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New heterocyclic analogues of 4-(2-chloro-5-methoxyanilino)quinazolines as potent and selective c-Src kinase inhibitors

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Abstract—A series of 5,7-disubstituted quinazolines, bearing 4-heteroaryl substituents such as 2-pyridinylamine or 2-pyrazinylamine, has been synthetised and evaluated as c-Src kinase inhibitors. Highly potent inhibition, high selectivity and physical properties suitable for oral dosing were achieved within this series: **23d** and **42** were identified as sub-0.1 μM inhibitors in a c-Src-driven cell proliferation assay and displayed adequate rat pharmacokinetics after oral administration.

The non-receptor tyrosine kinase c-Src is the best-understood member of a family of closely related kinases, that includes among its members c-Yes, Fyn and Lck. c-Src is expressed at low levels in most cell types and, in the absence of appropriate extracellular stimuli, is maintained in an inactive conformation. c-Src gene knock-out experiments in mice have shown that the only phenotypic consequence is osteopetrosis, a defect in osteoclast function.[1]

In contrast to its highly regulated role in normal cells, there is significant evidence demonstrating deregulated, increased kinase activity of c-Src in several human tumour types, most notably colon and breast tumours.[2] Recent data suggest deregulated c-Src tyrosine kinase activity is associated predominantly with adhesion and cytoskeletal changes in tumour cells, ultimately resulting in a change to a motile, invasive phenotype.[3] Increased c-Src tyrosine kinase activity results in break down of E-cadherin-mediated epithelial cell/cell adhesion,[4,5] which can be restored by Src inhibition.[5] c-Src activity is also known to be essential in the turnover of focal adhesions,[6] a critical cell motility component.

Evidence from the clinic also supports a link between deregulated c-Src activity and increased invasive potential of tumour cells. In colon tumours, increased c-Src kinase activity has been shown to correlate with tumour progression, with the highest activity found in metastatic tissue.[2] Increased Src activity in colon tumours has also been shown to be an indicator of poor prognosis.[7]

We have reported data on 6,7-substituted[8] and 5,7-substituted[9] 4-anilinoquinazolines bearing a 2-chloro-5-methoxy aniline or a 4-amino-5-chloro-1,3-benzodioxole (e.g., structures **1**–**5** pictured below in Fig. 1) as potent and selective c-Src inhibitors. In this communication, we describe the synthesis of heterocyclic

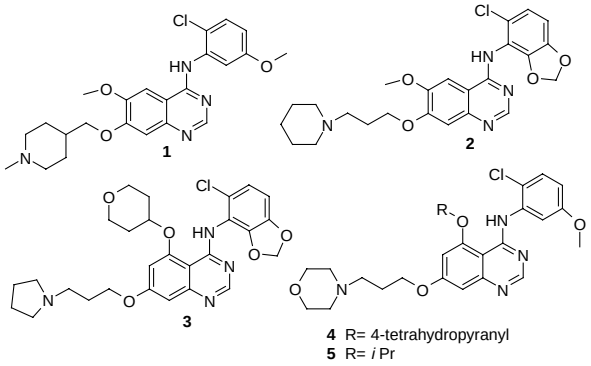

Figure 1. Stuctures of known Src inhibitors.

