RESEARCH ARTICLE

View Article Online
View Journal | View Issue Cite this: *Med. Chem. Commun.*,
2018, 9, 1289Received 10th March 2018,
Accepted 15th April 2018

DOI: 10.1039/c8md00134k

rsc.li/medchemcomm

Scaffold hopping from synthetic RXR modulators
by virtual screening and *de novo* design†Daniel Merk, ^a Francesca Grisoni, ^{ab} Lukas Friedrich, ^a
Elena Gelzinyte^a and Gisbert Schneider ^{*a}

The lack of potent subtype-selective modulators of retinoid X receptors (RXRs) has hindered their full exploitation as promising drug targets. Using computational similarity searching, target prediction and automated *de novo* design, we identified novel RXR ligands exhibiting innovative molecular frameworks, pronounced receptor-subtype preference and suitable properties for hit-to-lead expansion.

The ligand-activated transcription factors retinoid X receptors (RXRs)¹ fulfill a unique role among nuclear receptors by acting as universal heterodimer partners of numerous other members of this protein family. The RXR agonist bexarotene is approved for use in the treatment of certain cancers, but this drug is poorly tolerated.^{2,3} Evidence from animal models points to a considerable therapeutic value of RXR activation in neurodegenerative diseases,⁴ including multiple sclerosis⁵ and Alzheimer's disease.⁶ However, the lack of RXR modulators that selectively address the receptor subtypes RXR α , RXR β and RXR γ , their often poor drug-likeness and their toxicity have prevented further studies and the expanded therapeutic use of RXR ligands in humans.^{7,8}

The available RXR ligands beyond the putative endogenous agonist 9-*cis* retinoic acid⁹ (**1a**, Scheme 1) include several potent agonists and antagonists. However, their chemotype diversity is limited, and RXR agonists (e.g., **1a–c**) and antagonists (e.g., **2a–c**) share a small number of scaffolds (Fig. 1).¹⁰ 90% of the known RXR modulators with a potency (EC₅₀ or IC₅₀) up to 50 μ M described in ChEMBL¹¹ contain only seven distinct reduced scaffolds.^{12,13} Moreover, most of them differ only in the attachment positions and lengths of the linkers (0 or 1 atoms), thus confirming the narrow chemical diversity of the known RXR ligands.

Although the structure–activity relationship (SAR) of classical retinoids has been extensively studied, no remarkable improvements in drug-likeness or subtype selectivity have been achieved to date. The discovery of novel chemotypes of RXR ligands will be mandatory for future drug development. To

address this issue and identify RXR-targeting small molecules with novel molecular frameworks, we have employed virtual screening and computational *de novo* design as complementary approaches for scaffold hopping from known to new RXR modulators.

We computationally searched a collection of commercially available screening compounds (3.38 million compounds from selected providers) for potential RXR ligands by using two complementary techniques. First, the probability of RXR modulation was calculated for all compounds by the target prediction software SPiDER.¹⁴ This method is based on 2D pharmacophoric feature distributions and physicochemical properties. The compounds were then ranked according to the lowest *p*-values (false-positive potential) for RXR modulation. We used the *chemically advanced template search* (CATS)¹⁵ as the second *in silico* tool. CATS ranks all screening compounds by their 2D pharmacophore similarity to the known RXR ligands **1a–c**. The SPiDER and CATS rankings were then merged, and from this consolidated list, we selected compounds 3–19 for *in vitro* characterization, considering their individual ranks and scaffolds.

Scheme 1 RXR ligands: putative endogenous agonist 9-*cis* retinoic acid (**1a**), drug approved RXR agonist bexarotene (**1b**), the bexarotene analogue **1c** and RXR antagonists **2a–c**.

^a Department of Chemistry and Applied Biosciences, Swiss Federal Institute of Technology (ETH), Vladimir-Prelog-Weg 4, CH-8093 Zurich, Switzerland.
E-mail: gisbert.schneider@pharma.ethz.ch

^b Department of Earth and Environmental Sciences, University of Milano-Bicocca, P.za della Scienza, 1, IT-20126 Milan, Italy

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c8md00134k

Fig. 1 Scaffold analysis of ChEMBL annotated RXR ligands in terms of most-frequently occurring scaffolds and reduced scaffolds. 90% of known RXR modulators ($n = 521$, agonists and antagonists) with a potency (EC_{50} or IC_{50}) up to $50 \mu\text{M}$ share only seven different reduced scaffolds. The scaffolds display limited structural diversity, merely differing in linker length (0–1 atoms) and linker attachment positions.

