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Discovery of disubstituted xylylene derivatives as small molecule direct inhibitors of Keap1-Nrf2 protein-protein interaction

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ABSTRACT

The Keap1–Nrf2–ARE system represents a crucial antioxidant defense mechanism that protects cells against reactive oxygen species. Targeting Keap1–Nrf2 protein–protein interaction (PPI) has become a promising drug target for several oxidative stress-related and inflammatory diseases including pulmonary fibrosis, chronic obstructive pulmonary disorder (COPD) and cancer chemoprevention. For the development of a potential therapeutic agent, drug-like properties and potency are important considerations. In this work, we focused on the modification of **4** as a lead through a molecular dissection strategy in an effort to improve its metabolic stability, leading to the discovery of a series of new disubstituted xylylene derivatives. The preliminary SAR of **9a** indicated that compound **21a** containing *S*-methylated acetate moieties exhibited comparable potency to the lead compound **4** in a fluorescent polarization assay but with improved metabolic stability in the presence of human liver microsomes.

1. Introduction

The human body is continuously subjected to numerous electrophilic and oxidative chemicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS).[1–3] These oxidants can cause damage to cellular components and lead to cell death and disease. Fortunately, our body's antioxidant defense system can mitigate the damaging effects of these reactive species (ROS, RNS) by either directly reducing the reactive species through endogenous or dietary antioxidants or using a more efficient catalytic detoxification process via different antioxidant enzymes.[4,5] Inducible expression of these antioxidant cellular defense enzymes is under the control of the Keap1-Nrf2-ARE transcription pathway. Nuclear factor erythroid 2–related factor 2 (Nrf2) is a transcription factor which is an essential player in the inducible expression of cytoprotective enzymes.[6–9] Kelch-like ECH-associated protein 1 (Keap1) is a cytoplasmic protein that negatively regulates the activity of Nrf2 protein. Keap1 is an adaptor protein for Cullin3-dependent E3 ubiquitin ligase. Keap1 binds to and promotes the ubiquitination of Nrf2, which is followed by degradation of Nrf2 by the 26S proteasome.[10–12]

Inhibition of Keap1–Nrf2 protein–protein interaction (PPI) has become a promising therapeutic strategy for several oxidative stress conditions.[13,14] There are two types of inhibitors of the Keap1-Nrf2 PPI, namely indirect and direct inhibitors based on their mechanism of action. Examples of indirect inhibitors of Keap1–Nrf2 PPI include sulforaphane, an isothiocyanate compound found in cruciferous vegetables (e.g. broccoli);[15] bardoxolone methyl, a synthetic triterpenoid that has been under clinical trials for various diseases including diabetes and chronic kidney diseases;[16] and dimethyl fumarate, Tecfidera®, an FDA approved drug for the treatment of multiple sclerosis.[17] Although these indirect inhibitors of Keap1–Nrf2 PPI are effective Nrf2 activators, these electrophiles are not selective for Keap1 leading to possible off-target toxicity. An example of the lack of selectivity of currently known Nrf2 activators is bardoxolone methyl which entered a phase III clinical trial for the treatment of patients with type 2 diabetes and patients with chronic kidney disease. However, development of this drug was terminated due to its adverse cardiovascular effects.[18,19]

Recently, there has been a great interest in developing non-electrophilic direct inhibitors of Keap1-Nrf2 PPI that may be more selective for Nrf2 activation and thus have less off-target toxicities (Fig. 1).[1,2,20–24] High-throughput screening (HTS) of the MLPCN library using a fluorescence polarization (FP) assay developed in our lab identified the first-in-class small molecule direct inhibitor of the Keap1-Nrf2 PPI (LH601A, **1**, IC_{50} = 3 μM).[2] Other hits, identified by Silvian

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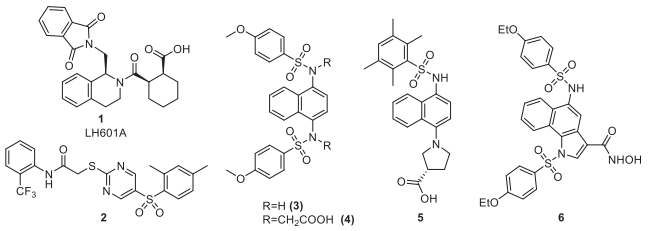


Fig. 1. Chemical structures of a few direct inhibitors of Keap1-Nrf2 PPI.

and colleagues at Biogen through HTS, are a thiopyrimidine derivative, **2** (IC_{50} = 118 μM) and a naphthalene derivative (compound **3**, IC_{50} = 2.7 μM).[1,18] The *bis*-acidic naphthalene derivative **4** derived from compound **3** was reported by You and colleagues as a potent inhibitor of the Keap1–Nrf2 PPI with IC_{50} of 28.6 nM in an FP assay.[1,25] Other examples of small molecules that bind directly to the Nrf2-interacting Kelch domain of Keap1 are **5** (RA839) with an IC_{50} = 0.14–0.2 μM (FP) and **6** with an IC_{50} = 0.2 μM (FP).[26]

Although compound **4** is a very potent inhibitor of Keap1-Nrf2 PPI, its metabolic stability was a concern. In this work, we report the design, synthesis, and evaluation of a series of xylylene-based *bis*-sulfonamides as direct inhibitors of Keap1-Nrf2 PPI with improved metabolic stability. The optimized compound **21a** exhibited an IC_{50} = 0.15 ± 0.016 μM which is comparable to the IC_{50} of 0.11 ± 0.01 μM for compound **4** under our FP assay conditions but with significantly higher metabolic stability.

2. Results and discussion

2.1. Design strategy

Compound **4** was reported as a potent inhibitor of Keap1-Nrf2 PPI, which exhibited an IC_{50} of 0.11 ± 0.01 μM under our FP assay conditions. The presence of the two nitrogen atoms directly attached to the 1,4-positions of the naphthalene ring as in **4** may cause metabolic stability concerns.[27] With the aim of identifying a potent and metabolically stable Keap1 − Nrf2 PPI, we explored the SAR on the naphthalene ring of compound **4.** Molecular dissection of **4** may help improve its lead-like properties with retention of the desired biological activity. In this approach, we removed one of the phenyl rings in the naphthalene moiety of lead **4** to give compound **9a**, along with its positional regioisomers **9b** and **9c** as shown in Fig. 2. Compound **14** was designed to add a phenyl ring back on the other side. In addition, we explored the importance of the sulfonamide linker in the design of compounds **18a-b** (Fig. 2).

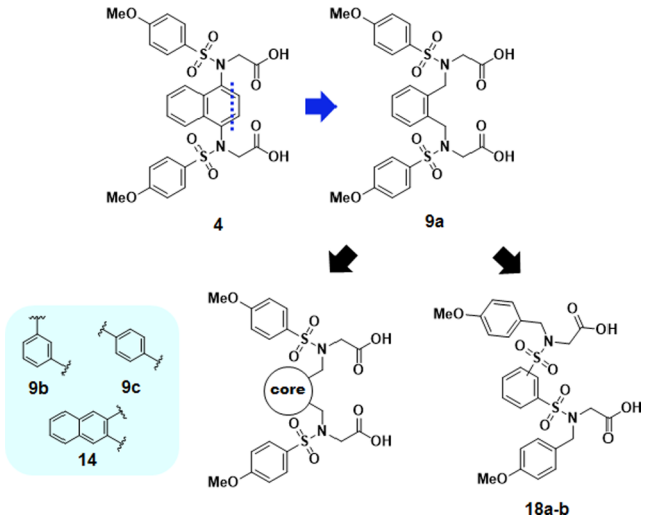
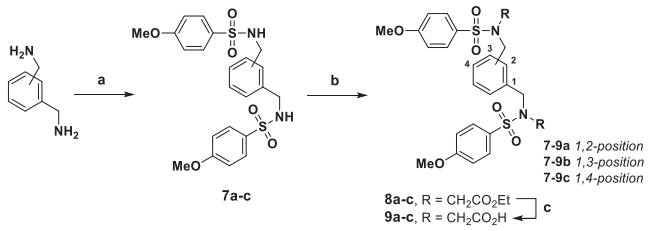


Fig. 2. Design of new analogs based on the modification of *bis*-acidic naphthalene inhibitor **4**.



Scheme 1. Synthesis of disubstituted xylylene derivatives **9a-c**[a]. [a]Reagents and conditions: (a) 4-methoxybenzenesulfonly chloride, TEA, DCM, rt, overnight, 75–82%; (b) ethyl 2-bromoacetate, K_2CO_3, DMF, rt, overnight, 80–87%; (c) NaOH, EtOH, H_2O, rt, 72–80%.

