CCR2 antagonists for the treatment of neuropathic pain

The discovery and development of AZD2423

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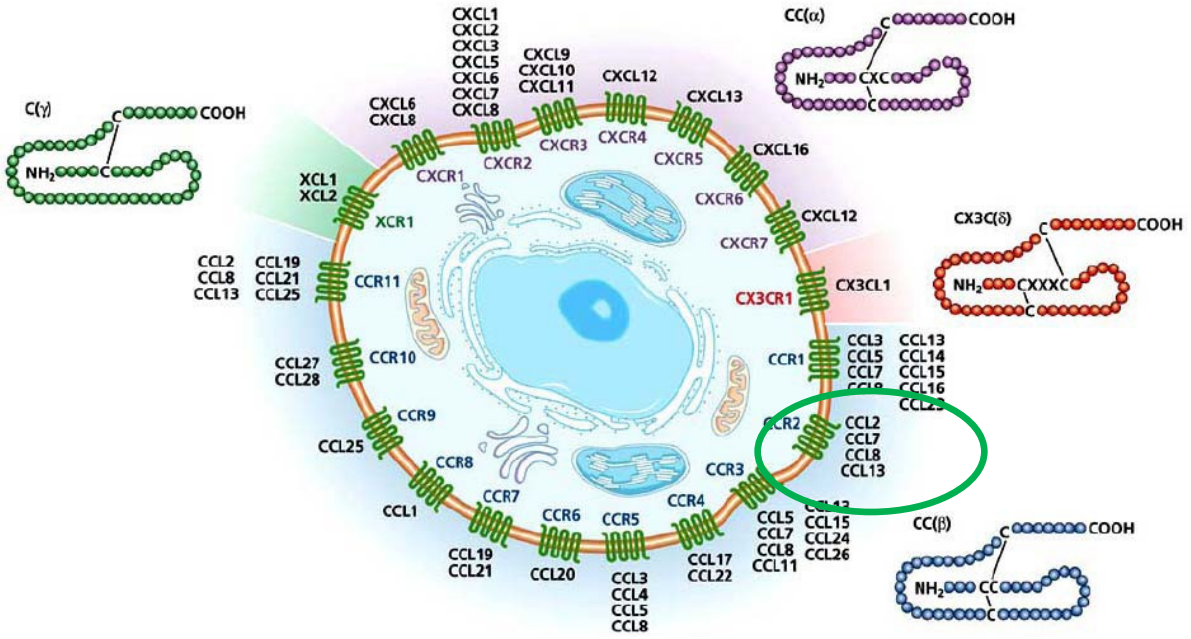
AstraZeneca

Outline

- Rationale: CCR2 and neuropathic pain
- Project objectives
- Medicinal chemistry
- Profile of AZD2423
- Pre-clinical efficacy
- Clinical development
- Conclusions

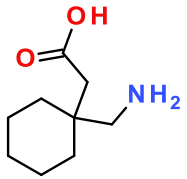
CCR2 and its ligand CCL-2 (MCP-1)

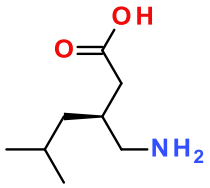


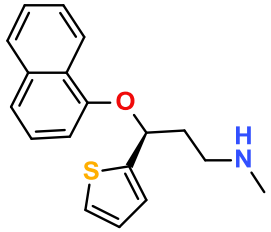
- CCR2 is the receptor for CCL-2, CCL-7, CCL-8 and CCL-13
  - CCR2 is only known receptor for CCL-2 (MCP-1) and CCL-13 (MCP-4)
  - CCL-2 binds preferentially to CCR2 with high affinity (K_i 2 nM)
- CCL-2 is chemotactic for monocytes, dentritic cells, memory T cells and basophils
- CCL-2 knock-out studies indicate that it has a non-redundant role for monocyte recruitment

Neuropathic pain

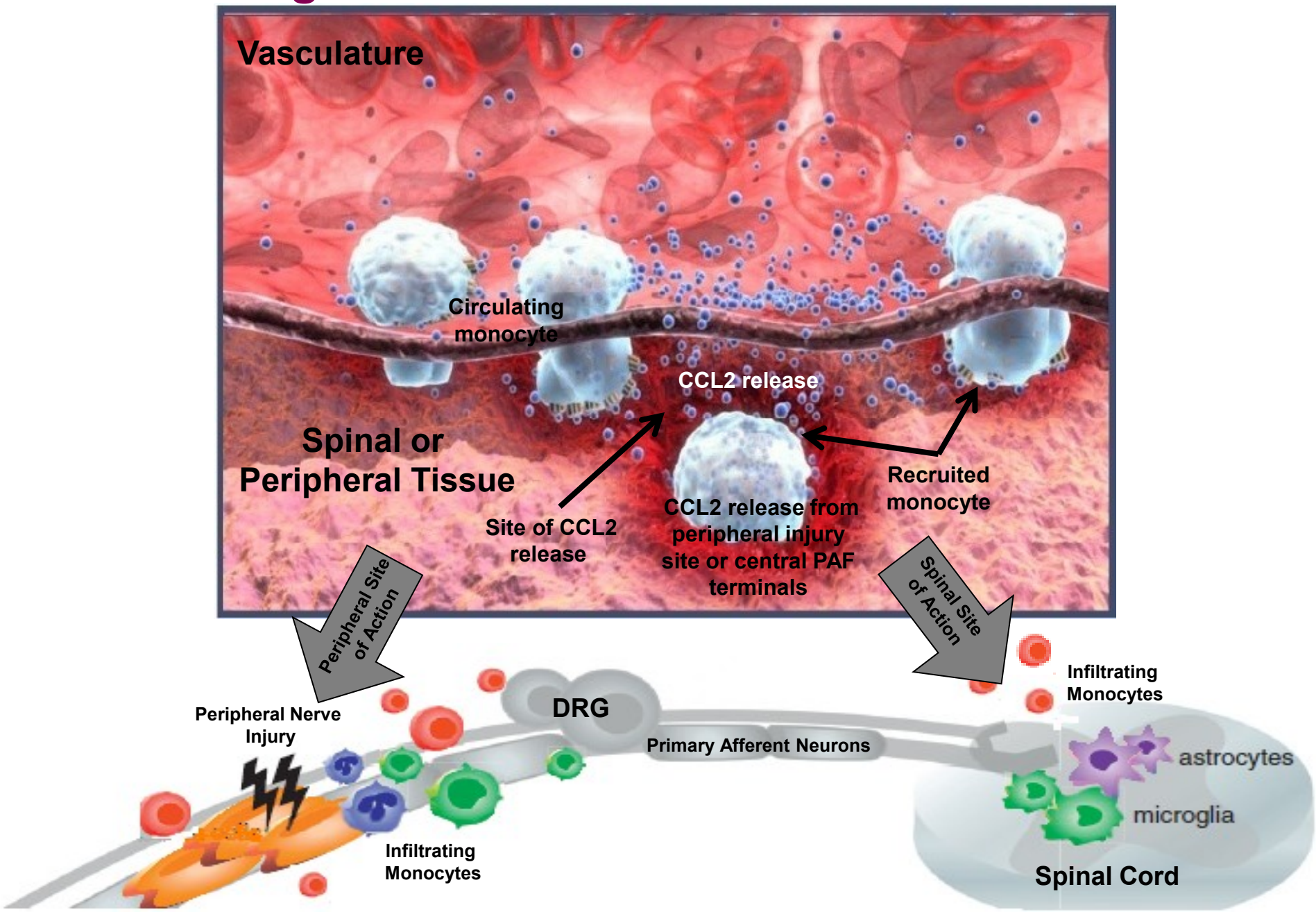
- Heterogeneous group of disorders having in common nerve injury
  - spinal nerve root pain (radicular pain)
  - pain after nerve trauma (post traumatic neuralgia, PTN)
  - painful diabetic neuropathy (PDN)
  - pain after shingles (post herpetic neuralgia, PHN)
- Total prevalence at least 1%
- Approved treatments: gabapentin, pregabalin, and duloxetine
- Limited efficacy (1/3 of subjects) and CNS side effects



