MINIREVIEW

Integrated Approaches for Genome-wide Interrogation of the Druggable Non-olfactory G Protein-coupled Receptor Superfamily

Published, JBC Papers in Press, June 22, 2015, DOI 10.1074/jbc.R115.654764
Bryan L. Roth¹ and Wesley K. Kroeze
From the Department of Pharmacology, University of North Carolina Chapel Hill School of Medicine, Chapel Hill, North Carolina 27514

G-protein-coupled receptors (GPCRs) are frequent and fruitful targets for drug discovery and development, as well as being off-targets for the side effects of a variety of medications. Much of the druggable non-olfactory human GPCR-ome remains under-interrogated, and we present here various approaches that we and others have used to shine light into these previously dark corners of the human genome.

G-protein-coupled receptors (GPCRs; 7-transmembrane domain receptors) historically have represented both the most abundant and the most popular gene superfamily for therapeutic drug discovery and development, with perhaps 30–40% of approved drugs targeting the non-olfactory GPCRs (1, 2). Typical estimates are that, at any given time, between 20 and 40% of candidate medications target non-olfactory GPCRs as their canonical or principal molecular targets (2–4). Indeed, in 2014, of the 41 new molecular entities approved by the Food and Drug Administration (FDA) (http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DrugInnovation/ucm20025676.htm), 9 had GPCRs as their canonical sites of action (Table 1). Of the GPCRs targeted by known drugs, the Family A aminergic GPCRs are by far the most popular (2–4) with aripiprazole, which has multiple biogenic amine GPCR targets and a complex mode of action (5), being the bestseller in 2014. As the olfactory GPCRs do not yet represent therapeutic targets, we will focus on the non-olfactory GPCRs here.

Not only do GPCRs represent the principal therapeutic site of action of many approved and candidate medications, but GPCRs also represent prominent “off-targets” for severe and potentially life-threatening side effects. Of these, drugs with 5-HT₂B serotonin receptor agonism have long been documented to induce severe, life-threatening valvular heart disease (6–8). Indeed, based on the potent 5-HT₂B agonist activity of certain ergot derivatives used in treating Parkinson disease and migraine headaches (e.g. pergolide, cabergoline, and dihydroergotamine), we correctly predicted that these medications would also induce valvular heart disease (7, 8). Two of these drugs (pergolide and cabergoline) were withdrawn from the international market following large-scale trials demonstrating their life-threating side effects (8, 9). In follow-up studies, we surveyed 2200 FDA-approved and investigational medications, finding that 27 had potentially significant 5-HT₂B agonism, of which 6 are currently FDA-approved (guanfacine, quinidine, xylometazoline, oxymetazoline, fenoldopam, and ropinirole) (10). Interestingly, of the 2200 drugs screened, around 30% displayed significant 5-HT₂B antagonist activity (10), indicating that 5-HT₂B receptors represent a “promiscuous target” for approved and candidate medications. Our discovery that ergotamine and other ergots displayed functional selectivity for β-arrestin over G-protein signaling at 5-HT₂B receptors (10) led to the first structure-based explanation of GPCR β-arrestin-biased signaling (11). The discovery that the 5-HT₂B receptor was responsible for the side effects of the appetite-suppressing medications fenfluramine and dexfenfluramine (6–8) was thus a seminal finding of immense public health importance, which ensures that drugs under development will now be counter-screened against the 5-HT₂B receptor for significant agonist activity before being advanced to clinical trials.

Simultaneously with the discovery that the side effects of fenfluramine were due to the 5-HT₂B agonist activity of its main metabolite norfenfluramine (6–8), it became clear that its therapeutic (anorectic) actions were due to norfenfluramine’s agonist activity at the closely related 5-HT₂C receptor (12). This led to the prediction that 5-HT₂C-selective agonists devoid of 5-HT₂B agonist activity would represent safe and effective appetite suppressants (13) and the discovery of the 5-HT₂C-preferring agonist lorcaserin, which was approved by the FDA as the first new obesity medication in nearly 20 years in 2012 (14–16). Taken together, this vignette underscores how an understanding of both on-target and off-target actions of drugs at a single subfamily of GPCRs, in this case the 5-HT₂ serotonin receptor family, can be crucial for successful drug discovery efforts.