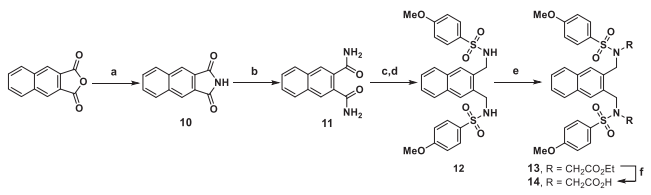
2.2. Chemistry

As outlined in Scheme 11,2–1,3- and 1,4-disubstituted xylylene analogs **9a-c** were synthesized using commercially available diamine starting material which was treated with 4-methoxybenzenesulfonyl chloride in the presence of triethylamine as a base in anhydrous dichloromethane at room temperature to give sulfonamide intermediates **7a-c**. Alkylation of sulfonamide intermediates with ethyl bromoacetate in DMF at room temperature followed by removal of the ester protecting group under basic condition to give final compounds **9a-c**.

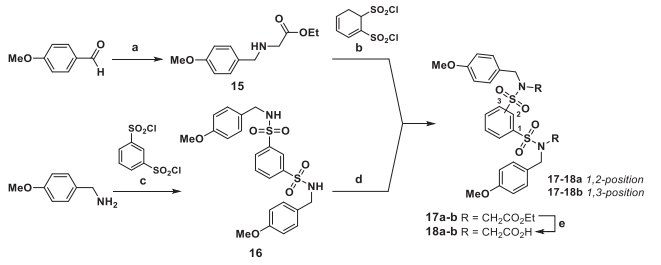
The naphthalene analog **14** was synthesized using commercially available 2,3-naphthalenedicarboxylic anhydride as starting material (Scheme 2). Treatment of anhydride with urea at 170 °C using a microwave reactor gave phthalimide intermediate **10** which was then treated with ammonia in DMF at 50 °C to give diamide intermediate **11**. Reduction of the diamide using $BH_3{\cdot}Me_2S$ in anhydrous THF under reflux gave diamine intermediate which was treated with 4-methoxybenzenesulfonyl chloride to give sulfonamide derivative **12**. Alkylation of the sulfonamide with ethyl bromoacetate followed by hydrolysis with 2 N NaOH gave final compound **14**.

The target compounds **18a-b** were prepared according to Scheme 3. Firstly, 1,2-disubstituted phenyl analogue **17a** was synthesized by reductive amination of 4-anisaldehyde with ethyl glycinate hydrochloride, followed by *N*-sulfonylation with benzene-1,2-disulfonyl chloride. The 1,3-disubstituted intermediate **17b** was obtained in two steps including *N*-sulfonylation of 4-methoxybenzylamine and subsequent alkylation of **16** with ethyl bromoacetate. The diacid analogs **18a-b** were finally synthesized by base-mediated hydrolysis of the two ethyl ester groups of **17a-b**.

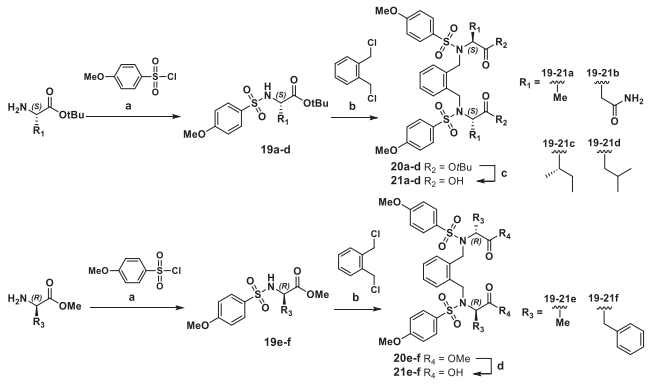
The preparation of compounds **21a-f** containing modified acetates is summarized in Scheme 4. Intermediates **19a-f** were first synthesized by treatment of L-amino acid *tert*-butyl ester or D-amino acid methyl ester starting materials with 4-methoxybenzenesulfonyl chloride. Nucleophilic substitution of **19a-f** with α,ά-dichloro-*o*-xylene afforded intermediates **20a-f**. While **21a-d** with *S,S*-configuration were obtained by TFA-mediated acid hydrolysis, **21e-f** with *R,R*-configuration were



Scheme 2. Synthesis of 1,2-disubstituted naphthalene derivative **14**[a]. [a]Reagents and conditions: (a) Urea, 170 °C (microwave), 2 h, 90%; (b) NH_3, DMF, rt to 50 °C, 8 h, 67%; (c) $BH_3{\cdot}Me_2S$ complex, THF, reflux, 2 d, 63%; (d) 4-methoxybenzenesulfonly chloride, TEA, DCM, rt, overnight, 63%; (e) ethyl 2-bromoacetate, K_2CO_3, DMF, rt, overnight, 87%; (f) NaOH, EtOH, H_2O, rt, 79%.



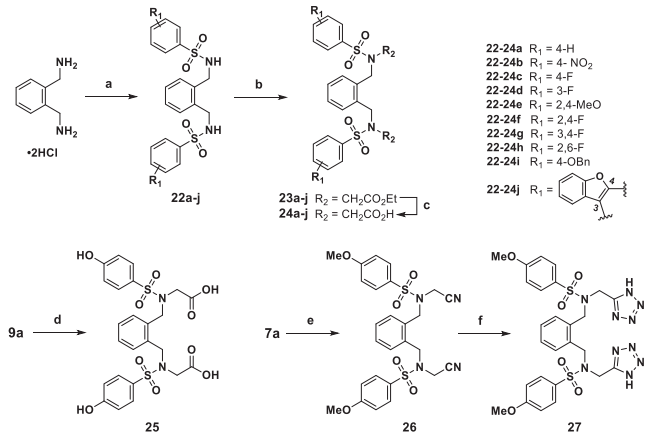
Scheme 3. Synthesis of compounds **18a-b**[a]. [a]Reagents and conditions: (a) ethyl glycinate HCl, $NaBH_3CN$, EtOH, rt, overnight, 51%; (b) K_2CO_3, DMF, rt, overnight, 71%; (c) TEA, DCM, rt, overnight, 81%; (d) ethyl 2-bromoacetate, K_2CO_3, DMF, rt, overnight, 79%; (e) NaOH, EtOH, H_2O, rt, 87%.



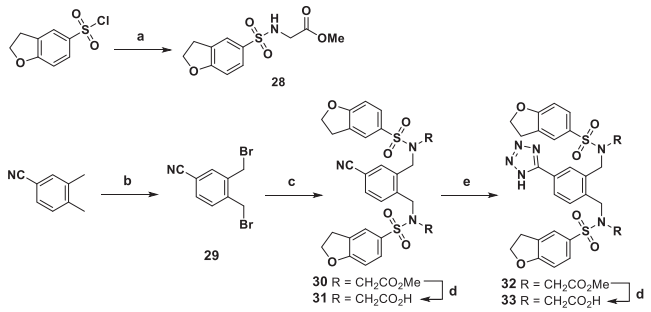
Scheme 4. Synthesis of compounds **21a-f**[a]. [a]Reagents and conditions: (a) TEA, DCM, rt, overnight, 83–92%; (b) K_2CO_3, DMF, 50 °C, overnight, 68–79%; (c) TFA, DCM, 0 °C to rt, 1 h, 72–77%; (d) NaOH, EtOH, H_2O, rt, 78–79%.

prepared by base-mediated hydrolysis using NaOH.

The synthetic route for target compounds **24a-j** and **25** bearing various substituents on the phenyl ring of the benzenesulfonamide moiety is outlined in Scheme 5. *o*-Xylylenediamine dihydrochloride, as a starting material, was treated with different benzenesulfonyl chlorides using triethylamine as a base in anhydrous dichloromethane or acetonitrile at room temperature to give sulfonamide intermediates **22a-j**. Alkylation of **22a-j** with ethyl bromoacetate in DMF at room temperature followed by removal of the ester protecting group under basic conditions gave final compounds **24a-j**. Compound **25** with



Scheme 5. Synthesis of compounds **24a-j**, and **25**-**27**[a]. [a]Reagents and conditions: (a) R_1-substituted benzenesulfonyl chloride, TEA, DCM or ACN, rt, overnight, 62–79%; (b) ethyl 2-bromoacetate, K_2CO_3, DMF, 50 °C, overnight, 69–83%; (c) NaOH, EtOH, H_2O, rt, 65–81%; (d) BBr_3, DCM, rt, overnight, 12%; (e) bromoacetonitrile, K_2CO_3, DMF, rt, overnight, 71%; (f) NaN_3, NH_4Cl, DMF, 120 °C, 4 h, 90%.



