Targeting the kynurenine pathway for tumour detection and characterization by PET and SPECT

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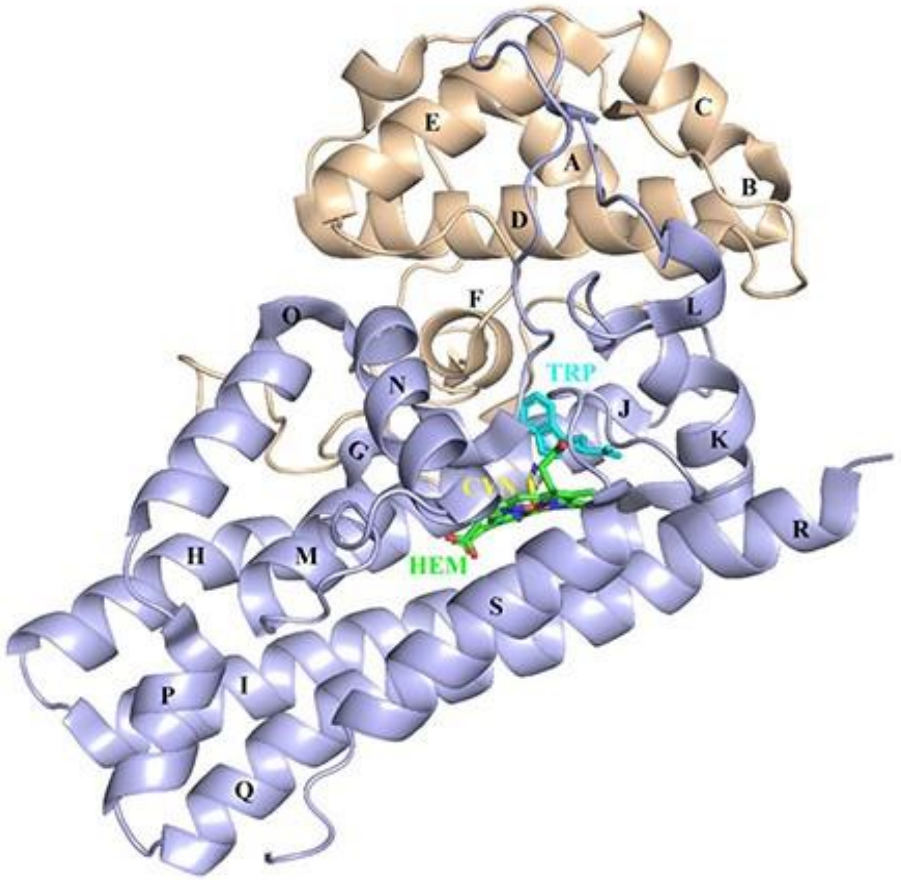

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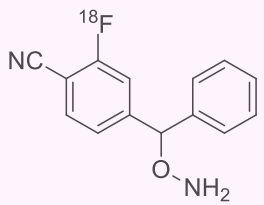
INTRODUCTION

KP blockade is an attractive approach for cancer immunotherapy and recent efforts have been focusing on the development of dual IDO1/TDO and/or pan-inhibitors.[1] To-date there are no means to effectively detect KP activation *in vivo*, which hampers the development of efficacious anti-cancer strategies. A successful KP-specific imaging probe would represent a step-change in our ability to:

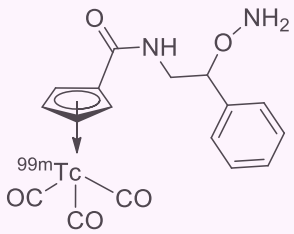
- Improve tumour diagnosis and characterization in oncology
- Allow non-invasive assessment of cancer immunotherapy response
- Understand the exact role of the implicated enzymes IDO1/2 and TDO in cancer

