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## ARTICLE

## The first indoleamine-2,3-dioxygenase-1 (IDO1) inhibitors containing carborane

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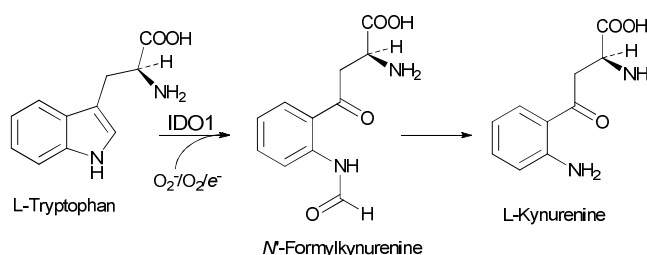
Indoleamine 2,3-dioxygenase 1 (IDO1) is a critical immunoregulatory enzyme responsible for the metabolism of tryptophan during inflammation and disease. Based upon a pyranonaphthoquinone framework, the first examples of indoleamine-2,3-dioxygenase-1 (IDO1) inhibitors containing a carborane cage are reported. The novel *closo*-1,2-carboranyl-*N*-pyranonaphthoquinone derivatives display low  $\mu\text{M}$  binding affinity for the human recombinant enzyme, with  $\text{IC}_{50}$  values ranging from 0.78 to 1.77  $\mu\text{M}$ .

### Introduction

A lack of structural diversity has been cited as a potential limitation to future drug design.<sup>1</sup> Whilst the element boron currently accounts for approximately 0.5% of framework heteroatoms within the CAS Registry,<sup>2</sup> the unique chemical properties of this element are worthy of further exploration in medicinal chemistry. Boron clusters, particularly the icosahedral dicarba-*closo*-dodecaboranes (commonly referred to as *closo*-carboranes), have attracted increasing interest from medicinal chemists in recent years as unique pharmacophores.<sup>3</sup> With a three-dimensional volume (70–80  $\text{\AA}^3$ ) that is comparable to the rotational sweep of a phenyl ring, low toxicity, high kinetic stability to hydrolysis and metabolic degradation, a tunable hydrophobic character, and the possibility of many versatile chemical modifications, carborane clusters offer medicinal chemists a powerful tool to alter shape and charge distribution in both existing and novel drug frameworks.<sup>3</sup> Due to the considerable hydrophobicity and unique shape of the *closo*-carboranes, enzyme targets possessing hydrophobic regions in their active sites have been a focus of recent carborane-based drug development.<sup>1</sup> With an active site dominated by two hydrophobic binding pockets,<sup>4</sup> the haemoprotein indoleamine-2,3-dioxygenase-1 (IDO1; EC 1.13.11.52) is an excellent candidate for a study of carborane/enzyme interactions.

IDO1 is responsible for the first catabolic step (oxidative cleavage of L-tryptophan to *N*-formylkynurenine, Figure 1) of the 'Kynurenine Pathway', the major pathway for tryptophan metabolism (*ca.* 90%) in mammals. An immunoregulatory enzyme controlled by inflammatory cytokines (principally interferon- $\gamma$ ); during infection and disease increased expression of IDO1 lowers cellular L-tryptophan levels, which elicits a range of innate and adaptive

immune responses. Most significantly, low tryptophan levels and tryptophan metabolites downstream of *N*-formylkynurenine affect T-lymphocyte behaviour and communication, resulting in T-cell tolerance and apoptosis.<sup>5, 6</sup> Evolved to suppress autoimmunity during periods of sustained inflammation, IDO1 activity is co-opted by cancer cells (among other pathological conditions) as a mechanism to evade immune detection.<sup>7, 8</sup> Thus, restoration of normal immune response *via* IDO1 enzyme inhibition has been the subject of intense study in recent years due to the potential use of such inhibitors as immunoregulatory chemotherapeutic agents.<sup>9</sup>