Scheme 6. Synthesis of compounds **31** and **33**[a]. [a]Reagents and conditions: (a) glycine methyl ester HCl, TEA, DCM, rt, overnight, 80%; (b) NBS, AIBN, CH_3Cl, 75 °C, overnight, 84%; (c) **28**, K_2CO_3, DMF, rt, overnight, 82%; (d) NaOH, EtOH, H_2O, rt, 74–84%; (e) NaN_3, NH_4Cl, DMF, 85 °C, overnight, 72%.

phenolic groups was prepared by demethylation of the methyl ethers of **9a**. In order to get a tetrazole analog, sulfonamide intermediate **7a** was treated with bromoacetonitrile in DMF at room temperature, and then the resulting alkylated sulfonamide **26** was treated with NaN_3 in presence of NH_4Cl in DMF at 120 °C to get the tetrazole analog **27** (Scheme 5).

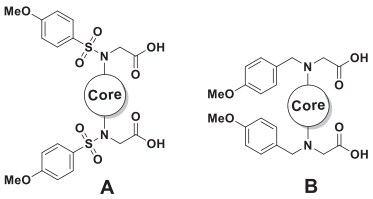
Synthesis of derivatives with cyano or tetrazole substitution on the central benzene ring (**31** or **33**) is summarized in Scheme 6. Sulfonamide intermediate **28** was first prepared by treatment of 2,3-dihydrobenzofuran-5-sulfonyl chloride with glycine methyl ester hydrochloride. Bromination of commercially available *o*-xylene with NBS/AIBN in chloroform as a solvent at 75 °C using a sealed tube gave brominated intermediate **29**, which was then treated with compound **28** using K_2CO_3 in DMF at room temperature to give compound **30.** Base-mediated hydrolysis using 2 N NaOH gave final compound **31**. Treatment of intermediate **30** with sodium azide at 85 °C followed by base-mediated hydrolysis gave final compound **33**.

2.3. Biological evaluation using an FP assay and structure-activity relationship (SAR)

All analogs synthesized were tested for their inhibitory effect against Keap1-Nrf2 PPI using our FP assay.[28] Initially, two or three concentrations (0.5, 5 and 50 μM) were used to compare the potency of all synthesized analogs. For more potent compounds, a full dose-response curve was generated to derive IC_{50} values. The inhibitory activity of xylylene (**9a-c** and **14**), naphthalene (**4**), and phenyl (**18a-b**) analogs against Keap1-Nrf2 PPI are summarized in Table 1 and Fig. 3. The IC_{50} for compound **4** under our FP assay conditions was 110 nM. 1,2-Disubstituted xylylene analog **9a** with IC_{50} of 2.3 ± 0.24 μM was more potent than 1,3- and 1,4-disubstituted compounds (**9b** and **9c**). Introducing a phenyl ring to the left side of the core-phenyl ring of compound **9a** gave compound **14** which demonstrated a decrease in inhibitory activity compared to compound **9a**. This decrease in activity may indicate that this substitution is not tolerated. Compounds **18a-b** containing *N*-benzyl linkers connected to the core benzene-1,2-disulfonyl moiety was no activity up to 50 μM, indicating the importance of the positioning of the sulfonamide linker. Therefore, we decided to maintain the arrangement of this moiety as in **4** in subsequent series. Compared to the naphthalene analog **4**, 1,2-disubstituted xylylene analog **9a** exhibited weaker inhibitory effect. Since our goal was to mitigate the metabolic stability concerns associated with two nitrogen-containing substituents at the para positions of a phenyl ring,[27] we decided to pursue further structure-activity relationship studies to optimize the 1,2-disubstituted xylylene analog **9a** with IC_{50} of 2.30 ± 0.24 μM, where the two nitrogen atoms are not directly attached to the phenyl ring.

Another important region to be explored is the acetate groups attached to sulfonamide moiety. The inhibitory activity of amino acid

Table 1
Inhibitory activity of different disubstituted xylylene, phenyl, and naphthalene analogs.[a]



Compd	Series	Core	% Inhibition		IC_{50} (μM)[b]
			50 μM	5 μM	
4	A		100	100	0.11 ± 0.01
9a (LH769)	A		101	79	2.30 ± 0.24
9b (LH770)	A		19	14	ND
9c (LH771)	A		10	6	ND
14 (LH791)	A		58	25	ND
18a (LH787)	B		15	14	ND
18b (LH788)	B		9	12	ND

[a] Inhibitory activity was determined by FP assay, and all experiments were performed in triplicates. ND: not determined.
[b] IC_{50} values are reported as an average of three replicates ± SEM.

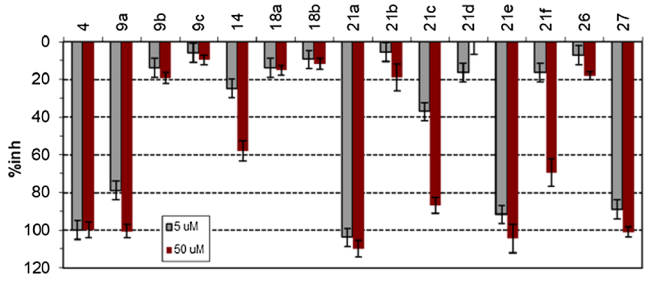
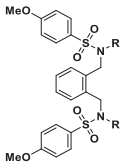


Fig. 3. The inhibitory activities of different disubstituted xylylene and naphthalene analogs. Data represents the average of three replicates ± SEM.

derived analogs **21a-f** on Keap-Nrf2 PPI was also evaluated using our FP assay (Table 2 and Fig. 3). Introducing a methyl group with *S,S*-configuration to the alpha carbon resulted in an improvement in the inhibitory activity by approximately 15-fold as in L-alanine derivative **21a**, compared with compound **9a** based on their IC_{50} values, while D-alanine derivative **21e** with *R,R*-configuration was less potent in comparison to compound **21a**. Subsequently, we increased the number of carbon atoms and maintained *S,S* stereochemistry as in L-isoleucine and L-leucine derivatives (**21c** and **21d**). This modification resulted in a decrease in activity of our analogs. In addition, L-asparagine **21b**, which had amide moieties with *S,S*-configuration, and D-phenylalanine analog **21f** with *R,R*-configuration were less potent than **9a**. The important point in this SAR is that introducing chiral centers resulted in a more potent compound **21a** suggesting that conformational restriction placed the carboxylic acid moieties in a more proper orientation for binding

Table 2
Inhibitory activity of different disubstituted xylylene analogs with modified acetate moieties.[a]



Compd	R	% inhibition			IC_{50} (μM)[b]
		50 μM	5 μM	0.5 μM	
9a (LH769)		101	79	ND	2.30 ± 0.24
21a (LH945)		110	104	101	0.15 ± 0.02
21b (LH968)		19	5	ND	ND
21c (LH946)		87	37	ND	ND
21d (LH947)		21	17	ND	ND
21e (LH967)		105	92	44	0.62 ± 0.03
21f (LH887)		70	16	ND	ND
26 (LH783)		18	7	ND	ND
27 (LH785)		101	89	ND	1.17 ± 0.50

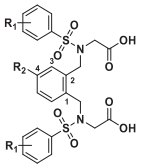
[a] Inhibitory activity was determined by FP assay, and all experiments were performed in triplicates. ND: not determined.
[b] IC_{50} values are reported as an average of three replicates ± SEM.

and/or the extra small methyl group enhanced its binding to Keap1 Kelch domain. Interestingly, the replacement of the two carboxylic acid with cyano groups as in **26** led to loss of most of its inhibitory potency, whereas bioisosteric replacement of carboxylic acid moiety with tetrazole as in **27** resulted in a slight improvement in activity with IC_{50} of 1.17 ± 0.5 μM (Table 2 and Fig. 3).

Next, we explored the 4-methoxyphenyl moiety. Modifications of this group might improve not only the Keap1 Kelch domain binding affinity, but also the physicochemical properties such as lipophilicity, leading to improvement in cell-based and *in vivo* activity. Different electron-donating and electron-withdrawing groups were introduced at varying positions on the phenyl ring linked to the sulfonamides. As shown in Table 3 and Fig. 4, removal of a methoxy or methyl group (**24a** or **25**) led to a reduction in activity (< 40% inhibition at 5 μM), suggesting the importance of the substitution on the phenyl ring. Replacement of a methoxy group with a nitro (**24b**) or fluoro group (**24c-d**) reduced the activity by at least 16% at 5 μM. Introducing hydrophobic moieties in the *para*-position of the aryl sulfonamide moiety as in **24i** (benzyloxy) and **24j** (dibenzofuran) resulted in improvements in the activity which may be due to π-π interactions with hydrophobic residues such as Phe525 and Phe577 in the binding site. Among disubstituted phenyl ring analogs such as 2,4-methoxy (**24e**) and difluoro groups (**24f-h**), only 2,6-difluoro analog (**24 h**) led to improvement in activity with IC_{50} of 1.78 ± 0.4 μM.

