Comprehensive exploration of chemical space using trisubstituted carboranes

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A total of 42 trisubstituted carboranes categorised into five scaffolds were systematically designed and synthesized by exploiting the different reactivities of the twelve vertices of o-, m-, and p-carboranes to cover all directions in chemical space. Significant inhibitors of hypoxia inducible factor transcriptional activitay were mainly observed among scaffold V compounds (e.g., Vi–m, and Vo), whereas anti-rabies virus activity was observed among scaffold V (Va–h), scaffold II (Iib–g), and scaffold IV (IVb) compounds. The pharmacophore model predicted from compounds with scaffold V, which exhibited significant anti-rabies virus activity, agreed well with compounds Iib–g with scaffold II and compound IVb with scaffold IV. Normalized principal moment of inertia analysis indicated that carboranes with scaffolds I–V cover all regions in the chemical space. Furthermore, the first compounds shown to stimulate the proliferation of the rabies virus were found among scaffold V carboranes.

Biomolecules play an important role in maintaining living systems in a crowded biological environment. The structure of biomolecules is three-dimensional, and these molecules interact with each other to construct complex biological networks and systems that comprise robust and redundant functionalities. Dysfunctions of such networks and systems cause diseases, and drugs help restore these networks and systems correct physiological functions. Therefore, drugs need to work in the three-dimensional chemical space of biomolecular interactions. More complex molecules are deemed to have the ability to access a larger chemical space1,2, and natural products meet this complexity requirement due to their enormous structural diversity3,4. In fact, many small molecular drugs on the market are derived from natural products5. Lovering et al. suggested that the higher the fraction of sp3 carbon centres (Fsp3) in a small molecule, the higher the probability that the small molecule will make its way from drug discovery through clinical trials to becoming a drug6–8. Diversity-oriented synthesis (DOS), a concept for synthesis to access structurally complex and diverse compounds9, has been developed with the goal of producing three-dimensional complex drug-like compounds and chemical modulators10–14. However, the systematic design of three-dimensional divergent molecules as a way to explore the chemical space has not been fully investigated yet8.

We focused on the icosahedral structure of carborane (dicarba-closo-dodecaborane). Notably, carborane consists of two carbon atoms, ten boron atoms, and ten hydrogen atoms, and three different isomers of this species are possible, depending on the relative position of two carbons: ortho (1,2), meta (1,7), and para (1,12)15,16. Given its three-dimensional hydrophobic features, over the past two decades, carborane has attracted much attention as a hydrophobic pharmacophore for drug discovery17–20. Indeed, by introducing substituents on the carbon centres of carborane, various promising drug candidates have been synthesised, including 17β-estrogen mimics17,21, antifolates22, HSP60 inhibitors23,24, COX-2 inhibitors25,26, vitamin D receptor ligands27,28, and nicotinamide phosphoribosyl-transferase inhibitors29,30. We assumed that, by introducing three substituents at arbitrary carbon positions on carborane, it would be possible to spatially cover all directions. In fact, five types

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Hüsgen cycloaddition reaction with benzyl azide or isobutyl azide in the presence of CuI and sodium ascorbate was unambiguously determined by X-ray structural analysis. Esterification of 3 with phenylacetyl chloride gave compound 4 in 90% yields over two steps. Finally, acetylene 5 was subjected to a Hüsgen cycloaddition reaction with benzyl azide or isobutyl azide in the presence of CuI and sodium ascorbate.

