ELSEVIER

Screening the receptorome: an efficient approach for drug discovery and target validation

Ryan T. Strachan, Gina Ferrara and Bryan L. Roth

Departments of Biochemistry, Neurosciences, Psychiatry and Oncology, Comprehensive Cancer Center and NIMH Psychoactive Drug Screening Program, Case Western Reserve University Medical School, Cleveland, OH 44106, USA

The receptorome, comprising at least 5% of the human genome, encodes receptors that mediate the physiological, pathological and therapeutic responses to a vast number of exogenous and endogenous ligands. Not surprisingly, the majority of approved medications target members of the receptorome. Several *in silico* and physical screening approaches have been devised to mine the receptorome efficiently for the discovery and validation of molecular targets for therapeutic drug discovery. Receptorome screening has also been used to discover, and thereby avoid, the molecular targets responsible for serious and unforeseen drug side effects.

Introduction

The plasma membrane plays host to more than 20 different families of receptors, including over 1000 different proteins [1], which we have dubbed the 'receptorome' [2,3]. The G-protein-coupled receptor (GPCR) superfamily represents the single largest slice of the receptorome [4], although the receptorome also includes toll-like receptors, integrin receptors, low-density lipoprotein receptors, receptor protein tyrosine kinases and phosphatases, cytokine receptors and even some ion channels that can function as receptors (http://receptome.stanford.edu/HPMR) [5] (Figure 1). The receptorome has evolved to 'capture' a vast and diverse universe of ligands, such as photons, odorants, tastants, pheromones, viruses, neurotransmitters, amino acids, polypeptides, hormones, nucleotides, ions, intermediary metabolites and catabolites, natural products and lipids [6].

The therapeutic exploitation of the interaction between extracellular ligands and cell surface receptors, which originated as the 'drug–receptor' concept, is considered to be one of the great ideas and insights in 20th-century biomedical science [7]. Not surprisingly, this conceptual insight has led to the discovery and development of a large number of drugs that target members of the receptorome. Indeed, because of continuing advances in target identification, screening technologies and target validation, receptorome-based drug discovery efforts are likely to be productive for many decades to come. Not surprisingly, most experts conclude that the receptorome accounts for the largest portion in the 'druggable genome', with GPCRs consistently leading the pack [6,8,9].

Deorphanizing the receptorome for target discovery and validation: focus on GPCRs

The largest portion of the receptorome comprises the seven transmembrane spanning receptors known as the GPCRs. The GPCR superfamily constitutes more than 50% of the receptorome and 2% of the human genome (Figure 1) and is responsible for mediating multiple cellular responses to a diverse set of endogenous and exogenous ligands, including biogenic amines, amino acids, peptides, lipids, odorants, nucleotides and photons. A recent phylogenetic analysis of the human GPCR superfamily has revealed more than 860 family members, which are divided into five distinct families: rhodopsin (A), secretin (B), glutamate (C), adhesion and frizzled/taste2 [10,11]. Of these five families, the rhodopsin family (A) is the largest, with an estimated 752 receptors, of which 258 are nonolfactory [4] and, therefore, highly druggable targets. Although olfactory receptors are, conceivably, druggable, they are not typically considered to be targets for therapeutic drugs because they are expressed mainly in nasal epithelia and function to detect odors.

Despite the large number of existing drug targets among GPCRs, a substantial proportion of them, termed orphan GPCRs (oGPCRs),

Corresponding author: Roth, B.L. (bryan.roth@case.edu)

1359-6446/06/$ - see front matter doi:10.1016/j.drudis.2006.06.012

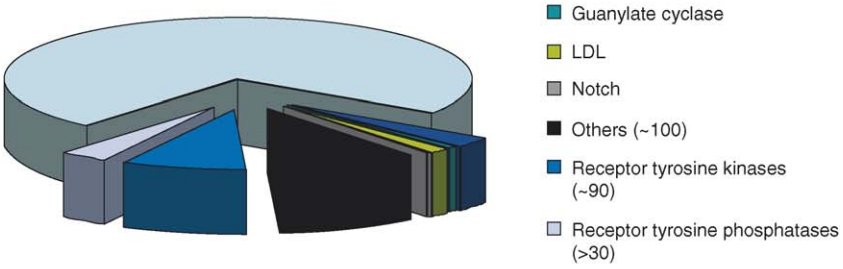

FIGURE 1

The human receptorome. Shown are the main members of the human receptorome. It can be seen that the GPCR superfamily represents the largest single member of the human receptorome.

are not characterized with respect to function and ligand preference [12]. It is estimated that 100–140 oGPCRs exist within the non-olfactory group of GPCRs, and in light of successful GPCR therapeutic targeting, these oGPCRs represent a potential gold mine of therapeutic targets [12]. The discovery of endogenous ligands for these oGPCRs, and their subsequent use in drug discovery and target validation via a process dubbed 'reverse pharmacology', underlies a substantial amount of current research (Box 1).

One of the classic examples of how reverse pharmacology has facilitated drug development was the discovery of a large number of oGPCRs that were subsequently identified as representing distinct 5-hydroxytryptamine (5-HT) receptors. 5-HT receptors now number 15 and are divided into seven main families, (5-HT1 to 5-HT7) [13], and all, with the exception of the 5-HT3, are GPCRs. This discovery of the extensive molecular diversity of 5-HT receptors led to the recognition that the plethora of functions ascribed to 5-HT was mediated by the interaction of 5-HT with functionally and structurally diverse receptors located in discrete anatomical sites [14].

Indeed, G-21 – the first oGPCR described in the scientific literature – was subsequently identified as the 5-HT1A receptor. The discovery that the 5-HT1A receptor was encoded by a distinct gene, which proved to be the molecular target for buspirone and the atypical anxiolytics, has led to the validation of the theory that 5-HT1A subtype-selective ligands might prove useful for treating anxiety and related disorders [15]. Ultimately, similar discoveries have led to the development of subtype-selective 5-HT receptor agonists and antagonists aimed at treating diseases as diverse as schizophrenia, obesity, viral infections and cancer [16–19].

