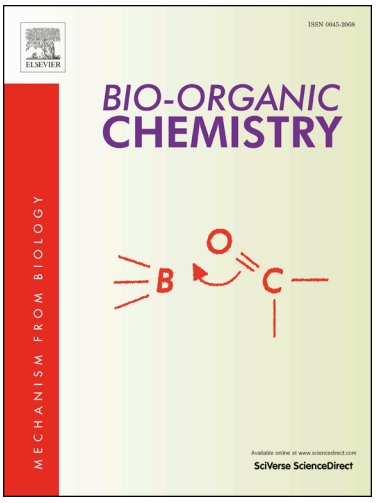

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1,2,4-Trisubstituted imidazolinones with dual carbonic anhydrase and p38 mitogen-activated protein kinase inhibitory activity

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Abstract: Various 1,2,4 trisubstituted imidazolin-5-one derivatives were synthesized and evaluated for their inhibitory activity against p38 mitogen-activated protein kinase (p38MAPK) and carbonic anhydrase (CA) enzymes aiming to explore potential dual inhibitors. Results revealed that compounds **3c, 3g, 3h, 4a, 6c** and **6d** were the most effective derivatives against p38αMAPK (IC_{50}= 0.14, 0.14, 0.056, 0.14, 0.13 and 0.14 μM, respectively) compared to sorafenib (IC_{50}= 1.58 μM) as standard drug. On the other hand, compound **4a** revealed the best inhibitory activity against all the tested carbonic anhydrase isoforms CA I, II, IV and IX with K_i values of 95.0, 0.83, 6.90 and 12.4 nM, respectively compared to acetazolamide with K_i values 250, 12.1, 74 and 12.8 nM, respectively. Therefore, compound **4a** can be considered as a potent dual p38αMAPK/CA inhibitor.

Keywords: Imidazoline-5-ones; sulphonamide derivatives; p38αMAPK inhibitors; carbonic anhydrase inhibitors.

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1. Introduction:

Carbonic anhydrases (CA) are zinc metalloenzymes that exist in 15 different enzyme isoforms and play a major role in catalyzing the interconversion of carbon dioxide and water to bicarbonate and protons [1]. They are involved in numerous physiological and pathological processes such as gluconeogenesis, lipogenesis, ureagenesis and tumorigenicity [2]. The human membrane-bound enzyme carbonic anhydrase hCA IX expression is usually induced by hypoxia in certain types of solid tumors, such as glioma, breast cancer and colon carcinoma [3-5]. Inhibition of hCA IX was strongly associated with significant suppression of the growth of both primary tumor stages as well as metastases which makes the enzyme a validated tumor hypoxia marker and anticancer drug target [6-8]. Added to the above, several benzene sulfonamide-containing compounds were reported as efficient CA inhibitors (CAIs), where the negatively charged nitrogen of SO_2NH^- coordinates with the positively charged metal ion [9,10]. However, selectivity of tumor associated isoform IX targets remains an obstacle towards a remarkable therapeutic progress in this area [5,10]. The binding mode of a series of arylimidzoline benzene sulfonamide hybrids as represented by (compound **I) (Figure 1)** denoted the coordination between the sulfamoyl group as the Zn binding group to the Zn ion present within the hCAs active site and an appended aryl group occupied in the hydrophobic part of the enzyme **(Figure 2a)** [11]. Likewise, CAIs should possess a Zn binding group (ZBG) and an appended hydrophobic tail for ideal enzyme interaction activity [2]. On the other hand, a serine/threonine protein kinase, p38 mitogen-activated protein kinase (p38MAPK), that is activated by several environmental stimuli such as stress or via the immune

response [12] plays an essential role in the signal transduction pathways leading to the biosynthesis of pro-inflammatory cytokines, interleukin-1ß (IL-1ß) and tumor necrosis factor-α (TNF-α) [13,14]. Accordingly, effective p38MAPK inhibitors, especially the strongly expressed p38αMAPK subtype [14-17] were investigated. Several successful p38αMAPK inhibitors possessed an imidazole moiety (SB203580 **II**, **III** and **IV** with IC_{50}= 0.29, 4.5 and 0.00024 µM, respectively) as a constituting core **(Figure 1)**. The binding of the inhibitor SB203580 **(II)** to the p38α kinase active site involves hydrogen bonding between the imidazole nitrogen and the amino group of Lys53. In addition, the 4-aryl ring of the inhibitor interacts with an additional hydrophobic pocket and the other aryl group at position 2 extends into the phosphate-binding region where a π–π-stacking with Tyr35 occurs **(Figure 2b)** [18].

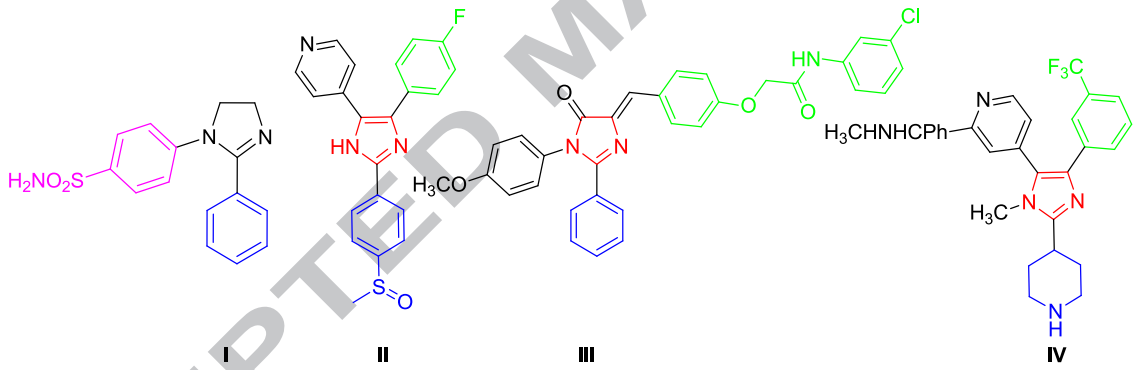
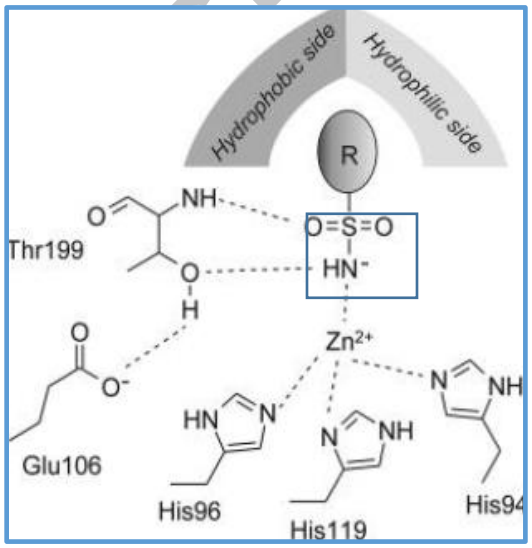
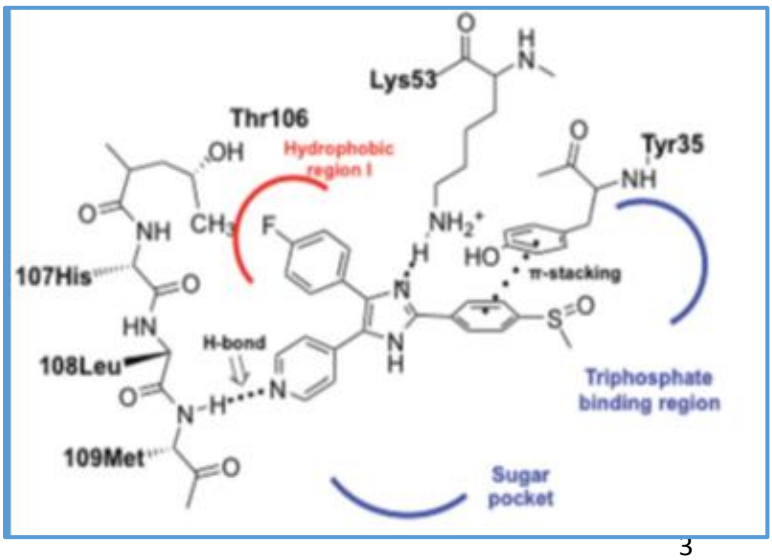


Figure 1: The structure of potent p38αMAPK/CA inhibitors





(a) **(b)**

Figure 2: (a) The key interactions between a sulfonamide inhibitor and hCA II active site (b) The key interactions between an imidazole inhibitor (II) and p38αMAPK active site

Nevertheless, surveys for dual p38αMAPK/CA inhibitors were supported by the fact that inhibition of MAPK enzyme reduces both CA IX promoter activity and CA IX protein expression in both hypoxia and high cell density [19]. Therefore, the scaffold design of the present dual p38αMAPK/CA inhibitor target compounds was based on combining the sulphamoyl group as the (ZBG) on the phenyl substituent at position 1 of the imidazole ring together with the hydrophobic appendage at position 2 of the scaffold being accommodated in the enzyme pocket in order to favor the CA inhibitory activity on one hand, while preserving the substituted imidazoline core to favor p38αMAPK inhibition on the other hand. **(Figure 3)**.

