DISCOVERY OF NOVEL, DRUG-LIKE FERROPTOSIS INHIBITORS WITH IN VIVO EFFICACY

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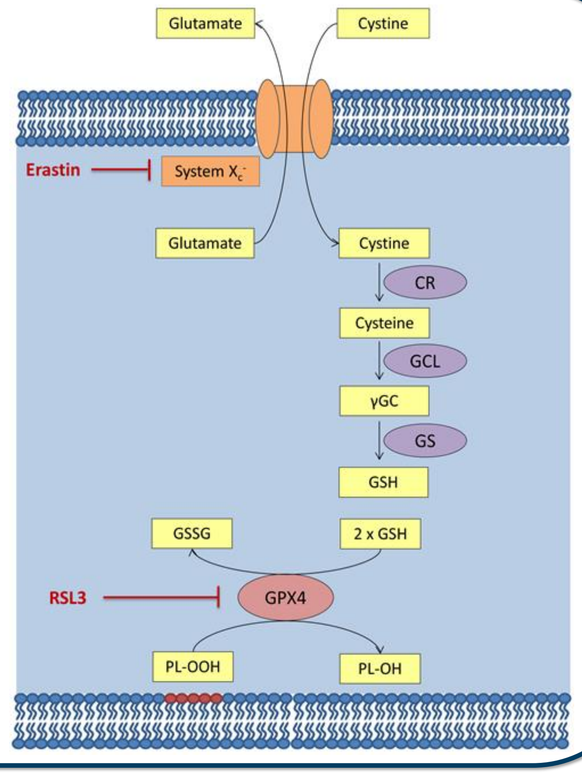
Introduction

Ferroptosis is an iron-catalyzed, nonapoptotic form of regulated necrosis that results in oxidative lipid damage in cell membranes. Ferroptosis may thus play a key role in several diseases where lipid hydroperoxide has been implicated. Interestingly, this form of cell death can be inhibited by different class of molecules.[1] Between those, Ferrostatin-1 (Fer-1) emerged as a novel potent radical-trapping antioxidant (RTAs) suffered from solubility issues.[2] The aim of this study is the design, synthesis and biological evaluation of a more stable and readily soluble series of Fer-1 analogues that potently inhibit ferroptosis enhancing the solubility.[3]



in vitro results

Table 1. Synthesized Fer-1 analogues and their antiferroptotic activity in response to Erastin-induced Ferroptosis in IMR-32 neuroblastoma cells

compd	R_1 or R_3	R_2	IC_{50} (nM)[a]	solubility (μM)[b]
1 (Fer-1)	-	-H	33	>200
UAMC-3240	morpholine	benzyl	24	50-100
UAMC-3234	−NH(Me)	benzyl	3	>200
UAMC-3206	−NH2	benzyl	12	>200
UAMC-3203	piperazine	benzyl	10	>200

[a] Reported IC50 values are calculated from measurements in triplicate. [b] Final test compound concentration range between 3.125 and 200 μM [4 μM DMSO solution in 196 μM buffer solution (10 mM PBS pH 7.4)].

Table 2. **ADME assays**: Microsomal and plasma stability

compd	microsomal stability (t1/2) (h)[a]			plasma stability (% recovery after 6h)[b]		
	human	rat	mouse	human	rat	mouse
1 (Fer-1)	0.109 ± 0.003	0	0	47.3	1.1	0
UAMC-3240	0.98 ± 0.35	0.173 ± 0.007	0.051 ± 0.002	100	100	100
UAMC-3234	13.21 ± 4.10	0.35 ± 0.01	0.45 ± 0.02	90.3	65.8	100
UAMC-3206	17.71 ± 2.04	2.05 ± 0.21	13.00 ± 4.15	100	100	100
UAMC-3203	20.53 ± 5.49	16.48 ± 4.66	3.46 ± 1.37	84.2	85.8	100

