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**Synthesis, docking study and kinase inhibitory activity of a number of new substituted pyrazolo[3,4-c]pyridines.**

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## Summary

A series of new pyrazolo[3,4-*c*]pyridines bearing various 1, 3, 5 or 1, 3, 7 pattern substitutions, were designed and synthesized. Some of them showed interesting inhibitory activity mainly against GSK3 $\alpha/\beta$  as well as against CLK1 and DYRK1A, with good selectivity and remarkable SARs, without being cytotoxic. Molecular simulations in correlation with biological data revealed the importance of the existence of *N*1-H as well as the absence of a bulky 7-substituent.

**Keywords:** pyrazolopyridine; kinase inhibition; GSK3 $\alpha/\beta$ ; purine analogues; molecular simulations

Neurodegenerative diseases are among the most challenging diseases with poorly known mechanism and lack of complete cure. Alzheimer's disease (AD) in particular, is the most prevalent cause of dementia, very devastating for the patients and their families, provided that current treatments offer only modest symptomatic relief. This severe mental disorder is mostly aging-associated and represents a global health problem, since it currently affects more than 30 million people worldwide, and its incidence is predicted to rise significantly, due to the increasing average life span.<sup>1)</sup> In an effort to explain the pathogenesis of AD, many hypotheses have been explored, among them neurotransmitter modulation, chronic inflammation, metal-induced oxidative stress, elevated cholesterol, and glycogen synthase kinase 3 (GSK-3) implication are of major importance.<sup>2)</sup> GSK-3 is a ubiquitous serine/threonine kinase, which was first described as the major regulator of glycogen metabolism. It was revealed that GSK-3 plays central roles in important cell signaling pathways, and its malfunction is associated with neurodegeneration and the pathogenesis of several diseases, including diabetes type II, immune and bipolar disorders, chronic inflammation, heart failure and cancer.<sup>3)</sup> In AD, GSK-3 promotes neuronal death and is a linker between the two histopathological hallmarks: the deposition of extracellular senile plaques composed of amyloid-beta (Ab) peptide, and the accumulation of hyperphosphorylated microtubule-binding tau protein, leading to tau aggregation and the formation of intracellular neurofibrillary tangles.<sup>4)</sup> On the other hand, substantial evidence exists to support the involvement of additional phylogenetically and physiologically relevant protein kinases in neurodegenerative disorders, and, most notably, cyclin dependent kinase 5 (CDK5),<sup>5)</sup> dual-specificity tyrosine phosphorylation-regulated kinase 1A (DYRK 1A),<sup>6)</sup> casein kinase 1 (CK1)<sup>7)</sup> and cdc2-like kinases CLK1 and CLK2<sup>8)</sup> are also investigated as potential targets for the development of novel AD therapeutics. Consequently, the exploitation of small molecule

inhibitors for potential inhibitory activity against a set of the above mentioned protein kinases could offer interesting information and assist in the understanding of AD and related tauopathies.

A great number of GSK3 inhibitors have been reported in the literature and some of them were found to ameliorate cognitive impairments<sup>9)</sup> and reduce the amount of amyloid-beta, together with the tau hyperphosphorylation in neuronal cells.<sup>10)</sup> Although a number of putative allosteric inhibitors appeared in the literature,<sup>11)</sup> most of the reported GSK3 inhibitors are ATP competitive, and are often characterized by the presence of a central purine-like heterocyclic scaffold, substituted with a characteristic carboxamide residue.<sup>12,13)</sup> Within this context and as part of a research program aimed to study modified purine analogues as potential protein kinase inhibitors, we have explored the pyrazolopyridine scaffold and present in this study the design, synthesis and evaluation of a number of new substituted pyrazolo[3,4-*c*]pyridines for inhibitory potency against a panel of eight protein kinases.

## Results and discussion

**Chemistry** For the preparation of the target compounds we have used both nitroderivatives of 2-aminopicoline which were synthesized according to reported procedures.<sup>14)</sup> 2-Amino-5-nitropicoline (**1**, Chart 1) was diazotized and the resulting pyridinone **2**<sup>15)</sup> was treated with phosphorus oxychloride and provided the chloride **3**.<sup>16)</sup> This chloride was reduced using tin chloride and the resulting aminoderivative **4** was acetylated to give the acetamide **5**.<sup>17)</sup> Compound **5** was treated with isoamyl nitrite in the presence of potassium acetate and acetic anhydride and this reaction furnished a mixture of the corresponding 1- and 2-acetylpyrazolo[3,4-*c*]pyridines, through a rearrangement of the intermediate N-nitroso compound.<sup>18)</sup> The acetamides were not isolated but the acetyl group was cleaved upon treatment with methanolic ammonia to result in the pyrazolopyridine **6**.<sup>17,19)</sup> The heterocyclic compound **6**

was then nitrated and the resulting 3-nitroderivative **7** was treated with methyl iodide, or 4-methoxybenzyl chloride in the presence of potassium carbonate, to provide both regio-isomers **8a,b** and **9a,b** respectively, which were chromatographically separated and identified by means of 2D NMR experiments. More specifically, in the corresponding NOE spectra we observed clear cross-peak correlation between the *N1* methyl group of **8a**, or the *N1* methylene group of **8b** with H-7, verifying unambiguously the *N1* substitution. This correlation was, as expected not detected in the case of compounds **9a** and **9b**. The chlorine atom of compounds **8a** and **8b** was subsequently displaced upon treatment with cyclohexylamine or aniline to result in nitroderivatives **10a-d**. This reaction was effected through a straightforward aromatic nucleophilic substitution when cyclohexylamine was used, however in the case of the weak nucleophile, aniline, the use of Buchwald-Hartwig coupling conditions was necessary.<sup>20)</sup> Compounds **10a-d** were then catalytically reduced to the 3-aminoderivatives **11a-d** and subsequently converted to the corresponding chloracetamides **12a-d**, which were used for the preparation of the target aminoderivatives **13a-l**. The 4-methoxybenzyl group of the compounds **13g-l** was then removed in acidic media and derivatives **14a-f** were prepared as well.

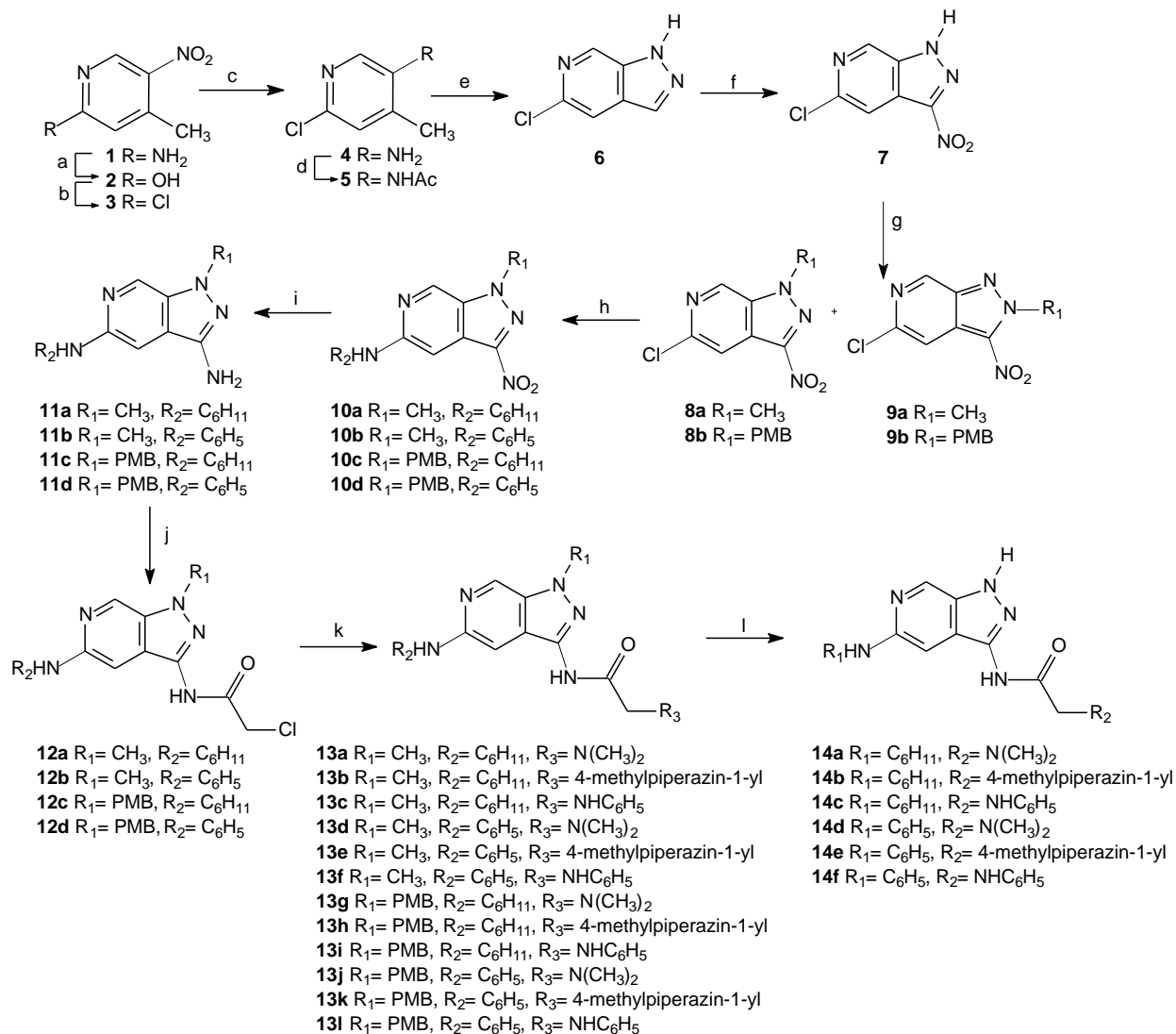


Chart 1. Reagents and conditions: a) NaNO<sub>2</sub>, H<sub>2</sub>SO<sub>4</sub>, H<sub>2</sub>O; b) POCl<sub>3</sub>, 110 °C; c) SnCl<sub>2</sub>·2H<sub>2</sub>O, HCl(c.), 55 °C; d) Ac<sub>2</sub>O, CH<sub>2</sub>Cl<sub>2</sub>, r.t.; e) (1): AcOK, Ac<sub>2</sub>O, isoamyl nitrite, benzene, reflux, (2): NH<sub>3</sub>(g), MeOH, r.t.; f) HNO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub>, 95 °C; g) (1): K<sub>2</sub>CO<sub>3</sub>, MeCN, 0 °C, (2): CH<sub>3</sub>I for **8a**, **9a** or 4-methoxybenzyl chloride for **8b**, **9b**, 50 °C; h) cyclohexylamine, DMSO, 150 °C for **10a** or cyclohexylamine, DMSO, microwave irradiation, 60 W, 158 °C for **10c** or aniline, X-Phos, Pd<sub>2</sub>dba<sub>3</sub>, CsCO<sub>3</sub>, toluene, reflux for **10b**, **10d**; i) Pd/C, H<sub>2</sub>, EtOH, 55 psi, r.t. for **11a-c** or Pd/C, Et<sub>3</sub>SiH, MeOH, r.t for **11d**; j) chloroacetylchloride, Et<sub>3</sub>N, THF, -40 °C for **12a**, **12c** or 0 °C for **12b**, **12d**; k) dimethylamine (5.6M solution in EtOH) for **13a**, **13d**, **13g**, **13j** or 1-methylpiperazine for **13b**, **13e**, **13h**, **13k** or aniline for **13c**, **13f**, **13i**, **13l**, EtOH, 80 °C; l) trifluoroacetic acid, 70°C.

In order to synthesize the corresponding isomeric analogues we used 2-amino-3-nitropicoline (**15**, Chart 2), which by a reaction sequence analogous to the above mentioned





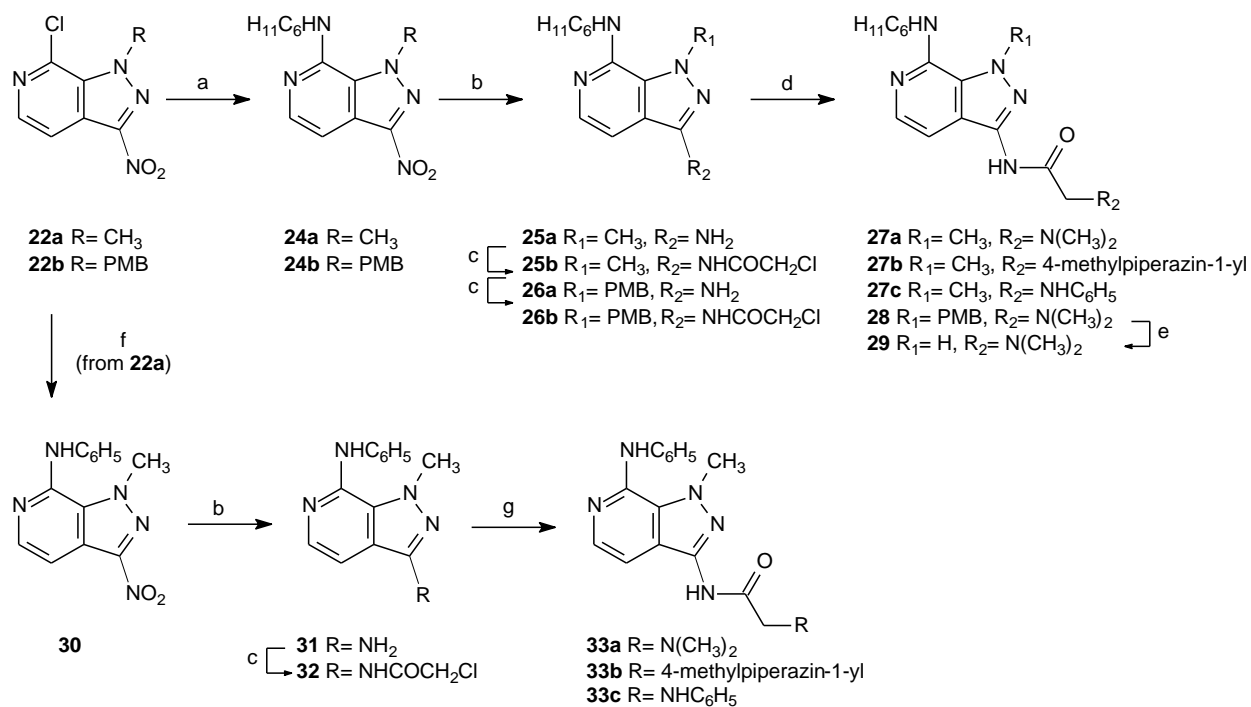


Chart 3. Reagents and conditions: a) cyclohexylamine, DMSO, 150 °C; b) Pd/C, H<sub>2</sub>, EtOH, 55 psi, r.t.; c) chloroacetylchloride, Et<sub>3</sub>N, THF, -40 °C; d) dimethylamine (5.6M solution in EtOH) for **27a**, **28** or 1-methylpiperazine for **27b**, or aniline for **27c**, EtOH, 80 °C; e) trifluoroacetic acid, 70 °C; f) aniline, 2-ethoxyethanol, 130 °C; g) dimethylamine (5.6M solution in EtOH) for **33a**, or 1-methylpiperazine for **33b**, or aniline for **33c**, EtOH, 80 °C.

Our attempts to substitute the chlorine atom of **22b** with aniline were unsuccessful, presumably due to steric hindrance provided by the bulky 1-benzyl group. Thus we used the nitroderivative **21** (Chart 4) which was easily substituted to provide the phenylamino analogue **34**. This compound was treated with 4-methoxybenzylchloride to provide selectively the corresponding *N*2-isomer **35**, which following a procedure analogous to the described above, provided the target compounds **38a,b** and **39a,b**.

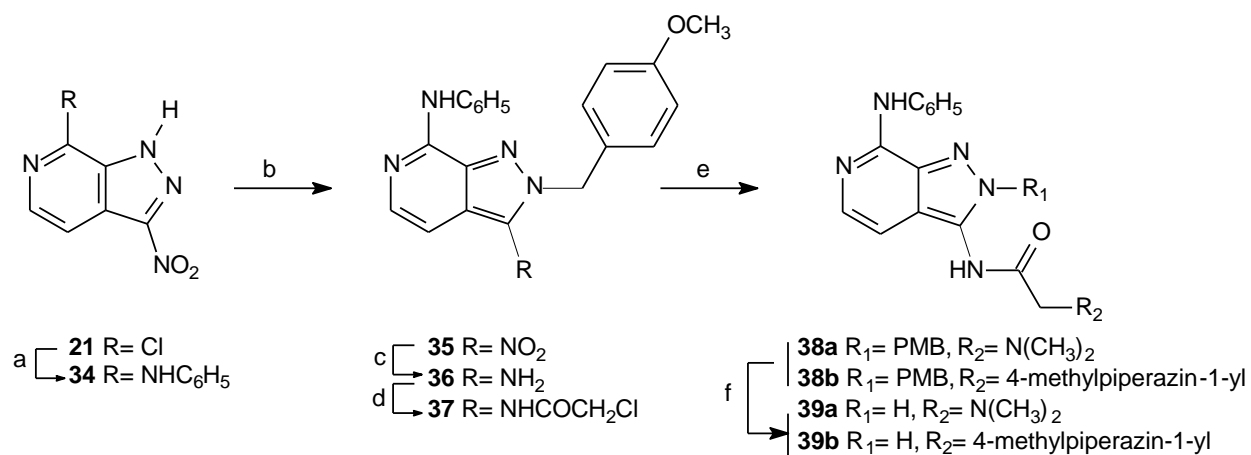


Chart 4. Reagents and conditions: a) aniline, 1,4-dioxane, reflux; b) (1):  $\text{K}_2\text{CO}_3$ , MeCN,  $0\text{ }^\circ\text{C}$ , (2): 4-methoxybenzyl chloride,  $50\text{ }^\circ\text{C}$ ; c) Pd/C,  $\text{H}_2$ , EtOH, 55 psi, r.t.; d) chloroacetylchloride,  $\text{Et}_3\text{N}$ , THF,  $0\text{ }^\circ\text{C}$ ; e) dimethylamine (5.6M solution in EtOH) for **38a** or 1-methylpiperazine for **38b**, EtOH,  $80\text{ }^\circ\text{C}$ ; f) trifluoroacetic acid,  $70\text{ }^\circ\text{C}$ .

**Biological evaluation** The kinase inhibitory activity of the new compounds was tested against a panel of protein kinases and the results concerning the derivatives found to possess  $\text{IC}_{50}$  values  $<10\text{ }\mu\text{M}$  are presented in Table 1. 6-Bromoindirubin-3'-oxime (6-BIO), a selective inhibitor of GSK-3, was used as a reference compound.<sup>21)</sup> It is remarkable to notice that even if the vast majority of the synthesized new compounds are not endowed with interesting inhibitory activity against most of the kinases tested, the substitution pattern around the central heterocyclic ring system plays a crucial role on the inhibitory activity when present. Note here that, the kinases affected in the tested panel are structurally related, as they are members of the same group of the kinome, the CMGC cluster (for CDK, MAPK, GSK3 and CLK).

**Table 1.** IC<sub>50</sub> values ( $\mu\text{M}$ ) of the most active compounds against protein kinases. Results are averages of at least two independent experiments.