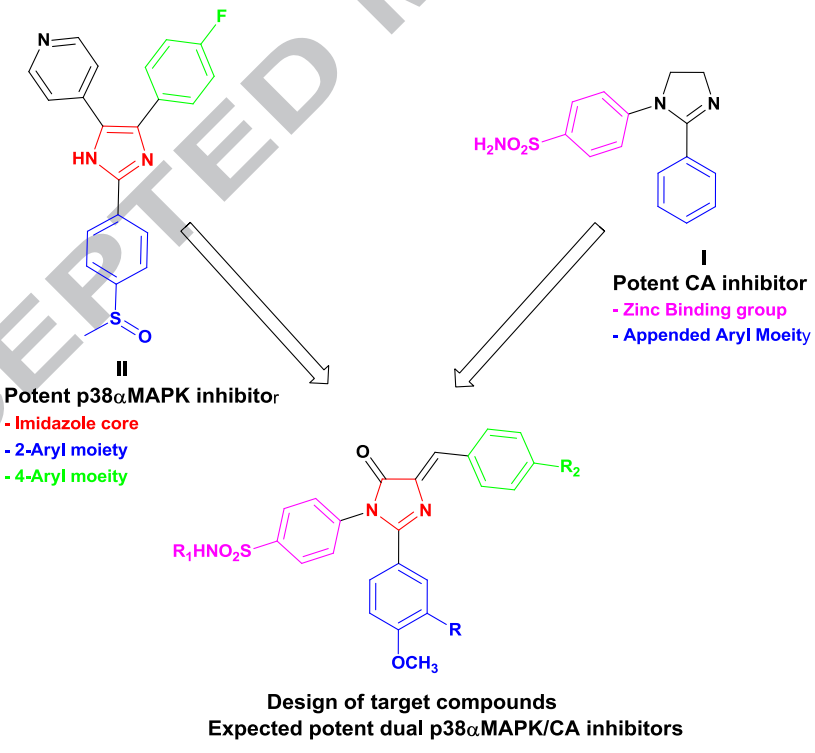


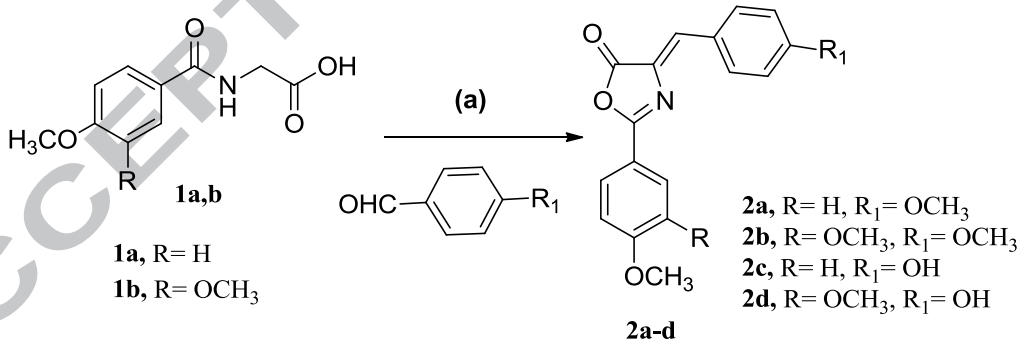
Figure 3: Design approach to 1,2,4-trisubstituted imidazolinone derivatives 3a-ł, 4a,b and 6a-f as dual p38αMAPK/CA inhibitors

2. Results and discussion

2.1. Chemistry

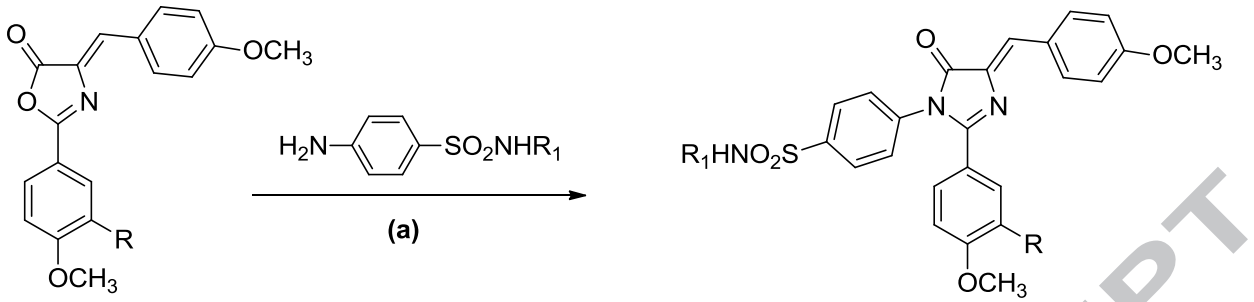
The target compounds **3a-ł**, **4a,b** and **6a-f** were synthesized according to schemes 1-3. Firstly in scheme 1, Erlenmyer reaction of methoxy/dimethoxy hippuric acid **(1a-b)** [20,21] with 4-hydroxybenzaldehyde or 4-methoxybenzaldehyde in acetic anhydride to yield *2*-(3-un/substituted-4-methoxyphenyl)-4-(4-hydroxy/methoxybenzylidene) **(2a-d)** according to the reported method [22,23]. Secondly, the imidazolinone derivatives (**3a-ł)** were obtained through the reaction of the oxazolone intermediates **(2a,b)** with different sulfonamide derivatives in glacial acetic acid in the presence of anhydrous sodium acetate as shown in scheme 2. The mechanism of this reaction proceeded through open intermediate formation, which were afterwards recyclized in the presence of glacial acetic acid to afford the imidazolinone derivatives (**3a-ł)** [17]. The structures of the prepared compounds were confirmed by different spectral data and elemental microanalyses. IR spectra revealed the disappearance of the characteristic C=O band of oxazolone at 1784-1780 cm^{-1} and the appearance of new C=O band of the imidazolone at 1718-1639 cm^{-1} along with the appearance of 2 stretching bands of SO_2 group at 1330-1303 cm^{-1}, 1180-1138 cm^{-1}, respectively. ^1H NMR spectra of compounds **3b**, **3d**, **3f**, **3h**, **3j** and **3l** bearing aliphatic CH_3 revealed a singlet signals at a range of 1.87-2.30 ppm and at 12.5-24.4 ppm in their

^{13}C NMR spectra. Likely, a characteristic signal corresponding to the H_4 proton of the oxazole ring occurred at 6.08 and 6.10 ppm in the ^{1}H NMR spectra of compounds **3d** and **3j** and at 95.9 and 95.8 ppm in their ^{13}C NMR spectra, respectively. Finally, scheme 3 starts with the reaction of compounds **2c,d** with sulfanilamide to give the 4-hydroxybenzylidene imidazolinones **4a,b** followed by introduction of a tail at the 4-OH group through etherification with the appropriate 2-chlorophenylacetamide **5a-c** [23,24] to furnish the target compounds **6a-f**. IR spectra of this series revealed the appearance of an additional carbonyl stretching band assigned to the phenylacetamido group at 1685-1635 cm^{-1} along with the disappearance of the stretching band corresponding to the OH group. ^{1}H NMR revealed the presence of a singlet signal integrated for the two protons corresponding to the CH_2 group at 3.86-4.86 ppm which was reflected as a signal at 65.3-67.5 ppm in their ^{13}C NMR spectra. Interestingly, another singlet signal corresponding to the 3 protons of the additional OCH_3 group of compounds **6b** and **6e** appeared at 3.76 and 3.79 ppm, respectively along with their carbon signals at 55.8-56.3 ppm, respectively.



Scheme 1. Synthesis of target compounds 2a-d

Reagents and reaction conditions: (a) acetic anhydride, fused sodium acetate, (100^{o} C)



2a,b

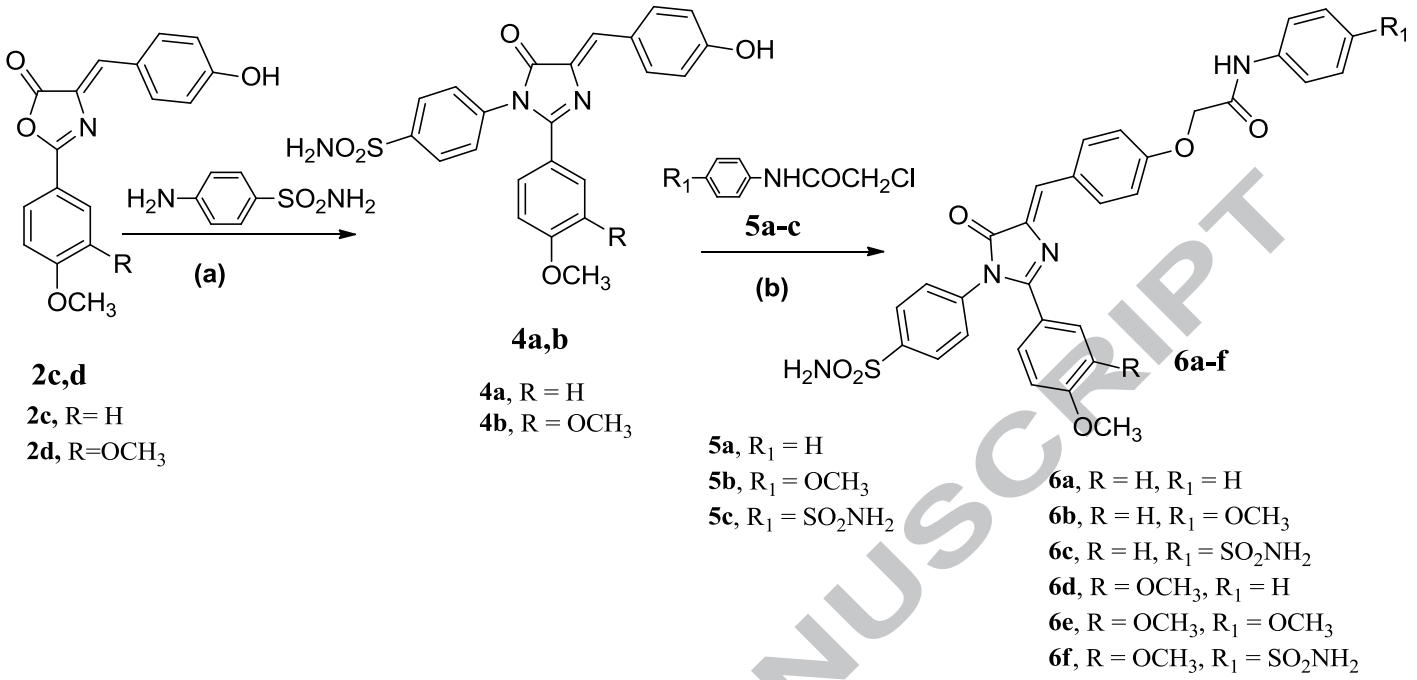
3a-*l*

2a, R= H
2b, R= OCH_3

3a, R = H, R_1 = H
3b, R = H, R_1 = 
3c, R = H, R_1 = 
3d, R = H, R_1 = 
3**e**, R = H, R_1 = 
3f, R = H, R_1 = 
3g, R = OCH_3, R_1 = H
3h, R =OCH_3, R_1 = 
3i, R = OCH_3, R_1 = 
3j, R = OCH_3, R_1 = 
3k, R = OCH_3, R_1 = 
3l, R = OCH_3, R_1 = 

Scheme 2. Synthesis of target compounds 3a-*l*

Reagents and reaction conditions: (a) glacial acetic acid, fused sodium acetate, water bath, (100^o C).



