Supporting Information

Rapid Discovery of a Novel Series of Abl Kinase Inhibitors by Application of an Integrated Microfluidic Synthesis and Screening Platform

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Platform Description and Operation

The platform consists of nine integrated sub-systems to enable fully automated hit to lead optimisation with closed-loop feedback. There are six hardware sub-systems; a reagent handler, a flow synthesis system, a HPLC purification system, an analysis and quantification system, a fraction capture and dilution system and a bioassay system, Figure S1. In addition there are three software sub-systems, one controls hardware and the overall process, one for informatics and one for design. These are not described in detail here.

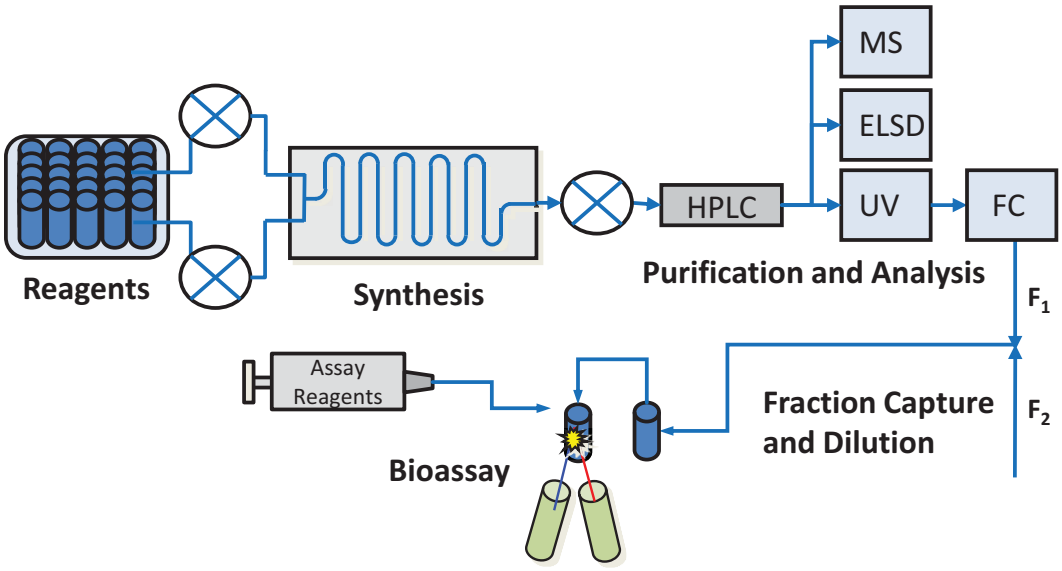


Figure S1. Schematic showing the continuous fluidic path taken by reagents and products on the platform.

Platform Components

Reagent Handler: The reagent handling system is based on a commercial xyz robot. Reagents are stored as solutions within glass vials accessible by the robots aspiration and dispense needle. An aliquot of a selected reagent can be transferred from the vial to any one of four reagent loop/valve assemblies on the synthesis system via injection ports.

Flow Synthesis System: The flow synthesis system utilises the Vapourtec R4 reactors and R2 pump modules with integrated valves and reagent loops controlled by Flow

Commander™software. Up to four reactors, pumps and valves are used depending on the complexity of the chemistry. The output from the final reactor flows to a HPLC injection valve enabling an aliquot of product to be injected onto the purification system. Loss of material due to dispersion in the synthesis system is minimised in several ways. Firstly, small bore tubing is used throughout the system as this minimises dispersion. Secondly, the reagent loop sizes are selected to ensure a steady state concentration of reactants and product is achieved in the reactor. Finally, the injection to HPLC is timed to ensure that an aliquot is taken at the point of maximum product concentration, i.e. under steady state conditions.

Purification System: Purification is carried out by analytical scale reverse phase HPLC on a Waters Acquity system. Gradient elution is performed using either an acetonitrile/water or methanol/water gradient with acid or base modifiers as appropriate.

Analysis and Quantification System: Analysis of the components eluted from the HPLC column is performed by ultraviolet light (UV) absorption spectrometry using a Waters Acquity UV detector, mass spectrometry (MS) using a Waters single quad mass spectrometer and evaporative light scattering detection (ELSD) using a PolymerLabs ELS detector. The Waters components are controlled using Waters MassLynx software. The MS extracted ion current is used to trigger capture of the expected product. The ELSD is used to quantify the amount of product going into the assay using a calibration method based on a literature procedure[2]. Hydroxyethyl theophylline, cortisone, dibenzyl phthalate and 9-phenyl anthracene were selected as standards due to their greater solubility. The accuracy of quantitation had been established to be +/- 30% across a large number of diverse chemical structures.

Fraction Capture and Dilution System: Following detection of the required synthesis product an aliquot of the product is captured using mass triggered fraction collection. This solution is immediately diluted using a microfluidic continuous flow methodology. The dilution ratio can be fine-tuned by varying the flow rates F_1 and F_2 of the confluent streams of product and diluent. Typically, a 100 fold dilution is sufficient to provide a 10μM to 60μM solution of product in assay buffer, containing <1% organic solvent.

Bioassay System: The bioassay system incorporates a collection station, a refrigerated reagent station, integrated liquid handling robotics, plate store and an embedded fluorescence plate reader. Utilising the liquid handling robotics an aliquot of the diluted product is transferred to a well-plate and mixed with biochemical reagents, resulting in a two-fold

dilution. A fifteen-point three-fold dilution series of inhibitor is then generated. Additional biochemical reagents are dispensed, the plate is incubated as required and the fluorescence signals measured on the plate reader, from which an IC_{50} value is calculated.

Platform Operation for Hit to Lead Optimisation: Prior to starting a hit to lead optimisation experiment the platform is set up with appropriate reagents. Experimental parameters for chemistry, design, purification, dilution and assay are loaded to the control software and the experiment started by the operator. Initially the control software calls the design algorithm to predict the first compound to synthesise and then passes the required information (e.g. which reagents are required) to the synthesis system. The selected compound is synthesised and screened, the informatics system is updated with the experimental data and the design model is updated with the IC_{50} value to complete the iteration. Subsequent iterations run automatically, without human intervention, in a serial process to build the SAR within the chemical space available on the platform.

Biology - Materials and Methods

General bioassay material and methods. Unless otherwise indicated all biochemical reagents were purchased from Sigma-Aldrich Chemical Company, Poole, Dorset, UK or ThermoFisher Scientific, Loughborough, Leicestershire, UK and were of analytical or higher grade. All solvents were purchased from ThermoFisher Scientific and of HPLC or analytical grade. Imatinib (Gleevec®), Ponatinib and VX-680 were purchased from Selleck Chemicals, Houston, TX, USA. All compounds were routinely made up to 10 mM in one hundred percent dimethylsulfoxide (DMSO) and diluted appropriately; ensuring the final DMSO concentration was less than 0.5% (vol./vol.).

All assays were routinely carried out at 25 ± 2°C. Manual data analysis was carried out using Prism software v 5.04 (GraphPad Inc., San Diego, CA, USA) using in-built standard Michaelis-Menten saturation model for substrate kinetics and the four parameter logistic-variable slope model for IC_{50} determinations. For all manual assays the sample size ("*n*") was four, for automated assays the sample size was one. Assays were routinely carried out in 384-well plates (Costar 3574; ThermoFisher) using either a SpectraMaxParadigm (TUNE cartridge) or SpectraMaxGemini XS plate reader employing SoftMax Pro v 6.2.1 or SoftMax Pro v 3.1.2 software respectively (Molecular Devices UK Ltd., Wokingham, Berkshire, UK).