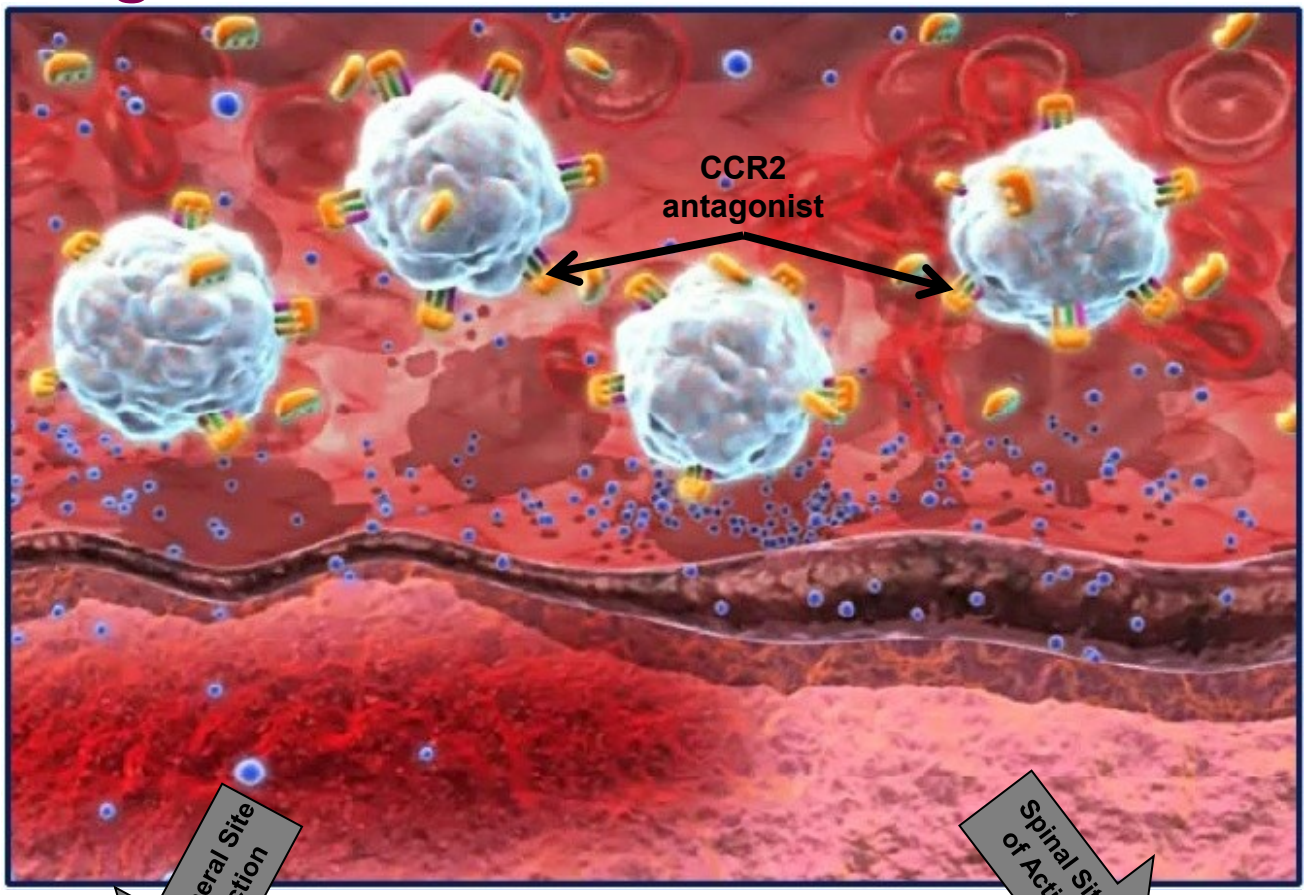




CCR2 antagonists inhibit chemotaxis and infiltration



CCR2 antagonists inhibit chemotaxis and infiltration



Peripheral Site of Action

Spinal Site of Action

Peripheral Nerve Injury

DRG

Primary Afferent Neurons

Infiltrating Monocytes

Attenuation of Immune Cell Infiltration and Release of Sensitising Factors

Attenuation of Immune Cell Infiltration and Release of Sensitising Factors

Monocytes

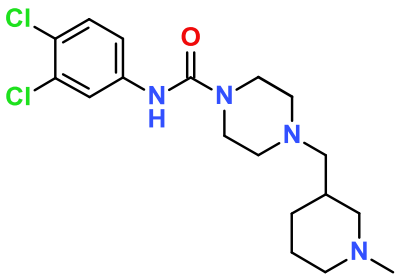
Normalisation of Peripheral and Spinal Sensitisation

Spinal Cord

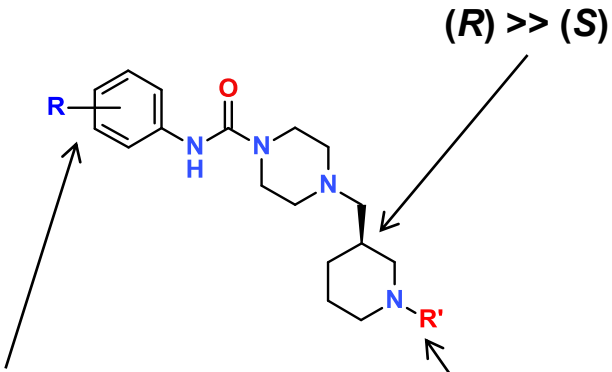
Project goals

- Potent CCR2 antagonist
- Selective vs other chemokine receptors and GPCRs (>100 x)
- Predicted human PK consistent with once daily oral dosing (< 1 mg/kg)
- hERG IC_{50} > 20 μM and hERG margin (vs free C_{max}) >300
  - Assuming human Cmax/Cmin = 3, requires *in vitro* margin CCR2b/hERG >3000x [>3.5 log units]
- Determination of CNS exposure
- Rodent or other non-human species potency to demonstrate activity in disease models and set target C_{ss}

Lead generation

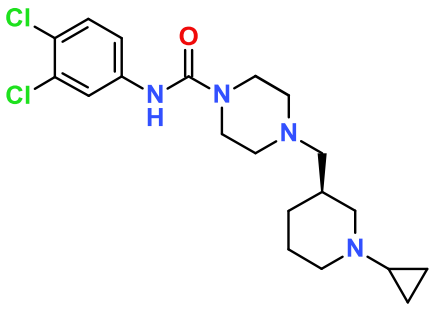


Compound 1
singleton HTS hit
CCR2 binding IC_{50} = 170 nM
CCR2 Ca^{2+} flux IC_{50} = 965 nM