Scheme 3. Synthesis of target compounds 4a,b and 6a-f

Reagents and reaction conditions: (a) glacial acetic acid, fused sodium acetate, water bath, (100^{o} C); **(b)** K_2CO_3, DMF, stir, rt, 30 min., then add **5a-c**, (100^{o} C).

2.2. Biological Evaluation:

2.2.1. In vitro p38αMAPK inhibitory assay:

The IC_{50} values of all the tested compounds against p38αMAPK expressed in (μM concentration) compared to the reference drug sorafenib are summarized in **Table 1**. From the results, it was found that compound **3h** was the most active compound (IC_{50} = 0.056 μM) with 28 fold the activity of sorafenib (IC_{50} = 1.58 μM). Moreover, compounds **3c**, **3g** and **4a** showed 11 fold the activity of the reference drug (IC_{50} = 0.14, 0.14 and 0.14 μM), respectively. On the other hand, compound **3e** showed 9 fold the activity of the reference drug (IC_{50} = 0.17 μM). While other compounds **3d**, **3f**, **3j**, **3k**, **3l** and **4b** revealed from 1.5 to 3 fold the

activity of the reference drug (IC_{50} = 0.63, 0.66, 0.53, 0.57, 0.90 and 0.99 μM), respectively. Finally, compounds **3a**, **3b** and **3i** were less active than the reference drug. Results of p38αMAPK inhibition activity revealed that, 1-sulfamoyl-2-(4-methoxyphenyl)imidazolinone derivative is a good scaffold for the enzyme inhibition. Replacement of 4-methoxybenzylidene with its 4-hydroxy congener led to decrease in the activity in case of 4-methoxyphenyl while the reverse is true in case of 3,4-dimethoxyphenyl as shown in compounds **3a** and **3g** compared to compounds **4a** and **4b**, respectively. Furthermore, the 3,4-dimethoxyphenyl analogue showed lower activity than the 4-methoxyphenyl one in case of substitution with guanidine moiety either opened or cyclized as shown in compounds **3c**, **3e** and **3f** compared to compounds **3i**, **3k** and **3***l*, respectively. On the other hand, in absence of the guanidino moiety the 3,4-dimethoxy analogues revealed better activity than the 4-methoxy ones as shown in compounds **3g**, **3h** and **3j** compared to compounds **3a**, **3b** and **3d**, respectively. Finally, elongation of the benzylidene moiety at position 4 of the imidazole ring favored the activity in the 3,4-dimethoxy derivatives than the 4-methoxy ones where compounds **6d** and **6e** were more active than their 4-methoxy analogues **6a** and **6b**.

2.2.2. In vitro carbonic anhydrase inhibitory assay:

Compounds **3a-l** and **4a,b** have been screened for their inhibition activity against hCA isoforms I,II, IV and IX and the inhibition results presented as K_i values (nM) compared to those of the standard sulfonamide inhibitor acetazolamide **(AAZ)**, are reported in **Table 1**, and have been used to delineate the following structure–activity relationship (SAR). As for CA I, inhibition data highlighted that substitutions on the primary sulfonamides to afford secondary sulfonamides are not well tolerated, leading to K_i values ranging from 7083 nM for

small substituent, such as acetoxy group **3b** and **3h**, to more than 10000 nM for more hindered functionalities **3c-3f** and **3i-3l**. As for primary sulfonamides, compound **4a** bearing hydroxybenzylidene moiety in position 5 and mono-methoxyphenyl in position 2 of the imidazolinone ring showed good inhibition potency against this isoform (95 nM). Modification of this structure such as the insertion of a further methoxyl group on the ring in position 2 in compound **4b** or the switch to methoxybenzylidene in position 5 as in compound **3a** led respectively to 8.2 and 8.7 potency decrease. The combinations of these modifications in compound **3g** led to a K_i value 93 fold lower when compared to the lead compound **4a**. Moreover, inhibition data against hCA II revealed that compound **4a** is the most potent within the series, with a K_i value of 0.83 nM. With respect to what happens for CA I, all the modifications to this scaffold on the aromatic rings in positions 5 and 2 did not negatively affect the inhibition potency against CA II, with K_i values in the low nanomolar range **4b**, **3a** and **3g**. Substitution of primary benzensulfonamides with acetoxyl or guanidine groups are tolerated too, mostly for compounds with di- methoxyphenyl in position 2 as in compounds **3h** and **3i** with K_i values = (20.3 nM and 47.3 nM, respectively). Conversely, introduction of 5-methylisoxazole **3d** and **3j**, pyrimidine **3e** and **3k** and 4-methylpyrimidine **3f** and **3l** substituents on the sulfonamide was detrimental for the inhibition activity. Surprisingly, the introduction of these hindered substituents on sulfonamide functionalization revealed to be well tolerated within CA IV active site. The potency seemed to be related also to the substitution of the aromatic ring in position 2, with best results obtained mostly from di-methoxy substituted rings **3h-3l** with K_i values spanning from 91.4 to 371.6 nM). Therefore, compounds **3d-3f** and **3j-3l** could be considered as selective inhibitors of CA IV. As for primary sulfonamides, compound **4a** remains the most potent one within the series (6.9

nM), and any modification of this structure was detrimental for the inhibition potency (K_i values from 656.4 to 5229.0 nM). Except for compound **4a** (12.4 nM), most of the tested compounds showed low inhibition potency against CA IX. Primary sulfonamides and some of secondary sulfonamides with small substituents were quite tolerated **4a-3c** and **3g** where compound **3c** was almost twice as selective for CA IX than for the other enzymes considered. Among the more hindered sulfonamides, compound **3j** was the only one that preserved good inhibitory activity against CA IX, with K_i value of 264.6 nM.

Table 1: **Inhibition data of human CA isoforms I, II, IV and IX by the tested compounds as K_i values in nM compared to acetazolamide (AZZ) and their IC_{50} values in µM compared to sorafenib aganist p38*α*MAPK enzyme**

Compound No.	K_i (nM) hCA I	K_i (nM) hCA II	K_i (nM) hCA IV	K_i (nM) hCA IX	IC_{50} (µM) p38αMAPK
3a	823.2	5.0	656.4	874.1	3.50
3b	7083.0	65.6	>10000	745.9	2.11
3c	>10000.	7317.0	5999.0	3771.0	0.14
3d	>10000	>10000	2753.0	>10000	0.63
3e	>10000	>10000	5659.0	>10000	0.17
3f	>10000	>10000	80.0	>10000	0.66
3g	8833.0	92.7	862.2	902.4	0.14
3h	8464.0	20.3	371.6	>10000	0.056
3i	>10000	47.3	325.0	>10000	4.41
3j	>10000	>10000	79.6	264.9	0.53
3k	>10000	>1000	91.4	>10000	0.57
3l	>10000	>10000	91.5	>10000	0.90
4a	95.0	0.83	6.9	12.4	0.14
4b	781.3	9.2	5229.0	354.3	0.99
6a	NT	NT	NT	NT	0.88
6b	NT	NT	NT	NT	0.57
6c	NT	NT	NT	NT	0.13
6d	NT	NT	NT	NT	0.14
6e	NT	NT	NT	NT	0.40
6f	NT	NT	NT	NT	1.00
AZZ	250	12.1	74	25.8	-
Sorafenib	-	-	-	-	1.58

*NT = not tested

3- Conclusion:

Twenty imidazolinone derivatives were synthesized and screened for their inhibitory activity against p38αMAPK and CA enzymes. The tested compounds revealed good inhibitory activity against p38αMAPK, especially compounds **3h**, **6c** and **4a** with IC_{50} values = 0.056, 0.13 and 0.14 μM, respectively. Moreover, compound **4a** revealed the best inhibitory activity against all the tested carbonic anhydrase isoforms CA I, II, IV and IX with K_i values of 95.0, 0.83, 6.90 and 12.4 nM, respectively. Therefore, compound **4a** can be considered as a potent dual p38αMAPK/CA inhibitor.

3- Experimental

4.1. Chemistry

4.1.1. General

Melting points were recorded on a Stuart SMP10 digital melting point apparatus and were uncorrected. Infrared (IR) Spectra were recorded as KBr disks using a Shimadzu FT-IR 8400S infrared spectrophotometer. Mass spectral data are given as m/z (Intensity %). The ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker 400 MHz FT-NMR spectrophotometer (^{1}H: 400, ^{13}C: 100 MHz) at Faculty of Pharmacy, Cairo University or Varian MERCURY 400 (^{1}H: 400,^{13}C: 100 MHz) at chemical warfare department, Ministry of Defense all in deuterated dimethylsulfoxide (DMSO-d_6). Chemical shifts are expressed in δ values (ppm) using the solvent peak as internal standard. All coupling constant (*J*) values are given in hertz. The abbreviations used are as follows: s, singlet; d, doublet; m, multiplet. Elemental analyses were carried out at the Regional Center for Mycology and Biotechnology, Al-Azhar University, Egypt. Reaction courses and product mixtures were routinely monitored by Thin Layer Chromatography (TLC) on silica gel precoated F254 Merck plates. Unless otherwise noted, all solvents and

reagents were commercially available and used without further purification. Compounds **1a,b** [20,21], **2a-d** [22,23] and **5a-c** [24,25] were prepared as reported in the literature.

4.1.2. General procedure for the synthesis of compounds (3a-l):

A mixture of compound **2a,b** (10 mmol), the appropriate sulfonamide derivative (12 mmol) and freshly prepared fused sodium acetate (1.23 g, 15 mmol) in glacial acetic acid (10 mL) was heated in a boiling water bath and the reaction time was monitored by TLC. The crystalline product separated on cooling was filtered off, washed with water, dried, and recrystallized from ethanol.