Kinase assays Unless otherwise indicated all the kinase assay reagents were purchased from Life Technologies, Paisley, Renfrewshire, UK. The Omnia® kinase activity assay technology was employed to monitor real-time kinase activity. The base assay buffer was 1 X Omnia Reaction Buffer containing 1 mM 2-mercaptoethanol (ThermoFisher), 100 µM DTT and 0.1% (wt./vol.) Brij 35 (B4184; Sigma). The final assay conditions for ABL1 were 10 nM ABL1 (P3049; Life Technologies), 17 µM Tyr 6 peptide substrate (K_M^{app}= 16.9 ± 1.5 µM) and 12 µM ATP (K_M^{app}= 11.7 ± 0.8 µM) in assay buffer. For ABL2 the final assay conditions were 10 nM ABL2 (PV3266; Life Technologies), 5 µM Tyr 6 peptide substrate (K_M^{app}= 4.8 ± 0.3 µM) and 40 µM ATP (K_M^{app}= 41.9 ± 2.1 µM) in assay buffer.

Manual assays For manual IC_{50} determinations, 25 µL enzyme solution was dispensed to the respective column plus an additional 25 µL to the top well. Inhibitor (≤2 µL) was added to the top well and the solution double diluted down the column. Assays were initiated by the addition of 25 µL substrate solution at concentration equivalent to twice

the K_M^{app}. Residual enzyme activity was monitored by fluorescence (excitation 360 nm; emission 485 nm) collecting data every 10 seconds for 15 minutes.

Automated bioassays

Automated bioassay hardware. The CIDP bioassay module consisted of a collection station, a refrigerated (12 – 15°C) reagent station, liquid handling robotics, plate store and an embedded plate reader (Gemini XS using SoftMax Pro v 3.1).

Bioassay process. At the appropriate time the control software initiated the bioassay process. Utilising the integrated liquid handling robotics a three-fold dilution series of inhibitor was generated to which was added enzyme solution (40 nM ABL1 or 40 nM ABL2 in assay buffer) followed by substrate solution (for ABL1: 68 μM Tyr 6 peptide substrate and 48 μM ATP in assay buffer; for ABL2: 20 μM Tyr 6 peptide substrate 160 μM ATP in assay buffer). Residual enzyme activity was monitored by fluorescence (excitation 360 nm; emission 485 nm); collecting data every 10 seconds for 10 minutes. The rate data for each assay was fitted by linear regression and the rate data auto-saved as a text file for processing by Matlab as described below.

Process control and data analysis. MatLab (MathWorks, Cambridge, U.K.) software was used to manage the automated processes using code written by Cyclofluidic Limited. Briefly, the MatLab management software monitored the various input/output signals via data acquisition cards (DAC) (PCIe-6259; National Instruments Corporation (U.K.) Ltd., Newbury, Berks., U.K.). Inputs as voltage signals from detectors or contact closures from components were sent to the DAC to be monitored by MatLab. Event-based signals were sent as outputs to the various components to execute sub-processes (*e.g.* valve switching, pumping rates, *etc*.). On saving the bioassay data file, theMatLab management software opened, processed and analysed the data. The data was analysed by a non-linear regression analysis, based on a four parameter logistic fit to determine the IC_{50} value. The span was fixed between zero (*i.e.* no activity rate) and the maximum observed positive control rate (*i.e.* row P). The initial compound concentration was determined from the ELSD data. To ensure consistent output, rules were set up to govern bioassay data integrity. In the first instance if no less than seventy-five percent activity or no greater than twenty-five percent activity were observed the data was rejected. This ensured that there was sufficient titration data for data analysis to be carried out.

Thereafter the quality of the fit was judged by the R-squared value. If this value fell below 0.85 then the data was rejected. In all cases rejection led to a bioassay failure tag.

Kinase panel screening Inhibition assays with ABL1 (H396P), ABL1 (M351T), ABL1 (Q252H), ABL1 (T315I), ABL1 (Y253F), ABL2/ARG and P38a/MAPK14were carried out as a service by Reaction Biology Corporation, Malvern, PA, USA.

Microsomal stability and PAMPA assays. In vitro human microsomal stability and parallel artificial membrane permeability assay (PAMPA) assays were carried out as a service by Cyprotex Discovery Ltd., Macclesfield, Cheshire, UK.

Chemistry: Synthetic methods for compounds in Tables 1, 2 and 3

Representative Experimental Procedures and Analytical Data (MS, HPLC, NMR) For Novel Templates

3-Iodo-4-methyl-N-(3-((4-methylpiperazin-1-yl)methyl)-5-(trifluoromethyl)phenyl)benzamide (8-1) (93%)

In a 50 mL single necked round bottom flask equipped with a magnetic stirrer bead and a glass stopper was prepared a solution of 3-iodo-4-methylbenzoyl chloride (1.05 g, 3.74 mmol) in tetrahydrofuran (10 mL). To this is added 3-((4-methylpiperazin-1-yl)methyl)-5-(trifluoromethyl)aniline (1.03 g, 3.74 mmol), DMAP (0.046g, 0.374 mmol) and diisopropylethylamine (1.3 ml, 7.49 mmol) in one portion, resulting in an off-white suspension. The suspension is allowed to stir for 1 hour at 25 ^{o}C. To the reaction mixture is added 50 mL water and the aqueous phase is extracted with ethylacetate (3 x 20 ml). The combined organic phase was separated, dried over anhydrous sodium sulphate, filtered under gravity and the filtrate is evaporated to dryness under reduced pressure (on a rotary evaporator) to give a solid residue. The solid is purified by flash column chromatography using 10 % MeOH in dichloromethane to give an off white solid (88 %, 1.7 g).

N-(4-((4-(2-Hydroxyethyl)piperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)-3-iodo-4-methylbenzamide (8-2) (69%)

1H NMR ($CDCl_3$): δ 8.30 (d, *J*= 4, 1H), 8.22 (s, 1H), 7.90 (m, 1H), 7.88 (m, 1H), 7.85 (m, 1H), 7.77 (m, 1H), 7.75 (m, 1H), 7.72 (s, 1H), 7.31 (d, *J* = 8, 1H), 3.65 (s, 2H), 3.63 (br, 2H), 2.54 (m, 8H), 2.54 (br, 2H), 2.48 (s, 3H)

MS: m/z 548 $[M+H]^+$

N-(4-((4-Hydroxypiperidin-1-yl)methyl)-3-(trifluoromethyl)phenyl)-3-iodo-4-methylbenzamide (8-3) (44%)

1H NMR (d_6-DMSO): δ 10.4 (s, 1H), 8.39 (d, *J*=4, 1H), 8.14 (m, 1H), 7.99 (dd, *J*=4, 4H, 1H), 7.87 (dd, *J*=4, 4H, 1H), 7.68 (d, *J*=8, 1H), 7.46 (d, *J*=8, 1H), 2.60-2.75 (m, 2H), 2.04-2.10 (m, 2H), 2.42 (s, 3H), 1.64-1.73 (m, 2H), 1.33-1.44 (m, 2H)

MS: m/z 519 $[M+H]^+$

N-(4-((1H-Imidazol-1-yl)methyl)-3-(trifluoromethyl)phenyl)-3-iodo-4-methylbenzamide (8-4) (82%)

1H NMR (d_6-DMSO): δ 10.5 (s, 1H), 8.40 (m, 1H), 8.25 (m, 1H), 7.99-8.02 (m, 1H), 7.88-7.90 (m, 1H), 7.76 (br, 1H), 7.47 (d, *J* = 8, 1H), 7.16 (br, 1H), 7.09 (d, *J*= 12 , 1H), 6.97 (br, 1H), 5.35 (s, 2H), 2.42 (s, 3H)

MS: m/z 486 $[M+H]^+$

3-Iodo-4-methyl-N-phenylbenzamide (8-5) (93%)

1H NMR (d_6-DMSO): δ 10.2 (s, 1H), 8.38 (d, *J*= 4, 1H), 7.87 (dd, *J*= 4, 4, 1H), 7.73 (d, *J*= 4, 2H), 7.45 (d, *J*= 4, 1H), 7.31 (t, *J*= 8, 2H), 7.07 (t, *J*= 8, 1H), 2.43 (s, 3H)

MS:m/z EI 338 $[M+H]^+$

N-(3-Iodo-4-methylphenyl)-4-((4-methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)benzamide (16-1)

A 50 mL single necked round bottom flask equipped with a magnetic stirrer bead and a glass condenser was charged with a solution of 4-(bromomethyl)-N-(3-iodo-4-methylphenyl)-3-(trifluoromethyl)benzamide (3 g, 6.02 mmol) in tetrahydrofuran (10 mL). To this was added 1-methyl piperazine (2.41 g, 24.08 mmol) and the resultant mixture heated at reflux for 3 hours. The reaction mixture was cooled and added to 100 mL of water. The aqueous phase

was extracted with dichloromethane (2 x 50 mL), then the combined organic phase separated, dried over anhydrous magnesium sulphate, filtered under gravity and the filtrate evaporated to dryness under reduced pressure (on a rotary evaporator) to give a solid residue. The solid is purified by flash column chromatography using 5% MeOH in dichloromethane to give an off white solid 1.5g, (46 %).