Keywords: c-Src; Kinase; Inhibitor; 5,7-Disubstituted quinazoline.
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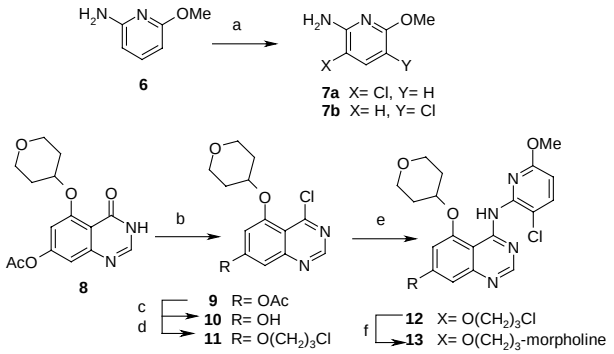
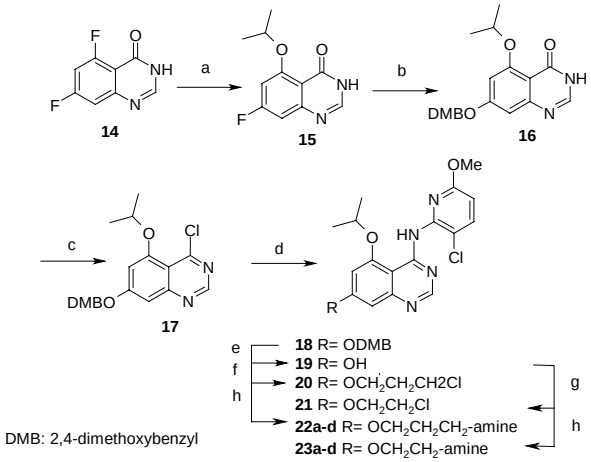


Scheme 1. Reagents and conditions: (a) NCS (1 equiv), $CHCl_3$, 0 °C (**7a**: 73%, **7b**: 11%); (b) PPh_3/CCl_4, $ClCH_2CH_2Cl$, 70 °C; (c) NH_3/MeOH, 0 °C (69% from **8**); (d) DTAD, PPh_3, $Cl(CH_2)_3OH$, CH_2Cl_2, 0 °C to rt, 31%; (e) NaHMDS (2 equiv), **7a** (1 equiv), THF, 0 °C to rt, 74%; (f) morpholine, KI, DMA, 90 °C, 70%.

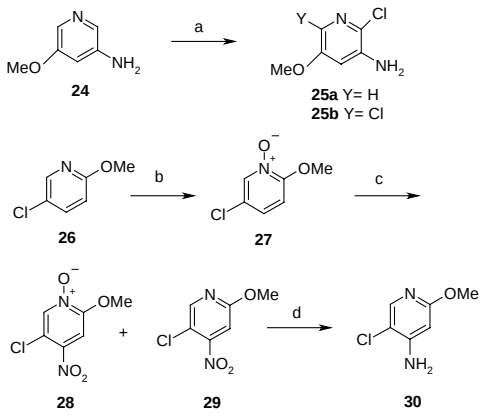
analogues of the 4-(2-chloro-5-methoxyanilino)quinazolines **4** and **5** such as 4-(pyridinylamino)quinazolines and discuss their biological activities.

The synthesis of the 2-aminopyridine analogue of **4** starts from **6**[10] (Scheme 1). Chlorination of **6** with NCS gave two separable regioisomers **7a** and **7b**. The previously described intermediate **8**[9] was chlorinated with triphenylphosphine/carbon tetrachloride to give the chloroquinazoline **9**, which afforded **10** after deprotection of the 7-acetoxy group with ammonia in methanol. **10** was reacted with 3-chloropropanol under Mitsunobu conditions to give **11**, which was coupled[11] with the anion of **7a** to give **12**. The chloro derivative **12** afforded **13** after heating with excess of morpholine in the presence of KI.

Although the 5-isopropoxy analogue **22a** could have been prepared by a route similar to **13**, an alternative procedure (Scheme 2) was devised based upon the sequential displacement of the C-5 and C-7 fluorine



Scheme 2. Reagents and conditions: (a) *i*PrOH, NaH, DMF, 5 °C to rt, 71%; (b) 2,4-(MeO)BnOH (3 equiv), *t*BuOK (6 equiv), THF, reflux, 68%; (c) $POCl_3$, $NEt*i*Pr_2$, $ClCH_2CH_2Cl$, 75 °C, 62%; (d) NaHMDS (2 equiv), **7a** (1 equiv), THF, 0 °C to rt, 76%; (e) 20% TFA, CH_2Cl_2, quant; (f) $Cl(CH_2)_3Br$, Cs_2CO_3, DMF, 60 °C, 55%; (g) $ClCH_2CH_2Cl$, K_2CO_3, DMF, 60 °C, 55%; (h) amine, KI, DMA, 90 °C, 38–79%.



