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Teaser'You make the compounds you design': this article describes a new way for chemistry undergraduates to learn about drug discovery.

A practical drug discovery project at the undergraduate level

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In this article, we describe a practical drug discovery project for third-year undergraduates. No previous knowledge of medicinal chemistry is assumed. Initial lecture workshops cover the basic principles; then students, in teams, seek to improve the profile of a weakly potent, insoluble phosphatidylinositide 3-kinase delta (PI3Kδ) inhibitor (1) through compound array design, molecular modelling, screening data analysis and the synthesis of target compounds in the laboratory. The project benefits from significant industrial support, including lectures, student mentoring and consumables. The aim is to make the learning experience as close as possible to real-life industrial situations. In total, 48 target compounds were prepared, the best of which (5b, 5j, 6b and 6ap) improved the potency and aqueous solubility of the lead compound (1) by 100–1000 fold and ≥tenfold, respectively.

This article is an account of a 'hands-on' drug discovery course that has been running for the past 3 years at the University of Nottingham. The purpose is fivefold: (i) to teach students, who are in the third year of a 4-year MSci degree course, how new medicines are discovered; (ii) to give an appreciation of the role of the chemist in that process; (iii) to give students practice in compound design and data interpretation; (iv) to use industry-standard equipment and methods in the laboratory; and (v) to develop communication, team-working and interpersonal skills. Key aspects of the course included the participation of scientists from GlaxoSmithKline (GSK) as lecturers and workshop mentors and, above all, in the practical application of drug discovery principles in the laboratory.

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From the perspective of GSK, the aim and guiding principle was to have individuals from the pharmaceutical industry train 'industry-ready' graduates for employment in medicinal chemistry. In this context, 'industry ready' meant students who have been taught applied medicinal chemistry as practised in industry as opposed to the less applied approaches usually found in academia. A substantial part of the course was taught not by professional teachers but by enthusiastic professional medicinal chemists. This provided an up-to-date perspective reflecting current industrial practice and the science of drug discovery, rather than a case history describing science and processes from the past.

The value of investigational or research-driven laboratory projects (of varying length and complexity) has been discussed widely elsewhere [1–3]. Advantages are that students feel more engaged, because projects are aligned to real-life problems [4], and there is not necessarily a 'right' answer, as in real research. Indeed, it has been shown that the type of laboratory work affects views on the nature of scientific enquiry itself [5]. At the University of Nottingham, laboratory experience over the first 2 years teaches technique through following a set of traditional 'recipe-following' laboratory experiments. By contrast, those staying on to complete the MSci fourth year research project are 'in at the deep end', having to comprehend primary literature, design experiments and break new ground, where uncertainty and failure are everyday experiences. Consequently, we sought to bridge this gap by developing projects[3] that were positioned somewhere between the 'inquiry-led' (students define the problem, design experiments and analyse the data generated) and the 'research-based' (working on a real problem with potential to advance knowledge) [6,7].

Another reason for embarking on this work was provided by a Higher Education Academy (HEA) Physical Sciences survey of chemistry graduates in 2010 [8]. The students were asked in retrospect to highlight 'skills deficits'; that is, those skills that the graduates wished they were proficient in, once employed. Top of the list were experiment design, team working, oral presentations and time management. Therefore, investigational projects could give much needed experience in these areas.

Course description: principles of medicinal chemistry, project background and target design

The course and accompanying laboratory work constituted ten credits each (students take 120 credits annually; one credit corresponding approximately to 2 hours of lectures and an equivalent amount of private study). No understanding of medicinal chemistry was assumed. Assessment of this part of the module was through a 90-min written examination. The principles were taught through five, 2-hour workshops that followed a relatively conventional form (a brief description is provided below).

- Introduction to the process of drug discovery: what it takes to discover a new medicine, emphasising the role of the chemist and using the discovery of salmeterol for the treatment of asthma as a case history of drug discovery.
- Discussion of physicochemical properties (particularly log *P* and p*K*a), including basic principles of pharmacokinetics and drug metabolism, such as tactics for blocking metabolism; lipid crossing; impact of acidity, basicity and/or lipophilicity on absorption and pharmacokinetics; and Lipinski's 'Rule of Five'.
- Structure–activity relationships and relation of structure to physicochemical properties, including hydrogen bonding and polar surface area; size; lipophilicity and electronic effects of substituents; outlines of pharmaceutical properties (such as drug solubility and stability); and drug structure and toxicity.
- Pharmacology: drug target and target classes, including receptor pharmacology (agonists and/or antagonists) and enzyme inhibitors, using kinases as an example.
- Computational chemistry: molecular interactions; (i.e. how drugs bind to targets); molecular similarity (i.e. the basis of bioisosterism); conformations of molecules and how to calculate them; and structure-based drug design.

After this, the course embarked on teaching skills in drug design. One workshop was used to introduce the research-based part of the course, comprising a brief description of the target disease (asthma) and the chosen therapeutic approach (kinase inhibition). Essential background on compound array design and screening was also provided. The importance of team skills was discussed (see below). The students were placed in teams of six, each with an experienced medicinal chemist as mentor, and were asked to design compounds that addressed the deficiencies of a lead compound (**1**) (Fig. 1). Three 2-hour workshops were devoted to this. The lead was a weakly potent ($pIC_{50} = 5.7$), lipophilic (clog $P = 3.9$) and poorly soluble (<12 μg/mL, aqueous pH 7) inhibitor of PI3Kδ, which is implicated in the pathology of asthma and chronic obstructive pulmonary disease [9].

PI3Ks are lipid kinases that phosphorylate the 3-position hydroxyl of phosphatidylinositides. Eight different kinases are known, of which the class IA subgroup (the α-, β- and δ-isoforms) generates phosphatidylinositol-3,4,5-triphosphate. Interest in PI3K inhibitors has been intense over recent years, and approximately 20 compounds (with varying degrees of selectivity for the various isoforms) are currently in clinical development [10]. For example, PI3Kα inhibitors are being progressed as potential anticancer agents [10].

The students worked on a research programme that was live at GSK. They did not work on the structural series being pursued by GSK (any intellectual property that results from the module is owned by the University of Nottingham), but this enabled the students to feel they were connected to an ongoing effort to discover a new medicine for treating severe asthma.

A primary focus was to progress the molecular series toward an improved drug-like profile (as opposed to results for publication) by paying close attention to physicochemical properties alongside potency. The project was specifically designed to ensure that the students experienced two iterations of the 'analyse–design–synthesis–test' cycle [11] (pivotal learning points in medicinal chemistry) despite their inexperience and the limited time available. There were three stages. First, the students devised two arrays of 20 target compounds; one comprising amine derivatives (**2**) (which should help improve aqueous solubility) and the other substituted aryl derivatives (**3**) (to improve potency) (Fig. 1). Lists of the building blocks (or monomers) used in the target compound arrays are detailed in Figs. 2 and 3.

Consideration of lipophilicity, molecular weight (MW) and hydrogen bond donor and/or acceptor count (Lipinski's 'Rule of

[3] New projects currently being trialled at the School of Chemistry will be reported elsewhere.

