Binding model for the interaction of anti-cancer arylsulfonamides with the p300 transcription co-factor

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Western blot analysis.

In an initial investigation, we examined whether we could use an affinity approach to determine if KCN1 could be used as bait in a cell extract to pull down p300. We used our SAR to identify a site on KCN1 amenable to the attachment of an aliphatic linker, and then developed a coupling method (see below). A cell extract of human LN229 glioma cells grown under hypoxic conditions was exposed to KCN1-coupled beads and the uncoupled beads used as a control. Following the incubation period the bound proteins were separated from the extract by centrifugation of the beads and separated by SDS polyacrylamide gel electrophoresis. To determine whether p300 was pulled-down using this method, the proteins separated by molecular weight in the gel were transferred to a nitrocellulose membrane and reacted with anti-p300 or anti-HIF-1α antibodies using a Western blot procedure.

Biosensor-Surface Plasmon Resonance (SPR).

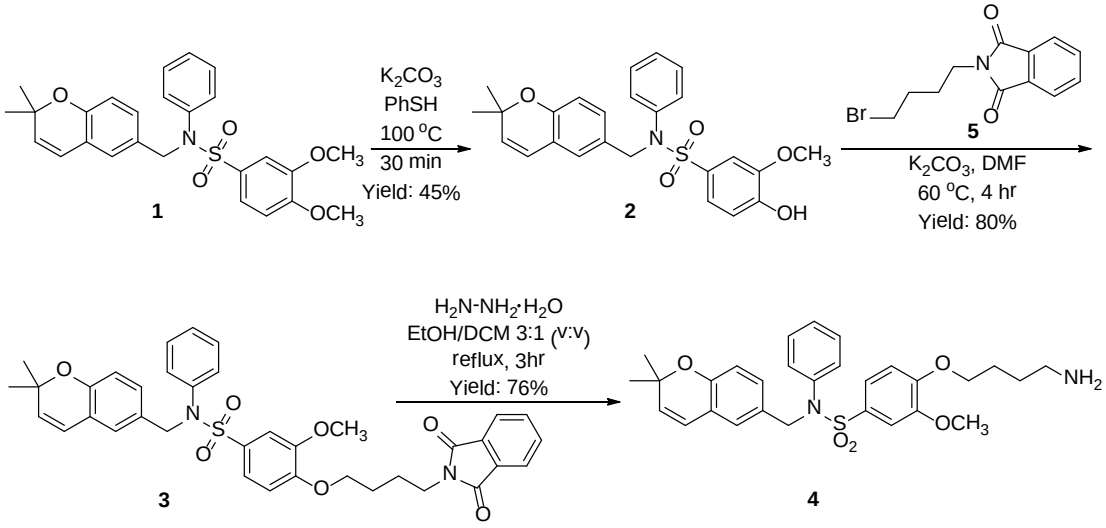
Binding and kinetics measurements were performed using a BIAcore T200 system and carboxylic acid-coated sensor chips (CM-5 chips from BIAcore). After activation of the surface with the amide coupling reagent EDC/NHS (1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride/N-hydroxysuccinimide; cf. http://www.piercenet.com/Objects/View.cfm?type=Page&ID=C0B555CC-0F33-4818-B09D-031E7FCF89FC, accessed 5/26/12) for two flow cells, KCN-amine (500 nM) in HBS buffer was immobilized on the surface of one flow cell by covalent capture and the other one was left as a control. After immobilization of KCN-amine, 1 M ethanolamine

(pH=8.5) was injected to both flow cells to block the remaining activated ester. Binding studies were conducted at 25 °C in P300 buffer (50 mM Tris-HCl (pH=8), 1.5 mM NaCl, 0.1 mM $ZnCl_2$, 10 mM $MgCl_2$ and 1mM DTT). P300 samples were injected at a flow rate of 50 μL/min. 1 M NaCl solution was used to dissociate the P300 from bound KCN for surface regeneration. The injection of the protein (association) was followed by injection of running buffer (binding dissociation). To reduce the probability of nonspecific binding to the chip surface, 50 μL/L of surfactant P20 was added to the P300 buffers in the binding experiments. All the curves were obtained after subtraction of control signal.