Another example of how reverse pharmacology leads to the identification of novel drug targets relates to the deorphanization of the oGPCR HFGAN72 (also known as orexin receptor 1) as the molecular target for a novel class of peptides called orexins. Subsequently, another GPCR was discovered (orexin receptor 2) by sequence homology. Further study of the orexin receptor and orexin peptide genes implicated a dysfunctional orexin neuropeptide system in the majority of human narcolepsy cases [20–22]. Furthermore, orexin receptor deorphanization has facilitated peptide ligand structure–activity studies at the orexin receptors, a step towards the rational design of peptidomimetic orexin receptor ligands. These receptor ligands could be useful for treating human narcolepsy. Further studies with this receptor class have suggested, surprisingly, that orexin receptor ligands might also be useful to treat obesity and disorders of human motivation [23]. Thus, even though the initial discovery of the orexin receptor pointed toward narcolepsy as the main therapeutic target, subsequent studies have expanded the potential therapeutic targets of this receptor class and have led to the validation of the orexin receptor as a therapeutic target for narcolepsy.

Continued efforts towards deorphanizing oGPCRs and other orphan receptors are likely to lead to the further identification of novel targets for therapeutic drug discovery. Most of the current approaches for exploiting the receptorome for drug-discovery purposes rely on the identification of a single molecular target as a potential therapeutic target, and the performance of high-throughput screening (HTS). These standard approaches are highly efficient at discovering and developing potential lead compounds, and will not be discussed here. Instead, we focus on massively parallel approaches to screening the receptorome (both *in silico* and physically) for target discovery and validation.

BOX 1

Reverse pharmacology

In reverse pharmacology, an orphan receptor (receptor with unknown natural ligand) is used as bait to discover the native ligand. The now deorphanized receptor and its ligand are then used for drug discovery purposes to find selective agonists and antagonists to define the *in vivo* and *in vitro* pharmacology of the receptor further.

Current receptoromics: physical screening approaches for the receptorome

In this section, we summarize available technologies for screening the receptorome in a parallel fashion. Ideally, one would simultaneously screen the entire complement of receptors in the genome with compound libraries to discover compounds with the requisite potency and selectivity to be used as lead compounds for therapeutic drug development [6]. Unfortunately, there is currently no single approach that can be used to perform such massively parallel and unbiased screening because of technological limitations related to the diversity of assay read-outs that are needed to screen multiple targets simultaneously.

The use of competition radioligand binding assays to screen the receptorome

Competition radioligand binding assays rely on the use of high specific activity radioligands that selectively target the receptor of interest. Competition radioligand binding assays, as typically carried out, provide a reliable estimate of drug affinities for particular molecular targets but do not give information related to efficacy (as either agonists, antagonists or partial agonists). Traditionally, pharmacologists have relied on competition radioligand binding assays to measure ligand affinities and receptor specificities, as well as ascribe physiological relevance to GPCRs (the reader is directed to the article by Allen *et al.* [24] for discussion of a nonradiometric binding assay alternative). Competition radioligand receptorome screens are amenable to near-HTS techniques because they can be performed in 96+ well plates, which have proven invaluable for the efficient screening of focused chemical libraries against an array of receptors [2,3,25] (Figure 1). Indeed, this approach led to the discovery of the κ-opioid receptor as the single molecular target responsible for the actions of the hallucinogen salvinorin A [26].

Physical screening of the receptorome using high-affinity receptor-specific radioligands, competitive small molecule libraries and heterologously expressed GPCRs, ion channels and transporters has been applied successfully to populate a receptorome screening database, the publicly available National Institute of Mental Health Psychoactive Drug Screening Program K_i Database (KiDB, http://kidb.case.edu; described by Vortherms and Roth [5] and Roth *et al.* [27]). The database currently has >40,000 K_i values, which have been generated using hundreds of ligands and receptors, and is searchable using several criteria (Figure 2). This and analogous databases can be used to develop tools for virtual screening and to predict the pharmacology of unknown compounds [28] (Figure 3).

Another approach for screening the receptorome with competition radioligand binding technologies has been described by a group at Pfizer [29]. The Pfizer group uses a commercial database (Cerep BioPrint Database) of 1567 compounds, which have been screened at 92 ligand-binding assays to generate a proprietary database of relative drug affinities at 92 different molecular targets. The Pfizer group has used hierarchical clustering of the >140,000 data points, along with chemical similarity predictions to link chemical structure with drug side effects [29].

The most powerful uses of such databases are for molecular target identification, validation and therapeutic drug discovery. A pertinent case in point is the recent discovery of the 5-HT2A receptor as the molecular target of the JC virus – the causative