Results and discussion

Synthesis of trisubstituted carboranes. We developed tricyclic three-dimensional scaffolds that are rich in sp³-hybridized carbon centres to access unexplored chemical spaces, and we used these scaffolds to design peptidomimetic molecules. The α-helix is one of the important elements of a protein’s secondary structure that affords the construction of three-dimensional tertiary structures of proteins. It is also a structure found in many protein–protein interactions (PPIs) and the α-helix plays an important role in biological networks. Although PPIs have been attracting the attention of researchers as drug targets, their contact surfaces are relatively large (750–1500 Å²) compared with their counterparts between proteins and small molecules (~ 300 Å²). Therefore, the development of small molecule inhibitors targeting PPIs remains a challenging proposition. Recently, the FDA approved Venetoclax (ABT-199) as a first-in-class small molecule-based PPI inhibitor targeting the Bcl-2/Bax interaction. The X-ray co-crystal structure of Bcl-xL with ABT-737 revealed that ABT-737 binds in the same cleft as the Bax native helix at the Bcl-xL/Bax interface. The protein–protein interfaces usually contain crucial residues for the PPI called ‘hot spots’, which are often composed of hydrophobic amino acid residues. In this study, benzyl (Bn) and isobutyl (iBu) groups were introduced into the carborane scaffolds as mimics of hydrophobic amino acid residues phenylalanine and leucine, respectively.

The approach to the synthesis of scaffold I (1,2,3-trisubstituted o-carboranes) implemented in this study is detailed in Scheme 1. We were concerned that a direct amide bond to the o-carborane cluster could cause degradation via deboronation induced by the amide group. Therefore, o-carborane was functionalized without introducing any amide bonds. Selective iodination at the 3-position of o-carborane was performed according to a literature procedure, and the resulting B3-iodocarborane was subjected to the Kumada–Tamao–Corriu cross coupling to produce 1 in 70% yield. After the lithiation on C1 of 1 with “BuLi followed by the addition to paraformaldehyde, the resulting hydroxy group was protected with the tert-butyldimethylsilyl (TBS) group. The modification of another hydroxy methyl group on C2 achieved in the same manner described for C1 afforded compound 2. Removing the trimethylsilyl (TMS) group of 2 with potassium carbonate produced a mixture of mono-alcohol 3 and di-alcohol 4 in 57% and 40% yields, respectively. Notably, the structure of 4 was unambiguously determined by X-ray structural analysis. Esterification of 3 with phenylacetyl chloride gave compound 5 in 46% yields over two steps. The TBS group was then removed, and the resulting alcohol was esterified with isovaleryl chloride to afford compound 6 in 90% yields over two steps. Finally, acetylene 6 was subjected to a Hüsgen cycloaddition reaction with benzyl azide or isobutyl azide in the presence of Cu and sodium ascorbate.

Figure 1. (a) The five types of trisubstituted carboranes that can cover all directions in a chemical space. (b) Synthetic strategy for the production of the described trisubstituted carboranes.

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to obtain the trisubstituted carboranes Ia and Ib in 45% and 88% yields, respectively. On the other hand, esterification of two hydroxy groups on 4 with the identical substituents afforded 7a and 7b in 72% and 74% yields, respectively; subjecting 7a and 7b to the Hüsgen cycloaddition reaction with benzyl azide or isobutyl azide gave Ic–f in yields ranging between 8 and 72%. Thus, the synthesis of 1,2,3-trisubstituted carboranes with different substituents on the three tethers was achieved in scaffold I.

Next, the synthesis of scaffold II (1,2,4-trisubstituted o-carboranes) was carried out according to the approach detailed in Scheme 2. Propargyl p-methoxybenzyl (PMB) ether (8) was made to react with decaborane in the presence of N,N-dimethylaniline under microwave (MW) irradiation in chlorobenzene to obtain the 1-substituted o-carborane 9 in 79% yield51. A carboxylic acid moiety was then introduced into the C2-position of 9 using nBuLi and carbon dioxide, which functioned as a directing group to afford the selective alkynylation of the B4-position via an approach developed by Xie and co-workers, producing compound 10 in 31% yield over two steps31. Notably, the carboxylic acid intermediate had to be immediately subjected to the alkynylation reaction to avoid the deprotection of the PMB group induced by the acidity of the intermediate. After introducing a hydroxy methyl group into the C2-position of compound 10 via an approach similar to that of step d in Scheme 1, the triisopropylsilyl (TIPS) group of the alkyne moiety was removed. The TBS protection of the hydroxy group of compound 11, followed by the introduction of a hydroxy methyl group into the terminal alkyne, afforded compound 12. The hydroxy group on compound 12 was converted into either a Bn group (13a, 83% yield) or an iBu group (13b, quant.). On the other hand, removal of the PMB group of compound 11 afforded dialcohol 14, whose structure was determined by X-ray structural analysis. (It should be noted that good quality crystals even made several measurements were not obtained due to the unnecessary reflections.). The PMB group of compound 13 was selectively removed using 2,3-dichloro-5,6-dicyano-p-benzoquinone (DDQ), and the subsequent esterification of the deprotected derivatives of compounds 13a and 13b with phenylacetyl chloride or isovaleryl chloride afforded compounds 15a–d. After removing the TBS group, Bn and iBu groups were introduced into the C2-position of 15 to obtain the 1,2,4-trisubstituted carboranes IIa–d in 43–89% yields. Furthermore, compounds 13a and 13b were treated with HCl in dioxane, and Bn and iBu groups were introduced into the resulting diols 16 to obtain compounds IIe–h in 23–88% yields.