1H NMR ($CDCl_3$) 8.10 (m, 1H), 8.08 (d, 1H, *J*=2), 8.05-8.08 (m, 1H), 8.0 (s, 1H), 7.95 (d, 1H), 7.55 (dd, *J*=4, 2, 1H), 7.20 (d, *J*=8, 1H), 3.75 (s, 2H) 2.4-2.6 (m, 8H), 2.4 (s, 3H), 2.3 (s, 3H)

MS:m/z 518 $[M+H]^+$

4-((4-(2-Hydroxyethyl)piperazin-1-yl)methyl)-N-(3-iodo-4-methylphenyl)-3-(trifluoromethyl)benzamide (16-2) (43%)

1H NMR ($CDCl_3$) 8.20 (s, 1H), 8.11(m, 1H), 8.08 (d, *J*=4, 1H) , 8.05-8.0 (m, 1H), 7.95 (d, 1H), 7.55 (dd, *J*=4, 2, 1H), 7.20 (d, *J*=8, 1H), 3.70 (s, 2H), 3.6 (t, 2H), 2.6-2.4 (m, 8H), 2.5 (t, *J*=4, 2H), 2.4 (s, 3H)

MS:m/z 548 $[M+H]^+$

4-((4-Hydroxypiperidin-1-yl)methyl)-N-(3-iodo-4-methylphenyl)-3-(trifluoromethyl)benzamide (16-3) (45%)

1H NMR (d_6-DMSO): δ 10.40 (s, 1H), 8.27 (d, *J*=4, 1H), 8.20 (m, 1H), 8.19 (m, 1H), 7.90 (d, *J*=8, 1H), 7.70 (dd, *J*=4, 2, 1H), 7.28 (d, *J*=8, 1H), 4.55 (d, 1H), 3.60 (s, 2H), 3.4-3.5 (m, 1H), 2.60-2.65 (m, 2H), 2.3 (s, 3H), 2.05-2.10 (m, 2H), 1.65-1.75 (m, 2H), 1.35-1.45 (m, 2H)

MS:m/z 519 $[M+H]^+$

4-((1H-Imidazol-1-yl)methyl)-N-(3-iodo-4-methylphenyl)-3-(trifluoromethyl)benzamide (16-4) (53%)

1H NMR (d_6-DMSO): δ 10.40 (s, 1H), 8.27 (m, 1H), 8.25 (d, *J*=4, 1H), 8.15 (m, 1H), 7.75 (s, 1H), 7.65 (dd, *J*=8,2, 1H), 7.30 (d, *J*=8, 1H), 7.17 (m, 1H), 7.05 (d, *J*=8, 1H), 6.95 (m, 1H), 5.47 (s, 2H), 2.30 (s, 3H)

MS:m/z 486 $[M+H]^+$

1-(5-Bromo-1-methyl-1H-pyrazol-3-yl)-3-(4-((4-methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)urea (19)

4-((4-methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)aniline (750 mg, 2.74 mmol) was dissolved in DMSO (5 ml) and carbonyldiimidazole (445 mg, 2.74 mmol) added in a single portion. After 30 minutes 5-bromo-1-methyl-1H-pyrazol-3-amine (483 mg, 2.74 mmol) was added and the reaction stirred for 1hour.The crude reaction mixture was poured into water and extracted with ethyl acetate. The organic layers were combined, dried ($MgSO_4$), filtered and evaporated to give the crude product. The crude compound was purified by column chromatography over silica using 10% MeOH / dichloromethane as eluent. The pure fractions were evaporated to give the product as an off white solid 530 mg, (40.6%).

1H NMR ($CDCl_3$): δ 8.49 (br, 1H), 8.27 (br, 1H), 7.74 (d, *J*=7.9, 1H), 7.72 (s, 1H), 7.54 (d, *J*=7.9, 1H), 6.08 (m, 1H), 3.82 (s, 3H), 3.68 (s, 2H), 2.80 (8H, m), 2.75 (s, 3H)

MS:m/z 475, 477 $[M+H]^+$ (bromide splitting)

General experimental procedure for alkynes

In a Biotage (Initiator Sixty) microwave reaction vial (2-5 mL) is prepared a suspension of the bromide or iodide (0.6 mmol), CuI (0.0057 g, 0.030 mmol), $PdCl_2(PPh_3)_2$ (0.0105 g, 0.015 mmol), diisopropylethylamine (0.194 g, 1.49 mmol) and ethynyltrimethylsilane (0.147 g, 1.49 mmol). Acetonitrile (2 mL) is used as a solvent. The reaction vessel is sealed with an aluminium crimp cap equipped with a rubber seal. The reaction vessel is irradiated with microwaves at 120 °C for 45 minutes. The reaction mixture contents from the reaction vial were transferred in a round bottom flask (50 mL) and the solvent was evaporated to dryness (on a rotary evaporator) to give a dark brown-black residue. The residue is re-dissolved in methanol (10 mL) and treated with potassium carbonate (0.2 g, 1.5 mmol). After stirring at room temperature for 45 minutes the crude reaction mixture is purified by flash column chromatography over silica using 10 % MeOH in dichloromethane as eluent.

Analytical Data for Novel Alkynes

Methyl 3-ethynylimidazo[1,2-a]pyridine-6-carbimidate (22-1) (19%)

1H NMR (d_6-DMSO): δ 9.41 (s, 1H), 8.76 (s, 1H), 8.01 (m, 1H), 7.89 (m, 1H), 7.87 (m, 1H) 5.15 (s, 1H), 3.83 (s, 3H)

MS:m/z 200 $[M+H]^+$

3-Ethynyl-6-(trifluoromethyl)imidazo[1,2-a]pyridine (22-3) (61%)

1H NMR (d_6-DMSO): δ 8.75 (m, 1H), 8.09(s, 1H), 7.86 (d, *J*=8, 1H), 7.60 (dd, *J*=8, 2, 1H), 5.15 (s, 1H)

MS:m/z 211 $[M+H]^+$

3-Ethynylimidazo[1,2-b]pyridazine (22-4) (25%)

1H NMR (d_6-DMSO): δ 8.64 (m, 1H), 8.20 (m, 1H), 7.32 (dd, *J*=4, 4, 1H), 4.93 (s, 1H)

MS:m/z 144 $[M+H]^+$

5-Ethynylimidazo[1,2-a]pyrazine (22-5) (55%)

1H NMR (d_6-DMSO): δ 9.13 (m, 1H), 8.19 (m, 2H), 7.95 (m, 1H), 5.39 (s, 1H, CH)

MS:m/z 144 $[M+H]^+$

3-Ethynylimidazo[1,2-a]pyrazine (22-6) (13%)

1H NMR (d_6-DMSO): δ 9.16 (s, 1H), 8.51 (d *J*=4, 1H), 8.15 (bm, 2H), 8.06 (d *J*=4, 1H), 5.19 (s, 1H)

