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Development of novel benzofuran-based SLC-0111 analogs as selective cancer-associated carbonic anhydrase isoform IX inhibitors

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Dedication: This paper is dedicated to the memory of our wonderful colleague Prof. Dr. Ghada Sameh Hassan, who sadly passed away on Wednesday 5th Aug 2020.

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Tail approach

ABSTRACT

In the present study, we describe the design of different series of benzofuran-based derivatives as potential carbonic anhydrase inhibitors (CAIs). The adopted design is based on bioisosteric replacement for the *p*-fluorophenyl **SLC-0111** tail with the lipophilic 2-methylbenzofuran or 5-bromobenzofuran tails to furnish the 2-methylbenzofuran (MBF) sulfonamides (MBFS; **9**, **11** and **13**) and 5-bromobenzofuran (BBF) sulfonamides (BBFS; **27a-b**, **28a-b** and **29a-c**), respectively. Thereafter, the urea spacer was either elongated to furnish MBFS (**17** and **19**), and BBFS (**30**) series, or replaced by a carbamate one to afford MBFS (**15**). All the designed compounds were synthesized and evaluated for their inhibitory activities against four human (*h*) CA isoforms: *h*CA I, II, IX and XII. MBFS (**11b** and **17**) and BBFS (**28b**, **29a** and **30**) efficiently inhibited the tumor-related CA IX isoform in the single-digit nanomolar range (K_Is = 8.4, 7.6, 5.5, 7.1 and 1.8 nM, respectively). In particular, MBFS **11b** and BBFS **28b** exhibited good selectivity toward hCA IX isoform over the main off-target hCA II isoform (*S.I.* = 26.4 and 58.9, respectively). As a consequence, **11b** and **28b** were examined for their anticancer and pro-apoptotic activities toward MDA-MB-231 and MCF-7 cancer cell lines.

1. Introduction

Carbonic anhydrases (CA, EC 4.2.1.1) are a group of zinc containing metalloenzymes that are widespread in all living organisms. The process of carbon dioxide hydration to produce bicarbonate and protons that is catalyzed by the CAs have critical role in various human physiological processes such as respiration, pH and CO_2 homeostasis, lipogenesis, gluconeogenesis and tumorigenicity [1]. Accordingly, CA inhibitors (CAIs) became an important class of therapeutics during last decades as topically acting antiglaucoma [2,3], anticonvulsants [4] and antitumor agents [5–7]. The α-class of CA is divided into fifteen isoforms, which exhibit distinct molecular attributes, protein structure, kinetics, localization and catalytic behavior [8,9].

CAs IX and XII are well-known transmembrane CA isoforms which have shown increased expression in hypoxia-induced tumor cells [10]. Such isozymes represent valuable biological targets for the design of CA inhibitors (CAIs) with many biomedical applications [6,8,11,12]. Human CA IX plays a great role in tumor cell proliferation, migration and adhesion, thus the inhibition of hCA IX activity lead to a decrease of these processes and metastatic cascade [13]. Selective targeting of membrane-bound isoform hCA

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IX has been advised as a promising strategy to halt the growth of different solid tumors through suppressing distinctive tumor survival mechanisms in hypoxic environment [10,13].

The most exploited structural element enhances potency and selectivity of sulfonamide-like CAIs is the "tail" that the zinc-binding inhibitors incorporate [14,15]. Several tails based on different chemical scaffolds have been employed to design selective CA IX and hCA XII inhibitors [16–21]. In particular, "tail" approach succeeded in development of the ureido benzenesulfonamide **SLC-0111** (Fig. 1) that is being evaluated in the clinical trials for management of different hypoxic tumors, with selective CA IX inhibitory action [22].

Recently, our research group has described the design and synthesis of new series of benzofuran-based carboxylic acids (Structure **I**, Fig. 1) featuring the carboxylic functionality, as a zinc anchoring group, that connected *via* an ureido linker to 2-methylbenzofuran and 5-bromobenzofuran tails, as a potential CAIs [23]. The results obtained from the stopped-flow CO_2 hydrase assay revealed that *h*CA IX was moderately inhibited by the developed benzofuran-based carboxylic acids (Structure **I**, Fig. 1) with K_Is ranging from 0.56 μM to 5.1 μM.

Moreover, two new sets of benzofuran-based sulfonamides were reported by Abdelrahman et al. [24] as promising CAIs. The synthesized benzofuran derivatives were designed to incorporate the zinc anchoring sulfamoyl functionality that linked to a benzofuran tail through hydrazino (Structure **II**) and hydrazido (Structure **III**) spacers, Fig. 1. The results elucidated that all the reported benzofuran-based sulfonamides possessed low-nanomolar inhibitory action toward *h*CA IX with K_Is range: 10.0–76.6 nM, for the hydrazino linker-bearing derivatives, and 27.7–97.5 nM, for the hydrazido linker-bearing derivatives.

Pursuing on our continued efforts to develop efficient *h*CA IX inhibitors [23–27], here we describe the design and synthesis of novel benzofuran-based **SLC-0111** analogs (Fig. 2). The design of herein reported target benzofuran-based CAIs is based on bioisosteric replacement for the *p*-fluorophenyl **SLC-0111** tail with the lipophilic 2-methylbenzofuran or 5-bromobenzofuran tails to furnish the 2-methylbenzofuran (MBF) sulfonamides (MBFS; **9a-b**, **11a-b** and **13a**) and 5-bromobenzofuran (BBF) sulfonamides (BBFS; **27a-b**, **28a-b** and **29a**), respectively, Fig. 2. Both fused lipophilic MBF and BBF tails is anticipated to achieve a significant hydrophobic interactions within the roomier *h*CA IX and XII binding sites.

Furthermore, the urea spacer was elongated by two-atoms spacer; ethylene ($-CH_2CH_2-$) or amide (−HN-C═O), to produce another series of MBFS **17** and **19**, and BBFS **30**, Fig. 2. In addition, the urea linker was replaced by a carbamate one to afford MBFS **15**, whereas the primary sulfamoyl group in MBFS **13a** and BBFS **29a** was substituted to yield the secondary sulfonamides **13b-c** and **29b-c**, respectively, in order to explore the role of the primary sulfamoyl functional group as a zinc-anchoring motif for the CA inhibitory action, Fig. 2.

All the herein designed MBFS (**9a-b**, **11a-b**, **13a-c**, **15**, **17** and **19**) and BBFS (**27a-b**, **28a-b**, **29a-c** and **30**) have been synthesized and explored for their CA inhibitory action against hCA I, II, IX and XII. Then, the anti-proliferation and the apoptosis induction effects of the most efficient *h*CA IX inhibitors (MBF **11b and** BBF **28b**) have been *in vitro* investigated.

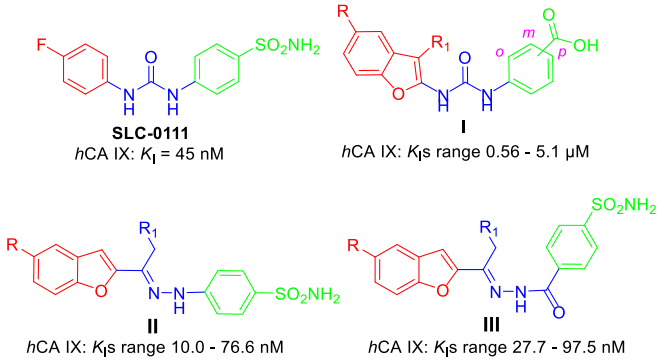


Fig. 1. Chemical structure for SLC-0111 and benzofuran-based CAIs **I-III**

2. Results and discussion

2.1. Chemistry

The preparation of MBF sulfonamides (**9a-b**, **11a-b**, **13a-c**, **15**, **17** and **19**) and BBF sulfonamides (**27a-b**, **28a-b**, **29a-c** and **30**) reported in this study is illustrated in Schemes 1–3. Ethyl 3-methylbenzofuran-2-carboxylate **4** was prepared according to the reported procedures [28]. Then, hydrazinolysis of ester **4**, to afford hydrazide **5**, was achieved through refluxing with hydrazine hydrate in isopropyl alcohol. Thereafter, 3-methylbenzofuran-2-carbohydrazide **5** was reacted with sodium nitrite in glacial acetic acid in an ice-bath to furnish the corresponding 3-methylbenzofuran-2-carbonyl azide **6** which was then subjected to Curtius rearrangement *via* heating in dry toluene to produce the key intermediate 2-isocyanato-3-methylbenzofuran **7**. The target MBFS ureides (**9a-b**, **11a-b**, **13a-c**, **17** and **19**) and MBFS carbamate (**15**) were obtained *via* the reaction of isocyanato-3-methylbenzofuran **7** with different amino-benzenesulfonamide derivatives (**8a-b**, **10a-b**, **12a-c**, **16** and **18**) and 4-hydroxybenzenesulfonamide (**14**), respectively, in refluxing toluene (Schemes 1 and 2).

In Scheme 3, ethyl 5-bromobenzofuran-2-carboxylate **23** was prepared as reported previously [29], then treated in a similar way as ester **4** to form the key intermediate **26**. Thereafter, 5-bromo-2-isocyanatobenzofuran **26** was reacted with different amino-benzenesulfonamide derivatives (**8a-b**, **10a-b**, **12a-c** and **18**) in refluxing toluene to furnish the corresponding target BBF sulfonamides (**27a-b**, **28a-b**, **29a-c** and **30**) (Scheme 3).

