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Synthesis and evaluation of (*E*)-2-(5-phenylpent-2-en-4-ynamido)cyclohex-1-ene-1-carboxylate derivatives as HCA2 receptor agonists

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ABSTRACT

Novel series of compounds consisting of 2-amidocyclohex-1-ene carboxylate and phenyl parts which are connected by enyne (compounds **2a**–**f**), but-1-yne (compounds **4a**–**j**), and phenylethylene (compounds **5a**–**f**) linkers as HCA2 full agonists were designed and their functional activity using cAMP assay and binding affinity using radioligand (^{3}H-niacin) binding assay were evaluated. In general, compounds of all three series exhibit similar HCA2 binding and activation profile. However, the activity is strongly dependent on the substituent at the aromatic part of the structure. Among the structures evaluated, the highest affinity and potency in all series were exhibited by compounds containing 4-hydroxy and/or 2-chloro or 2-fluoro substituents. The most active compounds in the enyne and but-1-yne series in the cAMP assay are 2-fluoro,4-hydroxy and 2-chloro,4-hydroxy phenyl derivatives **2f**, **4f**, and **4g** showing potency similar to the previously described 4-hydroxy-biphenyl analogue **5c**.

1. Introduction

Hydroxycarboxylic acid receptor 2 (HCA2) is a G-protein coupled receptor, highly expressed in adipocytes, immune cells such as macrophages, monocytes, neutrophils, and epidermal cells. The endogenous ligand of HCA2 is hydroxylated short chain fatty acid 3-hydroxybutyrate, which is the product of metabolic β-oxidation process in liver.[1]

The most well known HCA2 agonist is niacin, which is used in the treatment of lipid disorders since the 1950s. It was believed that antidyslipidemic properties of niacin were based on HCA2 activation in adipocytes with subsequent decrease of the plasma levels of low-density lipoproteins (LDL) and increase of high-density lipoproteins (HDL).[2] However, the study on mice lacking the HCA2 receptor and the clinical trial of HCA2 agonists MK-1903[3] and SCH900271[4] (Fig. 1) demonstrated that the niacin antilipolytic effect is independent of the HCA2 activation.[5] HCA2 mediates the decrease of the levels of free fatty acids in plasma, but has no effect on the decrease of LDL levels or increase of the HDL.

Atherosclerosis is chronic inflammatory disease in different stages of which macrophages and monocytes are involved.[6] Using the mouse model of atherosclerosis, it is shown that niacin inhibits disease progression through the HCA2 expressed by macrophages without an effect on HDL cholesterol plasma level.[7] The study on human monocytes showed that niacin and HCA2 agonist acipimox (Fig. 1) have anti-inflammatory activity, modulating function of immune cells that leads to antiatherogenic effect.[8]

While HCA2 agonists were mostly studied for the treatment of dyslipidemia and atherosclerosis, the beneficial effect of HCA2 agonists are found in some other conditions related to inflammation. For example, HCA2 agonists dimethyl fumarate and monomethyl fumarate are used in the treatment of psoriasis.[9] Dimethyl fumarate also showed protective effect in the mouse model of autoimmune disease multiple sclerosis with data suggesting that dimethyl fumarate acted through HCA2.[10] The HCA2 activation by endogenous and exogenous ligands has also been associated

Abbreviations: LDL, low-density lipoprotein; HDL, high-density lipoprotein; cAMP, 3′-5′-cyclic adenosine monophosphate; SAR, structure-activity relationship; RLB, radioligand binding; GPCR, G-protein coupled receptor; BSA, bovine serum albumin; DCM, dichloromethane; TEA, triethylamine; DIPEA, diisopropylethylamine; TBDMS, *tert*-butyldimethylsilyl; Pd(dppf)Cl_2, [1,1′-bis(diphenylphosphino)-ferrocene]dichloropalladium(II).

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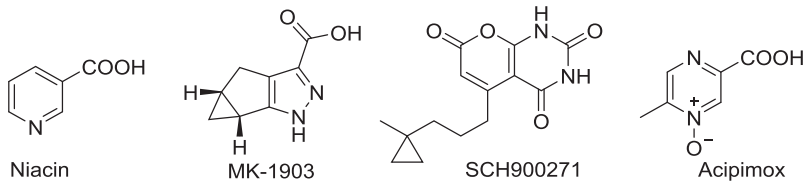


Fig. 1. Structures of selected HCA2 receptor agonists.

with anti-inflammatory effects in other diseases. In particular, studies report that niacin has the ability to reduce inflammation in sepsis, diabetic retinopathy, and renal disease.[11] These data suggest that HCA2 may be an important target for treating inflammatory processes, thus the design of new potent and selective HCA2 ligands may be important for determining other HCA2 functions as well as for the development of new drugs.

Merck chemists developed cyclohex-1-ene carboxylic acid derivatives **I** (Fig. 2) as potent and selective high affinity HCA2 full agonists.[12–14] They have shown that Ar in the molecules **I** preferentially should be aromatic bicycles or other higher fused systems which are beneficial for affinity.[15]

In our previous work we described the studies of cyclohex-1-ene carboxylic acid derivatives as HCA2 ligands and demonstrated that a change of a saturated ethylene linker to such rigidity elements as *E*-double bond, triple bond, or *trans*-substituted cyclopropane ring into the amide part of appropriate aryl substituted 2-amidocyclohex-1-enes **II** allowed modulating the potency and selectivity of these compounds towards the HCA2 receptor activation.[16] During these studies we confirmed that a presence of such aromatic bicycles as naphthalene and quinoline in molecules **II** is a prerequisite to construct active compounds as well. However, we found that simple (*E*)-2-acrylamidocyclohex-1-enecarboxylic acid derivative **1** containing a phenylethynyl group attached to the *E* double bond exhibits comparable activation of HCA2 with aromatic bicycle based compounds (Fig. 2).

In this paper, we report the synthesis and SAR of a series of novel cyclohex-1-ene carboxylic acid derivatives **2a–f**, **3**, **4a–h**, and **5a–f** (Table 1) whose structure design is based on hit structure **1**. All compounds were evaluated in a functional assay and a binding assay. In these assays, Flp-In-293 cells, which express the recombinant human HCA2, were utilized. For the functional assay, forskolin-stimulated cAMP accumulation assay (cAMP) was arranged. For the radioligand binding assay (RLB), displacement of radiolabeled niacin from the membrane preparation was measured. Almost all active analogues described in this paper had a maximum level of response E_{max} to niacin at least 90% in the cAMP assay and could be, therefore, characterized as full agonists (Table 1).

2. Synthesis

Compounds **2a–f** containing the enyne linker were synthesized using the Sonogashira reaction – Wittig reaction sequence (Scheme 1).

Arylhalides **6a–f** in the Sonogashira reaction with propargyl alcohol gave 3-phenylprop-2-yn-1-ols **7a–f** which were oxidized to aldehydes **8a–f** with MnO_2 or Dess-Martin periodinane. The aldehydes **8a–f** were utilized in the subsequent Wittig reaction with phosphorane, generated from previously described triphenylphosphonium bromide **9**,[16] to give *E*-alkenes **10a–f** contaminated with a minor amount of the corresponding *Z*-isomer. It is necessary to mention that the employed triphenylphosphonium bromide ethyl ester **9** contained some amount (*ca* 5%) of the related methyl ester impurity unavoidably coming from the synthesis method of the compound **9**. Compounds **10a–c**, **f** were converted into free phenols **11a–c**, **f** and the esters **10d**, **e** and **11a–c**, **f** were hydrolyzed into desired carboxylic acids **2a–f**.

The synthesis of compound **3** with the but-1-ene linker was performed as follows (Scheme 2). 3-Phenylpropanal (**12**) was subjected to the Wadsworth-Emmons reaction with methyl 2-(dimethoxyphosphoryl)acetate followed by the hydrolysis providing (*E*)-5-phenylpent-2-enoic acid (**13**).[17] The acid **13** was used for the preparation of the corresponding acid chloride which in turn was condensed with ethyl 2-aminocyclohex-1-ene-1-carboxylate to give ethyl ester of the acid **13**. The hydrolysis of the latter gave the desired compound **3**.

To obtain the necessary core of compounds **4a–j** containing the but-1-yne linker, we tried a strategy consisting of the synthesis of appropriate 5-arylpent-4-ynoic acid which was employed to acylate ethyl 2-aminocyclohex-1-ene-1-carboxylate. Thus, following this approach the Sonogashira reaction of 1-chloro-2-iodobenzene with pent-4-ynoic acid (**15**) afforded 5-(2-chlorophenyl)pent-4-ynoic acid (**16**) (Scheme 3). The acid **16** was converted into the corresponding acid chloride which in turn was treated with ethyl 2-aminocyclohex-1-ene-1-carboxylate to give desired amidoester **17d**. Unfortunately, the yield of the acid **16** was low due to a competing aryl-γ-methylene-γ-butyrolactone formation[18] and difficulties in the isolation of the pure product. All attempts to increase the yield of the acid **16** by changing catalysts, bases, and/or solvents failed. However, a prior formation of the propynyl amidoester **18** by acylation of ethyl 2-aminocyclohex-1-ene-1-carboxylate with the acid **15** chloride eliminated the above mentioned drawbacks connected with the preparation of 5-arylpent-4-ynoic acid **16**. The Sonogashira reaction of the amidoester **18** and appropriate aryl iodides proceeded as expected allowing efficient preparation of desired amidoesters **17a–c**, **e–j**. The hydrolysis of the amidoesters **17a–j** finished the preparation of the corresponding target compounds **4a–j** (Scheme 3).

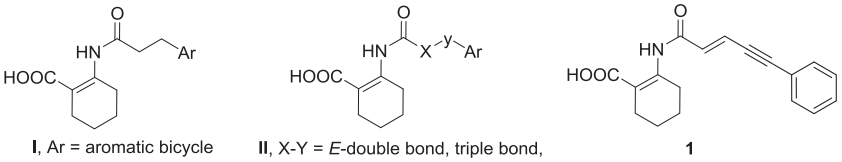
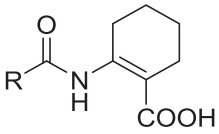
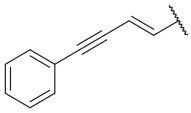
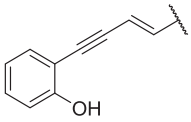
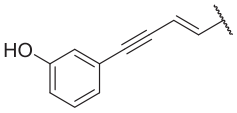
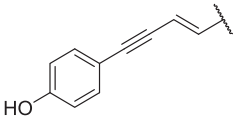
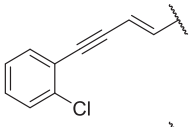
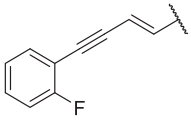
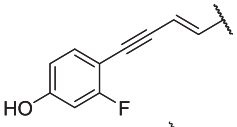
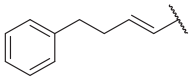
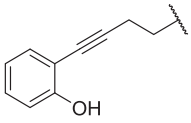
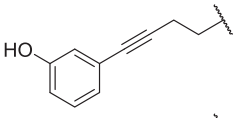
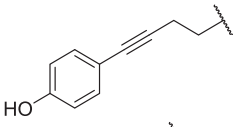
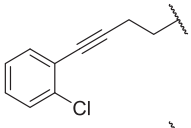
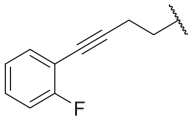


Fig. 2. Structures of known HCA2 receptor agonists **I, II**, and **1**.

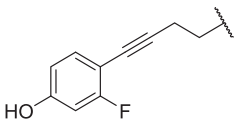
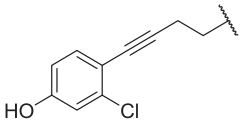
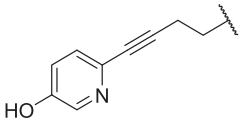
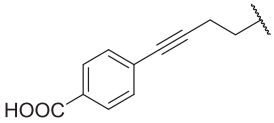
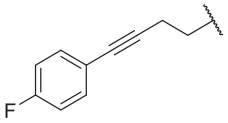
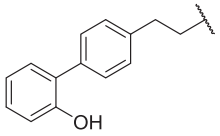
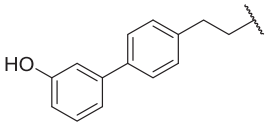
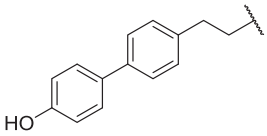
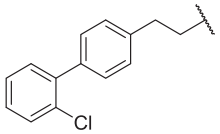
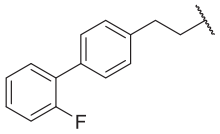
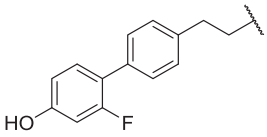
Table 1
Affinity and potency of the synthesized compounds in the HCA2 receptor RLB and cAMP assays.



En-try	No.	R	RLB IC_{50} ± SD, nM[a]	cAMP EC_{50} ± SD, nM[a]	E_{max} (%)[b]
1	**1**		327 ± 71	1010 ± 590	79 ± 4
2	**2a**		37 ± 11	250 ± 110	78 ± 6
3	**2b**		383 ± 68	390 ± 160	92 ± 3
4	**2c**		25 ± 4	130 ± 20	97 ± 3
5	**2d**		88 ± 24	270 ± 140	88 ± 6
6	**2e**		16 ± 1	450 ± 150	84 ± 6
7	**2f**		331 ± 81	110 ± 60	96 ± 5
8	**3**		2828 ± 927	19600 ± 3100	70 ± 3
9	**4a**		229 ± 89	720 ± 100	93 ± 5
10	**4b**		138 ± 9	2980 ± 490	89 ± 7
11	**4c**		13 ± 6	160 ± 60	91 ± 6
12	**4d**		56 ± 17	190 ± 80	95 ± 4
13	**4e**		67 ± 9	36 ± 12	90 ± 4

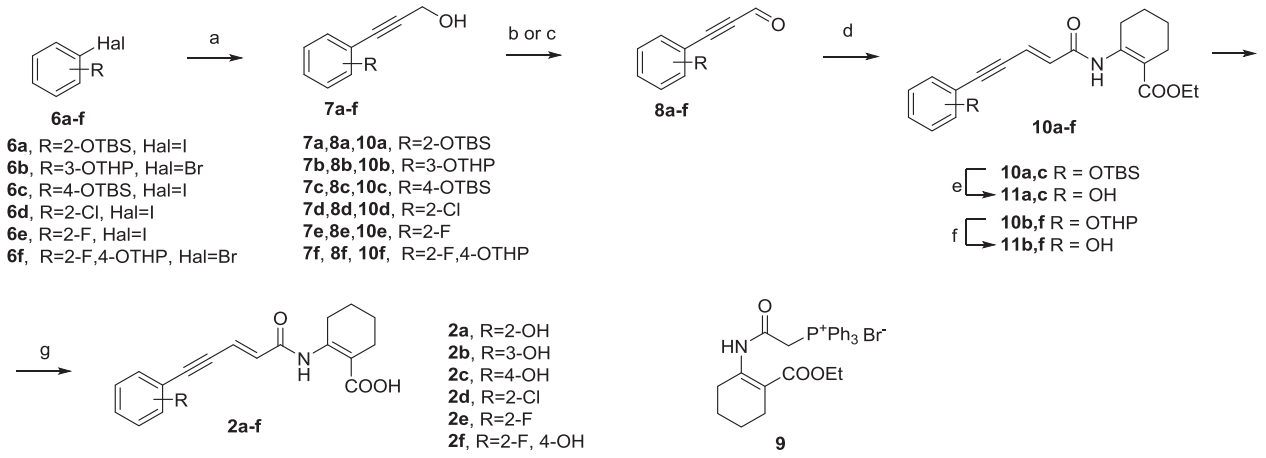
(*continued on next page*)

Table 1 (*continued*)

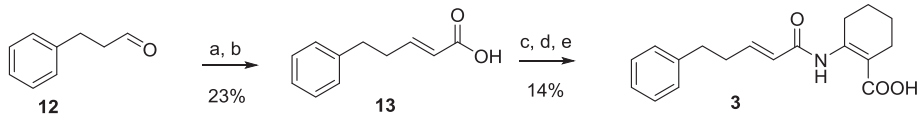
En-try	No.	R	RLB IC_{50} ± SD, nM[a]	cAMP EC_{50} ± SD, nM[a]	E_{max} (%)[b]
14	**4f**		28 ± 2	80 ± 10	93 ± 4
15	**4g**		76 ± 19	9 ± 2	94 ± 7
16	**4h**		665 ± 13	3370 ± 1390	91 ± 6
17	**4i**		3620 ± 542	5610 ± 1100	74 ± 5
18	**4j**		291 ± 78	630 ± 90	99 ± 4
19	**5a**		40 ± 5	410 ± 140	90 ± 8
20	**5b**		32 ± 8	230 ± 120	96 ± 2
21	**5c**		21 ± 4	70 ± 10	92 ± 5
22	**5d**		75 ± 7	130 ± 60	92 ± 7
23	**5e**		282 ± 49	190 ± 40	97 ± 2
24	**5f**		58 ± 9	260 ± 90	99 ± 3

[a] Each value represents the mean ± SD calculated from at least three experiments, each performed in duplicate.
[b] Maximal inhibition (%) of forskolin-stimulated cAMP.