*4.1.2.1. 4-[4-(4-Methoxybenzylidene)-2-(4-methoxyphenyl)-5-oxo-4,5-dihydro-1H-imidazol-1-yl]benzenesulfonamide **(3a)***. Reaction time: 6 h, yellow crystals; yield: 0.51 g (68 %); m.p. 260-263 ^{o}C; IR (KBr, cm^{-1}) υ_{max}: 3352, 3257 (NH_2), 3095 (CH aromatic), 2964 (CH aliphatic), 1685 (C=O), 1309, 1159 (SO_2); ^{1}H NMR: δ 3.78 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 6.96 (d, 2H, aromatic H, *J* = 8.7 Hz), 7.07 (d, 2H, aromatic H, *J* = 8.4 Hz), 7.21 (s, 1H, CH), 7.45-7.48 (m, 6H, 4H aromatic + 2H of NH_2, ex), 7.88 (d, 2H, aromatic H, *J* = 8.1 Hz), 8.32 (d, 2H, aromatic H, *J* = 8.4 Hz); ^{13}C NMR: δ 55.8, 55.9, 114.4, 114.5, 115.0, 121.0, 127.1, 127.6, 127.7, 128.6, 131.1, 134.7, 136.7, 138.1, 144.0, 158.9, 161.6, 162.1, 169.8; MS (m/z %): 464 (M+1, 29.36 %), 463 (100 %), Anal. Calcd. for $C_{24}H_{21}N_3O_5S$ (463.50): C, 62.19; H, 4.57; N, 9.07 %. Found: C, 62.33; H, 4.42; N, 9.03 %.

*4.1.2.2. 4-[4-(4-Methoxybenzylidene)-2-(4-methoxyphenyl)-5-oxo-4,5-dihydro-1H-imidazol-1-yl)phenyl)sulfonyl]acetamide (**3b**).* Reaction time: 6 h, yellow crystals; yield: 0.52 g (64 %); m.p. 236-239 ^{o}C; IR (KBr, cm^{-1}) υ_{max}: 3380 (NH), 3099 (CH aromatic), 2679 (CH aliphatic), 1638, 1685 (2C=Os), 1305, 1156 (SO_2); ^{1}H NMR: δ 1.87 (s, 3H, CH_3), 3.81 (s, 6H, 2 OCH_3), 6.49 (d, 2H, aromatic H, *J* = 6.6 Hz), 6.93-6.96 (m, 5H, 4H aromatic + 1H of CH), 7.42 (d, 2H, aromatic H, *J* = 7.2 Hz),

7.83-7.87 (m, 4H, aromatic H), 10.10 (s, ex, 1H, NH); ^{13}C NMR: δ 21.9, 55.7, 55.8, 112.4, 113.8, 114.9, 118.0, 125.7, 127.0, 127.8, 128.2, 129.2, 131.6, 132.2, 141.0, 162.5, 168.2, 172.4, 172.7, 175.4; MS (m/z %): 504 (M-1, 9.89 %), 309 (100 %), Anal. Calcd. for $C_{26}H_{23}N_3O_6S$ (505.54): C, 61.77; H, 4.59; N, 8.31%. Found: C, 61.35; H, 4.38; N, 7.94 %.

*4.1.2.3. N-Carbamimidoyl-4-[4-(4-methoxybenzylidene)-2-(4-methoxyphenyl)-5-oxo-4,5-dihydro-1H-imidazol-1-yl]benzenesulfonamide (**3c**).* Reaction time: 8 h, yellow crystals; yield: 0.46 g (56 %); m.p. 233-236 ^{o}C; IR (KBr, cm^{-1}) υ_{max}: 3446-3342 (NHs), 3095 (CH aromatic), 2900 (CH aliphatic), 1708 (C=O), 1320, 1174 (SO_2); ^{1}H NMR: δ 3.84 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 6.71 (s, ex, 2H, NH_2), 7.03-7.11 (m, 4H, aromatic H), 7.38 (d, 2H, aromatic H, *J* = 8.4 Hz), 7.44 (d, 2H, aromatic H, *J* = 8.7 Hz), 7.65 (s, 1H, CH), 7.80 (d, 2H, aromatic H, *J* = 8.7 Hz), 8.30 (d, 2H, aromatic H, *J* = 8.4 Hz), 9.94 (s, ex, 1H, NH), 10.30 (s, ex, 1H, NH); ^{13}C NMR: δ 55.8, 56.3, 114.5, 115.0, 121.0, 123.5, 126.7, 127.0, 131.1, 131.7, 134.7, 136.7, 138.4, 149.0, 157.9, 158.6, 158.9, 164.1, 165.4; MS (m/z %): 505 (M^+, 10.4 %), 463 (100 %), Anal. Calcd. for $C_{25}H_{23}N_5O_5S$ (505.54): C, 59.40; H, 4.59; N, 13.85 %. Found: C, 59.70; H, 4.60; N, 13.26 %.

*4.1.2.4. 4-[4-(4-Methoxybenzylidene)-2-(4-methoxyphenyl)-5-oxo-4,5-dihydro-1H-imidazol-1-yl]-N-(5-methylisoxazol-3-yl)benzene sulfonamide (**3d**).* Reaction time: 6 h, off-white crystals; yield: 0.55 g (62 %); m.p. 240-243 ^{o}C; IR (KBr, cm^{-1}) υ_{max}: 3232 (NH), 3105 (CH aromatic), 2924 (CH aliphatic), 1647 (C=O), 1323, 1168 (SO_2); ^{1}H NMR : δ 2.28 (s, 3H, CH_3), 3.77 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 6.08 (s, 1H, CH oxazole), 6.96 (d, 2H, aromatic H, *J* = 8.8 Hz), 7.05 (d, 2H, aromatic H, *J* = 8.8 Hz), 7.10 (s, 1H, CH), 7.59 (d, 2H, aromatic H, *J* = 8.7 Hz), 7.77 (d, 2H, aromatic H, *J* = 8.5 Hz), 7.88 (d, 2H, aromatic H, *J* = 8.5 Hz), 8.00 (d, 2H, aromatic H, *J* = 8.6 Hz), 9.93 (s, ex, 1H, NH); ^{13}C NMR: δ 12.5, 55.6, 55.9, 95.9, 114.0, 114.5, 120.0, 126.0, 127.0, 128.1, 129.0, 129.2, 130.3, 131.7, 134.2,

143.9, 158.8, 160.1, 162.5, 165.7, 165.9, 170.3; MS (m/z %): 545 (M+1, 35.5 %), 544 (100 %), Anal. Calcd. for $C_{28}H_{24}N_4O_6S$ (544.58): C, 61.76; H, 4.44; N, 10.29 %. Found: C, 61.22; H, 4.53; N, 10.59 %.

*4.1.2.5. 4-[4-(4-Methoxybenzylidene)-2-(4-methoxyphenyl)-5-oxo-4,5-dihydro-1H-imidazol-1-yl]-N-(pyrimidin-2-yl)benzenesulfonamide (**3e**)*. Reaction time: 6 h, yellow crystals; yield: 0.61 g (70%); m.p. 195-198 ^{o}C; IR (KBr, cm^{-1}) υ_{max}: 3300 (NH), 3120 (CH aromatic), 3080 (CH aliphatic), 1687 (C=O), 1327, 1149 (SO_2); ^{1}H NMR: δ 3.83 (s, 6H, $2OCH_3$), 5.94 (s, ex, 1H, NH), 6.50 (d, 2H, aromatic H, *J* = 8.7 Hz), 6.93-7.02 (m, 6H, aromatic H), 7.58 (d, 2H, aromatic H, *J* = 9 Hz), 7.81-7.90 (m, 4H, 3H aromatic + 1H of CH), 8.45 (d, 2H, aromatic H, *J* = 4.8 Hz); ^{13}C NMR: δ 55.8, 56.2, 112.5, 114.1, 114.6, 115.8, 124.5, 125.5, 128.9, 129.6, 130.2, 131.7, 132.2, 150.2, 153.3, 153.7, 157.8, 158.4, 158.6, 163.0, 167.6; MS (m/z %): 542 (M+1, 24.48 %), 309 (100 %), Anal. Calcd. For $C_{28}H_{23}N_5O_5S$ (541.58): C, 62.10; H, 4.28; N, 12.93 %. Found: C, 62.57; H, 4.44; N, 12.59 %.

*4.1.2.6. 4-[4-(4-Methoxybenzylidene)-2-(4-methoxyphenyl)-5-oxo-4,5-dihydro-1H-imidazol-1-yl]-N-(4-methylpyrimidin-2-yl)benzene sulfonamide (**3f**)*. Reaction time: 6 h, yellow crystals; yield: 0.49 g (55%); m.p. 218-222 ^{o}C; IR (KBr, cm^{-1}) υ_{max}: 3379 (NH), 3095 (CH aromatic), 2940 (CH aliphatic), 1685 (C=O), 1303, 1180 (SO_2); ^{1}H NMR: δ 2.30 (s, 3H, CH_3), 3.812 (s, 3H, OCH_3), 3.819 (s, 3H, OCH_3), 6.53 (d, 2H, aromatic H, *J* = 9 Hz), 6.85 (d, 2H, aromatic H, *J* = 4.8 Hz), 6.99 (d, 2H, aromatic H, *J* = 8.7 Hz), 7.60-7.65 (m, 3H, 2H aromatic + 1H of CH), 7.87 (d, 2H, aromatic H, *J* = 8.7 Hz), 8.28 (d, 2H, aromatic H, *J* = 5 Hz), 8.38 (d, 2H, aromatic H, *J* = 4.8 Hz), 11.3 (s, ex, 1H, NH); ^{13}C NMR: δ 23.7, 55.8, 56.3, 106.4, 108.0, 112.4, 114.2, 114.9, 115.2, 120.8, 122.0, 123.5, 125.4, 130.4, 131.7, 152.7, 153.3, 157.3, 158.0, 163.2, 167.5, 168.4, 176.6; MS (m/z %): 556 (M+1, 36.66 %), 555 (100 %), Anal. Calcd. for $C_{29}H_{25}N_5O_5S$ (555.61): C, 62.69; H, 4.54; N, 12.61 %. Found: C, 62.48; H, 4.68; N, 12.93 %.