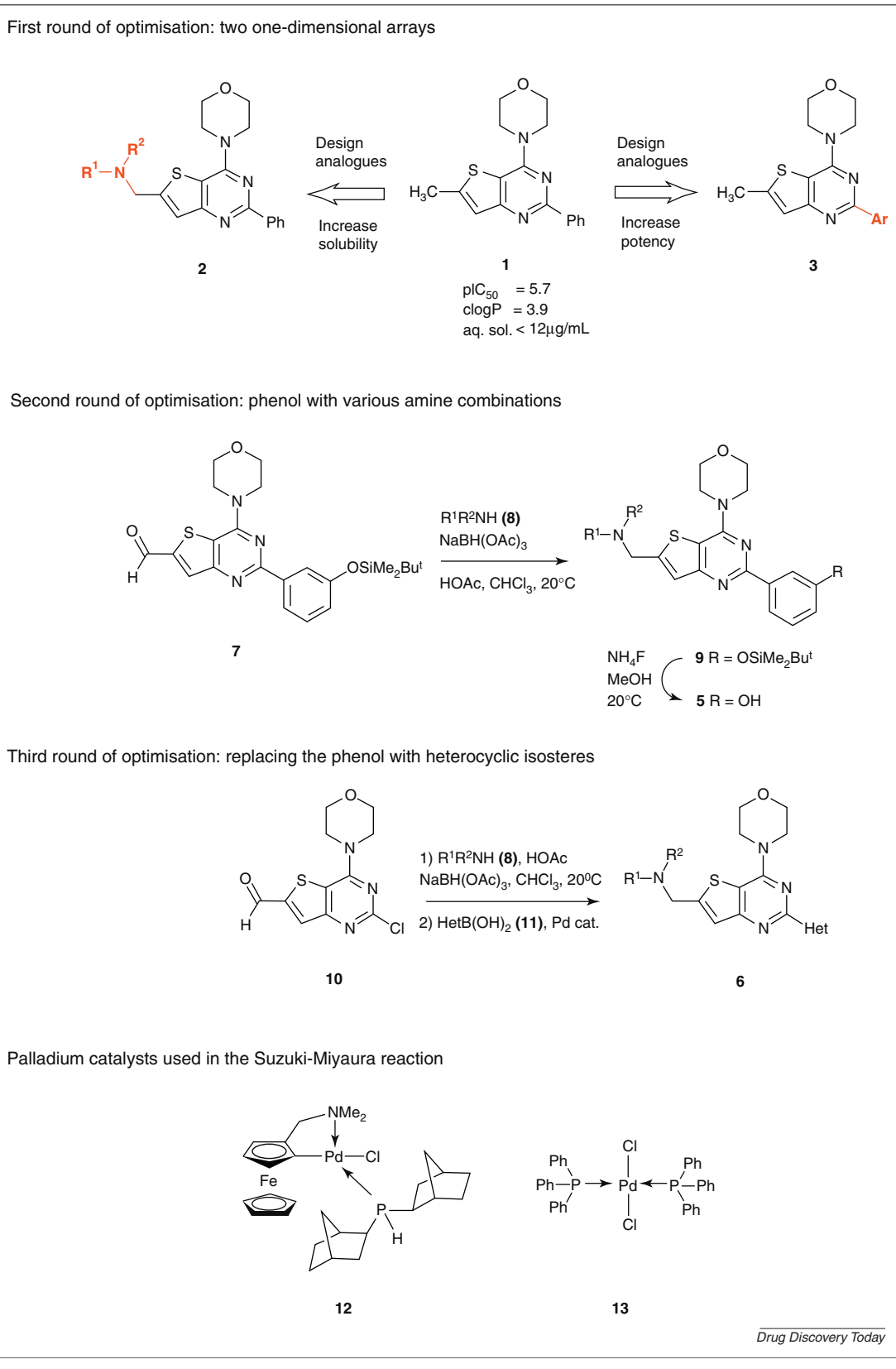


FIGURE 1

Structural changes and reaction conditions for the three rounds of inhibitor optimisation.

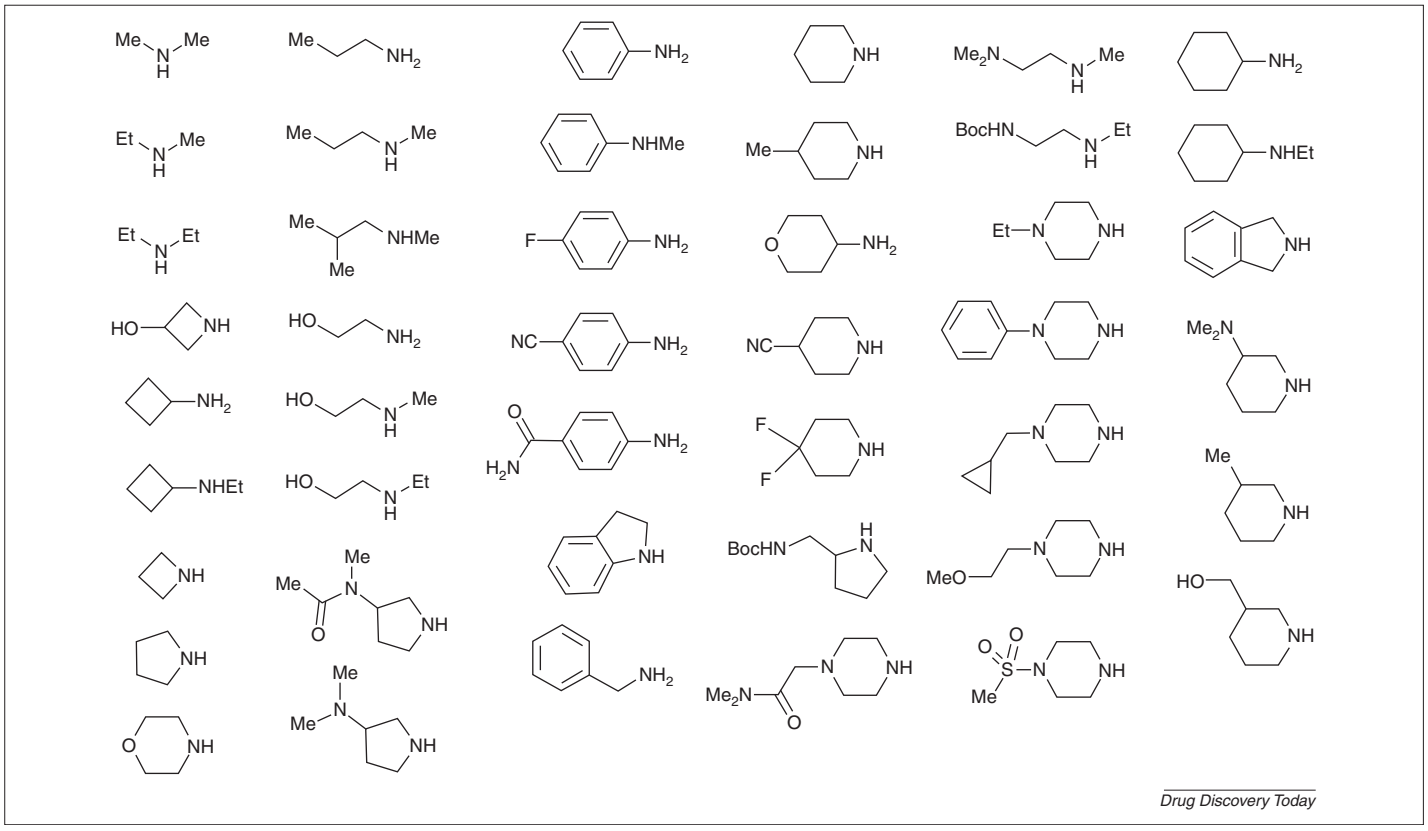


FIGURE 2

Amine building blocks (monomers) used for the synthesis of **2**.

Five') [12] drove the selection of amines, whereas the aryl derivatives were selected by interactive visualisation on the basis of their fit and potential interactions suggested from dockings into the enzyme binding site[4] derived from the parent structure established by X-ray crystallography [13,14].[5] Rotations about the pyrimidine–aryl bond were also evaluated in conjunction with a potential energy plot, to determine whether other attractive, low-energy binding modes existed. Calculated log *P* (clog *P*) and molecular weight data were also considered. Figure 4 shows compound **1** docked into the ATP binding site of PI3Kδ. The students found the assimilation of the wealth of data challenging, because they had to design their arrays in a 1-hour workshop. The presence of an experienced medicinal chemist with each group during its deliberations was important to ensure logical and not random choices were made. In the case of the amine analogues, presentation of their properties through a Spotfire™ plot[6] (Fig. 5) was particularly useful for visualising the chemical space. For example, initial choices could be marked on the plot to ensure an adequate diversity of structure in the array and, if necessary, re-examined before settling on the final array. Given that the compound arrays had already been prepared by scientists at GSK, the screening results [inhibition potency versus PI3Kδ (pIC_{50})] [15], aqueous solubility [16] and measured log *D* [17,18][7]) were available to the students in time for the next workshop and round of design.

