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Modulation of PPAR subtype selectivity. Part 2: Transforming PPARα/γ dual agonist into α selective PPAR agonist through bioisosteric modification ☆

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ABSTRACT

A novel series of oxime containing benzyl-1,3-dioxane-r-2-carboxylic acid derivatives (**6a–k**) were designed as selective PPARα agonists, through bioisosteric modification in the lipophilic tail region of PPARα/γ dual agonist. Some of the test compounds (**6a**, **6b**, **6c** and **6f**) showed high selectivity towards PPARα over PPARγ in vitro. Further, highly potent and selective PPARα agonist **6c** exhibited significant antihyperglycemic and antihyperlipidemic activity in vivo, along with its improved pharmacokinetic profile. Favorable in-silico interaction of **6c** with PPARα binding pocket correlate its in vitro selectivity profile toward PPARα over PPARγ. Together, these results confirm discovery of novel series of oxime based selective PPARα agonists for the safe and effective treatment of various metabolic disorders.

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors belonging to the nuclear hormone receptor superfamily. The PPAR subfamily consists of three isotypes: PPARα, PPARδ, and PPARγ. Physiologically, PPARs accounts for lipid and glucose homeostasis and thereby, it contributes significantly in metabolic disorders such as obesity, diabetes and cardiovascular complications. The PPARα is localized in heart, liver and muscles, where it plays an essential role in lipid metabolism by regulating cellular free fatty acid oxidation and cholesterol trafficking. Thus, activation of PPARα decreases serum triglycerides (TG) and increases serum high-density lipoprotein cholesterol (HDL-C), which together restore lipid and glucose homeostasis.[1]

Fibrates represent an important class of PPARα ligands, but usually administered at high doses to get the clinical benefits, mainly because of their relatively weak affinity for PPARα and poor subtype selectivity.[2] The PPARγ is highly distributed in skeletal muscle, liver and adipose tissue. Activation of PPARγ improves glycemic control by increasing insulin sensitivity, via activation of genes involved in the control of glucose production, transportation and utilization.[3] Pioglitazone and Rosiglitazone are selective PPARγ agonists, which are in practice since a decade for the treatment of diabetes. However side effects such as heart failure, fluid retention, and weight gain in patients treated with PPARγ selective agonists warrant development of newer drug with better efficacy and safety profile.[4]

In contrast to a selective PPARγ agonist, PPARα/γ dual agonist exhibit insulin sensitizing potential of PPARγ agonist along with beneficial lipid modulating activities of the PPARα agonist. Thus it was thought that PPARα/γ dual or PPARα/γ/δ pan agonist would simultaneously correct insulin resistance and lipid imbalance, thereby overcome the side effects of selective PPARγ agonist and might work as monotherapy for Type-2 diabetes treatment.[5] On the basis of this hypothesis, in past, several PPARα/γ dual agonists[6] and PPARα/γ/δ pan agonists[7] were developed and evaluated. However some of the developments were terminated because of concerns over cardiovascular safety in clinical trials and propensity to cause cancer in rodents.[8] The unsuccessful efforts to develop dual/pan agonists and the recent findings that the selective activation of PPARα lower triglycerides, elevate HDL, and exert insulin sensitizing effects[9] led to the discovery of several potent and selective PPARα agonist as a promising therapeutic option for the treatment of various metabolic disorders. In view of these opportunities, identification of structural components controlling the selectivity for PPAR isoforms would be advantageous in designing the next generation of PPARα agonist. Although some research groups have already

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disclosed potent and selective PPARα agonists,[10] these compounds are still under clinical evaluation.[11] Recently a potent and selective PPARα agonist **NS-220**[11] reported to exert antihyperglycemic and lipid modulating effects in animal models but its further development is discontinued for unknown reasons. Another compound **K111**[12], a relatively weak PPARα agonist is presently undergoing clinical trials for the treatment of Type-2 diabetes (Fig. 1).

As a part of our on-going research in the field of PPARs to develop novel therapeutic agents for the treatment of metabolic disorders,[13] earlier, several PPAR agonists were reported, which were developed by chemical modifications, either in the lipophilic tail part and/or the central spacer region (linker) to modulate agonistic activity and subtypes selectivity.[14,15,16] In the present communication our objective was to develop oxime containing benzyl 1,3-dioxane-r-2-carboxylic acid derivatives as highly potent and selective PPARα agonists designed by bioisosteric replacement of rigid oxazole ring of our previously reported PPARα/γ dual agonist (**I**)[13c] with a flexible lipophilic tail (Fig. 2). The in vitro PPAR agonistic activity and subtypes selectivity of all the test compounds (**6a–k**) was assessed using in vitro hPPAR transactivation assay. Furthermore, highly potent and selective test compound (**6c**) was subjected for in vivo study to assess its antihyperlipidemic and antihyperglycemic effects along with its pharmacokinetic study.

