This is an Accepted Manuscript of an article published by Taylor & Francis in **Journal of Asian Natural Products Research** *on 27.11.2019, available online:* **https://www.tandfonline.com/doi/abs/10.1080/10286020.2019.1680642?journalCode=ganp20**

New potent STS inhibitors based on fluorinated 4-(1-phenyl-1*H*-[1,2,3]triazol-4-yl)-phenyl sulfamates

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A series of fluorinated analogs based on the frameworks of 4-(1-phenyl-1*H*-[1,2,3]triazol-4-yl)-phenyl sulfamates have been synthesized as steroid sulfatase (STS) inhibitors. The design of chemical structures of new potential STS inhibitors was supported by molecular docking techniques to identify potential interactions between inhibitors and amino acid residues located in the STS active site. The STS inhibitory potency was evaluated on STS isolated from human placenta. We found that compounds substituted with fluorine atom at the meta position demonstrated the highest inhibitory effects in enzymatic STS assay. The most active analog **12e** – inhibited STS enzyme with the IC_{50} value of 36 nM.

Keywords: triazoles; steroid sulfatase; breast cancer; STS inhibitors; sulfamates

1. Introduction

Hormone-dependent breast cancer (HDBC) is a major cause of mortality, and there is pressing need to develop novel treatment methods. According to International Agency of Research on Cancer (IARC), 2.1 million cases of breast cancer will be diagnosed in 2018. Furthermore, the breast cancer will be a cause of death in 620 000 cases. Over the past decades, numerous reports have suggested the importance of biologically active hormone precursors in regulating the supply of estrogens to HDBC. One approach for treatment of HDBC involves inhibitors of enzymes responsible for the biosynthesis of estrogens in peripheral tissues [1].

There are different enzyme pathways responsible for the formation of active estrogens in the breast tissue, e.g., the aromatase enzyme complex (implicated in the conversion of androgens into estrogens during steroidogenesis) [2], the 17β-hydroxysteroid dehydrogenase (17β-HSD) (implicated in the interconversion of E1 and E2), the 3β-hydroxysteroid dehydrogenase/isomerase (3β-HSD) isoenzymes (implicated in the formation of all cases of active steroid hormones) [3] and steroid sulfatase enzyme (STS). Within these pathways, STS plays a major role in the formation of biologically active estrogens or androgens and acts by hydro-

lyzing aryl and alkyl steroid sulfates (including E1S and DHEAS) [1]. It is worth noting that STS expression is detected in 90% of breast tumors, with much higher activity than the aromatase complex [4]. Wide distribution of the STS enzyme throughout the body is an indication of its involvement in numerous physiological and pathological conditions [5].

In recent years, there has been intensive research toward finding novel effective inhibitors of STS. Approaches to design efficacious STS inhibitors include three different categories of compounds: alternative substrates (including competitive reversible inhibitors), reversible inhibitors, and irreversible inhibitors [6]. One of the strategies for designing of the STS inhibitors was a replacement of sulfate moiety, present in natural substrate, into the sulfamate group. One of the most promising synthesized compounds was EMATE **1** (Figure 1), which exhibited very high activity in MCF-7 cells, with an IC_{50} value of 65 pM [7]. The unique activity of this compound has since spawned a large library of such sulfamate-based STS inhibitors based around the aryl sulfamate pharmacophore [8, 9]. A series of nonsteroidal analogues with different functional groups have been synthesized, including - sulfamates, phosphates, phosphonates and thiophosphonates – as potent STS inhibitors [10-16]. The sulfamated coumarin derivatives (e.g., coumarin-7-*O*-sulfamate **2**) were proved to be an important class of compounds exhibiting a high inhibitory potency against STS. In contrast to EMATE, they demonstrated fewer adverse effects and much weaker estrogenic properties. The first potent inhibitor based on the coumarin scaffold was 4-methylcoumarin-7-*O*-sulfamate **3** (COUMATE), which exhibited good activity with an IC_{50} value of 380 nM, when evaluated against placental microsomes [17]. Further modifications of its structure led to a wide range of tricyclic coumarin derivatives that mimicked the ABC rings of steroids. These compounds displayed a significantly more potent inhibitory activity than COUMATE (e.g., 667-COUMATE **4** demonstrated potent activity toward STS with IC_{50} value of 8 nM) [18].

