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Parallel-Compound Synthesis: Methodology for Accelerating Drug Discovery

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Abstract—Parallel compound synthesis enables large numbers of individual compounds to be prepared simultaneously using semi-automated techniques. This fast and efficient methodology has an important role to play in accelerating lead optimisation and hence the whole drug discovery process. The potential of this strategy to rapidly optimise chemical leads and provide structure–activity relationship (SAR) information was demonstrated in two therapeutic areas, antiviral agents (herpes simplex virus), and neurokinin-2 receptor antagonists. Copyright © 1996 Elsevier Science Ltd

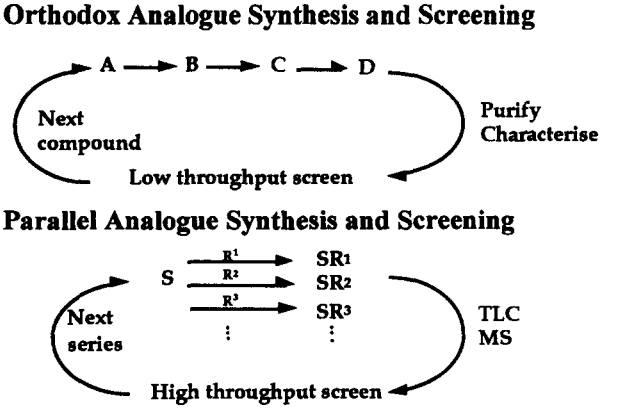
Introduction

The quest for new drug molecules in the pharmaceutical industry is historically a long and expensive procedure in an increasingly competitive environment. In particular, the process of lead optimisation, whereby a compound with weak biological activity is modified to increase both potency and selectivity, is often rate-limited by the speed of orthodox organic synthesis. Thus, any methodology enabling acceleration of analogue synthesis will have an important role to play in drug discovery. Much work in the area of new high speed chemical technology has recently focused on the use of combinatorial libraries for creating large numbers of drug-like products either as single compounds or in mixtures.[1] Although primarily focused on lead discovery, these methods may also have an impact on lead optimisation, especially if targeted *single* compounds are prepared in parallel.

We describe here our recent use of an automated approach to the parallel preparation of tens to hundreds of analogues of a biologically active substrate. The products are synthesised using reliable coupling and functional group interconversion chemistry and are progressed to screening after removal of solvent and volatile by-products. A schematic comparison of our parallel-analogue synthesis and screening methodology with an orthodox protocol is shown below (Scheme 1).

Orthodox synthesis often involves a multistep sequence (e.g. from A through to the final product D) that is purified and fully characterised before screening. The next analogue is then designed (guided by the biological activity of the previous compound) synthesised, and screened. This process is repeated to optimise activity and selectivity. Parallel-analogue synthesis involves reaction of a substrate S with multiple reactants, R^1, R^2, $R^3 \ldots R^n$, to produce a compound library of n individual products SR^1, SR^2, $SR^3 \ldots SR^n$. The library is screened without purification and with only minimal characterisation of the individual compounds, using rapid throughput screening techniques. If any active compounds are identified, they are resynthesised on a larger scale for purification, characterisation, and screening by traditional methods. If biological activity is confirmed, the newly discovered leads and structure–activity relationships (SAR) are used to design new substrate templates. Further libraries are then prepared, using the parallel-analogue synthesis methodology, focused on the new substrate. This process is thus a rapid and iterative fine-tuning similar to conventional lead optimisation, only faster and involving more analogues.

Typically, parallel synthesis involves one- or two-step reactions using reliable solution chemistry such as reductive aminations, acylations, and Suzuki couplings performed on a small scale ($\sim$10 μmol). Initially, trial reactions are carried out to optimise the reaction conditions, and this also dictates the vessels to be used



Scheme 1.

(e.g. reacti-vials™, 96-well plates, microwave vials). The reactant library (a selected set of reactants with appropriate functional groups, e.g. acid chlorides) necessary for the desired analogues is assembled, usually from commercial sources. The reactant set can either be targeted, using available SAR information, or non-targeted but selected to encompass a diverse range of physical and chemical properties, such as molecular size (M_R), lipophilicity (log D) and acidity (pK_a). Once selected, the reactants and substrate are dissolved in a suitable solvent (e.g. dichloromethane, acetonitrile, THF, DMF) to give solutions of known concentration (usually ~0.1 M). The required volume of the substrate solution and each of the reactant solutions is dispensed into the reaction vessels, along with the required quantity of any necessary reagent solutions, such as triethylamine. These reaction mixtures are subjected to appropriate conditions, such as heating or sonication, following which the crude products are analysed by thin-layer chromatography (TLC) and mass spectrometry (MS) to give an indication of the average extent of reaction and percentage of products formed. Solid-phase reagents such as polymer-bound coupling agents[2] are used where possible, since they enhance product purity due to the ease which with they can be separated by a simple filtration.[3]

Parallel-compound synthesis using hundreds of reactants lends itself ideally to automation. We have employed a Gilson 232XL solution dispenser for repetitive handling of substrates, reactants, reagents, and solvents. This has been beneficial both in terms of speed and accuracy and, furthermore, liberates chemists to design and develop new high-speed chemistry.

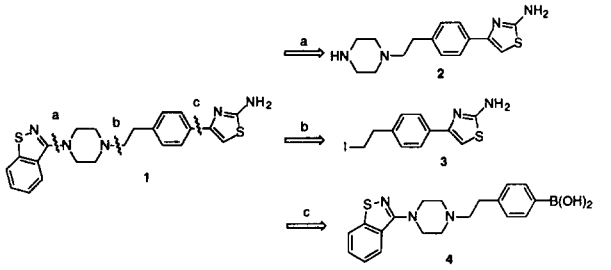
Results and Discussion

The utility of parallel synthesis methodology can best be demonstrated by real examples from our work. We present two examples: one from a program to discover compounds active against herpes simplex virus (HSV) and one from a program to find novel antagonists of the neurokinin-2 (NK_2) receptor.[4] Our examples focus only on the part of the programmes that involved the use of this methodology.

Example 1: HSV-1

The aminothiazole derivative **1** was discovered through in-house screening to have activity against herpes simplex virus (IC_{50} = 3 μM vs HSV-1 helicase ATPase and 11 μM antiviral activity in a HSV-1 plaque reduction assay). Our objective was to use parallel-synthesis techniques to rapidly explore the SAR of the lead compound and optimise the activity in both the HSV-helicase ATPase assay and the anti-viral plaque reduction assay.

