Aptamers ISSN 2514-3247 Aptamers, 2021, Vol 5, 15-21

RESEARCH ARTICLE

Chemical conjugations of Sgc8-c with the lymphoma drug dasatinib to generate selective biotherapeutics

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Received: 16 July 2021 | **Revised:** 13 October 2021 | **Accepted:** 18 October 2021 | **Published:** 18 October 2021

ABSTRACT

The conjugation of drugs to target therapeutics has become a promising method that could improve the efficacy of therapy and reduce side effects. Herein, we describe the efforts to covalently link the anti-lymphoma agent dasatinib to the truncated aptamer Sgc8-c, expecting the new hybrids to specifically damage lymphoma cells but with minimal toxicity towards non-target cells. Two linkages, ester and carbamate, with variable pH labilities were used to connect Sgc8-c with dasatinib. Different reaction conditions were studied by varying the solvent, time, temperature, heat source, pH and counter-ions. Each product from the reaction mixture was analysed by qualitative electrospray ionization time-of-flight mass spectrometry, identifying the nucleic acid modifications formed under the different experimental conditions. Among the reactions, depurinations from the 3´-extreme mainly occurred as lateral processes. Preparation of the carbamate-linked Sgc8-c–dasatinib hybrid **Sgc8-c-carb-da** was successful but the ester-linked hybrid only produced lateral undesired products. The potential biotherapeutic **Sgc8-c-carb-da** displayed the ability to trigger dasatinib at endosomal pH, which is optimal because this could be the aptamer's cellular uptake route.

KEYWORDS: biotherapeutics, Sgc8-c, dasatinib, drug delivery, synthesis, depurination

INTRODUCTION

The advantages of aptamers in terms of size, ease of production and ease of chemical modification (Breaker, 2004; Hwang et al, 2010) make them excellent candidates for the development of new biotechnological platforms to produce biotherapeutics (Famulok and Mayer, 1999; Hicke et al, 2006; Tong et al, 2010). Specifically, the use of aptamers as a vehicle for the selective delivery of drugs for clinical use has been described in the literature (Patil et al, 2005; Yazdian-Robati et al, 2017). The first examples included the use of DNA-intercalating drugs (Bagalkot et al, 2006; Taghdisi et al, 2010) such as doxorubicin and daunorubicin; however, these drugs could affect aptamer recognition by its target, leading to a loss of specificity. Advantages have been found using a series of covalent binding strategies between drug and aptamer at a site not relevant for recognition, thus avoiding loss of bioactivity (Zhao et al, 2015). However, the partially stable covalent bonds used, such as amides (Zhao et al, 2015), do not allow release of the drug under physiological conditions, potentially losing the activity provided by this component of the molecule.

The truncated DNA aptamer Sgc8-c, which has 41 bases and specifically binds to the PTK7 receptor (Shangguan et al, 2007), has been studied in our laboratory to develop tumour imaging agents (Calzada et al, 2017a; Calzada et al, 2017b; Sicco et al, 2018; Sicco et al, 2020). We found that

Sgc8-c was able to recognize PTK7 *in vivo* in murine melanoma and lymphoma models (Calzada et al, 2017a; Calzada et al, 2017b; Sicco et al, 2020). This behaviour provided us with the basis to develop potential therapeutic agents, using this truncated aptamer to direct anti-tumour drugs specifically to the site of action.