We then turned our attention to modifying the R_2 substituent at the 4-position on the central benzene ring (Table 3). The effect of introducing a cyano (**31**) or tetrazole (**33**) moiety on the activity of **9a** was evaluated using our FP assay (Table 3 and Fig. 4). These analogs possessed weaker activity by more than 44% at 5 μM as compared to **9a**. This indicated that substitutions at this position on the central

Table 3
Inhibitory activity of different disubstituted xylylene analogs.[a]



Compd	R_1	R_2	% inhibition		IC_{50}(μM)[b]
			50 μM	5 μM	
9a (LH769)	4-OMe	H	101	79	2.30 ± 0.24
24a (LH786)	4-H	H	76	38	ND
24b (LH790)	4-NO_2	H	96	63	ND
24c (LH811)	4-F	H	84	51	ND
24d (LH810)	3-F	H	87	39	ND
24e (LH805)	2,4-OMe	H	96	58	ND
24f (LH807)	2,4-F	H	92	69	ND
24 g (LH809)	3,4-F	H	94	38	ND
24 h (LH806)	2,6-F	H	98	87	1.78 ± 0.40
24i (LH886)	4-OBn	H	94	82	0.57 ± 0.04
24j (LH883)		H	98	96	0.42 ± 0.03
25 (LH813)	4-OH	H	84	36	ND
31 (LH884)	4-OMe	CN	86	35	ND
33 (LH885)	4-OMe		57	25	ND

[a] Inhibitory activity was determined by FP assay, and all experiments were performed in triplicates. ND: not determined.
[b] IC_{50} values are reported as an average of three replicates ± SEM.

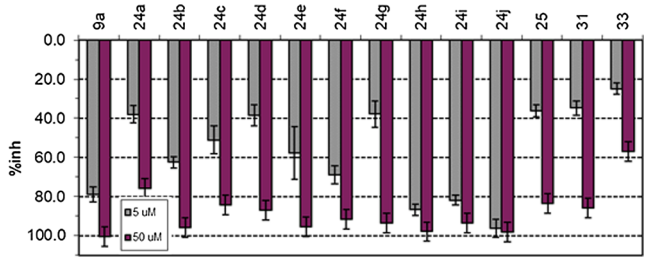


Fig. 4. The inhibitory activities of different disubstituted xylylene analogs. Data represents the average of three replicates ± SEM.

benzene ring may not be appropriate for binding to the Keap1 Kelch domain.

2.4. Metabolic stability of **9a** and **21a** in the presence of human liver microsomes

Metabolic liability is an important consideration in the early stage of drug discovery.[29] Therefore, we evaluated the metabolic stability of compound **4**, the 1,2-disubstituted xylene analog **9a**, and the most potent compound in this series compound **21a** in the presence of human liver microsomes. After 90 min of incubation, **9a** and **21a** were stable with only slight changes (97.7% and 98.2% remained, respectively), while the stability of **4** was moderate with only 56.9% remaining after 90 min of incubation as shown in Fig. 5. The greater stability of the xylylene derivatives in human liver microsomes suggests that this class of compounds may be preferable over the naphthalene derivative in terms of metabolic stability.

2.5. Molecular docking simulation

In an effort to model the effect of the *S*-methyl group on binding to

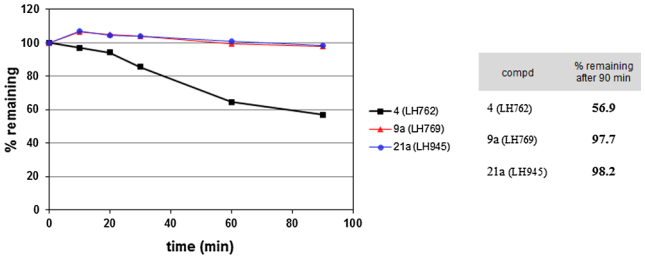


Fig. 5. The metabolic stability in human liver microsomes of compounds **4**, **9a** and **21a**.

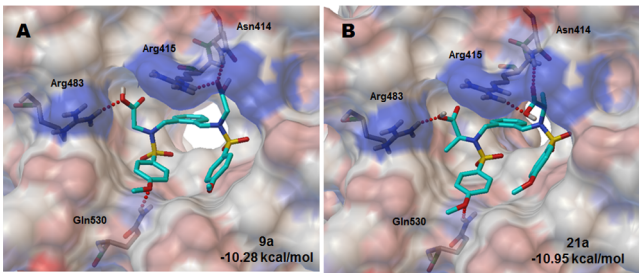


Fig. 6. Docking binding modes of disubstituted xylylene derivatives (represented in cyan sticks) bound to the Keap1 Kelch domain (derived from PDB code: 4XMB); (A) **9a**, (B) **21a**. The surface of Keap1 was colored according to the partial charge of the amino acid side chains (represented in red for the lipophilic areas, and blue for polar areas). Red dashed lines represent hydrogen bonds.

the Keap1 Kelch domain, we carried out molecular docking studies of disubstitued xylylene derivatives **9a** and **21a** to the Keap1 Kelch domain using its X-ray co-crystal structure with a small molecule ligand, 2,2′-(naphthalene-1,4-diylbis(((4-methoxyphenyl)sulfonyl)azanediyl)) diacetamide (PDB code: 4XMB).[18] As shown in Fig. 6, the binding poses with the highest binding scores suggested that **9a** and **21a** displayed a similar spatial orientation in the binding site of Keap1 Kelch domain through a combination of various binding interactions. The two acetate groups of the xylylene derivatives formed hydrogen bond interactions with the side chains of key polar residues (Asn414, Arg415 and Arg483), resulting in strong binding affinities to the Keap1 protein. Additionally, the methoxy group on the sulfonamide moiety made a hydrogen bond interaction with Gln530. Introduction of the *S*-methyl group of **21a** at α-carbon appeared to be associated with a good shape fit toward the pore, resulting in improved binding energy to the Keap1 Kelch domain over **9a** (−10.95 vs −10.28 kcal/mol) as along with a boost in the inhibitory activity against Keap1-Nrf2 PPI under our FP assay conditions (IC_{50} = 0.15 vs 2.3 μM).

3. Conclusions

We performed a detailed SAR study around lead **4** to determine the structural requirements for binding to Keap1 Kelch domain. Removal of one phenyl ring from the naphthalene in **4** led to a reduction in inhibitory activity of Keap1-Nrf2 PPI suggesting the importance of this core structure in Keap1 Kelch domain binding. Positional modification of the two substitutions on the xylylene core structure showed that 1,2-disubstitution (**9a**) was the optimal pattern of substitution, and that substitution with cyano (**31**) or tetrazole (**33**) at the 4-position resulted in weaker activity. These studies also showed that the position of the sulfonamide moieties was important for optimal activity. When a substitution was made on the aryl sulfonamide moieties, the hydrophobic substituents (**24i-j**) at the *para* position and the 2,6-difluoro substitution (**24h**) exhibited an improvement in activity. The SAR studies on the diacetate moiety indicated that replacement with tetrazole groups maintained potency. Importantly, introduction of the *S*-methyl group

(**21a**) on the alkyl side chain resulted in a promising increase in activity with IC_{50} of 0.15 ± 0.02 μM, which is similar to that of **4** under our FP assay conditions. Based on the docking results, the *S*-methyl group of 21a also served to fit this compound better in the binding site of the Keap1 Kelch domain, contributing to its stronger binding affinity in terms of binding energy. Lastly, compounds **9** and **21a** exhibited higher metabolic stability in human liver microsomes as compared to **4**. These xylylene compounds can be used as leads for further optimization in our search for novel and more potent direct inhibitors of Keap1-Nrf2 PPI.

