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# Design and synthesis of novel imidazo[1,2-*a*]quinoxalines as PDE4 inhibitors

Carine Deleuze-Masquéfa,[a] Grégori Gerebtzoff,[a] Guy Subra,[a] Jean-Roch Fabreguettes,[a] Annabel Ovens,[a] Maëlle Carraz,[a] Marie-Paule Strub,[b] Jacques Bompart,[a] Pascal George[c] and Pierre-Antoine Bonnet[a,*]

[a]*Pharmacochimie et Biomolécules, Laboratoire de Chimie Organique Pharmaceutique, Faculté de Pharmacie, 15, Av. Ch. Flahault, BP 14 491, 34093 Montpellier Cedex 5, France*
[b]*Centre de Biochimie Structurale, Faculté de Pharmacie, Montpellier, France*
[c]*Sanofi-Synthélabo Recherche, Département de Recherche Système Nerveux Central, 31, Av. P.-V. Couturier, 92220 Bagneux, France*

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**Abstract**—New imidazo[1,2-*a*]quinoxaline derivatives have been synthesised by condensation of an appropriate α-aminoalcohol with a quinoxaline followed by intramolecular cyclisation and nucleophilic substitutions. Their phosphodiesterase inhibitory activities have been assessed on a preparation of the PDE4 isoform purified from a human alveolar epithelial cell line (A549). These studies showed potent inhibitory properties that emphasize the importance of a methyl amino group at position 4 and a weakly hindered group at position 1.


## 1. Introduction

Cyclic AMP (cAMP) and cyclic GMP (cGMP) are ubiquitous second messengers inducing numerous intracellular responses by transduction of different inflammatory stimuli.[1] An elevated level of cAMP has been reported to lead to suppression of function in pro-inflammatory and immunocompetent cells.[1] Cyclic nucleotide phosphodiesterases (PDE) are the only enzymes which can hydrolyse cyclic nucleotides. Up to the present time, 11 families of PDE have been identified, sharing various properties (substrate specificities, enzymatic properties, responsiveness to inhibitors…).[2–4] PDE4 is the only isoform found in most pro-inflammatory and immunocompetent cells involved in airway inflammation (e.g., lymphocytes, mast cells, eosinophils, granulocytes),[1,5] therefore being the preferred target to control the cAMP intracellular level in diverse inflammatory disorders such as asthma or chronic obstructive pulmonary disease (COPD).[1,5–8]

As part of our ongoing efforts to develop novel inflammatory lead molecules through their action on PDE, and based on imidazo[1,2-*a*]quinoxalines as set out in a preceding paper,[9] we report here the design approach and synthesis of derivatives substituted in positions 1 and 4, and their inhibitory profile on purified HSPDE4 isoenzyme obtained from a human alveolar epithelial cell line (A549).

## 2. Chemistry

Compounds **4a** and **b** were prepared following precedents for such transformations as described in ref 9. Reaction of isovaleraldehyde or 3-phenylpropionaldehyde, for series **a** and **b** respectively, with sodium cyanide led to 2-hydroxy-4-methylpentanenitrile **1a** and 2-hydroxy-4-phenylbutanenitrile **1b** which, upon reduction with lithium aluminium hydride, produced 1-amino-4-methylpentan-2-ol **2a** and 1-amino-4-phenylbutan-2-ol **2b** in 87% and 81% overall yields (Scheme 1). Both compounds were condensed with 2,3-dichloroquinoxaline in the presence of triethylamine in dioxan to furnish the 1-[(3-chloroquinoxalin-2-yl)amino]-4-methylpentan-2-ol **3a** and 1-[(3-chloroquinoxalin-2-

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* Corresponding author. Tel.: +33-4-67-54-86-43; fax: +33-4-67-54-80-36; e-mail: pabonnet@univ-montp1.fr

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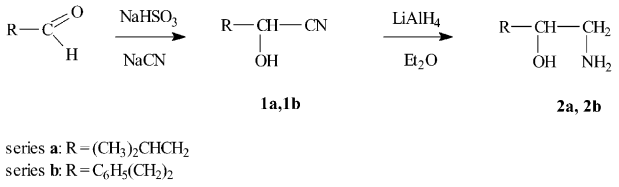


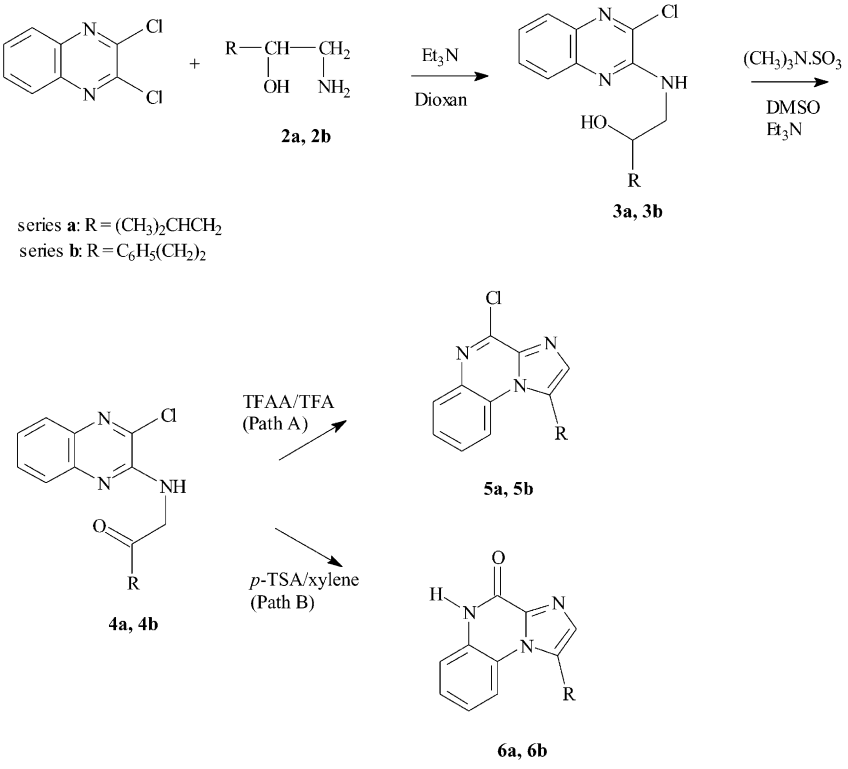

**Scheme 1.** Synthesis of the α-aminoalcohol **2a** and **b**.

yl)amino]-4-phenylbutan-2-ol **3b** intermediates which upon subsequent oxidation of their hydroxyl moiety, in presence of sulphur trioxide trimethylamine complex, gave rise to compounds **4a** and **b** (Scheme 2) with yields ranging from 50 to 60 for each intermediate step except for the synthesis of **4b** which yielded 90%.

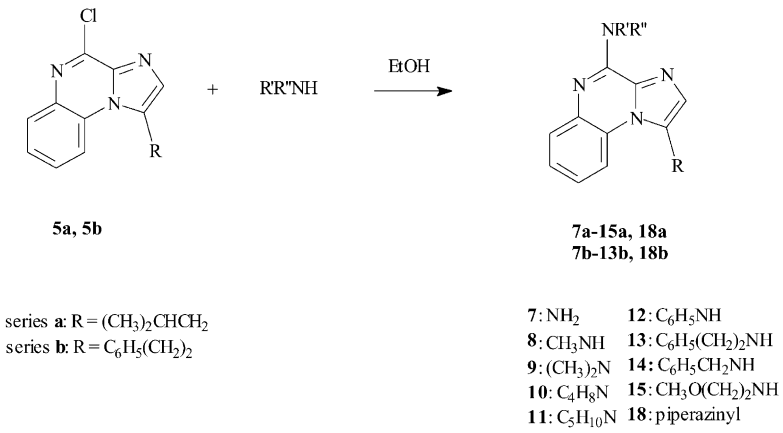
4-Chloro-1-isobutylimidazo[1,2-*a*]quinoxaline **5a** and 4-chloro-1-(2-phenylethyl)imidazo-[1,2-*a*]quinoxaline **5b**, the precursors of series **a** and **b** respectively, were obtained by intramolecular cyclisation,[10,11] a key step in imidazo[1,2-*a*]quinoxalines skeletal construction, of the corresponding 1-[(3-chloroquinoxalin-2-yl)amino]-4-methylpentan-2-ol **4a** and 1-[(3-chloroquinoxalin-2-yl)amino]-4-phenylbutan-2-ol **4b** (Scheme 2, step 3). In this synthesis the problem of ring closure was effectively controlled. Indeed, dehydration in trifluoroacetic anhydride/trifluoroacetic acid led to the exclusive products **5a** and **5b** with yields of 84% and 66% respectively (path A), whilst cyclising performed in the presence of *p*-toluene sulfonic acid/xylene favoured an alternate pathway generating lactams **6a** and **b** (path B) with an average yield of 85%. With compounds **5a** and **b** in hand, the next step in the synthesis plan required nucleophilic substitution of the chlorine in position 1 by various amines (Scheme 3). With the exception of compounds **7a** and **b**, a general protocol was used for all compounds, albeit compounds **17a** and **b** bearing an *N*-Boc group required a deprotection step to give compounds **18a** and **b** respectively. Briefly the appropriate amines were added to a solution of **5a** or **b** dissolved in Et2O, and the reaction mixtures were stirred either at room temperature (3–40 h) or under reflux (8 to 24 h), both with 80–90% yield for series **a** and 60–65% yield for series **b** respectively.