*4.1.2.7. 4-[2-(3,4-Dimethoxyphenyl)-4-(4-methoxybenzylidene)-5-oxo-4,5-dihydro-1H-imidazol-1-yl]benzenesulfonamide (**3g**.* Reaction time: 8 h, yellow crystals; yield: 0.42 g (58 %); m.p. 228-231 ^{o}C; IR (KBr, cm^{-1}) υ_{max}: 3356, 3262 (NH_2), 3095 (CH aromatic), 2942 (CH aliphatic), 1660 (C=O), 1330, 1165 (SO_2); ^{1}H NMR: δ 3.74 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 6.92 (s, 1H, aromatic H), 6.99 (d, 1H, aromatic H, *J* = 8.4 Hz), 7.06 (d, 1H, aromatic H, *J* = 8.8 Hz), 7.14 (d, 2H, aromatic H, *J* = 8.8 Hz), 7.20 (s, 1H, CH), 7.46 (d, 2H, aromatic H, *J* = 8.4 Hz), 7.71 (s, ex, 2H, NH_2), 7.88 (d, 2H, aromatic H, *J* = 8.8 Hz), 8.32 (d, 2H, aromatic H, *J* = 8.8 Hz); ^{13}C NMR: δ 55.6, 55.8, 56.1, 111.8, 112.3, 115.0, 120.8, 123.0, 127.1, 127.3, 128.9, 129.3, 134.8, 136.7, 138.2, 144.2, 148.4, 151.9, 158.8, 161.7, 169.8; MS (m/z %): 493 (M^+, 100 %), Anal. Calcd. for $C_{25}H_{23}N_3O_6S$ (493.53): C, 60.84; H, 4.70; N, 8.51 %. Found: C, 60.61; H, 4.57; N, 8.79 %.

*4.1.2.8. N-[(4-(2-(3,4-Dimethoxyphenyl)-4-(4-methoxybenzylidene)-5-oxo-4,5-di hydro-1H-imidazol-1-yl)phenyl)sulfonyl]acetamide (**3h**).* Reaction time: 12 h, yellow crystals; yield: 0.45 g (57 %); m.p. 215-218 ^{o}C; IR (KBr, cm^{-1}) υ_{max}: 3302 (NH), 3009 (CH aromatic), 2964 (CH aliphatic), 1718, 1639 (2C=Os), 1307, 1138 (SO_2); ^{1}H NMR: δ 2.03 (s, 3H, CH_3), 3.77 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 6.77 (s, 1H, aromatic H), 6.97 (d, 1H, aromatic H, *J* = 8.8 Hz), 7.04 (d, 2H, aromatic H, *J* = 9.2 Hz), 7.13 (s, 1H, CH), 7.24 (d, 1H, aromatic H, *J* = 8.4 Hz), 7.49 (d, 2H, aromatic H, *J* = 8.4 Hz), 7.78 (d, 2H, aromatic H, *J* = 8.4 Hz), 8.27 (d, 2H, aromatic H, *J* = 8.8 Hz), 10.13 (s, ex, 1H, NH); ^{13}C NMR: δ 24.4, 55.8, 55.9, 56.0, 111.9, 112.2, 113.6, 114.9, 117.9, 120.8, 123.0, 127.5, 128.3, 130.6, 134.7, 136.3, 140.7, 148.3, 151.8, 159.0, 161.5, 170.1, 174.6; MS (m/z %): 537 (M+2, 3 %), 165 (100 %), Anal. Calcd. for $C_{27}H_{25}N_3O_7S$ (535.57): C, 60.55; H, 4.71; N, 7.85 %. Found: C, 60.69; H, 4.61; N, 7.96 %.

*4.1.2.9. N-Carbamimidoyl-4-[2-(3,4-dimethoxyphenyl)-4-(4-methoxybenzylidene)-5-oxo-4,5-dihydro-1H-imidazol-1-yl]benzenesulfonamide (**3i**).* Reaction time: 8 h, brown crystals; yield: 0.49 g (62 %); m.p. 200-203 ^{o}C; IR (KBr, cm^{-1}) υ_{max}: 3430-3312 (NHs), 3175 (CH aromatic), 2970 (CH aliphatic), 1656 (C=O), 1320, 1174 (SO_2); ^{1}H NMR: δ 3.80 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 6.64 (s, ex, 2H, NH_2), 7.01 (d, 2H, aromatic H, *J* = 8.4 Hz), 7.06 (s, 1H, aromatic H), 7.53 (d, 2H, aromatic H, *J* = 8.4 Hz), 7.58 (d, 2H, aromatic H, *J* = 8.8 Hz), 7.65-7.69 (m, 3H, 2H aromatic + 1H of CH), 7.80 (d, 2H, aromatic H, *J* = 8.8 Hz), 9.90 (s, ex, 1H, NH), 10.33 (s, ex, 1H, NH); ^{13}C NMR: δ 55.6, 55.8, 56.0, 111.4, 112.3, 114.5, 119.7, 121.8, 123.6, 126.7, 127.0, 129.3, 131.7, 139.2, 142.3, 148.7, 153.0, 158.5, 160.1, 165.4, 165.9, 167.5; MS (m/z %): 535 (M^+, 8.75 %), 493 (100 %), Anal. Calcd. for $C_{26}H_{25}N_5O_6S$ (535.57): C, 58.31; H, 4.71; N, 13.08 %. Found: C, 58.20; H, 4.32; N, 12.91 %.

*4.1.2.10. 4-[2-(3,4-Dimethoxyphenyl)-4-(4-methoxybenzylidene)-5-oxo-4,5-dihydro-1H-imidazol-1-yl]-N-(5-methylisoxazol-3-yl)benzene sulfonamide (**3j**).* Reaction time: 12 h, yellow crystals; yield: 0.53 g (63 %); m.p. 210-213 ^{o}C; IR (KBr, cm^{-1}) υ_{max}: 3203 (NH), 3160 (CH aromatic), 2900 (CH aliphatic), 1639 (C=O), 1305, 1163 (SO_2); ^{1}H NM: δ 2.27 (s, 3H, CH_3), 3.75 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 6.10 (s, 1H, CH oxazole), 6.94 (d, 1H, aromatic H, *J* = 8.8 Hz), 7.05 (d, 1H, aromatic H, *J* = 8.8 Hz), 7.10 (s, 1H, CH), 7.23 (s, 1H, aromatic H), 7.65 (d, 2H, aromatic H, *J* = 8 Hz), 7.71 (d, 2H, aromatic H, *J* = 9.2 Hz), 7.77 (d, 2H, aromatic H, *J* = 9.2 Hz), 7.88 (d, 2H, aromatic H, *J* = 8.8 Hz), 9.93 (s, ex, 1H, NH); ^{13}C NMR: δ 12.5, 55.6, 56.0, 56.1, 95.8, 114.5, 115.1, 120.0, 121.8, 123.3, 126.0, 126.9, 128.2, 128.8, 133.4, 129.2, 131.7, 144.2, 148.7, 152.2, 158.0, 160.1, 165.7, 165.9, 170.7; MS (m/z %): 574 (M^+, 100 %), Anal. Calcd. for $C_{29}H_{26}N_4O_7S$ (574.60): C, 60.62; H, 4.56; N, 9.75 %. Found: C, 60.71; H, 4.65; N, 9.69 %.

*4.1.2.11. 4-[2-(3,4-Dimethoxyphenyl)-4-(4-methoxybenzylidene)-5-oxo-4,5-dihydro-1H-imidazol-1-yl]-N-(pyrimidin-2-yl)benzene sulfonamide (**3k**).* Reaction time: 8 h, yellow crystals; yield: 0.61g (72 %); m.p. 231-234 ^{o}C; IR (KBr, cm^{-1}) υ_{max}: 3356 (NH), 3037 (CH aromatic), 2937 (CH aliphatic), 1640 (C=O), 1325, 1155 (SO_2); ^{1}H NMR: δ 3.80 (s, 3H, OCH_3), 3.820 (s, 3H, OCH_3), 3.828 (s, 3H, OCH_3), 6.52 (d, 1H, aromatic H, *J* = 8.8 Hz), 5.94 (s, ex, 1H, NH), 6.95 (t, 1H, aromatic H, *J* = 8.4 Hz), 7.00 (d, 1H, aromatic H, *J* = 8.8 Hz), 7.17 (s, 1H, aromatic H), 7.25 (d, 2H, aromatic H, *J* = 8.4 Hz), 7.41 (d, 2H, aromatic H, *J* = 8.4 Hz), 7.57 (d, 2H, aromatic H, *J* = 8.8 Hz), 7.82-7.88 (m, 3H, 2H aromatic + 1H of CH), 8.43 (d, 2H, aromatic H, *J* = 4.8 Hz); ^{13}C NMR: δ 55.8, 56.0, 56.1, 111.4, 112.3, 112.5, 114.5, 115.6, 123.5, 125.7, 127.5, 128.4, 130.1, 132.1, 141.2, 148.7, 150.2, 152.9, 153.3, 154.1, 157.9, 158.6, 167.6; MS (m/z %): 571 (M^+, 29.59 %), 57 (100 %), Anal. Calcd. for $C_{29}H_{25}N_5O_6S$ (571.60): C, 60.94; H, 4.41; N, 12.25 %. Found: C, 60.95; H, 4.48; N, 12.47 %.

*4.1.2.12. 4-[2-(3,4-Dimethoxyphenyl)-4-(4-methoxybenzylidene)-5-oxo-4,5-dihydro-1H-imidazol-1-yl]-N-(4-methylpyrimidin-2-yl)benzenesulfonamide(**3l**).*

Reaction time: 8 h, yellow crystals; yield: 0.53 g (61 %); m.p. 176-179 ^{o}C; IR (KBr, cm^{-1}) υ_{max}: 3300 (NH), 3014 (CH aromatic), 2960 (CH aliphatic), 1653 (C=O), 1310, 1163 (SO_2). ; ^{1}H NMR : δ 2.30 (s, 3H, CH_3), 3.74 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 6.78 (s, 1H, aromatic H), 6.94 (d, 2H, aromatic H, *J* = 8.4 Hz), 7.10 (s, 1H, CH), 7.42 (d, 2H, aromatic H, *J* = 8.4 Hz), 7.55-7.66 (m, 4H, aromatic H), 8.02 (d, 2H, aromatic H, *J* = 8.4 Hz), 8.26-8.33 (m, 2H, aromatic H), 10.43 (s, ex, 1H, NH); ^{13}C NMR: δ 24.0, 55.1, 55.8, 56.0, 111.6, 112.2, 114.5, 115.0, 121.8, 126.9, 127.3, 128.3, 129.2, 131.7, 134.8, 136.7, 148.2, 151.8, 152.2, 158.8, 160.1, 161.7, 165.6, 165.9, 169.7; MS (m/z %): 585 (M^+, 100

%), Anal. Calcd. for $C_{30}H_{27}N_5O_6S$ (585.63): C, 61.53; H, 4.65; N, 11.96 %. Found: C, 61.78; H, 4.43; N, 11.78 %.

