Computational prediction and *in vitro* analysis of potential physiological ligands of the bile acid binding site in cytochrome *c* oxidase

Leann Buhrow, Carrie Hiser, Jeffrey R. Van Voorst, Shelagh Ferguson-Miller, and Leslie Kuhn
Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, Michigan

Supplemental Tables

Supplemental Table 1. *ROCS* candidates most similar to deoxycholate. *ROCS*-analyzed ligands scoring at least three standard deviations above the average TanimotoCombo score across Binding MOAD were selected as being significantly similar to deoxycholate. These ligands were grouped into dominant chemical classes with redundant ligands removed. (A) Bile acids including cholic acid derivatives. (B) Steroids excluding bile acids. (C) Retinoic acid derivatives. (D) Amino acid analogs. (E) Nucleotide and flavin analogs. (F) Thyroid hormone derivatives.

Supplemental Table 1A. *ROCS* highly ranked bile acids

Rank	Ligand ID	Conformer	Ligand name	Tanimoto Combo	Shape Tanimoto	Color Tanimoto
1	DXC	1	Deoxycholic acid	1.51	0.84	0.66
2	CHD	1	Cholic acid	1.49	0.92	0.57
3	CHC	8	Chenodeoxycholic acid	1.25	0.86	0.4
4	GCH	111	Glycocholic acid	1.24	0.82	0.42
6	TCH	308	Taurocholic acid	1.19	0.79	0.4
7	CHO	104	Glycochenodeoxycholic acid	1.14	0.81	0.34
8	TUD	302	Taurochenodeoxycholic acid	1.09	0.77	0.32
25	12H	1296	12-Hydroxydodecanoic acid	0.95	0.54	0.41
27	NPC	1478	4-Hydroxy-3-nitrophenylacetyl-ε-aminocaproic acid anion	0.95	0.67	0.28
52	T24	1015	(9z,11e,13s,15z)-13-Hydroxyoctadeca-9,11,15-trienoic acid	0.89	0.54	0.35

Supplemental Table 1B. *ROCS* highly ranked steroids

Rank	Ligand ID	Conformer	Ligand name	Tanimoto Combo	Shape Tanimoto	Color Tanimoto
5	TH2	115	Testosterone hemisuccinate	1.21	0.83	0.38
9	CI2	1	5β-Dihydroprogesterone	1.09	0.8	0.29
10	ANO	1	5β-Androstane-3,17-dione	1.07	0.78	0.29
11	HE7	67	Estradiol-17β-hemisuccinate	1.02	0.71	0.31
12	HCY	3	Hydrocortisone	1.01	0.81	0.2
14	C0R	4	Corticosterone	1	0.78	0.22
15	ANB	1	Androsta-1,4-diene-3,17-dione	0.99	0.72	0.27
16	ASD	1	4-Androstene-3-17-dione	0.99	0.72	0.27
17	STR	1	Progesterone	0.99	0.72	0.27
18	CLR	393	Cholesterol	0.98	0.71	0.27
19	ERG	18	Ergosterol	0.98	0.72	0.26
20	AS4	4	Aldosterone	0.97	0.76	0.21
21	HC3	12	25-Hydroxycholesterol	0.96	0.71	0.26
22	1CA	3	Desoxycorticosterone	0.96	0.73	0.23
23	HCR	16	7-Hydroxycholesterol	0.96	0.71	0.24
24	TES	1	Testosterone	0.96	0.71	0.25
28	FFA	1	Epitestosterone	0.94	0.61	0.33
29	SNL	3	Spironolactone	0.94	0.68	0.26
30	LAN	14	Lanosterol	0.94	0.69	0.25
32	R18	1	Metribolone	0.93	0.67	0.26
35	FUA	7	Fuscidic acid	0.93	0.61	0.32
36	HC2	4	20-Hydroxycholesterol	0.92	0.74	0.19
37	DHT	1	5-α-dihydrotestosterone	0.92	0.68	0.24
38	DEX	2	Dexamethasone	0.92	0.73	0.19
40	PLO	1	Pregnenolone	0.91	0.69	0.23
43	PDN	1	Cortisone	0.9	0.7	0.21
44	PO1	93	(9β,13ρα,14β,17α)-2-methoxyestra-1,3,5(10)-triene-3,17-diyl disulfamate	0.9	0.66	0.24
45	EST	1	Estradiol	0.9	0.7	0.21
50	EQU	1	Equilenin	0.9	0.69	0.21
60	17H	1	Tetrahydrogestrinone	0.89	0.66	0.23
51	667	46	6-Oxo-8,9,10,11-tetrahydro-7h-cyclohepta[c][1]benzopyran-3-O-sulfamate	0.89	0.68	0.22

