Toward the Comprehensive Systematic Enumeration and Synthesis of Novel Kinase Inhibitors Based on a 4-Anilinoquinazoline Binding Mode

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There are currently eight small-molecule kinase inhibitors approved as cancer treatments, and a significantly larger number of compounds are in the earlier stages of clinical development. Although kinase inhibitors are most commonly developed in a cancer setting, other disease areas have been targeted. The vast majority of reported kinase small-molecule inhibitors contain functionalities that interact with the adenosine triphosphate (ATP) binding site of the kinase. The 4-anilinoquinazolines have previously been reported as potent epidermal growth factor receptor (EGFR) inhibitors, binding at the 'hinge' region of the ATP site. Subsequently, this chemical series has been optimized against a number of different kinases including Src and Aurora B. Here, we detail the computational enumeration of ring systems that have the ability to make comparable interactions to the 4-anilinoquinazoline core. These were prioritized by computational, medicinal, and synthetic chemistry input, and a number of libraries were subsequently synthesized.

INTRODUCTION

Protein kinases play a fundamental role in the aberrant signaling that is a hallmark of the uncontrolled proliferation of a variety of human tumors. The search for potent, selective, and novel small-molecule inhibitors of diverse kinases continues to be the major focus of the oncology drug discovery community. Following their discovery through directed screening against the epidermal growth factor receptor (EGFR) tyrosine kinase in the early 1990s,[1] 4-anilinoquinazolines have emerged as a most versatile template for kinase inhibition. This class of inhibitors acts via direct competition with adenosine triphosphate (ATP), and the template has been heavily exploited in the search for new inhibitors of signal transduction mechanisms, both against EGFR, and for other diverse kinases.[2] Of the eight small-molecule kinase inhibitors currently marketed against oncology indications, three are from the 4-anilinoquinazoline class and target subsets of the EGFR kinase family: gefitinib **1**,[3] erlotinib,[4] and lapatinib **2**.[5] A survey of currently available data[6] indicates 17 agents belonging to this template are currently under active clinical development, targeting at least 9 primary kinase targets against an even greater number of potential disease indications. At the target level, analysis of the RSCB Protein Data Bank[7] highlights over 20 anilinoquinazoline-based inhibitors bound to 9 distinct kinase targets, further illustrating the importance and the versatility of this template for kinase inhibition.

The explosion of interest around this high-value kinase template has meant that opportunities for its continued exploitation against new and emerging targets is limited by the scope of existing disclosures. One medicinal chemistry approach to alleviate such issues is through the development of alternative ring systems, which bind similarly to the quinazoline core. Indeed, such a strategy has already demonstrated potential in a clinical setting—the use of 3-cyanoquinolines to replace the quinazoline is well documented,[8] and examples such as neratinib[9] (EGF, ErbB2) and bosutinib[10] (Src, Abl) have progressed into patient studies. Recently, Bristol Myers Squibb (BMS) has disclosed the pyrrolotriazine template exemplified by **5** to **8**,[11] which contains a bridgehead nitrogen embedded in a 6,5-ring system, and has gone on to exploit this against a range of both kinase and nonkinase targets, delivering agents with properties suitable for clinical exploitation; one such example being brivanib **7**[12] (vascular endothelial growth factor, VEGFR, and fibroblast growth factor receptors, FGFR). One versatile feature of the 4-anilinoquinazoline template is that much of the potency and selectivity is derived from the 4-anilino (or alternative) headgroup moiety. As such, discovery of this replacement has allowed BMS to rapidly gain chemical equity against diverse targets by assimilating the medicinal chemistry SAR undertaken elsewhere in the literature, the selective exploitation of which is illustrated in Table 1.

A recent publication highlighted a systematic approach to the generation of novel heterocyclic systems that might have utility in general drug discovery or in collection enhancement programs.[16] Independently, we report our efforts to comprehensively survey heterocyclic systems that have the potential to bind similarly to the anilinoquinazoline template. The process included systematic ring enumeration, filtering, synthetic tractability, medicinal chemistry considerations, and overall precedence through to synthesis of screening libraries based on representative cores. In this analysis, we simultaneously consider the quinazoline and quinoline-like templates

