



feature

Designed multiple ligands in metabolic disease research: from concept to platform

W. Gattrell^{1,*}, wgattrell@yahoo.co.uk, C. Johnstone², S. Patel³, C. Sambrook Smith⁴, A. Scheel² and M. Schindler⁵

Type 2 diabetes mellitus (T2DM) is a multifactorial disease, and drug monotherapy typically results in unsatisfactory treatment outcomes for patients. Even when used in combination, existing therapies lack efficacy in the long term. Designed multiple ligands (DMLs) are compounds developed to modulate multiple targets relevant to a disease. DMLs offer the potential to yield greater efficacy over monotherapies, either by modulating different biological pathways, or by boosting a single one. However, examples of DMLs progressing into clinical trials, or onto the market are rare; DML drug discovery is challenging, and perceived by some to be almost impossible. Nevertheless, with the judicious selection of biological targets, both from a biological and chemical perspective, it is possible to develop drug-like DMLs.

T2DM is a chronic condition in which glucose control deteriorates progressively over time. For most patients, lifestyle interventions alone are insufficient to maintain blood glucose within a target range over the long term, and pharmacotherapy is required [1]. Current international guidelines for the treatment of T2DM recommend the use of combination therapy when oral monotherapy fails to achieve or maintain sufficient blood glucose control, and combination therapy should be considered at the time of diagnosis for patients with poor blood glucose control [2]. Given that T2DM is a multifactorial disease, linked to both environmental and genetic factors, it might be expected that drug monotherapy will result in unsatisfactory treatment outcomes.

Combination therapy, sometimes referred to as 'poly-pharmacology', can yield increased

efficacy over monotherapy by modulating different biological pathways, or by boosting a single one. However, the pill burden of patients with T2DM can be high; patients often have comorbidities, such as hypertension or dyslipidemia, and are likely to be taking multiple medications before antihyperglycemic therapy is initiated. Combining two or more drug treatments in a single dosage form, a so-called 'fixed-dose combination', can reduce pill burden for patients, and has been shown to improve medication adherence [3]. Fixed-dose combinations of drugs are available for the treatment of disorders such as HIV, tuberculosis (TB) and hypertension. In some cases, such as in the treatment of TB, agents are combined to exploit synergistic effects [4], although current fixed-dose combinations for the treatment of T2DM

act *via* complementary mechanisms of action, through which additive, rather than synergistic effects, are observed [5]. Existing therapies to treat T2DM lack efficacy, particularly in long-term treatment, and there remains a significant unmet medical need [6].

The term 'DML' describes a single chemical entity developed to modulate multiple targets relevant to a disease [7]. As with combination therapy, DMLs offer the potential to yield greater efficacy compared with monotherapy approaches [8], and have several advantages over combination therapy; because only a single tablet is administered to the patient, there is a reduced pill burden and also a lower risk of drug–drug interactions. Also, for DMLs, unlike fixed-dose combinations, there is no requirement for the coformulation of two drug

components, with the inherent problems of achieving compatibility in the physicochemical, pharmacokinetic and pharmacodynamic properties.

Examples of DMLs progressing into clinical trials, or onto the market are rare [9]. One reason for this is that DML drug discovery is challenging, and perceived by some to be almost impossible. The multiparameter optimization of molecular properties is one of the key challenges in drug design and, because DMLs need to be optimized against multiple biological targets, this poses additional hurdles. Historically, DMLs have tended to be larger and more lipophilic than compounds designed to modulate a single target; in many cases, this is because pharmacophores have been linked together [10]. Scientists at Prosidion Ltd¹ showed that it is technically feasible to develop DMLs with good, drug-like properties. However, a crucial element in making DML drug discovery technically feasible lies in the careful selection of biological targets, both from a biological and a chemical perspective.

Target selection and validation

Experimental evaluation and validation of potential target pairs is required before labor-intensive medicinal chemistry initiatives begin. Many target combinations do not warrant further study because the effects achieved through the modulation of multiple targets can be less than additive. Typically, the effects of multitarget modulation compared with the respective single-target approach are initially explored *in vitro*, because this can enable the rapid and simultaneous evaluation of multitarget combinations. In such studies, cell lines derived from the relevant physiological tissue rather than engineered cell lines are typically used, because this ensures that the relevant signaling pathways are present and expressed at a physiological level. To explore the effects of simultaneous modulation of multiple targets, it is feasible to use molecular biology methods, such as the overexpression of target combinations (to stimulate target activity) or small interfering (si)RNA experiments (to achieve simultaneous knockdown).

