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Brief Article

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# γ-Aminobutyric Acid(C) ($GABA_C$) Selective Antagonists Derived from the Bioisosteric Modification of 4-Aminocyclopent-1-enecarboxylic Acid: Amides and Hydroxamates

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Supporting Information

**ABSTRACT:** Series of compounds were generated via the bioisosteric replacement of the carboxylate of 4-ACPCA (**2**) with hydroxamate or amide groups. All compounds from this study exhibited increased selectivity for $GABA_C$, the most potent being 4-ACPHA (**10a**, $IC_{50}$ = 13 μM) and 4-ACPAM (**11a**, $IC_{50}$ = 10 μM). This provides evidence that a zwitterionic structure is not essential for $GABA_C$ antagonists, rather the emphasis lies in appropriate heteroatoms to participate in hydrogen bonding.

## INTRODUCTION

GABA (**1**, Figure 1) is the major inhibitory neurotransmitter of the mammalian central nervous system (CNS), with its effects

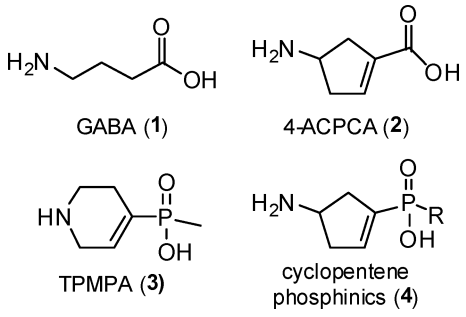


**Figure 1.** GABA and various conformationally restricted analogues: **2**, a $GABA_A$ agonist and $GABA_C$ antagonist, and **3** and **4**, selective $GABA_C$ antagonists.

mediated through the regulation of ionotropic $GABA_A$ and $GABA_C$ receptors as well as the metabotropic $GABA_B$.[1] There has been recent interest in the $GABA_C$ receptor and its implications on memory formation due to its long ion channel opening time,[2] location in the hippocampus,[3] and evidence of involvement in ammonia-induced apoptosis of hippocampal cells.[4] Their role in cognition has been supported by findings that $GABA_C$ antagonists can enhance learning in chicks[5] and mice.[6]

Few selective $GABA_C$ receptor antagonists have been identified, hence much of the structure−activity relationships at $GABA_C$ receptors remain unclear. This is due to the limited diversity of $GABA_C$ selective antagonists, where most are derived from phosphinic acids.[7] It is therefore crucial for a wider range of compounds to be identified in order to further the understanding of $GABA_C$ structure−activity relationships, as this may lead to the development of novel therapeutics.

As a result of GABA's flexible backbone, it is able to adopt a number of conformations when interacting with various macromolecular targets. This characteristic of GABA can be utilized to provide selective ligands through the generation of conformationally restricted analogues. These use structures such as double bonds, rings, or bulky substituents to restrict molecule into conformations of GABA favored by a subset of these sites. For instance, the introduction of a five-membered ring structure allows the compound to mimic the binding mode of GABA at $GABA_A$ and $GABA_C$ receptors, thus driving selectivity for these subtypes over that of $GABA_B$ receptors. Such compounds have been synthesized, an example being **2**, which displays antagonism at the $GABA_C$ receptor, however, it also has $GABA_A$ agonist activity.[8,9]

Previous work has investigated the effect of manipulating the acidic and basic moieties of these restricted analogues. This has led to the synthesis of a series of phosphinic acid derivatives (**4**)[10,11] based around the structures of **2** and **3**. The replacement of a carboxylic acid by a phosphinic acid retains hydrogen and ionic bonding capability but also allows for the introduction of alkyl substituents. It is thought that these alkyl side chains drive the $GABA_C$ selectivity, with no $GABA_A$ activity observed with this class of compounds. As a result, a lipophilic pocket is proposed at the $GABA_C$ receptor site which is able to accommodate these groups.[7,11] This raises the question as to whether similar groups included in the structure of **2** could also lead to potent and/or selective $GABA_C$ agents. Therefore, the present study sought to bioisosterically replace the carboxylate group of **2** with an alternate functional group to allow the introduction of alkyl and aryl groups at this position. It has previously been noted that a zwitterionic structure is important for activity at GABA receptors,[11−14] and hence stage one of the present study investigated a functional group expected to be partly ionized at physiological pH.