Retrosynthetic analysis of **1**, using the disconnections shown in Scheme 2, led to three intermediates, **2**–**4**, all



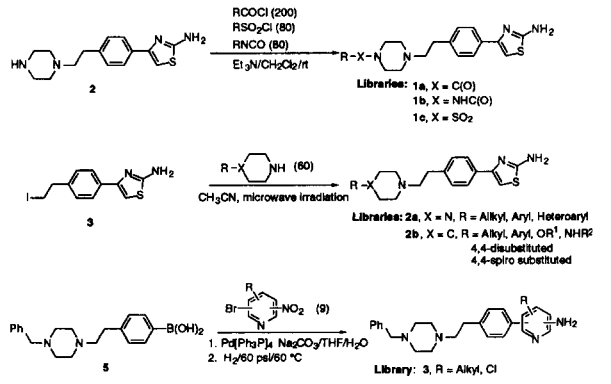
Scheme 2.

of which were candidate substrates for parallel synthesis.

Disconnection **a** gave the piperazine derivative **2** which should react readily with electrophiles. Disconnection **b** led to an electrophilic intermediate such as the iodide **3**, which should undergo nucleophilic substitution, and disconnection **c** suggested the boronic acid intermediate **4**, which under Suzuki[5] conditions reacts with heteroaryl halides to form biaryl derivatives. Previous SAR suggested that the aminothiazole was important for activity, so the first two disconnections retained this heterocycle. The third disconnection allowed replacements for the aminothiazole to be sought.

Parallel synthesis, based on the retrosynthetic analysis, was used to rapidly prepare (in one week) three compound libraries containing a total of more than 400 analogues of the lead compound **1** (Scheme 3). The piperazine substrate **2** was acylated with 200 different commercial acid chlorides and 80 isocyanates, and sulphonylated with 80 sulphonyl chlorides to give Library 1. The iodide **3** underwent smooth nucleophilic substitution with a series of 60 piperidines and piperazines to give Library 2. Microwave irradiation was used here as a quick and convenient method of heating multiple reaction flasks in parallel. Finally, palladium-mediated cross coupling of the phenyl boronic acid derivative **5** (the benzyl analogue of **4**) with bromonitroheterocycles (under Suzuki conditions) followed by catalytic hydrogenation with hydrogen gas at 60 psi, *without the addition of any further catalyst*, gave aminoheteroaryl phenyl analogues, Library 3.

The three libraries were screened in an HSV-1 helicase ATPase binding assay at a concentration of 40 μM for



Scheme 3.

each compound (calculated on the quantity of the starting substrate). Compounds with greater than 50% binding were titrated to determine their IC_{50} values. Compounds with $IC_{50} < 10$ μM were resynthesised using orthodox techniques, purified and fully characterised and their IC_{50}s determined. If activity was confirmed for the resynthesised compounds, they were tested for antiviral activity in the HSV-1 plaque reduction assay. Library 1 contained one confirmed hit, **6**, and Library 2 had three confirmed hits **7–9**. Library 3 showed no activity at 40 μM for any compound. The results for the four active compounds are shown in Table 1.

These results highlighted some key points concerning our methodology. Firstly, activity at the micromolar level was readily measured even on the crude library compounds; the presence of by-products such as triethylamine hydrochloride did not significantly affect the biological assay. By-products that are known to be formed in each reaction were assayed separately to confirm that they were inactive and did not interfere with the assay. Secondly, there was a clear consistency between the biological activity of the library samples and the resynthesised and purified samples, thus conferring confidence in the library results and suggesting that the chemical conversion during parallel synthesis was high, confirming the good yields implied by TLC and MS analysis. Thirdly, a range of activity was observed; many compounds were weaker than the lead compound, but some, such as **7**, were more potent. Furthermore, we have improved the antiviral potency of the compounds compared with **1**; for example, compound **9** is 18× more potent in the HSV helicase plaque reduction assay than **1**. This example demonstrated that we can rapidly begin to optimise potency and explore SAR of a lead compound using simple and straightforward parallel-synthesis methodology.

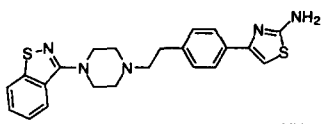
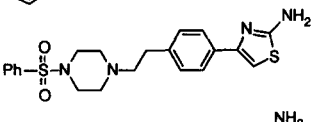
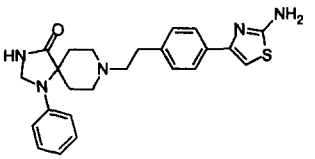
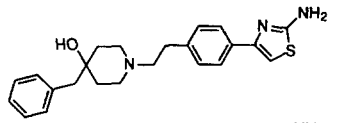
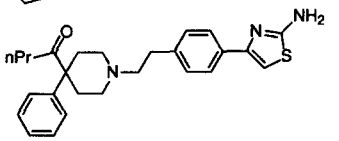
Example 2: NK_2 antagonists

A second example of the use of parallel synthesis for rapid SAR evaluation arose during work from a program to find novel antagonists of the NK_2 receptor. The known NK_2 antagonist,[6] **10**, is an ideal chemical lead for the application of parallel-synthesis methodology. Retrosynthetic analysis of **10** as shown in Scheme 4 led to four substrates, **11–14** (Scheme 5) that were suitable for SAR investigation and may lead to novel NK_2 antagonists.

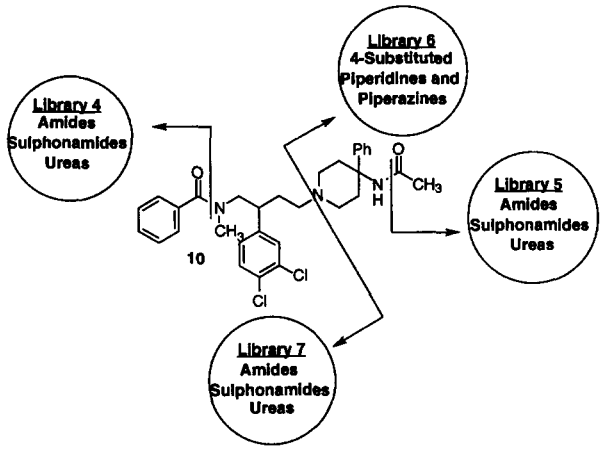
Substrates **11** and **12** were used to explore the SAR around the left-hand and right-hand amide linkages of **10**, and also to replace the amide bonds with ureas and sulphonamides (Libraries 4 and 5). Nucleophilic substitution of the mesylate **13** with piperidines and piperazines enabled rapid investigation of the SAR around the piperidine nucleus (Library 6). Finally, the piperidine substrate **14** was ideal to investigate the replacement of the entire left-hand side of **10** and allow the search for structurally novel NK_2 antagonists (Library 7).