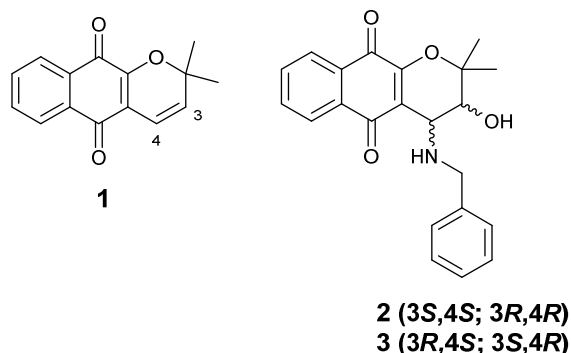


**Figure 1.** The first steps of the 'Kynurenine Pathway'. Indoleamine-2,3-dioxygenase-1 (IDO1) cleaves the 2,3-double bond of the L-tryptophan indole ring *via* incorporation of O<sub>2</sub> or O<sub>2</sub><sup>-</sup> to form *N*-formylkynurenine. *N*-formylkynurenine spontaneously (and *via* enzymatic cleavage) forms L-kynurenine.

Until the human IDO1 structure was determined by means of X-ray crystallography in 2006,<sup>4</sup> IDO1 inhibitor development was mainly based on alterations of the tryptophan-indole scaffold with the best inhibitors displaying  $\text{IC}_{50}$  affinities in the  $\mu\text{M}$  range.<sup>9</sup> However, through a greater understanding of the substrate active-site, which is characterised by a large hydrophobic pocket containing the haem *b* catalytic site and a smaller hydrophobic region near the entrance to the active site, significant developments in non-tryptophan related

frameworks have been made in recent years,<sup>10, 11</sup> thus improving the potency of IDO1 inhibitors for potential clinical trial assessment.

Following the discovery of pyranonaphthoquinones from marine natural products extracts with potent IDO1 inhibitory properties (Annulin B from *Garveia annulata*<sup>12</sup> and Exiguamine A from *Neopetrosia exigua*<sup>13</sup>), pyranonaphthoquinone frameworks have emerged as promising lead candidates for further synthetic development (**1**).<sup>14</sup> Interestingly, the *cis* diastereomers (*3S,4S/3R,4R*) of the benzylamino-pyranonaphthoquinone derivative (Figure 2, **2**) are reported to exhibit some of the most potent IDO1 inhibition observed to date.<sup>14</sup>



**Figure 2.** Dehydro- $\alpha$ -lapachone (**1**); a natural product containing the pyranonaphthoquinone framework which has demonstrated excellent IDO1 inhibition.<sup>14, 15</sup>

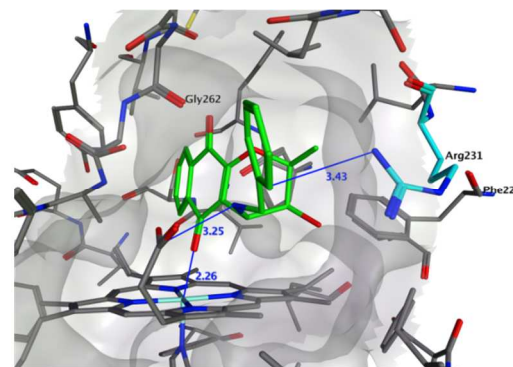
Inclusion of both benzylamino and hydroxyl groups (**2**, **3**) into the pyranonaphthoquinone framework was found to significantly increase IDO1 inhibitor potency over the parent compound **1** (Figure 2) and structure-activity relationship (SAR) studies of other pyran ring derivatives<sup>14</sup> revealed substitution at the 4-position of the pyran ring was readily tolerated. Given the potential for carboranes to positively modify existing drug frameworks,<sup>3</sup> bioisosteric replacement of the benzyl group in **2** and **3** with a *closo*-1,2-carborane cage and an assessment of carborane substitution on inhibiting IDO1 activity was the primary goal of this work. Herein we report the first carborane-based inhibitors which are designed to target the active site of IDO1 and inhibit its catalytic activity.