3-/4-/3,4- Cl, F, Me, CF_3, Br ↑
2-Cl, 3-/4- OMe, CN, SO_2Me ↓

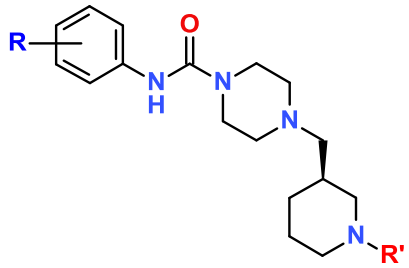
$(CH_2)_2Ph$ > c-Pr ~ i-Pr > Et ~
$(CH_2)_3Ph$ > CH_2Ph > Me > H

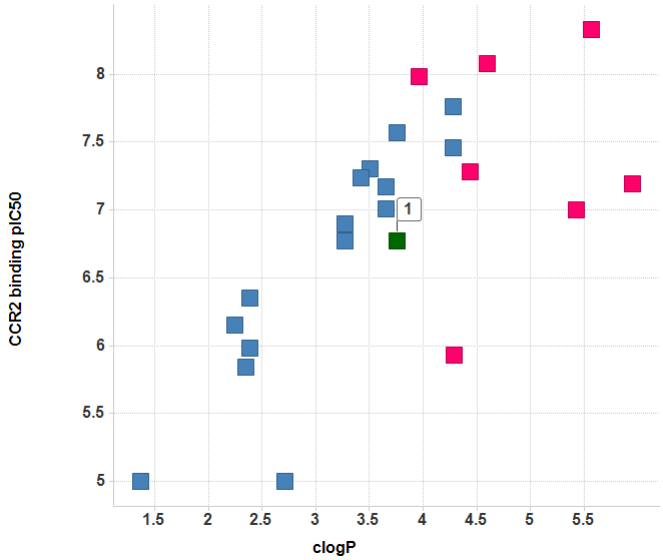


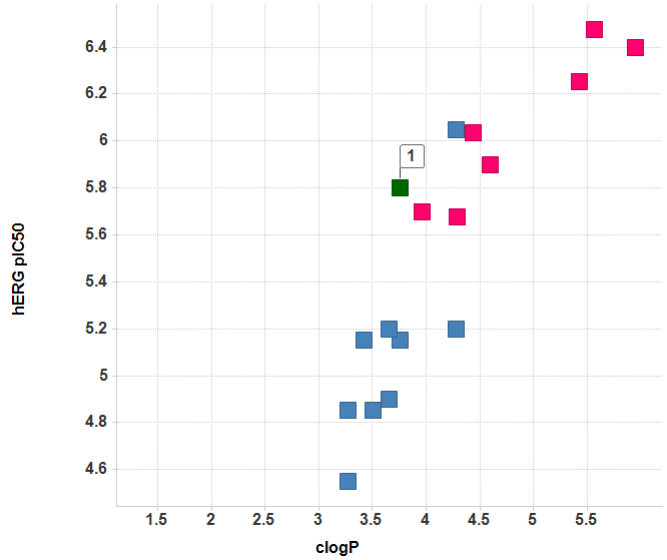
CCR2 binding IC_{50} = 10 nM
CCR2 Ca^{2+} flux IC_{50} = 64 nM
clog*P* = 4.0
hERG binding IC_{50} = 2.0 μM
Good solubility and rat PK
CYP 3A4/2D6 IC_{50} = 1.6/3.1 μM

Cumming, J. G.* *et al. Bioorg. Med. Chem. Lett.* 2012, *22*, 3895-3899.

Potency and hERG correlate with lipophilicity

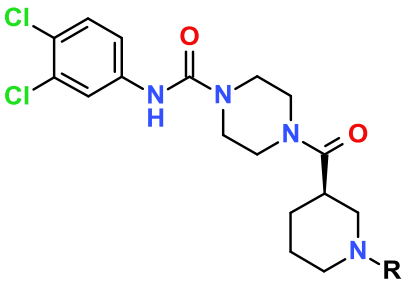


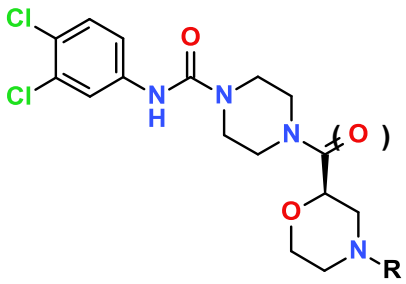


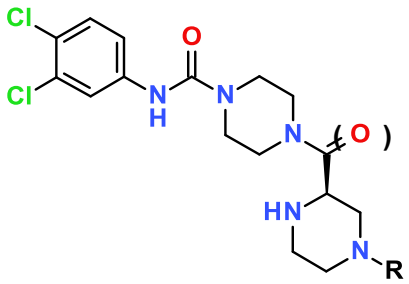


Cumming, J. G.* *et al. Bioorg. Med. Chem. Lett.* 2012, *22*, 3895-3899.

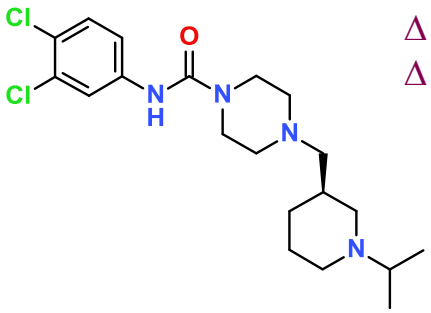
Improving hERG margin by modulating LLE



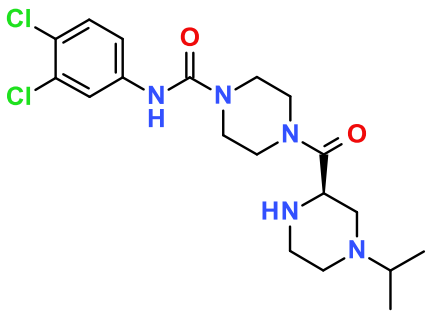




Δ CCR2	+0.4
Δ hERG	−1.1
Δ log*P*	−1.5
Δ log$D_{7.4}$	+0.6



pKa = 10.0



pKa = 7.8
Compound 2

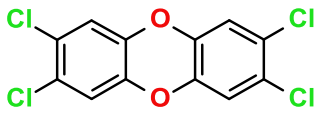
CCR2 binding IC_{50} = 3.5 nM
CCR2 Ca^{2+} flux IC_{50} = 5.8 nM
hERG binding IC_{50} = 17 μM
Good solubility and rat PK
CYP 3A4/2D6 IC_{50} > 10 μM
Rat CCR2 Ca^{2+} flux IC_{50} = 200 nM

- Elevation of CYP1A1 after 7 day rat oral tolerability study
- Flag for arylhydrocarbon receptor (AhR) agonism

Toxicities associated with AhR agonism

CYP1A1 mediated

- Drug-Drug Interactions
- Activation of carcinogens (PAHs)
- Increased metabolism of endogenous ligands?



