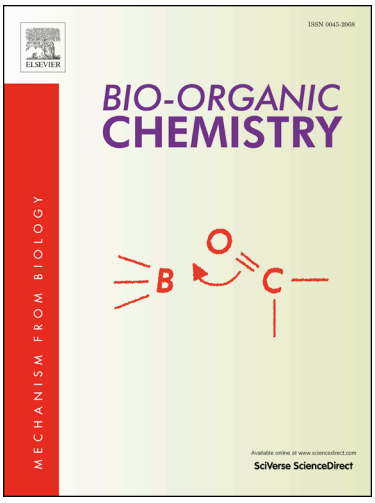


# Synthesis of novel isoindoline-1,3-dione-based oximes and benzenesulfonamide hydrazones as selective inhibitors of the tumor-associated carbonic anhydrase IX

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# Synthesis of novel isoindoline-1,3-dione-based oximes and benzenesulfonamide hydrazones as selective inhibitors of the tumor-associated carbonic anhydrase IX.

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**Abstract**. The synthesis, characterization and biological evaluation of a library of isoindoline-1,3-dione-based oximes and benzenesulfonamide hydrazones is disclosed. The set of hydroxyiminoethyl aromatic derivatives **10-18** was designed to assess the potentiality as zinc-binder for a feebly studied functional group in the field of carbonic anhydrase (CA, EC 4.2.1.1) inhibition. Analogue phenylphthalimmides were linked to benzenesulfonamide scaffold by hydrazone spacers in the second subset of derivatives **20-28** to further investigate the application of the "tail approach" as tool to afford CA selective inhibition profiles. The compounds were assayed for the inhibition of physiologically relevant isoforms of human carbonic anhydrases (hCA, EC 4.2.1.1), the cytosolic CA I and II, and the membrane-bound CA IV and tumor-associated CA IX. The new zinc-binders, both of the oxime and sulfonamide types, showed a striking selective activity against the target hCA IX over ubiquitous hCA I and II, with diverse inhibitory ranges and *ratio* differing the two subsets. With CA IX being a strongly current antitumor/antimetastatic drug target, these series of compounds may be of interest for the development of new, both conventional and unconventional anticancer drugs targeting hypoxia-induced CA isoforms such as CA IX with minimum ubiquitous CAs-related side effects.

***Keywords***: Isoindoline-1,3-dione; oxime; hydrazone; benzenesulfonamide; carbonic anhydrase inhibition, carbonic anhydrase IX, anti-tumor.

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## 1. Introduction

Carbonic anhydrases (CAs, EC 4.2.1.1) are a superfamily of zinc enzymes which catalyze the reversible hydration of $CO_2$ into $HCO_3$ and protons by a metal hydroxide nucleophilic mechanism.[1,2] Seven genetically distinct CA families (α-, β-, γ-, δ-, ζ-. η- and θ-CAs.) were identified to date.[1-3]. The 15 different α-CA isoforms isolated in humans (h) feature catalytic activity, sub-cellular localization and organ/tissue distribution. Among the catalytically active isoforms, some are cytosolic (CA I, CA II, CA III, CA VII and CA XIII), others are membrane bound (CA IV, CA IX, CA XII, CA XIV and CA XV), two of them are mitochondrial (CA VA and CA VB), and one isozyme is secreted in saliva (CA VI).[2] Considering the variety of human physiological-pathological processes showing abnormal levels or activities of these enzymes, CA isozymes are valuable targets for many pharmacological applications such as antiglaucoma drugs, diuretics, antiobesity, anticonvulsant and/or antitumor agents/diagnostic tools.[1] When inhibitors targeting hCA I are useful in retinal and cerebral edema, inhibitors targeting hCA II, IV, XII and XIV are used as diuretics, in the management edema, as antiglaucoma agents, antiepileptic drugs, and also for the treatment of altitude sickness.[1,2] With CA I and II being ubiquitous and physiologically relevant isoforms, their inhibition is often off-target.[2] CA IX, a transmembrane isoform, is involved in tumor growth, metastases formation with its inhibitors having shown applications as diagnostic tools for imaging hypoxic tumors and as antitumor/antimetastatic agents.[4-7] In hypoxic tumors (e.g. breast, brain, colorectal, etc.) hCA IX, whose expression is induced via the hypoxia inducible factor-1α (HIF-1α), maintains the external pH, supporting an acidic extracellular microenvironment suited for hypoxic tumor cell survival and proliferation, but detrimental to normal cells.[4-7] The above made hCA IX become an attractive target for the design of antineoplastic therapies.[1,2] The achievement of selective inhibition of some isoforms over others is a necessary endeavor to afford tools for the treatments of a plethora of pathologies endowed with minimum side effects.[1] In this contest many efforts have been made for the development of isoform-

selective CAIs, and some remarkable results have been achieved in the last 15 years since the introduction of the "tail approach" to most classical sulfonamide-like inhibitors [8-11] and identification of varied new CA inhibitory chemotypes.[8] Zinc-binding groups diverse from sulfonamides and their congeners have been discovered, among which carboxilates, hydroxamates, phosphonates, mono- and dithiocarbammates, xanthates, thioxanthates and boroles.[12-17] The use of heterocyclic tails is on the other hand the most exploited tools to seek for selectivity with sulfonamides inhibitors against target hCAs.[18-29] In this context, heterocycles of the isatin, phthalimide or quinoline types are undoubtedly amongst the most incorporated scaffolds as tail of zinc-binding group bearing molecules since they possess a variety pharmacological and biological activities, such as excellent anticancer profiles and inhibition profile for several CA isoforms.[18-29]

Herein synthesis and biological evaluation of novel isoindoline-1,3-dione-based oximes and benzenesulfonamide hydrazones is proposed to go forward into the design of heterocyclic compounds of potential pharmacologic interest. A set of hydroxyiminoethyl aromatic derivatives **10-18** was designed to assess the potentiality as zinc-binder for a feebly studied functional group in the field of carbonic anhydrase inhibition.[30,31] Analogue scaffolds were linked to benzenesulfonamide cores by hydrazone spacers **20-28** to further investigate the application of the tail approach as tool to address CA selective inhibition profiles, with the target tumor-associated hCA IX being screened beside the physiologically relevant hCA I, II and IV.

