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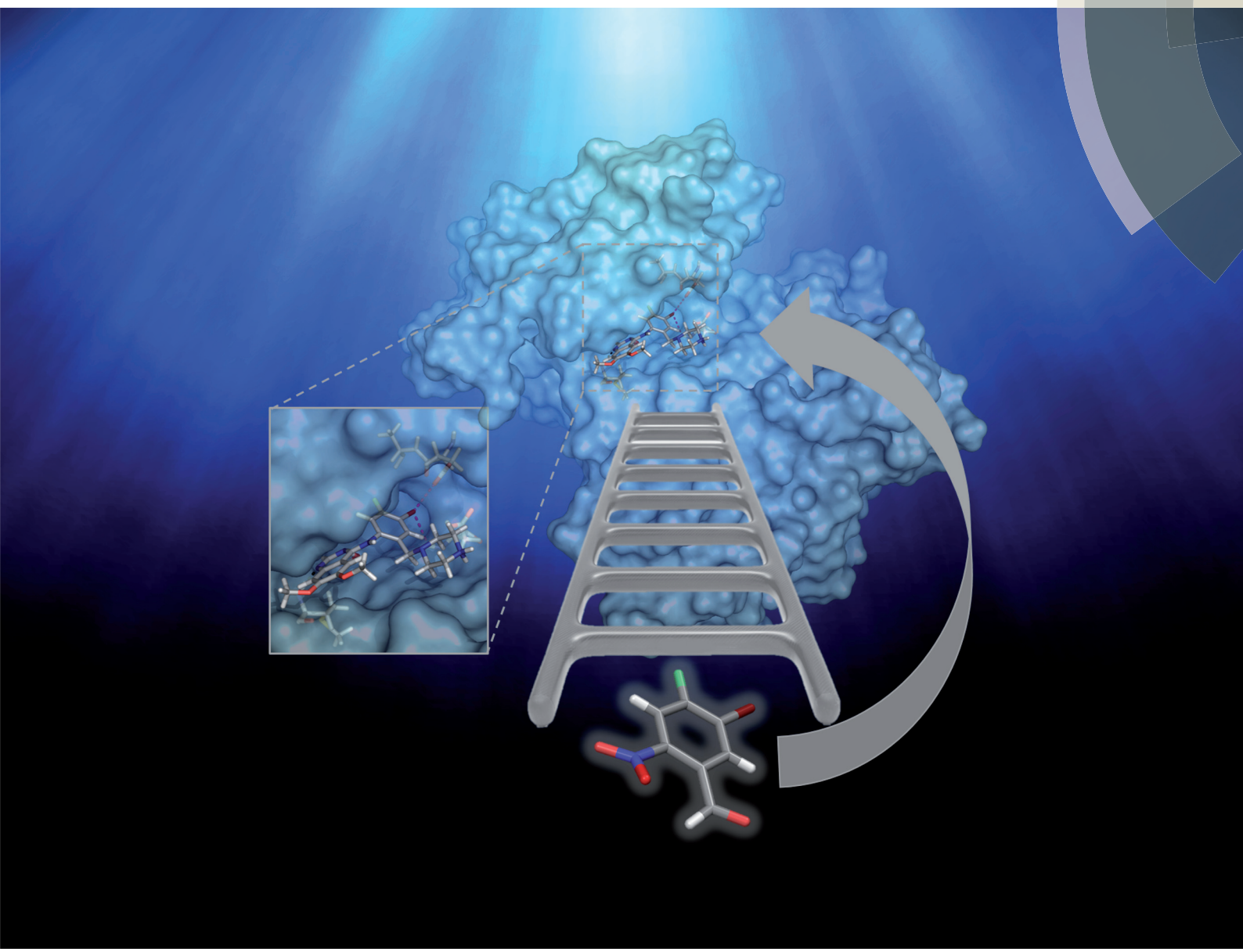

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Elaboration of tetra-orthogonally-substituted aromatic scaffolds towards novel EGFR-kinase inhibitors†

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Nitration of three regioisomers of bromo-fluorobenzaldehyde proceeds regioselectively, notably with H_2SO_4/HNO_3 at 0 °C. The thereby synthesized tetrasubstituted aromatics, endowed with orthogonal substituents, can be elaborated *via* Pd-catalysed coupling, reduction and reductive amination reactions. As a test-case, these compounds were converted into EGFR inhibitors related to Gefitinib, whose activity was rationalised by docking studies.

