Development of Potent Inhibitors of Receptor Tyrosine Kinases by Ligand-Based Drug Design and Target-Biased Phenotypic Screening

Samuel H. Myers,† Carolin Temps,‡ Douglas R. Houston,§ Valerie G. Brunton,† and Asier Unciti-Broceta*†§

†Cancer Research UK Edinburgh Centre, MRC Institute of Genetics and Molecular Medicine, University of Edinburgh, Crewe Road South, Edinburgh EH4 2XR, U.K.
§Institute of Quantitative Biology, Biochemistry and Biotechnology, University of Edinburgh, Edinburgh EH9 3BF, U.K.

ABSTRACT: Pyrazolopyrimidines with potent antiproliferative properties were developed by an adaptive strategy that applies ligand-based design and phenotypic screening iteratively and is informed by biochemical assays. To drive development toward specific oncopathways, compounds were tested against cancer cells that overexpress, or not, AXL kinase. Identified phenotypic hits were found to inhibit oncotargets AXL, RET, and FLT3. Subsequent optimization generated antiproliferative lead compounds with unique selectivity profiles, including selective AXL inhibitors and a highly potent inhibitor of FLT3.

INTRODUCTION

The R&D costs of new drug development are continuously increasing, with figures of $1.4 billion being estimated as the average out-of-pocket cost to bring a new drug into the market.1,2 In response to this challenging situation,3 many medicinal chemistry laboratories are exploring strategies that prioritize the search for phenotypic hits over target-based approaches to accelerate the earlier stages of drug discovery.4 Such strategies are particularly well suited to find multitargeted inhibitors against difficult-to-treat malignancies due to the heterogeneous nature of these cancers.5–7 Using a strategy that combines ligand-based design of focused compound libraries and phenotypic screening in an iterative manner, our lab has recently reported the discovery of novel anticancer kinase inhibitors with suitable drug-like properties in a fraction of the time and preclinical R&D costs typically required in the industry.7,8

The transmembrane protein AXL is a receptor tyrosine kinase (RTK) that belongs to the TAM (TYRO3, AXL, and MER) subfamily. The oncogenic role of AXL has come under the spotlight in recent years due to its correlation with multiple cancer-promoting processes.9 Incremented AXL signaling is associated with poor prognosis and drug resistance in both solid and hematological malignancies.10 This oncogenic effect can be mediated through AXL overexpression, upregulation of its ligand GAS6, or by interaction with other RTKs (e.g., EGFGR, HER2, FLT3),9,11,12 highlighting the relevant and complex role of AXL in drug resistance to both targeted therapies and chemotherapy. While several FDA-approved kinase inhibitors have been found to inhibit AXL activity as a secondary target,9 the absence of X-ray crystallographic data for the AXL kinase has limited the development of robust structure-based drug discovery activities until very recently (the first crystal structure of the AXL kinase domain in complex with an inhibitor was reported in 201713).

Because of the timely need of making small molecules able to interfere with AXL oncogenic pathways, we embarked on a medchem campaign to search for phenotypically active kinase inhibitors. Inspired by published literature on inhibitors of MER (a TAM family member),14 we investigated the development of focused libraries based on a pyrazolo[3,4-d]pyrimidine scaffold featuring an amino group at the C6 position (Figure 1). To drive development toward the discovery of kinase inhibitors that inhibit AXL signaling, cell viability assays were performed against two cancer cell lines: a drug resistant cancer cell line expressing AXL (positive discriminating cell model) and a cancer cell line that does not express AXL (negative discriminating cell model). After two rounds of synthesis and screening, followed by kinase inhibition profiling of selected hits showing preferential activity against the AXL-expressing cell line, novel phenotypically active inhibitors of AXL, RET, and FLT3 were discovered. Further optimization enabled the discovery of several lead compounds including eSM156, an FLT3 inhibitor with single-digit nM potency and high antiproliferative activity against acute myeloid leukemia (AML) cells.

RESULTS AND DISCUSSION

Design, Synthesis, and Screening of Compounds 7a–j. Wang and co-workers14 explored the development of trisubstituted pyrazolopyrimidines as MER inhibitors based
on a docking study with the cocrystal structure of MER and derivative S2.15 Because the TAM family kinases show remarkable similarities to one another, in addition to MER, all compounds were tested against AXL and TYRO3. Exploration at the C6 position with various alkylamino groups showed that the larger the alkyl, the higher the degree of selectivity for MER over AXL. Notably, the use of a methylamino group at that position resulted in a potent inhibitor exhibiting superior potency for AXL than for MER (Figure 1). This observation served as the starting point of the ligand-based design explored in this work.