Next, we synthesized 1,7,9-substituted carboranes (scaffold III) and 1,9,10-substituted carboranes (scaffold IV) using m-carborane as the starting building block (Scheme 3). Selective electrophilic iodination at the B9-position of m-carborane followed by the Kumada–Tamao–Corriu cross coupling were performed implementing a literature procedure50. A hydroxy methyl group was then introduced into the C1 position of the resulting 9-(trimethylsilyl)ethyl-m-carborane in a similar manner to that described for the synthesis of compounds 1

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**Scheme 1.** Synthesis of 1,2,3-trisubstituted o-carboranes (scaffold I). Reaction conditions: (a) KF, ethanol, reflux; then, HCl, (CH3)3NHCl, r.t., 87%; (b) nBuLi, diethyl ether, r.t.; then, BnI, toluene, r.t., 81%; (c) TMSiCl2MgBr, Pd(PPh3)2Cl2, THF, 80 °C, 70%; (d) iBuLi, (CHO)n, THF, −78 °C to 0 °C; (ii) TBSOTf, 2,6-lutidine, CH2Cl2, 0 °C to r.t., 20% in two steps; (e) BuLi, (CHO)n, THF, −78 °C to 0 °C, 20%; (f) K2CO3, methanol, r.t.; (g) BnCOCl, Et3N, CH2Cl2, r.t., 81%; (h) HCl-dioxane, CH2Cl2, r.t., 90%; (i) BuCOCl, Et3N, CH2Cl2, r.t., quant.; (j) BnN3 or iBuN3, CuI, Na-ascorbate, DMF/H2O, r.t.; TMS: trimethylsilyl; TBS: tert-butylidimethylsilyl; Bn: benzyl; ‘Bu: isobutyl; DMF: dimethylformamide; Et: ethyl; PPh3: triphenylphosphine; r.t.: room temperature; OTf: triflate.
to 2 (see Scheme 1), followed by TBS protection to give the 1,9-disubstituted \( m \)-carborane 17 in 42% yield over four steps. Afterwards, a carboxylic acid group was introduced into the C7-position of compound 17 using \( n \)BuLi and carbon dioxide, and the resulting carboxylic acid 18 was subjected to amidation with either benzyl amine or isobutyl amine in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI), 1-hydroxybenzotriazole monohydrate (HOBt), and \( N \),\( N \)-diisopropylethylamine (DIEA); the subsequent removal of the TMS group afforded compounds 19a and 19b in 16% and 55% yields over two steps, respectively. These two compounds were then subjected to Hüsgen cycloaddition reactions with benzyl azide or isobutyl azide implementing the approach detailed in Scheme 1 to give compounds 20a–d in 14–93% yields. Finally, removal of the TBS group followed by an esterification process afforded 1,7,9-substituted carboranes IIIa–h in 61% to quantitative yields over two steps. Scaffold IV was obtained according to the synthetic procedure depicted in Scheme 3b. The selective electrophilic diiodination at the 9,10-positions of \( m \)-carborane was achieved under microwave conditions.
irradiation to afford 9,10-diiodo-m-carborane 21 in 84% yield. Surprisingly, 5,9,10-triiodo-m-carborane 22, which has not been reported previously, was obtained as a by-product in 10% yield.

