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Synthesis of small molecule inhibitors of the orphan nuclear receptor steroidogenic factor-1 (NR5A1) based on isoquinolinone scaffolds

Joshua Roth,[a] Franck Madoux,[b] Peter Hodder[b,c] and William R. Roush[a,*]

[a]*Department of Chemistry, Scripps Florida, 5353 Parkside Drive, RE-2, Jupiter, FL 33458, USA*
[b]*Lead Identification, The Scripps Research Institute Molecular Screening Center, Scripps Florida, 5353 Parkside Drive, RF-1, Jupiter, FL 33458, USA*
[c]*Department of Molecular Therapeutics, Scripps Florida, 5353 Parkside Drive, RF-1, Jupiter, FL 33458, USA*

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Abstract—Three synthetic routes were developed for structure–activity relationship (SAR) studies of HTS-derived isoquinolinone inhibitor probes for the orphan nuclear receptor steroidogenic factor-1 (NR5A1). Among the new analogs reported herein, **31** and **32** have improved potency, lower cellular toxicity, and improved selectivity compared to the initial HTS-derived leads **1** and **2**.

Nuclear receptors are transcription factors that regulate gene expression through the binding of endogenous ligands.[1] Many nuclear receptors have been extensively studied, and several have proven to be important targets for treatment of a range of human diseases.[2] However, natural or unnatural ligands are not known for a subset of nuclear receptors, the so-called 'orphan' nuclear receptors, which have limited efforts to determine the pharmacology and therapeutic potential of these receptors.[1] As part of a broader program to develop an understanding of the pharmacology of relatively unexplored orphan receptors, the Scripps Research Institute's Molecular Library Screening Center has performed high-throughput screens of several orphan receptors, among them the steroidogenic factor-1 (SF-1, also known as NR5A1).[3]

Steroidogenic factor-1 (SF-1) has been implicated in sex determination during development and in formation of steroidogenic tissues.[4] SF-1 is involved in endocrine function throughout life with expression in the pituitary, testes, ovaries, and adrenal gland.[5] Knockout mice exhibit male to female sex reversal and impaired development of adrenals and gonads.[6] Due to the potential role SF-1 plays in the regulation of steroid hormone synthesis including adrenal androgen and gonadal testosterone synthesis, selective control of this receptor could result in therapeutic treatment of metastatic prostate cancer.[7] Additionally, the involvement of SF-1 in energy metabolism suggests relevancy in controlling obesity.[8]

Thus, the development of selective small-molecule biological probes of SF-1 is an important objective. Phospholipids have been found in the ligand-binding domain of human SF-1[9] and recently the first small molecules with the ability to modulate the activity of this transcription factor were described.[10]

Approximately 65,000 compounds were screened for SF-1 inhibition by the Molecular Library Screening Center Network (MLSCN) at The Scripps Research Institute.[3,11] All initial hits were counterscreened against the retinoic acid receptor-related orphan receptor α (RORα), a phylogenetically distant nuclear receptor,[2] in order to eliminate promiscuous as well as non-selective compounds. This led to the identification of two mid-nanomolar SF-1 selective inhibitors **1** (PubChem SID 7970631) and **2** (SID 7969543; Fig. 1).[3,11] Accordingly, isoquinolinones **1** and **2** were selected as starting points for the development of SF-1 small molecule probes. We have developed and report herein three

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* Corresponding author. Tel.: +1 561 799 8880; fax: +1 561 799 8955; e-mail: roush@scripps.edu

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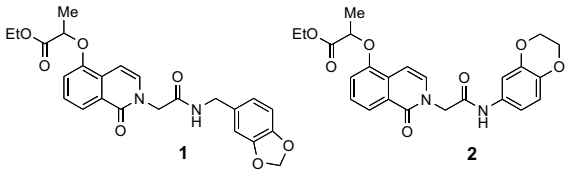