Conjugation of KCN-amine to solid support.

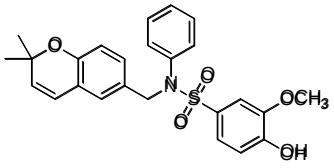
Pierce Reacti-Gel 6 × (0.75 ml, no. 20259, 1,1'-carbonyldiimadazole-activated 6% cross-linked beaded agarose, 50 umol/mL) was transferred to a fritted vial and washed with ice-cold water (three times, 2.5 ml). The resin was then suspended in 0.75 mL of aqueous K_2CO_3 (0.1 M) to which KCN-amine (20 mg, 38 umol) dissolved in 50 ml of DMF was added. The resulting mixture was shaken on a Burrel wrist action shaker (Model 75) for 12 hr at room temperature, followed by washing with PBS (five times, 5 mL each). The resin was capped with ethylamine (0.1 M) in aqueous K_2CO_3 (0.1 M) for 12 h, and washed again with PBS (five times, 2.5 mL each). The resulting derivatized resin was stored in PBS with 0.02% NaN_3 at 4°C. (Reid-Mooring, S.; Yin, S.; Mun, J.; Goodman, M.; Van Meir, E. G.; Wang, B., *in preparation*.)

Synthesis of KCN1 free amine for SPR analysis.



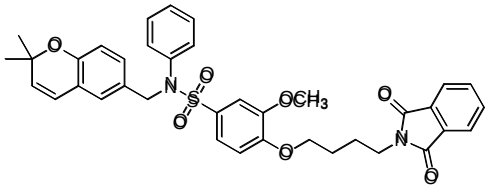
The KCN1 free amine was synthesized as shown in above. The methoxy group at the *para*-position was selectively demethylated by thiophenol and K_2CO_3 in *N*-Methyl-2-pyrrolidone (NMP) at 100 ºC. Then the hydroxyl compound **2** was alkylated with *N*-(4-bromobutyl) phthalimide and K_2CO_3 in dimethylformamide (DMF). The phthalimide KCN1 was deprotected to obtain the final KCN1 free amine by refluxing with hydrazine monohydrate in a mixture of ethanol and dichloromethane.

N-((2,2-dimethyl-2H-chromen-6-yl)methyl)-4-hydroxy-3-methoxy-*N*-phenylbenzenesulfonamide **2**.



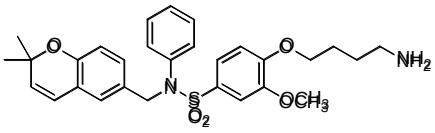
To a mixture of K_2CO_3 (445 mg, 3.2 mmol) in NMP (1 mL) was added thiophenol (PhSH) (344 μL, 3.2 mmol) at 100 ºC under Ar atmosphere. Then the reaction mixture was stirred for 10 minutes. The solution of compound **1** (300 mg, 0.65 mmol) in NMP (1 mL) was added into the reaction mixture. The reaction was stirred for 20 minutes. After pouring the light yellow solution into cold H_2O (10 mL), the aqueous solution was extracted with EtOAc (15 mL × 3). The combined organic layers was washed with water (15 mL × 6) to remove NMP. The organic layer was then dried over anhydrous Na_2SO_4. Solvent was removed in vacuo to yield a crude white solid, which was purified by flash chromatography on silica gel (Hexane/DCM = 1:1 (200 mL), pure DCM 200 mL, DCM/EtOAc = 50/1 (200 mL)) to yield white crystals **2** (131 mg, 45%).

N-((2,2-Dimethyl-2H-chromen-6-yl)methyl)-4-(4-(1,3-dioxoisoindolin-2-yl)butoxy)-3-methoxy-*N*-phenylbenzenesulfonamide **3**.