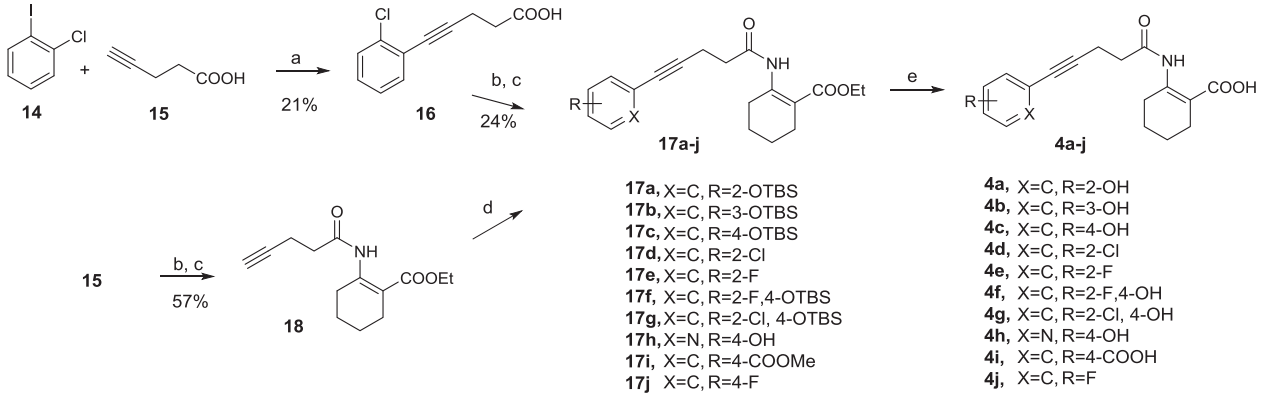
A series of compounds **5a–f** containing phenylethyl linker was synthesized from 3-(4-iodophenyl)propanoic acid[19] (**19**) by two similar protocols differing by the sequence of the employed chemical conversions (Scheme 4). According to the first approach, the iododerivative **19** was converted into boronate **20** using the Miyaura borylation reaction which was coupled with 1-chloro-2-iodobenzene by the Suzuki reaction forming the necessary C—C bond of biphenyl acid **21**. After the amide bond formation and basic hydrolysis the product **5d** was obtained. To minimize the number of the required synthetic steps, a modified synthetic route[20] was used for the synthesis of other biphenyl amidoesters **22a–c**, **e–f**. At first, ethyl 2-aminocyclohex-1-ene-1-carboxylate was condensed with the acid **19** chloride to give aryliodide **23**. The iodide **23** was coupled with appropriate aryl pinacolyl boro-



Scheme 1. Synthesis of compounds **2a–f**. Conditions: (a) propargyl alcohol, $Pd(PPh_3)_4$ (3 mol%), CuI (6 mol%), TEA, DMF, rt or 80 °C, 15–18 h; (b) Dess-Martin periodinane, DCM, rt, 2 h; (c) MnO_2, DCM, rt, 48–72 h; (d) phosphonium bromide **9**, *t*-BuOK, DMSO, rt, 1 h; (e) TBAF, THF, rt, 2 h; (f) 6 mol% pTSA, DCM, MeOH, rt, 6 h; (g) 2 N NaOH, THF/MeOH, 6–18 h.



Scheme 2. Synthesis of compound **3**. Conditions: (a) methyl 2-(dimethoxyphosphoryl)-acetate, *t*-BuOK, DMSO, rt, 1 h; (b) NaOH, MeOH, H_2O, rt; (c) oxalyl chloride, DMF (cat.), DCM, rt, 1 h; (d) ethyl 2-aminocyclohex-1-ene-1-carboxylate, TEA, DCM, 0 °C, 1 h; (e) 2 N NaOH, THF/MeOH.



Scheme 3. Synthesis of compounds **4a–j**. Conditions: (a) $Pd(PPh_3)_4$ (3 mol%), CuI (6 mol%), TEA, DMF, rt, 18 h; (b) oxalyl chloride, DMF, DCM, rt, 1 h; (c) ethyl 2-aminocyclohex-1-ene-1-carboxylate, TEA, DCM, 0 °C, 1 h; (d) appropriate aryl iodide, $PdCl_2(PPh_3)_2$ (5 mol%), CuI (5 mol%), TEA, MeCN, 2–18 h, rt; (e) 2 N NaOH, EtOH or THF/MeOH, rt, 15–24 h.

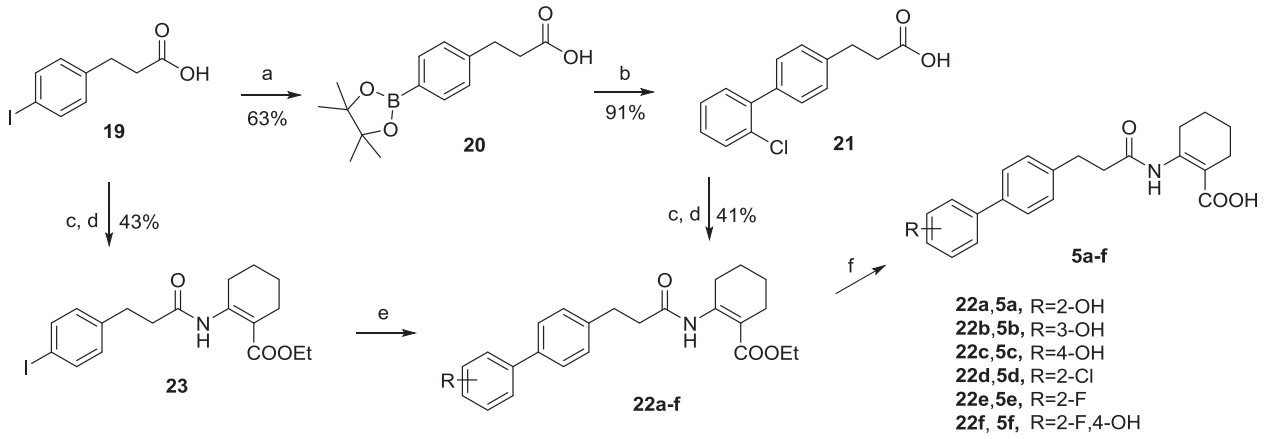
nates or boronic acids by the Suzuki protocol to give amidoesters **22a–c**, **e–f** which were converted into desired target compounds **5a–c**, **e–f** by the basic hydrolysis (Scheme 4).

3. Biological activity and structure-activity relationships (SAR)

The pharmacophoric description of compound **1** and analogues, synthesized in this paper, can be represented as consisting of cyclohexene and aromatic parts which are connected by more or less flexible linker. In the case of the compound **1**, the linker contains a combination of the triple and double bonds – enyne linker, which is completely planar and conformationally stable. The restriction of the conformation of the molecule could allow evaluation of an appropriate conformation of the ligand for binding with the receptor.

The non-substituted compound **1** exhibited EC_{50} 3600 ± 1600 nM in the cAMP assay in the presence of 2% bovine serum albumin (BSA) as it was determined earlier.[16] Albumin is a transport protein in plasma and is known to bind drugs, especially lipophilic molecules and carboxylic acids.[21] Since our target compounds were carboxylic acids, we were interested to establish the activity of the compound **1** without the presence of BSA, which could interfere with the assessment of EC_{50}. Indeed, the cAMP assay for the compound **1** without the BSA afforded a 3-fold lower EC_{50} value equal to 1010 ± 590 nM (Table 1, entry 1). Thus, in the following experiments the binding and functional assays were performed without the BSA additive.

To test the possibility to increase the activity of the compound **1** by adding substituents at the phenyl ring of the molecule, a series of analogues **2a–f** was prepared.



Scheme 4. Synthesis of compounds **5a–f.** Conditions: (a) bis(pinacolato)diboron, Pd(dppf)Cl_2 (5 mol%), KOAc, DMF, 80 °C, 2.5 h; (b) 1-chloro-2-iodobenzene, Pd(dppf)Cl_2 (5 mol%), K_2CO_3, dioxane/H_2O, 80 °C, 2 h; (c) oxalyl chloride, DMF (cat.), DCM, rt, 1 h; (d) ethyl 2-aminocyclohex-1-ene-1-carboxylate, TEA, DCM, 0 °C, 1 h; (e) appropriate arylboronic acid or arylboronic acid pinacolate ester, Pd(dppf)Cl_2 (5 mol%), K_2CO_3, dioxane/H_2O, 80 °C, 1–4 h; (f) 2 N NaOH, THF/MeOH, rt, 16–18 h.

In general, the introduction of a hydroxyl group or halide (F or Cl) in the aromatic part of the molecule improved activity both in the RLB and cAMP assays. The SAR studies by RLB method revealed that affinities of the analogues **2a–f** towards HCA2 decreased in the following order [substitution pattern (compound number, IC_{50})]: 2-F (**2e**, 16 nM) > 4-OH (**2c**, 25 nM) > 2-OH (**2a**, 37 nM) > 2-Cl (**2d**, 88 nM) > H (**1**, 327 nM) ≈ 2-F, 4-OH (**2f**, 331 nM) ≈ 3-OH (**2b**, 383 nM).

However, in the cAMP assay 2-fluoro-4-hydroxy derivative **2f** exhibited the highest potency (EC_{50} = 110 ± 60 nM), showing *ca* 9-fold increase of the activity compared to the parent substance **1** (EC_{50} = 1010 ± 590 nM). Similar potencies of 8 to 4-fold increase in the cAMP assay were shown by 4-hydroxy derivative **2c** (EC_{50} = 130 ± 20 nM), 2-hydroxy derivative **2a** (EC_{50} = 250 ± 110 - nM), and 2-chloro analogue **2d** (EC_{50} = 27 ± 140 nM). 3-Hydroxy and 2-fluoro analogues **2b** and **2e** exhibited slightly lower potencies (EC_{50} = 390 ± 160 nM and 450 ± 150 nM, accordingly) than the above mentioned compounds **2a, 2c, 2d,** and **2f**, however, still being 2–3-fold more active than the parent compound **1**. It is interesting to note, that the 2-fluoro, 4-hydroxy analogue **2f** having the highest cAMP activity exhibited quite modest affinity to HCA2 in the RLB assay comparable with the affinity of the compound **1**. On the contrary, the 2-fluoro analogue **2e** with 20-fold higher RLB affinity than the unsubstituted structure **1** did not exhibit such remarkable improvement in the potency in cAMP assay (EC_{50} = 450 ± 150 nM for **2e** *versus* EC_{50} = 1010 ± 590 nM for **1**).

To establish which bond (double or triple) of the enyne linker is more important for the activity of compounds of the series **2a–f**, we synthesized derivative **3** containing the double bond in a combination with a fully saturated triple bond (i.e., the ethylene group) in the linker part of the molecule. This modification resulted in a significant loss of the binding and functional activity comparing to the parent structure **1** (Table 1, entries 1 and 8). We assumed that the conformationally restricted linker part with the triple bond flanked by the ethylene group and the benzene ring is apparently essential for the activity of the compound **1** and likely for the analogues **2a–f** as well.

The saturation of the double bond of the enyne linker leaving the triple bond intact led to a series of compounds **4a–j** being on the average just as active as the series **2a–f** with the fully unsaturated and rigid enyne linker. Since the compounds **4a–j** do not have activated unsaturated bonds, they are more appropriate for biological systems than compounds **2a–f**. The affinities of the analogues **4a–j** towards HCA2 in RLB assay decreased as follows [substitution pattern (compound number, IC_{50})]: 4-OH (**4c**, 13 nM) > 2-F, 4-OH (**4f**, 28 nM) > 2-Cl (**4d**, 56 nM) > 2-F (**4e**, 67 nM) > 2-Cl, 4-OH (**4g**, 76 nM) ≈ 3-OH (**4b**, 138 nM) ≈ 2-OH (**4a**, 229 nM) ≈ 4-F (**4j**, 291 nM) > 4-OH, N(2) (**4h**, 665 nM) > 4-COOH (**4i**, 3620 nM).

The highest potencies in this series in the functional assay was exhibited by a group of compounds **4c, 4f, 4g** containing 4-hydroxy group at the benzene ring. In this case, the presence of an additional 2-fluoro or 2-chloro atom at the benzene ring for the structures **4f** and **4g**, accordingly, seems to be beneficial for the potency (**4f**, R = 2-F, 4-OH, EC_{50} = 80 ± 10 nM; **4g**, R = 2-Cl, 4-OH, EC_{50} = 90 ± 20 nM) if compared with the monosubstituted 4-hydroxy derivative **4c** (EC_{50} = 160 ± 60 nM). 2-Chloro and 2-fluoro monosubstituted derivatives **4d** and **4e** exhibited remarkable activities both in the RLB and cAMP assays with a tendency of possessing slightly reduced potency (EC_{50} = 190 ± 80 nM and 360 ± 120 nM, accordingly) in functional assay if compared with the 4-hydroxy substituted derivatives **4c**, **4g**, **4h**, nevertheless being superior to the 2-hydroxy analogue **4a**. It is noteworthy that 4-fluorobenzene **4j** exhibited distinctly lower activity in binding and functional assays than the corresponding 4-hydroxy analogue **4c**. Despite quite notable affinity of 3-hydroxy derivative **4b** to HCA2 receptor (IC_{50} = 138 ± 9 nM), the potency of this compound was only in a low micromolar range (EC_{50} = 2980 ± 490 nM). Quite surprisingly, compound **4h** with 4-hydroxy-2-pyridine in the aromatic part of the molecule, the analogue of the previously described Merck HCA2 agonist MK-6892[13], has lost the activity comparing to the 4-hydroxybenzene **4c**. 4-Carboxybenzene **4i** exhibited only weak activity both in RLB and cAMP tests. In addition, it seems to be a partial agonist of HCA2 (E_{max} = 74 ± 5%).

IJzerman et.al. recently described novel 4-hydroxybiphenyl cyclohexene derivative **5c** which showed remarkable affinity (IC_{50} = 108 ± 4 nM) towards HCA2 receptor and longer residence time profile than niacin.[20] Since the 4-hydroxyphenyl derivatives **2c** and **4c** of the series **2a–f** and **4a–j** were among the most active compounds both in the binding and functional assays, we decided to extend our study so that to cover biphenyl derivatives **5a–f** which formally can be regarded as consisting of the cyclohexene and benzene parts joined by a phenylethyl linker. The model calculations of the superimposed structures **2c, 4c**, and **5c** revealed that the biphenyl derivative **5c** is about 2Å longer than the analogues **2c** and **4c**. The affinities of the compounds **5a–f** towards HCA2 receptor in the binding assay decreased in the following order [substitution pattern (compound number, IC_{50}]: 4-OH (**5c**, 21 nM) > 3-OH (**5b**, 32 nM) > 2-OH (**5a**, 40 nM) > 2-F, 4-OH (**5f**, 58 nM) > 2-Cl (**5d**, 75 nM) ≈ 2-F (**5e**, 282 nM). Thus, the higher affinity in the series **5a–f** was exhibited by 4-hydroxy, 3-hydroxy, and 2-hydroxy

derivatives **5c**, **5b**, and **5a**. Then followed 2-fluoro-4-hydroxy, 2-chloro, and, finally, 2-fluoro analogues **5f**, **5d**, and **5e**, respectively. Although in the functional assay, similarly as in the case of the series **4a**–**j**, 4-hydroxy derivative **5c** showed the best potency (EC_{50} = 70 ± 10 nM, 2-chloro and 2-fluoro analogues **5d** and **5e** with EC_{50} = 130 ± 60 nM and 260 ± 90 nM exhibited the tendency to be more potent than the compounds **5b** and **5a** with 3-hydroxy and 2-hydroxy substituents (EC_{50} = 230 ± 120 nM and 410 ± 140 nM, accordingly). However, unlike the series with the enyne linker **2a**–**f** and the but-1-yne linker **4a**–**j**, an additional 2-fluoro substituent did not increase the potency of 4-hydroxy derivative and the corresponding 2-fluoro-4-hydroxy analogue **5f** showed moderate EC_{50} value of 260 ± 90 nM.

4. Conclusions

We have designed and synthesized three series of novel aryl derivatives of 2-amidocyclohex-1-ene carboxylate as HCA2 full agonists which can be described as consisting of cyclohexene and aromatic parts which are connected by a more or less flexible linker. The linker part of the first series of compounds **2a**–**f** contained a combination of the triple and double bond (enyne linker), the second series of compounds **4a**–**j** it was a combination of the triple bond and the ethylene group (pent-1-yne linker), and the third series included biphenyl derivatives **5a**–**f** which can formally be regarded as consisting of the cyclohexene and benzene parts joined by a 4-substituted phenylethyl linker.

In the case of enyne series **2a**–**f**, it was possible to evaluate the impact of a particular substitution pattern of the benzene ring on the activity towards HCA2 receptor by direct comparison with the unsubstituted structure **1**. Thus, the incorporation of a hydroxy group at the 2-, 3-, or 4-position or a halogen atom (Cl or F) at the 2-position of the phenyl group of the structure **1** increased the potency in all studied cases.

The presence of the 4-hydroxy group at the benzene ring boosted both the affinities and potencies of compounds belonging to all three series (structures **2c**, **4c**, **5c**). In the case of the series with enyne and pent-1-yne linkers, a tendency of further improvement of the potency of 4-hydroxyphenyl derivatives was observed by adding an additional 2-fluoro or 2-chloro atom at the benzene ring (compounds **2f**, **4f**, **4g**).