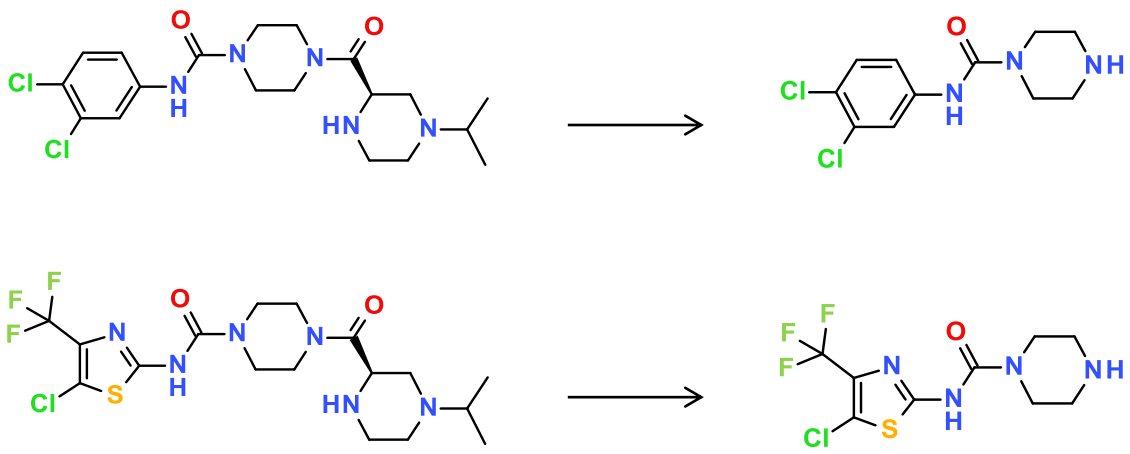
Dioxin (TCDD)

Via other mechanisms

- Characteristic wasting syndrome
- Chloracne
- Immunosuppression
- Teratogenicity
- Endocrine disturbances
- Neuro-behavioural effects

Yushchenko endured dioxin poisoning, likely by political foes, which, along with nearly killing him, left his skin severely disfigured. WILLisms.com

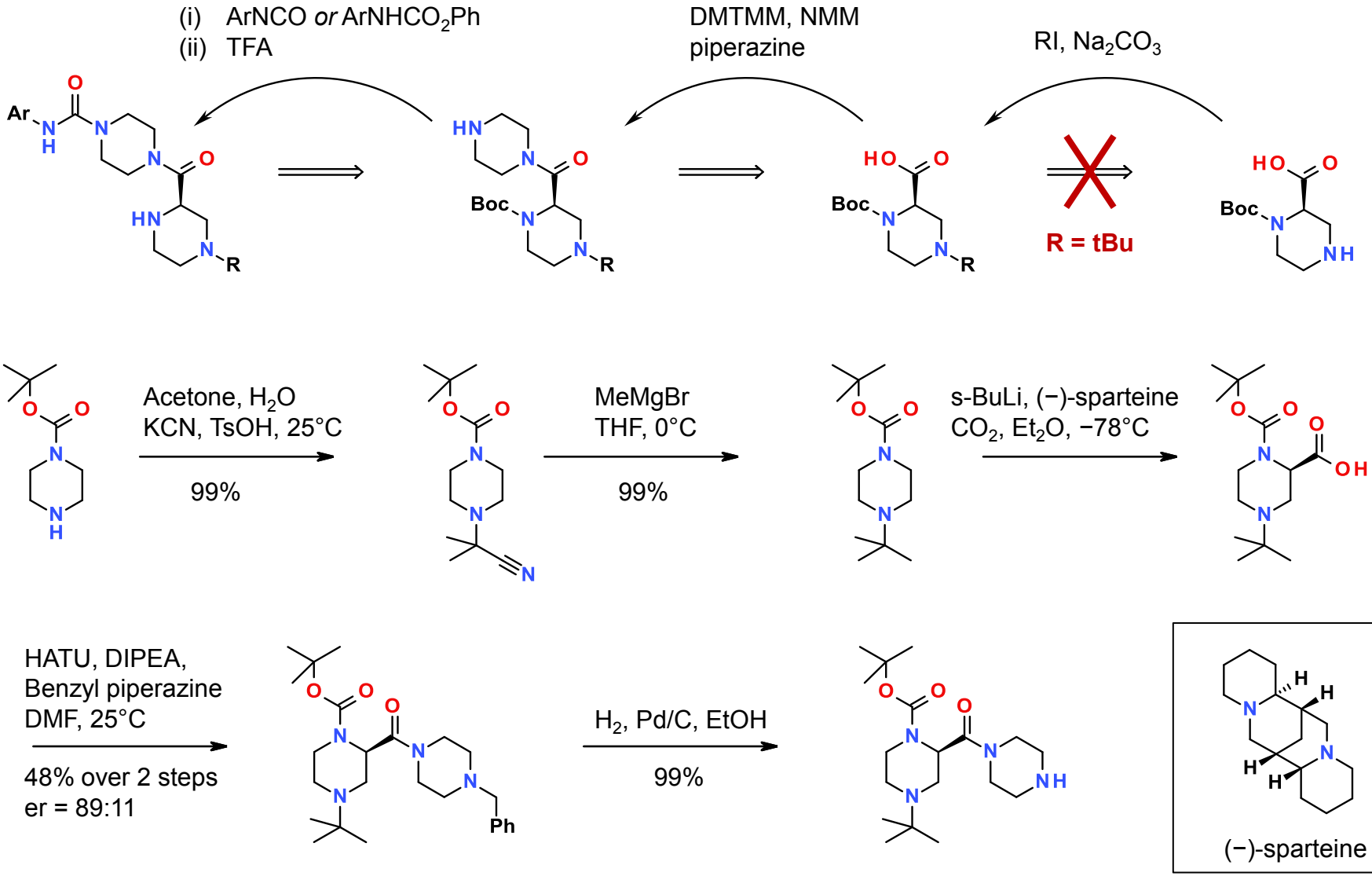
Mitigation of AhR agonism liability



- No adverse effects observed in-life or at pathology
- Piperazine fragments detected *in vivo* (~2% relative to parent)
  - induce CYP1A1 in rat but not human hepatocytes
  - weak activity in rat AhR agonism reporter assay
  - inactive vs human AhR
- Parent compounds showed no activity in any of the *in vitro* assays

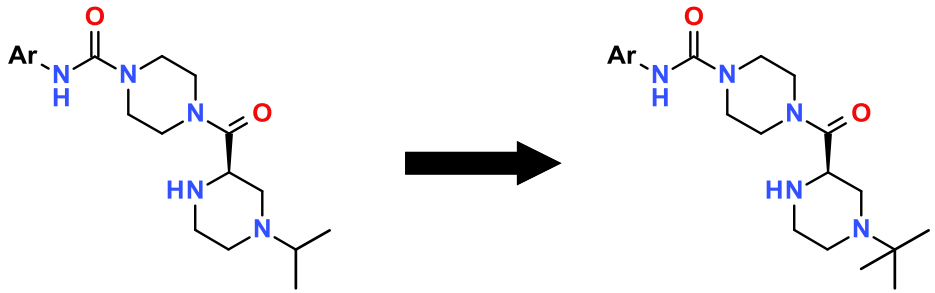
- **Effect appears to be rat-specific and low risk for development**
- **Straightforward to monitor in pre-clinical tox studies**