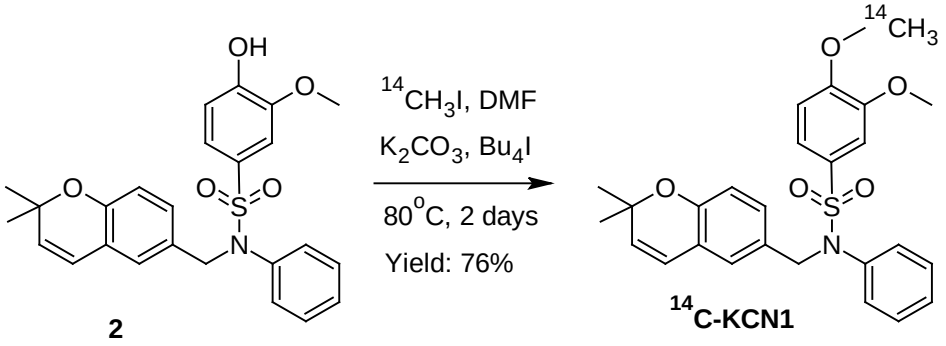
To a solution of compound **2** (320 mg, 0.71 mmol) in dried DMF (5 mL) was added *N*-(4-bromobutyl)phthalimide **5** (300 mg, 1.1 mmol) and K_2CO_3 (147 mg, 1.1 mmol) at 60 ºC under Ar atmosphere. The reaction was stirred for 4 hours. After pouring the solution into a separatory funnel with water and DCM, the aqueous solution was extracted with DCM (15 mL × 3). The combined organic layers was washed with water (15 mL × 3) and brine (15 mL × 3) before drying over anhydrous Na_2SO_4. Solvent was removed in vacuo to yield a crude colorless solid, which was purified by flash chromatography on silica gel (pure DCM 200 ml, DCM/EtOAc=50/1 200 ml) to yield a colorless solid **3** (368 mg, 80%).

4-(4-Aminobutoxy)-*N*-((2,2-dimethyl-2H-chromen-6-yl)methyl)-3-methoxy-*N*-phenylbenzenesulfonamide (KCN1 free amine **4**).



To a solution of compound **3** (140 mg, 0.21 mmol) in EtOH/DCM (3/1 v:v) (6 mL) was added hydrazine monohydrate (H_2N-NH_2·H_2O) (81 μL, 1.06 mmol) under Ar and at reflux. The resulting mixture was stirred for 3 hours. White solid appeared during the reaction. The reaction mixture was suction filtered and the filtrate was concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel (DCM/MeOH=20/1 (200 mL), 10/1 (200 mL), 5/1 (100 mL)) to yield colorless solid **4** (85 mg, 76%).

Synthesis of ^{14}C-KCN1.



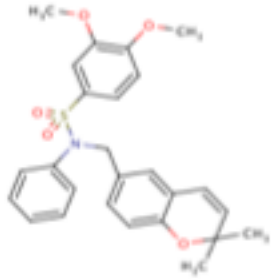
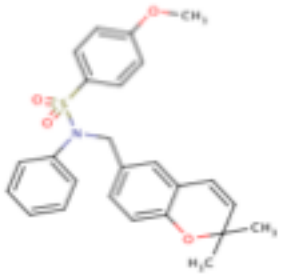
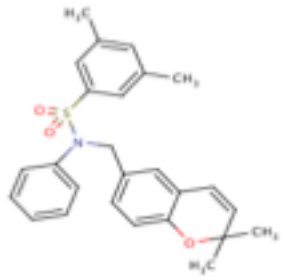
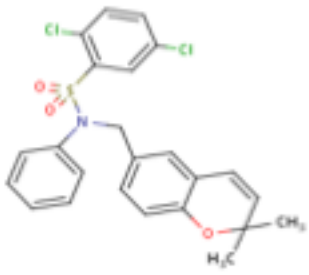
Synthesis of *N*-((2,2-dimethyl-*2H*-chromen-6-yl)methyl)-3-methoxy-4-[^{14}C]methoxy-*N*-phenylbenzenesulfonamide (^{14}C-KCN1).