The presence of a rigid triple bond or phenyl group next to the benzene ring according to the employed pharmacophoric model seems to be a prerequisite to design active molecules towards HCA2 receptor. Compound **3** containing the double bond in a combination with a fully saturated triple bond (i.e., the ethyl group) in the linker part of the molecule exhibited a significant loss of the binding and functional activity comparing to the parent structure **1**.

5. Experimental

5.1. Chemistry

Chemicals and reagents were obtained from commercial suppliers and were used without further purification. TLC analyses were performed with Merck F254 Alumina Silica Plates using UV visualization or staining. ^{1}H NMR and ^{13}C NMR spectra were recorded at ambient temperature with Bruker (300 MHz) or Varian (400 MHz) spectrometers. Chemical shifts are reported in parts per million and referenced to the residual solvent signal. LCMS was performed on a Waters Acquity UPLC system connected to the Micromass Q-TOF micro hybrid quadrupole time-of-flight mass spectrometer operating in the electrospray ionization (ESI) positive and negative ion mode and using reverse-phase Acquity UPLC BEH C18 column (1.7 μm, 2.1 × 50 mm) on a gradient of 10–95% MeCN in 0.1% aqueous HCOOH. HRMS (ESI) was performed on a Waters Synapt G2-Si Mass Spectrometer. Mass is reported in positive ionization mode unless stated otherwise. GCMS was performed on Agilent Series 5975C GC/MSD using Agilent Technologies 30 m × 0.250 mm column. The purity of the final compounds was confirmed to be ≥ 95% with Waters Alliance LC systems equipped with 2695 separation module with quaternary pump, degasser, autosampler, column thermostat, and LiChrospher PR Select 4.0 × 250 mm column. Waters 2489 dual absorbance detector was used for the analysis. Elemental analyses were obtained with Carlo Erba EA 1108 instrument. Chromatographical purifications of compounds were performed by column chromatography using silica gel, 0.035–0.070 mm or prepacked columns on a Biotage Isolera One Purification system. Reaction conditions and yields were not optimized.

5.1.1. *tert*-Butyl(2-iodophenoxy)dimethylsilane (**6a**)

2-Iodophenol (1.00 g, 4.54 mmol) and imidazole (0.62 g, 9.09 mmol) were dissolved in DMF (10 ml) under argon atmosphere. A solution of TBDMSCl (1.03 g, 6.81 mmol) in DMF (2 mL) was added dropwise at rt over 5 min and the obtained mixture was stirred for 2 h. The mixture was diluted with ice water, extracted with diethyl ether, the organic layer was washed 3 times with water, brine, and dried over Na_2SO_4. The solvent is removed under reduced pressure and the residue was chromatographed on silica gel (EtOAc in petroleum ether, 1:10) to give compound **6a** (1.50 g, 99%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (dd, J = 7.9, 1.7 Hz, 1H), 7.20 (ddd, J = 8.1, 7.3, 1.7 Hz, 1H), 6.83 (dd, J = 8.1, 1.4 Hz, 1H), 6.68 (td, J = 7.6, 1.4 Hz, 1H), 1.06 (s, 9H), 0.28 (s, 6H). GCMS m/z: 277.0 $[M\text{-}C_4H_9]^+$.

*5.1.1.1. tert-Butyl(4-iodophenoxy)dimethylsilane (**6c**).* Compound **2c** was prepared in the same manner as compound **6a** using 4-iodophenol (2.00 g, 9.08 mmol), imidazole (0.62 g, 9.11 mmol), and TBDMSCl (1.37 g, 9.09 mmol). The product **6c** (2.85 g, 92%) was obtained as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50 (d, J = 8.9, 2H), 6.61 (d, J = 8.9, 2H), 0.97 (s, 9H), 0.18 (s, 6H). GCMS m/z: 334 M^+.

*5.1.1.2. tert-Butyl(3-fluoro-4-iodophenoxy)dimethylsilane (**6g**).* Compound **2g** was prepared in the same manner as compound **6a** using 3-fluoro-4-iodophenol (1.05 g, 4.41 mmol), imidazole (0.60 g, 8.82 mmol), and TBDMSCl (1.00 g, 6.62 mmol). The product **6g** (1.51 g, 97%) was obtained as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53 (dd, J = 8.6, 7.7 Hz, 1H), 6.59 (dd, J = 9.5, 2.6 Hz, 1H), 6.45 (ddd, J = 8.6, 2.6, 0.7 Hz, 1H), 0.97 (s, 9H), 0.20 (s, 6H). GCMS m/z: 352.0 M^+.

*5.1.1.3. tert-Butyl(3-chloro-4-iodophenoxy)dimethylsilane (**6h**).* Compound **2h** was prepared in the same manner as compound **6a** using 3-chloro-4-iodophenol (1.30 g, 5.11 mmol), imidazole (0.70 g, 10.2 mmol), and TBDMSCl (1.16 g, 7.66 mmol). The product **6h** (1.78 g, 95%) was obtained as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.64 (d, J = 8.6 Hz, 1H), 6.97 (d, J = 2.7 Hz, 1H), 6.49 (dd, J = 8.6, 2.7 Hz, 1H), 0.97 (s, 9H), 0.20 (s, 6H). GCMS m/z: 368.0 M^+.

*5.1.1.4. 5-((tert-Butyldimethylsilyl)oxy)-2-iodopyridine (**6i**).* Compound **6i** was prepared in the same manner as compound **6a** using 6-iodopyridin-3-ol (0.39 g, 1.76 mmol), imidazole (0.24 g, 3.53 mmol), and TBDMSCl (0.40 g, 2.65 mmol). The product **6i** (0.59 g, 99%) was obtained as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.99 (dd, J = 4.5, 1.6 Hz, 1H), 7.11 (dd, J = 8.0, 4.5 Hz, 1H), 7.00 (dd, J = 8.0, 1.6 Hz, 1H), 1.07 (s, 9H), 0.29 (s, 6H). GCMS m/z: 277.9 $[M\text{-}C_4H_9]^+$.

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*5.1.1.5. tert-Butyl(3-iodophenoxy)dimethylsilane (**6j**).* Compound **6j** was prepared in the same manner as compound **6a** using 3-iodophenol (2.00 g, 9.08 mmol), imidazole (0.62 g, 9.11 mmol), and TBDMSCl (1.37 g, 9.09 mmol). The product **6j** (2.91 g, 94%) was obtained as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 (ddd, J = 7.8, 1.6, 1.0, 1H), 7.21 (dd, J = 2.3, 1.6, 1H), 6.94 (t, J = 8.0, 1H), 6.79 (ddd, J = 8.0, 2.4, 1.0, 1H), 0.98 (s, 9H), 0.20 (s, 6H). GCMS m/z: 334 M^+.

*5.1.2. 2-(3-Bromophenoxy)tetrahydro-2H-pyran (**6b**)*

p-Toluenesulfonic acid (10 mg, 0.06 mmol) was added to DCM (5 ml), the mixture was stirred for 10 min, and the solid was filtered off. 3-Bromophenol (1.00 g, 5.78 mmol) was dissolved in the filtrate and to the obtained solution dihydropyrane (0.53 ml, 5.78 mmol) was added dropwise. The mixture was stirred for 1.5 h at rt and quenched by the addition of saturated $NaHCO_3$ solution. The phases were separated and the aqueous phase was extracted with DCM. The combined organic phases were dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel (EtOAc in petroleum ether, 1:5, with 0.2% triethylamine) to give the product **6b** (0.97 g, 65%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.25–7.20 (m, 1H), 7.17–7.07 (m, 2H), 6.98 (dt, J = 7.0, 2.4 Hz, 1H), 5.40 (t, J = 3.2 Hz, 1H), 3.87 (ddd, J = 11.3, 9.6, 3.2 Hz, 1H), 3.66–3.57 (m, 1H), 2.07–1.89 (m, 1H), 1.89–1.79 (m, 2H), 1.77–1.55 (m, 3H). GCMS m/z: 172.0 [M-C_5H_8O]$^+$.

*5.1.2.1. 2-(4-Bromo-3-fluorophenoxy)tetrahydro-2H-pyran (**6f**).* Compound **6f** was prepared in the same manner as compound **6b** using 4-bromo-3-fluorophenol (0.80 g, 4.19 mmol), *p*-toluenesulfonic acid (39 mg, 0.21 mmol), and dihydropyrane (0.57 ml, 6.28 mmol). The product **6f** (0.50 g, 43%) was obtained as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.40 (t, J = 8.4 Hz, 1H), 6.88 (dd, J = 10.5, 2.6 Hz, 1H), 6.82–6.68 (m, 1H), 5.37 (unresolved t, 1H), 3.91–3.77 (m, 1H), 3.67–3.55 (m, 1H), 2.07–1.89 (m, 1H), 2.06–1.50 (m, 7H). LCMS (ESI): no ionization.

*5.1.3. 3-(2-((tert-Butyldimethylsilyl)oxy)phenyl)prop-2-yn-1-ol (**7a**)*

A solution of *tert*-butyl(2-iodophenoxy)dimethylsilane **(6a)** (0.80 g, 2.39 mmol) and triethylamine (3.35 ml, 23.92 mmol) in DMF (3 ml) was degassed by bubbling argon for 10 min, then propargyl alcohol (0.28 ml, 4.79 mmol) followed by [Pd(PPh_3)$_4$] (83 mg, 0.07 mmol) and CuI (27 mg, 0.14 mmol) were added. The reaction mixture was stirred for 7 h at rt, concentrated, diluted with water, and extracted with EtOAc. The organic extract was concentrated under reduced pressure and purified by column chromatography on silica gel (EtOAc in petroleum ether, 1:4) to give the product **7a** (0.36 g, 45%) as a yellow oil ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.37 (dd, J = 7.6, 1.7 Hz, 1H), 7.20 (td, J = 8.1, 1.7 Hz, 1H), 6.90 (td, J = 7.6, 1.1 Hz, 1H), 6.82 (dd, J = 8.1, 1.1 Hz, 1H), 4.50 (d, J = 6.2 Hz, 2H), 1.53 (t, J = 6.2 Hz, 1H), 1.04 (s, 9H), 0.23 (s, 6H). LCMS (ESI) m/z: 263.10 [M+H]$^+$.

*5.1.3.1. 3-(3-((Tetrahydro-2H-pyran-2-yl)oxy)phenyl)prop-2-yn-1-ol (**7b**).* Compound **7b** was prepared in the same manner as compound **7a** using bromide **6b** (0.60 g, 2.33 mmol), triethylamine (3.27 ml, 23.33 mmol), propargyl alcohol (0.28 ml, 4.67 mmol), [Pd(PPh_3)$_4$] (81 mg, 0.07 mmol), and CuI (27 mg, 0.14 mmol). The reaction mixture was stirred for 9 h at 70 °C. The product **7b** (0.30 g, 55%) was obtained as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.21 (t, J = 7.9 Hz, 1H), 7.15 (dd, J = 2.5, 1.3 Hz, 1H), 7.06 (dt, J = 7.7, 1.0 Hz, 1H), 7.02 (ddd, J = 8.2, 2.5, 1.1 Hz, 1H), 5.40 (t, J = 3.2 Hz, 1H), 4.49 (d, J = 6.2 Hz, 2H), 3.89 (ddd, J = 11.3, 9.4, 3.2 Hz, 1H), 3.65–3.66 (m, 1H), 2.05–1.89 (m, 1H), 1.91–1.76 (m, 1H), 1.73–1.55 (m, 3H), 1.61 (t, J = 6.2 Hz, 1H). LCMS (ESI) m/z: 233.06 [M+H]$^+$.

*5.1.3.2. 3-(4-((tert-Butyldimethylsilyl)oxy)phenyl)prop-2-yn-1-ol (**7c**).* Compound **7c** was prepared in the same manner as compound **7a** using iodide **6c** (0.50 g, 1.50 mmol), triethylamine (2.2 ml, 15.7 mmol), propargyl alcohol (0.11 ml, 1.79 mmol), [Pd(PPh_3)$_4$] (5 mg, 0.01 mmol), and CuI (3 mg, 0.02 mmol). The product **7c** (0.35 g, 88%) was obtained as an oil. ^{1}H NMR (400 MHz, $CDCl_3$) 7.36–7.29 (m, 2H), 6.81–6.74 (m, 2H), 4.48 (d, J = 6.2 Hz, 2H), 1.58 (t, J = 6.2 Hz, 1H), 0.97 (s, 9H), 0.20 (s, 6H). LCMS (ESI) m/z 263 [M+H]$^+$.

*5.1.3.3. 3-(2-Chlorophenyl)prop-2-yn-1-ol (**7d**).* Compound **7d** was prepared in the same manner as compound **7a** using 1-chloro-2-iodobenzene (0.80 g, 3.35 mmol), triethylamine (4.70 ml, 33.5 mmol), propargyl alcohol (0.40 ml, 6.71 mmol), [Pd(PPh_3)$_4$] (116 mg, 0.10 mmol), and CuI (60 mg, 0.20 mmol). The reaction mixture was stirred for 18 h at rt. The product **7d** (0.55 g, 98%) was obtained as a brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.51–7.44 (m, 1H), 7.44–7.36 (m, 1H), 7.30–7.17 (m, 2H), 4.56 (d, J = 6.2 Hz, 2H), 1.69 (t, J = 6.2 Hz, 1H). GCMS m/z: 166.0 M^+.

*5.1.3.4. 3-(2-Fluorophenyl)prop-2-yn-1-ol (**7e**).* Compound **7e** was prepared in the same manner as compound **7a** using 1-fluoro-2-iodobenzene (0.39 g, 1.76 mmol), triethylamine (2.46 ml, 17.57 mmol), propargyl alcohol (0.21 ml, 3.51 mmol), [Pd(PPh_3)$_4$] (61 mg, 0.05 mmol), and CuI (20 mg, 0.11 mmol). The reaction was stirred for 18 h at rt. The product **7e** (0.24 g, 91%) was obtained as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.43 (td, J = 7.6, 1.9 Hz, 1H), 7.36–7.26 (m, 1H), 7.15–7.01 (m, 2H), 4.54 (d, J = 6.2 Hz, 1H), 1.70 (t, J = 6.2 Hz, 1H). LCMS (ESI): no ionization.

*5.1.3.5. 3-(2-Fluoro-4-((tetrahydro-2H-pyran-2-yl)oxy)phenyl)prop-2-yn-1-ol (**7f**).* Compound **7f** was prepared in the same manner as compound **7a** using bromide **6f** (0.49 g, 1.78 mmol), triethylamine (1.24 ml, 8.91 mmol), propargyl alcohol (0.21 ml, 3.56 mmol), [Pd(PPh_3)$_4$] (103 mg, 0.09 mmol), and CuI (10 mg, 0.05 mmol). The reaction mixture was stirred for 24 h at 70 °C. The product **6f** (0.11 g, 25%) was obtained as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.32 (t, J = 8.6 Hz, 1H), 6.93–6.72 (m, 2H), 5.40 (t, J = 3.2 Hz, 1H), 4.51 (d, J = 6.0 Hz, 2H), 3.91–3.77 (m, 1H), 3.67–3.57 (m, 1H), 2.06–1.51 (m, 7 H). LCMS (ESI) m/z: 251.18 [M+H]$^+$.

*5.1.4. 3-(2-((tert-Butyldimethylsilyl)oxy)phenyl)propiolaldehyde (**8a**)*

To a solution of alcohol **7a** (0.28 g, 1.06 mmol) in DCM (1 ml) was added MnO_2 (0.93 g, 10.7 mmol), and the slurry was stirred at rt for 7 days. The mixture was filtered through a pad of silica, washed with DCM, the filtrate was concentrated and the residue was chromatographed on silica gel (EtOAc in petroleum ether, 1:10) to give the product **8a** (0.18 g, 43%) as a brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.42 (s, 1H), 7.50 (dd, J = 7.6, 1.8 Hz, 1H), 7.36 (ddd, J = 8.3, 7.4, 1.8 Hz, 1H), 6.97 (td, J = 7.6, 1.0 Hz, 1H), 6.90–6.84 (m, 1H), 1.05 (s, 9H), 0.26 (s, 6H). GCMS m/z: 203.1 [M-C_4H_9]$^+$.

*5.1.4.1. 3-(3-((Tetrahydro-2H-pyran-2-yl)oxy)phenyl)propiolaldehyde (**8b**).* Compound **8b** was prepared in the same manner as compound **8a** using propynol **7b** (0.30 g, 1.29 mmol) and MnO_2 (1.12 g, 12.9 mmol). The reaction mixture was stirred at rt for 4 days. The product **8b** (0.14 g, 46%) was obtained as a brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.42 (s, 1H), 7.36–7.21 (m, 3H), 7.18 (ddd, J = 8.2, 2.5, 1.4 Hz, 1H), 5.42 (t, J = 3.2 Hz, 1H), 3.87 (ddd, J = 11.4, 9.5, 3.2 Hz, 1H), 3.62 (dtd, J = 11.4, 4.1, 1.5 Hz, 1H), 2.09–1.91 (m, 1H), 1.93–1.81 (m, 1H), 1.77–1.55 (m, 4H). LCMS (ESI) m/z: 231.13 [M+H]$^+$.