Compounds 3–19 were analyzed in specific Gal4 hybrid reporter gene assays^{16,17} for the modulation of RXR α , RXR β and RXR γ .[‡] The compounds were tested for RXR activation and for RXR antagonism in competition with $1 \mu\text{M}$ bexarotene (**1b**). Single-point testing ($30 \mu\text{M}$) on RXR α , RXR β and RXR γ confirmed six of the 17 candidates as RXR modulators, including partial agonists (12, 13, 19) and antagonists (5, 14, 15). For the RXR activating compounds, control experiments without the hybrid receptor showed no transactivation of the reporter gene, thus confirming that the observed effects are RXR-mediated (Fig. S1A[†]). Moreover, their RXR activating activity could be blocked with the RXR antagonist HX531 (**2c**, $1 \mu\text{M}$) (Fig. S1B and C[†]). Full dose-response char-

[‡] RXR-Gal4 hybrid reporter gene assays were conducted in HEK293T cells (gift from the Steinhilber Laboratory, Frankfurt, Germany) using pFR-Luc (Stratagene, La Jolla, CA, USA) as reporter, pRL-SV40 (Promega Corp., Fitchburg, WI, USA) as internal control and Gal4-RXR expression plasmids coding for the hinge region and ligand binding domain of the respective canonical human RXR isoform (UniProt ID: hRXR α : P19793, residues 225–462; hRXR β : P28702-1, residues 294–533; hRXR γ : P48443-1, residues 229–463), which were constructed by integrating cDNA fragments obtained from PCR amplification of commercial cDNA (Source BioScience, Nottingham, UK) into the BamHI and an afore-inserted KpnI cleavage site of the pFA-CMV vector (Stratagene).¹⁶

acterization revealed intermediate micromolar potency, despite the low transactivation efficacy for the partial agonists, which possess unique molecular frameworks that previously were not associated with RXR modulation (Fig. 2). Notably, the uncharged structure of **19** enables RXR modulator development without the acidic residue typically contained in RXR ligands.¹⁸

The RXR antagonists **5**, **14** and **15** displayed low micromolar IC_{50} values (assay agonist: $1 \mu\text{M}$ bexarotene (**1b**)) and possessed unique scaffolds including the non-acidic structure of **5**. Interestingly, **14** and **15** revealed opposite subtype preferences, with **14** favoring RXR α and **15** being more potent for RXR β and RXR γ . Since **14** and **15** possess similar scaffolds but differ in their substitution patterns, their SAR may provide an opportunity for subtype-selective RXR ligand development (*cf.* Fig. S2[†]).

Among the newly identified RXR antagonists, compound **14** showed the highest efficacy, reducing the bexarotene-induced RXR activation to nearly baseline levels. We rescreened the collection of screening compounds for analogues

Fig. 2 Diversity of novel RXR modulators: (A) structures and Bemis-Murcko scaffolds of new RXR modulators. (B) AlogP and AlogS values²⁶ of newly identified RXR modulators compared to ChEMBL-annotated RXR ligands.

with similar molecular frameworks to obtain a preliminary SAR of this ligand class. 249 compounds that retained the central 1,2,3-substituted five-ring scaffold of **14** were retrieved and computationally ranked for their similarity to the known RXR antagonists **2a–c** using Morgan fingerprints (1024 bit, RDKit²⁰), CATS and LIQUID²¹ descriptors. The combination of these three computational metrics covers fundamental aspects of the molecular structure as well as the 2D and 3D pharmacophores. Six compounds, including the query antagonist **14**, appeared two or more times among the top-5 of these similarity rankings, thus confirming the validity of this approach. Of the remaining five compounds, four (**20–23**) were readily available and were characterized *in vitro* (Table 1).

Compounds **21–23** showed RXR antagonistic activity in the reporter gene assays, whereas compound **20** was inactive. The most linear analogue **21** possessed the highest potency ($IC_{50} = 2.0 \pm 0.8 \mu M$ on RXR α). The close analogues **22** and **23**, differing from **14** only in their aromatic ring substituents, were slightly less potent and displayed IC_{50} values of 10–20 μM on all receptor subtypes. Analogue **20**, with a 4-chlorophenyl residue comprising the largest 4-substituent at the 5-ring core, was inactive. Together, these results draw a preliminary SAR of 1,2,4-triazole based RXR antagonists, suggesting that small moieties in 4-position are preferred and that a linear extension of the lipophilic 5-substituent might enhance RXR antagonistic potency.