Introduction

Tetrasubstituted aromatics are commonplace in drug discovery yet regioselective routes towards these compounds, which often contain orthogonal groups, are rather scarce.[1] Kinase inhibitors are a rapidly growing class of anticancer agents and many of these comprise such tetrasubstituted scaffolds, often built around an adenine-like quinazoline scaffold, a solubilising group (*e.g.* ethers, morpholine or piperazine groups) and a tri or tetra-substituted aromatic, hydrophobic group that imparts selectivity towards particular classes of kinase (Fig. 1).[2–5]

To underline their importance, the synthesis of the recently approved irreversible EGFR (epidermal growth factor receptor tyrosine kinase family; *HER1*) inhibitor Osimertinib, starts with a somewhat simple, yet non-trivial, tetrasubstituted precursor, which comprises four orthogonal substituents (Fig. 2).[6]

We have recently reported procedures for forming poyl-substituted aromatics, mainly based on a MIDA boronate-substituted aryl scaffold.[7,8] Our aim was to synthesise analogues of the type I and II (Fig. 3) as useful 1,2,3,4- and 1,2,4,5-substituted building blocks.

Here, we report our investigation of the synthesis of related scaffolds, which do not include a MIDAboronate aryl scaffold but are substituted with bromo, fluoro and formyl substitu-

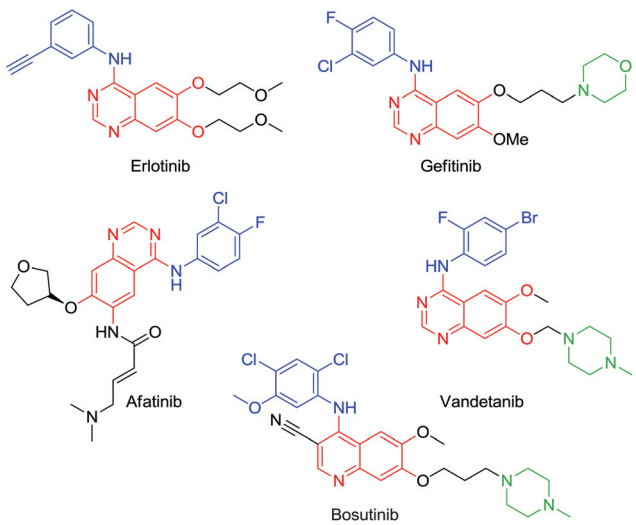

Fig. 1 Kinase inhibitors, highlighting the aromatic hydrophobic (blue), the adenine mimic (red) and solubilising group (green).

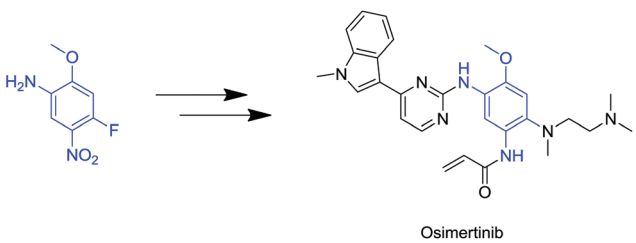

Fig. 2 Demonstration of a tetrasubstituted aromatic used as a building block in anticancer molecules.

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†Electronic supplementary information (ESI) available. CCDC 1485121–1485123. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c6ob01394e

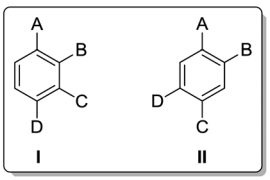


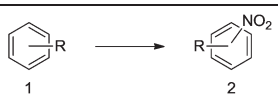
Fig. 3 Tetrasubstituted aromatic building blocks.

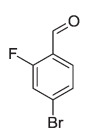
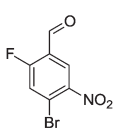
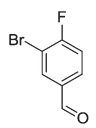
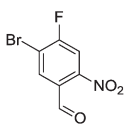
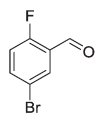
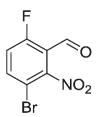
ents. We show that these orthogonal groups can be modified in order to furnish useful drug-like fragments as well as tetrasubstituted units that can be incorporated into potential kinase inhibitors.

