The First CNS-Active Carborane: A Novel $P2X_7$ Receptor Antagonist with Antidepressant Activity

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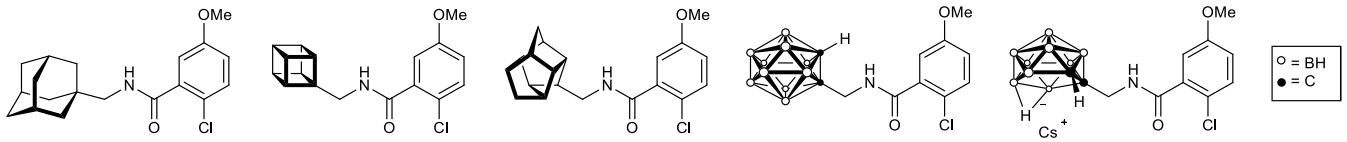
ABSTRACT: Relative to other polycyclic frameworks **(1-3),** a carborane cage (**4** and Cs•**5**) exerts a significant biological effect as an inhibitor of the purinergic $P2X_7$ receptor ($P2X_7R$) which allows one to target depression *in vivo* and thus demonstrate, for the first time, that a carborane has the capacity to modify CNS activity.



1 2 3 4 Cs•5

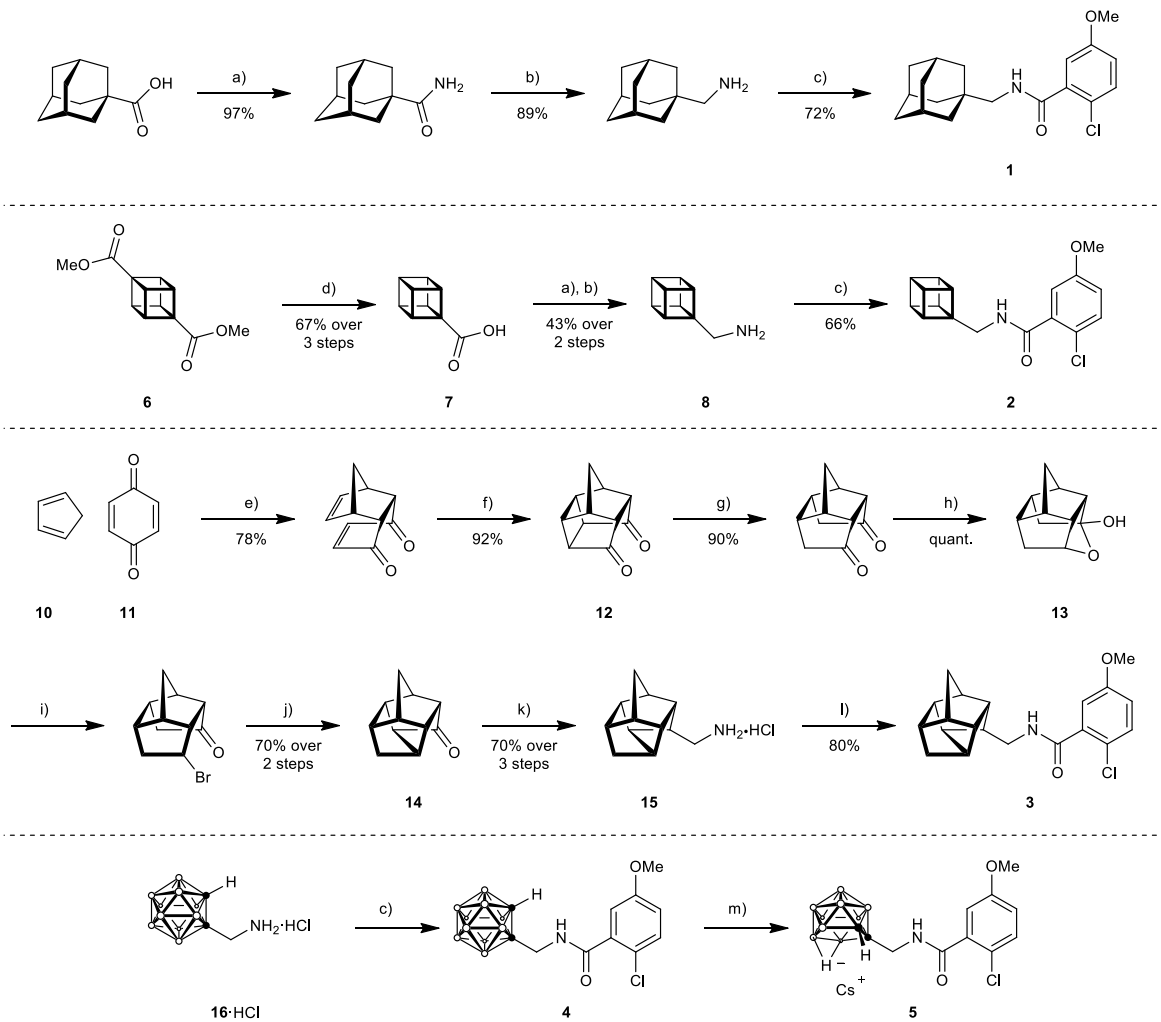
KEYWORDS: *Carborane, Blood-brain barrier, $P2X_7$, Polycycle, Antidepressant*