[a] Metabolism by microsomes (CYP450 and other NADP-dependent enzymes) was monitored and expressed as half-life (h). [b] Percentage of remaining parent compound

in vivo results

Table 3. Pretreatment with the compounds significantly decreases LDH levels after acute iron poisoning [a]

trigger	vehicle	plasma LDH (U/L)
	vehicle	484 ± 72
$FeSO_4$	vehicle	3572 ± 185
$FeSO_4$	1 (Fer-1)	2898 ± 178
$FeSO_4$	UAMC-3234	2093 ± 90
$FeSO_4$	UAMC-3206	2293 ± 148
$FeSO_4$	UAMC-3203	2346 ± 99

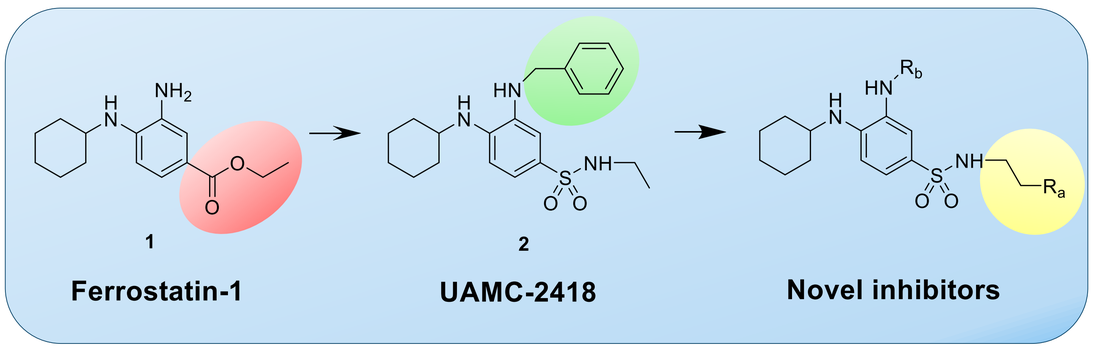
[a] Vehicle, Fer-1, compound UAMC 3234 – 3206 - 3203 was injected intravenously (20 μmol/kg) 15 min before intraperitoneal injection with 300 mg/kg iron sulfate. Two hours after IP injection, mice were sacrificed and blood was taken. Plasma levels of LDH are shown. Vehicle = 2% DMSO in 0.9% NaCl.

Table 4. Tissue distribution profile of compound UAMC-3203 in various organs (iv dose at 5 mg/kg; the internal organs were collected at necropsy at 24 h.) [a]

compd	liver	kidney	lung
Rat1	0.23	0.20	0.19
Rat2	0.22	0.35	0.48
Rat3	0.15	0.38	0.53

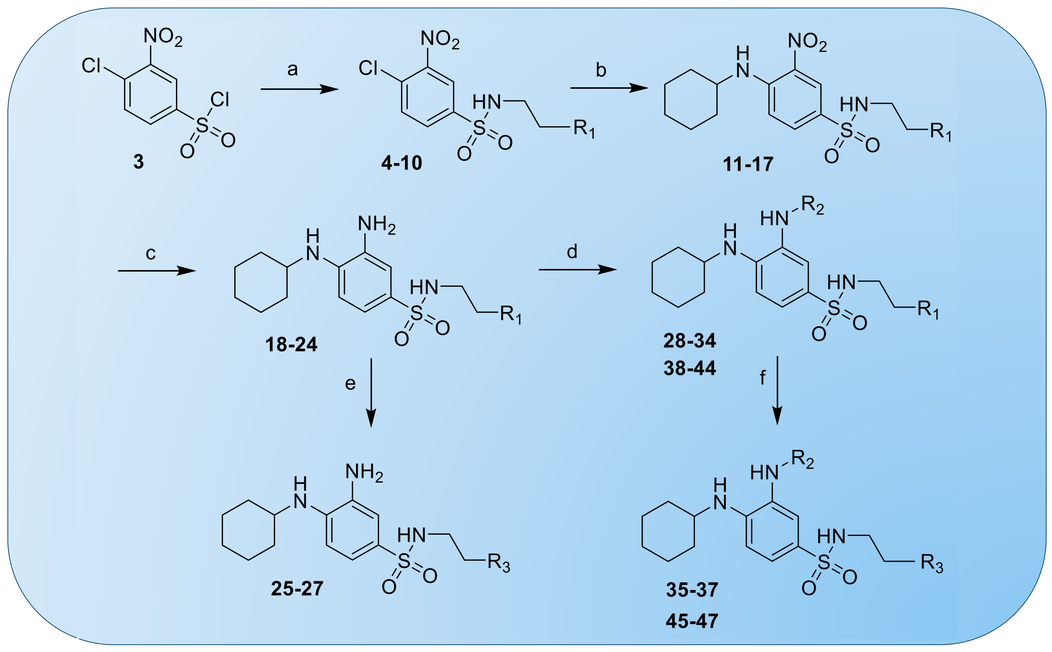
[a] Analysis of the samples was conducted by LC-MS