Parallel synthesis using these substrates gave four compound libraries containing a total of 1180 indivi-

Table 1. Structure and activity of inhibitors of HSV helicase

Compound (library)	Structure	IC_{50} vs HSV-1 helicase ATPase assay (μM)		HSV-1 plaque reduction assay in vitro IC_{50} (μM) [IC_{90} (μM)] pure sample
		Library sample	Pure sample	
1		NT[a]	3.0 ($n=5$)	11 ($n=5$)
6 (Library 1)		5	6.0 ($n=2$)	4.3 ($n=2$) (26.7 ($n=2$))
7 (Library 2)		1.0	1.1 ($n=3$)	1.6 ($n=3$) (4.9 ($n=3$))
8 (Library 2)		4.0	3.4 ($n=2$)	1.5 ($n=4$) (3.5 ($n=4$))
9 (Library 2)		4.0	1.4 ($n=4$)	0.6 ($n=3$) (1.0 ($n=3$))

[a]NT = not tested.

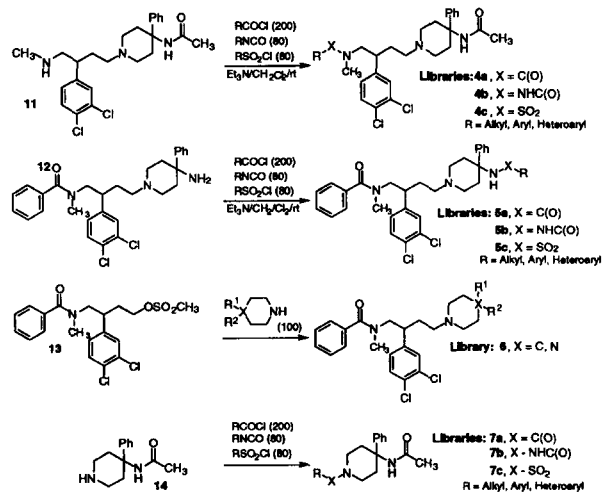


Scheme 4.

dual compounds. These libraries were screened against NK_2 receptors (isolated from rat duodenum membranes) at concentrations of 100, 10, and 1 nM. The number of active compounds found from screening the libraries at each of these concentrations are listed in Table 2.

Extensive SAR can be derived from these results. For example, Library 4 indicates that amides, but not ureas or sulphonamides, were well tolerated on the left-hand side of **10**, and Library 5 shows that amides were preferred on the right-hand-side. The 4,4-disubstituted piperidine moiety of **10** could be replaced with a broad range of piperidines and piperazines, as shown by the large number of potent compounds in Library 6. These compounds are undergoing further investigation. Finally, attempts to replace the 3,4-dichlorophenylbutylamine moiety, such as in Library 7, did not lead to potent compounds, showing the importance of this central section of **10** for NK_2 activity.

Five compounds, chosen to amplify the above screening results, were resynthesised, fully characterised, and screened against human NK_2 receptors. Data on these



Scheme 5.

Table 2. Number and type of compounds active against NK_2 at 100

Library no.	Compound type	Number of hits showing specified inhibition of NK_A binding		
		>50% at 100 nM	>50% at 10 nM	>30% at 1 nM
4a	Amides	73	4	5
4b	Ureas	18	0	NT[a]
4c	Sulphonamides	4	0	NT
5a	Amides	32	7	NT
5b	Ureas	4	0	NT
5c	Sulphonamides	40	1	NT
6	Secondary amines	27	10	16
7a	Amides	0	NT	NT
7b	Ureas	0	NT	NT
7c	Sulphonamides	3	NT	NT

[a]NT = not tested.

compounds are shown in Table 3. Compounds **15–17** confirmed both that the activity seen in the library compounds was retained when the compounds were fully purified, and that the SAR described above for Library 4 was confirmed by these three compounds. Compounds **18** and **19** also confirmed the translation in activity from the library compounds to purified compounds, as well as demonstrating that alkylamides as well as arylamides were tolerated on the left-hand side of **10**.

The time scale for the preparation of the 1180 compounds in four library sets using parallel synthesis methodology was about 6 weeks, and screening of these was carried out in a few days. This demonstrates that parallel-synthesis can considerably enhance the rate at which medicinal chemists can prepare analogues of lead compounds to determine SAR information.

Conclusions

We have described the parallel synthesis of libraries of single compounds, and illustrated the technique with two examples: one to optimise antiviral activity against herpes simplex virus, and one to investigate the SAR of NK_2 receptor binding. These examples demonstrate the power of parallel synthesis in rapid compound synthesis and SAR evaluation. It is a technique which when used together with conventional organic synthesis can have a major impact on lead optimisation, and potentially the whole drug discovery process.

Experimental

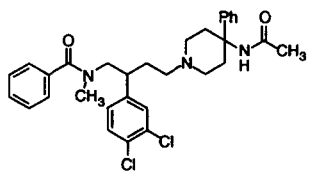
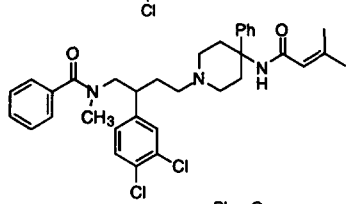
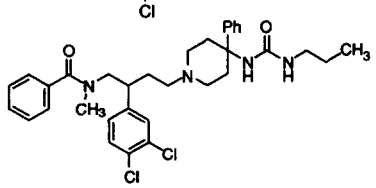
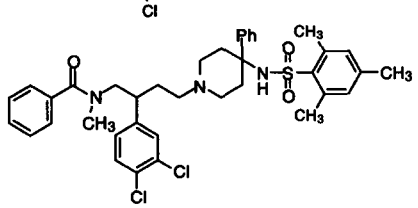
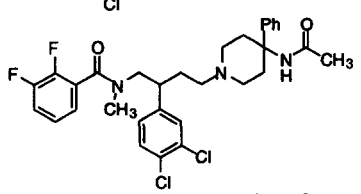
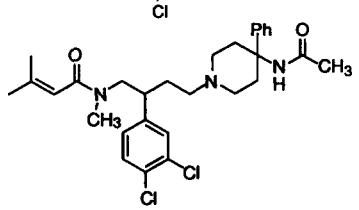
Chemistry

All melting points are uncorrected and were obtained in open ended glass capillary tubes on a Gallenkamp melting point apparatus. ^1H NMR spectra were recorded at 300 MHz using a Brucker AC 300 or GE QE-300 instrument. Elemental analysis were per-

formed on a Perkin Elmer Model 2400, series II, CHN analyser. Mass spectral data were obtained from a Fisons TRIO 1000 mass spectrometer. Flash column chromatography was performed on silica gel 60, 0.04–0.063 mm (E. Merck). Thin-layer chromatography was performed on precoated silica gel F-254 plastic plates (0.2 mm; E. Merck). Solvents were of reagent grade or higher. A Gilson 232XL solution dispenser was employed for the repetitive handling of substrate and reactant solutions during parallel synthesis, and a Savant AES2000 vacuum centrifuge was used for the parallel evaporation of multiple reaction mixtures. A CEM MDS-2000 microwave sample preparation system was used for microwave irradiation of samples.

