Exploiting Chemical Toolboxes for the Expedited Generation of Tetracyclic Quinolines as a Novel Class of PXR Agonists

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Supporting Information

ABSTRACT: The discovery of lead compounds relies on the iterative generation of structure–activity relationship data resulting from the synthesis and biological evaluation of hit analogues. Using traditional approaches, a significant time delay may occur from compound design to results, leading to slow and expensive hit-to-lead explorations. Herein, we have exploited the use of chemical toolboxes to expedite lead discovery and optimization. In particular, the integration of flow synthesizers, automation, process analytical technologies, and computational chemistry has provided a prototype system enabling the multicomponent flow synthesis, in-line analysis, and characterization of chiral tetracyclic quinolines as a novel class of PXR agonists. Within 29 compounds, a novel template **19b** (3aS,11R,11aS) was identified with an EC_{50} of 1.2 μ M (efficacy 119%) at the PXR receptor.

KEYWORDS: Automation, flow technologies, electronic circular dichroism, enantioresolution, integrated flow system, lead discovery, Pregnane X receptor

One of the major purposes of medicinal chemistry is the design and synthesis of new lead compounds for druggable targets.¹ Medicinal chemistry therefore plays a fundamental role in taking forward chemical biology, pharmacology, and medicine in order to define novel therapeutic approaches and discover new drugs. Medicinal chemistry is a complex process that relies on iterative learning cycles composed by molecular design, synthesis, testing, and structure–activity/structure–property relationship (SAR/SPR) analysis.^{2–4} Using traditional approaches, a significant time delay may occur from the design hypothesis to obtaining the results, thus limiting the number of compounds that can be advanced into clinical studies. At this regard, while the advent of computational tools and high-throughput screening (HTS) has enabled the design, testing, and analysis of large number of compounds, the synthesis of compound collections is not as efficient and represents an ongoing bottleneck in the process of drug discovery.

Medicinal chemistry toolboxes offer alternative solutions to solve limitations of chemical synthesis in terms of compound throughput and quality,⁵ and have shown great potential in

uncovering leads and drug candidates.⁶ In particular, the adoption of enabling chemical technologies as automation⁷ and flow systems⁸ coupled with bioassays and predictive tools, process control devices, and in-line analysis has provided autonomous platform capable of library building and characterization contributing to the shortening of medicinal chemistry discovery cycles.^{9–12}

In this Technology Note, we present our ongoing efforts devoted to the development of an integrated system designed for the automated synthesis, in-line analysis, purification, and stereochemical characterization of compound libraries generated by multicomponent reactions under continuous flow conditions. Besides the intrinsic atom economy and simple procedures, multicomponent reactions are ideally suited for preparing chemical libraries.¹³ However, although multi-

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component reactions have been around for many years, their employment on automated flow synthesis is still poor.

Our prototype system was validated for the generation by multicomponent Povarov reaction of chiral tetracyclic tetrahydroquinolines readily available for screening as ligands of the Pregnane X receptor (PXR, NR1I2), a master xenobiotic receptor involved in drug metabolism and disposition.^{14,15} As PXR plays many other roles, it is considered a valuable target for human diseases associated with inflammation and dysregulation of immune system. Specifically, selective PXR agonists can be useful as chemical probes to investigate the therapeutic relevance of the receptor for the prevention and treatment of gut barrier dysfunction, such as inflammatory bowel disease, colon cancer, irritable bowel syndrome, and autoimmune disorders.¹⁶ Several PXR ligands exist and could be developed as potential therapeutics (e.g., rifampicin, rifaximin); however, they are not selective ligands and plagued by chemical toxicity and/or potential off-target effects. There is therefore an urgent need to discover and develop compounds that selectively interfere with the receptor in intestine and immune cells. To this scope, we aimed at identifying a novel class of PXR agonists endowed with drug-like property and amenable for expedited hit-to-lead explorations.