Chemical Informatics-based Approaches for Genome-wide GPCR-based Discovery

The discovery of small molecule drug-like compounds that interact with GPCRs in a number of ways (e.g. as orthosteric, allosteric, or biased ligands) is now relatively straightforward and will not be reviewed in any detail here as there are a number of excellent and recent review articles (17–19). As these are important concepts for GPCR drug discovery, however, they will be briefly defined. Thus, orthosteric ligands are those that occupy the site(s) of the native or natural ligand, whereas allosteric ligands occupy a site distinct from the orthosteric site (18,
In the ChEMBL database and PDSP Ki database (KiDB), and, typically, their chemical descriptors along with the biological properties of small molecules. Table 2 lists a few of the more popular and widely used databases. Essentially, these databases have large lists of chemical compound names and, typically, their chemical descriptors along with the biological activity associated with these compounds. Most commonly, as in the ChEMBL database and PDSP Kᵢ database (KiDB), which rely mainly on published data, the activity is encoded as a Kᵢ or EC₅₀ value, whereas other databases (e.g., ChemBank and PubChem) provide the raw data as well as fitted data parameters. Utilizing the information from such databases, we and our collaborators have successfully predicted novel GPCR targets for known drugs (3, 20, 21) and have designed novel drugs targeting GPCRs entirely in silico (22).

In silico approaches for discovering GPCR modulators typically take advantage of large chemical databases that annotate the biological properties of small molecules. Table 2 lists a few of the more popular and widely used databases. Essentially, these databases have large lists of chemical compound names and, typically, their chemical descriptors along with the biological activity associated with these compounds. Most commonly, as in the ChEMBL database and PDSP Kᵢ database (KiDB), which rely mainly on published data, the activity is encoded as a Kᵢ or EC₅₀ value, whereas other databases (e.g., ChemBank and PubChem) provide the raw data as well as fitted data parameters. Utilizing the information from such databases, we and our collaborators have successfully predicted novel GPCR targets for known drugs (3, 20, 21) and have designed novel drugs targeting GPCRs entirely in silico (22).

Importantly, in these exemplars of this overall approach, the GPCR-centric predictions were extensively validated both in vitro and in vivo in model organisms such as worms (23), zebrafish (24), mice (3, 22), and most remarkably, in humans (21).

All of these resources rely upon accurately curated, precise data and, of the cited resources, ChEMBL and KiDB would appear to be the most useful as the main source of their data is from peer-reviewed publications. ChEMBL historically has drawn its data from medicinal chemistry publications, although the most recent version of ChEMBL also incorporates large amounts of data from PubChem. KiDB obtains its data mainly from non-medical chemistry publications (e.g., biochemistry, cell biology, pharmacology, neuroscience, and so on). Examining ChEMBL, which is the largest of these resources, we find that a large number of GPCR targets are under-annotated with respect to both their biological function and the chemical matter with which they may interact (Fig. 1, A and B). As can be seen, at least 50% of the non-olfactory GPCRs in the human genome have had few publications associated with them based on a search of PubMed conducted in mid-2013. Additionally, more than 50% of the non-olfactory GPCRs in the human genome had few annotated small molecules (Table 2; GPCR Safari ChEMBL release 3.0). Indeed, of the 159 “orphan” GPCRs in the ChEMBL database, only 5 had annotated small molecules with documented bioactivity. Significantly, although ChEMBL is a curated database, it misidentifies the synthetic ligand 3-[4-[4-(2-cyanophenyl)-1-piperazinyl]butyl]-1H-indole-5-carboxamide as the natural ligand for GPR35 (https://www.ebi.ac.uk/chembl/sarfari/gpcrsarfari/report/protein/266), even though kynurenic acid has been proposed as a naturally occurring ligand for GPR35 (25, 26). This example of GPR35 being misannotated illustrates three important points: first, the need for careful expert curation; second, the fact that all of these databases contain a significant number of errors that could lead investigators astray; and third, the value of orthogonal (i.e., assays for which the readouts are independent) assays to validate “hits” and presumed active compounds.

In Fig. 2, we show that most of the non-olfactory human GPCR-ome is un-interrogated with respect to the chemical matter as annotated in ChEMBL. The practical impact of this is that, when using a database such as ChEMBL for predicting on- and off-target actions of small molecules, most of the GPCR-ome is hidden from a cheminformatics perspective. GPCRs are not unique in that most of them are understudied, as a similar conclusion was reached for kinases a few years ago (27). Indeed, Isserlin et al. (28) have described what they have dubbed the “Harlow-Knapp (H-K) effect,” which they define as: “the propensity of the biomedical and pharmaceutical research communities to focus their activities, as quantified by the number of publications and patents, on a small fraction of the proteome.” Isserlin and colleagues (27, 28) noted that this was true for the targets they studied (kinases, nuclear hormone receptors, and ion channels) irrespective of whether they confined their bibliographic analysis to the “pre-genomic era” (i.e., prior to the publication of the draft human genome in 2000) or later dates (i.e., 2009). We performed a different type of analysis and re-interrogated the publication records for the druggable, non-olfactory GPCRs in 2014, and compared this with all publications predating 2013. As shown in Fig. 3, there was a similar although not identical trend, with most of the understudied GPCRs still being understudied and the more popular GPCRs continuing this trend.
For resources available to interrogate GPCRs from a chemical standpoint, such as PubChem and ChemBank, these databases will essentially supply raw screening data with (in many but not all instances) confirmatory concentration-response curves from which estimates of potency and efficacy are derived. For example, PubChem lists screens for a large number of GPCRs and, from these screens, results for a handful of orphan GPCRs have been published in peer-reviewed journals (29–31). These published findings have led to the discovery that pamoic acid is a potent agonist for GPR35 via arrestin signaling (31), as well as the discovery of novel agonists and antagonists for GPR55 (29, 30).