A 10-member library of derivatives of 1 was synthesized using the synthetic route described in Scheme 1 (see full details in the Supporting Information). Commercially available 6-chloro-1H-pyrazolo[3,4-d]pyrimidine, 3, was treated with N-iodosuccinimide to iodinate the C3 position of the heterocycle (94% yield) and thus generate an activated position for Suzuki–Miyaura cross-coupling. The chloro atom at the C6 position of 4 was then substituted via a SNAr with methylamine in THF to give 5 in moderate yield (55%). On the basis of in-house investigations suggesting that the presence of a methylene-linked saturated five-membered ring at the N1 position of pyrazolopyrimidine scaffolds enhances kinase inhibition activity, intermediate 5 was alkylated via reaction with iodomethylcyclopentane or 3-bromomethyl-1,3-dioxolane to give 6a,b in moderate yield (41%). 3-Aryl derivatives 7a–j were prepared via Suzuki coupling in moderate to good yields (29–72%).

Derivatives 7a–j were then tested against two breast cancer cell lines: BT474 cells (AXL−), which does not express AXL, and AXL-overexpressing mouse breast cancer cells (AXL+), a cell line created in-house which is resistant to the pan-HER inhibitor saptatinib. AXL+ and AXL− cells were used as a positive and negative discriminating cell model, respectively, with the aim to find compounds with preferential activity against AXL+ cells and thereby bias subsequent chemical design toward the inhibition of AXL-associated oncopathways, without ruling out that further proteomics differences between these cell lines could drive the design toward additional targets. Reduction of cell proliferation was employed as the primary screening output, using the pan-RTK inhibitor foretinib (which is reported to inhibit AXL at 11 nM)9 as a positive control. EC50 values were calculated using 8-point half-log dose–response assays (0.03–100 μM) and the results plotted in Figure 2. The phenotypic screen showed that the majority of the compounds in this series were more active against AXL+ cells than AXL−, with three compounds, 7f, 7g, and 7h, displaying EC50 values equal to or lower than 10 μM. The most potent compound from this series was 7f, which exhibited sub-μM activity (0.83 μM) against AXL+ cells and a 2-fold potency increase over the AXL− BT474 cell line (1.64 μM). Notably, the antiproliferative potency of its close analogue 7g (containing a dioxolane group instead of a cyclopentane) was an order of magnitude lower, indicating that the presence of one or more oxygen atoms in that moiety negatively affects activity. Because of the high potency of 7f and its promising
A substituted 1,2,3-triazole group (Scheme 2) was linked to the pyrazolopyrimidine. Because the presence of a methylene-incorporation of more complex moieties at the N1 position of the scaffold studied in this work have not yet been reported. A substituted 1,2,3-triazole group (Scheme 2) was thus selected to facilitate the preparation of different analogues by click chemistry, study novel structure-activity relationships (SAR), and add freedom-to-operate to the chemical space under exploration. The alkyne handle was introduced by copper-catalyzed azide-alkyne cycloadditions were carried out with seven different organic azides using copper iodide, sodium ascorbate, and triethylamine to give 9a−g in good yields (>66%). Palladium-catalyzed Suzuki cross-coupling of the triazole-containing derivatives 9a−g with 6-(1-piperazinyl)pyridyl-4-boronic acid pinacol ester provided final compounds 10a−g.

The antiproliferative activity of 10a−g was then tested against AXL− and AXL+ cells. As shown in Figure 3, the most potent derivatives of this series were 10a (R = benzyl) and 10d (R = 2-(methoxycarbonyl)thieryl), both of which exhibited superior antiproliferative activity against AXL+ cells. However, the potency of 10a and 10d was lower than that of the phenotypic hit 7f. The introduction of aliphatic rings (10b,c) at the 3’ position of the triazole ring led to a dramatic reduction in potency. The direct linkage of phenyl rings to the 3’ position of the triazole (10e−g) did not improve activity over the benzyl-containing derivative 10a.