Herein, we propose covalently incorporating an anti-tumour agent into the structure of Sgc8-c using molecular hybridization strategies. The selected anti-tumour agent was dasatinib, which is a BCR-ABL kinase inhibitor approved by the Food and Drug Administration (USA) for the treatment of chronic myelogenous leukaemia (D´Cruz and Uckun, 2013; McCLafferty et al, 2018) and Philadelphia chromosome-positive acute lymphoblastic leukaemia (Jabbour and Kantarjian, 2016; Sasaki et al, 2020). Dasatinib is also a potent inhibitor of five other critical oncogenic tyrosine kinase families: SRC, c-KIT, PDGF receptors, Bruton tyrosine kinase (BTK) and ephrin receptor kinases (Hantschel et al, 2007; Araujo and Logothetis, 2010; Umakanthan et al, 2019). For example, inhibition of BTK can lead to the downstream mitigation of cell growth, proliferation, adhesion, migration and survival of B-cell malignancies, including chronic lymphocytic leukaemia, mantle cell lymphoma, marginal zone lymphoma and Waldenström macroglobulinaemia (Moore and Thompson, 2021). In view of this, dasatinib was incorporated into Sgc8-c using different (i.e. ester and carbamate) covalent connectors. These connectors were considered because they display different abilities to be hydrolysed, chemically or enzymatically, and hence promote the release of the drug at the site of action (Babu et al, 2020). Therefore, these potential therapeutic agents will use the aptamer as a vehicle for the selective delivery of dasatinib in tumour cells with overexpression of PTK7.

MATERIALS AND METHODS

Chemicals

All chemicals necessary for the reactions and to prepare suitable buffers were purchased from Sigma-Aldrich (St. Louis, MO, USA). Water was purified and deionized (18MΩ/cm^2) in a Milli-Q water filtration system (Millipore Corp., Milford). Dasatinib was purchased from Hong Kong Guokang Bio-Technology Co., Ltd (Baoji City, China). The 5'-(6-aminohexyl)-modified Sgc8-c truncated aptamer (12813 Da, **Sgc8-c-NH$_2$**) was purchased commercially from IDT Technologies (Integrated DNA Technologies, Inc., IA, USA).

Synthesis of dasatinib intermediates

Dasatinib phenylcarbonate (2)

This intermediate was prepared according to Carpino et al (Method II, Carpino et al, 1973) using dasatinib (**1**) in alcohol (1 equiv) and phenylchloroformate (1 equiv), modifying the reaction time (2hr) and the base (trimethylamine, 2 equiv). The reaction was monitored by thin layer chromatography (TLC) (dichloromethane:methanol = 95:5). The final product (**2**) was structurally characterized using nuclear magnetic resonance spectroscopy (^{1}H and^{13}C) and heteronuclear single quantum coherence (HSQC) and heteronuclear multiple bond correlation (HMBC) experiments.

Chloroacetyl dasatinib (3)

This intermediate was prepared according to Baker and Bordwell (Method II, Baker and Bordwell, 1955) using **1** (1 equiv) and chloroacetyl chloride (1 equiv), modifying the base (trimethylamine, 3.5 equiv) and the solvent (dry dichloromethane). The reaction was monitored by TLC (dichloromethane:methanol = 95:5). The final product (**3**) was structurally characterized using nuclear magnetic resonance spectroscopy (^{1}H and^{13}C) and HSQC and HMBC experiments.

Synthesis of Sgc8-c-carb-da

First, commercial **Sgc8-c-NH$_2$** (0.5mg) was washed with Milli-Q water and reaction buffer using Microcon® centrifugal filters (10kDa cut-off) or a PD-10 column (GE Healthcare Life Sciences, Little Chalfont, UK).

Intermediate **2** (200equiv) dissolved in dimethylsulphoxide (DMSO; 400µl for each 0.5mg of aptamer) was added to washed **Sgc8-c-NH$_2$** (1equiv) dissolved in a mixture of equal volumes (50:50) of sodium phosphate buffer (0.1M) and sodium bicarbonate buffer (0.1M) at pH 8.3 (200µl for each 0.5mg of aptamer) (Sicco et al, 2018). This mixture was left to react for 1 h at 60°C and *N*,*N*-dimethylformamide (DMF; 100µl for each 0.5mg of aptamer) was added, with the reaction maintained at 60°C for an additional 47hr. The reaction was stopped by washing **Sgc8-c-carb-da** with Milli-Q water using Microcon® centrifugal filters (10kDa cut-off). The reaction was monitored by reversed-phase high-performance liquid chromatography (RP-HPLC; Agilent 1200 Series Infinity Star, Santa Clara, USA) with a 5µm C-18 Kinetex column (150 x 4.6 mm; Phenomenex) run with an aqueous solution of triethylamine (50mM, pH 7.5)/5% (v/v) acetonitrile (solvent A) and methanol (solvent B) at a flow rate of 1ml/min and an A:B gradient of 90:10 to 40:60 for 30min (UV detection). Purification of **Sgc8-c-carb-da** was performed in the same RP-HPLC conditions. The desired product and subproducts were characterized using electrospray time-of-flight mass spectrometry (ESI-TOF-MS; IDT Technologies).