Recently, the strategy based on the introduction of fluorine atoms to the chemical structure of coumarin derivatives has been used in designing of novel STS inhibitors. The introduction of fluorine atoms into the structure of biologically active compounds alters nearly all physical properties of the compounds and modifies their absorption, distribution, metabolism and excretion. As potent STS inhibitors, the fluorinated 3-phenylcoumarin-7-*O*-sulfamate derivatives have been synthesized [19]. Two fluorinated compounds, **5** and **6**, demonstrated the highest inhibitory activity in the enzymatic assay, with IC_{50} values of 270 nM in both cases. Furthermore, the introduction of additional fluorinated aromatic ring *via* amide moiety led to obtain a series of *N*-acylated derivatives of 3-(4-aminophenyl)-coumarin-7-*O*-sulfamate [20]. Compounds **7** and **8** inhibited the STS enzyme with IC_{50} values of 180 nM in both cases. The fluorination strategy has been used also in case of STS inhibitors based on *N*-acylated tyramine sulfamates [21]. The 4-(2-perfluoroundecanoylaminoethyl)-phenyl sulfamate **9** exhibited the greatest inhibitory effect, with an IC_{50} value of 2.18 μM.

In the present work we described a convenient method for the synthesis of compounds, based on nonsteroidal core of 4-(1-phenyl-1*H*-[1,2,3]triazol-4-yl)-phenyl sulfamate, as potent STS inhibitors. We found that the abovementioned nonsteroidal scaffold perfectly imitates the steroid core of the natural substrate and may undergo numerous electrostatic interactions affecting the stabilization of the inhibitor-enzyme complexes within the active site of STS. Furthermore, in the design and synthesis of the novel STS inhibitors, the fluorination strategy has been involved.

2. Results and discussion

2.1 Chemistry

In the course of our research, we synthesized newly designed STS inhibitors, based on fluori-

nated 4-(1-phenyl-1*H*-[1,2,3]triazol-4-yl)-phenyl sulfamates, according to click chemistry approach [22]. The detailed synthesis pathway is shown in Scheme 1. In the first step, 4-(trimethylsilyl)ethynyl)phenol **10** was obtained by the Sonogashira coupling between the 4-iodophenol and trimethylsilyl acetylene. Next, the commercially available aniline derivatives were transformed into azides [by the reaction with tert-butyl nitrite (t-BuONO) and azidotrimethylsilane ($TMSN_3$)] and then directly coupled to **10** in the presence of $CuSO_4$ (0.1 equiv.), ascorbic acid (0.2 equiv.) and tetrabutylammonium fluoride (TBAF) to bring about the deprotection of **10** in the reaction mixture. Finally, the OH groups of obtained 4-(1-phenyl-1*H*-[1,2,3]triazol-4-yl)-phenol derivatives **11a-n** were sulfamoylated. In these cases, the solutions of stable fluorinated derivatives of 4-(1-phenyl-1*H*-[1,2,3]triazol-4-yl)-phenol **11a-n** in *N*,*N*-dimethylacetamide (DMA) were treated with H_2NSO_2Cl, previously generated in the reaction of chlorosulfonyl isocyanate and formic acid in the presence of a catalytic amount of DMA. After the standard isolation procedure, we obtained desired compounds **12a-n**.

2.2 Molecular modeling

In order to determine the binding modes of proposed structures of fluorinated 4-(1-phenyl-1*H*-[1,2,3]triazol-4-yl)-phenyl sulfamate to the STS active site, the molecular docking approach was applied. The computational studies allowed us to identify potential interactions between specified functional groups, atoms of inhibitors and amino acid residues forming the binding site of STS. The docking procedures of the properly optimized ligands to the prepared structure of the STS enzyme (retrieved from Protein Data Bank – 1P49 accession code) were performed using Autodock Vina 1.1.2 software (Molecular Graphics Laboratory, The Scripps Research Institute, La Jolla, CA, USA).