As should be clear from the foregoing, cheminformatics-based approaches can be quite useful for predicting GPCR targets for both known drugs and other small, perhaps drug-like, molecules. Because the bulk of the GPCR-ome is relatively uncharted territory, i.e. because very few drug-like small molecules have been identified for a large number of human GPCRs, such studies are necessarily and unavoidably underpowered.

### Physical Approaches for Interrogating the GPCR-ome

In the past, we and others have used both radioligand binding and functional assays to elucidate the ligand-based pharmacology of non-orphan GPCRs. This approach, which we dubbed “receptorome screening,” and which has been extensively described in prior reviews (17, 32–34), has led us to a number of important discoveries including: the identification of the μ-opioid receptor as the site of action of the widely abused hallucinogen salvinorin A (35); the discovery that the 5-HT2B serotonin receptor is the valvulopathy receptor (6); identification of the remarkably complex pharmacology of antipsychotic drugs (36); large-scale validation of cheminformatics predictions (3, 22); identification of GPCR as high affinity off-targets of kinase inhibitors (37–39); and large-scale validation of computationally docked and crystallography-confirmed binding poses (11, 40–48).

As radioligand-based approaches require radioligands with high specific activity and high affinity for their targets, they are not useful for the vast majority of GPCRs, for which such radioligands are unavailable. Additionally, the physical, informatics,

---

**TABLE 2**

Useful cheminformatic, chemical biology, and pharmacology databases

| Database acronym* | URL | Typeb | Ref. | Downloadablec |
|-------------------|-----|-------|------|---------------|
| ChEMBL | https://www.ebi.ac.uk/chembl/ | C, T, ID | 71 | Y |
| KiDB, PDS P K, Database | http://pds.p.med.unc.edu/kidb.php | C, T, ID (limited) | 72 | Y |
| Chembank | http://chembank.broadinstitute.org/ | C, T, ID | 73 | Y in part |
| SEA; Similarity Ensemble Approach | http://sea.bkslab.org/ | C, T, ID | 3, 20 | N but predictions can be made |
| PubChem | https://pubchem.ncbi.nlm.nih.gov/ | C, T, ID | 74 | Y |
| IUPHAR GPCR Database | http://www.guidetopharmacology.org/GRAC/ReceptorFamiliesForward?type=GPCR | C, T | 7 | Y |

* Where applicable, name comes after semicolon.

b C, chemical; T, target; ID, chemical identifiers.

Y = yes; N = no.

---

**FIGURE 1. Many GPCRs are understudied.** A, shown is a graph of PubMed publications for the non-olfactory GPCRs. For this graph, the time period studied was up to and including August 2013. For searching for publications referencing a particular GPCR, either the International Union of Basic and Clinical Pharmacology (IUPHAR)-approved name or the genome identifier was used, depending upon which resulted in the largest number of publications. B, many GPCRs have no identified chemical modulators. Shown is a graph of CheMBL compounds culled from GPCR SARfari (https://www.ebi.ac.uk/chembl/sarfari/gpcrsarfari) using version 3.00.
and infrastructure requirements required to routinely screen more than a few GPCRs simultaneously using radioligand binding assays are beyond the resources of most academic and industrial laboratories. Fortunately, the National Institute of Mental Health’s Psychoactive Drug Screening Program (NIMH-PDSP), which is housed in the authors’ laboratory, provides screening as a free service to not-for-profit investigators, thereby making this resource available to a large part of the scientific community. Indeed, in the past 5 years, more than 500 investigators world-wide took advantage of the NIMH-PDSP for GPCR profiling of novel and candidate drug-like small molecules.

Functional screening methods are an alternative to radioligand binding-based approaches. Unfortunately, there are currently no published approaches suitable for interrogating the entire olfactory and non-olfactory GPCR-ome. Indeed, screening the entire druggable GPCR-ome is technically challenging due to the diverse G-protein-mediated signaling cascades used by GPCRs (e.g., Gs, Gi, Gq, or G12/13). In the past, forced coupling of Gs, Gi, and G12/13 G-proteins to a Gq-like Ca2+ readout has been frequently used (49, 50) to identify ligands for orphan and/or sparsely annotated GPCRs (17, 51). Approaches that
relly on native coupling to known G-proteins have been successful in identifying novel and selective ligands for orphan GPCRs (52). Additionally, many GPCRs couple to G_{12} and G_{13}. Interestingly, the G_{12/13}-dependent shedding of a membrane-bound reporter protein (53) has been reported as a potential “universal” approach for both orphan and non-orphan GPCRs.