A simpler and quicker methodology is the use of tool compound combinations, where each

¹ The biotechnology company Prosidion Ltd discovered and developed innovative molecular targeted therapies addressing major unmet medical needs in type 2 diabetes and obesity. Prosidion ceased all activities at its facility in Oxford, UK in March 2012.

tool compound is known to modulate selectively the target of interest. Clearly, this strategy can only apply to known targets where selective tool compounds are known. If tool compounds also have suitable pharmacokinetic properties, they can also be suitable for *in vivo* combination studies. Such considerations need to be taken into account when selecting which target combinations to explore, and precludes the combination of novel targets where tool compounds do not exist.

In diabetes research, one mechanism of interest is the stimulation of the release of hormones that improve glucose control, such as insulin or glucagon-like peptide-1 (GLP-1). For the identification of suitable target combinations that increase hormone levels, multiple cell models are available that can be utilized for evaluating DML concepts *in vitro*. Of course, such models are only useful if both targets are expressed in the cell model of choice and if target activation results in the functional release of the desired hormone, which restricts the number and types of combination that can be examined *in vitro*. The response to modulation of multiple targets can then be quantified for additive, synergistic or antagonistic effects compared with the addition of either single agent alone

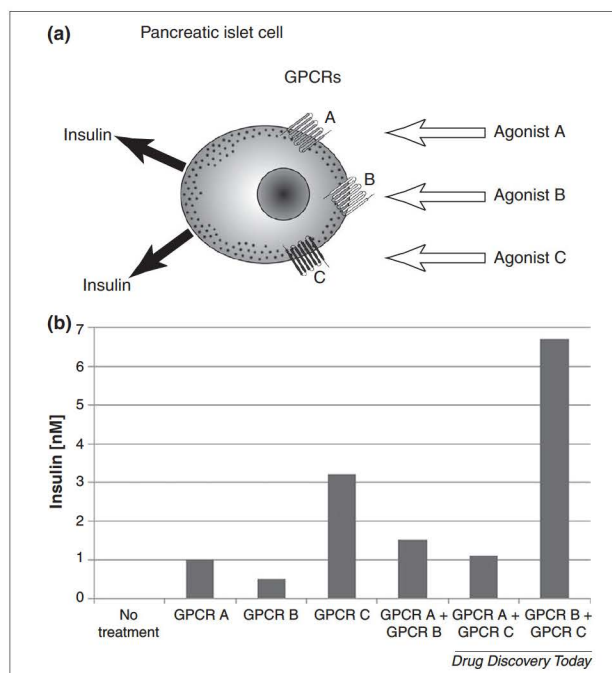


FIGURE 1

Insulin secretion in response to simultaneous modulation of multiple G protein-coupled receptors (GPCRs) (a), can be quantified to determine additive, synergistic or antagonistic effects compared with the addition of either single agent alone, (b).

(Fig. 1). In the example shown, simultaneous agonism of G protein-coupled receptors (GPCRs) A and B elicits a response, in this case insulin secretion, that is additive; whereas agonism of GPCRs A and C results in an effect that is less than additive. The most desirable outcome is in the case of agonism of GPCRs B and C, whereby the insulin secretion observed is greater than the sum of modulating the individual targets.

For target combinations where the individual targets are expressed in different tissues of the body, only an *in vivo* approach allows the thorough evaluation and validation of a combination. This is a significant obstacle, because it requires the identification of tool compounds that not only selectively modulate the individual target of interest, but also have suitable pharmacokinetic properties. Recently, DMLs were developed at Prosidion that combined agonism of GPR119 and inhibition of the serine protease dipeptidyl peptidase IV (DPP-IV) [11]. GPR119 agonists are being evaluated in clinical trials as oral antidiabetic agents and are understood to exert at least part of their antidiabetic effects through increasing the secretion of GLP-1 from intestinal L-cells [12,13]. GLP-1 has a half-life of less than 3 min, undergoing rapid cleavage by DPP-IV. Given that DPP-IV inhibitors such as