Results and discussion

Brominations on trisubstituted aromatics are an effective means for synthesising tetrasubstitued aromatic units containing orthogonal (*e.g.* MIDA and bromide) groups. These electrophilic substitutions work with excellent regioselectivity when complementary directing groups are combined in the trisubstituted precursor. We now disclose our results on the related nitrations of trisubstituted (non-MIDA) aromatics and resulting functional group conversions thereafter affording novel tetrasubstituted frameworks. In order to maintain our ethos of elaborating orthogonally substituted aromatics we refrained from attempting the bromination of bromoaromatics.

Table 1 Nitration of bromo-fluorobenzaldehydes



Entry	Starting material	Product	Yield (%)
1	 **1a**	 **2a**	88[a,c] 84[b,d] 94[b,f]
2	 **1b**	 **2b**	61[b,e] 74[b,f]
3	 **1c**	 **2c**	30[b,e] 27[b,f]

[a] NO_2BF_4 −20 °C – r.t., 16 h. [b] H_2SO_4/HNO_3 0 °C – r.t., 16 h. [c] 12 mmol scale. [d] 25 mmol scale. [e] 30 mmol scale. [f] 99 mmol scale.

We attempted the nitration of three regioiomers of bromo-fluorobenzaldehyde (Table 1). Compounds **2a** and **2b** have been previously synthesised, described in patents, *via* nitration, using varying amounts of nitric acid in sulphuric acid.[9,10] We required a generic, high yielding method for these three compounds and thus looked for alternative methods. Compound **1a** was used to probe different nitration conditions due to the complementarity of the directing groups, which would be expected to give one regioisomer. Initial attempts included the use of potassium nitrate in sulphuric acid[11] and $Mn(acac)_3$ in DCM with nitric acid,[12] neither of which gave good results, as analysed by crude ^{1}H-NMR spectra.

We were pleased that the NO_2BF_4 method showed comparable yields for the synthesis of **2a** (entry 1, Table 1) to that of the nitric acid methods, adopted from the patents. For the synthesis of **2b** and **2c**, yields were slightly lower. In general a nitric/sulphuric acid mixture gave the best results and NO_2BF_4 proved to be ineffective in the attempted nitration of **1b** and **1c**.[13]

The regiochemistry of the products **2a–2c** was confirmed by ^{1}H and ^{13}C NMR spectroscopy as well as by single crystal X-ray crystallography (Fig. 4).

Next, we sought a general method for reducing the nitro compounds **2** and we found that iron in acetic acid was an effective means for forming the anilines **3**. Compound **3a** has been synthesised previously using 10% platinum on carbon although we found it less effective than an alternative iron-mediated method (Scheme 1).[14]

Unfortunately, a whole range of methods proved ineffective in the attempted reduction of the regioisomer **2a** (Scheme 2).

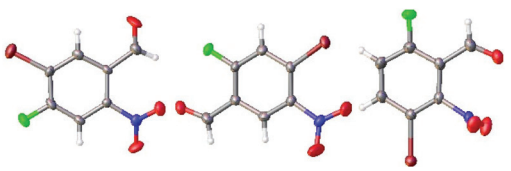
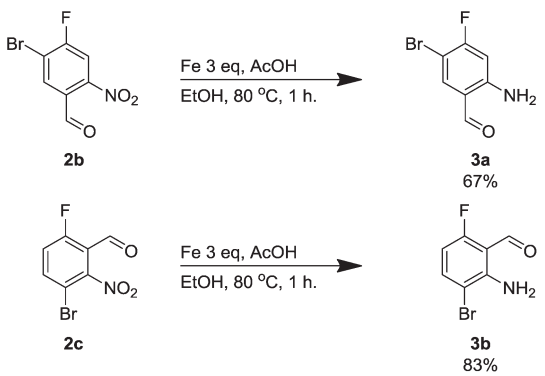
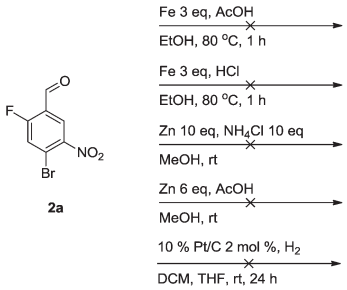


Fig. 4 ORTEP diagrams showing crystal structures of **2b** (left, CCDC 1485121), **2a** (middle, CCDC 1485122) and **2c** (right, CCDC 1485123). All are shown in Table 1. Red = oxygen blue = nitrogen, brown = bromine, green = fluorine, grey = carbon and white = hydrogen.



