ($CDCl_3$) δ 2.58 (s, 3H, CH_3), 3.89 (s, 3H, NCH_3), 6.77 (s, 1H, ArH), 7.25 (s, 1H, ArH), 7.30–7.64 (m, 5H, ArH), 7.86–7.94 (m, 4H, ArH), 8.13 (d, 1H, *J* = 15.6 Hz, CH), 8.53 (d, 1H, *J* = 15.6, CH). API-ES *m/z*: 366.1 (MH^+).

4.1.4.2. (*E*)-7-Chloro-2-methyl-5-(2-(1-methyl-4-phenyl-1*H*-imidazol-2-yl)vinyl)pyrazolo[1,5-*c*]quinazoline 8b. Yield 80%. ^{1}H NMR ($CDCl_3$) δ 2.59 (s, 3H, CH_3), 3.92 (s, 3H, NCH_3), 6.80 (s, 1H, ArH), 7.26–7.42 (m, 5H, ArH), 7.69 (d, 1H, *J* = 7.8 Hz, ArH), 7.82–7.89 (m, 3H, ArH), 8.28 (d, 1H, *J* = 15.4 Hz, CH), 8.52 (d, 1H, *J* = 15.4 Hz, CH). API-ES *m/z*: 400.1 (MH^+).

4.1.4.3. (*E*)-8-Chloro-2-methyl-5-(2-(1-methyl-4-phenyl-1*H*-imidazol-2-yl)vinyl)pyrazolo[1,5-*c*]quinazoline 8c. Yield 40%. ^{1}H NMR ($CDCl_3$) δ 2.57 (s, 3H, CH_3), 3.90 (s, 3H, NCH_3), 6.75 (s, 1H, ArH), 7.25–7.44 (m, 6H, ArH), 7.81–7.89 (m, 3H, ArH), 8.12 (d, 1H, *J* = 15.4 Hz, CH), 8.50 (d, 1H, *J* = 15.4 Hz, CH). API-ES *m/z*: 400.1 (MH^+).

4.1.4.4. (*E*)-9-Chloro-2-methyl-5-(2-(1-methyl-4-phenyl-1*H*-imidazol-2-yl)vinyl)pyrazolo[1,5-*c*]quinazoline 8d. Yield 90%. ^{1}H NMR ($CDCl_3$) δ 2.58 (s, 3H, CH_3), 3.89 (s, 3H, NCH_3), 6.75 (s, 1H, ArH), 7.26–7.56 (m, 6H, ArH), 7.78–7.89 (m, 3H, ArH), 8.10 (d, 1H, *J* = 15.6 Hz, CH), 8.53 (d, 1H, *J* = 15.6 Hz, CH). API-ES *m/z*: 400.1 (MH^+).

4.1.4.5. (*E*)-7-Methoxy-2-methyl-5-(2-(1-methyl-4-phenyl-1*H*-imidazol-2-yl)vinyl)pyrazolo[1,5-*c*]quinazoline 8e. Yield 95%. ^{1}H NMR ($CDCl_3$) δ 2.59 (s, 3H, CH_3), 3.91 (s, 3H, NCH_3), 4.09 (s, 3H, OCH_3), 6.77 (s, 1H, ArH), 7.07–7.89 (m, 9H, ArH), 8.21 (d, 1H, *J* = 15.6 Hz, CH), 8.55 (d, 1H, *J* = 15.6 Hz, CH). API-ES *m/z*: 396.1 (MH^+).

4.1.4.6. (*E*)-8-Methoxy-2-methyl-5-(2-(1-methyl-4-phenyl-1*H*-imidazol-2-yl)vinyl)pyrazolo[1,5-*c*]quinazoline 8f. Yield 82%. ^{1}H NMR ($CDCl_3$) δ 2.56 (s, 3H, CH_3), 3.89 (s, 3H, NCH_3), 3.94 (s, 3H, OCH_3), 6.65 (s, 1H, ArH), 7.08–7.89 (m, 9H, ArH), 8.12 (d, 1H, *J* = 15.6 Hz, CH), 8.50 (d, 1H, *J* = 15.6 Hz, CH). API-ES *m/z*: 396.1 (MH^+).

4.1.4.7. (*E*)-9-Methoxy-2-methyl-5-(2-(1-methyl-4-phenyl-1*H*-imidazol-2-yl)vinyl)pyrazolo[1,5-*c*]quinazoline 8g. Yield 72%. ^{1}H NMR ($CDCl_3$) δ 2.58 (s, 3H, CH_3), 3.88 (s, 3H, NCH_3), 3.94 (s, 3H, OCH_3), 6.74 (s, 1H, ArH), 7.18–7.44 (m, 6H, ArH), 7.80–7.89 (m, 3H, ArH), 8.06 (d, 1H, *J* = 15.6 Hz, CH), 8.49 (d, 1H, *J* = 15.6 Hz, CH). API-ES *m/z*: 396.1 (MH^+).