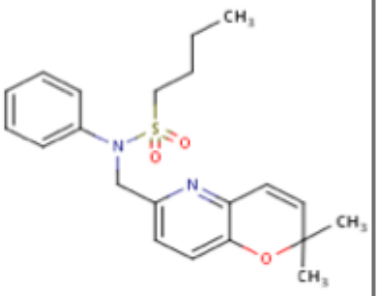
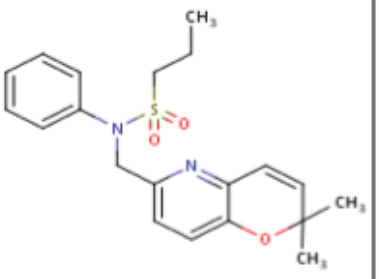
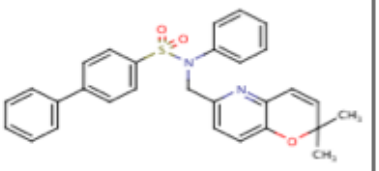
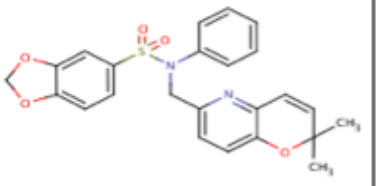
^{14}C-KCN1 was synthesized as shown above. *N*-((2,2-dimethyl-*2H*-chromen-6-yl)methyl)-4-hydroxy-3-methoxy-*N*-phenylbenzenesulfonamide (**2**) was methylated by $^{14}CH_3I$ and K_2CO_3 in DMF in the presence of phase transfer catalyst, tetrabutylammonium iodide, at 80°C for two days. To a mixture of *N*-((2,2-dimethyl-*2H*-chromen-6-yl)methyl)-4-hydroxy-3-methoxy-*N*-phenylbenzenesulfonamide (**2**) (5 mg, 11 µmole), K_2CO_3 (2 mg, 14 µmole), and tetrabutylammonium iodide (1 mg, 3 µmole) in DMF (60 µL) was added $^{14}CH_3I$ (322679, Sigma, 250 µCi, 40 ~ 60 mCi per mmole, 6.25 µmole) in DMF (150 µL) at -78°C in a 2 mL v-vial, which was then closed with a silicon-teflon liner and aluminum cap. The reaction mixture was stirred at 80°C for two days. After cooling, 0.5 mL of 1 N aqueous KOH solution was added to the reaction mixture, and then it was extracted with methylene chloride (1 mL X 3). The combined organic layer was washed with brine (1 mL) and dried with anhydrous magnesium sulfate. The crude product was purified by silica column chromatography with 100% of methylene chloride eluent using a disposable column (Kontes, Flex-column) to yield *N*-((2,2-dimethyl-*2H*-chromen-6-yl)methyl)-3-methoxy-4-[^{14}C]methoxy-*N*-phenylbenzenesulfonamide (^{14}C-KCN1) (2.2 mg, 76%) as a clear viscous liquid.

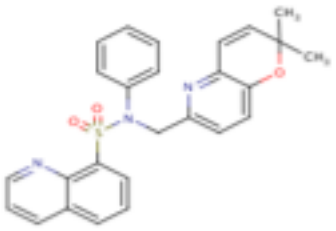
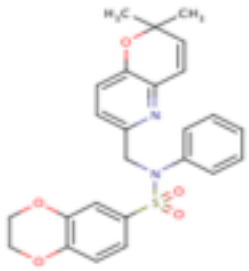
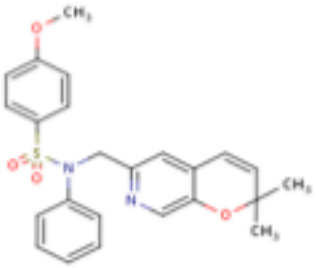
Thirty structures used[1] for the QSAR correlations.