Physicochemical characterization of Sgc8-c-carba-da

Thermal stability (Sicco et al, 2018)

Sgc8-c-carba-da was incubated in Milli-Q water at 25°C, 37°C, 45°C, 60°C and 75°C for 30min. Subsequently, the mixtures were filtered (0.22µm) and analysed by RP-HPLC (see previous conditions) and gel electrophoresis. Briefly, for gel electrophoresis, 1.0µg of the sample was suspended in Milli-Q water (10µl), heated at 75°C for 10min, chilled on ice for 10min and added to electrophoresis buffer (1 µl). The samples were loaded into the gel. The gel electrophoreses were performed on 15% (w/v) native polyacrylamide gel in 1X TAE buffer using a constant voltage of 100V and a current of 0.04A. Silver nitrate staining was used for visualization. BioRad 170-8201 (20bp) was used as a molecular weight marker. These tests were carried out in duplicate.

Storage stability (Sicco et al, 2018)

The stability of **Sgc8-c-carba-da** in Milli-Q water at -20°C and 4°C for 30 days was checked weekly. At these time points, aliquots of the reaction mixture were filtered (0.22µm) and

analysed using RP-HPLC (see previous conditions) and gel electrophoresis as described above. These tests were carried out in duplicate.

Lipophilicity

Sgc8-c-carba-da was placed in an Eppendorf tube with 500µl of 1X phosphate-buffered saline (PBS, pH 7.4) and 500µl of *n*-octanol, centrifuged at 13000 rpm for 10min at room temperature and 200µL aliquots were removed for quantification on a spectrophotometer at 260nm. This test was carried out five times.

Dasatinib-releasing ability studies

To study the *in vitro* releasing capacity of the carbamate group at different pH, **Sgc8-c-carba-da** was challenged against a mixture of equal volumes (50:50) of sodium phosphate buffer (0.1M) and sodium bicarbonate buffer (0.1M) adjusted to pH 5.0, 5.5 and 7.4 with hydrochloric acid. The solutions were kept at a constant temperature of 37°C and fractions were removed at different times (0.5, 3, 6, 24, 30 and 48hr) for analysis by RP-HPLC (see previous conditions).

RESULTS

Chemical conjugations

The syntheses of the differently designed Sgc8-c–dasatinib conjugates were planned as two-step procedures. First, we prepared two different dasatinib derivatives (**2** and **3**; Figure 1) in excellent yields to study two different releasing moieties (ester and carbamate) that give us different biological responses of the bio-conjugates. Then, using RP-HPLC, we analysed the optimal experimental conditions to link **Sgc8-c-NH$_2$** to **2** or **3**. For this, we initially studied the coupling of **Sgc8-c-NH$_2$** to carbonate **2** in order to test the reaction solvents, molar ratios of reactants, time, temperature, heat source and other reaction conditions (Sicco et al, 2017; Sicco et al, 2020).

According to previous results with the coupling of **Sgc8-c-NH$_2$** (Sicco et al, 2017), we selected an initial reactant molar ratio of 1:50 (aptamer:**2**) and room temperature to study the optimum reaction solvents. In this regard, we chose a mixture of equal volumes of sodium bicarbonate buffer (0.1M, pH 8.3) and sodium phosphate buffer (0.1M, pH 8.3) as the solvent for **Sgc8-c-NH$_2$** and different organic solvents were studied to dissolve **2**. The use of DMSO, triethylamine (Et_3N) or DMF did not produce consumption of **Sgc8-c-NH$_2$** in the first 24hr of reaction (runs 2–4, Table 1) whereas acetone produced complete **Sgc8-c-NH$_2$** decomposition (run 1, Table 1). However, when long reaction times were studied (*i.e.,* 120hr), the use of DMSO, Et_3N or DMF generated new compounds in very low yield, one of them being the product of interest, **Sgc8-c-carba-da** (see ESI-TOF-MS characterization below) (runs 5-7, Table 1). In order to improve the yield of **Sgc8-c-carba-da**, we studied a mixture of organic solvents and a selected mixture of buffers working at higher temperature. For example, at 60°C new products began to appear after 3hr of reaction (runs 8-10, Table 1). In these cases, the worst results were observed with the higher amount of Et_3N and the best results were obtained when a mixture of the four solvents was used (run 10, Table 1).