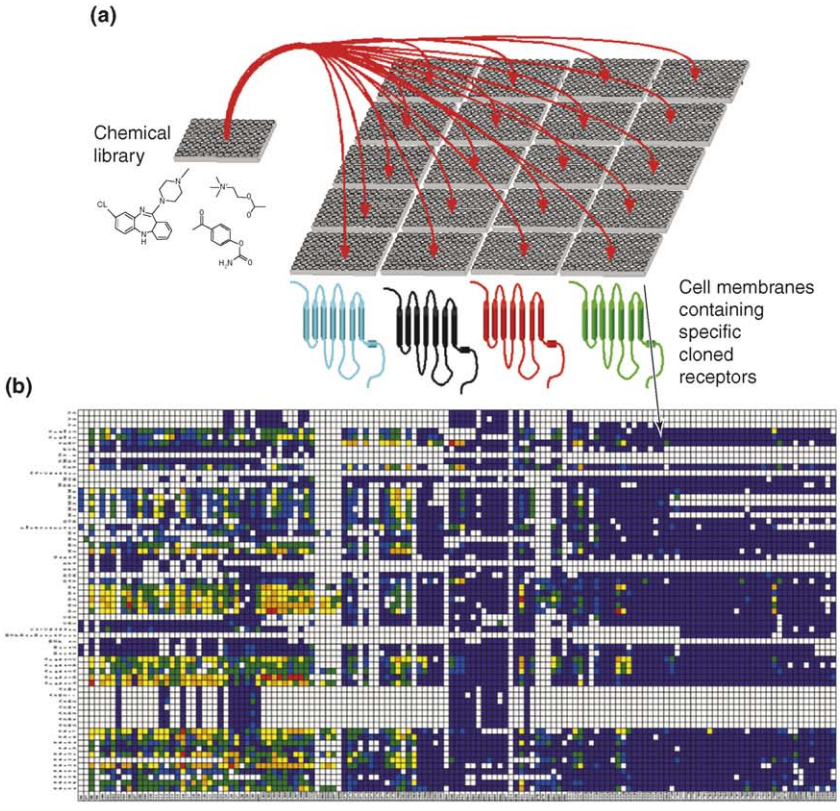

FIGURE 2

Massive parallel chemical library-based approaches to mine the receptorome. Shown is the radioligand-based approach to mine the receptorome. **(a)** Large numbers of cell lines, each expressing a different cloned human receptor, are used to prepare membranes, which are subsequently used in radioligand-binding assays. **(b)** Diverse chemical libraries are then used to 'mine' the receptorome by providing receptor-binding profiles, which can then be used to select drugs with the appropriate 'on-target' and 'off-target' pharmacologies. (b) shows the results of a typical receptorome screen, wherein affinity values for drugs at various targets are color coded with red and yellow, representing high affinities (0.1–10 nM); green, representing intermediate affinities (10–100 nM); and blue and purple, representing low affinities (100–10000+ nM affinities). Modified, with permission, from Ref. [6].

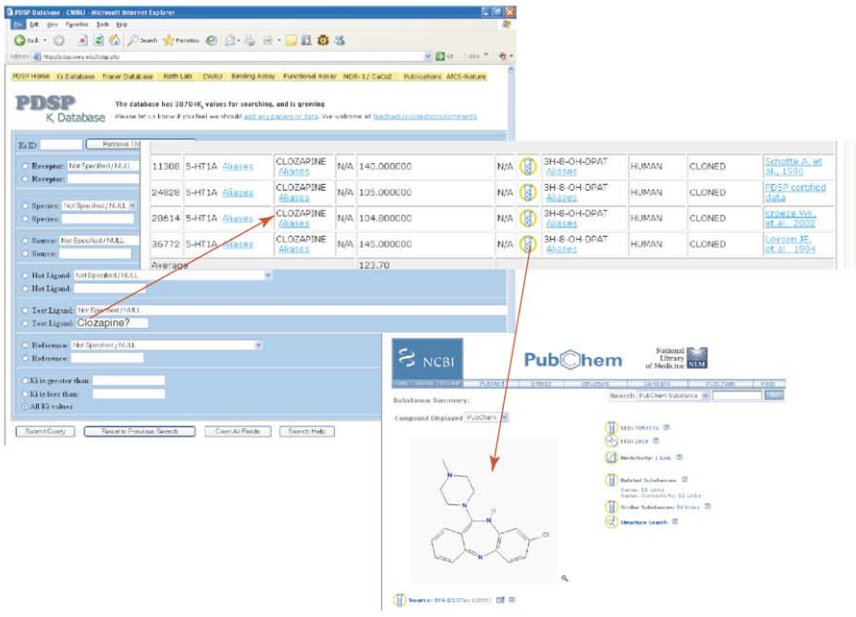

FIGURE 3

The KiDB offers a seamless, powerful web-based resource to mine the receptorome. Shown is a typical query result from KiDB (http://pdsp.cwru.edu/pdsp.php), wherein clozapine was queried and affinities for human, cloned 5-HT1A receptors are shown. The icons provide links to PubChem and PubMed. Chemical homology searching and pharmacological prediction for compounds of unknown pharmacology represent recent enhancements to KiDB.

agent of progressive multifocal leukoencephalopathy [18]. This receptor was identified based on the discovery that chlorpromazine – which blocks infection – has a high affinity for a large number of 5-HT receptors, including 5-HT2A [19]. Subsequently, mirtazepine was identified as a potential candidate medication for progressive multifocal leukoencephalopathy [19].

Radioligand screens are typically consistent across different cellular expression systems but, as usually performed, do not differentiate between agonists, partial agonists, antagonists and inverse agonists. Importantly, radioligand receptorome screens fail to detect responses that occur downstream of ligand binding, and thus might not be suited for deorphanizing oGPCRs because, by definition, these oGPCRs have unknown ligands. Additionally, radioligand-binding assays are, typically, biased for detecting ligand binding to the endogenous receptor site (orthosteric site), and therefore might not detect small molecule modulators that exert their effect at sites distinct from the endogenous site (allosteric site) [30] However, it is possible, with appropriate attention to experimental design, to perform radioligand-binding assays that will interrogate allosteric interactions, and by directly labeling allosteric sites with appropriate high-affinity labeled allosteric ligands or using multiple orthosteric ligands [30].

Information-rich receptoromics: high-throughput functional assays to screen the receptorome

In contrast to radioligand-binding assays, functional assays produce information-rich ligand profiles that reveal how ligands modulate GPCR signal transduction (i.e. agonist versus partial agonist). Functional GPCR screening relies on the detection of second messengers, which are produced as a result of receptor-specific signal transduction pathways. Typically, Ca^{++} is measured using fluorometric dyes and analyzed by automated fluorescent plate readers [31]. Although the activation of many GPCRs will not induce a Ca^{++} signal, the use of chimeric [32] and/or promiscuous G proteins enables most GPCRs to couple to Ca^{++} [33]. Chimeric G proteins, invented by Conklin and co-workers [32], have been engineered to couple Gi- or Gs-coupled GPCRs to phospholipase C and, thereby, to intracellular Ca^{++} mobilization; whereas promiscuous G proteins promiscuously couple many GPCRs to Ca^{++}. Other functional screening methods include GPCR-induced changes in melanophores (a pigmented cell from frogs that changes color when activated) [34], ion channel activity [35], cell growth [36], arrestin translocation [37] and transcription factor activation [6].

Unfortunately, there are currently no technological platforms enabling the simultaneous analysis of hundreds of receptors in parallel using functional readouts. Additionally, because a multiplicity of downstream signaling events can occur following receptor activation, even among members of a single receptor family (e.g. 5-HT receptors) [38], it is difficult to predict which of the signaling events is relevant physiologically and therefore useful as a readout. This is particularly problematic when searching for agonists and partial agonists because chemically distinct agonists frequently elicit functionally distinct readouts [39–41] – a phenomenon referred to as 'functional selectivity' or 'agonist-directed trafficking' [39].

