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Letter

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Integrated Synthesis and Testing of Substituted Xanthine Based DPP4 Inhibitors: Application to Drug Discovery

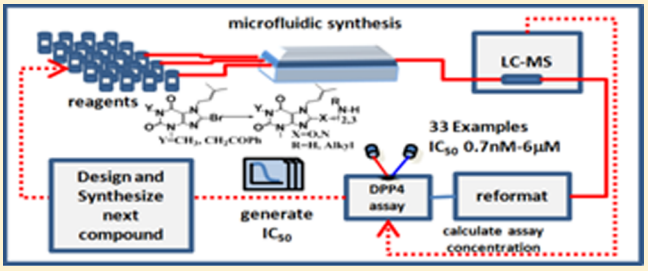
Werngard Czechtizky,[‡] Jüergen Dedio,[‡] Bimbisar Desai,[†] Karen Dixon,[†] Elizabeth Farrant,[†] Qixing Feng,[†] Trevor Morgan,[†] David M. Parry,[†] Manoj K. Ramjee,[†] Christopher N. Selway,[†] Thorsten Schmidt,[‡] Gary J. Tarver,*[,†] and Adrian G. Wright[†]

[†]Cyclofluidic Ltd., Biopark, Broadwater Road, Welwyn Garden City, Hertfordshire AL7 3AX, U.K.

[‡]Sanofi-Aventis, Industriepark Höchst, Gebäude K703, 65929 Frankfurt, Germany

Supporting Information

ABSTRACT: A novel integrated discovery platform has been used to synthesize and biologically assay a series of xanthine-derived dipeptidyl peptidase 4 (DPP4) antagonists. Design, synthesis, purification, quantitation, dilution, and bioassay have all been fully integrated to allow continuous automated operation. The system has been validated against a set of known DPP4 inhibitors and shown to give excellent correlation between traditional medicinal chemistry generated biological data and platform data. Each iterative loop of synthesis through biological assay took two hours in total, demonstrating rapid iterative structure−activity relationship generation.



KEYWORDS: *Closed loop drug design, flow synthesis, automated drug discovery, DPP4*

Medicinal chemistry is an iterative process of synthesis and screening of novel molecules to optimize both biological and physical properties to ultimately yield a candidate molecule for the clinic. We have previously reported[1] a closed loop integrated approach to elements of medicinal chemistry whereby the synthesis process is fully integrated with the biological assay enabling the rapid and automated generation of structure−activity relationship (SAR) data. This approach enables a true closed loop approach to medicinal chemistry to be undertaken where molecules are designed for synthesis and screening based upon the emerging SAR in a true serial iterative manner.

Data published in this letter demonstrates both the reproducibility and consistency of automated SAR generation for a series of compounds with known activity against dipeptidyl peptidase 4 (DPP4).

Several groups have implemented online screening of drug-like compounds. For example, Kool et al. used online bioaffinity analysis to screen a library of fragments for activity against acetylcholine binding protein, and Guetzoyan et al. used frontal affinity chromatography to screen a range of γ-aminobutyric acid (GABA) agonists synthesized in flow.[2,3] In addition, the opportunity to improve the speed, efficiency, and quality of medicinal chemistry hypothesis testing by the direct integration of compound synthesis with biology and design algorithms has been recognized by several groups.[4,5] The integrated platform described in this letter is designed to select a compound from a drug-like chemical space and synthesize, purify, and biologically test the compound. The SAR generated is then used to select the next compound for synthesis and test. This offers the advantage of fully automated, fast serial generation of SAR for drug discovery with each design iteration taking no more than two hours from the start of synthesis to biological readout.