## 3. Biology

The potential for the synthesised imidazo[1,2-*a*]quinoxalines to inhibit PDE4 activity was tested on the HSPDE4 isoform. This isoform was isolated from the human alveolar epithelial cell line A459 by sonication, ultracentrifugation and liquid chromatography purification using a Q-Sepharose Fast-Flow column. The presence of the PDE4 in the chromatography fractions was revealed by SDS-PAGE gel electrophoresis as a single band of ~90 kDa. In addition to the predominant isoform HSPDE4, A459 cells also contain a low concentration of PDE1 and a relatively large amount of PDE3,[6,12] whose active site has a similar structure to that of PDE4.[12] Since the characterisation of PDE4 inhibitors requires an enzymatic preparation containing no detectable amount of PDE3, we investigated the presumably pure PDE4 fractions for a possible contamination by these isoforms (Fig. 1). To that effect, we used rolipram, cilostamide and $Ca^{2+}$/Calmodulin ($Ca^{2+}$/CaM) as these are a selective inhibitor of PDE4, a selective inhibitor of PDE3 and a specific activator of




**Scheme 2.** Synthesis of the imidazo[1,2-*a*]quinoxaline heterocycle.




**Scheme 3.** Nucleophilic substitution of chlorine by the appropriate amines.

PDE1, respectively. Their activities on an ultracentrifuged crude extract and chromatography-purified PDE4 were compared (Fig. 1). The high inhibition activity of 100 μM rolipram on crude extract as well as on purified PDE confirmed a high prevalence of PDE4 in A549 cells. Cilostamide (2.5 μM) partially inhibited the crude extract, thus signalling the possible presence of PDE3 in A549 cells. After chromatography, results of inhibition activity are in favour of the elimination of significant PDE3 activity in the purified preparation. As an increase of enzymatic activity was observed with 0.1 mM $Ca^{2+}$/150 μM CaM between the crude extract and the purified PDE, a co-purification of PDE1 and PDE4 could not be excluded. Fortunately, PDE1, which is present at low concentration in A549 cells, is strongly inhibited in absence of $Ca^{2+}$.[13] Since our activity measurements of imidazo[1,2-*a*]quinoxalines on PDE4 were performed at a low concentration of $Ca^{2+}$ (50 μM), the possible presence of small amounts of PDE1 in the purified preparation should not significantly influence the characterisation of the synthesised compounds as PDE4 inhibitors. Besides, the experimental $IC_{50}$ value of rolipram (6.03 ± 0.84 μM) determined from the purified PDE4 preparation was similar to the published value[14] which would not be the case if PDE1 levels were present.

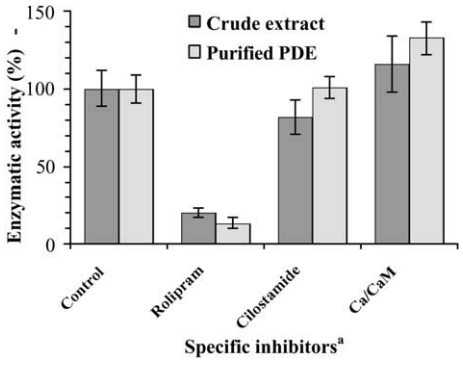



**Figure 1.** Characterization of the PDE preparation. [a]Control: Positive control, cAMP without inhibitor. Rolipram: cAMP + 100 μM rolipram (PDE4 inhibitor). Cilostamide: cAMP + 2.5 μM cilostamide (PDE3 inhibitor). Ca/CaM: cAMP + 0.1 mM $Ca^{2+}$ + 150 μM Calmodulin (PDE1 activator).

## 4. Results and discussion

Based on an early report from this laboratory,[9] we designed novel imidazo[1,2-*a*]quinoxalines with improved pharmacological profiles. SAR studies performed at that time clearly demonstrated that a substitution in position 1 and/or 2 has a strong influence on the targeted PDE isoform; a substitution in position 2 unambiguously led to a preferential inhibition of PDE3 whilst an imidazo[1,2-*a*]quinoxaline substitution in R1 exhibited selective PDE4 inactivation. Furthermore it was shown that the most potent PDE4 inhibitor possessed a hydrophobic side chain at R1. In the present work, we further explored the tolerance for steric hindrance in position 1 and synthesised two series of imidazo[1,2-*a*]quinoxaline of twelve and ten analogues each, in which R1 was either an *iso*-butyl (series **a**) or a 2-phenylethyl moiety (series **b**). In position 4, different groups were placed which had different electronic, hydrophobic and steric properties such as the chloro, methylamino, pyrolidyl, (2-methoxyethyl)amino, phenethylamino or piperazinyl functions which gave rise to compounds **5** through **18** presented in Table 1.

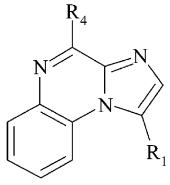
All derivatives were assessed for their inhibitory activity on HSPDE4 from human alveolar epithelial cell line A459. The residual enzyme activity in presence of 100 μM for each inhibitor is shown in Figure 2 for series **a**. In Table 1 are reported the residual enzyme activity in presence of 100 μM for all compounds and the concentration of compounds that inhibited 50% of the PDE activity ($IC_{50}$), only for compounds that showed a residual enzyme activity less than 50% at 100 μM (except for compound **11a** which showed ~52% of PDE activity at 100 μM).

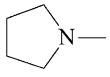
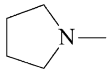
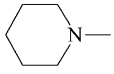
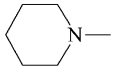
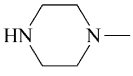
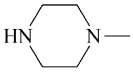
A rapid comparison of the residual PDE4 activity obtained for series **a** and **b** did not show any significant variation which could be correlated to the properties of the moiety in position R1. Of particular notice are compounds **18a** and **b**, for which less than 5% activity is retained. Nevertheless, for compounds **8** and **9**, and to a lesser extend compounds **5** and **7**, the isobutyl substitution appears to be more favorable than the phenylethyl group.

The different substitutions at R4 within the series **a** affected quite differently the PDE4 activity (Fig. 2). Whilst compounds **5a**, **6a**, **7a** and **8a** with respectively a chloro, a hydroxyl, a primary amino and an aminomethyl moiety group with a mesomer donor effect exhibited a potent PDE4 inhibitory activity (over 80% of inhibition) as expected,[9,15] substitution on the amino group led to a significant drop in activity. This decrease is remarkable not only because it closely parallels the increase of the length of the lipophylic side chain (compound **8a** and **15a**) but also the decrease of the length of aliphatic spacer between the amino and the phenyl moieties (compound **13a**, **14a** and **12a**). A similar profile was observed for compound **9a**, **10a** and **11a** when R4 was an aminodimethyl, a pyrolidyl and a piperidyl group, respectively; however substitution with a piperazyl group (compound **18a**) dramatically reduced PDE4 activity. These preliminary results suggested that it is not total steric volume *per se* which hampers PDE4 inhibition but the deficiency in accessibility of the amino group.

Further analysis of the most potent inhibitors (i.e., those producing more than 50% inhibition) in both series showed that the majority of the compounds tested have an $IC_{50}$ of the same order of magnitude as the reference drug rolipram with the exception of compounds **6a**, **9a** and **13a** which are 3 to 5 times less active. These differences might be related to the possible keto/enol form of compound **6a** on one hand and to the poor