4. Experimental section

4.1. Chemistry

Reagents purchased were ACS grade and used without further purification. Solvents purchased were either HPLC grade or ACS reagent grade. All reactions were monitored by thin-layer chromatography (TLC) using aluminum backed Silica G TLC plates and visualized with ultraviolet light, a Shimadzu 2010 LC-MS system, and/or Agilent 1200 HPLC system coupled with an Agilent 6140 Quadrupole MS system (Santa Clara, CA). Flash column chromatography was done on a Teledyne ISCO CombiFlash Companion using ethyl acetate, dichloromethane, hexane, and methanol as mobile phases with prepacked silica gel columns as the stationary phase. Lyophilization of the final compounds was performed on a VirTis freezemobile freeze dryer. ^{1}H NMR spectra (400 MHz) and ^{13}C NMR spectra (100 MHz) were recorded on Bruker 400 MHz Multinuclear NMR spectrometer using $CDCl_3$, acetone-d_6, methanol-d_4, and DMSO-d_6. NMR data is reported in parts per million (ppm) relative to the residual nondeuterated solvent signals. High-resolution mass spectra (HRMS) experiments were conducted by the Center for Integrative Proteomics Research (CIPR) at Rutgers University.

4.1.1. General procedure for basic hydrolysis of ethyl ester (method C1)

To a solution of alkylated sulfonamide in EtOH (2 mL), 4 N NaOH in water (2 mL) was added and the reaction mixture was stirred at room temperature. Upon completion, the reaction mixture was diluted with ethyl acetate, washed with 1 N HCl, water, and brine. Then, the organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to yield crude product, which was recrystallized using ethyl acetate/hexane, and washed with petroleum ether to afford pure final product.

4.1.2. General procedure for acidic hydrolysis of tert-butyl ester (method C2)

A solution of alkylated sulfonamide in DCM (0.5 mL) was cooled to 0 °C. A solution of TFA:DCM (1:2) was then added dropwise (1.5 mL). The reaction mixture was stirred at room temperature for 1 h. Upon completion, the reaction mixture was concentrated under reduced pressure to yield crude product, which was recrystallized using ethyl acetate/hexane or ethyl ether to afford pure final product.

4.1.3. 2,2′-((1,2-phenylenebis(methylene))bis(((4-methoxyphenyl) sulfonyl)azanediyl))diacetic acid (**9a**)

Prepared as described in the general procedure for basic hydrolysis of ethyl ester (Method C1) to get the title compound as a white solid (39 mg, 78% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.64 (s, 2H), 7.77 (d, J = 8.8 Hz, 4H), 7.29 (s, 2H), 7.25 (s, 2H), 7.11 (d, J = 8.8 Hz, 4H), 4.45 (s, 4H), 3.86 (s, 6H), 3.78 (s, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.8, 162.6, 134.4, 130.6, 129.4, 128.7, 127.6, 114.4, 55.7, 49.1, 48.3; HRMS (ESI) Calcd for $C_{26}H_{29}N_2O_{10}S_2$ $(M+H)^+$ 593.1258, found 593.1258.

4.1.4. 2,2′-((1,3-Phenylenebis(methylene))bis(((4-methoxyphenyl) sulfonyl)azanediyl))diacetic acid (**9b**)

Prepared as described in the general procedure for basic hydrolysis of ethyl ester (Method C1) to get the title compound as a white solid (36 mg, 72% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.74 (s, 2H), 7.76 (d, J = 8.8 Hz, 4H), 7.25 (t, J = 8.0 Hz, 1H), 7.12 (d, J = 8.8 Hz, 2H), 7.09 (d, J = 8.8 Hz, 4H), 6.96 (s, 1H), 4.33 (s, 4H), 3.84 (s, 6H), 3.78 (s, 4H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.8, 162.5, 136.0, 131.3, 129.3, 128.6, 128.0, 127.6, 114.3, 55.7, 50.9, 47.4; HRMS (ESI) Calcd for $C_{26}H_{28}N_2O_{10}S_2$ $(M+H)^+$ 593.1258, found 593.1254.

4.1.5. 2,2′-((1,4-Phenylenebis(methylene))bis(((4-methoxyphenyl) sulfonyl)azanediyl))diacetic acid (**9c**)

Prepared as described in the general procedure for basic hydrolysis of ethyl ester (Method C1) to get the title compound as a white solid (36 mg, 72% yield). ^{1}H NMR (400 MHz, MeOH-d_4): δ 7.79 (d, J = 9.0 Hz, 4H), 7.12 (s, 4H), 7.06 (d, J = 9.0 Hz, 4H), 4.36 (s, 4H), 3.84 (s, 6H), 3.64 (s, 4H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.5, 162.5, 135.6, 131.9, 129.5, 128.5, 114.4, 55.8, 50.6, 48.2; HRMS (ESI) Calcd for $C_{26}H_{28}N_2O_{10}S_2$ $(M+H)^+$ 593.1258, found 593.1252.

4.1.6. 2,2′-((Naphthalene-2,3-diylbis(methylene))bis(((4-methoxyphenyl) sulfonyl)azanediyl))diacetic acid (**14**)

Prepared as described in the general procedure for basic hydrolysis of ethyl ester (Method C1) to get the title compound as a yellow solid (51 mg, 79% yield). ^{1}H NMR (400 MHz, MeOH-d_4): δ 7.80 (d, J = 9.0 Hz, 4H), 7.74–7.72 (m, 4H), 7.47–7.44 (m, 2H), 7.02 (d, J = 9.0 Hz, 4H), 4.71 (s, 4H), 3.89 (s, 4H), 3.86 (s, 6H). ^{13}C NMR (100 MHz, MeOH-d_4): δ 164.7, 134.2, 133.3, 132.1, 130.9, 130.1, 128.6, 127.4, 115.3, 56.2, 51.5; HRMS (ESI) Calcd for Calcd for $C_{30}H_{30}N_2O_{10}S_2$ $(M+H)^+$ 643.1415, found 643.1401.

4.1.7. 2,2′-((1,2-Phenylenedisulfonyl) bis((4-methoxybenzyl)azanediyl)) diacetic acid (**18a**)

Prepared as described in the general procedure for basic hydrolysis of ethyl ester (Method C1) to get the title compound as a beige solid (11 mg, 87% yield). ^{1}H NMR (400 MHz, MeOH-d_4): δ 8.34–8.31 (m, 2H), 7.75–7.73 (m, 2H), 7.09 (d, J = 8.8 Hz, 4H), 6.38 (d, J = 8.8 Hz, 4H), 4.52 (s, 4H), 4.05 (s, 4H), 3.75 (s, 6H); ^{13}C NMR (100 MHz, MeOH-d_4): δ 172.4, 161.1, 140.2, 134.0, 133.4, 131.2, 128.2, 115.1, 55.7, 52.1; HRMS (ESI) Calcd for $C_{26}H_{28}N_2O_{10}S_2$ $(M+H)^+$ 591.1258, found 593.1254.

4.1.8. 2,2′-((1,3-Phenylenedisulfonyl)bis((4-methoxybenzyl)azanediyl)) diacetic acid (**18b**)

Prepared as described in the general procedure for basic hydrolysis of ethyl ester (Method C1) to get the title compound as an off-white solid (14 mg, 87% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.80 (s, 2H), 8.24 (s, 1H), 8.14 (d, J = 8.0 Hz, 2H), 7.80 (t, J = 8.0 Hz, 1H), 7.13 (d, J = 8.0 Hz, 4H), 6.86 (d, J = 8.0 Hz, 4H), 4.35 (s, 4H), 3.87 (s, 4H), 3.72 (s, 6H); ^{13}C NMR (100 MHz, DMSO- d_6): δ 169.7, 159.0, 140.9, 131.2, 130.5, 129.9, 126.9, 125.3, 113.9, 55.0, 50.6, 47.1; HRMS (ESI) Calcd for $C_{26}H_{28}N_2O_{10}S_2$ $(M+H)^+$ 593.1258, found 593.1253

4.1.9. (2S,2′S)-2,2′-((1,2-Phenylenebis(methylene))bis(((4-methoxyphenyl)sulfonyl)azanediyl))dipropio nic acid (**21a**)

Prepared as described in the general procedure for acidic hydrolysis of *tert*-butyl ester (Method *C*2) to get the title compound as a white solid (23 mg, 74% yield). ^{1}H NMR (400 MHz, $(CD_3)_2$ CO): δ 7.82 (d, J = 9.0 Hz, 4H), 7.53–7.51 (m, 2H), 7.23–7.21 (m, 2H), 7.05 (d, J = 9.0 Hz, 4H), 4.61 (s, 4H), 4.52 (q, J = 7.2 Hz, 4H), 3.89 (s, 6H), 1.31 (d, J = 7.2 Hz, 6H); ^{13}C NMR (100 MHz, $(CD_3)_2$ CO): δ 172.5, 163.7, 136.1, 132.7, 130.4, 129.2, 127.7, 114.8, 55.9, 55.9, 47.1, 16.3; HRMS (ESI) Calcd for $C_{28}H_{32}N_2O_{10}S_2$ $(M+H)^+$ 621.1571, found 621.1567.