Synthesis of title compounds **6a–k** was carried out as described in scheme 1, following modified literature procedure[13c] and all the starting materials, procured from commercial source. Various substituted oximes (**2a–k**) were synthesized by reacting the corresponding acetophenone (**1a–k**) with hydroxylamine hydrochloride. The geometrical structure of all the oximes (**2a–k**) prepared were considered to be (*E*)-isomer because the literature precedent reveals that the (*E*)-isomers are thermodynamically more stable than the corresponding (*Z*)-isomers.[17] Alkylation of oximes (**2a–k**) with 1,2-dibromoethane in presence of cesium carbonate (Cs_2CO_3) in dimethylformamide (DMF) gave the intermediate (**3a–k**) in good yield. Finally coupling of (**3a–k**) with **4**[13c] in presence of potassium carbonate (K_2CO_3) in DMF gave the esters (**5a–k**) which on hydrolysis under basic condition yielded title compounds **6a–k**. The stereochemistry of compound **6** is established as *cis* isomers, based on ^{1}H NMR data, wherein the chemical shifts data of compound **6** was found to be in accordance with the similar compounds reported earlier by us as well as other groups.[13c,18] The structure of all the title compounds and intermediates was confirmed by their physical, analytical and spectral data and the overall percentage yields were found to be in the range of 50–70%.[19]

In order to assess the potency and subtypes selectivity, all the test compounds synthesized (**6a–k**) were screened in vitro for hPPAR α, γ, and δ agonistic activity (PPAR transfected in HepG2 cells) according to the procedure described in our earlier publication.[13c] The **NS-220** (PPARα), **GW-501516** (PPARδ), **Rosiglitazone** (PPARγ), and compound **I** (PPARα/γ) were used as positive controls (standards) and the in vitro activity results (fold inductions vs vehicle control (DMSO; 1% solution)) are summarized in Table 1. As described earlier, our goal was to develop potent and selective PPARα agonist. In this attempt, we intended to replace the oxazole ring of dual agonist (compound **I**) with phenyl oxime group (Fig. 2) and the initial compound **6a** was found to be selective towards PPARα. Based upon our past experience, we envisioned that

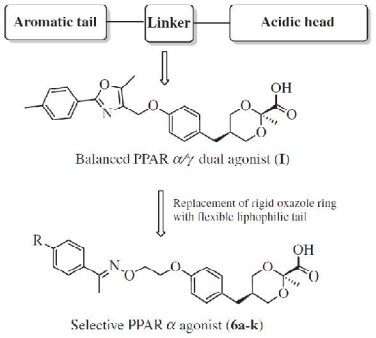


Figure 2. Identification of novel lipophilic part for selective PPARα agonistic activity.

substitution at *para* position of phenyl ring (**6a**) may play important role in the modulation of potency and selectivity of the test compounds. In previous communication we reported electron donating groups improve the PPARα selectivity over PPARγ.[15] Thus, substitution at the *para* position of compound **6a** with electron donating groups showed very good PPARα selectivity over PPARγ, as compounds **6b**, **6c**, and **6f** possessing methyl, methoxy and fluoro groups respectively exhibited excellent potency and selectivity towards PPARα subtype. These findings encouraged us to study the effect of substituents on both 3- and 4-position of the phenyl ring. In this regard, we chose methoxy group on both the positions and synthesized compound **6d**, but compound failed to retain its selectivity as well as potency. Substitution at *para* position with electron withdrawing groups exhibited detrimental effects in vitro, which is evident from the activity of **6e, 6g**, and **6h** possessing methane sulfonyl, trifluoromethyl and trifluoromethoxy groups respectively. Surprisingly compound **6h** showed PPARγ selectivity over PPARα. Furthermore, when the flexible substitution at *para* position was replaced with rigid bi-cyclic ring system (tetrahydronaphthyl (**6i**) and naphthyl (**6j**)), it resulted in a weak agonistic activity and poor affinity towards all subtypes tested. Similarly, compound **6k** prepared by replacing phenyl ring with 3-pyridyl ring showed weak agonistic activity as compared to **6a**. Together, these in vitro agonistic activity results across all the three PPAR subtypes clearly confirmed our hypothesis that the phenyl oxime group act as a bioisostere of oxazole ring system. Furthermore, structure–activity relationship (SAR) drawn from the in vitro study results indicated that electron donating groups at *para* position of phenyl ring is essential for PPARα selectivity.