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Hydroxamic acids, with p$K_a$ values in the order of 9, would be expected to be approximately 1% ionized under physiological conditions. *O*-Substitution changes the p$K_a$ only slightly,[15] hence the hydroxamic acid group appeared to fulfill the requirements of this study, namely a functional group that could be expected to be partially ionized at physiological pH, leaving open the possibility of ionic binding as well as serving as a point for the introduction of substituents.

To further explore the relative importance of an acidic group and consequently a zwitterionic structure for activity, stage two of this study involved replacing the carboxylic acid of **2** with an alternate group expected to be un-ionized at physiological pH. Amides typically have p$K_a$ values over 15[16] and so would be effectively un-ionized at physiological pH but would, however, be capable of forming similar hydrogen bonding to **2**. Therefore, it was thought that testing amide derivatives of **2** would provide more information on the importance of a zwitterionic structure for activity at GABA receptor sites. We also wished to further explore the possibility of introducing various substituents, the amide serving as a point for substitution. This may further characterize the proposed hydrophobic cavity at $GABA_A$ and $GABA_C$ receptors.

This study aimed to synthesize hydroxamic and amide analogues of **2**. Testing of the unsubstituted derivatives **10a** (4-ACPHA) and **11a** (4-ACPAM) would help to identify the influence that the bioisosteric replacement would have on activity when compared with the parent carboxylic acid, **2**. A series of substituted derivatives were also to be prepared (**10b–d** and **11b–e**) to assess the effect of alkyl and aryl substituents on GABA receptor activation.

## RESULTS AND DISCUSSION

The target compounds were synthesized according to Scheme 1. The route to intermediate **8** followed that reported previously.[17] In short, treatment of the commercially available lactam **5** (2-azabicyclo[2.2.1]hept-5-en-3-one) with methanol and thionyl chloride gave the methyl ester hydrochloride (**6**). Treatment of this intermediate with di-*tert*-butyl dicarbonate and triethylamine produced the protected amino ester (**7**), which was isomerized with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to give the conjugated methyl 4-(*tert*-butoxycarbonylamino)cyclopent-1-enecarboxylate (**8**) as a white, crystalline solid in good yield. The methyl ester **8** was selectively hydrolyzed with base to give **9**, a key intermediate in this study, as it was used to generate all final target compounds. The Boc protecting group of **9** was hydrolyzed to give parent compound **2** as a white crystalline solid after isolation using a Dowex 50 ($H^+$) ion exchange column.

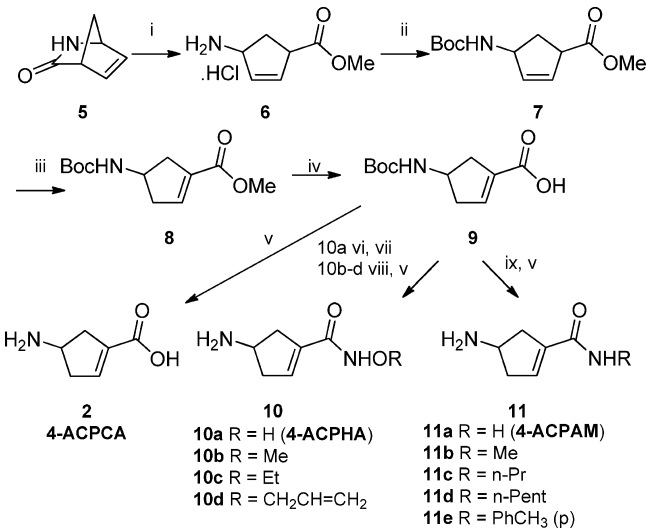
Hydroxamic acid derivatives (**10a–d**) were prepared via a mixed anhydride method. **9** was treated with *i*-butyl chloroformate in the presence of base and subsequent addition of the hydroxylamine of interest. In the case of the unsubstituted **10a**, a commercially available, pyran-protected hydroxylamine was used to limit attack on the mixed anhydride to that by the free amine position of the hydroxylamine. Acidic hydrolysis of protecting groups gave hydroxamates **10a–d**.