The integrated optimization platform (Figure 1) comprises a reagent autosampler and flow synthesis apparatus connected to a commercial high-performance liquid chromatography (HPLC) mass spectrometer. Liquid chromatography mass spectrometry (LC−MS) is used to purify and characterize synthesized material with an evaporative light-scattering detector (ELSD) to establish sample concentration.[6] Purified material is subsequently reformatted to the correct concentration for biological assay using proprietary hardware. Biological IC_{50} determination is achieved using a bespoke liquid handler coupled to a fluorescence plate reader. The IC_{50} is determined by fitting a curve to the measured data using in house software written in Matlab.[7] The integrated optimization platform is controlled from a workstation using an in-house designed software package.

In order to further validate the integrated synthesis and screening approach, a joint project was undertaken to replicate an existing data set in a blinded experiment. Sanofi-Aventis provided a series of compounds with known inhibitory activity against DPP4[8−10] for evaluation on the platform. The SAR

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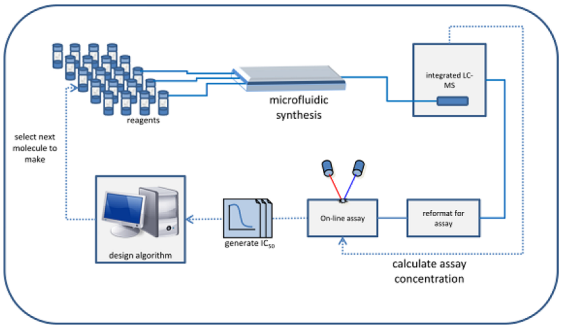
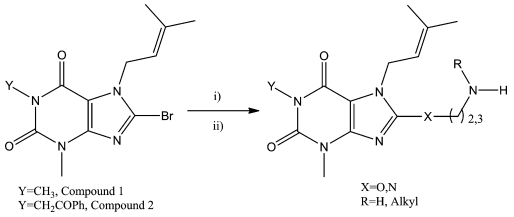


Figure 1. Cyclofluidic optimization platform.

Scheme 1. General Synthesis of DPP4 Inhibitors



Scheme 2. Synthesis of Diamino DPP4 Inhibitors

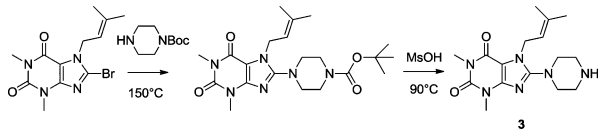


Table 1. Repeated Synthesis and Assay of Compound 3

loop number	DPP4 (human) nM column 1	DPP4 (human) nM column 2
1	76	115
2	84	104
3	86	83
4	120	110
5	103	72
6	76	128
7	82	105
8	166	158
9	122	131
10	124	128

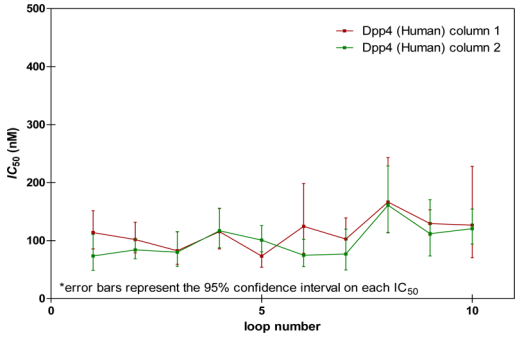


Figure 2. Graph of IC_{50} results for compound **3**.

generated would then be unblinded by Sanofi-Aventis to provide validation of the fully integrated approach.

The compounds to be synthesized are shown in Scheme 1. The first step of the synthesis used either boc-protected diamines or amino alcohols to displace 8-bromoxanthines. The in situ generated boc-protected intermediates were then deprotected to give the compound of interest for testing.

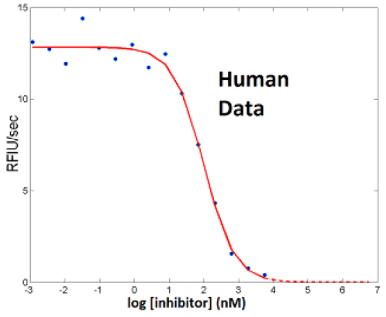


Figure 3. Example curve of human DPP4 inhibition.