**Table 1.** Imidazo[1,2-*a*]quinoxalines: formulas and phosphodiesterase 4 inhibitory activities




| Compd | $R_1$ | $R_4$ | mp (°C) | Formula | PDE4 residual activity (%)[a] | $IC_{50}$ (μM)[b] |
|---|---|---|---|---|---|---|
| **5a** | $(CH_3)_2$–CH–$CH_2$– | Cl– | 118 | $C_{14}H_{14}N_3Cl$ | 9.64±1.44 | 4.00±0.92 |
| **5b** | $C_6H_5$-$(CH_2)_2$– | Cl– | 122–124 | $C_{18}H_{14}N_3Cl$ | 24.00±4.07 | 3.00±0.63 |
| **6a** | $(CH_3)_2$–CH–$CH_2$– | HO– | >250 | $C_{14}H_{14}N_3O$ | 13.87±1.33 | 20.00±4.63 |
| **6b** | $C_6H_5$-$(CH_2)_2$– | HO– | >250 | $C_{18}H_{14}N_3O$ | 97.40±7.43 | — |
| **7a** | $(CH_3)_2$–CH–$CH_2$– | $NH_2$– | >250 | $C_{14}H_{16}N_4$ | 6.79±1.45 | 2.00±0.18 |
| **7b** | $C_6H_5$-$(CH_2)_2$– | $NH_2$– | 186 | $C_{18}H_{16}N_4$ | 28.05±2.40 | 10.00±1.00 |
| **8a** | $(CH_3)_2$–CH–$CH_2$– | $CH_3$-NH– | 126–127 | $C_{15}H_{18}N_4$ | 6.10±3.00 | 0.20±0.05 |
| **8b** | $C_6H_5$-$(CH_2)_2$– | $CH_3$-NH– | 148–149 | $C_{19}H_{18}N_4$ | 68.28±6.80 | — |
| **9a** | $(CH_3)_2$–CH–$CH_2$– | $(CH_3)_2$-N– | 118–119 | $C_{16}H_{20}N_4$ | 21.56±3.86 | 20.00±7.00 |
| **9b** | $C_6H_5$-$(CH_2)_2$– | $(CH_3)_2$-N– | 140 | $C_{20}H_{20}N_4$ | 61.76±9.45 | — |
| **10a** | $(CH_3)_2$–CH–$CH_2$– |  | 112–113 | $C_{18}H_{22}N_4$ | 62.04±7.58 | — |
| **10b** | $C_6H_5$-$(CH_2)_2$– |  | 128 | $C_{22}H_{22}N_4$ | 62.01±10.78 | — |
| **11a** | $(CH_3)_2$–CH–$CH_2$– |  | 60–61 | $C_{19}H_{24}N_4$ | 47.79±3.63 | — |
| **11b** | $C_6H_5$-$(CH_2)_2$– |  | 112–113 | $C_{23}H_{24}N_4$ | 53.34±2.51 | — |
| **12a** | $(CH_3)_2$–CH–$CH_2$– | $C_6H_5$-NH– | 119–120 | $C_{20}H_{20}N_4$ | 53.13±10.85 | — |
| **12b** | $C_6H_5$-$(CH_2)_2$– | $C_6H_5$-NH– | 126–128 | $C_{24}H_{20}N_4$ | 52.98±1.56 | — |
| **13a** | $(CH_3)_2$–CH–$CH_2$– | $C_6H_5$-$(CH_2)_2$-NH– | 105–106 | $C_{22}H_{24}N_4$ | 41.02±8.73 | 30.00±5.00 |
| **13b** | $C_6H_5$-$(CH_2)_2$– | $C_6H_5$-$(CH_2)_2$-NH– | 120 | $C_{26}H_{24}N_4$ | 63.48±5.96 | — |
| **14a** | $(CH_3)_2$–CH–$CH_2$– | $C_6H_5$–$CH_2$-NH– | 136–137 | $C_{21}H_{22}N_4$ | 50.90±11.72 | — |
| **15a** | $(CH_3)_2$–CH–$CH_2$– | $CH_3O$-$(CH_2)_2$-NH– | 98–99 | $C_{17}H_{22}N_4O$ | 18.09±1.58 | — |
| **18a** | $(CH_3)_2$–CH–$CH_2$– |  | >250 | $C_{18}H_{23}N_5$ | 3.36±1.42 | 3.00±0.84 |
| **18b** | $C_6H_5$-$(CH_2)_2$– |  | >250 | $C_{22}H_{23}N_5$ | 2.87±0.40 | 5.00±1.00 |

[a,b]The results presented are means±SEM of at least three experiments.
[a] All compounds were tested at 100 μM.
[b] $IC_{50}$ values were determined using a nonlinear regression curve fitting program for compounds which inhibited 50% or more of the PDE activity at 100 μM. Rolipram $IC_{50}$ value: 6.03±0.84 μM.

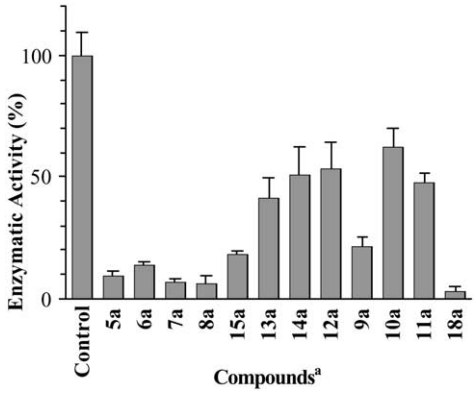



**Figure 2.** PDE4 activity without (Control) and with different imidazo[1,2-*a*]quinoxalines (100 μM). [a]Control: Positive control, cAMP without inhibitor. **5a**–**18a**: cAMP with 100 μM imidazo[1,2-*a*]-quinoxalines.

exposure of the amino group in the case of compounds **9a** and **13a**. Interestingly, compounds **5a** and **b**, which are the precursors of both series, show an $IC_{50}$ half of that of rolipram but no effect related to the steric or the electronic differences in R1 could be established, whereas a slight difference was noted for compounds **18** and **7**. Indeed in both cases, the **b** series was less active (40% for compound **18** and 20% for compound **7**) than the **a** series. It is also worth noticing that although compound **7a** was 3 times more potent than the reference compound, one should really consider compound **8a** which was 30 times more active than rolipram. Thus an amino group in R1 is essential for PDE4 inactivation and a single substitution with a methyl group is optimal. Whilst this confirms results reported previously[9,15] it would be interesting to further probe the role of the linker in compounds based on compound **18a**.

## 5. Conclusion

In the present paper, we describe the synthesis of imidazo[1,2-*a*]quinoxaline derivatives and their PDE4 inhibitory activities. The synthesis was based on intramolecular cyclisation of quinoxaline substituted by an aminoketone group, followed by nucleophilic substitution with appropriate amines. Their inhibitory activities were assayed on PDE4 extracted from a human alveolar epithelial cell line (A549). Substitution in position 1 was previously shown to be essential.[9] Comparative results obtained with either an alkyl or a phenylethyl group on this position show that the nature of the groups may in certain cases have a substantial effect on PDE4 inhibitory activity. Substitution in position 4 clearly shows the need of a weakly hindered amino group.

## 6. Experimental

### 6.1. Chemistry

**6.1.1. General procedures.** $^1H$ and $^{13}C$ NMR spectra were recorded using a Bruker AC 100 spectrometer. Chemical shifts are reported in parts per million (ppm) relative to TMS as internal standard. Coupling constants are reported in hertz (Hz), spectral splitting patterns are designed as follows: s, singlet; br, broad; d, doublet; t, triplet; m, multiplet. All melting points are uncorrected and were determined using a Köfler hot plate melting point apparatus. All reagents and solvents were of reagent grade and used without further purification unless indicated otherwise. Column chromatography was performed on Merck silica gel 60 (200–400 mesh). Elemental analysis was carried out at the Microanalytical Central Department, Vernaison, France. Its results were not corrected.

All reactions were monitored by TLC on 20×60 mm plastic sheets precoated with silica gel (HT-254 Merck) to a thickness of 0.25 mm and viewed at 254 nm UV-light.

**6.1.2. 2-Hydroxy-4-methylpentanenitrile (1a).** Isovaleraldehyde (10 g, 116 mmol) was added over 2–3 min to a stirred solution of 37% aqueous $NaHSO_3$ (25 mL, 116 mmol), at 0 °C. A white bisulphite precipitate was formed almost immediately. A solution of NaCN (5.7 g, 116 mmol) in $H_2O$ (30 mL) was then added dropwise, over 45 min. Stirring was maintained for 18 h at room temperature, during which time the precipitate was redissolved and two immiscible layers were formed. The mixture was extracted with $Et_2O$ (30 mL). The combined dried ($Na_2SO_4$) organic layers were evaporated to give a yellow oil which was used without further purification (12.21 g, 93%); $^1H$ NMR (100 MHz, $CDCl_3$) δ: 0.88 (d, $J=6$ Hz, 6H), 1.51–1.98 (m, 3H, H-3 + H-4), 4.20 (s, br, OH), 4.45 (t, $J=7$ Hz, 1H); $^{13}C$ NMR (25 MHz, DMSO-$d_6$) δ: 21.6, 22.1, 23.8, 43.1, 58.3, 121.3. Anal. calcd for $C_6H_{11}NO$: C, 63.68; H, 9.80; N, 12.38. Found: C, 63.56; H, 9.75; N, 12.42.

**6.1.3. 2-Hydroxy-4-phenylbutanenitrile (1b).** **1b** was prepared from 3-phenylpropionaldehyde following the protocol described for **1a**; 3-phenylpropionaldehyde (11.14 g, 83.2 mmol), $NaHSO_3$ (17.7 mL of 37% aqueous solution, 83 mmol), NaCN (4.08 g, 83.2 mmol) in $H_2O$ (18 mL). The product, a yellow oil, was used without further purification (12.4 g, 93%); $^1H$ NMR (100 MHz, $CDCl_3$) δ: 2.02–2.25 (m, 2H), 2.76–2.91 (m, 2H), 3.98 (s, 1H), 4.39 (t, $J=8$ Hz, 1H), 7.26 (d, $J=3$ Hz, 5H); $^{13}C$ NMR (25 MHz, $CDCl_3$) δ: 30.49, 36.37, 59.92, 120.04, 126.28, 128.28, 128.49, 139.60. Anal. calcd for $C_{10}H_{11}NO$: C, 74.51; H, 6.88; N, 8.69. Found: C, 74.38; H, 6.97; N, 8.42.