4.1.4.8. (*E*)-2,7-Dimethyl-5-(2-(1-methyl-4-phenyl-1*H*-imidazol-2-yl)vinyl)pyrazolo[1,5-*c*]quinazoline 8h. Yield 40%. ^{1}H NMR ($CDCl_3$) δ 2.58 (s, 3H, CH_3), 2.79 (s, 3H, CH_3), 3.93 (s, 3H, NCH_3), 6.77 (s, 1H, ArH), 7.26–7.44 (m, 6H, ArH), 7.78–7.89 (m, 3H, ArH), 8.24 (d, 1H, *J* = 15.4 Hz, CH), 8.46 (d, 1H, *J* = 15.6 Hz, CH). API-ES *m/z*: 380.1 (MH^+).

4.1.4.9. (*E*)-2,9-Dimethyl-5-(2-(1-methyl-4-phenyl-1*H*-imidazol-2-yl)vinyl)pyrazolo[1,5-*c*]quinazoline 8i. Yield 75%. ^{1}H NMR ($CDCl_3$) δ 2.53 (s, 3H, CH_3), 2.58 (s, 3H, CH_3), 3.90 (s, 3H, NCH_3), 6.75 (s, 1H, ArH), 7.26–7.44 (m, 6H, ArH), 7.73–7.89 (m, 3H, ArH), 8.12 (d, 1H, *J* = 15.6 Hz, CH), 8.52 (d, 1H, *J* = 15.8 Hz, CH). API-ES *m/z*: 380.1 (MH^+).

4.1.5. General procedure V. Method A. Synthesis of 2-methyl-5-(2-(1-methyl-4-phenyl-1*H*-imidazol-2-yl)ethyl)pyrazolo[1,5-*c*]-quinazolines 1i,m–q

A mixture of the appropriate (*E*)-2-methyl-5-(2-(1-methyl-4-phenyl-1*H*-imidazol-2-yl)vinyl)pyrazolo[1,5-*c*]quinazoline **8** (1 equiv, 0.76 mmol) and 10% Pd/C (0.1 equiv, 0.071 mmol) in THF (35 ml) was hydrogenated at room temperature at 45 psi for 6 h. The suspension was filtered over Celite® and the solution concentrated. Analytically pure product was isolated after purification by FC (petroleum ether/AcOEt 1:1, 2:8, AcOEt) and trituration with AcOEt.

4.1.5.1. 2-Methyl-5-(2-(1-methyl-4-phenyl-1*H*-imidazol-2-yl)-ethyl)pyrazolo[1,5-*c*]quinazoline 1i. Yield 50%. Mp 151–153 °C. ^{1}H NMR ($CDCl_3$) δ 2.54 (s, 3H, CH_3), 3.44 (t, 2H, *J* = 7.0 Hz, CH_2), 3.78 (s, 3H, NCH_3), 3.84 (t, 2H, *J* = 7.0 Hz, CH_2), 6.77 (s, 1H, ArH), 7.08 (s, 1H, ArH), 7.18–7.37 (m, 3H, ArH), 7.51–7.96 (m, 6H, ArH). API-ES *m/z*: 368.1 (MH^+). Anal. ($C_{23}H_{21}N_5$) C, H, N.

4.1.5.2. 7-Methoxy-2-methyl-5-(2-(1-methyl-4-phenyl-1*H*-imidazol-2-yl)ethyl)pyrazolo[1,5-*c*]quinazoline 1m. Yield 40%. Mp 203–205 °C. ^{1}H NMR ($CDCl_3$) δ 2.45 (s, 3H, CH_3), 3.47 (t, 2H, *J* = 7.2 Hz, CH_2), 3.77 (s, 3H, NCH_3), 3.90 (t, 2H, *J* = 7.2 Hz, CH_2), 3.96 (s, 3H, OCH_3), 6.68 (s, 1H, ArH), 7.01 (m, 1H, ArH), 7.19 (s, 1H, ArH), 7.24–7.70 (m, 7H, ArH). API-ES *m/z*: 398.1 (MH^+). Anal. ($C_{24}H_{23}N_5O$) C, H, N.

4.1.5.3. 8-Methoxy-2-methyl-5-(2-(1-methyl-4-phenyl-1*H*-imidazol-2-yl)ethyl)pyrazolo[1,5-*c*]quinazoline 1n. Yield 58%. Mp 188–192 °C. ^{1}H NMR ($CDCl_3$) δ 2.52 (s, 3H, CH_3), 3.43 (t, 2H, *J* = 6.8 Hz, CH_2), 3.73 (s, 3H, NCH_3), 3.82 (t, 2H, *J* = 6.8 Hz, CH_2), 3.94 (s, 3H, OCH_3), 6.64 (s, 1H, ArH), 7.09 (s, 1H, ArH), 7.10–7.37 (m, 6H, ArH), 7.72 (d, 1H, *J* = 6.8 Hz, ArH), 7.84 (d, 1H, *J* = 7.2 Hz, ArH). API-ES *m/z*: 398.1 (MH^+). Anal. ($C_{24}H_{23}N_5O$) C, H, N.