Comp	<i>Hs</i> _CDK5	<i>Mm</i> _	<i>Rn</i> _DYR	<i>Ssc</i> _GS	<i>Ssc</i> _CK	<i>Hs</i> _HAS	<i>Hs</i> _AUR	<i>Hs</i> _RIP
	/p25	CLK1	K1A	K3 $\alpha$ / $\beta$	1 $\delta$ / $\epsilon$	PIN	KB	K3
<b>14a</b>	>10	>10	>10	1	>10	>10	>10	>10
<b>14b</b>	>10	1.3	4.5	>10	>10	>10	>10	>10
<b>14c</b>	3.4	7	>10	0.5	>10	>10	>10	>10
<b>14d</b>	>10	>10	>10	0.55	>10	>10	>10	>10
<b>14e</b>	>10	1.1	4	0.4	>10	>10	>10	>10
<b>14f</b>	>10	6	>10	1	>10	>10	>10	>10
<b>6BIO</b>	0.14	ND	1.30	0.014	1.90	ND	ND	ND

In general, the 7-substituted pyrazolopyridines, possessing either a phenylamino- or a cyclohexylamino-group, did not show any activity regardless the decoration of the rest of the scaffold. On the contrary, the corresponding 5-substituted analogues presented quite interesting SARs. Among this group, compounds bearing methyl or 4-methoxybenzyl substitution at position 1, were again found inactive, with the exception of **13d**, which appear to demonstrate a slight inhibitory effect (IC<sub>50</sub>: 2.8  $\mu\text{M}$ ) against CDK5. However, the majority of the corresponding 1-unsubstituted analogues **14a-f**, have shown interesting activity and various degree of specificity. More precisely, compounds **14a**, **14c** and **14d-e** inhibited GSK3 $\alpha$ / $\beta$  with IC<sub>50</sub> values within 0.4-1  $\mu\text{M}$  and among them, **14a** and **14d**, both containing the 3-dimethylaminoacetyl amino substitution are selective towards this kinase. Compounds **14b** and **14e**, bearing a 3-(4-methylpiperazin-1-yl)aminoacetyl amino substitution, show inhibitory

activity against CLK1 and DYRK1A with  $IC_{50}$  values in the range of 1.1-4.5  $\mu\text{M}$ , while **14e** is simultaneously active against GSK3 $\alpha/\beta$  as mentioned before, being the most active analogue in the series showing  $IC_{50}$  value 0.4  $\mu\text{M}$ . Compound **14c**, which possess an aromatic group at position 3- has shown moderate inhibition against CDK5 and CLK 1, while its 5-phenylamino-counterpart **14f**, demonstrated also a slight activity against CLK1. As a remark, the presence of a 5-alkyl or aryl group although important, influence equally the activity, nevertheless the contribution of a pyrazole NH is crucial for inhibitory activity of this type of compounds, mainly against GSK3 $\alpha/\beta$  and in a lesser extent against CLK1 and DYRK1A.

With the aim to estimate the potential antiproliferative activity of compounds **14a-f**, we have also evaluated their cytotoxicity against four breast cancer cell lines, namely MCF7, T47D, MDA-MB-231 and HCC1954 (data not shown). All derivatives presented  $IC_{50}$  values  $\geq 10 \mu\text{M}$ , providing evidence that they do not possess remarkable cytotoxic properties.

The aforementioned findings could argue in favor of a potential use of this type of derivatives against neurodegenerative diseases and this possibility is currently under investigation.

**Theoretical calculations** To gain insight to the putative interaction mode between the active pyrazolopyridine derivatives **14a-f** and GSK3 $\alpha/\beta$ , docking calculations were performed by implementing the Glide SP algorithm and post-docking flexible minimization utilizing MacroModel.<sup>22-26)</sup> The co-crystal structure of GSK3 $\beta$  with indirubin was used as a template for docking calculations (pdb. id: 1UV5).<sup>21)</sup> The predicted binding mode of the pyrazolopyridine scaffold is depicted in Fig. 1. The inhibitors bind the active site of the kinase through a typical type-I interaction mode (Fig. 1A). Two hydrogen bonds are formed between the pyrazole

nitrogens of positions 1 and 2 and the D133 carbonyl of the hinge region, while a third bond is formed between the exocyclic amide nitrogen of position 3 and the V135 backbone amide. An additional hydrogen bond between the nitrogen position 3-substituent and the sidechain of Y134 further stabilizes the complex (Fig. 1B). This interaction mode is in excellent agreement with the two basic SAR notions derived from experiment. Indeed, capping of *N1* diminishes activity as one of the hydrogen bonds would be excluded while the geometry of the rest would be moderately perturbed, as evident for analogues **13a-f**. Moreover, the lack of activity for analogues carrying a bulky group on position 7 can be explained by the existence of extensive steric clashes with the hinge backbone and the gatekeeper sidechain (for example, derivative **39a** as compared to **14a** and **14d** as well as **39b** to **14b** and **14e**). Within the series of active compounds **14a-f**, the preference for a more flexible substitution in position 5 such as a cyclohexyl instead of a phenyl ring can be explained by the enhanced capacity of the aliphatic group to fit to the plasticity of the cavity entrance as defined by the Gly-loop.

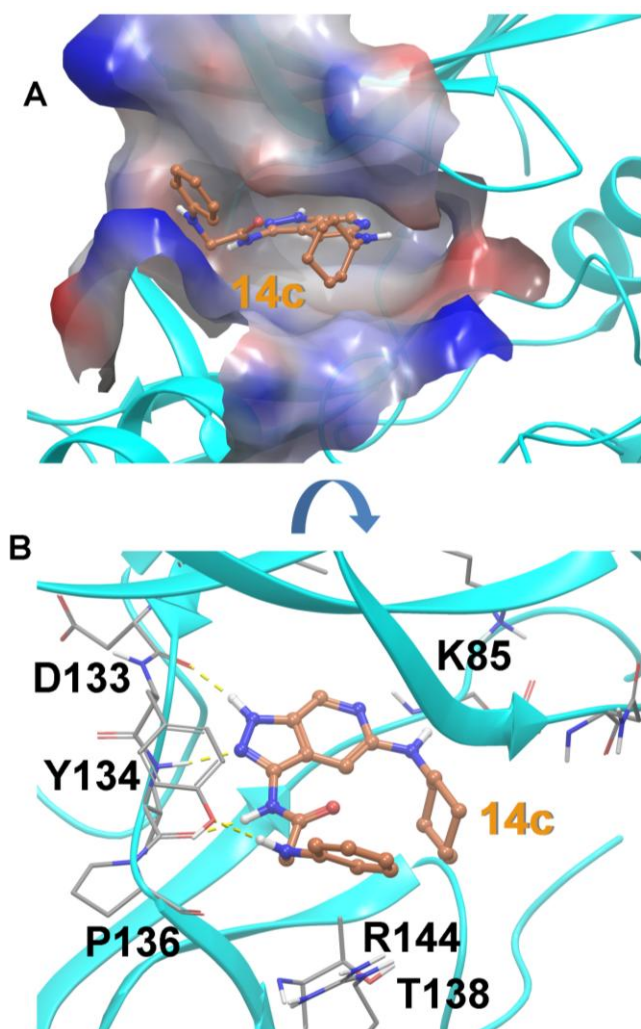


Fig. 1. A. The predicted type-I binding mode of the pyrazolopyridine analogue **14c** in the active site of GSK3 $\beta$  kinase, depicted as an electrostatic potential-coloured molecular surface. B. The pyrazolopyridine **14c** (shown in a ball-and-stick representation) is stabilized in the ATP-binding pocket of GSK3 $\beta$  by four hydrogen bonds (yellow dashed lines) formed between the inhibitor heterocyclic or exocyclic nitrogens and the enzyme.

## Conclusion

In conclusion we have designed and synthesized a number of new *N*-substituted (pyrazolo[3,4-*c*]pyridin-3-yl)acetamides, possessing a variety of substituents on the heterocyclic ring system, in order to study their kinase inhibitory profile. Among the derivatives, only compounds that bear 5-aryl or alkyl substituents and retain the pyrazole nitrogens unsubstituted,

have shown inhibition of GSK3 $\alpha/\beta$ , with adequate selectivity, without being cytotoxic. Docking calculations showed that the aforementioned combination of substituents can provide a rationale for the further development of this original versatile lead into highly potent and selective inhibitors of kinase GSK3 $\alpha/\beta$ .

## Experimental

**Chemistry** Melting points were determined on a Büchi apparatus and are uncorrected.  $^1\text{H}$  NMR spectra and 2D spectra were recorded on a Bruker Avance III 600 or a Bruker Avance DRX 400 instrument, whereas  $^{13}\text{C}$  NMR spectra were recorded on a Bruker Avance III 600 or a Bruker AC 200 spectrometer in deuterated solvents and were referenced to TMS ( $\delta$  scale). The signals of  $^1\text{H}$  and  $^{13}\text{C}$  spectra were unambiguously assigned by using 2D NMR techniques:  $^1\text{H}^1\text{H}$  COSY, NOESY, HMQC, and HMBC. Mass spectra were recorded with a LTQ Orbitrap Discovery instrument, possessing an Ionmax ionization source. Flash chromatography was performed on Merck silica gel 60 (0.040–0.063 mm). Analytical thin layer chromatography (TLC) was carried out on precoated (0.25 mm) Merck silica gel F-254 plates. The purity of all the synthesized compounds was >95% as ascertained by elemental analysis. Elemental analyses were undertaken using a PerkinElmer PE 240C elemental analyzer (Norwalk, CT, U.S.) and the measured values for C, H, and N were within  $\pm 0.4\%$  of the theoretical values.

**5-Chloro-3-nitro-1*H*-pyrazolo[3,4-*c*]pyridine (7)** Chloro derivative **6** (490 mg, 3.19 mmol) was dissolved into concentrated sulfuric acid (9.50 mL) at 0 °C and then a mixture of concentrated sulfuric acid (0.49 mL) and nitric acid (0.49 mL) was added dropwise into this solution. This reaction mixture was heated at 95 °C for 1h. Upon cooling, it was poured into crashed ice, neutralized with an aqueous solution of  $\text{NH}_3$  and the product was extracted with

ethyl acetate (3 x 100 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated under reduced pressure to provide 600 mg of pure **7** as a yellow solid. Yield 94%, mp: 195–7 °C (EtOAc). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ: 8.10 (1H, d, H-4, *J*=1.0 Hz), 9.16 (1H, d, H-7, *J*=1.0 Hz). <sup>13</sup>C NMR (50 MHz, DMSO-*d*<sub>6</sub>) δ: 113.3 (C-4), 122.1 (C-3a), 137.1 (C-7), 137.8 (C-7a), 144.0 (C-3). *Anal.* Calcd for C<sub>6</sub>H<sub>3</sub>ClN<sub>4</sub>O<sub>2</sub>: C, 36.29; H, 1.52; N, 28.22. Found: C, 36.43; H, 1.61; N, 28.45.

**General procedure for the synthesis of 1-substituted derivatives 8a–b and 2-substituted derivatives 9a–b** Potassium carbonate (530 mg, 3.84 mmol) was added into a solution of nitro derivative **7** (540 mg, 2.72 mmol) in anhydrous acetonitrile (20 mL) at 0 °C and under argon, and this solution was stirred for 30 min, followed by dropwise addition of iodomethane (0.22 mL, 3.53 mmol) or 4-methoxybenzyl chloride (0.41 mL, 3mmol). This reaction mixture was heated at 50 °C for 5 h. Upon completion of the reaction, the solvent was evaporated and the residue was extracted with ethyl acetate (3 x 100 mL) and water (100 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated under reduced pressure. The crude product was then purified by column chromatography (silica gel) to provide pure compounds **8a–b** and **9a–b**.

**5-Chloro-1-methyl-3-nitro-1*H*-pyrazolo[3,4-*c*]pyridine (8a) and 5-chloro-2-methyl-3-nitro-2*H*-pyrazolo[3,4-*c*]pyridine (9a)** These compounds were prepared according to the general procedure described above. Purification was effected using a mixture of cyclohexane / ethyl acetate (7/3, v/v) as the eluent.

Data for **8a**: Yield 80%. Pale yellow solid, mp: 172 °C (Et<sub>2</sub>O). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 4.34 (3H, s, CH<sub>3</sub>), 8.16 (1H, d, H-4, *J*=1.0 Hz), 8.89 (1H, d, H-7, *J*=1.0 Hz). <sup>13</sup>C NMR (50



MHz, CDCl<sub>3</sub>)  $\delta$ : 38.0 (CH<sub>3</sub>), 114.4 (C-4), 123.3 (C-3a), 134.2 (C-7), 137.5 (C-7a), 145.9 (C-3).

*Anal.* Calcd for C<sub>7</sub>H<sub>5</sub>ClN<sub>4</sub>O<sub>2</sub>: C, 39.55; H, 2.37; N, 26.35. Found: C, 39.49; H, 2.47; N, 26.52.

Data for **9a**: Yield 14%. Pale yellow solid, mp: 177 °C (Et<sub>2</sub>O). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ : 4.67 (3H, s, CH<sub>3</sub>), 8.03 (1H, d, H-4, *J*=1.0 Hz), 9.13 (1H, d, H-7, *J*=1.0 Hz). <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>)  $\delta$ : 43.8 (CH<sub>3</sub>), 112.7 (C-4), 122.6 (C-3a), 136.9 (C-7a), 141.5 (C-5), 144.8 (C-7), 147.3 (C-3). *Anal.* Calcd for C<sub>7</sub>H<sub>5</sub>ClN<sub>4</sub>O<sub>2</sub>: C, 39.55; H, 2.37; N, 26.35. Found: C, 39.66; H, 2.42; N, 26.14.

**5-Chloro-1-(4-methoxybenzyl)-3-nitro-1H-pyrazolo[3,4-*c*]pyridine (8b) and 5-chloro-2-(4-methoxybenzyl)-3-nitro-2H-pyrazolo[3,4-*c*]pyridine (9b)** These compounds were prepared according to the general procedure described above. Purification was effected using a mixture of cyclohexane / ethyl acetate / dichloromethane (9/0.5/0.5, v/v) as the eluent.

Data for **8b**: Yield 80%. Yellow solid, mp: 185 °C (EtOAc). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ : 3.79 (3H, s, OCH<sub>3</sub>), 5.70 (2H, s, CH<sub>2</sub>), 6.90 (2H, d, H-3', H-5', *J*=8.7 Hz), 7.30 (2H, d, H-2', H-6', *J*=8.7 Hz), 8.18 (1H, d, H-4, *J*=1.0 Hz), 8.67 (1H, d, H-7, *J*=1.0 Hz). <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>)  $\delta$ : 55.3 (OCH<sub>3</sub>), 55.9 (CH<sub>2</sub>), 114.5 (C-4), 114.8 (C-3', C-5'), 123.8 (C-3a), 124.7 (C-1'), 129.5 (C-2', C-6'), 134.8 (C-7), 136.9 (C-7a), 145.7 (C-3), 160.4 (C-4'). *Anal.* Calcd for C<sub>14</sub>H<sub>11</sub>ClN<sub>4</sub>O<sub>3</sub>: C, 52.76; H, 3.48; N, 17.58. Found: C, 52.95; H, 3.60; N, 17.33.

Data for **9b**: Yield 7%. Yellow solid, mp: 157 °C (EtOAc). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ : 3.75 (3H, s, OCH<sub>3</sub>), 6.09 (2H, s, CH<sub>2</sub>), 6.83 (2H, d, H-3', H-5', *J*=8.7 Hz), 7.36 (2H, d, H-2', H-6', *J*=8.7 Hz), 7.99 (1H, s, H-4), 9.16 (1H, s, H-7). <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>)  $\delta$ : 55.3 (OCH<sub>3</sub>), 58.7 (CH<sub>2</sub>), 112.9 (C-4), 114.4 (C-3', C-5'), 122.9 (C-3a), 125.3 (C-1'), 130.1 (C-2', C-6'),

136.9 (C-7a), 141.6 (C-5), 145.1 (C-7), 147.3 (C-3), 160.2 (C-4'). *Anal.* Calcd for C<sub>14</sub>H<sub>11</sub>ClN<sub>4</sub>O<sub>3</sub>: C, 52.76; H, 3.48; N, 17.58. Found: C, 53.01; H, 3.69; N, 17.44.

***N*-Cyclohexyl-1-methyl-3-nitro-1*H*-pyrazolo[3,4-*c*]pyridin-5-amine (10a)**

Cyclohexylamine (1.14 mL, 10 mmol) was added under argon into a solution of the 5-chloro-pyrazolopyridine **8a** (425 mg, 2 mmol) in anhydrous DMSO (3 mL) and this mixture was heated at 140 °C for 14h. Upon completion of the reaction, the mixture was diluted with water (100 mL) and extracted with dichloromethane (3 x 100 mL). The combined organic layers were washed with water (200 mL), then with a saturated aqueous solution of NaCl (200 mL) and finally were dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated under reduced pressure. The residue was purified by column chromatography (silica gel) using a mixture of cyclohexane / dichloromethane (1/9, v/v) as the eluent, to provide pure **10a** (275 mg, yield 50%) as an orange colored solid. mp: 148–9 °C (EtOAc). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 1.25-1.34 (3H, m, cyclohexyl H), 1.46 (2H, m, cyclohexyl H), 1.68 (1H, m, cyclohexyl H), 1.80 (2H, m, cyclohexyl H), 2.09 (2H, m, cyclohexyl H), 3.50 (1H, m, cyclohexyl H-1'), 4.22 (3H, s, CH<sub>3</sub>), 5.24 (1H, brs, D<sub>2</sub>O exch., NH), 6.96 (1H, s, H-4), 8.69 (1H, s, H-7). <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>) δ: 24.8 (cyclohexyl C-3', C-5'), 25.8 (cyclohexyl C-4'), 32.8 (cyclohexyl C-2', C-6'), 38.0 (CH<sub>3</sub>), 51.3 (cyclohexyl C-1'), 91.1 (C-4), 125.6 (C-3a), 132.4 (C-7), 133.8 (C-7a), 146.0 (C-3), 155.0 (C-5). *Anal.* Calcd for C<sub>13</sub>H<sub>17</sub>N<sub>5</sub>O<sub>2</sub>: C, 56.72; H, 6.22; N, 25.44. Found: C, 56.49; H, 6.02; N, 25.57.

**1-Methyl-3-nitro-*N*-phenyl-1*H*-pyrazolo[3,4-*c*]pyridin-5-amine (10b)** Aniline (0.99 mL, 10.86 mmol) was added under argon to a solution of the 5-chloro-pyrazolopyridine **8a** (900 mg, 4.24 mmol) in dry toluene (70 mL), followed by addition of cesium carbonate (7.3 g, 18.96 mmol), 2-dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl (X-phos, 172 mg, 0.36 mmol) and tris(dibenzylideneacetone)dipalladium(0) (207 mg, 0.23 mmol) and the resulting mixture

was refluxed for 20 h. Upon completion of the reaction, the mixture was filtered through a celite pad, the filtrate was vacuum-evaporated, extracted with ethyl acetate – water and the organic extracts were dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated to dryness. The crude product was purified by column chromatography (silica gel) using a mixture of dichloromethane / ethyl acetate (97/3, v/v) as the eluent, to provide pure compound **10b** (670 mg, 75%) as a red solid. mp: 171–2 °C (EtOAc). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ: 4.27 (3H, s, CH<sub>3</sub>), 6.94 (1H, t, H-4', *J*=7.2 Hz), 7.30 (2H, t, H-3', H-5', *J*=7.3 Hz), 7.38 (1H, d, H-4, *J*=1.0 Hz), 7.55 (2H, d, H-2', H-6', *J*=8 Hz), 9.07 (1H, d, H-7, *J*=1.0 Hz), 9.29 (1H, s, D<sub>2</sub>O exch., NH). <sup>13</sup>C NMR (50 MHz, DMSO-*d*<sub>6</sub>) δ: 37.8 (CH<sub>3</sub>), 93.9 (C-4), 118.2 (C-2', C-6'), 120.9 (C-4'), 123.4 (C-3a), 128.7 (C-3', C-5'), 134.3 (C-7a), 134.9 (C-7), 141.5 (C-1'), 144.9 (C-3), 152.9 (C-5). *Anal.* Calcd for C<sub>13</sub>H<sub>11</sub>N<sub>5</sub>O<sub>2</sub>: C, 57.99; H, 4.12; N, 26.01. Found: C, 58.22; H, 4.16; N, 25.73.