MS:m/z 144 $[M+H]^+$

2-Ethynylquinoxaline (22-10) (66%)

1H NMR ($CDCl_3$): δ 8.92 (s, 1H), 8.10 (m, 1H), 7.81 (m, 1H), 3.44 (s, 1H)

MS:m/z 155 $[M+H]^+$

2-Ethynyl-5H-pyrrolo[2,3-b]pyrazine (22-11) (25%)

1H NMR (d_6-DMSO): δ 12.26 (s, 1H), 8.37 (s, 1H), 7.95 (d, *J*=4, 1H), 6.60 (d, *J*=4, 1H), 4.33 (s, 1H)

MS:m/z 144 $[M+H]^+$

3-Ethynyl-1,5-naphthyridine (22-12) (29%)

1H NMR (d_6-DMSO): δ 9.02 (dd, *J*=4,2, 1H) 9.00 (d, *J*=4, 1H), 8.52 (d, *J*=2, 1H), 8.42 (m,1H), 7.80 (dd, *J*=4,2, 1H), 4.65(s, 1H)

MS:m/z 155 $[M+H]^+$

6-Ethynylthiazolo[5,4-b]pyridine (22-13) (15%)

1H NMR ($CDCl_3$): δ 9.12 (s, 1H), 8.64 (d, *J*=4, 1H), 8.36 (d, *J*=4, 1H), 3.61(s, 1H)

MS:m/z 161 $[M+H]^+$

5-Ethynyl-N-phenylpyrimidin-2-amine (22-22) (56%)

1H NMR (d_6-DMSO): δ 8.85 (s, 2H), 6.95-7.0 (m, 5H), 3.85 (s, 1H)

MS:m/z 196 $[M+H]^+$

6-((Trimethylsilyl)ethynyl)imidazo[1,2-a]pyrimidine (22-25) (96%)

Used as Silyl protected alkyne in reaction. The silylated compound was consistent with the expected mass as characterized by LC-MS, m/z : 216 $[M+H]^+$

7-((Trimethylsilyl)ethynyl)-1H-imidazo[4,5-c]pyridine (22-26) (93%)

1H NMR (d_6-DMSO): δ9.15 (s, 1H), 8.50 (m, 1H), 8.14 (s, 1H), 8.05 (m, 1H), 5.19 (s, 1H)

MS:m/z 144 $[M+H]^+$

5-Ethynyl-2-methylthiazole (**22-27**) (17%)

Used as Silyl protected alkyne in reaction. The silylated compound was consistent with the expected mass as characterized by LC-MSMS:m/z 196 $[M+H]^+$

Flow synthesis of Imatinib (1)

Three stock solutions were made up: - 6-methyl-N1-(4-(pyridin-3-yl)pyrimidin-2-yl)benzene-1,3-diamine (100 mg, 0.361 mmol) and pyridine (0.146 ml, 1.803 mmol) were dissolved in

NMP (2 ml) to make stock 1. 4-(chloromethyl)benzoyl chloride (102 mg, 0.541 mmol) was dissolved in NMP (2 ml) to make stock 2 and 1-methylpiperazine (0.200 ml, 1.803 mmol) was dissolved in NMP (2.000 ml) to make stock 3. These stocks were automatically loaded to 300ul injection loops on a Vapourtec R4 synthesiser (Figure S2) from a Gilson 215 sample handler. The Vapourtec instrument was run with NMP as solvent and then the loops loaded from stock. Solutions 1 and 2 were mixed at 40°C in a 2ml coil with a residence time of 20 minutes. Next the loop loaded from stock 3 was added and the reaction heated at 80°C running through a second 2ml coil with a retention time of 13.2 minutes.

The peak of interest was captured purified and analyzed as described in the general experimental.

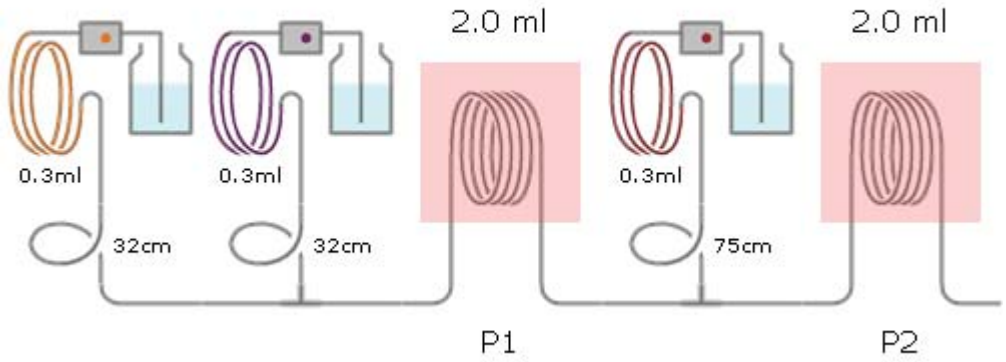
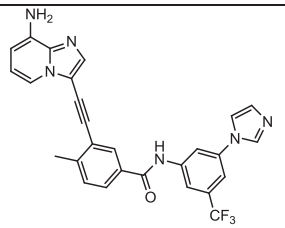
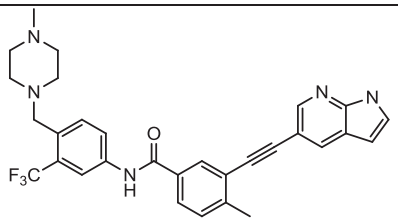
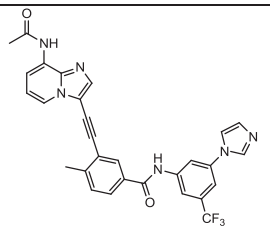
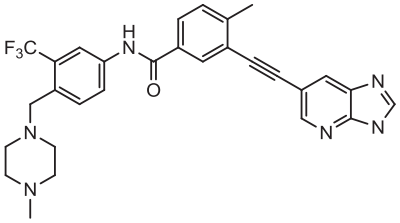
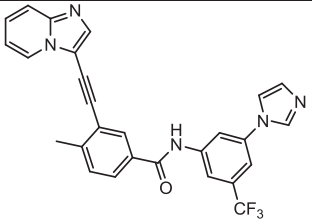
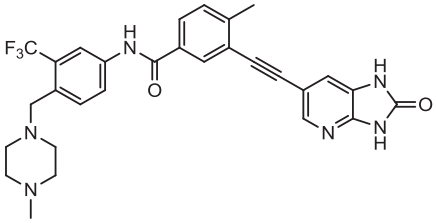
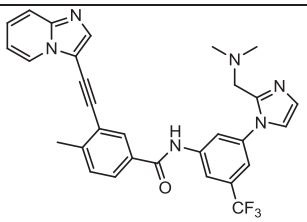
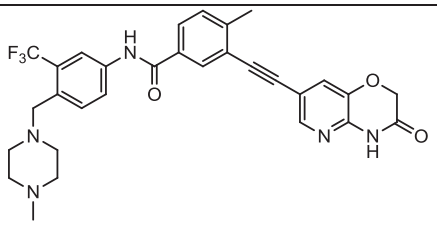
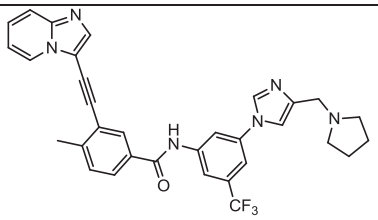
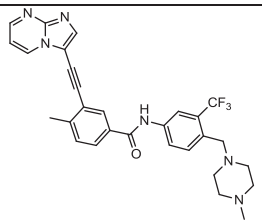
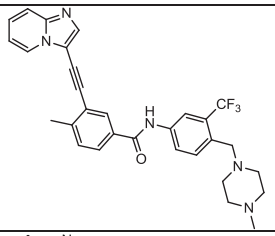
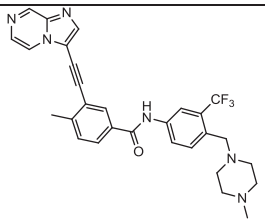
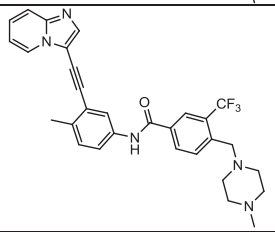
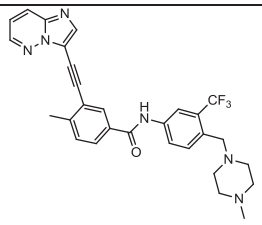
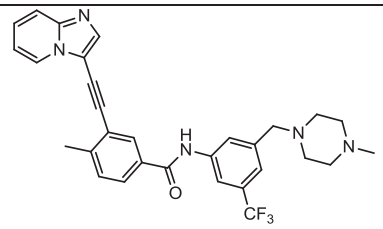
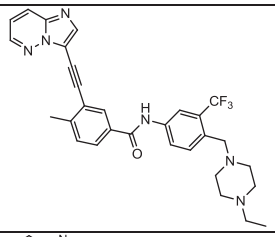
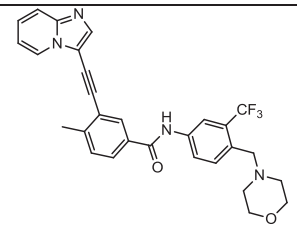
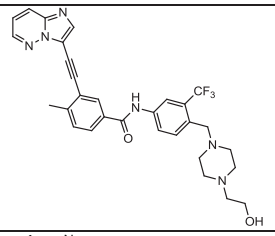
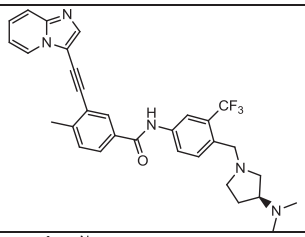
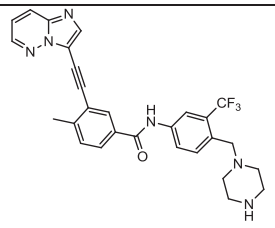
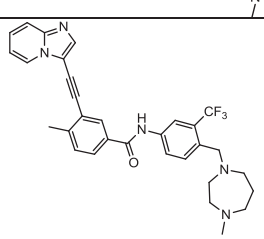
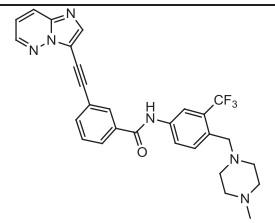
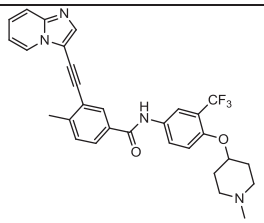
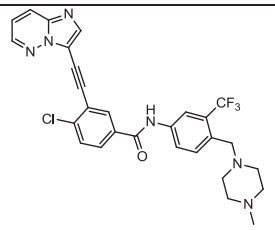
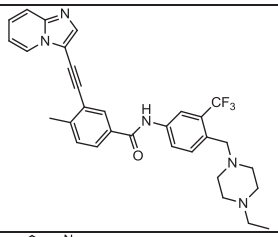
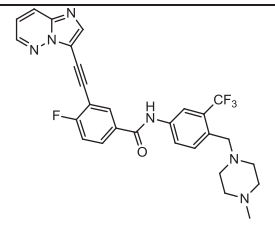
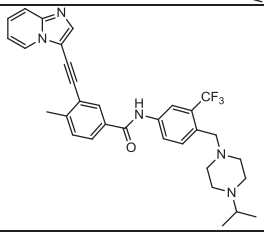
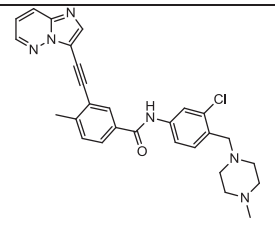