*5.1.5. 3-(4-Hydroxyphenyl)propiolaldehyde (**8c**)*

To a solution of alcohol **7c** (0.35 g, 1.31 mmol) in DCM (5 ml) Dess-Martin periodinane (15% solution in DCM, 5.0 ml, 1.80 mmol) was added. After stirring at rt for 1 h, the reaction mixture was diluted with DCM, washed with 10% aqueous sodium thiosulfate solution, saturated $NaHCO_3$ solution, brine, dried over Na_2SO_4, and concentrated. The residue was chromatographed on silica gel (EtOAc in petroleum ether, 1:9) to give the product **8c** (0.32 g, 93%) as a brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) 9.40 (s, 1H), 7.55–7.47 (m, 2H), 6.89–6.80 (m, 2H), 0.98 (s, 9H), 0.23 (s, 6H). GCMS *m*/*z*: 260.0 M^+.

*5.1.5.1. 3-(2-Chlorophenyl)propiolaldehyde (**8d**).* Compound **8d** was prepared in the same manner as compound **8c** using alcohol **7d** (0.55 g, 3.30 mmol) and Dess-Martin periodinane (15% solution in DCM, 12.1 ml, 4.29 mmol). The product **8d** (0.51 g, 94%) was obtained as a brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.49 (s, 1H), 7.63 (dd, *J* = 7.7, 1.6 Hz, 1H), 7.48 (dd, *J* = 8.1, 1.5 Hz, 1H), 7.42 (ddd, *J* = 8.1, 7.7, 1.6 Hz, 1H), 7.30 (td, *J* = 7.5, 1.5 Hz, 1H). GCMS *m*/*z*: 164.0 M^+.

*5.1.5.2. 3-(2-Fluorophenyl)propiolaldehyde (**8e**).* Compound **8e** was prepared in the same manner as compound **8c** using alcohol **7e** (0.24 g, 1.60 mmol) and Dess-Martin periodinane (15% solution in DCM, 4.97 ml, 1.76 mmol). The product **8e** (0.19 g, 80%) was obtained as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.45 (s, 1H), 7.58 (td, *J* = 7.2, 1.8 Hz, 1H), 7.54–7.44 (m, 1H), 7.19 (td, *J* = 7.6, 1.0 Hz, 1H), 7.15 (t, *J* = 8.8 Hz, 1H). LCMS (ESI): no ionization.

*5.1.5.3. 3-(2-Fluoro-4-((tetrahydro-2H-pyran-2-yl)oxy)phenyl)propiolaldehyde (**8f**).* Compound **8f** was prepared in the same manner as compound **8c** using alcohol **7f** (106 mg, 0.42 mmol) and Dess-Martin periodinane (15% solution in DCM, 1.38 ml, 0.55 mmol). The product **8f** (88 mg, 84%) was obtained as a brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.42 (s, 1H), 7.54–7.41 (m, 1H), 6.92–6.81 (m, 2H), 5.46 (t, *J* = 3.0 Hz, 1H), 3.81 (ddd, *J* = 11.2, 10.0, 3.0 Hz, 1H), 3.63 (dtd, *J* = 11.2, 4.1, 1.4 Hz, 1H), 2.04–1.80 (m, 2H), 1.82–1.55 (m, 4H). LCMS (ESI) *m*/*z*: 249.11 $[M+H]^+$.

*5.1.6. Ethyl (E)-2-(5-(2-((tert-butyldimethylsilyl)oxy)phenyl)pent-2-en-4-ynamido)cyclohex-1-ene-1-carboxylate (**10a**)*

To a solution of (2-((2-(ethoxycarbonyl)cyclohex-1-en-1-yl) amino)-2-oxoethyl)triphenylphosphonium bromide (**9**) (0.36 g, 0.65 mmol) and *t*-BuOK (60 mg, 0.54 mmol) in DMSO (4 ml) was added a solution of aldehyde **8a** (0.14 g, 0.54 mmol) in DMSO (1.5 ml) and the resulting mixture was stirred at rt for 2 h. The reaction mixture was diluted with water and extracted with EtOAc. The organic extract was washed successively with water, brine, dried over Na_2SO_4, concentrated, and purified by column chromatography on silica gel (EtOAc in petroleum ether, 1:10) to afford the product **10a** (0.21 g, 65%) as a brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 11.87 (s, 1H), 7.39 (dd, *J* = 7.7, 1.7 Hz, 1H), 7.25–7.18 (m, 1H), 6.96 (d, *J* = 15.4 Hz, 1H), 6.96–6.90 (m, 1H), 6.83 (d, *J* = 7.8 Hz, 1H), 6.40 (d, *J* = 15.4 Hz, 1H), 4.20 (q, *J* = 7.1 Hz, 2H), 3.12–2.97 (m, 2H), 2.40–2.27 (m, 2H), 1.69–1.57 (m, 4H), 1.31 (t, *J* = 7.1 Hz, 3H), 1.05 (s, 9H), 0.23 (s, 6H). LCMS (ESI) *m*/*z*: 454.31 $[M+H]^+$.

*5.1.6.1. Ethyl (E)-2-(5-(3-((tetrahydro-2H-pyran-2-yl)oxy)phenyl) pent-2-en-4-ynamido)cyclohex-1-ene-1-carboxylate (**10b**).* Compound **10b** was prepared in the same manner as compound **10a** using aldehyde **8b** (0.16 g, 0.70 mmol), phosphonium bromide **9** (0.46 g, 0.83 mmol), and *t*-BuOK (78 mg, 0.70 mmol). The product **10b** (0.24 g, 87%) was obtained as a yellow oil which contained the corresponding methyl carboxylate (*ca* 6%, LCMS (ESI) *m*/*z*: 410.28 $[M+H]^+$). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 11.88 (s, 1H), 7.24 (t, *J* = 7.9 Hz, 1H), 7.20–7.16 (m, 1H), 7.10 (dt, *J* = 7.6, 1.1 Hz, 1H), 7.05 (ddd, *J* = 8.2, 2.5, 1.1 Hz, 1H), 6.93 (d, *J* = 15.4 Hz, 1H), 6.44 (d, *J* = 15.4 Hz, 1H), 5.42 (t, *J* = 3.3 Hz, 1H), 4.20 (q, *J* = 7.1 Hz, 2H), 3.90 (ddd, *J* = 11.3, 9.3, 3.3 Hz, 1H), 3.69–3.56 (m, 1H), 3.10–2.97 (m, 2H), 2.39–2.29 (m, 2H), 2.08–1.87 (m, 1H), 1.90–1.82 (m, 2H), 1.73–1.57 (m, 7H), 1.31 (t, *J* = 7.1 Hz, 3H). LCMS (ESI) *m*/*z*: 424.30 $[M+H]^+$. In addition, the corresponding *Z*-isomer of compound **10b** (17 mg, 6%) after column chromatography was isolated. **(*Z*)–10b**, ^{1}H NMR (300 MHz, $CDCl_3$) δ: 11.92 (s, 1H), 7.21 (t, *J* = 7.9 Hz, 1H), 7.17 (dd, *J* = 2.5, 1.5 Hz, 1H), 7.11 (dt, *J* = 7.6, 1.3 Hz, 1H), 7.03 (ddd, *J* = 8.2, 2.5, 1.1 Hz, 1H), 6.25 (d, *J* = 11.5 Hz, 1H), 6.17 (d, *J* = 11.5 Hz, 1H), 5.39 (t, *J* = 3.2 Hz, 1H), 4.09 (q, *J* = 7.1 Hz, 2H), 3.87 (ddd, *J* = 11.3, 9.4, 3.1 Hz, 1H), 3.65–3.54 (m, 1H), 3.13–2.99 (m, 2H), 2.39–2.27 (m, 2H), 2.07–1.90 (m, 1H), 1.90–1.79 (m, 2H), 1.76–1.49 (m, 7H), 1.23 (t, *J* = 7.1 Hz, 3H). LCMS (ESI) *m*/*z*: 424.30 $[M+H]^+$.

*5.1.6.2. Ethyl (E)-2-(5-(2-chlorophenyl)pent-2-en-4-ynamido)cyclohex-1-ene-1-carboxylate (**10d**).* Compound **10d** was prepared in the same manner as compound **10a** using aldehyde **8d** (0.105 g, 0.64 mmol), phosphonium bromide **9** (0.39 g, 0.71 mmol), and *t*-BuOK (72 mg, 0.64 mmol). The product **10d** (0.18 g, 79%) was obtained as a yellow solid which contained the corresponding methyl carboxylate (*ca* 4%, LCMS (ESI) *m*/*z*: 344.24 $[M+H]^+$, ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.75 (s)) and the *Z*-isomer (*ca* 6%, LCMS (ESI) *m*/*z*: 358.26 $[M+H]^+$, ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.31 (d, *J* = 11.5 Hz), 6.23 (d, *J* = 11.5 Hz)) as impurities.^{1}H NMR (300 MHz, $CDCl_3$) δ: 11.89 (s, 1H), 7.55–7.45 (m, 1H), 7.47–7.37 (m, 1H), 7.35–7.18 (m, 2H), 6.98 (d, *J* = 15.4 Hz, 1H), 6.50 (d, *J* = 15.4 Hz, 1H), 4.20 (q, *J* = 7.1 Hz, 2H), 3.14–2.97 (m, 2H), 2.40–2.28 (m, 2H), 1.72–1.56 (m, 4H), 1.31 (t, *J* = 7.1 Hz, 3H). LCMS (ESI) *m*/*z*: 358.24 $[M+H]^+$.

*5.1.6.3. Ethyl (E)-2-(5-(2-fluorophenyl)pent-2-en-4-ynamido)cyclohex-1-ene-1-carboxylate (**10e**).* Compound **10e** was prepared in the same manner as compound **10a** using aldehyde **8e** (0.10 g, 0.68 mmol), phosphonium bromide **9** (0.45 g, 0.81 mmol), and *t*-BuOK (76 mg, 0.68 mmol). The product **10e** (0.20 g, 87%) was obtained as a yellow solid which contained the corresponding methyl carboxylate (*ca* 5%, LCMS (ESI) *m*/*z*: 328.21 $[M+H]^+$, ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.75 (s)) and the *Z*-isomer (*ca* 8%, LCMS (ESI) *m*/*z*: 342.24 $[M+H]^+$, ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.29 (d, *J* = 11.5 Hz), 6.22 (d, *J* = 11.5 Hz)) as impurities. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 11.90 (s, 1H), 7.46 (td, *J* = 7.4, 1.8 Hz, 1H), 7.35 (tdd, *J* = 7.8, 5.3, 1.8 Hz, 1H), 7.17–7.06 (m, 2H), 6.96 (d, *J* = 15.4 Hz, 1H), 6.49 (d, *J* = 15.4 Hz, 1H), 4.20 (q, *J* = 7.1 Hz, 2H), 3.12–2.98 (m, 2H), 2.41–2.29 (m, 2H), 1.72–1.56 (m, 4H), 1.31 (t, *J* = 7.1 Hz, 3H). LCMS *m*/*z*: 342.37 $[M+H]^+$.

*5.1.6.4. Ethyl (E)-2-(5-(2-fluoro-4-((tetrahydro-2H-pyran-2-yl)oxy) phenyl)pent-2-en-4-ynamido)cyclohex-1-ene-1-carboxylate (**10f**).* Compound **10f** was prepared in the same manner as compound **10a** using aldehyde **8f** (85 mg, 0.34 mmol), phosphonium bromide **9** (0.23 g, 0.41 mmol), and *t*-BuOK (42 mg, 0.38 mmol). The product **10f** (0.14 g, 93%) was obtained as a yellow solid, which contained the corresponding methyl carboxylate (*ca* 3%, LCMS (ESI) *m*/*z*: 428.32 $[M+H]^+$, ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.75 (s)) and the *Z*-isomer (*ca* 7%, LCMS (ESI) *m*/*z*: 442.32 $[M+H]^+$, ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.27 (d, *J* = 11.5 Hz), 6.16 (d, *J* = 11.5 Hz)) as impurities. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 11.87 (s, 1H), 7.39–7.31 (m, 1H), 6.95 (d, *J* = 15.4 Hz, 1H), 6.88–6.75 (m, 2H), 6.44 (d, *J* = 15.4 Hz, 1H), 5.42 (t, *J* = 3.1 Hz, 1H), 4.20 (q, *J* = 7.1 Hz, 2H), 3.84 (ddd, *J* = 11.1, 9.6, 3.1 Hz, 1H), 3.67–3.56 (m, 1H), 3.09–2.98 (m, 2H), 2.40–2.27 (m, 2H), 2.07–1.80 (m, 1H), 1.88–1.83 (m,

2H), 1.77–1.56 (m, 7H), 1.31 (t, J = 7.1 Hz, 3H). LCMS (ESI) m/z: 442.31 $[M+H]^+$.

*5.1.7. Ethyl (E)-2-(5-(4-((tert-butyldimethylsilyl)oxy)phenyl)pent-2-en-4-ynamido)cyclohex-1-ene-1-carboxylate (**10c**)*

A mixture of aldehyde **10c** (0.17 g, 0.64 mmol), phosphonium bromide **9** (0.42 g, 0.76 mmol), K_2CO_3 (0.11 g 0.76 mmol), and 18-crown-6 (6 mg, 0.02 mmol) in DCM (8 ml) was stirred for 16 h at rt. The mixture was diluted with DCM (20 ml) and washed successively with saturated NH_4Cl solution, water, brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (EtOAc in petroleum ether, 0–20%) to give the product **10c** (0.24 g, 83%) as an oil. ^1H NMR (400 MHz, $CDCl_3$) δ: 11.85 (s, 1H), 7.41–7.31 (m, 2H), 6.93 (d, J = 15.4 Hz, 1H), 6.84–6.75 (m, 2H), 6.40 (d, J = 15.4 Hz, 1H), 4.20 (q, J = 7.1 Hz, 2H), 3.07–3.02 (m, 2H), 2.37–2.31 (m, 2H), 1.72–1.56 (m, 4H), 1.31 (t, J = 7.1 Hz, 3H), 0.98 (s, 9H), 0.21 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.2, 163.2, 157.0, 152.3, 133.7, 133.2, 123.2, 120.5, 115.3, 105.7, 98.3, 86.3, 60.5, 28.7, 25.8, 24.5, 22.0, 21.8, 18.4, 14.4, -4.3. LCMS (ESI) m/z: 454.32 $[M+H]^+$.

*5.1.8. Ethyl (E)-2-(5-(2-hydroxyphenyl)pent-2-en-4-ynamido)cyclohex-1-ene-1-carboxylate (**11a**)*

To a solution of TBDMS-ether **10a** (0.19 g, 0.42 mmol) in THF (4 ml) was added 1 M solution of tetra-*n*-butylammonium fluoride in THF (0.46 ml, 0.46 mmol) by cooling the reaction flask in a cold water bath and the reaction mixture was stirred at rt for 10 min. The reaction mixture was diluted with ethyl acetate, successively washed with water, saturated NH_4Cl solution, brine, and dried over Na_2SO_4. The solvents were removed and the residue was purified by column chromatography on silica gel (EtOAc in petroleum ether, 1:4) to give the product **11a** (0.12 g, 84%) as a yellow solid which contained the corresponding methyl carboxylate (*ca* 4%, LCMS (ESI) m/z: 326.33 $[M+H]^+$, ^1H NMR (300 MHz, $CDCl_3$) δ: 3.76 (s)) as an impurity. ^1H NMR (300 MHz, $CDCl_3$) δ: 11.94 (s, 1H), 7.38 (dd, J = 7.7, 1.3 Hz, 1H), 7.36–7.26 (m, 1H), 7.06–6.86 (m, 3H), 6.49 (d, J = 15.5 Hz, 1H), 5.71 (s, 1H), 4.22 (q, J = 7.1 Hz, 2H), 3.13–3.00 (m, 2H), 2.42–2.31 (m, 2H), 1.75–1.58 (m, 4H), 1.33 (t, J = 7.1 Hz, 3H). LCMS (ESI) m/z: 340.22 $[M+H]^+$.

*5.1.9. Ethyl (E)-2-(5-(3-hydroxyphenyl)pent-2-en-4-ynamido)cyclohex-1-ene-1-carboxylate (**11b**)*

p-Toluenesulfonic acid (5 mg, 0.03 mmol) was added to a suspension of compound **10b** (0.23 g, 0.54 mmol) in a mixture of DCM (5 mL) and methanol (2.5 mL). After stirring at rt for 6 h, the solvent was evaporated. The residue was diluted with water, extracted with EtOAc, the organic the extract was washed with water, brine, dried over Na_2SO_4, and concentrated to give the product **11b** (0.18 g, 98%) as a yellow solid. ^1H NMR (300 MHz, $CDCl_3$) δ: 11.90 (s, 1H), 7.22 (t, J = 7.9 Hz, 1H), 7.05 (dt, J = 7.6, 1.1 Hz, 1H), 6.95 (dd, J = 2.5, 1.4 Hz, 1H), 6.94 (d, J = 15.4 Hz, 1H), 6.85 (ddd, J = 8.1, 2.5, 0.9 Hz, 1H), 6.44 (d, J = 15.4 Hz, 1H), 5.07 (s, 1H), 4.20 (q, J = 7.1 Hz, 2H), 3.10–2.99 (m, 2H), 2.40–2.30 (m, 2H), 1.72–1.56 (m, 4H), 1.31 (t, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.2, 163.3, 156.0, 152.0, 134.0, 129.9, 124.5, 123.5, 123.2, 118.7, 117.0, 106.5, 97.8, 86.7, 60.7, 28.7, 24.5, 22.0, 21.7, 14.4. R_f 0.4 (EtOAc in petroleum ether, 1:4). LCMS (ESI) m/z: 340.23 $[M+H]^+$.