Supplemental Table 1C. *ROCS* highly ranked retinoic acid analogs

Rank	Ligand ID	Conformer	Ligand name	Tanimoto Combo	Shape Tanimoto	Color Tanimoto
13	RE9	44	Synthetic retinoic acid	1.01	0.65	0.36
26	564	107	6-(5,5,8,8-tetramethyl-5,6, 7,8 tetrahydro-naphthalene-2-carbonyl)-naphthalene-2 carboxylic acid	0.95	0.64	0.31
34	184	11	6-[hydroxy-(5,5,8,8-tetra-methyl-5,6,7,8-tetrahydro-naphthalen-2-yl)-methyl]-naphthalene-2-carboxylic acid	0.93	0.61	0.32
39	156	36	4-[3-oxo-3-(5,5,8,8-tetra-methyl-5,6,7,8-tetrahydro-naphthalen-2-yl)-propenyl] -benzoic acid	0.92	0.61	0.31
58	R13	31	3-Methyl-7-(5,5,8,8-tetramethyl-5,6,7,8-tetra hydro-naphthalen-2-yl) -octa-2,4,6-trienoic acid	0.89	0.63	0.26

Supplemental Table 1D. *ROCS* highly ranked amino acid analogs

Rank	Ligand ID	Conformer	Ligand name	Tanimoto Combo	Shape Tanimoto	Color Tanimoto
31	BP5	15	3-(2,2'-Bipyridin-5-yl)-L-alanine	0.93	0.65	0.28
33	CCL	785	N-6-[(Cyclopentyloxy) carbonyl]-D-lysine	0.93	0.68	0.25
42	NHL	166	(4s)-4-(2-Naphthyl methyl)-D-glutamic acid	0.91	0.63	0.28
46	PDE	795	Para-Nitrophenyl phospho-nobutanoyl D-alanine	0.9	0.7	0.2
48	MP2	702	N-[(benzyloxy)carbonyl]-L-cysteinylglycine	0.9	0.65	0.25
53	DNS	554	N-6-{[5-(Dimethylamino)-1-naphthyl]sulfonyl}-L-lysine	0.89	0.64	0.25
55	F6F	272	2-{[4-(Trifluoromethoxy) benzoyl]amino} ethyl dihydrogen phosphate	0.89	0.68	0.21
56	HEP	510	Phenyl[1-(Nsuccinyl-amino)pentyl]phosphonate	0.89	0.62	0.27

Supplemental Table 1E. *ROCS* highly ranked nucleotide and flavin analogs

Rank	Ligand ID	Conformer	Ligand name	Tanimoto Combo	Shape Tanimoto	Color Tanimoto
41	P1S	4	(6ar,12ar)-3-(Hydroxy-methyl)-6h [1,3]dioxolo [5,6][1]benzofuro[3,2-C]chromen-6a(12ah)-Ol	0.91	0.7	0.21
47	EMA	1215	(Adenin-9-yl-ethoxy methyl)-hydroxyphos phinyl-diphosphate	0.9	0.66	0.24
49	RMB	116	N1-(5'-Phospho- α-ribosyl)-5-methylbenzimidazole	0.9	0.7	0.2
54	GCQ	548	Gemcitabine diphosphate	0.89	0.67	0.22
57	RPD	128	(C8-R)-hydantocidin 5'-phosphate	0.89	0.66	0.22
59	IMU	144	Phosphoric acid mono-[5-(2-amino-4-oxo-4,5-dihydro-3h-pyrrolo[3,2-D]pyrimidin-7-yl)-3,4-dihydroxypyrrolidin-2-ylmethyl] ester	0.89	0.63	0.25

Supplemental Table 1F. *ROCS* highly ranked thyroid hormone analogs

Rank	Ligand ID	Conformer	Ligand name	Tanimoto Combo	Shape Tanimoto	Color Tanimoto
61	IH5	1	{3,5-Dichloro-4-[4-hydroxy-3- (propan-2-yl) phenoxy]phenyl}acetic acid	0.89	0.68	0.2
63	T3	21	T3 thyroid hormone	0.85	0.6	0.25