The in vivo antihyperlipidemic and antihyperglycemic activities of lead compound **6c** (a highly potent and PPARα selective in vitro) was evaluated in *db/db* mice. In this model, mice were dosed orally with either compound **6c** or standard compound (**NS-220**) at 3 and 30 mpk (mg/kg/day) respectively for 7 days and serum triglycerides (TG) and serum glucose (Glucose) were measured. As shown in Table 2, compound **6c** showed significantly reduced TG and glucose and it was found to be comparable with positive controls (**NS-220**), at 10-fold lower dose. Similarly, in high cholesterol (HC) fed

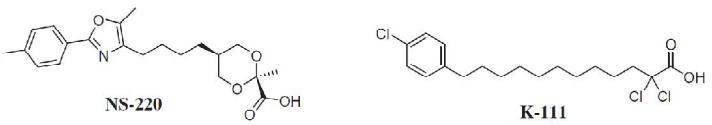
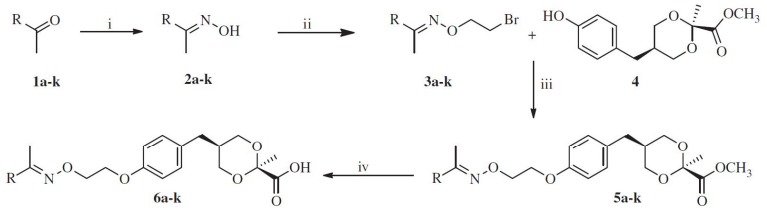
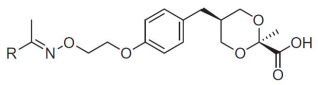


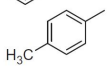
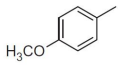
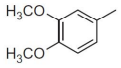
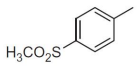
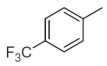
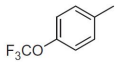
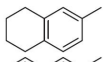
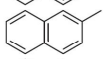
Figure 1. Selective PPARα agonist.



Scheme 1. Reagents and conditions: (i) $NH_2OH{\cdot}HCl$, NaOAc, EtOH, H_2O, reflux, 2 h, 48–98%; (ii) 1,2-Dibromoethane, Cs_2CO_3, DMF, heat, 80 °C, 20 h, 19–84%; (iii) K_2CO_3, DMF, heat, 80 °C, 20 h, 49–93%; (iv) $LiOH{\cdot}H_2O$, CH_3OH, THF, H_2O, 30 °C, 20 h, 50–70%.

Table 1
In vitro hPPAR transactivation activity of test compounds (**6a–k**)



Compd	R	hPPAR transactivation[a,b]		
		α (10 μM)	γ (0.2 μM)	δ (10 μM)
6a		10.79	1.47	1.58
6b		10.81	3.05	2.78
6c		15.04	1.44	IA
6d		3.61	1.75	1.68
6e		1.37	IA	1.23
6f		10.49	1.20	1.62
6g		5.79	2.83	1.31
6h		3.48	7.39	1.46
6i		1.63	4.36	1.25
6j		1.76	3.87	IA
6k		2.36	1.13	1.31
Vehicle		1.0	1.0	1.0
I		14.72	7.90	IA
NS-220		12.72	IA	2.52
GW-501516		ND	ND	9.24
Rosiglitazone		IA	7.22	IA

[a] HepG2 cells transfected with pSG5 vector containing cDNA of hPPARα/γ/δ and cotransfected with PPRE3-TK-luc. The Luciferase activity determined using fire-fly luciferase assay and β-galactosidase activity in ELISA reader. Activities presented as fold induction of PPARα,γ, and δ activation, wrt vehicle control (DMSO; 1%).
[b] IA denotes inactive compounds.

rat model (Table 3), compound **6c** exhibited excellent reduction in TG, total cholesterol (TC) and LDL-cholesterol (LDL-C), along with increased in the levels of HDL-cholesterol (HDL-C). As observed

Table 2
Antihyperlipidemic and antihyperglycemic activities in *db*/*db* mice[a].

Compd	Dose (mpk/day)	% Change	
		TG	Glucose
6c	3	−53	−45
NS-220	30	−54	−43

[a] Male *db*/*db* mice (6–8 weeks old) were dosed with test compounds daily for 7 days and serum triglycerides (TG) and serum glucose (Glucose) were measured. Values reported as % change of compound-treated group versus vehicle control ($n = 6$).