The calculated free binding energies (Table 1), for the newly designed candidates

12a-n, were in the range from −5.3 to −7.1 kcal mol^{-1} and in almost every cases were significantly lower than the free binding energy values for the reference inhibitors: coumarin-7-*O*-sulfamate (−2.9 kcal mol^{-1}), **COUMATE** (−4.1 kcal mol^{-1}), **667-COUMATE** (−5.4 kcal mol^{-1}). The best docking results were determined for compounds **12d** (−6.7 kcal mol^{-1}), **12e** (−6.7 kcal mol^{-1}), **12f** (−6.7 kcal mol^{-1}) and **12h** (−7.1 kcal mol^{-1}). It is worth noting that in the structures of compounds **12d**, **12e**, **12f** and **12h** the terminal aromatic ring is substituted with fluorine atom or trifluoromethyl group at the *meta* position. Our docking analysis, for the newly designed STS inhibitor candidates **12a-n**, showed that these compounds could be well associated with the active site of STS.

The binding modes of compounds **12a-n** were similar to the mode of the reported STS inhibitor **667-COUMATE**. The Figure 2 shows structures of compounds **12d**, **12e**, **12f** and the reference inhibitor **667-COUMATE** docked to the active site of the STS enzyme. We found that the sulfamate functional groups are located very close to the catalytic amino acid residue FGly75 (~3.0 Å) coordinated to the Ca^{2+} ion. Furthermore, they are surrounded by the catalytically essential residues (Asp35, Asp36, Arg79, Lys134, His136 and Lys368). The tricyclic cores of the designed candidates **12a-n** are well accommodated in the binding cavity formed by the lipophilic amino acids (Arg98, Leu103, Phe104, Leu167, Val 177, Phe178, Phe182, Leu185, Phe230, Phe233, Phe237, Thr484, Val486, Phe488, Trp550 and Phe553). In the course of our docking studies, we noticed that the central triazole rings are located close to Thr484 residue indicating the possibility to create a putative electrostatic interaction. Furthermore, we detected that fluorine atoms of a terminal aromatic ring of designed candidates **12a-n** are able to electrostatically interact with Arg98 residue. Taking the example of compound **12f** (Figure 2, *right side*), we noticed that the shortest distance between the fluorine atom and the Arg98 residue occurs, when the fluorine atom is at the *meta*

position. This observation may explain the lowest free binding energy values calculated for analogues with fluorinated substituent at the *meta* position (**12d**, **12e**, **12f** and **12h**). Additionally, this may allow us to suppose that the more favorable stabilization of the inhibitor-enzyme complexes and more effective inhibition of the enzyme is possible in cases of analogues with fluorinated substituent at the *meta* position.

2.3 STS enzyme assays

In order to evaluate the STS inhibitory activities of synthesized compounds **12a-n**, we performed an enzymatic assay according to the previously reported methods [23, 24] with STS enzyme isolated from human placenta. As a direct source of the STS enzyme we used the fraction after purification by a 3-step chromatographic procedure. The summarized results of the enzymatic assay, for newly synthesized fluorinated 4-(1-phenyl-1*H*-[1,2,3]triazol-4-yl)-phenyl sulfamate derivatives **12a-n** and reference compounds (coumarin-7-O-sulfamate **2**, COUMATE **3** and 667-COUMATE **4**), are presented in Table 1.

In the course of our investigations, we found that newly synthesized compounds **12a-n** are very potent STS inhibitors. 4-[1-(3,4-difluorophenyl)-1*H*-1,2,3-triazol-4-yl]-phenyl sulfamate **12d**, 4-[1-(3,5-difluorophenyl)-1*H*-1,2,3-triazol-4-yl]-phenyl sulfamate **12e** and 4-[1-(2,3,4-trifluorophenyl)-1*H*-1,2,3-triazol-4-yl]-phenyl sulfamate **12f** exhibited the highest inhibitory activities (the IC_{50} values of 0.063 μM, 0.036 μM and 0.058 μM, respectively). This results are in agreement with the data obtained from molecular docking studies that showed the most favorable free binding energy values for compounds with fluorine atom at the *meta* position of terminal aromatic ring. Furthermore, the inhibitory potency of the most active derivative 4-[1-(3,5-difluoro-phenyl)-1*H*-1,2,3-triazol-4-yl]-phenyl sulfamate **12e** (the IC_{50} value of 0.036 μM) was comparable with **667 COUMATE** (the IC_{50} value of 0.025 μM), which is known as potent STS inhibitor.