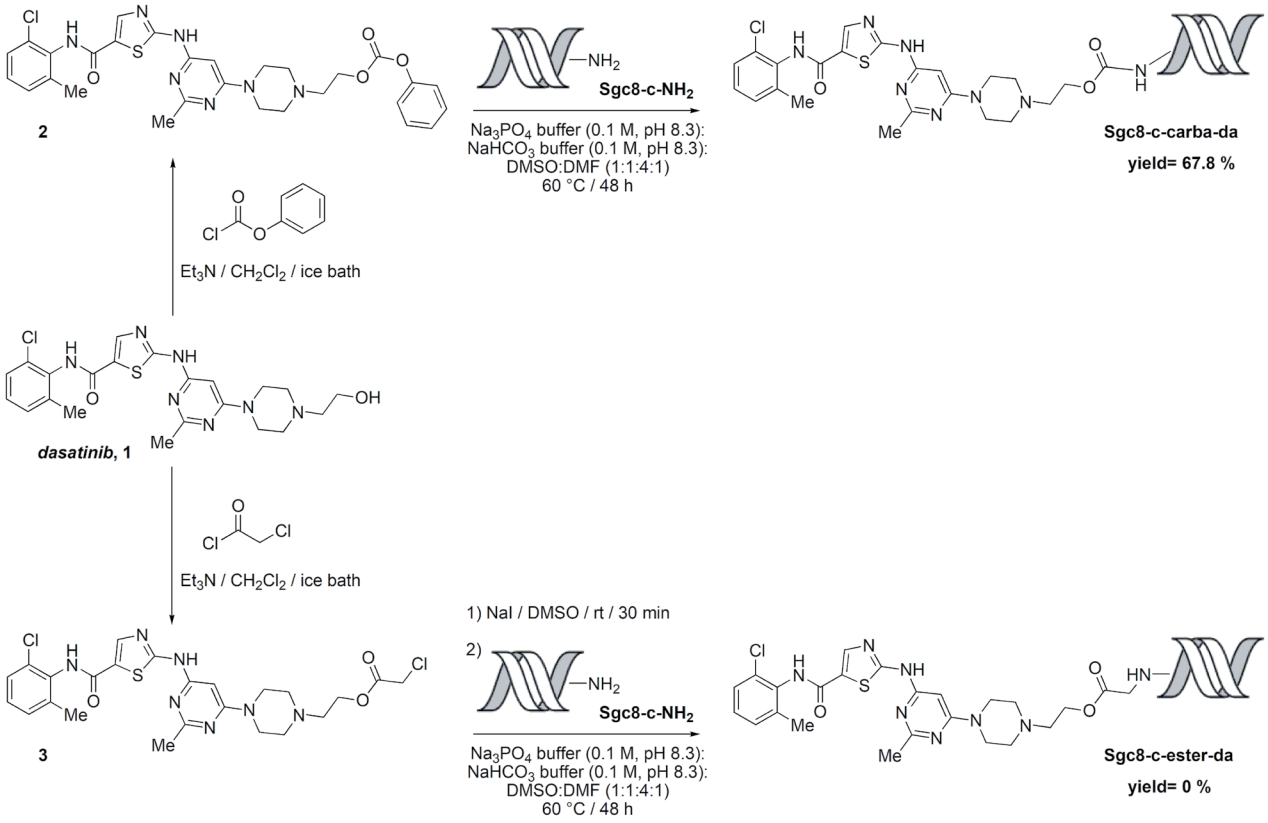

Figure 1. Schematic procedures designed and studied for the preparation of **Sgc8-c-carba-da** and **Sgc8-c-ester-da**.