Supplemental Table 2. *ROCS* nucleotide candidates most similar to deoxycholate. Nucleotide ligands have been suggested to be physiological regulators of C*c*O via their interactions at the bile acid site [Tsukihara, T., Aoyama, H., Yamashita, E., Tomizaki, T., Yamaguchi, H., Shinzawa-Itoh, K., Nakashima, R., Yaono, R., and Yoshikawa, S. (1996) The whole structure of the 13-subunit oxidized cytochrome *c* oxidase at 2.8 angstrom. *Science* 272, 1136-1144.]. For this reason, a broader set of nucleotides meeting a less stringent scoring threshold (two standard deviations above the mean) is included, relative to the data in Supplemental Table 1E.

Supplemental Table 2. *ROCS* highly ranked flavins, nicotinamides, and nucleotides

Ligand ID	Conformer	Ligand name	Tanimoto Combo	Shape Tanimoto	Color Tanimoto
TMP	131	Thymidine-5'-phosphate	0.85	0.65	0.2
FAD	175	Flavin-adenine dinucleotide	0.85	0.68	0.17
NAI	53	1,4-Dihydronicotinamide adenine dinucleotide	0.83	0.64	0.2
IDP	892	Inosine-5'-diphosphate	0.82	0.66	0.17
GDP	359	Guanosine-5'-diphosphate	0.82	0.57	0.25
TTP	1	Thymidine-5'-triphosphate	0.82	0.61	0.21
UMP	133	2'-Deoxyuridine 5'-monophosphate	0.82	0.61	0.2
ATP	2943	Adenosine-5'-triphosphate	0.81	0.59	0.22
CDP	162	Cytidine-5'-diphosphate	0.81	0.62	0.19
CMP	11	Adenosine-3',5'-cyclicmonophosphate	0.81	0.67	0.13
UDP	112	Uridine-5'-diphosphate	0.79	0.61	0.18
GMP	10	Guanosine	0.79	0.61	0.18
UTP	1494	Uridine 5'-triphosphate	0.79	0.62	0.17
ADP	130	Adenosine-5'-diphosphate	0.78	0.58	0.21
GTP	109	Guanosine-5'-triphosphate	0.78	0.51	0.27

Supplemental Table 3. *SimSite3D* high-ranking protein site matches. *SimSite3D* ligand binding sites scoring at least two standard deviations below the average *SimSite3D* score for Binding MOAD sites were selected as similar. These binding sites were grouped into dominant chemical classes and redundant protein binding sites were removed.

Supplemental Table 3A. *SimSite3D* high-ranking flavin site matches

Rank	PDB	Protein	Ligand Code	*SimSite3D* Score
12	1FFU	Carbon monoxide dehydrogenase	FAD	-2.81
18	2R9Z	Glutathione amide reductase	FAD	-2.72
22	1QO8	Flavocytochrome *c*3 fumarate reductase	FAD	-2.63
26	1E1K	Adrenodoxin reductase	FAD	-2.61
33	1FEA	*Crithidia fasciculata* trypanothione reductase	FAD	-2.56
36	1PBD	P-Hydroxybenzoate hydroxylase	FAD	-2.54
37	1VDV	Bovine milk xanthine dehydrogenase Y-700	FAD	-2.54
49	1XAN	Human glutathione reductase	FAD	-2.46
54	1QX4	S127P mutant of cytochrome *b*5 reductase	FAD	-2.41
82	1JEH	Yeast e3 lipoamide dehydrogenase	FAD	-2.31
92	1DOE	4-OH Benzoate hydroxylase	FAD	-2.29
94	2NVK	Thioredoxin reductase	FAD	-2.28
100	1XKK	Epidermal growth factor receptor	FMM	-2.27
107	2FJA	Adenosine 5'-phosphosulfate reductase	FAD	-2.24
125	1CC2	Cholesterol oxidase	FAD	-2.21
138	3CGD	Bacillus anthracis coenzyme a-disulfide reductase	FAD	-2.18
179	1DS7	FMN-dependent nitroreductase	FMN	-2.08
190	3MDD	Acyl-coA dehydrogenase	FAD	-2.06
195	1AN9	D-amino acid oxidase	FAD	-2.05
202	2FJD	Adenosine-5-phosphosulfate reductase	SFD	-2.04
207	2J07	Thermus DNA photolyase	FAD	-2.03
214	2Q0K	Oxidized thioredoxin reductase	FAD	-2.02
226	2VNK	Ferredoxin-NADP(H) reductase	FAD	-2.00
231	2A8X	Lipoamide dehydrogenase	FAD	-2.00