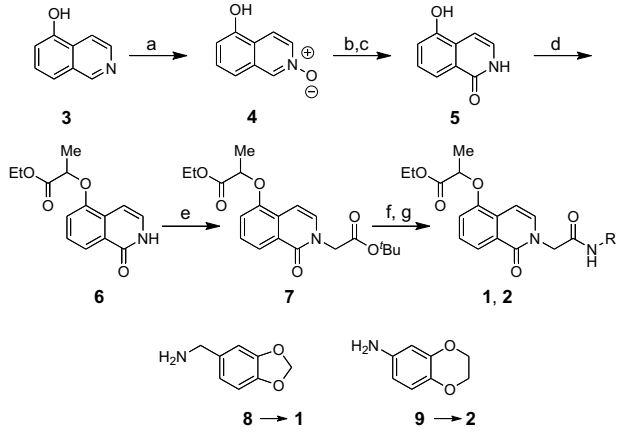
Figure 1. SF-1 inhibitors identified via ultra high-throughput screening of the MLSCN library.

routes for the synthesis of **1** and **2** that enable different aspects of the SAR of this SF-1 inhibitor series to be examined. Among the analogs reported here, **31** and **32** have improved SF-1 inhibitor potency, lower cellular toxicity, and possess improved selectivity compared to the initial leads **1** and **2**.

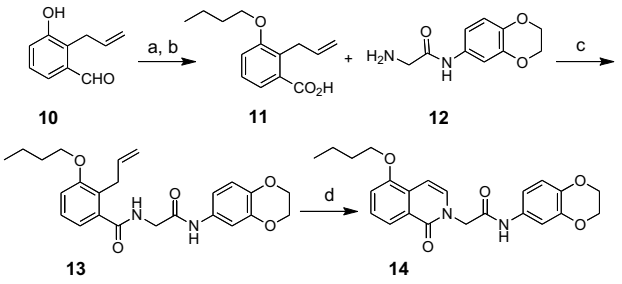
Our initial strategy for synthesis of analogs of **1** and **2** focused on sequential alkylations of an isoquinolinone core (Scheme 1). Treatment of commercially available 5-hydroxyquinoline with peroxyacetic acid provided *N*-oxide **4**. Heating of the *N*-oxide **4** in acetic anhydride gave a diacyl derivative that was not isolated but converted directly to 5-hydroxyisoquinolinone **5**.[12] Selective alkylation of **5** at the phenolic position by using ethyl (±)-2-bromopropionate (NaH, DMF, 25 °C, 80%) gave propionic ester **6**. Subsequent N-alkylation of **6** with *tert*-butyl bromoacetate (Cs_2CO_3, DMF, 25 °C, 80%) provided diester **7**. Acidic deprotection of the *tert*-butyl ester and subsequent coupling (EDC, DMAP, CH_2Cl_2) of the derived carboxylic acid with either benzyl amine **8** or aniline **9** provided authentic samples of **1** and **2**, which were used to confirm the results of the MLSCN SF-1 inhibitor screen.

While the sequence summarized in Scheme 1 permitted us to synthesize a number of analogs, this route proved less than ideal especially for synthesis of isoquinolinone ether analogs owing to its linear nature. Especially problematic is that the chemoselectivity of alkylations of **5** with agents less reactive than α-halopropionate was poor, with N-alkylation of **5** competing with phenol O-alkylation with alkyl halides. This led us to develop the more convergent second-generation synthesis summarized for **14** in Scheme 2.

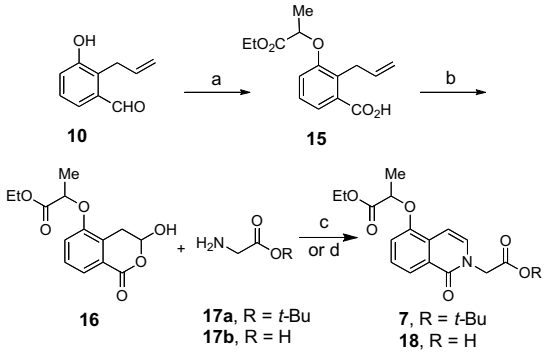
Aldehyde **10**, available from the Claisen rearrangement of *m*-salicaldehyde allyl ether,[13] was alkylated in high