Since the discovery by Davies *et al.*[1] in 1964 that 1-aminoadamantane (amantadine) displayed antiviral effects, polycyclic hydrocarbon cage compounds, including adamantane, trishomocubane and cubane, have made unique contributions to medicinal chemistry.[2] Their rigid scaffold provides compounds with improved metabolic stability and allows substituents to be precisely positioned in three dimensions. More importantly, they facilitate transport across the blood-brain barrier (BBB) which is a major challenge in central nervous system (CNS) drug development.

A recently-introduced polycycle in medicinal chemistry is the carborane cluster.[3] Carboranes are pseudo-aromatic polyhedral clusters consisting of boron, carbon and hydrogen atoms which have been utilized as a rich source of boron for boron neutron capture therapy (BNCT).[4] Carboranes can also act as useful bioisosteres for phenyl rings and adamantane as their size and lipophilicity are comparable but their unique structures allow new frontiers to be explored in drug development.[3, 5] An additional feature of carboranes for application in medicinal chemistry is the ability to convert the neutral, lipophilic *closo* cluster into the anionic, hydrophilic *nido* species in a single synthetic step and still preserve much of the steric features of the *closo* cluster. The incorporation of carboranes as a unique bioisostere in medicinal chemistry is still in its infancy but there is significant interest in the exploitation of this polycycle in recent years which can, in part, be attributed to an expanding field of synthetic methodology, their remarkable stability toward moisture and biological degradation, and their low toxicity. Only recently has it been conclusively demonstrated that carboranes can cross the BBB[6] but never before has it been reported that carboranes can elicit a CNS-modifying effect. Herein we report the first example of a carborane-containing compound with the ability to target the purinergic $P2X_7$ receptor ($P2X_7R$) *in vivo* resulting in the observation of antidepressant activity in mice.

The $P2X_7R$ is a non-sensitizing, cation-selective ion channel which is directly gated by ATP. However, upon repetitive or prolonged agonist exposure, the $P2X_7R$ forms a non-selective pore which is permeable by cations up to 900 Da.[7] Formation of this pore results in apoptosis and the production and release of the cytokine interleukin 1β (IL-1β). High levels of IL-1β in the brain have been implicated in depression,[8] hyperalgesia[9] and neurodegeneration.[10] The development of a $P2X_7R$ antagonist is thus presumed to have antidepressant, analgesic and/or neuroprotective properties.

In recent years, we and others have comprehensively explored the chemical space around AstraZeneca's adamantanyl benzamide series.[11] Removing or flattening the adamantane polycycle to a simpler cycloalkane was detrimental to $P2X_7R$ antagonism thereby suggesting that occupancy of a globular hydrophobic pocket was found to be central to potent $P2X_7R$ antagonism.[12] To examine this concept further, we explored the effect of substituting the adamantane of lead benzamide **1** with a variety of polycyclic cages (Scheme 1). This series comprised a cubanyl **2**, trishomocubanyl **3** and the first use of carboranes within this class, *viz: closo*-1,2-carboranyl **4** and *nido*-1,2-carboranyl benzamide **5**.