## Results and Discussion

### Molecular Modelling

The essential structural features of IDO1 which govern tolerance for variation at the 4-position of the pyranonaphthoquinone framework are not well understood. Molecular modelling of a potent 4-benzyl substituted pyranonaphthoquinone IDO1 inhibitor ((*3S,4S*)-**2**)<sup>14</sup> was thus initially undertaken to assess the feasibility of *closo*-1,2-carborane substitution at this position. In order to preclude expulsion of the (*3S,4S*)-**2** ligand from the IDO1 active-site during energy minimisations performed as part of a stochastic conformational search, a low-mode molecular dynamics (LMMD) ‘what-if’ docking was investigated. This method employed a short (*ca.* 1 ps) low-mode molecular dynamics simulation followed by energy

minimisation to achieve novel ligand-protein conformations – a process successfully utilised in previous studies to dock other types of IDO1 inhibitors.<sup>11</sup> Briefly, poses employing both quinone oxygen atoms (5-oxo, 10-oxo) of (*3S,4S*)-**2** were created as haem ligands. The starting position of each LMMD docking run was created by including (*3S,4S*)-**2** within the active site of IDO1. (*3S,4S*)-**2** was then energy minimised without influence from the IDO1 protein to form a low energy conformation. This conformation was then minimised within the active site while holding the protein and haem group rigid. Finally, the active site and ligand were relaxed through energy minimisation. The resulting relaxed docking pose for (*3S,4S*)-**2** within the active site of IDO1 was found to maintain considerable similarity with the starting structure. The ensuing conformation was then used as the starting position of the next pose iteration. LMMD simulations were allowed 700 attempts at finding binding positions. These calculations resulted in 51 unique structures for the 5-oxo (*3S,4S*)-**2** ligation pose and 23 structures for the 10-oxo (*3S,4S*)-**2** ligation pose (see Supplementary Data). The more likely 5-oxo (*3S,4S*)-**2** ligation pose allows the functionalised 3,4-pyran ring to interact with both the haem propanoate arm (*via* the benzylamino group) and the Arg231 residue (*via* both hydroxyl and amino groups) located near the entrance to the IDO1 active-site (Figure 3). Indeed, in this pose, the 4-benzyl group of (*3S,4S*)-**2** extends into the void of the active-site entrance, indicating some degree of clearance for a variety of sterically-bulky groups (*e.g.* a carborane cage) at this position and is in good agreement with the observed data from comprehensive SAR studies.<sup>14</sup>



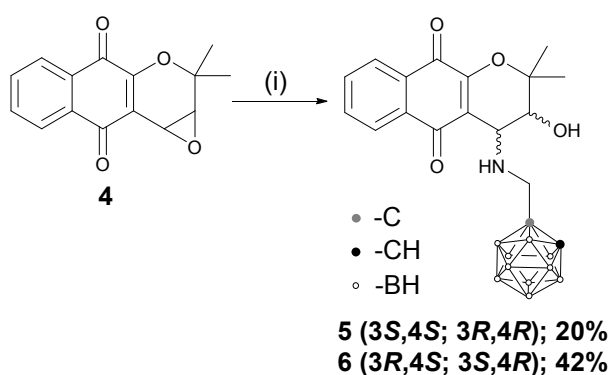
**Figure 3.** Example of *in silico* docking of (*3S,4S*)-**2** (green) within the active site of IDO1. Atomic distances (Å) – dark blue. Atom colours: grey – IDO1 protein carbon, yellow – sulfur, dark blue – nitrogen, red – oxygen, light blue – iron. Arg231 is highlighted in light blue (right).

Like many other computer-aided molecular design programs,<sup>3</sup> Molecular Operating Environment (2011.10 - the software used to perform LMMD docking) does not possess a parameter set for hexavalent boron (a critical atomic property of *closo*-carboranes).<sup>16</sup> As a result, computational modelling of a carborane containing pyranonaphthoquinone was not conducted. However, the modelling of a 4-adamantyl derivative of (*3S,4S*)-**2** in which the carbon polycycle possesses similar steric bulk and lipophilicity to a *closo*-carborane cage showed similar docking poses to the benzyl-substituted (*3S,4S*)-**2** (see ESI). Indeed, modelling of both the benzyl- and adamantyl-derivatives confirmed that bulky 4-position substituents can be accommodated readily within the IDO1 active-

site entrance void. Given that the molecular environment predicted for pyranonaphthoquinone inhibitors within the IDO1 active-site appears conducive to carborane cage substitution, the synthesis of new *N*-methylamino-*closo*-1,2-carboranylpyranonaphthoquinone derivatives was subsequently undertaken and their *in vitro* inhibition of IDO1 was investigated.

