NOVEL UAMC-3203 ANALOGUES WITH IMPROVED PHARMACOKINETICS PROPERTIES TO INHIBIT FERROPTOSIS FOR CNS APPLICATION

Camilla Scarpellini[1]*, Greta Klejborowska[1], Caroline Lanthier[1], Geraldine Veeckmans[2], Magali Walravens[2], Emily Van San[2], Pieter Van der Veken[1], Behrouz Hassannia[2,3,4], Tom Vanden Berghe[2,3,4], and Koen Augustyns[1]

[1] Laboratory of Medicinal Chemistry, Faculty of Pharmaceutical, Biomedical and Veterinary Sciences, University of Antwerp, Antwerp, Belgium
[2] Cell Death Signalling Lab, Department of Biomedical Sciences, University of Antwerp, Antwerp, Belgium
[3] VIB Center for Inflammation Research, Ghent, Belgium
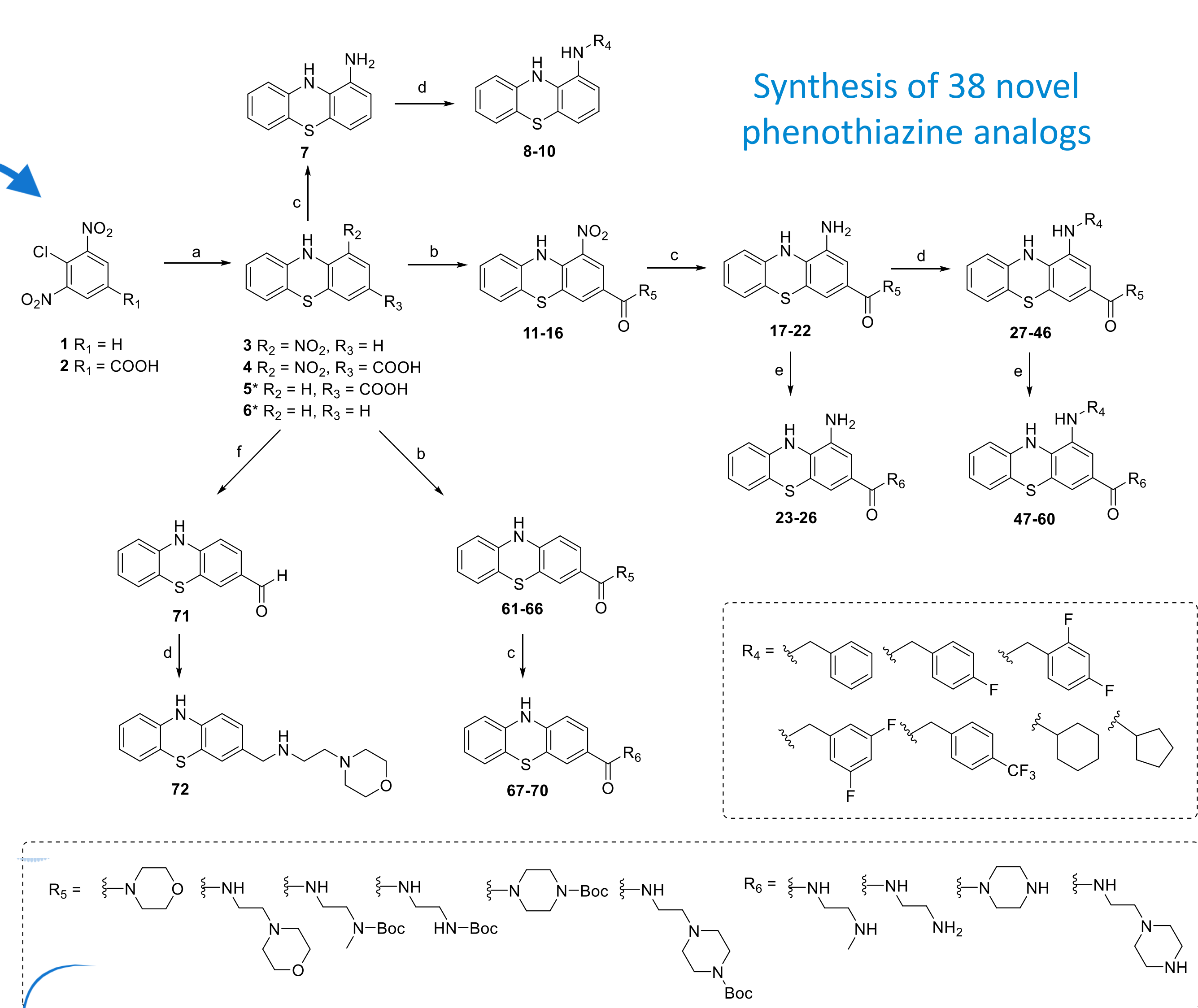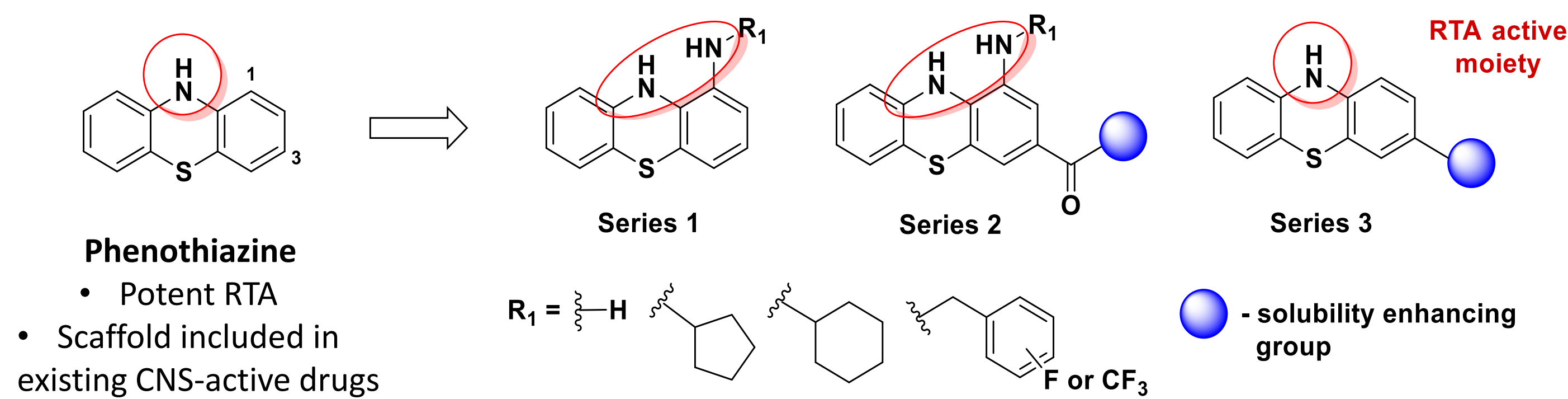[4] Department of Biomedical Molecular Biology, Ghent University, Ghent, Belgium