Scheme 1 Iron-mediated reductions of compounds **2b** and **2c**.

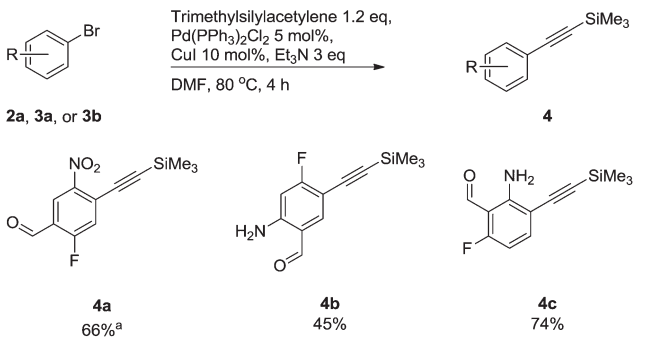


Scheme 2 Nitro group reductions attempted on compound **2a**.

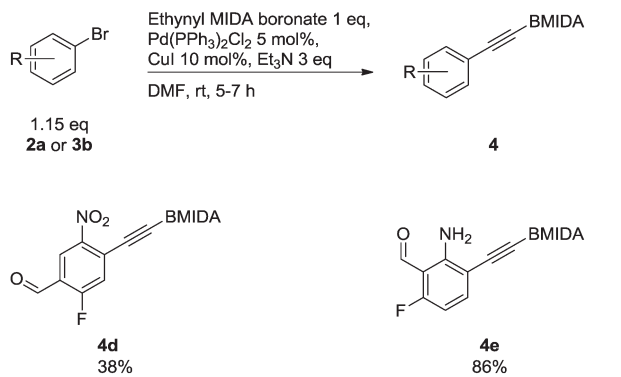
Nevertheless, by changing the reaction sequence (*vide infra*, Scheme 5) this issue can be circumvented and elaborated tetra-substituted units can be synthesised.

Alkyne groups are present in a number of kinase inhibitors such as Erlotinib (Fig. 1) and can also be used in *e.g.* indole forming reactions.[15–17] We carried out Sonogashira couplings on **2a**, **3a** and **3b** and obtained the silyl-protected alkynyl-benzenes **4** in moderate to good yields (Scheme 3).

To show a broader synthetic scope, ethynyl MIDA phenyl-boronate derivatives were also included, although a slightly modified protocol was employed, since an excess of aryl halide was required as the halide could be removed with ease on silica gel (Scheme 4).[18]

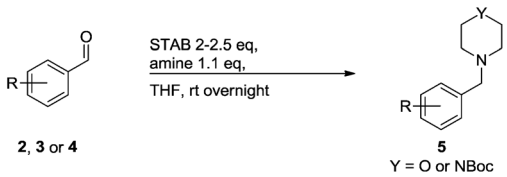


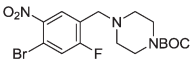
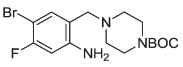
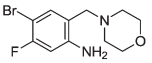
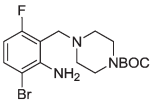
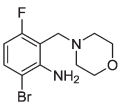
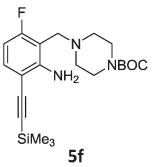
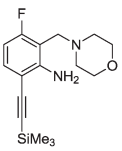
Scheme 3 Compounds synthesised *via* Sonogashira cross-coupling reactions of trimethylsilylacetylene. [a] Reaction performed at rt for 1 hour.



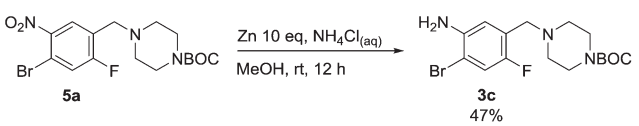
Scheme 4 Compounds synthesised *via* Sonogashira cross-coupling reactions of ethynyl MIDAphenylboronates.