Postulated structures for the newly synthesized MBF and BBF sulfonamides were in full agreement with their spectral analyses data.

2.2. Biological evaluation

2.2.1. Carbonic anhydrases inhibition

The newly synthesized benzofuran-based sulfonamides (MBFS; **9a-b**, **10a-b** and **13a-c**) and (BBFS; **27a-b**, **28a-b** and **29a-c**) were evaluated for their ability to inhibit the physiologically relevant hCA isoforms, hCA I, II (cytosolic), and hCA IX and XII (trans membrane, cancer-related isoforms), utilizing the stopped-flow CO_2 hydration assay [30]. SLC-0111 and acetazolamide (AAZ) were included in the experiments as standard inhibitors. The inhibition data for the examined molecules against the tested CA isoforms are summarized in Table 1.

(i) The ubiquitous cytosolic *h*CA I isoform was inhibited by the herein reported benzofuran-based sulfonamides in a variable degree. The sulfonamides **30** and **27b** displayed moderate potency with inhibition constant (K_I) values of 164.4 and 92.2 nM, respectively, whereas **9a, 13a, 15, 17, 19, 27a, 28b, 29a** and **5b** potently inhibited *h*CA I isoform with K_I values between 27.5 and 71.8 nM. Contrariwise, *h*CA I was weakly inhibited by **9b, 11a, 11b** and **28a** with K_I values of 200.1, 495.8, 290.7 and 280.1 nM, respectively. Furthermore, **13b**

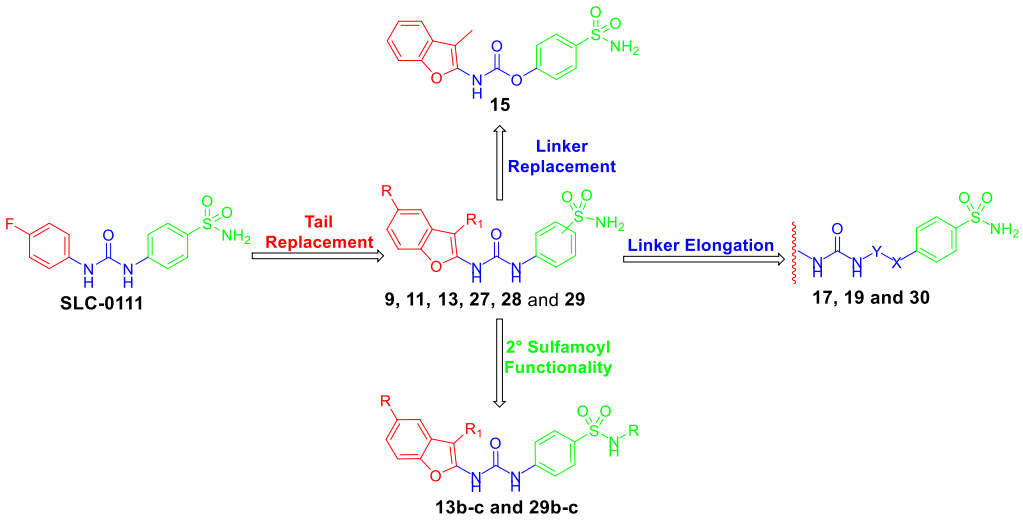
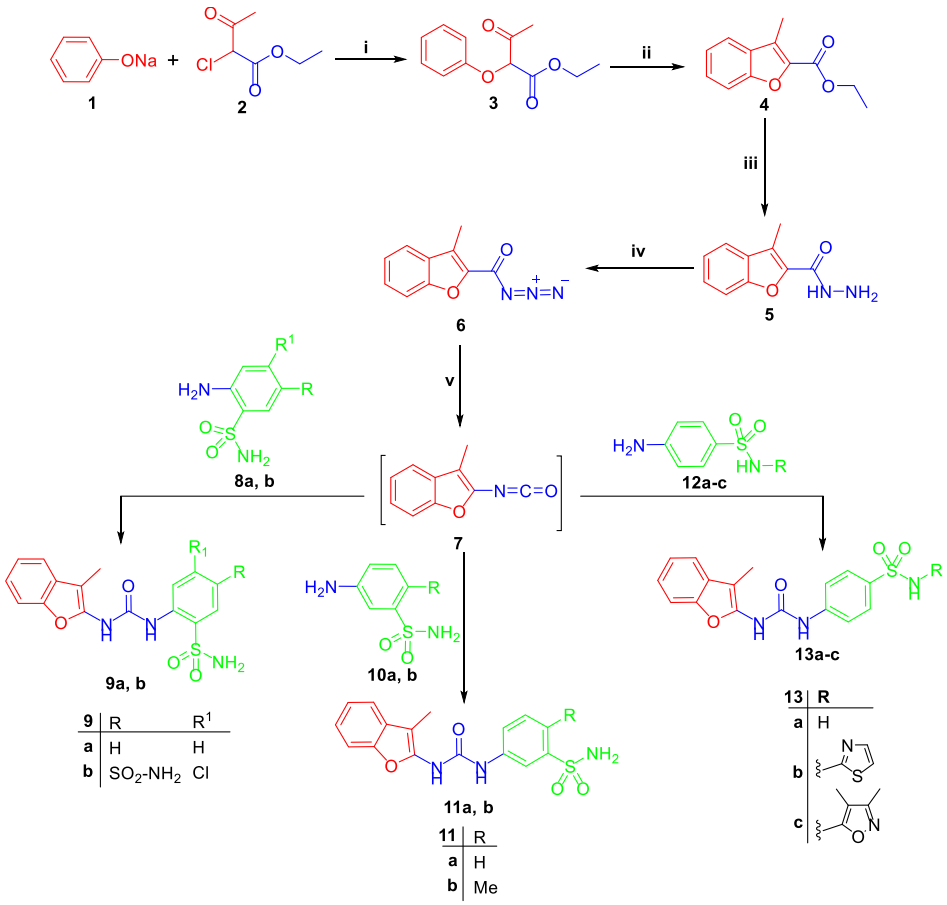


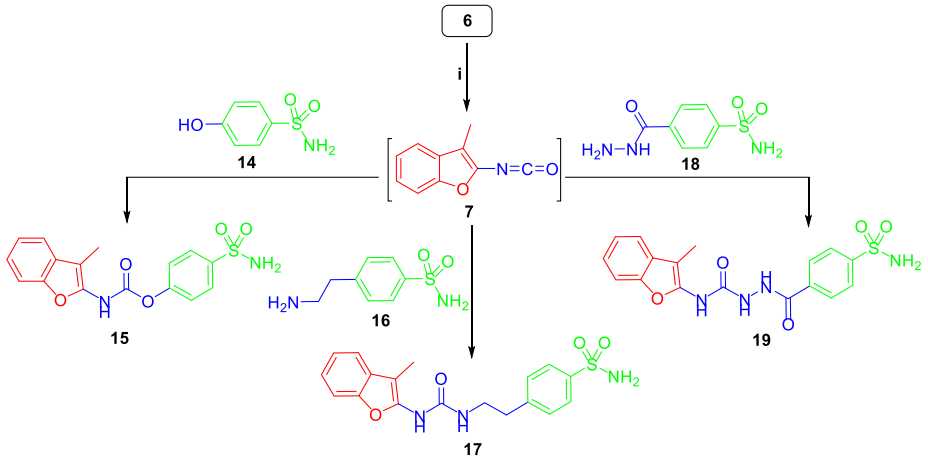
Fig. 2. Design of target 2-methylbenzofuran sulfonamides (MBFS; **9a-b**, **11a-b**, **13a-c**, **15**, **17** and **19**) and 5-bromobenzofuran sulfonamides (BBFS; **27a-b**, **28a-b**, **29a-c** and **30**).