During the second stage, analysis of the screening data clearly revealed phenol **4** (Fig. 6) to be the best aryl derivative, because it increased potency by approximately 100-fold, although its solubility was practically unchanged compared with compound **1**. Interpretation of the results from the amines was less clear-cut because there was a spread of potency and solubility; students were asked to select one amine derivative each (**5**) to make in conjunction with the *meta* phenol group substituent (Fig. 1). From a table of data, hydroxy amines and diamines stood out as substituents that enhanced not only the solubility, but also the potency (up to tenfold) while targeting an attractive log *D* range (2–3.5). At this stage, the teams prepared and delivered a presentation of their results so far and their plans for the compounds they would prepare in the laboratory.

Lastly, in the final workshop, the students were presented with a further challenge. They were informed that the phenol might be

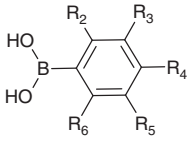
[4] MOE (Molecular Operating Environment) is a suite of drug discovery software sold by Chemical Computing Group, Montreal, Quebec, Canada, see http://www.chemcomp.com/ (accessed 20.06.13).

[5] For X-ray crystal structures of PI3Kδ and inhibitors, see [14]. Protein Data Bank structure number 2WXP.

[6] Spotfire is a data analysis tool from TIBCO Spotfire, 212 Elm Street, Somerville, MA 02144, USA. http://spotfire.tibco.com/en/discover-spotfire.aspx (accessed 20.06.13). The compounds of the amine array were plotted according to clogP (*X*-axis), molecular weight (*Y*-axis) and each colour- and shape-coded according to the number of hydrogen bond acceptors and donors, respectively.

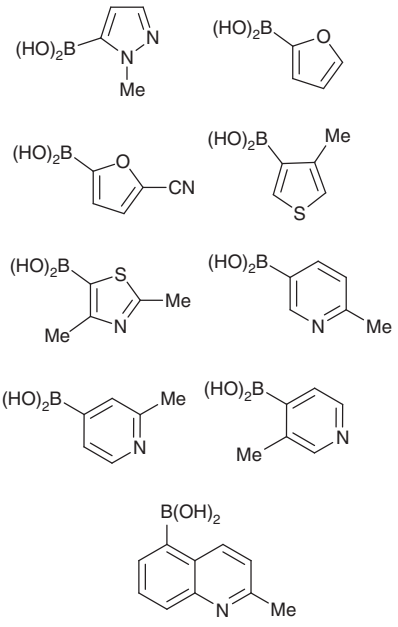
[7] LogD was measured by a reverse-phase chromatographic method as described by [17].

Aryl



R^2	R^3	R^4	R^5	R^6
Me	H	H	H	H
Et	H	H	H	H
i-Pr	H	H	H	H
CF_3	H	H	H	H
OH	H	H	H	H
OMe	H	H	H	H
OPr^i	H	H	H	H
CO_2Et	H	H	H	H
H	F	H	H	H
H	Cl	H	H	H
H	CF_3	H	H	H
H	Me	H	H	H
H	i-Pr	H	H	H
H	OMe	H	H	H
H	NH_2	H	H	H
H	OH	H	H	H
H	CN	H	H	H
H	CO_2H	H	H	H
H	H	F	H	H
H	H	Me	H	H
H	H	OMe	H	H
H	H	NH_2	H	H
H	H	CN	H	H
H	H	$CONH_2$	H	H
F	F	H	H	H
F	H	F	H	H
Me	H	Me	H	H
F	H	Cl	H	H
OMe	H	Cl	H	H
F	H	H	Me	H
Cl	H	H	F	H
Me	H	H	H	Me
H	F	Me	H	H
H	Me	Me	H	H
H	OMe	OMe	H	H

Heteroaryl



Drug Discovery Today

FIGURE 3

Building blocks (monomers), $ArB(OH)_2$/$HetB(OH)_2$, used for the synthesis of **3**.

good for potency, but that it is a group with susceptibility to rapid metabolism; therefore, it would be prudent to prepare some bioisosteres of the phenol [11,19]. Thus, by examining the fit of a set of heterocyclic analogues in the enzyme active site, clog *P* values and using the Rule of Five, the students then planned to make further target compounds (**6**) that combined the best amine and heterocyclic groups (Fig. 1).

Each team then carried out their synthesis plans in the laboratory. They had 40 hours laboratory time, spread over 4 weeks. During this time, each team typically attempted the preparation of

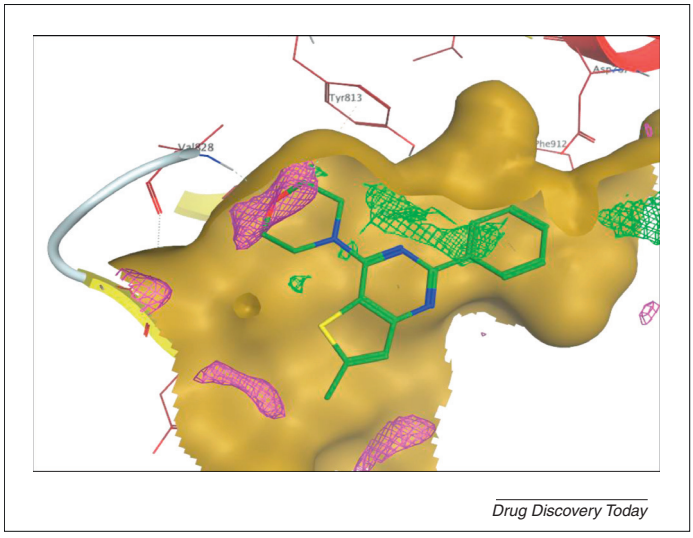


FIGURE 4

The reference binding mode for compound **1** was obtained from the crystal structure 2WXP [15]. The binding site was surfaced and the hydrophobic (green) and polar (purple) interaction potentials were mapped by using MOE [13] to help identify features of interest in the binding site and to indicate where it might be possible to make new interactions with the receptor.