Supplemental Table 3B. *SimSite3D* high-ranking lipidic site matches

Rank	PDB	Protein	Ligand Code	*SimSite3D* Score
5	2DYS	Bovine heart cytochrome *c* oxidase	TGL	-2.99
27	1JCS	Rat protein farnesyltransferase	FII	-2.61
43	1S63	Human protein farnesyltransferase	FPP	-2.50
51	3DST	Rab GGTase	GRG	-2.45
55	2E8V	*S. cerevisiae* geranylgeranyl pyrophosphate synthase	GRG	-2.41
63	2E2X	Sec14 homology module of neurofibromin	PEV	-2.37
75	1Q16	Nitrate reductase a	AGA	-2.34
77	1ND2	Rhinovirus 16	MYR	-2.33
103	1N4Q	Protein geranylgeranyltransferase type-I	MGM	-2.26
106	1YOK	NR5 orphan receptors sf-1 and lrh-1	P6L	-2.25
112	2R40	Ecdysone receptor	EPH	-2.24
135	1O5M	Tricyclic farnesyl protein transferase	FPP	-2.18
136	1FK7	Maize lipid-transfer protein complexes	RCL	-2.18
148	1QBQ	Rat farnesyl protein transferase	HFP	-2.16
151	3BDQ	Sterol carrier protein-2 like-2	PLM	-2.15
168	1PPJ	Bovine cytochrome *bc*1 complex	PEE	-2.11
178	1FK4	Maize lipid-transfer protein complexes	STE	-2.09
181	2E3R	Cert start domain	18C	-2.08
185	1FK2	Maize lipid-transfer protein complexes	MYR	-2.07
189	1LN1	Human phosphatidylcholine transfer protein	DLP	-2.06
215	3DSU	Rab geranylgeranyl transferase	FPP	-2.02

Supplemental Table 3C. *SimSite3D* high-ranking nicotinamide site matches

Rank	PDB	Protein	Ligand Code	*SimSite3D* Score
1	1NNU	Enoyl-acyl-carrier-protein reductase	NAD	-3.14
2	1KY8	Non-phosphorylating glyceraldehyde-3-phosphate dehydrogenase	NAP	-3.13
4	1IY8	Levodione reductase	NAD	-3.02
6	2JL1	Citrobacter sp. triphenylmethane reductase	NAP	-2.99
7	1E6W	3-Hydroxyacyl-coA dehydrogenase	NAD	-2.98
8	1AHI	7 α-Hydroxysteroid dehydrogenase	NAD	-2.93
10	1VHD	Iron containing alcohol dehydrogenase	NAP	-2.85
13	1D8A	*E. coli* enoyl reductase	NAD	-2.79
14	2ID2	CoA-independent ALDH	NAP	-2.76
16	1H5Q	Mannitol dehydrogenase	NAP	-2.74
19	1M8F	*M. thermoautotrophicum* nicotinamide mononucleotide adenylyltransferase R11A	NAD	-2.72
20	1V35	Eoyl-ACP reductase	NAI	-2.71
21	1PS9	*E. coli* 2,4-dienoyl CoA reductase	NAP	-2.69
24	2IMP	Lactaldehyde dehydrogenase	NAI	-2.62
25	1G0O	Trihydroxynaphthalene reductase	NDP	-2.61
29	1X1T	d-3-Hydroxybutyrate dehydrogenase	NAD	-2.59
38	2BD0	*Chlorobium tepidum* sepiapterin reductase	NAP	-2.53
42	1QRR	SQD1 protein	NAD	-2.50
45	1T90	Methylmalonate semialdehyde dehydrogenase	NAD	-2.48
47	1GZ6	(3R)-Hydroxyacyl-coA dehydrogenase fragment	NAI	-2.48
61	1IB0	NADH-dependent cytochrome b5 reductase	NAD	-2.38
65	1XHL	Tropinone reductase-II	NDP	-2.36
66	1YVE	Acetohydroxy acid isomeroreductase	NDP	-2.36
67	1CD2	*Pneumocystis carinii* dihydrofolate reductase	NAP	-2.36
69	1J3I	*Plasmodium falciparum* dihydrofolate reductase-thymidylate synthase	NDP	-2.36
70	1OJZ	C3stau2	NAD	-2.36