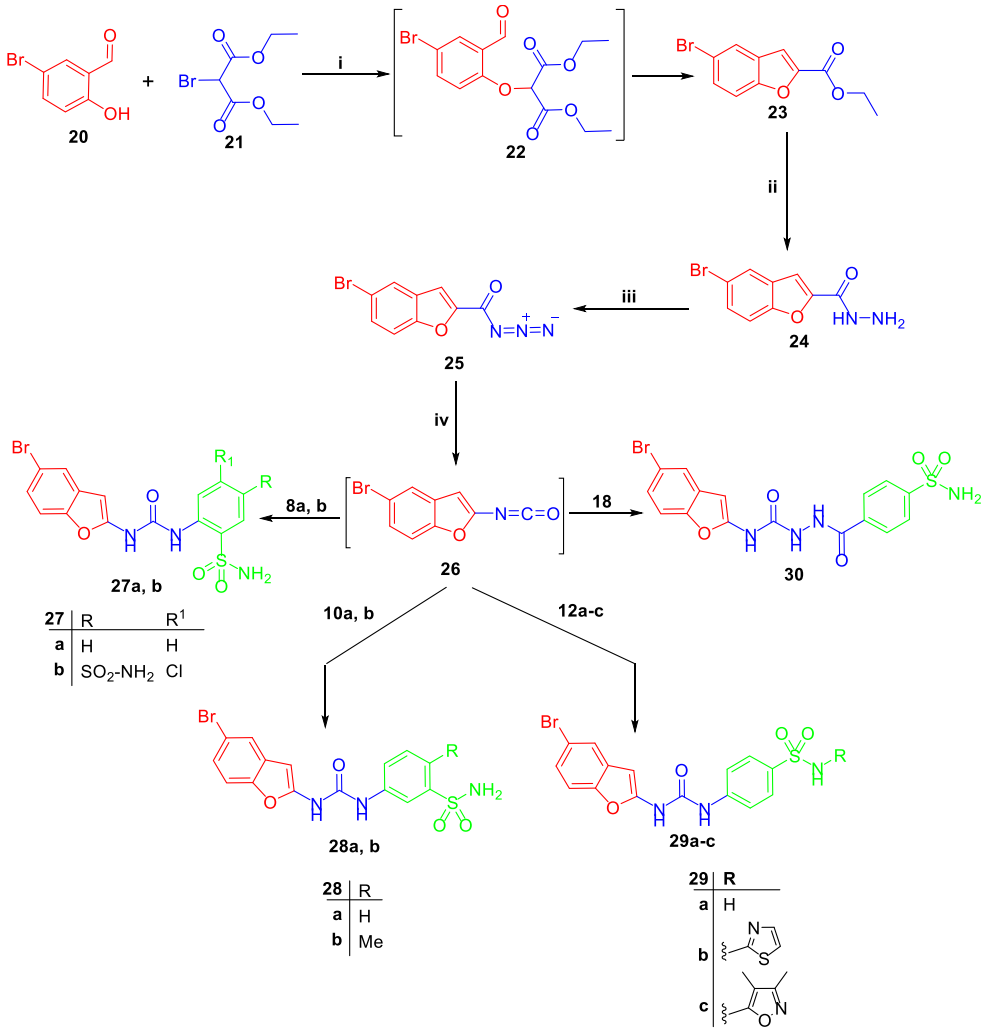
Scheme 1. *Reagent and conditions:* (**i**) dry toluene, reflux 4 h; (**ii**) H_2SO_4, stirring 2 h (0–5 °C); (**iii**) Isopropyl alcohol, $NH_2NH_2 \cdot H_2O$, reflux 3 h; (**iv**) Sodium nitrite, acetic acid, stirring 1 h (0 °C) then 1.5 h (r.t.); (**v**) Toluene, reflux 6 h.

displayed high K_I value of 54.0 μM while **13c, 29b** and **29c** showed no inhibitory activity on *h*CA I isoform.

(ii) The *in vitro* kinetic data listed in Table 1 revealed that the physiologically dominant cytosolic hCA II isoform was inhibited in a similar fashion to *h*CA I inhibition profile except for **30** which showed low K_I value of 8.0 nM. While, **9a**, **13a**, **15**, **17**, **19**, **27a**, **28b** and **29a** effectively inhibited hCA II (K_Is: 4.8–54.3 nM) with comparable activity to the standard drug AAZ (K_I = 12 nM), sulfonamides **9b, 11a, 11b, 27b, 28a** and **28b** displayed moderate inhibitory activity with K_Is spanning in the high nanomolar range: 135.3–324.2 nM.



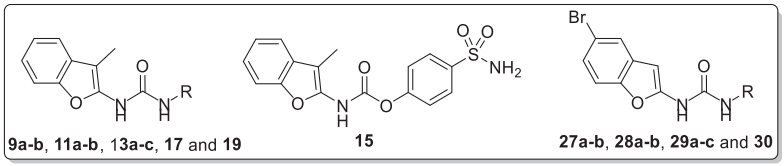
Scheme 2. *Reagent and conditions:* **(i)** Toluene, reflux 6 h.

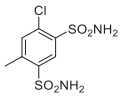
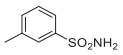
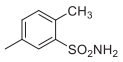
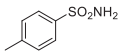
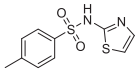
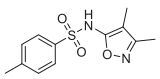
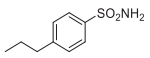
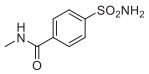
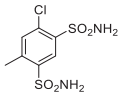
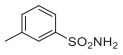
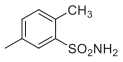
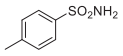
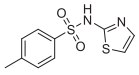
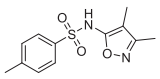
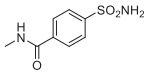


Scheme 3. *Reagent and conditions:* **(i)** Potassium carbonate, DMF, reflux 10 h; (**ii**) Isopropyl alcohol, $NH_2NH_2 \cdot H_2O$, reflux 3 h; (**iii**) Sodium nitrite, acetic acid, stirring 1 h (0 °C) then 1.5 h (r.t.); (**iv**) Toluene, reflux 6 h.

(iii) The tumor-associated hCA IX isoform was efficiently inhibited by the herein reported benzofuran-based sulfonamides (**9a**,**b**, **11a**,**b**, **13a**, **15**, **17**, **19**, **27a**,**b**, **28a**,**b**, **29a** and **30**) with K_I values in the nanomolar range from 1.8 to 68.2 nM, Table 1. Superiorly, sulfonamides **11b**, **13a**, **17**, **19**, **28b**, **29a** and **30** displayed more potent hCA IX inhibitory activity than the

Table 1
Inhibition data of human CA isoforms hCA I, II, IX and XII for the target sulfonamides, using (AAZ) as a standard drug.



Comp.	*R*	K_I (nM)[a]			
		hCA I	hCA II	hCA IX	hCA XII
9a		40.1	28.2	68.2	24.4
9b		200.1	182.1	53.9	23.6
11a		495.8	135.3	25.3	17.1
11b		290.7	221.5	8.4	51.4
13a		47.6	15.0	10.4	32.3
13b		54.0 μM	4.9 μM	0.65 μM	0.52 μM
13c		>100 μM	31.3 μM	0.56 μM	0.97 μM
15	-	67.0	22.8	34.5	57.0
17		27.5	4.8	7.6	15.3
19		38.3	28.4	12.6	9.6
27a		71.8	54.3	35.8	31.6
27b		92.2	146.9	22.8	16.5
28a		280.1	140.2	20.2	8.1
28b		46.3	324.2	5.5	30.7
29a		66.8	18.3	7.1	19.1
29b		>100 μM	7.5 μM	0.93 μM	0.40 μM
29c		>100 μM	47.7 μM	2.3 μM	1.5 μM
30		164.4	8.0	1.8	24.2
AAZ	**AAZ**	250.0	12.0	25.0	5.7

[a] Mean from 3 different assays, by a stopped flow technique (errors were in the range of ± 5–10% of the reported values).

(**iv**) The data listed in Table 1 ascribed to the newly synthesized benzofuran-based sulfonamides (**9a**,**b**, **11a**,**b**, **13a**, **15**, **17**, **19**, **27a**,**b**, **28a**,**b**, **29a** and **30**) potent efficiency in inhibiting the transmembrane tumor-associated hCA XII isoform. The target sulfonamides possessed activity with K_I values spanning in the nanomolar range: 8.1–57.0 nM, Table 1. Compound **28a** was the most potent hCA XII inhibitor in this study with K_I value of 8.1 nM.

standard drug AAZ (K_I = 25 nM). Also, compounds **11a**, **15, 27a, 27b** and **28a** displayed potent inhibitory activity toward hCA IX isoform with K_I values equal 25.3, 37.5, 35.8, 22.8, and 20.2 nM, respectively. It is worth stressing that switching of the primary sulfamoyl functionality from *ortho*- and *meta*-positions to *para*-position in both MBFS (**9a** and **11a**; K_Is = 68.2 and 25.3 *vs.* **13a**; K_I = 10.4 nM) and BBFS (**27a** and **28a**; K_Is = 35.8 and 20.2 *vs.* **29a**; K_I = 7.1 nM) led to an enhancement of the *h*CA IX inhibitory activity as shown in Table 1.

Furthermore, elongation of urea linker in MBFS **13a** with ethylene (−CH_2CH_2−) spacer and in BBFS **29a** with amide (−HN-C═O) spacer led to compounds MBFS **17** and BBFS **30**, respectively, with increased activity (K_Is = 7.6 and 1.8 nM, respectively) towards *h*CA IX isoform. On the contrary, replacement of the urea linker in MBFS **13a** (K_I = 10.4 nM) with carbamate linker in MBFS **15** (K_I = 34.5 nM) was not more advantageous for the inhibitory activity against the tumor-associated *h*CA IX isoform.

It is worth emphasizing that substitution of sulfonamide amino group either with thiazole ring (**13b** and **29b**) or 3,4-dimethyl-1,2-oxazole ring (**13c** and **29c**) abolished the carbonic anhydrase inhibitory activity.

2.2.2. Anticancer activity

2.2.2.1. In vitro anti-proliferative activity. The obtained results from the CA assay (Table 1) showed that MBFS **11b** and BBFS **28b** are efficient single-digit nanomolar CA IX inhibitors (K_Is = 8.4 and 5.5 nM, respectively), in addition to their significant selectivity toward hCA IX isoform over the main off-target hCA II isoform (*S.I.* = 26.4 and 58.9, respectively), Table 2. As a consequence, MBFS **11b** and BBFS **28b** were chosen to be evaluated for their potential anti-proliferative activities toward breast cancer MDA-MB-231 and MCF-7 cell lines, by using the protocol of the MTT assay [31]. Staurosporine has been coassayed as a reference anticancer drug.