Table 3
In vivo antihyperlipidemic activity of compound **6c** in HC fed SD rats[a].

Compd	Dose (mpk, po/day)	% Change			
		TG	TC	LDL-C	HDL-C
6c	3	−54	−56	−68	68
NS-220	30	−51	−49	−58	62

[a] Male Sprague–Dawely (SD) rats were fed with diet containing high cholesterol (HC) for 15 days then dosed with vehicle or the indicated doses of test compounds, daily for 14 days by oral gavage (po). Serum triglycerides (TG), total cholesterol (TC), LDL-cholesterol (LDL-C) and HDL-cholesterol (HDL-C) were measured. Values represent % change versus vehicle control ($n = 9$).

Table 4
Mean pharmacokinetic parameters[a] of **6c** in fasted male *wistar* rats.

Compd	T_{max} (h)	C_{max} (ng/ml)	$T_{1/2}$ (h)	AUC (0–α) (h ng/ml)
6c	0.61 ± 0.02	119.61 ± 1.01	13.96 ± 1.91	1420.01 ± 18.31
NS-220	0.65 ± 0.10	40.32 ± 1.19	1.99 ± 0.76	99.96 ± 11.41

[a] Test compounds were administered orally at 30 mpk, in fasted male *wistar* rats ($n = 6$) and plasma concentration was analyzed by LC–MS, values indicate mean ± SD.

in *db*/*db* mice, compound **6c** showed comparable antihyperlipidemic activity in HC fed rat model at 10-fold lower dose. Further to assess the rational behind superior in vivo efficacy of compound **6c** over **NS-220**, single dose (30 mpk, po) comparative pharmacokinetic study was carried out in male *wistar* rats and the data is summarized in Table 4. The compound **6c** showed rapid T_{max}, good C_{max} and AUC along with extended half-life. Compared to **NS-220**, compound **6c** showed three-fold higher Cmax, fourteen-fold improvement in AUC and seven-fold longer half-life. Thus, improved pharmacokinetic profile of compound **6c** justifies its excellent pharmacodynamic effects in animal models at a lower dose.

The molecular docking analysis of **6c** was carried out to infer its selectivity profile and critical interactions with the active site of PPARα and PPARγ binding pockets, using GLIDE version 5.6.[20] Briefly, the automated docking program implemented in the Schrodinger package. The geometry of docked compound (**6c**) was

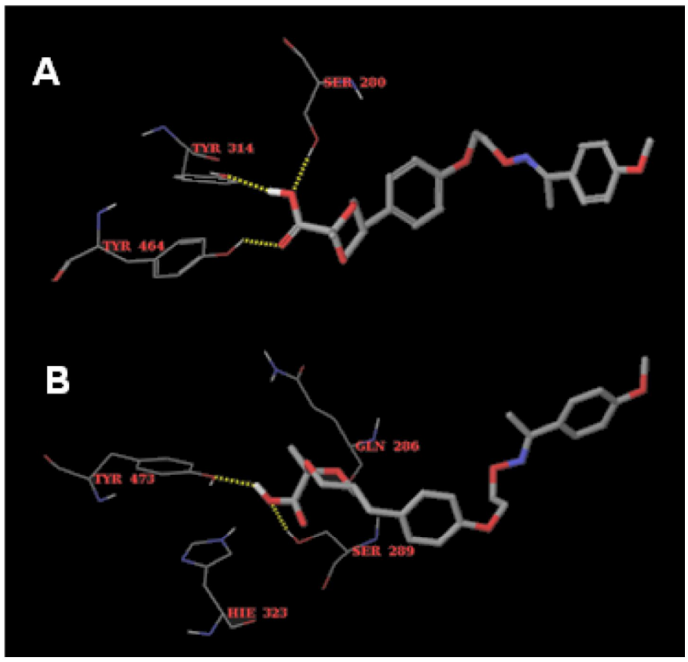


Figure 3. Molecular docking of **6c** into PPARα (A) and γ (B) binding pockets: H-bond interactions with amino acids are shown in dashed lines.