Amide derivatives (**11a–e**) were prepared using a similar method. The *N*-Boc acid (**9**) was treated with *i*-butyl chloroformate to generate the mixed anhydride and subsequent addition of the desired amine, followed by deprotection to give **11a–e**. The activity of compounds **2**, **10a–d**, and **11a–e** was assessed using the two-electrode voltage clamp method in *Xenopus laevis* oocytes expressing human recombinant $GABA_A$ ($\alpha_1\beta_2\gamma_{2L}$), $GABA_{B(1b,2)}$, and $\rho_1$ $GABA_C$ receptors. All compounds were initially screened alone at a concentration of 300 $\mu$M and then at a concentration of 300 $\mu$M in the presence of a submaximal dose of GABA ($EC_{50}$) to detect either agonist or antagonist activity, respectively. Compounds displaying the most potent effects at $GABA_C$ receptors underwent further evaluation and a current concentration–response curve generated in the presence of an $EC_{50}$ GABA concentration (1 $\mu$M).

All newly synthesized hydroxamate derivatives (**10a–d**) displayed antagonist profiles at $GABA_C$ receptors (Table 1). These compounds exhibited reduced activities at both $GABA_A$ and $GABA_B$ receptors when compared with the parent carboxylic acid **2**. In particular, the unsubstituted hydroxamic acid, **10a**, displayed similar potency as an antagonist at $GABA_C$ receptors but with superior selectivity when compared with the parent **2**. This indicates that the bioisosteric replacement of the carboxylic acid moiety of **2** with a hydroxamic acid to give **10a** is well tolerated at the binding site and has resulted in a selective $GABA_C$ antagonist. This is in spite of the fact that a hydroxamic acid group would be expected to be approximately only 1% ionized at physiological pH. While this is a small proportion, it still leaves a possibility that it is the ionized form of **10a–d** that participates in binding at the receptor site. When contrasting the activity of **10a** with the other hydroxamates of the series (**10b–d**) that incorporate an *O*-substituent, it can be seen that *O*-substitution has led to a 7–12-fold reduction in potency at the $GABA_C$ site. This shows that alkyl groups at this position are not well tolerated at the receptor site, presumably from interference with important binding interactions between the hydroxamic acid and receptor. This is in contrast to findings for the phosphinic acid derivatives, where alkyl groups are thought to increase binding affinity at the site.[12,13]

Compounds **11a–e** were generated through the bioisosteric introduction of an amide group at the carboxylic acid position

**Scheme 1**[a]




[a]Reagents and conditions: (i) $SOCl_2$, MeOH, 0 °C; (ii) $(Boc)_2O$, $Et_3N$, THF, 0 °C to rt; (iii) DBU, THF, rt; (iv) 0.5 M NaOH, THF; (v) HCl, EtOAc; (vi) (1) *i*-BuOCOCl, TEA, −20 °C, (2) *O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine, −20 °C to rt; (vii) 6 M HCl, MeOH; (viii) (1) *i*-BuOCOCl, TEA, −20 °C, (2) NHOR, −20 °C to rt; (ix) (1) *i*-BuOCOCl, TEA, −20 °C, (2) $H_2NR$, −20 °C to rt.