*4.1.10. (2S,2′S)-2,2′-((1,2-Phenylenebis(methylene))bis(((4-methoxyphenyl)sulfonyl)azanediyl))bis(4-amino-4-oxobutanoic acid) (**21b**)*

Prepared as described in the general procedure for acidic hydrolysis of ethyl ester (Method *C*2) to get the title compound as a white solid (33 mg, 77% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.77 (d, J = 8.4 Hz, 4H), 7.30–7.28 (m, 4H), 7.17–7.14 (m, 2H), 7.06 (d, J = 8.8 Hz, 4H), 6.85 (br, 2H), 4.54–4.51 (m, 2H), 4.48–4.24 (m, 4H), 3.84 (s, 4H), 2.80–2.73 (m, 2H), 2.21–2.16 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.1, 170.5, 162.5, 134.4, 130.9, 129.6, 128.5, 127.1, 114.2, 56.1, 55.6, 47.0, 35.1; HRMS (ESI) Calcd for $C_{30}H_{34}N_4O_{12}S_2$ $(M+H)^+$ 707.1687, found 707.1663.

*4.1.11. (2S,2′S,3S,3′S)-2,2′-((1,2-Phenylenebis(methylene))bis(((4-methoxyphenyl)sulfonyl)azanediyl)) bis(3-methylpentanoic acid) (**21c**)*

Prepared as described in the general procedure for basic hydrolysis of *tert*-butyl ester (Method C1) to get the title compound as a white solid (32 mg, 72% yield). ^{1}H NMR (400 MHz, $(CD_3)_2$ CO): δ 7.74 (d, J = 8.8 Hz, 4H), 7.56–7.53 (m, 2H), 7.14–7.12 (m, 2H), 6.98 (d, J = 8.8 Hz, 4H), 4.90 (d, J = 16.8 Hz, 2H), 4.74 (d, J = 16.8 Hz, 2H), 4.22 (d, J = 10.0 Hz, 2H), 3.87 (s, 6H), 1.65–1.58 (m, 4H), 0.96–0.88 (m, 2H), 0.82 (d, J = 6.8 Hz, 6H), 0.57 (t, J = 7.6 Hz, 6H); ^{13}C NMR (100 MHz, $(CD_3)_2CO$): δ 171.9, 163.9, 136.4, 132.9, 130.6, 129.5, 127.3, 114.8, 65.5, 56.1, 45.8, 35.4, 26.9, 16.0, 11.0; HRMS (ESI) Calcd for $C_{34}H_{44}N_2O_{10}S_2$ $(M+H)^+$ 705.2510, found 705.2510.

*4.1.12. (2S,2′S)-2,2′-((1,2-Phenylenebis(methylene))bis(((4-methoxyphenyl)sulfonyl)azanediyl))bis (4methylpentanoic acid) (**21d**)*

Prepared as described in the general procedure for basic hydrolysis of ethyl ester (Method C1) to get the title compound as a white solid; (15 mg, 72% yield). ^{1}H NMR (400 MHz, $(CD_3)_2$ CO): δ 7.86 (d, J = 8.8 Hz, 4H), 7.67–7.64 (m, 2H), 7.32–7.29 (m, 2H), 7.11 (d, J = 8.8 Hz, 2H), 4.79–4.61 (m, 4H), 4.62–4.59 (m, 2H), 3.96 (s, 6H), 1.76–1.60 (m, 4H), 1.52–1.45 (m, 2H), 0.95 (d, J = 6.4 Hz, 6H), 0.75 (d, J = 6.4 Hz, 6H); ^{13}C NMR (100 MHz, $(CD_3)_2$ CO): δ 172.6, 163.9, 136.6, 132.7, 130.6, 129.4, 127.6, 115.0, 58.9, 56.1, 46.7, 40.2, 25.7, 22.6, 22.2; HRMS (ESI) Calcd for $C_{34}H_{44}N_2O_{10}S_2$ $(M+H)^+$ 705.2510, found 705.2505.

*4.1.13. (2R,2′R)-2,2′-((1,2-Phenylenebis(methylene))bis(((4-methoxyphenyl)sulfonyl)azanediyl)) dipropionic acid (**21e**)*

Prepared as described in the general procedure for basic hydrolysis of ethyl ester (Method C1) to get the title compound as a white solid (31 mg, 79% yield). ^{1}H NMR (400 MHz, CD_3CN): δ 7.76–7.73 (m, 4H), 7.41–7.38 (m, 2H), 7.22–7.19 (m, 2H), 7.02–6.99 (m, 4H), 4.59–4.39 (m, 6H), 3.85 (s, 6H), 1.22 (d, J = 7.2 Hz, 6H); ^{13}C NMR (100 MHz, CD_3CN): δ. 172.6, 163.9, 135.9, 132.1, 130.4, 129.3, 127.9, 114.9, 56.2, 55.9, 47.4, 15.9; HRMS (ESI) Calcd for $C_{28}H_{32}N_2O_{10}S_2$ $(M+H)^+$ 621.1571, found 621.1577.

*4.1.14. (2R,2′R)-2,2′-((1,2-Phenylenebis(methylene))bis(((4-methoxyphenyl)sulfonyl)azanediyl))bis(3-phenylpropanoic acid) (**21f**)*

Prepared as described in the general procedure for basic hydrolysis of ethyl ester (Method C1) to get the title compound as a white solid (19 mg, 78% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.90 (s, 2H), 7.84 (m, 4H), 7.25–7.21 (m, 9H), 7.13–7.06 (m, 9H), 4.62–4.44 (m, 6H), 3.83–3.79 (m, 6H), 3.09–3.01 (m, 2H), 2.69–2.59 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.8, 162.7, 137.1, 134.7, 131.0, 129.7, 129.6, 129.2, 129.02, 128.95, 128.5, 128.2, 128.1, 128.0, 126.7, 126.5, 114.4, 114.3, 114.0, 60.8, 55.7, 55.6, 55.5, 45.9, 45.8, 36.0, 35.7; HRMS (ESI) Calcd for $C_{40}H_{40}N_2O_{10}S_2$ $(M+H)^+$ 773.2197, found 773.2190.

*4.1.15. 2,2′-((1,2-Phenylenebis(methylene))bis((phenylsulfonyl) azanediyl))diacetic acid (**24a**)*

Prepared as described in the general procedure for basic hydrolysis of ethyl ester (Method C1) to get the title compound as a white solid; (19 mg, 72% yield). ^{1}H NMR (400 MHz, MeOH-d_4): δ 7.86–7.84 (m, 4H), 7.63–7.53 (m, 6H), 7.30–7.22 (m, 4H), 4.58 (s, 4H), 3.84 (s, 4H); ^{13}C NMR (100 MHz, MeOH-d_4): δ 171.9, 140.6, 135.6, 134.0, 130.9, 130.3, 129.3, 128.6, 50.6, 48.8; HRMS (ESI) Calcd for $C_{24}H_{24}N_2O_8S_2$ $(M+H)^+$ 533.1047, found 533.1031.

*4.1.16. 2,2′-((1,2-Phenylenebis(methylene))bis(((4-nitrophenyl)sulfonyl) azanediyl))diacetic acid (**24b**)*

Prepared as described in the general procedure for basic hydrolysis of ethyl ester (Method C1) to get the title compound as a yellow solid; (23 mg, 79% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.35 (d, J = 8.8 Hz, 4H), 8.08 (d, J = 8.8 Hz, 4H), 7.26 (s, 4H), 4.52 (s, 4H), 3.79 (s, 4H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.6, 149.8, 144.5, 133.8, 128.6, 128.3, 127.9, 124.4, 49.1, 48.3; HRMS (ESI) Calcd for $C_{24}H_{22}N_4O_{12}S_2$ $(M+H)^+$ 623.0748, found 623.0743.

*4.1.17. 2,2′-((1,2-Phenylenebis(methylene))bis(((4-fluorophenyl) sulfonyl)azanediyl))diacetic acid (**24c**)*

Prepared as described in the general procedure for basic hydrolysis of ethyl ester (Method C1) to get the title compound as a white solid (11 mg, 76% yield). ^{1}H NMR (400 MHz, MeOH-d_4): δ 7.93–7.89 (m, 4H), 7.33–7.25 (m, 8H), 4.59 (s, 4H), 3.87 (s, 4H); ^{13}C NMR (100 MHz, MeOH-d_4): δ 171.8, 167.9, 165.5, 137.0, 135.5, 131.7, 131.6, 130.9, 129.4, 117.3, 117.1, 50.5, 48.4; ^{19}F NMR (377 MHz, MeOH-d_4): δ – 107.9 (s, 2F); HRMS (ESI) Calcd for $C_{24}H_{22}$ $F_2N_2O_8S_2$ $(M+H)^+$ 569.0858, found 569.0851.