From isopropyl to tert-butyl: synthesis



McDermott, B. P., Campbell, A. D., Ertan, A. *Syn. Lett.* 2008, *6*, 875-879

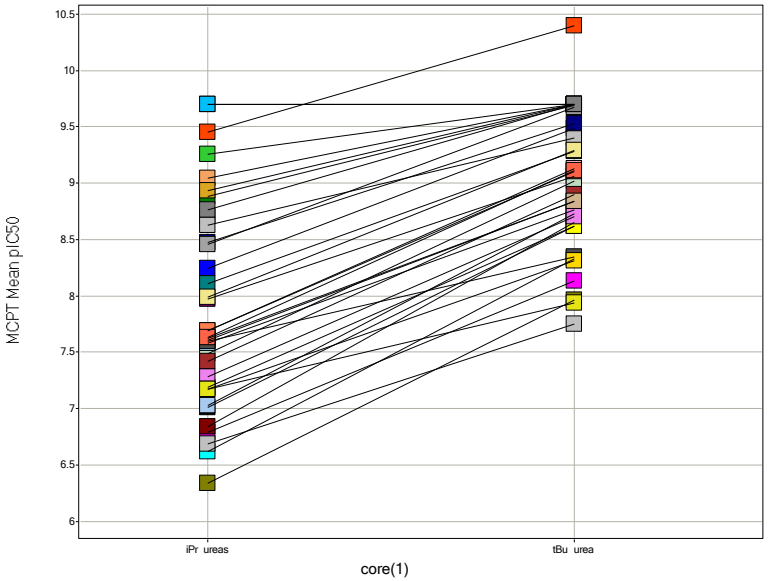
From isopropyl to tert-butyl: SAR



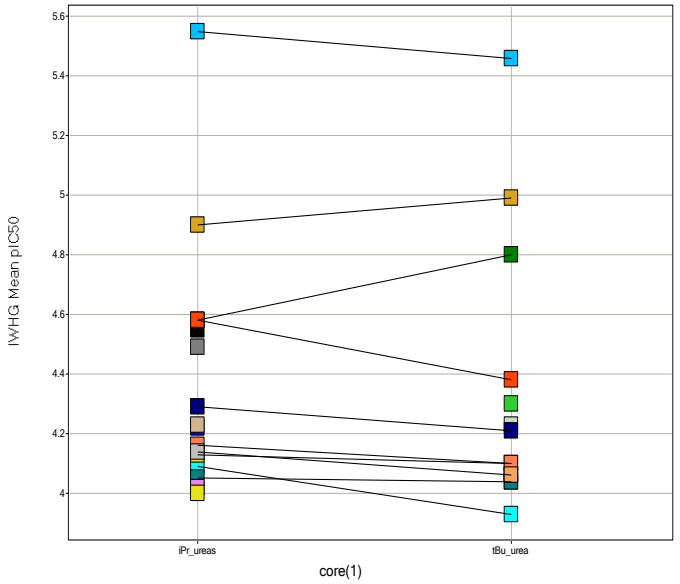
$\Delta \log D_{7.4}$ = +0.13

Matched pair analyses:

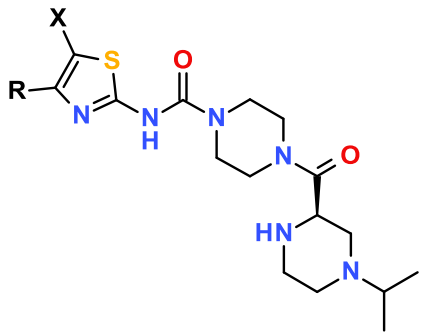
potency



hERG

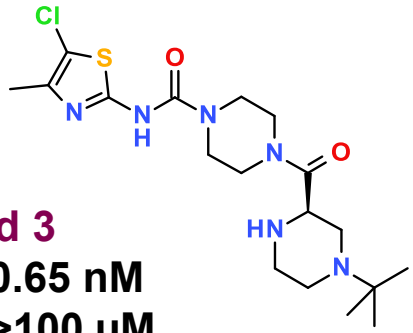


Thiazole series SAR

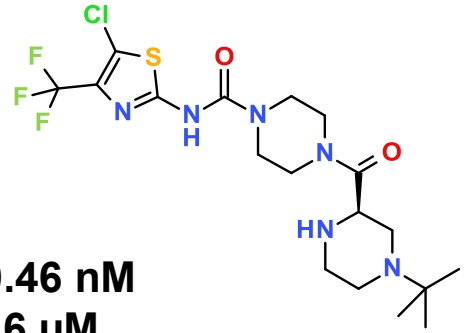


R	X	CCR2 Ca^{2+} flux (nM)	hERG (μM)
^{t}Bu	H	330	74
H	Cl	160	>100
CF_3	H	150	>100
H	Br	68	>100
H	Ph	68	30
CF_3	Me	20	>100
Me	Cl	7.8	>100
Ph	H	2.7	22
CF_3	Cl	1.3	26

*i*Pr → *t*Bu

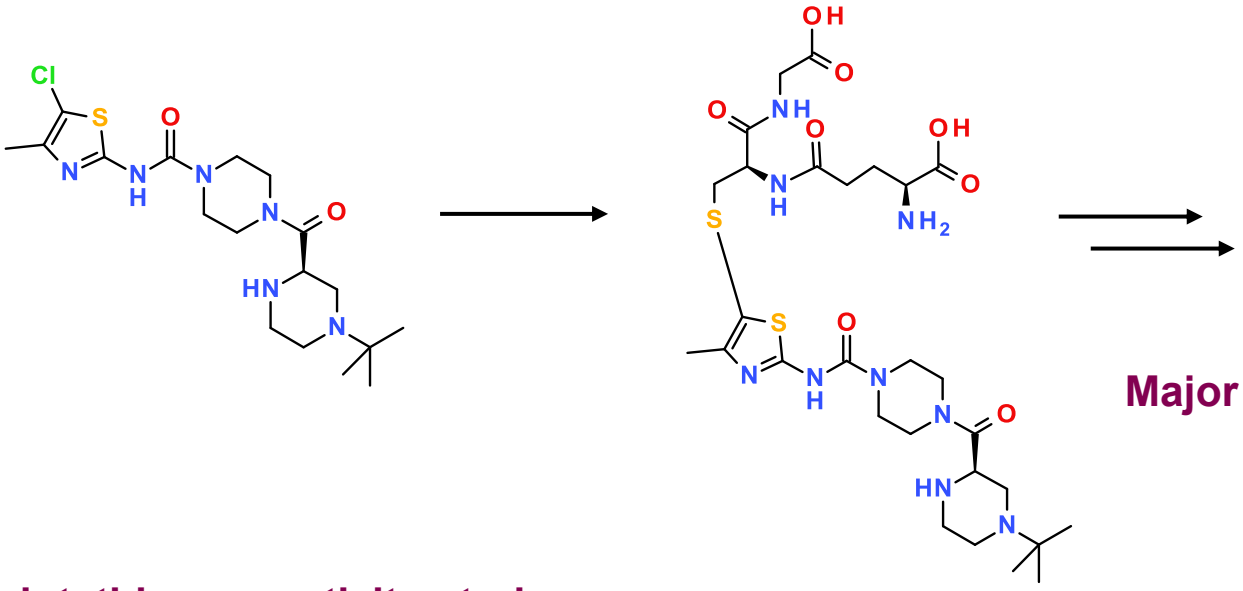


Compound 3
CCR2 0.65 nM
hERG >100 μM



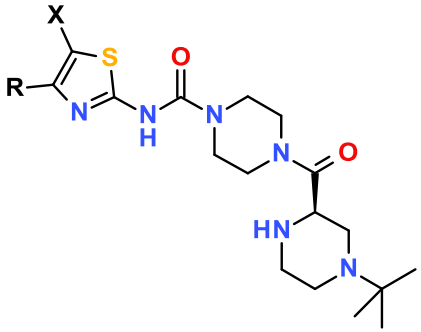
AZ889
CCR2 0.46 nM
hERG 16 μM
Rat CCR2 1.3 ±0.2 nM
Rat tool compound

Unexpected chemical reactivity (1)