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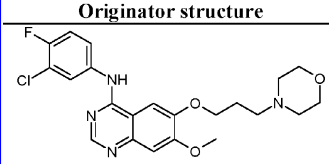
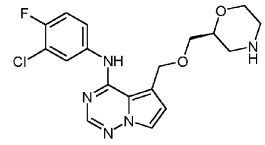
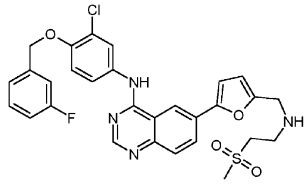
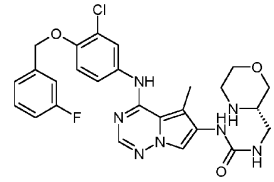
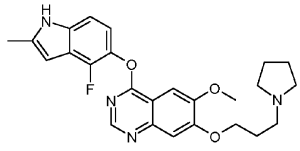
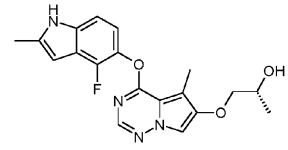
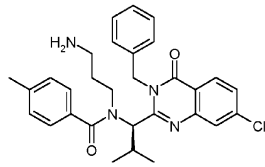
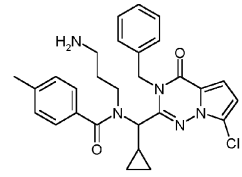
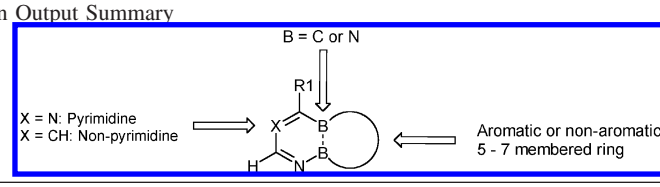
Table 1. Pyrrolotriazene Core As a Alternative to the Quinazoline

Originator structure	Name	Target	Pyrrolotriazene structure
 **1**	*gefitinib*[a]	EGFR	 **5**[13]
 **2**	*lapatinib*[b]	EGFR ErbB2	 **6**[14]
 **3**	*cediranib*[a]	VEGFR	 **7**
 **4**	*ispinesib*[c]	KSP (Eg5)	 **8**[15]

[a] AstraZeneca. [b] GlaxoSmithKline. [c] Cytokinetics and GlaxoSmithKline.

Table 2. Ring System Enumeration Output Summary



ring system	valence filter	substructural filter	chemistry database hits	prioritized[a]	literature known[b]	kinase reference[c]
6,5 aromatic pyrimidine	109	109	58	33	24	13
6,5 nonaromatic pyrimidine	171	171	13	10	4	2
6,5 aromatic nonpyrimidine	527	527	38	58	35	15
6,5 nonaromatic nonpyrimidine	342	342	6	12	3	1
6,6 aromatic pyrimidine	209	209	30	29	19	5
6,6 nonaromatic pyrimidine	826	103	12	39	10	3
6,6 aromatic nonpyrimidine	1076	1076	40	30	12	4
6,6 nonaromatic nonpyrimidine	1656	105	10	40	4	0
6,7 nonaromatic pyrimidine	3898	166	0	45	2	0
6,7 nonaromatic nonpyrimidine	7796	166	0	47	1	0
total	16 610	2974	207	343	114	43

[a] Consensus selection from a team of AstraZeneca medicinal chemists. [b] Substructural query in SciFinder. [c] At least one associated reference details a kinase study. No assessment was made as to whether this was within the context of a 4-anilinoquinazoline binding mode.

and the possibility of bridgehead nitrogen at either available position on enumerated 6−5, 6−6, and 6−7 fused ring systems.

RESULTS AND DISCUSSION

Ring Enumeration and Filtering. The key protein−inhibitor interactions of the 4-anilinoquinazoline binding mode can be assessed by protein bound crystal structures[17] and by studying the reported SAR of the series. The focus of this enumeration was to maintain the essential interaction points and the substituent positions, while varying the chemistry of these ring systems to develop novel kinase hinge-region binders. In the ring enumeration scheme (Table 2), the position of the key nitrogen hydrogen-bond acceptor to the hinge was fixed, along with the neighboring unsubstituted carbon. It has been highlighted that the hydrogen on this

carbon may form a weak hydrogen bond with the hinge region,[18] so to ensure compatibility with the hinge region, it was preserved. An additional risk of placing groups at this position is that it may result in a steric clash with the hinge.

The ring atoms, which compose the highlighted 5−7 membered ring systems, were exhaustively enumerated along with the nonhinge region binding nitrogen on the pyrimidine ring. A chlorine atom was positioned at the substituent point indicated by R1, as it is a preferred library building block directed toward the kinase selectivity pocket. In this paper, the selectivity pocket is defined as the region of the active site occupied by the anilino substituent (C-4 position) of anilinoquinazoline-based inhibitors, such as gefitinib.