subsequently optimized using the LigPrep version 2.6.[21] The complexed X-ray crystal structure of the ligand binding domain (LBD) of PPARα with GW409544 (1k7l.pdb) and PPARγ with Rosiglitazone (2PRG.pdb) were obtained from RCSB Protein Data Bank. As depicted in Fig. 3, when **6c** was docked into PPARα binding pocket, the most stable docking models of **6c** adopted a conformation that allows the carboxylic group to form hydrogen bonds with Tyr314, Tyr464, and Ser280 (Fig. 3; A), which have been reported to be essential interaction for PPARα selective compounds.[22] The literature precedence suggests that interaction of the ligand with Ser289, His323, Tyr473, and His449 are important for PPARγ affinity, as this H-bonding network could stabilize the AF-2 helix in a conformation which favors the binding of co-activators to PPARγ and consequently, enhance their recruitment.[22] When **6c** was docked into PPARγ binding pocket the most stable docking model of **6c** adopted a conformation that only allows the carboxylic group to form hydrogen bonds with Tyr473 and Ser289. However, other important residues such as His449 and His323 which are crucial for PPARγ selectivity were found to be away from the ligand and no H-bond interactions of these amino acids with the carboxylate group of compound **6c** were observed (Fig. 3; B). Thus favorable in-silico interaction of compound **6c** with PPARα binding pocket and partial interaction with PPARγ binding pocket correlates its in vitro selectivity profile toward PPARα over PPARγ.

In summary a novel series of oxime containing benzyl-1,3-dioxane-*r*-2-carboxylic acid derivatives with electron donating groups on *para* position of phenyl ring emerged as selective PPARα agonists. Lead compound **6c** showed excellent antihyperglycemic and antihyperlipidemic effects in animal models, along with improved oral bioavailability and in-silico docking studies results were found be in conformity with in vitro PPARα selectivity. Our preliminary study results confirmed that highly potent and selective PPARα agonist could be viable approach for the safe and effective regulation of lipid and glucose homeostasis in obese and diabetic patients. Further evaluation of this lead compound (chronic pharmacodynamic studies and toxicological evaluation) is currently under progress and will be communicated subsequently.