Major species *in vivo*

In vitro glutathione reactivity study



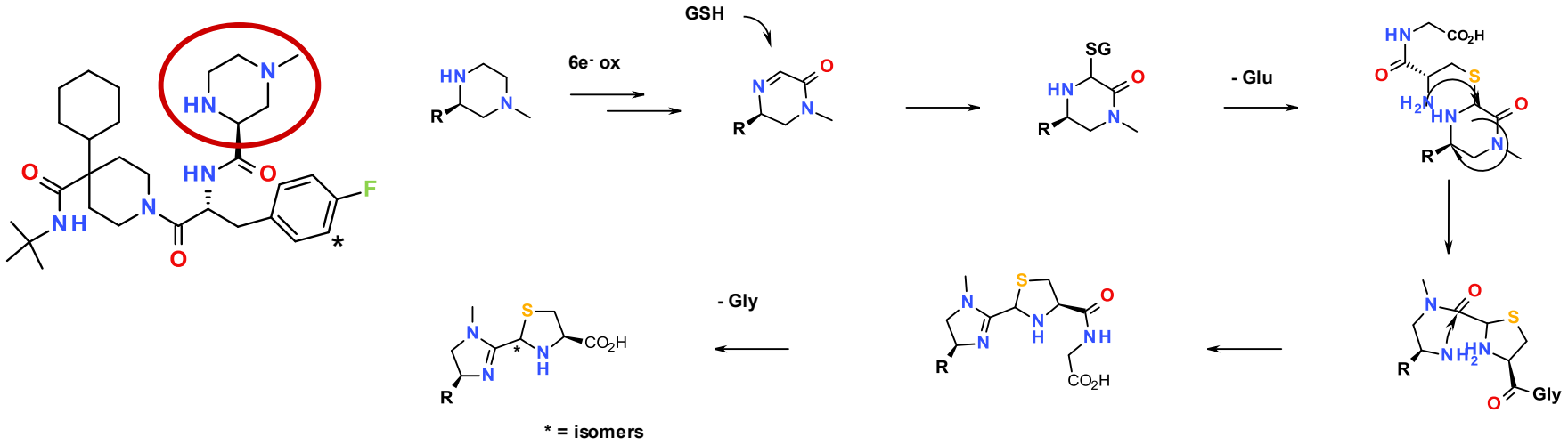
reactivity: X = Br > Cl > F

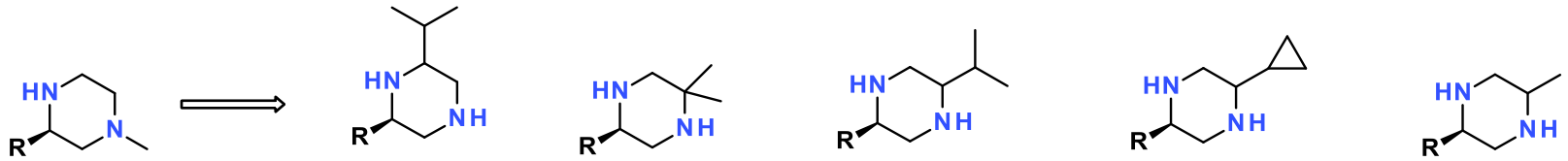
R (X = Cl)	t½ (h)
Me	17
Et	23.5
^{i}Pr	30
^{t}Bu	33
cPr	2
H	no reaction
CF_3	no reaction

S_NAr mechanism?

Metabolism study identified reactive metabolites

- Parent −4H + cysteine observed in rat urine
- *N*-tBu > *N*-iPr > *N*-Et/N-Me (*in vitro* microsomal GSH trapping)
- Consistent with Merck MC4R agonist (MB243) metabolism





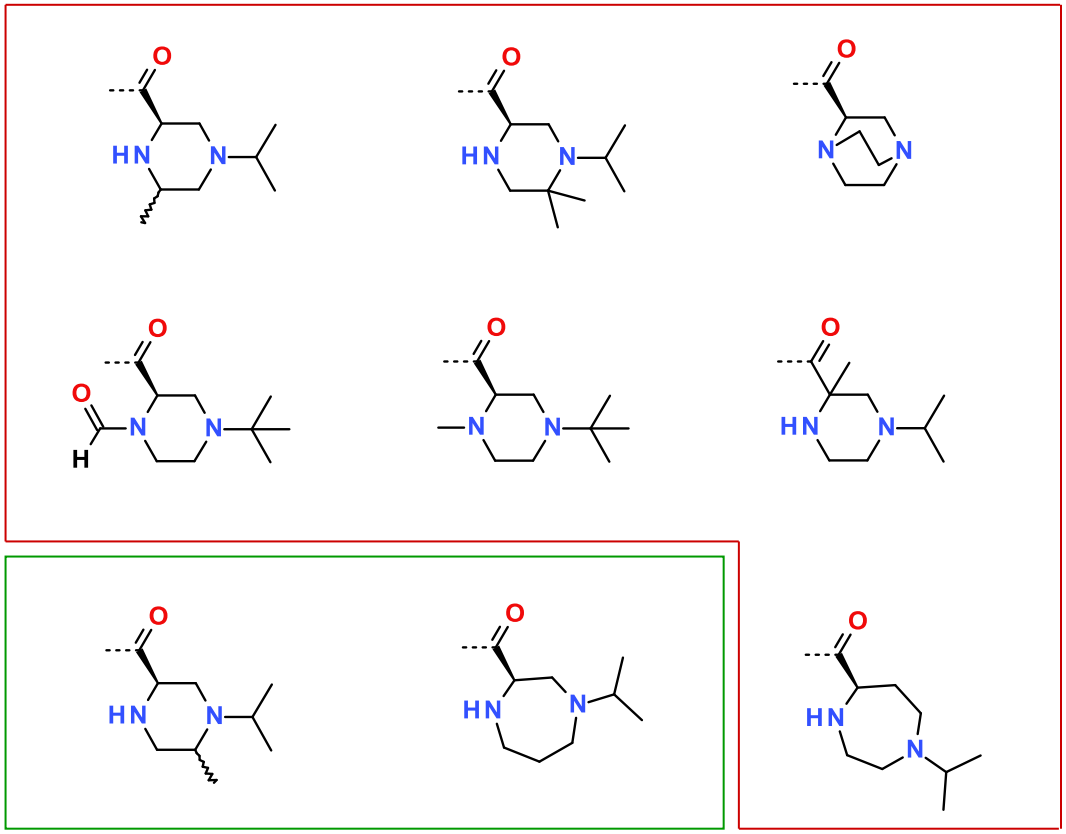
2100 216 149 107 103 50

pmol equiv/mg protein

Doss, G. A. *et al. Chem. Res. Toxicol.* 2005, *18*, 271-276

Can we ‘design out’ reactive metabolites?