Supplemental Table 3C Continued. *SimSite3D* high-ranking nicotinamide site matches

Rank	PDB	Protein	Ligand Code	*SimSite3D* Score
73	1A71	Horse liver alcohol dehydrogenase	NAD	-2.35
88	1EK6	Human UDP-galactose 4-epimerase	NAI	-2.30
95	1KVQ	UDP-galactose 4-epimerase	NAD	-2.28
97	1HE2	Human biliverdin IX β reductase	NAP	-2.28
98	1GJR	Ferredoxin-NADP+ reductase	NAP	-2.28
111	2GZ1	Aspartate semialdehyde dehydrogenase	NAP	-2.24
127	1B15	Alcohol dehydrogenase	NAE	-2.21
128	1B2L	Alcohol dehydrogenase	NDC	-2.21
130	2JD1	1-Deoxy-D-xylulose 5-phosphate reductoisomerase	NDP	-2.20
133	1Y7T	NAD(H)-depenent malate dehydrogenase	NDP	-2.19
140	1WNB	*E. coli* YDCW aldehyde dehydrogenase	NAD	-2.17
143	1JAY	Coenzyme F420H2:NADP+ oxidoreductase	NAP	-2.16
144	1RKX	CDP-D-Glucose 4,6-dehydratase	NAD	-2.16
157	2DTE	*T. acidophilum* aldohexose dehydrogenase	NAI	-2.13
159	1UXT	Non-phosphorylating glyceraldehyde-3-phosphate dehydrogenase (GAPN)	NAD	-2.13
161	1IE3	R153C *E. coli* malate dehydrogenase	NAD	-2.12
169	2FZI	DHFR complexes	NAP	-2.11
171	1RPN	GDP-D-Mannose 4,6-dehydratase	NDP	-2.10
174	3CE6	*M. tuberculosis* S-adenosyl-L-homocysteine hydrolase	NAD	-2.10
192	1E7W	Leishmania pteridine reductase	NDP	-2.06
197	1DIG	Human methylenetetrahydrofolate dehydrogenase / cyclohydrolase	NAP	-2.05
200	1DYR	*P. carinii* dihydrofolate reductase	NDP	-2.05
206	2DFV	Hyperthermophilic threonine dehydrogenase	NAD	-2.03
212	1B16	Alcohol dehydrogenase	NAQ	-2.02
217	2DT5	TTHA1657 (AT-rich DNA-binding protein)	NAD	-2.02

Supplemental Table 3D. *SimSite3D* high-ranking nucleotide site matches

Rank	PDB	Protein	Ligand Code	*SimSite3D* Score
9	2PTR	*E. coli* adenylosuccinate lyase mutant H171A	2SA	-2.88
11	2GQR	*E. coli* phosphoribosylaminoimidazole succinocarboxamide synthetase	ADP	-2.84
28	2A14	Human indolethylamine N-methyltransferase	SAH	-2.60
31	1ATP	CAMP-Dependent protein kinase	ATP	-2.57
39	1S7N	Ribosomal L7/L12 α-N-protein acetyltransferase	COA	-2.52
44	3ELW	Wesselsbron virus methyltransferase	SAM	-2.49
53	2HK9	Shikimate dehydrogenase	ATR	-2.41
76	2H7C	Human carboxylesterase	COA	-2.34
86	2PX5	Murray Valley Encephalitis virus NS5 2'-O methyltransferase domain	SAH	-2.30
90	3TMK	Yeast thymidylate kinase	T5A	-2.29
91	1QZ5	Rabbit actin	ATP	-2.29
99	2Q4V	Thialysine n-acetyltransferase (SSAT2)	ACO	-2.27
101	1Q99	*S. cerevisiae* SR protein kinase	ANP	-2.27
102	2FF3	WH2/β-Thymosin motif-containing proteins	ATP	-2.26
105	1L3R	Transition state mimic of the catalytic subunit of cAMP-dependent protein kinase	ADP	-2.26
109	1O6K	PKB kinase domain S474D	ANP	-2.24
129	1V0P	*P. falciparum* protein kinase	PVB	-2.21
137	3MCT	Vaccinia methyltransferase VP39	SAH	-2.18
147	2JI8	Oxalyl-coA decarboxylase	ADP	-2.16
150	2BZG	Thiopurine S-methyltransferase	SAH	-2.15
176	1AV5	Protein kinase C interacting protein (PKCI)	AP2	-2.09
183	1DR2	Chicken liver dihydrofolate reductase	TAP	-2.08
185	1DTP	Catalytic domain of diphtheria toxin	APU	-2.08