Table 2 Compounds formed by reductive aminations

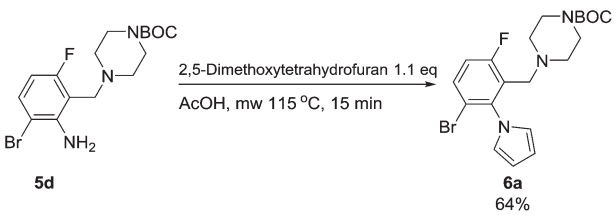


Entry	Starting material	Product	Yield (%)
1[e]	**2b**	 **5a**	47[a] 77[b]
2[f]	**3a**	 **5b**	83[a] 91[c]
3[f]	**3a**	 **5c**	97[a]
4[e]	**3b**	 **5d**	82[a] 95[d]
5[e]	**3b**	 **5e**	95[a]
6[e]	**4c**	 **5f**	28[a]
7[e]	**4c**	 **5g**	63[a]

[a] 0.5 mmol scale. [b] 8 mmol scale. [c] 3 mmol scale. [d] 6.3 mmol scale. [e] 2 eq. of STAB. [f] 2.5 eq. of STAB.



Scheme 5 Zinc-mediated nitro reductions of compound **5a**.



Scheme 6 Clauson-Kaas pyrrole synthesis under microwave conditions on compound **5d**.

A STAB (sodium triacetoxyborohydride)-mediated reductive amination of the formyl group in a selection of the compounds **2–4** was performed in order to form benzyl-substituted piperazine or morpholine derivatives (Table 2). Yields in general were good and these reactions proceeded selectively in the presence of an aniline substituent *e.g.* entries 2–5 (Table 2). Deprotected analogues of *e.g.* **5d**, may prove to be useful synthons for further derivatisation or as novel Ro3 (rule of three) fragments in drug discovery projects.[19,20]

Nitro reduction of **5a** was achieved, in moderate yield, using zinc in the presence of ammonium chloride, in order to maintain the acid-sensitive Boc protecting group. This represents an indirect route for obtaining the product **3c** since it is a derivative of the recalcitrant nitro precursor **2a.**

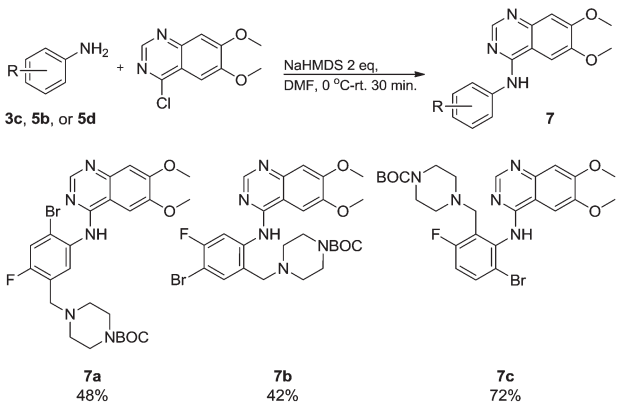
Having synthesized a range of tetrasubstituted anilines we wished to perform further modification of the aniline moiety. Firstly a Clauson-Kaas pyrrole synthesis was performed on the aniline **5d**, which led to the pyrrole **6a** (Scheme 6). Gratifyingly, the Boc-protecting group withstood the harsh microwave and acidic conditions.

As a proof of principle, based on structural similarity to the EGFR inhibitor Gefitinib and with the emergence of tetra-substituted scaffolds leading to the development of anticancer therapeutics (*e.g.* Osimertinib), we rationalised that compounds **7–9** are likely to target the receptor tyrosine kinase, EGFR. In order to assess the biological activity of our panel of tetra-substituted small-molecules, we carried out a dual-pass biological evaluation including *in vitro* and cellular-based assays. Compound potency against EGFR (wild-type, Exon 20) was established using a homogeneous time-resolved fluorescence (HTRF) kinase assay, which measures the extent of internal tyrosine phosphorylations. The hERG-CHO cell line over-expresses the human Ether-à-go-go Related Gene (hERG), which is a gene (*KCNH2*) that encodes a K^+ channel ($K_v11.1$) and is a red light toxicity alert in many drug discovery programmes (Table 3).