The Kumada–Tamao–Corriu cross coupling between 21 and trimethylsilylelactenylene was performed under reaction conditions similar to those described in Scheme 2 to give 9,10-bis(trimethylsilyl)elactenylene 23 in 94% yield. After introducing a carboxylic acid into the C1-position of 23, the resulting carboxylic acid was subjected to amidation reactions with benzyl amine or isobutyl amine using EDCI to afford compounds IVa–d in 29–82% yields over two steps. Notably, although we were able to synthesize 1,9,10-trisubstituted carboranes, we found it difficult to introduce different substituents at the 9,10-positions of scaffold IV. The structures of 18 (scaffold III), 5,9,10-triiodo-m-carborane 22, and compound 25 (scaffold IV) were determined by X-ray structural analysis.

Finally, the synthesis of 1,2,12-substituted carborane was carried out using p-carborane (Scheme 4). The electrophilic iodination at the B2-position of p-carborane followed by the Sonogashira–Hagihara cross coupling with propargyl PMB ether produced B2-substituted carborane 26 in 49% yield over two steps. By introducing a hydroxymethyl group into 26 via the lithiation with ‘BuLi followed by the addition to paraformaldehyde, 1,2-disubstituted and 1,7-disubstituted carboranes were generated as regioisomers. After a TBS group was introduced to protect the hydroxy group, a carboxylic acid group was introduced into another carbon atom; subsequently,
a separation procedure was implemented using preparative HPLC to obtain the 1,7,12-trisubstituted carborane 27 and 1,2,12-trisubstituted carborane 28 in 21% and 16% yields, respectively, over three steps. The carboxylic acids 27 and 28 were subjected to amidation reactions with benzyl amine or isobutyl amine in a manner similar to that described in Scheme 3; subsequently, removal of the PMB group followed by the Dess–Martin oxidation produced aldehydes 31a,b and 35a,b in 15–75% yields over three steps. These aldehydes were then subjected to Pinnick–Kraus oxidations, and the resulting carboxylic acids were again subjected to amidation with benzyl amine or isobutyl amine followed by removal of the TBS group to afford 32a–d and 36a–d in 55–85% yields over three steps. Finally, esterification with phenylacetyl chloride against methanol gave compounds Va–p in 25% to quantitative yields. The absolute structures of the two isomers were confirmed by X-ray crystallography to be 32d and 36d (see Scheme 4).

**Biological evaluation of trisubstituted carborane library.** In order to validate the biological activity of the thus synthesized trisubstituted carborane library, we performed cell-based assays to determine: hypoxia

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**Scheme 4.** Synthesis of scaffold V. Reaction conditions: (a) I$_2$, HNO$_3$, H$_2$SO$_4$, AcOH, 80 °C, 99%; (b) propargyl PMB ether, PdCl$_2$(PPh$_3$)$_2$, CuI, toluene, piperidine, 80 °C, 49%; (c) i—BuLi, (CHO)$_n$, THF, −78 °C to r.t.; ii—BuLi, CO$_2$, THF, −78 °C to r.t.; (d) EDCI, HOBt, DIEA, BnNH$_2$, CH$_2$Cl$_2$, r.t.; (e) DDQ, NaH$_2$PO$_4$, CH$_2$Cl$_2$, H$_2$O, r.t.; (f) Dess–Martin periodinane, CH$_2$Cl$_2$, r.t.; (g) i—NaClO$_2$, NaH$_2$PO$_4$, 2-methyl-2-butene, acetonitrile/H$_2$O, r.t.; ii—EDCI, HOBt, DIEA, BnNH$_2$, or BuNH$_2$, THF, r.t.; (h) HCl–dioxane, CH$_2$Cl$_2$, r.t.; (i) BuCOCl, Et$_3$N, THF, r.t.; (j) BnCOCl or BuCOCl, Et,N, CH$_2$Cl$_2$, r.t. AcO: acetate; Bn: benzyl; Bu: butyl; Et: ethyl; Ph: phenyl; DDQ: 2,3-dichloro-5,6-dicyano-p-benzoquinone; PMB: p-methoxybenzyl TBS: tert-butylimethyldimethylsilyl; r.t.: room temperature; EDCI: 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; HOBt: 1-hydroxybenzotriazole monohydrate; DIEA: N,N-diisopropylethylamine.
The same scaffold, compound \( \text{Va} \), exhibited significant antiviral activity (IC\(_{50} = 3.87 \mu M\)), whereas \( \text{Vi} \) activated the proliferation of the rabies virus (Fig. 2). Notably compounds \( \text{Va} \) and \( \text{Vi} \) comprise the same substituents (Bn groups). A similar divergence of activity was observed between compounds \( \text{Va–h} \) and compounds \( \text{Vi–l} \). In addition, the intracellular viral protein accumulation and the virus titer were measured using \( \text{Vp} \). As shown in Fig. S4, the intracellular viral proteins treated with \( \text{Vp} \) was significantly increased without affecting the virus titer. Since viral the ribonucleoproteins (RNPs) are formed from the replicated genomic RNA and Rabies N proteins are released from the cell to infecting other cells, these results suggest that \( \text{Vp} \) may have inhibited the formation of viral ribonucleoproteins, resulting in increased accumulation of viral proteins in the cell.