2-Amino-4-(4-(2-(piperazinyl)-ethyl)-phenyl)thiazole trihydrochloride (2). To a stirred soln of 2-amino-4-(4-(2-chloroethyl)-phenyl)thiazole hydrobromide[7] (9.8 g, 31 mmol) and triethylamine (9 mL, 65 mmol) in DMF (100 mL) was added *tert*-butyl 1-piperazinecarboxylate (5.7 g, 31 mmol) and the mixture heated at 120 °C for 5 h. The reaction mixture was evapd in vacuo and the residue partitioned between EtOAc and H_2O. The EtOAc layer was washed with H_2O, dried ($MgSO_4$), and evapd to dryness under red pres. The brown oily residue was purified by flash CC eluting with EtOAc to give 2-amino-4-(4-(2-(*N-tert*-butyloxycarbonyl-piperazin-1-yl)-ethyl)-phenyl)thiazole as a brown foam, which was recrystallised from toluene to afford an off-white solid (yield: 3.3 g, 27%), mp 179–180 °C, MS (*m/z*) 389 [M+1], (found C, 62.11; H, 7.41; N, 14.24, calcd for $C_{20}H_{28}N_4O_2S$, C, 61.86; H, 7.22; N, 14.43%); ^{1}H NMR (DMSO): δ 1.38 (s, 9H), 2.39 (m, 2H), 2.55 (m, 4H), 2.72 (m, 2H), 3.30 (m, 4H), 6.91 (s, 1H), 7.02 (br s, 2H), 7.20 (d, J=8 Hz, 2H), 7.68 (d, J=8 Hz, 2H).

Table 3. Structure and affinity of NK_2 ligands

Compound (library)	Structure	Library compounds: % inhibition vs. NK_2 receptors from rat duodenum	Resynthesised compounds: IC_{50} vs. human NK_2 receptors expressed in CHO cells (nM)
10		86% at 100 nM 2% at 10 nM 14% at 1 nM	2.4
15 (4a)		78% at 100 nM 37% at 10 nM	3.1
16 (4b)		66% at 100 nM 21% at 10 nM	6.9
17 (4c)		0% at 100 nM	700
18 (5a)		44% at 10 nM 36% at 1 nM	2.5
19 (5a)		82% at 10 nM 29% at 1 nM	11

[a]NT = not tested.

Hydrogen chloride gas was bubbled into a suspension of 2-amino-4-(4-(2-(*N-tert*-butyloxycarbonyl-piperazin-1-yl)-ethyl)-phenyl)thiazole (2.8 g, 7.2 mmol) in dry CH_2Cl_2, at 0 °C in an ice bath, until a clear solution was obtained. The mixture was stirred for 1 h and the ppt. separated by filtration, washed with CH_2Cl_2 and dried to give the title compound as an off-white solid, (yield: 2.8 g, 96%), MS (*m/z*) 289 [M+1], (found C, 44.77; H, 6.25; N, 13.66, calcd for $C_{15}H_{20}N_4S \cdot 3HCl$, C, 44.84; H, 5.75; N, 13.94%); ^{1}H NMR (DMSO): δ 3.20 (m, 2H), 3.50 (br m, 6H), 3.85 (m, 4H), 7.20 (s, 1H), 7.35 (d, J=8 Hz, 2H), 7.75 (d, J=8 Hz, 2H), 9.76 (s, 1H).

2-Amino-4-(4-(2-iodoethyl)-phenyl)thiazole (3). To a stirred soln of 2-amino-4-(4-(2-chloroethyl)-phenyl) thiazole (0.41 g, 1.7 mmol) in butan-2-one was added NaI (2 g, 13 mmol) and the mixture heated at reflux temperature for 16 h, cooled, and evapd to dryness in vacuo. The residue was dissolved in EtOAc (100 mL), and the solution washed with H_2O (50 mL) and dilute aqueous sodium thiosulphate solution (50 mL), dried ($MgSO_4$), and evapd to dryness under red. pres. to give a solid (Yield: 490 mg, 87%). A small sample was recrystallized from EtOAc:hexane, mp 155–156 °C, MS (*m/z*) 331 [M+1], (found C, 40.42; H, 3.14; N, 8.50, calcd for $C_{11}H_{11}N_2SI$, C, 40.01; H, 3.36; N, 8.48%); ^{1}H NMR ($CDCl_3$): δ 3.19, (t, J=7 Hz, 2H), 3.37 (t, J=7 Hz, 2H), 5.14 (br s, 2H), 6.71 (s, 1H), 7.19 (d, J=8 Hz, 2H), 7.75 (d, J=8 Hz, 2H).

General procedure for the parallel synthesis of 90 compounds

The substrate (1 mmol) is dissolved in a suitable solvent (10 mL, 0.1 M solution) and an aliquot (100 μL, 10 μmol) transferred to each of 90 reaction vessels. Each reactant (0.1 mmol) is dissolved in the chosen solvent (1 mL, 0.1 M soln) and an aliquot (110 μL, 11 μmol, 1.1 equiv) of each reactant is transferred to the 90 reaction vessels, a different reactant to each vessel. An aliquot of any additional reagents necessary for the reaction is also added to the reaction vessels, which are then subjected to the chosen reaction conditions. The crude products are analysed by TLC and MS to give an indication of the average extent of reaction and number of products formed. The solvent is removed using a vacuum centrifuge and the residues dissolved in DMSO prior to automated screening.