Then, we employed the computational *de novo* design software design of genuine structures (DOGS)²² to obtain new chemotypes. The software constructs new molecules from a collection of commercially available building blocks and a set of synthetic reaction schemes. DOGS has previously been proven to generate synthetically accessible, isofunctional mimetics of the given template.²³ We used seven known RXR ligands from the literature and our in-house library of nuclear receptor modulators as templates for DOGS and retrieved a total of 2308 *de novo* designs. The designs were then ranked *in silico* using the SPiDER and CATS software. Additionally, the frequency of their generation from different templates was analyzed. From the consolidated ranking, we

Scheme 2 Synthesis of *de novo* designs **24–26**: reagents & conditions: (a) $Pd(PPh_3)_4$, Cs_2CO_3 , toluene/EtOH, reflux, 16 h, 41%; (b) $C_6H_5-B(OH)_2$ (**31**), $Pd(dppf)Cl_2$, Cs_2CO_3 , toluene, 80 °C, 16 h, 31%; (c) hexane-2,5-dione (**33**), montmorillonite K10, μW , 90 °C, 30 min, 89%.

selected designs **24–26** for synthesis by considering their individual ranks and building block availability.

Compounds **24–26** were accessible in single-step procedures (Scheme 2). Suzuki reaction of 1-bromo-3,5-di-*tert*-butylbenzene (**28**) with 4-boronobenzoic acid (**29**) and of 3-(5-(4-chlorophenyl)oxazol-2-yl)propanoic acid (**30**) with phenylboronic acid (**31**) yielded **24** and **25**, respectively. **26** was available through Paal-Knorr reaction of 5-amino-1-(3-chlorophenyl)-1H-pyrazole-4-carbonitrile (**32**) with hexane-2,5-dione (**33**).

The computationally generated compounds **24–26** were then characterized in reporter gene assays, which confirmed designs **24** and **26** as RXR agonists, while **25** partially antagonized the RXR transactivation (Table 2). However, a scaffold similar to **25** has been associated with luciferase inhibition;²⁴ therefore, the activities of **25** should be interpreted cautiously. Agonist **24** displayed low micromolar potency for all three subtypes but revealed remarkable subtype preference for RXR β and RXR γ in terms of transactivation efficacy. It behaved as a moderate partial agonist for RXR α but a strong full agonist for RXR β and RXR γ . By exploiting this particular selectivity profile, **24** can serve as a starting point for the development of subtype-specific RXR modulators.

Using virtual screening and an automated *de novo* design, we have identified a total of twelve novel RXR modulators comprising ten different scaffolds. Although the query/template compounds contained only a few molecular frameworks, we retrieved novel RXR modulator chemotypes by both

Table 1 *In vitro* activity of RXR modulators obtained by virtual screening (mean $\pm$ SEM, $n \geq 4$ independent experiments)

ID	RXR α	RXR β	RXR γ
5	$IC_{50} = 7 \pm 2 \mu M$	$IC_{50} = 10.9 \pm 0.3 \mu M$	$IC_{50} = 8.2 \pm 0.8 \mu M$
12	$EC_{50} = 21 \pm 1 \mu M$ (2.9 $\pm$ 0.1-fold act.)	Inactive (30 μM)	Inactive (30 μM)
13	$EC_{50} = 21 \pm 3 \mu M$ (3.0 $\pm$ 0.5-fold act.)	$EC_{50} = 42 \pm 1 \mu M$ (8.2 $\pm$ 0.1-fold act.)	$EC_{50} = 51 \pm 9 \mu M$ (12 $\pm$ 4-fold act.)
14	$IC_{50} = 4.8 \pm 0.7 \mu M$	$IC_{50} = 12.3 \pm 0.4 \mu M$	$IC_{50} = 18.1 \pm 0.1 \mu M$
15	$IC_{50} = 18 \pm 4 \mu M$	$IC_{50} = 6 \pm 1 \mu M$	$IC_{50} = 4.9 \pm 0.5 \mu M$
19	$EC_{50} = 27 \pm 3 \mu M$ (6.2 $\pm$ 1.0-fold act.)	$EC_{50} = 37 \pm 1 \mu M$ (3.3 $\pm$ 0.1-fold act.)	$EC_{50} = 24 \pm 6 \mu M$ (2.3 $\pm$ 0.1-fold act.)
20	Inactive (30 μM)	Inactive (30 μM)	Inactive (30 μM)
21	$IC_{50} = 2.0 \pm 0.8 \mu M$	$IC_{50} = 18 \pm 2 \mu M$	$IC_{50} = 1.8 \pm 0.1 \mu M$
22	$IC_{50} = 12 \pm 7 \mu M$	$IC_{50} = 14 \pm 4 \mu M$	$IC_{50} = 17 \pm 8 \mu M$
23	$IC_{50} = 12 \pm 4 \mu M$	$IC_{50} = 11 \pm 2 \mu M$	$IC_{50} = 18 \pm 1 \mu M$
1b ¹⁹	$EC_{50} = 33 \pm 2 nM$	$EC_{50} = 24 \pm 4 nM$	$EC_{50} = 25 \pm 2 nM$