## 2 Results and discussion

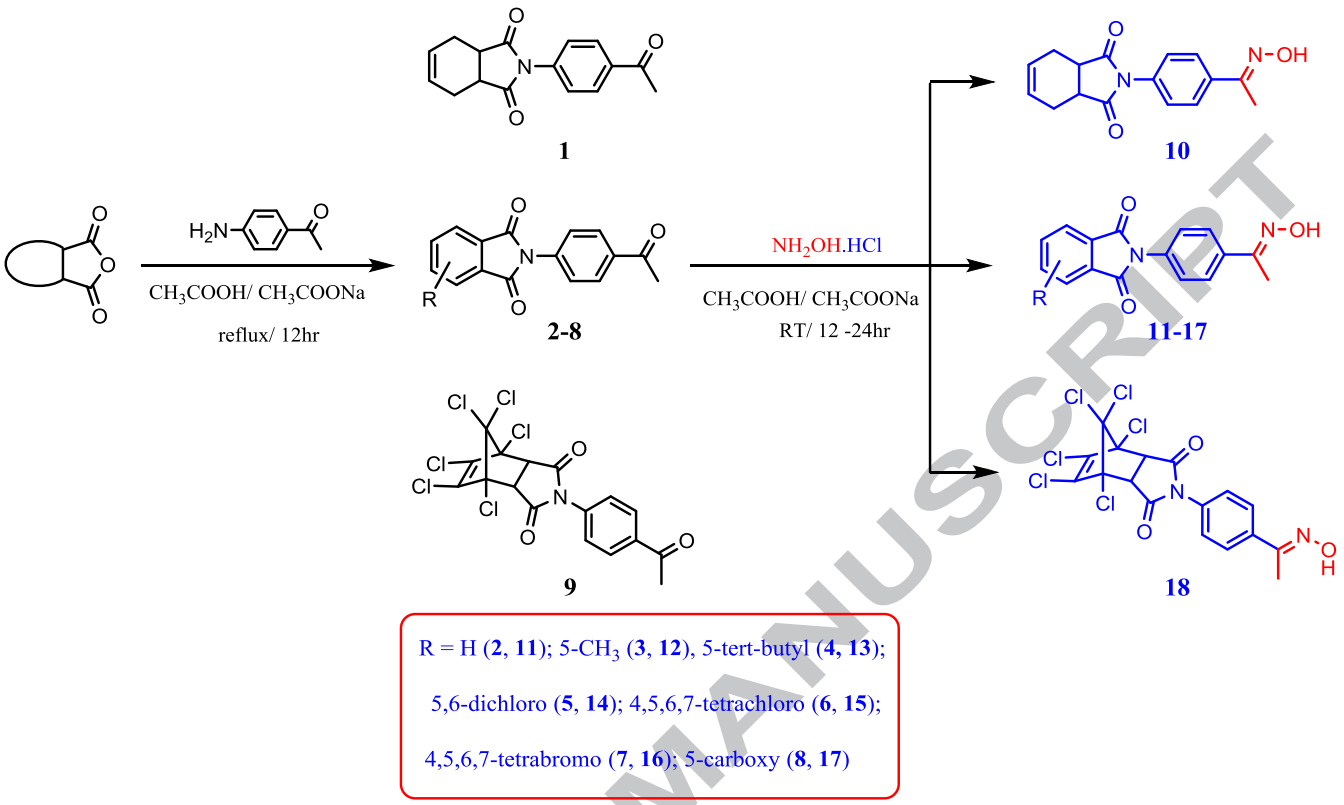
### 2.1. Chemistry

The present work possesses a dual rationale: hydroxyiminoethyl moieties were place onto variously substituted phthalimmidophenyl scaffolds to explore the potentiality of isoindoline-1,3-dione-based oximes to inhibit CAs in a selective manner. Equal scaffolds were appended to benzenesulfonamide through hydrazone linkers. Hydrazone group is a urea-bioisoster, with this latter being the most

commonly incorporated tailing linker in benzenesulfonamides derivatives. A ureido-sulfonamide CA IX selective inhibitor (**SLC-0111**) entered in Phase II clinical trials for the treatment of patients with advanced solid, metastatic tumors over-expressing CA IX.[32,33] Ureido bioisosterism has been significantly applied to **SLC-0111**.[2,8,34] Varied substituents at the isoindoline-1,3-dione groups considered in the present study allowed to study the compounds interactions at the entrance of the CA active site cavity. Steric and lipophilic contributions of halogens as well as their ability to form stabilizing interactions, such as halogen bonding, hydrogen bonding and multipolar interactions were also exploited to seek for isoform-selectivity.[35,36] Since that region is the most diverse one among the different α-CAs, selective inhibition may be achieved through particular interactions between the tail of the inhibitor and this region of the cavity.[2]

The synthesis of isoindoline-1,3-dione-based oximes **10-18** and benzenesulfonamide hydrazones **20-28** was performed as highlighted in *Schemes 1* and *2*, starting from common intermediates 2-(4-acetylphenyl)-isoindoline-1,3-diones **1-9.**

2-(4-Acetylphenyl)-isoindoline-1,3-diones derivatives **1-9** were obtained in 80-95% yield by heating of 4-aminoacetophenone with the corresponding acid anhydride in glacial acetic acid containing anhydrous sodium acetate [19-29] (Scheme 1). The structures of obtained compounds were confirmed by their elemental and spectral analyses. The infrared (IR) spectrum of compound **1-9** exhibited characteristic bands at 1683-1730 $cm^{-1}$ due to the presence of carbonyl (C=O) groups. The $^{1}H$ NMR spectrum of compounds **1-9** was verified by the presence of new signal due to acetyl group ($CO\underline{CH_3}$) at 2.59-2.66 ppm. Additionally, new peaks were observed at 26.68-27.63 ppm due to acetyl group ($CO\underline{CH_3}$), and 162.80-178.78 ppm for carbonyl (C=O) group due to anhydride moiety as well as characteristic peak of carbonyl ($\underline{CO}CH_3$) group at 197.30-197.80 ppm respectively in $^{13}C$ NMR spectrum.

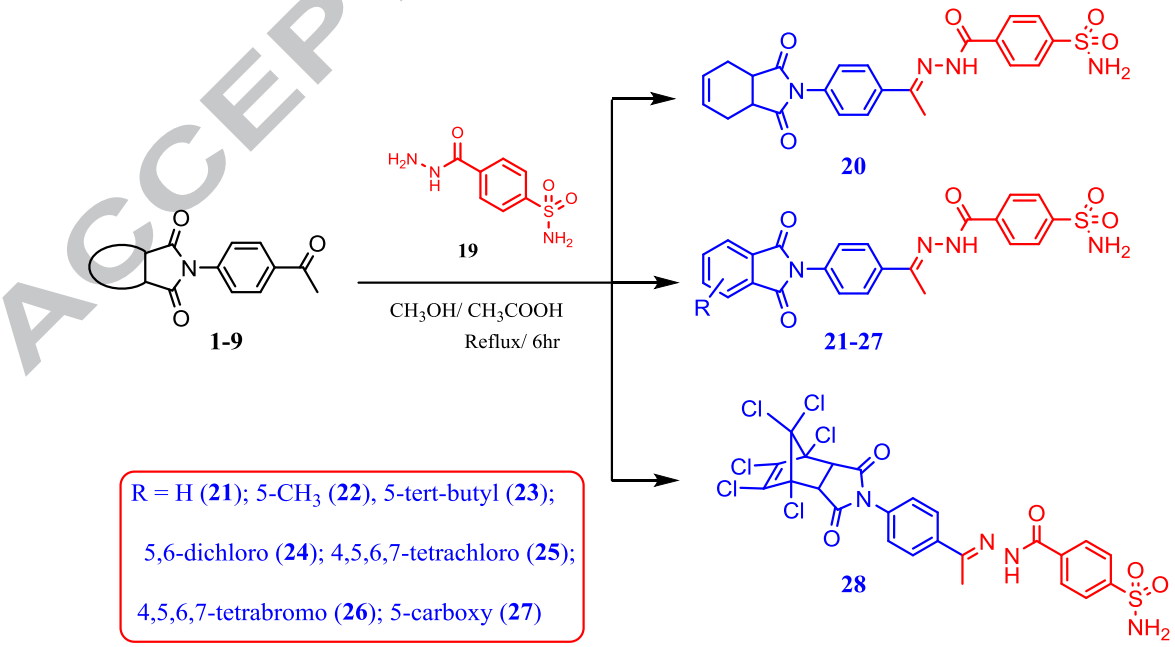



**Scheme 1.** Synthesis of 2-(4-acetylphenyl)isoindoline-1,3-diones (**1-9**) and 2-(4-(1-(hydroxyimino)ethyl)phenyl)isoindoline-1,3-diones (**10-18**)

2-[4-(1-(Hydroxyimino)ethyl)phenyl] derivatives **10-18** were produced in 83-96% yield by stirring of the isoindoline-1,3-diones **1-9** and hydroxylamine hydrochloride in glacial acetic acid containing anhydrous sodium acetate (Scheme 1). The structures of new synthesized compounds were confirmed by their elemental and spectral data. IR spectra of compounds **10-18** were used to verify their structures through the appearance of characteristic absorption bands due to hydroxyl group of oxime and carbonyl (C=O) groups at 3245-3471 and 1700-1783 $cm^{-1}$ respectively. $^{1}H$ NMR spectra of compounds **10-18** were characterized by the disappearance of acetyl group ($CO\underline{CH_3}$) at 2.59-2.66 ppm and appearance of new singlet peaks of hydroxyl group ($CH_3C{=}N\text{-}\underline{OH}$), as well as ethylimine group ($\underline{CH_3}C{=}N\text{-}OH$) of oxime moiety at 11.14-11.58 and 1.91-2.41 ppm respectively. Moreover, $^{13}C$ NMR spectra of compounds **10-18** showed the disappearance of acetyl ($CO\underline{CH_3}$), and carbonyl ($\underline{CO}CH_3$) groups at

26.68-27.63 and 197.30-197.80 ppm respectively and presence of new peak for ($CH_3C$=N-OH) group at 11.99-12.76 ppm.

Benzenesulfonamides **20-28** were obtained in 84-93% yield by heating of isoindoline-1,3-diones **1-9** and 4-(hydrazinecarbonyl)benzenesulfonamide (**19**) in methanol containing catalytic amount of acetic acid (Scheme 2). Compounds **20-28** were confirmed by their elemental and spectral analyses. The IR spectrum of compounds **20-28** were characterized by the presence of absorption bands at 3513-3260 and 1785-1708 $cm^{-1}$ representing (NH) and (C=O) groups, respectively. The $^{1}H$ NMR spectrum of compounds **20-28** were verified by the disappearance of acetyl group ($COCH_3$) at 2.59-2.66 ppm and appearance of new singlet peaks of amide group ($CH_3C$=N-NH-CO-R), as well as ethylimine group ($CH_3C$=N-NH-COR) due to ethylidenehydrazine moiety at 11.84-11.0 and 2.47-2.36 ppm respectively, in addition to aromatic protons of benzensulfonamide moiety at aromatic region. Furthermore, $^{13}C$ NMR spectra of compounds **20-28** showed the disappearance of acetyl ($COCH_3$), and carbonyl ($COCH_3$) groups at 26.68-27.63 and 197.30-197.80 ppm respectively and presence of new peak for ethylimine ($CH_3C$=N-NH-COR) group at 15.26-15.21 ppm.