*4.1.18. 2,2′-((1,2-Phenylenebis(methylene))bis(((3-fluorophenyl) sulfonyl)azanediyl))diacetic acid (**24d**)*

Prepared as described in the general procedure for basic hydrolysis of ethyl ester (Method C1) to get the title compound as a white solid (13 mg, 70% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.68 (br, 2H), 7.68–7.60 (m, 6H), 7.56–7.53 (m, 2H), 7.23–7.22 (m, 4H), 4.51 (s, 4H), 3.98–3.86 (m, 4H); ^{13}C NMR^{13}C NMR (100 MHz, DMSO-d_6): δ 169.7, 168.8, 163.0, 160.6, 141.1, 141.0, 134.0, 133.9, 131.7, 131.54, 131.46, 128.8, 128.6, 127.8, 127.7, 123.4, 120.4, 120.2, 120.0, 114.4, 114.1, 51.8, 49.2, 49.1, 48.5, 48.4; HRMS (ESI) Calcd for $C_{24}H_{22}$ $F_2N_2O_8S_2$ $(M+H)^+$ 569.0858, found 569.0852.

*4.1.19. 2,2′-((1,2-Phenylenebis(methylene))bis(((2,4-dimethoxyphenyl) sulfonyl)azanediyl))diacetic acid (**24e**)*

Prepared as described in the general procedure for basic hydrolysis of ethyl ester (Method C1) to get the title compound as a white solid (13 mg, 81% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.53 (s, 2H), 7.60 (d, J = 8.8 Hz, 2H), 7.17 (s, 4H), 6.67 (d, J = 2.0 Hz, 4H), 6.55 (dd, J = 8.8, 2.0 Hz, 2H), 4.46 (s, 4H), 3.86 (s, 4H), 3.83 (s, 6H), 3.81 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.1, 164.3, 158.2, 134.5, 131.7, 128.5, 127.3, 119.9, 104.6, 99.3, 56.0, 55.7, 48.3, 47.9; HRMS (ESI) Calcd for $C_{28}H_{32}N_2O_{12}S_2$ $(M+H)^+$ 653.1469 found 653.1460.

*4.1.20. 2,2′-((1,2-Phenylenebis(methylene))bis(((2,4-difluorophenyl) sulfonyl)azanediyl))diacetic acid (**24f**)*

Prepared as described in the general procedure for basic hydrolysis of ethyl ester (Method C1) to get the title compound as a white solid (16 mg, 72% yield). ^{1}H NMR (400 MHz, MeOH-d_4): δ 7.85–7.79 (m, 2H), 7.29–7.24 (m, 4H), 7.19–7.14 (m, 2H), 7.06–7.02 (m, 2H), 4.65 (s, 4H), 3.95 (s, 4H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.8, 168.6, 168.5, 166.1, 166.0, 162.8, 162.6, 160.2, 160.1, 135.4, 133.6, 133.5, 130.9, 129.44, 129.35, 126.2, 126.1, 126.00, 125.97, 112.79, 112.76, 112.6, 112.5, 106.9, 106.6, 106.4, 65.8, 48.8; ^{19}F NMR (377 MHz, MeOH-d_4): δ – 103.6 (d, J = 12 Hz, 1F), – 103.7 (d, J = 12 Hz, 1F); HRMS (ESI) Calcd for $C_{24}H_{20}$ $F_4N_2O_8S_2$ $(M+H)^+$ 605.0670, found 605.0661.

*4.1.21. 2,2′-((1,2-Phenylenebis(methylene))bis(((3,4-difluorophenyl) sulfonyl)azanediyl))diacetic acid **(24g)***

Prepared as described in the general procedure for basic hydrolysis of ethyl ester (Method C1) to get the title compound as a white solid (9 mg, 65% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.67–59 (m, 6H), 7.55–7.51 (m, 2H), 7.23 (s, 4H), 4.49 (s, 4H), 3.98 (s, 1H), 3.84 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.6, 168.7, 163.0, 162.9, 160.5, 160.4, 141.1, 141.0, 140.64, 140.58, 140.5, 134.0, 133.89, 133.86, 133.8, 131.6, 131.5, 131.4, 131.3, 128.7, 128.5, 127.71, 127.65, 127.5, 123.3, 120.3, 120.0, 119.8, 114.3, 114.0, 51.7, 49.2, 49.1, 49.0, 48.9, 48.4, 48.3, 48.2; HRMS (ESI) Calcd for $C_{24}H_{20}F_4N_2O_8S_2$ $(M+H)^+$ 605.0670, found 605.0700.

*4.1.22. 2,2′-((1,2-Phenylenebis(methylene))bis(((2,6-difluorophenyl) sulfonyl)azanediyl))diacetic acid **(24h)***

Prepared as described in the general procedure for basic hydrolysis of ethyl ester (Method C1) to get the title compound as an off-white solid (16 mg, 72% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.75–7.67 (m, 2H), 7.27–7.23 (m, 8H), 4.63 (s, 4H), 3.97 (s, 4H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.0, 161.9, 159.3, 137.54, 137.45, 136.8, 136.7, 136.6, 133.6, 129.0, 128.8, 128.2, 128.0, 124.9, 124.8, 114.3, 114.0, 55.4; HRMS (ESI) Calcd for $C_{24}H_{20}F_4N_2O_8S_2$ $(M+H)^+$ 605.0670, found 605.0669.

*4.1.23. 2,2′-((1,2-Phenylenebis(methylene))bis(((4-(benzyloxy)phenyl) sulfonyl)azanediyl))diacetic acid **(24i)***

Prepared as described in the general procedure for basic hydrolysis of ethyl ester (Method C1) to get the title compound as an off-white solid (17 mg, 76% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.76 (d, J = 9.2 Hz, 4H), 7.48–7.46 (m, 4H), 7.42–7.35 (m, 6H), 7.28–7.25 (m, 2H), 7.21–7.16 (m, 6H), 5.19 (s, 4H), 4.44 (s, 4H), 3.75 (s, 4H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.9, 161.7, 136.3, 134.3, 130.8, 129.4, 128.6, 128.5, 128.1, 127.9, 127.5, 115.1, 69.7, 49.0, 48.3; HRMS (ESI) Calcd for $C_{38}H_{36}N_2O_{10}S_2$ $(M+H)^+$ 745.1884, found 745.1872.

*4.1.24. 2,2′-((1,2-Phenylenebis(methylene))bis((dibenzo[b,d]furan-2-ylsulfonyl)azanediyl))diacetic acid **(24j)***

Prepared as described in the general procedure for basic hydrolysis of ethyl ester (Method C1) to get the title compound as a beige solid (14 mg, 78% yield). ^{1}H NMR (400 MHz, Acetone-d_6): δ 8.65 (d, J = 1.2 Hz, 2H), 8.25 (d, J = 8.0 Hz, 2H), 8.05 (dd, J = 8.8, 2.0 Hz, 2H), 7.77 (d, J = 8.8 Hz, 2H), 7.70 (d, J = 8.8 Hz, 2H), 7.62–7.58 (m, 2H), 7.46–7.42 (m, 2H), 7.40–7.38 (m, 2H), 7.24–7.23 (m, 2H), 4.74 (s, 4H), 4.02 (s, 4H); ^{13}C NMR (100 MHz, Acetone-d_6): δ 170.2, 158.9, 157.8, 135.54, 135.49, 130.3, 129.4, 128.8, 127.8, 125.5, 124.5, 124.2, 122.7, 122.2, 112.9, 112.7, 50.3, 48.7; HRMS (ESI) Calcd for $C_{36}H_{28}N_2O_{10}S_2$ $(M+H)^+$ 713.1258, found 713.1252.

*4.1.25. 2,2′-((1,2-Phenylenebis(methylene))bis(((4-hydroxyphenyl) sulfonyl)azanediyl))diacetic acid **(25)***

To a suspension of compound 9a (65 mg, 0.1 mmol) in dry DCM (1 mL), 1 mL solution of BBr_3 (1 M in DCM) was added, and the reaction was stirred at room temperature for overnight. The crude mixture was quenched with water, diluted with DCM, and then washed with water and brine. The organic layer was dried over sodium sulfate, and the residue was purified by flash column chromatograph (0–20% MeOH/ DCM) to get the title compound (6.8 mg, 12% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.69 (d, J = 8.8 Hz, 4H), 7.23 (s, 4H), 7.86 (d, J = 8.8 Hz, 4H), 4.47 (s, 4H), 3.52 (s, 4H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.0, 161.7, 134.6, 129.6, 128.8, 128.7, 127.6, 115.8, 49.1, 48.4; HRMS (ESI) Calcd for $C_{24}H_{24}N_2O_{10}S_2$ $(M+H)^+$ 565.0945, found 565.0936.