**6.1.4. 1-Amino-4-methylpentan-2-ol (2a).** A solution of **1a** (12.13 g, 107 mmol) dissolved in $Et_2O$ (50 mL), was added dropwise, over 45 min, under gentle reflux to a stirred slurry of $LiAlH_4$ (8.12 g, 214 mmol) in $Et_2O$ (160 mL). The mixture was further refluxed for 90 min. After cooling to 0–5 °C, the excess of $LiAlH_4$ was quenched by dropwise addition of $H_2O$ (8 mL), 15% aqueous NaOH (8 mL) and $H_2O$ (40 mL). The mixture was stirred until all $LiAlH_4$ was consumed and a white precipitate was formed. The mixture was filtered and the precipitate was washed with $Et_2O$. The organic fraction was dried (KOH pellets) and the solvent was removed

under reduced pressure to yield an orange oil which was used without further purification (11.78 g, 94%); $^1$H NMR (100 MHz, $CDCl_3$) δ: 0.74 (d, $J=7$ Hz, 6H), 1.04 (m, 2H, H-3), 1.56 (m, $J=7$ Hz, 1H, H-4), 2.41 (m, 5H, H-1, OH, NH2), 3.40 (m, $J=4$ Hz, 1H, H-2); $^{13}$C NMR (25 MHz, DMSO-$d_6$) δ: 21.90, 23.20, 24.30, 43.80, 47.80, 69.90. Anal. calcd for $C_6H_{15}NO$: C, 61.49; H, 12.90; N, 11.95. Found: C, 61.70; H, 12.53; N, 11.67.

**6.1.5. 1-Amino-4-phenylbutan-2-ol (2b).** **2b** was prepared from **1b** following the protocol described for **2a**; $LiAlH_4$ (6.2 g, 163 mmol), $Et_2O$ (120 mL), **1b** (12.4 g, 77 mmol), $H_2O$ (6.2 mL), 15% aqueous NaOH (6.2 mL) and $H_2O$ (18 mL). The product, an orange oil, was used without further purification (11 g, 87%); $^1$H NMR (100 MHz, $CDCl_3$) δ: 1.80 (m, 3H), 2.65 (m, 4H), 3.62 (m, 3H), 7.21 (s, 5H); $^{13}$C NMR (25 MHz, $CDCl_3$) δ: 33.98, 36.25, 70.92, 125.46, 128.04, 141.74. Anal. calcd for $C_{10}H_{15}NO$: C, 72.69; H, 9.15; N, 8.48. Found: C, 72.45; H, 9.33; N, 8.24.

**6.1.6. 1-[(3-Chloroquinoxalin-2-yl)amino]-4-methylpentan-2-ol (3a).** 2,3-dichloroquinoxaline (18.05 g, 90.7 mmol) was added to a solution of crude **2a** (11.7 g, 99.8 mmol) and $Et_3N$ (19 mL, 13.8 g, 136 mmol) in dioxan (210 mL). The resulting solution was refluxed (under $N_2$) for 6 h, whereupon it was cooled to room temperature. $Et_3N$, HCl was removed by filtration and the filtrate was concentred under reduced pressure. The dark orange residue was purified by column chromatography on silica gel eluting with $CH_2Cl_2$/MeOH (100:0→99:1) to yield a yellow solid (12.14 g, 48%); $^1$H NMR (100 MHz, $CDCl_3$) δ: 0.72 (d, 3H), 0.78 (d, 3H), 1.60 (m, 3H), 3.68 (m, 4H), 5.82 (t, 1H), 7.58 (m, 4H); $^{13}$C NMR (25 MHz, $CDCl_3$) δ: 22.17, 23.35, 24.60, 44.32, 48.62, 69.67, 125.30, 125.62, 127.91, 130.36, 136.52, 137.92, 140.59, 148.62. Anal. calcd for $C_{14}H_{18}N_3OCl$: C, 60.10; H, 6.49; N, 15.02. Found: C, 59.87; H, 6.62; N, 15.25.

**6.1.7. 1-[(3-Chloroquinoxalin-2-yl)amino]-4-phenylbutan-2-ol (3b).** **3b** was prepared from **2b** following the protocol described for **3a**; 2,3-dichloroquinoxaline (9.95 g, 50 mmol), **2b** (9.02 g, 55 mmol) in dioxan (250 mL) with $Et_3N$ (7.57 g, 75 mmol). The crude product was purified by column chromatography on silica gel eluting with $CH_2Cl_2$/MeOH (98:2) to yield a yellow solid (10 g, 61%); $^1$H NMR (100 MHz, $CDCl_3$) δ: 1.76–1.97 (m, 2H), 2.70–2.90 (m, 2H), 3.53–4.05 (m, 4H), 5.96 (t, $J=5$ Hz, 1H), 7.23–7.82 (m, 9H). Anal. calcd for $C_{18}H_{18}N_3OCl$: C, 65.95; H, 5.53; N, 12.82. Found: C, 66.16; H, 5.43; N, 12.58.

**6.1.8. 1-[(3-Chloroquinoxalin-2-yl)amino]-4-methylpentan-2-one (4a).** A mixture of **3a** (6.96 g, 24.8 mmol), 28.4 mL $Et_3N$ and sulphur trioxide trimethylamine complex (7.9 g, 56.8 mmol) in 28.4 mL DMSO, was stirred overnight (under $N_2$) at room temperature, whereupon ice-cold water (50 mL) was added. The aqueous layer was extracted with $CH_2Cl_2$ (3×30 mL). The combined organic phases were dried ($CaCl_2$) and the solvents removed under reduced pressure. The residues were purified by column chromatography on silica gel eluting with $C_6H_{12}/Et_2O$ (85:15) to give a beige solid (4.18 g, 61%); $^1$H NMR (100 MHz, $CDCl_3$) δ: 0.93 (s, 3H), 1.00 (s, 3H), 2.34 (m, 1H), 2.40 (s, 2H), 4.38 (d, 2H), 6.39 (t, 1H), 7.59 (m, 4H); $^{13}$C NMR (25 MHz, $CDCl_3$) δ: 22.56, 25.01, 49.28, 51.55, 125.34, 125.87, 127.98, 130.14, 136.65, 137.92, 140.99, 147.30, 205.01. Anal. calcd for $C_{14}H_{16}N_3OCl$: C, 60.54; H, 5.81; N, 15.13. Found: C, 60.32; H, 6.06; N, 14.95.

**6.1.9. 1-[(3-Chloroquinoxalin-2-yl)amino]-4-phenylbutan-2-one (4b).** **4b** was prepared from **3b** following the protocol described for **4a**; **3b** (4.7 g, 14.4 mmol), 14.4 mL DMSO, 14.4 mL $Et_3N$, and $Me_3N.SO_3$ (4 g, 28.8 mmol). The product was purified by column chromatography on silica gel eluting with $C_6H_{12}/Et_2O$ (80:20) to give a yellow solid (4.51 g, 96%); $^1$H NMR (100 MHz, $CDCl_3$) δ: 2.82–3.01 (m, 4H), 4.38 (d, $J=5$ Hz, 2H), 6.4 (m, 1H), 7.23–7.84 (m, 9H). Anal. calcd for $C_{18}H_{16}N_3OCl$: C, 66.36; H, 4.95; N, 12.90. Found: C, 66.53; H, 5.23; N, 12.78.

**6.1.10. 4-Chloro-1-isobutylimidazo[1,2-*a*]quinoxaline (5a).** **4a** (16.39 g, 23 mmol) was dissolved in a mixture of trifluoroacetic anhydride (100 mL) and trifluoroacetic acid (1 mL) and stirred (under $N_2$) for 24 h at room temperature. The solvent was then removed under reduced pressure, and the crude concentrate was dissolved in $CH_2Cl_2$ (300 mL). The organic fraction was successively washed with 5% $NaHCO_3$ (75 mL) and water, dried ($Na_2SO_4$) and concentrated under vacuum to yield a dark orange oil which was purified by column chromatography on silica gel eluting with $C_6H_{12}/Et_2O$ (95:5) to give a cream solid, (5.04 g, 84%); $^1$H NMR (100 MHz, $CDCl_3$) δ: 1.07 (d, $J=6$–7 Hz, 6H), 2.16 (m, 1H), 3.11 (d, $J=6$–7 Hz, 2H), 7.53–7.64 (m, 3H), 7.96–8.16 (m, 2H); $^{13}$C NMR (25 MHz, $CDCl_3$) δ: 22.41, 26.88, 36.66, 115.39, 126.50, 128.41, 128.94, 130.04, 132.02, 134.37, 135.61, 136.60, 143.66. Anal. calcd for $C_{14}H_{14}N_3Cl$: C, 64.74; H, 5.43; N, 16.18. Found: C, 64.66; H, 5.55; N, 15.86.