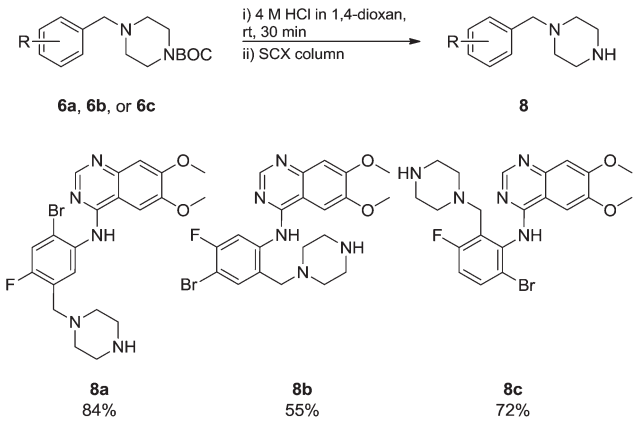
As a cautionary note, we did not embark on a drug discovery programme; for example, the biological evaluation (*vide infra*) was performed a long time after the synthesis of the compounds, preventing for example, any new syntheses and fine tuning of the products to address pharmacokinetics or off-target issues or to further investigate SAR (structure activity relationships).

Commercially available 4-chloro-6,7-dimethoxyquinazoline was reacted with anilines **3c**, **5b**, and **5d** to afford **7a–7c** in moderate to good yields (Scheme 7). We found that the use of sodium bis(trimethylsilyl)amide (NaHDMS) gave the product without deprotection of the Boc piperazine, unlike commonly used acid catalysed protocols.[21] Boc-deprotection was achieved by the addition of hydrochloric acid in 1,4-dioxane (Scheme 8) and purification was achieved by simply using solid phase extraction (strong cation exchange, SCX, column), giving **8a–8c**.

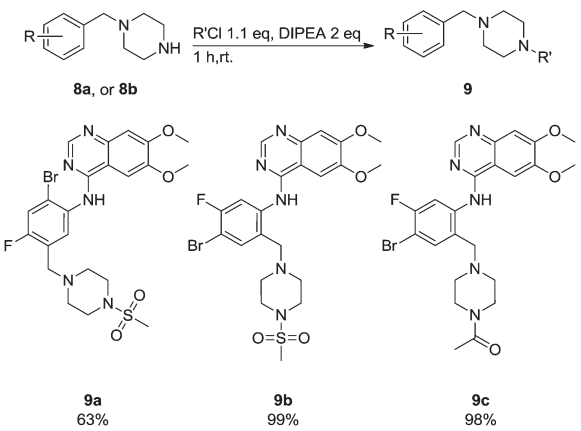
These newly synthesised secondary amines could be easily functionalised to sulphonamides or amides in high yields particularity in the case of **9b** and **9c** (Scheme 9).



Scheme 7 Linking of the quinazoline unit.



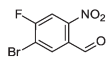
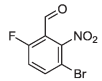
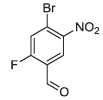
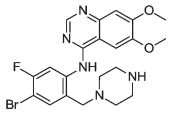
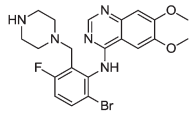
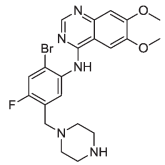
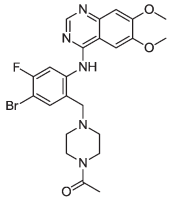
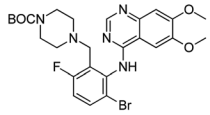
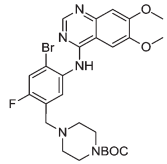
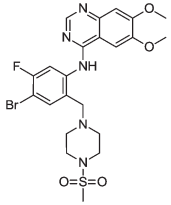
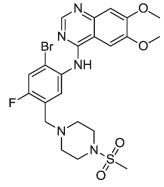
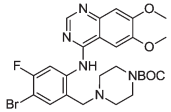
Scheme 8 Boc cleavage.



Scheme 9 Functionalization of the piperazine unit.