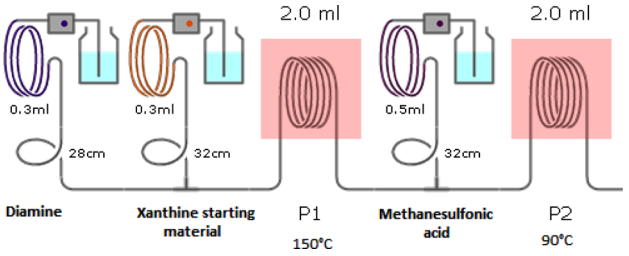
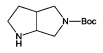
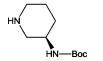
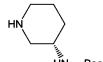
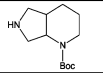
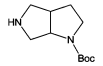
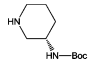
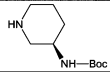
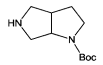
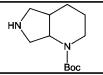
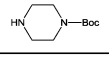
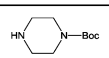
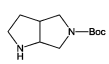


Figure 4. Flow reactor setup for diamine reaction.

Table 2. Results of Diamine Synthesis and Testing

Building block	Diamine	Activity (nM) DPP4 Human	Activity (nM) DPP4 Porcine
2		11	1.6
2		1	0.7
1		8	6
2		1640	269
1		7000	6000
2		2	4
1		5	11
2		645	57
1		>24500	>24500
1		117	173
2		72	11
2		100	132

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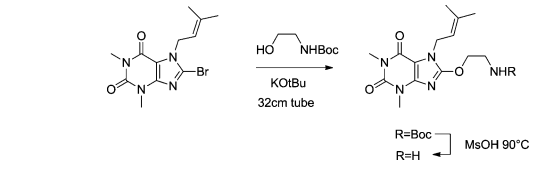
Scheme 3. Synthesis of Amino-Alcohol Modified Xanthines



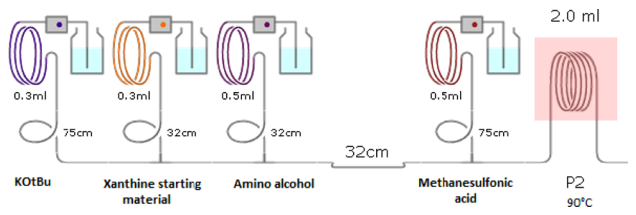


Figure 5. Reaction setup for amino-alcohol reaction.

The first evaluation was to establish consistency between cycles by repeatedly synthesizing and screening a single compound **3** (Scheme 2) ten times on the integrated platform. For each individual compound synthesis, the sample was assayed twice in alternate assay plate columns against human DPP4 to assess variance in the assay. By way of control, a discrete solid sample was assayed manually to provide comparator data.

The data from the experiment is displayed in Table 1, and Figure 2 clearly indicates that, with over 20 hours and ten cycles of both synthesis and screening on the platform, the data is consistent within normal error; the activity for a solid sample was 106 nM. This clearly illustrates that as well as giving very little variance, the IC_{50} is in complete agreement with the discrete sample determined manually.

An example IC_{50} curve for compound **3** generated during the run is shown in Figure 3 showing excellent fit.

With reproducibility now confirmed, a series of diamine modified xanthines were synthesized via a two-step synthetic protocol using a Vapourtec R4 flow chemistry system. An example reaction is shown in Scheme 2.