*5.1.9.1. Ethyl (E)-2-(5-(2-fluoro-4-hydroxyphenyl)pent-2-en-4-ynamido)cyclohex-1-ene-1-carboxylate (**11f**).* Compound **11f** was prepared in the same manner as compound **11b** using THP-ether **10f** (0.14 g, 0.32 mmol) and *p*-toluenesulfonic acid (3 mg, 0.02 mmol). The product **11f** (92 mg, 81%) was obtained as a yellow solid which contained the corresponding methyl carboxylate (*ca* 5%, LCMS (ESI) m/z: 344.19 $[M+H]^+$, ^1H NMR (300 MHz, $CDCl_3$) δ: 3.76 (s)) and the *Z*-isomer (*ca* 4%, LCMS (ESI) m/z: 358.21 $[M+H]^+$, ^1H NMR (300 MHz, $CDCl_3$) δ: 6.29 (d, J = 11.5 Hz), 6.17 (d, J = 11.5 Hz)) as impurities. ^1H NMR (300 MHz, $CDCl_3$) δ: 11.89 (s, 1H), 7.31 (t, J = 8.1 Hz, 1H), 6.94 (d, J = 15.4 Hz, 1H), 6.66–6.69 (m, 2H), 6.42 (d, J = 15.4 Hz, 1H), 6.06 (s, 1H), 4.21 (q, J = 7.1 Hz, 2H), 3.09–2.99 (m, 2H), 2.40–2.29 (m, 2H), 1.71–1.55 (m, 4H), 1.32 (t, J = 7.1 Hz, 3H). R_f 0.4 (EtOAc in petroleum ether, 1:4). LCMS (ESI) m/z: 358.21 $[M+H]^+$.

*5.1.10. (E)-2-(5-(2-Hydroxyphenyl)pent-2-en-4-ynamido)cyclohex-1-ene-1-carboxylic acid (**2a**)*

To a solution of ethyl carboxylate **11a** (0.12 g, 0.53 mmol) in a mixture of THF (2 ml) and MeOH (1 ml) 2 N NaOH solution (0.53 ml, 1.06 mmol) was added and the mixture was stirred for 6 h at rt. The mixture was acidified with aqueous 1 N HCl to pH 4, concentrated in vacuo to 1 ml, the precipitated solid was filtered, and dried in vacuo over P_2O_5. This residue was chromatographed on silica gel (MeOH in DCM, 1:20 to 1:10) to give the product **2a** (59 mg, 54%) as a yellow solid. ^1H NMR (400 MHz, DMSO-d_6) δ: 12.66 (b s, 1H), 11.70 (s, 1H), 10.12 (b s, 1H), 7. 35 (dd, J = 7.6, 1.6 Hz, 1H), 7.25 (ddd, J = 8.3, 7.4, 1.6 Hz, 1H), 6.91 (dd, J = 8.3, 1.0 Hz, 1H), 6.84 (d, J = 15.5 Hz, 1H), 6.82 (td, J = 7.5, 1.0 Hz, 1H), 6.49 (d, J = 15.5 Hz, 1H), 2.90–2.84 (m, 2H), 2.29–2.23 (m, 2H), 1.62–1.50 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.0, 161.9, 158.8, 149.9, 133.5, 133.2, 131.2, 121.9, 119.2, 115.7, 108.9, 107.0, 95.1, 90.3, 28.1, 24.5, 21.5, 21.3. LCMS (ESI) m/z: 310.12 $[M-H]^-$. Anal. Calcd for $C_{18}H_{17}NO_4 \times 0.13\ CHCl_3$ (4.7%): C 66.62, H 5.28, N 4.29. Found C 66.54, H 5.25, N 4.26.

*5.1.10.1. (E)-2-(5-(3-Hydroxyphenyl)pent-2-en-4-ynamido)cyclohex-1-ene-1-carboxylic acid (**2b**).* Compound **2b** was prepared in the same manner as compound **2a** using ethyl carboxylate **11b** (0.17 g, 0.50 mmol) and 2 N NaOH solution (0.75 ml, 1.50 mmol) in water. The product **2b** (79 mg, 51%) was obtained as a yellow solid. ^1H NMR (400 MHz, DMSO-d_6) δ: 11.75 (b s, 1H), 9.80 (b s, 1H), 7.22 (t, J = 7.9 Hz, 1H), 6.95 (dt, J = 7.7, 1.1 Hz, 1H), 6.89–6.83 (m, 2H), 6.82 (d, J = 15.5 Hz, 1H), 6.55 (d, J = 15.5 Hz, 1H), 2.89–2.82 (m, 2H), 2.30–2.22 (m, 2H), 1.63–1.50 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.0, 161.8, 157.4, 149.6, 134.7, 130.1, 122.6, 122.3, 121.3, 117.9, 117.2, 107.3, 97.2, 86.4, 28.1, 24.6, 21.5, 21.3. HRMS (ESI) m/z: calcd. for $C_{18}H_{18}NO_4$ $[M+H]^+$ 312.1230, found 312.1242.

*5.1.10.2. (E)-2-(5-(4-Hydroxyphenyl)pent-2-en-4-ynamido)cyclohex-1-ene-1-carboxylic acid (**2c**).* Compound **2c** was prepared in the same manner as compound **2a** using ethyl carboxylate **10c** (95 mg, 0.21 mmol) and NaOH (0.42 ml, 0.84 mmol). The product **2c** (37 mg, 57%) was obtained as a yellow solid. ^1H NMR (400 MHz, DMSO-d_6) δ: 12.50 (b s, 1H), 11.71 (s, 1H), 10.10 (b s, 1H), 7.36 (d, J = 8.6 Hz, 2H), 6.81 (d, J = 15.3 Hz, 1H), 6.80 (d, J = 8.6 Hz, 2H), 6.45 (d, J = 15.3 Hz, 1H), 2.91–2.81 (m, 2H), 2.30–2.21 (m, 2H), 1.63–1.48 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.0, 162.0, 158.9, 149.9, 133.6, 133.1, 121.9, 115.9, 111.6, 107.0, 98.5, 85.6, 28.1, 24.1, 21.5, 21.3. LCMS (ESI) m/z: 312.10 $[M+H]^+$. Anal. Calcd for $C_{18}H_{17}NO_4$: C 69.44, H 5.50, N 4.50. Found C 68.97, H 5.49, N 4.37.

*5.1.10.3. (E)-2-(5-(2-Chlorophenyl)pent-2-en-4-ynamido)cyclohex-1-ene-1-carboxylic acid (**2d**).* Compound **2d** was prepared in the same manner as compound **2a** using ethyl carboxylate **10d** (0.18 g, 0.50 mmol) and 2 N NaOH (0.75 ml, 1.51 mmol). The product **2d** (63 mg, 38%) was obtained as a yellow solid. ^1H NMR (400 MHz, DMSO-d_6) δ: 12.70 (b s, 1H), 11.67 (s, 1H), 7.66 (dd, J = 7.4, 1.7 Hz, 1H), 7.60 (dd, J = 7.8, 1.1 Hz, 1H), 7.48 (td, J = 7.8, 1.7 Hz, 1H), 7.41 (td, J = 7.4, 1.1 Hz, 1H), 6.88 (d, J = 15.5 Hz, 1H), 6.63 (d, J = 15.5 Hz, 1H), 2.90–2.80 (m, 2H), 2.31–2.21 (m, 2H), 1.64–1.48

(m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 170.9, 161.5, 149.5, 135.6, 134.8, 133.8, 131.2, 129.5, 127.5, 121.3, 120.7, 107.5, 93.0, 91.6, 28.1, 24.6, 21.5, 21.3. LCMS (ESI) m/z: 328.22 $[M-H]^-$. Anal. Calcd for $C_{18}H_{16}ClNO_3$: C 65.56, H 4.89, N 4.25. Found: C 65.04, H 4.91, N 4.22.

*5.1.10.4. (E)-2-(5-(2-Fluorophenyl)pent-2-en-4-ynamido)cyclohex-1-ene-1-carboxylic acid (**2e**).* Compound **2e** was prepared in the same manner as compound **2a** using ethyl carboxylate **10e** (0.20 g, 0.59 mmol) and 2 N NaOH (0.88 ml, 1.76 mmol). The product **2e** (70 mg, 38%) was obtained as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 11.61 (s, 1H), 10.23 (b s, 1H), 7.52–7.44 (m, 1H), 7.40–7.30 (m, 1H), 7.16–7.06 (m, 2H), 7.00 (d, J = 15.5 Hz, 1H), 6.45 (d, J = 15.5 Hz, 1H), 3.13–3.04 (m, 2H), 2.45–2.36 (m, 2H), 1.75–1.58 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 174.6, 162.9 (d, J_{CF} = 253.6 - Hz), 162.8, 155.1, 134.1, 133.9, 131.2 (d, J_{CF} = 8.1 Hz), 124.3 (d, J_{CF} = 3.8 Hz), 123.2, 115.8 (d, J_{CF} = 20.8 Hz), 111.2 (d, J_{CF} = 15.6 Hz), 104.9, 91.6 (d, J_{CF} = 3.2 Hz), 91.3, 29.1, 24.6, 21.9, 21.7. LCMS (ESI) m/z: 312.14 $[M-H]^-$. Anal. Calcd for $C_{18}H_{16}FNO_3$: C 69.00, H 5.15, N 4.47. Found: C 68.28, H 5.08, N 4.35.

*5.1.10.5. (E)-2-(5-(2-Fluoro-4-hydroxyphenyl)pent-2-en-4-ynamido)-cyclohex-1-ene-1-carboxylic acid (**2f**).* Compound **2f** was prepared in the same manner as compound **2a** using ethyl carboxylate **11f** (92 mg, 0.26 mmol) and 2 N NaOH (0.39 ml, 0.77 mmol). The product **2f** (32 mg, 38%) was obtained as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.67 (b s, 1H), 11.70 (s, 1H), 10.60 (b s, 1H), 7.43–7.38 (m, 1H), 6.83 (d, J = 15.5 Hz, 1H), 6.70–6.65 (m, 2H), 6.49 (d, J = 15.5 Hz, 1H), 2.89–2.83 (m, 2H), 2.29–2.23 (m, 2H), 1.62–1.50 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.0, 163.1 (d, J_{CF} = 249.8 Hz), 161.8, 160.8 (d, J_{CF} = 11.8 Hz), 149.7, 134.6 (d, J_{CF} = 2.9 Hz), 133.8, 121.4, 112.5 (d, J_{CF} = 2.6 Hz), 107.2, 103.1 (d, J_{CF} = 22.9 Hz), 100.0 (d, J_{CF} = 15.8 Hz), 91.4, 90.1 (d, J_{CF} = 2.9 Hz), 28.2, 24.6, 21.5, 21.3. LCMS (ESI) m/z: 328.10 $[M-H]^-$. Anal. Calcd for $C_{18}H_{16}FNO_4$: C 65.65, H 4.90, N 4.25. Found: C 65.29, H 4.83, N 4.22.

*5.1.11. (E)-5-Phenylpent-2-enoic acid (**13**)*

To a solution of methyl 2-(dimethoxyphosphoryl)acetate (0.63 ml, 4.45 mmol) in DMSO (2.5 ml) was added *t*-BuOK (0.46 g, 4.10 mmol) and the mixture was stirred at rt for 10 min. To this mixture 3-phenylpropanal (**12**) (0.50 g, 3.73 mmol) was added and stirring was continued for 1 h. The reaction mixture was diluted with water and extracted with EtOAc, the organic layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (EtOAc in petroleum ether, 1:7) to give a mixture of *E* and *Z* isomers of methyl 5-phenylpent-2-enoate (0.27 g, 38%) as a colorless oil. Methyl (*E*)-5-phenylpent-2-enoate: ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.34–7.11 (m, 5H), 7.01 (dt, J = 15.7, 6.8 Hz, 1H), 5.85 (dt, J = 15.7, 1.4 Hz, 2H), 3.72 (s, 3H), 2.84–2.71 (m, 2H), 2.60–2.46 (m, 2H). The oil was dissolved in a mixture of THF (1.5 ml) and MeOH (1 ml), to the obtained solution was added 2 N NaOH (2.5 ml, 5.00 mmol) and the resulting mixture was stirred at rt for 4 h. The solvents were evaporated, the residue was dissolved in water and acidified to pH 2 with 1 N HCl. The mixture was extracted with DCM, the extract was dried over Na_2SO_4, and concentrated. The residue was purified by Biotage on reverse phase column (MeCN in water, 20–100%) to give a mixture of *E* and *Z* isomers of the product **13** (0.18 g, 61%) (NMR ratio 1:0.21) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 11.00 (b s, 1H), 7.52–6.88 (m, 6H), 5.86 (d, J = 15.6 Hz, 1H), 2.87–2.72 (m, 2H), 2.66–2.49 (m, 2H). LCMS (ESI) m/z: 177.03 $[M+H]^+$ (*E* isomer, content 65%), 177.05 $[M+H]^+$ (*Z* isomer, content 23%).

*5.1.12. (E)-2-(5-Phenylpent-2-enamido)cyclohex-1-ene-1-carboxylic acid (**3**)*

5-Phenylpent-2-enoic acid (**13**) (0.18 g, 1.02 mmol) in DCM (2 ml) under argon atmosphere at rt was treated with a catalytic amount of DMF (1 drop) and oxalyl chloride (0.26 ml, 3.06 mmol). The mixture was stirred for 40 min and the volatiles were evaporated. The residue was dissolved in DCM (2 ml) and to the obtained solution at 0 °C a mixture of ethyl 2-aminocyclohex-1-ene-1-carboxylate (0.26 g, 1.54 mmol) and triethylamine (0.14 ml, 1.00 mmol) in DCM (1 ml) was added dropwise over 5 min. The mixture was stirred for 40 min at rt, then the mixture was supplemented with DCM, washed with saturated NH_4Cl solution, brine, dried over Na_2SO_4, and concentrated. The residue was chromatographed on silica gel (EtOAc in petroleum ether, 1:10 to 1:6) to give crude ethyl (*E*)-2-(5-phenylpent-2-enamido)cyclohex-1-ene-1-carboxylate (0.21 g) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 11.71 (s, 1H), 7.33–7.16 (m, 5H), 6.92 (dt, J = 15.3, 6.8 Hz, 1H), 5.92 (dt, J = 15.3, 1.5 Hz, 1H), 4.19 (q, J = 7.1 Hz, 1H), 3.09–2.98 (m, 2H), 2.85–2.69 (m, 2H), 2.59–2.45 (m, 2H), 2.39–2.25 (m, 2H), 1.73–1.56 (m, 4H), 1.30 (t, J = 7.1 Hz, 3H). LCMS (ESI) m/z: 328.37 $[M+H]^+$. The oil was dissolved in a mixture of THF (2 ml) and EtOH (1 ml) and to the obtained solution 2 N NaOH (0.92 ml, 1.84 mmol) was added. The mixture was stirred for 16 h at rt followed by stirring for 5 h at 50 °C. The reaction mixture was cooled to rt and acidified to pH 4 with 1 N HCl. The solvents were evaporated and the residue was partitioned between DCM and water. The organic layer was dried over Na_2SO_4, evaporated, and the residue was chromatographed on silica gel (MeOH in DCM, 1:20) to give an oily solid. The solid was triturated with EtOAc petroleum ether mixture (1:4), filtered, and dried in vacuo to give the product **3** (44 mg, 14%, calculated with respect to **13**) as white crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 11.47 (s, 1H), 10.40 (b s, 1H), 7.33–7.25 (m, 2H), 7.23–7.16 (m, 3H), 6.93 (dt, J = 15.3, 6.9 Hz, 1H), 5.98 (dt, J = 15.3, 1.3 Hz, 1H), 3.10–3.01 (m, 2H), 2.83–2.74 (m, 2H), 2.59–2.49 (m, 2H), 2.42–2.33 (m, 2H), 1.73–1.56 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 174.6, 164.2, 155.6, 146.1, 141.0, 128.6, 128.5, 126.3, 125.7, 103.7, 34.7, 34.1, 29.1, 24.6, 21.9, 21.8. HRMS (ESI) m/z: calcd. for $C_{18}H_{22}NO_3$ $[M+H]^+$ 300.1594, found 300.1587.

*5.1.13. 5-(2-Chlorophenyl)pent-4-ynoic acid (**16**)*

Compound **16** was prepared in the same manner as compound **7a** using 1-chloro-2-iodobenzene (**14**) (0.50 g, 2.10 mmol), triethylamine (2.92 ml, 20.96 mmol), pent-4-ynoic acid (**15**) (0.21 ml, 3.51 mmol), $[Pd(PPh_3)_4]$ (73 mg, 0.06 mmol), and CuI (24 mg, 0.13 mmol). The reaction was stirred for 18 h at rt. The product **16** (92 mg, 21%) was obtained as a brown solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 10.15 (b s, 1H), 7.44–7.40 (m, 1H), 7.39–7.34 (m, 1H), 7.24–7.13 (m, 2H), 2.85–2.74 (m, 2H), 2.77–2.70 (m, 2H). LCMS (ESI) m/z: 207.03 $[M-H]^-$.