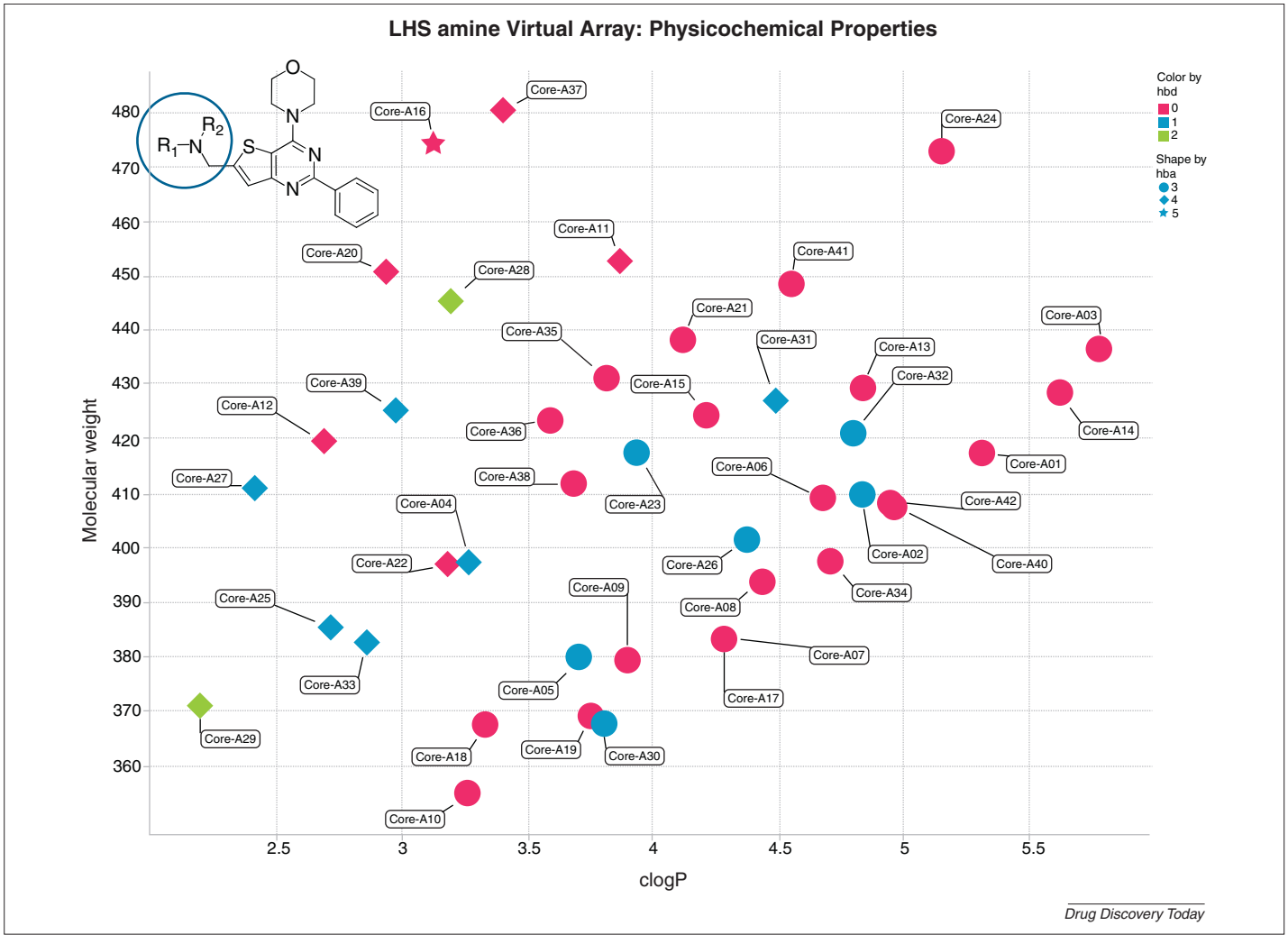


FIGURE 5

Spotfire™ plot (molecular weight versus calculated log *P*) for the array of amines. Each spot corresponds to a different target compound (amines A01–42), with the shape and colour of the spots coded according to the number of hydrogen bond acceptors and donors, respectively. Students discussed target choice in relation to Lipinski's 'Rule of Five'.

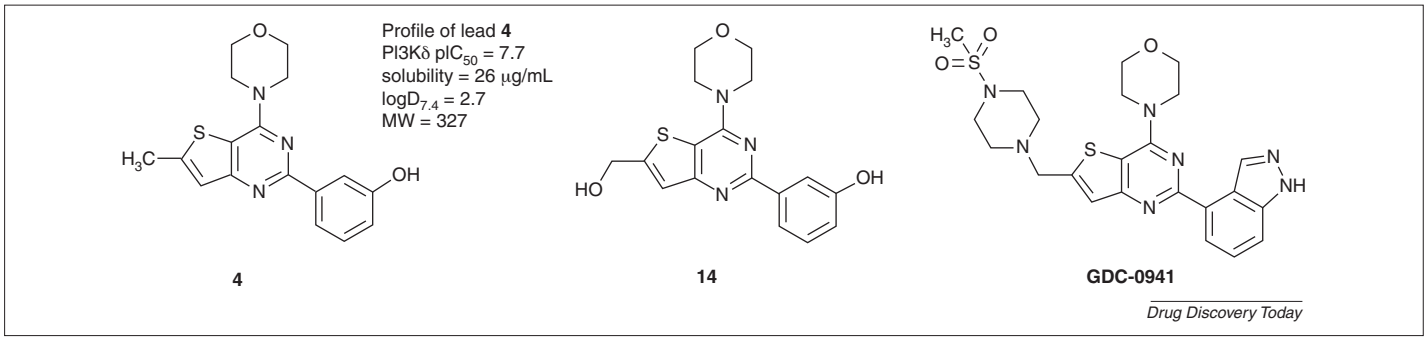


FIGURE 6

Structures of compound 4 (best aryl derivative from round one optimisation), 14 (reductive amination side product) and clinical candidate GDC-0941.

approximately 15 target compounds. Given that the teams selected targets independently of each other, some targets appeared in the plans of more than one team.

The way in which the teams were selected and coached deserves comment. Teamwork was emphasised strongly because it is so important for success in the pharmaceutical industry. Part of one workshop was devoted to describing how effective teams are assembled. To do this, we used the Belbin Team Role questionnaire, which helps identify preferred ways of working in teams (http://www.belbin.com/rte.asp?id=396). This enabled the students to develop increased self-knowledge and a clearer appreciation of their strengths and weaknesses, which could then feed into organisation of their presentations and laboratory activities. Each team was selected to have a blend of the nine characteristics. In addition, we took into consideration gender and academic ability. Teams were also encouraged to develop a relationship with the industrial mentors that was distinct from the usual student–academic one in which assessment provides a backdrop. At the end of the course, the students visited the GSK laboratories at Stevenage and had the opportunity to see the biological and

BOX 1

Experimental details

General experimental details

Spectroscopic data were recorded on Bruker Tensor 27 IR and Bruker AV400 (^{1}H NMR 400 MHz), Bruker DPX300 (^{1}H NMR 300 MHz), Jeol EX270 (^{1}H NMR 270 MHz) instruments. All reactions were conducted under a positive pressure of dry nitrogen unless stated otherwise. Amines **8a–m** and boronic acids **11a–l** were obtained from commercial sources. Amines **8n** and **8o** were purchased as their *N*-Boc derivatives. Compounds **7** and **10** were prepared by GSK according to the methods of Folkes *et al.* [20] Palladium catalysts **12** and **13** were purchased from Sigma–Aldrich and Alfa Aesar, respectively. Column chromatography was performed on silica gel or aminopropyl-modified silica gel (Biotage Isolute™ or SNAP™ cartridges) with a Jones Chromatography Flashmaster II automated system. Kieselgel 60 F_{254} plates from E. Merck and aminopropyl-modified plates from Biotage were used for TLC, and compounds were visualised using ultraviolet light, anisaldehyde solution or 0.5% aqueous potassium permanganate solution. Petrol refers to petroleum ether (bp 40–60°C) unless stated otherwise.

Hazards

Chloroform is an irritant and harmful if ingested. It also is a cancer suspect agent in humans (IARC class 2B). Dioxane is an irritant, highly flammable and might form explosive peroxides. It also is a cancer suspect agent. Acetic acid is corrosive and flammable. Ethanol is flammable. Toluene is flammable and an irritant and can cause lung damage if ingested. It is also a potential teratogen. Sodium triacetoxyborohydride can liberate hydrogen gas in the presence of water. Ammonium fluoride is toxic on skin contact, inhalation or if ingested. Potassium phosphate is a skin irritant and can cause serious eye damage. The various amines used in the project are flammable and corrosive, and further information on their hazards can be obtained from commercial suppliers. Although the palladium catalysts, 2-(dimethylaminomethyl)ferrocen-1-yl-palladium (II) chloride dinorbornylphosphine complex (**12**) and $PdCl_2(PPh_3)_2$ (**13**), do not have hazards listed, they should be assumed to be toxic.