Receptoromics approaches to discover and characterize selectively nonselective drugs

It is appreciated that drugs that interact with multiple molecular targets (e.g. selectively nonselective drugs; 'magic shotguns') are frequently more effective for treating complex diseases (e.g. schi-

zophrenia, depression, cancer) than are target-selective drugs (e.g. magic bullets) [16,42]. For example, clozapine, which interacts with dozens of receptors [16], is not only more effective than other antipsychotic drugs at treating schizophrenia [43] but is also the only antipsychotic drug that can prevent suicidality [44] – perhaps owing to its affinity for 5-HT7 receptors (Figure 4). Unfortunately, clozapine has several serious and potentially life-threatening side effects, including agranulocytosis, seizures, weight gain and adverse metabolic side effects [45–47]. At least some of the side effects of clozapine, particularly weight gain, are a result of off-target interactions with receptors not involved in their therapeutic actions [48]. Ideally, one would like to design selectively nonselective drugs that interact only with therapeutic targets and avoid molecular targets associated with side effects. Although the rational design of such magic shotguns is not a trivial endeavor, it is feasible, as recent successes demonstrate [49,50]. One can thus envisage the parallel screening of hundreds of receptors simultaneously with small molecule libraries to identify lead-like compounds with the predicted polypharmacy. Such compounds could then be optimized simultaneously for interactions with selected high-priority targets and with potentially toxic targets, which could be eliminated before animal testing.

Because it is currently impossible to screen hundreds of receptors simultaneously in any HTS-like fashion, other approaches need to be used to discover selectively nonselective drugs. Phenotypic-based, high-content screening is an approach that might be ideally suited for discovering such magic shotguns. This approach combines multiple cell types, cellular environments and cellular readouts with the aim of measuring compound profiles that are characteristic of its therapeutic potential in the modeled biologies [51,52]. This approach identifies hits with prequalified drug-like activities, and can bypass the need for initial target identification and validation. The utility of this approach is limited to therapeutic areas that are amenable to simulation in tissue culture models (i.e. inflammation, metabolic disorders and cancer but not neuropsychiatric disorders). It is also limited to mechanisms and targets that can be represented in cultured cells. Kunkel *et al.* [53] recently used this multicellular systems approach, which they defined as biologically multiplexed activity profiling (abbreviated to BioMAP), to functionally characterize the activity of various classes of anti-inflammatory drugs. Multiplexing is a process whereby multiple molecular targets are screened simultaneously. This implies that multiplexed activity profiling in complex cellular systems has the ability to characterize mechanisms and pathways of novel compounds, irrespective of their target specificities. In theory, one could identify selectively nonselective compounds using such high-content screening platforms, and then use receptoromics profiling to identify those lead-like compounds with the fewest interactions with potentially toxic targets.

Computationally screening the receptorome: facilitating HTS drug discovery using *in silico* techniques

Computational methods have become a crucial component of many drug discovery programs, from hit identification to lead optimization and beyond, and approaches such as ligand- or

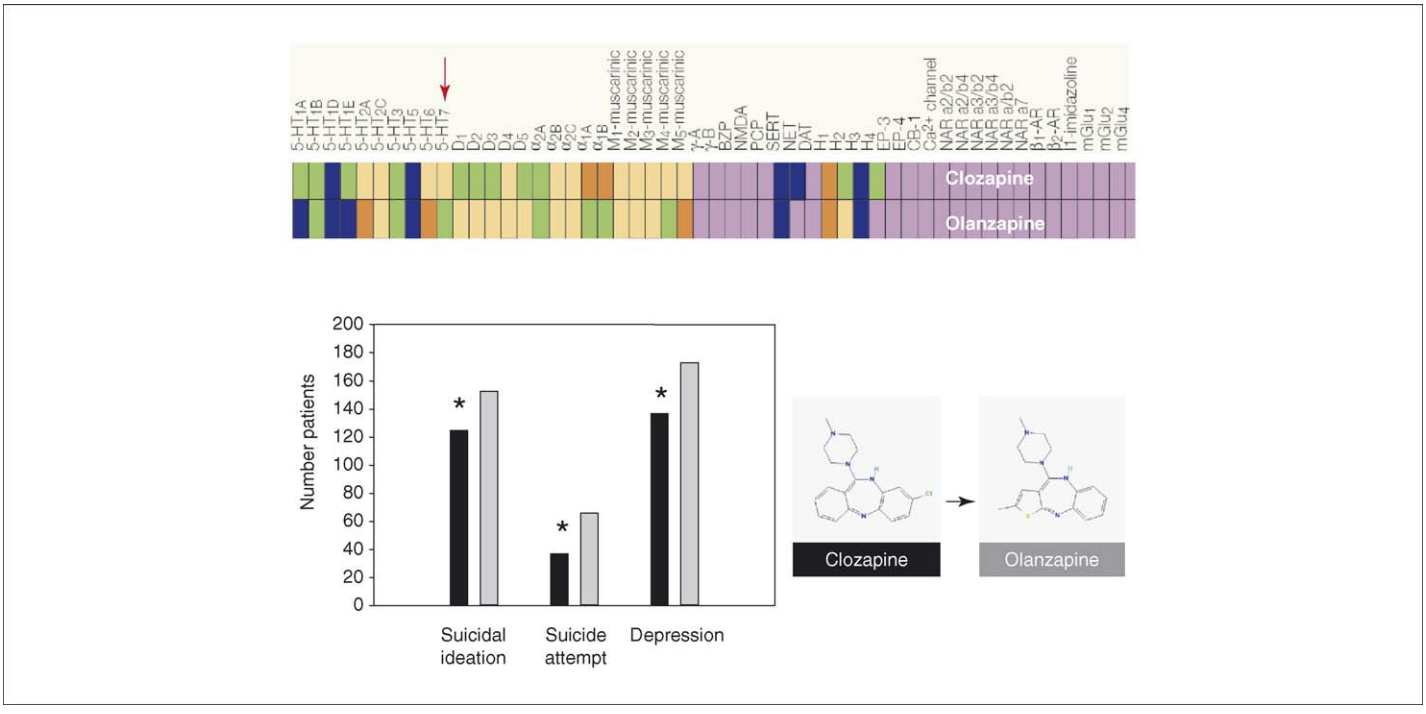

FIGURE 4

Using receptorome binding to discover molecular targets responsible for drug-specific actions. One current question is: is the 5-HT7 receptor involved in the unique actions of clozapine? It is currently unknown why clozapine and olanzapine differ with respect to the ability to prevent suicide. We performed a receptorome screen to determine if differences in receptor pharmacology could account for differential clinical efficacy. Shown on the upper portion of the panel are the receptorome profiles for clozapine and olanzapine – two atypical antipsychotic drugs with similar structures but differential abilities to prevent suicide [44]. Clozapine differs from olanzapine by having higher affinity for 5-HT7 receptors – a target recently implicated as a target for antidepressant drug discovery [73,74]. These results imply that differential affinity for 5-HT7 receptors might underlie the superior efficacy of clozapine in preventing suicide.

structure-based virtual screening techniques are widely used in many discovery efforts [54]. In fact, various computational methods have been applied to facilitate the experimental deorphanization of oGPCRs by identifying potential cognate ligands and putative signaling pathways [52].