**6.1.11. 4-Chloro-1-(2-phenylethyl)imidazo[1,2-*a*]quinoxaline (5b).** **5b** was prepared from **4b** following the protocol described for **5a**; **4b** (4 g, 12.2 mmol), trifluoroacetic anhydride (100 mL), trifluoroacetic acid (4 mL). The product was purified by column chromatography on silica gel eluting with $CH_2Cl_2$/MeOH (98:2) to give a yellow solid (2.5 g, 66%); $^1$H NMR (100 MHz, $CDCl_3$) δ: 3.20–3.31 (m, 2H), 3.53–3.68 (m, 2H), 7.32 (s, 5H), 7.54–7.63 (m, 3H), 7.99–8.31 (m, 2H); $^{13}$C NMR (25 MHz, $CDCl_3$) δ: 30.05, 33.80, 115.34, 126.54, 126.66, 128.19, 128.46, 128.70, 129.94, 129.20, 133.00, 139.72. Anal. calcd for $C_{18}H_{14}N_3Cl$: C, 70.24; H, 4.58; N, 13.65. Found: C, 70.04; H, 4.96; N, 13.82.

**6.1.12. 1-Isobutylimidazo[1,2-*a*]quinoxaline-4(5H)-one (6a).** *p*-Toluene sulfonic acid (30 mg, 0.15 mmol) was added, to a stirred solution of **4a** (0.5 g, 1.8 mmol) under reflux in xylene (300 mL). Refluxing in a Dean-Stark was maintained for 24 h. The solvent was eliminated under reduced pressure and the residue was dissolved in $CH_2Cl_2$ (500 mL). The organic fraction was then successively washed with 5% $Na_2CO_3$ (5 mL) and water (5 mL) prior to be dried ($Na_2SO_4$) and concentred to give

the crude product which was purified by column chromatography on silica gel eluting with $CH_2Cl_2$/MeOH (90:10) to yield a yellow solid (0.35 g, 82%); $^1$H NMR (100 MHz, $CDCl_3$) δ: 1.07 (d, 6H), 2.16 (m, 1H), 3.11 (d, 2H), 7.53–7.64 (m, 3H), 7.96–8.16 (m, 2H); $^{13}$C NMR (25 MHz, $CDCl_3$) δ: 22.41, 26.88, 36.66, 115.39, 126.50, 128.41, 128.94, 130.04, 132.02, 134.37, 135.61, 136.60, 143.66. Anal. calcd for $C_{14}H_{14}N_3O$: C, 69.69; H, 6.27; N, 17.415. Found: C, 70.01; H, 6.01; N, 17.70.

**6.1.13. 1-(2-Phenylethyl)-imidazo[1,2-*a*]quinoxaline-4(5H)-one (6b).** **6b** was prepared from **4b** following the protocol described for **6a**; **4b** (0.5 g, 1.5 mmol). The product was purified by column chromatography as specified for **4a** to give a yellow solid (0.38 g, 88%); $^1$H NMR (100 MHz, DMSO-$d_6$) δ: 3.04–3.19 (m, 2H), 3.46–3.61 (m, 2H), 7.13–7.39 (m, 9H), 8.10 (d, $J$ = 7.6 Hz, 1H), 11.87 (s, 1H); $^{13}$C NMR (25 MHz, DMSO-$d_6$) δ: 28.53, 52.93, 116.25, 116.53, 122.45, 123.00, 134.90, 135.19, 128.12, 128.74, 131.21, 131.91, 140.21, 152.25. Anal. calcd for $C_{18}H_{14}N_3O$: C, 74.72; H, 5.23; N, 14.52. Found: C, 74.65; H, 5.14; N, 14.75.

**6.1.14. 1-Isobutylimidazo[1,2-*a*]quinoxaline-4-amine (7a).** **5a** (1 g, 3.85 mmol) was heated to 120 °C for 4 h in the presence of ammonia (60 mL of a 30% (w/v) aqueous solution, 0.5 mmol). The reaction mixture was allowed to cool to room temperature and filtered. The precipitate was washed with $H_2O$ (10 mL), dissolved in $CH_2Cl_2$ (25 mL), and the organic fraction was dried ($Na_2SO_4$) and concentred under reduced pressure. The crude product was purified by column chromatography on silica gel eluting with $CH_2Cl_2$/MeOH (90:10) to give a yellow solid (0.75 g, 81%); $^1$H NMR (100 MHz, DMSO-$d_6$ ) δ: 2.07 (m, $J$ = 6-7 Hz, 1H), 3.09 (d, $J$ = 6-7 Hz, 2H), 7.08–8.05 (m, 7H). Anal. calcd for $C_{14}H_{16}N_4$: C, 69.97; H, 6.71; N, 23.32. Found: C, 70.13; H, 6.97; N, 23.07.

**6.1.15. 4-Chloro-1-(2-phenylethyl)imidazo[1,2-*a*]quinoxaline (7b).** **7b** was prepared from **5b** following the protocol described for **7a**. **5b** (0.8 g, 2.6 mmol); ammonia (48 mL of a 30% (w/v) aqueous solution, 0.4 mmol). A yellow solid was obtained after column chromatography purification as specified for **7a** (0.465 g, 62%); $^1$H NMR (100 MHz, $CDCl_3$) δ: 3.10–3.24 (m, 2H), 3.48–3.63 (m, 2H), 5.74 (s, 2H), 7.20–7.48 (m, 8H), 7.69 (d, $J$ = 7.2 Hz, 1H), 8.05 (d, $J$ = 7.9 Hz, 1H); $^{13}$C NMR (25 MHz, $CDCl_3$) δ: 29.75, 34.00, 115.01, 123.09, 125.94, 126.33, 126.78, 128.08, 128.46, 130.51, 130.89, 137.50, 140.04, 148.44. Anal. calcd for $C_{18}H_{16}N_4$: C, 74.98; H, 5.59; N, 19.43. Found: C, 74.86; H, 5.75; N, 19.37.

**6.1.16. 1-Isobutyl-*N*-methyl-imidazo[1,2-*a*]quinoxaline-4-amine (8a).** Methylamine (0.6 mL of a 40% (w/v) aqueous solution, 6.93 mmol) was added dropwise to a stirred solution of **5a** (0.6 g, 2.31 mmol) in absolute EtOH (15 mL) at room temperature. After 40 h, another portion of methylamine (0.6 mL of a 40% (w/v) aqueous solution, 6.93 mmol) was added and stirring was maintained for an additional 3 h. The solvent was removed under reduced pressure and the residue was dissolved in $CH_2Cl_2$ (50 mL). The organic fraction was successively washed with 5% $NaHCO_3$ (30 mL) and $H_2O$ (30 mL), dried ($Na_2SO_4$) and concentred under reduced pressure. The product was purified by column chromatography on silica gel eluting with $C_6H_{12}$/EtOAc (70:30) to yield a cream solid (0.49 g, 83%); $^1$H NMR (100 MHz, $CDCl_3$) δ: 1.04 (d, $J$ = 6.2 Hz, 6H), 1.90–2.35 (m, 1H), 3.03 (d, $J$ = 7.0 Hz, 2H), 3.20 (d, $J$ = 4.9 Hz, 3H), 6.10–6.40 (m, 1H), 7.10–7.55 (m, 3H), 7.60–8.00 (m, 2H); $^{13}$C NMR (25 MHz, $CDCl_3$) δ: 22.43, 26.93, 27.37, 36.65, 115.15, 122.49, 125.93, 126.63, 127.38, 130.43, 131.32, 134.01, 138.23, 148.41. Anal. calcd for $C_{15}H_{18}N_4$: C, 70.84; H, 7.13; N, 22.03. Found: C, 71.12; H, 7.44; N, 22.21.

**6.1.17. *N*-Methyl-1-(2-phenylethyl)imidazo[1,2-*a*]quinoxaline-4-amine (8b).** **8b** was prepared from **5b** following the protocol described for **8a**; methylamine (0.260 mL of a 40% (w/v) aqueous solution, 3 mmol), **5b** (0.307 g, 1 mmol). The crude product was purified by column chromatography eluting with $CH_2Cl_2$/MeOH (90:10) to yield a yellow solid (0.2 g, 66%); $^1$H NMR (100 MHz, $CDCl_3$) δ: 3.07–3.22 (m, 5H), 3.42–3.58 (m, 2H), 6.56 (d, $J$ = 5.6 Hz, 1H), 7.12–7.45 (m, 7H), 7.73 (d, $J$ = 9.1 Hz, 1H), 7.98 (d, $J$ = 7.9 Hz, 1H); $^{13}$C NMR (25 MHz, $CDCl_3$) δ: 27.27, 29.76, 33.96, 114.93, 122.28, 125.79, 126.33, 127.05, 129.16, 129.49, 129.79, 130.51, 133.72, 133.97, 140.24, 149.14. Anal. calcd for $C_{19}H_{18}N_4$: C, 75.47; H, 6.00; N, 18.43. Found: C, 75.68; H, 6.15; N, 18.51.