In this work, we report on the pre-clinical development of ^{18}F-PET tracers **1** and **2** and ^{99m}Tc-SPECT **3** tracer as possible tools for the non-invasive imaging of KP in cancer with potential applications for tumour detection and characterization, based on the pharmacophore structure of diaryl hydroxylamines whose potential for dual and/or pan inhibition was recently shown.[2]

1a



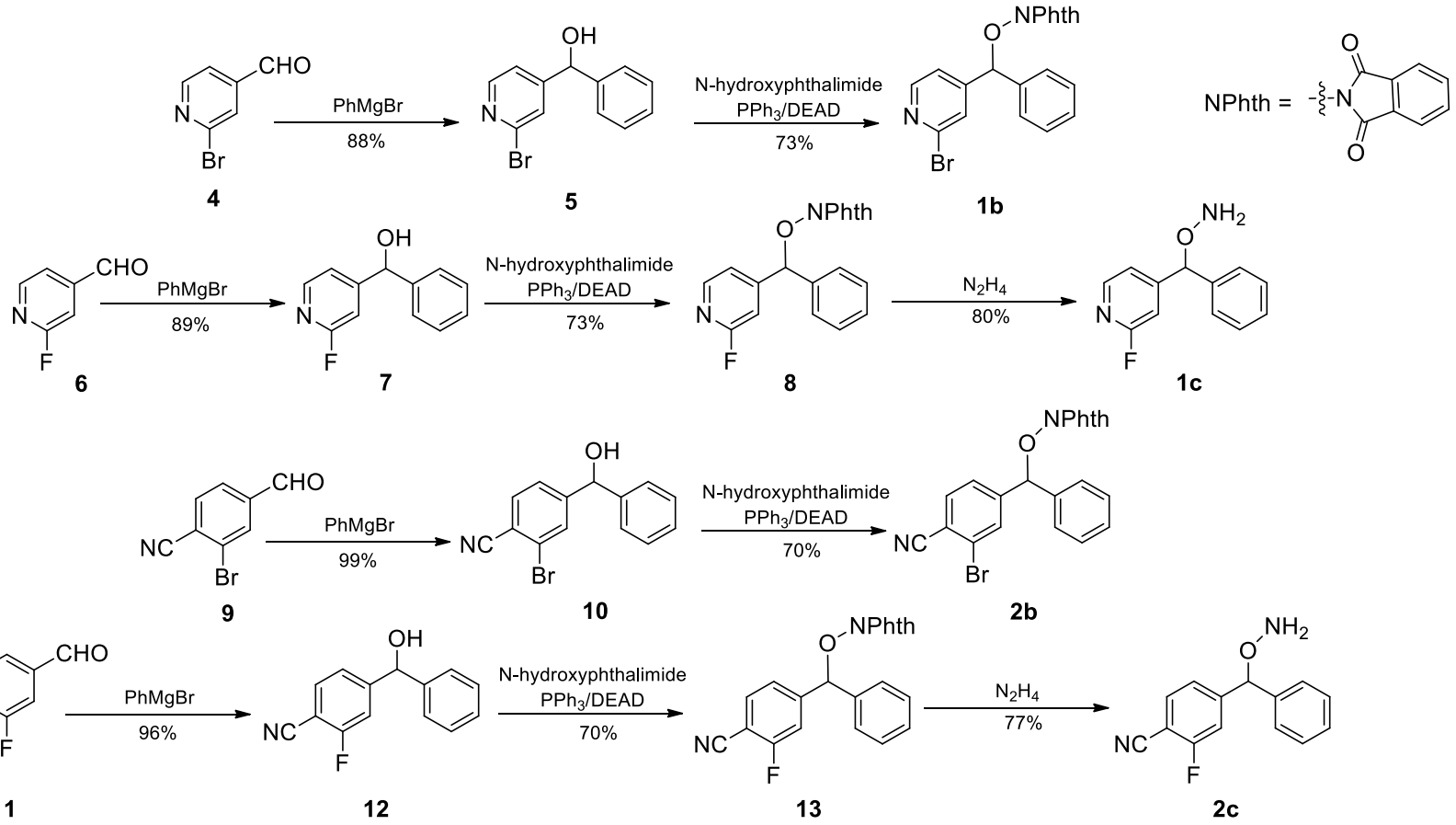
2a



3a

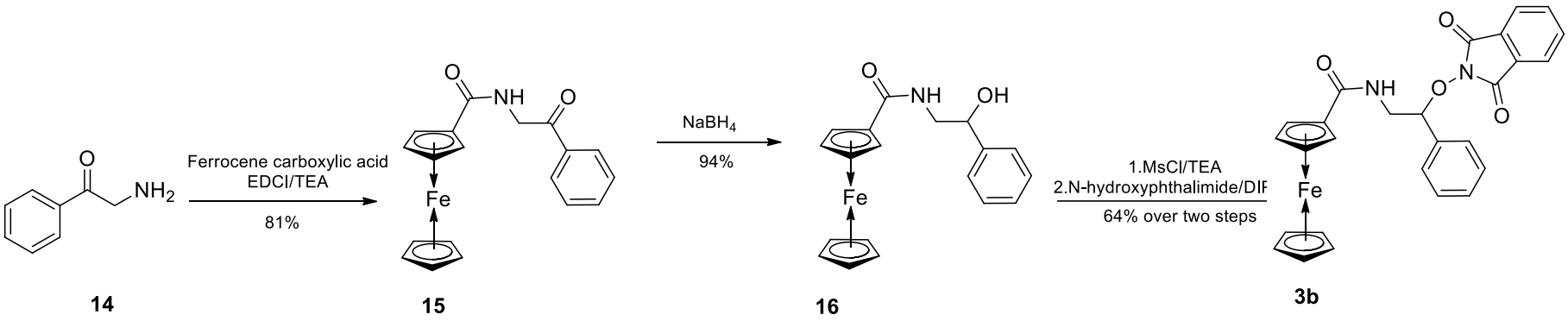