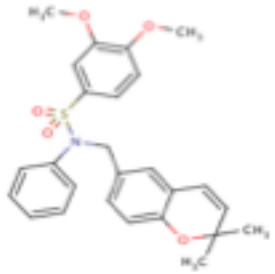
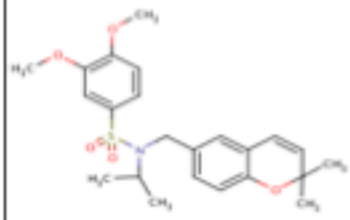
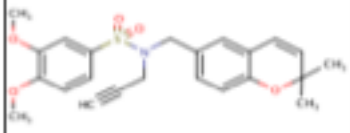
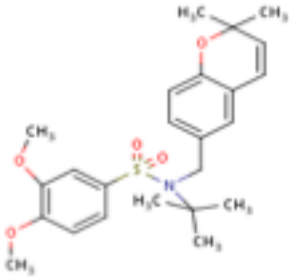
KCN1 - 1
Group 1 – Numbers 510-3601
Group 2 – Numbers 501-2609

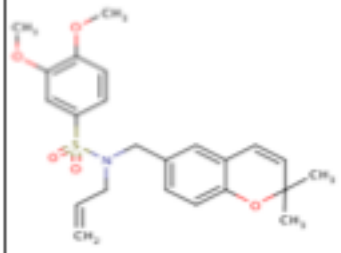
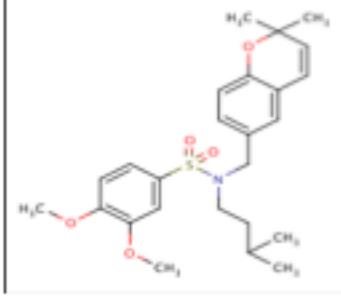
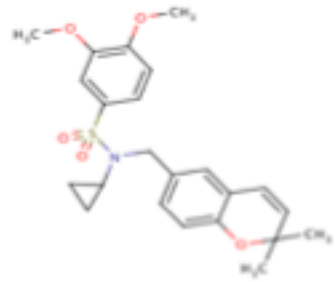
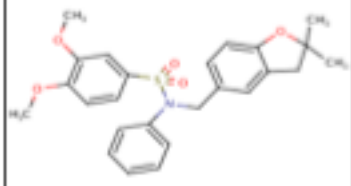
[1] Tan, C.; de Noronha, R. G.; Devi, N. S.; Jabbar, A. A.; Kaluz, S.; Liu, Y.; Mooring, S. R.; Nicolaou, K. C.; Wang, B.; Van Meir, E. G. Sulfonamides as a new scaffold for hypoxia inducible factor pathway inhibitors *Bioorg. Med. Chem. Lett.* **2011**, 21, 5528−5532.

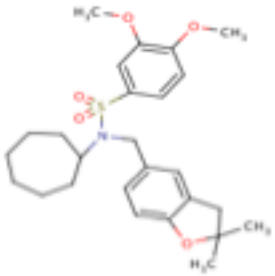
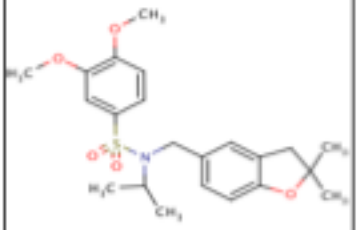
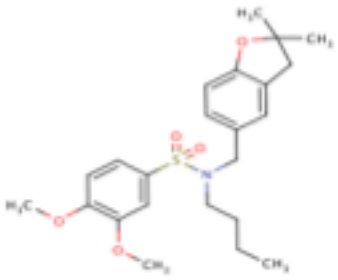
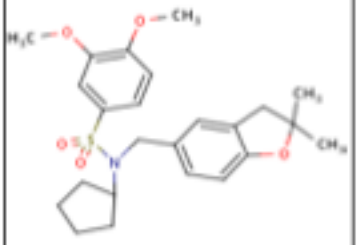
Structure	Name	Group	MMGBSA_Site1 (kcal/mol)	MMGBSA_Site2 (kcal/mol)	IC50 (uM)	IC50KCN1 (uM)
	1	I	-27.5	-28.5	0.7	0.7
	510	I	-23.4	-29.7	0.6	1.2
	511	I	-22.3	-27.3	0.5	1.2
	512	I	-24.9	-28.6	2.1	1.7