Scheme 1. Synthesis of adamantane benzamide **1** and its polycyclic analogues **2-5.** Conditions: **a)** CDI, THF, rt, 1 h, then 28% $NH_{3(aq)}$, rt, 4 h; **b)** $LiAlH_4$, THF, reflux, 20 h; **c)** 2-chloro-5-methoxybenzoic acid **9**, $(COCl)_2$, THF, rt, 1 h, then amine, THF, Et_3N, rt, 16 h; **d)**i) NaOH (1 equiv.), MeOH, THF, rt, 16 h, then $HCl_{(aq)}$, ii) $(COCl)_2$, CH_2Cl_2, rt, 1 h, then 2-mercaptopyridine *N*-oxide sodium salt, *hv*, DMAP, $CHCl_3$, reflux, 1 h, iii) NaOH, MeOH, reflux, 1 h; **e)** PhMe, -10 °C to rt, 3 h, 78%; **f)** *hv*, Me_2CO/hexanes, 8 h, 92%; **g)** Zn, AcOH, rt, 5 h, 90%; **h)** $NaBH_4$, EtOH/H_2O, rt, 3 h, quant.; **i)** 33% HBr in CH_3CO_2H, sealed tube, 100 °C, 16 h; **j)** *t*-BuOK, Et_2O, rt, 16 h, 70% over 2 steps; **k)**i) TosMIC, *t*-BuOK, $(MeOCH_2)_2$, EtOH, 5-35 °C, ii) $LiAlH_4$, Et_2O, reflux, 16 h, iii) anhyd. HCl, Et_2O; **l)** 2-chloro-5-methoxybenzoic acid **9**, EDC.HCl, HOBt, NMM, CH_2Cl_2, 0 °C to rt, 16 h; **m)** CsF (3 equiv.), EtOH, reflux, 24 h.

RESULTS AND DISCUSSION

The smallest polycycle of the series is the cubanyl benzamide **2** prepared from the commercially-available methyl cubane-1,4-diester **6** *via* a decarboxylation to yield cubane carboxylic acid **7** which was converted to cubanylmethylamine **8**, through its carboxamide, and subsequently coupled to 2-chloro-5-methoxybenzoic acid **9**. Its X-ray crystal structure (Figure 1) illustrates the highly-strained 90° bond angles associated with this polycycle.

The polycyclopentyl framework of D_3-trishomocubanyl benzamide **3** was prepared in 10 steps initiated by the Diels-Alder cycloaddition reaction between cyclopentadiene **10** and benzoquinone **11**. A 2+2 photocyclization furnishes Cookson's diketone **12** which is reduced over 2 steps to open the cyclobutane ring and generate the lactol **13**. Bromination followed by base-catalyzed cyclization affords the D_3-trishomocubanone **14**. This ketone **14** was subjected to a Van Leusen reaction using TosMIC with the corresponding nitrile reduced with $LiAlH_4$ to the precursory D_3-trishomocubanylmethylamine **15**. The amine **15** was then coupled to benzoic acid **9** to afford the trishomocubanyl benzamide **3**.

The *closo*-1,2-carboranyl **4** was synthesized in 64% yield from the amide coupling reaction between *closo*-1,2-carboranylmethylamine hydrochloride[13] **16**·HCl and 2-chloro-5-methoxybenzoic acid **9**. A single crystal X-ray structure of **4** was successfully obtained (Figure 1) which confirms the presence of the *closo*-1,2-carborane cage. The *closo*-1,2-carboranyl benzamide **4** was subsequently converted to the *nido*-7,8-carborane analogue Cs·**5** by means of a mild deboronation reaction (Scheme 1) with CsF/EtOH in a single, high-yielding step (95%).

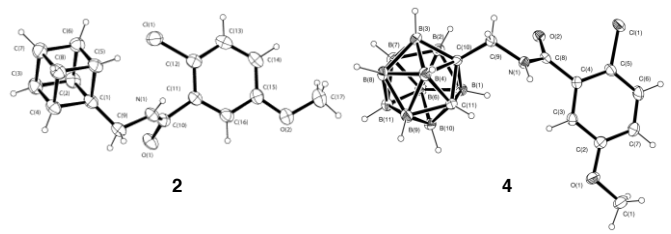


Figure. 1. ORTEP depictions of **2** and **4** shown with 50% displacement ellipsoids.