4.1.3. General procedure for the synthesis of compounds (4a,b):

A mixture of compound **2c,d** (10 mmol), sulfanilamide (2.07 g, 12 mmol) and freshly prepared fused sodium acetate (1.23 g, 15 mmol) in glacial acetic acid (10 mL) was heated in a boiling water bath for 6 h. The crystalline product separated on cooling was filtered off, washed with water, dried and recrystallized from ethanol.

*4.1.3.1. 4-[4-(4-Hydroxybenzylidene)-2-(4-methoxyphenyl)-5-oxo-4,5-dihydro-1H-imidazol-1-yl]benzenesulfonamide (**4a**).* Yellow crystals; yield: 0.49 g (64 %); m.p. 191-194; IR (KBr, cm^{-1}) υ_{max}: 3363 (OH), 3257, 3200 (NH_2), 3103 (CH aromatic), 2960 (CH aliphatic), 1730 (C=O), 1311, 1161 (SO_2); ^{1}H NMR : δ 3.84 (s, 3H, OCH_3), 6.74 (d, 2H, aromatic H, *J* = 7.8 Hz), 6.88 (d, 2H, aromatic H, *J* = 8.7 Hz), 7.04 (d, 2H, aromatic H, *J* = 7.8 Hz), 7.47 (d, 2H, aromatic H, *J* = 8.7 Hz), 7.77 (s, 1H, CH), 7.99 (d, 2H, aromatic H, *J* = 8.1 Hz), 8.21 (d, 2H, aromatic H, *J* = 7.8 Hz), 9.79 (s, ex, 2H, NH_2), 10.29 (s, ex, 1H, OH); ^{13}C NMR: δ 55.8, 113.6, 114.0, 114.4, 114.5, 115.9, 119.8, 122.4, 126.8, 128.3, 130.2, 130.5, 131.0, 131.9, 144.7, 155.1, 165.6; MS (m/z %): 449 (M^+, 100 %), Anal. Calcd. for $C_{23}H_{19}N_3O_5S$ (449.48): C, 61.46; H, 4.26; N, 9.35 %. Found: C, 60.97; H, 4.25; N, 9.19 %.

*4.1.3.2. 4-[2-(3,4-Dimethoxyphenyl)-4-(4-hydroxybenzylidene)-5-oxo-4,5-dihydro-1H-imidazol-1-yl]benzenesulfonamide (**4b**).* Yellow crystals; yield: 0.46 g (63 %); m.p. 230-233^{o}C; IR (KBr, cm^{-1}) υ_{max}: 3346 (OH), 3317, 3259 (NH_2), 3097 (CH aromatic), 2937 (CH aliphatic), 1722 (C=O), 1346, 1160 (SO_2); ^{1}H NMR: δ 3.74 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 6.87 (d, 2H, aromatic H, *J* = 9.2 Hz), 6.98 (d, 2H, aromatic H, *J* = 8.8 Hz), 7.15 (s, 1H, aromatic H), 7.45-7.47 (m, 4H, aromatic H + NH_2 ex + 1H of CH), 7.87-7.89 (m, 3H, aromatic H + 1H of CH), 8.21 (d, 2H,

aromatic H, *J* = 8.8 Hz), 10.24 (s, ex, 1H, OH),; ^{13}C NMR: δ 55.6, 56.1, 110.0, 111.8, 112.3, 116.4, 120.9, 123.0, 125.9, 127.1, 128.8, 135.1, 135.9, 138.3, 144.1, 148.4, 151.7, 158.2, 160.6, 169.8; MS (m/z %): 479 (M^+, 100 %), Anal. Calcd. for $C_{24}H_{21}N_3O_6S$ (479.50): C, 60.12; H, 4.41; N, 8.76 %. Found: C, 60.23; H, 4.44; N, 8.47 %.

4.1.4. General procedure for the synthesis of compounds (6a-f):

To a solution of compound **4a,b** (10 mmol) in dry DMF (15 mL), K_2CO_3 (1.38 g, 10 mmol) was added and the solution was stirred at room temperature for 30 min. The appropriate 2-chloro-*N*-un/substituted phenylacetamide **5a-c** (10 mmol) was then added and the mixture was heated under reflux for 24 h. The reaction mixture was poured onto ice/water and the precipitated solid was filtered off, washed with water, dried and crystallized from ethanol.

*4.1.4.1. 2-[4-((2-(4-Methoxyphenyl)-5-oxo-1-(4-sulfamoylphenyl)-1,5-dihydro-4H-imidazol-4-ylidene)methyl)phenoxy]-N-phenylacetamide (**6a**).* Yellow crystals; yield: 0.41 g (65 %); m.p. 259-262 ^{o}C; IR (KBr, cm^{-1}) υ_{max}: 3278-3200 (NHs), 3090 (CH aromatic), 2981 (CH aliphatic), 1720, 1685 (2 C=Os), 1307, 1168 (SO_2); ^{1}H NMR: δ 3.80 (s, 3H, OCH_3), 4.86 (s, 2H, CH_2), 6.98 (d, 4H, aromatic H, *J* = 8.8 Hz), 7.05 (d, 2H, aromatic H, *J* = 8.8 Hz), 7.29 (t, 3H, aromatic H, *J* = 8 Hz), 7.55 (d, 2H, aromatic H, *J* = 8.8 Hz), 7.74 (s, 1H, CH), 7.86 (d, 4H, aromatic H, *J* = 8.8 Hz), 7.96 (d, 2H, aromatic H, *J* = 8.4 Hz), 10.17 (s, ex, 1H, NH), 12.58 (s, ex, 2H, NH_2); ^{13}C NMR: δ 55.8, 66.2, 114.2, 114.5, 119.7, 121.8, 123.4, 123.9, 129.2, 131.7, 132.0, 138.9, 163.2, 163.8, 165.5, 165.9, 167.4; MS (m/z %): 583 (M+1, 5.79 %), 57 (100 %), Anal. Calcd. for $C_{31}H_{26}N_4O_6S$ (582.63): C, 63.91; H, 4.50; N, 9.62 %. Found: C, 63.78; H, 4.80; N, 9.73 %.

4.1.4.2. N-[4-Methoxyphenyl)-2-(4-((2-(4-methoxyphenyl)-5-oxo-1-(4-sulfamoyl phenyl)-1,5-dihydro-4H-imidazol-4-ylidene)methyl)phenoxy]acetamide ***(6b)***. Yellow crystals; yield: 0.42 g (63 %); m.p. 260-263 ^{o}C; IR (KBr, cm^{-1}) υ_{max}: 3520-3360 (NHs), 3095 (CH aromatic), 2920 (CH aliphatic), 1699, 1636 (2 C=Os), 1369, 1164 (SO_2); ^{1}H NMR: δ 3.75 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.86 (s, 2H, CH_2), 6.86 (d, 2H, aromatic H, *J* = 8.8 Hz), 6.98 (d, 4H, aromatic H, *J* = 9.2 Hz), 7.13-7.16 (m, 2H, aromatic H), 7.33 (s, ex, 2H, NH_2), 7.43 (d, 2H, aromatic H, *J* = 8.8 Hz), 7.49 (s, ex, 1H, NH), 7.86-7.88 (m, 3H, aromatic H + 1H of CH), 8.14 (d, 2H, aromatic H, *J* = 8.4 Hz), 8.20 (d, 2H, aromatic H, *J* = 8.4 Hz); ^{13}C NMR: δ 55.8, 56.1, 66.0, 114.4, 114.5, 115.3, 116.4, 121.1, 125.3, 127.0, 128.4, 128.7, 130.2, 130.3, 130.5, 131.0, 134.9, 135.9, 143.9, 158.3, 160.6, 162.0, 163.7, 167.8, 169.8; MS (m/z %): 610 (M-2, 0.1 %), 449 (100 %), Anal. Calcd. for $C_{32}H_{28}N_4O_7S$ (612.66): C, 62.74; H, 4.61; N, 9.15 %. Found: C, 63.01; H, 4.76; N, 9.40 %.

4.1.4.3. 2-[4-((2-(4-Methoxyphenyl)-5-oxo-1-(4-sulfamoylphenyl)-1,5-dihydro-4H-imidazol-4-ylidene)methyl)phenoxy]-N-(4-sulfamoylphenyl)acetamide ***(6c)***. Yellow crystals; yield: 0.45 g (63 %); m.p. 257-260 ^{o}C; IR (KBr, cm^{-1}) υ_{max}: 3460-3300 (NHs), 3100 (CH aromatic), 2880 (CH aliphatic), 1705, 1635 (2 C=Os), 1340, 1164 (SO_2); ^{1}H NMR: δ 3.76 (s, 3H, OCH_3), 3.86 (s, 2H, CH_2), 6.87 (d, 2H, aromatic H, *J* = 8.8 Hz), 6.94 (d, 2H, aromatic H, *J* = 8.8 Hz), 7.14 (s, 1H, CH), 7.33 (d, 2H, aromatic H, *J* = 8.8 Hz), 7.42-7.50 (m, 4H, aromatic H), 7.73 (s, ex, 1H, NH), 7.86 (d, 2H, aromatic H, *J* = 8.8 Hz), 8.20 (d, 2H, aromatic H, *J* = 8.8 Hz), 8.30 (d, 2H, aromatic H, *J* = 8.8 Hz), 10.24 (s, ex, 4H, NHs); ^{13}C NMR: δ 55.8, 65.5 114.4, 114.5, 115.3, 116.4, 116.5, 121.1, 125.9, 127.1, 128.4, 128.6, 128.7, 130.2, 130.5, 131.0, 135.1, 135.9, 138.2, 143.9, 158.3, 160.6, 162.0, 169.8; MS (m/z %): 660 (M-1, 0.46 %), 57 (100 %), Anal. Calcd. for $C_{31}H_{27}N_5O_8S_2$ (661.70): C, 56.27; H, 4.11; N, 10.58 %. Found: C, 56.33; H, 4.08; N, 10.49 %.