METHODS

Synthesis of reference compounds and precursors for radiolabelling 1b-c and 2b-c

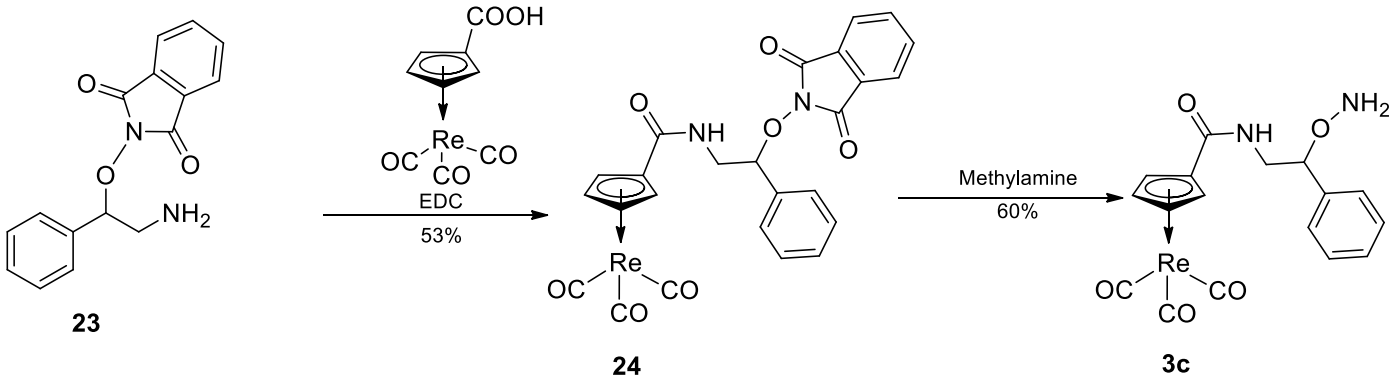


The O-arylhydroxylamine derivatives were synthesized from the corresponding alcohols using the Mitsunobu reaction with N-hydroxyphthalimide and subsequent deprotection of the phthalimide group with hydrazine. The alcohol precursors were usually synthesized by adding phenylmagnesium bromide to the appropriate aldehyde.