Other approaches have relied on platforms that take advantage of G-protein-independent β-arrestin recruitment because nearly all GPCRs induce arrestin translocation (54). Many methods have emerged to quantify GPCR-β-arrestin interactions, including high content screening (HCS) (55), bioluminescence resonance energy transfer (BRET) (56), and transcriptional activation following arrestin translocation (TANGO) (57). We have found the TANGO-based approach to be quite useful for chemical interrogation of GPCRs (11, 40, 43, 58, 59).

Indeed, we have recently devised a genome-wide approach using a TANGO-based readout to screen nearly all of the druggable GPCR-ome in a facile, simultaneous, and parallel manner (47).

Conclusions and Recommendations

As we have shown, although GPCRs represent a useful and important target class for therapeutic drug discovery and biochemical study, most are under-interrogated. In part, this stems from the lack of robust and scalable ways to assess their activities. New technological platforms are becoming available that allow for unbiased interrogation of the druggable GPCR-ome (47), and when these are made freely available, they will likely begin to have a transformative effect on the study of GPCRs. Additionally, because of the “Harlow-Knapp effect,” many GPCRs will likely remain understudied despite their potential importance from both a basic science as well as a translational perspective.

Author Contributions—B. L. R. and W. K. K. conceived and wrote the paper. B. L. R. and W. K. K. performed the bibliographic analysis. Both authors approved the results and the final version of the manuscript.

Acknowledgments—We thank Seva Katritch and Ray Stevens for the use of an unannotated version of their GPCR tree.