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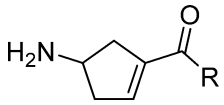
**Table 1. Pharmacological Data**



| compd | R = | $GABA_A$ ($\alpha_1\beta_2\gamma_{2L}$) activity: % $GABA_{max}$ response (at 300 $\mu$M)[a] | $GABA_A$ ($\alpha_1\beta_2\gamma_{2L}$) activity: % inhibition (at 300 $\mu$M against GABA $EC_{50}$ dose)[b] | $GABA_{B(1b,2)}$ activity: % $GABA_{max}$ response (at 300 $\mu$M)[a] | $GABA_{B(1b,2)}$ activity: % inhibition (at 300 $\mu$M against GABA $EC_{50}$ dose)[b] | $GABA_C$ $\rho_1$ activity: $IC_{50}$[c] or % inhibition (at 300 $\mu$M against GABA $EC_{50}$ dose)[b] |
|---|---|---|---|---|---|---|
| **2** | OH | 59.7 ± 2.7% | | 75.3 ± 7.5% | | 6.6 ± 0.7 $\mu$M |
| **10a** | NHOH | 4.3 ± 0.1% | | 23.0 ± 3.8% | | 12.9 ± 1.1 $\mu$M |
| **10b** | $NHOCH_3$ | 6.3 ± 0.7% | | 31.0 ± 3.3% | | 81.5 ± 2.9 $\mu$M |
| **10c** | $NHOCH_3$ | 2.5 ± 0.1% | | 16.3 ± 2.2% | | 153.0 ± 15.6 $\mu$M |
| **10d** | $NHOCH_2CH{=}CH_2$ | 1.7 ± 0.9% | | 33.0 ± 5.5% | | 115.3 ± 9.7 $\mu$M |
| **11a** | $NH_2$ | | 23.3 ± 7.8% | 5.2 ± 0.7% | | 9.6 ± 0.9 $\mu$M[20] |
| **11b** | $NHCH_3$ | NA[d] at 300 $\mu$M | NA at 300 $\mu$M | 3.2 ± 2.0% | | 408.3 ± 57.6 $\mu$M |
| **11c** | $NH(CH_2)_2CH_3$ | NA at 300 $\mu$M | NA at 300 $\mu$M | 9.0 ± 2.3% | | 570.0 ± 54.8 $\mu$M |
| **11d** | $NH(CH_2)_4CH_3$ | NA at 300 $\mu$M | NA at 300 $\mu$M | 3.3 ± 1.2% | | 20.5 ± 1.1% |
| **11e** | $NHPhCH_3(p)$ | NA at 300 $\mu$M | NA at 300 $\mu$M | | 27.0 ± 7.5% | 11.3 ± 3.1% |

[a]Current produced by 300 $\mu$M of compound. Data are mean ± SEM ($n$ = 3–5 oocytes). [b]Percentage inhibition by 300 $\mu$M of compound of the current produced by a submaximal dose of GABA ($EC_{50}$). $EC_{50}$ values for GABA for $GABA_A$ ($\alpha_1\beta_2\gamma_{2L}$), $GABA_{B(1b,2)}$, and $\rho_1$ $GABA_C$ are 30, 1, and 1 $\mu$M, respectively. Data are mean ± SEM ($n$ = 3–4 oocytes). [c]Compound concentration which inhibits a submaximal dose of GABA (1 $\mu$M, $EC_{50}$). Data are mean ± SEM ($n$ = 3–5 oocytes). [d]NA is no activity observed up to given concentration.

of **2**. In a similar manner to the hydroxamates, the unsubstituted amide **11a** displayed the greatest potency as an antagonist at $GABA_C$ receptors ($IC_{50}$ = 9.6 $\mu$M). **11a** also has similar potency to that of the parent compound **2** but with reduced activity at $GABA_B$ receptors. At $GABA_A$ receptors, **11a** displayed very weak antagonist properties (23.3% inhibition at 300 $\mu$M), whereas **2** is a weak agonist at $GABA_A$ receptors (59.7% of the $GABA_{max}$ response at 300 $\mu$M). Hence, the simple bioisosteric replacement of a carboxylic acid (**2**) predominantly ionized at physiological pH by a neutral amide (**11a**) has given a $GABA_C$ selective antagonist. This provides clear evidence that a zwitterionic structure is not essential for potent antagonist action at $GABA_C$ receptors. The *N*-alkyl and *N*-aryl substituted amide derivatives **11b–e** were also found to act as $GABA_C$ antagonists but with greater than 40-fold reduction in activity compared to the unsubstituted amide **11a**, for instance, **11e** displays only 11.3% inhibition at 300 $\mu$M. This indicates that substitution at this position does not appear to be well tolerated at this site as it may sterically interfere with important binding interactions.