In the present work, we described the synthesis and biological evaluation of a series of new

STS inhibitors **12a-n** based on fluorinated 4-(1-phenyl-1*H*-[1,2,3]triazol-4-yl)-phenyl sulfamate core. Additionally, the computational analyses were carried out as a support in designing the chemical structures of potential inhibitors. In the course of our docking studies, we found that the fluorinated derivatives of 4-(1-phenyl-1*H*-[1,2,3]triazol-4-yl)-phenyl sulfamate **12a-n** are well accommodated in the binding cavity of STS. According to the computational research, we noticed that the most favorable free binding energy values were calculated for derivatives with a fluorine atom at the *meta* position of terminal aromatic ring (**12d**, **12e**, **12f** and **12h**). This result may be explained by the shortest distances between fluorine atoms at the *meta* position and the Arg98 residue located in the active site of STS. The molecular modeling results were confirmed by the biological research. In the course of our biological evaluation studies, we found that the newly synthesized compounds based on fluorinated 4-(1-phenyl-1*H*-[1,2,3]triazol-4-yl)-phenyl sulfamate core **12a-n** exhibited very high inhibitory potency. The highest inhibitory activities were demonstrated by compounds contained the fluorine atom at the *meta* position of terminal aromatic ring: 4-[1-(3,4-difluorophenyl)-1*H*-1,2,3-triazol-4-yl]-phenyl sulfamate **12d**, 4-[1-(3,5-difluoro-phenyl)-1*H*-1,2,3-triazol-4-yl]-phenyl sulfamate **12e** and 4-[1-(2,3,4-trifluorophenyl)-1*H*-1,2,3-triazol-4-yl]-phenyl sulfamate **12f** (the IC_{50} values of 0.063 μM, 0.036 μM and 0.058 μM, respectively). Furthermore, the inhibitory potency of the most active derivative 4-[1-(3,5-difluoro-phenyl)-1*H*-1,2,3-triazol-4-yl]-phenyl sulfamate **12e** (the IC_{50} value of 0.036 μM) was comparable with 667 COUMATE (the IC_{50} value of 0.025 μM).

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

We gratefully acknowledge the National Science Centre (Poland) for financial support

(PRELUDIUM grant no. 2015/19/N/NZ7/00938 and ETIUDA fellowship no. 2017/24/T/NZ7/00271). Furthermore, we gratefully acknowledge the Foundation for Polish Science (FNP) for financial support in the form of the fellowship START for one of the coauthors.

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Figures.