Compounds **1**-**4** and Cs·**5** were assessed *in vitro* for their ability to inhibit human P2X$_7$R (hP2X$_7$R) pore formation by using a functional dye uptake assay in THP-1 cells (Figure 2).[14] The P2X$_7$R pore formation was determined by means of an agonist-induced uptake of YO-PRO®-1, a large, cationic dye molecule (629 Da). 3'-Benzoylbenzoyl adenosine-5'-triphosphate (BzATP), a synthetic and more potent analogue of ATP at P2X$_7$R, was used (100 μM) to induce P2X$_7$R pore-formation. Each compound was assessed over a concentration range of 1 nM to 10 μM with the pIC$_{50}$ values (Table 1) determined by spectrofluorimetry to quantify the amount of YO-PRO®-1 uptake into the cells following pore formation.

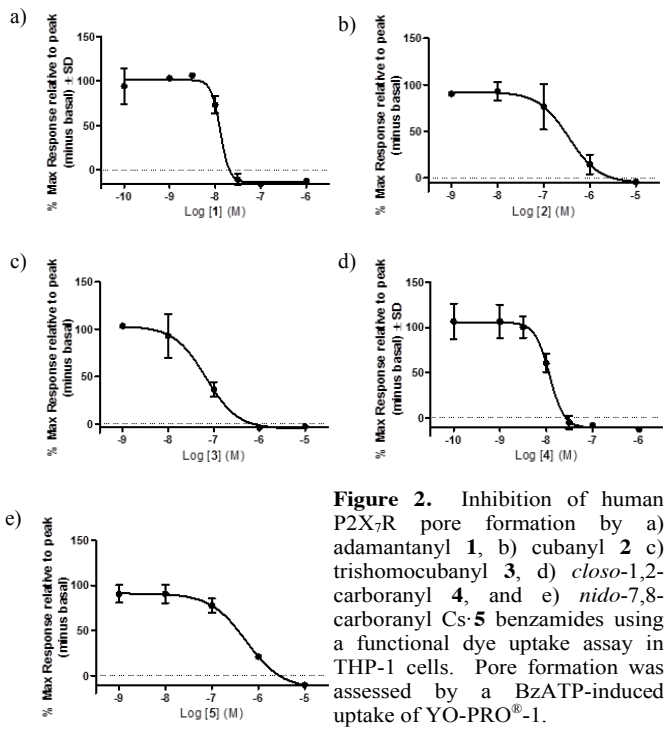


Figure 2. Inhibition of human P2X$_7$R pore formation by a) adamantanyl **1**, b) cubanyl **2** c) trishomocubanyl **3**, d) *closo*-1,2-carboranyl **4**, and e) *nido*-7,8-carboranyl Cs·**5** benzamides using a functional dye uptake assay in THP-1 cells. Pore formation was assessed by a BzATP-induced uptake of YO-PRO®-1.

A trend was observed between the potency of hP2X$_7$R inhibition and the size of the polycyclic cage with those molecules possessing larger polycycles, i.e. **1**, **3** and **4**, causing greater inhibition of hP2X$_7$R pore formation. Indeed, the lead adamantanyl benzamide **1** demonstrated potent hP2X$_7$R antagonism (pIC$_{50}$ = 7.98 ± 0.15). Contracting the size of the adamantane cage in **1** by the use of a D_3-trishomocubane group (**3**) reduced potency by approximately one-third, whilst reducing the polycycle volume even further to a cubane (**2**) resulted in a decrease of over one-order of magnitude in hP2X$_7$R inhibition. The *closo*-1,2-carborane cage is equivalent in size to adamantane but is more lipophilic due to the presence of low-polarity B-H bonds.[15] Interestingly, the *closo*-1,2-carboranyl benzamide **4** displayed a slight improvement in hP2X$_7$R antagonism over **1**. The *nido*-7,8-carboranyl benzamide Cs·**5** was prepared with the aim of retaining potency through its polycyclic cage, whilst simultaneously reducing the lipophilic properties due to its anionic charge. Indeed, the anionic charge of Cs·**5** resulted in a dramatic diminution in hP2X$_7$R pore inhibition when compared to **4**. It is worthy to note that the Hill slope calculated for the adamantanyl **1** and *closo*-1,2-carboranyl benzamide **4** is -4.3 and -2.7 respectively (whereas the remaining benzamides equate to -1). We speculate this is due to cooperativity, which is known to occur for related adamantane antagonists of the P2X$_7$R.[16] Future work involving radioligand binding is intended to elucidate the mechanistic aspects of these ligands.