The first synthetic displacement was conducted on a 2 mL volume stainless steel coil heated at 150 °C with injections of 250 μL of the 8-bromosubstituted xanthine in *N*-methyl-2-pyrrolidone (NMP) and 250 μL solution of two equivalents of the desired boc-protected diamine in NMP. The flow rate for step 1 was 50 μL/min per pump to give a residence time of 20 min. Next, the boc-protected intermediate was mixed with 500 μL of 30% methanesulfonic acid in water added at 50 μL/min to deprotect the boc group. This reaction was conducted in a second 2 mL coil at 90 °C and the product subsequently run through a 20 μL injection loop into the LC−MS purification system switched at the point of maximum concentration. NMP was used as the system and wash solvent. A schematic of the setup is shown in Figure 4.

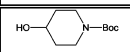
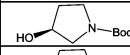
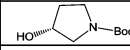
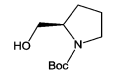
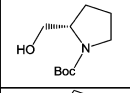
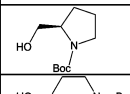
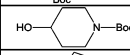
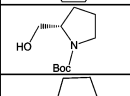
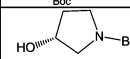
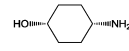
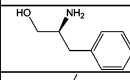
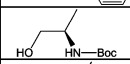
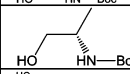
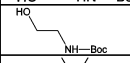
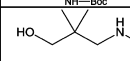
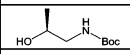
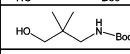
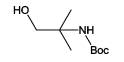
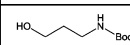
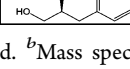
The total synthesis time per compound to purification was 50 min, and the total cycle time per sample was 120 min.

All 12 compounds were successfully synthesized and tested in 24 h total platform time. Yields varied between 3% and 38%. It should be noted that even low yields provided enough material for the compounds to be purified and assayed.

The results are displayed in Table 2.

Next, the synthesis and testing of a series of amino alcohols was undertaken. An example synthetic route is shown in Scheme 3.

Table 3. Results of Amino-Alcohol Synthesis and Testing

Building block	Amino-alcohol	Activity (nM) DPP4 Human	Activity (nM) DPP4 Porcine
2		>1000	275
2		910	147
1		>8000	>8000
2		715	144
1		>7000	>7000
1		1400	1600
2		>5000 10,000	705 580
1		>5000	>5000
2		287 82	16 2.6
1		>7000	>7000
2		no data[a]	no data[a]
1		814	430
2		no data[a]	no data[a]
1		2000	780
2		no data[b]	no data[b]
1		280	260
2		2000	420
2		70	6
2		>2500 >8700 6500	800 1500 940
2		166	22
2		no data[a]	no data[a]

[a]Synthesis failed. [b]Mass spec. failed to give expected molecular ion. Several of the compounds have repeated values to further demonstrate consistency.

In this case, the initial reaction was very fast using KO^{t}Bu as base and could be conducted in a 32 cm long 1 mm ID tube in ∼3 min. Subsequent deprotection of the boc group was again achieved using 30% methanesulfonic acid in water at 90 °C with yields for the two step process of between 0% and 23%. The overall synthesis success for this process was a respectable 82%.

A schematic of the reaction setup is shown in Figure 5.

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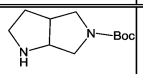
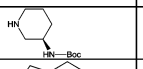
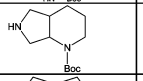
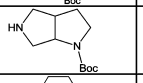
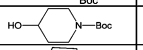
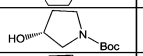
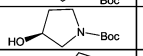
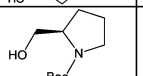
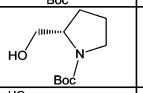
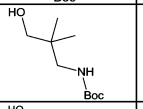
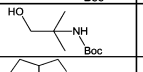
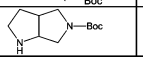
Table 4. Comparison of Sanofi-Aventis and Integrated Platform Data[a]

Building block	Diamine or amino-alcohol	IC_{50} DPP4 (P) Sanofi (nM)	IC_{50} DPP4 (P) Platform (nM)	IC_{50} DPP4 (H) Sanofi (nM)	IC_{50} DPP4 (H) Platform (nM)
2		1	1.6	9	11
2		<40	0.73	1	1.1
2		0.048	0.269	No data	1.6
2		17	57.2	No data	645
2		1100	275	No data	>1000
2		84	147	No data	910
2		334	145	No data	715
2		2500	705	No data	> 5000
2		17	16	No data	290
2		9,13,15	6.5	120	73
2		550	800,1500	No data	>2500 >8700
1		210	130	No data	100

[a](P) = porcine data; (H) = human data.