Table 1. Selected initial experimental conditions assayed to optimize the preparation of **Sgc8-c-carba-da.**

run	solvent	reactants molar ratio (Sgc8-c-NH_2:2)	temperature (°C)	time (hr)	yield of Sgc8-c-carba-da	yield of secondary products[a]
1	mix buf[b]:acetone (30:1)	1:50	25	24	0	0
2	mix buf:DMSO (30:1)[c]					
3	mix buf:Et_3N (30:1)					
4	mix buf:DMF (30:1)					
5	mix buf:DMSO (30:1)			120	1.3	0.6
6	mix buf:Et_3N (30:1)				0.9	0
7	mix buf:DMF (30:1)				0	0.7
8	mix buf:DMF:Et_3N (4:1:5.5)		60	3	0.3	0.4
9	mix buf:DMF:Et_3N (3:4:1)				2.3	0
10	mix buf:DMSO:DMF:Et_3N (3:3:1:1)				6.1	0

[a]See (ESI-TOF-MS characterization); [b]Mixture of equal volumes of sodium bicarbonate buffer (0.1M, pH 8.3) and sodium phosphate buffer (0.1M, pH 8.3); [c]volume:volume

Table 2. Selected experimental conditions assayed to optimize the preparation of **Sgc8-c-carba-da** when varying the ratio of reactants and reaction time.

run	solvent	reactants molar ratio (Sgc8-c-NH_2:2)	temperature (°C)	time (hr)	yield of Sgc8-c-carba-da	yield of secondary products[ab]
1	mix buf[cc]:DMSO:DMF (2:4:1) (v:v:v)	1:2	60	24	0	0
2		1:10			0.8	0
3		1:50			4.2	0
4		1:100			8.0	2.4
5		1:200			48.8	2.9
6		1:300			39.0	0
7		1:400			22.0	0.7
8		1:200	60	2	3.0	0
9				4	7.0	0
10				6	11.6	0
11				8	17.0	0
12				30	51.8	1.9
13				**48**	**67.8**	**2.9**
14				72	49.4	0

[a]See (ESI-TOF-MS characterization); [b]Unreacted **Sgc8-c-NH_2** completes the 100% yield; [c]Mixture of equal volumes of sodium bicarbonate buffer (0.1M, pH 8.3) and sodium phosphate buffer (0.1M, pH 8.3)

For these reasons, our next goal was to optimize the amount of reactants and the reaction time using as solvent the mixture of buffer together with DMSO and DMF (Table 2). With reference to the amount of reactants (runs 1-7, Table 2), working at 60°C for 24hr the best result was obtained with a ratio of 1:200 (aptamer:**2**) (run 5, Table 2), a result that was completely in agreement with our previous findings with other organic reagents instead of **2** (Sicco et al, 2017). Regarding reaction times (runs 5 and 8-14, Table 2), working with the reactant ratio of 1:200 at 60°C the optimal time was 48hr of incubation. Other analyses were also performed, such as variation of temperature, microwave irradiation as mode of heating, different reaction conditions (e.g. variation in pH), incorporation of additives (i.e. propylene glycol, Tween-20 or $MgCl_2$) and reagent **2** addition scheme (see Supporting Information, Table S1). All these analyses demonstrated that the best conditions to obtain **Sgc8-c-carba-da** were to use reactants in the ratio 1:200 (aptamer:**2**) and a mixture of buffer:DMSO:DMF (2:4:1, v:v:v) as solvent at 60°C for 48hr.

For the desired product **Sgc8-c-ester-da**, designed by the reaction between **Sgc8-c-NH_2** and intermediate **3** (Figure 1), the best conditions described above for **Sgc8-c-carba-**

da were used. In the first stage, sodium iodide was added to increase the reactivity of chloroacetyl chloride. Unfortunately, under the studied experimental conditions it was not possible to generate **Sgc8-c-ester-da**. The main products detected (see ESI-TOF-MS characterization below) were mainly the result of acetylations of the desired product, showing that the reactivity of the ester group of intermediate **3** (or the corresponding iodo-analogue) is very high compared with the aptamer-nucleophilic moieties (see below).