Preparation of analogues of 2-amino-4-(4-(2-(piperazin-1-yl)-ethyl)-phenyl)thiazole (2) by parallel-synthesis

Analogue library 1a. Prepared by acylation of **2** with a series of aliphatic and aromatic acid chlorides in the presence of triethylamine using dichloromethane as solvent, for 18 h at ambient temperature. The products were analysed by TLC and MS; the majority showed a single new product on TLC and the expected molecular ion by MS.

Analogue library 1b. Prepared by acylation of **2** with a series of aliphatic and aromatic isocyanates using dichloromethane as solvent for 18 h at ambient temperature. The products were analysed by TLC and MS; the majority showed a single new product on TLC and the expected molecular ion by MS.

Analogue library 1c. Prepared by sulphonylation of **2** with a series of aliphatic and aromatic sulphonyl chlorides in the presence of triethylamine using CH_2Cl_2 as solvent, for 18 h at ambient temperature. The products were analysed by TLC and MS; the majority showed a single new product on TLC and the expected molecular ion by MS.

2-Amino-4-[4-(2-(4-phenylsulphonyl) piperazin-1-yl) ethylphenyl]thiazole (6). Prepared by the reaction of 2-amino-4-(4-(2-(piperazin-1-yl)-ethyl)-phenyl)thiazole (**2**) with benzenesulphonyl chloride. Recrystallized from ethanol as yellow plates (yield: 1.01 g, 35%), mp 219–220 °C, MS (*m/z*) 429 [M+1], (found C, 58.75; H, 5.61; N, 12.93, calcd for $C_{21}H_{24}N_4O_2S_2$, C, 58.85; H, 5.64; N, 13.07%); ^{1}H NMR (DMSO): δ 2.60 (m, 10H), 2.81 (m, 2H), 6.84 (s, 1H), 7.01 (s, 2H), 7.10 (d, J=8 Hz, 2H), 7.61 (d, J=8 Hz, 2H), 7.72 (m, 5H).

Preparation of analogues of 2-amino-4-(4-(2-iodoethyl)-phenyl)thiazole (3) by parallel synthesis

Analogue libraries 2a and b. Prepared by reaction of **3** with 4-substituted piperazines (27 compounds) and 4-substituted piperidines (21 compounds), using acetonitrile as solvent and heating the reaction mixtures in sealed polypropylene vials under microwave irradiation for 4 h. The products were analysed by TLC and MS; the majority showed a single new product on TLC and the expected molecular ion by MS.

General procedure for the reaction of 2-amino-4-(4-(2-iodoethyl)-phenyl)thiazole (3) with substituted piperidines to give 2-amino-4-(4-(2-piperidinylethyl)-phenyl)-thiazoles (7–9)

A solution of 2-amino-4-(4-(2-iodoethyl)phenyl)thiazole (**3**) (0.5 g, 1.51 mmol) and the piperidine (3 mmol, 2 equiv.) in acetonitrile (15 mL) was heated at 100 °C for 16 h and evapd to dryness under red pres. The residue was dissolved in CH_2Cl_2 (200 mL), and the solution washed with H_2O (50 mL) and aq sodium bicarbonate solution (50 mL), dried ($MgSO_4$), filtered and evapd to dryness under red pres. The residue was purified by flash CC eluting with a gradient of MeOH in CH_2Cl_2 (1–4%). The oily products were converted to their hydrochloride salts by treatment with a solution of HCl chloride in Et_2O at 0 °C.

2-Amino-4-[4-(2-(1-phenyl-1,3,8-triazaspiro-(4,5)-decan-4-one)ethyl)phenyl]thiazole (7). Colourless solid, obtained as the dihydrochloride; mp 210 °C (dec), (yield: 230 mg, 30%); MS (*m/z*) 434 [M+1], (found C, 57.08; H, 5.81; N, 13.12, calcd for $C_{24}H_{27}N_5OS \cdot 2HCl$, C, 56.92; H, 5.77; N, 13.82%); ^{1}H NMR (DMSO): δ

3.01 (m, 10H), 3.19 (m, 2H), 4.59 (s, 2H), 6.77 (t, $J=7$ Hz, 1H), 7.05 (d, $J=8$ Hz, 2H), 7.19 (m, 3H), 7.39 (d, $J=8$ Hz, 2H), 7.75 (d, $J=8$ Hz, 2H), 9.00 (s, 1H).

2-Amino-4-[4-(2-(4-hydroxy-4-phenymethylpiperidin-1-yl)ethyl)phenyl]thiazole (8). Colourless solid, obtained as the dihydrochloride; mp softens at 140–150 °C, (yield: 280 mg, 39%); MS (*m/z*) 394 [M+1], (found C, 58.75; H, 6.56; N, 8.85, calcd for $C_{23}H_{27}N_3OS\cdot 2HCl$, C, 59.23; H, 6.27; N, 9.01%); ^{1}H NMR ($CDCl_3$): δ 1.27 (br s, 1H), 5.55 (br d, $J=$ 10 Hz, 2H), 1.82 (br t, $J=10$ Hz, 2H), 2.41 (br t, $J=10$ Hz, 2H), 2.65 (m, 2H), 2.81 (m, 6H), 5.05 (br s, 2H), 6.68 (s, 1H), 7.15 (d, $J=8$ Hz, 2H), 7.30 (m, 5H), 7.70 (d, $J=8$ Hz, 2H).

2-Amino-4-[4-(2-(4-phenyl-4-(butanoyl)piperidin-1-yl) ethyl)phenyl]thiazole (9). Colourless solid, obtained as the dihydrochloride; mp 180 °C (dec.), (yield: 110 mg, 14%); MS (*m/z*) 434 [M+1], (found C, 61.60; H, 6.78; N, 7.77, calcd for $C_{26}H_{31}N_3OS\cdot 2HCl$, C, 61.66; H, 6.57; N, 8.30%); ^{1}H NMR ($CDCl_3$): δ 0.66 (t, J = 7 Hz, 3H), 1.45 (m, 2H), 2.05 (br t, $J=10$ Hz, 2H), 2.15 (br t, $J=7$ Hz, 2H), 2.36 (br t, $J=10$ Hz, 2H), 2.59 (m, 4H), 2.80 (m, 4H), 5.00 (br s, 2H), 6.69 (s, 1H), 7.15 (d, $J=8$ Hz, 2H), 7.30 (m, 5H), 7.68 (d, $J=8$ Hz, 2H).