References

1. Hopkins, A. L., and Groom, C. R. (2002) The druggable genome. *Nat. Rev. Drug. Discov.* 1, 727–730
2. Overington, J. P., Al-Lazikani, B., and Hopkins, A. L. (2006) How many drug targets are there? *Nat. Rev. Drug. Discov.* 5, 993–996
3. Keiser, M. J., Setola, V., Irwin, J. J., Laggner, C., Abbas, A. I., Hufeisen, S. J., Jensen, N. H., Kuijer, M. B., Matos, R. C., Tran, T. B., Whaley, R., Glennon, R. A., Hert, J., Thomas, K. L., Edwards, D. D., Shoichet, B. K., and Roth, B. L. (2009) Predicting new molecular targets for known drugs. *Nature* 462, 175–181
4. Rask-Andersen, M., Almén, M. S., and Schiöth, H. B. (2011) Trends in the exploitation of novel drug targets. *Nat. Rev. Drug. Discov.* 10, 579–590
5. Shapiro, D. A., Renock, S., Arrington, E., Chioldo, L. A., Liu, L. X., Sibley, D. R., Roth, B. L., and Mailman, R. (2003) Aripiprazole, a novel atypical antipsychotic drug with a unique and robust pharmacology. *Neuropsycopharmacology* 28, 1400–1411
6. Rothman, R. B., Baumann, M. H., Savage, J. E., Rauser, L., McBride, A., Hufeisen, S. J., and Roth, B. L. (2000) Evidence for possible involvement of 5-HT_{2A} receptors in the cardiac valvulopathy associated with fenfluramine and other serotoninergic medications. *Circulation* 102, 2836–2841
7. Setola, V., Hufeisen, S. J., Grande-Allen, K. J., Vesely, I., Glennon, R. A., Blough, B., Rothman, R. B., and Roth, B. L. (2003) 3,4-Methylenedioxymethamphetamine (MDMA, “Ecstasy”) induces fenfluramine-like proliferative actions on human cardiac valvular interstitial cells in vitro. *Mol. Pharmacol.* 63, 1223–1229
8. Roth, B. L. (2007) Drugs and valvular heart disease. *N. Engl. J. Med.* 356, 6–9
9. Zanettini, R., Antonini, A., Gatto, G., Gentile, R., Tesei, S., and Pezzoli, G. (2007) Valvular heart disease and the use of dopamine agonists for Parkinson’s disease. *N. Engl. J. Med.* 356, 39–46
10. Huang, X. P., Setola, V., Yadav, P. N., Allen, J. A., Rogan, S. C., Hanson, B. J., Revankar, C., Robers, M., Doucette, C., and Roth, B. L. (2009) Parallel functional activity profiling reveals valvulopathogens are potent 5-hydroxytryptamine_{2C} receptor agonists: implications for drug safety assessment. *Mol. Pharmacol.* 76, 710–722
11. Wacker, D., Wang, C., Katritch, V., Han, G. W., Huang, X. P., Vardy, E., McCorry, D. J., Jiang, Y., Chu, M., Siu, F. Y., Liu, W., Xu, H. E., Cherezov, V., Roth, B. L., and Stevens, R. C. (2013) Structural features for functional selectivity at serotonin receptors. *Science* 340, 615–619
12. Vickers, S. P., Clifton, P. G., Dourish, C. T., and Tecott, L. H. (1999) Reduced satiating effect of D-fenfluramine in serotonin 5-HT_{2C} receptor mutant mice. *Psychopharmacology* 143, 309–314
13. 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
14. Meltzer, H. Y., and Roth, B. L. (2013) Lorcanerin and pinamvaserin: emerging selectivity of serotonin receptor subtype-targeted drugs. *J. Clin. Invest.* 123, 4986–4991
15. Thomsen, W. J., Grottick, A. J., Menzaghi, F., Reyes-Saldana, H., Espitia, S., Yuskin, D., Whelan, K., Martin, M., Morgan, M., Chen, W., Al-Shamma, H., Smith, B., Chalmers, D., and Behan, D. (2008) Lorcanerin, a novel selective human 5-hydroxytryptamine_{2C} agonist: in vitro and in vivo pharmacological characterization. *J. Pharmacol. Exp. Ther.* 325, 577–587
16. Smith, S. R., Weissman, N. J., Anderson, C. M., Sanchez, M., Chuang, E., Stubbe, S., Bays, H., Shanahan, W. R., and the Behavioral Modification and Lorcanerin for Overweight and Obesity Management (BLOOR) Study Group (2010) Multicenter, placebo-controlled trial of lorcanerin for weight management. *N. Engl. J. Med.* 363, 245–256
17. Allen, J. A., and Roth, B. L. (2011) Strategies to discover unexpected targets for drugs active at G protein-coupled receptors. *Annu. Rev. Pharmacol. Toxicol.* 51, 117–144
18. Wootten, D., Christopoulos, A., and Sexton, P. M. (2013) Emerging paradigms in GPCR allosterics: implications for drug discovery. *Nat. Rev. Drug. Discov.* 12, 630–644
19. Violin, J. D., Crombie, A. L., Soergel, D. G., and Lark, M. W. (2014) Biased ligands at G-protein-coupled receptors: promise and progress. *Trends Pharmacol. Sci.* 35, 308–316
20. Keiser, M. J., Roth, B. L., Armbruster, B. N., Ernsberger, P., Irwin, J. J., and Shoichet, B. K. (2007) Relating protein pharmacology by ligand chemistry. *Nat. Biotechnol.* 25, 197–206
21. Lounkine, E., Keiser, M. J., Whitebread, S., Mikhailov, D., Hamon, J., Jenkins, J. L., Lavan, P., Werner, E., Doak, A. K., Côté, S., Shoichet, B. K., and Urban, L. (2012) Large-scale prediction and testing of drug activity on side-effect targets. *Nature* 486, 361–367
22. Besnard, J., Ruda, G. F., Setola, V., Abecasis, K., Rodriguez, R. M., Huang, X. P., Norval, S., Sassano, M. F., Shin, A. I., Webster, L. A., Simeons, F. R., Stojanovski, L., Prat, A., Seidah, N. G., Constam, D. B., Bickerton, G. R., Reid, K. D., Wetsel, W. C., Gilbert, I. H., Roth, B. L., and Hopkins, A. L. (2012) Automated design of ligands to polypharmacological profiles. *Nature* 492, 215–220
23. Lemieux, G. A., Keiser, M. J., Sassano, M. F., Laggner, C., Mayer, F., Bain, J. R., Werb, Z., Roth, B. L., Shoichet, B. K., and Ashrafii, K. (2013) In silico molecular comparisons of *C. elegans* and mammalian pharmacology identify distinct targets that regulate feeding. *PLoS Biol.* 11, e1001712
24. Laggner, C., Kokel, D., Setola, V., Tolia, A., Lin, H., Irwin, J. J., Keiser, M. J., Cheung, C. Y., Minor, D. L., Roth, B. L., Peterson, R. T., and Shoichet, B. K. (2010) Discovery of a novel selective activator of serotonin 5-HT_{2C} receptors in the cardiac valvulopathy associated with fenfluramine and other serotoninergic medications. *Circulation* 102, 2836–2841
MINIREVIEW: Genome-wide Interrogation of the GPCR-ome

B. K. (2012) Chemical informatics and target identification in a zebrafish phenotypic screen. Nat. Chem. Biol. 8, 144–146

25. Wang, J., Simonavicius, N., Wu, X., Swaminath, G., Reagan, I., Tian, H., and Ling, L. (2006) Kynurenine acid as a ligand for orphan G protein-coupled receptor GPR35. J. Biol. Chem. 281, 22021–22028

26. Jenkins, L., Alvarez-Curto, E., Campbell, K., de Munnik, S., Canals, M., Schlyer, S., and Milligan, G. (2011) Agonist activation of the G protein-coupled receptor GPR35 involves transmembrane domain III and is transduced via Gαi3 and β-arrestin-2. Br. J. Pharmacol. 162, 733–748