To shed light over the pharmacodynamic profile responsible for the antiproliferative properties of phenotypic hits 7f,g,h and 10a,d, kinase inhibition activities were evaluated against a panel of 12 protein kinases. The panel, which included the three members of the TAM family, was selected based on the selectivity profile frequently found for other AXL inhibitors. Kinase inhibition studies were performed by Reaction Biology Corporation, USA, by measuring 32P incorporation on the corresponding kinase substrate relative to DMSO. Calculated half-maximal inhibitory concentration (IC50) values are shown in Table 1.

The results from the kinase screenings confirmed that the most potent phenotypic hits from both libraries inhibited AXL kinase activity at low μM to sub-μM levels. However, much like many reported AXL inhibitors, in most cases the primary target was not the AXL kinase, with FLT3 and RET being the most potently inhibited kinases across the screen. Of note, all the hits showed selectivity over the TAM family members TYRO3 and MER, proving the importance of using a methylamino group at the C6 position of the scaffold to achieve subfamily selectivity. In agreement with the cell-based assays, derivative 7f displayed the greatest activity against AXL, FLT3, and RET: 0.12, 0.02, and 0.05 μM, respectively. Arguably, the most interesting hit from this screening was the 3’-benzyltriazolyl derivative 10a, which exhibited the second highest potency against AXL (0.4 μM) and, importantly, was the only inhibitor to display superior activity for AXL than for AXL+ cells.

Figure 2. EC50 values of 7a−j and the positive control foretinib against AXL− (red) and AXL+ (blue) cells. Cell viability assay: PrestoBlue reagent at day 5. Error bars: ±SD from n = 2.

Figure 3. EC50 values of 10a−g and reference hit 7f against AXL− (red) and AXL+ (blue) cells. Cell viability assay: PrestoBlue reagent at day 5. Error bars: ±SD from n = 2.
Table 1. IC_{50} Values (in μM) for 7f,g,h and 10a,d against a Selection of Recombinant Tyrosine Kinases

| kinase/hit | 7f | 7g | 7h | 10a | 10d |
|------------|----|----|----|-----|-----|
| AKT        | >10| >10| >10| >10 | >10 |
| Aurora A   | 0.16| 0.66| 0.56| 1.8 | 0.79|
| AXL \textsuperscript{a} | 0.12| 0.53| 1.3 | 0.40| 0.78|
| KIT        | 1.1 | 7.5 | >10| >10 | >10 |
| MER \textsuperscript{a} | 0.64| 6.2 | 8.7| 2.3 | 8.9 |
| MET        | 2.1 | >10| >10| >10 | >10 |
| SRC        | 1.1 | 0.89| 7.2| 1.0 | >10 |
| FLT3       | 0.02| 0.10| 0.25| 0.53| 0.86|
| VEGFR2     | 0.69| 4.5 | 7.5| >10 | >10 |
| mTOR       | >10| >10| >10| >10 | >10 |
| RET        | 0.05| 0.16| 1.2| 0.60| 0.43|
| TYRO3 \textsuperscript{a} | >10| >10| >10| >10 | >10 |

\textsuperscript{a}Member of TAM subfamily.

FLT3 and RET. This preferential selectivity against AXL was not observed with the close derivative 10d, suggesting that the flexible benzyl group provides a certain degree of conformational freedom that is advantageous for binding to the AXL protein but less favorable for FLT3 and RET.

Design, Synthesis, and Screening of Compounds 12a–f. On the basis of the cell assays and the kinase screening, six new derivatives of 10a were prepared to search for improved AXL inhibition. 12a–f were prepared in moderate yields (27–58%) by Suzuki cross-coupling of intermediate 9a with six different arylboronate/boronic acids. The diversity of the moieties incorporated at the C3 position (Figure 4) aimed to evaluate the importance of the H-donor and acceptors of the 6-(1-piperazinyl)-pyrid-3-yl moiety of 10a.

Following the protocol described above, 12a–f were tested against AXL\textsuperscript{−} and AXL\textsuperscript{+} cells and the resulting EC_{50} values plotted in Figure 5. Whereas none of the new analogues improved the activity of 10a against the AXL\textsuperscript{+} cell line, 12b showed a superior EC_{50}(AXL\textsuperscript{−}))/EC_{50}(AXL\textsuperscript{+}) ratio, potentially indicating enhanced AXL selectivity. Interestingly, the seemingly minor substitution of the pyridine N atom by a CH led to an inversion of the cell activity ratio, pointing out off-target effects. It was also noticeable that the attachment of large groups at the piperazinyl NH (12c,d) reduced antiproliferative activity against the AXL\textsuperscript{+} cell line. The substitution of the piperazinyl group by methylamino (12f) resulted in a completely inactive derivative.