ESI-TOF-MS characterization of reaction products

To establish the identity of the final products of the reaction and consequently isolate the desired product, the different entities generated under particular conditions were separated by RP-HPLC and characterized by ESI-TOF-MS, with gel electrophoresis additionally used as a complementary evaluation. For example, Figure S1A (Supplementary Data) shows the reaction mixture for the preparation of **Sgc8-c-carba-da** in the conditions of run 11 of Table 2, whereas Figure S1C (Supplementary Data) shows the reaction mixture for the attempt to prepare this biotherapeutic in acetic acid (see Supplementary Data, Table S1, run S11), where a very different compound appeared as the main product. After isolation from the reaction mixture and ESI-TOF-MS characterization of each compound, the following conclusions could be reached (see Supplementary Figure S2): (i) the compound with a retention time (t_R) near to 11min (peak **A** in Supplementary Figure S1A) corresponded to **Sgc8-c-NH$_2$**; (ii) the compounds with t_R near to 12 and 14min (peaks **B** and **C** in Supplementary Figure S1A corresponded to the desired product, but with 3´-depurinations (**B)** possibly by the loss of deoxyadenosine and OH; **C** possibly by the loss of formyladenine and four H); (iii) the compound with t_R near to 21min (peak **D** in Supplementary Figure S1A) corresponded to the desired product but with fragmentation/hydrolysis of the pyrimidine ring from 3´-adenine and the addition of PO_2; (iv) the product with t_R near to 24min (peak **E** in Supplementary Figure S1A) corresponded to the desired product, **Sgc8-c-carba-da** (ESI-TOF-MS spectrum in Supplementary Figure S1D); and (v) the product in acidic milieu with t_R near to 26min (peak **F** in Supplementary Figure S1C) could correspond to the desired product but has a new purine fragment added (deoxyadenosine 3´-diphosphite) (see Supplementary Figure S2). The analyses by gel electrophoresis were completely in agreement with the MS studies (Figure 2).

The same analysis procedure was performed for the reaction mixtures used to obtain **Sgc8-c-ester-da**. In these cases, none of the isolated products corresponded to the desired one. We were unable to identify the product **Sgc8-c-ester-da** from the reaction mixtures**,** the isolated compounds being the result of both 3´-depurinations and iodo- or chloroacetylation processes (see analysis of the most relevant products in Supplementary Figure S3). As mentioned above, the iodo- or chloroacetyl esters were so reactive that they promoted iodo- or chloroacetylations as side reactions on phosphates or nucleophilic bases of the aptamer. Attempts to improve the reaction conditions were not performed.

The desired product, **Sgc8-c-carba-da,** was purified by RP-HPLC (t_R = 24.4min; see Materials and Methods, chromatographic conditions, peak **E** in Supporting Information, Figure S1A) in 67.8% yield and with purity higher than 99% (see Supplementary Figure S1B).

Physicochemical characterization of Sgc8-c-carba-da

The physical stability of **Sgc8-c-carba-da** was analysed in Milli-Q water at different temperatures and under different storage conditions over time. The RP-HPLC and gel electrophoresis results confirmed integrity in all cases. Furthermore, the electrophoresis profiles were consistent with the absence of low-molecular-weight fragmentation. **Sgc8-c-carba-da** was stable in water up to 75°C for 30min of incubation and under the different storage conditions analysed (dissolved in Milli-Q water and lyophilized) (Figure 3).

The lipophilicity of **Sgc8-c-carba-da**, calculated as $logD_{7.5}$, was -1.35 ± 0.04. The potential biotherapeutic **Sgc8-c-carba-da** was hydrophilic and a little more lipophilic than other Sgc8-c-probes previously developed by us (Table 3) (Calzada et al, 2017a, Calzada et al, 2017b; Sicco et al, 2020). The slightly less hydrophilic character could be the result of incorporation of the apolar framework from dasatinib. The $logD_{7.5}$ value, of the same order as for other probes, could indicate that the biological behaviour of **Sgc8-c-carba-da** was similar to that of other imaging agents.