**6.1.18. 1-Isobutyl-*N*,*N*-dimethylimidazo[1,2-*a*]quinoxaline-4-amine (9a).** **9a** was prepared from **5a** following the protocol described for **8a**; dimethylamine (1.3 mL of a 40% (w/v) aqueous solution, 11.55 mmol), **5a** (1.03 g, 3.85 mmol) in absolute EtOH (10 mL). The crude product was purified by column chromatography on silica gel eluting with $CH_2Cl_2$/MeOH (97:3) to give a white solid (0.8 g, 78%); $^1$H NMR (100 MHz, $CDCl_3$) δ: 1.03 (d, $J$ = 6-7 Hz, 6H), 2.16 (m, $J$ = 6–7 Hz, 1H), 3.03 (d, $J$ = 6–7 Hz, 2H), 3.58 (s, 6H), 7.15–7.41 (m, 3H), 7.62–7.72 (m, 1H), 7.86–7.95 (m, 9H); $^{13}$C NMR (25 MHz, $CDCl_3$) δ: 22.42, 26.75, 36.89, 40.00, 114.86, 122.05, 125.80, 126.50, 126.80, 126.98, 129.51, 131.08, 134.74, 137.74, 149.15. Anal. calcd for $C_{16}H_{20}N_4$: C, 71.61; H, 7.51; N, 20.88. Found: C, 71.77; H, 7.23; N, 20.64.

**6.1.19. N,N-Dimethyl-1-(2-phenylethyl)imidazo[1,2-*a*]quinoxaline-4-amine (9b).** **9b** was prepared from **5b** following the protocol described for **9a**; **5b** (0.32 g, 1 mmol), dimethylamine (0.44 mL of a 40% (w/v) aqueous solution, 3.90 mmol). The product was purified by column chromatography as specified for **9a** and yield a beige solid (0.2 g, 65%); $^1$H NMR (100 MHz, $CDCl_3$) δ: 3.09–3.26 (m, 2H), 3.42–3.54 (m, 2H), 3.59 (s, 6H), 7.14–7.42 (m, 8H), 7.70 (d, $J$ = 6.5 Hz, 1H), 8.00 (d, $J$ = 6.9 Hz, 1H); $^{13}$C NMR (25 MHz, $CDCl_3$) δ: 30.41, 34.30, 40.12, 114.99, 125.25, 126.02, 126.58, 127.07, 128.43, 128.75, 129.89, 130.02, 134.84, 137.82, 140.57, 149.21. Anal. calcd for $C_{20}H_{20}N_4$: C, 75.92; H, 6.37; N, 17.71. Found: C, 75.82; H, 6.15; N, 17.57.

**6.1.20. (1-Isobutyl-4-pyrrolidin-1-yl)imidazo[1,2-*a*]quinoxaline (10a).** Pyrrolidine (0.58 mL, 0.49 g, 6.93 mmol) was added dropwise to a stirred solution of **5a** (0.6 g, 2.31

mmol) in absolute EtOH (15 mL) at room temperature. Stirring was maintained for 3 h. The solvent was removed under reduced pressure and the residue was dissolved in $CH_2Cl_2$ (50 mL). The organic phase was successively washed with 5% $Na_2CO_3$ (30 mL) and $H_2O$ (30 mL), dried ($Na_2SO_4$) and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel eluting with $C_6H_{12}$/EtOAc (85:15) to yield a cream solid (0.6 g, 88%); $^1$H NMR (100 MHz, $CDCl_3$) δ: 1.03 (d, $J$ = 6.5 Hz, 6H), 1.85–2.30 (m, 5H), 3.04 (d, $J$ = 6.7 Hz, 2H), 4.00–4.25 (m, 4H), 7.05–7.45 (m, 3H), 7.60–7.95 (m, 2H); $^{13}$C NMR (25 MHz, $CDCl_3$) δ: 22.38, 25.41, 26.73, 36.83, 49.24, 114.84, 121.36, 125.74, 126.22, 126.26, 129.53, 131.34, 134.90, 138.50, 147.33. Anal. calcd for $C_{18}H_{22}N_4$: C, 73.44; H, 7.53; N, 19.03. Found: C, 73.24; H, 7.68; N, 19.31.

**6.1.21. [1-(2-Phenylethyl)-4-pyrrolidin-1-yl]imidazo[1,2-*a*]quinoxaline (10b).** **10b** was prepared from **5b** following the protocol described for **10a**; pyrrolidine (0.37 mL, 0.32 g, 4.5 mmol), **5b** (0.46 g, 1.5 mmol). The product was purified by column chromatography as specified for **10a** and yield a yellow solid (0.32 g, 62%); $^1$H NMR (100 MHz, $CDCl_3$) δ: 1.93–2.06 (m, 4H), 3.08–3.26 (m, 2H), 3.40–3.60 (m, 2H), 4.05–4.17 (m, 4H), 7.11–7.41 (m, 8H), 7.67 (d, $J$ = 6.8 Hz, 1H), 7.98 (d, $J$ = 8.2 Hz, 1H); $^{13}$C NMR (25 MHz, $CDCl_3$) δ: 25.55, 30.36, 34.35, 49.11, 115.30, 121.62, 126.01, 126.56, 126.81, 128.43, 128.75, 130.19, 134.80, 138.65, 140.61, 147.48. Anal. calcd for $C_{22}H_{22}N_4$: C, 77.16; H, 6.48; N, 16.36. Found: C, 77.48; H, 6.96; N, 16.22.

**6.1.22. (1-Isobutyl-4-piperidin-1-yl)imidazo[1,2-*a*]quinoxaline (11a).** **11a** was prepared from **5a** following the protocol described for **10a**; piperidine (0.7 mL, 0.59 g, 6.93 mmol); **5a** (0.6 g, 2.31 mmol) in absolute EtOH (15 mL). The product was purified by column chromatography on silica gel eluting with $C_6H_{12}$/$Et_2O$ (90:10) to yield a white solid (0.68 g, 96%); $^1$H NMR (100 MHz, $CDCl_3$) δ: 1.04 (d, $J$ = 6.2 Hz, 6H), 1.60–1.90 (m, 6H), 1.90–2.40 (m, 1H), 3.04 (d, $J$ = 6.6 Hz, 2H), 4.10–4.40 (m, 4H), 7.10–7.45 (m, 2H), 7.60–8.05 (m, 3H); $^{13}$C NMR (25 MHz, $CDCl_3$) δ: 22.41, 25.02, 26.22, 26.78, 36.92, 47.90, 114.82, 122.33, 125.72, 126.67, 127.21, 129.40, 131.01, 134.70, 137.57, 148.79. Anal. calcd for $C_{19}H_{24}N_4$: C, 73.99; H, 7.84; N, 18.17. Found: C, 73.61; H, 7.72; N, 18.43.

**6.1.23. [1-(2-Phenylethyl)-4-piperidin-1-yl]imidazo[1,2-*a*]quinoxaline (11b).** **11b** was prepared from **5b** following the protocol described for **10a**; piperidine (0.7 mL, 0.59 g, 6.93 mmol), **5b** (0.7 g, 2.3 mmol). The product was purified by column chromatography as specified for **11a** to yield a yellow solid (0.62 g, 75%); $^1$H NMR (100 MHz, $CDCl_3$) δ: 1.76 (s, 6H), 3.11–3.30 (m, 2H), 3.47–3.65 (m, 2H), 4.28 (s, 4H), 7.14–7.46 (m, 8H), 7.72 (d, $J$ = 8.4 Hz, 1H), 8.05 (d, $J$ = 8.7 Hz, 1H); $^{13}$C NMR (25 MHz, $CDCl_3$) δ: 25.11, 26.36, 30.30, 34.17, 47.83, 53.79, 115.81, 122.29, 125.78, 126.41, 126.97, 128.40, 128.61, 129.62, 129.97, 137.63, 140.74, 148.90. Anal. calcd for $C_{23}H_{24}N_4$: C, 77.50; H, 6.79; N, 15.72. Found: C, 77.33; H, 6.91; N, 15.48.

**6.1.24. 1-Isobutyl-*N*-phenylimidazo[1,2-*a*]quinoxaline-4-amine (12a).** Aniline (0.63 mL, 0.645 g, 6.93 mmol) was added dropwise to a stirred solution of **5a** (0.6 g, 2.31 mmol) in absolute EtOH (15 mL) at room temperature. Stirring was maintained for 67 h. The solvent was removed under reduced pressure and the residue was dissolved in $CH_2Cl_2$ (50 mL). The organic fraction was washed successively with 5% $Na_2CO_3$ (30 mL) and $H_2O$ (30 mL), dried ($Na_2SO4$) and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel eluting with $C_6H_{12}$/EtOAc (80:20) to yield a white solid (0.41 g, 56%); $^1$H NMR (100 MHz, $CDCl_3$) δ: 1.07 (d, $J$ = 6.8 Hz, 6H), 1.95–2.45 (m, 1H), 3.07 (d, $J$ = 7.0 Hz, 2H), 6.95–7.55 (m, 5H), 7.70–8.30 (m, 5H); $^{13}$C NMR (25 MHz, $CDCl_3$) δ: 22.456, 26.95, 36.67, 115.11, 119.33, 122.73, 123.82, 126.06, 127.09, 128.38, 128.96, 131.04, 131.44, 133.60, 137.31, 139.49, 144.65. Anal. calcd for $C_{20}H_{20}N_4$: C, 75.92; H, 6.37; N, 17.71. Found: C, 75.89; H, 6.03; N, 17.95.