*e-mail: camilla.scarpellini@uantwerpen.be
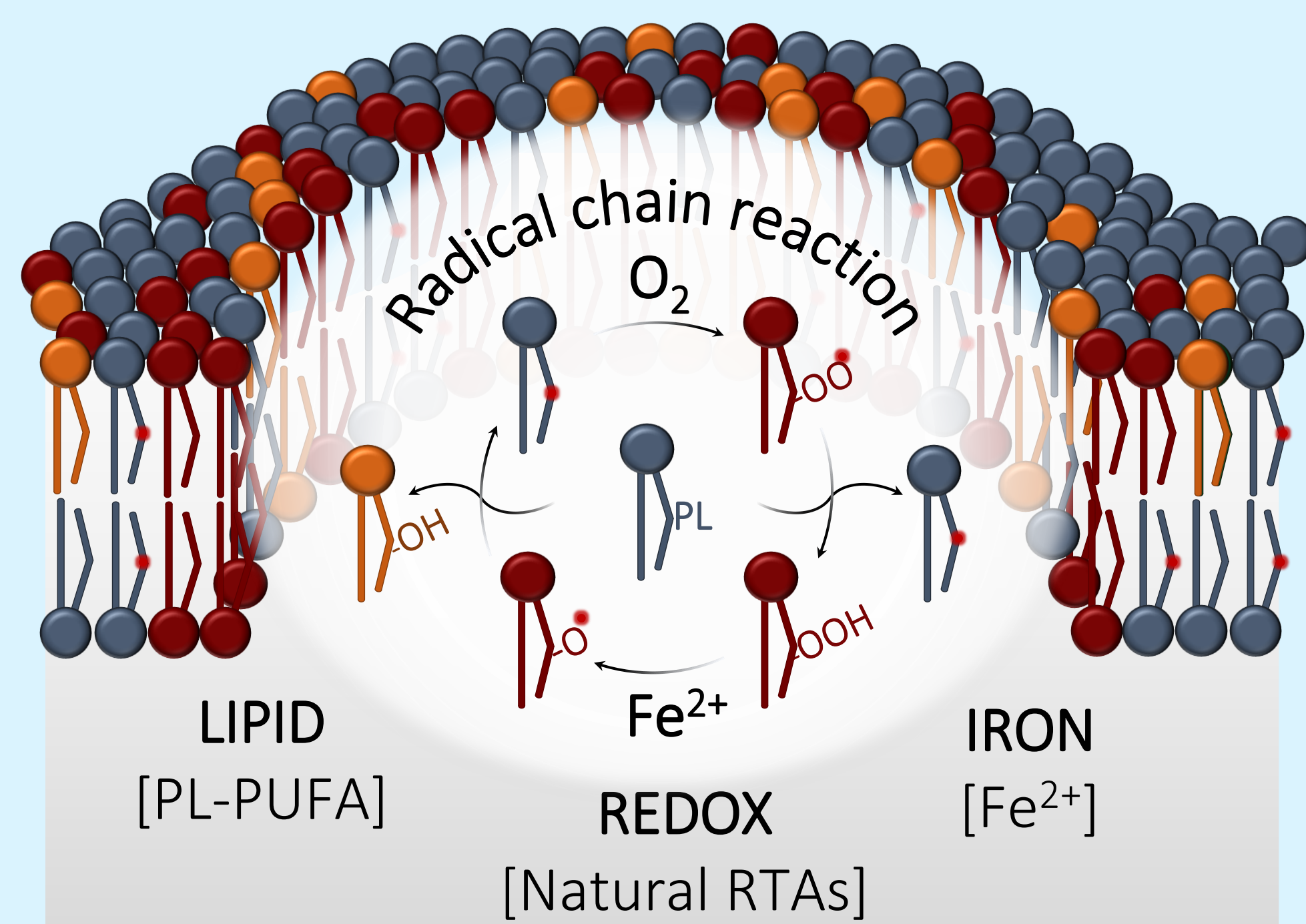
FERROPTOSIS CONTRIBUTES TO NEURODEGENERATIVE DISORDERS