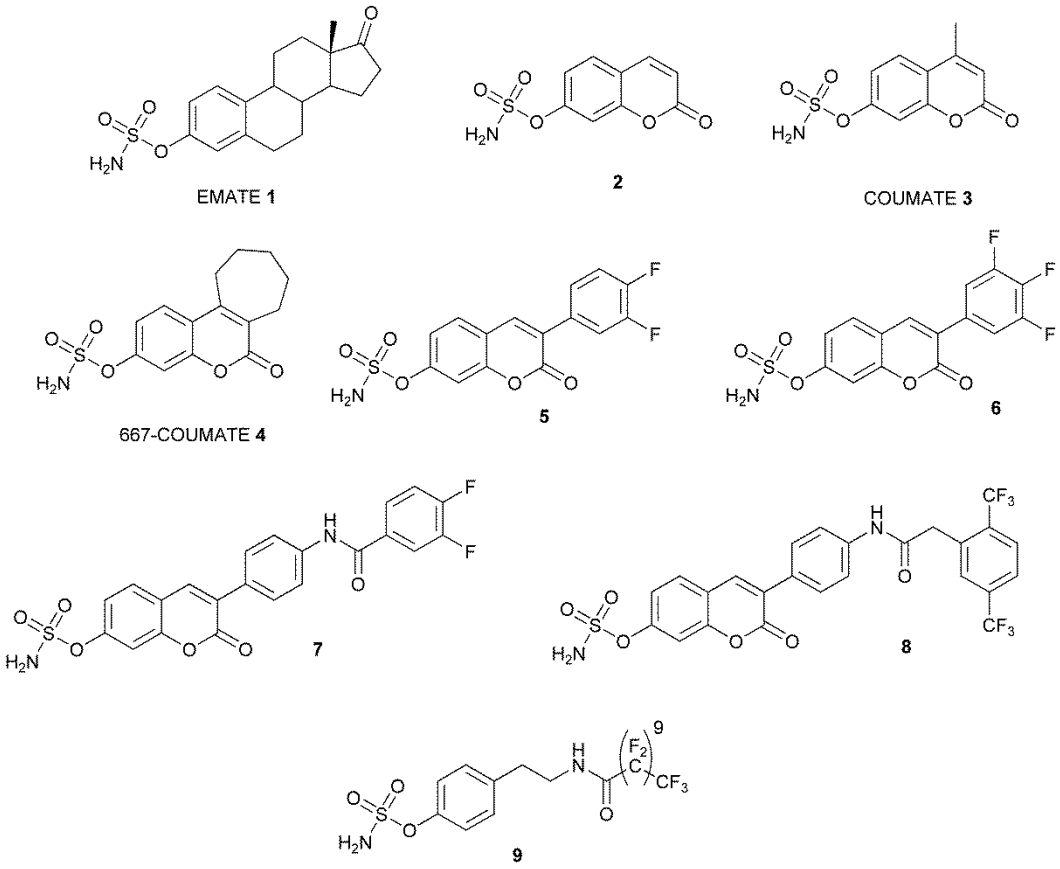


Figure 1. Chemical structures of STS inhibitors **1-9**.

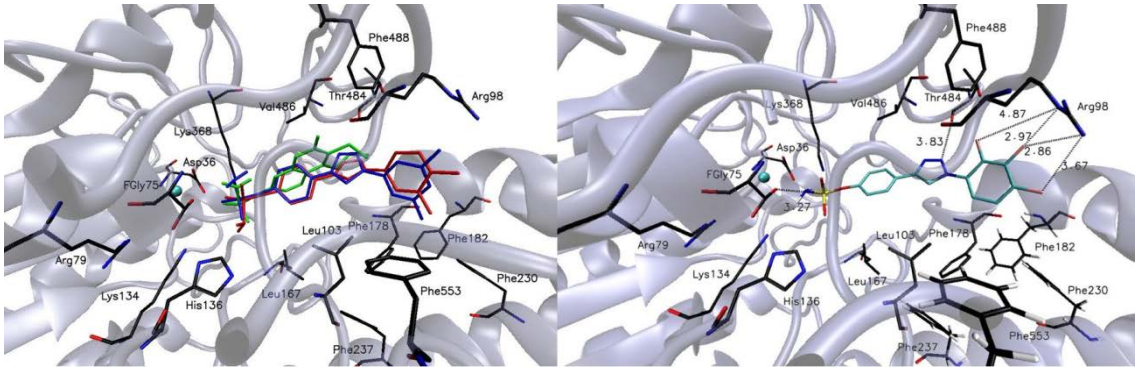
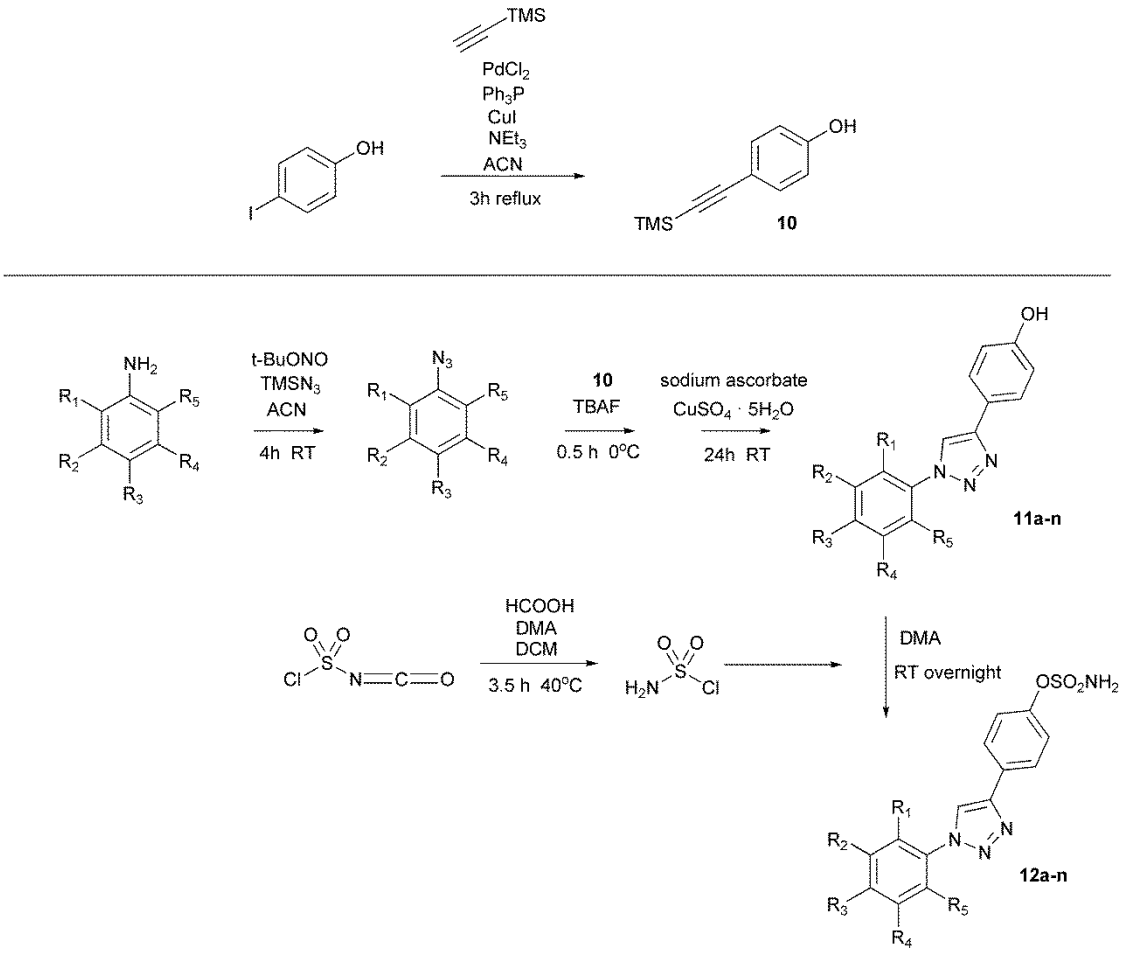