**Scheme 2.** Synthesis of hydrazones based on isoindoline-1,3-dione incorporating benzenesulfonamide **20-28**

### 2.2. Carbonic anhydrase inhibition

The CA inhibitory properties of oximes **10-18** and sulfonamides **20-28** against human isoforms CA I, II, IV and IX were measured by a stopped flow $CO_2$ hydrase assay using acetazolamide (**AAZ**) as standard inhibitor.[37] Inhibition profiles were displayed in comparison to **SLC-0111**. The following structure–activity relationships (SAR) were gathered from the inhibition data reported in Table 1.

**Table 1**: Inhibition data of human CA isoforms hCA I, II, IV and IX with isoindoline-1,3-dione-based oximes **10-18**, benzenesulfonamide hydrazones **20-28** and the standard sulfonamide inhibitor acetazolamide (**AAZ**) by a stopped flow $CO_2$ hydrase assay.[37]

| Cmpd | R | $K_I$ (nM)* | | | |
|---|---|---|---|---|---|
| | | hCA I | hCA II | hCA IV | hCA IX |
| **10** | - | >100000 | 74880 | 38700 | 2038 |
| **11** | H | >100000 | 84790 | 23180 | 318.9 |
| **12** | 5-$CH_3$ | >100000 | 66150 | 15850 | 280.5 |
| **13** | 5-$C(CH_3)_3$ | >100000 | 24110 | 24640 | 1328 |
| **14** | 5,6-diCl | >100000 | 56250 | 36900 | 318.4 |
| **15** | 4,5,6,7-tetraCl | >100000 | 86570 | 31910 | 169.3 |
| **16** | 4,5,6,7-tetraBr | >100000 | 58040 | 43960 | 323.9 |
| **17** | 5-COOH | 9670 | 5585 | 3660 | 240.1 |
| **18** | - | >100000 | 97840 | 46530 | 19900 |
| **20** | - | 6901 | 795.2 | 2030 | 33.9 |
| **21** | H | 9283 | 834.9 | 4322 | 31.4 |
| **22** | 5-$CH_3$ | 6922 | 632.7 | 4065 | 30.3 |
| **23** | 5-$C(CH_3)_3$ | 8302 | 787.3 | 1869 | 83.8 |
| **24** | 5,6-diCl | 5551 | 670.7 | 2103 | 22.9 |
| **25** | 4,5,6,7-tetraCl | 21090 | 707.1 | 3092 | 17.3 |
| **26** | 4,5,6,7-tetraBr | 38090 | 663.9 | 4758 | 23.1 |
| **27** | 5-COOH | 6113 | 369.1 | 406.1 | 22.6 |
| **28** | - | 63000 | 539.8 | 24410 | 164.3 |
| **AAZ** | - | 250 | 12 | 74 | 25 |
| **SLC-0111** | - | 5080 | 960 | 286 | 45 |

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

(i) Oximes **10-18** did not inhibit the cytosolic hCA I up to 100 µM and showed feeble hCA II and IV inhibition profiles. Compound **17** stood out amongst the others as unique low micromolar hCA I, II and IV inhibitors, with inhibition constants ($K_I$s) of 9667.5, 5585.4 and 3660.4 nM, respectively. This is

probably due to presence of a carboxylic group on the phthalimide aromatic portion. Carboxy derivatives have been shown to inhibit several hCAs in a low micromolar range. The other hydroxyiminoethyl derivatives showed $K_I$s ranging between 16 and 98 μM for the two off-target isoforms hCA II and IV. It may be observed that CA IX, overexpressed in hypoxic tumors, was significantly sensitive to inhibition by isoindoline-1,3-dione-featuring oximes with $K_I$s mainly reaching a medium-low nanomolar range (169.3-2038.4 nM). The most effective CAI **15** bore the fully chlorinated phthalimmide scaffold. The loss of aromaticity in the isoindoline-1,3-dione core decreased the inhibition potency of **10** against hCA IX by almost ten-fold with respect to **11** ($K_I$s of 2038.4 vs 318.9 nM). The 5-COOH-bearing derivative **17** was also an effective hCA IX inhibitor showing an inhibition constant of 240.3 nM. Incorporation of a bulky hexa-halogenated moiety such as in **18** elicited a remarkable drop of efficacy in inhibiting the tumor-associated isozyme. Most oximes **10-18** showed a markedly selective action against the hCA IX over the off-targer hCA I and XII (over two orders of magnitude) with the standard **AAZ** showing a more potent but promiscuous activity versus the screened isozymes.

(ii) The inhibition profiles of isoindoline-1,3-diones **20-28** changed dramatically compared to compounds **10-18** owing to the incorporation of hydrazone benzenesulfonamides in place of hydroxyiminoethyl moieties, though a striking preferential effectiveness against hCA IX was kept. The sulfonamides are the most effective zinc-binding group in CA inhibition due to concomitant important features in terms of ligand-target interactions; the presence of a negatively charged nitrogen which coordinates the zinc ion and the presence of a hydrogen atom on it which enables the sulfonamide to form two H-bonds with diverse portion of residue Thr199, present in all catalytically active human isozymes.[2] As a result, the different CA inhibition profiles flattened out, with hCA II exhibiting the greater enhancement of inhibition with sulfonamides **20-28** ($K_I$s ranging between 369.1 and 834.9 nM). The cytosolic hCA I and the membrane-bound hCA IV were moderately inhibited by sulfonamide

reported here, with inhibition constants spanning the low micromolar range between 1869.1 and 9283.3 nM. Inhibition of CA I was more significantly affected by incorporation of bulkier substituents on the tails with $K_I$s dropping to 21, 38 and 63 μM for **25**, **26** and **28** respectively. Compound **27**, that bear a 5-carboxy moiety, arose as the unique sub-micromolar inhibitor of hCA IV. hCA II was inhibited in a rather narrow nanomolar range by **20-28** ($K_I$s of 539.8-834.9 nM). Again, **27** stood out as the best hCA II inhibitor. In a similar manner with the first subset of derivatives, namely oximes **10-18**, sulfonamides **20-28** showed a selective activity to the tumor-associated hCA IX ($K_I$s of 17.3-164.3 nM), since $K_I$s ranged 20-fold below those observed against hCA II. In detail, hCA IX was inhibited in the narrow 17.3-33.9 nM range by all sulfonamide, except **23** and **28**, that possess differently bulkier carbon or halogen groups at the isoindoline-1,3-dione scaffolds and shows $K_I$s of 83.8 and 169.3 nM, respectively.

(iii) Noteworthy, when **20-22** and **24-27** demonstrated the same hCA IX inhibition efficacy of the standard **AAZ**, they showed comparable or better IX/II selective efficacy than lead **SLC-0111**. Inhibition data in Table 1 highlighted that a hydrazone linker as connection between the benzenesulfonamide fragment and phthalimmide tails addressed the inhibitory activity versus hCA IX with respect to hCA I, II or IV. Aromatic oximes of the hydroxyiminoethyl type **10-18** were shown to act as selective hCA IX zinc-binders, owing to more than two orders of magnitude CA IX/II *ratio*.

(iv) The present phthalimmidobenzenesulfonamides showed remarkable inhibition profiles in terms of selective action against CA IX over CA I/II in comparison to a wealth of heterocycle-tailed benzenesulfonamides previously reported.[18-29] These data make some such derivatives interesting leads or tools to design novel anti-cancer therapies.

## 3. Conclusion

The present work reported the synthesis, characterization and biological evaluation of a library of isoindoline-1,3-dione-based oximes and benzenesulfonamide hydrazones. The inhibitory profiles of the

hydroxyiminoethyl aromatic derivatives **10-18** allowed to assess the potentiality as zinc-binder for a rarely studied moiety in the field of carbonic anhydrase inhibition. Isoindoline-1,3-dione hydrazone benzenesulfonamides **20-28** showed instead the aptitude of hydrazone and phatlimmides to act as the ureido-bioisoster linker and tailing components of the "tail approach". The new zinc-binders, both of the oxime and sulfonamide types, showed a remarkable selective activity against the target hCA IX over ubiquitous hCA I and II, with diverse inhibitory ranges and *ratio* differing the two subsets. With CA IX being a strongly current antitumor/antimetastatic drug target, these series of compounds may be of interest for the development of new, both conventional and unconventional anticancer drugs targeting hypoxia-induced CA isoforms such as CA IX.