One of the most popular computational *in silico* approaches is virtual screening – a process by which ligands are screened against molecular targets *in silico*. Virtual ligand screening endeavors to use molecular and atomic descriptors of molecular structure and chemical properties to reduce chemical library size rationally. These descriptors include 2D substructures (molecular fragments), 3D pharmacophore models, specialized molecular descriptors (i.e. similarities in molecular shape, topology or electronic features), molecular fingerprints (i.e. diverse aspects of molecular structure and properties), compound clustering or partitioning, and compound filtering [55]. Virtual screening methods complement HTS efforts and incorporate protein structure-based compound screening (docking) and chemical similarity searching based on small molecules. Typically, virtual screening techniques are used in a parallel fashion with early-stage HTS-based screening assays. Importantly, current *in silico* efforts rely heavily upon ligand-based methods because little structural information is available for many members of the receptorome, despite the recent successful design of thyroid hormone receptor antagonists [56]. Virtual screening has been successful in identifying subtype-selective nonpeptidic somatostatin agonists and urotensin II antagonists (reviewed by Klabunde and Hessler [57]), as well as receptor tyrosine kinases [58].

When such *in silico* approaches are applied to the receptorome, millions of compounds can be computationally screened against thousands of potential ligand-binding sites – including both orthosteric and allosteric sites. *In silico* screening can be used to enrich a library of compounds that are subsequently physically screened against the receptorome. Indeed, such an approach has been applied successfully to the biogenic amine family of GPCRs [59,60] to discover compounds with novel pharmacologies. Theoretically, one could also enrich compound libraries for drugs with potential activities at multiple, selected molecular targets that could then be used for conventional screening campaigns. Several bioinformatics sites are available to the general public; these are summarized in Table 1. These sites might be of use for receptorome-based computational drug-discovery efforts.

Case studies of applied receptoromics

Mining the receptorome for cancer therapeutics: targeting receptor tyrosine kinases

In principle, it should also be possible to screen other members of the receptorome in a parallel fashion for drug discovery and drug development efforts. One target class that has received particular interest is the receptor tyrosine kinase family, which includes many anticancer therapeutic targets, including epidermal growth factor receptors, platelet-derived growth factor receptors, fibroblast growth factor receptors and vascular endothelial growth factor receptors [1]. Recently, as part of a larger study aimed at kinases in general, Fabian *et al.* [61] profiled clinically approved

TABLE 1

Public-sector informatics resources to screen the receptorome

Name	URL	Description
KiDB	http://kidb.case.edu	The K_i database serves as a data warehouse for published and internally derived K_i, or affinity, values for a large number of drugs and drug candidates at an expanding number of GPCRs, ion channels, transporters and enzymes.
GPCRDB	http://www.gpcr.org/7tm	A useful database that offers information on sequences and sequence similarities between GPCRs. Limited pharmacological information is available.
AffinDB	http://www.agklebe.de/affinity	An affinity database for ligand–protein complexes, including receptors, for which structures are available via the Protein Data Bank
DrugBank	http://redpoll.pharmacy.ualberta.ca/drugbank	A useful database of approved and experimental drugs with links to potential targets.
GLIDA	http://gdds.pharm.kyoto-u.ac.jp:8081/glida	A relational database of GPCRs, their ligands and access to other web-based resources for GPCRs.
IUPHAR receptor and ion channels database	http://www.iuphar-db.org/GPCR http://www.iuphar-db.org/iuphar-ic/index.html	A database listing approved nomenclature for GPCRs and ion channels. This database is not searchable but is a useful source for information that can be used to populate relational databases.
PubChem	http://pubchem.ncbi.nlm.nih.gov	A searchable database containing chemical information on a large number of compounds, enabling flexible structure-based homology search. Is easily linked to other receptor-based databases.
ChemBank	http://chembank.broad.harvard.edu	ChemBank is a freely available collection of data about small molecules and resources for studying their properties, especially their effects on biology. It is being developed to assist biologists who wish to identify small molecules that can be used to perturb a particular biological system and chemists designing novel compounds or libraries, and serves as a source of data for cheminformatic analyses.
Human receptome database	http://receptome.stanford.edu/HPMR	A highly useful database listing the various members of the human receptome or receptorome. Phylogenic information and similarity searching is provided.