Initially, a virtual screening experiment was carried out on an internal library using the *Schrodinger Suite 2018-1* (Schrodinger, LLC, New York, NY, 2018). In particular, the Glide software was set to standard precision (SP) mode to dock molecules into the receptor grid generated by the PXR-T0901317 protein complex (pdb code: 2O9I).¹⁷ As a result, a tetracyclic tetrahydroquinoline compound was identified as virtual hit and tested using AlphaScreen assay (UPF-2365, **1**, Figure 1). Noteworthy, a review of the literature indicated that

Figure 1. (A) Structure of UPF-2365 (**1**) and focused hit exploration. (B) AlphaScreen results of **1** at 20 μ M using T0901317 (EC_{50} = 50 nM) as reference compound. (C) Criteria for hit compound selection.

the use of compound **1** as a nuclear receptor modulator has not been reported. Moreover, **1** showed compliance to rule-of-five and properties (MW, clogP) in the range of reported PXR agonists and most importantly we were captured by its synthetic feasibility (Figure 1C). Analogues of **1** can be indeed easily obtained by multicomponent Povarov reaction,^{18,19} a chemical transformation that has been applied in natural products synthesis and that is well suited for compound library generation as it allows to modify the core size, substituents type, and stereochemistry. Besides these considerations, the nucleus of UPF-2365 (**1**) recalls the cyclopentaperhydrophe-

nanthrene scaffold of steroidal PXR ligands, while allowing a wider range of structural variations.

Starting from the hit **1**, initially we planned a chemical strategy aimed at exploring ring D size and type, and the aryl substituent at ring C (Figure 1A). Thus, 5,6,7,8-tetrahydro-1-naphthylamine (**2**), aldehydes (**3–6**), and dienophiles (**7–10**) solubilized in a solution of 5% (v/v) of polyethylene glycol (PEG) 300 in MeCN were mixed in a four way-connector and flowed with a total rate of 0.8 mL min⁻¹ through the reactor-coil at 25 °C (Figure 2).

Figure 2. Flow setup for the continuous automated synthesis and purification of quinolines **1** and **11–22**. AFC, automated silica gel flash chromatography; BPR, back pressure regulator; FC, fraction collector; P1–6, pumps; R, 10 mL reactor coil; S, liquid–liquid membrane separator; UV-d, UV detector.

The output was combined with a stream of H₂O (1.2 mL min⁻¹) and Et₂O (0.4 mL min⁻¹). The organic phase was separated from the aqueous solution using a liquid–liquid membrane separator. The outflow was monitored by in-line UV detector and collected into a fraction collector. The ethereal solution was concentrated and submitted to automatic silica gel flash chromatography assisted by UV detector for fraction collection. Reactions were performed in a sequential, automated flow-through process washing the lines with MeCN. Each isolated compound was racemic but diastereomerically pure. Overall, the synthesis and purification resulted very efficient affording the desired products with a throughput of two compounds per 80 min (Table 1). Compounds **17a–20a** and **22a** were then submitted to trifluoroacetic acid (TFA) treatment to obtain the corresponding derivatives **23a–27a** in 10–47% yield over two steps.

All the synthesized compounds were tested as racemic mixtures at 20 μ M in AlphaScreen assays (Table 1). Although premature, the results allowed to depict a preliminary SAR profile for this first set of compounds. As a general trend, it can be stated that (a) five-membered ring-D was preferred over the six-membered one, (b) *cis* compounds characterized by the furan substructure at ring-D were more potent than the *trans* isomers, (c) thiophenyl group was the most effective substituent, and (d) removal of the *tert*-butoxy carbonyl (Boc) at ring-D led to inactive compounds. Among these, three compounds (**14a**, **17a**, and **19b**) showed a good range of