Ring systems were enumerated exhaustively in a SMILES[19] format using an in-house software Boomslang.[20] Each atom was independently varied between C, N, O, and S. Additionally, methyl substituents on carbon and nitrogen were included, since these would also lead to validated ring systems that might show inherently more stable chemical properties compared to that of less substituted analogues. Ring carbonyls and sulphones were also explicitly included. Only C and N atoms were allowed in either bridgehead position. After ring systems with invalid valences were removed, 16 610 unique examples were identified for the specified enumeration scheme (Table 2). The enumerated cores were labeled as 6,5′, 6,6, or 6,7 fused ring templates to aid subsequent analysis. SMARTS[21] based filters also further classified the ring systems into examples with a pyrimidine or nonpyrimidine hinge binding motif. In addition, the ring systems were defined as aromatic or nonaromatic using the Openeye definition of aromaticity.[22] The number of ring systems was then reduced to 2974 through the use of defined substructural filters based on input from a team of medicinal chemists, indicated in Table 2 as 'substructural filter'. This automated filtering step removed chemically feasible but medicinally undesirable functionality, such as S−S and O−O bonds and isolated ketone groups. As the lipophilicity of kinase inhibitors can be relatively high, we were interested to see how likely we were to identify ring systems of a lower lipophilicity than the quinazoline core. For this analysis, ClogP (version 4.3) of the chloro-substituted enumerated ring systems was calculated, and of the 2974 rings described, only 379 (13%) were more lipophillic than the quinazoline reference with ClogP = 1.8 (mean ClogP of this set was 0.37, ranges from −3.2 to 4.4). To identify ring systems where suitable building blocks may be commercially available or routes known, the enumerated structures were duplicated to also include fluorine and bromine at R1, in addition to chlorine. Flush[23] was then used to search for exact matches of these ring systems in our in-house and commercial reagent databases.[24] These enumerated potential scaffolds were also searched for in a range of chemistry-focused databases, including GVKBio[25] and PubChem[26] to assess if there may be other relevant literature available. Finally, the chlorine at R1 was converted into an oxygen, nitrogen, and sulfur group for each of the enumerated ring systems. These structures were then searched for in a number of databases (corporate collection, commercially available compounds, GVKBio, and PubChem) using in-house software[27] to look for compounds, which contained these substructures, that may suggest a synthetic route is possible. The combined number of hits from these exact match and substructural searches is shown in the 'chemistry database hits' column in Table 2. In total, only 207 of these ring systems (i.e., 7% of the filtered list) had hits in either of these searches. The entire list of rings from the substructural filter (2794) was subjected to a further round of filtering and prioritizing by a team of medicinal chemists with extensive experience in drug design generally and by kinase inhibitor programs more specifically. This team of medicinal chemists was able to exploit the information from the described chemistry database searches, although they also viewed the novel structures, as the aim was not to restrict the scope to known rings. This process naturally introduces an element of subjectivity and bias into the prioritization process but is necessary, in order to reduce the number of rings to a manageable level and to allow focus on only those ring systems believed to have the greatest chance of both being synthesized and acting as hinge region binders. Generally, a parent ring system was chosen over its more substituted homologues, except where ring stability was felt to be a potential issue. Rings with an excess (typically three or more) of heteroatoms were avoided, and preference was given to those ring systems that might be able to effectively position other substituents known to be important in quinazolines and related series. These included, for example, the ability to introduce a solubilizing side chain or to mimic the donor−acceptor motif seen in many kinase inhibitors. The column headed 'prioritized' in Table 2 indicates the number of rings in a given class (as a subset of those passing the substructural filters) that achieved consensus among the medicinal chemistry team as worthy of synthesis, should a route be available. A number of manual substructural searches were then carried out on these prioritized ring systems using SciFinder.[28] For each ring classification, the number of prioritized rings that had any reference associated is captured in the 'literature known' column. Of these, the number of rings that had at least one associated kinase-related reference is captured in the final column of Table 2.

The breakdown of the searching and the filtering in this way is informative. A combination of computationally automated enumeration and filtering followed by medicinal chemistry prioritization highlighted 343 ring systems as potential targets for synthetic chemistry work. Of these, only 114 (33%) had any precedent in the chemical literature, and furthermore, only 43 rings (13%) had any reference linking them to a kinase target. Row five in Table 2 is particularly illustrative in that the classification 6,6 aromatic pyrimidine describes the class in which quinazoline itself belongs. Of the 29 rings that were prioritized in house, 19 (66%) were known in the chemical literature, but only 5 (17%) had a kinase reference associated with them. Ring systems that have no associated references carry the greatest risk in terms of the ability to synthesize them but potentially greatest rewards in terms of differentiation in intellectual property terms. Ring systems that are associated with kinase references offer the least risk in terms of validity, and it may be possible through tuning of pendant groups that suitable potency and freedom to operate can be obtained. Those in between these extremes, namely where literature precedent for a ring exists but where, to date, exploitation within the field of kinase inhibition has yet to be reported perhaps, offer the optimal balance—synthetic precedent combined with potential novel compounds. It is to this category that the pyrrolotriazene