With respect to $GABA_C$ potency, the introduction of *N*-substituents to the amide derivatives (**11b–e**) was much less successful than the *O*-substitution to give the hydroxamate derivatives (**10b–d**). This observation may be explained by the fact that *N*-substituents of the amide group compared with the *O*-substituents of the hydroxamate are placed in different relative positions. While both functional groups can adopt both *E* and *Z* conformations, the hydroxamate places the *O*-substituent one atom further away from the C=O. This may allow the group to access different residues available at the receptor site compared to the amide derivatives.

$GABA_C$ receptor ligand docking studies were performed to help elucidate the molecular interactions underlying these results using a TPMPA minimized *N*-terminal ligand-binding domain homology model of the $\rho_1$ $GABA_C$ receptor developed previously by others in our group.[7] Docking trials were performed for both optical isomers of **2**, **10a**, and **11a** using the state of ionization predominant at physiological pH. Structures were docked, and solutions were selected as detailed in the Supporting Information. All compounds were found to dock in a similar way. They displayed hydrogen bond interactions with residues at the receptor site. Docking results of key structures are shown in Figure 2A–D. Results are in strong concordance with observed experimental data.

In agreement with previous findings,[7] the amino moiety of each compound interacted with the tyrosine 198 residue (Y198) while the carbonyl region in each displayed discrete bonding arrangements. The oxygens of the carboxylic acid **2** (Figure 2A) formed two H-bonds to arginine 104 (R104), one to serine 243 (S243), and another to Y198. The hydroxamic acid **10a** and amide **11a** did not appear to interact with Y198 but forms additional bonds with other residues. In the case of **10a**, it was found to interact more strongly with R104, with the two oxygen atoms forming a total of three H-bonds with this residue as shown in Figure 2B. The terminal oxygen formed an addition bond with S243. It should also be pointed out that this hydroxamic acid did appear to reside in the *Z* geometry as predicted by previous studies.[18,19] In contrast, amide **11a** (Figure 2C) formed the bulk of bonds with the carbonyl group, forming two bonds to R104 and another to S243. Also interestingly, the amide $NH_2$ formed a hydrogen bond with aspartate 164 (D164), a residue not utilized by either **2** or **10a**. This change may be explained by the difference in orientation of the carboxamide group of **11a** (seen to be approximately perpendicular to the plane of the page in Figure 2C) when compared with the carbonyl group of **2** (seen to be approximately in the plane of the page in Figure 2A). Further evidence for a different binding mode of **11a** has been noted in a previous study from our group involving $\rho$1 $GABA_C$ Y102 receptor mutants.[20] These receptors are constitutively active, allowing an investigation of the binding preference of various $GABA_C$ agents for closed versus open receptor states. Of all the cyclopentene agents tested (**2**, 3-ACPBPA (3-aminopropyl-methyl-phosphinic acid) and **11a**), **11a** was the only compound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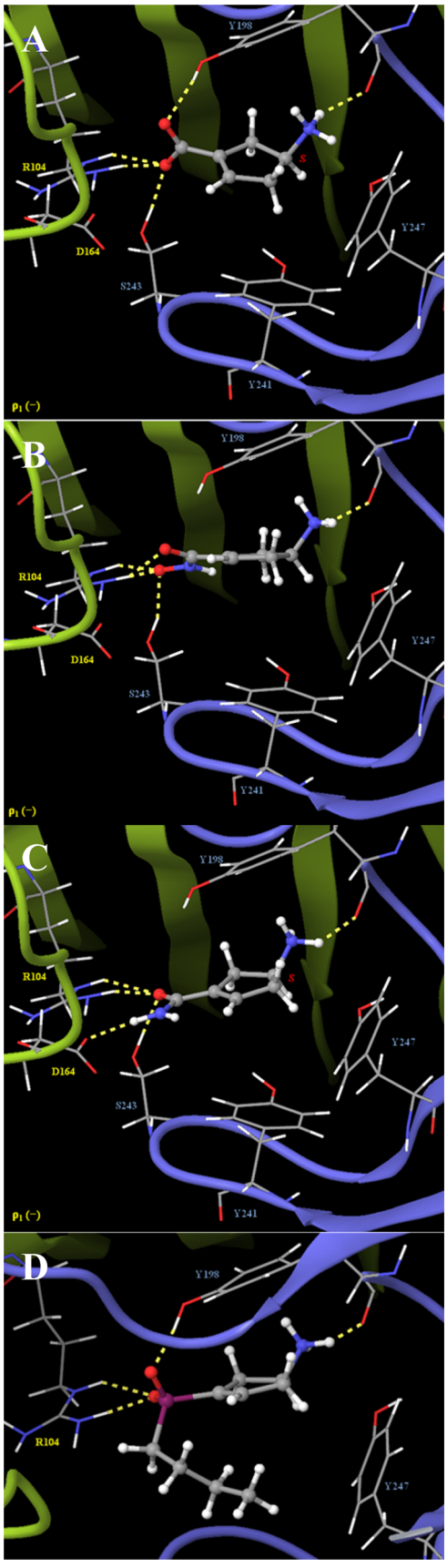