Table 1. Results of Tetracyclic Quinolines Synthesis and AlphaScreen Assay

Cmpd ^a	Ar	X	n	Yield (%) ^b	PXR activity ^c
1	2-furyl	O	1	36	++
11b				29	—
12a	phenyl	O	1	67	+
12b				15	—
13a	2-thiophenyl	O	1	51	++
13b				18	—
14a	3-thiophenyl	O	1	51	+++
14b				29	+
15a	2-furyl	O	2	27	++
15b				31	—
16a	phenyl	O	2	26	—
16b				41	—
17a	2-furyl	N-Boc	1	46	+++
17b				20	+
18a	phenyl	N-Boc	1	57	++
18b				21	—
19a	3-thiophenyl	N-Boc	1	54	++
19b				18	+++
20a	2-furyl	N-Boc	2	26	+
20b				42	+
21a	phenyl	N-Boc	2	25	—
21b				43	—
22a	3-thiophenyl	N-Boc	2	14	++
22b				50	—
23a	2-furyl	NH	1	34 ^d	—
24a	phenyl	NH	1	47 ^d	—
25a	3-thiophenyl	NH	1	42 ^d	—
26a	2-furyl	NH	2	20 ^d	—
27a	3-thiophenyl	NH	2	10 ^d	—

^aCompounds were tested as pure diastereoisomers but in their racemic form. The relative *cis* and *trans* configurations were determined by ¹H NMR and 2D-NMR (NOESY) analysis. ^bIsolated yield after silica gel flash chromatography. ^cActivity was assayed by AlphaScreen assay, and target compounds were tested at a single dose (20 μM) in triplicate using T0901317 (EC₅₀ = 50 nM) as reference compound. +++, ++, +, and — denote that PXR efficacy in response to 20 μM of the target compound is respectively 75–100%, 50–75%, 25–50%, and <25% of the reference compound. ^dIsolated yield over two steps.

activity between 75 and 100% of efficacy (Table 1). For these compounds, we determined the relative EC₅₀ values with **19b** being the most potent with an EC₅₀ of 1.6 μM and an efficacy of 82% (Table 2).

We next decided to separate and test as PXR ligands single enantiomers of **19b** in order to evaluate the effect of the configuration at the chiral carbon centers. As crystallization and chemical resolution of **19b** were problematic, we sought to combine chiral high pressure liquid chromatography (HPLC) to analyze and isolate pure enantiomers, with experimental and *in silico* simulated electronic circular dichroism ECD spectra obtained by time-dependent density functional theory (TD-DFT) calculations to assign the relative configuration. To this aim, we needed to evaluate enantiomers of both **19b** and the parent *cis* analog **19a**. Thus, a suitable enantioselective

Table 2. PXR Activity of Compounds **14a**, **17a**, and **19b**^a

Cmpd	PXR ^b	
	EC ₅₀	Efficacy ^c
3.5±0.3	42±2
1.5±0.2	53±4
1.6±0.5	82±15

^aData represent mean values SD of at least three independent experiments. ^bUnits are μM for EC₅₀ and % versus T0901317. ^cEfficacy values are here used as measure of AlphaScreen signal change.

chromatographic method was employed to analyze the reaction mixture before silica gel chromatography and to isolate pure enantiomers in the amount needed for both ECD evaluation and biological screenings. With the aim of selecting a unique chiral stationary phase (CSP) for the contemporary chemo- and enantioselective determination of compounds **19a–b**, five polysaccharide-based CSPs (CSPs 1–5, see Supporting Information for details) were first scrutinized. The comparative evaluation revealed CSP-4 (with a cellulose *tris*(3,5-dimethylphenylcarbamate) chiral selector) as the best performing chiral medium also producing good levels of chemoselectivity (Figure 3). Therefore, CSP-4 was utilized to optimize the analysis of test compounds **19a–b**. The excellent chromatographic results coupled with the high volatility of the employed normal-phase eluent systems, facilitated the collection of all stereoisomeric species for the following ECD study. Notably, only few micrograms of each isomer (less than 5 μg obtained with a single HPLC run) were required for the spectroscopic investigation.