***N*-Cyclohexyl-1-(4-methoxybenzyl)-3-nitro-1*H*-pyrazolo[3,4-*c*]pyridin-5-amine (10c)**

A mixture of compound **8b** (2.50 g, 7.85 mmol) and cyclohexylamine (9 mL, 78.47 mmol) in dimethylsulfoxide (8 mL) was irradiated at 158 °C (60 W, Milestone Start E) for 2 h. Upon cooling, the mixture was poured into water (300 mL) and extracted with dichloromethane (3 x 200 mL). The combined organic layers were washed with water (500 mL), then with a saturated aqueous solution of NaCl (400 mL) and finally were dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated under reduced pressure. The crude product was purified by column chromatography (silica gel) using a mixture of cyclohexane / ethyl acetate (1/1, v/v) as the eluent, to provide pure **10c** (2 g, 66%) as an orange colored solid. mp: 125–6 °C (CHCl<sub>3</sub>/Et<sub>2</sub>O). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 1.26 (3H, m, cyclohexyl H), 1.44 (2H, m, cyclohexyl H), 1.66 (1H, m, cyclohexyl H), 1.78 (2H, m, cyclohexyl H), 2.07 (2H, m, cyclohexyl H), 3.48 (1H, m, cyclohexyl H-1'), 3.79 (3H, s, OCH<sub>3</sub>), 4.90 (1H, brs, D<sub>2</sub>O exch., NH), 5.58 (2H, s, CH<sub>2</sub>), 6.87 (2H, d, H-3'', H-5'', *J*=8.7 Hz), 6.91

(1H, d, H-4,  $J=1.0$  Hz), 7.28 (2H, d, H-2'', H-6'',  $J=8.7$  Hz), 8.40 (1H, d, H-7,  $J=1.0$  Hz).  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ )  $\delta$ : 24.6 (cyclohexyl C-3', C-5'), 25.7 (cyclohexyl C-4'), 32.7 (cyclohexyl C-2', C-6'), 51.1 (cyclohexyl C-1'), 55.3 (OCH<sub>3</sub>), 55.6 (CH<sub>2</sub>), 91.0 (C-4), 114.7 (C-3'', C-5''), 125.6 (C-1''), 125.8 (C-3a), 129.4 (C-2'', C-6''), 132.8 (C-7a), 133.1 (C-7), 145.4 (C-3), 155.0 (C-5), 160.2 (C-4''). *Anal.* Calcd for  $\text{C}_{20}\text{H}_{23}\text{N}_5\text{O}_3$ : C, 62.98; H, 6.08; N, 18.36. Found: C, 63.22; H, 6.19; N, 18.09.

**1-(4-Methoxybenzyl)-3-nitro-*N*-phenyl-1*H*-pyrazolo[3,4-*c*]pyridin-5-amine (10d)** This compound was prepared following a procedure analogous to that described for the synthesis of **10b**, starting from **8b**. The product was purified by column chromatography (silica gel) using a mixture of cyclohexane / ethyl acetate (7/3, v/v) as the eluent. Yield 68%. Red solid, mp: 179 °C (EtOAc).  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO-}d_6$ )  $\delta$ : 3.72 (3H, s, OCH<sub>3</sub>), 5.79 (2H, s, CH<sub>2</sub>), 6.92 (3H, m, H-3'', H-5'', H-4'), 7.29 (2H, t, H-3', H-5',  $J=7.5$  Hz), 7.40 (3H, m, H-4, H-2'', H-6''), 7.54 (2H, d, H-2', H-6',  $J=7.5$  Hz), 9.15 (1H, d, H-7,  $J=1.0$  Hz), 9.31 (1H, s,  $\text{D}_2\text{O}$  exch., NH).  $^{13}\text{C}$  NMR (50 MHz,  $\text{DMSO-}d_6$ )  $\delta$ : 53.8 (CH<sub>2</sub>), 55.0 (OCH<sub>3</sub>), 94.3 (C-4), 114.2 (C-3'', C-5''), 118.3 (C-2', C-6'), 121.0 (C-4'), 123.8 (C-3a), 126.9 (C-1''), 128.7 (C-3', C-5'), 129.7 (C-2'', C-6''), 133.6 (C-7a), 134.7 (C-7), 141.4 (C-1'), 145.6 (C-3), 152.9 (C-5), 159.2 (C-4''). *Anal.* Calcd for  $\text{C}_{20}\text{H}_{17}\text{N}_5\text{O}_3$ : C, 63.99; H, 4.56; N, 18.66. Found: C, 64.06; H, 4.67; N, 18.58.

***N*<sup>5</sup>-Cyclohexyl-1-methyl-1*H*-pyrazolo[3,4-*c*]pyridine-3,5-diamine (11a)** A solution of nitro derivative **10a** (400 mg, 1.45 mmol) in absolute ethanol (40 mL) was hydrogenated in the presence of 10% Pd/C (80 mg) under a pressure of 55 psi at room temperature for 3h. The solution was filtered through a celite pad to remove the catalyst and the filtrate was evaporated to dryness. The residue was purified by column chromatography (silica gel) using a mixture of dichloromethane / methanol (99/1, v/v) as the eluent, to provide pure **11a** (280 mg, 79%) as a

yellow solid. mp: 179–180 °C (EtOAc). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 1.11-1.24 (3H, m, cyclohexyl H), 1.32 (2H, m, cyclohexyl H), 1.60 (1H, m, cyclohexyl H), 1.72 (2H, m, cyclohexyl H), 2.02 (2H, m, cyclohexyl H), 3.29 (1H, m, cyclohexyl H-1'), 3.80 (3H, s, CH<sub>3</sub>), 4.18 (3H, brs, D<sub>2</sub>O exch., NH<sub>2</sub>, NH), 6.33 (1H, d, H-4, *J*=1.0 Hz), 8.28 (1H, d, H-7, *J*=1.0 Hz). <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>) δ: 24.9 (cyclohexyl C-3', C-5'), 25.8 (cyclohexyl C-4'), 33.1 (cyclohexyl C-2', C-6'), 35.3 (CH<sub>3</sub>), 51.7 (cyclohexyl C-1'), 91.5 (C-4), 122.5 (C-3a), 130.3 (C-7), 134.3 (C-7a), 145.4 (C-3), 150.9 (C-5). *Anal.* Calcd for C<sub>13</sub>H<sub>19</sub>N<sub>5</sub>: C, 63.65; H, 7.81; N, 28.55. Found: C, 63.97; H, 8.08; N, 28.29.

**1-Methyl-N<sup>5</sup>-phenyl-1H-pyrazolo[3,4-*c*]pyridine-3,5-diamine (11b)** This compound was prepared following an analogous procedure to that described for the synthesis of **11a**, starting from the nitro derivative **10b**. The product was purified by column chromatography (silica gel) using a mixture of cyclohexane / ethyl acetate (from 5/5 up to 2/8, v/v) as the eluent, to provide pure **11b** as a green solid, in 84% yield. mp: 178–9 °C (EtOAc). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ: 3.75 (3H, s, CH<sub>3</sub>), 5.61 (2H, brs, D<sub>2</sub>O exch., NH<sub>2</sub>), 6.78 (1H, t, H-4', *J*=7.2 Hz), 7.21 (3H, m, H-3', H-5', H-4), 7.37 (2H, dd, H-2', H-6', *J*=8.0 Hz, *J*=0.9 Hz), 8.56 (2H, m, H-7, NH-aniline). <sup>13</sup>C NMR (50 MHz, DMSO-*d*<sub>6</sub>) δ: 35.1 (CH<sub>3</sub>), 97.9 (C-4), 116.2 (C-2', C-6'), 118.8 (C-4'), 121.1 (C-3a), 128.6 (C-3', C-5'), 130.4 (C-7), 134.8 (C-7a), 143.5 (C-1'), 146.4 (C-5), 147.3 (C-3). *Anal.* Calcd for C<sub>13</sub>H<sub>13</sub>N<sub>5</sub>: C, 65.26; H, 5.48; N, 29.27. Found: C, 65.03; H, 5.74; N, 29.49.

**N<sup>5</sup>-Cyclohexyl-1-(4-methoxybenzyl)-1H-pyrazolo[3,4-*c*]pyridine-3,5-diamine (11c)**

This compound was prepared following an analogous procedure to that described for the synthesis of **11a**, starting from the nitro derivative **10c**. The product was purified by column chromatography (silica gel) using a mixture of dichloromethane/ethyl acetate (8/2, v/v) as the eluent, providing **11c** as a yellow solid, in 85% yield. mp: 142–3 °C (Et<sub>2</sub>O). <sup>1</sup>H NMR (400 MHz,

CDCl<sub>3</sub>)  $\delta$ : 1.20 (3H, m, cyclohexyl H), 1.33 (2H, m, cyclohexyl H), 1.60 (1H, m, cyclohexyl H), 1.72 (2H, m, cyclohexyl H), 2.02 (2H, m, cyclohexyl H), 3.29 (1H, m, cyclohexyl H-1'), 3.72 (3H, s, OCH<sub>3</sub>), 4.14 (2H, brs, D<sub>2</sub>O exch., NH<sub>2</sub>), 5.21 (2H, s, CH<sub>2</sub>), 6.32 (1H, d, H-4,  $J=1.0$  Hz), 6.78 (2H, d, H-3'', H-5'',  $J=8.7$  Hz), 7.13 (2H, d, H-2'', H-6'',  $J=8.7$  Hz), 8.20 (1H, d, H-7,  $J=1.0$  Hz). <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>)  $\delta$ : 25.0 (cyclohexyl C-3', C-5'), 25.9 (cyclohexyl C-4'), 33.2 (cyclohexyl C-2', C-6'), 51.6 (cyclohexyl C-1'), 52.6 (CH<sub>2</sub>), 55.3 (OCH<sub>3</sub>), 91.2 (C-4), 114.1 (C-3'', C-5''), 123.0 (C-3a), 128.8 (C-2'', C-6''), 129.2 (C-1''), 131.4 (C-7), 133.9 (C-7a), 145.8 (C-3), 151.4 (C-5), 159.2 (C-4''). *Anal.* Calcd for C<sub>20</sub>H<sub>25</sub>N<sub>5</sub>O: C, 68.35; H, 7.17; N, 19.93. Found: C, 68.08; H, 7.04; N, 20.22.

**1-(4-Methoxybenzyl)-N<sup>5</sup>-phenyl-1H-pyrazolo[3,4-c]pyridine-3,5-diamine (11d)** 10% Pd/C (5 mg) and triethylsilane (0.34 mL, 2.13 mmol) were added into a solution of the nitro derivative **10d** (80 mg, 0.21 mmol) in anhydrous methanol (5 mL), under argon, and the resulting solution was stirred at room temperature for 12h. The solution was filtered through a celite pad to remove the catalyst and the filtrate was evaporated to dryness. The residue was purified by column chromatography (silica gel) using a mixture of cyclohexane / ethyl acetate (8/2, v/v) as the eluent, to provide pure **11d** (60 mg, 82%) as a brown solid. mp: 148 °C (Et<sub>2</sub>O). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ : 3.78 (3H, s, OCH<sub>3</sub>), 4.64 (2H, brs, D<sub>2</sub>O exch., NH<sub>2</sub>), 5.27 (2H, s, CH<sub>2</sub>), 6.84 (2H, d, H-3'', H-5'',  $J=8.6$  Hz), 7.06 (2H, m, H-4', H-4), 7.18 (2H, d, H-2'', H-6'',  $J=8.6$  Hz), 7.23 (2H, d, H-2', H-6',  $J=7.6$  Hz), 7.32 (2H, t, H-3', H-5',  $J=7.6$  Hz), 7.58 (1H, brs, D<sub>2</sub>O exch., NH), 8.20 (1H, s, H-7). <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>)  $\delta$ : 53.1 (CH<sub>2</sub>), 55.3 (OCH<sub>3</sub>), 96.7 (C-4), 114.3 (C-3'', C-5''), 120.6 (C-2', C-6'), 123.5 (C-4'), 124.2 (C-3a), 127.0 (C-7), 127.9 (C-1''), 128.9 (C-2'', C-6''), 129.6 (C-3', C-5'), 133.2 (C-7a), 140.1 (C-1'), 146.2 (C-3),

146.5 (C-5), 159.6 (C-4''). *Anal.* Calcd for C<sub>20</sub>H<sub>19</sub>N<sub>5</sub>O: C, 69.55; H, 5.54; N, 20.28. Found: C, 69.29; H, 5.37; N, 20.46.

**General procedure for the synthesis of derivatives 12a–d** Chloroacetyl chloride (96  $\mu$ L, 1.2 mmol) and triethylamine (153  $\mu$ L, 1.1 mmol) were added into a solution of the amines **11a–d** (1 mmol) in anhydrous THF (15 mL), under argon, at -40 °C for amines **11a**, **11c** or at 0 °C for amines **11b**, **11d**. The resulting solution was allowed to reach room temperature and stirring was continued for one more h. The solvents were then vacuum-evaporated and the residue was used immediately to the next step, without any further purification.

**General procedure for the synthesis of derivatives 13a–l** Dimethylamine (5.6 M solution in ethanol, 0.89 mL, 5mmol) or 1-methylpiperazine (0.22 mL, 2 mmol) or aniline (0.36 mL, 4 mmol) was added into a solution of the intermediate chlorides **12a–d** (1 mmol) in absolute ethanol (10 mL), under argon, and the resulting solution was heated at 80 °C for 4 h. Upon completion of the reaction solvents were evaporated and the residue was purified by column chromatography to provide pure amides **13a–l**.

***N*-(5-(Cyclohexylamino)-1-methyl-1*H*-pyrazolo[3,4-*c*]pyridin-3-yl)-2-(dimethylamino)acetamide (13a)** This compound was prepared according to the general procedure described above, upon reaction of the chloride **12a** with dimethylamine (5.6 M solution in ethanol). The product was purified by column chromatography (silica gel) using a mixture of dichloromethane/methanol (100/1, v/v) as the eluent to provide pure **13a** as a green oil. Yield: 52%. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ : 1.22 (3H, m, cyclohexyl H), 1.40 (2H, m, cyclohexyl H), 1.61 (1H, m, cyclohexyl H), 1.73 (2H, m, cyclohexyl H), 2.05 (2H, m, cyclohexyl H), 2.42 (6H, s, N(CH<sub>3</sub>)<sub>2</sub>), 3.17 (2H, s, CH<sub>2</sub>), 3.44 (1H, m, cyclohexyl H-1'), 3.94 (3H, s, CH<sub>3</sub>–pyrazole), 6.83 (1H, s, H-4), 8.39 (1H, s, H-7), 9.55 (1H, s, D<sub>2</sub>O exch., NHCO). <sup>13</sup>C NMR (50

MHz, CDCl<sub>3</sub>)  $\delta$ : 24.8 (cyclohexyl C-3', C-5'), 25.9 (cyclohexyl C-4'), 33.2 (cyclohexyl C-2', C-6'), 35.7 (CH<sub>3</sub>-pyrazole), 46.0 (N(CH<sub>3</sub>)<sub>2</sub>), 51.2 (cyclohexyl C-1'), 63.0 (CH<sub>2</sub>), 93.8 (C-4), 123.9 (C-3a), 131.3 (C-7), 134.2 (C-7a), 136.9 (C-3), 152.0 (C-5), 168.6 (CO). HR-MS (ESI)  $m/z$ : Calcd for C<sub>17</sub>H<sub>27</sub>N<sub>6</sub>O: [M1+H]<sup>+</sup> =331.2241, found 331.2247. *Anal.* Calcd for C<sub>17</sub>H<sub>26</sub>N<sub>6</sub>O: C, 61.79; H, 7.93; N, 25.43. Found: C, 61.69; H, 7.76; N, 25.55.

***N*-(5-(Cyclohexylamino)-1-methyl-1*H*-pyrazolo[3,4-*c*]pyridin-3-yl)-2-(4-methylpiperazin-1-yl)acetamide (13b)** This compound was prepared according to the general procedure described above, upon reaction of the chloride **12a** with 1-methylpiperazine. The product was purified by column chromatography (silica gel) using a mixture of dichloromethane/methanol (94/6, v/v) as the eluent to provide pure **13b** as a yellow solid. Yield: 38%, mp: 154 °C (dec.) (Et<sub>2</sub>O). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ : 1.23 (3H, m, cyclohexyl H), 1.40 (2H, m, cyclohexyl H), 1.62 (1H, m, cyclohexyl H), 1.74 (2H, m, cyclohexyl H), 2.06 (2H, m, cyclohexyl H), 2.39 (3H, s, CH<sub>3</sub>-piperazine), 2.63 (4H, brs, piperazine H-3'', H-5''), 2.77 (4H, brs, piperazine H-2'', H-6''), 3.24 (2H, s, CH<sub>2</sub>), 3.45 (1H, m, cyclohexyl H-1'), 3.96 (3H, s, CH<sub>3</sub>-pyrazole), 6.78 (1H, d, H-4, *J*=1.0 Hz), 8.41 (1H, d, H-7, *J*=1.0 Hz), 9.40 (1H, s, D<sub>2</sub>O exch., NHCO). <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>)  $\delta$ : 24.9 (cyclohexyl C-3', C-5'), 26.0 (cyclohexyl C-4'), 33.3 (cyclohexyl C-2', C-6'), 35.8 (CH<sub>3</sub>-pyrazole), 45.7 (CH<sub>3</sub>-piperazine), 51.3 (cyclohexyl C-1'), 53.1 (piperazine C-2'', C-6''), 55.0 (piperazine C-3'', C-5''), 61.5 (CH<sub>2</sub>), 93.6 (C-4), 124.0 (C-3a), 131.6 (C-7), 134.3 (C-7a), 136.8 (C-3), 152.1 (C-5), 168.2 (CO). HR-MS (ESI)  $m/z$ : Calcd for C<sub>20</sub>H<sub>32</sub>N<sub>7</sub>O: [M1+H]<sup>+</sup> =386.2663, found 386.2665. *Anal.* Calcd for C<sub>20</sub>H<sub>31</sub>N<sub>7</sub>O: C, 62.31; H, 8.11; N, 25.43. Found: C, 62.56; H, 8.22; N, 25.29.