Preparation of 4-(2-(4-benzyl) piperazin-1-yl) ethyl) phenyl boronic acid (5). To a solution of 4-bromophenethyl alcohol[8] (9.7 g, 48 mmol) and pyridine (15 mL, 190 mmol) in CH_2Cl_2 (100 mL) stirred at 0 °C under a nitrogen atmosphere was added methanesulphonyl chloride in a dropwise manner over 10 min. The mixture was stirred overnight at ambient temperature, washed sequentially with aq 2 N HCl, H_2O and brine, dried ($MgSO_4$), filtered and evapd to dryness under red. pres. to give 4-bromophenethyl methanesulphonate as a colourless solid (yield: 12.8 g, 95%); MS (*m/z*) 296, 298, and 299 [MNH_4^+].

The mesylate (8 g, 28 mmol), 4-benzylpiperazine[8] (5 g, 28 mmol) and K_2CO_3 (3.9 g, 28 mmol) were heated together at reflux temperature in acetonitrile (80 mL) for 7 h, cooled, evapd to dryness in vacuo and redissolved in CH_2Cl_2 (250 mL). The solution was washed with H_2O, dried ($MgSO_4$), filtered, and evapd to dryness under red pres. The residue was purified by CC eluting with hexane:EtOAc (1:1). The fractions containing pure product were combined and evapd to dryness to give to give 1-(4-bromophenethyl)-4-benzylpiperazine as an oil (yield: 7.7 g, 75%); MS (*m/z*) 360 [M+1], (found C, 63.64; H, 6.57; N, 7.62, calcd for $C_{19}H_{23}BrN_2$, C, 63.52; H, 6.45; N, 7.80%); ^{1}H NMR ($CDCl_3$): δ 2.50 (m, 10H), 2.75 (m, 2H), 3.55 (s, 2H), 7.05 (d, $J=8$ Hz, 2H), 7.31 (m, 5H), 7.40 (d, $J=8$ Hz, 2H).

A solution of 1-(4-bromophenethyl)-4 benzylpiperazine (7 g, 20 mmol) stirred at −70 °C under a nitrogen atmosphere was treated with butyllithium (8.5 mL of a 2.5 M solution in hexane, 22 mmol) in a dropwise manner over 10 min. The mixture was stirred at −70 °C for 15 min and freshly distilled trimethyl borate (2.5 mL, 22 mmol) added in a dropwise manner. The clear soln was stirred at −70 °C for 45 min and quenched with satd NH_4Cl solution (10 mL). The pptd mixture was stirred at ambient temperature for 2 days and extracted with EtOAc (3 × 80 mL). The extracts were washed with brine, dried ($MgSO_4$), filtered, and evapd to dryness under red. pres. to give a white solid. The solid was suspended in H_2O (80 mL), heated for 2 h on a steam bath and extracted with EtOAc (3 × 80 mL). The extracts were washed with brine, dried ($MgSO_4$), filtered and evapd to dryness under red. pres. to give a white solid. The solid was triturated with EtOAc and filtered to give 4-(2-(4-benzyl)piperazin-1-yl)ethyl)phenyl boronic acid (**5**) (contains a trace of the mono-methyl borate) as a colourless solid (yield: 3.4 g, 53%); MS (*m/z*) 325 [M+1] and 339 [M+14].

Procedure for the sequential palladium-catalysed cross-coupling of boronic acids with substituted halonitrobenzenes and reduction of the nitro group to an amine

Analogue library 3. Prepared by reaction of 4-(2-(4-benzyl)piperazin-1-yl)ethyl)phenyl boronic acid (**5**) (10 μmol for each reaction) with substituted bromonitrobenzenes (9 compounds, 10 μmol of each) as follows. To a THF soln of the boronic acid (0.1 M, 100 μL, 10 μmol) was added a THF solution of a halonitrobenzene (0.1 M, 100 μL, 10 μmol) and an aq soln of sodium bicarbonate (0.4 M, 50 μL, 20 μmol), followed by tetrakis(triphenylphosphine)palladium(0) (5 mol%). The mixture stood at room temperature for 4 h, and then each reaction mixture hydrogenated with hydrogen gas at 60 psi at 60 °C for 8 h. The products were analysed by TLC and MS, the majority showed a single new product on TLC and the expected molecular ion by MS.

***N*-Methyl-2-(3,4-dichlorophenyl)-4-(4-acetamido-4-phenylpiperidinyl)butylamine (11)**. A mixture of *N*-methyl-2-(3,4-dichlorophenyl)-4-hydroxybutylamine (3.7 g 15 mmol) and di-*tert*-butyl dicarbonate (3.9 g, 18 mmol) in CH_2Cl_2 was stirred at 20 °C for 16 h and the solvent evapd to dryness. The residue was purified by CC eluting with a gradient of MeOH in CH_2Cl_2 (1–4%). The fractions containing pure product were combined and evapd to dryness to give *N-tert*-butyl oxycarbonyl-*N*-methyl-2-(3,4-dichlorophenyl)-4-hydroxybutylamine as an oil (yield: 5.2 g, 100%); MS (*m/z*) 349 [M+1]; ^{1}H NMR ($CDCl_3$): δ 1.35 (s, 9H), 1.80 (m, 2H), 2.69 (m, 2H), 3.10 (m, 1H), 3.41 (m, 4H), 3.60 (m, 1H), 7.02 (br s, 1H), 7.26 (br s, 1H), 7.35 (d, $J=8$ Hz, 1H).

A cold (0 °C) solution of *N-tert*-butyl oxycarbonyl-*N*-methyl-2-(3,4-dichlorophenyl)-4-hydroxybutylamine (5.0 g, 14.3 mmol) and triethylamine (1.74 g, 17 mmol) in CH_2Cl_2 (70 mL) was treated in a dropwise manner with methanesulphonyl chloride (1.97 g, 17 mmol). The mixture was stirred at 0 °C for 1 h, washed with water, satd sodium bicarbonate solution, dried ($MgSO_4$), filtered, and evapd to dryness under red. pres. The residue was purified by CC eluting with a gradient