Table 2
Selectivity ratios for the inhibition of hCA IX and XII over hCA I and II for targeted MBFS (**9a-b**, **10a-b** and **13a-c**) and BBFS (**27a-b**, **28a-b** and **29a-c**).

Cmpd	I/IX	II/IX	I/XII	II/XII
9a	0.6	0.4	1.6	1.2
9b	3.7	3.4	8.5	7.7
11a	19.6	5.3	29.0	7.9
11b	34.6	**26.4**	5.7	4.3
13a	4.6	1.4	1.5	0.5
13b	NA	55.9	NA	32.3
13c	1.9	0.7	1.2	0.4
15	1.9	0.7	1.2	0.4
17	3.6	0.6	1.8	0.3
19	3.0	2.3	4.0	3.0
27a	2.0	1.5	2.3	1.7
27b	4.0	6.4	5.6	8.9
28a	13.9	6.9	34.6	17.3
28b	8.4	**58.9**	1.5	10.6
29a	9.4	2.6	3.5	1.0
29b	NA	8.1	NA	18.8
29c	NA	20.7	NA	31.8
30	91.3	4.4	6.8	0.3
AAZ	10.0	0.5	43.9	2.1

(v) The calculated selectivity indexes (SIs) displayed in Table 2 undeniably ascribed to the *m*-sulfonamides **11a,b** and **28a,b** excellent selectivity towards hCA IX over hCA I (SIs ranges: 19.6, 34.6, 13.9 and 8.4, respectively) and over hCA II (SIs ranges: 5.3, 26.4, 6.9 and 58.9, respectively) and also excellent selectivity towards hCA XII over hCA I (SIs ranges: 29.5, 5.7, 34.6 and 1.5, respectively) and weak selectivity over hCA II (SIs ranges: 7.9, 4.3, 17.3 and 1.6, respectively). Besides, sulfonamide **30** displayed excellent selectivity towards hCA IX over hCA I (SI: 91.3) and weak selectivity over towards hCA XII over hCA I (SI: 6.8).

Table 3
Anti-proliferative activities of MBFS **11b** and BBFS **28b** towards MDA-MB-231 and MCF-7 cancer cell lines.

Compound	IC_{50} (μM)[a]	
	MDA-MB-231	MCF-7
11b	6.27 ± 0.13	6.45 ± 0.12
28b	14.16 ± 0.3	13.79 ± 0.27
Staurosporine	6.75 ± 0.27	3.67 ± 0.07

The obtained results have been expressed as IC_{50} values and represented in Table 3.

As displayed in Table 3, MBFS **11b** showed excellent anti-proliferative activity against both the examined MDA-MB-231 and MCF-7 cancer cell lines with IC_{50s} of 6.27 ± 0.13 μM and 6.45 ± 0.12 μM, respectively, compared to reference drug staurosporine that possessed IC_{50s} of 6.75 ± 0.27 and 3.67 ± 0.07 μM, respectively. On the other hand, BBFS **28b** exerted moderate anti-proliferation impact against MDA-MB-231 and MCF-7 cancer cell lines with IC_{50} of 14.16 ± 0.3 μM in MDA-MB-231 cell line and IC_{50} of 13.79 ± 0.27 μM in MCF-7 cell line, Table 3.

*2.2.2.2. Impact of MBFS **11b** on apoptotic markers Bax, Bcl-2, and caspase-3.* Herein in the present study, the levels of certain mitochondria-related apoptotic proteins; Bax (a pro-apoptosis protein), Bcl-2 (an anti-apoptosis protein), and caspase-3 (a key executioner protease) have been determined in MDA-MB-231 and MCF-7 cells upon incubation with MBFS **11b** (Fig. 3).

The results depicted in Fig. 3, revealed that expression levels for Bax protein have been increased by 1.9-fold and 3.4-fold in **11b**-treated MDA-MB-231 and MCF-7 cells, respectively, in comparison to untreated control. In addition, treatment of MDA-MB-231 and MCF-7 cells with MBFS **11b** resulted in a significant elevation in the expression levels for active caspase-3 by 4.6-fold and 8.7-fold, respectively, compared to untreated control (Fig. 3). On the other hand, the expression levels of the anti-apoptotic Bcl-2 protein has been efficiently suppressed by 3.4-fold and 2.8-fold in **11b**-treated MDA-MB-231 and MCF-7 cells, respectively, in comparison to untreated control.

2.3. Molecular modeling studies

In order to reveal the binding modes and the relationships between structural features and inhibition activity of the synthesized compounds, docking and MM-GBSA-based refinements within hCA isozymes IX and XII (PDB 5FL4 [32] and 4WW8 [33]) were performed. As anticipated, docking poses of **11b** and **28b** revealed that the benzenesulfonamide fits deeply into the active site region of both isozymes, with the negatively charged nitrogen coordinating the zinc atom.

The docking study into the active site of hCA IX showed that **11b** forms three H-bonds (the NH^- group with T200, the S═O with T199, and the NH of the urea linker with H64). In addition, the benzenesulfonamide accommodates into the hydrophobic area defined by V121, L141, V143, and L198. Similarly, **28b** makes three H-bonds; the S═O with T199, the S═O with T200 and the NH of the urea linker with H64, as well as the hydrophobic interaction between the benzenesulfonamide ring and the hydrophobic area defined by V121, L141, V143, L198 and V207 (Fig. 4). Furthermore, additional hydrophobic interaction between the benzofuran ring of **28b** and L60 was observed.

Regarding hCA XII, the docking study disclosed that **11b** makes three H-bonds; T198 (S═O−NH) and T199 (S═O···NH and NH···O), while **28b** is at H-bond distance from T198 (S═O···NH), T199 (S═

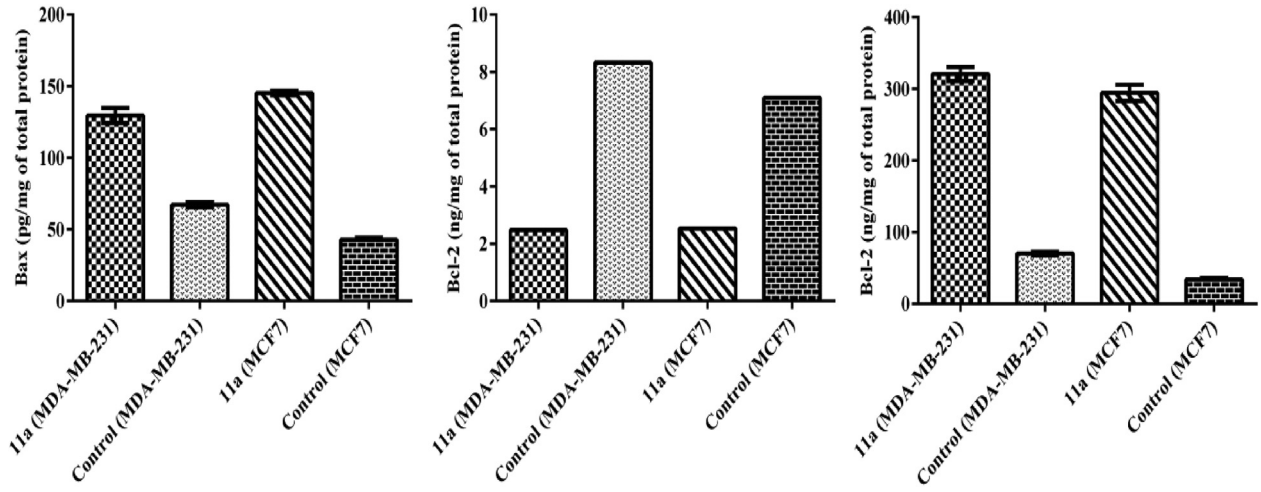


Fig. 3. Effect of MBFS **11b** on the expression levels of Bax, Bcl-2 and Caspase-3 in MDA-MB-231 and MCF-7 cells.