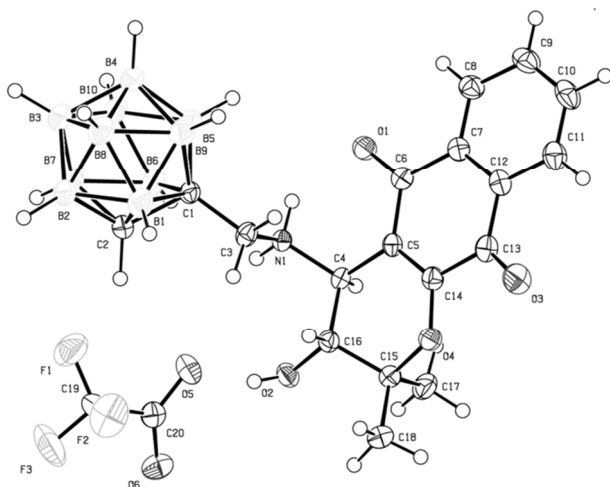
### Synthesis

Derivatization of the pyran ring at the 3,4-position of **1** was performed by means of an epoxide ring-opening reaction involving nucleophilic attack of 1-aminomethyl-*closo*-1,2-carborane<sup>17</sup> (or benzylamine) on the epoxide **4** in the presence of a catalytic quantity of InCl<sub>3</sub> in CH<sub>2</sub>Cl<sub>2</sub> to afford both *cis* and *trans* diastereomers **5** and **6** after column chromatography and normal phase-HPLC (Scheme 1) in 20% and 42% yield, respectively.<sup>14, 18</sup>



**Scheme 1.** The synthesis of *N*-methylamino-*closo*-1,2-carboranylpyranonaphthoquinones. Reagents: (i) 1-aminomethyl-*closo*-1,2-carborane<sup>17</sup>, InCl<sub>3</sub> (5 mol%), CH<sub>2</sub>Cl<sub>2</sub>.

Assignment of the *cis/trans* geometry in these compounds was made by a <sup>1</sup>H NMR coupling constant analysis, and the absolute configuration of (3*R*,4*S*)-**6** was determined by a single-crystal X-ray diffraction study of its *N*-protonated trifluoroacetate salt (Fig. 4 and Table 1). <sup>11</sup>B{<sup>1</sup>H} NMR studies confirmed that the *closo*-carborane cage remained intact during the preparation of **5** and **6** and no conversion to the *nido* species was observed (*vide infra*).



**Figure 4.** ORTEP representation of (3*R*,4*S*)-**6** in its *N*-protonated form with trifluoroacetate as a counter-ion.

**Table 1.** Crystal data for (3*R*,4*S*)-**6**

Crystal data and structure refinement for (3 <i>R</i> ,4 <i>S</i> )- <b>6</b>	
Identification code	CCDC-967701
Empirical formula	C <sub>20</sub> H <sub>28</sub> B <sub>10</sub> O <sub>6</sub> NF <sub>3</sub>
Formula weight	543.53
Temperature/K	150
Crystal system	Orthorhombic
Space group	Pbca
<i>a</i> /Å	12.9860(7)
<i>b</i> /Å	19.7953(11)
<i>c</i> /Å	21.0341(12)
<i>α</i> /°	90.00
<i>β</i> /°	90.00
<i>γ</i> /°	90.00
Volume/Å <sup>3</sup>	5407.1(5)
<i>Z</i>	8
$\rho_{\text{calc}}$ /mg/mm <sup>3</sup>	1.335
$\mu$ /mm <sup>-1</sup>	0.101
<i>F</i> (000)	2240.0
Crystal size/mm <sup>3</sup>	0.2 × 0.1 × 0.1
2 $\theta$ range for data collection	3.88 to 52.1°
Index ranges	-13 ≤ <i>h</i> ≤ 15, -24 ≤ <i>k</i> ≤ 24, -25 ≤ <i>l</i> ≤ 25
Reflections collected	37551
Independent reflections	5324[R(int) = 0.0445]
Data/restraints/parameters	5324/0/364
Goodness-of-fit on <i>F</i> <sup>2</sup>	1.012
Final <i>R</i> indexes [ <i>I</i> ≥ 2 $\sigma$ ( <i>I</i> )]	<i>R</i> <sub>1</sub> = 0.0449, <i>wR</i> <sub>2</sub> = 0.1070
Final <i>R</i> indexes [all data]	<i>R</i> <sub>1</sub> = 0.0686, <i>wR</i> <sub>2</sub> = 0.1203
Largest diff. peak/hole / e Å <sup>-3</sup>	0.36/-0.26