As shown in Table 2, derivative 12b exhibited superior AXL inhibition (0.38 μM) and an increased degree of selectivity over FLT3 and RET in comparison to reference hit 10a. In silico studies provided preliminary insights into the binding mode of 12b in AXL and RET (see the Supporting Information). It is important to note that 10a displayed higher antiproliferative activity against AXL\textsuperscript{+} cells (Figure 5), which may be caused by superior cell permeant properties or by unidentified off-target activities. The rest of the compounds of this series were not as potent as 12b and showed poorer selectivity profiles. Derivative 12a, which was previously found to display a EC_{50}(AXL\textsuperscript{−}))/EC_{50}(AXL\textsuperscript{+}) < 1, did not elicit inhibition to any of the kinases tested in the screen, further proof that its “inversed” antiproliferative properties are a consequence of targeting proteins unrelated to AXL-associated oncopathways. Remarkably, the screening revealed that the supposedly AXL-selective
inhibitor BGB324 actually inhibits both AXL and FLT3 kinases with equivalent potency. This information, which to our knowledge is reported herein for the first time, further illustrates the difficulty of designing selective AXL inhibitors due to the structural similarities of the catalytic domains of AXL and other RTKs (e.g., FLT3 and RET). Furthermore, it underlines the value of discovering cell-active AXL kinase-selective inhibitors such as 10a and 12b.

Design, Synthesis, and Screening of Compounds 13a–d. At this point of the campaign, only 7f exhibited sub-
μM antiproliferative activity against the AXL* breast cancer cell model. According to the kinase screen (Table 1), 7f inhibited AXL with high potency (0.12 μM) and displayed even higher potency against FLT3 and RET (0.02 and 0.05 μM, respectively). These two RTKs have been strongly linked to various malignancies. FLT3 is upregulated in acute lymphoblastic leukemia,17 and the FLT3-ITD mutation is found in 30–40% of adult AML patients and associated with low survival rates. Because of its prognostic importance, the WHO recommends ascertaining the mutational status of FLT3 in AML patients.18 RET has been linked to numerous cancer types, including pancreatic,19 breast cancer,20 and AML.21 RET is associated with metastasis and cancer relapse, being a predictor of acquired resistance and poor patient prognosis. Because AXL has also been associated with AML,22 following the lab’s ethos of evolving development in response to inhibitors’ properties to accelerate the discovery of lead compounds, the program was steered toward AML.

Four derivatives featuring a cyclopentylmethyl moiety at N1 position (as 7f) were prepared by Suzuki coupling of intermediate 6a with the corresponding arylboration/boronic acids. The substituents at the C3 position of 13b–d (Figure 6) were the same as the ones previously used for 12b–d (Figure 4). However, because derivative 12a displayed no inhibition of AXL, FLT3, or RET (see Table 2), a N-methyl derivative of the 4-pyrazinylphenyl group used in 12a was incorporated at C3 on derivative 13a.

The antiproliferative properties of 13a–d were tested against the AML cell lines MV4-11 (homozygous FLT3/ITD mutant23 and MOLM-13 (heterozygous for FLT3/ITD mutation)), using the dual AXL/FLT3 inhibitor BGB324 as a positive control. As shown in Figure 7, derivatives 13a and 13b displayed sub-μM EC50 values, highlighting the potent antiproliferative activity of derivative 13a, which displayed in both cell lines superior potency than BGB324, an inhibitor that is currently in clinical development for the treatment of AML.23

Given the fairly similar MW and cLogP of derivatives 13a–d (Figure 6), it was anticipated that the different antiproliferative activity observed for each member of this series were caused by variations in on-target potency. Biochemical assays were then performed for 13a–d against the 12 recombinant kinases tested cells. Derivative 13a, the most potent inhibitor of the series, displayed 1.4 nM activity (IC50) against FLT3 and IC50 values ranging from 6 to 13 nM for Aurora A, RET, and AXL. To attain a more detailed picture of the selectivity of the best hits, 13a,b and BGB324 were further screened against a selection of eight kinases commonly inhibited by FLT3 inhibitors: ALK, Aurora A, DDR1, EGFR, PDGFRα, and FLT3 mutants FLT ITD and FLT3 835Y. Derivative 13a showed selectivity over EGFR and PDGFRα, exhibited moderate potency against Aurora B and DDR1, and high potency against ALK, FLT ITD, and FLT3 835Y. The same trend was observed for 13b, although displaying a potency reduction of 4–8-fold relative to 13a. In comparison with the clinical candidate BGB324, the potent inhibitor 13a displayed 2-fold lower potency against RET, FLT3, and its mutants and 4-fold superior potency against Aurora A. However, it exhibited a 20-fold reduction in activity against AXL. It is important to note that 13a showed superior potency against MV4-11 and MOLM-13 cells, which may be due to improved membrane permeant properties or to the inhibition of other targets such as Aurora A.