of MeOH in CH_2Cl_2 (1–4%). The frs containing pure product were combined and evapd to dryness to give 1-*tert*-butyloxycarbonyl-2-(3,4-dichlorophenyl)-4-methanesulphonyloxy-1-methyl-butylamine as an oil (yield: 5.0 g, 90%); MS (m/z) 427 [M+1]. A mixture of this mesylate (860 mg, 2 mmol), 4-phenyl-4-acetamidopiperidine[9] **14** (400 mg, 1.83 mmol) and K_2CO_3 (560 mg, 4 mmol) were heated together at reflux temperature under a N_2 atmosphere for 17 h. The mixture was evapd to dryness and the residue purified by CC eluting with MeOH in CH_2Cl_2 (5%). The fractions containing pure product were combined and evapd to dryness to give tert-butyl *N*-methyl-*N*-[2-(3,4-dichlorophenyl)-4-methanesulphonylbutyl]carbamate as an oil (Yield: 600 mg, 60%); MS (*m/z*) 549 [M+1]; ^{1}H NMR ($CDCl_3$): δ 1.28 (s, 9H), 1.77 (m, 1H), 1.85 (m, 1H), 2.00 (s, 3H), 2.18 (m, 8H), 2.35 (m, 2H), 2.70 (m, 4H), 2.98 (m, 1H), 3.40 (m, 1H), 5.53 (s, 1H), 7.03 (m, 1H), 7.30 (m, 7H). The *tert*-butyloxycarbonyl protecting group was removed by treatment with TFA in CH_2Cl_2 (50%) (10 mL) at room temperature for 16 h. The reaction mixture was evapd to dryness and dissolved in CH_2Cl_2. The solution was washed with satd sodium bicarbonate, dried ($MgSO_4$), filtered, and evaporated to dryness under red. pres. The residue was purified by CC eluting with MeOH in CH_2Cl_2 (5%). The fractions containing pure product were combined and evapd to dryness to give the title compound as a foam (yield: 340 mg, 76%); MS (*m/z*) 449 [M+1]; ^{1}H NMR ($CDCl_3$): δ 1.65 (m, 1H), 1.90 (m, 1H), 2.00 (s, 3H), 2.17 (m, 8H), 2.37 (s, 3H), 2.78 (m, 5H), 3.48 (s, 1H), 5.49 (br s, 1H), 7.05 (dd, *J*=8, 1 Hz, 1H), 7.31 (m, 7H).

***N*-Methyl-*N*-[2-(3,4-dichlorophenyl)-4-(4-amino-4-phenylpiperidyl)butyl]benzamide (12).** The mesylate **13** (1.34 g, 3.1 mmol) and 4-phenyl-4-*tert*-butyloxycarbonylaminopiperidine (860 mg, 3.1 mmol) and K_2CO_3 (430 mg, 3.1 mmol) were heated together in acetonitrile at reflux temperature for 16 h, cooled, and evapd to dryness. The residue was purified by CC, eluting with MeOH in CH_2Cl_2 (5%). The fractions containing product were combined and evapd to dryness to give a *N*-methyl-*N*-[2-(3,4-dichlorophenyl)-4-(4-*tert*-butyloxycarbonylamino-4-phenylpiperidyl) butyl]benzamide as foam (yield: 1.25 g, 80%); MS (*m/z*) 611 [M+1]. The *tert*-butyloxycarbonyl protecting group was removed by treatment with TFA in CH_2Cl_2 (50%) (10 mL) at room temperature for 16 h. The reaction mixture was evapd to dryness and dissolved in CH_2Cl_2. The solution was washed with satd sodium bicarbonate, dried ($MgSO_4$), filtered, and evapd to dryness under red. pres. The residue was dissolved in MeOH:Et_2O (1:2, 10 mL) and treated with ethereal HCl. The resulting ppt. was sepd by filtration and dried in vacuo to give the title compound as a colourless solid (yield: 700 mg, 76%); MS (*m/z*) 511 [M+1]; ^{1}H NMR ($CDCl_3$): δ 1.80 (m, 4H), 2.71 (s, 3H), 3.05 (s, 2H), 3.36 (m, 2H), 3.50 (br m, 6H), 3.70 (m, 2H), 4.00 (m, 1H), 7.30 (br m, 13H).

Analogue libraries 4a, 5a and 7a. Prepared by acylation of **11**, **12** and **14**, respectively, with a series of aliphatic and aromatic acid chlorides in the presence of triethylamine using CH_2Cl_2 as solvent, for 18 h at ambient temperature. The products were analysed by TLC and MS; the majority showed a single new product by TLC and the expected molecular ion by MS.

Analogue libraries 4b, 5b and 7b. Prepared by acylation of **11**, **12** and **14**, respectively, with a series of aliphatic and aromatic isocyanates using dichloromethane as solvent for 18 h at ambient temperature. The products were analysed by TLC and MS; the majority showed a single new product by TLC and the expected molecular ion by MS.

Analogue libraries 4c, 5c and 7c. Prepared by sulphonylation of **11**, **12** and **14**, respectively, with a series of aliphatic and aromatic sulphonyl chlorides in the presence of triethylamine using CH_2Cl_2 as solvent, for 18 h at ambient temperature. The products were analysed by TLC and MS; the majority showed a single new product by TLC and the expected molecular ion by MS.

***N*-Methyl-*N*-[2-(3,4-dichlorophenyl)-4-methanesulphonylbutyl]benzamide (13).** Methanesulphonyl chloride (900 mg, 606 μL, 7.3 mmol) was added in a dropwise manner over 5 min to a solution of *N*-methyl-*N*-[2-(3,4-dichlorophenyl)-4-hydroxybutyl]benzamide[9] (2.3 g, 6.5 mmol) and triethylamine (790 mg, 1.1 mL, 7.3 mmol) in CH_2Cl_2 (60 mL) stirred at 0 °C under a nitrogen atmosphere. The mixture was stirred at 0 °C for 1 h, washed with H_2O, satd sodium bicarbonate soln, dried ($MgSO_4$), filtered, and evapd to dryness under red. pres. The residue was purified by CC eluting with a gradient of methanol in CH_2Cl_2 (1–4%). The fractions containing pure product were combined and evapd to dryness to give the title compound as an oil (yield: 2.8 g, 100%); MS (*m/z*) 431 [M+1]; ^{1}H NMR ($CDCl_3$): δ 1.58 (s, 3H), 2.00 (m, 1H), 2.24 (m, 1H), 2.95 (m, 3H), 3.35 (m, 1H), 3.50 (m, 2H), 4.00 (m, 1H), 4.21 (m, 1H), 6.90 (m, 1H), 7.05 (m, 1H), 7.38 (m, 6H).

Analogue library 6. Prepared by reaction of **13** with 4-substituted piperazines, 4-substituted piperidines, and secondary alicyclic amines (86 reactants in total) using acetonitrile as solvent and heating the reaction mixtures in sealed reacti-vials™. The products were analysed by TLC and MS; the majority showed a single new product on TLC and the expected molecular ion by MS.