Procedure for reductive amination of aldehyde (7) followed by desilylation

2-(3-((*Tert*-butyldimethylsilyl)oxy)phenyl)-4-morpholinothieno[3,2-d]pyrimidine-6-carbaldehyde (152 mg, 0.33 mmol) was dissolved in chloroform (5 mL) under nitrogen at room temperature. The amine **8** (0.66 mmol) and acetic acid (0.5 M in chloroform, 1.32 mL, 0.66 mmol) were added and the resulting solution was stirred for 15–30 min. Sodium triacetoxyborohydride (210 mg, 0.99 mmol) was then added as a solid in one portion, and the reaction mixture was stirred until TLC indicated that the reaction was complete (between 30 min and 16 hours). Saturated aqueous sodium bicarbonate (10 mL) was added to the solution and the organic layer separated. The aqueous layer was extracted with chloroform (10 mL) and the combined organic solutions were dried ($MgSO_4$) and concentrated under reduced pressure to give the crude amine silyl ether **9**.

The crude silyl ether **9** (assumed to be pure, 1.0 equivalent) was treated with a solution of ammonium fluoride (5.0 equivalent) in methanol (5–10 mL/mmol silyl ether) under nitrogen at room temperature. The mixture was stirred until TLC indicated that the reaction was complete (typically <30 min). The solvent was removed under reduced pressure and the residue was taken up in 1–2 mL dichloromethane (some ammonium fluoride did not dissolve). The solution was then applied to the top of a Biotage Isolute™ silica gel cartridge (20 g) and eluted using the Flashmaster apparatus (gradient elution with hexane, ethyl acetate, methanol and triethylamine or dichloromethane, methanol and ammonia). Fractions containing product were identified by TLC, and evaporated under reduced pressure to give the product. For those

products that were not obtained as solids, treatment with a small volume of hot ethyl acetate and scratching caused the product to crystallise. The suspension was filtered and dried to give the desired product **5**.
Alternatively, the desilylation step could be performed by using a solution of tetrabutylammonium fluoride in THF (1.1 equivalent) at room temperature.
For those amines that were purchased as the hydrochloride salts, the acetic acid was omitted and replaced by a molar equivalent amount of anhydrous sodium acetate.
Data for 6-hydroxymethyl-2-(3-hydroxyphenyl)-4-morpholinothieno[3,2-d]pyrimidine (compound **14**): ^{1}H NMR (270 MHz, DMSO-d_6) δ_H 9.49 (1H, s), 7.84 (1H, s), 7.83 (1H, d, *J* 8 Hz), 7.31 (1H, s), 7.26 (1H, t, *J* 8 Hz), 6.24 (1H, d, *J* 8 Hz), 5.91 (1H, t, *J* 5.7 Hz), 4.85 (2H, d, *J* 5.7 Hz), 3.98 (4H, m), 3.80 (4H, m).

Preparation of heterocyclic derivatives (6) via Suzuki reaction

The procedure for reductive amination of chloropyrimidine (**10**) was the same as above, omitting the desilylation step, but was generally conducted on 2.5–3.5 mmol scale.

Suzuki reaction: method A

The chloropyrimidine (0.25 mmol, 1.0 equivalent), boronic acid (**11**) (0.375–0.5 mmol) and potassium phosphate (3.0 equivalent) were weighed into a microwave vial equipped with a stir-bar at room temperature. The catalyst, 2-(dimethylaminomethyl)ferrocen-1-yl-palladium (II) chloride dinorbornylphosphine complex (0.1 equivalent) was added. A mixture of dioxane and water (2:1, 4 mL/mmol of chloropyrimidine) was then added carefully and the vial was flushed with nitrogen. The vial was sealed and heated in a microwave reactor for 10 min at 140°C. The cooled reaction mixture was sampled by TLC (silica gel and aminopropyl silica gel) and, if the reaction was not complete, another 1 equivalent boronic acid and/or ester added, and reaction continued for another 10–20 min at 140°C. The mixture was filtered through a pad of kieselguhr, washing with ethyl acetate (10 mL). The filtrate was washed with saturated aqueous sodium bicarbonate (5 mL), dried ($MgSO_4$) and the solvent removed under reduced pressure. The residue was taken up in approximately 1 mL dichloromethane and applied to the top of an aminopropyl silica gel cartridge (10 or 20 g) and eluted using the Flashmaster apparatus (gradient elution with hexane, ethyl acetate and methanol; typically 0–100% ethyl acetate in hexane over 15 min followed by 0–15% methanol in ethyl acetate over 15 min, flow rate 15–20 mL/min). Fractions containing product were identified by TLC, and evaporated under reduced pressure to give the product as a gum. Treatment with a small volume of hot ethyl acetate and scratching caused the product to crystallise. The suspension was filtered and dried to give the desired product (**6**). Occasionally, products that refused to crystallise were converted to their hydrochloride salts to obtain a solid sample.

Suzuki reaction: method B

The chloropyrimidine (0.22 mmol, 1.0 equivalent), boronic acid (**11**) (0.44 mmol), and sodium carbonate (0.77 mmol, 3.5 equivalent) were weighed into a microwave vial equipped with a stir-bar at room temperature. The catalyst, *bis*(triphenylphosphine)palladium (II) chloride (0.02 mmol, 0.1 equivalent) was added. Then, toluene (1.2 mL), ethanol (0.6 mL) and water (0.3 mL) were added carefully and the vial was flushed with nitrogen. The vial was sealed and heated in a microwave reactor for 90 min at 130°C. Work-up and purification was as described above.

Compound assays

Methods for measuring PI3Kδ inhibitor potency, aqueous solubility and logD by HPLC are described in [15–18].

physicochemical assays in action. Students welcomed the opportunity to ask about applied research and careers in industry. Indeed, one student from the 2010 group is now employed by GSK, after completing a successful summer internship.

Box 1 provides a more detailed description of the chemistry. Once the compounds had been made and characterised, they were sent for screening at GSK. The results were returned to the students for incorporation into individual reports, which formed part of the formal assessment. The reports were approximately eight pages long and followed the general structure of a paper from *Bioorganic and Medicinal Chemistry Letters*. This gave the students the opportunity to demonstrate their understanding of the project, its objectives and to what extent the compounds they made moved the project toward those objectives.

Target compound synthesis and screening results

The project chemistry was designed to enable rapid synthesis of a wide range of target compounds. Thus, intermediate aldehydes **7** [20] and **10** [21,22] that are not commercially available were prepared and donated by GSK. The project provides a showcase for two mechanistically important reactions frequently used in the pharmaceutical industry: reductive amination [23] and the Suzuki–Miyaura reaction [24] for biaryl coupling. Preliminary investigations of the chemistry by GSK following the procedures described by Folkes *et al.* [20] revealed some problems. Consequently, to give the best chance for many analogues to be completed, the students were provided with modified reaction procedures, and were not encouraged to vary them. Although it would have enhanced the learning experience for students to select their own reaction conditions based on reviewing available literature, preliminary work indicated that this would increase the risk of failure considerably. Given that the main objective was to make compounds for their screening data, it was felt necessary to direct the students closely on this point. If we had had more time, as in an MSci project, exploration of the synthetic route would have provided a valuable learning opportunity. Thus, in the reductive amination to make phenol derivatives **5**, the phenol was protected with a *tert*-butyldimethylsilyl group because it led to faster reactions. The reduction was achieved with sodium triacetoxyborohydride in chloroform because 1,2-dichloroethane was deemed too hazardous. The various amines chosen by the students (**8a–o**) are shown in Table 1. The intermediate **9** is desilylated, conveniently and rapidly, with ammonium fluoride [25,26][8] and then purified by column chromatography to give **5**. Table 1 shows the analogues prepared (with screening data).