*5.1.14. Ethyl 2-(5-(2-chlorophenyl)pent-4-ynamido)cyclohex-1-ene-1-carboxylate (**17d**)*

Acid **16** (0.10 g, 0.48 mmol) in DCM (2 ml) under argon atmosphere at rt was treated with a catalytic amount of DMF (1 drop) and oxalyl chloride (0.12 ml, 1.44 mmol). The mixture was stirred for 20 min and the volatiles were evaporated. The residue was dissolved in DCM (2 ml) and cooled to 0 °C, to this solution a mixture of ethyl 2-aminocyclohex-1-ene-1-carboxylate (0.12 g, 0.72 mmol) and triethylamine (67 µl, 0.48 mmol) in DCM (1 ml) was added dropwise over 5 min. The mixture was stirred for 40 min at rt, diluted with DCM, washed with NH_4Cl, brine, dried over Na_2SO_4, and concentrated. The residue was chromatographed on silica gel (EtOAc in petroleum ether, 1:10 to 1:6) to give the product **17d** (92 mg, 53%) as a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 11.72 (s, 1H), 7.47–7.34 (m, 2H), 7.25–7.13 (m, 2H), 4.18 (q,

J = 7.1 Hz, 2H), 3.05–2.96 (m, 2H), 2.88–2.79 (m, 2H), 2.72–2.64 (m, 2H), 2.37–2.30 (m, 2H), 1.72–1.57 (m, 4H), 1.31 (t, *J* = 7.1 Hz, 3H). LCMS (ESI) *m*/*z* 360.28 $[M+H]^+$.

*5.1.15. Ethyl 2-(pent-4-ynamido)cyclohex-1-ene-1-carboxylate (**18**)*

4-Pentynoic acid (**15**) (1.00 g, 10.19 mmol) in DCM (20 ml) under argon atmosphere at rt was treated with DMF (39 µl, 0.51 mmol) and oxalyl chloride (2.22 ml, 25.48 mmol). The mixture was stirred for 40 min and the volatiles were evaporated. The residue was dissolved in DCM (3 ml) and the obtained solution was added to a mixture of ethyl 2-aminocyclohex-1-ene-1-carboxylate (0.10 g, 0.59 mmol) and DIPEA (1.76 ml, 10.2 mmol) in DCM (15 ml) at −5 °C to 2 °C under argon atmosphere. The mixture was stirred for 1 h at 0 °C and warmed to rt. The mixture was diluted with DCM, washed with saturated NH_4Cl, brine, and dried over Na_2SO_4. The solvent was evaporated and the residue was chromatographed on silica gel (EtOAc in petroleum ether, 1:20 to 1:10) to afford a white solid. Recrystallization from *n*-heptane gave the product **18** (1.20 g). The filtrate after crystallization was concentrated and the residue was chromatographed on Biotage (EtOAc in petroleum ether, 2–20%) to give the product (0.25 g); the combined yield was 1.45 g, 57%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 11.66 (s, 1H), 4.18 (q, *J* = 7.1 Hz, 2H), 3.03–2.92 (m, 2H), 2.65–2.49 (m, 4H), 2.36–2.25 (m, 2H), 1.97 (t, *J* = 2.3 Hz, 1H), 1.70–1.54 (m, 4H), 1.30 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.1, 169.6, 152.1, 104.9, 82.8, 69.2, 60.4, 37.2, 28.7, 24.3, 22.0, 21.8, 14.6, 14.4. LCMS (ESI) *m*/*z* 250.18 $[M+H]^+$.

*5.1.16. Ethyl 2-(5-(2-((tert-butyldimethylsilyl)oxy)phenyl)pent-4-ynamido)cyclohex-1-ene-1-carboxylate (**17a**)*

To a degassed solution of *tert*-butyl(2-iodophenoxy)dimethylsilane (**6a**) (0.21 g, 0.63 mmol), ethyl 2-(pent-4-ynamido)cyclohex-1-ene-1-carboxylate (**18**) (0.13 g, 0.52 mmol), and triethylamine (0.36 ml, 2.61 mmol) in MeCN (1 ml) under argon atmosphere $PdCl_2[PPh_3]_2$ (18 mg, 0.03 mmol) and CuI (5 mg, 0.03 mmol) were added. The mixture was stirred for 15 h at rt. The mixture was concentrated, diluted with saturated NH_4Cl solution, extracted with DCM (20 ml), dried over Na_2SO_4, and concentrated. After a silica gel chromatography on Biotage (EtOAc in petroleum ether, 3–30%) the product **17a** (0.12 g, 52%) was obtained as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 11.69 (s, 1H), 7.32 (dd, *J* = 7.6, 1.8 Hz, 1H), 7.14 (ddd, *J* = 8.1, 7.6, 1.8 Hz, 1H), 6.86 (td, *J* = 7.6, 1.1 Hz, 1H), 6.78 (dd, *J* = 8.2, 1.1 Hz, 1H), 4.16 (q, *J* = 7.1 Hz, 2H), 3.05–2.88 (m, 2H), 2.86–2.70 (m, 2H), 2.70–2.54 (m, 2H), 2.37–2.20 (m, 2H), 1.68–1.54 (m, 4H), 1.29 (t, *J* = 7.1 Hz, 3H), 1.03 (s, 9H), 0.21 (s, 6H). LCMS (ESI) *m*/*z*: 456.46 $[M+H]^+$.

*5.1.16.1. Ethyl 2-(5-(3-((tert-butyldimethylsilyl)oxy)phenyl)pent-4-ynamido)cyclohex-1-ene-1-carboxylate (**17b**).* Compound (**17b**) was prepared in the same manner as compound **17a** using iodide **6j** (0.21 g, 0.63 mmol), acetylene **18** (0.13 g, 0.52 mmol), triethylamine (0.36 ml, 2.61 mmol), $PdCl_2[PPh_3]_2$ (18 mg, 0.03 mmol), and CuI (5 mg, 0.03 mmol). The product **17b** (0.19 g, 79%) was obtained as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 11.70 (s, 1H), 7.11 (t, *J* = 7.9 Hz, 1H), 6.97 (dt, *J* = 7.6, 1.2 Hz, 1H), 6.85 (d, *J* = 2.2 Hz, 1H), 6.75 (ddd, *J* = 8.1, 2.5, 1.0 Hz, 1H), 4.16 (q, *J* = 7.1 Hz, 2H), 3.03–2.93 (m, 2H), 2.80–2.70 (m, 2H), 2.68–2.57 (m, 2H), 2.36–2.26 (m, 2H), 1.69–1.54 (m, 4H), 1.28 (t, *J* = 7.1 Hz, 3H), 0.97 (s, 9H), 0.18 (s, 6H). LCMS (ESI) *m*/*z*: 456.41 $[M+H]^+$.

*5.1.16.2. Ethyl 2-(5-(4-((tert-butyldimethylsilyl)oxy)phenyl)pent-4-ynamido)cyclohex-1-ene-1-carboxylate (**17c**).* Compound **17c** was prepared in the same manner as compound **17a** using iodide **6c** (0.16 g, 0.48 mmol), acetylene **18** (0.10 g, 0.40 mmol), triethylamine (0.28 ml, 2.00 mmol), $PdCl_2[PPh_3]_2$ (14 mg, 0.02 mmol), and CuI (4 mg, 0.02 mmol). The product **17c** (94 mg, 51%) was obtained as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 11.69 (s, 1H), 7.25 (d, *J* = 8.7 Hz, 2H), 6.73 (d, *J* = 8.7 Hz, 2H), 4.16 (q, *J* = 7.1 Hz, 2H), 3.02–2.94 (m, 2H), 2.77–2.69 (m, 2H), 2.65–2.58 (m, 2H), 2.35–2.26 (m, 2H), 1.70–1.55 (m, 4H), 1.29 (t, *J* = 7.1 Hz, 3H), 0.97 (s, 9H), 0.18 (s, 6H). LCMS (ESI) *m*/*z*: 456.44 $[M+H]^+$.

*5.1.16.3. Ethyl 2-(5-(2-fluorophenyl)pent-4-ynamido)cyclohex-1-ene-1-carboxylate (**17e**).* Compound **17e** was prepared in the same manner as compound **17a** using 1-fluoro-2-iodobenzene (56 mg, 0.25 mmol), acetylene **18** (55 mg, 0.22 mmol), triethylamine (0.15 ml, 1.07 mmol), $PdCl_2[PPh_3]_2$ (8 mg, 0.01 mmol), and CuI (2 mg, 0.01 mmol). The product **17e** (54 mg, 71%) was obtained as a brown solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 11.70 (s, 1H), 7.37 (td, *J* = 7.6, 1.9 Hz, 2H), 7.29–7.19 (m, 1H), 7.09–6.98 (m, 2H), 4.16 (q, *J* = 7.1 Hz, 2H), 3.01–2.95 (m, 2H), 2.85–2.76 (m, 2H), 2.71–2.60 (m, 2H), 2.35–2.27 (m, 2H), 1.68–1.53 (m, 4H), 1.28 (t, *J* = 7.1 Hz, 4H). LCMS (ESI) *m*/*z*: 344.23 $[M+H]^+$.

*5.1.16.4. Ethyl 2-(5-(4-((tert-butyldimethylsilyl)oxy)-2-fluorophenyl)pent-4-ynamido)cyclohex-1-ene-1-carboxylate (**17f**).* Compound **17f** was prepared in the same manner as compound **17a** using iodide **6g** (0.22 g, 0.63 mmol), acetylene **18** (0.13 g, 0.52 mmol), triethylamine (0.36 ml, 2.61 mmol), $PdCl_2[PPh_3]_2$ (18 mg, 0.03 mmol), and CuI (5 mg, 0.03 mmol). The product **17f** (0.15 g, 62%) was obtained as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 11.68 (s, 1H), 7.21 (t, *J* = 8.5 Hz, 1H), 6.56–6.49 (m, 2H), 4.16 (q, *J* = 7.1 Hz, 2H), 3.01–2.95 (m, 2H), 2.78 (t, *J* = 7.3 Hz, 2H), 2.67–2.60 (m, 2H), 2.35–2.27 (m, 2H), 1.67–1.55 (m, 4H), 1.29 (t, *J* = 7.1 Hz, 3H), 0.96 (s, 9H), 0.19 (s, 6H). LCMS (ESI) *m*/*z*: 358.00 $[M-C_6H_{15}Si]^+$.

*5.1.16.5. Ethyl 2-(5-(4-((tert-butyldimethylsilyl)oxy)-2-chlorophenyl)pent-4-ynamido)cyclohex-1-ene-1-carboxylate (**17g**).* Compound **17g** was prepared in the same manner as compound **17a** using iodide **6h** (0.23 g, 0.63 mmol), acetylene **18** (0.13 g, 0.52 mmol), triethylamine (0.36 ml, 2.61 mmol), $PdCl_2[PPh_3]_2$ (18 mg, 0.03 mmol), and CuI (5 mg, 0.03 mmol). The product **17g** (94 mg, 37%) was obtained as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 11.69 (s, 1H), 7.27 (d, *J* = 8.4 Hz, 1H), 6.85 (d, *J* = 2.4 Hz, 1H), 6.64 (dd, *J* = 8.4, 2.4 Hz, 1H), 4.16 (q, *J* = 7.1 Hz, 2H), 3.04–2.93 (m, 2H), 2.85–2.73 (m, 2H), 2.68–2.61 (m, 2H), 2.37–2.25 (m, 2H), 1.68–1.54 (m, 4H), 1.29 (t, *J* = 7.1 Hz, 2H), 0.96 (s, 9H), 0.19 (s, 6H). LCMS (ESI) *m*/*z*: 490.39 $[M+H]^+$.

*5.1.16.6. Ethyl 2-(5-(5-hydroxypyridin-2-yl)pent-4-ynamido)cyclohex-1-ene-1-carboxylate (**17h**).* Compound **17h** was prepared in the same manner as compound **17a** using iodide **6i** (0.23 g, 0.68 mmol), acetylene **18** (0.13 g, 0.52 mmol), triethylamine (0.36 ml, 2.61 mmol), $PdCl_2[PPh_3]_2$ (18 mg, 0.03 mmol), and CuI (5 mg, 0.03 mmol). The product **17h** (74 mg, 41%) was obtained as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 11.74 (s, 1H), 8.47 (dd, *J* = 4.8, 1.3 Hz, 1H), 7.65 (dt, *J* = 8.3, 1.1 Hz, 1H), 7.13 (dd, *J* = 8.3, 4.8 Hz, 1H), 6.67 (d, *J* = 0.8 Hz, 1H), 4.16 (q, *J* = 7.1 Hz, 1H), 3.20 (t, *J* = 7.5 Hz, 2H), 3.00–2.91 (m, 2H), 2.80 (t, *J* = 7.5 Hz, 1H), 2.36–2.24 (m, 2H), 1.69–1.53 (m, 4H), 1.29 (t, *J* = 7.1 Hz, 2H). LCMS (ESI) *m*/*z*: 343.16 $[M+H]^+$.

5.1.16.7. Methyl 4-(5-((2-(ethoxycarbonyl)cyclohex-1-en-1-yl)amino)-5-oxopent-1-yn-1-yl)benzoate (17i). Compound **17i** was prepared in the same manner as compound **17a** using methyl 4-iodobenzoate (0.16 g, 0.63 mmol), acetylene **18** (0.13 g, 0.52 mmol), triethylamine (0.36 ml, 2.61 mmol), $PdCl_2[PPh_3]_2$ (18 mg, 0.03 mmol), and CuI (5 mg, 0.03 mmol). The product **17i** (0.18 g, 90%) was obtained as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 11.72 (s, 1H), 7.94 (d, *J* = 8.6 Hz, 2H), 7.42 (d, *J* = 8.6 Hz, 1H), 4.15 (q, *J* = 7.1 Hz, 2H), 3.91 (s, 3H), 3.03–2.93 (m, 2H), 2.84–

2.72 (m, 2H), 2.70–2.58 (m, 2H), 2.36–2.26 (m, 2H), 1.70–1.55 (m, 4H), 1.28 (t, J = 7.1 Hz, 3H). LCMS (ESI) m/z: 384.39 $[M+H]^+$.

*5.1.16.8. Ethyl 2-(5-(4-fluorophenyl)pent-4-ynamido)cyclohex-1-ene-1-carboxylate (**17j**).* Compound **17j** was prepared in the same manner as compound **17a** using 1-fluoro-4-iodobenzene (0.14 g, 0.63 mmol), acetylene **18** (0.13 g, 0.52 mmol), triethylamine (0.36 ml, 2.61 mmol), $PdCl_2[PPh_3]_2$ (18 mg, 0.03 mmol), and CuI (5 mg, 0.03 mmol). The product **17j** (0.14 g, 78%) was obtained as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 11.70 (s, 1H), 7.40–7.30 (m, 2H), 7.02–6.91 (m, 2H), 4.16 (q, J = 7.1 Hz, 2H), 3.04–2.92 (m, 2H), 2.81–2.68 (m, 2H), 2.68–2.56 (m, 2H), 2.37–2.26 (m, 2H), 1.71–1.55 (m, 4H), 1.29 (t, J = 7.1 Hz, 3H). LCMS (ESI) m/z: 344.29 $[M+H]^+$.

*5.1.17. 2-(5-(2-Hydroxyphenyl)pent-4-ynamido)cyclohex-1-ene-1-carboxylic acid (**4a**)*

To a solution of ethyl carboxylate **17a** (0.12 g, 0.26 mmol) in THF (1 ml) and EtOH (1 ml) 2 N NaOH solution (0.40 ml, 0.80 mmol) was added and the mixture was stirred for 70 h at rt. The mixture was acidified with 1 N HCl to pH 2, concentrated in vacuo to ~ 2 ml, extracted with DCM, dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed on Biotage reverse phase column (MeCN in water, 10–100%) to give the product **4a** (23 mg, 28%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.46 (b s, 1H), 11.66 (s, 1H), 9.65 (b s, 1H), 7.19 (dd, J = 7.6, 1.7 Hz, 1H), 7.12 (ddd, J = 8.2, 7.3, 1.7 Hz, 1H), 6.83 (dd, J = 8.2, 1.1 Hz, 1H), 6.72 (td, J = 7.5, 1.1 Hz, 1H), 2.87–2.78 (m, 2H), 2.66 (t, J = 6.9 Hz, 2H), 2.58–2.52 (m, 2H) 2.26–2.19 (m, 2H), 1.59–1.47 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.2, 169.1, 158.1, 150.4, 132.9, 129.2, 118.9, 115.4, 110.3, 105.1, 92.2, 77.7, 36.8, 28.1, 24.4, 21.5, 21.4, 15.2. HRMS (ESI) m/z: calcd. for $C_{18}H_{20}NO_4$ $[M+H]^+$ 314.1387, found 314.1393.