Ferroptosis is a form of regulated cell death characterized by the accumulation of phospholipid hydroperoxides (PLOOHs) and iron-dependent oxidative damage to cell membranes, along with a loss of glutathione peroxidase 4 (GPX4) reducing capacity. Increasing evidence suggests that the activation of the ferroptosis pathway is involved in neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis. Neurons, which require iron to meet their high energy demands and contain high levels of polyunsaturated fatty acids (PUFAs), are particularly susceptible to ferroptosis. In mice, the neuronal ablation of GPX4 leads to cognitive impairment and neurodegeneration. Currently, lipophilic radical-trapping antioxidants (RTAs) are considered the most potent ferroptosis inhibitors. However, the tools to study ferroptosis in CNS remain limited. Therefore, our aim was to develop a ferroptosis inhibitor with improved BBB permeability.

DESIGN AND SYNTHESIS OF PHENOTHIAZINE ANALOGS, IN VITRO ASSESSMENT OF FERROPTOTIC INHIBITORY ACTIVITY AND PHYSICOCHEMICAL PROPERTIES OF SELECTED COMPOUNDS

Phenothiazine
• Potent RTA
• Scaffold included in existing CNS-active drugs

$R_1 = $ -H

- solubility enhancing group

Synthesis of 38 novel phenothiazine analogs

Scheme 1. Reagents and conditions: *commercially available; (a) 2-aminothiophenol, NaOH, rt → 65-85°C, 12-72 h; (b) HATU, DIPEA, corresponding amine, DMF or THF, 0°C → rt, 12-72 h; (c) Pd(OH)$_2$/C, H$_2$, MeOH, rt, 12-72 h; (d) AcOH, corresponding aldehyde or ketone, NaBH$_3$CN, THF, rt, 12-16 h; (e) DCM, 4M HCl in dioxane, rt, 2 h, (f) AcOH, HMT, reflux, 6h.