*4.1.26. N,N'-(1,2-Phenylenebis(methylene))bis(N-(cyanomethyl)-4-methoxybenzene-sulfonamide) **(26)***

Prepared as described in the general procedure for synthesis of alkylated sulfonamides (method B) to get the title compound as an oily product; (119 mg, 71% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.86 (d, J = 8.8 Hz, 4H), 7.45–7.38 (m, 4H), 7.08 (d, J = 8.8 Hz, 2H), 4.51 (s, 4H), 4.03 (s, 4H), 3.89 (s, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.0, 132.8, 130.3, 130.1, 129.4, 127.8, 55.7, 48.6, 34.9; HRMS (ESI) Calcd for $C_{26}H_{26}N_4O_6S_2$ $(M+H)^+$ 555.1367, found 555.1362.

*4.1.27. N-((1H-Tetrazol-5-yl)methyl)-N-(2-(((N-((1H-tetrazol-5-yl) methyl)-4-methoxyphenyl)sulfon-amido)methyl)benzyl)-4-methoxybenzenesulfonamide **(27)***

To a solution of N,N'-(1,2-Phenylenebis(methylene))bis(N-(cyanomethyl)-4-methoxybenzene sulfonamide) (26) (50 mg, 0.09 mmol) in DMF (1.5 mL) were added ammonium chloride (37 mg, 0.27 mmol) and sodium azide (35 mg, 0.45 mmol) at room temperature. The reaction mixture was stirred at 120 °C for 4 hr. The crude mixture was acidified with 1 N HCl, extracted with ethyl acetate, and then washed with water and brine. The organic layer was dried over sodium sulfate and concentrated under reduced pressure. The residue was purified by a flash column chromatography using 0–20% MeOH/ DCM to get the title compound (52 mg, 90% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.73 (d, J = 8.8 Hz, 4H), 7.30–7.28 (m, 2H), 7.17–7.16 (m, 2H), 7.06 (d, J = 8.8 Hz, 4H), 4.50 (s, 4H), 3.84 (s, 4H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.9, 153.2, 132.9, 129.8, 129.4, 128.8, 55.8, 41.4, 29.8; HRMS (ESI) Calcd for $C_{26}H_{28}N_{10}O_6S_2$ $(M+H)^+$ 640.1707, found 641.1687.

*4.1.28. 2,2′-(((4-Cyano-1,2-phenylene)bis(methylene))bis(((2,3-dihydrobenzofuran-5-yl)sulfonyl)-azanediyl))diacetic acid **(31)***

Prepared as described in the general procedure for basic hydrolysis of ethyl ester (Method C1) to get the title compound as a beige solid (16 mg, 84% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.71–7.66 (m, 4H), 7.58–7.54 (m, 3H), 6.90–6.87 (m, 2H), 4.64 (t, J = 8.8 Hz, 4H), 4.47 (s, 2H), 4.45 (s, 2H), 3.83 (s, 2H), 3.79 (s, 2H), 3.24 (t, J = 8.8 Hz, 4H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.1, 169.9, 163.50, 163.47, 140.6, 136.5, 131.7, 130.9, 129.9, 129.8, 129.3, 129.1, 128.9, 128.7, 128.6, 124.7, 124.6, 118.7, 109.9, 109.1, 72.3, 49.6, 49.5, 49.3, 49.2, 28.4; HRMS (ESI) Calcd for $C_{29}H_{27}N_3O_{10}S_2$ $(M+H)^+$ 642.1211, found 642.1211.

*4.1.29. N-(2-(((N-(Carboxymethyl)-2,3-dihydrobenzofuran)-5-sulfonamido)methyl)-4-(1H-tetrazol-5-yl) benzyl)-N-((2,3-dihydrobenzofuran-5-yl)sulfonyl)glycine **(33)***

Prepared as described in the general procedure for basic hydrolysis of ethyl ester (Method C1) to get the title compound as a yellow solid (14 mg, 78% yield). ^{1}H NMR (400 MHz, Acetone-d_6): δ 7.97 (d, J = 1.2 Hz, 1H), 7.91 (dd, J = 8.0, 1.6 Hz, 1H), 7.67–7.58 (m, 5H), 6.84 (d, J = 8.8 Hz, 1H), 6.77 (d, J = 8.8 Hz, 1H), 4.66–4.59 (m, 8H), 3.99 (s, 2H), 3.88 (s, 2H), 3.26–3.24 (m, 4H); ^{13}C NMR (100 MHz, Acetone-d_6): δ 172.3, 172.1, 165.64, 165.56, 139.6, 137.6, 131.8, 131.7, 131.5, 130.42, 130.38, 130.3, 130.2, 129.2, 127.5, 126.1, 126.0, 125.1, 110.3, 110.2, 73.62, 73.57, 51.1, 29.9; HRMS (ESI) Calcd for $C_{29}H_{28}N_6O_{10}S_2$ $(M+H)^+$ 685.1381, found 685.1370.

4.2. Fluorescence polarization competition assay

Fluorescence polarization assay was performed on a Wallac Victor 3 V multi-label counter/plate reader (PerkinElmer, Shelton, CT) using 484 nm excitation and 535 nm emission filters for the fluorophore used in the binding experiment as previously reported.[28] The plate used for the FP measurement was Corning 384-well plate (product #3575), loaded with 40 μL of assay solution per well. The assay buffer used was 10 mM HEPES buffer, pH 7.4, containing 150 mM NaCl, 50 mM EDTA, and 0.005% Tween-20. The fluorescent probe used in this assay is the 9-mer Nrf2 ETGE motif derived peptide, FITC-LDEETGEFL-NH_2. In each well, the final volume of 40 μL consisted of 10 μL of 400 nM Keap1 Kelch domain protein, and 20 μL of 20 nM FITC-9mer Nrf2 peptide amide, and 10 μL of the inhibitor compound at different concentrations

in triplicate. Then, the plate was centrifuged for 2 min, covered, and incubated with shaking for 30 min at room temperature. After the incubation, the plate was centrifuged for 2 min prior to FP measurements. The FP was determined by measuring the parallel and perpendicular fluorescence intensity ($F_{||}$ and $F^{\perp}$) with respect to the linearly polarized excitation light. The IC_{50} was derived from the plot of % inhibition against inhibitor concentration using Sigma Plot 12.3.

4.3. Metabolic stability

The incubation mixture consisted of 0.5 mg protein/mL human liver microsomes (50-donor pools, XenoTech, Kansas City, KS), NADPH (2 mM) and 50 mM phosphate buffer (pH 7.4). Following pre-incubation for 10 min at 37 °C, reactions were initiated by the addition of test compounds (0.1 mM). Samples (20 μL) were withdrawn at 0, 10, 20, 30, 60 and 90 min. The reaction was terminated by the addition of 40 μL acetonitrile:methanol (2/1, v/v). The resulting mixture was centrifuged at 9000 rpm for 5 min, and then the supernatant was analyzed with LC/MS (Agilent Technologies LC/MS system).

4.4. Molecular docking simulation.

Computational molecular docking studies were conducted using the AutoDock 4.0 software. The co-crystal structure of a small molecule ligand bound to the Keap1 protein was obtained from the RCSB protein data bank (4XMB).[15] In preparing the protein, all water molecules were removed from the structure file. Missing hydrogen atoms were added, and Kollman charges were calculated using the AutoDock tool. The ligand structures were built in MOE and prepared for docking using AutoDock. The docking grid was centered on the observed binding pocket of the ligand, and the number of grid points in each direction was set to X, Y, Z = 40, 40, 40 with spacing of 0.375 Å resolution. The prepared ligands were docked into the binding sites of the Keap1 Kelch domain. For a docking parameter file, Lamarckian genetic algorithm was used, the number of GA runs was 200, and all other parameters were set to default values. The results of the docking experiments were reported in kcal/mol, and the docked conformations were ranked according to their binding energy. To validate the docking method, the prepared ligand was re-docked using the same method, and then compared with the corresponding X-ray crystal structure. As a result, the top-scoring docked pose was similar to the crystal structure of the ligand with a low RMSD value of 0.94 Å. This demonstrated that the docking method was reliable and could be used for docking simulation of other compounds within the binding pocket of the Keap1 Kelch domain.

Declaration of Competing Interest

The authors declare that L.H. and D.A. are inventors of a patent application based on some of the compounds reported in this paper.

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