**Figure 2.** $GABA_C$ receptor docking results for (A) **2**, (B) **10a**, (C) **11a**, (D) **4** (R = *n*-butyl).

found to explicitly act on receptors in the closed state. To identify possible explanations for the loss of activity observed for the alkyl and aryl derivatives **10b–d** and **11b–e**, fitting results were compared with those obtained for **4** (R = *n*-butyl) (Figure 2D), a previously described $GABA_C$ selective antagonist. The *n*-butyl group of this compound is thought to be accommodated by a lipophilic pocket available at the binding site, serving to increase binding affinity. If the relative position of this group is compared to the docking results obtained for **10a** and **11a**, it can be seen that if terminal substituents were introduced to the hydroxamic acid or amide groups, this would place them in a different orientation relative to that of the *n*-butyl group. This would render them unable to access the lipophillic pocket and may in fact have introduced steric hindrance, reducing binding affinity. This is echoed by results obtained for all substituted derivatives in this study, **10b–d** and **11b–e**, showing reduced potencies in all cases.

Overall, docking results have provided a rationale for the observed potencies of synthesized compounds. They also provide further evidence that a zwitterionic structure is not essential for activity at the $GABA_C$ receptor site. For strong ligand–receptor interactions, the requirement is that a compound has appropriate heteroatoms in the correct orientation to participate in hydrogen bonding with residues available at the receptor site and that at the nitrogen binding site an ionic interaction is not essential.

## CONCLUSION

This study identified two new classes of $GABA_C$ analogues through the bioisosteric replacement of the carboxylic acid moiety of **2** with either a hydroxamic acid (**10a–d**) or amide (**11a–e**) group. These functional groups were chosen to allow an investigation of the importance of a zwitterionic structure for potent actions at $GABA_C$ receptors, as a hydroxamic acid would be expected to be only partially ionized at physiological pH while an amide would be expected to be nonionized.

All compounds from this study exhibited increased selectivity for the $GABA_C$, the most potent compounds being **10a** ($IC_{50}$ = 13 $\mu$M) and **11a** ($IC_{50}$ = 10 $\mu$M). While the potency of compounds may sit just beyond that useful for clinical applications, they serve as important pharmacological tools to probe binding interactions at the $GABA_C$ site. **11a** is of particular interest, as it provides clear proof that for strong ligand–receptor interactions at the $GABA_C$ site a zwitterionic structure is not essential.