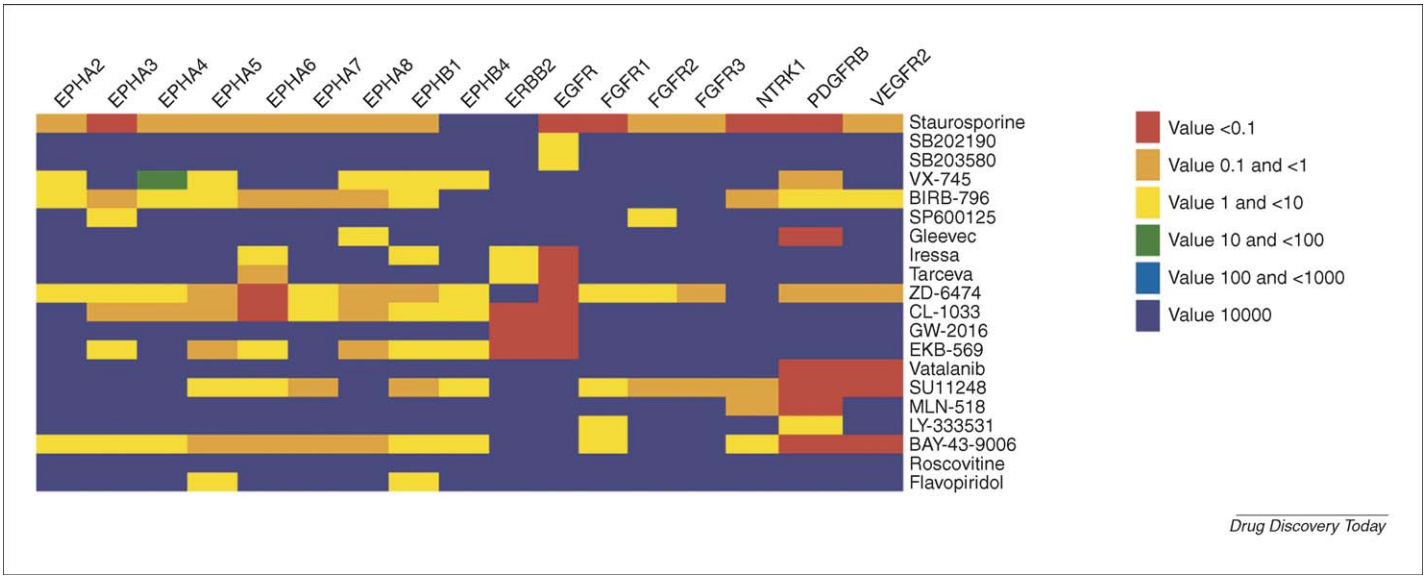

FIGURE 5

'Selective' receptor tyrosine kinase inhibitors are not necessarily selective. Shown is a heat map of data derived from the study by Fabian *et al.*, [61] wherein a large number of kinases were used for kinase inhibitor mapping. Here, we display the data for the receptor tyrosine kinases that were screened by Fabian *et al.* [61] As can be seen, several putatively selective receptor tyrosine kinase inhibitors display appreciable off-target affinities for unrelated receptor tyrosine kinases.

and investigational kinase inhibitors at receptor tyrosine kinases and other nonreceptor kinases. These authors [61], as well as others [42], have recently noted that kinase inhibitors that target more than a single protein kinase appear to be clinically superior to highly selective kinase inhibitors. We have replotted data presented in the Fabian *et al.* paper with a focus on receptor tyrosine kinases (Figure 5). As can be seen, the tested kinase inhibitors varied widely with respect to specificities for their intended targets. Indeed, two compounds designed to be targeted against the epidermal growth factor (Iressa and Tarceva) display nM affinities for other receptor tyrosine kinases, whereas Gleevec (targeted to the platelet-derived growth factor receptor) has nM affinity for an ephrin receptor (EPHA8) (Figure 5). As suggested by Fabian *et al.* [61], comprehensive screening of putatively selective receptor tyrosine kinase inhibitors against other kinases should 'greatly facilitate and accelerate the drug discovery process' [61].

Prader–Willi-syndrome, heart valves and obesity: a receptorome success story

Fenfluramine, one of the most widely prescribed appetite suppressants used for long-term management of obesity, was taken off the market in 1997 after it was found to increase the risk of developing pulmonary hypertension and valvular heart disease (VHD) [62]. In a broad-based receptorome screen performed by the National Institute of Mental Health Psychoactive Drug Screening Program, the molecular target responsible for fenfluramine-induced valvular heart disease was identified as being the human 5-HT2B receptor [19,25,63]. It was subsequently discovered that pergolide, cabergoline and dihydroergotamine, drugs previously reported to induce valvular heart disease in humans [64–66], also activate 5-HT2B receptors [2,67]. Later, Launay *et al.* [68] discovered that 5-HT2B receptor activation is also responsible for fenfluramine-induced primary pulmonary hypertension. These studies led to the validation of the 5-HT2B receptor as the target for cardiovascular side effects of many approved drugs.

It was subsequently discovered that the anorectic actions of fenfluramine are mediated, at least in part, through direct activation of 5-HT2C receptors via norfenfluramine, the major metabolite of fenfluramine [69,70]. These results agreed with prior findings that genetic deletion of the 5-HT2C receptor induces obesity in mice [71], and with many other studies demonstrating that 5-HT2C agonists are anorectic [69]. More recently, it was discovered that patients with Prader–Willi syndrome, a condition involving pathological overeating, have altered 5-HT2C receptor mRNA expression due to aberrant mRNA splicing. Taken together, these results indicate that 5-HT2C-selective drugs devoid of appreciable activity at 5-HT2B receptors and 5-HT2A receptors (because they represent the site of action of lysergic acid diethylamide [26]) would represent safe and effective appetite-suppressants. Taken together, these studies validated the 5-HT2C receptor as a target for anorectic drug discovery.

Several groups have synthesized 5-HT2C-selective agonists [17,72], one of which (APD 356) has shown efficacy in a Phase IIb clinical trial without evidence of cardiovascular side effects, including valvulopathy or pulmonary hypertension (http://www.arenapharm.com/wt/page/apd356). Taken together, these results demonstrate that screening the receptorome can quickly yield validated molecular targets for therapeutic drug discovery. Additionally, receptorome screening enables the simultaneous avoidance of various targets related to serious drug toxicities and side effects.

Conclusions

Receptorome screening provides an unbiased and highly efficient approach for molecular target discovery and validation. Development of novel screening technologies and improved chemoinformatics resources will greatly enhance our ability to mine the receptorome for therapeutic drug discovery.

Acknowledgements

This work was supported in part by the NIMH Psychoactive Drug Screening Program and by grants RO1MH57635, RO1MH61887, RO1DA01766 and KO2MH01366 to B.L.R.