*4.1.4.4. 2-[4-((2-(3,4-Dimethoxyphenyl)-5-oxo-1-(4-sulfamoylphenyl)-1,5-dihydro-4H-imidazol-4-ylidene)methyl)phenoxy]-N-phenylacetamide (**6d**).* Yellow crystals; yield: 0.39 g (62 %); m.p. 275-278 ^{o}C; IR (KBr, cm^{-1}) υ_{max}: 3373-3250 (NHs), 3095 (CH aromatic), 2840 (CH aliphatic), 1716, 1640 (2C=Os), 1340, 1150 (SO_2); ^{1}H NMR: δ 3.89 (s, 3H, OCH3), 3.90 (s, 3H, OCH3), 4.84 (s, 2H, CH_2), 6.94 (s, ex, 1H, NH), 7.01 (d, 1H, aromatic H, *J* = 8.4 Hz), 7.09 (t, 1H, aromatic H, *J* = 7.1 Hz), 7.15-7.20 (m, 4H, aromatic H), 7.34 (t, 2H, aromatic H, *J* = 7.5 Hz), 7.50 (d, 2H, aromatic H, *J* = 7.4 Hz), 7.57 (s, 1H, CH), 7.64 (d, 2H, aromatic H, *J* = 7.6 Hz), 7.73 (d, 1H, aromatic H, *J* = 7.1 Hz), 7.91 (d, 1H, aromatic H, *J* = 8.1 Hz), 8.31 (d, 1H, aromatic H, *J* = 8.3 Hz), 8.36 (d, 1H, aromatic H, *J* = 8.3 Hz), 10.18 (s, ex, 2H, NH2); ^{13}C NMR: δ 56.1, 56.3, 67.5, 110.4, 112.3, 115.6, 115.7, 117.7, 118.9, 120.1, 122.7, 124.1, 127.1, 128.9, 129.2, 129.9, 131.6, 134.6, 138.8, 149.5, 160.6, 166.5; MS (m/z %): 612 (M^+, 0.11 %), 479 (100 %), Anal. Calcd. for $C_{32}H_{28}N_4O_7S$ (612.66): C, 62.74; H, 4.61; N, 9.15 %. Found: C, 62.81; H, 4.52; N, 9.45 %.

*4.1.4.5. 2-[4-((2-(3,4-Dimethoxyphenyl)-5-oxo-1-(4-sulfamoylphenyl)-1,5-dihydro-4H-imidazol-4-ylidene)methyl)phenoxy]-N-(4-methoxyphenyl)acetamide (**6e**).* Yellow crystals; yield: 0.41 g (63 %); m.p. 270-273 ^{o}C; IR (KBr, cm^{-1}) υ_{max}: 3440-3370 (NHs), 3086 (CH aromatic), 2974 (CH aliphatic), 1668, 1650 (2 C=Os), 1360, 1155 (SO_2); ^{1}H NMR: δ 3.79 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 4.61 (s, 2H, CH_2), 6.90-6.93 (m, 3H, aromatic H), 7.02 (d, 2H, aromatic H, *J* = 8.2 Hz), 7.17-7.20 (m, 2H, aromatic H), 7.48 (d, 2H, aromatic H, *J* = 8.2 Hz), 7.73 (d, 2H, aromatic H, *J* = 8.4 Hz), 7.90 (s, 1H, CH), 8.18 (d, 2H, aromatic H, *J* = 8.6 Hz), 8.24 (d, 2H, aromatic H, *J* = 8.6 Hz), 10.28 (s, ex, 2H, NH_2), 10.41 (s, ex, 1H, NH_2); ^{13}C NMR: δ 56.1, 56.2, 56.3, 65.3, 111.8, 112.3, 116.5, 120.9, 123.0, 125.9, 127.1, 128.5, 128.9, 135.1, 135.9, 138.3, 144.1, 148.4, 151.8, 158.3, 160.6, 169.5; MS (m/z %): 639 (M-3, 0.02 %), 479 (100 %), Anal.

Calcd. for $C_{33}H_{30}N_4O_8S$ (642.68): C, 61.67; H, 4.71; N, 8.72 % Found: C, 61.55; H, 4.82; N, 8.94 %.

4.1.4.6. 2-[4-((2-(3,4-dimethoxyphenyl)-5-oxo-1-(4-sulfamoylphenyl)-1,5-dihydro-4H-imidazol-4-ylidene)methyl)phenoxy]-N-(4-sulfamoylphenyl)acetamide ***(6f)***. Yellow crystals; yield: 0.44 g (62%); m.p. 277-281 ^{o}C; IR (KBr, cm^{-1}) υ_{max}: 3360-3280 (NHs), 3100 (CH aromatic), 2880 (CH aliphatic), 1714, 1643 (2 C=Os), 1342, 1170 (SO_2); ^{1}H NMR: δ 3.89 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 4.68 (s, 2H, CH_2), 6.92 (s, ex, 4H, 2 NH_2), 7.03-7.09 (m, 4H, aromatic H), 7.18-7.23 (m, 3H, aromatic H), 7.48 (d, 1H, aromatic H, *J* = 7.8 Hz), 7.56-7.74 (m, 3H, aromatic H), 7.90 (s, 1H, CH), 8.18 (d, 2H, aromatic H, *J* = 8.3 Hz), 8.25 (d, 2H, aromatic H, *J* = 8.4 Hz), 12.5 (s, ex, 1H, NH); ^{13}C NMR: δ 56.1, 56.3, 65.3, 111.8, 112.3, 116.3, 120.9, 123.0, 125.9, 127.1, 128.4, 128.9, 135.1, 135.9, 138.3, 144.1, 148.4, 151.8, 153.6, 160.6, 169.9; MS (m/z %): 692 (M+1, 0.1 %), 479 (100 %), Anal. Calcd. for $C_{32}H_{29}N_5O_9S_2$ (691.73): C, 55.56; H, 4.23; N, 10.12 %. Found: C, 55.31; H, 4.16; N, 9.83 %.

4.2. Biological Evaluation:

4.2.1. In vitro p38αMAPK *assay:*

The inhibitory activity was measured according to the method of Forrer *et al.* [26] where the well plates were coated with 50 µl ATF-2- solution (10 µg/ml) for 1 h at 37°C. Plates were then washed three times and 50 µl kinase mixture which consists of (50 mM Tris–HCl, 10 mM $MgCl_2$, 10 mM ß-glycerol phosphate, 100 µg/ml BSA, 1 mM DTT, 100 µM ATP, 100 µM Na_3VO_4 and 10 ng p38α activated with or without an inhibitor) was added to the wells and incubated for 1 h at 37°C. After three washes, plates were incubated with phospho-ATF-2 antibody (1:2000) for 1 h at 37°C. After washing the plates three times, alkaline phosphatase labeled goat antirabbit IgG (1:2000) was added for 1 h at 37°C then finally after

three washes, alkaline phosphatase substrate solution (3 mM 4-NPP, 50 mM $NaHCO_3$, 50 mM $MgCl_2$, 100 µl/well) was added for 1.5 h at 37°C. The formation of 4-nitrophenolate was measured at 410 nm using a microtiter plate reader and IC_{50} values were calculated.

4.2.2. In vitro carbonic anhydrase inhibitory assay:

An applied photophysics stopped-flow instrument has been used for assaying the CA catalyzed CO_2 hydration activity [27] where phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 20 mM Hepes (pH 7.5) as buffer, and 20 mM Na_2SO_4 following the initial rates of the CA-catalyzed CO_2 hydration reaction for a period of 10–100 s. For each inhibitor at least six traces of the initial 5-10 % of the reaction have been used for determining the initial rate. Stock solutions of the inhibitor (0.1 mM) were prepared in distilled-deionized water and dilutions up to 0.01 nM were done thereafter with the assay buffer. Inhibitor and enzyme solutions were preincubated together for 15 min at room temperature prior to assay, in order to allow for the formation of the E-I complex. The inhibition constants were then obtained by non-linear least-squares methods using PRISM 3 and the Cheng-Prusoff equation.

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References:

[1] C.T. Supuran, Carbonic anhydrases: novel therapeutic applications for inhibitors and activators, Nat. Rev. Drug Discov., 7 (2008) 168-181. https://doi.org/10.1038/nrd2467.

[2] V. Alterio, A. D. Fiore, K. D'Ambrosio, C.T. Supuran, G. De Simone, Multiple binding modes of inhibitors to carbonic anhydrases: how to design specific drugs targeting 15 different isoforms?, Chem. Rev., 112 (2012) 4421-4468. https://doi.org/10.1021/cr200176r.

[3] J. Pastorek, S. Pastorekova, Hypoxia-induced carbonic anhydrase IX as a target for cancer therapy: from biology to clinical use, Semin. Cancer Biol., Elsevier, 2015, pp. 52-64. https://doi.org/10.1016/j.semcancer.2014.08.002.

[4] C.T. Supuran, Carbonic anhydrase inhibition and the management of hypoxic tumors, Metabolites, 7 (2017) 1-13 https://doi.org/10.3390/metabo7030048.

[5] D. Neri, C.T. Supuran, Interfering with pH regulation in tumours as a therapeutic strategy, Nat. Rev. Drug Discov., 10 (2011) 767-777. https://doi.org/10.1038/nrd3554.

[6] G. De Simone, C.T. Supuran, Carbonic anhydrase IX: Biochemical and crystallographic characterization of a novel antitumor target, Biochim. Biophys. Acta, 1804 (2010) 404-409. https://doi.org/10.1016/j.bbapap.2009.07.027.

[7] F. Pacchiano, F. Carta, P.C. McDonald, Y. Lou, D. Vullo, A. Scozzafava, S. Dedhar, C.T. Supuran, Ureido-substituted benzenesulfonamides potently inhibit carbonic anhydrase IX and show antimetastatic activity in a model of breast cancer metastasis, J. Med. Chem., 54 (2011) 1896-1902. https://doi.org/10.1021/jm101541x.

[8] R.G. Gieling, M. Babur, L. Mamnani, N. Burrows, B.A. Telfer, F. Carta, J.-Y. Winum, A. Scozzafava, C.T. Supuran, K.J. Williams, Antimetastatic effect of sulfamate carbonic anhydrase IX inhibitors in breast carcinoma xenografts, J. Med. Chem, 55 (2012) 5591-5600. https://doi.org/10.1021/jm300529u.

[9] C. T. Supuran, Carbon-versus sulphur-based zinc binding groups for carbonic anhydrase inhibitors?, J. Enzyme. Inhib. Med. Chem. 33 (2018) 485-495. https://doi.org/10.1080/14756366.2018.1428572.

[10] A. Cincinelli, T. Martellini, A. Innocenti, A. Scozzafava, C. T. Supuran, Purification and inhibition studies with anions and sulfonamides of an α-carbonic anhydrase from the Antarctic seal Leptonychotes weddellii, Bioorg. Med. Chem. 19 (2011) 1847-1851. https://doi.org/10.1016/j.bmc.2011.02.015.