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19. *Spectroscopic analysis of the compounds* **6a**–**k**. Compound **6a**: *2-Methyl-c-5-(4-(2-(((E)-(1-phenylethylidene)amino)oxy)ethoxy)benzyl)-1,3-dioxane-r-2-carboxylic acid*. White solid; yield: 70.0%; mp: 82–84 °C; purity: 98.79%; IR (KBr): 3414, 3018, 2939, 2663, 2532, 1713, 1612, 1510, 1445, 1369, 1271, 1145, 959 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.55 (s, 3H), 2.24 (s, 3H), 2.27 (s, 3H), 3.53 (t, *J* = 10.8 Hz, 2H), 3.93 (dd, *J* = 12 and 3.6 Hz, 2H), 4.25 (t, *J* = 5.2 Hz, 2H), 4.52 (t, *J* = 4.4 Hz, 2H), 6.86 (d, *J* = 8.8 Hz, 2H), 7.00 (d, *J* = 8.8 Hz, 2H),7.34–7.38 (m, 2H), 7.62–7.64 (m, 2H); ^{13}C NMR: (100 MHz, $CDCl_3$): δ 13.13, 25.93, 33.91, 35.21, 66.79, 68.30, 72.58, 98.22, 115.00, 126.24, 128.53, 129.29, 129.66, 130.13, 136.66, 155.77, 157.70, 175.21; ESI/MS *m*/*z*:414.20 $(M+H)^+$; **6b**: *2-Methyl-c-5-(4-(2-(((E)-(1-(p-tolyl)ethylidene)amino)oxy)ethoxy)benzyl)-1,3-dioxane-r-2-carboxylic acid*: white solid; yield: 67.0%; mp: 92–94 °C; purity: 99.68%; IR (KBr): 3414, 3290, 2924, 2870, 1740, 1718, 1639, 1512, 1460, 1367, 1151, 1033, 655 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.55 (s, 3H), 2.22 (s, 3H), 2.30 (s, 3H), 2.35 (s, 3H), 3.52 (t, *J* = 10.4 Hz, 3H), 3.91 (dd, *J* = 12 and 3.6 Hz, 2H), 4.24 (t, *J* = 4.8 Hz, 2H), 4.50 (t, *J* = 4.4 Hz, 2H), 6.86 (d, *J* = 8.4 Hz, 2H), 6.99 (d, *J* = 8.4 Hz, 2H), 7.16 (d, *J* = 8.0 Hz, 2H), 7.53 (d, *J* = 8.0 Hz, 2H); ^{13}C NMR: (100 MHz, $CDCl_3$): δ 13.08, 21.38, 25.91, 33.90, 35.20, 66.79, 68.27, 72.47, 98.23, 114.98, 126.12, 129.22, 129.64, 130.09, 133.80, 139.30, 155.73, 157.70, 174.83; ESI/MS *m*/*z*: 428.21 $(M+H)^+$. Compound **6c**: *c-5-(4-(2-(((E)-(1-(4-Methoxyphenyl)ethylidene)amino)oxy)ethoxy)benzyl)-2-methyl-1,3-dioxane-r-2-carboxylic acid*: off white solid; yield: 63.0%; mp: 108–109 °C; purity: 98.79%; IR (KBr): 3348, 2995, 2852, 1764, 1720, 1606, 1512, 1190 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.55 (s, 3H), 2.17 (s, 3H), 2.21-2.30 (m, 3H), 3.52 (t, *J* = 10.56 Hz, 2H), 3.82 (s, 3H), 3.93 (dd, *J* = 12.32 and 5.2 Hz, 2H), 4.25 (t, *J* = 5.13 Hz, 2H), 4.49 (t, *J* = 4.71 Hz, 2H), 6.87 (dd, *J* = 8.82 and 5.25 Hz, 4H), 7.00 (d, *J* = 8.49 Hz, 2H), 7.59 (t, *J* = 8.85 Hz, 2H); ^{13}C NMR: (100 MHz, $CDCl_3$): δ 21.43, 21.58, 25.88, 29.83, 33.93, 35.23, 66.62, 68.20, 72.71, 98.22, 114.96, 128.04, 128.12, 128.25, 128.94, 129.07, 129.44, 129.63, 130.10, 130.24, 133.43, 133.73, 136.77, 139.06, 139.63, 157.64, 157.78, 175.00; ESI/MS *m*/*z*: 444.2 $(M+H)^+$. Compound **6d**: *c-5-(4-(2-(((E)-(1-(3,4-Dimethoxyphenyl)ethylidene)amino)oxy)ethoxy)benzyl)-2-methyl-1,3-dioxane-r-2-carboxylic acid*: oil; yield: 61.0%; purity: 99.0%; IR ($CHCl_3$): 3385, 3018, 2937, 1739, 1610, 1512, 1406, 1249, 1057, 929, 869 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.54 (s, 3H), 2.22 (s, 3H), 2.29 (s, 3H), 3.53 (t, *J* = 10.4 Hz, 2H), 3.90 (m, 8H), 4.24 (s, 2H), 4.50 (s, 2H), 6.83–6.87 (m, 3H), 6.99 (d, *J* = 7.2 Hz, 2H), 7.14 (d, *J* = 8.0 Hz, 1H), 7.28 (s, 1H); ^{13}C NMR: (100 MHz, $CDCl_3$): δ 12.98, 25.88, 33.90, 