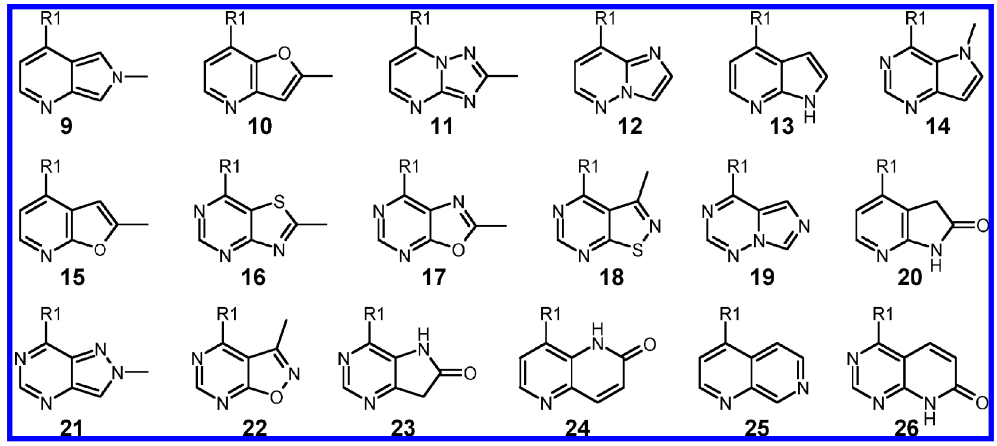


Figure 1. Examples of enumerated ring systems that are known in the literature to be associated with kinase inhibition.

template exploited by BMS belonged, with reports on the suitability of this ring system as an adenosine pocket binding group emerging in the years preceding BMS' disclosure.[29]

Figure 1 illustrates some of the ring systems generated using this approach, which are both known in the literature and have at least one associated kinase reference. Although a number of these ring systems may bind similarly to the 4-anilinoquinazoline, this will not be the case for all of the rings. A further subset was identified which contains cores that have been exemplified in the literature but, to date, have not been linked to kinase inhibition. Most of the reports on compounds in this grouping are associated with a nonkinase biological study or a patent, but examples were also found whose sole precedent lay in the synthetic organic chemistry literature. The final grouping was the rings that were unknown at the time of writing in the chemical literature. It is informative to look at how what is known about the rings identified in this study has changed since these searches were undertaken (2007). Compounds with ring scaffolds **12**, **16**, **17**, and **21**−**26** inclusive, now known to be associated with kinase inhibition, were originally classified as being known ring systems but without described kinase activity. Similarly, a number of the previously unreported enumerated ring systems have now been reported in the literature, albeit often with no associated kinase activity as of yet. It is perhaps a degree of validation of the strategy adopted that rings identified in one grouping have moved through to greater precedent and to increasing kinase relevance in a relatively short period.

Certain ring systems that were found to be available commercially were purchased. For the remainder, literature routes were followed or modified, or synthetic routes derived from first principles by in-house chemistry effort toward as many of the prioritized rings as was feasible within the time and resource constraints applied. In-house designs were realized into chemical libraries through a collaboration with an external partner. In certain cases, the route development work highlighted synthetic routes to novel building blocks outside the scope of the initial enumeration, and these were included. From the final selection of enumerated cores, 29 ring systems were ultimately followed-up with a synthetic chemistry effort, resulting in compound libraries being synthesized. Potential ring synthetic candidates were combined at R1 (typically referred to as the C-4 position of an anilinoquinazoline) with reagent sets, including anilines, phenols, benzylamines, secondary amines, and directly linked aryl groups for the R1 substituent, many of which have precedence as a quinazoline substituent. In total 8479 compounds classified into 29 chemical libraries were synthesized and registered into the AstraZeneca compound collection from this work.

Table 3. Summary of Kinase Activity from the Synthesised Compounds

	compound		library[a]	
	number	percent, %	number	percent, %
total synthesized	8479		29	
tested in ≥1 kinase assays	756		27	
$pIC_{50} \leq 4$ (inactive)	315	42		
$4 < pIC_{50} \leq 5$	265	35	27	100
$5 < pIC_{50} \leq 6$	132	17	23	85
$6 < pIC_{50} \leq 7$	25	3	15	56
$pIC_{50} > 7$	19	3	12	44

[a] At least one member of a library (scaffold) demonstrating activity at the indicated range.

Activity Analysis of Synthesized Libraries Across the Company. At the time of writing, 756 (9%) of the 8479 synthesized compounds have been tested in a dose response enzyme assay against one or more kinases, and of these, 441 compounds (58%) have shown activity of $pIC_{50} > 4$ (i.e., < 100uM) in at least one kinase assay. For compounds that have been tested in one or more kinases, the mean number tested against was 3.4, and the mean number of these the active compounds showed activity against was 2.8. Table 3 shows the numbers of actives from the synthesized compounds at certain defined pIC_{50} ranges. The data is presented from both a compound and a library perspective (where members of a library share a common core scaffold). It can be seen that all libraries tested have shown kinase activity, even where only a small proportion of compounds from a given library have inhibition data. Of those tested, 6% have a $pIC_{50} > 6$ in any kinase, an encouraging level of activity. The typical top concentration of the assays used in this study is 100uM, therefore, the accuracy of the assignment of compounds into these activity bins should be relatively high. All dose responses curves are also commonly viewed individually to identify potential issues with poorly soluble compounds. For all compounds tested, a 'hit rate' was defined as the percentage of the number of assays a compound was active in (most commonly $IC_{50} < 100$uM) compared to the number of assays tested in. This data is presented by binning the hit rate and shown for the tested