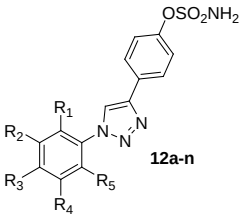
Figure 2. Docked binding modes and distances to Arg98 and Thr484 of compounds **12d** (red), **12e** (blue), **12f** (CPK coloured) and **667 COUMATE** (green).



Scheme 1. Synthesis of 4-(1-phenyl-1*H*-[1,2,3]triazol-4-yl)-phenyl sulfamate derivatives **12a-n** (R_1, R_2, R_3, R_4, R_5 = H, F, CF_3, OCF_3).

Tables

Table 1. Activities of the synthesized compounds 12a-n, and reference inhibitors in the STS enzyme assays.



12a-n

No.	R_1	R_2	R_3	R_4	R_5	Free binding energy [kcal mol^{-1}]	IC_{50} [µM]*
12a	H	H	H	H	H	−6.1	0.35 ± 0.05
12b	H	H	F	H	H	−6.5	0.30 ± 0.04
12c	H	F	H	H	H	−6.3	0.072 ± 0.01
12d	H	F	F	H	H	−6.7	0.063 ± 0.01
12e	H	F	H	F	H	−6.7	0.036 ± 0.004
12f	F	F	F	H	H	−6.7	0.058 ± 0.007
12g	H	H	CF_3	H	H	−5.9	0.14 ± 0.05
12h	H	CF_3	H	H	H	−7.1	0.18 ± 0.06
12i	CF_3	H	H	H	H	−6.2	0.18 ± 0.07
12j	H	CF_3	H	CF_3	H	−6.6	0.82 ± 0.05
12k	F	H	CF_3	H	H	−5.9	0.35 ± 0.05
12l	CF_3	H	F	H	H	−6.4	0.45 ± 0.06
12m	H	H	OCF_3	H	H	−6.2	0.24 ± 0.03
12n	OCF_3	H	H	H	H	−5.3	1.5 ± 0.2
2	-	-	-	-	-	−2.9	1.04 ± 0.32
3	-	-	-	-	-	−4.1	0.3 ± 0.07
4	-	-	-	-	-	−5.4	0.025 ± 0.008

* All measurements were performed in triplicate