In a similar manner, reductive amination of **10**, typically on 0.75–1.0 g scale, with selected amines (approximately three per team) gave stocks of intermediate chloropyrimidines, sufficient for several Suzuki coupling reactions with various boronic acids (**11a–l**) (Fig. 7) on 0.25 mmol scale. Two sets of reaction conditions,

[8] TBAF (tetrabutylammonium fluoride) deprotection also works [26].

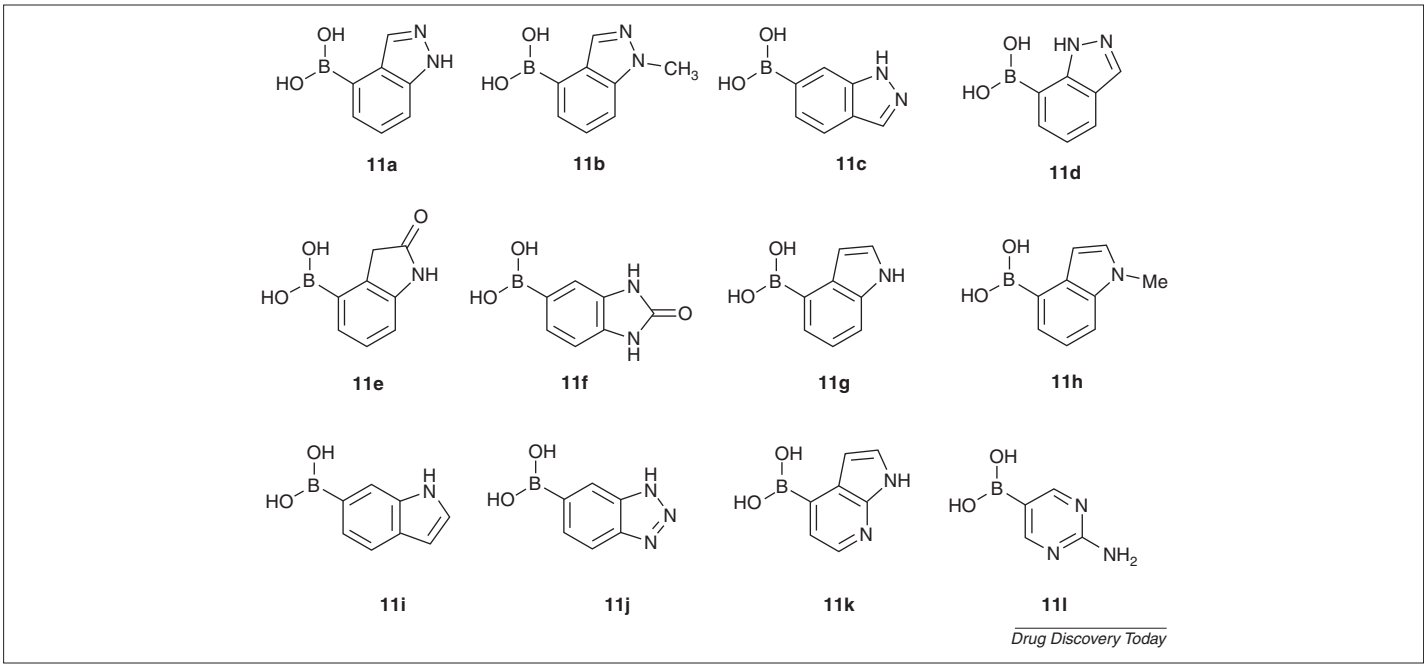
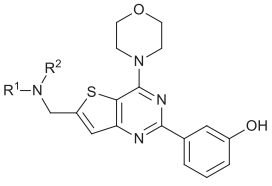


FIGURE 7

Boronic acids [HetB(OH)$_2$] used in the Suzuki.

TABLE 1

Phenol analogues prepared, with screening results



5 a-j

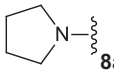
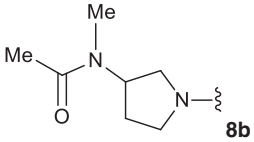
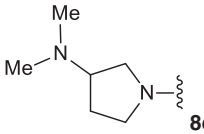
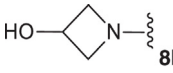
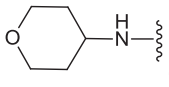
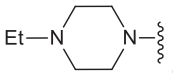
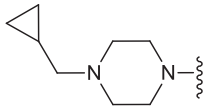
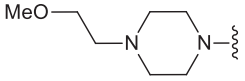
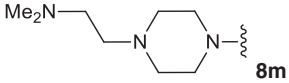
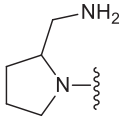
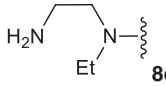
Compound	Amine R^1R^2N-	Yield[a] (%)	pIC_{50}	BEI[b]	Solubility[c]	log $D_{7.4}$
5a	 **8a**	47, 21	6.9	17.4	++	2.7
5b	 **8b**	58, 53, 39, 21, 16, F	8.9	18.8	+++	2.0
5c	 **8c**	59, 42, 33, 17	8.1	18.5	++	1.3
5d	$Me_2NCH_2CH_2NMe$ **8d**	61, 60, 33, 22	7.9	18.5	+++	1.2
5e	$HOCH_2CH_2NH$ **8e**	11, 9, F	7.6	19.7	+++	1.2
5f	$HOCH_2CH_2NMe$ **8f**	63,[d] 58,[d] 39,[d] 20, F, F, F	7.9	19.7	+++	1.7
5g	$CH_3CH_2CH_2NH$ **8g**	53	7.3	19.0	++	2.4
5h	 **8h**	69,[d] 16, 9	7.4	18.6	++	1.3
5i	 **8i**	24, 31, 39	7.7	18.1	++	2.1

TABLE 1 (*Continued*)

Compound	Amine R^1R^2N-	Yield[a] (%)	pIC_{50}	BEI[b]	Solubility[c]	log $D_{7.4}$
5j	 **8j**	57, 33, 10, F	8.6	19.6	+++	1.6
5k	 **8k**	24	8.2	17.6	++	2.3
5l	 **8l**	78, 59, 51, 44	8.3	17.7	++	1.9
5m	 **8m**	40	8.3	17.2	+++	1.2
5n	 **8n**	46[e]	7.8	18.3	+++	1.3
5o	 **8o**	71[e]	7.2	17.4	++	1.3

[a] Yields: each figure corresponds to a separate experiment; F corresponds to a failed reaction in which no product was successfully isolated following chromatography. All compounds were solids and were characterised by melting point, ^{1}H NMR and IR spectroscopy. In addition, all compounds tested by GSK underwent quality assessment by LC–MS and all were determined to be ≥90% pure.

[b] BEI: $pIC_{50} \times 1000/MW$.

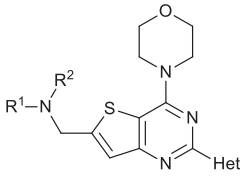
[c] Solubility was measured by a turbidometric (precipitative) method [16]. The compound (10 mM in DMSO) was added to aqueous buffer (pH 7.4) and the sample centrifuged; the concentration of compound was measured in the supernatant by using quantitative Chemiluminescent Nitrogen Detection (CLND). Compounds were ranked as +++ (soluble, ⩾100 μg/mL), ++ (poorly soluble, 10–100 μg/mL) and ++ (very insoluble, ⩽10 μg/mL).

[d] Yield improved by using five equivalents of amine.

[e] Reductive aminations were performed with 2-(*N*-Boc-aminomethyl)pyrrolidine (**8n**) and *N*-Boc-N′-ethyl-1,2-diaminoethane (**8o**). After desilylation and purification, the Boc group was removed by treatment with 4N hydrogen chloride in dioxane at 0°C.