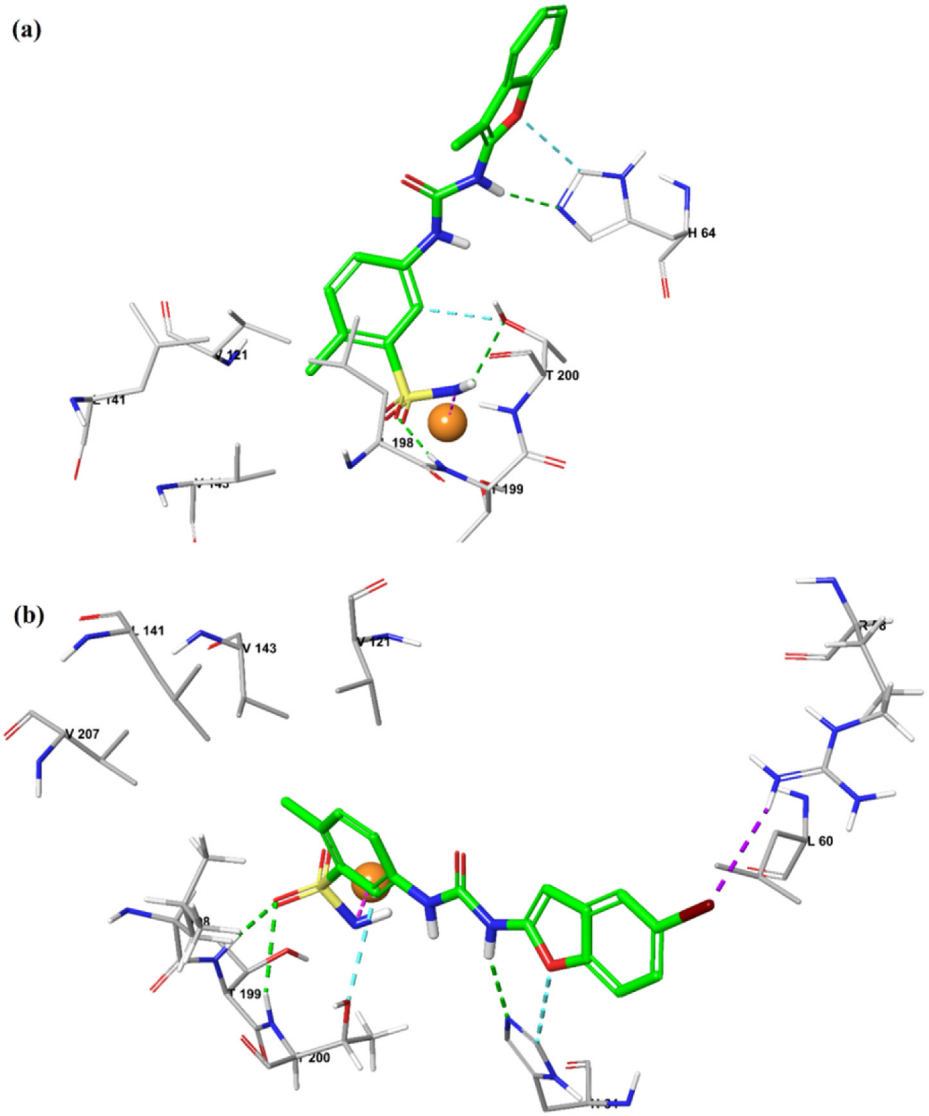


Fig. 4. Docking of A) **11b** and B) **28b** in hCA IX.

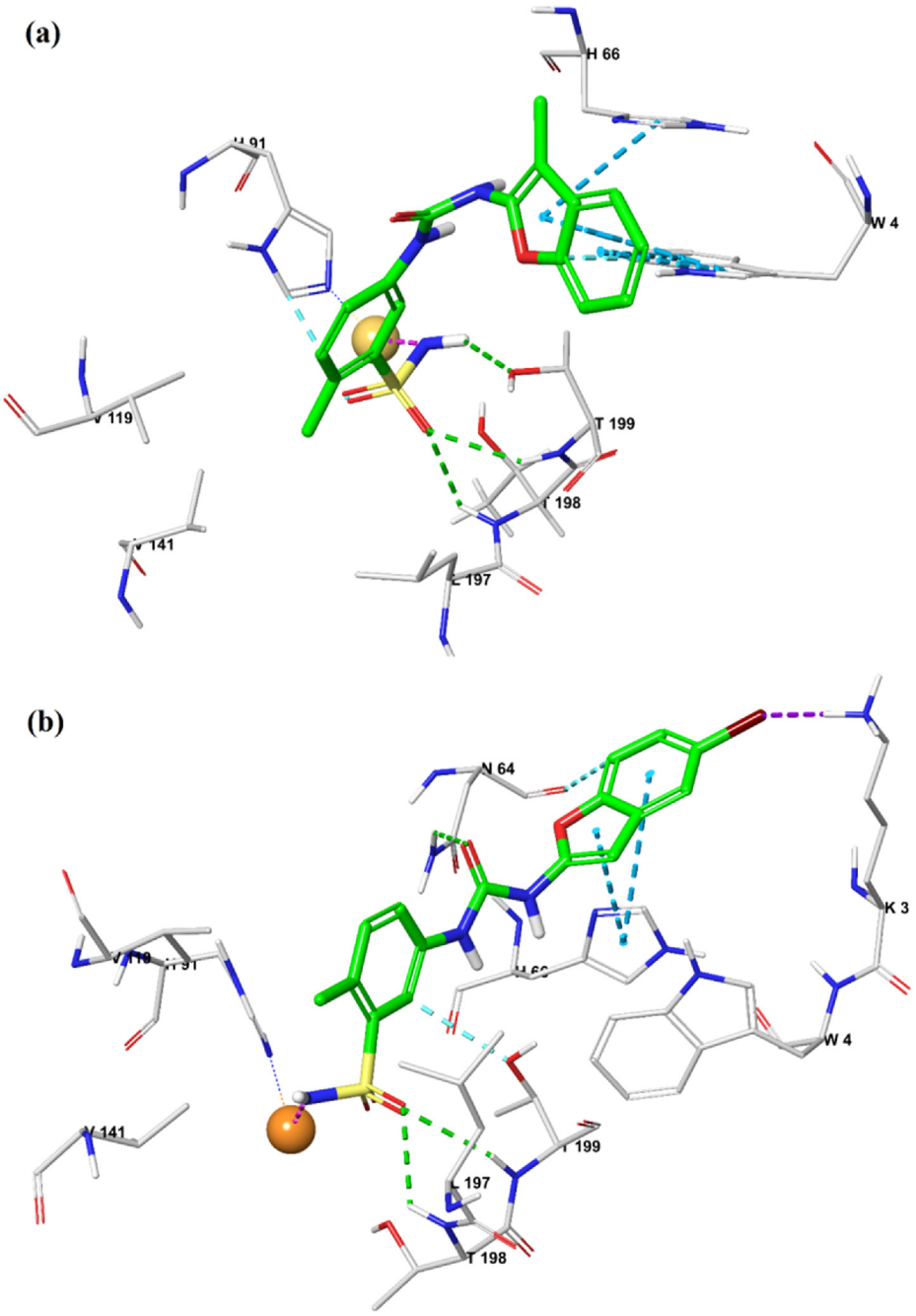


Fig. 5. Docking of A) **11b** and B) **28b** in hCA XII.

O···NH) and N64 (C═O···NH). In addition, the hydrophobic interaction of the benzenesulfonamide ring with residues H91, V119, V141 and L197 is observed for both compounds. The benzofuran ring of **11b** is stabilized by π-π interaction with W4 and H66, while that of **28b** interacts similarly with only H66 (Fig. 5).

The comparable observed *in vitro* inhibitory activity of **11b** and **28b** on each isozyme is in coherence with the narrow range of docking scores and prime MMGBSA dG binding energy as shown in Table 4.

Table 4
The docking scores and prime MMGBSA energy of **11b** and **28b** into hCA IX and XII.

	hCA IX		hCA XII	
	Docking score	MMGBSA dG bind	Docking score	MMGBSA dG bind
11b	−7.807	−45.91	−6.474	−33.24
28b	−7.438	−42.70	−5.825	−27.43

3. Conclusion

In the current work, different novel series of benzofuran-based sulfonamides (MBFS; **9a-b**, **11a-b**, **13a-c**, **15**, **17** and **19**) and (BBFS; **27a-b**, **28a-b**, **29a-c** and **30**) were designed, synthesized, characterized and explored for their CA inhibitory action against hCA I, II,

IX and XII. Most of the newly reported MBFS and BBFS efficiently inhibited the herein examined tumor-related isoforms *h*CA IX and XII with K_Is in the 1.8–68.2 nM and 8.1–57.0 nM ranges, respectively. Superiorly, MBFS (**11b** and **17**) and BBFS (**28b**, **29a** and **30**) efficiently inhibited the tumor-related CA IX isoform in the single-digit nanomolar range (K_Is = 8.4, 7.6, 5.5, 7.1 and 1.8 nM, respectively). In particular, MBFS **11b** and BBFS **28b** exhibited good selectivity toward hCA IX isoform over the main off-target hCA II isoform (*S.I.* = 26.4 and 58.9, respectively). While, MBFS **11b** elicited potent anti-proliferative activity against MDA-MB-231 cancer cells (IC_{50} = 6.27 ± 0.13 μM) and MCF-7 cancer cells (IC_{50} = 6.45 ± 0.12 μM), BBFS **28b** exerted moderate activity against both MDA-MB-231 cells (IC_{50} = 14.16 ± 0.3 μM) and MCF-7 cells (IC_{50} = 13.79 ± 0.27 μM). Furthermore, treatment of both MDA-MB-231 and MCF-7 cells with MBFS **11b** resulted in up-regulation of the expression levels of pro-apoptotic Bax and Caspase-3 proteins, as well as down-regulation of the expression level of anti-apoptotic Bcl-2 protein.

4. Experimental

4.1. Chemistry

4.1.1. General

All reaction solvents and reagents were purchased from commercial suppliers and used without further purification. Melting points were measured with a Stuart melting point apparatus and were uncorrected. The NMR spectra were obtained on Bruker Avance 400 (400 MHz ^{1}H and 100 MHz ^{13}C NMR). ^{1}H NMR spectra were referenced to tetramethylsilane (δ = 0.00 ppm) as an internal standard and were reported as follows: chemical shift, multiplicity (b = broad, s = singlet, d = doublet, t = triplet, dd = doublet of doublet, m = multiplet). IR spectra were recorded with a Bruker FT-IR spectrophotometer. Reaction courses and product mixtures were routinely monitored by thin layer chromatography (TLC) that carried out using glass sheets pre-coated with silica gel 60 F_{254} purchased by Merk. Compounds **4**–**7** and **23**–**26** were prepared according to the reported methods [23].