Additionally, we attempted to prepare the anionic *nido*-7,8-carboranyl species from the *cis* and *trans closo*-1,2-carboranyl compounds **5** and **6**, respectively, in order to assess how a dramatic alteration of the hydrophobicity of the carborane cage, whilst preserving much of its steric bulk, affected its interaction with the IDO1 active-site. However, under the conditions of the (mild) deboronation reaction<sup>19</sup> involving CsF and EtOH, the pyranonaphthoquinone ring system was found to be extensively degraded.

Inhibitory constants (IC<sub>50</sub>) for the new carboranyl derivatives **5** and **6**, along with **1**, **2** and **3**, were determined using recombinant human IDO1<sup>20, 21</sup> in a cell-free assay system, as devised by Takikawa *et al.* with some modifications (Table 2).<sup>22</sup> Briefly, ascorbic acid and methylene blue provided the reductive environment necessary for the activation of IDO1 *i.e.* reduction of the Fe<sup>3+</sup> inactive form to the active Fe<sup>2+</sup> form of IDO1 that is involved in O<sub>2</sub> binding and metabolism of L-tryptophan to *N*'-formylkynurenine and subsequent conversion to L-kynurenine.<sup>23</sup> Compounds **1**, **2** and **3** were chosen as controls as they represented: the basic pyranonaphthoquinone framework without 4-position modification (**1**) and the equivalent

diastereomeric benzyl- substituted pyranonaphthoquinones (**2** and **3**, respectively) to the synthesized *closo*-1,2-carboranyl compounds **5** and **6**.

**Table 2.** Inhibitory activities (IC<sub>50</sub>) of pyranonaphthoquinone derivatives towards IDO1.<sup>a</sup>

Compound	IC <sub>50</sub> (μM)
<b>1</b>	1.22 ± 0.08
<b>2</b>	0.35 ± 0.04
<b>3</b>	0.50 ± 0.03
<b>5</b>	0.78 ± 0.02
<b>6</b>	1.77 ± 0.03

<sup>a</sup> IC<sub>50</sub> (inhibitory concentration 50%) determined with L-tryptophan as the substrate at a concentration of 100 μM. Under the conditions of our assay, the IC<sub>50</sub> values of **1**, **2** and **3** were found to be *ca.* 2 to 6-fold higher than those previously reported for the same compounds.<sup>14</sup>

In accordance with previous studies,<sup>14</sup> the racemic *cis* diastereomer **2** was found to be a significantly more potent inhibitor of IDO1 than the *trans* racemate **3**. The same trend was observed for the *closo*-1,2-carboranyl series (**5** and **6**), indicating that *cis/trans* isomers of both the lipophilic benzyl and *closo*-carboranyl derivatives interact with the IDO1 active site in a similar manner with low μM affinity (Table 2). A minor increase in the IC<sub>50</sub> value with *closo*-carborane substitution (*e.g.* *ca.* 2-fold loss of inhibitor potency for **5** when compared to **2**) was observed in our hands. This observation is consistent with the *closo*-1,2-carborane cage slightly diminishing the inhibitor-IDO1 interaction when compared with the analogous benzylamino derivatives **2** and **3**, most likely due to the loss of a cation-π interaction between Arg231 and the benzyl ring, as observed in the molecular modelling for **2** (Figure 3). Nevertheless, the fact that both *closo*-carboranes **5** and **6** inhibited IDO1 activity at low μM levels (and *closo*-carborane substitution (**5**) enhanced inhibition over the basic pyranonaphthoquinone framework (**1**)) and given the known limited inhibition of IDO1 activity by clinical compounds (*e.g.* 1-methyl-D-tryptophan<sup>24</sup> (IC<sub>50</sub> > 2.5 mM, Clinical Trial I.D.: NCT00567931) these results clearly show that second-generation carborane derivatives based on these compounds are an excellent lead for study as potential IDO1 inhibitors.