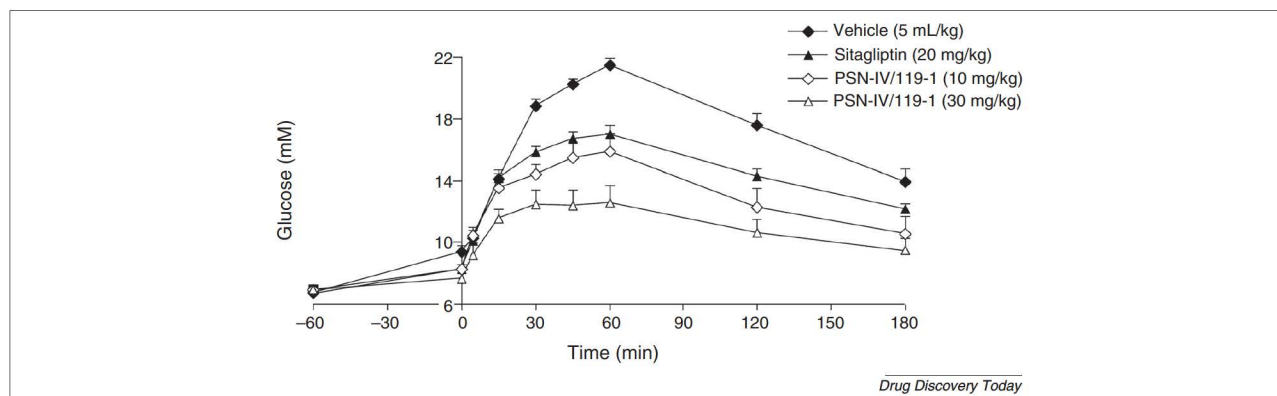


FIGURE 2

Results from an oral glucose tolerance test, comparing oral dosing of a dual dipeptidyl peptidase IV (DPP-IV) inhibitor and G protein-coupled receptor 119 (GPR119) agonist (PSN-IV/119-1) with sitagliptin in a rodent model of diabetes.

sitagliptin act primarily by preventing the degradation of GLP-1, a combination of a GLP-1 secretagogue (a GPR119 agonist) and a GLP-1 protecting agent (a DPP-IV inhibitor) could lead to greater levels of GLP-1 compared with administration of either agent alone. This hypothesis has been confirmed in studies carried out in animal models of diabetes using co-administration of a GPR119 agonist and a DPP-IV inhibitor to modulate each target individually [14,15].

Subsequently, a DML was developed that combined both pharmacologies in a single chemical entity. In a key proof-of-concept study, a DML (PSN-IV/119-1) with dual DPP-IV inhibition and GPR119 agonism was tested head-to-head in an oral glucose tolerance test with sitagliptin in a rodent model of diabetes (Fig. 2) [11]. At a dose of 10 mg/kg, PSN-IV/119-1 displayed a comparable reduction in plasma glucose (area under curve, 0–180 min) to sitagliptin (39% and 22%, respectively), despite only achieving moderate levels (approximately 50%) of DPP-IV inhibition as measured in the plasma of treated animals compared with 80% inhibition achieved by sitagliptin. At a dose of 30 mg/kg, PSN-IV/119-1 caused similar levels of plasma DPP-IV inhibition as sitagliptin, but showed a superior reduction in plasma glucose levels ($P < 0.05$). These data support the hypothesis that incorporating DPP-IV inhibition and GPR119 agonism in a single molecule could provide a therapy that leads to greater glucose lowering compared with a DPP-IV inhibitor alone. Superior glucose control was also seen in comparison with dosing of a selective GPR119 agonist (data not shown). PSN-IV/119-1 was able to achieve impressive *in vivo* results, despite modest *in vitro* potency against the targets in question: for DPP-IV, $IC_{50} = 0.2 \mu\text{M}$, and for GPR119, $EC_{50} = 2.24 \mu\text{M}$ [16].

Lead generation

Established lead generation techniques, such as high-throughput screening and, more recently, fragment-based approaches, are inherently focused on single targets. In some cases, for example in the area of kinase inhibitors, cross-screening of existing inhibitors from previous kinase projects has been used successfully to identify new chemical start points with the desired multikinase activity. In effect, chemotypes from one project are recycled as medicinal chemistry starting points for another. Key to this success is the fact that kinase inhibitors often bind at a site sharing a high degree of homology (the ATP-binding site). For DML lead generation, cross-screening can prove valuable but does rely on access to an appropriate compound collection and associated biological data for the targets of interest. Furthermore, alternative approaches might be required to address multiple targets that share low sequence homology.