35.20, 56.02, 66.80, 68.22, 72.46, 98.30, 108.84, 110.70, 114.96, 119.37, 129.39, 129.64, 130.13, 148.91, 150.23, 155.33, 157.68, 174.81; ESI/MS *m*/*z*: 474.2 $(M+H)^+$. Compound **6e**: *2-Methyl-c-5-(4-(2-(((E)-(1-(4-(methylsulfonyl)phenyl)ethylidene)amino)oxy)ethoxy)benzyl)-1,3-dioxane-r-2-carboxylic acid*: oil; yield: 64.0%; purity: 99.64%; IR ($CHCl_3$): 3020, 2976, 1724, 1610, 1512, 1385, 1317, 1246, 1153, 1041, 955 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.54 (s, 3H), 2.26 (s, 3H), 2.28–2.32 (m, 3H), 3.06 (s, 3H) 3.52 (t, *J* = 10.8 Hz, 2H), 3.89 (dd, *J* = 12.8 and 4.4 Hz, 2H), 4.26 (t, *J* = 4.8 Hz, 2H), 4.56 (t, *J* = 4.8 Hz, 2H), 6.85 (d, *J* = 8.4 Hz, 2H), 7.00 (d, *J* = 8.4 Hz, 2H), 7.83 (d, *J* = 8.4 Hz, 2H), 7.93 (d, *J* = 8.4 Hz, 2H); ^{13}C NMR: (100 MHz, $CDCl_3$): δ 12.85, 25.89, 33.86, 35.20, 44.61, 66.65, 68.23, 73.09, 98.26, 115.50, 127.00, 127.60, 129.68, 130.29, 140.65, 141.85, 153.85, 157.54, 174.60; ESI/MS *m*/*z*: 514.13 $(M+Na)^+$. Compound **6f**: *c-5-(4-(2-(((E)-(1-(4-Fluorophenyl)ethylidene)amino)oxy)ethoxy)benzyl)-2-methyl-1,3-dioxane-r-2-carboxylic acid*: white solid; yield: 70.0%; mp: 80–81 °C; purity: 98.0%; IR (KBr): 3061, 2935, 2866, 1739, 1610, 1512, 1371, 1240, 1105, 935, 837 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.55 (s, 3H), 2.22 (s, 3H), 2.24–2.32 (m, 3H), 3.53 (t, *J* = 10.8 Hz, 2H), 3.91 (dd, *J* = 12.4 and 4 Hz, 2H), 4.24 (t, *J* = 5.2 Hz, 2H), 4.51 (t, *J* = 4.4 Hz, 2H), 6.86 (d, *J* = 8.8 Hz, 2H), 6.99 (d, *J* = 8.8 Hz, 2H), 7.02–7.07 (m, 2H), 7.60–7.65 (m, 2H); ^{13}C NMR: (100 MHz, $CDCl_3$): δ 13.08, 25.94, 33.90, 35.21, 66.75, 68.31, 72.62, 98.20, 114.98, 115.37, 115.59, 128.01, 129.67, 130.15, 132.75, 154.70, 157,68, 162.29, 164.76, 175.21; ESI/MS *m*/*z*: 432.20 $(M+H)^+$. Compound **6g**: *2-Methyl-c-5-(4-(2-(((E)-(1-(4-(trifluoromethyl)phenyl)-ethylidene)amino)oxy)ethoxy)benzyl)-1,3-dioxane-r-2-carboxylic acid*: oil; yield: 70%; purity: 98.50%; IR ($CHCl_3$): 3580, 3018, 2976, 2930, 1724, 1610, 1512, 1458, 1385, 1327, 1215, 1130, 1059, 930 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.55 (s, 3H), 2.25 (s, 3H), 2.28 (s, 3H), 3.53 (t, *J* = 10.8 Hz, 2H), 3.91 (dd, *J* = 9.6 and 4.0 Hz, 2H), 4.25 (t, *J* = 4.8 Hz, 2H), 4.54 (t, *J* = 4.4 Hz, 2H), 6.86 (d, *J* = 8.4 Hz, 2H), 7.01 (d, *J* = 8.8 Hz, 2H), 7.61 (d, *J* = 8.4 Hz, 2H), 7.75 (d, *J* = 8.4 Hz, 2H); ^{13}C NMR: (100 MHz, $CDCl_3$): δ 12.92, 25.90, 33.92, 35.20, 66.71, 68.21, 72.92, 98.22, 114.97, 125.44, 126.48, 129.69, 130.22, 139.97, 154.34, 157.64, 174.38; ESI/MS *m*/*z*: 482.18 $(M+H)^+$. Compound **6h**: *2-Methyl-c-5-(4-(2-(((E)-(1-(4-(trifluoromethoxy)phenyl)ethylidene)amino)oxy)ethoxy)benzyl)-1,3-dioxane-r-2-carboxylic acid* : white solid; yield: 56.0%; mp: 68–70 °C; purity: 98.52%; IR (KBr): 3550, 3149, 2934, 2868, 1736, 1610, 1512, 1452, 1371, 1249, 1157, 1103, 949, 748 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.55 (s, 3H), 2.23 (s, 3H), 2.25–2.31 (m, 3H), 3.53 (t, *J* = 10.8 Hz, 2H), 3.93 (dd, *J* = 14 and 4.0 Hz, 2H), 4.25 (t, *J* = 5.2 Hz, 2H), 4.52 (t, *J* = 4.8 Hz, 2H), 6.86 (d, *J* = 8.4 Hz, 2H), 7.00 (d, *J* = 