IC$_{50}$, kinetic solubility, CNS MPO, and mouse microsomal stability and RTA potency in FENIX assay were evaluated for all the compounds. For selected compounds, cytotoxicity on the HepG2 cell line, human microsomal stability, experimental logD, and MDCK-MDR1 assays were also performed.

Name	Structure	IC$_{50}$ (nM)[a]	Cytotox. EC$_{50}$(µM)[b] [SI][c]	Kin. Solubility (µM)[d]	CNS MPO Score[e]	Cl$_{int}$ (µL/min/mg protein)[f] mouse	Cl$_{int}$ (µL/min/mg protein)[f] human	logD[g] calc	logD[g] exp	MDCK Permeability Dynamic[h] A2B P$_{app}$ (10^{-6} cm/s)	MDCK Permeability Dynamic[h] Efflux ratio
UAMC-5242		0.461 ± 0.689	n.d.	50-100	3.6	n.d.	n.d.	3.8	n.d.	n.d.	n.d.
UAMC-3203		9.22 ± 6.29	26.60 [2885]	>200	2.4	36.6	8.27	1.2	n.d.	not permeable	-
UAMC-4763		7.07 ± 2.17	177.4 [25092]	>200	4.9	37.2	6.84	2.0	2.55	1.43	46.0
UAMC-4757		11.7 ± 7.39	207.8 [17760]	>200	4.3	<5.5	<5.5	0.7	0.9	0.323	9.28
UAMC-4841		2.40 ± 0.792	127.8 [53250]	>200	5.0	26.8	22.1	1.1	1.55	0.898	31.0
UAMC-4842		3.58 ± 2.61	26.49 [7397]	100-200	3.6	21.6	<5.5	2.7	4.0	0.534	130
UAMC-5172		10.3 ± 0.794	178.6 [17339]	>200	5.5	50.6	18.4	1.7	2.0	3.43	18.5
UAMC-5173		15.5 ± 0.194	160.6 [10361]	>200	4.3	14.8	<5.5	1.1	1.5	1.37	7.73

n.d. = not determined; [a]IC$_{50}$ values are calculated using a sigmoidal dose-response curve. The mean values are calculated from a minimum of duplicate measurements in ML162-induced ferroptosis in HT-1080 cells reported with their corresponding standard deviations. [b]EC$_{50}$ values are calculated using a sigmoidal dose-response curve. The mean values are calculated from a minimum of duplicate measurements in HT-1080 cells. [c]SI – selectivity index is calculated as a ratio between cytotoxicity and antiferroptotic activity (SI = EC$_{50}$/IC$_{50}$). [d] The kinetic solubility was measured in a concentration range between 3.13 and 200 µM in PBS buffer, pH 7. [e]CNS MPO - central nervous system multiparameter optimization calculated with Collaborative Drug Discovery (CDD)©. [f]Human Cl$_{int}$ = human intrinsic clearance in liver microsomes, low Cl$_{int}$ ≤ 8.6 µL/min/mg protein; high Cl$_{int}$ > 47.0 µL/min/mg protein; verapamil, and dextromethorphan were used as controls; mouse Cl$_{int}$ = mouse intrinsic clearance in liver microsomes, low Cl$_{int}$ < 13.1 µL/min/mg protein; high Cl$_{int}$ > 71.1 µL/min/mg protein; diazepam and diphenhydramine were used as controls. [g]logD calc = calculated logD with CDD; logD exp = experimental value form logD shake flask assay. [h]P$_{app}$ – apparent permeability coefficient. A2B – transport of the compound from the apical compartment to the basolateral compartment. Efflux ratio - P-gp mediated drug efflux.

UAMC-5172 CROSSES THE BLOOD-BRAIN BARRIER AND HAS HIGH ORAL BIOAVAILABILITY

| In vivo pharmacokinetic profile	PO (10 mg/kg)	IV (10 mg/kg)
t$_{½}$ (h)	NC	0.89# 3.66*
T$_{max}$ (h)	0.5	-
C$_{max}$ (ng/mL)	230 ± 39.2	-
C$_0$ (ng/mL)	-	946
AUC$_{0-last}$ (hr*ng/mL)	1211 ± 454	1432
AUC$_{0-inf}$ (hr*ng/mL)	NC	1434
F %	85 ± 31.7	-
CL (mL/min/kg)	-	116
V$_{ss}$ (L/kg)	-	19.0