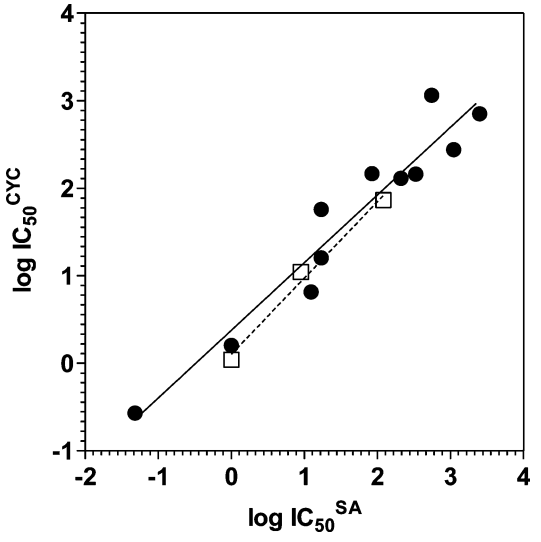


Figure 6. Correlation between Sanofi-Aventis and platform data.

The results from 21 iterations of the system are shown in Table 3.

Biological assays were carried out in 40 mM tris·HCl, pH 7.4 at 25 ± 2 °C, in a total assay volume of 50 μL using 384-well plates (Costar 3574). Routinely, compounds were titrated in assay buffer to which was added either enzyme (0.82 mU/mL porcine DPP4 (Sigma) or 34 U/mL human DPP4 (Sigma). Residual enzyme activity was monitored by adding substrate (H-Gly-Pro-AMC; Bachem) to give a final concentration equivalent to the K_M^{app} (K_M^{app} = 25 μM for human DPP4 and K_M^{app} = 35 μM for porcine DPP4). Data was analyzed by nonlinear regression using a four parameter logistic variable slope model for IC_{50} determinations.

The integrated platform data was compared against Sanofi-Aventis data as shown in Table 4. The porcine data matched closely validating the technique (Figure 6). The historical human DPP4 data was limited, but where available, the platform data is also in agreement with previously reported values.

Overall, the data obtained by the platform is fully consistent with reference data provided by Sanofi-Aventis using traditional means.

The synthesis was generally robust, and the integrated approach enables biological data to be measured from low yielding reactions. This additionally minimizes the optimization time needed to achieve a reliable, generic, flow synthesis method and ensures results are obtained from reagents with a range of reactivity.

The biological assay has also been shown to be both robust and stable over an extended period of time, essential for an automated environment.

A plot of the porcine (closed circle) and human (open square) Sanofi-Aventis ($\log IC_{50}^{SA}$) and platform ($\log IC_{50}^{CYC}$) inhibition data presented in Table 4 is shown in Figure 6. The lines represent the best fit linear regression analysis of the data.

In conclusion, a series of DPP4 inhibitors have been successfully synthesized, purified, and tested using the platform with an overall chemistry success rate of 93%. Each compound was synthesized and tested in two hours total. It took less than three days to synthesize and test the total of 29 compounds. Close correlation between integrated platform data and data generated within the corresponding traditional medicinal chemistry approach has validated the technique (R^2 = 0.9253 and 0.9893 for porcine and human data, respectively; Figure 6). Further work on a series of synthetic routes and various target classes is currently underway.