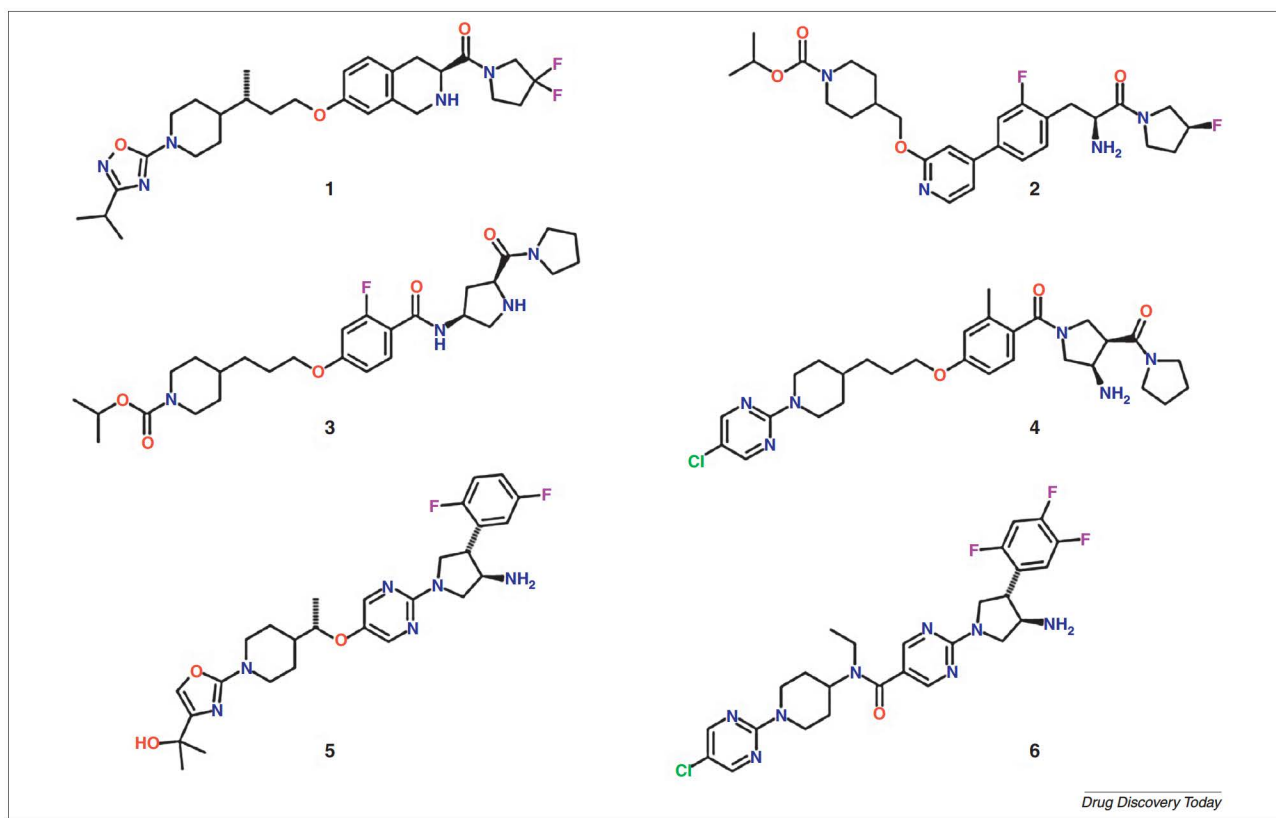
When modulating multiple targets simultaneously, it becomes obvious that synergistic effects might be required in reality, because DMLs are frequently less potent than single-target ligands. The lower ligand efficiency that is obtained for DMLs compared with modulating the individual targets is observed frequently. Moreover, the most suitable target pairs need substantial overlap in the pharmacophores of their respective ligands, to achieve dual activity within a single DML of acceptable molecular weight. Thus, on initial examination of target pairs, it is crucial to assess whether separate pharmacophores are compatible, which requires substantial medicinal and computational chemistry expertise, and creative thinking.