Compounds **7–9** were tested for inhibition of wild-type EGFR (Table 3). These results show that all of the compounds containing a BOC protecting groups were inactive except for **7c**, which had a high IC_{50} (21.5 μM). None of the compounds with regiochemistry resulting from the **2a** series

Table 3 Biological studies of the synthesized molecules

Compounds synthesised from **2b**				Compounds synthesised from **2c**				Compounds synthesised from **2a**			
											
Compound	EGFR IC_{50} [a]	Mean hERG inhibition[b] (%)	Dock score[c]	Compound	EGFR IC_{50} [a]	Mean hERG inhibition[b] (%)	Dock score[c]	Compound	EGFR IC_{50} [a]	Mean hERG inhibition[b] (%)	Dock score[c]
 **8b**	0.0184	61.1	−7.849	 **8c**	0.935	44.5	−8.895	 **8a**	>21.3	33.7	−5.986
 **9c**	0.377	96.5		 **7c**	21.5	21.5		 **7a**	27.1	46.4	
 **9b**	0.546	55.6						 **9a**	>30	43.9	
 **7b**	>30	19.5									

[a] EGFR Ex20 WT HTRF CR GMean IC_{50} (μM). [b] hERG Hu CHO IF EPhs SS GD mean, at 10 μM. [c] Using Schrodinger Glide.

i.e. **8a**, **7a** and **9a** showed any appreciable activity. The most active compound was **8b** (entry 8) which gave an IC_{50} of 18.4 nM. All of the compounds in the **2b** series gave good results *i.e.* all IC_{50}'s were less than 1 μM, with the exception of the BOC protected compound **7b**, which showed no activity. Unfortunately the percentage hERG inhibition increased as the activity of the compounds improved, as exemplified by **8b** (Table 3). There are ways in which to design out hERG inhibitions *e.g.* by reducing lipophilicity, reducing the pK_a of the nitrogen atoms, increasing steric hindrance around the nitrogen atoms or decreasing the number of hydrogen bond acceptors, but, as mentioned above, this was not addressed.[22]

The biological data suggest that the solubilising group piperazine *ortho* to the aniline moiety (linking to the quinazoline unit) gave compounds of higher activity *i.e.* compounds in the **2b** and **2c** series. However, the series where the aniline and the piperazine groups are mutually *meta*, *i.e.* the **2a** series, leads to a loss of activity. To look in to the relationship between the *meta* and *ortho* compounds we modelled the binding mode of the compounds **8a–8c** and produced docking poses (Table 3/Fig. 5).

To explore the binding mode of **8a–c** in EGFR we performed docking studies using the structure of the Gefitinib-EGFR complex.[23] We found that **8c** was able to bind in a way similar to Gefitinib forming archetypal hydrogen bonds between the backbone N–H of Met793 and a structural water from the nitrogen atoms of the quinazoline core (Fig. 5). An additional hydrogen bond was formed from the protonated piperazine and the carbonyl of Asp855. The halogenated phenyl group was accommodated in the hydrophobic pocket however, halogen substitution in **8c** was not aligned so as to form the halogen bonding exhibited in Gefitinib between the Cl···C═O of Leu788.

Compound **8b**, with halogen substitution *meta*- and *para*- to the aniline group, was docked similarly with the quinazoline core and phenyl ring slightly shifting to form Br···H–N (Asp855) and Br···O═C (Glu762) halogen bonds. The key interaction between Met793 and the quinazoline nitrogen was formed but the bond with the structural water was not conserved. An additional interaction between Arg841 and the protonated piperazine was formed.