4.1.2. General procedures for preparation of target MBF and BBF sulfonamides

The 3-methylbenzofuran-2-carbonyl azide **6** (0.1 g, 0.50 mmol) or 5-bromobenzofuran-2-carbonyl azide **25** (0.13 g, 0.50 mmol) were dissolved in a dry toluene (10 mL), then the resulting solution was heated under reflux temperature for 1 h. Thereafter, an equimolar amount of the appropriate benzenesulfonamide derivatives (**8a-b**, **10a-b**, **12a-c**, **14**, **16** and **18**) was added to the previously prepared hot toluene solution. The reaction mixture was stirred under reflux temperature for 6 h. The precipitated solid was collected by filtration while hot, washed with cold ethanol and petroleum ether then recrystallized from acetonitrile to produce the target MBF and BBF sulfonamides.

4.1.2.1. 3-(3-Methyl-1-benzofuran-2-yl)-1-(2-sulfamoylphenyl)urea (9a). Compound **9a** was obtained following the general procedure mentioned-above using 2-aminobenzenesulfonamide **8a** (0.086 g, 0.50 mmol). 63% yield; m. p. 219–221 °C; IR (KBr, ν cm^{-1}): 3386, 3294 (NH, NH_2), 1663 (C=O) and 1341, 1158 (SO_2); ^{1}H NMR (DMSO-d_6) δ *ppm*: 2.15 (s, 3H, CH_3), 7.18–7.31 (m, 3H, Ar–3H), 7.47 (d, 1H, Ar–H, J = 7.6 Hz), 7.56 (t, 2H, Ar–2H, J = 7.6, 8.4 Hz), 7.69 (s, 2H, NH_2, D_2O exchangeable of $-SO_2NH_2$), 7.84 (d, 1H, Ar–H, J = 8 Hz), 8.16 (d, 1H, Ar–H, J = 8.4 Hz), 8.86 (s, 1H, NH, D_2O exchangeable), 8.82 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ *ppm*: 8.07, 105.86, 111.04, 119.58, 122.73, 122.92, 122.95, 124.28, 128.05, 130.14, 131.69, 133.14, 136.45, 144.34, 151.25, 152.71; Anal. Calcd. For $C_{16}H_{15}N_3O_4S$: C, 55.64; H, 4.38; N, 12.17; found C, 55.79; H, 4.34; N, 12.11.

4.1.2.2. 1-(5-Chloro-2,4-disulfamoylphenyl)-3-(3-methyl-1-benzofuran-2-yl)urea (9b). Compound **9a** was obtained following the general procedure mentioned-above using 4-amino-6-chlorobenzene-1,3-disulfonamide **8b** (0.14 g, 0.50 mmol). 61% yield; m. p. 263–264 °C; IR (KBr, ν cm^{-1}): 3327, 3265 (NH, NH_2), 1685 (C=O) and 1322, 1147 (SO_2); ^{1}H NMR (DMSO-d_6) δ *ppm*: 2.13 (s, 3H, CH_3), 7.22–7.30 (m, 4H, Ar–H), 7.46 (s, 2H, NH_2, D_2O exchangeable of $-SO_2NH_2$), 7.48 (s, H, Ar–H), 7.53 (s, 2H, NH_2, D_2O exchangeable of $-SO_2NH_2$), 7.56 (s, H, Ar–H), 9.09 (s, 2H, 2NH, D_2O exchangeable); Anal. Calcd. for $C_{16}H_{15}ClN_4O_6S_2$: C, 41.88; H, 3.29; N, 12.21; found C, 42.17; H, 3.32; N, 12.25.

4.1.2.3. 3-(3-Methyl-1-benzofuran-2-yl)-1-(3-sulfamoylphenyl)urea (11a). Compound **9a** was obtained following the general procedure mentioned-above using 3-aminobenzenesulfonamide **10a** (0.086 g, 0.50 mmol). 70% yield; m. p. 216–218 °C; IR (KBr, ν cm^{-1}): 3355, 3271 (NH, NH_2), 1666 (C=O) and 1323, 1151 (SO_2); ^{1}H NMR (DMSO-d_6) δ *ppm*: 2.13 (s, 3H, CH_3), 7.21–7.33 (m, 2H, Ar–H), 7.36 (s, 2H, NH_2, D_2O exchangeable of $-SO_2NH_2$), 7.43–7.52 (m, 3H, Ar–H), 7.56 (d, 1H, Ar–H, J = 7.2 Hz), 7.64 (d, 1H, Ar–H, J = 6.8 Hz), 8.07 (s, 1H, Ar–H), 8.86 (s, 1H, NH, D_2O exchangeable), 9.29 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ *ppm*: 8.10, 106.30, 111.03, 116.15, 119.59, 119.77, 122.11, 122.88, 124.31, 129.85, 130.17, 140.39, 144.40, 145.06, 151.26, 153.10; Anal. Calcd. for $C_{16}H_{15}N_3O_4S$: C, 55.64; H, 4.38; N, 12.17; found C, 55.91; H, 4.33; N, 12.09.

4.1.2.4. 3-(3-Methyl-1-benzofuran-2-yl)-1-(4-methyl-3-sulfamoylphenyl)urea (11b). Compound **9a** was obtained following the general procedure mentioned-above using 5-amino-2-methylbenzenesulfonamide **10b** (0.093 g, 0.50 mmol). 73% yield; m. p. 223–224 °C; IR (KBr, ν cm^{-1}): 3379, 3257 (NH, NH_2), 1662 (C=O) and 1322, 1154 (SO_2); ^{1}H NMR (DMSO-d_6) δ *ppm*: 2.12 (s, 3H, CH_3), 2.53 (s, 3H, CH_3), 7.22–7.32 (m, 3H, Ar–H), 7.36 (s, 2H, NH_2, D_2O exchangeable of $-SO_2NH_2$), 7.56 (d, 1H, Ar–H, J = 8.0 Hz), 7.59 (d, 1H, Ar–H, J = 7.2 Hz), 7.61 (d, 1H, Ar–H, J = 8.4 Hz), 8.06 (s, 1H, Ar–H), 8.77 (s, 1H, NH, D_2O exchangeable), 9.19 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ *ppm*: 8.11, 19.59, 106.16, 111.01, 118.09, 119.55, 122.19, 122.85, 124.24, 129.54, 130.21, 132.95, 137.92, 142.64, 144.56, 151.24, 153.11; Anal. Calcd. for $C_{17}H_{17}N_3O_4S$: C, 56.81; H, 4.77; N, 11.69; found C, 57.02; H, 4.79; N, 11.77.

4.1.2.5. 3-(3-Methyl-1-benzofuran-2-yl)-1-(4-sulfamoylphenyl)urea (13a). Compound **9a** was obtained following the general procedure mentioned-above using 4-aminobenzenesulfonamide **12a** (0.086 g, 0.50 mmol). 76% yield; m. p. 241–243 °C; IR (KBr, ν cm^{-1}): 3361, 3274 (NH, NH_2), 1680 (C=O) and 1322, 1151 (SO_2); ^{1}H NMR (DMSO-d_6) δ *ppm*: 2.12 (s, 3H, CH_3), 7.34 (s, 2H, NH_2, D_2O exchangeable of $-SO_2NH_2$), 7.40–7.46 (m, 2H, Ar–H), 7.50 (d, 1H, Ar–H, J = 7.6 Hz), 7.54 (d, 2H, Ar–H, J = 7.6 Hz), 7.59 (d, 1H, Ar–H, J = 7.6 Hz), 7.71 (d, 2H, Ar–H, J = 8 Hz), 8.91 (s, 1H, NH, D_2O exchangeable), 9.47 (s, 1H, NH, D_2O exchangeable); Anal. Calcd. for $C_{16}H_{15}N_3O_4S$: C, 55.64; H, 4.38; N, 12.17; found C, 55.48; H, 4.42; N, 12.25.

4.1.2.6. 3-(3-Methyl-1-benzofuran-2-yl)-1-(4-[(1,3-thiazol-2-yl)sulfamoyl]phenyl)urea (13b). Compound **9a** was obtained following the general procedure mentioned-above using 4-amino-*N*-(thiazol-2-yl)benzenesulfonamide **12b** (0.127 g, 0.50 mmol). 72% yield; m. p. 248–250 °C; IR (KBr, ν cm^{-1}): 3356, 3292, 3102 (NH, NH_2), 1713 (C=O) and 1318, 1144 (SO_2); ^{1}H NMR (DMSO-d_6) δ *ppm*: 2.11 (s, 3H, CH_3), 6.81 (d, 1H, Ar–H, J = 8 Hz), 7.19–7.32 (m, 3H, Ar–H), 7.46 (d, 1H, Ar–H, J = 7.6 Hz), 7.54 (d, 1H, Ar–H, J = 7.6 Hz), 7.63 (d, 2H,

Ar−H, J = 8 Hz), 7.74 (d, 2H, Ar−H, J = 8 Hz), 8.88 (s, 1H, NH, D_2O exchangeable), 9.35 (s, 1H, NH, D_2O exchangeable), 12.6 (s, 1H, NH, D_2O exchangeable of $-SO_2NH$); Anal. Calcd. for $C_{19}H_{16}N_4O_4S_2$: C, 53.26; H, 3.76; N, 13.08; found C, 53.11; H, 3.8; N, 12.99.