Block oxidation on piperazine (c.f. Merck paper)

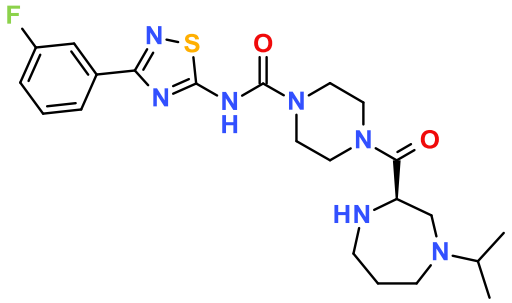


Lose potency

Potency OK

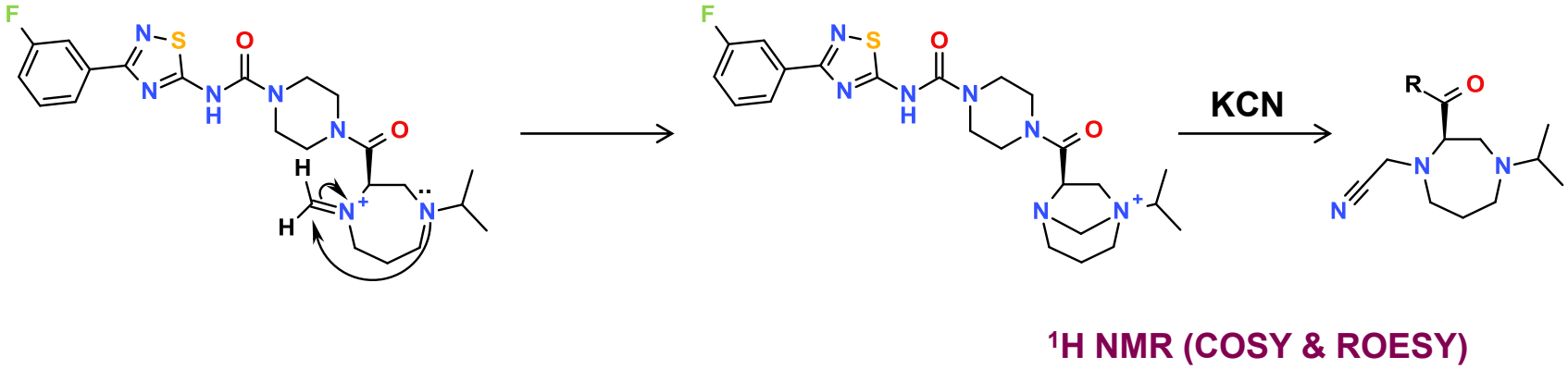
expand to homopiperazine

Unexpected chemical reactivity (2)



$[M+H]^+$ = 476.2238
Major species in blood and urine *m/z* = 488.2238 $[M+C+H]^+$
Found in pre-dose urine spiked with compound

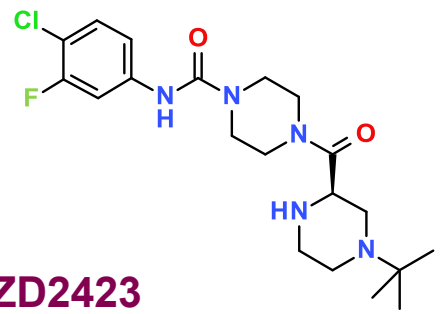
Formaldehyde (0.1 mM in rat blood)



^{1}H NMR (COSY & ROESY)

Martin, S. *et al. Drug Metabolism and Disposition* 2012, *40*, 1478-1486

Profile of AZD2423



AZD2423

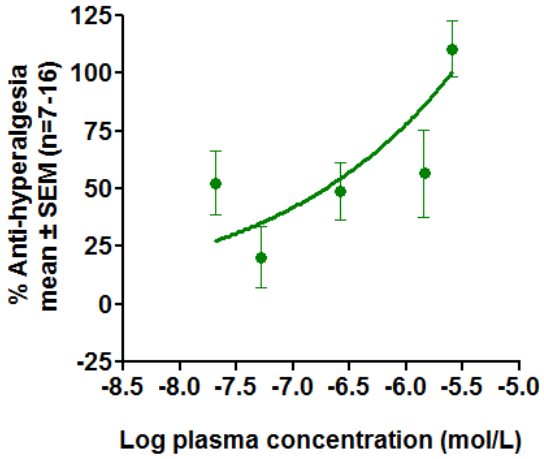
CCR2 binding IC_{50}	2.6 nM
CCR2 Ca^{2+} flux IC_{50}	1.2 nM
CCR2 chemotaxis IC_{50}	4.4 nM
Human whole blood A_2	1.3 nM
CCR5 chemotaxis IC_{50}	316 nM
Rat CCR2 Ca^{2+} flux IC_{50}	607 nM
GPCR selectivity	>100x
hERG IC_{50}	90 µM
MWt	426
Solubility (pH 7.4 buffer)	13.2 mM
Stable crystalline material	
log$D_{7.4}$	1.85
p*K*a	7.9
PPB (% free) [rat, dog, human]	42, 35, 47
CL [rat, dog]	21, 4 ml/min/kg
Bioavailability (%) [rat, dog]	16, 71
Caco-2 [A2B / B2A / ER]	2.5 / 10 / 4
Predicted human dose	12-24 mg qd

Potent and selective inhibitor of CCR2 with properties suitable for development and testing of clinical hypothesis

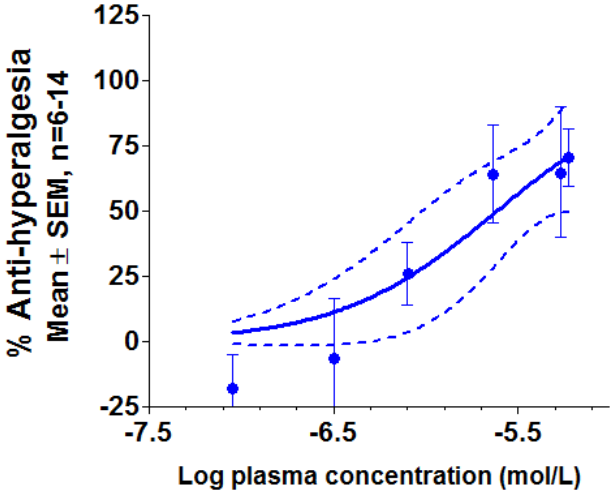
Pre-clinical rat neuropathic pain models

Chronic constriction injury (CCI)-induced mechanical hypersensitivity

AZ889

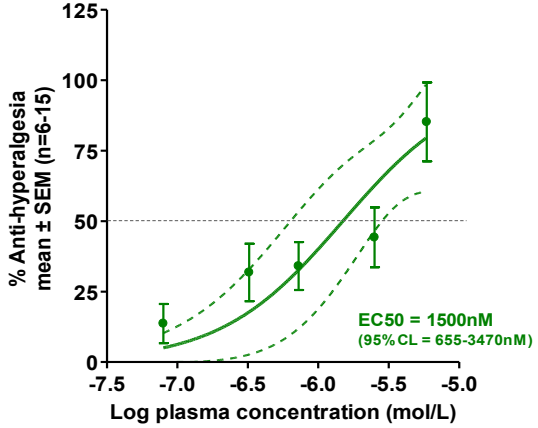


AZD2423



E_{max} >100%
EC_{50} plasma = 191 nM (total), 33 nM (free)
EC_{50} brain = 63 nM (total), 3.8 nM (free)