## 4. Experimental

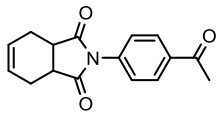
### 4.1. Chemistry

Melting points (uncorrected) were recorded on Barnstead 9100 Electrothermal melting apparatus. IR spectra were recorded on a FT-IR Perkin-Elmer spectrometer. $^{1}$H NMR and $^{13}$C NMR were recorded in DMSO-$d_6$ and $CDCl_3$ on Bruker 500 & 700 and 125 & 176 MHz instrument, respectively, using TMS as internal standard (chemical shifts in δ ppm). Mass spectra were recorded on a Agilent 6320 Ion Trap mass spectrometers. Elemental analysis was carried out for C, H and N at the Research Centre of College of Pharmacy, King Saud University and the results are within ± 0.4% of the theoretical values. Compounds **2** and **19** were prepared according to their reported procedure [38].

**2.1.1. General procedure for the synthesis of isoindoline-1,3-diones 1-9 (Scheme 1).**

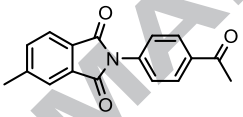
A mixture of 4-aminoacetophenone (10.0 mmol), anhydrous sodium acetate (1.38g, 10.0 mmol) and an appropriate acid anhydride (10.0 mmol) in glacial acetic acid (50 mL) was heated under reflux for 12 hr. After cooling of the reaction mixture, the precipitate obtained was filtered, washed with water, dried and re-crystallised from acetic acid.

***2.1.1.1. 2-(4-Acetylphenyl)-3a,4,7,7a-tetrahydro-1H-isoindole-1,3(2H)-dione (1)***



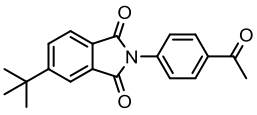
Yield, 80%; melting point (mp): 270-271 °C; IR (KBr, $cm^{-1}$) *ν*: 1715, 1698 (C=O), 1591 (C=C), 1176 (C-C); $^{1}$H NMR (500 MHz, $CDCl_3$): $\delta$ 8.05 (d, 2H, *J* = 8.5 Hz), 7.42 (d, 2H, *J* = 8.5 Hz), 6.00 (s, 2H), 3.30 (t, 2H, *J*= 9.5, 7.5 Hz), 2.73 (d, 2H, *J*= 14.5 Hz), 2.62 (s, 3H), 2.34 (d, 2H, *J*= 13.5 Hz); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 23.75, 26.69, 39.33, 126.35, 127.85, 129.08, 136.10, 136.62, 178.78, 197.03; $C_{16}H_{15}NO_3$: m/z (269).

***2.1.1.2. 2-(4-Acetylphenyl)-5-methylisoindoline-1,3-dione (3)***



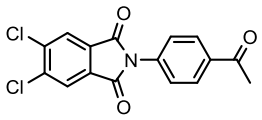
Yield, 93%; melting point (mp): 220-221 °C; $^{1}$H NMR (500 MHz, $CDCl_3$): $\delta$ 8.11 (d, 2H, *J*= 8.5 Hz), 7.86 (d, 1H, *J*= 7.5 Hz), 7.78 (s, 1H), 7.63 (t, 3H, *J*= 8.5, 7.0 Hz), 2.66 (s, 3H), 2.58 (s, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 22.12, 26.69, 123.88, 124.43, 126.04, 128.97, 129.13, 131.94, 135.31, 135.91, 136.13, 146.18, 166.84, 166.97, 197.11; $C_{17}H_{13}NO_3$: m/z (279).

***2.1.1.3. 2-(4-Acetylphenyl)-5-(tert-butyl)isoindoline-1,3-dione (4)***



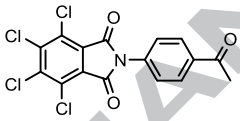
Yield, 82%; melting point (mp): 175-177 °C; IR (KBr, $cm^{-1}$) *ν*: 1710, 1697 (C=O), 1601 (C=C), 1079 (C-C); $^{1}$H NMR (500 MHz, $CDCl_3$): $\delta$ 8.10 (d, 2H, *J*= 8.0 Hz), 8.01 (s, 1H), 7.89 (d, 1H, *J*= 7.5 Hz), 7.84 (t, 1H, *J*= 6.5, 7.5 Hz), 7.63 (d, 2H, *J*= 8.5 Hz), 2.65 (s, 3H), 1.42 (s, 9H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 26.68, 31.15, 35.89, 121.10, 123.79, 126.07, 128.87, 129.14, 131.78, 135.90, 136.16, 159.52, 166.80, 167.23, 197.08; $C_{20}H_{19}NO_3$: m/z (321).

***2.1.1.4. 2-(4-Acetylphenyl)-5,6-dichloroisoindoline-1,3-dione (5)***



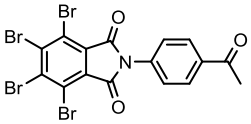
Yield, 88%; melting point (mp): 259-260 °C; IR (KBr, $cm^{-1}$) *ν*: 1708, 1683 (C=O), 1591 (C=C), 1087 (C-C); $^{1}$H NMR (700 MHz, DMSO-$d_6$): $\delta$ 8.34 (s, 2H), 8.12 (d, 2H, *J*= 9.0 Hz), 7.63 (d, 2H, *J*= 8.5 Hz), 2.64 (s, 3H); $^{13}$C NMR (125 MHz, DMSO-$d_6$): $\delta$ 27.34, 126.15, 127.44, 129.34, 132.05, 136.13, 136.50, 138.10, 165.41, 197.80; $C_{16}H_9C_{l2}NO_3$: m/z (334).

***2.1.1.5. 2-(4-Acetylphenyl)-4,5,6,7-tetrachloroisoindoline-1,3-dione (6)***



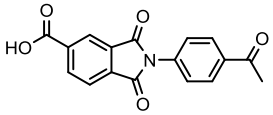
Yield, 95%; melting point (mp): 249-250 °C; IR (KBr, $cm^{-1}$) *ν*: 1717, 1700 (C=O), 1598 (C=C), 1114 (C-C); $^{1}$H NMR (700 MHz, DMSO-$d_6$): $\delta$ 8.14 (d, 2H, *J*= 8.5 Hz), 7.61 (d, 2H, *J*= 8.5 Hz), 2.65 (s, 3H); $^{13}$C NMR (176 MHz, DMSO-$d_6$): $\delta$ 27.36, 127.73, 128.82, 128.86, 129.44, 135.66, 136.85, 138.93, 162.81, 197.79; $C_{16}H_7Cl_4NO_3$: m/z (403).

***2.1.1.6. 2-(4-Acetylphenyl)-4,5,6,7-tetrabromoisoindoline-1,3-dione (7)***



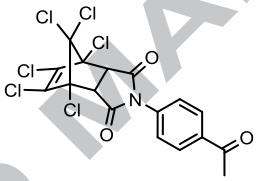
Yield, 93%; melting point (mp): 290-292 °C; IR (KBr, $cm^{-1}$) *ν*: 1713, 1698 (C=O), 1595 (C=C), 1117 (C-C); $^{1}$H NMR (700 MHz, DMSO-$d_6$): $\delta$ 8.13 (d, 2H, *J*= 8.5 Hz), 7.60 (d, 2H, *J*= 8.5 Hz), 2.65 (s, 3H); $^{13}$C NMR (176 MHz, DMSO-$d_6$): $\delta$ 27.36, 121.35, 127.82, 129.35, 131.52, 136.00, 136.78, 137.19, 163.14, 172.69, 197.80; $C_{16}H_7Br_4NO_3$: m/z (580).

***2.1.1.7. 2-(4-Acetylphenyl)-1,3-dioxoisoindoline-5-carboxylic acid (8)***



Yield, 82%; melting point (mp): 330-232 °C; IR (KBr, $cm^{-1}$) $\nu$: 3050 (br. OH), 1730, 1711, 1643 (C=O), 1594 (C=C), 1093 (C-C); $^{1}$H NMR (700 MHz, DMSO-$d_6$): $\delta$13.81 (s, 1H), 8.42 (dd, 1H, *J*= 6.3, 7.7 Hz), 8.31 (s, 1H), 8.13 (d, 2H, *J*= 8.5 Hz), 8.10 (d, 1H, *J*= 7.7 Hz), 7.65 (d, 2H, *J*=8.5 Hz), 2.64 (s, 3H); $^{13}$C NMR (176 MHz, DMSO-$d_6$): $\delta$27.32, 123.95, 124.39, 127.45, 129.29, 132.46, 135.29, 136.05, 136.27, 136.40, 137.04, 166.26, 166.39, 166.41, 197.78; $C_{17}H_{11}NO_5$: m/z (309).