Scheme 3. Reagents and conditions: (a) 12 N HCl, H_2O_2 (1.5 equiv) added dropwise, 70 °C (**25a**: 27%, **25b**: 9%); (b) H_2O_2, Ac_2O, $(CF_3CO)_2O$, 0 °C; then **26**, 0–80 °C, 53%; (c) H_2SO_4, HNO_3; 0–80 °C (**28**: 11%, **29**: 9%); (d) Raney Ni, EtOAc/MeOH, 77%.

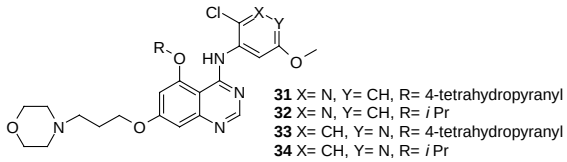


Figure 2. Other regioisomeric purid as Src inhibitors.

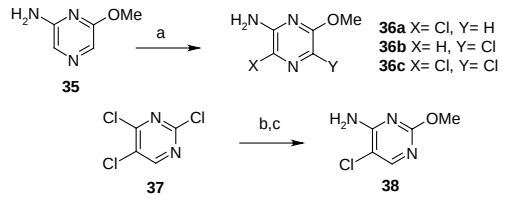
atoms of the previously described quinazolone **14**[9] with alkoxides. Treatment with sodium isopropoxide followed by reaction with potassium 2,4-dimethoxybenzyl oxide gave the quinazolone **16**, which was chlorinated with $POCl_3$. Reaction of **17** with the anion of **7a** followed by functional group manipulation at C-7 gave compounds **22a**–**d** and **23a**–**d**.

The other regioisomeric pyridines were prepared according to Scheme 3. Chlorination of **24**[12] gave the expected chloropyridine **25a** and the dichloro adduct **25b**. N-Oxidation of pyridine **26** with peroxytrifluoroacetic acid gave **27**, which was nitrated to give the 4-nitropyridine-*N*-oxide **28** and some 4-nitropyridine **29**. This mixture was reduced to the expected 4-aminopyridine **30** with Raney nickel.

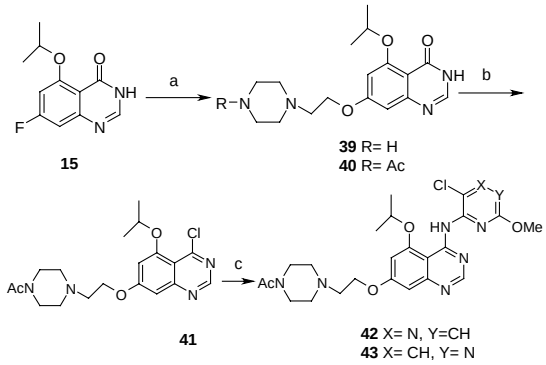
The two anilines **25a** and **30** were transformed into **31**–**34** (Fig. 2) via similar procedures[13] as described in Schemes 1 and 2.

We also prepared the corresponding pyrazine and pyrimidine as other heterocyclic replacements of the 2-chloro-5-methoxyaniline. Chlorination of **35**[14] gave the expected pyrazine **36a**, along with the regioisomer **36b** and the dichloro adduct **36c**. Reaction of **37**[15] with ammonia in methanol followed by sodium methoxide gave the expected pyrimidine **38** (Scheme 4).

The corresponding quinazolines **42** and **43** were assembled as described below (Scheme 5): reaction of **15** with potassium piperazine ethoxide gave **39**, which was acetylated to give **40**. Chlorination of **40** followed by coupling with the anion of the corresponding aminoheterocycle gave the expected quinazolines **42** and **43**.