8.4 Hz, 2H), 7.20 (d, *J* = 8.4 Hz, 2H), 7.67 (d, *J* = 8.8 Hz, 2H); ^{13}C NMR: (100 MHz, $CDCl_3$): δ 12.99, 25.94, 33.88, 35.20, 66.71, 68.32, 72.75, 98.19, 114.91, 119.25, 120.87, 121.81, 127.70, 129.67, 130.19, 135.22, 149.91, 154.40, 157.64, 174.38; ESI/MS *m*/*z*: 498.18 $(M+H)^+$. Compound **6i**: *2-Methyl-c-5-(4-(2-(((E)-(1-(5,6,7,8-tetrahydronaphthalen-2-yl)ethylidene)amino)oxy)ethoxy)benzyl)-1,3-dioxane-r-2-carboxylic acid*: oil; yield: 64.0%; purity: 99.47%; IR ($CHCl_3$): 3300, 3018, 2932, 1724, 1512, 1385, 1246, 1145, 1041, 929 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.55 (s, 3H), 1.77–1.80 (m, 4H), 2.20 (s, 3H), 2.26–2.30 (m, 3H), 2.76 (d, *J* = 6.0 Hz, 4H), 3.52 (t, *J* = 10.8 Hz, 2H), 3.92 (dd, *J* = 14.4 and 4.0 Hz, 2H), 4.24 (t, *J* = 4.8 Hz, 2H), 4.51 (t, *J* = 4.8 Hz, 2H), 6.86 (d, *J* = 8.4 Hz, 2H), 6.99 (d, *J* = 8.4 Hz, 2H), 7.05 (d, *J* = 8.0 Hz, 1H), 7.32–7.35 (m, 2H); ^{13}C NMR: (100 MHz, $CDCl_3$): δ 13.16, 23.25, 25.86, 29.37, 29.59, 33.34, 35.35, 60.82, 68.27, 72.43, 98.21, 115.09, 123.36, 126.85, 129.28, 129.63, 130.04, 133.85, 137.28, 137.47, 138.64, 155.96, 157.73, 174.81; ESI/MS *m*/*z*: 468.34 $(M+H)^+$. Compound **6j**: *2-Methyl-c-5-(4-(2-(((E)-(1-(naphthalen-2-yl)ethylidene)amino)oxy)ethoxy)benzyl)-1,3-dioxane-r-2-carboxylic acid*: white solid; yield: 53.0%; mp: 136–137 °C; purity: 99.21%; IR (KBr):3500, 3059, 2965, 2853, 1751, 1610, 1510, 1454, 1387, 1246, 1149, 1120, 1041, 964, 868 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.55 (s, 3H), 2.27–2.52 (m, 3H), 2.35 (s, 3H) 3.52 (t, *J* = 10.8 Hz, 2H), 3.93 (dd, *J* = 10.8 and 4.0 Hz, 2H), 4.29 (t, *J* = 5.2 Hz, 2H), 4.57 (t, *J* = 4.8 Hz, 2H), 6.89 (d, *J* = 8.4 Hz, 2H), 7.00 (dd, *J* = 8.4 and 2.8 Hz, 2H,), 7.47–7.51 (m, 2H), 7.79–7.87 (m, 3H), 7.90 (dd, *J* = 8.8 and 1.6 Hz, 1H), 7.99 (s, 1H); ^{13}C NMR: (100 MHz, $CDCl_3$): δ 12.89, 25.94, 33.90, 35.10, 66.83, 68.34, 72.71, 98.13, 115.01, 123.60, 125.97, 126.48, 126.73, 127.77, 128.13, 128.60, 129.67, 130.12, 133.23, 133.80, 133.96, 155.52, 157.72, 174.83; ESI/MS *m*/*z*: 486.19 $(M+Na)^+$. Compound **6k**: *2-Methyl-c-5-(4-(2-(((E)-(1-(pyridin-3-yl)ethylidene)amino)oxy)ethoxy)benzyl)-1,3-dioxane-r-2-carboxylic acid*: oil; yield: 62.0%; purity: 99.05%; IR ($CHCl_3$): 3387, 3018, 2976, 1739, 1612, 1512, 1385, 1246, 1151, 1043, 929 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.55 (s, 3H), 2.25 (s, 3H), 2.28–2.35 (m, 1H), 2.41 (d, *J* = 6.4 Hz, 1H), 3.61 (t, *J* = 11.6 Hz, 2H), 3.90 (dd, *J* = 12.4 and 4.4 Hz, 2H), 4.31(t, *J* = 4.8 Hz, 2H), 4.55 (t, *J* = 4.8 Hz, 2H), 6.83 (d, *J* = 8.8 Hz, 2H), 6.98 (dd, *J* = 10.4 and 2.0 Hz, 2H), 7.40 (dd, *J* = 8.0 and 4.8 Hz, 1H), 7.96 (dd, *J* = 9.6 and 1.6 Hz, 1H), 8.57 (d, *J* = 4.0 Hz, 1H), 8.82 (s, 1H); ^{13}C NMR: (100 MHz, $CDCl_3$): δ 12.57, 26.06, 33.97, 35.28, 66.63, 68.07, 73.02, 98.64, 115.17, 124.09, 129.75, 130.48, 133.24, 135.05, 145.72, 148.00, 152.31, 157.45, 173.68; ESI/MS *m*/*z*: 415.21 $(M+H)^+$.
20. GLIDE 5.6, Schrodinger, LLC, New York, NY, 2010.
21. LigPrep 2.6, Schrodinger, LLC, New York, NY, 2010.
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