Dasatinib-releasing ability of Sgc8-c-carba-da

The hydrolysis ability of the carbamate moiety in **Sgc8-c-carba-da,** releasing dasatinib from the biotherapeutic, was evaluated *in vitro* at different pH. The selected values

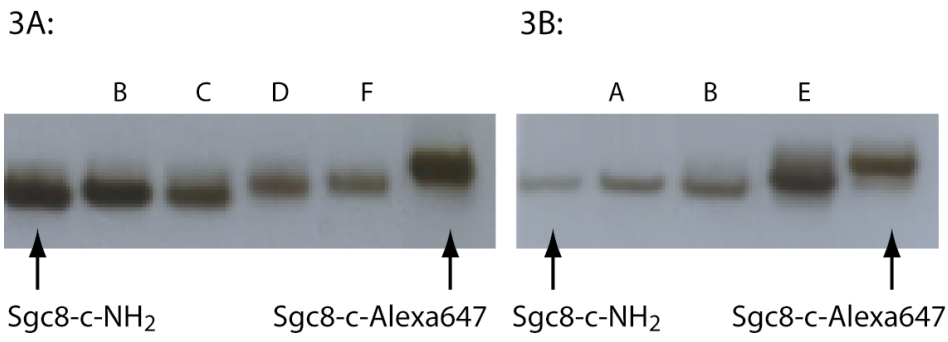

Figure 2. Gel electrophoresis analyses. Examples of two different electrophoretic analyses, including the products isolated from the reaction mixtures shown in Supporting Information, Figures S1A and S1C (isolated peaks **A**–**F**). In both runs, standards were included: **Sgc8-c-NH$_2$** and **Sgc8-c-Alex647** (MW = 13678D) (Calzada et al, 2017a; Sicco et al, 2018).

Table 3. Lipophilicities of different **Sgc8-c-NH$_2$** derivatives.

	Sgc8-c-NH$_2$ derivative				
	Sgc8-c-Alex647	**Sgc8-c-HYNIC-^{99m}Tc**	**Sgc8-c-DOTA-^{67}Ga**	**Sgc8-c-NOTA-^{67}Ga**	**Sgc8-c-carba-da**
LogD$_{7.5}$	- 1.90±0.30	- 2.36±0.29	- 1.87±0.05	- 2.41±0.11	- 1.35±0.04
Reference	Calzada et al, 2017a		Calzada et al, 2017b	Sicco et al, 2020	This paper

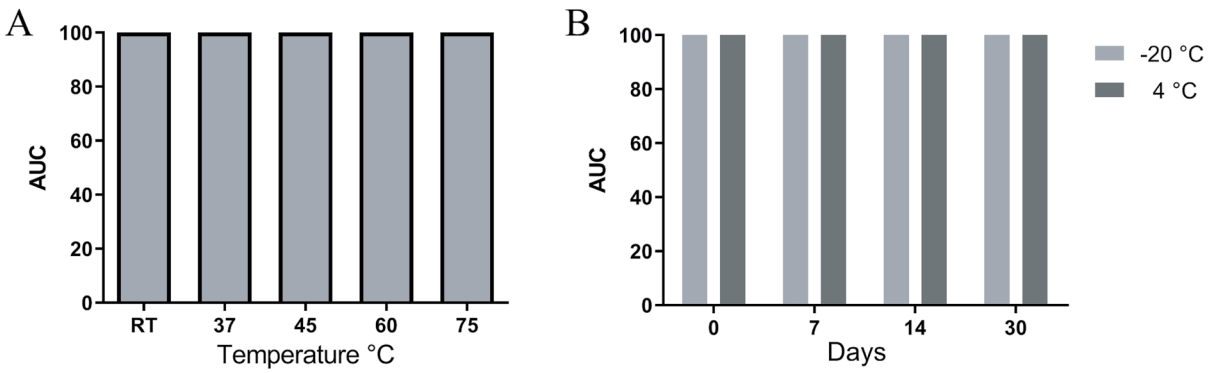

Figure 3. Stability studies. (**A**) **Sgc8-c-carba-da** was stable at different temperatures when incubated for 30min. (**B**) **Sgc8-c-carba-da** was stable under different storage conditions (dissolved in Milli-Q water or lyophilized, both at −20°C and 4°C) for 30 days. AUC: area under the curve from the RP-HPLC analysis. The measurements were carried out in duplicate.