***N*-(5-(Cyclohexylamino)-1-methyl-1*H*-pyrazolo[3,4-*c*]pyridin-3-yl)-2-(phenylamino)acetamide (13c)** This compound was prepared according to the general



procedure described above, upon reaction of the chloride **12a** with aniline. The product was purified by column chromatography (silica gel) using a mixture of dichloromethane/methanol (100/1, v/v) as the eluent to provide pure **13c** as a yellow solid. Yield: 35%, mp: 175–6 °C (Et<sub>2</sub>O). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 1.20-1.31 (3H, m, cyclohexyl H), 1.42 (2H, m, cyclohexyl H), 1.66 (1H, m, cyclohexyl H), 1.78 (2H, m, cyclohexyl H), 2.07 (2H, m, cyclohexyl H), 3.44 (1H, m, cyclohexyl H-1'), 3.91 (3H, s, CH<sub>3</sub>), 4.00 (2H, s, CH<sub>2</sub>), 4.57 (1H, brs, D<sub>2</sub>O exch., NH-cyclohexylamine), 6.71 (2H, d, H-2'', H-6'', *J*=7.9 Hz), 6.74 (1H, s, H-4), 6.85 (1H, t, H-4'', *J*=7.3 Hz), 7.24 (2H, t, H-3'', H-5'', *J*=7.5 Hz), 8.38 (1H, s, H-7), 9.10 (1H, brs, D<sub>2</sub>O exch., NHCO). <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>) δ: 24.8 (cyclohexyl C-3', C-5'), 25.9 (cyclohexyl C-4'), 33.1 (cyclohexyl C-2', C-6'), 35.7 (CH<sub>3</sub>), 49.1 (CH<sub>2</sub>), 51.2 (cyclohexyl C-1'), 93.2 (C-4), 113.4 (C-2'', C-6''), 119.5 (C-4''), 124.4 (C-3a), 129.5 (C-3'', C-5''), 131.1 (C-7), 134.0 (C-7a), 136.4 (C-3), 147.0 (C-1''), 151.9 (C-5), 169.2 (CO). HR-MS (ESI) *m/z*: Calcd for C<sub>21</sub>H<sub>27</sub>N<sub>6</sub>O: [M1+H]<sup>+</sup> =379.2241, found 379.2244. *Anal.* Calcd for C<sub>21</sub>H<sub>26</sub>N<sub>6</sub>O: C, 66.64; H, 6.92; N, 22.20. Found: C, 66.48; H, 6.69; N, 22.43.

**2-(Dimethylamino)-N-(1-methyl-5-(phenylamino)-1H-pyrazolo[3,4-c]pyridin-3-yl)acetamide (13d)** This compound was prepared according to the general procedure described above, upon reaction of the chloride **12b** with dimethylamine (5.6 M solution in ethanol). The product was purified by column chromatography (silica gel) using a mixture of dichloromethane/methanol (100/2, v/v) as the eluent to provide pure **13d** as a green solid. Yield: 86%, mp: 166–7 °C (EtOH). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 2.41 (6H, s, N(CH<sub>3</sub>)<sub>2</sub>), 3.15 (2H, s, CH<sub>2</sub>), 4.00 (3H, s, CH<sub>3</sub>-pyrazole), 6.64 (1H, s, D<sub>2</sub>O exch., NH-aniline), 6.95 (1H, t, H-4', *J*=7.2 Hz), 7.30 (4H, m, H-2', H-3', H-5', H-6'), 7.57 (1H, d, H-4, *J*=1.2 Hz), 8.54 (1H, d, H-7, *J*=1.2 Hz), 9.59 (1H, s, D<sub>2</sub>O exch., NHCO). <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>) δ: 35.7 (CH<sub>3</sub>-pyrazole), 46.0

(N(CH<sub>3</sub>)<sub>2</sub>), 63.0 (CH<sub>2</sub>), 98.7 (C-4), 117.8 (C-2', C-6'), 121.1 (C-4'), 123.2 (C-3a), 129.2 (C-3', C-5'), 131.5 (C-7), 135.0 (C-7a), 137.5 (C-3), 142.1 (C-1'), 147.9 (C-5), 168.8 (CO). HR-MS (ESI) *m/z*: Calcd for C<sub>17</sub>H<sub>21</sub>N<sub>6</sub>O: [M+H]<sup>+</sup> =325.1771, found 325.1778. *Anal.* Calcd for C<sub>17</sub>H<sub>20</sub>N<sub>6</sub>O: C, 62.95; H, 6.21; N, 25.91. Found: C, 63.04; H, 6.26; N, 25.78.

***N*-(1-Methyl-5-(phenylamino)-1*H*-pyrazolo[3,4-*c*]pyridin-3-yl)-2-(4-methylpiperazin-1-yl)acetamide (13e)** This compound was prepared according to the general procedure described above, upon reaction of the chloride **12b** with 1-methylpiperazine. The product was purified by column chromatography (silica gel) using a mixture of dichloromethane/methanol (9/1, v/v) as the eluent to provide pure **13e** as a yellow solid. Yield: 93%, mp: 196–7 °C (EtOH). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$ : 2.18 (3H, s, CH<sub>3</sub>-piperazine), 2.38 (4H, brs, piperazine H-3'', H-5''), 2.57 (4H, brs, piperazine H-2'', H-6''), 3.20 (2H, s, CH<sub>2</sub>), 4.01 (3H, s, CH<sub>3</sub>-pyrazole), 6.80 (1H, t, H-4', *J*=7.2 Hz), 7.21 (3H, m, H-3', H-5', H-4), 7.45 (2H, d, H-2', H-6', *J*=8.0 Hz), 8.74 (1H, s, D<sub>2</sub>O exch., NH-aniline), 8.80 (1H, d, H-7, *J*=1.0 Hz), 10.14 (1H, brs, D<sub>2</sub>O exch., NHCO). <sup>13</sup>C NMR (50 MHz, DMSO-*d*<sub>6</sub>)  $\delta$ : 35.5 (CH<sub>3</sub>-pyrazole), 45.6 (CH<sub>3</sub>-piperazine), 52.6 (piperazine C-2'', C-6''), 54.5 (piperazine C-3'', C-5''), 60.8 (CH<sub>2</sub>), 98.4 (C-4), 116.5 (C-2', C-6'), 119.2 (C-4'), 123.2 (C-3a), 128.6 (C-3', C-5'), 131.8 (C-7), 134.2 (C-7a), 137.2 (C-3), 143.1 (C-1'), 147.8 (C-5), 168.2 (CO). HR-MS (ESI) *m/z*: Calcd for C<sub>20</sub>H<sub>26</sub>N<sub>7</sub>O: [M+H]<sup>+</sup> =380.2193, found 380.2200. *Anal.* Calcd for C<sub>20</sub>H<sub>25</sub>N<sub>7</sub>O: C, 63.30; H, 6.64; N, 25.84. Found: C, 63.41; H, 6.79; N, 25.73.

***N*-(1-Methyl-5-(phenylamino)-1*H*-pyrazolo[3,4-*c*]pyridin-3-yl)-2-(phenylamino)acetamide (13f)** This compound was prepared according to the general procedure described above, upon reaction of the chloride **12b** with aniline. The product was purified by column chromatography (silica gel) using a mixture of dichloromethane/ethyl acetate (1/1, v/v)

as the eluent to provide pure **13f** as a yellow solid. Yield: 70%, mp: 180–1 °C (EtOH). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ: 3.95 (2H, d, CH<sub>2</sub>, *J*=6.0 Hz), 4.01 (3H, s, CH<sub>3</sub>), 6.04 (1H, t, CH<sub>2</sub>-NH-, *J*=6.0 Hz), 6.60 (3H, m, H-2'', H-4'', H-6''), 6.80 (1H, t, H-4', *J*=7.2 Hz), 7.11 (2H, t, H-3'', H-5'', *J*=7.7 Hz), 7.20 (3H, m, H-3', H-5', H-4), 7.42 (2H, d, H-2', H-6', *J*=7.8 Hz), 8.72 (1H, s, D<sub>2</sub>O exch., NH-aniline), 8.79 (1H, d, H-7, *J*=0.8 Hz), 10.50 (1H, brs, D<sub>2</sub>O exch., NHCO). <sup>13</sup>C NMR (50 MHz, DMSO-*d*<sub>6</sub>) δ: 36.1 (CH<sub>3</sub>), 47.0 (CH<sub>2</sub>), 98.9 (C-4), 112.7 (C-2'', C-6''), 116.9 (C-4''), 117.0 (C-2', C-6'), 119.7 (C-4'), 123.6 (C-3a), 129.1 (C-3', C-5'), 129.4 (C-3'', C-5''), 132.4 (C-7), 134.7 (C-7a), 137.9 (C-3), 143.7 (C-1'), 148.3 (C-5), 148.7 (C-1''), 169.9 (CO). HR-MS (ESI) *m/z*: Calcd for C<sub>21</sub>H<sub>21</sub>N<sub>6</sub>O: [M1+H]<sup>+</sup> =373.1771, found 373.1772. *Anal.* Calcd for C<sub>21</sub>H<sub>20</sub>N<sub>6</sub>O: C, 67.73; H, 5.41; N, 22.57. Found: C, 67.94; H, 5.56; N, 22.28.

***N*-(5-(Cyclohexylamino)-1-(4-methoxybenzyl)-1*H*-pyrazolo[3,4-*c*]pyridin-3-yl)-2-(dimethylamino)acetamide (13g)** This compound was prepared according to the general procedure described above, upon reaction of the chloride **12c** with dimethylamine (5.6 M solution in ethanol). The product was purified by column chromatography (silica gel) using a mixture of dichloromethane/methanol (100/1, v/v) as the eluent to provide pure **13g** as a yellow oil. Yield: 57%. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 1.18 (3H, m, cyclohexyl H), 1.36 (2H, m, cyclohexyl H), 1.57 (1H, m, cyclohexyl H), 1.69 (2H, m, cyclohexyl H), 2.01 (2H, m, cyclohexyl H), 2.36 (6H, s, N(CH<sub>3</sub>)<sub>2</sub>), 3.11 (2H, s, NHCOCH<sub>2</sub>), 3.42 (1H, m, cyclohexyl H-1''), 3.70 (3H, s, OCH<sub>3</sub>), 4.30 (1H, brs, D<sub>2</sub>O exchang., NH-cyclohexylamine), 5.32 (2H, s, CH<sub>2</sub>-pyrazole), 6.77 (2H, d, H-3', H-5', *J*=8.6 Hz), 6.84 (1H, s, H-4), 7.11 (2H, d, H-2', H-6', *J*=8.6 Hz), 8.28 (1H, s, H-7), 9.55 (1H, s, D<sub>2</sub>O exchang., NHCO). <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>) δ: 24.6 (cyclohexyl C-3'', C-5''), 25.7 (cyclohexyl C-4''), 33.0 (cyclohexyl C-2'', C-6''), 45.9 (N(CH<sub>3</sub>)<sub>2</sub>), 50.9 (cyclohexyl C-1''), 52.7 (CH<sub>2</sub>-pyrazole), 55.0 (OCH<sub>3</sub>), 62.9 (NHCOCH<sub>2</sub>), 93.9 (C-4), 114.0 (C-3', C-5'),

124.2 (C-3a), 128.1 (C-1'), 128.5 (C-2', C-6'), 131.6 (C-7), 133.5 (C-7a), 137.3 (C-3), 151.8 (C-5), 159.2 (C-4'), 168.5 (CO). HR-MS (ESI)  $m/z$ : Calcd for  $C_{24}H_{33}N_6O_2$ :  $[M+H]^+ = 437.2660$ , found 437.2666. *Anal.* Calcd for  $C_{24}H_{32}N_6O_2$ : C, 66.03; H, 7.39; N, 19.25. Found: C, 66.21; H, 7.52; N, 19.01.

***N*-(5-(Cyclohexylamino)-1-(4-methoxybenzyl)-1*H*-pyrazolo[3,4-*c*]pyridin-3-yl)-2-(4-methylpiperazin-1-yl)acetamide (13h)** This compound was prepared according to the general procedure described above, upon reaction of the chloride **12c** with 1-methylpiperazine. The product was purified by column chromatography (silica gel) using a mixture of dichloromethane/methanol (96/4, v/v) as the eluent to provide pure **13h** as a yellow solid. Yield: 98%, mp: 157–8 °C (CHCl<sub>3</sub>/Et<sub>2</sub>O). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ : 1.21 (3H, m, cyclohexyl H), 1.40 (2H, m, cyclohexyl H), 1.62 (1H, m, cyclohexyl H), 1.74 (2H, m, cyclohexyl H), 2.04 (2H, m, cyclohexyl H), 2.47 (3H, s, CH<sub>3</sub>-piperazine), 2.74 (4H, brs, piperazine H-3''', H-5'''), 2.84 (4H, brs, piperazine H-2''', H-6'''), 3.26 (2H, s, NHCOCH<sub>2</sub>), 3.44 (1H, m, cyclohexyl H-1''), 3.77 (3H, s, OCH<sub>3</sub>), 4.29 (1H, brs, D<sub>2</sub>O exchang., NH-cyclohexylamine), 5.37 (2H, s, CH<sub>2</sub>-pyrazole), 6.80 (1H, d, H-4,  $J=1.0$  Hz), 6.83 (2H, d, H-3', H-5',  $J=8.7$  Hz), 7.16 (2H, d, H-2', H-6',  $J=8.7$  Hz), 8.31 (1H, d, H-7,  $J=1.0$  Hz), 9.34 (1H, s, D<sub>2</sub>O exchang., NHCO). <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>)  $\delta$ : 24.9 (cyclohexyl C-3'', C-5''), 26.0 (cyclohexyl C-4''), 33.3 (cyclohexyl C-2'', C-6''), 45.4 (CH<sub>3</sub>-piperazine), 51.2 (cyclohexyl C-1''), 52.7 (piperazine C-2''', C-6'''), 53.1 (CH<sub>2</sub>-pyrazole), 54.8 (piperazine C-3''', C-5'''), 55.4 (OCH<sub>3</sub>), 61.5 (NHCOCH<sub>2</sub>), 94.0 (C-4), 114.4 (C-3', C-5'), 124.6 (C-3a), 128.4 (C-1'), 128.9 (C-2'', C-6''), 132.1 (C-7), 133.8 (C-7a), 137.3 (C-3), 152.2 (C-5), 159.6 (C-4''), 168.0 (CO). HR-MS (ESI)  $m/z$ : Calcd for  $C_{27}H_{38}N_7O_2$ :  $[M+H]^+ = 492.3081$ , found 492.3085. *Anal.* Calcd for  $C_{27}H_{37}N_7O_2$ : C, 65.96; H, 7.59; N, 19.94. Found: C, 65.78; H, 7.51; N, 20.20.

***N*-(5-(Cyclohexylamino)-1-(4-methoxybenzyl)-1*H*-pyrazolo[3,4-*c*]pyridin-3-yl)-2-(phenylamino)acetamide (13i)** This compound was prepared according to the general procedure described above, upon reaction of the chloride **12c** with aniline. The product was purified by column chromatography (silica gel) using a mixture of dichloromethane/methanol (100/1, v/v) as the eluent to provide pure **13i** as a yellow solid. Yield: 46%, mp: 152 °C (CHCl<sub>3</sub>/Et<sub>2</sub>O). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 1.24 (3H, m, cyclohexyl H), 1.40 (2H, m, cyclohexyl H), 1.64 (1H, m, cyclohexyl H), 1.75 (2H, m, cyclohexyl H), 2.05 (2H, m, cyclohexyl H), 3.44 (1H, m, cyclohexyl H-1''), 3.74 (3H, s, OCH<sub>3</sub>), 3.97 (2H, d, COCH<sub>2</sub>NH, *J*=5.2 Hz), 4.32 (1H, brs, D<sub>2</sub>O exchange., NH-cyclohexylamine), 4.45 (1H, t, D<sub>2</sub>O exchange., COCH<sub>2</sub>NH, *J*=5.2 Hz), 5.31 (2H, s, CH<sub>2</sub>-pyrazole), 6.70 (2H, d, H-2''', H-6''', *J*=7.7 Hz), 6.76 (1H, s, H-4), 6.78 (2H, d, H-3', H-5', *J*=8.5 Hz), 6.84 (1H, t, H-4''', *J*=7.2 Hz), 7.11 (2H, d, H-2', H-6', *J*=8.5 Hz), 7.23 (2H, t, H-3''', H-5''', *J*=7.5 Hz), 8.30 (1H, s, H-7), 9.05 (1H, s, D<sub>2</sub>O exchange., NHCO). <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>) δ: 24.8 (cyclohexyl C-3'', C-5''), 25.9 (cyclohexyl C-4''), 33.0 (cyclohexyl C-2'', C-6''), 49.3 (NHCOCH<sub>2</sub>), 51.2 (cyclohexyl C-1''), 53.2 (CH<sub>2</sub>-pyrazole), 55.3 (OCH<sub>3</sub>), 94.1 (C-4), 113.5 (C-2''', C-6'''), 114.3 (C-3', C-5'), 119.7 (C-4'''), 125.0 (C-3a), 127.9 (C-1'), 128.8 (C-2', C-6'), 129.6 (C-3''', C-5'''), 130.9 (C-7), 133.3 (C-7a), 136.9 (C-3), 146.9 (C-1'''), 151.6 (C-5), 159.5 (C-4'), 169.0 (CO). HR-MS (ESI) *m/z*: Calcd for C<sub>28</sub>H<sub>33</sub>N<sub>6</sub>O<sub>2</sub>: [M+H]<sup>+</sup> =485.2660, found 485.2663. *Anal.* Calcd for C<sub>28</sub>H<sub>32</sub>N<sub>6</sub>O<sub>2</sub>: C, 69.40; H, 6.66; N, 17.34. Found: C, 69.63; H, 6.80; N, 17.12.

**2-(Dimethylamino)-*N*-(1-(4-methoxybenzyl)-5-phenylamino-1*H*-pyrazolo[3,4-*c*]pyridin-3-yl)acetamide (13j)** This compound was prepared according to the general procedure described above, upon reaction of the chloride **12d** with dimethylamine (5.6 M solution in ethanol). The product was purified by column chromatography (silica gel) using a mixture of

dichloromethane/ethyl acetate (6/4, v/v) as the eluent to provide pure **13j** as a pale green solid. Yield: 70%, mp: 143 °C (Et<sub>2</sub>O). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 2.36 (6H, s, N(CH<sub>3</sub>)<sub>2</sub>), 3.12 (2H, s, NHCOCH<sub>2</sub>), 3.72 (3H, s, OCH<sub>3</sub>), 5.36 (2H, s, CH<sub>2</sub>-pyrazole), 6.82 (2H, d, H-3'', H-5'', J=8.7 Hz), 6.90 (2H, m, H-4', NH-aniline), 7.17 (2H, d, H-2'', H-6'', J=8.7 Hz), 7.26 (4H, m, H-2', H-3', H-5', H-6'), 7.59 (1H, d, H-4, J=1.0 Hz), 8.45 (1H, d, H-7, J=1.0 Hz), 9.69 (1H, brs, D<sub>2</sub>O exch., NHCO). <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>) δ: 46.0 (N(CH<sub>3</sub>)<sub>2</sub>), 53.0 (CH<sub>2</sub>-pyrazole), 55.3 (OCH<sub>3</sub>), 63.0 (NHCOCH<sub>2</sub>), 99.1 (C-4), 114.3 (C-3'', C-5''), 117.9 (C-2', C-6'), 121.1 (C-4'), 123.8 (C-3a), 128.1 (C-1''), 128.9 (C-2'', C-6''), 129.2 (C-3', C-5'), 131.9 (C-7), 134.5 (C-7a), 138.1 (C-3), 142.2 (C-1'), 148.1 (C-5), 159.5 (C-4''), 168.9 (CO). HR-MS (ESI) *m/z*: Calcd for C<sub>24</sub>H<sub>27</sub>N<sub>6</sub>O<sub>2</sub>: [M+H]<sup>+</sup> =431.2190, found 431.2191. *Anal.* Calcd for C<sub>24</sub>H<sub>26</sub>N<sub>6</sub>O<sub>2</sub>: C, 66.96; H, 6.09; N, 19.52. Found: C, 67.11; H, 6.24; N, 19.39.