Because of the high potency of 13a in vitro and in cells, an expanded kinome inhibition study was performed (single dose

Figure 7. EC50 values of 13a–d and BGB324 against MV4-11 and MOLM-13 cells. Cell viability assay: PrestoBlue reagent at day 5. Error bars: ±SD from n = 2.
of 1 μM, in duplicate) against 369 wild-type kinases. Enzymatic inhibition was determined to be >9% pure by HPLC.

**Synthesis of 3-ido-N-methyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (6a).** N-iodosuccinimide (1.9 g) was added to a suspension of 6-chloro-1H-pyrazolo[3,4-d]pyrimidine (1.0 g) in DMF (15 mL) and microwave-heated at 120 °C for 1 h. H2O (50 mL) and EtOAc (50 mL × 3) were added, and the organic layers were collected, washed with H2O (30 mL × 2) and brine (20 mL), dried over anhydrous MgSO4, and concentrated in vacuo. Flash chromatography (MeOH/DCM 0–2%) yielded 4 as a yellow solid (1.64 g, 91%). *1H NMR (500 MHz, DMSO) δ 14.64 (1 H, s, NH), 9.02 (1 H, s, CH). LRMS (+ve) m/z [M + 1] 281.00. 4 (500 mg) was dissolved in THF (3 mL), added to CH3NH2 (12 mL), and microwave heated at 150 °C for 1 h. A white solid precipitated out, dried by vacuum filtration, and washed with H2O (3 mL × 3) to yield 5 as a beige solid (270 mg, 55%). *1H NMR (500 MHz, DMSO) δ 7.58 (1 H, s, NH), 7.00 (2 H, d, J = 7.5), 4.25 (2 H, s), 1.63 (9 H, m). *13C NMR (126 MHz, DMSO) δ 162.5, 155.8, 154.2, 112.0, 92.3, 50.7, 32.1, 30.1, 28.3, 24.8. HRMS (ESI +ve) m/z [M + 1] 275.9737.

**Synthesis of 1-(Cyclopentylmethyl)-N-methyl-3-(4-[(4-methylpiperazin-1-yl)phenyl]-1H-pyrazolo[3,4-d]pyrimidin-6-amine (13a).** 6a (265 mg) in DMF (3 mL) was added NaH (34 mg) and stirred until gas evolution subsided. (Iodomethyl)cyclopentane (0.182 mL) was added dropwise and the resulting mix microwave-heated for 1.5 h at 150 °C. H2O (50 mL) and EtOAc (50 mL × 3) were added, and the organic layers were collected, washed with water (30 mL × 2) and brine (20 mL), dried over anhydrous MgSO4, and concentrated in vacuo. The crude was purified by flash chromatography MeOH/DCM (0–5%) to give 6a as a yellow solid (133 mg, 40%). *1H NMR (500 MHz, DMSO) δ 8.44 (1 H, s, NH), 7.61 (1 H, s), 4.11 (2 H, s), 2.86 (3 H, s), 1.65–1.21 (9 H, m). *13C NMR (126 MHz, DMSO) δ 162.5, 155.8, 154.2, 112.0, 92.3, 50.7, 32.1, 30.1, 28.3, 24.8. HRMS (ESI +ve) m/z [M + 1] 358.0523. 6a (60 mg), 4-(4-methylpiperazin-1-yl)phenylboronic acid (76 mg), K2CO3 (34 mg), PPh3 (20%mol), and Pd(OAc)2 (5%mol) were dissolved in 9:1 dioxane/water (5 mL) and microwave-heated at 120 °C for 1 h. H2O (50 mL) and EtOAc (50 mL × 3) were added, and the organic layers were collected, washed with water (30 mL × 2) and brine (20 mL), dried over anhydrous MgSO4, and concentrated in vacuo. The crude was purified by flash chromatography (MeOH/DCM 0–10%) to yield 13a as a yellow solid (35 mg, 45%). *1H NMR (500 MHz, CDCl3) δ 8.93 (1 H, s), 7.83 (2 H, m), 7.04 (2 H, m), 4.25 (2 H, d, J = 7.5), 3.34 (5 H, m), 3.09 (3 H, d, J = 5.0), 2.65 (6 H, d, J = 6.0), 1.41 (4 H, s), 1.41 (2 H, d, J = 5.5), 1.28 (3 H, s). *13C NMR (126 MHz, CDCl3) δ 161.4, 156.0, 153.5, 151.3, 143.9, 127.8, 115.9, 54.9, 50.8, 48.5, 46.1, 40.2, 30.3, 28.6, 24.9. HRMS (ESI +ve) m/z [M + 1] 406.2132.