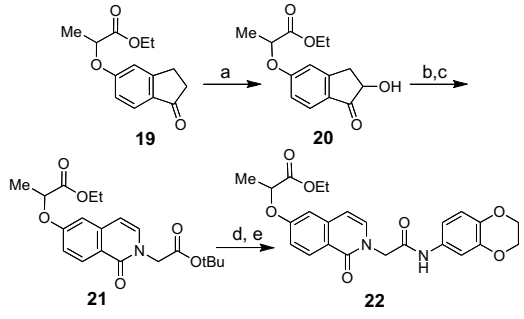
Scheme 1. Reagents and conditions: (a) peroxyacetic acid, CH_2Cl_2 (99%); (b) acetic anhydride, 140 °C, 4 h, then concentrate; (c) NaOMe, MeOH (60%, 2 steps); (d) NaH, DMF, 30 min, then ethyl (±)-2-bromopropionate (80%); (e) Cs_2CO_3, DMF, *tert*-butyl bromoacetate (80%); (f) 2 M HCl in Et_2O and EtOH; (g) EDC, DMAP, CH_2Cl_2, **8** or **9** (45%, 2 steps).



Scheme 2. Reagents and conditions: (a) iodobutane, acetone, K_2CO_3, 60 °C, 4 h (60–85%); (b) 1.5 equiv H_2CrO_4, acetone, 1 h (98%); (c) **9**, EDC, DMAP, CH_2Cl_2 (83%); (d) i—O_3, CH_2Cl_2, −78 °C; ii—PPh_3, iii—cat. I_2, CH_2Cl_2 (45%).



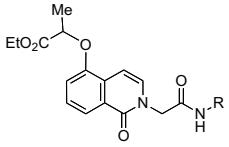
Scheme 3. Reagents and conditions: (a) ethyl (±)-2-bromopropionate, K_2CO_3, acetone, 60 °C, 4 h; (b) i—O_3, CH_2Cl_2, −78 °C; ii—Me_2S, 1 h, 23 °C; (c) *tert*-butyl glycine hydrochloride (**17a**), 3 equiv Et_3N, AcOH to achieve pH 3–5, benzene, 100 °C, sealed tube, 12 h (67%); (d) glycine (**17b**, 3 equiv), benzene, 100 °C, 12 h, sealed tube (94%).



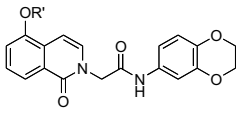
Scheme 4. Reagents and conditions: (a) i—TBSOTf, Et_3N, THF; ii—OsO_4 (2%), NMO, *t*-BuOH, acetone; (b) $NaIO_4$, acetone, H_2O, THF, *t*-BuOH (78% from **19**); (c) glycine *tert*-butyl ester hydrochloride, benzene, Et_3N and AcOH until pH 3–5, 100 °C sealed tube 12 h (21%); (d) 2 M HCl in Et_2O and EtOH (99%); (e) **9**, EDC, DMAP, CH_2Cl_2 (55%).

yield with a variety of electrophiles (use of iodobutane is illustrated in Scheme 2). The resulting product was then oxidized using chromic acid to give acid **11**.[14] Standard peptide coupling of **11** with glycinamide **12** (EDC, DMAP, CH_2Cl_2, 83%) provided diamide **13**, which was then converted to SF-1 inhibitor analog **14** via

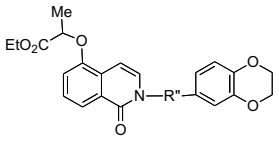
Table 1. SF-1 inhibitor activity of analogs of **1** and **2** (all IC_{50} data are in μM)



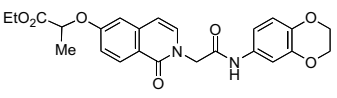
Compound	R	IC_{50} data[a] (μM)			
		SF-1	RORα	VP16	Cell tox[b]
1	See Figure 1	0 26 ± 0.06	>33	>33	>33
2	See Figure 1	0.76 ± 0.10	>33	>33	Inactive
23	$–(CH_2)_3CH_3$	9.71 ± 5.63	Inactive	Inactive	Inactive
24	Cyclohexyl	1.77 ± 0.16	Inactive	Inactive	Inactive
25	Benzyl	1.06 ± 0.03	3.22 ± 0.34	Inactive	Inactive
26	*p*-Fluorobenzyl	0.68 ± 0.12	1.68 ± 0.43	Inactive	Inactive
27	$–CH_2$-2-furanyl	4.71 ± 0.86	>33	Inactive	Inactive
28	phenylethyl	0.36 ± 0.03	>33	Inactive	Inactive
29	*p*-Nitrophenyl	0.53 ± 0.20	Inactive	Inactive	Inactive
30	*p*-Methylphenyl	0.34 ± 0.02	36.5 ± 6.0	>33	40.2 ± 3.7
31	*p*-Methoxyphenyl	0.20 ± 0.04	Inactive	Inactive	Inactive
32	*p*-(F_3C)Ophenyl	0.20 ± 0.11	Inactive	Inactive	Inactive
33	*p*-Ethoxyphenyl	0.19 ± 0.01	22.6 ± 2.0	24.2 ± 5.6	37.0 ± 0.7
34	*p*-Ethylphenyl	0.11 ± 0.04	25.0 ± 3.9	>33	>33
35	*p*-*n*-Butylphenyl	0.36 ± 0.05	30.7 ± 10.7	Inactive	Inactive