Table 1. hP2X$_7$R inhibition functional assay and lipophilic evaluation of the benzamide series **1**-**4** and Cs·**5**.

Benzamide	pIC$_{50}$[a]	cLog$P$[b]	LogD$_{7.4}$[c]	LLE[d]
Adamantanyl **1**	7.98 ± 0.15	4.12	4.30	3.68
Cubanyl **2**	6.36 ± 0.12	1.46	3.42	2.94
Trishomocubanyl **3**	7.49 ± 0.19	2.98	4.31	3.18
Closo-carboranyl **4**	8.07 ± 0.19	-[e]	4.29	3.78
Nido-carboranyl Cs·**5**	6.43 ± 0.10	-[e]	1.44	4.99

[a]pIC$_{50}$ values were derived from concentration-response curves (n > 4) ± SD. [b]Predicted using Spartan 10 (V1.1.0) using H-F calculations with a 6-31G* basis set. [c]Experimentally determined by HPLC method. [d]Ligand-Lipophilicity efficiency (LLE) = pIC$_{50}$ – logD. [e] Could not be predicted with software.

Animal behavioral tests were performed in order to assess the *in vivo* efficacy of benzamides **1**-**4** and Cs·**5**, evaluate their BBB penetration, and also validate their CNS activity. The antidepressant potential of the benzamides **1**-**4** and Cs·**5** were evaluated by means of a Forced-Swim Test (FST) which induces depressive behavior (Figure 2).[17] The FST paradigm utilizes the observation that rodents initially engage in escape-directed behavior when placed in an inescapable scenario, but will eventually develop a passive immobile posture which can be measured as a model of depression.[18] It has also been recently shown that P2X$_7$R knockout (KO) mice show resilience in the FST (i.e. increased mobility compared to WT mice), an observation which both validates the use of such a behavioral model for this target and also provides a maximal response benchmark for P2X$_7$R antagonism.[19]

Prior to the FST, a drug tolerability test was performed on the mice with doses up to 20 mg/kg showing no adverse effects to body temperature, locomotor activity, or body weight (see Supplementary Material). The mice were then injected with 20 mg/kg of

1-4 and Cs·**5** or vehicle and subjected to a 6 min FST. Compared to wild-type (WT) mice, $P2X_7R$ KO mice remained 32 ± 14% more mobile in the FST thus demonstrating their antidepressant-like phenotype. Remarkably, the trend observed in the functional cell assay was reversed in the *in vivo* behavioral assay, i.e. compounds **1**, **3** and **4** exerted no significant antidepressant behavior. In contrast, despite **2** and Cs·**5** having the lowest pIC_{50} values in the series, these compounds were shown to impart significant antidepressant activity (16% and 13% more mobile than WT, respectively). It should be noted that the FST experiments were all performed with mass concentrations of 20 mg/kg and so the relative administered concentration for benzamide Cs·**5** (43 μmol/kg) was lower than the other benzamides **1**-**4** (59-66 μmol/kg) assessed in this study due to its significantly higher MW, i.e. Cs·**5** inferred significant antidepressant activity of the series despite it being assessed at the lowest dose concentration. Furthermore, despite a very limited collection of studies that demonstrate the capacity of carboranes to cross the BBB,[6b] this is the first study which has demonstrated the ability of a carborane to modify CNS activity.