TABLE 2

Heterocyclic analogues prepared with screening results



6

Compound	R^1R^2NH	Het	Yield %[a] (Method)	pIC_{50}	BEI[b]	Solubility[c]	log $D_{7.4}$
6a	**8a**	**11a**	5 (A)	6.9	16.4	++	NA
6b	**8b**	**11a**	78 (A) 31 (A) 57 (B) 20 (B)	7.8	15.9	++	2.0
6c	**8c**	**11a**	52 (A) 58 (A) 8 (A)	7.5	16.7	+++	1.3
6d	**8d**	**11a**	45 (A) 27 (A) 13 (A)	7.1	15.7	++	1.3
6e	**8f**	**11a**	50 (A) 42 (A) 12 (B)	6.9	16.2	++	1.7
6f	**8h**	**11a**	51 (A)	6.4	15.2	++	1.4
6g	**8i**	**11a**	21 (B) 8 (B)	7.7	15.6	+++	2.0

TABLE 2 (*Continued*)

Compound	R^1R^2NH	Het	Yield %[a] (Method)	pIC_{50}	BEI[b]	Solubility[c]	log $D_{7.4}$
6h	**8j**	**11a**	41 (A) 8 (B) 5 (A)	7.7	16.4	++	1.7
6i	**8l**	**11a**	8 (B) 22 (B)	7.8	15.8	+++	2.0
6j	**8b**	**11b**	37 (B)	6.6	13.0	+++	2.6
6k	**8j**	**11b**	9 (A) F (A)	6.2	13.3	++	2.2
6l	**8a**	**11c**	27 (A)	5.4	12.9	+	2.7
6m	**8j**	**11c**	F (A)				
6n	**8b**	**11d**	53 (A)	6.8	13.8	+	2.9
6o	**8c**	**11d**	F (A)				
6p	**8j**	**11d**	10 (A)	6.4	13.8	++	2.6
6q	**8b**	**11e**	24 (A)	6.2	12.2	+++	1.7
6r	**8c**	**11e**	F (A)				
6s	**8d**	**11e**	15	5.8	12.4	+++	1.1
6t	**8j**	**11e**	13 (A) 79 (A) 19 (B) 39 (B) F (A)	6.3	13.2	+++	1.5
6u	**8l**	**11e**	30 (B)	6.1	12.9	+++	1.7
6v	**8c**	**11f**	F (A)				
6w	**8j**	**11f**	59 (A)	<4.5	<9.6	+++	NA
6x	**8c**	**11g**	F (A)				
6y	**8d**	**11g**	27 (A) F (A)	6.9	15.3	+	1.6
6z	**8j**	**11g**	F (A)				
6aa	**8c**	**11h**	5 (A)	6.6	13.9	111	2.6
6ab	**8a**	**11i**	F (A)				
6ac	**8b**	**11i**	15 (B) 7 (A)	6.3	12.8	++	2.6
6ad	**8c**	**11i**	29 (A)	6.2	13.4	+++	1.9
6ae	**8d**	**11i**	48 (A) 9 (A)	7.1	15.8	++	NA
6af	**8h**	**11i**	23 (A)	5.5	13.0	++	1.9
6ag	**8j**	**11i**	14 (A)	6.0	13.0	++	2.3
6ah	**8l**	**11i**	11 (B)	5.9	12.0	+++	2.6
6ai	**8m**	**11i**	2 (B)	5.9	11.7	+++	NA
6aj	**8b**	**11j**	F (A)				
6ak	**8j**	**11j**	F (A)				
6al	**8d**	**11k**	18 (A)	6.7	14.9	+++	1.1
6am	**8f**	**11k**	50 (A)	5.5	13.0	+++	1.7
6an	**8h**	**11k**	8 (A)	6.1	14.5	++	1.1
6ao	**8d**	**11l**	8 (A)	7.7	18.0	+++	0.6
6ap	**8c**	**11l**	9 (A)	7.9	18.0	++	0.7
6aq	**8f**	**11l**	8 (A)	7.2	18.0	++	1.0

[a] Yields: each figure corresponds to a separate experiment; F corresponds to a failed reaction in which no product was successfully isolated following chromatography. All compounds are solids and were characterised by melting point, ^{1}H NMR and IR spectroscopy. In addition, all compounds tested by GSK underwent quality assessment by LC–MS and all were determined to be ≥90% pure.

[b] BEI: $pIC_{50} \times 1000/MW$.

[c] Compounds were ranked as +++ (soluble, >100 μg/mL), ++ (poorly soluble, 10–100 μg/mL), and + (very insoluble, <10 μg/mL).

with different palladium catalysts **12** and **13** (Fig. 1) under microwave heating, were used (for full details, see Box 1). Conditions with conventional heating were not investigated. The advantage of catalyst **12** was shortened reaction times. Following work-up, the products **6** were purified by column chromatography. Table 2 shows the analogues prepared (with screening data).

To ensure that the experiments were conducted safely, the following precautions were taken. Students wore standard personal protective equipment (i.e. lab coats, safety spectacles and gloves) and conducted all reactions and work-ups in a fume cupboard. The hazards associated with the aldehydes **7** and **10** and boronic acids were not established, but standard precautions were adequate to limit exposure. The hazards associated with the other chemicals are listed in Box 1. Certain chemicals, particularly dioxane and chloroform, were undesirable owing to their toxicity and environmental impact, and efforts are being made to eliminate them through modification of the experimental protocols. Demonstrators instructed the students in setting up the Suzuki reactions in microwave vials, in particular to ensure that all solids were below the level of the solvent. The demonstrators were responsible for sealing the vials and operating the microwave reactors.

Resources

GSK committed substantial resources in setting up the course. These included selecting the appropriate live in-house programme, the appropriate molecular template for inhibiting PI3Kδ that enabled iterative cycles of medicinal chemistry, choreographing the iterations to fit the time available, development of robust synthetic chemistry procedures and purification protocols, bulk synthesis of intermediates for the students to use, the synthesis and testing of large numbers of compounds so that data could be rapidly provided to the students after various iterations, the preparation of material to be taught in workshop modules, molecular modelling visualisations of potential targets in the active site, data analysis visualisation plots, and multiple visits for mentoring the students. For example, each GSK mentor spent approximately 1–2 weeks preparing and then another week teaching and mentoring. To date, over 20 employees at GSK in Stevenage have contributed.

Discussion

Extensive informal feedback was obtained from the students through individual conversations. They particularly appreciated the problem-based approach, especially because it so sharply contrasted with their laboratory experience from the previous year. Other aspects that drew favourable comments were that theory was linked to practice and a real-life problem, and the significant input from scientists from industry. The students found the team skills training interesting and not like anything they had experienced before; they discovered that success is not just about academic ability, which gave personal insights into what qualities potential employers might expect. It was interesting to observe that, despite the process to assign students to teams, not all teams performed equally well. Those that did some planning ahead of each practical session (independently of a tutor or demonstrator) were generally better organised and able to make the best use of their time in the laboratory. Aspects of the course workshops, such as discussion and practicing concepts (calculating hydrophobicities and molecular modelling) immediately helped cement learning.

The following are quotes from students, taken from the Student Evaluation of Module and Teaching forms of the University of Nottingham.

- 'The best part of the module was being taught by people who have extensive experience in the pharmaceutical industry; this was beneficial as they often related new material to projects they had worked on previously.'
- 'I really enjoyed how the module combined everything we had learnt in lectures to allow us to see and be part of the drug discovery process. I believe it gave a real insight into medicinal chemistry/pharmaceutical industry, through practical drug development and seeing how drugs get to market, and showed us what we could achieve after leaving university. I thoroughly enjoyed the module and would recommend it to any aspiring medicinal chemist.'
- 'What I liked about the GSK module is that it brought my degree into practice and helped bring the pharmacological aspects that we had studied together with the chemistry. I think the module could have been improved by having more time in the design process of compounds.'
- 'Really enjoyable module, made me want to do my 4th year project in medicinal chemistry.'
- 'Very engaging, lots of advice and support, excellent notes, good fun.'
- 'Free-thinking encouraged.'
- 'The interactive workshops challenged thought processes.'
- 'It allows me to put the theory learnt into practice. Conducted in small groups increasing teamwork and communication skills.'
- 'Made you think. Relevant to drug design.'
- 'Loved having actual researchers from industry, made the module interesting and enjoyable.'
- 'I like the variety of assessments types. I think the link with the lab modules is very good because it enables a better idea of the practical issues facing medicinal chemists.'
- 'The workshops were well structured and encouraged participation. The relevance to the real world also helped retain my interest.'
- 'I enjoy working in a team, and it was nice working with people I haven't worked with before. It was also really good having speakers coming in from GSK and the mentors were brilliant at providing assistance and willing to answer questions.'