**6.1.25. *N*-Phenyl-1-(2-phenylethyl)imidazo[1,2-*a*]quinoxaline-4-amine (12b).** **12b** was prepared from **5b** following the protocol described for **10a**; aniline (0.63 mL, 0.645 g, 6.93 mmol), **5b** (0.7 g, 2.3 mmol). The product was purified by column chromatography as specified for **12a** to yield a yellow solid (0.376 g, 45%); $^1$H NMR (100 MHz, $CDCl_3$) δ: 3.13–3.30 (m, 2H), 3.50–3.64 (m, 2H), 6.66–6.82 (m, 1H), 7.10–7.50 (m, 10H), 7.85–9.23 (m, 5H); $^{13}$C NMR (25 MHz, $CDCl_3$) δ: 29.99, 34.12, 115.06, 119.46, 119.23, 122.70, 123.94, 126.00, 126.55, 127.00, 128.30, 128.69, 128.93, 129.21, 130.16, 131.28, 133.66, 137.21, 139.42, 140.26, 144.53. Anal. calcd for $C_{24}H_{20}N_4$: C, 79.10; H, 5.53; N, 15.37. Found: C, 79.35; H, 5.36; N, 15.69.

**6.1.26. 1-Isobutyl-*N*-(2-phenylethyl)imidazo[1,2-*a*]quinoxaline-4-amine (13a).** 2-Phenylethyl-amine (0.8 mL, 0.84 g, 6.93 mmol) was added dropwise to a stirred solution of **5a** (0.6 g, 2.31 mmol) in absolute EtOH (15 mL) at room temperature. Stirring was maintained for 23 h and then the reaction mixture was refluxed for an additional 24 h. The solvent was removed under reduced pressure and the residue was dissolved in $CH_2Cl_2$ (50 mL). The organic fraction was successively washed with 5% $Na_2CO_3$ (30 mL) and $H_2O$ (30 mL), dried ($Na_2SO_4$) and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel eluting with $C_6H_{12}$/EtOAc (85:15) to yield a cream solid (0.7 g, 88%); $^1$H NMR (100 MHz, $CDCl_3$) δ: 1.05 (d, $J$ = 6.5 Hz, 6H), 1.90–2.30 (m, 1H), 2.95–3.15 (m, 4H), 3.80–4.05 (m, 2H), 6.26 (t, $J$ = 5.7 Hz, 1H), 7.10–7.50 (m, 8H), 7.70–8.00 (m, 2H); $^{13}$C NMR (25 MHz, $CDCl_3$) δ: 22.45, 26.95, 35.81, 36.67, 41.87, 115.15, 122.59, 125.94, 126.38, 126.70, 127.50, 128.57, 128.81, 130.49, 131.36, 137.91, 139.26, 147.59. Anal. calcd for $C_{22}H_{24}N_4$: C, 76.71; H, 7.52; N, 16.27. Found: C, 77.05; H, 7.47; N, 16.21.

**6.1.27. *N*,1-bis(2-Phenylethyl)imidazo[1,2-*a*]quinoxaline-4-amine (13b).** **13b** was prepared from **5b** following the protocol described for **13a**; 2-Phenylethylamine (0.87 mL, 0.84 g, 6.93 mmol), **5b** (0.7 g, 2.3 mmol). The crude

product was purified by column chromatography as specified for **13a** and yield a yellow solid (0.56 g, 62%); $^1$H NMR (100 MHz, $CDCl_3$) δ: 3.02 (m, 4H), 3.48 (m, 2H), 3.87 (m, 2H), 6.21 (m, 1H), 7.27 (s, 13H), 7.23 (d, $J$ = 7.1 Hz, 1H), 7.99 (d, $J$ = 7.8 Hz, 1H); $^{13}$C NMR (25 MHz, $CDCl_3$) δ: 29.83, 34.04, 35.59, 41.73, 114.94, 122.39, 125.80, 126.19, 126.39, 127.27, 129.19, 129.39, 129.53, 129.65, 129.85, 130.59, 133.62, 137.99, 139.12, 140.29, 147.39. Anal. calcd for $C_{26}H_{24}N_4$: C, 79.56; H, 6.16; N, 14.27. Found: C, 79.91; H, 6.22; N, 14.33.

**6.1.28. 1-Isobutyl-*N*-benzylimidazo[1,2-*a*]quinoxaline-4-amine (14a).** Benzylamine (0.76 mL, 0.743 g, 6.93 mmol) was added dropwise to a stirred solution of **5a** (0.6 g, 2.31 mmol) in absolute EtOH (15 mL) at room temperature. Stirring was continued for 19.5 h and the reaction mixture was then refluxed for an additional 8 h. The solvent was removed under reduced pressure and the residue was dissolved in $CH_2Cl_2$ (50 mL). The organic fraction was successively washed with 5% $NaHCO_3$ (10 mL) and $H_2O$ (10 mL), dried ($Na_2SO_4$) and concentrated under reduced pressure. The crude product was purified by column chromatography as specified for **13a** to yield a white solid (0.5 g, 66%); $^1$H NMR (100 MHz, $CDCl_3$) δ: 1.05 (d, $J$ = 6.6 Hz, 6H), 1.90–2.45 (m, 1H), 3.03 (d, $J$ = 6.8 Hz, 2H), 4.87 (d, $J$ = 5.7 Hz, 2H), 6.35–6.65 (m, 1H), 7.05–7.55 (m, 8H), 7.65–8.05 (m, 2H); $^{13}$C NMR (25 MHz, $CDCl_3$) δ: 22.43, 26.91, 36.63, 44.51, 115.12, 122.67, 125.93, 126.79, 127.29, 127.53, 127.99, 128.53, 130.53, 131.39, 133.80, 138.06, 137.78, 147.47. Anal. calcd for $C_{21}H_{22}N_4$: C, 76.33; H, 6.71; N, 16.96. Found: C, 76.23; H, 6.99; N, 16.90.

**6.1.29. 1-Isobutyl-*N*-(2-methoxyethyl)imidazo[1,2-*a*]quinoxaline-4-amine (15a).** 2-Methoxyethyl-amine (0.603 mL, 0.521 g, 6.93 mmol) was added dropwise to a stirred solution of **5a** (0.60 g, 2.31 mmol) in absolute EtOH (15 mL) at room temperature. Stirring was maintained for 22 h and then the reaction mixture was refluxed for 24 h. The solvent was removed under reduced pressure and the residue was dissolved in $CH_2Cl_2$ (50 mL). The organic fraction was successively washed with 5% $NaHCO_3$ (10 mL) and $H_2O$ (10 mL), dried ($Na_2SO_4$) and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel eluting with $C_6H_{12}$/EtOAc (70:30) to yield a cream solid (0.61 g, 89%); $^1$H NMR (100 MHz, $CDCl_3$) δ: 1.03 (d, $J$ = 6.7 Hz, 6H), 1.95–2.35 (m, 1H), 3.02 (d, $J$ = 6.9 Hz, 2H), 3.39 (s, 3H), 3.65 (t, $J$ = 4.8 Hz, 2H), 3.75–4.00 (m, 2H), 6.35–6.60 (m, 1H), 7.05–7.50 (m, 3H), 7.60–8.00 (m, 2H); $^{13}$C NMR (25 MHz, $CDCl_3$) δ: 2.37, 26.87, 36.59, 40.06, 58.76, 71.13, 115.08, 122.51, 125.84, 126.66, 127.32, 130.41, 131.40; 133.81, 138.02, 147.63, 175.15. Anal. calcd for $C_{17}H_{22}N_4O$: C, 68.43; H, 7.43; N, 18.78. Found: C, 68.71; H, 7.24; N, 18.83.

**6.1.30. *t*-Butyl-4-piperazine (16).** Compound **16** was prepared as described in the litterature.[16]

**6.1.31. *t*-Butyl-4-(1-isobutylimidazo[1,2-*a*]quinoxalin-4-yl)-piperazine-1-carboxylate (17a).** **16** (0.86 g, 4.62 mmol) was added to a stirred solution of **5a** (0.60 g, 2.31 mmol) in absolute EtOH (15 mL). After 22 h of reflux, the solvent was removed under reduced pressure. The residue was dissolved in $CH_2Cl_2$ (50 mL). The organic fraction was successively washed with 5% $NaHCO_3$ (10 mL) and $H_2O$ (10 mL), dried ($Na_2SO_4$) and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel eluting with $C_6H_{12}$/EtOAc (85:15) to yield a white solid (0.88 g, 93%); $^1$H NMR (100 MHz, $CDCl_3$) δ: 1.05 (d, $J$ = 6.9 Hz, 6H), 1.47 (s, 9H), 2.00–2.40 (m, 1H), 3.06 (d, $J$ = 7.0 Hz, 2H), 3.50–3.70 (m, 4H), 4.20–4.40 (m, 4H), 7.15–7.50 (m, 3H), 7.65–8.05 (m, 2H); $^{13}$C NMR (25 MHz, $CDCl_3$) δ: 22.46, 26.82, 28.43, 36.90, 43.78, 46.59, 79.82, 114.95, 123.18, 125.94, 126.88, 127.56, 129.73, 131.23, 134.42, 137.07, 148.44, 154.90.