One technique that has been utilized is the exploitation of common structural motifs within ligands for both targets of interest. By merging

suitable ligands from each target, it was possible to obtain potent dual MCH-1R antagonists and DPP-IV inhibitors, an example of the modulation of two targets that span different protein classes [17]. In the design of ligands combining inhibition of DPP-IV and agonism of GPR119, the crystal structure of the DPP-IV enzyme active site was used to assess the tolerance of incorporating the GPR119 pharmacophore [18]. For GPR119 agonists, there are two key pharmacophores, linked *via* a spacer, and the ligand is required to adopt an extended conformation [19]. Analysis of the crystal structure of DPP-IV indicated an extensive cavity in a position suitable to accommodate the elongated GPR119 pharmacophore. This expectation was later confirmed by X-ray crystallography of a DPP-IV/GPR119 DML bound to the DPP-IV enzyme.

Examples of ligands with dual DPP-IV inhibition and GPR119 agonism taken from the patent literature can be seen in Fig. 3 [20–25]. The two key pharmacophores for GPR119 agonists are a piperidine ‘capped’ with a carbamate or isosteric heterocycle at one end of the ligand, and at the other terminus, an aryl or hetero-aryl ring substituted with a H-bond acceptor group [19]. All the ligands shown have an appropriately substituted piperidine ring and, in the case of ligands 1–4, a carbonyl oxygen is placed in a suitable position to act as a H-bond acceptor for GPR119. In the case of ligands 5 and 6, the fluorine atom proximal to the pyrrolidine ring might be able to act as the acceptor group.

Compounds 1–6 all have a basic primary or secondary amine. This feature is present in most DPP-IV inhibitors, where it forms electrostatic interactions and H-bonds with the protein [18]. Another common feature of DPP-IV inhibitors is a hydrophobic S1 group that binds in the P1 pocket of the protease. In the case of compounds 1–4, it is

**FIGURE 3**

Examples of dual dipeptidyl peptidase IV inhibitors/G protein-coupled receptor 119 (DDP-IV/GPR119) agonists developed at Prosidion reported in the patent literature. See main text for more details.

likely that the pyrrolidine ring provides this key hydrophobic interaction. Compounds **5** and **6** might represent a significant leap in DML design, whereby a substituent on the S1 group (the proximal fluorine substituent) could provide a key pharmacophore for GPR119. This represents a greater merging of the pharmacophores for each target, and has enabled the overall length of ligands **5** and **6** to be shortened compared with ligands **1–4**. In addition, several different compound classes were obtained, with significant *in vitro* potency (GPR119 $EC_{50} < 1 \mu M$ and DPP-IV $IC_{50} < 1 \mu M$, for examples **3**, **5** and **6**), despite these targets being from different protein families. In fact, this program culminated with the nomination of a clinical development candidate, PSN491.

Concluding remarks

There is increasing recognition that the low-hanging fruit has already been picked for the single-drug single-target approach to drug discovery. Indeed, many drugs originally designed to have activity against one target have since been shown to be active against multiple

targets, and it is likely that this spectrum of activity contributes to their overall efficacy [26]. The necessity for more efficacious treatments for T2DM has been the driver to undertake what is perceived to be a challenging drug discovery approach. However, with the appropriate strategies in place, it is possible to develop drug-like DMLs, and the *in vivo* profile of the ligands obtained has justified the endeavor.

Conflict of interest

All the authors are former employees of Prosidion.

Acknowledgements

The authors would like to thank the former employees of Prosidion.

References

- (1998) UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet* 352, 837–853
- Rodbard, H.W. *et al.* (2009) Statement by an American Association of Clinical Endocrinologists/American College of Endocrinology consensus panel on type 2 diabetes mellitus: an algorithm for glycemic control. *Endocr. Pract.* 15, 540–559
- Thayer, S. *et al.* (2010) Adherence to a fixed-dose combination of rosiglitazone/glimepiride in subjects switching from monotherapy or dual therapy with a thiazolidinedione and/or a sulfonylurea. *Ann. Pharmacother.* 44, 791–799
- Moulding, T. *et al.* (1995) Fixed-dose combinations of antituberculous medications to prevent drug resistance. *Ann. Intern. Med.* 122, 951–954
- Blonde, L. and San Juan, Z.T. (2012) Fixed-dose combinations for treatment of type 2 diabetes mellitus. *Adv. Ther.* 29, 1–13
- Kahn, S.E. *et al.* (2006) Glycemic durability of rosiglitazone, metformin, or glyburide monotherapy. *N. Engl. J. Med.* 355, 2427–2443
- Morphy, R. *et al.* (2004) From magic bullets to designed multiple ligands. *Drug Discov. Today* 9, 641–651
- Hopkins, A.L. (2008) Network pharmacology: the next paradigm in drug discovery. *Nat. Chem. Biol.* 4, 682–690
- Costantino, L. and Barlocco, D. (2012) Designed multiple ligands: basic research vs clinical outcomes. *Curr. Med. Chem.* 19, 3353–3387
- Morphy, R. and Rankovic, Z. (2006) The physicochemical challenges of designing multiple ligands. *J. Med. Chem.* 49, 4961–4970
- Swain, S. *et al.* (2009) A Novel Dual DPP-IV Inhibitor and GPR119 Agonist that Exhibits Superior Glucose