*5.1.17.1. 2-(5-(3-Hydroxyphenyl)pent-4-ynamido)cyclohex-1-ene-1-carboxylic acid (**4b**).* Compound **4b** was prepared in the same manner as compound **4a** using ethyl carboxylate **17b** (0.17 g, 0.37 mmol) and 2 N NaOH (0.55 ml, 1.10 mmol). The product **4b** (55 mg, 47%) was obtained as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.67 (s, 1H), 9.61 (pl s, 1H), 7.11 (td, J = 7.5, 1.2 Hz, 1H), 6.77 (dt, J = 7.5, 1.2 Hz, 1H), 6.75–6.71 (m, 1H), 6.72 (d, J = 1.2 Hz, 1H), 2.86–2.80 (m, 2H), 2.64 (t, J = 6.8 Hz, 2H), 2.54 (t, J = 6.8 Hz, 2H), 2.27 – 2.19 (m, 2H), 1.60–1.46 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.2, 169.0, 157.2, 150.2, 129.6, 123.9, 122.1, 117.8, 115.5, 105.2, 88.6, 81.0, 36.6, 28.1, 24.4, 21.5, 21.4, 14.9. HRMS (ESI) m/z: calcd. for $C_{18}H_{20}NO_4$ $[M+H]^+$ 314.1387, found 314.1388.

*5.1.17.2. 2-(5-(4-Hydroxyphenyl)pent-4-ynamido)cyclohex-1-ene-1-carboxylic acid (**4c**).* Compound **4c** was prepared in the same manner as compound **4a** using ethyl carboxylate **17c** (90 mg, 0.20 mmol) and 2 N NaOH (0.31 ml, 0.62 mmol). The product **4c** (26 mg, 41%) was obtained as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.55 (b s, 1H), 11.65 (s, 1H), 9.72 (b s, 1H), 7.23–7.12 (m, 2H), 6.74–6.65 (mz, 2H), 2.87–2.80 (m, 2H), 2.61 (t, J = 6.8 Hz, 2H), 2.54–2.49 (m, 2H, overlapped with DMSO), 2.26–2.18 (m, 2H), 1.62–1.44 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.1, 169.1, 157.3, 150.4, 132.7, 115.5, 113.2, 105.0, 86.5, 81.2, 36.9, 28.1, 24.4, 21.5, 21.4, 15.0. HRMS (ESI) m/z: calcd. for $C_{18}H_{20}NO_4$ $[M+H]^+$ 314.1387, found 314.1402.

*5.1.17.3. 2-(5-(2-Chlorophenyl)pent-4-ynamido)cyclohex-1-ene-1-carboxylic acid (**4d**).* Compound **4d** was prepared in the same manner as compound **4a** using ethyl carboxylate **17d** (90 mg, 0.25 mmol) and 2 N NaOH (0.38 ml, 0.75 mmol). The product **4d** (38 mg, 46%) was obtained as white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 11.45 (s, 1H), 10.10 (pl s, 1H), 7.43–7.39 (m, 1H), 7.37–7.33 (m, 1H), 7.20 (td, J = 7.5, 2.1 Hz, 1H), 7.16 (td, J = 7.5, 1.7 Hz, 1H), 3.06–2.99 (m, 2H), 2.83 (t, J = 7.0 Hz, 2H), 2.66 (t, J = 7.0 Hz, 2H), 2.39–2.32 (m, 2H), 1.70–1.55 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 174.4, 169.8, 155.0, 135.9, 133.5, 129.2, 128.9, 126.4, 123.5, 103.9, 93.8, 78.5, 37.5, 29.0, 24.4, 21.9, 21.7, 15.9. HRMS (ESI) m/z: calcd. for $C_{18}H_{19}ClNO_3$ $[M+H]^+$ 332.1048 $[M+H]^+$, found 332.1049.

*5.1.17.4. 2-(5-(2-Fluorophenyl)pent-4-ynamido)cyclohex-1-ene-1-carboxylic acid (**4e**).* Compound **4e** was prepared in the same manner as compound **4a** using ethyl carboxylate **17e** (95 mg, 0.28 mmol) and 2 N NaOH (0.42 ml, 0.84 mmol). The product **4e** (26 mg, 30%) was obtained as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 11.44 (s, 1H), 10.33 (b s, 1H), 7.37 (td, J = 7.6, 1.9 Hz, 1H), 7.29–7.20 (m, 1H), 7.08–7.04 (m, 1H), 7.04–6.99 (m, 1H), 3.06–2.95 (m, 2H), 2.85–2.75 (m, 2H), 2.70–2.60 (m, 2H), 2.42–2.26 (m, 2H), 1.72–1.51 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 174.4, 169.8, 162.9 (d, J_{CF} = 250.7 Hz), 154.9, 133.7, 129.6 (d, J_{CF} = 8.0 Hz), 123.9 (d, J_{CF} = 3.7 Hz), 115.5 (d, J_{CF} = 21.0 Hz), 112.1 (d, J_{CF} = 15.9 Hz), 103.8, 93.6 (d, J_{CF} = 3.4 Hz), 75.0, 37.5, 29.0, 24.4, 21.9, 21.7, 15.8. HRMS (ESI) m/z: calcd. for $C_{18}H_{19}FNO_3$ $[M+H]^+$ 316.1343, found 316.1344.

*5.1.17.5. 2-(5-(2-Fluoro-4-hydroxyphenyl)pent-4-ynamido)cyclohex-1-ene-1-carboxylic acid (**4f**).* Compound **4f** was prepared in the same manner as compound **4a** using ethyl carboxylate **17f** (0.15 g, 0.32 mmol) and 2 N NaOH (0.48 ml, 0.96 mmol). The product **4f** (46 mg, 44%) was obtained as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.51 (b s, 1H), 11.63 (s, 1H), 10.24 (b s, 1H), 7.21 (t, J = 8.7 Hz, 1H), 6.62–6.64 (m, 2H), 2.87–2.78 (m, 2H), 2.65 (t, J = 7.1 Hz, 2H), 2.58–2.50 (m, 2H, overlapped with DMSO), 2.27–2.18 (m, 2H), 1.61–1.46 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.1, 169.0, 162.9 (d, J_{CF} = 247.5 Hz), 159.0 (d, J_{CF} = 11.7 Hz), 150.3, 134.0 (d, J_{CF} = 3.4 Hz), 111.9 (d, J_{CF} = 2.8 Hz), 105.0, 102.8 (d, J_{CF} = 23.2 Hz), 101.3 (d, J_{CF} = 15.8 Hz), 91.9, 74.4, 36.6, 28.1, 24.4, 21.5, 21.4, 15.0. HRMS (ESI) m/z: calcd. for $C_{18}H_{19}FNO_4$ $[M+H]^+$ 332.1293, found 332.1303.

*5.1.17.6. 2-(5-(2-Chloro-4-hydroxyphenyl)pent-4-ynamido)cyclohex-1-ene-1-carboxylic acid (**4g**).* Compound **4g** was prepared in the same manner as compound **4a** using ethyl carboxylate **17g** (94 mg, 0.19 mmol) and 2 N NaOH (0.29 ml, 0.58 mmol). The product **4g** (29 mg, 44%) was obtained as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.55 (b s, 1H), 11.67 (s, 1H), 10.21 (b s, 1H), 7.27 (d, J = 8.5 Hz, 1H), 6.85 (d, J = 2.4 Hz, 1H), 6.69 (dd, J = 8.5, 2.4 Hz, 1H), 2.90–2.78 (m, 2H), 2.67 (t, J = 7.0 Hz, 2H), 2.53 (t, J = 7.0 Hz, 2H, overlapped with DMSO), 2.29–2.16 (m, 2H), 1.62–1.43 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.1, 169.0, 158.1, 150.4, 135.2, 134.2, 115.9, 114.7, 112.9, 105.0, 91.8, 77.8, 36.8, 28.1, 24.4, 21.5, 21.4, 15.2. HRMS (ESI) m/z: calcd. for $C_{18}H_{19}ClNO_4$ $[M+H]^+$ 348.0997, found 348.0988.

*5.1.17.7. 2-(5-(5-Hydroxypyridin-2-yl)pent-4-ynamido)cyclohex-1-ene-1-carboxylic acid (**4h**).* Compound **4h** was prepared in the same manner as compound **4a** using ethyl carboxylate **17h** (73 mg, 0.21 mmol) and 2 N NaOH (0.32 ml, 0.64 mmol). The product **4h** (18 mg, 27%) was obtained as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.57 (b s, 1H), 11.65 (s, 1H), 8.49 (b s, 1H). 7.91 (d, J = 8.3 Hz, 1H), 7.25 (dd, J = 8.3, 4.6 Hz, 1H), 6.80 (s, 1H), 3.10 (t, J = 7.3 Hz, 2H), 2.86–2.70 (m, 4H), 2.26–2.15 (m, 2H), 1.62–1.45 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.1, 169.1, 162.5, 150.3, 148.3, 146.9, 145.4, 118.4, 117.5, 105.2, 103.4, 34.6, 28.1, 24.4, 23.7, 21.4, 21.3. HRMS (ESI) m/z: calcd. for $C_{17}H_{19}N_2O_4$ $[M+H]^+$ 315.1339, found 315.1340.

*5.1.17.8. 4-(5-((2-Carboxycyclohex-1-en-1-yl)amino)-5-oxopent-1-yn-1-yl)benzoic acid (**4i**).* Compound **4i** was prepared in the same manner as compound **4a** using ethyl carboxylate **17i** (0.18 g, 0.47 mmol) and 2 N NaOH (0.70 ml, 1.40 mmoil). The product **4i** (86 mg, 54%) was obtained as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.81 (s, 2H), 11.65 (s, 1H), 7.88 (d, J = 8.4 Hz, 2H), 7.46 (d, J = 8.4 Hz, 2H), 2.87–2.79 (m, 2H), 2.74–2.66 (m, 2H), 2.62–2.54 (m, 2H), 2.27–2.19 (m, 2H), 1.61–1.45 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.1, 169.0, 166.7, 150.3, 131.4, 130.0, 129.4, 127.4, 105.1, 92.5, 80.3, 36.4, 28.1, 24.4, 21.5, 21.4, 15.0. HRMS (ESI) m/z: calcd. for $C_{19}H_{20}NO_5$ $[M+H]^+$ 342.1336, found 342.1314.

*5.1.17.9. 2-(5-(4-Fluorophenyl)pent-4-ynamido)cyclohex-1-ene-1-carboxylic acid (**4j**).* Compound **4j** was prepared in the same manner as compound **4a** using ethyl carboxylate **17j** (0.13 g, 0.38 mmol) and 2 N NaOH (0.57 ml, 1.14 mmol). The product **4j** (63 mg, 53%) was obtained as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 11.50 (b s, 1H), 11.43 (s, 1H), 7.38–7.31 (m, 2H), 7.00–6.90 (m, 2H), 3.06–2.97 (m, 2H), 2.79–2.70 (m, 2H), 2.67–2.58 (m, 2H), 2.40–2.27 (m, 2H), 1.70–1.56 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 174.5, 169.9, 162.3 (d, J_{CF} = 248.5 Hz), 155.1, 133.5 (d, J_{CF} = 8.3 Hz), 119.7 (d, J_{CF} = 3.4 Hz), 115.5 (d, J_{CF} = 21.8 Hz), 103.7, 87.8, 80.6, 37.6, 29.0, 24.4, 21.9, 21.7, 15.6. HRMS (ESI) m/z: calcd. for $C_{18}H_{19}FNO_3$ $[M+H]^+$ 316.1343, found 316.1349.

*5.1.18. 3-(4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl) propanoic acid (**20**)*

A suspension of 3-(4-iodophenyl)propanoic acid (**19**) (0.50 g, 1.81 mmol), bis(pinacolato)diboron (0.69 g, 2.72 mmol), and KOAc (0.71 g, 7.24 mmol) in DMF (1 ml) was degassed by bubbling argon through the mixture for 15 min, then Pd(dppf)Cl_2 (66 mg, 0.09 mmol) was added to this mixture. The reaction was heated to 80 °C for 2.5 h. The reaction mixture was cooled to rt, filtered through a pad of silica gel, and washed with EtOAc. The filtrate was concentrated under reduced pressure and purified by column chromatography on silica gel (EtOAc in petroleum ether, 1:2) to give the product **20** (0.32 g, 63%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 10.43 (b s, 1H), 7.75 (d, J = 8.0 Hz, 2H), 7.22 (d, J = 8.0 Hz, 2H), 2.98 (t, J = 7.8 Hz, 2H), 2.68 (t, J = 7.8 Hz, 2H), 1.34 (s, 12H). LCMS (ESI) m/z: 277.09 $[M+H]^+$.

*5.1.19. 3-(2′-Chloro-[1,1′-biphenyl]-4-yl)propanoic acid (**21**)*

A solution of boronate **20** (0.16 g, 0.58 mmol) and 1-chloro-2-iodobenzene (0.14 g, 0.59 mmol) in dioxane (2.4 ml) was degassed by bubbling argon through the mixture for 15 min, then 2 M K_2CO_3 (0.87 ml, 1.74 mmol) and Pd(dppf)Cl_2 (21 mg, 0.03 mmol) under argon atmosphere were added. The reaction flask was sealed and stirred for 2 h at 80 °C. The mixture was cooled to rt, diluted with saturated NH_4Cl solution, extracted with EtOAc, and the organic phase was dried over Na_2SO_4. The extract was concentrated and the residue was purified by column chromatography on silica gel (MeOH in DCM, 1:20) to give the product **21** (0.14 g, 91%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 10.70 (b s, 1H), 7.53–7.23 (m, 8H), 3.03 (t, J = 7.8 Hz, 2H), 2.76 (t, J = 7.8 Hz, 2H). LCMS (ESI) m/z: 259.04 $[M+H]^+$.

*5.1.20. Ethyl 2-(3-(2′-chloro-[1,1′-biphenyl]-4-yl) propanamido)cyclohex-1-ene-1-carboxylate (**22d**)*

Compound **22d** was prepared in the same manner as compound **18** using acid **21** (0.13 g, 0.50 mmol), oxalyl chloride (0.13 ml, 1.50 mmol), ethyl 2-aminocyclohex-1-ene-1-carboxylate (0.10 g, 0.59 mmol), and triethylamine (0.07 ml, 0.50 mmol). The product **22d** (0.14 g) was obtained as a colorless oil which contained starting amine (^{1}H NMR ratio **22d**:amine = *ca* 1:0.3) as an impurity, calc. yield 58%. The crude product **22d** was used in the next step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 11.67 (s, 1H), 7.51–7.27 (m, 8H), 4.19 (q, J = 7.1 Hz, 3H), 3.11–3.03 (m, 2H), 3.03–2.96 (m, 2H), 2.74–2.65 (m, 2H), 2.36–2.29 (m, 2H), 1.74–1.53 (m, 4H), 1.31 (t, J = 7.1 Hz, 3H). LCMS (ESI) m/z: 412.21 $[M+H]^+$.

*5.1.21. Ethyl 2-(3-(4-iodophenyl)propanamido)cyclohex-1-ene-1-carboxylate (**23**)*

Compound **23** was prepared in the same manner as compound **18** using 3-(4-iodophenyl)propanoic acid (**19**) (0.79 g, 2.86 mmol), oxalyl chloride (0.62 ml, 7.15 mmol), ethyl 2-aminocyclohex-1-ene-1-carboxylate (0.73 g, 4.29 mmol), and triethylamine (0.40 ml, 2.86 mmol). The product **23** (0.53 g, 43%) was obtained as a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 11.60 (s, 1H), 7.62–7.56 (m, 2H), 7.06–6.89 (m, 2H), 4.16 (q, J = 7.1 Hz, 2H), 3.00–2.85 (m, 4H), 2.60 (t, J = 7.7 Hz, 2H), 2.35–2.26 (m, 2H), 1.66–1.52 (m, 4H), 1.29 (t, J = 7.1 Hz, 2H). LCMS (ESI) m/z: 428.14 $[M+H]^+$.

*5.1.22.1. Ethyl 2-(3-(2′-hydroxy-[1,1′-biphenyl]-4-yl)propanamido)-cyclohex-1-ene-1-carboxylate (**22a**).* Compound **22a** was prepared in the same manner as compound **21** using iodide **23** (0.16 g, 0.37 mmol), 2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol (0.11 g, 0.49 mmol), 2 M K_2CO_3 (0.56 ml, 1.12 mmol), and Pd(dppf)Cl_2 (14 mg, 0.02 mmol). The product **28a** (0.11 g, 77%) was obtained as a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 11.62 (s, 1H), 7.39 (d, J = 8.3 Hz, 2H), 7.34 (d, J = 8.3 Hz, 2H), 7.28–7.23 (m, 1H), 7.22 (dd, J = 7.9, 1.8 Hz, 1H), 6.98 (td, J = 7.5, 1.2 Hz, 1H), 6.98 (dd, J = 8.0, 1.2 Hz, 1H), 5.22 (s, 1H), 4.17 (q, J = 7.1 Hz, 2H), 3.11–3.00 (m, 2H), 3.01–2.93 (m, 2H), 2.73–2.64 (m, 2H), 2.35–2.26 (m, 2H), 1.67–1.55 (m, 4H), 1.29 (t, J = 7.1 Hz, 3H). LCMS (ESI) m/z: 394.37 $[M+H]^+$.