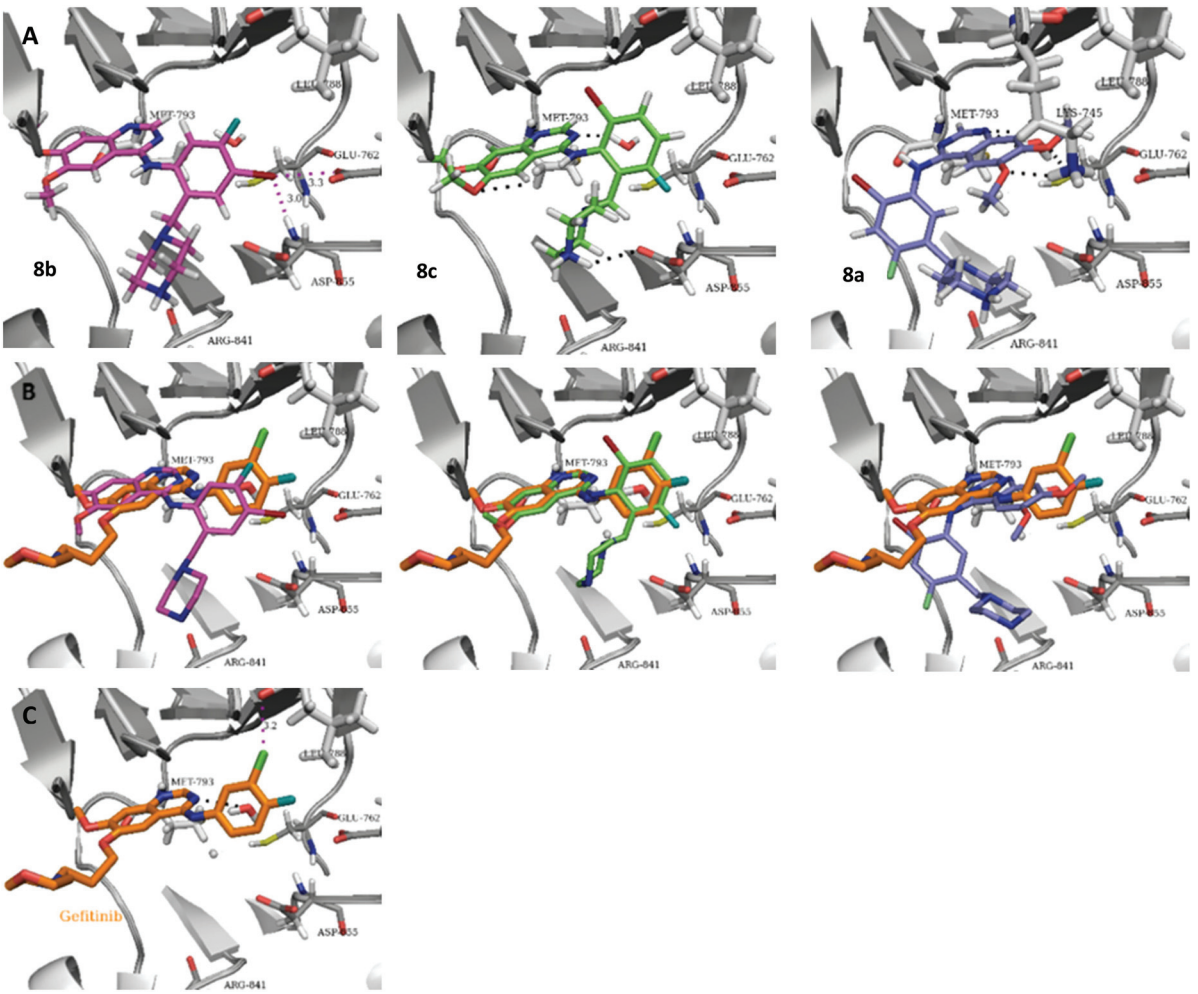


Fig. 5 Docked **8a–c** and comparison to Gefitinib, using Schrodinger Glide. (A) Docking poses of **8a–c**. (B) Comparison of docked **8a–c** with cocrystalized Gefitinib. (C) Cocrystal structure of gefitinib in EGFR (PDB code: 2ITY).

Compound **8a**, with the piperazine *meta*- to the aniline cannot be docked in a mode similar to gefitinib and forces an alternative binding mode whereby the methoxy-groups of the quinazoline are forced into the hydrophobic pocket forming hydrogen bonds between the oxygen of the MeO– and the H–N of Lys745. This suggests, and is in agreement, that **8a** will be inactive or show a much lower potency towards EGFR.

Conclusions

A library of tetrasubstituted aromatics has been synthesized starting with robust nitration chemistry. The library has been elaborated into a series of useful potential intermediates for drug discovery and final drug-like entities as exemplified by the formation of a range of EGFR inhibitors that display low nM inhibition.

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AstraZeneca (ACJ) and the University of Sussex (AJC, RNJ) are thanked for PhD studentship funding. Worldwide Cancer Research (grant no. 14-1002) partly funded this work through the provision of Schrodinger Glide software. The EPSRC UK National Mass Spectrometry Facility at Swansea University is thanked for assistance.

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