UAMC-5172 10 mg/kg
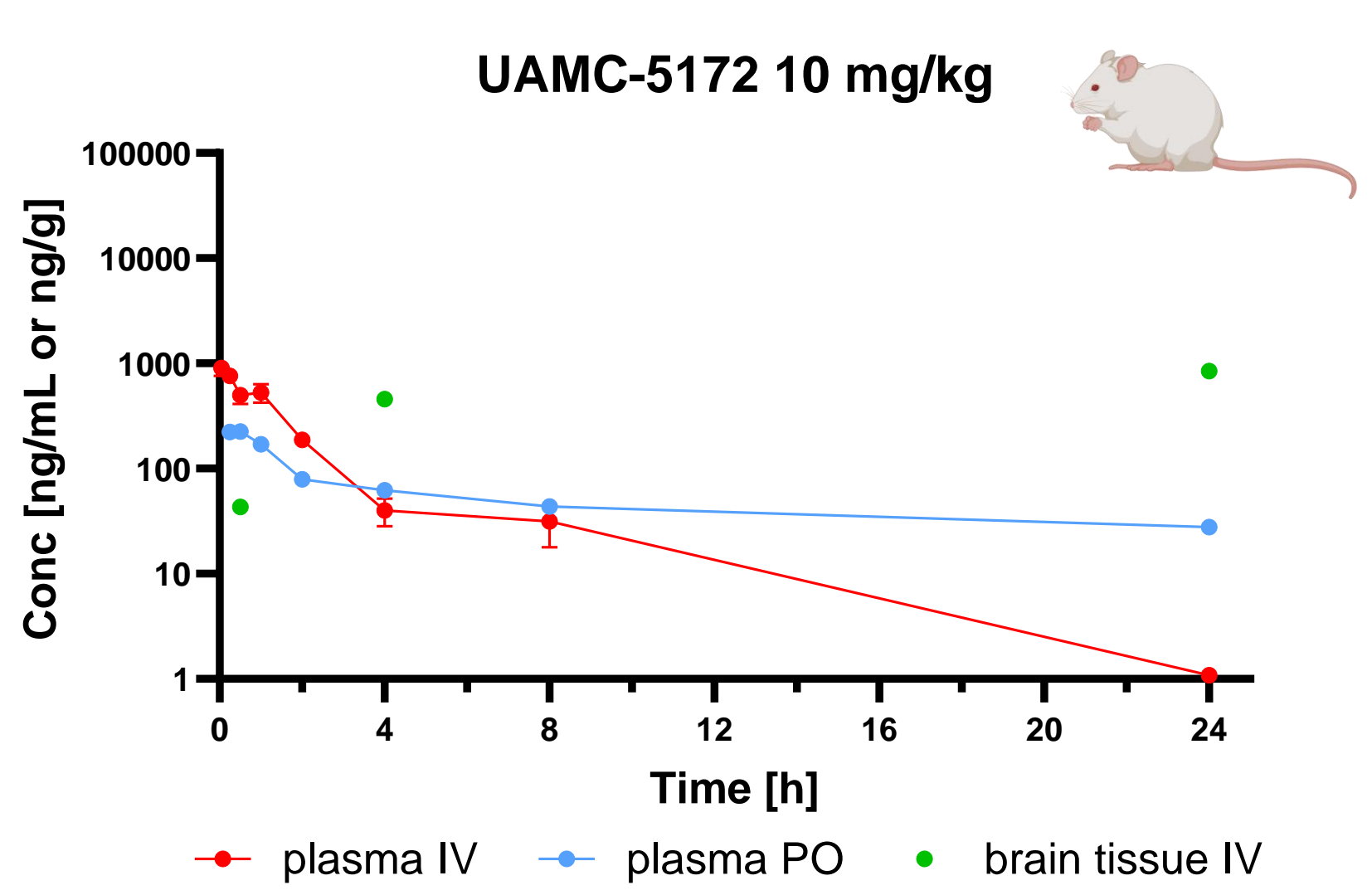
• **high oral bioavailability of 85 %,**
• **detected in cerebrospinal fluid** 2 h post-administration,
• **high concentrations in brain tissue** at 4, and 24 h post-administration— **142 to 263 times higher than IC$_{50}$ = 10.3 ± 0.794 nM,**
• UAMC-5172 will be employed as a **tool compound** to study ferroptosis in ND

