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Supporting Information

A 3-(4-Nitronaphthen-1-yl) amino-benzoate analog as a Bifunctional AKR1C3 Inhibitor with AR Antagonist Activity: Head to Head Comparison with Other Advanced AKR1C3 Targeted Therapeutics

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Synthesis of the following compounds:

3-((4-nitronaphthalen-1-yl)amino)benzoic acid (**1**)

3-(naphthalen-1-ylamino)benzoic acid (**2**)

3-((4,5-dinitronaphthalen-1-yl)amino)benzoic acid (**3**)

3-((4-acetylnaphthalen-1-yl)amino)benzoic acid (**4**)

5-((4-acetylnaphthalen-1-yl)amino)isophthalic acid (**5**)

3-((4-cyanonaphthalen-1-yl)amino)benzoic acid (**6**)

3-((4-(trifluoromethyl)naphthalen-1-yl)amino)benzoic acid (**7**)

3-((4-fluoronaphthalen-1-yl)amino)benzoic acid (**8**)

3-(quinolin-4-ylamino)benzoic acid (**9**)

4-(naphthalen-1-ylamino)benzoic acid (**10**)

4-((4-acetylnaphthalen-1-yl)amino)benzoic acid (**11**)

2-(naphthalen-1-ylamino)benzoic acid (**12**)

4-methoxy-2-(naphthalen-1-ylamino)benzoic acid (**13**)

5-acetyl-2-(naphthalen-1-ylamino)benzoic acid (**14**)

5-acetyl-2-((4-nitronaphthalen-1-yl)amino)benzoic acid (**15**)

5,5-dimethyl-3-(4-nitronaphthalen-1-yl)imidazolidine-2,4-dione (**16**)

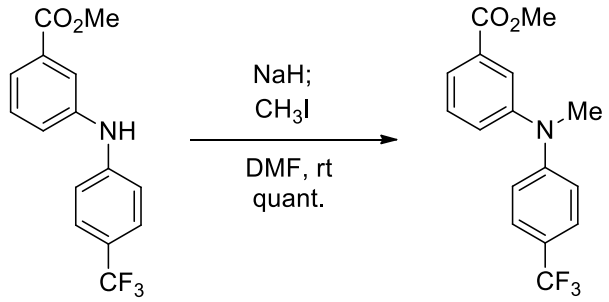
5,5-dimethyl-3-(4-nitronaphthalen-1-yl)-2-thioxoimidazolidin-4-one (**17**)

Table 1S. Gridbox parameters for docking experiments

Synthesis of Compounds 1-17

General Procedure

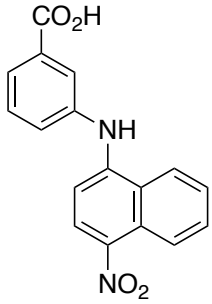
General procedure for synthesis of new compounds: The AKR1C3 compounds were prepared according to the two-step Buchwald-Hartwig coupling/ester saponification sequence as described in Adeniji, *et. al*., 2012, J. Med. Chem. 55:2311-23. The exception was that diglyme was used instead of toluene as the solvent for the Buchwald-Hartwig couplings.



To a solution of the *N*-phenyl aniline (35 mg, 0.119 mmol) in DMF (1 mL) at room temperature was added a suspension of NaH in mineral oil (60% wt/wt, 77 mg, 0.193 mmol), followed by CH_3I (15 μL, 0.233 mmol) dropwise. The reaction mixture was stirred at room temperature overnight. Water was added, and the mixture was extracted with ethyl acetate. The combined organic layers were dried (Na_2SO_4) and concentrated to give the *N*-methyl aniline as a red oil (37 mg, quant.) which did not require purification.

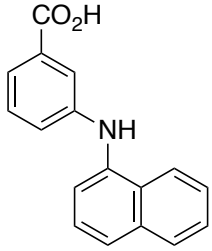
In a subsequent step, the ester was saponified to the corresponding acid following the protocol reported in Adeniji, *et. al*., 2012, J. Med. Chem. 55:2311-23 (0.2M KOH, EtOH, 100°C, 6h). All compounds were > 95% pure based on chromatography and characterized by ^{1}H-NMR (500 MHz).

3-((4-nitronaphthalen-1-yl)amino)benzoic acid (1)



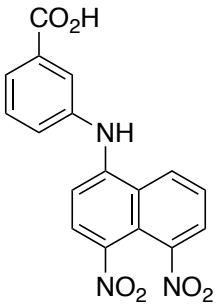
^{1}H NMR (500 MHz, DMSO-d_6): δ 13.00 (bs, 1H), 9.45 (s, 1H), 8.79 (d, J = 8.6 Hz, 1H), 8.53 (d, J = 8.3 Hz, 1H), 8.38 (d, J = 8.9 Hz, 1H), 7.93 (s, 1H), 7.84 (m, 1H), 7.68–7.76 (m, 2H), 7.63 (m, 1H), 7.55 (m, 1H), 7.13 (d, J = 8.8 Hz, 1H).