AZD2423 in Chung heat hyperalgesia model

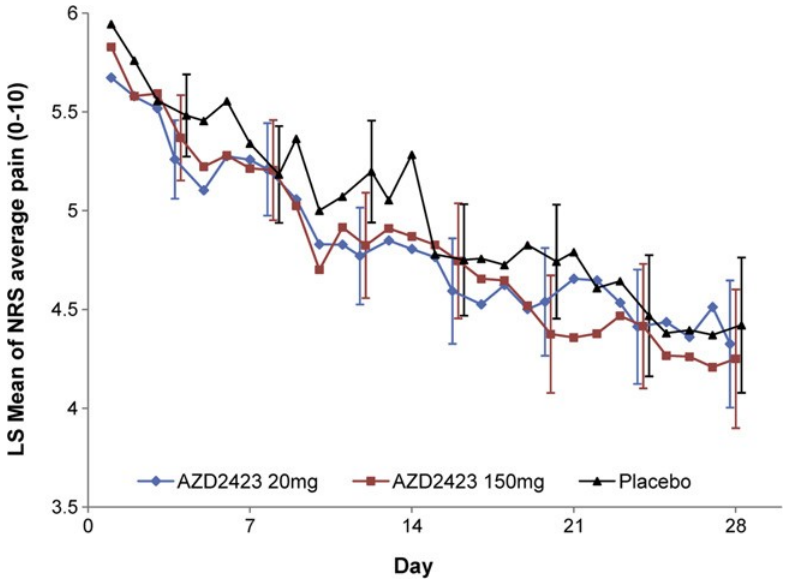


Serrano, A. *et al. Molecular Pain* 2010, *6*, 90

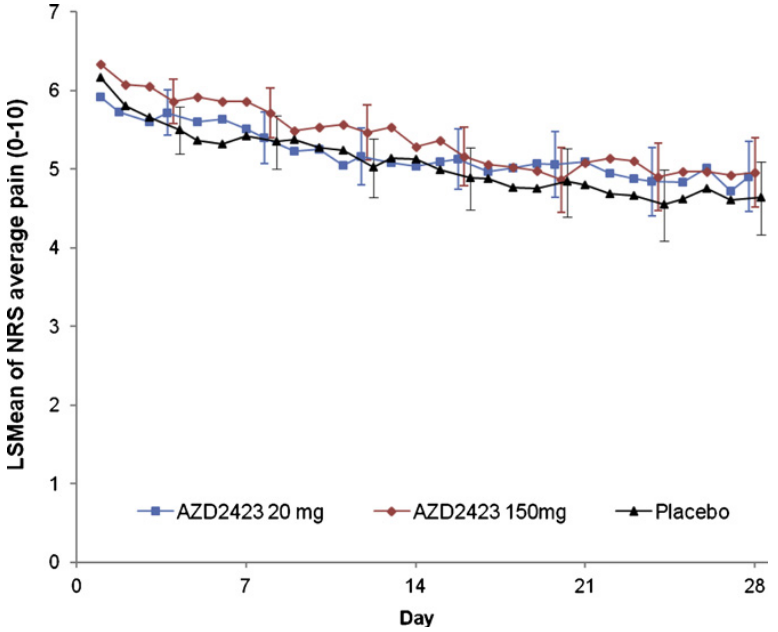
AZD2423 Ph2a trials in neuropathic pain patients

- Randomized, double-blind, placebo-controlled multi-centre trials
- 20 mg or 150 mg AZD2423 or placebo, once daily for 28 days

PTN 133 patients



PDN 134 patients



Kalliomäki, J. *et al. Pain* 2013, *154*, 761-767
Kalliomäki, J. *et al. Scandinavian Journal of Pain* 2013, *4*, 77-83

Summary of AZD2423 receptor occupancy

AZD2423 is a non-competitive negative allosteric modulator

		Peripheral	Central
Rat pain model (80% reversal of hyperalgesia in CCI)	Estimated based on IC_{50} and exposure	80%	17%
PDN and PTN Phase 2a studies ($C_{ss,avg}$ 150 mg)	Inhibition of CCL_2 clearance *in vivo*	96%	-
	Binding *ex vivo* in peripheral blood monocytes	93 %	-
	Calculated based on K_B and exposure	97%	90%

- **Both exposure and receptor occupancy exceed efficacious levels in rat pain models**
- **Therefore conclude that the hypothesis that AZD2423 is analgesic in PDN and/or PTN patients has been tested**

Conclusions

- Novel, potent and selective series of CCR2 antagonists identified from screening of ‘GPCR ligand motif’ library
- Separation of CCR2 and hERG activity achieved through structural modifications without increasing molecular weight
- CYP1A1 induction and reactive metabolite liabilities investigated
- Unexpected chemical reactivities observed within series
- AZD2423 selected as candidate for clinical development
- Good efficacy in pre-clinical models of neuropathic pain
- Two Phase 2a trials in neuropathic pain patients failed to show efficacy against primary endpoints despite good evidence of target engagement

Acknowledgements

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David Waterson
Jon Winter
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Colin Fielding
Julian Hudson
Natalie Huther
Mike James
Scott Lamont
Ben McDermott
Alan Reid
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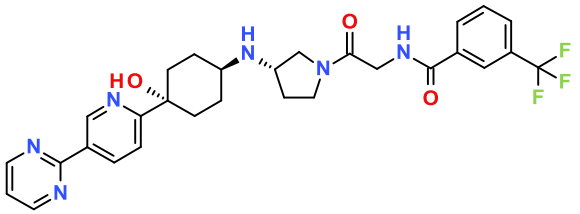
CCR2 literature validation for neuropathic pain

- CCL-2 induces pain when injected peripherally or into spinal cord
- Over-expression of CCL-2 in the spinal cord induces pain
- CCR2/CCL-2 elevated in the spinal cord after nerve injury
- CCL-2 can directly activate DRG neurons
- CCL-2/CCR2 key regulators of microglia recruitment/biology
- CCR2 KO mice show absence of hyperalgesia after nerve injury (Merck)
- Ablation of CCR2 centrally & peripherally is required for analgesia (Ji Zhang)
- A CCR2 antagonist is active in mouse neuropathic pain model (Merck)

CCR2 antagonists in clinical development

Highest phase: Ph2



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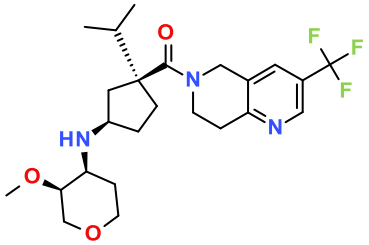

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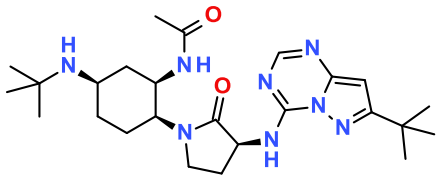

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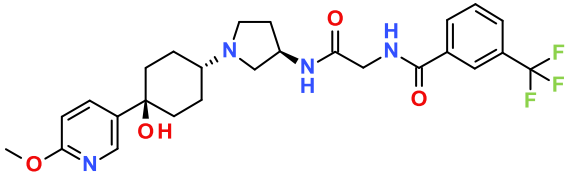

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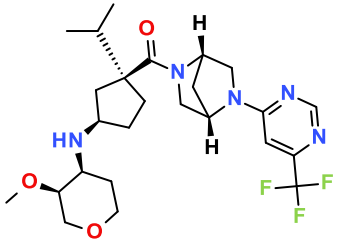

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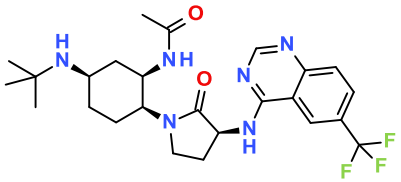
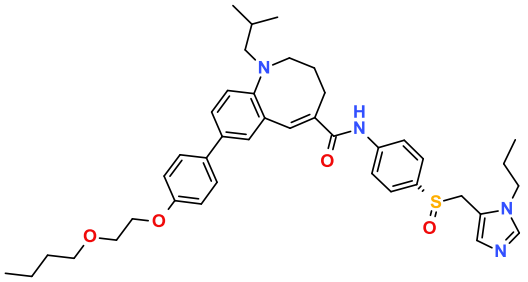
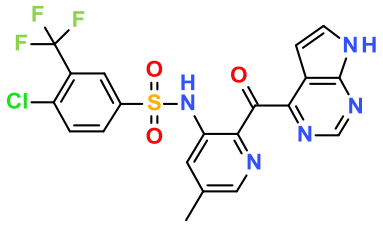


BMS-741672



Cenicriviroc (CCR2+CCR5)



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