Figure S2. Schematic showing the fluidic path through the injection loops and two reactors on the flow synthesiser.

Table S1. Prior Knowledge Data[1]

Compound	Abl1 IC_{50}nM		Compound	Abl1 IC_{50}nM
	2.3			19
	6.4			18
	26			6.9
	45			42
	26			69
	9			2.3
	9			8.6

Compound	Abl1 IC_{50}nM		Compound	Abl1 IC_{50}nM
	24			14
	78			12
	15			2.3
	13			31
	9.1			41
	23			42
	56			2.4

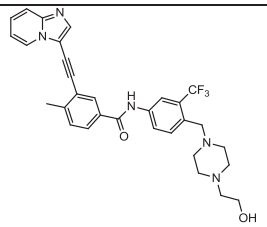
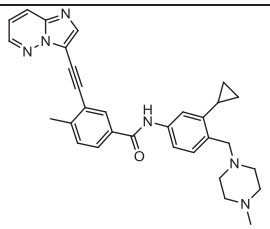
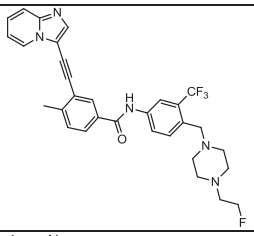
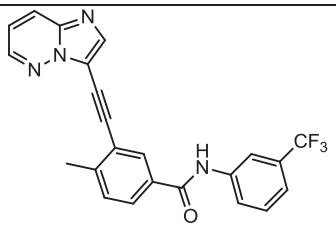
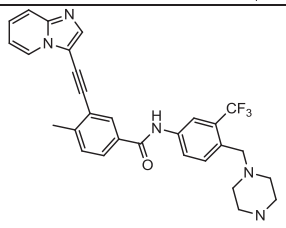
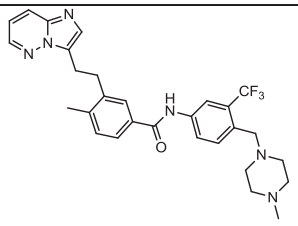
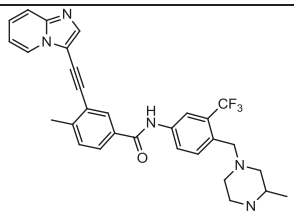
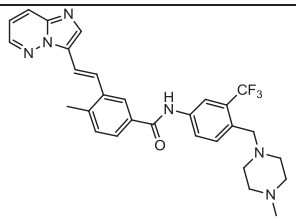
Compound	Abl1 IC_{50}nM		Compound	Abl1 IC_{50}nM
	19			5.3
	49			19
	14			19
	22			54

Table S2. Chemical and biological data generated on the automated platform.