***N*-Methyl-*N*-[2-(3,4-dichlorophenyl)-4-(4-(3,3-dimethylpropenoyl) amido-4-phenylpiperidyl) butyl] benzamide (15).** Prepared by reaction of **12** with 3,3-dimethylpropenoyl chloride. Product isolated as the hydrochloride salt (yield: 290 mg, 68%); mp 80 °C, MS (*m/z*) 593 [M+1], (found C, 64.61; H, 6.62; N, 6.34, calcd for $C_{34}H_{39}N_3Cl_2O_2 \cdot HCl$, C, 64.92; H, 6.41; N, 6.68%); ^{1}H NMR ($CDCl_3$): δ 1.3 (s, 6H), 1.98 (m, 6H), 2.10 (s,

2H), 2.31 (m, 2H), 2.90 (m, 8H), 5.80 (s, 1H), 6.60 (s, 1H), 7.4 (br m, 13H).

***N*-Methyl-*N*-[2-(3,4-dichlorophenyl)-4-(4-propylaminocarbonylamino-4-phenylpiperidyl)butyl]benzamide (16).** Prepared by the reaction of **12** with *n*-propylisocyanate. Product isolated as the hydrochloride salt (yield: 256 mg, 59%); mp 143 °C, MS (*m/z*) 596 [M+1], (found C, 62.41; H, 6.67; N, 8.63, calcd for $C_{33}H_{40}N_4Cl_2O_2 \cdot HCl$, C, 62.71; H, 6.54; N, 8.86%); ^1H NMR ($CDCl_3$): δ 0.85 (m, 3H), 1.24 (m, 5H), 1.82 (m, 6H), 3.20 (m, 12H), 6.70 (br s, 1H), 7.30 (m, 13H).

***N*-Methyl-*N*-[2-(3,4-dichlorophenyl)-4-(4-mesitylenesulphonamido-4-phenylpiperidyl)butyl]benzamide (17).** Prepared by reaction of **12** with mesitylenesulphonyl chloride. Product isolated as the hydrochloride salt (yield: 275 mg, 55%); mp 234–236 °C, MS (*m/z*) 693 [M+1], (found C, 62.69; H, 5.94; N, 5.58, calcd for $C_{38}H_{43}N_3Cl_2O_3S \cdot HCl$, C, 62.59; H, 6.08; N, 5.76%); ^1H NMR ($CDCl_3$): δ 1.80 (s, 6H), 2.21 (m, 4H), 2.35 (m, 5H), 2.50 (m, 8H), 3.40 (br m, 4H), 5.90 (s, 1H), 6.52 (m. 2H), 7.00 (m, 5H), 7.31 (m, 8H).

***N*-Methyl-*N*-[2-(3,4-dichlorophenyl)-4-(4-acetamido-4-phenylpiperidyl)butyl]-2,3-difluororobenzamide (18).** Prepared by the reaction of **11** with 2,4-difluorobenzoyl chloride. Converted to the hydrochloride salt and isolated as a foam, (yield: 310 mg, 91%); mp 130 °C, MS (*m/z*) 589 [M+1], (found C, 58.25; H, 5.76; N, 6.48, calcd for $C_{31}H_{33}N_3F_2Cl_2O_2 \cdot HCl \cdot H_2O$, C, 57.91; H, 5.64; N, 6.53%); ^1H NMR ($CDCl_3$): δ 1.78 (m, 4H), 2.05 (m, 3H), 3.30 (br s, 3H), 3.69 (s, 3H), 3.05 (br m, 4H), 3.35 (br m, 4H), 4.00 (br s, 1H), 6.90 (br m, 3H), 7.32 (m, 8H).

***N*-Methyl-*N*-[2-(3,4-dichlorophenyl)-4-(4-acetamido-4-phenylpiperidyl)butyl]-3,3-dimethylpropenamide (19).** Prepared by the reaction of **11** with 3,3-dimethylpropenoyl chloride. Converted to the hydrochloride salt and isolated as a foam (Yield: 262 mg, 85%); mp 130 °C, MS (*m/z*) 531 [M+1], (found C, 59.14; H, 6.74; N, 7.09, calculated for $C_{29}H_{37}N_3Cl_2O_2.HCl.H_2O$, C, 59.54; H, 6.89; N, 7.18%); ^1H NMR ($CDCl_3$): δ 1.79 (s, 6H), 2.05 (s, 3H), 2.20 (br m, 2H), 2.70 (br m, 10H), 3.20 (br m, 4H), 3.35 (m, 2H), 3.89 (m, 1H), 5.71 (s, 1H), 7.31 (br m, 8H).

Pharmacology

HSV-1 helicase DNA dependant ATPase assay. HSV-1 helicase inhibition was determined in a high throughput colorimetric DNA dependent ATPase assay. 96-well plates containing helicase, test compounds, and a reaction cocktail were incubated at 37 °C for 1 h and an aliquot of colour development solution (a filtered mixture of three parts 0.045% Malachite Green solution to one part 4.2% ammonium molybdate solution in 4 N HCl) added to each well. Reactions were quenched with 40% citric acid and the plates read at 650 nm and absorbence values determined. Percentage inhibition levels were calculated using an analysis program.

HSV-1 helicase plaque reduction assay, in vitro. Antiviral activity was determined in an in vitro plaque reduction assay. A VERO cell monolayer in a multiwell tissue culture dish is infected with a predetermined number of plaque forming units of herpes simplex virus and these are incubated at 37 °C at 5% CO_2 for 2 days in the presence of test and control compounds over a range of dilution. The plaques are counted under an inverted microscope without staining. The reduction in the number of plaques compared to a positive control gives a measure of the antiviral activity. A linear regression plot of number of plaques vs. concentration is drawn and an IC_{50} value determined. Acyclovir[10] was used as a standard for antiviral activity.

NK_2 Receptor binding assay

Library compounds. NK_2 receptor binding experiments on library compounds were carried out according to the method of Bergstrom[11] on rat duodenum membranes. Data is analysed using programs to calculate percentage specific binding for each sample.

Resynthesised compounds. Competition binding experiments were performed in an NK_2 receptor assay using labelled ^{125}I NK_A binding to human NK_2 receptors expressed in Chinese hamster ovary (CHO) cells. Incubations containing ^{125}I NK_A, the receptor preparation and test compounds are carried out at room temperature for 2 h with shaking before filtration and washing on a Brandel cell harvester and GF/C filters. Discs are punched from the filter mats, using a modification of the Brandel ADD system, into plastic tubes. Radioactivity remaining on the filter discs is determined using a gamma counter. Data is analysed using programs to calculate percentage specific binding for each sample and IC_{50} values after applying curve fitting routines.

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