[11] S. Kalinin, S. Kopylov, T. Tuccinardi, A. Sapegin, D. Dar'in, A. Angeli, C.T. Supuran, M. Krasavin, Lucky Switcheroo: Dramatic Potency and Selectivity Improvement of Imidazoline Inhibitors of Human Carbonic Anhydrase VII, ACS Med. Chem. Lett. 8 (2017) 1105-1109. https://doi.org/10.1021/acsmedchemlett.7b00300.

[12] X. Jin, Q. Mo, Y. Zhang, Y. Gao, Y. Wu, J. Li, X. Hao, D. Ma, Q. Gao, P. Chen, The p38 MAPK inhibitor BIRB796 enhances the antitumor effects of VX680 in cervical cancer, Cancer Biol. Ther., 17 (2016) 566-576. https://doi.org/10.1080/15384047.2016.1177676.

[13] B. Kaminska, MAPK signalling pathways as molecular targets for anti-inflammatory therapy-From molecular mechanisms to therapeutic benefits, Biochim. Biophys. Acta, 1754 (2005) 253-262. https://doi.org/10.1016/j.bbapap.2005.08.017.

[14] M. Getlik, C. Grutter, J. R. Simard, H.D. Nguyen, A.Robubi, B. Aust, W. A. V. Otterlo, D. Rauh, Structure-based design, synthesis and biological evaluation of *N*-pyrazole, *N'*-thiazole urea inhibitors of MAP kinase p38α, Eur. J. Med. Chem., 48 (2012) 1-15. https://doi.org/10.1016/j.ejmech.2011.11.019.

[15] A. L. Gill, M. Frederickson, A. Cleasby, S. J. Woodhead, M. G. Carr, A. J. Woodhead, M. T. Walker, M. S. Congreve, L. A. Devine, D. Tisi, M. O 'reilly, L. C. A. Seavers, D. J. Davis, J. Curry, R. Anthony, A. Padova, C. W. Murray, R. A. E. Carr, H. Jhoti, Identification of novel p38αMAP kinase inhibitors using fragment-based lead generation, J. Med. Chem., 48 (2005) 414-426. https://doi.org/10.1021/jm049575n.

[16] N. J. Liverton, J.W. Butcher, C. F. Claiborne, D. A. Claremon, B. E. Libby, K. T. Nguyen, S. M. Pitzenberger, H. G. Selnick, G. R. Smith, A. Tebben, J. P. Vacca, S. L. Varga, L. Agarwal, K. Dancheck, A. J. Forsyth, D. S. Fletcher, B. Frantz, W. A. Hanlon, C. F. Harper, S. J. Hofsess, M. Kostura, J. Lin, S. Luell, E. A. O'Neill, C. J. Orevillo, M. Pang, J. Parsons, A. Rolando, Y. Sahly, D. M. Visco, S. J. O'Keefe, Design and synthesis of potent, selective, and orally bioavailable tetrasubstituted imidazole inhibitors of p38 mitogen-activated protein kinase, J. Med. Chem., 42 (1999) 2180-2190. https://doi.org/10.1021/jm9805236.

[17] F. M. Awadallah, S. M. Abou-Seri, M. M. Abdulla, H. H. Georgey, Design and synthesis of potent 1,2,4-trisubstituted imidazolinone derivatives with dual p38αMAPK and ERK1/2 inhibitory activity, Eur. J. Med. Chem., 94 (2015) 397-404. https://doi.org/10.1016/j.ejmech.2015.03.008.

[18] J. F. Poon, J. P. Alao, P. Sunnerhagen, P. Dinér, Azastilbenes: a cut-off to p38 MAPK inhibitors, Org. Biomol. Chem., 11 (2013) 4526-4536. https://doi.org/ 10.1039/c3ob27449g

[19] J. Kopaceka, M. Barathovaa, F. Dequiedtc, J. Sepelakova, R. Kettmann, J. Pastorek, S. Pastorekov, MAPK pathway contributes to density-and hypoxia-induced expression of the tumor-associated carbonic anhydrase IX, Biochim. Biophys. Acta, 1729 (2005) 41-49. https://doi.org/10.1016/j.bbaexp.2005.03.003.

[20] D. A. Booth, R. Brettle, A. M. Harris, The synthesis of some acylglycines and related oxazolones, J. Chem. Soc, (1960) 3457-3461. https://doi.org/10.1039/JR9600003457.

[21] R. C. Tandel, D. Mammen, Synthesis and study of some compounds containing oxazolone ring, showing biological activity, Indian J. Chem.-Sect. B Org. Med. Chem., 47 (2008) 932-937 http://nopr.niscair.res.in/handle/123456789/1744.

[22] G. Mariappan, B. P. Saha, S. Datta, D. Kumar, P. K. Haldar, Design, synthesis and antidiabetic evaluation of oxazolone derivatives, J. Chem. Sci., 123 (2011) 335-341. https://doi.org/10.1007/s12039-011-0079-2.

[23] A. A. Beloglazkina, B. Wobith, E. S. Barskaia, N. A. Zefirov, A. G. Majouga, E. K. Beloglazkina, N. V. Zyk, S. A. Kuznetsov, O. N. Zefirova, Synthesis and biological testing of (5*Z*)-2-aryl-5-arylmethylidene-3,5-dihydro-4*H*-imidazol-4-ones as antimitotic agents, Med. Chem. Res., 25 (2016) 1239-1249. https://doi.org/ 10.1007/s00044-016-1566-2.

[24] A. Tomar, S. Kumar, M. Mall, Synthesis, computational studies and preliminary pharmacological evaluation of new acetamides: Potential antipsychotics, Der pharma chem., 4 (2012) 85-92.

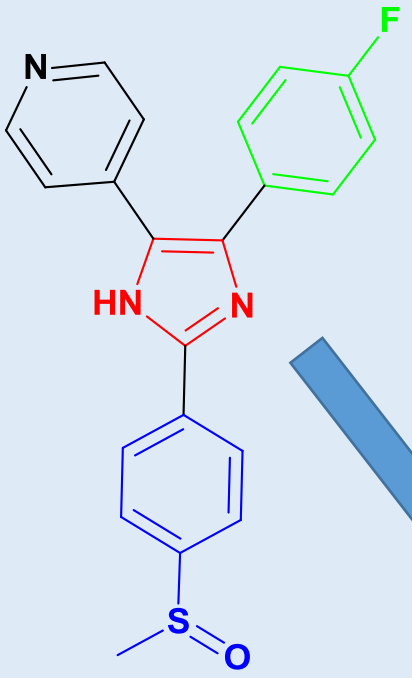
[25] G. B. Wang, L. F. Wang, C. Z. Li, J. Sun, G. M. Zhou, D. C. Yang, A facile and efficient method for the selective deacylation of *N*-arylacetamides and 2-chloro-*N*-arylacetamides catalyzed by $SOCl_2$, Res. Chem. Intermed. 38 (2012) 77-89. https://doi.org/10.1007/s11164-011-0327-6.

[26] P. Forrer, R. Tamaskovic, R. Jaussi, "Enzyme-linked immunosorbent assay for measurement of JNK, ERK, and p38 kinase activities", Biol. Chem., 379 (1998) 1101-1111. https://doi.org/10.1515/bchm.1998.379.8-9.1101.

[27] R.G. Khalifah, The carbon dioxide hydration activity of carbonic anhydrase I. Stop-flow kinetic studies on the native human isoenzymes B and C, J. Biol. Chem., 246 (1971) 2561-2573 (PMID:4994926).

Highlights

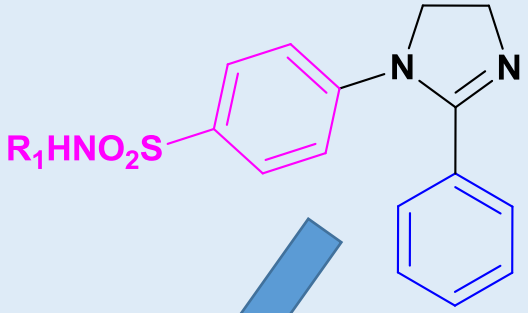
- Twenty 1,2,4-trisubstituted imidazolin-5-one derivatives were synthesized.
- Target compounds were evaluated as dual p38αMAPK/CA enzyme inhibitors
- The best p38αMAPK inhibition results were for compounds **3c, 3g, 3h, 4a, 6c** and **6d**
- Compound **4a** has IC_{50} against p38αMAPK= 0.14 μM
- The best hCA inhibition result was for compound **4a**
- Ki values of **4a** against hCA I, II, IV and IX = 95.0, 0.83, 6.90 and 12.4 nM, respectively
- **4a** was discovered as a potent dual p38αMAPK/CA inhibitor in this work



II

Potent p38aMAPK inhibitor

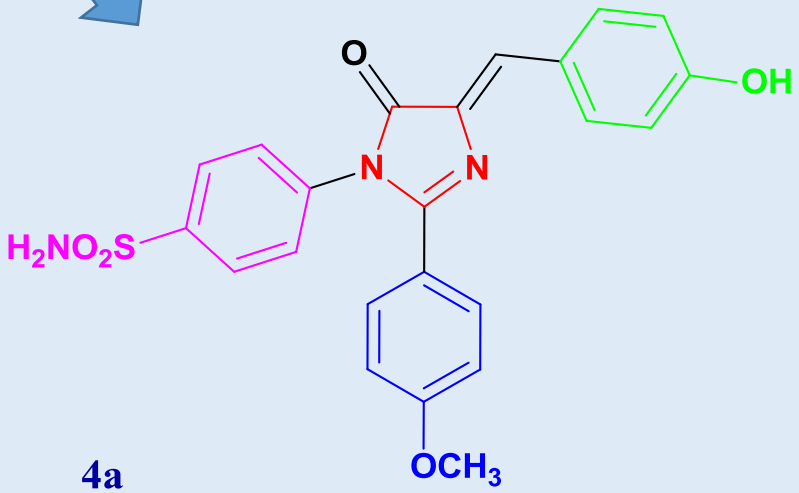
- Imidazole core
- 2-Aryl moiety
- 4-Aryl moeity



I

Potent CA inhibitor

- Zinc Binding Group (ZBG)
- Appended aryl moeity



4a

IC_{50} against p38aMAPK = 0.14 mM

K_i against CA I, II, IV and IX = 95.0, 0.83, 6.90 and 12.4 nM

Potent dual p38aMAPK/CA inhibitor