- Lowering to Sitagliptin in Diabetic ZDF Rats. American Diabetes Association 69th Scientific Sessions, New Orleans, USA. June 5–9. Abstract 453-P. Available at: http://professional.diabetes.org/Abstracts_Display.aspx?TYP=1&CID=73240 Accessed February 2013
- 12 Ohishi, T. and Yoshida, S. (2012) The therapeutic potential of GPR119 agonists for type 2 diabetes. *Expert Opin. Invest. Drugs* 21, 321–328
- 13 Lan, H. *et al.* (2012) Agonists at GPR119 mediate secretion of GLP-1 from mouse enteroendocrine cells through glucose-independent pathways. *Br. J. Pharmacol.* 165, 2799–2807
- 14 Chu, Z.-L. *et al.* Arena Pharmaceuticals, Inc.. Combination therapy for the treatment of diabetes and conditions related thereto and for the treatment of conditions ameliorated by increasing a blood GLP-1 level. US2006/0154866
- 15 Mark, M. *et al.* BoehringerIngelheim International GmbH. Combination therapy for the treatment of diabetes and related conditions. US2011/0263617
- 16 Litwack, G. (2010) Elsevier
- 17 Gattrell, W.T. *et al.* (2012) An example of designed multiple ligands spanning protein classes: dual MCH-1R antagonists/DPP-IV inhibitors. *Bioorg. Med. Chem. Lett.* 22, 2464–2469
- 18 Kuhn, B. *et al.* (2007) Molecular recognition of ligands in dipeptidyl peptidase IV. *Curr. Top. Med. Chem.* 7, 609–619
- 19 Shah U, E.S., Szewczyk, J. (2012) Recent Advances in the Discovery of GPR119 Agonists. In *New Therapeutic Strategies for Type 2 Diabetes* (Jones, R., ed.), Chapter 7, pp. 177–214, The Royal Society of Chemistry
- 20 Barba, O. *et al.* Prosidion Ltd. 3-Substituted 5-(Pyrrolidine-1-carbonyl) Pyrrolidine and its derivatives for use in the treatment of metabolic disorders. WO2011128394
- 21 Barba, O. *et al.* Prosidion Ltd. Cycloamino derivatives as GPR119 agonists. WO2011147951
- 22 Barba, O. *et al.* Prosidion Ltd. 1,4-Disubstituted pyrrolidine-3-yl-amine derivatives and their use for the treatment of metabolic disorders. WO2012066077
- 23 Barba, O. *et al.* Prosidion Ltd. N-Substituted 3-amino 4-(pyrrolidine-1-carbonyl)pyrrolidine and its derivatives for use in the treatment of metabolic disorders. WO2011128395
- 24 Barba, O. *et al.* Prosidion Ltd. Compounds for the treatment of metabolic disorders. WO2010103333
- 25 Barba, O. *et al.* Prosidion Ltd. Compounds for the treatment of metabolic disorders. WO2009034388
- 26 Roth, B.L. *et al.* (2004) Magic shotguns versus magic bullets: selectively non-selective drugs for mood disorders and schizophrenia. *Nat. Rev. Drug Discov.* 3, 353–359

**W. Gattrell^{1*}, C. Johnstone²,
S. Patel³, C. Sambrook Smith⁴
A. Scheel², M. Schindler⁵**

¹Research Evaluation Unit, Oxford PharmaGenesis, Oxford, UK

²Evotec (UK), Milton Park, Abingdon OX14 4SA, UK

³GlaxoSmithKline (UK), Medicines Research Centre, Stevenage SG1 2NY, UK

⁴Sygnature Discovery, BioCity, Pennyfoot Street, Nottingham NG1 1GF, UK

⁵AstraZeneca Research and Development, Mölndal, Pepparedsleden 1, Mölndal SE-431 83, Sweden