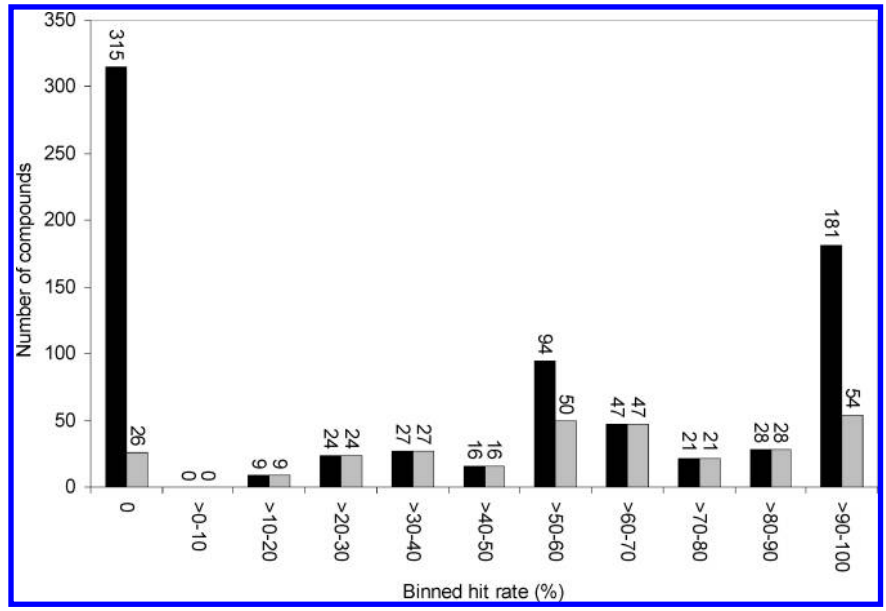


Figure 2. Binned hit rate (%) for compounds tested in dose response kinase assays. Black bars show hit rates for all for tested compounds. Gray bars show only the data for compounds which have been tested in three or more kinases.

Table 4. Binned Activity Data For a 140 Compound Subset Tested against 8 Kinases[a]

	CDK2	IGFR	FGFR	KDR	PKA	p38α	PI3Kα	ROCK
hit rate % ($pIC_{50} > 4$)	28	21	52	60	30	75	51	22
$pIC_{50} \leq 4$ (inactive)	101	110	67	56	98	35	68	109
$4 < pIC_{50} \leq 5$	35	27	58	54	30	94	53	22
$5 < pIC_{50} \leq 6$	4	3	11	26	8	11	18	6
$pIC_{50} > 6$	0	0	4	4	4	0	1	3

[a] Compound activity data used in the analysis is from the average of two independent assay experiments.

compounds in Figure 2. Also in Figure 2, the same analysis has additionally been performed on a subset of compounds tested in three or more kinases. Compounds in higher percentage bins may include less selective, potentially pan-kinase inhibitors, although the assay numbers examined are small compared to that of the kinome.

To provide further context to these observations, it is important to describe how these compounds would have been selected for IC_{50} testing in a kinase enzyme assay at AstraZeneca. One source of compound selection is from random or subset selection for testing against one or more kinases. Some of the compounds, however, will have been selected for IC_{50} testing based on a single concentration activity from a high-throughput or directed screen. A number of these compounds have shown activity in single point inhibition screens, but as these have been run at different concentrations and as assay quality can be more variable, a broader analysis of this data was not performed. A compound may also have been selected due to activity of a close analogue in an IC_{50} or single concentration assay. Alternatively, a compound may have shown activity in one kinase and, therefore, subsequently been tested in multiple kinase assays to assess selectivity. As a consequence, the hit rates shown in Figure 2 should not be considered an unbiased hit rate of the library, such as that which might be seen when screening a library against a new target.

Activity Analysis of Compounds in a Panel of Eight Diverse Kinases. The previous analyses demonstrate that compounds synthesized have shown activity in kinase assays, but we wanted to look more specifically at how the different enumerated ring systems behaved. To analyze the data further, a subset of 140 compounds were picked and screened against a panel of 8 diverse kinase biochemical assays. All assays were run at K_m [ATP] for each kinase, the top concentration being 100uM. The selection criteria involved choosing examples of each ring system elaborated with the most abundant R1 groups in the data set to allow comparison across the cores. Due to chemistry attrition, not all R1 groups were present in the same frequency in the synthesized libraries. The compounds in this data set include examples from all of the 29 synthesized cores, with relatively simple R1 groups. Table 4 summarizes the activity data of the 140 compound subset against a panel of 8 diverse kinases. Hit rates against individual kinases range from 21−75%, defining a hit as a compound with a measurable activity in the assay (i.e., $pIC_{50} > 4$). Again, this hit rate was encouraging, and examples were also identified with $pIC_{50} > 6$ in 5 out of the 8 kinases tested. Perhaps unsurprisingly, as two kinases known to bind 4-anilinoquinazolines, p38α and kinase domain region (KDR) showed the highest hit rates to the compound set of 75 and 60%, respectively. However, of these two, submicromolar actives were only identified against KDR. The AGC kinases (PKA and ROCK) showed a lower overall hit rate, but submicromolar actives were found against both targets. CDK2 and IGFR also produced modest hit rates (28 and 21%, respectively), with no submicromolar actives identified against either kinase. The hit rate observed from screening a random compound set, such as by high-throughput screening (HTS), often falls into the range of 1−4%,[30] varying with the choice of target and the compound