References

1 Ben-Shlomo, I. *et al.* (2003) Signaling receptome: a genomic and evolutionary perspective of plasma membrane receptors involved in signal transduction. *Sci. STKE* RE9
2 Setola, V. *et al.* (2003) 3,4-Methylenedioxymethamphetamine (MDMA, 'ecstasy') induces fenfluramine-like proliferative actions on human cardiac valvular interstitial cells *in vitro*. *Mol. Pharmacol.* 63, 1223–1229
3 Rothman, R.B. *et al.* (2003) *In vitro* characterization of ephedrine-related stereoisomers at biogenic amine transporters and the receptorome reveals selective actions as norepinephrine transporter substrates. *J. Pharmacol. Exp. Ther.* 307, 138–145
4 Kroeze, W.K. *et al.* (2003) G-protein-coupled receptors at a glance. *J. Cell Sci.* 116, 4867–4869
5 Vortherms, T.A. and Roth, B.L. (2005) Receptorome screening for CNS drug discovery. *IDrugs* 8, 491–496
6 Armbruster, B.N. and Roth, B.L. (2005) Mining the receptorome. *J. Biol. Chem.* 280, 5129–5132
7 Maehle, A.H. *et al.* (2002) The emergence of the drug receptor theory. *Nat. Rev. Drug Discov.* 1, 637–641
8 Hopkins, A.L. and Groom, C.R. (2002) The druggable genome. *Nat. Rev. Drug Discov.* 1, 727–730
9 Russ, A.P. and Lampel, S. (2005) The druggable genome: an update. *Drug Discov. Today* 10, 1607–1610
10 Fredriksson, R. and Schioth, H.B. (2005) The repertoire of G-protein coupled receptors in fully sequenced genomes. *Mol. Pharmacol.* 67, 1414–1425
11 Fredriksson, R. *et al.* (2003) The G-protein-coupled receptors in the human genome form five main families. Phylogenetic analysis, paralogon groups, and fingerprints. *Mol. Pharmacol.* 63, 1256–1272
12 Howard, A.D. *et al.* (2001) Orphan G-protein-coupled receptors and natural ligand discovery. *Trends Pharmacol. Sci.* 22, 132–140
13 Hoyer, D. *et al.* (1994) International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). *Pharmacol. Rev.* 46, 157–203
14 Kroeze, W.K. *et al.* (2002) Molecular biology of serotonin receptors structure and function at the molecular level. *Curr. Top. Med. Chem.* 2, 507–528
15 Fargin, A. *et al.* (1988) The genomic clone G-21 which resembles a beta-adrenergic receptor sequence encodes the 5-HT1A receptor. *Nature* 335, 358–360
16 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
[17] Sard, H. *et al.* (2005) SAR of psilocybin analogs: discovery of a selective 5-HT(2C) agonist. *Bioorg Med Chem Lett* 15, 4555–4559
18 Elphick, G.F. *et al.* (2004) The human polyomavirus, JCV, uses serotonin receptors to infect cells. *Science* 306, 1380–1383
19 O'Connor, K.A. and Roth, B.L. (2005) Finding new tricks for old drugs: an efficient route for public-sector drug discovery. *Nat. Rev. Drug Discov.* 4, 1005–1014
20 Chemelli, R.M. *et al.* (1999) Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. *Cell* 98, 437–451
21 Lin, L. *et al.* (1999) The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. *Cell* 98, 365–376
22 Hara, J. *et al.* (2001) Genetic ablation of orexin neurons in mice results in narcolepsy, hypophagia, and obesity. *Neuron* 30, 345–354
23 Scammell, T.E. and Saper, C.B. (2005) Orexin, drugs and motivated behaviors. *Nat. Neurosci.* 8, 1286–1288
24 Allen, M. *et al.* (2002) A homogeneous high throughput nonradioactive method for measurement of functional activity of Gs-coupled receptors in membranes. *J. Biomol. Screen.* 7, 35–44
25 Rothman, R.B. *et al.* (2000) Evidence for possible involvement of 5-HT(2B) receptors in the cardiac valvulopathy associated with fenfluramine and other serotonergic medications. *Circulation* 102, 2836–2841
26 Roth, B.L. *et al.* (2002) Salvinorin A: a potent naturally occurring nonnitrogenous kappa opioid selective agonist. *Proc. Natl. Acad. Sci. U. S. A.* 99, 11934–11939
27 Roth, B.L. *et al.* (2004) Screening the receptorome to discover the molecular targets for plant-derived psychoactive compounds: a novel approach for CNS drug discovery. *Pharmacol. Ther.* 102, 99–110
28 Zhang, J. *et al.* (2004) Development of KiBank, a database supporting structure-based drug design. *Comput. Biol. Chem.* 28, 401–407
29 Fliri, A.F. *et al.* (2005) Biological spectra analysis: linking biological activity profiles to molecular structure. *Proc. Natl. Acad. Sci. U. S. A.* 102, 261–266
30 Christopoulos, A. (2002) Allosteric binding sites on cell-surface receptors: ovel targets for drug discovery. *Nat. Rev. Drug Discov.* 1, 198–210
31 Sullivan, E. *et al.* (1999) Measurement of [Ca2+] using the fluorometric imaging plate reader (FLIPR). *Methods Mol. Biol.* 114, 125–133
32 Coward, P. *et al.* (1999) Chimeric G proteins allow a high-throughput signaling assay of Gi-coupled receptors. *Anal. Biochem.* 270, 242–248
33 Offermanns, S. and Simon, M.I. (1995) G alpha 15 and G alpha 16 couple a wide variety of receptors to phospholipase C. *J. Biol. Chem.* 270, 15175–15180
34 Potenza, M.N. *et al.* (1999) Effects of regulators of G protein-signaling proteins on the functional response of the mu-opioid receptor in a melanophore-based assay. *J. Pharmacol. Exp. Ther.* 291, 482–491
35 Reinscheid, R.K. *et al.* (2003) High-throughput real-time monitoring of Gs-coupled receptor activation in intact cells using cyclic nucleotide-gated channels. *Eur. J. Pharmacol.* 478, 27–34
36 Messier, T.L. *et al.* (1995) High throughput assays of cloned adrenergic, muscarinic, neurokinin, and neurotrophin receptors in living mammalian cells. *Pharmacol. Toxicol.* 76, 308–311
37 Barak, L.S. *et al.* (1997) Internal trafficking and surface mobility of a functionally intact beta2-adrenergic receptor-green fluorescent protein conjugate. *Mol. Pharmacol.* 51, 177–184
38 Raymond, J.R. *et al.* (2001) Multiplicity of mechanisms of serotonin receptor signal transduction. *Pharmacol. Ther.* 92, 179–212
39 Lawler, C.P. *et al.* (1999) Interactions of the novel antipsychotic aripiprazole (OPC-14597) with dopamine and serotonin receptor subtypes. *Neuropsychopharmacology* 20, 612–627
40 Shapiro, D.A. *et al.* (2003) Aripiprazole, a novel atypical antipsychotic drug with a unique and robust pharmacology. *Neuropsychopharmacology* 28, 1400–1411
41 Kenakin, T. (1995) Agonist-receptor efficacy. II. Agonist trafficking of receptor signals. *Trends Pharmacol. Sci.* 16, 232–238
42 Frantz, S. (2005) Drug discovery: playing dirty. *Nature* 437, 942–943
43 Kane, J. *et al.* (1988) Clozapine for the treatment-resistant schizophrenic. *Arch. Gen. Psychiatry* 45, 789–796
44 Meltzer, H.Y. *et al.* (2003) Clozapine treatment for suicidality in schizophrenia: International Suicide Prevention Trial (InterSePT). *Arch. Gen. Psychiatry* 60, 82–91
45 Meltzer, H.Y. (1989) Clozapine: clinical advantages and biological mechanisms. In *Schizophrenia: A Scientific Focus* (Schulz, S.C. and Tamminga, C., eds), pp. 302–309, Oxford University Press
46 Cohen, S. *et al.* (1990) Weight gain associated with clozapine. *Am. J. Psychiatry* 147, 503–504
47 Allison, D.B. *et al.* (1999) Antipsychotic-induced weight gain: a comprehensive research synthesis. *Am. J. Psychiatry* 156, 1686–1696
48 Kroeze, W.K. *et al.* (2003) H1-histamine receptor affinity predicts short-term weight gain for typical and atypical antipsychotic drugs. *Neuropsychopharmacology* 28, 519–526
49 Morphy, R. *et al.* (2004) From magic bullets to designed multiple ligands. *Drug Discov. Today* 9, 641–651
50 Morphy, R. and Rankovic, Z. (2005) Designed multiple ligands. An emerging drug discovery paradigm. *J. Med. Chem.* 48, 6523–6543
51 Ding, S. and Schultz, P.G. (2004) A role for chemistry in stem cell biology. *Nat. Biotechnol.* 22, 833–840
52 Klekota, J. *et al.* (2005) Identifying biologically active compound classes using phenotypic screening data and sampling statistics. *J Chem. Inf. Model.* 45, 1824–1836
53 Kunkel, E.J. *et al.* (2004) An integrative biology approach for analysis of drug action in models of human vascular inflammation. *FASEB J.* 18, 1279–1281
54 Kitchen, D.B. *et al.* (2004) Docking and scoring in virtual screening for drug discovery: methods and applications. *Nat. Rev. Drug Discov.* 3, 935–949
55 Bajorath, J. (2002) Integration of virtual and high-throughput screening. *Nat. Rev. Drug Discov.* 1, 882–894
56 Schapira, M. *et al.* (2003) Discovery of diverse thyroid hormone receptor antagonists by high-throughput docking. *Proc. Natl. Acad. Sci. U. S. A.* 100, 7354–7359
57 Klabunde, T. and Hessler, G. (2002) Drug design strategies for targeting G-protein-coupled receptors. *Chembiochem.* 3, 928–944