Supplemental Table 3D Continued. *SimSite3D* high-ranking nucleotide site matches

Rank	PDB	Protein	Ligand Code	*SimSite3D* Score
185	1DTP	Catalytic domain of diphtheria toxin	APU	-2.08
187	2VBQ	Aminoglycoside N-acetyltransferases	BSJ	-2.07
192	2CGJ	L-Rhamnulose kinase	ADP	-2.06
197	2A94	*P. falciparum* lactate dehydrogenase	AP0	-2.05
204	1NW5	β Class N6-adenine DNA methyltransferase	SAM	-2.04
210	1E8X	Phosphoinositide 3-kinase	ATP	-2.02
224	1M2G	Sir2 homologue	APR	-2.01
78	1S4O	Yeast α 1,2-mannosyltransferase Kre2p/Mnt1p	GDP	-2.33
96	1R27	Catalytic and electron-transfer subunits (NarGH) of the integral membrane protein, respiratory nitrate reductase (Nar)	MGD	-2.28
104	1RDS	Ribonuclease MS	GPC	-2.26
122	2G9X	Thr 160 phosphorylated CDK2/cyclin A	NU5	-2.22
153	1GUA	Human RAP1A	GNP	-2.14
209	2OM2	Human G[α]i1	GDP	-2.03
214	3DAG	[Fe]-Hydrogenase holoenzyme (HMD)	FEG	-2.02
217	1H1S	Human THR160-phospho CDK2/cyclin A	4SP	-2.02
57	2BZN	Guanosine monophosphate reductase 2	IMP	-2.40
62	1G93	α-1,3-Galactosyltransferase	UPG	-2.38
72	1UQT	Trehalose-6-phosphate	U2F	-2.35
143	1I2B	Mutant T145A SQD1 protein complex	UPG	-2.16
189	2NOM	Terminal uridylyl transferase 4	DUT	-2.06
219	2IV7	WAAG Glycosyltransferase	UDP	-2.01

Supplemental Table 3E. *SimSite3D* high-ranking steroid site matches

Rank	PDB	Protein	Ligand Code	*SimSite3D* Score
202	2AA2	Mineralocorticoid Receptor	AS4	-2.12
210	1ZHY	Yeast oxysterol binding protein Osh4	CLR	-2.11
223	2A3I	Mineralocorticoid Receptor	C0R	-2.09
268	2Q1H	Corticoid Receptor	AS4	-2.02
286	1F3B	Murine class alpha glutathione S-transferase	GBX	-2.01