*5.1.22.2. Ethyl 2-(3-(3′-hydroxy-[1,1′-biphenyl]-4-yl)propanamido)-cyclohex-1-ene-1-carboxylate (**22b**).* Compound **22b** was prepared in the same manner as compound **21** using iodide **23** (0.14 g, 0.33 mmol), (3-hydroxyphenyl)boronic acid (59 mg, 0.43 mmol), 2 M K_2CO_3 (0.49 ml, 0.98 mmol), and Pd(dppf)Cl_2 (12 mg, 0.02 mmol). The product **22b** (96 mg, 75%) was obtained as a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 11.65 (s, 1H), 7.48 (d, J = 8.1 Hz, 2H), 7.34–7.22 (m, 3H), 7.14 (ddd, J = 7.8, 1.5, 0.9 Hz, 1H), 7.04 (dd, J = 2.4, 1.5 Hz, 1H), 6.80 (ddd, J = 8.0, 2.4, 0.9 Hz, 1H), 5.02 (s, 1H), 4.16 (q, J = 7.1 Hz, 2H), 3.07–2.92 (m, 4H), 2.67 (t, J = 7.8 Hz, 2H), 2.34–2.25 (m, 2H), 1.70–1.52 (m, 4H), 1.28 (t, J = 7.1 Hz, 3H). LCMS (ESI) m/z: 394.38 $[M+H]^+$.

*5.1.22.3. Ethyl 2-(3-(4′-hydroxy-[1,1′-biphenyl]-4-yl)propanamido)-cyclohex-1-ene-1-carboxylate (**22c**).* Compound **22c** was prepared in the same manner as compound **21** using iodide **23** (0.14 g, 0.33 mmol), (4-hydroxyphenyl)boronic acid (59 mg, 0.43 mmol), 2 M K_2CO_3 (0.49 ml, 0.98 mmol), and Pd(dppf)Cl_2 (12 mg, 0.02 mmol). The product **22c** (0.11 g, 82%) was obtained as a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 11.65 (s, 1H), 7.48–7.41 (m, 4H), 7.28–7.22 (m, 2H overlapped with $CHCl_3$), 6.91–6.85 (m, 2H), 5.10 (s, 1H), 4.17 (q, J = 7.1 Hz, 2H), 3.08–2.93 (m, 4H), 2.73–2.61 (m, 2H), 2.35–7.26 (m, 2H), 1.71–1.52 (m, 4H), 1.29 (t, J = 7.1 Hz, 3H). LCMS (ESI) m/z: 394.36 $[M+H]^+$.

*5.1.22.4. Ethyl 2-(3-(2′-fluoro-[1,1′-biphenyl]-4-yl)propanamido)cyclohex-1-ene-1-carboxylate (**22e**).* Compound **22e** was prepared in the same manner as compound **21** using iodide **23** (0.14 g, 0.33 mmol), 2-(2-fluorophenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (0.10 g, 0.45 mmol), 2 M K_2CO_3 (0.49 ml, 0.98 mmol), and Pd(dppf)Cl_2 (19 mg, 0.03 mmol). The product **22e** (0.10 g) was obtained as a colorless oil, which contained unidentified impurities and was used in the next step without further purification. ^{1}H

NMR (300 MHz, $CDCl_3$) δ: 11.65 (s, 1H), 7.52–7.10 (m, 8H), 4.17 (q, J = 7.1 Hz, 2H), 3.10–2.90 (m, 4H), 2.72–2.63 (m, 2H), 2.36–2.24 (m, 2H), 1.70–1.55 (m, 4H), 1.29 (t, J = 7.1 Hz, 3H). LCMS (ESI) m/z: 396.43 $[M+H]^+$.

*5.1.22.5. Ethyl 2-(3-(2′-fluoro-4′-hydroxy-[1,1′-biphenyl]-4-yl) propanamido)cyclohex-1-ene-1-carboxylate (**22f**).* Compound **22f** was prepared in the same manner as compound **21** using iodide **23** (0.18 g, 0.42 mmol), 3-fluoro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol (0.13 g, 0.55 mmol), 2 M K_2CO_3 (0.63 ml, 1.26 mmol), and Pd(dppf)Cl_2 (25 mg, 0.03 mmol). The product **22f** (0.15 g, 87%) was obtained as a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 11.66 (s, 1H), 7.40 (dd, J = 8.3, 1.7 Hz, 2H), 7.30–7.21 (m, 3H), 6.70–6.62 (m, 2H), 5.34 (s, 1H), 4.17 (q, J = 7.1 Hz, 2H), 3.06–2.94 (m, 4H), 2.73–2.62 (m, 2H), 2.36–2.26 (m, 2H), 1.69–1.54 (m, 4H), 1.29 (t, J = 7.1 Hz, 3H). LCMS (ESI) m/z: 412.29 $[M+H]^+$.

*5.1.23. 2-(3-(2′-Chloro-[1,1′-biphenyl]-4-yl)propanamido)cyclohex-1-ene-1-carboxylic acid (**5d**)*

To a solution of crude ethyl carboxylate **22d** (0.13 g, 0.32 mmol) in THF (1 ml) and EtOH (1 ml) 2 N NaOH solution (0.47 ml, 0.95 mmol) was added and the mixture was stirred for 8 h at 50 °C. The mixture was acidified with 1 N HCl to pH 5, concentrated to ~ 2 ml, and extracted with $CHCl_3$. The extract was dried over Na_2SO_4, concentrated and the residue was chromatographed on Biotage reverse phase column (MeCN in water, 10–100%) to give the product **5d** (71 mg, 40%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 11.38 (s, 1H), 10.38 (b s, 1H), 7.48–7.43 (m, 1H), 7.40–7.35 (m, 2H), 7.33–7.23 (m, 5H), 3.08–2.96 (m, 4H), 2.73–2.63 (m, 2H), 2.38–2.30 (m, 2H), 1.70–1.54 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 174.6, 171.0, 155.1, 140.4 140.0, 137.5, 132.6, 131.5, 130.1, 129.8, 128.54, 128.2, 126.9, 103.6, 40.1, 31.1, 29.0, 24.4, 21.9, 21.7. LCMS (ESI) m/z: 382.10 $[M-H]^-$. Anal. Calcd for $C_{22}H_{22}ClNO_3$: C 68.84, H 5.78, N 3.65. Found: C 68.82, H 5.78, N 3.62.

*5.1.23.1. 2-(3-(2′-Hydroxy-[1,1′-biphenyl]-4-yl)propanamido)cyclohex-1-ene-1-carboxylic acid (**5a**).* Compound **5a** was prepared in the same manner as compound **5d** using ethyl carboxylate **22a** (0.11 g, 0.28 mmol) and 2 N NaOH (0.42 ml, 0.84 mmol). The product **5a** (38 mg, 37%) was obtained as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.54 (b s, 1H), 11.65 (s, 1H), 9.45 (b s, 1H), 7.45 (d, J = 8.3 Hz, 2H), 7.24 (d, J = 8.3 Hz, 2H), 7.22 (dd, J = 7.7, 1.7 Hz, 1H), 7.03 (ddd, J = 8.1, 7.3, 1.7 Hz, 1H), 6.92 (dd, J = 8.1, 1.2 Hz, 1H), 6.85 (td, J = 7.4, 1.2 Hz, 1H), 2.88 (t, J = 7.6 Hz, 2H), 2.87–2.78 (m, 2H), 2.62 (t, J = 7.6 Hz, 2H), 2.26–2.18 (m, 2H), 1.60–1.45 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.2, 169.9, 154.3, 150.6, 138.9, 136.3, 130.2, 129.0, 128.2, 127.8, 127.5, 119.4, 116.0, 104.7, 39.0, 30.1, 28.2, 24.4, 21.5, 21.4. LCMS (ESI) m/z: 364.18 $[M-H]^-$. Anal. Calcd for $C_{22}H_{23}NO_4$: C 72.31, H 6.34, N 3.83. Found: C 72.04, H 6.37, N 4.08.

*5.1.23.2. 2-(3-(3′-Hydroxy-[1,1′-biphenyl]-4-yl)propanamido)cyclohex-1-ene-1-carboxylic acid (**5b**).* Compound **5b** was prepared in the same manner as compound **5d** using ethyl carboxylate **22b** (95 mg, 0.24 mmol) and 2 N NaOH (0.36 ml, 0.72 mmol). The product **5b** (32 mg, 36%) was obtained as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.53 (b s, 1H), 11.63 (s, 1H), 9.48 (s, 1H), 7.50 (d, J = 8.2 Hz, 2H), 7.29 (d, J = 8.3 Hz, 2H), 7.23 (t, J = 7.9 Hz, 1H), 7.03 (ddd, J = 7.7, 1.6, 0.9 Hz, 1H), 6.98 (t, J = 2.0 Hz, 1H), 6.74 (ddd, J = 8.0, 2.5, 0.9 Hz, 1H), 2.89 (t, J = 7.5 Hz, 2H), 2.85–2.78 (m, 2H), 2.61 (t, J = 7.5 Hz, 2H), 2.25–2.17 (m, 2H), 1.61–1.45 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.2, 169.9, 157.8, 150.6, 141.4, 139.9, 138.1, 129.9, 128.8, 126.5, 117.3, 114.2, 113.3, 104.7, 30.0, 28.2, 24.3, 21.6, 21.4. LCMS (ESI) m/z: 364.23 $[M-H]^-$. Anal. Calcd for $C_{22}H_{23}NO_4 \times 0.15\ H_2O$ (0.7%): C 71.78, H 6.38, N 3.80. Found: C 71.71, H 6.20, N 3.75.

*5.1.23.3. 2-(3-(4′-Hydroxy-[1,1′-biphenyl]-4-yl)propanamido)cyclohex-1-ene-1-carboxylic acid (**5c**).* Compound **5c** was prepared in the same manner as **5d** using ethyl carboxylate **22c** (0.11 g, 0.28 mmol) and 2 N NaOH (0.40 ml, 0.80 mmol). The product **5c** (60 mg, 62%) was obtained as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.53 (b s, 1H), 11.66 (b s, 1H), 9.48 (s, 1H), 7.51–7.41 (m, 4H), 7.29–7.22 (m, 2H), 6.85–6.79 (m, 2H), 2.87 (t, J = 7.5 Hz, 2H), 2.85–2.77 (m, 2H), 2.64–2.56 (m, 2H), 2.25–2.17 (m, 2H), 1.61–1.45 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.2, 169.9, 156.9, 150.5, 138.8, 138.0, 130.8, 128.7, 127.5, 125.9, 115.7, 104.8, 39.0, 30.0, 28.1, 24.4, 21.5, 21.4. LCMS (ESI) m/z: 364.20 $[M-H]^-$. Anal. Calcd for $C_{22}H_{23}NO_4$: C 72.31, H 6.34, N 3.83. Found: C 71.62, H 6.28, N 3.64.

*5.1.23.4. 2-(3-(2′-Fluoro-[1,1′-biphenyl]-4-yl)propanamido)cyclohex-1-ene-1- carboxylic acid (**5e**).* Compound **5e** was prepared in the same manner as compound **5d** using ethyl carboxylate **22e** (0.12 g, 0.30 mmol) and 2 N NaOH (0.46 ml, 0.92 mmol). The product **5e** (59 mg, 53%) was obtained as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 11.37 (s, 1H), 10.37 (pl s, 1H), 7.51–7.45 (m, 2H), 7.41 (td, J = 7.7, 1.9 Hz, 1H), 7.33–7.25 (m, 3H), 7.23–7.15 (m, 1H), 7.13 (ddd, J = 10.8, 8.0, 1.3 Hz, 1H), 3.07–2.95 (m, 4H), 2.72–2.63 (m, 2H), 2.38–2.28 (m, 2H), 1.70–1.54 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 174.4, 170.9, 159.9 (d, $^1J_{CF}$ = 247.6 Hz), 155.1, 140.2, 133.9 (J_{CF} = 1.0 Hz), 130.8 (d, J_{CF} = 3.5 Hz), 129.3 (d, J_{CF} = 3.0 Hz), 128.9 (d, J_{CF} = 8.5 Hz), 128.6, 128.1 (d, J_{CF} = 1.8 Hz), 124.4 (d, J_{CF} = 3.7 Hz), 116.2 (d, J_{CF} = 22.6 Hz), 103.6, 40.2, 31.0, 29.0, 24.4, 21.9, 21.7. LCMS (ESI) m/z: 366.22 $[M-H]^-$. Anal. Calcd for $C_{22}H_{23}FNO_3$: C 71.95, H 6.04, N 3.81. Found: C 71.95, H 6.06, N 3.67.

*5.1.23.5. 2-(3-(2′-Fluoro-4′-hydroxy-[1,1′-biphenyl]-4-yl)propanamido) cyclohex-1-ene-1-carboxylic acid (**5f**).* Compound **5f** was prepared in the same manner as compound **5d** using ethyl carboxylate **22f** (0.15 g, 0.36 mmol) and 2 N NaOH (0.55 ml, 1.10 mmol). The product **5f** (80 mg, 57%) was obtained as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.54 (b s, 1H), 11.65 (s, 1H), 9.97 (b s, 1H), 7.41–7.23 (m, 5H), 6.69 (dd, J = 8.3, 2.5 Hz, 1H), 6.64 (dd, J = 12.8, 2.2 Hz, 1H), 2.88 (t, J = 7.6 Hz, 2H). 2.84–2.78 (m, 2H), 2.61 (t, J = 7.6 Hz, 2H), 2.26–2.17 (m, 2H), 1.60–1.46 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.0, 169.8, 159.6 (d, J_{CF} = 245.0 Hz), 158.3 (d, J_{CF} = 11.7 Hz), 150.4, 139.5, 133.2 (d, J_{CF} = 1.9 Hz), 130.9 (d, J_{CF} = 5.6 Hz), 128.4, 128.3 (d, J_{CF} = 3.0 Hz), 118.7 (d, J_{CF} = 13.4 - Hz), 112.1 (d, J_{CF} = 2.7 Hz), 104.8, 103.0 (d, J_{CF} = 25.1 Hz), 38.9, 30.1, 28.1, 24.4, 21.5, 21.4. LCMS (ESI) m/z: 382.21 $[M-H]^-$. Anal. Calcd for $C_{22}H_{22}FNO_4$: C 68.92, H 5.78, N 3.65. Found: C 68.51, H 5.71, N 3.52.

5.2. Biology

5.2.1. Intracellular cAMP assay

Flp-In-293 cells (Invitrogen) stably expressing human HCA2 receptor were grown in DMEM medium supplemented with 10% fetal bovine serum, penicillin, streptomycin and hygromycin (100 μg/ml each). For cAMP assay cells were distributed into a 384-well white microplate at a density 12,000 cells /well and stimulated with the indicated compounds in the presence of 3 μM forskolin for 30 min. Cells and the compounds were diluted in 1X phosphate-buffered saline (PBS) supplemented with 0.5 mM 3-isobutyl-1-methylxanthine (Sigma-Aldrich). Intracellular cAMP levels were measured using a Lance cAMP kit (Perkin Elmer) according to the manufacturer's instructions. Plates were read on Victor3V™ multilabel reader (Perkin-Elmer). The cAMP was quantitated for

each sample by comparison to a standard curve of known amounts of cAMP provided in the kit. Data was analyzed using the GraphPad Prism version 5.00 for Windows (GraphPad Software, La Jolla, CA).

5.2.1.1. Cell membrane preparation. HCA2 receptor expressing Flp-In-293 cells were grown to confluence in 150 cm^2 culture dishes. Cells were rinsed twice with PBS and harvested by centrifugation at 1000*g* for 5 min at 4 °C. Cell pellet was resuspended in ice- cold 10 mM Tris-HCl, 0.1 mM EDTA, pH 7.6 (TE buffer), supplemented with 1× Halt protease inhibitor cocktail (Thermo Scientific) before disruption by Covaris acoustic cell disrupter. Cell debris was removed by centrifugation at 1000*g* for 5 min and the supernatant was subsequently centrifuged at 21,000*g* for 30 min at 4 °C. The final pellet was re-suspended in TE buffer and stored at −80 °C. Protein concentrations were determined by Pierce BCA protein assay kit (Thermo Scientific).

5.2.1.2. Radioligand binding assay. Cell membranes were diluted in binding buffer 50 mM Tris-HCl, 1 mM $MgCl_2$, 0.02% CHAPS pH 7.6 and aliquoted (25 μg/well) into 96-well plates. Competition binding assays were conducted in the presence of 20 nM (5,6-^{3}H)-nicotinic acid (50 Ci/mmol) (American Radiolabeled Chemicals, USA) and increasing concentrations of unlabelled compounds. After 2 h incubation at room temperature, membrane-bound radioligand was separated from unbound by filtration of the samples through GF/B filters (Perkin Elmer) and washed three times with ice-cold binding buffer using a FilterMate harvester (Perkin Elmer). Radioactivity was counted using a Microbeta[2] counter (Perkin Elmer) and data analysed with a software GraphPad Prism version 5.00. The binding assays were performed in duplicates and repeated three times for each compound.

5.3. Computational

Schrödinger[22] LigPrep was used to produce low energy 3D structures of compounds. Conformations were minimized using OPLS3 force field. The ionization/tautomeric states were generated using Epik.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2017.06.028. These data include MOL files and InChiKeys of the most important compounds described in this article.

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