***2.1.1.8. 2-(4-Acetylphenyl)-4,5,6,7,8,8-hexachloro-3a,4,7,7a-tetrahydro-1H-4,7-methanoisoindole-1,3(2H)-dione (9)***

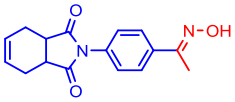


Yield, 89%; melting point (mp): 274-275 °C; $^{1}$H NMR (500 MHz, $CDCl_3$/DMSO-$d_6$): $\delta$8.06 (d, 2H, *J*= 8.5 Hz), 7.27 (d, 2H, *J*= 8.5 Hz), 4.18 (s, 2H), 2.59 (s, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$/DMSO-$d_6$): $\delta$27.06, 52.68, 79.41, 104.18, 126.98, 129.45, 131.02, 135.20, 137.37, 169.54, 196.88; $C_{17}H_9Cl_6NO_3$: m/z (487).

**2.1.2. General procedure for the synthesis of oxime based isoindoline-1,3-diones 10-18 (Scheme 1).**

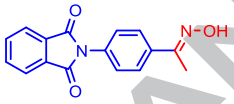
A mixture of an appropriate isoindoline-1,3-diones **1-9** (5.0 mmol), anhydrous sodium acetate (0.69g, 5.0 mmol) and hydroxylamine hydrochloride (0.56 g, 8 mmol) in glacial acetic acid (15 mL) was stirred at room temperature for 12-24 hr. After cooling the reaction mixture, the precipitate obtained was filtered, washed with water, dried and re-crystallised from acetic acid.

***2.1.2.1. 2-(4-(1-(Hydroxyimino)ethyl)phenyl)-3a,4,7,7a-tetrahydro-1H-isoindole-1,3(2H)-dione (10)***



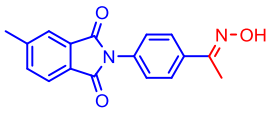
Yield, 98%; melting point (mp): 186-170 °C; IR (KBr, $cm^{-1}$) $\nu$: 3421 (OH), 1734, 1710 (C=O), 1654 (C=N), 1617, 1508 (C=C), 1171 (C-C), 927 (N-O); $^{1}$H NMR (700 MHz, DMSO-$d_6$): $\delta$ 11.32 (s, 1H), 7.75 (s, 2H), 7.23 (s, 2H), 5.95 (s, 2H), 3.31 (s, 2H), 2.48 (d, 2H, *J*= 14.0 Hz), 2.28 (d, 2H, *J*= 12.6 Hz), 2.17 (s, 3H); $^{13}$C NMR (176 MHz, DMSO-$d_6$): $\delta$ 11.99, 23.73, 39.31, 126.48, 127.18, 128.19, 129.07, 133.04, 137.33, 152.85, 179.66; $C_{16}H_{16}N_2O_3$: m/z (284).

***2.1.2.2. 2-(4-(1-(hydroxyimino)ethyl)phenyl)isoindoline-1,3-dione (11)***



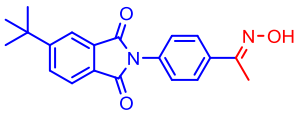
Yield, 94%; melting point (mp): 290-291 °C; IR (KBr, $cm^{-1}$) $\nu$: 3245 (OH), 1732, 1706 (C=O), 1685 (C=N), 1615, 1528 (C=C), 1268, 1100 (C-C), 819 (N-O); $^{1}$H NMR (700 MHz, DMSO-$d_6$): $\delta$ 11.34 (s, 1H), 7.96 (s, 2H), 7.91 (s, 2H), 7.80 (s, 2H), 7.48 (s, 2H), 2.21 (s, 3H); $^{13}$C NMR (176 MHz, DMSO-$d_6$): $\delta$ 12.03, 123.91, 126.44, 127.57, 129.23, 131.98, 132.54, 135.20, 135.32, 137.05, 152.94, 167.37; $C_{16}H_{12}N_2O_3$: m/z (280).

***2.1.2.3. 2-(4-(1-(Hydroxyimino)ethyl)phenyl)-5-methylisoindoline-1,3-dione (12)***



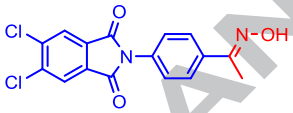
Yield, 91%; melting point (mp): 300-302 °C; IR (KBr, $cm^{-1}$) $\nu$: 3413 (OH), 1768, 1718 (C=O), 1518 (C=C), 1275, 1128 (C-C), 929 (N-O); $^{1}$H NMR (700 MHz, DMSO-$d_6$): $\delta$ 11.36 (s, 1H), 7.85 (d, 1H, *J*= 8.3 Hz), 7.79 (t, 3H, *J*= 8.8 Hz), 7.70 (d, 1H, *J*=7.6 Hz), 7.46 (d, 2H, *J*= 8.5 Hz), 2.52 (s, 3H), 2.20 (s, 3H); $^{13}$C NMR (176 MHz, DMSO-$d_6$): $\delta$ 12.04, 21.88, 123.85, 124.31, 126.39, 127.52, 129.35, 132.32, 132.58, 135.58, 136.91, 146.21, 152.91, 167.34, 167.44; $C_{17}H_{14}N_2O_3$: m/z (294).

***2.1.2.4. 5-(tert-Butyl)-2-(4-(1-(hydroxyimino)ethyl)phenyl)isoindoline-1,3-dione (13)***



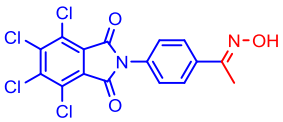
Yield, 86%; melting point (mp): 215-216 °C; IR (KBr, $cm^{-1}$) $\nu$: 3471 (OH), 1787, 1716 (C=O), 1678 (C=N), 1601, 1512 (C=C), 1268, 1122 (C-C), 829 (N-O); $^{1}$H NMR (700 MHz, DMSO-$d_6$): $\delta$ 11.34 (s, 1H), 7.94 (s, 2H), 7.89 (d, 1H, *J*= 7.7 Hz), 7.78 (d, 2H, *J*= 7.7 Hz), 7.45 (d, 2H, *J*= 7.0 Hz), 2.20 (s, 3H), 1.38 (s, 9H); $^{13}$C NMR (176 MHz, DMSO-$d_6$): $\delta$ 12.03, 31.25, 35.99, 120.71, 123.86, 126.43, 127.46, 127.56, 129.48, 132.08, 132.28, 132.64, 137.00, 152.94, 159.01, 167.22, 167.53; $C_{20}H_{20}N_2O_3$: m/z (336).

***2.1.2.5. 5,6-Dichloro-2-(4-(1-(hydroxyimino)ethyl)phenyl)isoindoline-1,3-dione (14)***



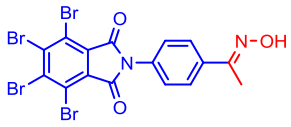
Yield, 94%; melting point (mp): 310-312 °C; IR (KBr, $cm^{-1}$) $\nu$: 3421 (OH), 1775, 1719 (C=O), 1654 (C=N), 1603, 1517 (C=C), 1219, 1103 (C-C), 829 (N-O); $^{1}$H NMR (700 MHz, DMSO-$d_6$): $\delta$ 11.34 (s, 1H), 8.30 (d, 2H, *J*= 14 Hz), 7.81 (s, 2H), 7.47 (s, 2H), 2.20 (s, 3H); $^{13}$C NMR (176 MHz, DMSO-$d_6$): $\delta$ 12.03, 126.03, 126.51, 127.47, 132.06, 132.22, 137.29, 138.01, 152.91, 165.63; $C_{16}H_{10}Cl_2N_2O_3$: m/z (349).

***2.1.2.6. 4,5,6,7-Tetrachloro-2-(4-(1-(hydroxyimino)ethyl)phenyl)isoindoline-1,3-dione (15)***



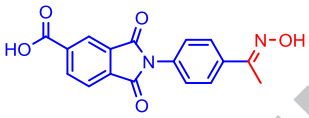
Yield, 96%; melting point (mp): 316-318 °C; IR (KBr, $cm^{-1}$) $\nu$: 3411 (OH), 1734, 1700 (C=O), 1654 (C=N), 1606, 1508 (C=C), 1297, 1099 (C-C), 824 (N-O); $^{1}$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 11.58 (s, 1H), 8.01 (d, 2H, *J*= 8.5 Hz), 7.65 (d, 2H, *J*= 8.0 Hz), 2.41 (s, 3H); $^{13}$C NMR (125 MHz, DMSO-$d_6$): $\delta$ 12.27, 121.50, 126.77, 128.01, 131.77, 137.29, 163.60; $C_{16}H_8Cl_4N_2O_3$: m/z (418).