***N*-(1-(4-Methoxybenzyl)-5-(phenylamino)-1*H*-pyrazolo[3,4-*c*]pyridin-3-yl)-2-(4-methylpiperazin-1-yl)acetamide (13k)** This compound was prepared according to the general procedure described above, upon reaction of the chloride **12d** with 1-methylpiperazine. The product was purified by column chromatography (silica gel) using a mixture of dichloromethane/methanol (98/2, v/v) as the eluent to provide pure **13k** as a pale green solid. Yield: 70%, mp: 134 °C (CH<sub>2</sub>Cl<sub>2</sub>/Et<sub>2</sub>O). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 2.31 (3H, s, CH<sub>3</sub>-piperazine), 2.52 (4H, brs, piperazine H-3''', H-5'''), 2.68 (4H, brs, piperazine H-2''', H-6'''), 3.18 (2H, s, NHCOCH<sub>2</sub>), 3.74 (3H, s, OCH<sub>3</sub>), 5.40 (2H, s, CH<sub>2</sub>-pyrazole), 6.74 (1H, brs, NH), 6.82 (2H, d, H-3'', H-5'', J=8.7 Hz), 6.92 (1H, m, H-4'), 7.17 (2H, d, H-2'', H-6'', J=8.7 Hz), 7.26 (4H, m, H-2', H-3', H-5', H-6'), 7.51 (1H, d, H-4, J=1.0 Hz), 8.42 (1H, d, H-7, J=1.0 Hz), 9.51 (1H, brs, D<sub>2</sub>O exch., NHCO). <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>) δ: 45.8 (CH<sub>3</sub>-piperazine), 53.1 (CH<sub>2</sub>-pyrazole), 53.3 (piperazine C-2''', C-6'''), 54.9 (piperazine C-3''', C-5'''), 55.3 (OCH<sub>3</sub>),

61.4 (NHCOCH<sub>2</sub>), 98.7 (C-4), 114.3 (C-3'', C-5''), 118.0 (C-2', C-6'), 121.2 (C-4'), 123.9 (C-3a), 128.0 (C-1''), 128.8 (C-2'', C-6''), 129.2 (C-3', C-5'), 132.0 (C-7), 134.5 (C-7a), 137.8 (C-3), 142.1 (C-1'), 148.1 (C-5), 159.5 (C-4''), 168.4 (CO). HR-MS (ESI) *m/z*: Calcd for C<sub>27</sub>H<sub>32</sub>N<sub>7</sub>O<sub>2</sub>: [M1+H]<sup>+</sup> =486.2612, found 486.2619. *Anal.* Calcd for C<sub>27</sub>H<sub>31</sub>N<sub>7</sub>O<sub>2</sub>: C, 66.78; H, 6.43; N, 20.19. Found: C, 66.94; H, 6.33; N, 20.07.

***N*-(1-(4-Methoxybenzyl)-5-(phenylamino)-1*H*-pyrazolo[3,4-*c*]pyridin-3-yl)-2-**

**(phenylamino)acetamide (13l)** This compound was prepared according to the general procedure described above, upon reaction of the chloride **12d** with aniline. The product was purified by column chromatography (silica gel) using a mixture of dichloromethane/methanol (100/1, v/v) as the eluent to provide pure **13l** as a beige solid. Yield: 68%, mp: 208–9 °C (CHCl<sub>3</sub>/Et<sub>2</sub>O). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ: 3.70 (3H, s, OCH<sub>3</sub>), 3.93 (2H, brs, NHCOCH<sub>2</sub>), 5.50 (2H, s, CH<sub>2</sub>-pyrazole), 6.00 (1H, brs, CH<sub>2</sub>NH), 6.58 (1H, t, H-4''', *J*=7.2 Hz), 6.61 (2H, d, H-2''', H-6''', *J*=7.8 Hz), 6.79 (1H, t, H-4', *J*=7.2 Hz), 6.88 (2H, d, H-3'', H-5'', *J*=8.5 Hz), 7.10 (2H, t, H-3''', H-5''', *J*=7.5 Hz), 7.19 (3H, m, H-3', H-5', H-4), 7.27 (2H, d, H-2'', H-6'', *J*=8.5 Hz), 7.41 (2H, d, H-2', H-6', *J*=8.2 Hz), 8.73 (1H, s, D<sub>2</sub>O exch., NH-aniline), 8.86 (1H, s, H-7), 10.57 (1H, s, D<sub>2</sub>O exch., NHCO). <sup>13</sup>C NMR (50 MHz, DMSO-*d*<sub>6</sub>) δ: 46.4 (NHCOCH<sub>2</sub>), 51.6 (CH<sub>2</sub>-pyrazole), 55.0 (OCH<sub>3</sub>), 98.7 (C-4), 112.2 (C-2''', C-6'''), 113.9 (C-3'', C-5''), 116.3 (C-4'''), 116.6 (C-2', C-6'), 119.2 (C-4'), 123.5 (C-3a), 128.6 (C-3', C-5'), 128.9 (C-3''', C-5'''), 129.0 (C-1''), 129.2 (C-2'', C-6''), 131.9 (C-7), 133.6 (C-7a), 138.0 (C-3), 143.0 (C-1'), 147.9 (C-5), 148.1 (C-1'''), 158.8 (C-4''), 169.4 (CO). HR-MS (ESI) *m/z*: Calcd for C<sub>28</sub>H<sub>27</sub>N<sub>6</sub>O<sub>2</sub>: [M1+H]<sup>+</sup> =479.2190, found 479.2194. *Anal.* Calcd for C<sub>28</sub>H<sub>26</sub>N<sub>6</sub>O<sub>2</sub>: C, 70.28; H, 5.48; N, 17.56. Found: C, 70.03; H, 5.39; N, 17.66.

**General procedure for the synthesis of derivatives 14a–f.** A solution of the compounds **13g–13l** (0.2 mmol) in trifluoroacetic acid (5 mL) was heated at 70 °C for 96h. Then the solvent was vacuum-evaporated and the residue was neutralized with sodium bicarbonate and extracted with ethyl acetate (3 x 50 mL). The combined organic extracts were dried (Na<sub>2</sub>SO<sub>4</sub>), concentrated under vacuum and the residue was purified by column chromatography to provide pure target compounds **14a–f**.

***N*-(5-(Cyclohexylamino)-1*H*-pyrazolo[3,4-*c*]pyridin-3-yl)-2-(dimethylamino)acetamide (14a)** This compound was prepared according to the general procedure described above, starting from **13g**. The product was purified by column chromatography (silica gel) using a mixture of dichloromethane/methanol (95/5, v/v) as the eluent to provide pure **14a** as a green solid. Yield: 80%, mp: 132–3 °C (Et<sub>2</sub>O/*n*-pentane). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 1.18 (3H, m, cyclohexyl H), 1.32 (2H, m, cyclohexyl H), 1.57 (1H, m, cyclohexyl H), 1.68 (2H, m, cyclohexyl H), 2.01 (2H, m, cyclohexyl H), 2.38 (6H, s, N(CH<sub>3</sub>)<sub>2</sub>), 3.17 (2H, s, CH<sub>2</sub>), 3.40 (1H, m, cyclohexyl H-1'), 4.23 (1H, brs, D<sub>2</sub>O exchange., NH–cyclohexylamine), 6.80 (1H, s, H-4), 8.37 (1H, s, H-7), 9.62 (1H, brs, D<sub>2</sub>O exchange., NHCO), 11.62 (1H, brs, D<sub>2</sub>O exchange., NH–pyrazole). <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>) δ: 24.7 (cyclohexyl C-3', C-5'), 25.8 (cyclohexyl C-4'), 33.0 (cyclohexyl C-2', C-6'), 46.0 (N(CH<sub>3</sub>)<sub>2</sub>), 51.2 (cyclohexyl C-1'), 63.0 (CH<sub>2</sub>), 93.3 (C-4), 123.3 (C-3a), 132.6 (C-7), 134.2 (C-7a), 138.4 (C-3), 151.8 (C-5), 169.2 (CO). HR-MS (ESI) *m/z*: Calcd for C<sub>16</sub>H<sub>25</sub>N<sub>6</sub>O: [M1+H]<sup>+</sup> =317.2084, found 317.2088. *Anal.* Calcd for C<sub>16</sub>H<sub>24</sub>N<sub>6</sub>O: C, 60.74; H, 7.65; N, 26.56. Found: C, 61.01; H, 7.80; N, 26.33.

***N*-(5-(Cyclohexylamino)-1*H*-pyrazolo[3,4-*c*]pyridin-3-yl)-2-(4-methylpiperazin-1-yl)acetamide (14b)** This compound was prepared according to the general procedure described



above, starting from **13h**. The product was purified by column chromatography (silica gel) using a mixture of dichloromethane/methanol (10/1, v/v) as the eluent to provide pure **14b** as a pale yellow solid. Yield: 88%, mp: 145 °C (Et<sub>2</sub>O). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 1.23 (3H, m, cyclohexyl H), 1.40 (2H, m, cyclohexyl H), 1.63 (1H, m, cyclohexyl H), 1.74 (2H, m, cyclohexyl H), 2.06 (2H, m, cyclohexyl H), 2.33 (3H, s, CH<sub>3</sub>), 2.54 (4H, brs, piperazine H-3'', H-5''), 2.72 (4H, brs, piperazine H-2'', H-6''), 3.24 (2H, s, CH<sub>2</sub>), 3.44 (1H, m, cyclohexyl H-1'), 4.33 (1H, brs, D<sub>2</sub>O exchang., NH– cyclohexylamine), 6.80 (1H, s, H-4), 8.44 (1H, s, H-7), 9.55 (1H, s, D<sub>2</sub>O exchang., NHCO), 10.52 (1H, brs, D<sub>2</sub>O exchang., NH–pyrazole). <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>) δ: 24.8 (cyclohexyl C-3', C-5'), 25.9 (cyclohexyl C-4'), 33.2 (cyclohexyl C-2', C-6'), 45.9 (CH<sub>3</sub>), 51.2 (cyclohexyl C-1'), 53.5 (piperazine C-2'', C-6''), 55.1 (piperazine C-3'', C-5''), 61.5 (CH<sub>2</sub>), 93.3 (C-4), 123.6 (C-3a), 132.4 (C-7), 134.2 (C-7a), 139.0 (C-3), 152.2 (C-5), 168.5 (CO). HR-MS (ESI) *m/z*: Calcd for C<sub>19</sub>H<sub>30</sub>N<sub>7</sub>O: [M+H]<sup>+</sup> =372.2506, found 372.2511. *Anal.* Calcd for C<sub>19</sub>H<sub>29</sub>N<sub>7</sub>O: C, 61.43; H, 7.87; N, 26.39. Found: C, 61.19; H, 7.76; N, 26.48.

***N*-(5-(Cyclohexylamino)-1*H*-pyrazolo[3,4-*c*]pyridin-3-yl)-2-(phenylamino)acetamide**

**(14c)** This compound was prepared according to the general procedure described above, starting from **13i**. The product was purified by column chromatography (silica gel) using a mixture of dichloromethane/methanol (96/4, v/v) as the eluent to provide pure **14c** as a yellow solid. Yield: 74%, mp: 166 °C (CHCl<sub>3</sub>). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 1.23 (3H, m, cyclohexyl H), 1.41 (2H, m, cyclohexyl H), 1.64 (1H, m, cyclohexyl H), 1.75 (2H, m, cyclohexyl H), 2.05 (2H, m, cyclohexyl H), 3.41 (1H, m, cyclohexyl H-1'), 4.01 (2H, s, CH<sub>2</sub>), 4.49 (1H, s, D<sub>2</sub>O exchang., CH<sub>2</sub>NH), 6.69 (1H, s, H-4), 6.72 (2H, d, H-2'', H-6'', *J*=8.0 Hz), 6.85 (1H, t, H-4'', *J*=7.2 Hz), 7.24 (2H, t, H-3'', H-5'', *J*=7.7 Hz), 8.42 (1H, s, H-7), 9.07 (1H, s, D<sub>2</sub>O exchang., NHCO), 10.19 (1H, brs, D<sub>2</sub>O exchang., NH–pyrazole). <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>) δ: 24.8 (cyclohexyl

C-3', C-5'), 25.9 (cyclohexyl C-4'), 33.1 (cyclohexyl C-2', C-6'), 49.2 (CH<sub>2</sub>), 51.2 (cyclohexyl C-1'), 93.0 (C-4), 113.5 (C-2'', C-6''), 119.7 (C-4''), 123.9 (C-3a), 129.6 (C-3'', C-5''), 132.2 (C-7), 134.0 (C-7a), 138.6 (C-3), 146.8 (C-1''), 152.1 (C-5), 169.4 (CO). HR-MS (ESI) *m/z*: Calcd for C<sub>20</sub>H<sub>25</sub>N<sub>6</sub>O: [M1+H]<sup>+</sup> =365.2084, found 365.2090. *Anal.* Calcd for C<sub>20</sub>H<sub>24</sub>N<sub>6</sub>O: C, 65.91; H, 6.64; N, 23.06. Found: C, 66.12; H, 6.77; N, 22.89.

**2-(Dimethylamino)-N-(5-(phenylamino)-1H-pyrazolo[3,4-c]pyridin-3-yl)acetamide**

**(14d)** This compound was prepared according to the general procedure described above, starting from **13j**. The product was purified by column chromatography (silica gel) using a mixture of dichloromethane/methanol (100/2, v/v) as the eluent to provide pure **14d** as a pale green solid. Yield: 89%, mp: 194 °C (EtOAc). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$ : 2.33 (6H, s, N(CH<sub>3</sub>)<sub>2</sub>), 3.16 (2H, s, CH<sub>2</sub>), 6.78 (1H, t, H-4', *J*=7.2 Hz), 7.20 (3H, m, H-3', H-5', H-4), 7.46 (2H, d, H-2', H-6', *J*=7.8 Hz), 8.66 (1H, d, H-7, *J*=0.8 Hz), 8.69 (1H, s, D<sub>2</sub>O exchang., NH-aniline), 10.14 (1H, brs, D<sub>2</sub>O exchang., NHCO), 12.87 (1H, s, D<sub>2</sub>O exchang., NH-pyrazole). <sup>13</sup>C NMR (50 MHz, DMSO-*d*<sub>6</sub>)  $\delta$ : 45.2 (N(CH<sub>3</sub>)<sub>2</sub>), 62.1 (CH<sub>2</sub>), 98.5 (C-4), 116.4 (C-2', C-6'), 119.0 (C-4'), 123.0 (C-3a), 128.6 (C-3', C-5'), 132.0 (C-7), 134.4 (C-7a), 138.6 (C-3), 143.2 (C-1'), 147.7 (C-5), 168.7 (CO). HR-MS (ESI) *m/z*: Calcd for C<sub>16</sub>H<sub>19</sub>N<sub>6</sub>O: [M1+H]<sup>+</sup> =311.1615, found 311.1619. *Anal.* Calcd for C<sub>16</sub>H<sub>18</sub>N<sub>6</sub>O: C, 61.92; H, 5.85; N, 27.08. Found: C, 62.11; H, 5.98; N, 27.17.

**2-(4-Methylpiperazin-1-yl)-N-(5-(phenylamino)-1H-pyrazolo[3,4-c]pyridin-3-**

**yl)acetamide (14e)** This compound was prepared according to the general procedure described above, starting from **13k**. The product was purified by column chromatography (silica gel) using a mixture of dichloromethane/methanol (100/5, v/v) as the eluent to provide pure **14e** as a green solid. Yield: 78%, mp: 186 °C (EtOAc). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$ : 2.18 (3H, s, CH<sub>3</sub>), 2.40 (4H, brs, piperazine H-3'', H-5''), 2.58 (4H, brs, piperazine H-2'', H-6''), 3.20 (2H, s,

CH<sub>2</sub>), 6.78 (1H, t, H-4', *J*=7.2 Hz), 7.20 (3H, m, H-3', H-5', H-4), 7.45 (2H, d, H-2', H-6', *J*=8.0 Hz), 8.66 (1H, s, H-7), 8.70 (1H, s, D<sub>2</sub>O exchang., NH–aniline), 10.12 (1H, s, D<sub>2</sub>O exchang., NHCO), 12.88 (1H, s, D<sub>2</sub>O exchang., NH–pyrazole). <sup>13</sup>C NMR (50 MHz, DMSO-*d*<sub>6</sub>) δ: 45.5 (CH<sub>3</sub>), 52.5 (piperazine C-2'', C-6''), 54.4 (piperazine C-3'', C-5''), 60.7 (CH<sub>2</sub>), 98.5 (C-4), 116.5 (C-2', C-6'), 119.0 (C-4'), 122.8 (C-3a), 128.5 (C-3', C-5'), 132.0 (C-7), 134.5 (C-7a), 138.6 (C-3), 143.2 (C-1'), 147.7 (C-5), 168.2 (CO). HR-MS (ESI) *m/z*: Calcd for C<sub>19</sub>H<sub>24</sub>N<sub>7</sub>O: [M1+H]<sup>+</sup> =366.2037, found 366.2044. *Anal.* Calcd for C<sub>19</sub>H<sub>23</sub>N<sub>7</sub>O: C, 62.45; H, 6.34; N, 26.83. Found: C, 62.28; H, 6.19; N, 26.99.

**2-(Phenylamino)-N-(5-(phenylamino)-1*H*-pyrazolo[3,4-*c*]pyridin-3-yl)acetamide (14f)**

This compound was prepared according to the general procedure described above, starting from **13l**. The product was purified by column chromatography (silica gel) using a mixture of cyclohexane/ethyl acetate (1/1, v/v) as the eluent to provide pure **14f** as a beige solid. Yield: 84%, mp: 126 °C (dec.) (EtOAc). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 4.00 (2H, s, CH<sub>2</sub>), 4.40 (1H, brs, D<sub>2</sub>O exchang., CH<sub>2</sub>NH), 6.53 (1H, brs, D<sub>2</sub>O exchang., NH–aniline), 6.71 (2H, d, H-2'', H-6'', *J*=7.8 Hz), 6.85 (1H, t, H-4'', *J*=7.2 Hz), 7.00 (1H, t, H-4', *J*=7.1 Hz), 7.23 (2H, t, H-3'', H-5'', *J*=7.7 Hz), 7.28 (2H, d, H-2', H-6', *J*=7.7 Hz), 7.33 (2H, t, H-3', H-5', *J*=7.6 Hz), 7.49 (1H, s, H-4), 8.59 (1H, s, H-7), 9.09 (1H, s, D<sub>2</sub>O exchang., NHCO), 9.95 (1H, brs, D<sub>2</sub>O exchang., NH–pyrazole). <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>) δ: 49.3 (CH<sub>2</sub>), 97.9 (C-4), 113.5 (C-2'', C-6''), 118.4 (C-2', C-6'), 119.8 (C-4''), 121.7 (C-4'), 123.4 (C-3a), 129.4 (C-3', C-5'), 129.6 (C-3'', C-5''), 132.4 (C-7), 135.1 (C-7a), 139.8 (C-3), 141.6 (C-1'), 146.6 (C-1''), 148.4 (C-5), 169.3 (CO). HR-MS (ESI) *m/z*: Calcd for C<sub>20</sub>H<sub>19</sub>N<sub>6</sub>O: [M1+H]<sup>+</sup> =359.1615, found 359.1622. *Anal.* Calcd for C<sub>20</sub>H<sub>18</sub>N<sub>6</sub>O: C, 67.03; H, 5.06; N, 23.45. Found: C, 66.89; H, 5.01; N, 23.62.