Scheme 4. Reagents and conditions: (a) NCS (1 equiv); $CHCl_3$, rt, (**36a**: 45%, **b**: 16%, **c**: 10%); (b) NH_3, MeOH, rt, 76%; (c) MeONa, MeOH, 70 °C, 97%.



Scheme 5. Reagents and conditions: (a) piperazine-*N*-CH_2CH_2OH (1.5 equiv), *t*BuOK (6 equiv), THF, reflux; (b) Ac_2O, 44% from **15**; (c) PPh_3, CCl_4, $ClCH_2CH_2Cl$, 75 °C, 62%; (d) NaHMDS (2 equiv), **36a** or **38** (1 equiv), THF, 0 °C to rt, 20–63%.

As shown in Tables 1–3, the regioisomeric pyridines display different ranges of potency versus Src and selectivity versus KDR. The 3-pyridine (**31** and **32**) shows good potency at the Src level with significant activity against KDR, while the 4-pyridine (**33** and **34**) shows poorer potency for Src and yet significant activity for KDR. On the other hand, the 2-pyridine (**13** and **22a**) is the most Src potent heterocycle both at the enzymatic and the cellular levels without significant activity for KDR.

The isopropoxy group in C-5 of the quinazoline shows better or at least equal potency compared to the 4-tetrahydropyranyloxy group: better potency for the 4-pyridine (see **34** vs **33**), similar potency for the 2- or the 3-pyridine (see **22a** vs **13** and **32** vs **31**).

Table 1. Biological activity of regioisomeric pyridines

Compound	IC_{50} (μM)			
	Enzyme inhibition		Cell inhibition	
	Src[a]	KDR [b]	Src 3T3[c]	A549[d]
4	0.01	0.4	0.2	0.08
5	0.04	0.03	0.15	0.4
13	0.005	5.5	0.07	0.16
22a	<0.004	1.9	0.03	0.09
31	0.007	0.03	0.14	0.29
32	0.005	0.03	0.15	n.d.
33	0.4	0.08	n.d.	n.d.
34	0.035	<0.002	0.35	n.d.

These biological tests are described in Ref. 8. n.d. stands for 'not determined'.

[a] Inhibition of c-Src enzyme activity.

[b] Inhibition of KDR enzyme activity.

[c] Inhibition of constitutively active Src-transfected 3T3 cell proliferation.

[d] Inhibition of A549 cell migration.

Table 2. Structure–activity relationship for the C–7 side chains

Compound	Amine	IC_{50} (μM)			
		Enzyme inhibition		Cell inhibition	
		Src[a]	KDR[b]	Src 3T3[c]	A549[d]
22a	Morpholine	<0.004	1.9	0.03	0.09
23a	Morpholine	0.020	2.9	0.12	0.13
22b	Pyrrolidine	0.006	0.9	0.07	0.14
23b	Pyrrolidine	0.015	3.6	0.12	0.08
22c	Piperazine-*N*-Me	<0.004	0.68	0.06	0.09
23c	Piperazine-*N*-Me	0.007	0.57	0.07	0.17
22d	Piperazine-*N*-Ac	<0.004	2.7	0.02	0.09
23d	Piperazine-*N*-Ac	0.010	6.3	0.06	0.09

[a,b,c,d]See details in Table 1.

Table 3. Biological activity of diazines **42–43**

Compound	IC_{50} (μM)			
	Enzyme inhibition		Cell inhibition	
	Src[a]	KDR[b]	Src 3T3[c]	A549[d]
42	0.008	2.8	0.07	0.14
43	0.015	15	0.28	0.27

[a,b,c,d]See details in Table 1.

Optimisation of the C-4 and C-5 substituents culminated with **22a**, which is significantly more potent than the parent aniline **5**.