Table 2 *In vitro* activity of *de novo* designs **24–26** (results are mean $\pm$ SEM, $n \geq 4$ independent experiments)

ID	RXR α	RXR β	RXR γ
24	$EC_{50} = 11.8 \pm 0.7 \mu M$ (5.7 $\pm$ 0.4-fold act.)	$EC_{50} = 11.7 \pm 0.1 \mu M$ (70.6 $\pm$ 0.2-fold act.)	$EC_{50} = 14.1 \pm 0.1 \mu M$ (58.3 $\pm$ 0.2-fold act.)
25	$IC_{50} = 11 \pm 8 \mu M$	$IC_{50} = 22.4 \pm 0.4 \mu M$	$IC_{50} = 6 \pm 1 \mu M$
26	$EC_{50} = 19 \pm 5 \mu M$ (3.6 $\pm$ 0.4-fold act.)	$EC_{50} = 16 \pm 2 \mu M$ (11.1 $\pm$ 0.3-fold act.)	$EC_{50} = 17 \pm 1 \mu M$ (8.1 $\pm$ 0.2-fold act.)
1b ¹⁹	$EC_{50} = 33 \pm 2 nM$	$EC_{50} = 24 \pm 4 nM$	$EC_{50} = 25 \pm 2 nM$

in silico approaches. The new RXR modulators are markedly different from the ChEMBL-annotated RXR agonists and antagonists ($IC_{50}/EC_{50} < 50 \mu M$, $N = 521$) in terms of their scaffolds, with the average Jaccard–Tanimoto similarity (extended connectivity fingerprints,²⁵ radius = 0 to 4 bonds, 1024 bit) below 0.20 ± 0.02 (Fig. S3†). *In vitro* characterization indicated that several of the new ligands possess subtype preference, thus facilitating the development of subtype-selective RXR modulators. Most of the new RXR ligands appear superior to the ChEMBL-annotated compounds in terms of both lipophilicity and solubility, as indicated by their lower AlogP and higher AlogS values (Fig. 2B).

Our computational scaffold hopping approach yielded several new lead compounds that expand the SAR of RXR modulators. These chemical entities provide a variety of innovative molecular frameworks to address key challenges in RXR targeting by enabling improved drug-like properties and greater receptor subtype selectivity. The concept of combined computational similarity searching and *de novo* design has been corroborated as a viable strategy for chemical scaffold hopping.

Conflicts of interest

G. S. declares a potential financial conflict of interest in his role as life-science industry consultant and cofounder of inSili.com GmbH, Zurich.

Acknowledgements

This research was financially supported by the Swiss National Science Foundation (grant no. IZSEZ0_177477). D. M. was supported by an ETH Zurich Postdoctoral Fellowship (grant no. 16-2 FEL-07).