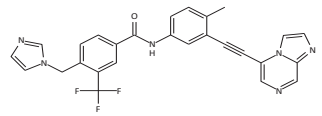
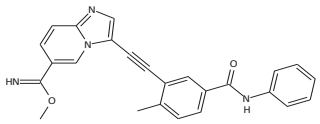
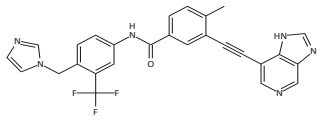
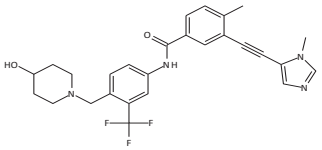
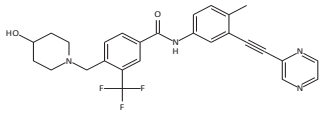
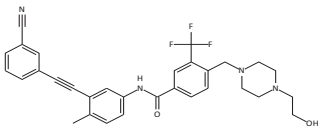
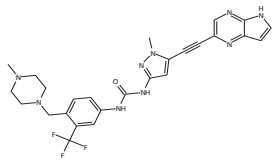
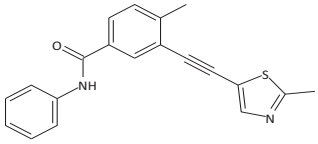
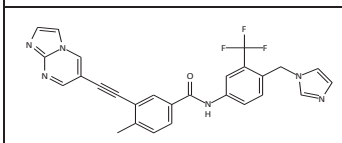
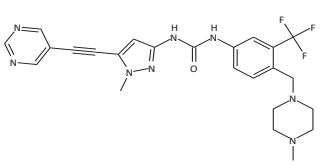
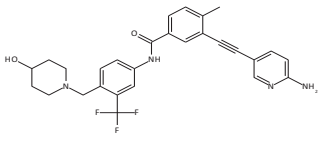
Compound	ELN ID (Compound No)	Synthesis Order [a]	ABL1 IC_{50} nM	ABL2 IC_{50} nM	HPLC Purity % [b]	Synthesis Yield (%) [c]	MS [d]: m/z $(M+H)^+$
	996-006-001-001	1	16	28	100	32	501
	996-006-002-001	2	>10,000	>10,000	78	N/R [e]	409
	996-006-003-001	3	N/A [f]	N/A		Failed Synthesis	
	996-006-004-001	4	190	900	100	N/R	497
	996-006-005-001	5	N/A	N/A		Failed Synthesis	
	996-006-006-001	6	N/A	N/A	100	Failed Synthesis	547
	996-006-007-001	7	9	43	100	25	538
	996-006-008-001	8	8000	>10,000	100	41	333
	996-006-009-001	9	N/A	N/A		Failed Synthesis	
	996-006-010-001	10	7	27	100	N/R	499
	996-006-011-001	11	180	1000	100	13	509

Table S2. Chemical and biological data generated on the automated platform.

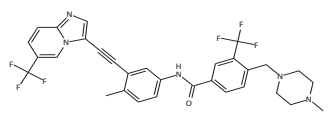
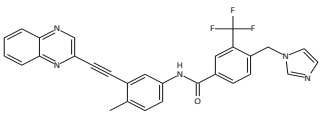
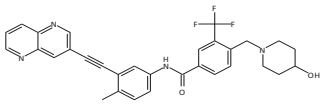
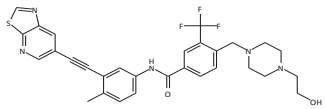
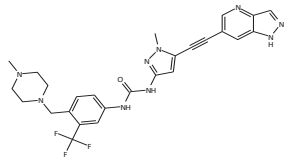
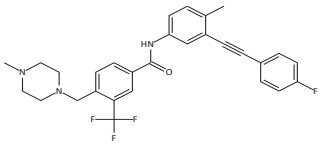
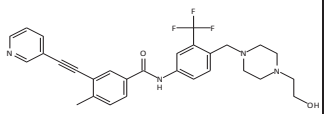
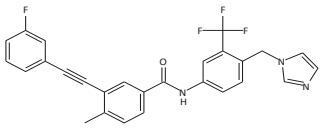
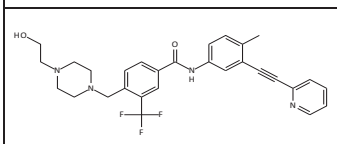
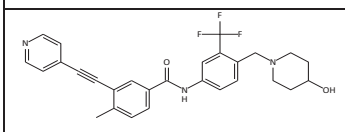
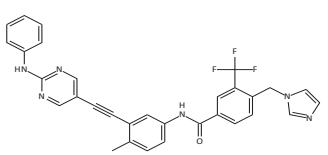
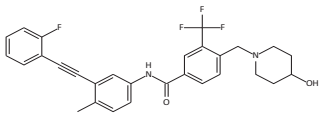
Compound	ELN ID (Compound No)	Synthesis Order [a]	ABL1 IC_{50} nM	ABL2 IC_{50} nM	HPLC Purity % [b]	Synthesis Yield (%) [c]	MS [d]: m/z $(M+H)^+$
	996-006-012-001	12	24	86	100	28	600
	996-006-013-001	13	130	450	100	32	512
	996-006-014-001	14	180	1000	97	N/R	545
	996-006-015-001	15	60	130	68	37	580
	996-006-016-001	16	6	14	100	N/R	538
	003-0222-001-001	17	1700	>10,000	100	N/R	510
	003-0225-001-001	18	N/A	N/A		Failed Synthesis	
	003-0225-002-001	19	>10,000	>10,000	100	34	478
	003-0225-003-001	20	6000	21000	100	42	523
	003-0225-004-001	21	>10,000	>10,000	100	12	494
	003-0225-005-001 (**27**)	22	230	>10,000	100	8	553
	003-0225-006-001	23	>10,000	>10,000	100	35	511

Table S2. Chemical and biological data generated on the automated platform.

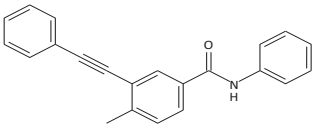
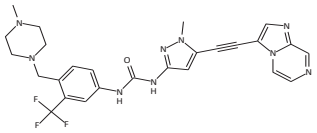
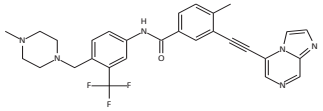
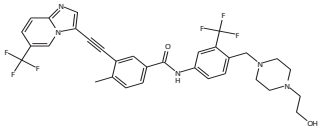
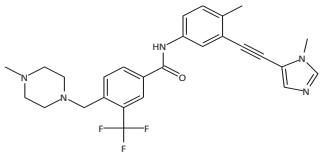
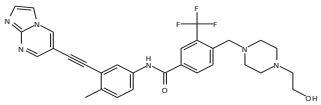
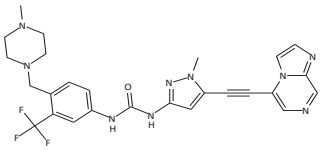
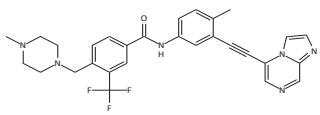
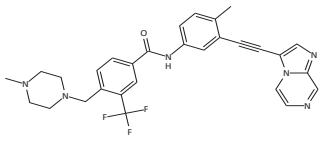
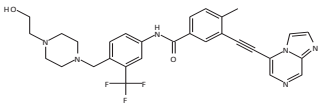
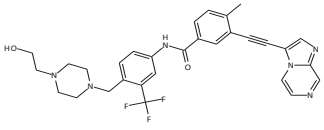
Compound	ELN ID (Compound No)	Synthesis Order [a]	ABL1 IC_{50} nM	ABL2 IC_{50} nM	HPLC Purity % [b]	Synthesis Yield (%) [c]	MS [d]: m/z $(M+H)^+$
	003-0225-007-001	24	>10,000	>10,000	100	16	312
	003-0225-008-001 (**25**)	25	0.4	3.3	97	26	538
	003-0225-009-001 (**24**)	26	0.2	2.3	100	45	533
	003-0225-010-001	27	59	95	100	22	630
	003-0225-011-001	28	11	19	100	38	496
	003-0225-012-001	29	N/A	N/A		Failed Synthesis	563
	003-0227-001-001 (**23**)	30	2	4.1	100	N/R	538
	003-0227-002-001	31	N/A	N/A		Failed Synthesis	
	003-0233-002-001	32	1	3	83	18	533
	003-0233-003-001	33	6	17	100	27	563
	003-0233-004-001	34	3	8	100	11	563

Table S2. Chemical and biological data generated on the automated platform.