27. Edwards, A. M., Isserlin, R., Bader, G. D., Frye, S. V., Willson, T. M., and Yu, F. H. (2011) Too many roads not taken. Nature 470, 163–165

28. Isserlin, R., Bader, G. D., A., E., Frye, S., Willson, T., and Yu, F. (2011) The human genome and drug discovery after a decade: roads (still) not taken. arXiv:1102.0448

29. Kotsikorou, E., Sharir, H., Shore, D. M., Hurst, D. P., Lynch, D. L., Madri, A., Isserlin, R., Bader, G. D., Frye, S. V., Willson, T. M., and Yu, F. H. (2011) The genetic design of signaling cascades to record TGFβ receptor bound to an antimotaur agent. Nature 497, 338–343

30. Wang, C., Wu, H., Katritch, V., Hansen, S. L., Foster, D. L., Gao, X., Zhao, X. E., Melcher, K., Zhang, C., Bai, F., Yang, H., Yang, L., Jiang, H., Roth, B. L., Cherezov, V., Stevens, R. C., and Xu, H. E. (2013) Structural basis for molecular recognition at serotonin receptors. Science 340, 610–614

31. Edwards, E. D., Frankowski, J. D., W., Hurst, D., Katritch, V., Westkaemper, R. B., Aube, J., Stevens, R. C., and Roth, B. L. (2013) Chemotype-selective modes of action of κ-opioid receptor agonists. J. Biol. Chem. 288, 34470–34483

32. Kroeze, W. K., Sassano, M. F., Huang, X. P., Lansu, K., McCorvy, J. D., Giguère, P. M., Sciaky, N., and Roth, B. L. (2015) PRESTO-Tango as an open-source resource for interrogation of the druggable human GPCR-ome. Nat. Struct. Mol. Biol. 22, 362–369

33. Thompson, A. L., Liu, W., Chen, E., Katritch, V., Wu, H., Vardy, E., Huang, X. P., Trapella, C., Guerrini, R., Calo, G., Roth, B. L., Cherezov, V., and Stevens, R. C. (2012) Structure of the nociceptin/orphanin FQ receptor in complex with a peptide mimetic. Nature 485, 395–399

34. Coward, P., Chan, S. D., Wada, H. G., Humphries, G. M., and Conklin, B. R. (1999) Chimeric G proteins allow a high-throughput signaling assay of Gαi-coupled receptors. Anal. Biochem. 270, 242–248

35. Coward, P., Wada, H. G., H., Feng, P. S., Akih, H., and Conklin, B. R. (1998) Controlling signaling with a specifically designed Gαi-coupled receptor. Proc. Natl. Acad. Sci. U.S.A. 95, 352–357

36. Beets, I., Lindemans, M., Janssen, T., and Verleyen, P. (2011) Deorphanzing G protein-coupled receptors by a calcium mobilization assay. Methods Mol. Biol. 789, 377–391

37. Jin, C., Decker, A. W., Huang, X. P., Gilmour, B. P., Blough, B. E., Roth, B. L., Hu, Y., Gill, J. B., and Zhang, X. P. (2014) Synthesis, pharmacological characterization, and structure-activity relationship studies of small molecular agonists for the orphan GPR88 receptor. ACS Chem. Neurosci. 5, 576–587

38. Inoue, A., Ishiguro, J., Kitamura, H., Arima, N., Okutani, M., Shuto, A., Higashiyama, S., Ohwada, T., Arai, H., Makide, K., and Aoki, I. (2012) Molecular agonists for the orphan GPR88 receptor. Mol. Biol. Cell 23, 5129–5132

39. Jensen, N. H., and Roth, B. L. (2008) Massively parallel screening of the receptorome. Comb. Chem. High Throughput Screen. 11, 420–426

40. Strachan, R. T., Ferrara, G., and Roth, B. L. (2006) Screening the receptorome: an efficient approach for drug discovery and target validation. Drug Discov. Today 11, 708–716

41. Roth, B. L., Baner, K., Westkaemper, R., Siebert, D., Rice, K. C., Steinberg, S., Ennsberger, P., and Rothman, R. B. (2002) Salvinorin A: a potent naturally occurring nonnigrogenous κ-opioid selective agonist. Proc. Natl. Acad. Sci. U.S.A. 99, 11934–11939

42. Roth, B. L., Sheffer, D. J., and Kroeze, W. K. (2004) Magic shotguns versus magic bullets: selectively non-selective drugs for mood disorders and schizophrenia. Nat. Rev. Drug Discov. 3, 353–359