3-(naphthalen-1-ylamino)benzoic acid (2)



^{1}H NMR (500 MHz, DMSO-d_6) δ 8.41 (s, 1H), 8.13 (d, J = 8.0 Hz, 1H), 7.98 – 7.89 (m, 1H), 7.67 – 7.57 (m, 2H), 7.56 – 7.47 (m, 2H), 7.44 (t, J = 7.8 Hz, 1H), 7.40 – 7.35 (m, 2H), 7.31 (t, J = 7.8 Hz, 1H), 7.21 (dd, J = 8.0, 3.1 Hz, 1H).

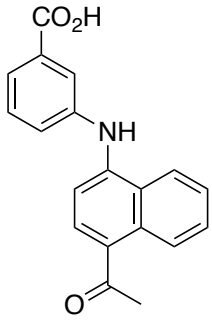
3-((4,5-dinitronaphthalen-1-yl)amino)benzoic acid (3)



^{1}H NMR (500 MHz, DMSO-d_6) δ 13.09 (s, 1H), 9.67 (s, 1H), 8.85 (dd, J = 8.6, 1.3 Hz, 1H), 8.43 (dd, J = 7.6, 1.2 Hz, 1H), 8.32 (d, J = 8.9 Hz, 1H), 7.92 (t, J = 2.0 Hz, 1H), 7.86 (dd, J = 8.7, 7.6

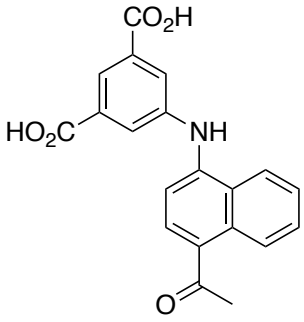
Hz, 1H), 7.75 (dt, *J* = 7.7, 1.4 Hz, 1H), 7.65 (ddd, *J* = 8.1, 2.4, 1.2 Hz, 1H), 7.56 (t, *J* = 7.8 Hz, 1H), 7.19 (d, *J* = 8.9 Hz, 1H).

3-((4-acetylnaphthalen-1-yl)amino)benzoic acid (4)



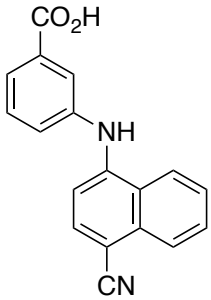
^{1}H NMR (500 MHz, DMSO-d_6) δ 12.95 (s, 1H), 9.04 (dd, *J* = 8.7, 1.2 Hz, 1H), 8.97 (s, 1H), 8.35 (d, *J* = 8.4 Hz, 1H), 8.13 (d, *J* = 8.2 Hz, 1H), 7.85 (t, *J* = 2.1 Hz, 1H), 7.64 (ddd, *J* = 8.4, 6.8, 1.4 Hz, 1H), 7.60 – 7.54 (m, 2H), 7.53 – 7.49 (m, 1H), 7.46 (t, *J* = 7.8 Hz, 1H), 7.22 (d, *J* = 8.2 Hz, 1H), 2.63 (s, 3H).

5-((4-acetylnaphthalen-1-yl)amino)isophthalic acid (5)



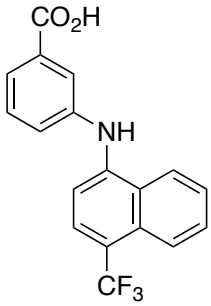
^{1}H NMR (500 MHz, DMSO-d_6) δ 9.11 (s, 1H), 8.96 (d, *J* = 8.7 Hz, 1H), 8.29 (d, *J* = 8.5 Hz, 1H), 8.14 (d, *J* = 8.1 Hz, 1H), 8.04 (s, 1H), 7.98 (s, 2H), 7.64 (t, *J* = 7.7 Hz, 1H), 7.56 (t, *J* = 7.6 Hz, 1H), 7.31 (d, *J* = 8.1 Hz, 1H), 2.64 (s, 3H).

3-((4-cyanonaphthalen-1-yl)amino)benzoic acid (6)



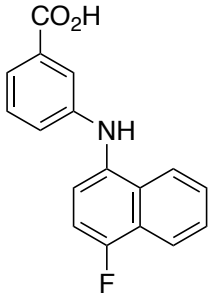
^{1}H NMR (500 MHz, DMSO-d_6) δ 9.21 (s, 1H), 8.52 (d, J = 8.5 Hz, 1H), 8.09 (d, J = 8.3 Hz, 1H), 7.97 (d, J = 8.2 Hz, 1H), 7.92 (t, J = 2.0 Hz, 1H), 7.84 (t, J = 7.6 Hz, 1H), 7.73 (t, J = 7.6 Hz, 1H), 7.69 (d, J = 7.7 Hz, 1H), 7.65 – 7.59 (m, 1H), 7.55 (t, J = 7.8 Hz, 1H), 7.24 (d, J = 8.1 Hz, 1H).