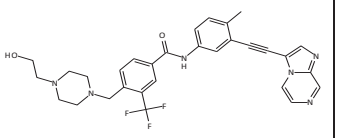
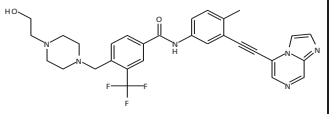
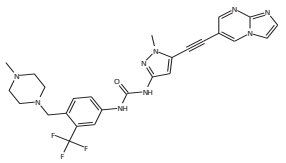
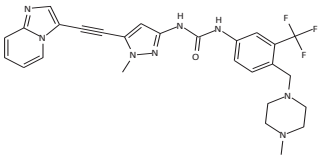
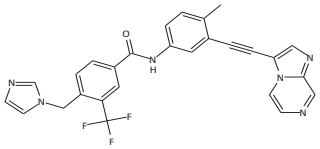
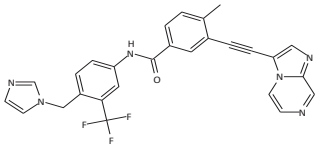
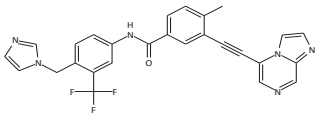
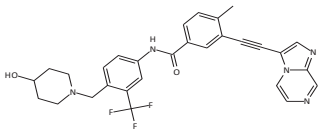
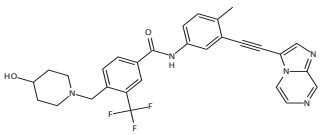
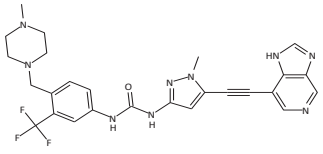
Compound	ELN ID (Compound No)	Synthesis Order [a]	ABL1 IC_{50} nM	ABL2 IC_{50} nM	HPLC Purity % [b]	Synthesis Yield (%) [c]	MS [d]: m/z $(M+H)^+$
	003-0233-005-001	35	2	3	93	16	563
	003-0234-001-001	36	5	5	100	34	563
	003-0234-002-001	37	N/A	N/A		Failed Synthesis	
	003-0234-003-001 (**28**)	38	3000	>10,000		12	537
	003-0234-004-001	39	7	40	100	17	501
	003-0234-005-001	40	N/A	N/A		Failed Synthesis	
	003-0234-006-001	41	17	36	100	29	501
	003-0234-007-001	42	N/A	N/A		Failed Synthesis	
	003-0234-008-001	43	N/A	N/A		Failed Synthesis	
	003-0234-009-001	44	3	9		8	538

Table S2. Chemical and biological data generated on the automated platform.

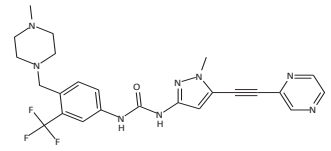
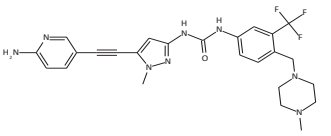
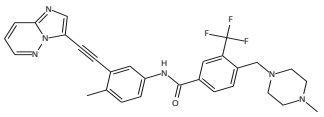
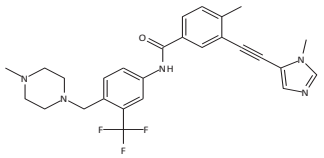
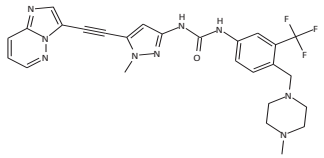
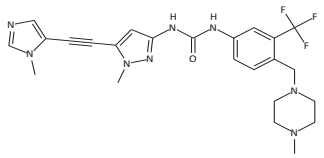
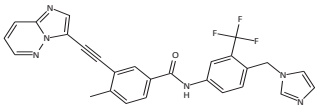
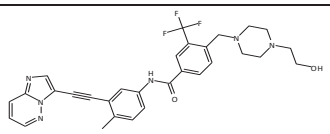
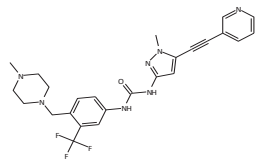
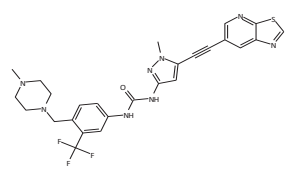
Compound	ELN ID (Compound No)	Synthesis Order [a]	ABL1 IC_{50} nM	ABL2 IC_{50} nM	HPLC Purity % [b]	Synthesis Yield (%) [c]	MS [d]: m/z $(M+H)^+$
	003-0236-001-001	45	N/A	N/A		Failed Synthesis	499
	003-0236-002-001 (**30**)	46	42	69	100	28	513
	003-0236-003-001 (**31**)	47	45	39	100	62	533
	003-0236-004-001	48	90	120	100	42	496
	003-0236-005-001 (**26**)	49	27	47	100	30	538
	003-0236-006-001	50	38	55	100	26	501
	003-0236-007-001	51	N/A	N/A		Failed Synthesis	
	003-0236-008-001	52	30	41	100	56	563
	003-0236-009-001 (**29**)	53	33	66	100	32	498
	003-0236-010-001	54	110	500	100	8	555

Table S2. Chemical and biological data generated on the automated platform.

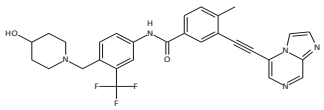
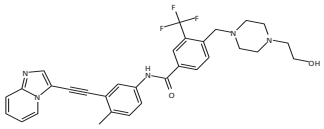
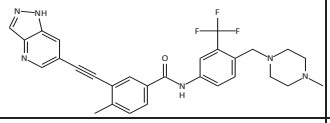
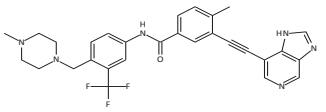
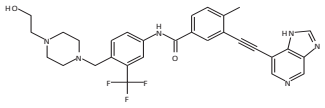
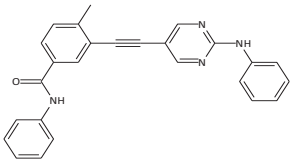
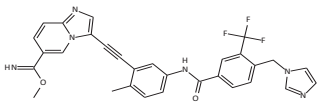
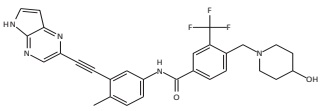
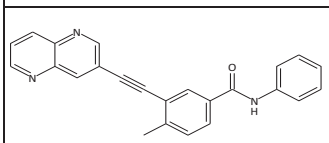
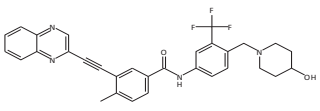
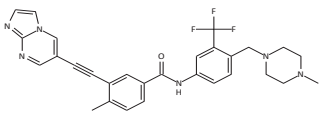
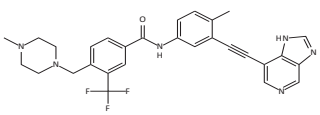
Compound	ELN ID (Compound No)	Synthesis Order [a]	ABL1 IC_{50} nM	ABL2 IC_{50} nM	HPLC Purity % [b]	Synthesis Yield (%) [c]	MS [d]: m/z $(M+H)^+$
	003-0236-011-001	55	45	82	100	32	534
	003-0236-012-001 (**32**)	56	27	41	95	21	562
	003-0236-013-001	57	N/A	N/A		Failed Synthesis	
	003-0236-014-001	58	13	18	100	12	533
	003-0236-015-001	59	14	26		N/R	563
	003-0237-001-001	60	N/A	N/A		Failed Synthesis	
	003-0237-002-001	61	N/A	N/A		Failed Synthesis	
	003-0237-003-001	62	220	300	100	38	534
	003-0237-004-001	63	6000	>10,000	100	11	364
	003-0237-005-001	64	220	590	100	16	545
	003-0237-007-001	65	N/A	N/A		Failed Synthesis	
	003-0238-001-001	66	16	14	100	8	533

Table S2. Chemical and biological data generated on the automated platform.