## Experimental

<sup>1</sup>H, <sup>13</sup>C{<sup>1</sup>H} and <sup>11</sup>B{<sup>1</sup>H} NMR spectra were recorded at 300 K on a Bruker AVANCE 400 MHz DRX spectrometer (<sup>1</sup>H at 400 MHz, <sup>13</sup>C at 101 MHz, and <sup>11</sup>B at 160 MHz). All NMR signals are reported in ppm. <sup>1</sup>H NMR spectra were referenced to TMS (0 ppm). <sup>11</sup>B NMR spectra were referenced according to the IUPAC unified scale (frequency ratio (Ξ) 32.083974). Coupling constants (*J*) are reported in Hz. Peak multiplicities are abbreviated as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet - unassignable multiplicity or overlapping signals), b (broad). High-resolution ESI-MS were recorded on a Bruker 7T FTICR mass spectrometer in the positive

ESI mode. Normal-phase HPLC was performed on a Waters 2525 binary gradient module equipped with a ZORBAX® RX-SIL column (5 μm, 21.2 mm × 250 mm) and a Waters 2996 photodiode array detector. The results were analysed by means of Waters MassLynx 4.1 software. Solvents and reagents were dried according to literature methods.<sup>25</sup> All reactions were performed under an inert atmosphere of nitrogen unless otherwise stated.

### General procedure for the synthesis of *N*-methylamino-*closo*-1,2-carboranylpyranonaphthoquinones

To a solution of 2,2-dimethyl-3,4-epoxy-2H-naphtho[2,3-*b*]pyran-5,10-dione (**4**) (52.7 mg, 0.22 mmol) in dichloromethane (10 mL) was added 1-aminomethyl-*closo*-1,2-carborane hydrochloride<sup>17</sup> (86 mg, 0.43 mmol) and sodium bicarbonate (36 mg, 0.43 mmol). Subsequently, a catalytic amount of indium(III) chloride (4 mg, 0.018 mmol) was added to the reaction mixture which was left to stir overnight at room temperature. After 14 h, the solvent was removed under reduced pressure to yield a yellow residue that was partially purified *via* flash chromatography (30% EtOAc/hexane) to yield a mixture of two diastereomers (crude 60 mg) which were further separated by preparative normal phase -HPLC (isocratic *n*-hexane:isopropanol:trifluoroacetic acid (97:3:0.1 v/v)).

(*3S,4S/3R,4R*)-4-(1-aminomethyl-*closo*-1,2-carboranyl)-3-hydroxy-2,2-dimethyl-3,4-dihydro-2H-benzo[*g*]chromene-5,10-dione (**5**).

Compound **5** was synthesized using the general procedure outlined above in 20% yield (19 mg); mp = 110-112 °C; TLC *R*<sub>f</sub> = 0.3 (30% EtOAc/*n*-hexane); *R*<sub>t</sub> NP-HPLC (isocratic: *n*-hexane:isopropanol:trifluoroacetic acid (97:3:0.1 v/v)): 33 min (7 mL/min); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ: 8.15-8.08 (m, 2H, ArH), 7.82 -7.71 (m, 2H, ArH), 4.15 (d, *J* = 4.5 Hz, 1H, HOCH), 4.07, (bs, 1H, BCH), 3.80 (m, 3H, NHCH<sub>2</sub>; OH), 3.64 (d, *J* = 13.6 Hz, 1H, NHCH), 3.50 (br s, 1H, NH), 3.0-1.3 (br m, 10H, BH), 1.66 (s, 3H, CH<sub>3</sub>), 1.33 (s, 3H; CH<sub>3</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ: 186.3 (C=O), 178.3 (C=O), 156.2 (O=C<sub>CO</sub>), 134.9 (ArC), 134.4 (ArC), 131.7 (ArC), 130.9 (ArC), 127.1 (ArC), 126.7 (ArC), 112.7 (O=C<sub>CCNH</sub>), 82.1 (HNCH<sub>2</sub>), 66.5 (HOCH), 60.2 (C<sub>2</sub>H<sub>6</sub>CO), 53.3 (BCCH<sub>2</sub>), 51.5 (BCH), 29.8 (CCNH), 24.2 (CH<sub>3</sub>), 23.1 (CH<sub>3</sub>); <sup>11</sup>B NMR (CDCl<sub>3</sub>) δ: -2.30 (2B), -4.66 (2B), -9.21 (4B), -12.51 (2B); HR-ESI-MS Calcd for [M+H]<sup>+</sup> *m/z* 431.29797, Found 431.29799.