***2.1.2.7. 4,5,6,7-Tetrabromo-2-(4-(1-(hydroxyimino)ethyl)phenyl)isoindoline-1,3-dione (16)***



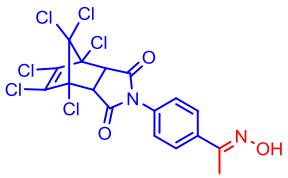
Yield, 95%; melting point (mp): 322-324 °C; $^{1}$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 11.41 (s, 1H), 7.81 (d, 2H, *J*= 8.5 Hz), 7.45 (d, 2H, *J*= 8.0 Hz), 2.20 (s, 3H); $^{13}$C NMR (125 MHz, DMSO-$d_6$): $\delta$ 11.99, 126.58, 127.67, 128.76, 128.82, 131.79, 137.62, 138.84, 152.82, 162.99, 172.49; $C_{16}H_8Br_4N_2O_3$: m/z (595).

***2.1.2.8. 2-(4-(1-(Hydroxyimino)ethyl)phenyl)-1,3-dioxoisoindoline-5-carboxylic acid (17)***



Yield, 83%; melting point (mp): 295-296 °C; IR (KBr, cm$^{-1}$) $\nu$: 3447 (OH), 1783, 1727 (C=O), 1654 (C=N), 1592 (C=C), 1209, 1128 (C-C), 742 (N-O); $^{1}$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 11.36 (s, 1H), 8.43-8.41 (m, 1H), 8.31 (d, 1H, *J*= 4.5 Hz), 8.13-8.07 (m, 1H), 7.81 (d, 2H, *J*= 8.5 Hz), 7.49 (d, 2H, *J*= 8.5 Hz), 2.21 (s, 3H); $^{13}$C NMR (125 MHz, DMSO-$d_6$): $\delta$ 12.04, 123.87, 123.96, 124.30, 126.47, 127.53, 129.28, 132.36, 132.49, 135.37, 135.97, 136.94, 137.19, 152.92, 166.28, 166.66; $C_{17}H_{12}N_2O_5$: m/z (324).

***2.1.2.9. 4,5,6,7,8,8-Hexachloro-2-(4-(-1-(hydroxyimino)ethyl)phenyl)-3a,4,7,7a-tetrahydro-1H-4,7-methanoisoindole-1,3(2H)-dione (18)***

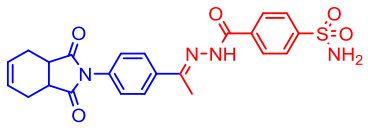


Yield, 91%; melting point (mp): 265-257 °C; 1H NMR (500 MHz, $CDCl_3$/DMSO-$d_6$): $\delta$ 11.14 (s, 1H), 7.71 (d, 2H, *J*= 9.0 Hz), 7.09 (d, 2H, *J*= 8.5 Hz), 4.12 (s, 2H), 2.16 (s, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$/DMSO-$d_6$): $\delta$ 12.76, 52.53, 79.41, 104.18, 126.58, 126.60, 130.96, 131.26, 152.42, 169.67; $C_{17}H_{10}Cl_6N_2O_3$: m/z (502).

**2.1.3. General procedure for the synthesis of hydrazone based isoindoline-1,3-diones 20-28 (Scheme 2).**

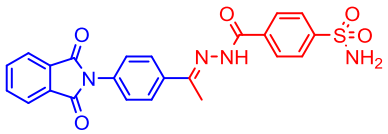
A mixture of an appropriate isoindoline-1,3-diones **1-9** (5.0 mmol), and 4-(hydrazinecarbonyl)benzenesulfonamide (**19**) (1.08 g, 5.0 mmol) in methanol (15 mL) and glacial acetic acid (0.5 mL) was heated under reflux for 6 hr. After cooling the reaction mixture, the precipitate obtained was filtered, dried and re-crystallised from methanol.

***2.1.3.1. 4-(2-(1-(4-(1,3-Dioxo-1,3,3a,4,7,7a-hexahydro-2H-isoindol-2-yl)phenyl)ethylidene)hydrazine-1-carbonyl)benzenesulfonamide (20)***



Yield, 90%; melting point (mp): 288-290 °C; IR (KBr, $cm^{-1}$) $\nu$: 3369, 3275 (NH), 1785, 1725 (C=O), 1655 (C=N), 1593, 1534 (C=C), 1096 (C-C) 1376, 1259 (O=S=O); $^{1}$H NMR (700 MHz, DMSO-$d_6$): $\delta$ 11.19 (s, 0.22H), 11.00 (s, 0.78 H), 8.07 (d, 2H, *J*= 7.7 Hz), 7.98-7.95 (m, 4H), 7.56 (s, 1.5 H), 7.50 (s, 0.5 H), 7.31 (2, 2H, *J*= 7.7 Hz), 5.97 (s, 2H), 3.33 (s, 2H), 2.48 (s, 2H), 2.42 (s, 2.25H), 2.36 (s, 0.75H), 2.29 (d, 2H, *J*= 12.6 Hz); $^{13}$C NMR (176 MHz, DMSO-$d_6$): δ 15.22, 23.74, 39.36, 125.42, 126.05, 127.18, 127.34, 127.47, 128.25, 129.13, 130.42, 133.88, 137.41, 138.24, 146.97, 155.54, 163.61, 179.68; $C_{23}H_{22}N_4O_5S$: m/z (466).

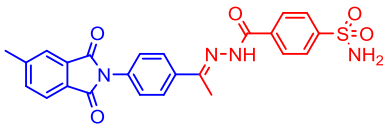
***2.1.3.2. 4-(2-(1-(4-(1,3-Dioxoisoindolin-2-yl)phenyl)ethylidene)hydrazine-1-carbonyl)benzenesulfonamide (21)***



Yield, 91%; melting point (mp): 350-352 °C; IR (KBr, $cm^{-1}$) $\nu$: 3513, 3424, 3137 (NH), 1784, 1725 (C=O), 1653 (C=N), 1550 (C=C), 1103 (C-C) 1360, 1266 (O=S=O); $^{1}$H NMR (700 MHz, DMSO-$d_6$): $\delta$ 11.19 (s, 0.25H), 11.09 (s, 0.75H), 8.11 (d, 1.5H, *J*= 7.7 Hz), 8.07 (d, 1.5H, *J*= 7.0 Hz), 8.03 (d, 1.5H, *J*= 7.7 Hz), 7.97 (d, 2H, *J*= 7.0 Hz), 7.93-7.92 (t, 3H, *J*= 4.2 & 3.5 Hz), 7.65 (d, 1.5H, *J*= 7.7 Hz), 7.57 (s, 3H), 2.45 (s, 2.4 H), 2.40 (s, 0.6 H); $^{13}$C NMR (176 MHz, DMSO-$d_6$): $\delta$ 15.24, 123.95, 124.03,

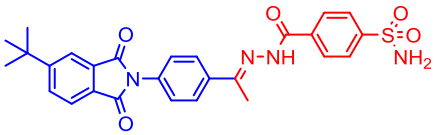
126.06, 127.42, 127.53, 129.25, 131.96, 132.00, 135.25, 135.33, 136.26, 136.48, 146.96, 155.73, 163.60, 167.13, 167.36; $C_{23}H_{18}N_4O_5S$: m/z (462).

***2.1.3.3. 4-(2-(1-(4-(5-Methyl-1,3-dioxoisoindolin-2-yl)phenyl)ethylidene)hydrazine-1-carbonyl)benzenesulfonamide (22)***



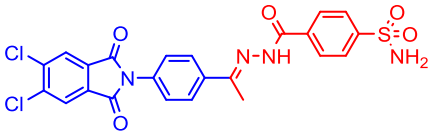
Yield, 89%; melting point (mp): 312-313 °C; IR (KBr, $cm^{-1}$) *ν*: 1730, 1717 (C=O), 1640 (C=N), 1598 (C=C), 1114 (C-C) 1354, 1197 (O=S=O); $^1$H NMR (700 MHz, DMSO-$d_6$): *δ* 11.17 (s, 0.25H), 11.01 (s, 0.75 H), 8.10 (d, 0.5H, *J*= 12.6 Hz), 8.08 (d, 1.5H, *J*= 9.8 Hz), 8.02 (d, 1.5H, *J*= 10.5 Hz), 7.96 (d, 2.5H, *J*= 11.2 Hz), 7.86 (q, 1H, *J*= 7.5, 4.0 Hz), 7.80 (s, 1H), 7.72 (d, 1.5H, *J*= 10.5 Hz), 7.63 (d, 0.5H, *J*= 11.9 Hz), 7.55 (s, 3H), 2.52 (s, 3H),2.44 (s, 3H); $^{13}$C NMR (176 MHz, DMSO-$d_6$): *δ* 15.24, 21.90, 123.88, 123.96, 124.33, 124.41, 126.06, 127.42, 129.14, 129.22, 129.34, 132.31, 135.62, 146.26, 146.37, 146.96, 155.71, 167.30, 167.40; $C_{24}H_{20}N_4O_5S$: m/z (476).