3-((4-(trifluoromethyl)naphthalen-1-yl)amino)benzoic acid (7)



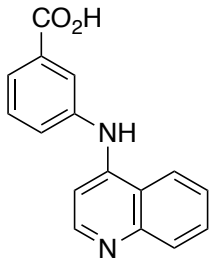
^{1}H NMR (500 MHz, DMSO-d_6) δ 12.72 (s, 1H), 9.24 – 9.06 (m, 1H), 8.88 (s, 1H), 8.45 – 8.31 (m, 1H), 8.14 (d, J = 8.2 Hz, 1H), 7.83 (t, J = 1.9 Hz, 1H), 7.65 (ddd, J = 8.7, 6.7, 1.4 Hz, 1H), 7.57 (ddt, J = 8.4, 5.0, 1.8 Hz, 2H), 7.50 (ddd, J = 8.1, 2.4, 1.3 Hz, 1H), 7.45 (t, J = 7.8 Hz, 1H), 7.26 (d, J = 8.2 Hz, 1H).

3-((4-fluoronaphthalen-1-yl)amino)benzoic acid (8)



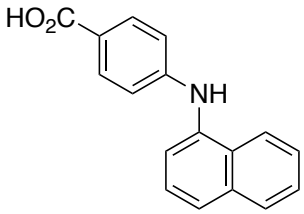
^{1}H NMR (500 MHz, DMSO-d_6) δ 12.72 (s, 1H), 8.31 (s, 1H), 8.14 – 8.09 (m, 1H), 8.08 – 8.01 (m, 1H), 7.65 (ddd, J = 8.4, 6.9, 1.4 Hz, 1H), 7.61 (ddd, J = 8.2, 6.8, 1.6 Hz, 1H), 7.46 (t, J = 2.1 Hz, 1H), 7.36 – 7.22 (m, 4H), 7.09 (ddd, J = 7.9, 2.6, 1.2 Hz, 1H).

3-(quinolin-4-ylamino)benzoic acid (9)



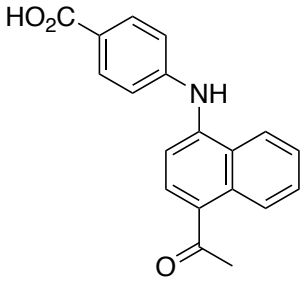
^{1}H NMR (500 MHz, DMSO-d_6) δ 9.13 (s, 1H), 8.51 (d, J = 5.2 Hz, 1H), 8.38 (dd, J = 8.5, 1.4 Hz, 1H), 7.92 (t, J = 2.1 Hz, 1H), 7.90 (dd, J = 8.5, 1.3 Hz, 1H), 7.72 (ddd, J = 8.4, 6.8, 1.5 Hz, 1H), 7.68 (dd, J = 7.6, 1.5 Hz, 1H), 7.63 (dd, J = 7.7, 3.1 Hz, 1H), 7.58 – 7.50 (m, 2H), 7.01 (d, J = 5.3 Hz, 1H).

4-(naphthalen-1-ylamino)benzoic acid (10)



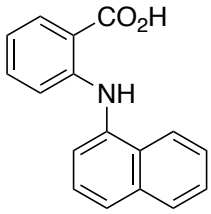
^{1}H NMR (500 MHz, DMSO-d_6) δ 8.77 (s, 1H), 8.06 (d, J = 8.2 Hz, 1H), 7.94 (d, J = 8.0 Hz, 1H), 7.76 (d, J = 8.4 Hz, 2H), 7.70 (d, J = 8.0 Hz, 1H), 7.58 – 7.44 (m, 4H), 6.93 (d, J = 8.5 Hz, 2H).

4-((4-acetylnaphthalen-1-yl)amino)benzoic acid (11)



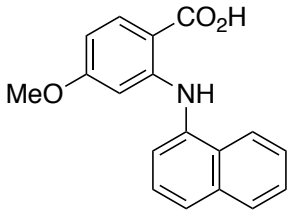
^{1}H NMR (500 MHz, DMSO-d_6) δ 12.46 (s, 1H), 9.15 (s, 1H), 8.95 (d, J = 8.5 Hz, 1H), 8.28 (d, J = 8.7 Hz, 1H), 8.16 (d, J = 8.2 Hz, 1H), 7.90 – 7.81 (m, 2H), 7.68 – 7.62 (m, 1H), 7.61 – 7.52 (m, 1H), 7.44 (d, J = 8.1 Hz, 1H), 7.24 (d, J = 8.9 Hz, 2H), 2.66 (s, 3H).