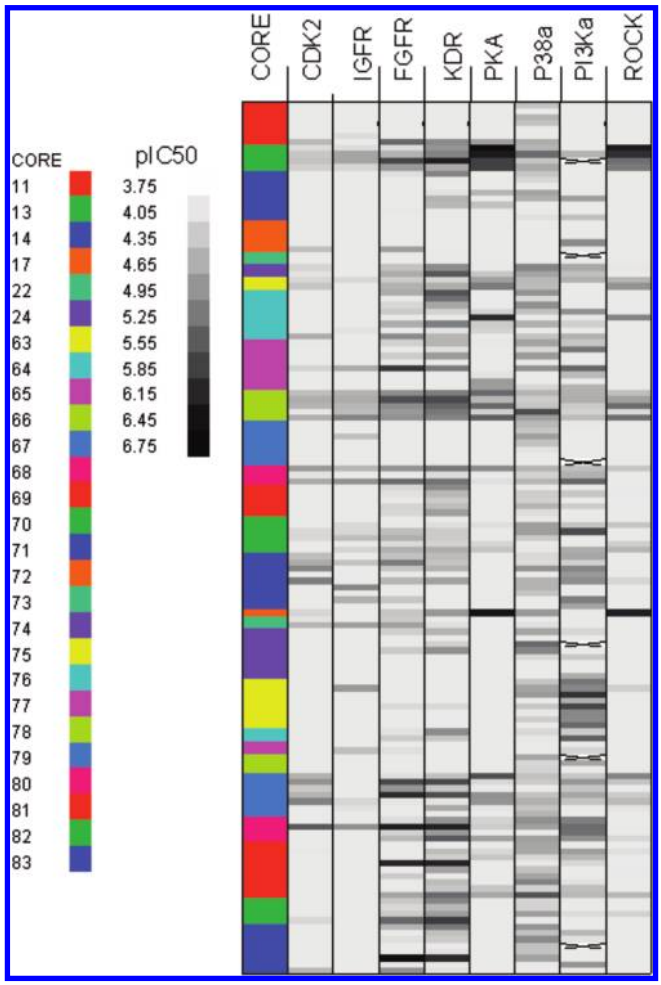


Figure 3. Heat map of activity data for the 140 compound subset against a panel of 8 kinases. Color intensity signifies level of potency, according to legend. Data are grouped and annotated by core, and each line represents a unique compound varying by R1. Crosses indicated no data available. Activity data shown is an average of at least two experiments.

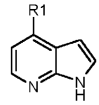
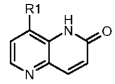
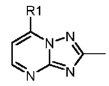
collection screened. Significant work has been carried out by pharmaceutical companies to enrich their compound collections with potential kinase inhibitors, often resulting in a higher observed hit rate when screening kinases than for other protein families.

Figure 3 illustrates this data at the compound level in the form of a heat map. In this plot, compounds are grouped by the enumerated core, and the shading intensity indicates relative potency. Examination of the data in this way quickly helps to identify rings that appear to enrich against a particular kinase or a group of kinases and will also highlight kinases that exhibit an inhibition profile similar to another kinase. Scaffold **66** appears to be among the least selective of those examined, showing active members across the whole panel. Kinases IGFR and CDK2 appear least susceptible to inhibition by this analysis, although the latter does pick up activity with templates **71** and **79** (see later). Scaffold **13** gives potent inhibition of PKA and ROCK, a well established feature of this particular hinge binding group, and a number of scaffolds including **80**, **81**, and **83** appear to be capable of providing potent inhibition against FGFR and KDR. Template **75** would appear to be relatively selective for PI3Kα inhibition, showing little activity against other panel members, and few other cores inhibit PI3Kα to the same extent. Analysis by kinase appears to show that FGFR and KDR demonstrate a similar inhibition profile, similarly for PKA and ROCK. This would suggest that achieving selectivity within each pairing might be challenging.

Analysis of Ring Systems with Fixed Substituent. While the previous overview is useful, we also wanted to compare activity across the different cores with a fixed R1 group. Within this data set, one of the most frequently occurring groups, *meta*-chloroaniline as R1 substituent, was combined with 14 of the 29 enumerated ring systems. This group was also combined with quinazoline itself to give **84**, which acts as a benchmark against which cores may be assessed. Table 5 shows the kinase data against each panel member for a variety of the ring systems when combined with *meta*-chloro aniline. Unfortunately, only rings systems which have previously reported kinase activity in the literature can have their chemical structures released in this report, for the others, the 'enumeration scheme' is shown. Quinazoline **84** shows significant potency against p38α kinase ($pIC_{50} = 5.3$), with more modest activity against KDR ($pIC_{50} = 4.8$) and PI3Kα ($pIC_{50} = 4.7$). We were particularly interested in finding ring systems with the same selectivity pocket group, different hinge region binding group, but equal or better activity in a target kinase. Exhibiting a similar overall binding mode may also be beneficial to facilitate learning of SAR from anilinoquinazolines and from other series related by binding mode.