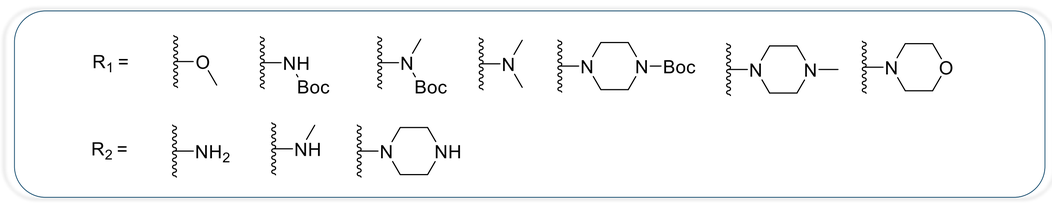
New series of Fer-1 analogues: design and synthesis



Design of novel compounds:

1) The replacement of the labile ester moiety with a sulfonamide greatly improve stability as well as potency;
2) The cyclohexyl moiety was deemed to be the most ideal substituent with regard to both potency and lipophilicity;
3) The introduction of an aromatic group on the 3-amino position greatly improved potency but also further decreased the solubility of the compounds;
4) Solubility enhancing groups were introduced in the terminal position of the aliphatic chain on the sulfonamide moiety (R_a) because these molecules have to act in the highly lipophilic environment of membranes.



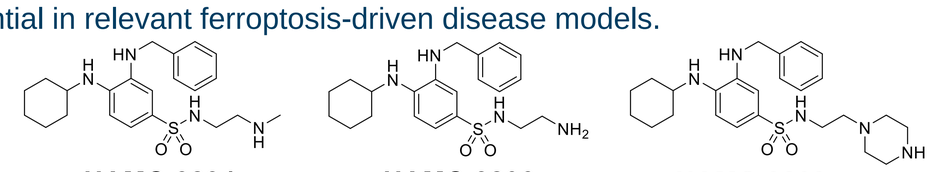


Reagents and conditions: *(a)* aliphatic amine analogue, triethylamine, THF, 1 h, −40° C; *(b)* cyclohexylamine, K_2CO_3, DMSO, 18 h, 60 ° C; *(c)* palladium hydroxide on carbon, H_2, methanol, 18 h, rt; *(d)* benzyl bromide or 4-(bromomethyl)pyridine hydrobromide, K_2CO_3, DMF; (e) HCl in dioxane, DCM, in case Boc protection was present.

Conclusion

- Novel ferroptosis inhibitors have been synthesized by introducing a solubility enhancing group and a sulfonamide moiety to the Fer-1 scaffold;
- The new series of molecules are more potent than Fer-1 while improving solubility and stability compare to Fer-1;
- Compounds **UAMC-3206** and **UAMC-3203** showed great recovery from plasma after 6 h, with microsomal half lives of multiple hours across three species;
- Compounds **UAMC-3234, UAMC-3206** and **UAMC-3203** were significantly more potent than Fer-1 against multiorgan injury in mice, which illustrate their efficacy in vivo;
- No toxicity was observed in mice after daily injection of **UAMC-3203** for 4 weeks. [4]

This novel compounds represent novel lead compounds with therapeutic potential in relevant ferroptosis-driven disease models.



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