■ ASSOCIATED CONTENT

Supporting Information

General synthetic and analytical purity determination procedures for all synthesized compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*(G.J.T.) Tel: +44 (0)1707358673. E-mail: gary.tarver@cyclofluidic.co.uk.

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Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Desai, B.; Dixon, K.; Farrant, E.; Feng, Q.; Gibson, K. R.; van Hoorn, W. P.; Mills, J.; Morgan, T.; Parry, D. M.; Ramjee, M. K.; Selway, C. N.; Tarver, G. J.; Whitlock, G.; Wright, A. G. Rapid discovery of a novel series of Abl kinase inhibitors by application of an

integrated microfluidic synthesis and screening platform. *J. Med. Chem.* **2013**, *56* (7), 3033−3047.
(2) Kool, J.; de Kloe, G. E.; Bruyneel, B.; de Vlieger, J. S.; Retra, K.; Wijtmans, M.; van Elk, R.; Smit, A. B.; Leurs, R.; Lingeman, H.; de Esch, I. J. P.; Irth, H. *J. Med. Chem.* **2010**, *53*, 4720−4730.
(3) Guetzoyan, L.; Nikbin, N.; Baxendale, I. R.; Ley, S. V. Flow chemistry synthesis of zolpidem, alpidem and other GABAA agonists and their biological evaluation through the use of in-line frontal affinity chromatography. *Chem. Sci.* **2013**, *4*, 764−769.
(4) Wong-Hawkes, S. Y. F.; Matteo, J. C.; Warrington, B. H.; White, J. D. MicroReactors as New Tools For Drug Discovery and Development. In *New Avenues to Efficient Chemical Synthesis: Emerging Technologies*; Seeberger, P. H., Blume, T., Eds.; Springer-Verlag: Heidelberg, Germany, 2007; p 39.
(5) Pickett, S. D.; Green, D. V. S.; Hunt, D. L.; Pardoe, D. A.; Hughes, I. Automated lead optimization of MMP-12 inhibitors using a genetic algorithm. *ACS Med. Chem. Lett.* **2011**, *2* (1), 28−33.
(6) Squibb, A. W.; Taylor, M. R.; Parnas, B. L.; Williams, G.; Girdler, R.; Waghorn, P.; Wright, A. G.; Pullen, F. S. Application of parallel gradient high performance liquid chromatography with ultra-violet, evaporative light scattering and electrospray mass spectrometric detection for the quantitative quality control of the compound file to support pharmaceutical discovery. *J. Chromatogr., A* **2008**, *1189* (1−2), 101−108.
(7) MATLAB. http://www.mathworks.co.uk/products/matlab/.
(8) Himmelsbach, F.; Mark, M.; Eckhardt, M.; Langkopf, E.; Maier, R.; Lotz, R. Preparation of xanthine derivatives as dipeptidylpeptidase-IV inhibitors. *PCT Int. Appl.* WO 2002068420 A1 20020906, 2002.
(9) Schoenafinger, K.; Jaehne, G.; Defossa, E.; Buning, C.; Tschank, G.; Werner, U.Preparation of 1*H*-purine-2,6-diones as dipeptidyl peptidase IV inhibitors for the treatment of type 2 diabetes. Patent Appl. WO2006018117, 2006.
(10) Eckhardt, M.; Langkopf, E.; Mark, M.; Tadayyon, M.; Thomas, L.; Nar, H.; Pfrengle, W.; Guth, B.; Lotz, R.; Sieger, P.; Fuchs, H.; Himmelsbach, F. 8-(3-(*R*)-Aminopiperidin-1-yl)-7-but-2-ynyl-3-methyl-1-(4-methyl-quinazolin-2-ylmethyl)-3,7-dihydropurine-2,6-dione (BI 1356), a highly potent, selective, long-acting, and orally bioavailable DPP-4 inhibitor for the treatment of type 2 diabetes. *J. Med. Chem.* **2007**, *50* (26), 6450−6453.