Compound	R′	SF-1	RORα	VP16	Cell tox[b]
14	$–CH_2(CH_2)_2CH_3$	13.7 ± 1.3	21.5 ± 3.5	24.1 ± 1.9	>33
36	$–CH_2CH_3CH_2CH_3$	7.86 ± 0.22	16.5 ± 2.2	17.5 ± 1.9	Inactive
37	$–CH_2CH_3(CH_2)_3CH_3$	0.86 ± 0.09	23.1 ± 6.1	20.7 ± 8.0	Inactive
38	$–C(CH_3)_2CO_2Et$	0.59 ± 0.12	Inactive	>33	Inactive



Compound	R″	SF-1	RORα	VP16	Cell tox[b]
40	$–CH_2CH_2CONH–$	8.46 ± 1.67	Inactive	Inactive	Inactive
41	–CH(Me)CONH–	Inactive	Inactive	Inactive	Inactive
42	$–CH(CH_2Ph)CONH–$	Inactive	Inactive	Inactive	Inactive
43	$–CH_2CON(Me)–$	3.57 ± 0.89	Inactive	Inactive	Inactive



Compound	SF-1	RORα	VP16	Cell tox[b]
22	Inactive	Inactive	Inactive	Inactive

[a] IC_{50} value >33 indicates that curve did not fit, and that IC_{50} was manually assigned to be >33 μM. 'Inactive' means <50% inhibition at 100 μM.
[b] Cellular toxicity determined by the CellTiterGlo assay (Ref. 18).

ozonolysis of the vinyl group and dehydration of the hemiaminal that is formed in situ. In some cases, the targeted isoquinolinone was obtained directly from standard workup of the ozonolysis reaction. However, in other cases the dehydration of the hemiaminal did not occur spontaneously. Therefore, as a standard procedure, a catalytic amount of I_2 iodine was added to a solution of the crude hemiaminal in CH_2Cl_2 to promote dehydration and aromatization.

In order to probe the SAR of the glycine spacer connected to the isoquinolinone nitrogen, we demonstrated that condensation of hydroxylactone **16** (accessible from ozonolysis of acid **15**) and glycine *tert*-butyl ester **17a** provides ester **7** (Scheme 3). Deprotection of **7** provides carboxylic acid **18**, which is an intermediate in the sequence summarized in Scheme 1. Alternatively, amino acids such as glycine (**17b**), alanine and phenylalanine can be coupled directly with **16** to give **18**, thereby obviating the need for use of the *tert*-butyl ester protecting group. Subsequent coupling of carboxylic acid **18** with a range of amines and anilines, using the conditions summarized in Scheme 1, greatly facilitated SAR studies of this amide substituent.

Finally, in order to probe the substitution pattern of the isoquinolinone nucleus of **1** and **2**, a third synthetic strategy was developed starting from commercially available hydroxyindanones (Scheme 4); the synthesis of **22** starting from **19** is illustrative. Thus, after alkylation of 5-hydroxyindanone with ethyl (±)-2-bromopropionate (K_2CO_3, acetone, 60 °C, 4 h), indanone **19** was treated with TBSOTf and Et_3N[15] to give the silyl enol ether which, without isolation, was treated with *N*-methylmorpholine *N*-oxide (NMO) and catalytic osmium tetroxide.[16] The resulting α-hydroxyindanone **20** was cleaved using $NaIO_4$ to give the hydroxylactone, which was condensed with glycine *tert*-butyl ester under conditions summarized in Scheme 3. Subsequent coupling of the carboxylic acid derived from deprotection of **21** with aniline **9** provided the regioisomeric isoquinolinone analog **22**.