**7-Chloro-3-nitro-1*H*-pyrazolo[3,4-*c*]pyridine (21)** This compound was prepared following an analogous synthetic procedure to that described for the synthesis of the nitroderivative **7**, upon nitration of pyrazolopyridine **20**, in 94% yield. The nitroderivative **21** was obtained pure, as a yellow solid. mp: 172–3 °C (EtOAc). <sup>1</sup>H NMR (600 MHz, DMSO-*d*<sub>6</sub>) δ: 8.03 (1H, d, H-4, *J*=5.6 Hz), 8.27 (1H, d, H-5, *J*=5.6 Hz). <sup>13</sup>C NMR (151 MHz, DMSO-*d*<sub>6</sub>) δ: 113.9 (C-4), 122.1 (C-3a), 136.4 (C-7a), 137.5 (C-3), 141.5 (C-5), 149.6 (C-7). *Anal.* Calcd for C<sub>6</sub>H<sub>3</sub>ClN<sub>4</sub>O<sub>2</sub>: C, 36.29; H, 1.52; N, 28.22. Found: C, 36.50; H, 1.72; N, 27.94.

**7-Chloro-1-methyl-3-nitro-1*H*-pyrazolo[3,4-*c*]pyridine (22a) and 7-chloro-2-methyl-3-nitro-2*H*-pyrazolo[3,4-*c*]pyridine (23)** These compounds were prepared following an analogous synthetic procedure to that described for the synthesis of the nitroderivatives **8a** and **9a**, starting from pyrazolopyridine **21** (540 mg, 2.72 mmol). Purification was effected using a mixture of cyclohexane / ethyl acetate (8/2, v/v) as the eluent, to provide the pure isomers **22a** (45% yield) and **23** (10% yield).

Data for **22a**: Beige solid, mp: 160–1 °C (EtOAc). <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ: 4.57 (3H, s, CH<sub>3</sub>), 8.08 (1H, d, H-4, *J*=5.6 Hz), 8.30 (1H, d, H-5, *J*=5.6 Hz). <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ: 41.1 (CH<sub>3</sub>), 114.5 (C-4), 123.6 (C-3a), 135.2 (C-7a), 135.4 (C-3), 142.5 (C-5), 146.8 (C-7). *Anal.* Calcd for C<sub>7</sub>H<sub>5</sub>ClN<sub>4</sub>O<sub>2</sub>: C, 39.55; H, 2.37; N, 26.35. Found: C, 39.74; H, 2.43; N, 26.17.

Data for **23**: Pale yellow solid, mp: 202–3 °C (EtOAc). <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ: 4.70 (3H, s, CH<sub>3</sub>), 7.93 (1H, d, H-4, *J*=5.9 Hz), 8.27 (1H, d, H-5, *J*=5.9 Hz). <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ: 43.9 (CH<sub>3</sub>), 113.2 (C-4), 122.3 (C-3a), 129.0 (C-7a), 140.2 (C-3), 143.5 (C-5), 144.6 (C-7). *Anal.* Calcd for C<sub>7</sub>H<sub>5</sub>ClN<sub>4</sub>O<sub>2</sub>: C, 39.55; H, 2.37; N, 26.35. Found: C, 39.82; H, 2.49; N, 26.21.

**7-Chloro-1-(4-methoxybenzyl)-3-nitro-1H-pyrazolo[3,4-c]pyridine (22b)** This compound was prepared following an analogous synthetic procedure to that described for the synthesis of the derivatives **8b** and **9b**, starting from pyrazolopyridine **21** (540 mg, 2.72 mmol). Purification was effected using a mixture of cyclohexane / ethyl acetate (8/2, v/v) as the eluent, to provide pure **22b** as a pale yellow solid, in 65% yield. mp: 142–4 °C (CH<sub>2</sub>Cl<sub>2</sub>/Et<sub>2</sub>O). <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ: 3.75 (3H, s, OCH<sub>3</sub>), 6.03 (2H, s, CH<sub>2</sub>), 6.84 (2H, d, H-2'', H-6'', *J*=8.5 Hz), 7.29 (2H, d, H-3'', H-5'', *J*=8.5 Hz), 8.10 (1H, d, H-4, *J*=5.5 Hz), 8.30 (1H, d, H-5, *J*=5.8 Hz). <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ: 55.4 (OCH<sub>3</sub>), 55.8 (CH<sub>2</sub>), 114.5 (C-2'', C-6''), 114.6 (C-4), 123.9 (C-3a), 126.8 (C-7a), 129.2 (C-3'', C-5''), 134.6 (C-1''), 135.0 (C-3), 142.4 (C-5), 147.4 (C-7), 160.0 (C-4''). *Anal.* Calcd for C<sub>14</sub>H<sub>11</sub>ClN<sub>4</sub>O<sub>3</sub>: C, 52.76; H, 3.48; N, 17.58. Found: C, 53.02; H, 3.59; N, 17.26.

**N-Cyclohexyl-1-methyl-3-nitro-1H-pyrazolo[3,4-c]pyridin-7-amine (24a)** This compound was prepared following an analogous synthetic procedure to that described for the synthesis of the derivative **10a**, upon reaction of the chloroderivative **22a** with cyclohexylamine for 2 h, in 89% yield. The product was purified by column chromatography using a mixture of cyclohexane/ethyl acetate (from 7/3 up to 6/4, v/v) as the eluent, to provide **24a** as a yellow solid. mp: 171–3 °C (EtOAc/Et<sub>2</sub>O). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 1.20-1.31 (3H, m, cyclohexyl H), 1.41-1.50 (2H, m, cyclohexyl H), 1.62-1.68 (1H, m, cyclohexyl H), 1.71-1.78 (2H, m, cyclohexyl H), 2.10-2.15 (2H, m, cyclohexyl H), 4.12-4.24 (1H, m, cyclohexyl H-1'), 4.45 (3H, s, CH<sub>3</sub>), 4.78 (1H, brs, D<sub>2</sub>O exchang., NH), 7.33 (1H, d, H-4, *J*=5.8 Hz), 7.95 (1H, d, H-5, *J*=5.8 Hz). <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>) δ: 25.0 (cyclohexyl C-3', C-5'), 25.9 (cyclohexyl C-4'), 33.4 (cyclohexyl C-2', C-6'), 41.1 (CH<sub>3</sub>), 49.8 (cyclohexyl C-1'), 104.0 (C-4), 123.0 (C-3a), 128.8

(C-7a), 142.7 (C-5), 145.2 (C-3), 147.0 (C-7). *Anal.* Calcd for C<sub>13</sub>H<sub>17</sub>N<sub>5</sub>O<sub>2</sub>: C, 56.72; H, 6.22; N, 25.44. Found: C, 56.93; H, 6.34; N, 25.29.

***N*-Cyclohexyl-1-(4-methoxybenzyl)-3-nitro-1*H*-pyrazolo[3,4-*c*]pyridin-7-amine (24b)**

This compound was prepared following an analogous synthetic procedure to that described for the synthesis of the derivative **10a**, upon reaction of the chloroderivative **22b** with cyclohexylamine for 3 h, in 70 % yield. The product was purified by column chromatography using a mixture of cyclohexane/ethyl acetate (90/10, v/v) as the eluent, to provide **24b** as an orange colored solid. mp: 152–4 °C (CH<sub>2</sub>Cl<sub>2</sub>/Et<sub>2</sub>O). <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ: 0.82-0.92 (3H, m, cyclohexyl H), 1.08-1.18 (2H, m, cyclohexyl H), 1.30-1.40 (1H, m, cyclohexyl H), 1.57-1.65 (2H, m, cyclohexyl H), 1.82-1.88 (2H, m, cyclohexyl H), 3.81 (3H, s, OCH<sub>3</sub>), 3.88-3.96 (1H, m, cyclohexyl H-1'), 4.64 (1H, d, D<sub>2</sub>O exchang., NH-cyclohexylamine, *J*=6.4 Hz), 5.82 (2H, s, CH<sub>2</sub>), 6.94 (2H, d, H-2'', H-6'', *J*=8.7 Hz), 7.09 (2H, d, H-3'', H-5'', *J*=8.7 Hz), 7.36 (1H, d, H-4, *J*=5.8 Hz), 7.94 (1H, d, H-5, *J*=5.8 Hz). <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ: 25.1 (cyclohexyl C-3', C-5'), 25.9 (cyclohexyl C-4'), 33.4 (cyclohexyl C-2', C-6'), 49.4 (cyclohexyl C-1'), 55.4 (OCH<sub>3</sub>), 57.7 (CH<sub>2</sub>), 101.7 (C-4), 114.4 (C-2'', C-6''), 122.4 (C-3a), 126.6 (C-7a), 129.7 (C-3'', C-5''), 134.7 (C-1''), 137.0 (C-3), 145.4 (C-5), 150.2 (C-7), 160.0 (C-4''). *Anal.* Calcd for C<sub>20</sub>H<sub>23</sub>N<sub>5</sub>O<sub>3</sub>: C, 62.98; H, 6.08; N, 18.36. Found: C, 62.78; H, 6.01; N, 18.55.

***N*<sup>7</sup>-Cyclohexyl-1-methyl-1*H*-pyrazolo[3,4-*c*]pyridine-3,7-diamine (25a)** This compound was prepared following an analogous synthetic procedure to that described for the synthesis of the aminoderivative **11a**, starting from the nitroderivative **24a** (600 mg, 2.18 mmol), in 82% yield. The product was purified by column chromatography using a mixture of cyclohexane/ethyl acetate (from 6/4 up to 3/7, v/v) as the eluent, to provide pure **25a** as a beige solid. mp: 144–6 °C (CH<sub>2</sub>Cl<sub>2</sub>/petroleum ether). <sup>1</sup>H NMR (600 MHz, DMSO-*d*<sub>6</sub>) δ: 1.15-1.25 (3H, m, cyclohexyl H),

1.30-1.40 (2H, m, cyclohexyl H), 1.58-1.65 (1H, m, cyclohexyl H), 1.70-1.78 (2H, m, cyclohexyl H), 1.96-2.10 (2H, m, cyclohexyl H), 3.92 (3H, s, CH<sub>3</sub>), 3.93-3.98 (1H, m, cyclohexyl H-1'), 5.27 (2H, brs, D<sub>2</sub>O exchang., NH<sub>2</sub>), 5.62 (1H, d, D<sub>2</sub>O exchang., NH, *J*=7.3 Hz), 6.82 (1H, d, H-4, *J*=5.7 Hz), 7.44 (1H, d, H-5, *J*=5.7 Hz). <sup>13</sup>C NMR (151 MHz, DMSO-*d*<sub>6</sub>)  $\delta$ : 24.9 (cyclohexyl C-3', C-5'), 25.7 (cyclohexyl C-4'), 32.3 (cyclohexyl C-2', C-6'), 37.8 (CH<sub>3</sub>), 49.0 (cyclohexyl C-1'), 104.0 (C-4), 118.2 (C-3a), 128.3 (C-7a), 134.5 (C-5), 144.9 (C-3), 148.0 (C-7). HR-MS (ESI) *m/z*: Calcd for C<sub>13</sub>H<sub>20</sub>N<sub>5</sub>: [M+H]<sup>+</sup> =246.1713, found 246.1715. *Anal.* Calcd for C<sub>13</sub>H<sub>19</sub>N<sub>5</sub>: C, 63.65; H, 7.81; N, 28.55. Found: C, 63.90; H, 8.01; N, 28.09.

***N*<sup>7</sup>-Cyclohexyl-1-(4-methoxybenzyl)-1*H*-pyrazolo[3,4-*c*]pyridine-3,7-diamine (26a)**

This compound was prepared following an analogous synthetic procedure to that described for the synthesis of the aminoderivative **11a**, starting from the nitroderivative **24b** (720 mg, 1.89 mmol), in 80% yield. The product was purified by column chromatography using a mixture of cyclohexane/ethyl acetate (from 1/1 up to 3/7, v/v) as the eluent, to provide pure **26a** as a grey solid. mp: 130–2 °C (CH<sub>2</sub>Cl<sub>2</sub>/*n*-hexane). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$ : 1.10-1.25 (3H, m, cyclohexyl H), 1.30-1.40 (2H, m, cyclohexyl H), 1.52-1.65 (1H, m, cyclohexyl H), 1.66-1.74 (2H, m, cyclohexyl H), 1.86-1.94 (2H, m, cyclohexyl H), 3.70 (3H, s, OCH<sub>3</sub>), 3.94 (1H, brs, cyclohexyl H-1'), 5.31 (2H, s, CH<sub>2</sub>), 6.14 (2H, brs, D<sub>2</sub>O exchang., NH<sub>2</sub>), 6.31 (1H, brs, D<sub>2</sub>O exchang., NH), 6.69 (1H, d, H-4, *J*=6.1 Hz), 6.88 (2H, d, H-2'', H-6'', *J*=8.5 Hz), 7.06-7.16 (3H, m, H-5, H-3'', H-5''). <sup>13</sup>C NMR (50 MHz, DMSO-*d*<sub>6</sub>)  $\delta$ : 25.0 (cyclohexyl C-3', C-5'), 25.3 (cyclohexyl C-4'), 32.5 (cyclohexyl C-2', C-6'), 48.6 (cyclohexyl C-1'), 50.3 (CH<sub>2</sub>), 55.1 (OCH<sub>3</sub>), 103.0 (C-4), 108.4 (C-3a), 113.8 (C-2'', C-6''), 128.6 (C-3'', C-5''), 128.9 (C-7a, C-1''), 135.2 (C-5), 141.0 (C-3), 147.8 (C-7), 158.6 (C-4''). *Anal.* Calcd for C<sub>20</sub>H<sub>25</sub>N<sub>5</sub>O: C, 68.35; H, 7.17; N, 19.93. Found: C, 68.21; H, 7.10; N, 20.12.

**2-Chloro-*N*-(7-(cyclohexylamino)-1-methyl-1*H*-pyrazolo[3,4-*c*]pyridin-3-yl)acetamide (25b)** and **2-chloro-*N*-(7-(cyclohexylamino)-1-(4-methoxybenzyl)-1*H*-pyrazolo[3,4-*c*]pyridin-3-yl)acetamide (26b)** These compounds were prepared following an analogous synthetic procedure to that described for the synthesis of the compounds **12a** and **12c**, starting from the amines **25a** and **26a**, respectively. These intermediates were used immediately to the next step, with no further purification.

***N*-(7-(Cyclohexylamino)-1-methyl-1*H*-pyrazolo[3,4-*c*]pyridin-3-yl)-2-(dimethylamino)acetamide (27a)** This compound was prepared following an analogous synthetic procedure to that described for the synthesis of the compounds **13a-l**, upon reaction of the chloride **25b** with dimethylamine (5.6 M solution in ethanol). The product was purified by column chromatography (silica gel) using a mixture of dichloromethane/methanol (from 100/5 up to 100/15, v/v) as the eluent to provide pure **27a** as a beige solid. Yield: 97%, mp: 145–7 °C (Et<sub>2</sub>O). <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ: 1.21-1.29 (3H, m, cyclohexyl H), 1.43-1.51 (2H, m, cyclohexyl H), 1.61-1.67 (1H, m, cyclohexyl H), 1.70-1.76 (2H, m, cyclohexyl H), 2.09-2.15 (2H, m, cyclohexyl H), 2.40 (6H, s, N(CH<sub>3</sub>)<sub>2</sub>), 3.15 (2H, s, CH<sub>2</sub>), 4.06-4.14 (1H, m, cyclohexyl H-1'), 4.22 (3H, s, CH<sub>3</sub>- pyrazole), 4.64 (1H, d, D<sub>2</sub>O exchange., NH-cyclohexylamine, *J*=6.1 Hz), 7.00 (1H, d, H-4, *J*=5.9 Hz), 7.63 (1H, d, H-5, *J*=5.9 Hz), 9.43 (1H, brs, D<sub>2</sub>O exchange., NHCO). <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ: 24.9 (cyclohexyl C-3', C-5'), 25.9 (cyclohexyl C-4'), 33.4 (cyclohexyl C-2', C-6'), 39.2 (CH<sub>3</sub>-pyrazole), 46.1 (N(CH<sub>3</sub>)<sub>2</sub>), 49.4 (cyclohexyl C-1'), 63.1 (CH<sub>2</sub>), 105.6 (C-4), 121.7 (C-3a), 128.4 (C-7a), 136.7 (C-5), 138.0 (C-3), 145.1 (C-7), 168.9 (CO). HR-MS (ESI) *m/z*: Calcd for C<sub>17</sub>H<sub>27</sub>N<sub>6</sub>O: [M1+H]<sup>+</sup> =331.2241, found 331.2247. *Anal.* Calcd for C<sub>17</sub>H<sub>26</sub>N<sub>6</sub>O: C, 61.79; H, 7.93; N, 25.43. Found: C, 61.96; H, 7.99; N, 25.31.



***N*-(7-(Cyclohexylamino)-1-methyl-1*H*-pyrazolo[3,4-*c*]pyridin-3-yl)-2-(4-methylpiperazin-1-yl)acetamide (27b)** This compound was prepared following an analogous synthetic procedure to that described for the synthesis of the compounds **13a-1**, upon reaction of the chloride **25b** with 1-methylpiperazine. The product was purified by column chromatography (silica gel) using a mixture of dichloromethane/methanol (from 100/5 up to 100/20, v/v) as the eluent to provide pure **27b** as a yellow oil. Yield: 96%. <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ: 1.20-1.30 (3H, m, cyclohexyl H), 1.40-1.50 (2H, m, cyclohexyl H), 1.60-1.68 (1H, m, cyclohexyl H), 1.69-1.77 (2H, m, cyclohexyl H), 2.05-2.15 (2H, m, cyclohexyl H), 2.32 (3H, s, CH<sub>3</sub>-piperazine), 2.50-2.60 (4H, brs, piperazine H-3'', H-5''), 2.65-2.80 (4H, brs, piperazine H-2'', H-6''), 3.19 (2H, s, CH<sub>2</sub>), 4.06-4.14 (1H, m, cyclohexyl H-1'), 4.24 (3H, s, CH<sub>3</sub>-pyrazole), 4.60 (1H, d, D<sub>2</sub>O exchange., NH-cyclohexylamine, *J*=7.0 Hz), 6.94 (1H, d, H-4, *J*=5.9 Hz), 7.65 (1H, d, H-5, *J*=5.9 Hz), 9.31 (1H, brs, D<sub>2</sub>O exchange., NHCO). <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ: 24.9 (cyclohexyl C-3', C-5'), 26.0 (cyclohexyl C-4'), 33.5 (cyclohexyl C-2', C-6'), 39.2 (CH<sub>3</sub>-pyrazole), 45.9 (CH<sub>3</sub>-piperazine), 49.3 (cyclohexyl C-1'), 53.4 (piperazine C-2'', C-6''), 55.1 (piperazine C-3'', C-5''), 61.5 (CH<sub>2</sub>), 105.4 (C-4), 121.7 (C-3a), 128.4 (C-7a), 137.1 (C-5), 137.8 (C-3), 145.2 (C-7), 168.5 (CO). HR-MS (ESI) *m/z*: Calcd for C<sub>20</sub>H<sub>32</sub>N<sub>7</sub>O: [M+H]<sup>+</sup> =386.2663, found 386.2665. *Anal.* Calcd for C<sub>20</sub>H<sub>31</sub>N<sub>7</sub>O: C, 62.31; H, 8.11; N, 25.43. Found: C, 62.63; H, 8.29; N, 25.21.