To modulate the physical properties of **22a**, C-7 variations were explored. The C-7 propoxy side chains appear slightly more active than the ethoxy analogues at the enzymatic level (**22a–d** vs **23a–d**) and possibly at the cellular level (Src 3T3) too. Both moderately and highly basic side chains are tolerated at C-7. Overall, improvements of physical properties were seen in the 2-pyridine series compared to the parent aniline series. As an example, **23d** is 8% free in rat plasma, whereas the C-7 acetylpiperazineethoxy analogue of **5** is only 0.5% free; solubility in phosphate buffer pH 7.4 is respectively 43 μM compared to 3 μM.

Replacement of the 2-pyridine with the corresponding pyrazine (see **42**) and pyrimidine (see **43)** gives also potent and selective c-Src kinase inhibitors both at the enzymatic and the cellular levels. The pyrazine **42** reaches comparable potency with the 2-pyridine analog **23d**.

Finally, **23d** and **42** were evaluated in rat for pharmacokinetic properties (rat cassette dosing of 5 compounds orally, 2 mg/kg each). Both compounds gave good blood levels (c_{max} of 0.31 and 0.42 μM, and $AUC_{0\text{-}6h}$ of 0.64 and 0.61 μM h, respectively for **23d** and **42**).

In conclusion, we have shown that 5,7-disubstituted 4-heteroarylaminoquinazolines such as 2-pyridinylamino- or 2-pyrazinylamino- give good c-Src inhibition both on the enzyme and in cells with a high degree of

selectivity associated with suitable physical properties for oral dosing. Further evaluation of these compounds for inhibiting c-Src in vivo is underway.

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11. Representative procedure for the coupling of aminoheterocycles to chloroquinazolines (synthesis of **18**): NaHMDS (5 ml, 1 M in THF, 5 mmol) was added dropwise to an ice-cooled solution of **17** (981 mg, 2.5 mmol) and **7a** (400 mg, 2.5 mmol) in THF (13 ml). The mixture was stirred at 0 °C for 5 min, then at rt for 3 h. After completion of the reaction, acetic acid (5 mmol) was added. After evaporation of the solvents, the residue was partitioned between CH_2Cl_2 and water and the pH adjusted to 7. The organic layer was extracted with CH_2Cl_2 and dried over $MgSO_4$. After evaporation of the solvents, the residue was purified by chromatography on silica gel (eluant: 2–5% MeOH in CH_2Cl_2). Evaporation of the fractions gave **18** (980 mg, 76%) as a solid: ^{1}H NMR spectrum ($CDCl_3$) δ 8.62 (s, 1H), 7.60 (d, 1H, $J = 8$ Hz), 7.34 (d, 1H, $J = 8$ Hz), 6.99 (s, 1H), 6.60 (s, 1H), 6.52 (m, 2H), 5.15 (s, 2H), 4.81 (m, 1H), 3.94 (s, 3H), 3.85 (s, 3H), 3.83 (s, 3H), 1.53 (s, 3H), 1.52 (s, 3H); MS (ES) 513, 511.
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13. The 3-aminopyridine **25a** was coupled to **17** and **9** in *i*PrOH at reflux in 86 and 79% yield instead of the conditions (described in Ref. 11) used for the aminopyridines **7a** and **30** where the anion of the aminoheterocycle is coupled to the chloroquinazoline. Attempts of coupling anilines **7a** and **30** with a chloroquinazoline in *i*PrOH at reflux were unsuccessful, probably because of the stronger basicity of these anilines hence protonation under these conditions. For the synthesis of **31**, we have modified slightly the synthetic sequence: after aniline coupling (reaction of **25a** with **9**) and subsequent deprotection of the 7-acetoxy in 7 N NH_3/MeOH (78% yield from **8** over 3 steps), the resulting phenol was coupled with 1-bromo-3-chloropropane in the presence of Cs_2CO_3 in DMA (70%) and the chloro was displaced with morpholine in DMA in the presence of KI (85%).
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