The compound concentration required to inhibit the relative light units by 50% (IC\(_{50}\)) was determined based on semi-logarithmic dose–response plots. All the samples were tested in triplicate. [a] YC-1 was used as positive control with IC\(_{50}\) of \( 0.17 \mu M \). [b] Favipiravir was used as a control with IC\(_{50}\) of \( 38 \mu M \).

**Assessment of trisubstituted carboranes for comprehensive chemical space search.** Finally, we investigated the morphologies of the herein synthesized compounds using the normalized principal moment of inertia (PMI)\(^5\), which is an approach commonly employed to assess the molecular shape of compound libraries in comparison to 2-butyne (rod), benzene (disc), and adamantane (sphere). For all compounds, 25 conformations were generated in each compound using the ‘iCon fast’ option of LigandScout 4.4, and the normalized PMI values calculated with the aid of the RDKit\(^6\) were plotted (Fig. 3; see also Fig. S5). Notably, a higher number of compounds characterized by scaffolds I and II (green and blue dots in Fig. 3) occupies the sphere-like shape section of the graph in Fig. 3 than compounds characterized by other scaffolds. On the other hand, compounds characterized by scaffold V tended to occupy the rod–disc area of the graph, as a result of the presence of the two substituents in para positions to each other aligned in a linear fashion. Scaffold III and IV-based compounds, derived from \( m \)-carborane, occupied the middle section of the morphology graph. The results of the PMI analysis indicate, therefore, that the herein synthesized carboranes characterized by scaffolds I–V cover all the regions of the described three-dimensional shape space.

**Conclusion**

We successfully synthesized a total of 42 trisubstituted carboranes characterized by five basic of scaffolds that were found to occupy the entire rod–disc–sphere chemical morphology space; we achieved this goal by exploiting the different reactivities of the twelve vertices of \( o, \), \( m, \), and \( p \)-carboranes. The typical three-dimensional structures of each scaffold type of trisubstituted carboranes, 4, 14, 18, 25, 32d, and 36d, were unambiguously determined by X-ray structural analysis. The synthesized compounds were utilized in cell-based biological assays performed to determine the effects of various carboranes on HIF-1 transcriptional activity and anti-rabies virus activity.
Table 1. Results of the evaluation of the biological activities of various members of the trisubstituted carborane compound library. The compound concentration required to inhibit the relative light units by 50% (IC50) was determined based on semi-logarithmic dose–response plots. All the samples were tested in triplicate. [a] YC-1 was used as positive control with IC50 of 0.17 µM. [b] Favipiravir was used as a control with IC50 of 38 µM.