The theoretical chiroptical properties of the four enantiomers were determined optimizing a standard protocol for stereochemical characterization using TD-DFT calculations.²⁰ One compound per enantiomeric pairs was submitted to a computational workflow composed by (a) drawing the selected enantiomer in the *Maestro* graphical interface, (b) running a molecular mechanic conformational search with *Macromodel*, (c) performing quantum mechanics geometrical optimization with *Jaguar* and TD-DFT calculations using the *Orca* software,²¹ (d) *in silico* prediction of the ECD spectra by the *SpecDis* program,²² and (e) determining the enantiomeric elution order (EEO) by comparison of the experimental and theoretical ECD spectra (detailed procedures are provided in Supporting Information). In Figure 3, the excellent chemo- and enantioseparation of all the four isomers as well as the

Figure 3. (A) Experimental and theoretical ECD spectra for enantiomers **19b**. (B) Experimental and theoretical ECD spectra for enantiomers **19a**. (C) Enantioresolution and PXR activity of pure enantiomers **19b** (3aR,11S,11aR), **19a** (3aR,11R,11aR), **19a** (3aS,11S,11aS), and **19b** (3aS,11R,11aS). PXR activity was assayed by AlphaScreen assay in the presence of T0901317 (50 nM) as positive reference, and all the experiments were run in triplicates. Color code is used to better understand the assignment of the experimental EEO and the underlying compound stereochemistry.

elevated matching between the experimental and the theoretical ECD spectra of **19a** and **19b** isomers are depicted.

Having assigned the correct configuration, the pure single enantiomers **19a** (*R,R,R*), **19a** (*S,S,S*), **19b** (*R,S,R*), and **19b** (*S,R,S*) were submitted to AlphaScreen assay (Figure 3C). While **19a** (*R,R,R*), **19a** (*S,S,S*), and **19b** (*R,S,R*) behaved as partial agonists, the **19b** (3aS,11R,11aS) isomer was able to activate the receptor with an EC_{50} of 1.2 μ M and an efficacy of 119%. The putative binding pose of **19b** (*S,R,S*) was investigated with a docking study into the PXR-T0901317 binding pocket (Figure 4). As the top scored binding pose, results showed **19b** interacting with the receptor through extensive hydrophobic contacts with residues Leu206, Leu209, Val211, Tyr306, Met323, Leu324, Phe326, Leu411, and Ile414. Although the binding of **19b** (3aS,11R,11aS) was

Figure 4. Compound **19b** (*S,R,S*) putative binding pose in the PXR receptor. (A) Binding site residues are labeled and colored depending on their features: hydrophobic (green), hydrophilic (cyan), or polar (blue). The π -stacking interaction is shown in cyan dashed line. (B) Ligand interaction diagram. (C–D) Binding site surfaces surrounding ring A and Boc group.

mainly driven by hydrophobic forces, a T-shaped π -stacking interaction was also observed between the thiophene moiety and the side chain of W299 (Figure 4B).

In conclusion, our study shows the potential of exploiting and integrating chemical toolboxes to accelerate the preparation and characterization of compound libraries readily available for biological testing. In particular, for the first time we have demonstrated the profitable integration of flow technologies with HPLC and ECD analysis assisted by quantum mechanical calculations for the rapid synthesis, purification, and stereochemical characterization of chiral products prepared by multicomponent reactions. Products were synthesized in an automated fashion and under continuous flow conditions by Povarov reaction and readily screened as ligands of the PXR receptor. Among the tested compounds, **19b** (3aS,11R,11aS) was found to activate the receptor with an EC_{50} of 1.2 μ M (efficacy: 119%) and represents a promising compound for further optimization. Finally, we strongly believe that this approach stands to provide an ideal system for integrated, self-controlled continuous flow system designed to generate multicomponent compound libraries for lead discovery and optimization. The proposed workflow can be indeed applied to chemical structures featuring similar degrees of freedom and atoms to expedite medicinal chemistry programs and method development studies.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmmedchemlett.8b00459.

General methods for synthesis, HPLC analysis, computational methods and biology are available. Characterization data for compounds **1**, **11**–**27**, copies of NMR spectra and HPLC chromatograms, and HRMS key compounds **14a**, **17a**, and **19b** (PDF)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

Boc, *tert*-butoxy carbonyl; CSP, chiral stationary phase; ECD, electronic circular dichroism; EEO, enantiomeric elution order; HPLC, high pressure liquid chromatography; HTS, high-throughput screening; IP, intellectual properties; LBD, ligand binding domain; PEG, polyethylene glycol; PXR, Pregnane x receptor; SAR, structure–activity relationship; SPR, structure–property relationship; TD-DTF, time-dependent density functional theory.

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