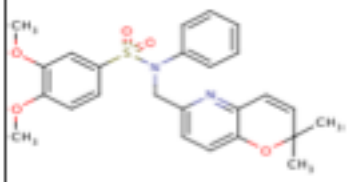
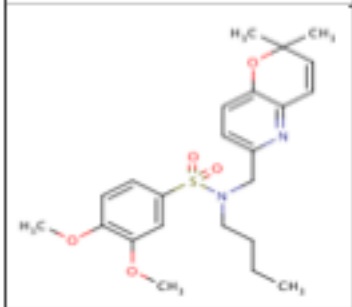
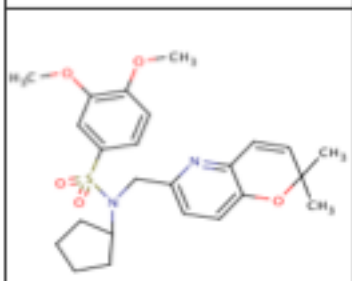
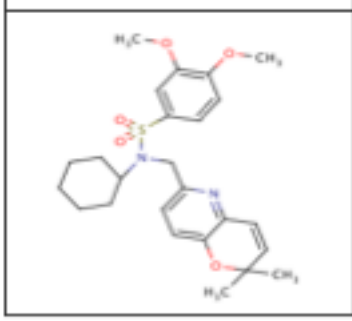
	2614	I	-24.2	-26.5	5.0	0.5
	2615	I	-20.6	-24.1	6.4	0.5
	2617	I	-26.1	-24.7	3.4	0.5
	2618	I	-25.8	-25.6	6.5	0.5

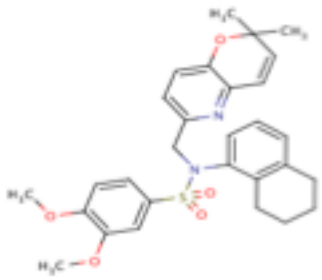
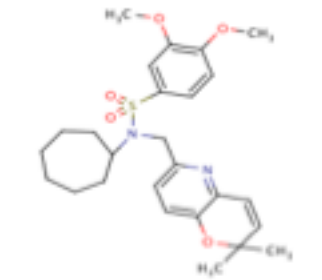
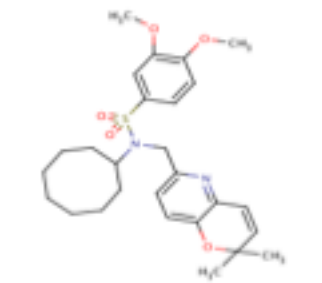
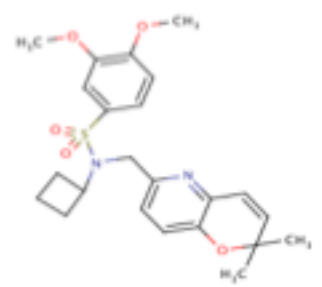
	2619	I	-18.8	-23.7	0.9	0.3
	2620	I	-19.7	-24.1	0.9	0.3
	3601	I	-20.5	-25.9	1.4	0.5

Structure	Name	Group	MMGBSA_Site1 (kcal/mol)	MMGBSA_Site2 (kcal/mol)	IC50 (uM)	IC50KCN1 (uM)
	1	II	-27.5	-28.5	0.7	0.7
	501	II	-20.0	-24.5	3.1	0.5
	502	II	-21.4	-24.0	1.3	0.5
	504	II	-20.0	-27.2	3.5	1.9

	505	II	-23.3	-28.3	3.4	1.9
	506	II	-20.3	-27.2	1.6	0.5
	508	II	-20.6	-26.6	1.5	0.5
	1601	II	-28.0	-29.1	0.5	1.2

	1602	II	-23.6	-32.0	9.1	0.3
	1603	II	-21.5	-24.9	1.5	0.3
	1604	II	-19.5	-27.3	0.6	0.3
	1606	II	-21.3	-29.7	0.4	0.5

	2601	II	-25.7	-29.2	1.3	1.4
	2602	II	-22.7	-26.6	0.9	1.4
	2604	II	-22.5	-33.2	0.6	1.4
	2605	II	-23.0	-29.8	0.8	1.4

	2606	II	-23.2	-28.5	6.2	0.7
	2607	II	-22.6	-32.1	6.6	0.7
	2608	II	-21.1	-31.8	0.7	0.4
	2609	II	-26.5	-29.7	0.3	0.6