***N*-(7-(Cyclohexylamino)-1-methyl-1*H*-pyrazolo[3,4-*c*]pyridin-3-yl)-2-(phenylamino)acetamide (27c)** This compound was prepared following an analogous synthetic procedure to that described for the synthesis of the compounds **13a-1**, upon reaction of the chloride **25b** with aniline. The product was purified by column chromatography (silica gel) using a mixture of chloroform/methanol (100/2, v/v) as the eluent to provide pure **27c** as a pale yellow solid. Yield: 51%. mp: >300 °C (EtOAc). <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ: 1.20-1.30 (3H, m,

cyclohexyl H), 1.42-1.52 (2H, m, cyclohexyl H), 1.60-1.68 (1H, m, cyclohexyl H), 1.69-1.78 (2H, m, cyclohexyl H), 2.06-2.14 (2H, m, cyclohexyl H), 3.94 (2H, s, CH<sub>2</sub>), 4.06-4.14 (1H, m, cyclohexyl H-1'), 4.16 (3H, s, CH<sub>3</sub>), 4.62 (1H, brs, D<sub>2</sub>O exchang., NH-cyclohexylamine), 4.83 (1H, brs, D<sub>2</sub>O exchang., NH-aniline), 6.67 (2H, d, H-2'', H-6'', *J*=7.9 Hz), 6.81 (1H, t, H-4'', *J*=7.3 Hz), 6.91 (1H, d, H-4, *J*=5.9 Hz), 7.20 (2H, t, H-3'', H-5'', *J*=7.8 Hz), 7.58 (1H, d, H-5, *J*=5.9 Hz), 9.06 (1H, brs, D<sub>2</sub>O exchang., NHCO). <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ: 24.9 (cyclohexyl C-3', C-5'), 25.9 (cyclohexyl C-4'), 33.3 (cyclohexyl C-2', C-6'), 39.3 (CH<sub>3</sub>), 49.2 (CH<sub>2</sub>), 49.8 (cyclohexyl C-1'), 105.4 (C-4), 113.5 (C-2'', C-6''), 119.4 (C-4''), 122.0 (C-3a), 128.1 (C-7a), 129.6 (C-3'', C-5''), 135.7 (C-5), 137.6 (C-3), 144.8 (C-1''), 147.1 (C-7), 169.7 (CO). HR-MS (ESI) *m/z*: Calcd for C<sub>21</sub>H<sub>27</sub>N<sub>6</sub>O: [M+H]<sup>+</sup> =379.2241, found 379.2245. *Anal.* Calcd for C<sub>21</sub>H<sub>26</sub>N<sub>6</sub>O: C, 66.64; H, 6.92; N, 22.20. Found: C, 66.87; H, 7.00; N, 22.11.

***N*-(7-(Cyclohexylamino)-1-(4-methoxybenzyl)-1*H*-pyrazolo[3,4-*c*]pyridin-3-yl)-2-(dimethylamino)acetamide (28)** This compound was prepared following an analogous synthetic procedure to that described for the synthesis of the compounds **13a-1**, upon reaction of the chloride **26b** with dimethylamine (5.6 M solution in ethanol). The product was purified by column chromatography (silica gel) using a mixture of ethyl acetate/methanol (from 100/5 up to 100/10, v/v) as the eluent to provide pure **28** as a white solid. Yield: 76%, mp: 138–9 °C (Et<sub>2</sub>O). <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ: 0.78-0.88 (3H, m, cyclohexyl H), 1.00-1.10 (2H, m, cyclohexyl H), 1.26-1.35 (1H, m, cyclohexyl H), 1.47-1.58 (2H, m, cyclohexyl H), 1.77-1.85 (2H, m, cyclohexyl H), 2.39 (6H, s, N(CH<sub>3</sub>)<sub>2</sub>), 3.14 (2H, s, NHCOCH<sub>2</sub>), 3.75 (3H, s, OCH<sub>3</sub>), 3.82-3.90 (1H, m, cyclohexyl H-1''), 4.41 (1H, d, D<sub>2</sub>O exchang., NH-cyclohexylamine, *J*=7.2 Hz), 5.61 (2H, s, CH<sub>2</sub>-pyrazole), 6.85 (2H, d, H-2', H-6', *J*=8.7 Hz), 7.01-7.06 (3H, m, H-4, H-3', H-5'), 7.64 (1H, d, H-5, *J*=5.9 Hz), 9.51 (1H, brs, D<sub>2</sub>O exchang., NHCO). <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>)

$\delta$ : 24.7 (cyclohexyl C-3'', C-5''), 25.86 (cyclohexyl C-4''), 33.0 (cyclohexyl C-2'', C-6''), 46.1 (N(CH<sub>3</sub>)<sub>2</sub>), 49.1 (cyclohexyl C-1''), 55.1 (CH<sub>2</sub>-pyrazole), 55.4 (OCH<sub>3</sub>), 63.2 (NHCOCH<sub>2</sub>), 105.5 (C-4), 114.8 (C-2', C-6'), 122.2 (C-3a), 127.5 (C-3', C-5'), 128.2 (C-7a), 128.9 (C-1'), 137.2 (C-5), 138.4 (C-3), 145.0 (C-7), 159.8 (C-4'), 168.9 (CO). HR-MS (ESI)  $m/z$ : Calcd for C<sub>24</sub>H<sub>33</sub>N<sub>6</sub>O<sub>2</sub>: [M1+H]<sup>+</sup> = 437.2660, found 437.2668. *Anal.* Calcd for C<sub>24</sub>H<sub>32</sub>N<sub>6</sub>O<sub>2</sub>: C, 66.03; H, 7.39; N, 19.25. Found: C, 66.33; H, 7.54; N, 18.98.

***N*-(7-(Cyclohexylamino)-1*H*-pyrazolo[3,4-*c*]pyridin-3-yl)-2-**

**(dimethylamino)acetamide (29)** This compound was prepared following an analogous synthetic procedure to that described for the synthesis of the compounds **14a-f**, starting from the derivative **28**. The product was purified by column chromatography (silica gel) using a mixture of dichloromethane/methanol (9/1, v/v) as the eluent to provide pure **29** as a colorless oil. Yield: 99%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$ : 1.15-1.18 (3H, m, cyclohexyl H), 1.21-1.42 (2H, m, cyclohexyl H), 1.58-1.64 (1H, m, cyclohexyl H), 1.71-1.79 (2H, m, cyclohexyl H), 1.97-2.05 (2H, m, cyclohexyl H), 2.35 (6H, s, N(CH<sub>3</sub>)<sub>2</sub>), 3.21 (2H, s, CH<sub>2</sub>), 3.93-4.05 (1H, m, cyclohexyl H-1'), 6.55 (1H, brs, D<sub>2</sub>O exchang., NH-cyclohexylamine), 6.80 (1H, d, H-4, *J*=5.9 Hz), 7.49 (1H, d, H-5, *J*=5.9 Hz), 10.11 (1H, brs, D<sub>2</sub>O exchang., NHCO), 12.77 (1H, brs, D<sub>2</sub>O exchang., NH-pyrazole). <sup>13</sup>C NMR (50 MHz, DMSO-*d*<sub>6</sub>)  $\delta$ : 24.6 (cyclohexyl C-3', C-5'), 25.5 (cyclohexyl C-4'), 32.7 (cyclohexyl C-2', C-6'), 45.1 (N(CH<sub>3</sub>)<sub>2</sub>), 48.4 (cyclohexyl C-1'), 61.9 (CH<sub>2</sub>), 104.0 (C-4), 119.0 (C-3a), 127.8 (C-7a), 136.0 (C-5), 139.8 (C-3), 144.4 (C-7), 168.2 (CO). HR-MS (ESI)  $m/z$ : Calcd for C<sub>16</sub>H<sub>25</sub>N<sub>6</sub>O: [M1+H]<sup>+</sup> = 317.2084, found 317.2087. *Anal.* Calcd for C<sub>16</sub>H<sub>24</sub>N<sub>6</sub>O: C, 60.74; H, 7.65; N, 26.56. Found: C, 60.43; H, 7.52; N, 26.75.

**1-Methyl-3-nitro-*N*-phenyl-1*H*-pyrazolo[3,4-*c*]pyridin-7-amine (30)** Aniline (0.17 mL, 1.87 mmol) was added into a solution of the chlorocompound **22a** (80 mg, 0.37 mmol) in 2-

ethoxyethanol (2 mL), under argon, and the resulting solution was heated at 130 °C for 3h. The solvent was then vacuum-evaporated and the residue was purified by column chromatography (silica gel) using dichloromethane as the eluent, to give pure **30** (60 mg, 59%) as a yellow solid. mp: 197–8 °C (EtOAc). <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ: 4.34 (3H, s, CH<sub>3</sub>), 7.14 (1H, t, H-4', J=7.3 Hz), 7.28 (2H, d, H-2', H-6', J=7.6 Hz), 7.36 (2H, t, H-3', H-5', J=7.5 Hz), 7.57 (1H, brs, H-4), 7.88 (1H, brs, H-5). <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ: 41.4 (CH<sub>3</sub>), 107.7 (C-4), 119.6 (C-3a), 120.7 (C-2', C-6'), 124.6 (C-4'), 129.9 (C-3', C-5'), 130.6 (C-7a), 134.9 (C-5), 140.9 (C-3), 142.6 (C-1'), 147.3 (C-7). *Anal.* Calcd for C<sub>13</sub>H<sub>11</sub>N<sub>5</sub>O<sub>2</sub>: C, 57.99; H, 4.12; N, 26.01. Found: C, 58.14; H, 4.22; N, 25.84.

**1-Methyl-N<sup>7</sup>-phenyl-1H-pyrazolo[3,4-c]pyridine-3,7-diamine (31)** This compound was prepared following an analogous synthetic procedure to that described for the synthesis of the compound **11a**, upon hydrogenation of the nitroderivative **30**. The product was purified by column chromatography (silica gel) using a mixture of cyclohexane/ethyl acetate (from 5/5 up to 2/8, v/v) as the eluent to provide pure **31** as an orange colored solid. Yield: 75%, mp: 120–2 °C (CH<sub>2</sub>Cl<sub>2</sub>/*n*-hexane). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ: 3.93 (3H, s, CH<sub>3</sub>), 5.52 (2H, brs, D<sub>2</sub>O exchang., NH<sub>2</sub>), 6.90 (1H, t, H-4', J=7.3 Hz), 7.20 (1H, d, H-4, J=5.6 Hz), 7.25 (2H, t, H-3', H-5', J=7.8 Hz), 7.44 (2H, d, H-2', H-6', J=7.8 Hz), 7.60 (1H, d, H-5, J=5.6 Hz), 8.37 (1H, brs, D<sub>2</sub>O exchang., NH). <sup>13</sup>C NMR (50 MHz, DMSO-*d*<sub>6</sub>) δ: 37.6 (CH<sub>3</sub>), 108.3 (C-4), 118.8 (C-2', C-6'), 119.7 (C-3a), 120.6 (C-4'), 128.4 (C-3', C-5'), 129.8 (C-7a), 134.0 (C-5), 141.6 (C-3), 142.2 (C-1'), 148.4 (C-7). *Anal.* Calcd for C<sub>13</sub>H<sub>13</sub>N<sub>5</sub>: C, 65.25; H, 5.48; N, 29.27. Found: C, 65.37; H, 5.60; N, 29.03.

**2-Chloro-N-(1-methyl-7-(phenylamino)-1H-pyrazolo[3,4-c]pyridin-3-yl)acetamide**

**(32)** This compound was prepared following an analogous synthetic procedure to that described

for the synthesis of the compounds **12b** and **12d**, starting from the amine **31**. This intermediate was used immediately to the next step, with no further purification.

**2-(Dimethylamino)-N-(1-methyl-7-(phenylamino)-1H-pyrazolo[3,4-c]pyridin-3-yl)acetamide (33a)** This compound was prepared following an analogous synthetic procedure to that described for the synthesis of the compounds **13a-1**, upon reaction of the chloride **32** with dimethylamine (5.6 M solution in ethanol). The product was purified by column chromatography (silica gel) using a mixture of dichloromethane/methanol (from 100/0.5 up to 100/5, v/v) as the eluent to provide pure **33a** as a beige solid, in 97% yield. mp: 140–2 °C (EtOH/Et<sub>2</sub>O). <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ: 2.44 (6H, s, N(CH<sub>3</sub>)<sub>2</sub>), 3.19 (2H, s, CH<sub>2</sub>), 4.09 (3H, s, CH<sub>3</sub>–pyrazole), 6.77 (1H, brs, D<sub>2</sub>O exchang., NH–aniline), 7.02 (1H, t, H-4', *J*=7.3 Hz), 7.21 (2H, d, H-2', H-6', *J*=7.6 Hz), 7.29 (2H, t, H-3', H-5', *J*=7.7 Hz), 7.45 (1H, brs, H-4), 7.79 (1H, brs, H-5), 9.62 (1H, brs, D<sub>2</sub>O exchang., NHCO). <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ: 38.9 (CH<sub>3</sub>–pyrazole), 46.1 (N(CH<sub>3</sub>)<sub>2</sub>), 63.1 (CH<sub>2</sub>), 111.0 (C-4), 119.2 (C-2', C-6'), 122.7 (C-3a, C-4'), 129.5 (C-3', C-5'), 130.6 (C-7a), 136.6 (C-5), 138.7 (C-3), 141.9 (C-1'), 142.3 (C-7), 168.9 (CO). HR-MS (ESI) *m/z*: Calcd for C<sub>17</sub>H<sub>21</sub>N<sub>6</sub>O: [M+H]<sup>+</sup> = 325.1771, found 325.1773. *Anal.* Calcd for C<sub>17</sub>H<sub>20</sub>N<sub>6</sub>O: C, 62.95; H, 6.21; N, 25.91. Found: C, 63.11; H, 6.28; N, 25.72.

**N-(1-Methyl-7-(phenylamino)-1H-pyrazolo[3,4-c]pyridin-3-yl)-2-(4-methylpiperazin-1-yl)acetamide (33b)** This compound was prepared following an analogous synthetic procedure to that described for the synthesis of the compounds **13a-1**, upon reaction of the chloride **32** with 1-methylpiperazine. The product was purified by column chromatography (silica gel) using a mixture of dichloromethane/methanol (from 100/2 up to 100/10, v/v) as the eluent to provide pure **33b** as a white solid, in 99% yield. mp: 202–4 °C (EtOH/Et<sub>2</sub>O). <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ: 2.34 (3H, s, CH<sub>3</sub>–piperazine), 2.50-2.60 (4H, brs, piperazine H-3'', H-5''), 2.65-2.80

(4H, brs, piperazine H-2'', H-6''), 3.23 (2H, s, CH<sub>2</sub>), 4.10 (3H, s, CH<sub>3</sub>-pyrazole), 6.58 (1H, brs, D<sub>2</sub>O exchang., NH-aniline), 7.03 (1H, t, H-4', *J*=7.3 Hz), 7.22 (2H, d, H-2', H-6', *J*=7.8 Hz), 7.30 (2H, t, H-3', H-5', *J*=7.9 Hz), 7.46 (1H, d, H-4, *J*=5.8 Hz), 7.85 (1H, d, H-5, *J*=5.8 Hz), 9.49 (1H, brs, D<sub>2</sub>O exchang., NHCO). <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ: 38.9 (CH<sub>3</sub>-pyrazole), 46.1 (CH<sub>3</sub>-piperazine), 53.7 (piperazine C-2'', C-6''), 55.3 (piperazine C-3'', C-5''), 61.7 (CH<sub>2</sub>), 111.1 (C-4), 119.1 (C-2', C-6'), 122.7 (C-4'), 122.8 (C-3a), 129.5 (C-3', C-5'), 130.6 (C-7a), 136.9 (C-5), 138.7 (C-3), 141.8 (C-7), 142.1 (C-1'), 168.7 (CO). HR-MS (ESI) *m/z*: Calcd for C<sub>20</sub>H<sub>26</sub>N<sub>7</sub>O: [M+H]<sup>+</sup> =380.2193, found 380.2198. *Anal.* Calcd for C<sub>20</sub>H<sub>25</sub>N<sub>7</sub>O: C, 63.30; H, 6.64; N, 25.84. Found: C, 63.55; H, 6.84; N, 25.59.

***N*-(1-Methyl-7-(phenylamino)-1*H*-pyrazolo[3,4-*c*]pyridin-3-yl)-2-**

**(phenylamino)acetamide (33c)** This compound was prepared following an analogous synthetic procedure to that described for the synthesis of the compounds **13a-1**, upon reaction of the chloride **32** with aniline. The product was purified by column chromatography (silica gel) using a mixture of dichloromethane/ethyl acetate (1/1, v/v) as the eluent to provide pure **33c** as a beige solid, in 55% yield. mp: 180–1 °C (EtOH/Et<sub>2</sub>O). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ: 3.94 (2H, s, CH<sub>2</sub>), 4.22 (3H, s, CH<sub>3</sub>), 6.05 (1H, m, D<sub>2</sub>O exchang., NHCH<sub>2</sub>), 6.56-6.65 (3H, m, H-2'', H-4'', H-6''), 6.94 (1H, t, H-4', *J*=7.4 Hz), 7.09-7.15 (3H, m, H-4, H-3', H-5'), 7.28 (2H, t, H-3'', H-5'', *J*=7.9 Hz), 7.48 (2H, d, H-2', H-6', *J*=8.1 Hz), 7.65 (1H, d, H-5, *J*=5.7 Hz), 8.48 (1H, brs, D<sub>2</sub>O exchang., NH-aniline), 10.45 (1H, brs, D<sub>2</sub>O exchang., NHCO). <sup>13</sup>C NMR (50 MHz, DMSO-*d*<sub>6</sub>) δ: 38.8 (CH<sub>3</sub>), 46.6 (CH<sub>2</sub>), 109.4 (C-4), 112.3 (C-2'', C-6''), 116.5 (C-4''), 119.4 (C-2', C-6'), 121.2 (C-4'), 122.5 (C-3a), 128.5 (C-3'', C-5''), 128.9 (C-3', C-5'), 129.4 (C-7a), 135.4 (C-5), 138.4 (C-3), 141.7 (C-1''), 142.3 (C-7), 148.3 (C-1'), 169.7 (CO). HR-MS (ESI)

*m/z*: Calcd for C<sub>21</sub>H<sub>21</sub>N<sub>6</sub>O: [M1+H]<sup>+</sup> =373.1771, found 373.1774. *Anal.* Calcd for C<sub>21</sub>H<sub>20</sub>N<sub>6</sub>O: C, 67.73; H, 5.41; N, 22.57. Found: C, 67.81; H, 5.44; N, 22.47.

**3-Nitro-*N*-phenyl-1*H*-pyrazolo[3,4-*c*]pyridin-7-amine (34)** This compound was prepared following an analogous synthetic procedure to that described for the synthesis of **30**, upon treatment of **21** with aniline in 1,4-dioxane, at 100 °C for 12h. The product was purified by column chromatography (silica gel) using a mixture of dichloromethane/methanol (98/2, v/v) as the eluent, to provide pure **34**, in 95% yield as a yellow solid. mp: 260–262 °C (EtOAc). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ: 7.11 (1H, t, H-4', *J*=7.1 Hz), 7.38-7.44 (3H, m, H-4, H-3', H-5'), 7.82 (2H, d, H-2', H-6', *J*=7.8 Hz), 7.95 (1H, brs, H-5), 9.30 (1H, brs, D<sub>2</sub>O exchang., NH–aniline). <sup>13</sup>C NMR (50 MHz, DMSO-*d*<sub>6</sub>) δ: 104.6 (C-4), 120.0 (C-2', C-6'), 120.5 (C-3a), 123.2 (C-4'), 129.1 (C-3', C-5'), 130.1 (C-7a), 139.2 (C-3, C-1'), 142.8 (C-5), 148.8 (C-7). *Anal.* Calcd for C<sub>12</sub>H<sub>9</sub>N<sub>5</sub>O<sub>2</sub>: C, 56.47; H, 3.55; N, 27.44. Found: C, 56.66; H, 3.61; N, 27.31.