4.1.2.7. 1-(4-[(3,4-Dimethyl-1,2-oxazol-5-yl)sulfamoyl]phenyl)-3-(3-methyl-1-benzofuran-2-yl)urea (13c). Compound **9a** was obtained following the general procedure mentioned-above using 4-amino-*N*-(3,4-dimethylisoxazol-5-yl)benzenesulfonamide **12c** (0.133 g, 0.50 mmol). 75% yield; m. p. 169−171 °C; IR (KBr, ν cm^{-1}): 3342, 3290 (NH, NH_2), 1681 (C═O) and 1341, 1162 (SO_2); ^{1}H NMR (DMSO-d_6) δ *ppm*: 1.05 (d, 3H, CH_3), 1.66 (s, 3H, CH_3), 2.11 (d, 3H, CH_3), 7.27 (m, 2H, Ar−H), 7.47 (d, 1H, Ar−H, J = 7.6 Hz), 7.55 (d, 1H, Ar−H, J = 7.6 Hz), 7.69 (d, 4H, Ar−H, J = 8 Hz), 8.59 (s, 1H, NH, D_2O exchangeable), 9.49 (s, 1H, NH, D_2O exchangeable), 10.9 (s, 1H, NH, D_2O exchangeable of $-SO_2NH$); Anal. Calcd. for $C_{21}H_{20}N_4O_5S$: C, 57.26; H, 4.58; N, 12.72; found C, 57.53; H, 4.55; N, 12.78.

4.1.2.8. 4-Sulfamoylphenyl N-(3-methyl-1-benzofuran-2-yl)carbamate (15). Compound **9a** was obtained following the general procedure mentioned-above using 4-hydroxybenzenesulfonamide **14** (0.086 g, 0.50 mmol). 60% yield; m. p. 259−260 °C; IR (KBr, ν cm^{-1}): 3250, 3167 (NH, NH_2), 1668 (C═O) and 1346, 1188 (SO_2); ^{1}H NMR (DMSO-d_6) δ *ppm*: 2.13 (s, 3H, CH_3), 6.90 (d, 2H, Ar−H, J = 8 Hz), 7.12 (s, 2H, NH_2, D_2O exchangeable of $-SO_2NH_2$), 7.21−7.34 (m, 2H, Ar−H), 7.47 (d, 1H, Ar−H, J = 8 Hz), 7.54 (d, 1H, Ar−H, J = 7.2 Hz), 7.67 (d, 2H, Ar−H, J = 8 Hz), 9.23 (s (b), 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ *ppm*: 8.08, 106.62, 111.05, 115.33, 115.65, 119.61, 122.82, 124.32, 128.30, 128.58, 128.68, 130.12, 135.00, 144.56, 151.29, 153.68, 160.79; Anal. Calcd. for $C_{16}H_{14}N_2O_5S$: C, 55.48; H, 4.07; N, 8.09; found C, 55.63; H, 4.05; N, 8.02.

4.1.2.9. 3-(3-Methyl-1-benzofuran-2-yl)-1-[2-(4-sulfamoylphenyl) ethyl]urea (17). Compound **9a** was obtained following the general procedure mentioned-above using 4-(2-aminoethyl)benzenesulfonamide **16** (0.1 g, 0.50 mmol). 68% yield; m. p. 238−239 °C; IR (KBr, ν cm^{-1}): 3421, 3356, 3280 (NH, NH_2), 1691 (C═O) and 1334, 1153 (SO_2); ^{1}H NMR (DMSO-d_6) δ *ppm*: 2.05 (s, 3H, CH_3), 2.85 (t, 2H, $-CH_2CH_2$-, J = 8 Hz), 3.35 (t, 2H, $-CH_2CH_2$-, J = 8 Hz), 6.48 (t, 1H, Ar−H, J = 5.4 Hz) 7.19−7.29 (m, 4H, Ar−H, NH_2, D_2O exchangeable of $-SO_2NH_2$), 7.43 (d, 3H, Ar−H, J = 8 Hz, NH, D_2O exchangeable), 7.50 (d, 1H, Ar−H, J = 6.8 Hz), 7.78 (d, 2H, Ar−H, J = 8 Hz), 8.55 (s, 1H, NH, D_2O exchangeable); Anal. Calcd. for $C_{18}H_{19}N_3O_4S$: C, 57.9; H, 5.13; N, 11.25; found C, 58.09; H, 5.07; N, 11.28.

4.1.2.10. N-([(3-methyl-1-benzofuran-2-yl)carbamoyl]amino)-4-sulfamoylbenzamide (19). Compound **9a** was obtained following the general procedure mentioned-above using 4-(hydrazinecarbonyl)benzenesulfonamide **18** (0.11 g, 0.50 mmol). 76% yield; m. p. 252−253 °C; IR (KBr, ν cm^{-1}): 3420, 3352, 3254 (NH, NH_2), 1692 (C═O) and 1327, 1151 (SO_2); ^{1}H NMR (DMSO-d_6) δ *ppm*: 2.11 (d, 3H, CH_3, J = 14 Hz), 7.19−7.33 (m, 3H, Ar−H), 7.46 (t, 1H, Ar−H, J = 8.4 Hz), 7.53 (s, 2H, NH_2, D_2O exchangeable of $-SO_2NH_2$), 7.55 (d, 1H, Ar−H, J = 8.4 Hz), 7.94 (d, 2H, Ar−H, J = 8 Hz), 8.09 (d, 1H, Ar−H, J = 8.4 Hz), 8.67 (s, 1H, NH, D_2O exchangeable), 9.09 (d, 1H, NH, D_2O exchangeable, J = 14 Hz), 10.56 (s, 1H, NH, D_2O exchangeable); . Anal. Calcd. for $C_{17}H_{16}N_4O_5S$: C, 52.57; H, 4.15; N, 14.43; found C, 52.69; H, 4.19; N, 14.35.

4.1.2.11. 3-(5-Bromo-1-benzofuran-2-yl)-1-(2-sulfamoylphenyl)urea (27a). Compound **9a** was obtained following the general procedure mentioned-above using 2-aminobenzenesulfonamide **8a** (0.086 g, 0.5 mmol). 77% yield; m. p. 189−191 °C; IR (KBr, ν cm^{-1}): 3467, 3259, 3180 (NH, NH_2), 1691 (C═O) and 1333, 1156 (SO_2); ^{1}H NMR (DMSO-d_6) δ *ppm*: 2.15 (s, 3H, CH_3), 7.18−7.31 (m, 3H, Ar−H), 7.47 (d, 1H, Ar−H, J = 7.6 Hz), 7.56 (t, 2H, Ar−H, J = 7.6, 8.4 Hz), 7.69 (s, 2H, NH_2, D_2O exchangeable of $-SO_2NH_2$), 7.84 (d, 1H, Ar−H, J = 8 Hz), 8.16 (d, 1H, Ar−H, J = 8.4 Hz), 8.86 (s, 1H, NH, D_2O exchangeable), 8.82 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ *ppm*: 8.07, 105.86, 111.04, 119.58, 122.73, 122.92, 122.95, 124.28, 128.05, 130.14, 131.69, 133.14, 136.45, 144.34, 151.25, 152.71 . Anal. Calcd. for $C_{15}H_{12}BrN_3O_4S$: C, 43.92; H, 2.95; N, 10.24; found C, 44.17; H, 2.93; N, 10.17.

4.1.2.12. 3-(5-Bromo-1-benzofuran-2-yl)-1-(5-chloro-2,4-disulfamoylphenyl)urea (27b). Compound **9a** was obtained following the general procedure mentioned-above using 4-amino-6-chlorobenzene-1,3-disulfonamide **8b** (0.14 g, 0.50 mmol). 75% yield; m. p. 221−223 °C; IR (KBr, ν cm^{-1}): 3407, 3314, 3195 (NH, NH_2), 1702 (C═O) and 1327, 1136 (SO_2); ^{1}H NMR (DMSO-d_6) δ *ppm*: 6.56 (s, 1H, Ar−H), 6.62 (s, 2H, Ar−H), 6.99 (s, 1H, Ar−H), 7.30 (d, H, Ar−H, J = 8.0 Hz), 7.36 (s, 2H, NH_2, D_2O exchangeable of $-SO_2NH_2$), 7.45 (d, 1H, Ar−H, J = 8.0 Hz), 7.52 (s, 2H, NH_2, D_2O exchangeable of $-SO_2NH_2$), 7.72 (s, 1H, NH, D_2O exchangeable), 8.17 (s, 1H, NH, D_2O exchangeable); Anal. Calcd. for $C_{15}H_{12}BrClN_4O_6S_2$: C, 34.4; H, 2.31; N, 10.7; found C, 34.51; H, 2.33; N, 10.64.