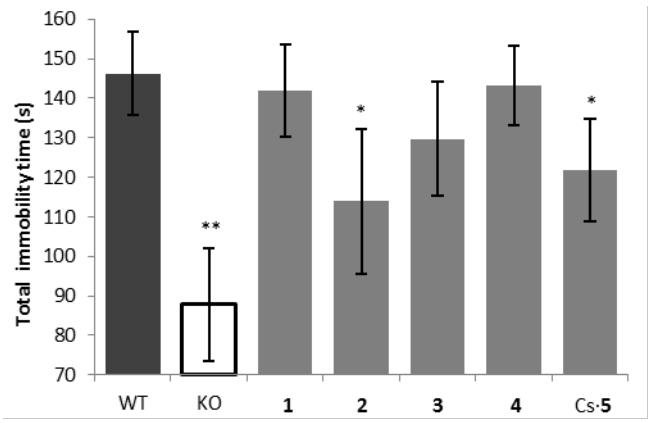


Figure. 2. Average immobility time (s) in which mice remained stationary over the final 3 min of a 6 min Forced-Swim Test (FST). Wild-type mice were injected (20 mg/kg) with either **1**, **2**, **3**, **4**, Cs·**5** or vehicle (WT) 20 min prior to a 6 min FST. $P2X_7R$ knockout mice (KO) were injected with vehicle. Error bars denote SEM. *P<0.05, **P<0.01, Mann–Whitney U-test against WT.

The failure of benzamides **1**, **3**, and **4** to demonstrate antidepressant activity in mice despite their potent $hP2X_7R$ inhibition values suggests a likely inability of these compounds to cross the BBB and/or poor interspecies crossover. Calculated physicochemical properties (Table 1) of the benzamide series advocate lipophilic penalties may indeed prevent crossing of the BBB.

To validate the BBB penetration with lipophilicity, the $logD_{7.4}$ values of the benzamides were determined experimentally by using a HPLC method (Table 1, column 3).[20] The three benzamides **1**, **3** and **4**, which showed no antidepressant behavior in the FST, were shown to have $logD_{7.4}$ values much greater than the range reported for successful CNS drugs (cLog*P* < 3).[21] These values indicate that a high lipophilicity may be inhibiting BBB penetration, which consequently eliminated their efficacy in the FST. Interestingly, substituting adamantane for *closo*-1,2-carborane in the benzamides does not increase the lipophilicity of the drug as previously reported in literature.[15] The $logD_{7.4}$ of **4** is almost equal to that of **1**. However, if **4** is converted to Cs·**5**, a significant drop in lipophilicity by three orders of magnitude is observed and one can thus readily convert a BBB-impenetrable molecule into a CNS-active drug in a single synthetic step.

The Ligand-Lipophilicity efficiency (LLE)[22] evaluation of the benzamide series (Table 1. Column 4) establishes the *nido*-7,8-carborane Cs·**5** as a good lead candidate (LLE ≥ 5). Despite its equivalent *in vitro* and *in vivo* results, the higher lipophilicity of cubanyl **2** suggests it will be less bioavailable and may be more susceptible to metabolism. Regardless of its adverse lipophilicity, the high potency of the *closo*-1,2-carboranyl **4** makes it the second best candidate by LLE analysis. However, based on its inactivity *in vivo*, further structure-activity studies of the aryl moiety would need to be undertaken before this compound could be considered for any further development.

CONCLUSION

In conclusion, this study makes a significant contribution to the emerging area of utilizing carboranes as pharmacophores in drug development. The equivalent size and lipophilicity of the *closo*-carborane and the adamantane cages led to identical biological results in this work. However, *closo*-1,2-carborane has a distinct advantage over adamantane due to its robust exogenous scaffold providing excellent metabolic stability and the ability to be converted readily to its corresponding anionic *nido*-carborane cluster. The significant *in vivo* antidepressant activity of Cs·**5** is consistent with CNS $P2X_7R$ inhibition. This study represents the first account of a carborane molecule possessing CNS-modifying activity thereby pioneering the application of carboranes in future CNS drug development strategies.

ASSOCIATED CONTENT

Supporting Information

Full experimental details and data for both chemical and biological studies. This material is available free of charge *via* the Internet at http://pubs.acs.org.

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Author Contributions

S.M.W. synthesized benzamides **1**-**5** and prepared drafts of this article. H.G. assisted in the synthesis of **2** and **3**. M.B. performed the functional cell assays. A.B. and M.M. carried out the animal behavior testing which was directed by I.S.M. P.T. determined the X-ray crystal structure of **2** and **4**. D.E.M. prepared the carborane precursor **16**•HCl and performed statistical analysis on the biological results. L.M.R. supplied carborane precursors and directed synthetic work involving carboranes. M.K. directed synthetic and *in vitro* work. M.R.B., M.K. and L.M.R. jointly conceived this project. The manuscript was prepared by M.K. and L.M.R. based on drafts written by S.M.W.

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Notes

The authors declare no competing financial interest.

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