58 Aparna, V. *et al.* (2005) Virtual screening of 4-anilinoquinazoline analogues as EGFR kinase inhibitors: importance of hydrogen bonds in the evaluation of poses and scoring functions. *J. Chem. Inf. Model.* 45, 725–738
59 Freyhult, E. *et al.* (2005) Unbiased descriptor and parameter selection confirms the potential of proteochemometric modelling. *BMC Bioinformatics* 6, 50
60 Lapinsh, M. *et al.* (2002) Proteochemometrics modeling of the interaction of amine G-protein coupled receptors with a diverse set of ligands. *Mol. Pharmacol.* 61, 1465–1475
61 Fabian, M.A. *et al.* (2005) A small molecule–kinase interaction map for clinical kinase inhibitors. *Nat. Biotechnol.* 23, 329–336
62 Connolly, H.M. *et al.* (1997) Valvular heart disease associated with fenfluramine-phentermine. *N. Engl. J. Med.* 337, 581–588
63 Fitzgerald, L.W. *et al.* (2000) Possible role of valvular serotonin 5-HT(2B) receptors in the cardiopathy associated with fenfluramine. *Mol. Pharmacol.* 57, 75–81
64 Creutzig, A. (1992) *Dtsch. Med. Wochenschr.* 117, 1736
65 Pritchett, A.M. *et al.* (2002) Valvular heart disease in patients taking pergolide. *Mayo Clin. Proc.* 77, 1280–1286
66 Flowers, C.M. *et al.* (2003) The US Food and Drug Administration's registry of patients with pergolide-associated valvular heart disease. *Mayo Clin. Proc.* 78, 730–731
67 Newman-Tancredi, A. *et al.* (2002) Differential actions of antiparkinson agents at multiple classes of monoaminergic receptor. III. Agonist and antagonist properties at serotonin, 5-HT(1) and 5-HT(2), receptor subtypes. *J. Pharmacol. Exp. Ther.* 303, 815–822
68 Launay, J.M. *et al.* (2002) Function of the serotonin 5-hydroxytryptamine 2B receptor in pulmonary hypertension. *Nat. Med.* 8, 1129–1135
69 Curzon, G. *et al.* (1997) Appetite suppression by commonly used drugs depends on 5-HT receptors but not on 5-HT availability. *Trends Pharmacol. Sci.* 18, 21–25
70 Vickers, S.P. *et al.* (1999) Reduced satiating effect of d-fenfluramine in serotonin 5-HT(2C) receptor mutant mice. *Psychopharmacology (Berl.)* 143, 309–314
71 Tecott, L.H. *et al.* (1995) Eating disorder and epilepsy in mice lacking 5-HT2c serotonin receptors. *Nature* 374, 542–546
72 Vickers, S.P. and Dourish, C.T. (2004) Serotonin receptor ligands and the treatment of obesity. *Curr. Opin. Investig. Drugs* 5, 377–388
73 Guscott, M. *et al.* (2005) Genetic knockout and pharmacological blockade studies of the 5-HT7 receptor suggest therapeutic potential in depression. *Neuropharmacology* 48, 492–502
74 Hedlund, P.B. *et al.* (2005) 5-HT7 receptor inhibition and inactivation induce antidepressantlike behavior and sleep pattern. *Biol. Psychiatry* 58, 831–837