correspond to physiological (7.4), intratumoural (5.5) and endosomal (5.0) pH. Complete **Sgc8-c-carba-da** hydrolysis was observed at 30hr after incubation but only at endosomal pH was dasatinib released in a short time (from 0.5hr). For cytoplasmatic and intratumoural pH, it was observed that dasatinib release began at times close to 24hr but this was not complete at 30hr.

CONCLUSIONS

Aptamers are promising molecular drug-delivery vehicles (Tang et al, 2020; Sameiyan et al, 2021), possessing optimal properties that include a ligand-specific response, short sequence, low immunogenic potential and easy functionalization. In our previous studies, we modified the Sgc8-c aptamer to generate imaging probes (Calzada et al, 2017a, Calzada et al, 2017b; Sicco et al, 2018, 2020). Synthesized and evaluated *in vivo*, Sgc8-c-fluorescent and -radioactive agents showed the ability to recognize different tumoural systems (*i.e.*, melanoma and lymphoma). Herein, a Sgc8-c-dasatinib hybrid was designed, synthesized and studied as a potential dasatinib-releasing biotherapeutic. A 5'-amino derivative of Sgc8-c was used to conjugate to dasatinib using two different linkages: carbamate and ester. The biotherapeutic with a carbamate connector was efficiently prepared with near to 70% yield under optimal conditions (*i.e.*, using a 200-fold excess of dasatinib derivative **2** in a mixture of adequate solvents). The biotherapeutic was successfully purified using RP-HPLC to obtain a product with high purity (near to 100%). The physicochemical properties of the biotherapeutic, such as stability and lipophilicity, are adequate for its use as a future drug. Additionally, the ability to release dasatinib was pH-dependent, the endosome being the best place to release the drug as cytoplasmatic and intratumoural pH gives only slow and incomplete hydrolysis. These data are very interesting: (i) as we have previously reported that probes based on the Sgc8-c aptamer have rapid tumour uptake and blood clearance (Calzada et al, 2017a, Calzada et al, 2017b; Sicco et al, 2020), when **Sgc8-c-carba-da** is evaluated *in vivo* it will be expected to reach the site of action with minimal hydrolysis at the non-target sites; and (ii) as the route of some aptamers' internalization has been confirmed as endosomal (Xiao et al, 2008; Porciani et al, 2018), when **Sgc8-c-carba-da** penetrates the tumour cell via the endosome the release of dasatinib will take place.

The findings reported here highlight the potential of a simple aptamer as an anti-tumour delivery vehicle. Experiments related to the bioactivity of **Sgc8-c-carba-da** are currently underway.

ACKNOWLEDGEMENTS

This work was supported by grants from the Agencia Nacional de Investigación e Innovación (ANII, Uruguay) and the PEDECIBA Química (Uruguay). E. Sicco was funded by a scholarship from the ANII (POS_NAC_2017_1_140364).

COMPETING INTERESTS

None declared.

LIST OF ABBREVIATIONS

DMF: *N*,*N*-**Dim**ethyl**f**ormamide
DMSO: **Dim**ethyl**s**ulf**o**xide
ESI-TOF: **E**lectros**p**ray **I**onization **T**ime-**o**f-**F**light
FDA: **F**ood and **D**rug **A**dministration
HMBC: **H**eteronuclear **M**ultiple **B**ond **C**orrelation

HPLC: **H**igh **P**erformance **L**iquid **C**hromatography
HSQC: **H**eteronuclear **S**ingle **Q**uantum **C**oherence
MeOH: **Meth**an**o**l
MS: **M**ass **S**pectrometry
PBS: **P**hosphate **B**uffered **S**aline
PTK7: **P**rotein **T**yrosine **K**inase **7**
RP: **R**everse **P**hase
TAE: **T**ris base, **a**cetic acid and **E**DTA
TLC: **T**hin **L**ayer **C**hromatography

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