The data in Table 5 illustrates that identification of alternative cores to compound **84**, which maintain the activity in p38α, is possible. Examples such as **24**, **80**, **83**, and **85** have similar potencies and overall selectivity profile to **84** across the panel. An additional consideration when looking for chemical start points is their comparative lipophilicity, where high lipophilicity may result in issues, such as poor solubility and CyP450 and hERG ion-channel inhibition. Template **24**, for example, shows an order of magnitude drop in lipophilicity, with ClogP = 3.5 compared to 4.6 for the anilinoquinazoline reference **84**. It is interesting to note that **13** and **66** show p38α activity but have additional activity against PKA and ROCK, both members of the AGC family, against which quinazoline **84** is inactive. Both of these hinge groups have an additional hydrogen-bond donor that is able to interact with the ATP site. This observation is perhaps consistent with reported data of examples of these and related hinge region binding groups, active against AGC kinases through a change in binding mode, where the heterocycle is flipped through 180° such that the pyrrole of **13** does not overlay the quinazoline phenyl ring.[31] Activity against the lipid kinase PI3Kα is seen in examples **80** and **85**, a pharmacology observed to some extent in the quinazoline **84**. Compound **79** is one of the few compounds to demonstrate activity against CDK2. It is tempting to speculate on a possible alternative binding mode for **79** and for other similar compounds such as **11** and **81**, which have a nitrogen in the five position of the anilinoquinazoline ring or in the equivalent position on a five-membered ring (Figure 4, upper panel). This allows compounds to bind with the aniline group in the solvent channel as opposed to the selectivity pocket and may be considered an 'olomoucine-like' binding mode. A compound binding, as described, may be expected to have a different selectivity profile than through the 4-anilinoquinazoline binding mode.[32]

Table 5. Kinase Panel Data for Key *meta*-Chloro Aniline Selectivity Pocket Examples

ID	Core[a]	CDK2	IGFR	FGFR	KDR	PKA	p38α	PI3Kα	ROCK
84		< 4.0	< 4.0	4.1	4.8	< 4.0	5.3	4.7	< 4.0
85	6,5 Aromatic Pyrimidine	< 4.0	< 4.0	< 4.0	4.5	< 4.0	5.6	5.0	< 4.0
13		4.4	4.8	4.6	5.1	6.3	5.4	4.6	6.0
83	6,5 Aromatic Non-Pyrimidine	< 4.0	< 4.0	4.1	4.4	< 4.0	5.1	4.1	< 4.0
24		4.3	< 4	4.5	5.0	< 4.0	5.1	4.7	< 4.0
80	6,6 Aromatic Non-Pyrimidine	< 4.0	< 4.0	4.2	5.0	4.3	5.0	5.2	< 4.0
66	6,5 Aromatic Pyrimidine	4.7	5.1	5.0	5.1	5.5	4.9	4.5	5.4
70	6,5 Aromatic Pyrimidine	< 4.0	4.2	< 4.0	4.2	4.1	4.9	4.4	4.4
82	6,6 Aromatic Pyrimidine	< 4.0	< 4.0	4.4	4.9	< 4.0	4.9	4.2	4.3
81	6,6 Aromatic Non-Pyrimidine	< 4.0	< 4.0	4.0	4.5	4.2	4.7	4.9	4.2
79	6,5 Aromatic Non-Pyrimidine	5.2	4.2	4.1	4.1	5.0	4.6	4.4	4.5
11		< 4.0	< 4.0	< 4.0	< 4.0	< 4.0	4.6	< 4.0	< 4.0
63	6,5 Aromatic Pyrimidine	4.2	4.2	4.4	4.3	4.8	4.5	4.7	4.6
69	6,5 Aromatic Pyrimidine	< 4.0	< 4.0	4.1	4.6	< 4.0	4.3	4.4	< 4.0
14		< 4.0	< 4.0	< 4.0	< 4.0	< 4.0	4.2	< 4.0	< 4.0

[a] R1 = *meta*-chloroanilino. All assays run at K_m [ATP] for each kinase. Data is sorted versus decreasing p38 potency after quinazoline **c84**. Activity values quoted are an average of a minimum of two assay experiments.