4.1.5.4. 9-Methoxy-2-methyl-5-(2-(1-methyl-4-phenyl-1*H*-imidazol-2-yl)ethyl)pyrazolo[1,5-*c*]quinazoline 1o. Yield 71%. Mp 194–196 °C. ^{1}H NMR ($CDCl_3$) δ 2.52 (s, 3H, CH_3), 3.41 (t, 2H,

J = 6.8 Hz, CH_2), 3.73 (s, 3H, NCH_3), 3.80 (t, 2H, J = 6.8 Hz, CH_2), 3.92 (s, 3H, OCH_3), 6.72 (s, 1H, ArH), 7.07 (s, 1H, ArH), 7.14–7.37 (m, 5H, ArH), 7.69–7.80 (m, 3H, ArH). API-ES *m/z*: 398.1 (MH^+). Anal. ($C_{24}H_{23}N_5O$) C, H, N.

4.1.5.5. 2,7-Dimethyl-5-(2-(1-methyl-4-phenyl-1*H*-imidazol-2-yl)ethyl)pyrazolo[1,5-*c*]quinazoline 1p. Yield 75%. Mp 185 °C. ^1H NMR ($CDCl_3$) δ 2.53 (s, 3H, CH_3), 2.70 (s, 3H, CH_3), 3.46 (t, 2H, J = 7.2 Hz, CH_2), 3.73 (s, 3H, NCH_3), 3.82 (t, 2H, J = 7.2 Hz, CH_2), 6.74 (s, 1H, ArH), 7.09 (s, 1H, ArH), 7.15–7.43 (m, 5H, ArH), 7.71–7.80 (m, 3H, ArH). API-ES *m/z*: 382.2 (MH^+). Anal. ($C_{24}H_{23}N_5$) C, H, N.

4.1.5.6. 2,9-Dimethyl-5-(2-(1-methyl-4-phenyl-1*H*-imidazol-2-yl)ethyl)pyrazolo[1,5-*c*]quinazoline 1q. Yield 74%. Mp 185–187 °C. ^1H NMR ($CDCl_3$) δ 2.52 (s, 6H, 2 × CH_3), 3.44 (t, 2H, J = 7.2 Hz, CH_2), 3.74 (s, 3H, NCH_3), 3.82 (t, 2H, J = 7.2 Hz, CH_2), 6.72 (s, 1H, ArH), 7.07 (s, 1H, ArH), 7.17–7.46 (m, 5H, ArH), 7.72 (m, 3H, ArH). API-ES *m/z*: 382.2 (MH^+). Anal. ($C_{24}H_{23}N_5$) C, H, N.

4.1.6. Method B. Synthesis of 2-methyl-5-(2-(1-methyl-4-phenyl-1*H*-imidazol-2-yl)ethyl)pyrazolo[1,5-*c*]quinazolines 1j–l

Compounds **1j–l** were obtained and purified following the above general procedure, performing the catalytic hydrogenation over PtO_2 (Adam's catalyst, 0.1 equiv, 0.071 mmol).

4.1.6.1. 7-Chloro-2-methyl-5-(2-(1-methyl-4-phenyl-1*H*-imidazol-2-yl)ethyl)pyrazolo[1,5-*c*]quinazoline 1j. Yield 90%. Mp 185–187 °C. ^1H NMR ($CDCl_3$) δ 2.54 (s, 3H, CH_3), 3.50 (t, 2H, J = 6.8 Hz, CH_2), 3.78 (s, 3H, NCH_3), 3.91 (t, 2H, J = 7.0 Hz, CH_2), 6.77 (s, 1H, ArH), 7.08 (s, 1H, ArH), 7.17–7.43 (m, 5H, ArH), 7.64–7.84 (m, 3H, ArH). API-ES *m/z*: 402.1 (MH^+). Anal. ($C_{23}H_{20}ClN_5$) C, H, N.

4.1.6.2. 8-Chloro-2-methyl-5-(2-(1-methyl-4-phenyl-1*H*-imidazol-2-yl)ethyl)pyrazolo[1,5-*c*]quinazoline 1k. Yield 55%. Mp 155–157 °C. ^1H NMR ($CDCl_3$) δ 2.53 (s, 3H, CH_3), 3.42 (t, 2H, J = 7.6 Hz, CH_2), 3.77 (s, 3H, NCH_3), 3.85 (t, 2H, J = 7.2 Hz, CH_2), 6.74 (s, 1H, ArH), 7.09 (s, 1H, ArH), 7.18–7.88 (m, 8H, ArH). API-ES *m/z*: 402.1 (MH^+). Anal. ($C_{23}H_{20}ClN_5$) C, H, N.