SF-1 inhibition activity of analogs of **1** and **2** is summarized in Table 1, along with comparative inhibition data versus RORα. All assays were performed as described in Ref. 3. Briefly, luciferase-format functional assays were used to measure inhibition of constitutively active SF-1(LBD)-Gal4 or RORα(LBD)-Gal4 chimeric constructs. Compounds **1**, **2**, **22**–**35** and **40**–**43** were studied as racemates, or as mixtures of diastereomers (**41**, **42**).

Initial efforts focused on replacing the oxygenated benzylic amide or anilide units of **1** and **2**, but introduction of alkyl amides as in **23** and **24** led to significant loss of SF-1 activity. Replacement of the highly oxygenated benzylic amide unit of **1** with potentially more metabolically stable benzyl (**25**) or *p*-fluorobenzyl (**26**) amides led to 5- to 50-fold loss of SF-1 activity. However, introduction of a 2-phenylethyl amide (**28**) yielded a 300 nM SF-1 inhibitor. Still better results were obtained by replacing the potentially metabolically sensitive anilide unit of **2** with other aniline amides, such as the *p*-methylphenyl (**30**), *p*-methoxyphenyl (**31**), *p*-trifluoromethoxyphenyl (**32**), and *p*-ethoxyethyl **(33)** amides. These analogs displayed SF-1 inhibitor activity in the 100–200 nM range, a 3- to 6-fold improvement over **2**.

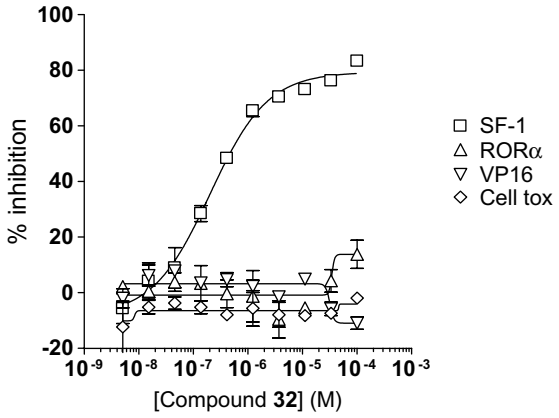


Figure 2. Titration curves for **32** versus SF-1 (□), RORα (Δ), VP16 (∇), and the cytotoxicity assays (◊). Data represent means of three independent experiments with the calculated standard deviation.

All substitutions of the R′ unit spanning the isoquinolinone and amide units led to substantial or complete loss of SF-1 activity (analogs **40**–**43**), as did moving the alkoxy substituent from the meta position in **1**–**2** to the para position in **22**. However, clear evidence of a productive SAR for the isoquinolinone alkoxy substituent has been demonstrated by analogs **14** and **36**–**39**. The stereochemistry of the ether substituent in **1** and **2** does not appear to be critical as **38**, with a $-C(Me_2)CO_2Et$ ether at this position, is more potent than **2**. In addition, an analog of **2** with a $-CH_2CO_2Et$ aryl ether (**39**, not shown), was deemed to be 'inactive' in the original HTS campaign.[3] These data suggest that hydrophobicity at this position is important for SF-1 inhibitor activity.

All analogs were further assessed in another transactivation assay (Gal4-VP16)[17] to confirm that any SF-1 activity measured was specific to that receptor and not due to transactivation assay artifacts (e.g., promiscuous inhibition of the reporter system); cellular cytotoxicity was also determined (CellTiterGlo assay[18]; see Table 1).[3] The titration curves for compound **32** are shown in Figure 2. This compound showed significant activity only in the SF-1 assay; no activity in the RORα, Gal4-VP16 and cytotoxicity assays were detected at concentrations up to 99 μM.

In summary, we have developed three routes for the synthesis of substituted isoquinolinones that has enabled us to explore the SAR of isoquinolinone inhibitors of the orphan nuclear receptor steroidogenic factor-1 (SF-1). Of those analogs described herein, **31** and **32** have improved potency and selectivity versus other nuclear receptor targets compared to the HTS leads **1** and **2**.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.03.027.

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