43. Lin, X., Huang, X. P., Chen, G., Whaley, R., Peng, S., Wang, Y., Zhang, G., Wang, S. X., Wang, S., Roth, B. L., and Huang, N. (2012) Life beyond kinases: structure-based discovery of sorafenib as nonamolant antagonist of 5-HT receptors. J. Med. Chem. 55, 5749–5759

44. Lin, H., Sassano, M. F., Roth, B. L., and Shoichet, B. K. (2013) A pharmacological organization of G protein-coupled receptors. Nat. Methods 10, 140–146

45. Gregori-Puigjané, E., Setola, V., Hert, J., Crews, B. A., Irwin, J. J., Lounkine, E., Marnett, L., Roth, B. L., and Shoichet, B. K. (2012) Identifying mechanism-of-action targets for drugs and probes. Proc. Natl. Acad. Sci. U.S.A. 109, 11178–11183

46. Carlsson, J., Coleman, R. G., Setola, V., Irwin, J. J., Fan, H., Schlessinger, A., Sali, A., Roth, B. L., and Shoichet, B. K. (2011) Ligand discovery from a dopamine D2 receptor homology model and crystal structure. Nat. Chem. Biol. 7, 769–778

47. Weiss, D. R., Ahn, S., Sassano, M. F., Kleist, A., Zhu, X., Strachan, R., Roth, B. L., Leffkowitz, R. J., and Shoichet, B. K. (2013) Conformation guides molecular efficacy in docking screens of activated β2-adrenergic G protein-coupled receptor. ACS Chem. Biol. 8, 1018–1026

48. Wang, C., Wu, H., Evron, T., Vardy, E., Han, G. W., Huang, X. P., Hufreisen, S. J., Mangan, T. J., Urban, D. J., Katritch, V., Cherezov, V., Caron, M. G., Roth, B. L., and Stevens, R. C. (2014) Structural basis for Smoothened receptor modulation and chemoresistance to anticaner drugs. Nat. Comm. 5, 4355

49. Wu, H., Wacker, D., Mileni, M., Katritch, V., Han, G. W., Vardy, E., Liu, W., Thompson, A. A., Huang, X. P., Carroll, F. I., Mascarell, S. W., Westkaemper, R. B., Mosier, P. D., Roth, B. L., Cherezov, V., and Stevens, R. C. (2012) Structure of the human κ-opioid receptor in complex with JDTic. Nature 485, 327–332

50. Wang, C., Wu, H., Katritch, V., Han, G. W., Huang, X. P., Liu, W., Siu, F. Y., Roth, B. L., Cherezov, V., and Stevens, R. C. (2013) Structure of the human smoothened receptor bound to an antiauxin agent. Nature 497, 338–343

51. Wang, C., Jiang, Y., Ma, J., Wu, H., Wacker, D., Katritch, V., Han, G. W., Liu, W., Huang, X. P., Vardy, E., McCorvy, J. D., Gao, X., Zhou, X. E., Melcher, K., Zhang, C., Bai, F., Yang, H., Yang, L., Jiang, H., Roth, B. L., Cherezov, V., Stevens, R. C., and Xu, H. E. (2013) Structural basis for molecular recognition at serotonin receptors. Science 340, 610–614

52. Vardy, E., Mosier, P. D., Frankowski, J. D., Wu, H., Katritch, V., Westkaemper, R. B., Aube, J., Stevens, R. C., and Roth, B. L. (2013) Chemotype-selective modes of action of κ-opioid receptor agonists. J. Biol. Chem. 288, 34470–34483

53. Kroeze, W. K., Sassano, M. F., Huang, X. P., Lansu, K., McCorvy, J. D., Giguère, P. M., Sciaky, N., and Roth, B. L. (2015) PRESTO-Tango as an open-source resource for interrogation of the druggable human GPCR-ome. Nat. Struct. Mol. Biol. 22, 362–369
controlled multicentre trials. *Lancet* **373**, 482–491

61. Bartholini, J., Constantinidis, J., Puig, M., Tissot, R., and Pletscher, A. (1975) The stereoisomers of 3,4-dihydroxyphenylserine as precursors of norepinephrine. *J. Pharmacol. Exp. Ther.* **193**, 523–532

62. Kaufmann, H., Freeman, R., Biaggioni, I., Low, P., Pedder, S., Hewitt, L. A., Mauney, I., Feurtig, M., and Mathias, C., on behalf of NOH301 Investigators (2014) Droxidopa for neurogenic orthostatic hypotension: a randomized, placebo-controlled, phase 3 trial. *Neurology* **83**, 328–335

63. Bush, M. A., Matthews, J. E., De Boever, E. H., Dobbins, R. L., Hodge, R. J., Walker, S. E., Holland, M. C., Gutierrez, M., and Stewart, M. W. (2009) Safety, tolerability, pharmacodynamics and pharmacokinetics of albiglutide, a long-acting glucagon-like peptide-1 mimic in healthy subjects. *Diabetes Obes. Metab.* **11**, 498–505