| Scaffold | Compound | HIF-1 | RABV | HeLa cells | N2a cells |
|---------|----------|-------|------|------------|-----------|
| I       | Ia       | > 30  | > 9  | > 30       | > 30      |
|         | Ib       | > 30  | > 9  | > 30       | > 30      |
|         | Ib       | > 30  | > 9  | > 30       | > 30      |
|         | Id       | > 30  | > 9  | > 30       | > 30      |
|         | Ie       | > 30  | > 9  | > 30       | > 30      |
|         | If       | > 30  | > 9  | > 30       | > 30      |
|         | IIa      | > 30  | > 9  | > 30       | > 30      |
|         | IIb      | > 30  | > 9  | > 30       | > 30      |
|         | IIc      | > 30  | > 9  | > 30       | > 30      |
|         | IIId     | 23.0  | > 9  | > 30       | > 30      |
|         | IIe      | > 30  | > 9  | > 30       | > 30      |
|         | IIIf     | > 30  | > 9  | > 30       | > 30      |
|         | IIg      | > 30  | > 9  | > 30       | > 30      |
|         | IIh      | > 30  | > 9  | > 30       | > 30      |
|         | IIIa     | > 30  | > 9  | > 30       | > 30      |
|         | IIIb     | 26.3  | > 9  | > 30       | > 30      |
|         | IIIc     | > 30  | > 9  | > 30       | > 30      |
|         | IIIId    | 23.0  | > 9  | > 30       | > 30      |
|         | IIIe     | > 30  | > 9  | > 30       | > 30      |
|         | IIIIf    | > 30  | > 9  | > 30       | > 30      |
|         | IIIg     | > 30  | > 9  | > 30       | > 30      |
|         | IIIh     | > 30  | > 9  | > 30       | > 30      |
|         | IVa      | > 30  | > 9  | > 30       | > 30      |
|         | IVb      | 17.4  | > 9  | > 30       | > 30      |
|         | IVc      | > 30  | > 30 | > 30       | > 30      |
|         | IVd      | > 30  | > 30 | > 30       | > 30      |
|         | Vb       | > 30  | 5.74 | > 30       | > 30      |
|         | Ve       | > 30  | 6.87 | > 30       | > 30      |
|         | Vd       | > 30  | 3.30 | > 30       | > 30      |
|         | Vc       | > 30  | 8.14 | > 30       | > 30      |
|         | Vf       | > 30  | 5.78 | > 30       | > 30      |
|         | Vg       | > 30  | 8.37 | > 30       | > 30      |
|         | Vh       | > 30  | > 9  | > 30       | > 30      |
|         | Va       | > 30  | 3.87 | > 30       | > 30      |
|         | Vb       | > 30  | 4.44 | > 30       | > 30      |
|         | Vc       | > 30  | 3.95 | > 30       | > 30      |
|         | Vd       | > 30  | 2.46 | > 30       | > 30      |
|         | Ve       | > 30  | 2.56 | > 30       | > 30      |
|         | Vf       | > 30  | 7.08 | > 30       | > 30      |
|         | Vg       | > 30  | 4.00 | > 30       | > 30      |
|         | Vh       | > 30  | > 9  | > 30       | > 30      |
|         | Vi       | 5.46  | Activation | > 30   | > 30      |
|         | Vj       | 5.24  | Activation | > 30   | 22.6      |
|         | Vk       | 5.64  | Activation | 15.0   | 21.2      |
|         | VI       | 18.1  | Activation | > 30   | 25.2      |
|         | Vm       | 23.2  | > 9  | > 30       | > 30      |
|         | Vn       | > 30  | > 9  | > 30       | > 30      |
|         | Vo       | 9.21  | Activation | 20.9   | 7.90      |
|         | Vp       | > 30  | Activation | > 30   | 8.00      |
Compounds characterized by scaffold V (e.g., Vi–m, and Vo) were mainly observed to exhibit significant inhibition of HIF-1 transcriptional; by contrast, anti-rabies virus activity was observed not only for compounds with scaffold V (compounds Va–h) but also for carboranes with scaffold II (compounds IIb–g) and IV (compound IVb). The pharmacophore model predicted by the scaffold V carboranes, which exhibited significant anti-rabies virus activities, agreed well especially with compounds IIb and IID with scaffold IVb and compound IVb with scaffold IV. Furthermore, the first compounds to have ever been found to stimulate the proliferation of the rabies virus were identified in the present study, and they were determined to be scaffold V compounds. Therefore, we believe that our strategy for the systematic design of three-dimensionally divergent molecules based on five different types of trisubstituted carboranes has great potential to satisfy all directions in the chemical space and to afford the identification of novel biologically active molecules that have not been recognised by other drug discovery approaches.