**2-(4-Methoxybenzyl)-3-nitro-*N*-phenyl-2*H*-pyrazolo[3,4-*c*]pyridin-7-amine (35)** This compound was prepared following an analogous synthetic procedure to that described for the synthesis of the compound **22b**. The product was purified by column chromatography (silica gel) using a mixture of cyclohexane/ethyl acetate/dichloromethane (from 90/10/3 up to 85/15/4, v/v) as the eluent, to provide pure **34** as an orange colored solid, in 45% yield. mp: 175–7 °C (MeOH). <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ: 3.78 (3H, s, OCH<sub>3</sub>), 6.04 (2H, s, CH<sub>2</sub>), 6.87 (2H, d, H-2'', H-6'', *J*=8.6 Hz), 7.12 (1H, t, H-4', *J*=7.3 Hz), 7.29 (1H, d, H-4, *J*=6.0 Hz), 7.35 (2H, d, H-3'', H-5'', *J*=8.6 Hz), 7.41 (2H, t, H-3', H-5', *J*=7.8 Hz), 7.70 (1H, brs, D<sub>2</sub>O exchang., NH), 7.89 (2H, d, H-2', H-6', *J*=7.8 Hz), 8.05 (1H, d, H-5, *J*=6.0 Hz). <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ: 55.5 (OCH<sub>3</sub>), 58.0 (CH<sub>2</sub>), 104.5 (C-4), 114.5 (C-2'', C-6''), 119.9 (C-2', C-6'), 122.2 (C-3a), 123.4 (C-4'), 126.4 (C-7a), 129.2 (C-3', C-5'), 129.9 (C-3'', C-5''), 134.7 (C-1''), 137.3 (C-3), 139.1

(C-1'), 144.7 (C-5), 147.8 (C-7), 160.2 (C-4''). *Anal.* Calcd for C<sub>20</sub>H<sub>17</sub>N<sub>5</sub>O<sub>3</sub>: C, 63.99; H, 4.56; N, 18.66. Found: C, 64.34; H, 4.87; N, 18.33.

**2-(4-Methoxybenzyl)-N<sup>7</sup>-phenyl-2H-pyrazolo[3,4-c]pyridine-3,7-diamine (36)** This compound was prepared following an analogous synthetic procedure to that described for the synthesis of the compound **11a**, upon hydrogenation of the nitroderivative **34**. The product was purified by column chromatography (silica gel) using a mixture of cyclohexane/ethyl acetate (from 7/3 up to 5/5, v/v) as the eluent to provide pure **35** as a brown solid, in 96% yield. mp: 54–6 °C (CH<sub>2</sub>Cl<sub>2</sub>/*n*-hexane). <sup>1</sup>H NMR (600 MHz, DMSO-*d*<sub>6</sub>) δ: 3.71 (3H, s, OCH<sub>3</sub>), 5.40 (2H, s, CH<sub>2</sub>), 6.23 (2H, brs, D<sub>2</sub>O exchang., NH<sub>2</sub>), 6.87-6.91 (3H, m, H-4', H-2'', H-6''), 6.95 (1H, d, H-4, *J*=5.9 Hz), 7.17 (2H, d, H-3'', H-5'', *J*=8.5 Hz), 7.25 (2H, t, H-3', H-5', *J*=7.8 Hz), 7.31 (1H, d, H-5, *J*=5.9 Hz), 8.04 (2H, d, H-2', H-6', *J*=7.7 Hz), 8.57 (1H, brs, D<sub>2</sub>O exchang., NH). <sup>13</sup>C NMR (151 MHz, DMSO-*d*<sub>6</sub>) δ: 50.4 (CH<sub>2</sub>), 55.1 (OCH<sub>3</sub>), 106.0 (C-4), 109.3 (C-3a), 113.8 (C-2'', C-6''), 118.9 (C-2', C-6'), 120.7 (C-4'), 128.2 (C-3', C-5', C-7a), 128.6 (C-3'', C-5''), 128.9 (C-1''), 131.0 (C-5), 135.8 (C-3), 141.3 (C-1'), 145.8 (C-7), 158.6 (C-4''). *Anal.* Calcd for C<sub>20</sub>H<sub>19</sub>N<sub>5</sub>O: C, 69.55; H, 5.54; N, 20.28. Found: C, 69.23; H, 5.31; N, 20.49.

**2-Chloro-N-(2-(4-methoxybenzyl)-7-(phenylamino)-2H-pyrazolo[3,4-c]pyridin-3-yl)acetamide (37)** This compound was prepared following an analogous synthetic procedure to that described for the synthesis of the compounds **12b** and **12d**, starting from the amine **36**. This intermediate was used immediately to the next step, with no further purification.

**2-(Dimethylamino)-N-(2-(4-methoxybenzyl)-7-(phenylamino)-2H-pyrazolo[3,4-c]pyridin-3-yl)acetamide (38a)** and **N-(2-(4-methoxybenzyl)-7-(phenylamino)-2H-pyrazolo[3,4-c]pyridin-3-yl)-2-(4-methylpiperazin-1-yl)acetamide (38b)** These compounds



were prepared following an analogous synthetic procedure to that described for the synthesis of the compounds **13a-l**, upon reaction of the chloride **37** with dimethylamine (5.6 M solution in ethanol) or 1-methylpiperazine, respectively. The product was purified by column chromatography using a mixture of dichloromethane/methanol (95/5, v/v) as the eluent to provide pure **38a** or **38b**.

Data for **38a**: Yield 98%. Beige solid, mp: 183–5 °C (CH<sub>2</sub>Cl<sub>2</sub>/Et<sub>2</sub>O). <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ: 2.32 (6H, s, N(CH<sub>3</sub>)<sub>2</sub>), 3.11 (2H, s, NHCOCH<sub>2</sub>), 3.76 (3H, s, OCH<sub>3</sub>), 5.45 (2H, s, CH<sub>2</sub>-pyrazole), 6.83-6.87 (3H, m, H-4, H-2'', H-6''), 7.02 (1H, t, H-4', *J*=7.3 Hz), 7.12 (2H, d, H-3'', H-5'', *J*=8.5 Hz), 7.35 (2H, t, H-3', H-5', *J*=7.8 Hz), 7.68 (1H, d, H-5, *J*=6.1 Hz), 7.87 (2H, d, H-2', H-6', *J*=7.9 Hz), 8.70-9.40 (1H, brs, D<sub>2</sub>O exchang., NHCO). <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ: 46.1 (N(CH<sub>3</sub>)<sub>2</sub>), 54.3 (CH<sub>2</sub>-pyrazole), 55.4 (OCH<sub>3</sub>), 62.8 (NHCOCH<sub>2</sub>), 104.7 (C-4), 114.4 (C-2'', C-6''), 118.1 (C-3a), 119.4 (C-2', C-6'), 122.2 (C-4'), 127.1 (C-7a), 128.0 (C-1''), 128.7 (C-3'', C-5''), 129.0 (C-3', C-5'), 136.1 (C-3), 136.6 (C-5), 140.1 (C-1'), 147.3 (C-7), 159.7 (C-4''), 169.5 (CO). HR-MS (ESI) *m/z*: Calcd for C<sub>24</sub>H<sub>27</sub>N<sub>6</sub>O<sub>2</sub>: [M+H]<sup>+</sup> =431.2190, found 431.2198. *Anal.* Calcd for C<sub>24</sub>H<sub>26</sub>N<sub>6</sub>O<sub>2</sub>: C, 66.96; H, 6.09; N, 19.52. Found: C, 67.17; H, 6.18; N, 19.44.

Data for **38b**: Yield 97%. White solid, mp: 159–160 °C (EtOAc/Et<sub>2</sub>O). <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ: 2.27 (3H, s, CH<sub>3</sub>), 2.30-2.40 (4H, brs, piperazine H-3''', H-5'''), 2.55-2.59 (4H, brs, piperazine H-2''', H-6'''), 3.17 (2H, s, NHCOCH<sub>2</sub>), 3.77 (3H, s, OCH<sub>3</sub>), 5.49 (2H, s, CH<sub>2</sub>-pyrazole), 6.79 (1H, d, H-4, *J*=6.1 Hz), 6.86 (2H, d, H-2'', H-6'', *J*=8.7 Hz), 7.02 (1H, t, H-4', *J*=7.4 Hz), 7.07 (2H, d, H-3'', H-5'', *J*=8.7 Hz), 7.35 (2H, t, H-3', H-5', *J*=8.0 Hz), 7.58 (1H, brs, D<sub>2</sub>O exchang., NH-aniline), 7.70 (1H, d, H-5, *J*=6.1 Hz), 7.88 (2H, d, H-2', H-6', *J*=7.6 Hz), 9.00 (1H, brs, D<sub>2</sub>O exchang., NHCO). <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ: 46.0 (CH<sub>3</sub>), 53.6

(piperazine C-2''', C-6'''), 54.3 (CH<sub>2</sub>-pyrazole), 55.0 (piperazine C-3''', C-5'''), 55.4 (OCH<sub>3</sub>), 61.3 (NHCOCH<sub>2</sub>), 104.4 (C-4), 114.6 (C-2'', C-6''), 118.2 (C-3a), 119.3 (C-2', C-6'), 122.3 (C-4'), 127.3 (C-7a), 127.9 (C-1''), 128.3 (C-3'', C-5''), 129.0 (C-3', C-5'), 136.2 (C-3), 137.0 (C-5), 140.1 (C-1'), 147.3 (C-7), 159.7 (C-4''), 169.0 (CO). HR-MS (ESI) *m/z*: Calcd for C<sub>27</sub>H<sub>32</sub>N<sub>7</sub>O<sub>2</sub>: [M+H]<sup>+</sup> =486.2612, found 486.2616. *Anal.* Calcd for C<sub>27</sub>H<sub>31</sub>N<sub>7</sub>O<sub>2</sub>: C, 66.78; H, 6.43; N, 20.19. Found: C, 67.05; H, 6.54; N, 19.98.

**2-(Dimethylamino)-N-(7-(phenylamino)-1H-pyrazolo[3,4-*c*]pyridin-3-yl)acetamide (39a)** and **2-(4-methylpiperazin-1-yl)-N-(7-(phenylamino)-1H-pyrazolo[3,4-*c*]pyridin-3-yl)acetamide (39b)** These compounds were prepared following an analogous synthetic procedure to that described for the synthesis of the compounds **14a-f**, upon reaction of the derivatives **38a** and **38b** with trifluoroacetic acid, respectively. The product was purified by column chromatography using a mixture of dichloromethane/methanol (9/1, v/v) as the eluent to provide pure **39a** or **39b**.

Data for **39a**: Yield 92%. Beige gum. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ: 2.32 (6H, s, N(CH<sub>3</sub>)<sub>2</sub>), 3.16 (2H, s, CH<sub>2</sub>), 6.97 (1H, t, H-4', *J*=7.3 Hz), 7.12 (1H, d, H-4, *J*=5.8 Hz), 7.34 (2H, t, H-3', H-5', *J*=8.0 Hz), 7.69 (1H, d, H-5, *J*=5.8 Hz), 7.89 (2H, d, H-2', H-6', *J*=8.0 Hz), 8.95 (1H, brs, D<sub>2</sub>O exchang., NH-aniline), 10.17 (1H, brs, D<sub>2</sub>O exchang., NHCO), 12.90 (1H, brs, D<sub>2</sub>O exchang., NH-pyrazole). <sup>13</sup>C NMR (50 MHz, DMSO-*d*<sub>6</sub>) δ: 45.3 (N(CH<sub>3</sub>)<sub>2</sub>), 62.2 (CH<sub>2</sub>), 107.4 (C-4), 116.3 (C-3a), 118.4 (C-2', C-6'), 119.9 (C-7a), 121.3 (C-4'), 128.7 (C-3', C-5'), 135.3 (C-5), 140.8 (C-3), 141.7 (C-7), 157.9 (C-1'), 168.7 (CO). HR-MS (ESI) *m/z*: Calcd for C<sub>16</sub>H<sub>19</sub>N<sub>6</sub>O: [M+H]<sup>+</sup> =311.1615, found 311.1617. *Anal.* Calcd for C<sub>16</sub>H<sub>18</sub>N<sub>6</sub>O: C, 61.92; H, 5.85; N, 27.08. Found: C, 61.68; H, 5.71; N, 27.37.

Data for **39b**: Yield 95%. Beige foam.  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO-}d_6$ )  $\delta$ : 2.31 (3H, s,  $\text{CH}_3$ ), 2.52-2.70 (8H, brs, piperazine H-2'', H-3'', H-5'', H-6''), 3.25 (2H, s,  $\text{CH}_2$ ), 6.97 (1H, t, H-4',  $J=7.3$  Hz), 7.12 (1H, d, H-4,  $J=5.8$  Hz), 7.33 (2H, t, H-3', H-5',  $J=7.8$  Hz), 7.69 (1H, d, H-5,  $J=5.8$  Hz), 7.93 (2H, d, H-2', H-6',  $J=7.8$  Hz), 9.13 (1H, brs,  $\text{D}_2\text{O}$  exchang., NH-aniline), 10.20 (1H, brs,  $\text{D}_2\text{O}$  exchang., NHCO), 13.11 (1H, brs,  $\text{D}_2\text{O}$  exchang., NH-pyrazole).  $^{13}\text{C}$  NMR (50 MHz,  $\text{DMSO-}d_6$ )  $\delta$ : 45.0 ( $\text{CH}_3$ ), 51.9 (piperazine C-2'', C-6''), 54.2 (piperazine C-3'', C-5''), 60.5 ( $\text{CH}_2$ ), 107.4 (C-4), 116.3 (C-3a), 118.4 (C-2', C-6'), 119.7 (C-7a), 121.3 (C-4'), 128.7 (C-3', C-5'), 135.3 (C-5), 140.9 (C-3), 141.9 (C-7), 158.1 (C-1'), 168.2 (CO). HR-MS (ESI)  $m/z$ : Calcd for  $\text{C}_{19}\text{H}_{24}\text{N}_7\text{O}$ :  $[\text{M}+\text{H}]^+ = 366.2037$ , found 366.2044. *Anal.* Calcd for  $\text{C}_{19}\text{H}_{23}\text{N}_7\text{O}$ : C, 62.45; H, 6.34; N, 26.83. Found: C, 62.71; H, 6.48; N, 26.59.

**Theoretical calculations** The crystal structure of human GSK3 $\beta$  (pdb id: 1UV5) was utilized for docking calculations using the modules of Schrodinger Small-Molecule Drug Discovery Suite 2016.<sup>21, 26</sup> Protein preparation was performed by the corresponding routine as implemented in Maestro. Prior to calculations, the studied compounds were prepared in terms of correct protonation states, tautomerism and stereoisomerism using the LigPrep routine of Maestro. Rigid docking was performed using the Glide-SP algorithm. The Van der Waals atom radii scaling was set to 0.8 for both the protein and docked ligands. The best poses were redocked by utilizing a flexible representation of the active site residues in a 5Å sphere around the ligand using MacroModel. The OPLS2005 force-field, an implicit GB/SA water solvent model and the TNCG minimization algorithm were used.

**Kinase activity evaluation** Kinase activities were assayed for each protein kinase in the appropriate buffers (listed below), with either a protein or a peptide as substrate in the presence of 15  $\mu\text{M}$  [ $\gamma$ - $^{33}\text{P}$ ] ATP (3,000 Ci/mmol; 10 mCi/mL) in a final volume of 30  $\mu\text{L}$  following an

assay previously described.<sup>27)</sup> Controls were performed with appropriate dilutions of dimethylsulfoxide. Full-length kinases are used unless specified. Peptide substrates were obtained from Proteogenix (Oberhausbergen, France).

Buffers:

(A) 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 1 mM DTT, 25 mM Tris-HCl pH 7.5, 50 μg/mL heparin, 0.15 mg/mL of BSA + 0.23 mg/mL of DTT

(B) 60 mM β-glycerophosphate, 30 mM p-nitrophenylphosphate, 25 mM MOPS (pH 7), 5 mM EGTA, 15 mM MgCl<sub>2</sub>, 1 mM DTT, 0.1 mM sodium orthovanadate

(D) 25 mM MOPS, pH7.2, 12.5 mM β-glycerolphosphate, 25 mM MgCl<sub>2</sub>, 5 mM EGTA, 2 mM EDTA, 0.25 mM DTT

(H) MOPS 25 mM pH 7.5, 10 mM MgCl<sub>2</sub>

(K) Tris 50 mM pH 7.5, 20 mM MgCl<sub>2</sub>, 2 mM MnCl<sub>2</sub>

(R) 5 mM MOPS pH 7.2, 2.5 mM β-glycerophosphate, 4 mM MgCl<sub>2</sub>, 2.5 mM MnCl<sub>2</sub>, 1 mM EGTA, 0.4 mM EDTA, 50 μg/mL BSA, 0.05 mM DTT

Each protein kinase was tested as follow:

HsRIPK3 (human, recombinant, expressed in Sf9 insect cells infected with 6xHIS:HsRIPK3 specific baculoviruses) was assayed in buffer R with 0.1 μg/μL of MBP (Myelin Basic Protein) as substrate.

HsHaspin-kd (human, kinase domain, amino acids 470 to 798, recombinant, expressed in bacteria) was assayed in buffer H with 0.007 μg/μL of Histone H3 (1-21) peptide (ARTKQTARKSTGGKAPRKQLA) as substrate.

HsCDK5/p25 (human, recombinant, expressed in bacteria) was assayed in buffer B, with 0.8 μg/μL of histone H1 as substrate.

HsAuroraB (human, recombinant, expressed by baculovirus in Sf9 insect cells, SignalChem, product #A31-10G) was assayed in buffer D with 0.2  $\mu\text{g}/\mu\text{L}$  of MBP as substrate.

SscGSK3 $\alpha/\beta$  (glycogen synthase kinase-3, porcine brain, native, affinity purified) was assayed in buffer A with 0.010  $\mu\text{g}/\mu\text{L}$  of GS-1 peptide, a GSK-3-selective substrate (YRRAAVPPSPSLSRHSSPHQSpEDEEE, “Sp” stands for phosphorylated serine).

SscCK1 $\delta/\epsilon$  (casein kinase 1 $\delta/\epsilon$ , porcine brain, native, affinity purified) was assayed in buffer B, with 0.022  $\mu\text{g}/\mu\text{L}$  of the following peptide: RRKHAAIGSpAYSITA as CK1-specific substrate.

RnDYRK1A-kd (*Rattus norvegicus*, amino acids 1 to 499 including the kinase domain, recombinant, expressed in bacteria, DNA vector kindly provided by Dr. W. Becker, Aachen, Germany) was assayed in buffer A with 0.033  $\mu\text{g}/\mu\text{L}$  of the following peptide: KKISGRLSPIMTEQ as substrate.

MmCLK1 (from *Mus musculus*, recombinant, expressed in bacteria) was assayed in buffer A with 0.027  $\mu\text{g}/\mu\text{L}$  of the following peptide: GRSRSRSRSRSR.

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**Conflict of Interest** The authors declare no conflict of interest.

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