64. Matthews, J. E., Stewart, M. W., De Boever, E. H., Dobbins, R. L., Hodge, R. J., Walker, S. E., Holland, M. C., Bush, M. A., and the Albiglutide Study Group (2008) Pharmacodynamics, pharmacokinetics, safety, and tolerability of albiglutide, a long-acting glucagon-like peptide-1 mimic, in patients with type 2 diabetes. *J. Clin. Endocrinol. Metab.* **93**, 4810–4817

65. Becker, R. C., Moliterno, D. J., Jennings, L. K., Pieper, K. S., Pei, J., Niedermaier, A., Ziada, K. M., Berman, G., Stone, J., Joseph, D., Mahaffey, K. W., Van de Werf, F., Veltri, E., Harrington, R. A., and TRA-PCI Investigators (2009) Safety and tolerability of SCH 530348 in patients undergoing non-urgent percutaneous coronary intervention: a randomised, double-blind, placebo-controlled phase II study. *Lancet* **373**, 919–928

66. Bouyssou, T., Casarosa, P., Naline, E., Pestel, S., Konetzki, I., Devillier, P., and Schnapp, A. (2010) Pharmacological characterization of olodaterol, a novel inhaled β2-adrenoceptor agonist exerting a 24-hour-long duration of action in preclinical models. *J. Pharmacol. Exp. Ther.* **334**, 53–62

67. Cox, C. D., Breslin, M. J., Whitman, D. B., Schreier, J. D., McGaughey, G. B., Bogusky, M. J., Roecker, A. J., Mercer, S. P., Bednar, R. A., Lemaire, W., Bruno, J. G., Reiss, D. R., Harrell, C. M., Murphy, K. L., Garson, S. L., Doran, S. M., Pueksaritanont, T., Anderson, W. B., Tang, C., Roller, S., Cabalu, T. D., Cui, D., Hartman, G. D., Young, S. D., Koblan, K. S., Winrow, C. J., Renger, J. J., and Coleman, P. J. (2010) Discovery of the dual orexin receptor antagonist [(7R)-4-(5-chloro-1,3-benzoxazol-2-yl)-7-methyl-1,4-diazepan-1-yl][5-methyl-2-(2H-1,2,3-triazol-2-yl)phenyl] methanone (MK-4305) for the treatment of insomnia. *J. Med. Chem.* **53**, 5320–5332

68. Chey, W. D., Webster, L., Sostek, M., Lappalainen, J., Barker, P. N., and Tack, J. (2014) Naloxegol for opioid-induced constipation in patients with noncancer pain. *N. Engl. J. Med.* **370**, 2387–2396

69. Glaesner, W., Vick, A. M., Millican, R., Ellis, B., Tschang, S. H., Tian, Y., Bokvis, K., Brenner, M., Koester, A., Forksen, N., Etgen, H., and Bromol, T. (2010) Engineering and characterization of the long-acting glucagon-like peptide-1 analogue LY2189265, anFc fusion protein. *Diabetes Metab. Res. Rev.* **26**, 287–296

70. Gralla, R. J., Bosnjak, S. M., Hontsa, A., Balser, C., Rizzi, G., Rossi, G., Borroni, M. E., and Jordan, K. (2014) A phase III study evaluating the safety and efficacy of NEPA, a fixed-dose combination of netupitant and palonosetron, for prevention of chemotherapy-induced nausea and vomiting over repeated cycles of chemotherapy. *Ann. Oncol.* **25**, 1333–1339

71. Gaulton, A., Bellis, L. J., Bento, A. P., Chambers, J., Davies, M., Hersey, A., Light, Y., McGlinchey, S., Michalovich, D., Al-Lazikani, B., and Overington, J. P. (2012) ChEMBL: a large-scale bioactivity database for drug discovery. *Nucleic Acids Res.* **40**, D1100–1107

72. Roth, B. L., Lopez, E., Patel, S., and Kroeze, W. K. (2000) The multiplicity of serotonin receptors: uselessly diverse molecules or an embarrassment of riches? *Neuroscientist* **6**, 252–262, 10.1177/107385840000600408

73. Seiler, K. P., George, G. A., Happ, M. P., Bodycombe, N. E., Carrinski, H. A., Norton, S., Brudz, S., Sullivan, J. P., Muhlich, J., Serrano, M., Ferraiolo, P., Tolliday, N. J., Schreiber, S. L., and Clemons, P. A. (2008) ChemBank: a small-molecule screening and cheminformatics resource database. *Nucleic Acids Res.* **36**, D351–359

74. Wang, Y., Xiao, J., Suzek, T. O., Zhang, J., Wang, J., and Bryant, S. H. (2009) PubChem: a public information system for analyzing bioactivities of small molecules. *Nucleic Acids Res.* **37**, W623–633