***2.1.3.4. 4-(2-(1-(4-(5-tert-Butyl-1,3-dioxoisoindolin-2-yl)phenyl)ethylidene)hydrazine-1-carbonyl)benzenesulfonamide (23)***



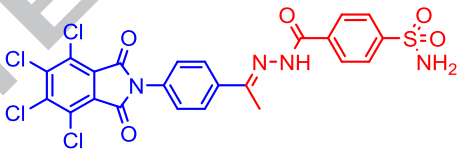
Yield, 86%; melting point (mp): 263-265 °C; IR (KBr, $cm^{-1}$) *ν*: 3260 (NH), 1729, 1708 (C=O), 1599, 1503 (C=C), 1088 (C-C) 1380, 1270 (O=S=O); $^1$H NMR (700 MHz, DMSO-$d_6$): *δ* 11.17 (s, 0.25H), 11.01 (s, 0.75H), 8.12-7.90 (m, 9H), 7.63-7.44 (m, 4H), 2.44 (s, 3H), 1.38 (s, 9H); $^{13}$C NMR (176 MHz, DMSO-$d_6$): *δ* 15.26, 31.26, 36.02, 167.50, 120.77, 123.89, 126.06, 127.41, 127.51, 129.13, 129.24, 129.49, 132.11, 132.28, 137.44, 137.86, 146.96, 155.74, 159.00, 163.58, 167.19; $C_{27}H_{26}N_4O_5S$: m/z (518).

***2.1.3.5. 4-(2-(1-(4-(5,6-Dichloro-1,3-dioxoisoindolin-2-yl)phenyl)ethylidene)hydrazine-1-carbonyl)benzenesulfonamide (24)***



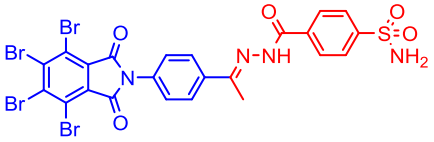
Yield, 92%; melting point (mp): 361-362 °C; IR (KBr, $cm^{-1}$) $\nu$: 1725, 1713 (C=O), 1639 (C=N), 1507 (C=C), 1162 (C-C) 1366, 1207 (O=S=O); $^{1}$H NMR (700 MHz, DMSO-$d_6$): $\delta$ 11.84 (s, 0.2H), 11.01 (s, 0.8H), 8.32 (d, 2H, *J*= 10.5 Hz),), 8.12 (d, 0.5H *J*= 11.9 Hz), 8.07 (d, 1.5H, *J*= 10.5 Hz), 8.03 (d, 1.5H, *J*= 11.2 Hz), 7.96 (d, 2H, *J*= 10.5Hz), 7.62 (d, 0.5H, *J*= 11.9 Hz), 7.55-7.45 (m, 4H), 2.47 (s, 3H); $^{13}$C NMR (176 MHz, DMSO-$d_6$): $\delta$ 15.23, 126.05, 126.13, 127.39, 127.49, 129.13, 129.33, 132.01, 132.06, 136.12, 136.48, 138.02, 146.97, 155.57, 163.59, 165.38, 165.59; $C_{23}H_{16}Cl_2N_4O_5S$: m/z (531).

***2.1.3.6. 4-(2-(1-(4-(4,5,6,7-Tetrachloro-1,3-dioxoisoindolin-2-yl)phenyl)ethylidene)hydrazine-1-carbonyl)benzenesulfonamide (25)***



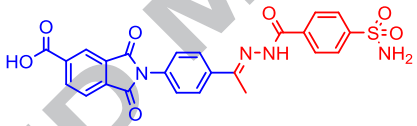
Yield, 94%; melting point (mp): 351-353 °C; $^{1}$H NMR (700 MHz, DMSO-$d_6$): $\delta$ 11.19 (s, 0.2H), 11.02 (s, 0.8H), 8.05 (d, 3H, *J*= 10.5 Hz), 7.95 (d, 3H, *J*= 10.5 Hz), 7.55-7.53 (m, 4H), 2.45 (s, 3H); $^{13}$C NMR (176 MHz, DMSO-$d_6$): $\delta$ 15.21, 125.46, 126.05, 127.16, 127.70, 127.61, 128.79, 128.82, 128.89, 129.13, 129.43, 130.44, 132.66, 137.34, 138.53, 138.86, 146.97, 155.47, 162.99, 163.64; $C_{23}H_{14}Cl_4N_4O_5S$: m/z (600).

***2.1.3.7. 4-(2-(1-(4-(4,5,6,7-Tetrabromo-1,3-dioxoisoindolin-2-yl)phenyl)ethylidene)hydrazine-1-carbonyl)benzenesulfonamide (26)***



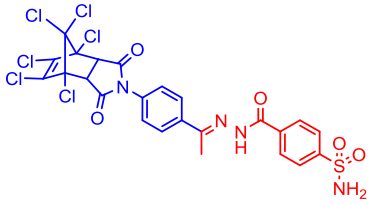
Yield, 93%; melting point (mp): 344-345 °C; IR (KBr, $cm^{-1}$) $\nu$: 3260 (NH), 1728, 1708 (C=O), 1637 (C=N), 1548 (C=C), 1090 (C-C) 1327, 1163 (O=S=O); $^{1}$H NMR (700 MHz, DMSO-$d_6$): $\delta$ 11.20 (s, 0.25H), 11.03 (s, 0.75H), 8.13 (d, 0.5H, $J$= 8.4 Hz), 8.07-8.03 (m, 3H), 7.96-7.89 (m, 3H), 7.60 (d, 0.5H, $J$= 8.4 Hz), 7.56-7.52 (m, 3H), 2.45 (s, 3H); $^{13}$C NMR (176 MHz, DMSO-$d_6$): $\delta$ 15.23, 121.28, 121.36, 126.05, 126.15, 127.51, 127.71, 127.81, 129.15, 129.35, 131.53, 133.00, 137.09, 138.45, 146.99, 155.50, 163.13, 163.35, 163.63; $C_{23}H_{14}Br_4N_4O_5S$: m/z (778).

***2.1.3.8. 1,3-Dioxo-2-(4-(1-(2-(4-sulfamoylbenzoyl)hydrazono)ethyl)phenyl)isoindoline-5-carboxylic acid (27)***



Yield, 84%; melting point (mp): 358-359 °C; IR (KBr, $cm^{-1}$) $\nu$: 2987 (br. OH), 1731, 1711 (C=O), 1640 (C=N), 1547 (C=C), 1097 (C-C) 1388, 1286 (O=S=O); $^{1}$H NMR (700 MHz, DMSO-$d_6$): $\delta$ 13.46 (s, 1H), 11.19 (s, 0.2 H), 11.03 (s, 0.8 H), 8.43 (s, 1H), 8.32 (s, 1H), 8.09 (s, 3H), 8.04 (s, 1H), 7.96 (s, 3H), 7.57 (s, 4H), 2.45 (s, 3H); $^{13}$C NMR (176 MHz, DMSO-$d_6$): $\delta$ 15.24, 123.89, 124.32, 126.06, 127.45, 129.14, 132.50,133.26, 135.35, 136.00, 136.97, 137.43, 138.08, 146.96, 155.64, 163.61, 166.30, 166.62, 166.63; $C_{24}H_{18}N_4O_7S$: m/z (506).

***2.1.3.9. 4-(2-(-1-(4-(-4,5,6,7,8,8-hexachloro-1,3-dioxo-1,3,3a,4,7,7a-hexahydro-2H-4,7-methanoisoindol-2-yl)phenyl)ethylidene)hydrazine-1-carbonyl)benzenesulfonamide (28)***



Yield, 91%; melting point (mp): 338-340 °C; $^{1}$H NMR (700 MHz, DMSO-$d_6$): $\delta$ 11.19 (s, 0.25H), 11.03 (s, 0.75H), 8.07 (d, 1.5H, *J*= 7.0 Hz), 8.03 (d, 1.5H, *J*= 7.0 Hz), 7.96 (d, 2.5H, *J*= 7.7 Hz), 7.75 (s, 0.5H), 7.55 (s, 2H), 7.22 (d, 1.5H, *J*= 7.0 Hz), 7.11 (s, 0.5H), 4.27 (s, 2H), 2.42 (s, 3H); $^{13}$C NMR (176 MHz, DMSO-$d_6$): $\delta$ 15.23, 52.61, 79.44, 104.20, 126.05, 126.96, 127.99, 129.16, 131.10, 132.29, 137.36, 139.31, 146.99, 155.19, 163.66, 170.06, 172.53; $C_{24}H_{16}Cl_6N_4O_5S$: m/z (685).