**6.1.32. *t*-Butyl-4-[1-(2-phenylethyl)imidazo[1,2-*a*]quinoxalin-4-yl]piperazine-1-carboxylate (17b). 17b** was prepared from **5b** following the protocol described for **17a**; **16** (0.74 g, 3 mmol), **5b** (0.62 g, 2 mmol), absolute EtOH (15 mL). The crude product was purified by column chromatography as specified for **17a** to give a white solid (0.54 g, 59%); $^1$H NMR (100 MHz, $CDCl_3$) δ: 1.43 (s, 9H), 3.04–3.22 (m, 2H), 3.43–3.61 (m, 6H), 4.19–4.28 (t, 2H), 7.11–7.34 (m, 3H + 5H), 7.64–7.71 (m, 1H), 7.96–8.03 (m, 1H).

**6.1.33. (1-Isobutyl-4-piperazin-1-yl)imidazo[1,2-*a*]quinoxaline (18a).** A solution of **17a** (0.82 g, 2 mmol) in TFA/$CH_2Cl_2$ (1:1) (50 mL) was stirred at room temperature for 30 min during which time a yellow solution resulted. The volatiles were removed under reduced pressure to leave a yellow oil which was dissolved in $CH_2Cl_2$ (30 mL), washed with 5% $NaHCO_3$ (30 mL) and then extracted with 1 M HCl (2×20 mL). The pH of the combined aqueous layers was adjusted to 10 with 1 M $Na_2CO_3$. The precipitate formed was collected by filtration, washed with a small amount of ice-cold water and dried to constant weight in a drying pistol to yield a white solid which was not further purified (0.46 g, 74%); $^1$H NMR (100 MHz, $CDCl_3$) δ: 1.04 (d, $J$ = 6.7 Hz, 6H), 1.95–2.35 (m, 1H), 3.06 (d, $J$ = 6.9 Hz, 2H), 3.25–3.60 (m, 4H), 4.50–4.85 (m, 4H), 6.10 (s, br, 1H), 7.15–7.50 (m, 3H), 7.60–8.10 (m, 2H); $^{13}$C NMR (25 MHz, $CDCl_3$) δ: 22.45, 26.80, 36.82, 43.48, 43.71, 114.99, 124.05, 126.07, 127.10, 127.94, 130.03, 131.43, 133.88, 136.46, 147.40. Anal. calcd for $C_{18}H_{23}N_5$: C, 69.87; H, 7.49; N, 22.63. Found: C, 70.15; H, 7.13; N, 23.01.

**6.1.34. [1-(2-phenylethyl)-4-piperazin-1-yl]imidazo[1,2-*a*]-quinoxaline (18b). 18b** was prepared from **17b** following the protocol described for **18a**; **17a** (0.54 g, 1.18 mmol), TFA/$CH_2Cl_2$ (1:1) (25 mL). The product obtained, a white solid, was not further purified (0.2 g, 47%); $^1$H NMR (100 MHz, MeOD) δ: 3.16–3.61 (m, 6H), 4.40–4.54 (m, 4H), 7.22–7.51 (m, 8H), 7.70–7.78 (m, 1H), 8.18–8.25 (m, 1H); $^{13}$C NMR (25 MHz, MeOD) δ: 30.75, 35.14, 45.08, 44.66, 116.73, 125.85, 127.49, 128.42, 129.00, 129.52, 129.67, 131.69, 132.35, 137.69, 141.81, 149.10. Anal. calcd for $C_{22}H_{23}N_5$: C, 73.92; H, 6.49; N, 19.59. Found: C, 74.29; H, 6.11; N, 19.36.

### 6.2. Biochemistry: measurement of PDE4 inhibitory activity

**6.2.1. Materials.** A549 cells were from the American Type Culture Collection, Rockville, MD, USA. T75 culture flasks were obtained from Costar. Dulbecco's modified Eagle's Medium (DMEM)-F12 came from Gibco-BRL, and L-glutamine, penicillin, streptomycin and phosphate-buffered saline (PBS) from BioMedia. Foetal calf serum (FCS) was supplied by Bio Whittaker Europe. Dimethylsulfoxyde (DMSO) was provided by ICN Biomedicals. Tris-2-amino-2-(hydroxymethyl)-1,3-propanediol pH 7.5 was from Q-Biogene. AG-50 Wx8 (200–400 mesh) and Poly-Prep (0.8×4 cm) columns were from Bio-Rad. [3,8-$^3$H]cAMP and Q-Sepharose Fast-Flow were from Amersham Biosciences. All other chemicals were from Sigma-Aldrich except when otherwise stated. Ultima Gold MV was supplied by Packard. Radioactivity was measured in a Beckman LS-3801 liquid scintillation β-counter. The A549 cells were counted in a Casy cell-counter. Protein concentration was determined with a commercial kit (Coomassie Plus Protein Assay Reagent®, Pierce), using bovine serum albumin (BSA) as a standard.

**6.2.2. A549 cells culture and HSPDE4 extraction.** A549 cells were cultured to confluence in T75 culture flasks, at 37 °C in 5% $CO_2$ in DMEM-F12 medium supplemented with 2 mM L-glutamine, 100 U/mL penicillin/streptomycin and 10% heat-inactivated FCS whereupon they were trypsinated for 4 min, collected by centrifugation at room temperature at 2000 *g* for 5 min and washed twice with PBS without calcium and magnesium and stored at −80 °C.

The pellets were thawed on ice and resuspended to a density of $10^7$ cells/mL in 10 mL ice-cold lysis buffer (10 mM Tris–HCl pH 7.5, 2 mM $MgCl_2$, 5 mM β-mercaptoethanol, 10 μM antipain, 10 μM leupeptin, 10 μM pepstatin A, 200 μM PMSF, 1 mM EDTA, 0.2 mM EGTA, 10 mM NaF, and 10 μM TLCK) and disrupted by sonication (ten pulses, 30 seconds each, on ice, at 60 W). The lysat was clarified by centrifugation at 100 000 *g* for 1 h at 4 °C and the supernatant was applied on a Q-Sepharose Fast-Flow (1.6×10 cm) column pre-equilibrated with 10 mM Tris–HCl pH 7.5, 2 mM $MgCl_2$ and 5 mM β-mercaptoethanol. The column was developed at a flow rate of 1 mL/min, with a non-linear sodium acetate gradient (100 min at 0 M, 50 min from 0.5 to 0.6 M, 60 min from 0.6 to 0.9 M, 25 min at 1 M, and 20 min at 0 M), 1 mL fractions were collected. The protein containing fractions were determined by 12.5% SDS–PAGE and the presence of PDE4 by enzymatic activity measurements, with or without rolipram, as described below. The active fractions were pooled and either kept on ice for at most one month or stored frozen at −80 °C after addition of 20% v/v glycerol. All subsequent enzymatic determinations were performed using this batch of PDE4.

### 6.3. PDE4 activity determination, PDE4 characterization and PDE4 inhibitor studies

PDE activity was measured as described previously[12,17] with minor modifications. Briefly, Poly-Prep columns were filled with 0.5 mL AG-50 Wx8 mesh resuspended in water. Prior to use, the columns were washed with 1 mL of 1 N NaOH, two times 1 mL of 1 N HCl, and four times 1 mL of $H_2O$.

The reaction mixture (100 μL) routinely contained 40 mM Tris–HCl, pH 8.0, 0.05 mM $CaCl_2$, 3 mM $MgCl_2$, 0.1 mg/mL BSA and 1% DMSO. The reaction, carried out at 37 °C, was started by addition of [$^3$H]cAMP (15 000 to 25 000 cpm, $0.1\times10^{-6}$ and $1\times10^{-3}$ M) and stopped with 0.5 mL of 5% TCA containing 1 mM AMP and 1 mM cAMP. The reaction mixtures were applied on the columns which were washed three times with 0.4 mL and four times with 1 mL $H_2O$, [$^3$H]AMP was eluted with five successive additions of 0.6 mL 0.4 M sodium citrate pH 7.5, collected directly in scintillation vials and immediately measured by scintillation spectroscopy after addition of 12 mL of Ultima Gold MV.

The purity of the PDE4 isoenzyme prepared was assessed by (i) its sensitivity to 100 μM rolipram, a selective PDE4 inhibitor, (ii) the effect of 2.5 μM cilostamide, a PDE3 selective inhibitor and (iii) the response to $Ca^{2+}$/Calmodulin (0.1 mM/150 μM) stimulation, a PDE1 specific activator.

Stock solutions for the inhibitors were prepared in 100% DMSO. The final concentration of DMSO in the enzyme assay was 1%.

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