4.1.2.13. 3-(5-Bromo-1-benzofuran-2-yl)-1-(3-sulfamoylphenyl)urea (28a). Compound **9a** was obtained following the general procedure mentioned-above using 3-aminobenzenesulfonamide **10a** (0.086 g, 0.50 mmol). 69% yield; m. p. 181−183 °C; IR (KBr, ν cm^{-1}): 3358, 3271 (NH, NH_2), 1704 (C═O) and 1339, 1157 (SO_2); ^{1}H NMR (DMSO-d_6) δ *ppm*: 6.52 (s, 1H, Ar−H), 7.28 (d, 1H, Ar−H, J = 8 Hz), 7.36−7.46 (m, 4H, Ar−H, NH_2, D_2O exchangeable of $-SO_2NH_2$), 7.51 (d, 1H, Ar−H, J = 6 Hz), 7.62 (d, 1H, Ar−H, J = 6 Hz), 7.69 (s, 1H, Ar−H), 8.13 (d, 1H, Ar−H, J = 8 Hz), 9.22 (s, 1H, NH, D_2O exchangeable), 10.02 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ *ppm*: 87.04, 112.42, 115.77, 115.97, 120.10, 122.02, 122.33, 124.78, 130.03, 132.49, 139.82, 145.18, 148.48, 150.84, 151.63; Anal. Calcd. for $C_{15}H_{12}BrN_3O_4S$: C, 43.92; H, 2.95; N, 10.24; found C, 44.21; H, 2.96; N, 10.31.

4.1.2.14. 3-(5-Bromo-1-benzofuran-2-yl)-1-(4-methyl-3-sulfamoylphenyl)urea (28b). Compound **9a** was obtained following the general procedure mentioned-above using 5-amino-2-methylbenzenesulfonamide **10b** (0.093 g, 0.50 mmol). 71% yield; m. p. 196−198 °C; IR (KBr, ν cm^{-1}): 3415, 3274, 3210 (NH, NH_2), 1695 (C═O) and 1331, 1142 (SO_2); ^{1}H NMR (DMSO-d_6) δ *ppm*: 2.54 (s, 3H, CH_3), 6.50 (s, 1H, Ar−H), 7.21−7.34 (m, 2H, Ar−H), 7.40 (s, 2H, NH_2, D_2O exchangeable of $-SO_2NH_2$), 7.42 (d, 1H, Ar−H, J = 8.4 Hz), 7.58 (d, 1H, Ar−H, J = 8.4 Hz), 7.68 (s, 1H, Ar−H), 8.08 (s, 1H, Ar−H), 9.12 (s, 1H, NH, D_2O exchangeable), 9.93 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ *ppm*: 19.60, 86.86, 112.38, 115.95, 117.90, 122.04, 122.26, 124.70, 128.67, 129.97, 133.12, 137.30, 142.80, 148.44, 150.83, 151.74; Anal. Calcd. for $C_{16}H_{14}BrN_3O_4S$: C, 45.3; H, 3.33; N, 9.9; found C, 45.18; H, 3.32; N, 9.85.

4.1.2.15. 3-(5-Bromo-1-benzofuran-2-yl)-1-(4-sulfamoylphenyl)urea (29a). Compound **9a** was obtained following the general procedure mentioned-above using 4-aminobenzenesulfonamide **12a** (0.086 g, 0.50 mmol). 74% yield; m. p. 230−231 °C; IR (KBr, ν cm-1): 3327, 3238 (NH, NH_2), 1696 (C═O) and 1330, 1150 (SO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ *ppm*: 6.53 (s, 1H, Ar−H) 7.27 (s, 2H, NH_2, D_2O exchangeable of $-SO_2NH_2$) 7.28 (d, 1H, Ar−H, J = 8 Hz), 7.43 (d, 1H, Ar−H, J = 8 Hz), 7.67 (t, 3H, Ar−H, J = 8 Hz), 7.78 (d, 2H, Ar−H, J = 8 Hz), 9.25 (s, 1H, NH, D_2O exchangeable), 10.02 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ *ppm*: 87.16, 112.43, 115.98, 118.45, 118.45, 122.37, 124.85, 127.34, 127.34, 132.44, 138.09, 142.44, 148.48, 150.70, 151.47; Anal. Calcd. for $C_{15}H_{12}BrN_3O_4S$: C, 43.92; H, 2.95; N, 10.24; found C, 44.09; H, 2.93; N, 10.29.

4.1.2.16. 3-(5-Bromo-1-benzofuran-2-yl)-1-(4-[(1,3-thiazol-2-yl)sulfamoyl]phenyl)urea (29b). Compound **9a** was obtained following the general procedure mentioned-above using 4-amino-*N*-(thiazol-2-yl)benzenesulfonamide **12b** (0.127 g, 0.50 mmol). 68% yield; m. p. 233–234 °C; IR (KBr, ν cm^{-1}): 3367, 3318, 3250 (NH, NH_2), 1718 (C=O) and 1329, 1136 (SO_2); ^{1}H NMR (DMSO-d_6) δ *ppm*: 6.50 (s, 1H, Ar–H), 6.82 (d, 1H, Ar–H, J = 4.8 Hz), 7.25 (d, 1H, Ar–H, J = 4.8 Hz), 7.28 (d, 1H, Ar–H, J = 8.4 Hz), 7.44 (d, 2H, Ar–H, J = 8.8 Hz), 7.63 (d, 2H, Ar–H, J = 8.4 Hz), 7.69 (s, 1H, NH, D_2O exchangeable), 7.76 (d, 2H, Ar–H, J = 8.4 Hz), 9.24 (s, 1H, NH, D_2O exchangeable of $-SO_2NH$), 10.02 (s, 1H, NH, D_2O exchangeable); Anal. Calcd. for $C_{18}H_{13}BrN_4O_4S_2$: C, 43.82; H, 2.66; N, 11.36; found C, 44.12; H, 2.64; N, 11.41.

4.1.2.17. 3-(5-Bromo-1-benzofuran-2-yl)-1-(4-[(3,4-dimethyl-1,2-oxazol-5-yl)sulfamoyl]phenyl)urea (29c). Compound **9a** was obtained following the general procedure mentioned-above using 4-amino-*N*-(3,4-dimethylisoxazol-5-yl)benzenesulfonamide **12c** (0.133 g, 0.50 mmol). 72% yield; m. p. 219–221 °C; IR (KBr, ν cm^{-1}): 3345, 3307, 3244 (NH, NH_2), 1713 (C=O) and 1323, 1159 (SO_2); ^{1}H NMR (DMSO-d_6) δ *ppm*: 1.66 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 6.52 (s, 1H, Ar–H), 7.28 (d, 1H, Ar–H, J = 8.4 Hz), 7.44 (d, 1H, Ar–H, J = 8.8 Hz), 7.71 (s, 6H, Ar–H, NH, D_2O exchangeable of $-SO_2NH_2$), 9.37 (s, 1H, NH, D_2O exchangeable), 10.10 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ *ppm*: 6.33, 10.78, 87.32, 105.50, 112.46, 115.99, 118.65, 118.65, 122.42, 124.91, 128.53, 128.53, 132.40, 133.44, 143.90, 148.51, 150.63, 151.36, 156.09, 161.89; Anal. Calcd. for $C_{20}H_{17}BrN_4O_5S$: C, 47.54; H, 3.39; N, 11.09; found C, 47.41; H, 3.42; N, 11.17.

4.1.2.18. N-([(5-Bromo-1-benzofuran-2-yl)carbamoyl]amino)-4-sulfamoylbenzamide (30). Compound **9a** was obtained following the general procedure mentioned-above using 4-(hydrazinecarbonyl)benzenesulfonamide **18** (0.11 g, 0.50 mmol). 78% yield; m. p. 211–213 °C; IR (KBr, ν cm^{-1}): 3361, 3331, 3268 (NH, NH_2), 1682 (C=O) and 1333, 1163 (SO_2); ^{1}H NMR (DMSO-d_6) δ *ppm*: 6.47 (s, 1H, Ar–H), 7.26 (d, 1H, Ar–H, J = 8 Hz) 7.40 (d, 1H, Ar–H, J = 8.8 Hz), 7.52 (d, 2H, Ar–H, J = 8.8 Hz), 7.68 (s, 1H, Ar–H), 7.89 (d, 1H, Ar–H, J = 8.4 Hz), 7.88–8.00 (m, 3H, Ar–H, NH_2, D_2O exchangeable of $-SO_2NH_2$), 8.09 (d, 1H, Ar–H, J = 8.4 Hz), 8.98 (s, 1H, NH, D_2O exchangeable), 10.37 (s, 1H, NH, D_2O exchangeable), 10.59 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ *ppm*: 112.33, 115.89, 122.29, 124.71, 126.13, 126.13, 128.10, 128.10, 128.83, 132.43, 135.84, 136.71, 146.58, 147.28, 148.48, 165.98; Anal. Calcd. for $C_{16}H_{13}BrN_4O_5S$: C, 42.4; H, 2.89; N, 12.36; found C, 42.58; H, 2.87; N, 12.43.

4.2. Biological evaluation

All adopted procedures for the conducted *in vitro* biological assays were performed as described earlier; CA (stopped-flow [30,34]), cytotoxicity (MTT [35,36]), and assessment of apoptotic markers [37–39] assays, and were mentioned in the Supporting Materials.

4.3. Molecular modelling

The utilized procedures within the docking experiments for MBFS **11b** and BBFS **28b** in hCA IX (pdb 5FL4) and hCA XII (pdb 4WW8) active sites are provided in the supplementary materials.

Declaration of competing interest

The authors have declared no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2021.113283.

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