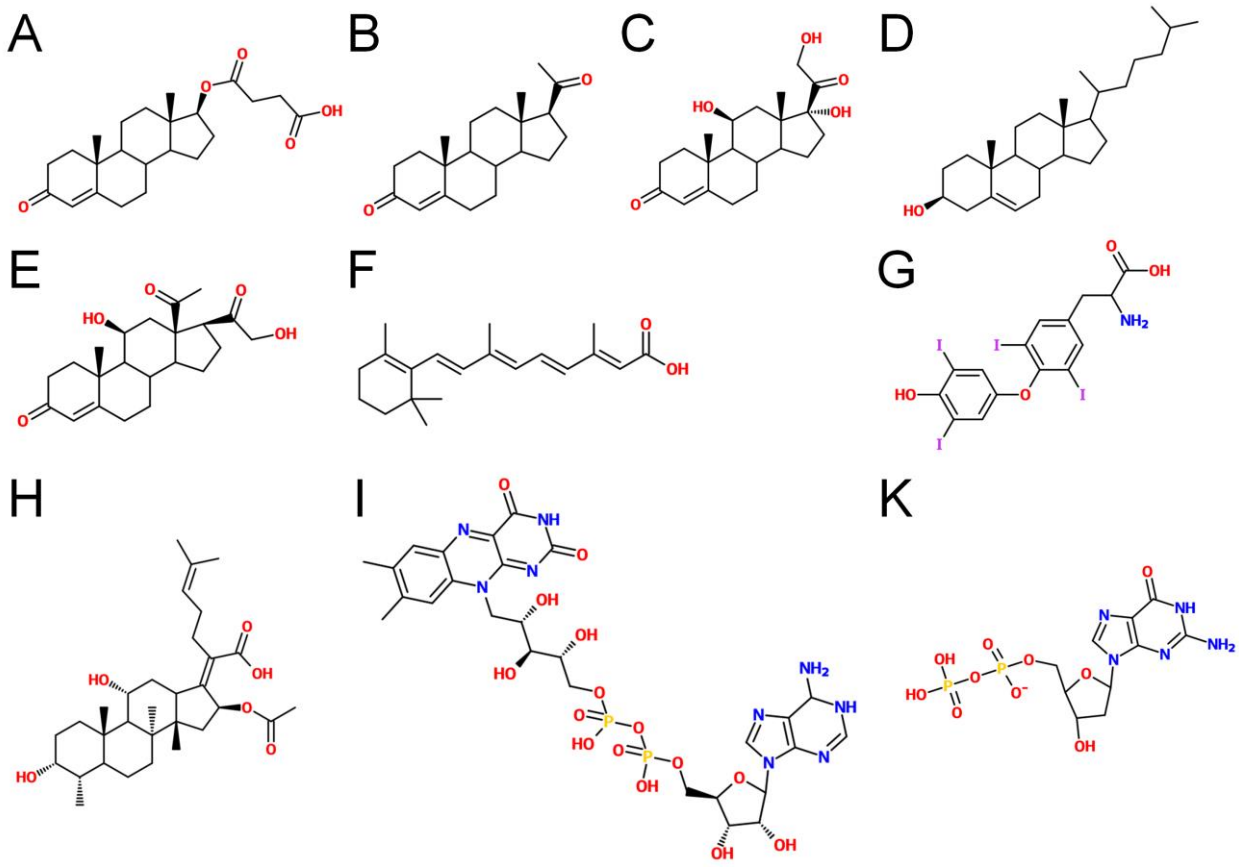
Supplemental Table 4. Kinetic values of *Rs*C*c*O inhibition. Kinetic inhibition values were determined by Graphpad Prism version 6 mixed inhibition least squares fitting in the presence of each candidate ligand. R^2 describes how well mixed inhibition least squares regression represents the data, with 1.00 representing a perfect fit.

Supplemental Table 4. Kinetic values of *Rs*C*c*O inhibition.

Ligand	Vmax (e^-/sec/aa_3)	K_m cytochrome *c* (μM)	K_i ligand	R^2
ADP	1386	2.1	3.1 mM	0.98
ATP analog	1351	1.9	1.5 mM	0.98
GDP	1364	2.0	1.7 mM	0.98
Fusidic acid	1350	1.9	2.5 mM	0.98
CHS	1351	1.9	290 μM	0.98
Retinoic acid	1282	1.8	140 μM	0.93
T3	1351	1.9	30 μM	0.98

Supplemental Figures

Supplemental Figure 1. 2D structures of top-scoring *ROCS* results. The comparison of the *Rs*C*c*O bound deoxycholate and Binding MOAD suggested steroidal ligands were significantly similar in shape and chemistry. The top ranked steroids which were purchasable and could be solubilized at high concentration included (A) testosterone hemisuccinate, (B) progesterone, (C) hydrocortisone, (D) cholesterol, and (E) aldosterone. Other highly similar ligands included (F) retinoic acid, (G) thyroid hormone, and (H) fusidic acid. *ROCS* also highly ranked nucleotides and flavins as matches for deoxycholate. The most similar examples of these ligands to the crystallographic conformation of deoxycholate, as determined by *ROCS*, are (I) FAD and (K) GDP. Ligand structures were depicted using eMolecules (Solano Beach, CA) molecular structure search tool.



Supplemental Figure 2. Hydrophobic steroids do not affect the activity of *Rs*C*c*O. Candidate ligands from *ROCS* ligand comparisons and *SimSite3D* protein binding site comparisons include steroids. However, these steroids failed to affect the protein activity as compared to their solvent, ethanol, alone.