**CONCLUSIONS**

Using an adaptive approach that combines ligand-based design of small libraries of pyrazolopyrimidines, target-informative cancer cell assays, and kinase screenings, two selective AXL inhibitors and a potent FLT3 inhibitor have been developed. The discovery of cell active AXL inhibitors 10a (eSM119) and 12b (eSM134) is relevant due to the lack of inhibitors that targets AXL with selectivity over TAM family members MER and TYRO3 and other RTKs (e.g., RET, FLT3). The merit of developing these lead compounds is further highlighted by the finding that BGB324, the only inhibitor in clinical development presumed to be selective for AXL, is actually a dual AXL/FLT3 inhibitor. By steering the program toward AML, the potent FLT3 inhibitor 13a (eSM156) was discovered, which displays single-digit nM IC50 against FLT3 and its mutants and low sub-μM EC50 against AML cells. Given its remarkable biological properties, eSM156 represents an attractive lead for future optimization against FLT3 and other proteins discovered in the kinome screening.

**EXPERIMENTAL SECTION**

**General.** Chemicals were purchased from Fisher, Sigma-Aldrich, or VWR. Microwave reactions were carried out in a Biotage Initiator. NMR spectra were recorded at rt on a 500 MHz Bruker Avance III spectrometer. Chemical shifts are reported in ppm relative to solvent peak. Analytical TLC was performed on Merck TLC Silica Gel 60 F254 plates and visualized by UV light. Purifications were performed by flash chromatography with commercially available silica gel and solvents. All compounds used in the biological screenings were determined to be >95% pure by HPLC.

**Journal of Medicinal Chemistry**

Figure 8. Map of the human kinome screened for 13a at 1 μM. Green circles denote <65% inhibition. Red circles denote >65% inhibition (>98% for the largest one).

Values listed in Supporting Information, Table 1. The study gave a S-score of 0.22 and, although not as potent as FLT3, most hits (>65% inhibition) belonged to the tyrosine kinase family. Besides FLT3, the tyrosine kinase JAK3 was also inhibited at levels superior to 98%. This is relevant as JAK3 is a proto-oncogene associated with different leukemias. Other strongly inhibited proteins from different families included ARKS, MAP4K family kinases, LRRK2, MINK1, RSK4, TNIK, TRKC, and TYK2.

**ASSOCIATED CONTENT**

S Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.7b01605.

Characterization of compounds, docking studies, and biological methods (PDF)

Molecular formula strings (CSV)

**AUTHOR INFORMATION**

**Corresponding Author**

Phone: (44)1317773584. Fax: (44)1317773520. E-mail: Asier.Unciti-Broceta@igmmed.ed.ac.uk.

**ORCID**

Asier Unciti-Broceta: 0000-0003-1029-2855

**Author Contributions**

All authors approved the final version of the manuscript.
concentration; RTK, receptor tyrosine kinase; SNAr, nucleophilic aromatic substitution; TAM, TYRO3, AXL and MER

AML, acute myeloid leukemia; IC50, half-maximal inhibitory concentration; RTK, receptor tyrosine kinase; SNAr, nucleophilic aromatic substitution; TAM, TYRO3, AXL and MER