UAMC-5172

t$_{½}$ - apparent terminal elimination half-life only reported if three or more time points in the elimination phase – excluding the T$_{max}$ - were used for linear regression, and if r$_{adj}$²>0.9, and if % of AUC extrapolation to infinity is lower than 20%; T$_{max}$ – time at which the highest concentration is detected; C$_{max}$ - maximum observed concentration, occurring at T$_{max}$; C$_0$ - initial concentration; AUC$_{0-}$ $_{last}$ - area under the plasma concentration versus time curve up to last quantifiable concentration, and/or up to infinity; AUC$_{0-inf}$ is calculated according to the linear up log down method. F – bioavailability; CL - total clearance determined after IV administration; V$_{ss}$ - apparent volume of distribution at equilibrium determined after IV administration. #αt1/2 (initial phase) - predominant; *βt1/2 (terminal phase). NC = not calculated, r²<0.9. ND - late T$_{max}$ (insufficient data points post C$_{max}$). Data is expressed as the mean ± SD (n= 3).

ACKNOWLEDGEMENTS
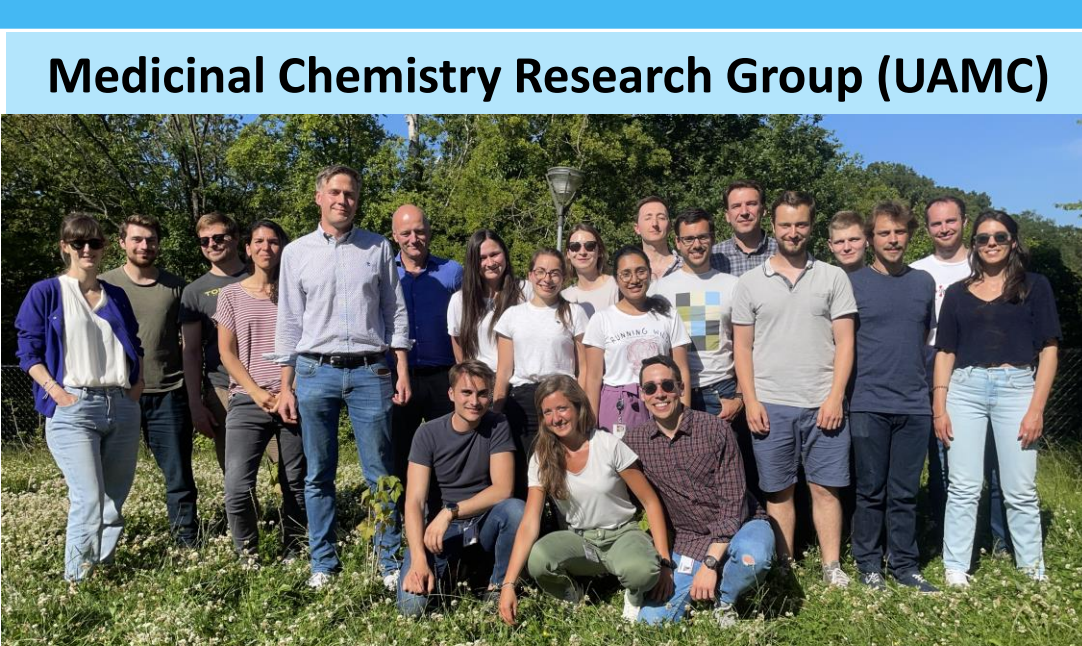
REFERENCES

Stockwell, B. R. ‖ Ferroptosis turns 10 : Emerging mechanisms, physiological functions, and therapeutic applications. Cell 185, 2401–2421 (2022);

Scarpellini C. et al., Beyond ferrostatin-1: a comprehensive review of ferroptosis inhibitors. TIPS 44, 902-916 (2023);

Veeckmans G., Van San E., Vanden Berghe T. A guide to ferroptosis, the biological rust of cellular membranes. The FEBS Journal 291 2767–2783 (2024);

Van San E. et al., Ferroptosis contributes to multiple sclerosis and its pharmacological targeting suppresses experimental disease progression. Cell Death & Differentiation 30:2092 – 2103 (2023);

Berndt C. et al., Ferroptosis in health and disease. Redox Biology 75, 103211 (2024);

Augustyns K. et al., Substituted phenothiazines as ferroptosis inhibitors. WO2024062043 (2024).