(*3R,4S/3S,4R*)-4-(1-aminomethyl-*closo*-1,2-carboranyl)-3-hydroxy-2,2-dimethyl-3,4-dihydro-2H-benzo[*g*]chromene-5,10-dione (**6**)

Compound **6** was synthesized using the general procedure outlined above in 41% yield (36 mg); mp = 151-153 °C; TLC *R*<sub>f</sub> = 0.3 (30% EtOAc/Hexane); *R*<sub>t</sub> NP-HPLC (isocratic: *n*-hexane:isopropanol:trifluoroacetic acid (97:3:0.1 v/v)): 18 min (7 mL/min); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ: 8.16-8.13 (m, 2H, ArH), 7.89 - 7.79 (m, 2H, ArH), 4.28 (d, *J* = 14.1 Hz, 1H, HOCH), 4.16 (m, 3H, NHCH<sub>2</sub>; BCH), 3.90 (br s, 1H, OH), 3.82 (d, *J* = 14.2 Hz, 1H, NHCH), 3.50 (br s, 1H, NH), 3.0 - 1.3 (broad m, 10H, BH), 1.64 (s, 3H, CH<sub>3</sub>), 1.33 (s, 3H; CH<sub>3</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ: 186.7 (C=O), 177.5 (C=O), 156.7 (O=C<sub>CO</sub>), 135.2 (ArC), 134.9 (ArC), 131.1

(ArC), 130.6 (ArC), 127.2 (ArC), 126.9 (ArC), 110.2 (O=C $\overline{C}$ CNH), 84.0 (HN $\overline{C}$ H $\overline{2}$ ), 69.4 (HO $\overline{C}$ H), 69.1 (C $\overline{2}$ H $\overline{6}$ CO), 60.1 (B $\overline{C}$ CH $\overline{2}$ ), 58.7 (BCH), 52.1 (C $\overline{C}$ NH), 25.5 (CH $\overline{3}$ ), 18.7 (CH $\overline{3}$ );  $^{11}\text{B}$  NMR (CDCl $\overline{3}$ )  $\delta$ : -1.64 (2B), -3.62 (2B), -8.77 (4B), -12.76 (2B); HR-ESI-MS Calcd for [M+H] $^{+}$   $m/z$  431.29797, Found 431.29799.

### X-ray Diffraction Studies

Single crystals of (3*R*,4*S*)-**6** were grown by means of liquid-liquid diffusion using a DCM/*n*-hexane mixture. A suitable crystal was selected *via* Paratone-N on a Bruker ApexII diffractometer. The crystal was kept at 150 K during data collection. Using Olex2,<sup>26</sup> the structure was solved with the ShelXS<sup>27</sup> structure solution program using Direct Methods and refined with the ShelXL<sup>28</sup> refinement package using Least Squares minimisation.

### Molecular Modelling (Docking) Studies

*In silico* docking studies were performed on (3*S*,4*S*)-**2** (or the related 4-adamantyl-substituted pyranonaphthoquinone).<sup>14</sup> All docking was performed on the 2D0T crystal structure.<sup>4</sup> All molecular mechanics techniques used the CHARMM22 force-field and the Born solvation model, as implemented Molecular Operating Environment version 2011.10 (MOE, Chemical Computing Group, Montreal, Canada). Dielectric constants were set to 4 and 80 for inside and outside the protein, respectively.

The LMMD methodology was employed using MOE's Conformational Search protocol. Default parameters were used except the iteration limit for finding new conformations was 700. The RMSD for defining two poses as equivalent was 0.25 Å and fixed atoms were excluded from this measurement. Flexible species were the ligand, the propanoate arm of haem, and IDO1 amino acid residues: T126, C129, V130, F163, F164, S167, F226, L230, R231, L234, S235, A260, S263, A264, F291, H346, I354 and L384. All backbone atoms of the protein were held fixed. During the 'What-If' analysis<sup>11</sup>, the restraint boundaries between iron and the ligating atom were 2.1 and 2.2 Å with a weighting constant of 20 kcal/mol. RMSD for energy minimisation was 0.005.