2-(naphthalen-1-ylamino)benzoic acid (12)



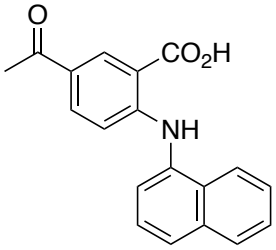
^{1}H NMR (500 MHz, DMSO-d_6) δ 13.16 (s, 1H), 10.03 (s, 1H), 8.19 – 7.86 (m, 3H), 7.75 (dd, *J* = 6.6, 2.7 Hz, 1H), 7.58 – 7.40 (m, 4H), 7.30 (ddd, *J* = 8.7, 7.0, 1.8 Hz, 1H), 6.89 (d, *J* = 8.4 Hz, 1H), 6.81 – 6.64 (m, 1H).

4-methoxy-2-(naphthalen-1-ylamino)benzoic acid (13)



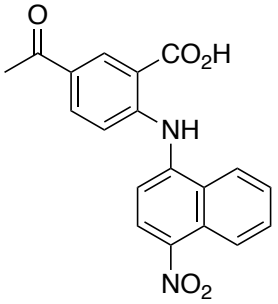
^{1}H NMR (500 MHz, DMSO-d_6) δ 12.81 (s, 1H), 10.20 (s, 1H), 7.96 (td, *J* = 6.8, 3.6 Hz, 2H), 7.92 – 7.83 (m, 1H), 7.76 (d, *J* = 7.9 Hz, 1H), 7.61 – 7.43 (m, 4H), 6.38 (d, *J* = 2.6 Hz, 1H), 6.36 (s, 1H), 3.61 (s, 3H).

5-acetyl-2-(naphthalen-1-ylamino)benzoic acid (14)



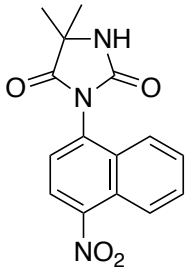
^{1}H NMR (500 MHz, DMSO-d_6) δ 10.42 (s, 1H), 8.55 (d, *J* = 2.3 Hz, 1H), 8.01 (dd, *J* = 7.4, 2.4 Hz, 1H), 7.96 – 7.86 (m, 2H), 7.83 (dd, *J* = 8.9, 2.4 Hz, 1H), 7.56 (td, *J* = 6.7, 2.4 Hz, 4H), 6.72 (d, *J* = 9.0 Hz, 1H), 2.46 (s, 3H).

5-acetyl-2-((4-nitronaphthalen-1-yl)amino)benzoic acid (15)



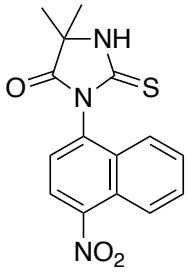
^{1}H NMR (500 MHz, DMSO-d_6) δ 12.78 (s, 1H), 8.69 (d, J = 8.8 Hz, 1H), 8.62 (d, J = 2.6 Hz, 1H), 8.42 (d, J = 8.7 Hz, 1H), 8.32 (d, J = 8.6 Hz, 1H), 7.98 (dd, J = 8.7, 2.5 Hz, 1H), 7.86 (t, J = 7.8 Hz, 1H), 7.76 (t, J = 7.8 Hz, 1H), 7.68 (d, J = 8.7 Hz, 1H), 7.53 (d, J = 8.7 Hz, 1H), 2.54 (s, 3H).

5,5-dimethyl-3-(4-nitronaphthalen-1-yl)imidazolidine-2,4-dione (16)



^{1}H NMR (500 MHz, Chloroform-d) δ 8.57 (dt, J = 8.9, 1.1 Hz, 1H), 8.26 (d, J = 8.1 Hz, 1H), 7.85 – 7.73 (m, 1H), 7.73 – 7.68 (m, 2H), 7.53 (d, J = 8.1 Hz, 1H), 5.85 (s, 1H), 1.72 (s, 3H), 1.65 (s, 3H).

5,5-dimethyl-3-(4-nitronaphthalen-1-yl)-2-thioxoimidazolidin-4-one (17)



^{1}H NMR (500 MHz, DMSO-d_6) δ 11.04 (s, 1H), 8.47 (d, J = 8.1 Hz, 1H), 8.41 (dd, J = 8.7, 1.1 Hz, 1H), 7.93 – 7.87 (m, 2H), 7.85 – 7.79 (m, 2H), 1.66 (s, 3H), 1.60 (s, 3H).

Table 1S. Gridbox parameters for docking experiments

Target	Coordinate Centers		
	X	Y	Z
AKR1C3 (PDB: 4DBS)	24.228	6.772	6.007
AR-LBD S-1 (PDB: 2AXA) and W741 L (PDB: 1Z95)	26.787	2.027	9.129