Several clear themes emerge from feedback from the GSK staff who have been involved in this programme:

- Most GSK staff particularly enjoyed the interactions with the students. Several of the GSK mentors felt that they developed more effective time-management skills based on juggling both their GSK and university workloads.
- Many were struck by the challenge of delivering the aims of the module to inexperienced students in the limited timeframe. Other challenges included: (i) preparing (and sometimes relearning) the teaching material at the right level; (ii) requiring the students to prepare pure, characterised and, in some cases, novel compounds for test; and (iii) learning how to coach the student teams in decision making, and to manage group dynamics.
- Thinking about the best way to present the results from the various iterations to the students also made one mentor think carefully about how to present data most effectively at GSK.

- Everyone involved from GSK was surprised how clearly the course aims and scope were expected to be. For example, it was essential that examinable and nonexaminable material was made explicit.

Chemistry achievements and challenges

Considering the scientific achievements of the course, most of the experiments were successful, and students were able to isolate good-quality samples. Some of the yields were rather low, which was probably because of student inexperience. Failures in the reductive aminations could be attributed to difficulties in dispensing accurately small quantities of liquid amines (Gilson pipettes were used), and the unreactive nature of some of the amines (particularly the amino alcohols). The main side product was the primary alcohol **14** (Fig. 6), which sometimes misled students into thinking that this was the desired product, though nuclear magnetic resonance (NMR) spectroscopy readily identified it. Yields for the reductive aminations on a 2–3-mmol scale were invariably higher than those on a 0.3 mmol scale. The literature suggests that improved yields might be expected with sodium cyanoborohydride in methanol [27,28], but this was considered too hazardous for undergraduates. The first time the project was run, some protected diamines (e.g. **8n** and **8o**) were included. Although the reactions worked, the extra step involved in removing the protection made the overall procedure long, although feasible for more able students. Some of the students responded positively to the challenge of a failed reaction and discussed how conditions might be altered before putting the reaction on again. Exposure to industry-standard equipment (microwave reactors and automated chromatography) and methodology was welcomed.

The ^{1}H NMR spectra of the target compounds were challenging to interpret because of features that were sometimes present. First, the relatively slow bond rotation of the morpholine ring (and piperazine ring, where present) sometimes caused signal broadening. This was also observed with the hydrochloride salts of certain derivatives. Finally, derivatives of the tertiary amide **8b** displayed two sets of signals in an unequal ratio owing to slow rotation about the amide bond. Most of the students were unfamiliar with these concepts from their lecture courses.

The two methods for the Suzuki coupling worked equally; catalyst **12** was more active but also more expensive than catalyst **13**. More failures were observed than with the reductive amination: these seemed to be more related to the boronic acid selected, although it was difficult to be sure about this.

On average, each student performed five reactions and three chromatographic separations, and succeeded in making two test compounds. The students' medicinal chemistry reports were generally well written, demonstrating good understanding of the project objectives, although some had difficulty with the correct use of scientific terminology and ensuring accurate drawing of chemical structures.

Some of the compounds had significantly improved profiles compared with compound **4** ($pIC_{50} = 7.7$). In particular, the pyrrolidines **5b** and **5c**, and piperazines **5j–m** combined pIC_{50} values $\geqslant 8$ with much improved aqueous solubility and reduced lipophilicity (log D in the range 1.3–2.3). The best of these was the tertiary amide **5b**, although **5j** had the best combination of potency and binding efficiency index (BEI) [29]. It is of note that **5f** was almost as potent and has the equal highest BEI, suggesting that derivatives are worth investigation. From the wide range of phenol bioisosteres examined, the ones with a 1H-indazol-4-yl group, in combination with the preferred amines (**6b**, **6c**, **6h** and **6i**) were the most potent, although none could quite match the potency of the corresponding phenols. However, good aqueous solubility was achieved while maintaining a drug-like log D. Only one 1H-indol-4-yl analogue (**6y**) was prepared successfully, and had similar potency to **6d**, suggesting that further analogues should be made. However, azaindoles **6al–an** were disappointing. By contrast, compounds based on isomeric indazoles (**11c** and **11d**) or *N*-methyl heterocycles (**11b** and **11h**) had modest potency and were also some of the most lipophilic compounds made ($\log D_{7.4} \geqslant 2.5$). Interesting results were obtained with the aminopyrimidines **6ao** and **6ap**, which achieved good potency and BEI at low lipophilicity. Incorporation of this heterocycle into further derivatives would enable a search for more potency through more lipophilic amines, without departing from the ideal log D range or increasing the MW beyond 500, as has recently been described by Genentech–Piramed [30].

It is interesting that, as yet, none of the students have proposed making GDC-0941 (Fig. 6) [20], which was the PI3Kα inhibitor taken into development. This is because the sulfonylpiperazine did little to improve aqueous solubility and, thus, is an unattractive choice of amine. GDC-0941 also breaks Lipinski's MW rule and has ten hydrogen bond acceptors. What cannot be predicted is that, whereas the 1H-indazol-4-yl compound in most cases is less potent than the corresponding phenol analogues, potency is fully retained in the case of the sulfonylpiperazine analogue [20,31].[9] Presumably, the surprising discovery of high potency combined with good pharmacokinetics in an indazole derivative was sufficient for Piramed scientists to make a compromise on the aqueous solubility. Development candidates frequently emerge from such compromises (and in usually more than three design cycles), so it is hardly surprising that the students, following guidance dutifully, did not select GDC-0941.

Concluding remarks

In this article, we described the development of a practical medicinal chemistry course, suitable for third-year undergraduates. In the course, the students learned about balancing three important properties (potency, solubility and lipophilicity) and successfully designed more potent and soluble analogues, such as **5b**, **5j**, **6b**, **6c**, **6h**, **6i**, **6ao** and **6ap**.

It is evident that other universities might not be able to reproduce this project without the same level of funding, access to equipment and screening facilities. However, one might be able to take it as a template, comprising:

- An enzyme target with a published X-ray crystal structure, with a way to conduct molecular modelling and docking of target compounds.
- A simple assay for enzyme inhibitors with commercially available enzyme and fluorogenic substrate. Aqueous solubility can be measured by eye (turbidometric method), and the

[9] The same potency enhancing cooperativity is observed where an indole replaces the indazole, see [31].

hydrophobicity index requires access to an analytical high performance liquid chromatography (HPLC) with reverse-phase column.

- A lead chemical series with opportunities for derivatisation through one to three chemical steps. Key intermediates should be commercially available or readily synthesised.

A recent paper in the *Journal of Chemical Education* [32] described a project to prepare and screen a series of antibiotics, which were made from readily available 6-aminopenicillanic acid in one step. In this case, the students made and screened the compounds and analysed their structure–activity relationships. Although the medicinal chemistry elements of structure-based and physiochemical property design were lacking, this project also served as a practical and simple way to explore medicinal chemistry with an undergraduate class.

Given that the current module was limited to 18 chemistry students, consideration is now being given to devising alternative projects that would be less expensive, less reliant on industry support (e.g. for intermediates and compound screening), and potentially open to more students.

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