Experimental section

Synthesis of trisubstituted carboranes. The synthetic procedures and characterization were provided in "Supplementary information S1".

X-ray crystallography. All data generated or analyzed were provided in "Supplementary information S1". CCDC 2110331, 2110336–2110341.
Cell culture. Human epithelioid cervical carcinoma (HeLa) cells were obtained from the Cell Resource Center for Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University (Sendai, Japan). The cells were incubated with RPMI-1640 medium (FUJIFILM Wako Pure Chemical) containing 10% fetal bovine serum (Gibco; Thermo Fisher Scientific) and 1% penicillin/streptomycin (FUJIFILM Wako Pure Chemical). Cells were incubated in a cell incubator with 5% CO₂ at 37 °C. Neuro 2a (N2a) cells were incubated with D-MEM medium (FUJIFILM Wako Pure Chemical) containing 10% fetal bovine serum (Gibco; Thermo Fisher Scientific) and 1% penicillin/streptomycin (FUJIFILM Wako Pure Chemical). Cells were incubated in a cell incubator with 5% CO₂ at 37 °C.

MTT assay. HeLa cells or N2a cells (5 × 10⁵ cells per well of a 96-well plate) were incubated under 5% CO₂ at 37 °C in RPMI-1640 media (for HeLa cells) or D-MEM media (for N2a cells) containing 10% fetal bovine serum, 1% penicillin/streptomycin, and various concentrations of compound (10 mM in DMSO) for 72 h. After the incubation, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) in PBS (5 mg/mL, 10 μL) were added, and the cells were further incubated at 37 °C for 2 h. After removal of the medium, DMSO (100 μL) was added and the absorbance at 590 nm was measured with a microplate reader. The IC₅₀ value was determined from semilogarithmic dose–response plots.

Luciferase reporter gene assay for HIF transcriptional activity. HeLa cells transfected with hypoxia response element dependent firefly luciferase reporter construct (HRE-Luc) were seeded in a 96-well plate (2.5 × 10⁴ cells per well) and were incubated under 5% CO₂ at 37 °C under 5% CO₂ for 48 h with RPMI-1640 media containing 10% fetal bovine serum, 1% penicillin/streptomycin. The luciferase reporter gene assay was performed with Luciferase Assay System (Promega, Madison, WI, USA) according to the manufacturer’s instructions. The compound concentration required to reduce relative luminescence units (RLU) by 50% (IC₅₀) was determined from semilogarithmic dose–response plots.

Luciferase reporter gene assay for antiviral activity against rabies virus. The recombinant rabies virus strain 1088 expressing gausia luciferase (1088/GLuc) was generated as described previously⁴. The virus titer was determined using focus assay as reported⁴ and expressed as focus forming units (FFU). N2a cells (4 × 10⁴ cells per well) and 1088/GLuc (4 × 10⁴ FFU per well) were prepared in E-MEM containing 10% fetal bovine serum and antibiotics, and the mixed solution was applied to a 96-well black plate with clear bottoms (Greiner). The substrate solution was formed using Pierce Gaussia Luciferase Glow Assay Kit (Thermo Fisher Scientific). The substrate solution was added to each well, and RLU was immediately measured using a microplate luminometer LuMate (Awareness Technology). Based on the RLU value, IC₅₀ was determined from semilogarithmic dose–response plots.

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Author contributions
Y.A. and S.H. synthesized the compounds, evaluated inhibitory activity toward HIF-1α and cytotoxicity, and analysed data. Y.A. and A.Y. performed computational analysis of the compounds. K.Y. and A.K. evaluated inhibitory activity toward RABV. H.N. and H.K. supervised the whole project. Y.A. and H.N. wrote the manuscript.
Competing interests
The authors declare no competing interests.

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