4.1.6.3. 9-Chloro-2-methyl-5-(2-(1-methyl-4-phenyl-1*H*-imidazol-2-yl)ethyl)pyrazolo[1,5-*c*]quinazoline 1l. Yield 55%. Mp 176–178 °C. ^1H NMR ($CDCl_3$) δ 2.53 (s, 3H, CH_3), 3.41 (t, 2H, J = 7.2 Hz, CH_2), 3.78 (s, 3H, NCH_3), 3.86 (t, 2H, J = 7.5 Hz, CH_2), 6.74 (s, 1H, ArH), 7.09 (s, 1H, ArH), 7.18–7.36 (m, 4H, ArH), 7.53 (dd, 1H, J_m = 2.2 Hz, J_o = 8.8 Hz, ArH), 7.68–7.80 (m, 2H, ArH), 7.88 (d, 1H, J = 2.2 Hz, ArH). API-ES *m/z*: 402.1 (MH^+). Anal. ($C_{23}H_{20}ClN_5$) C, H, N.

4.2. Biology experimental

4.2.1. PDE10A enzyme

PDE10A can be prepared in different cell types, for example, insect cells or *Escherichia coli*. Catalytically active PDE10A was obtained as follows: human PDE10A (amino acids 14–779 from the sequence with accession number NP_006652) was amplified from total human brain total RNA by standard RT-PCR and cloned into the BamH1 and Not1 sites of the pFastBac-HTb (Invitrogen). Expression in Sf9 cells using the Bac-to-Bac® Baculovirus Expression System (Gibco). Sf9 cells were grown at 27 °C in Sf-900 II serum free medium containing 50 units/ml penicillin and 50 μg/ml streptomycin and were infected with 1 ml of virus for 25 ml of media. At 72 h, cells were harvested and disrupted in lysis buffer (50 mM Tris8.0 + 1 mM $MgCl_2$ + PI + 0.5%triton) for 15 min. on ice and then centrifuged at 20,000*g* for 20 min. PDE10A was partially purified on Q sepharose and the most active fractions were pooled.

4.2.2. PDE10A inhibition assay

A PDE10A assay was performed as follows: the assay was performed in 60 μL samples containing a fixed amount of the relevant PDE enzyme (sufficient to convert 20–25% of the cyclic nucleotide substrate), a buffer (50 mM HEPES 7.6; 10 mM $MgCl_2$; 0.02% Tween20), 0.1 mg/ml BSA, 225 pCi of ^3H-labelled cyclic nucleotide substrate, tritium labelled cAMP to a final concentration of 5 nM and varying amounts of inhibitors. Reactions were initiated by addition of the cyclic nucleotide substrate, and reactions were allowed to proceed for 1 h at room temperature before being terminated through mixing with 15 μL 8 mg/ml yttrium silicate SPA beads (Amersham). The beads were allowed to settle for 1 h in the dark before the plates were counted in a Wallac 1450 Microbeta counter. The measured signals were converted to activity relative to an uninhibited control (100%) and IC_{50} values were calculated using the Xlfit extension to EXCEL.

4.2.3. Brain/plasma distribution ratio (B/P-ratio) in NMRI mice

The purpose of this assay is to assess brain penetration by determining the brain/plasma distribution ratio (B/P-ratio) of a given compound. When this assay is combined with an assay that determines the unbound fraction of a compound in the brain it is often possible to predict the dose needed for effect in an in vivo pharmacological study. For each B/P-ratio three animals were used and plasma and brain samples were collected 30 min after dosing. Brain samples were homogenised and extracted. After protein precipitation both plasma and brain samples were analysed using UPLC-MS/MS. The obtained brain concentrations were thus total drug concentration, that is, both bound and unbound concentrations. An average B/P-ratio and corresponding standard deviation (SD) were calculated. Approximately 2% of the brain is blood and therefore it is only a B/P-ratio value above 0.02 that show that the compound is distributed to the brain.

Compound **A** was dosed 2 mg/kg po using as vehicle 10% captisol at pH 2. The mean plasma concentration was measured to 629 ng/ml (SEM = ±20) and the mean brain concentration was below detection limit, that is, <25 ng/g resulting in a B/P-ratio <0.04.

Compound **1i** had a low bioavailability and the dosing was therefore chosen to be sc. Compound **1i** was dosed 5 mg/kg using as vehicle 25% cremophor at pH 4. The mean plasma concentration was measured to 150 ng/ml (SEM = ±20) and the mean brain concentration was 98 ng/g (SEM = ±6) resulting in a B/P-ratio = 0.65 (SEM = ±0.05).

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