Earlier, it was highlighted that scaffold **75** was one of the examples tested showing reasonable inhibition of PI3Kα with a range of R1 groups, the most active in this case being a directly linked *para*-methylphenyl group (pIC_{50} = 5.1 in PI3Kα, and no significant activity elsewhere in the panel). The positioning of the methyl group on the five-membered ring of the scaffold may hinder the binding via a typical 4-anilinoquinazoline binding mode. Again, it is possible that an alternative binding mode could be accessible, one in which binding to the hinge is mediated via an alternative nitrogen (Figure 4, lower panel). This mechanism appears to have precedent in p38α itself,[33] and indeed, analysis of Figure 3 indicates that after PI3Kα, p38α is the next most susceptible kinase to inhibition by scaffold **75**. Despite this, no activity is seen in p38α from this specific compound, suggesting that the R1 substituent is not accommodated in p38α. Another explanation is that the compound detailed with scaffold **75** binds through the specified nitrogen in the 'alternative binding mode' in Figure 4 but with the rest of the molecule flipped over 180°, so the *para*-methylphenyl group is positioned toward the solvent, as opposed to the selectivity pocket.

Although alternative binding modes may explain some differences in selectivity profiles between compounds in the data set above, it is unlikely to be the only explanation. No appreciable activity is seen with **14**, **63**, and **69** against p38α, indicating these are less suitable in the context of this kinase, with this R1 substituent. These examples would appear to

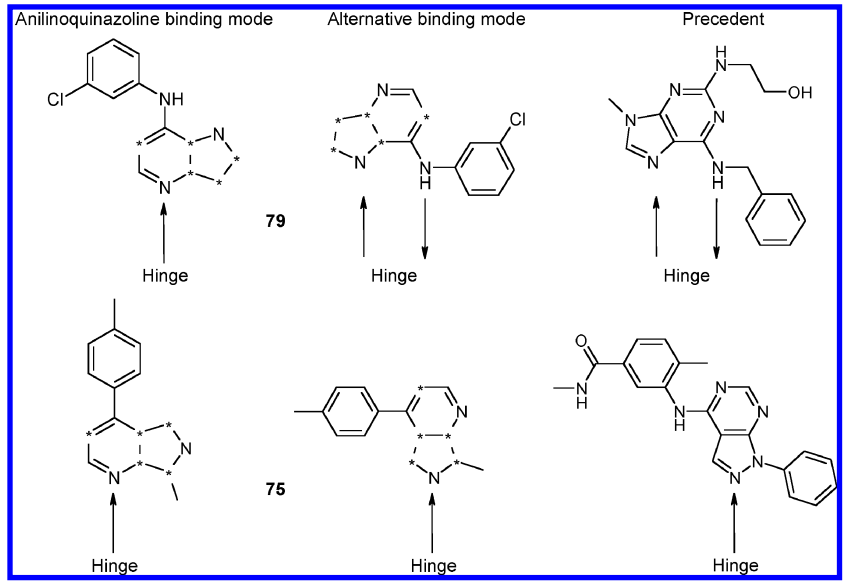


Figure 4. Alternative binding modes are potentially accessible. The specific structures of cores **75** and **79** cannot be released. (a) Upper panel: an olomoucine-like alternative binding mode for compounds such as **79** (R1 = *meta*-chloroanilino) with an appropriate 1,4 donor−acceptor relationship. Olomoucine binding mode shown upper right. (b) Lower panel: an alternative binding mode proposed for scaffold **75** (R1 = *para*-methylphenyl) in PI3Kα, which has precedent in p38α (example PDB: 3CG2 lower right).

have the required key interactions to bind similarly to a 4-anilinoquinazoline, but little activity was observed. It appears that these relatively low molecular weight compounds are able to affect the kinase selectivity profile through their different hinge region binding groups or through an adverse effect on the conformation of the R1 group. Although this region of kinases has a high degree of conservation, there are key residues immediately around the hinge region where different cores might achieve different selectivity profiles, while binding to the same primary target.

CONCLUSION

This paper has described a computational method for enumerating and prioritizing kinase hinge region motifs based on an anilinoquinazoline binding mode for subsequent synthesis. From an analysis exploiting computational, medicinal, and synthetic chemistry input, 8479 compounds were synthesized around this broad binding motif. The main goal for this was a desire to enrich the AstraZeneca compound collection in compounds that might provide useful start points in future collection screens. A total of 29 potential hinge binding groups were generated and elaborated in chemical libraries. These compounds are relatively new to the corporate collection, however, 441 of these have already shown kinase activity out of the 756 compounds tested. When a 140 compound subset was tested against a panel of 8 diverse kinases, we observed hit rates of between 21−75%. As a selected example, with the *meta*-chloro-4-anilinoquinazoline cores used as a benchmark, we identified a number of templates which showed activity against KDR and p38α. In addition to identifying ring systems with comparable activity (and selectivity), it proved possible to find more active hinge region binding groups as well as additional examples with likely alternative binding modes, thereby producing different selectivity profiles. Specifically, some examples produced activity against CDK2, and others were active against PI3Kα. High lipophillicity is a common issue with kinase hinge region binding groups, but we were able to identify less lipophillic cores compared to that of the quinazoline, which maintained activity. For some compounds synthesized in this study, we have also seen activity against nonkinase targets where the key interactions conserved in the enumeration overlap with the pharmacophore of another target or target class.

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