Notes and references

- 1 P. Germain, P. Chambon, G. Eichele, R. M. Evans, M. A. Lazar, M. Leid, A. R. De Lera, R. Lotan, D. J. Mangelsdorf and H. Gronemeyer, *Pharmacol. Rev.*, 2006, **58**, 760.
- 2 K. Eshagh, L. S. Romero, J. K. So and X. F. Zhao, *Cutis*, 2017, **99**, E30.
- 3 K. Liby and M. Sporn, *Curr. Top. Med. Chem.*, 2017, **17**, 721.
- 4 J. Riancho, M. T. Berciano, J. Berciano and M. Lafarga, *J. Neurol.*, 2016, **263**, 177.
- 5 J. K. Huang, A. A. Jarjour, B. Nait Oumesmar, C. Kerninon, A. Williams, W. Krezel, H. Kagechika, J. Bauer, C. Zhao, A. Baron-Van Evercooren, P. Chambon, C. Ffrench-Constant and R. J. M. Franklin, *Nat. Neurosci.*, 2011, **14**, 45.
- 6 K. P. Koster, C. Smith, A. C. Valencia-Olvera, G. R. J. Thatcher, L. M. Tai and M. J. LaDu, *Curr. Top. Med. Chem.*, 2017, **17**, 708.
- 7 S. Yamada and H. Kakuta, *Expert Opin. Ther. Pat.*, 2014, **24**, 443.
- 8 A. R. De Lera, W. Bourguet, L. Altucci and H. Gronemeyer, *Nat. Rev. Drug Discovery*, 2007, **6**, 811.
- 9 A. R. De Lera, W. Krezel and R. Rühl, *ChemMedChem*, 2016, **11**, 1027.
- 10 M. Dominguez, S. Alvarez and A. R. De Lera, *Curr. Top. Med. Chem.*, 2017, **17**, 631.
- 11 A. P. Bento, A. Gaulton, A. Hersey, L. J. Bellis, J. Chambers, M. Davies, F. A. Krüger, Y. Light, L. Mak, S. McGlinchey, M. Nowotka, G. Papadatos, R. Santos and J. P. Overington, *Nucleic Acids Res.*, 2014, **42**, D1083.
- 12 S. Renner and G. Schneider, *ChemMedChem*, 2006, **1**, 181.
- 13 G. W. Bemis and M. A. Murcko, *J. Med. Chem.*, 1996, **39**, 2887.
- 14 D. Reker, T. Rodrigues, P. Schneider and G. Schneider, *Proc. Natl. Acad. Sci. U. S. A.*, 2014, **111**, 4067.
- 15 M. Reutlinger, C. P. Koch, D. Reker, N. Todoroff, P. Schneider, T. Rodrigues and G. Schneider, *Mol. Inf.*, 2013, **32**, 133.
- 16 D. Flesch, S.-Y. Cheung, J. Schmidt, M. Gabler, P. Heitel, J. S. Kramer, A. Kaiser, M. Hartmann, M. Lindner, K. Lüddens-Dämgen, J. Heering, C. Lamers, H. Lüddens, M. Wurglics, E. Proschak, M. Schubert-Zsilavecz and D. Merk, *J. Med. Chem.*, 2017, **60**, 7199.
- 17 J. Schmidt, M. Rotter, T. Weiser, S. Wittmann, L. Weizel, A. Kaiser, J. Heering, T. Goebel, C. Angioni, M. Wurglics, A. Paulke, G. Geisslinger, A. Kahnt, D. Steinhilber, E. Proschak and D. Merk, *J. Med. Chem.*, 2017, **60**, 7703.
- 18 E. Proschak, P. Heitel, L. Kalinowsky and D. Merk, *J. Med. Chem.*, 2017, **60**, 5235.
- 19 M. Boehm, L. Zhang, B. Badea, S. White, D. Mais, E. Berger, C. Suto, M. Goldman and R. Heyman, *J. Med. Chem.*, 1994, **37**, 2930.
- 20 RDKit: Open-source cheminformatics, <http://www.rdkit.org>.
- 21 Y. Tanrikulu, M. Nietert, U. Scheffer, E. Proschak, K. Grabowski, P. Schneider, M. Weidlich, M. Karas, M. Göbel and G. Schneider, *ChemBioChem*, 2007, **8**, 1932.
- 22 M. Hartenfeller, H. Zettl, M. Walter, M. Rupp, F. Reisen, E. Proschak, S. Weggen, H. Stark and G. Schneider, *PLoS Comput. Biol.*, 2012, **8**, e1002380.
- 23 T. Rodrigues and G. Schneider, *Synlett*, 2014, **25**, 170.
- 24 N. Thorne, M. Shen, W. A. Lea, A. Simeonov, S. Lovell, D. S. Auld and J. Inglese, *Chem. Biol.*, 2012, **19**, 1060.
- 25 D. Rogers and M. Hahn, *J. Chem. Inf. Model.*, 2010, **50**, 742.
- 26 I. V. Tetko and V. Y. Tanchuk, *J. Chem. Inf. Comput. Sci.*, 2002, **42**, 1136.