Synthesis of reference compounds and precursors for radiolabelling 3b-c.

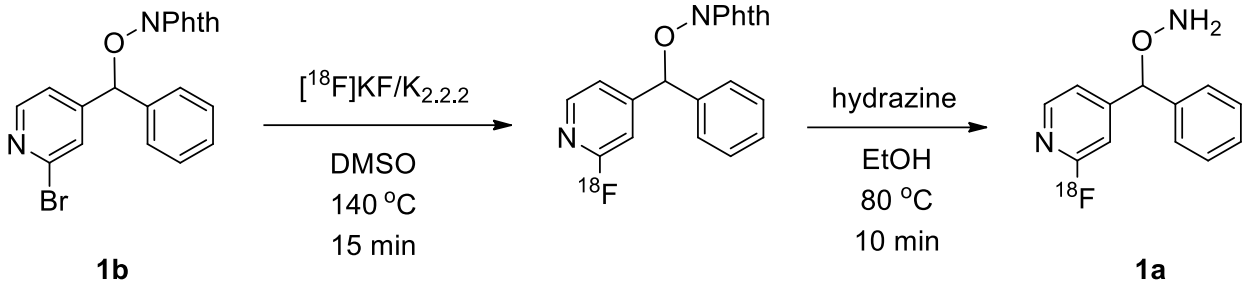


Amine **14**[3] was coupled with ferrocene carboxylic acid to give the corresponding amide **15** which was reduced to alcohol **16**. The latter was converted via its intermediate mesylate to **precursor 3b**.



Amine **23**[4] which was coupled with $Re(CO)_3CpCOOH$[5] to give the corresponding amide **24**. Methylaminolysis of **24** afforded the desired **reference 3c**.

Radiolabelling of compound 1a.



Preliminary radiolabelling of **1a** was accomplished via nucleophilic aromatic substitution using bromo-precursor **1a**. Reaction of **1b** with [^{18}F]KF/Kryptofix in DMSO at 140 °C afforded the radiolabelled intermediate which after hydrazinolysis yielded tracer **1a**.

Enzyme-based IDO1, IDO2 and TDO inhibition assays

Compound IC_{50} values were assessed from single point dilution series. Following the TCA fixation step, the supernatants were transferred to a roundbottomed 96-well plate and incubated at 65 °C for 15 min. The plates were then centrifuged at 1250xg for 10 min, and 100 mL of clarified supernatant was transferred to a new flat-bottomed 96-well plate and mixed at equal volume with 2% (w/v) p-dimethylaminobenzaldehyde in acetic acid. The yellow reaction was measured at 490 nm using a Synergy HT microtiter plate reader (Bio-Tek, Winooski, VT). Graphs of inhibition curves with IC_{50} values were generated using Prism v.5.0 (GraphPad Software, Inc.)

RESULTS

Enzyme inhibition data for compounds 1-3c.

Compound	IDO1 (μM)	TDO (μM)
1c	0.37	16.1
2c	0.57	9.9
3c	10	-

- Enzyme-based assays identified the references **1c** and **2c** as potent **dual IDO1/TDO inhibitors**, while **3c** was identified as an IDO1 inhibitor.
- Cell-based assays for IDO1 support these results.
- Preliminary efforts afforded tracer **1a** in 5% isolated radiochemical yield (decay corrected). Radiochemical purity was greater than 95% and specific activity was 57 GB/μmol.

CONCLUSIONS

The designed KP tracers act dual IDO1/TDO or IDO1 inhibitors and show promise for imaging KP activation. On-going efforts are focusing on the radiosynthesis of **2a** and **3a** and the *in vivo* evaluation of **1-3** in tumour bearing mice

ACKNOWLEDGMENTS

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