The discovery of two novel series of $GABA_C$ antagonists serves to widen the current scope for compounds acting at this site beyond those of phosphinic acid derivatives. Such information is invaluable when piecing together a detailed view of the structure–activity relationships and may lead to the development of novel therapeutics that target the $GABA_C$ receptor.

## EXPERIMENTAL SECTION

For full details see Supporting Information. Purity of tested compounds was assessed using analytical HPLC and found to be ≥95%.

**Representative Procedure for the Synthesis of Derivatives via the Mixed Anhydride Method.** *(±)-4-Aminocyclopent-1-enecarboxamide (**11a**).* Triethylamine (304 mg, 3 mmol) was added to a solution of (±)-4-*tert*-butoxycarbonylaminocyclopent-1-enecarboxylic acid (**9**, 341 mg, 1.5 mmol) in THF (30 mL) at 0 °C. *i*-Butylchoroformate (338 mg, 2.5 mmol) was added dropwise, and the solution left to stir for 15 min. Gaseous ammonia was bubbled through


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the solution for 20 min and the reaction left to stir at 0 °C for a further 2 h. The reaction was concentrated in vacuo, diluted with ethyl acetate (30 mL), and washed with aqueous sodium hydroxide (1M, 10 mL), saturated citric acid (10 mL), and brine (10 mL). The organic fraction was dried over sodium sulfate, and solvent was removed under reduced pressure. The product was isolated using flash chromatography, eluting with ethyl acetate/dichloromethane (10:1) to give (±)-*tert*-butyl 3-carbamoylcyclopent-3-enylcarbamate (315 mg, 92% yield). *tert*-Butyl 3-carbamoylcyclopent-3-enylcarbamate (315 mg, 1.39 mmol) was dissolved in a saturated solution of hydrochloric acid in ethyl acetate and the resulting solution allowed to stir for 4 h. Solvent was removed in vacuo and the product isolated using an ion-exchange column of Dowex 50W ($H^+$) (10 mL), eluting the amino amide with ammonia (2M). This gave (±)-4-aminocyclopent-1-enecarboxamide (**10a**, 156 mg, 89% yield). $R_f$ = 0.27 (4:1:1 *n*-butanol/acetic acid/water). $^1$H NMR (300 MHz, $D_2O$): $\delta$ 6.32 (1H, s, CH═), 4.03−3.93 (1H, m, C*H*$NH_2$), 3.01−2.84 (2H, m, cyclopentene C*H*H and CH*H*), 2.56−2.43 (2H, m, cyclopentene CH*H* and CH*H*). $^{13}$C NMR (300 MHz, $D_2O$): $\delta$ 171.1 (C═O), 140.2 (CH═), 136.0 (═C), 50.9 ($CHNH_2$), 42.3 (cyclopentene $CH_2$), 40.5 (cyclopentene $CH_2$). ESI-MS m/*z* positive ion mode, 127 (55%, $MH^+$), 110 (5%, $MH^+$-$NH_3$); negative ion mode, 126 (20%, $M^+ - H$). HPLC: $t_R$ = 3.61 min.

## ■ ASSOCIATED CONTENT

### Ⓢ Supporting Information

Full details of synthetic methods, spectroscopic data for compounds, in vitro pharmacological testing, and molecular modeling. This material is available free of charge via the Internet at http://pubs.acs.org.

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### Notes

The authors declare no competing financial interest.

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## ■ ABBREVIATIONS USED

4-ACPCA, 4-aminocyclopent-2-enecarboyxlic acid; 4-ACPHA, 4-amino-*N*-hydroxycyclopent-1-enecarboxamide; 4-ACPAM, 4-aminocyclopent-1-enecarboxamide; TPMPA, 1,2,5,6-tetrahydropyridin-4-yl(methylphosphinic acid)

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