REFERENCES

(1) Avorn, J. The $2.6 Billion pill — methodologic and policy considerations. *N. Engl. J. Med.* 2015, 372, 1877–1879.
(2) DiMasi, J. A.; Grabowski, H. G.; Hansen, R. W. Innovation in the pharmaceutical industry: New estimates of R&D costs. *J. Health. Econ.* 2016, 47, 20–33.
(3) Kola, I.; Landis, J. Can the pharmaceutical industry reduce attrition rates? *Nat. Rev. Drug Discovery* 2004, 3, 711–716.
(4) Lee, J. A.; Uhlik, M. T.; Moxham, C. M.; Tomandl, D.; Sall, D. J. Modern phenotypic drug discovery is a viable, neoclassic pharmacology. *J. Med. Chem.* 2012, 55, 4527–4538.
(5) Knight, Z.; Lin, H.; Shokat, K. M. Targeting the cancer kinase DFG-in through polypharmacology. *Nat. Rev. Cancer* 2010, 10, 130–137.
(6) Carragher, N.; Unciti-Broceta, A.; Cameron, D. Advancing drug discovery towards more agile development of targeted combination therapies. *Future Med. Chem.* 2012, 4, 87–105.
(7) Fraser, C.; Carragher, N. O.; Unciti-Broceta, A. eCF309: a potent, selective and cell-permeable mTOR inhibitor. *MedChemComm* 2016, 7, 471–477.
(8) Fraser, C.; Dawson, J. C.; Dowling, R.; Houston, D. R.; Weiss, J. T.; Munro, A.; Muir, M.; Harrington, L.; Webster, S. P.; Frame, M.; Brunton, V.; Patton, E. E.; Carragher, N. O.; Unciti-Broceta, A. Rapid discovery and structure—activity relationships of pyrazolopyrimidines that potently suppress breast cancer cell growth via SRC kinase inhibition with exceptional selectivity over ABL kinase. *J. Med. Chem.* 2016, 59, 4697–4710.
(9) Myers, S. H.; Brunton, V. G.; Unciti-Broceta, A. AXL inhibitors in cancer: A medicinal chemistry perspective. *J. Med. Chem.* 2016, 59, 3593–3608.
(10) Gay, C. M.; Balaji, K.; Byers, L. A. Giving AXL the axe: targeting AXL in human malignancy. *Br. J. Cancer* 2017, 116, 415–423.
(11) Park, I. K.; Mundy-Bosse, B.; Whitman, S. P.; Zhang, X.; Warner, S. L.; Bearss, D. J.; Blum, W.; Marcucci, G.; Caligiuri, M. A. Receptor tyrosine kinase Axl is required for resistance of leukemic cells to FLT3-targeted therapy in acute myeloid leukemia. *Leukemia* 2015, 29, 2382–2389.
(12) Schoumacher, M.; Burbridge, M. Key roles of AXL and MER receptor tyrosine kinases in resistance to multiple anticancer therapies. *Curr. Oncol. Rep.* 2017, 19, 19.
(13) Gajiwala, K. S.; Grodsky, N.; Bolanos, B.; Feng, J.; Ferre, R.; Timofeevski, S.; Xu, M.; Murray, B. W.; Johnson, T. W.; Stewart, A. The Axl kinase domain in complex with a macrocyclic inhibitor offers first structural insights into an active TAM receptor kinase. *J. Biol. Chem.* 2017, 292, 15705–15716.
(14) Liu, J.; Yang, C.; Simpson, C.; DeRyckere, D.; Van Deussen, A.; Miley, M. J.; Kireev, D.; Norris-Driouin, J.; Sather, S.; Hunter, D.; Korboukh, V. K.; Patel, H. S.; Janzen, W. P.; Machius, M.; Johnson, G. L.; Earp, H. S.; Graham, D. K.; Frye, S. V.; Wang, X. Discovery of small molecule mer kinase inhibitors for the treatment of pediatric acute lymphoblastic leukemia. *ACS Med. Chem. Lett.* 2012, 3, 129–134.
(15) Huang, X.; Finerty, P., Jr.; Walker, J. R.; Butler-Cole, C.; Vedadi, M.; Schapira, M.; Parker, S.; Turk, B.; Thompson, D. A.; Dhe-Paganon, S. Structural insights into the inhibited states of the Mer receptor tyrosine kinase. *J. Struct. Biol.* 2009, 165, 88–96.

We thank Scottish Power and CRUK for funding. C.T. thanks the CMVM for a Principal’s Scholarship.

Abbreviations Used

AML, acute myeloid leukemia; IC50, half-maximal inhibitory concentration; RTK, receptor tyrosine kinase; SNAr, nucleophilic aromatic substitution; TAM, TYRO3, AXL and MER