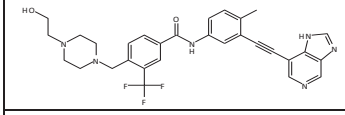
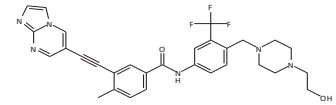
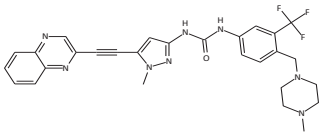
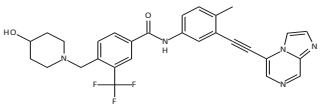
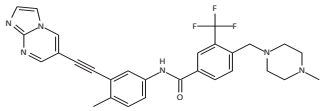
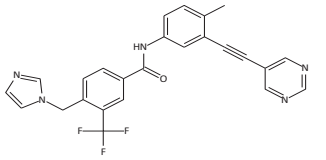
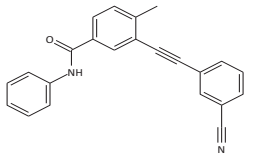
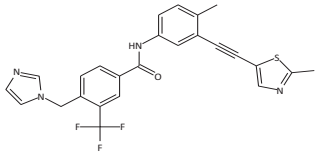
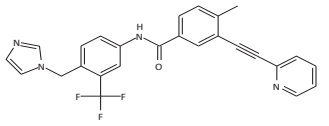
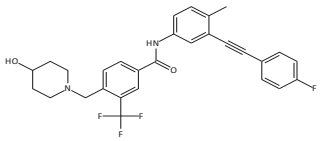
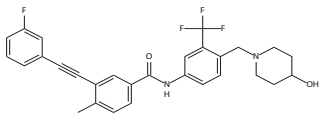
Compound	ELN ID (Compound No)	Synthesis Order [a]	ABL1 IC_{50} nM	ABL2 IC_{50} nM	HPLC Purity % [b]	Synthesis Yield (%) [c]	MS [d]: m/z $(M+H)^+$
	003-0238-002-001	67	17	30	100	7	563
	003-0238-003-001	68	N/A	N/A		Failed Synthesis	
	003-0238-004-001	69	23	83	100	8	549
	003-0238-005-001	70	46	57	100	32	534
	003-0238-006-001	71	N/A	N/A		Failed Synthesis	
	003-0239-001-001 (**33**)	72	230	160	100	9	462
	003-0239-002-001	73	>10,000	>10,000	100	20	337
	003-0239-003-001	74	N/A	N/A		Failed Synthesis	
	003-0239-004-001	75	>10,000	93000	100	59	461
	003-0239-005-001	76	>10,000	>10,000	100	19	511
	003-0239-006-001	77	>10,000	>10,000	100	8	511

Table S2. Chemical and biological data generated on the automated platform.

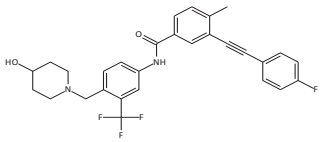
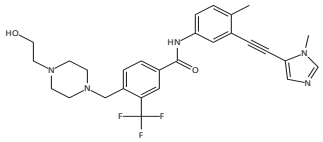
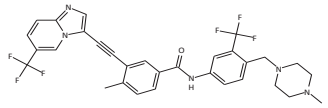
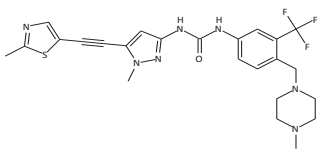
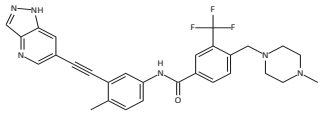
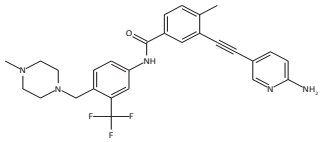
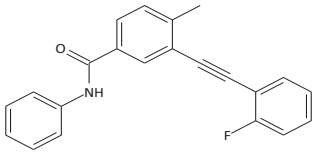
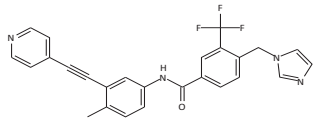
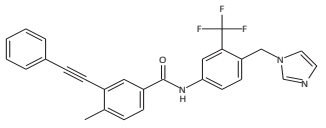
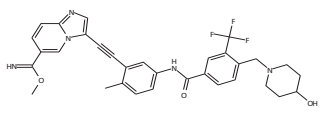
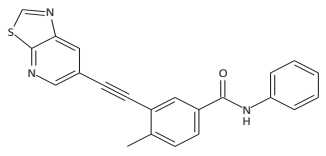
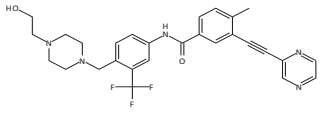
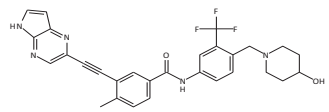
Compound	ELN ID (Compound No)	Synthesis Order [a]	ABL1 IC_{50} nM	ABL2 IC_{50} nM	HPLC Purity % [b]	Synthesis Yield (%) [c]	MS [d]: m/z $(M+H)^+$
	003-0239-007-001	78	>10,000	>10,000	100	8	511
	003-0240-001-001	79	51	93	100	42	526
	003-0240-002-001	80	N/A	N/A		Failed Synthesis	
	003-0240-003-001	81	N/A	N/A		Failed Synthesis	
	003-0240-004-001	82	N/A	N/A		Failed Synthesis	
	003-0240-006-001	83	88	190	100	22	508
	003-0240-007-001	84	>10,000	>10,000		N/R	330
	003-0241-001-001	85	>10,000	>10,000	100	9	461
	003-0241-002-001	86	>10,000	>10,000	100	26	460
	003-0241-003-001	87	N/A	N/A		Failed Synthesis	590
	003-0241-004-001	88	>10,000	>10,000	100	56	370

Table S2. Chemical and biological data generated on the automated platform.

Compound	ELN ID (Compound No)	Synthesis Order [a]	ABL1 IC_{50} nM	ABL2 IC_{50} nM	HPLC Purity % [b]	Synthesis Yield (%) [c]	MS [d]: m/z $(M+H)^+$
	003-0241-005-001	89	N/A	N/A		Failed Synthesis	
	003-0241-006-001	90	280	470	100	41	534

[a] This is the chronological sequence in which synthesis and screening was attempted. If synthesis and screening was successful the design model was updated with the Abl1 activity prior to selecting the next compound to attempt.

[b] Purity was determined by HPLC/UV analysis of the fraction collected for screening.

[c] Yield was determined by ELSD analysis.

[d] This is the molecular ion used to determine that the expected compound was present and also used to trigger the fraction collection.

[e] N/A = Not Applicable (since synthesis failed).

[f] N/R = Not recorded in database due to a software or hardware glitch.

References

[1] Huang, W. S.; Metcalf, C. A.; Sundaramoorthi, R.; Wang, Y. Z.; Thomas, R. M.; Zhu, X.; Cai, L.; Wen, D.; Liu, S.; Romero, J.; Qi, J.; Chen, I.; Banda, G.; Lentini, S. P.; Das, S.; Xu, Q.; Keats, J.; Wang, F.; Wardwell, S.; Ning, Y.; Snodgrass, J. T.; Broudy, M. I.; Russian, K.; Zhou, T.; Commodore, L.; Narasimhan, N. I.; Mohemmad, Q. K.; Iuliucci, J.; Rivera, V. M.; Dalgarno, D. C.; Sawyer, T. K.; Clackson, T.; Shakespeare, W. C. Discovery of 3-[2-(Imidazo[1,2-b]pyridazin-3-yl)ethynyl]-4-methyl-N-{4-[(4-methylpiperazin-1-yl)methyl]-3-(trifluoromethyl)phenyl}benzamide (AP24534), a Potent, Orally Active Pan-Inhibitor of Breakpoint Cluster Region-Abelson (BCR-ABL) Kinase Including the T315I Gatekeeper Mutant. *J. Med. Chem.* **2010**, 53, 4701–4719.

[2] A.W. Squibb, M. R. Taylor, B. L. Parnas, G. Williams, R. Girdler, P. Waghorn, A.G. Wright and F.S. Pullen J.Chromatogr.A. 1189 (2008) 101-108.