### IDO1 Inhibition Assays

#### Recombinant Human IDO1

Recombinant human IDO1 was expressed and purified according to literature procedures.<sup>20,21</sup>

#### General Assay Protocol

The standard reaction mixture (200  $\mu\text{L}$ ) contained 50 mM potassium phosphate buffer (pH 6.5), 10 mM ascorbic acid (neutralised with NaOH), 100  $\mu\text{g}/\text{mL}$  catalase, 10  $\mu\text{M}$  methylene blue, 100  $\mu\text{M}$  L-tryptophan, and 10 nM IDO1. The assay was conducted at 37 °C for 20 min (assay initiated ( $T_{\text{min}} = 0$ ) on the addition of 20 mM ascorbic acid to the pre-warmed mixture) and stopped by means of addition of 40  $\mu\text{L}$  of 30% (w/v) trichloroacetic acid. The mixture was then

heated at 65 °C for 15 min and 125  $\mu\text{L}$  of the reaction mixture was transferred into a well of a 96-well microtitre plate and mixed with 125  $\mu\text{L}$  of 2% (w/v) *p*-dimethylaminobenzaldehyde in glacial acetic acid. The yellow pigment derived from reaction with kynurenine was measured at 480 nm using a Spectramax M5 microtitre plate reader (Molecular Devices).

### Inhibitor Assessment

For inhibitor assessment, compounds **1**, **2**, **3**, **5** and **6** were added to the standard reaction mixture over a linear concentration range (10  $\mu\text{M}$  – 0.0195  $\mu\text{M}$  for compounds **5** and **6**; 2  $\mu\text{M}$  – 0.0039  $\mu\text{M}$  for compounds **1**, **2** and **3**) in DMSO (final concentration of DMSO in standard reaction mixture 2%). DMSO (2%) alone was used as the carrier control (i.e. 0  $\mu\text{M}$  inhibitor). IC $_{50}$  values were determined by means of non-linear regression analysis employing GraphPad Prism 4<sup>®</sup> (GraphPad Software).

### Conclusion

We have reported a new class of boronated agents that can effectively inhibit IDO1 at low  $\mu\text{M}$  concentrations. The *closo*-carboranes **5** and **6** display IDO1 binding characteristics that are comparable to their corresponding benzyl analogues. As the carborane cage is well tolerated by the IDO1 enzyme in cell-free assays, future work will focus on design of other carborane-based derivatives which engage the large lipophilic IDO1 active site pocket. The results of this work will be reported in due course.

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### Notes and references

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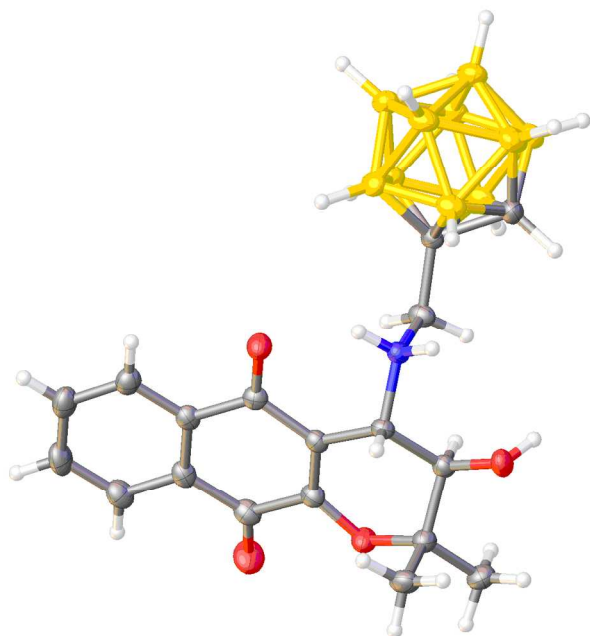
<sup>c</sup> Faculty of Pharmacy, The University of Sydney, Sydney, NSW 2006, Australia.

† Electronic Supplementary Information (ESI) available: X-ray diffraction details of **6** (CCDC 967701). see DOI: 10.1039/c000000x/

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## TOC Entry



The first examples of indoleamine-2,3-dioxygenase-1 (IDO1) inhibitors containing a carborane cage are reported.