### 4.2 Carbonic anhydrase inhibition

An Applied Photophysics stopped-flow instrument has been used for assaying the CA catalyzed $CO_2$ hydration activity.[37] Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 20 mMHepes (pH 7.5) as buffer, and 20 mM $Na_2SO_4$ (for maintaining constant the ionic strength), following the initial rates of the CA-catalyzed $CO_2$ hydration reaction for a period of 10–100 s. The $CO_2$ concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and inhibition constants. For each inhibitor at least six traces of the initial 5–10% of the reaction have been used for determining the initial velocity. The uncatalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of 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 obtained by non-linear least-squares methods using PRISM 3 and the Cheng–Prusoff equation, as reported earlier,[39] and represent the mean from at least three different determinations. All CA isoforms were recombinant ones obtained in-house as reported earlier.[40,41]

### Acknowledgments

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**References**

[1] C.T. Supuran, Carbonic anhydrases: novel therapeutic applications for inhibitors and activators. Nat. Rev. Drug Discovery 7 (2008) 168-181.

[2] (a) V. Alterio, A. Di 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; (b) S. Del Prete, D. Vullo, G.M. Fisher, K.T. Andrews, S. Poulsen, C. Capasso, C.T. Supuran. Discovery of a new family of carbonic anhydrases in the malaria pathogen Plasmodium falciparum. The η-carbonic anhydrases. Bioorg. Med. Chem. Lett. 18 (2014) 4389- 4396.

[3] S. Kikutani, K. Nakajima, C. Nagasato, Y. Tsuji, A. Miyatake, Y. Matsuda. Thylakoid luminal theta-carbonic anhydrase critical for growth and photosynthesis in the marine diatom phaeodactylum tricornutum. Proc. Natl. Acad. Sci. 113 (2016) 9828-9833.

[4] Neri, C.T. Supuran, Interfering with pH regulation in tumours as a therapeutic strategy. Nat. Rev. Dr ug Discov. 10 (2011) 767-777; (b) C.T. Supuran, Carbonic Anhydrase Inhibition and the Management o f Hypoxic Tumors. Metabolites. 7 (2017) E48.

[5] G. De Simone, C.T. Supuran, Carbonic anhydrase IX: biochemical and crystallographic characterizat ion of a novel antitumor target. Biochim. Biophys. Acta 1804 (2010) 404-409.

[6] J. Pastorek, S. Pastorekova, Hypoxia-induced carbonic anhydrase IX as a target for cancer therapy: F rom biology to clinical use. Semin. Cancer Biol. 31 (2015) 52−64;

[7] S.M. Monti, C.T. Supuran, G. De Simone, Anticancer carbonic anhydrase inhibitors: a patent review (2008 - 2013). Expert Opin. Ther. Pat. 23 (2013) 737-749.

[8] C.T. Supuran, How many carbonic anhydrase inhibition mechanisms exist? J. Enzyme Inhib. Med. C hem. 31 (2016) 345-360.

[9] A. Scozzafava, L. Menabuoni, F. Mincione, F. Briganti, G. Mincione, C.T. Supuran, Carbonic anhy drase inhibitors. Synthesis of water-soluble, topically effective, intraocular pressure-lowering aromatic/h eterocyclic sulfonamides containing cationic or anionic moieties: Is the tail more important than the ring ?, J. Med. Chem. 42 (1999) 2641−2650.

[10] A. Nocentini, M. Ferraroni, F. Carta, M. Ceruso, P. Gratteri, C. Lanzi, E. Masini, C.T. Supuran, Benzenesulfonamides Incorporating Flexible Triazole Moieties Are Highly Effective Carbonic Anhydrase Inhibitors: Synthesis and Kinetic, Crystallographic, Computational, and Intraocular Pressure Lowering Investigations. J. Med. Chem. 59 (2016) 10692-10704.

[11] A. Nocentini, S. Bua, C.L. Lomelino, R. McKenna, M. Menicatti, G. Bartolucci, B. Tenci B, L. Di Cesare Mannelli, C. Ghelardini C, P. Gratteri, C.T. Supuran. Discovery of New Sulfonamide Carbonic Anhydrase IX Inhibitors Incorporating Nitrogenous Bases. ACS Med. Chem. Lett. 8 (2017), 1314-1319.

[12] A. Nocentini, D. Vullo, S. Del Prete, S.M. Osman, F. Alasmary, Z. AlOthman, C. Capasso, F. Carta F, P. Gratteri, C.T. Supuran CT. Inhibition of the β-carbonic anhydrase from the dandruff-producing fungus Malassezia globosa with monothiocarbamates. J. Enzyme Inhib. Med. Chem. 32 (2017) 1064-1070.

[13] D. Vullo, S. Del Prete, A. Nocentini, S.M. Osman, Z. AlOthman, C. Capasso, M. Bozdag, F. Carta, P. Gratteri, C. T. Supuran. Dithiocarbamates effectively inhibit the β-carbonic anhydrase from the dandruff-producing fungus Malassezia globosa. Bioorg. Med. Chem. 25 (2017) 1260-1265.

[14] A. Nocentini, D. Vullo, S. Del Prete, S.M. Osman, F. Alasmary, Z. AlOthman, C. Capasso, F. Carta F, P. Gratteri, C.T. Supuran CT. Inhibition of the β-carbonic anhydrase from the dandruff-producing fungus Malassezia globosa with monothiocarbamates. J. Enzyme Inhib. Med. Chem. 32 (2017) 1064-1070.

[15] A. Nocentini, R. Cadoni, S. Del Prete, C. Capasso, P. Dumy, P. Gratteri, C.T. Supuran, J.Y. Winum. Benzoxaboroles as Efficient Inhibitors of the β-Carbonic Anhydrases from Pathogenic Fungi: Activity and Modeling Study. ACS Med. Chem. Lett. 8 (2017) 1194-1198.

[16] F. Carta, M. Aggarwal, A. Maresca, A. Scozzafava, R. McKenna, C.T. Supuran, Dithiocarbamates: a new class of carbonic anhydrase inhibitors. Crystallographic and kinetic investigations. Chem. Commun. (Camb) 48 (2012) 1868-1870.

[17] V. Alterio, R. Cadoni, D. Esposito, D. Vullo, A. Di Fiore, S.M. Monti, A. Caporale, M. Ruvo, M. Sechi, P. Dumy, C.T. Supuran, G. De Simone, J.Y. Winum. Benzoxaborole as a new chemotype for carbonic anhydrase inhibition. Chem Commun (Camb) 5 (2016) 11983-11986.

[18] W.M. Eldehna, M.F. Abo-Ashour, A. Nocentini, P. Gratteri, I.H. Eissa, M. Fares, O.E. Ismael, H.A. Ghabbour, M.M. Elaasser, H.A. Abdel-Aziz, C.T. Supuran, Novel 4/3-((4-oxo-5-(2-oxoindolin-3-ylidene)thiazolidin-2-ylidene)amino) benzenesulfonamides: Synthesis, carbonic anhydrase inhibitory activity, anticancer activity and molecular modelling studies. Eur. J. Med. Chem. 139 (2017), 250-262.

[19] A.A.-M Abdel-Aziz, K.E.H. ElTahir, Y.A. Asiri, Synthesis, anti-inflammatory activity and COX-1/COX-2 inhibition of novel substituted cyclic imides. Part 1: Molecular docking study. Eur. J. Med. Chem. 46 (2011) 1648-1655.

[20] A.A.-M. Abdel-Aziz, Novel and versatile methodology for synthesis of cyclic imides and evaluation of their cytotoxic, DNA binding, apoptotic inducing activities and molecular modeling study Eur. J. Med. Chem. 42 (2007) 612-626.

[21] M. A.Mohamed, A.A.-M. Abdel-Aziz, H. M. Sakr, A. S. El-Azab, S. Bua, C.T. Supuran, Synthesis and human/bacterial carbonic anhydrase inhibition with a series of sulfonamides incorporating phthalimido moieties, Bioorg. Med. Chem. 25 (2017) 2524–2529.

[22] A. A.-M. Abdel-Aziz, A. Angeli, A. S. El-Azab, M. A. Abu El-Enin, C. T. Supuran, Synthesis and biological evaluation of cyclic imides incorporating benzenesulfonamide moieties as carbonic anhydrase I, II, IV and IX inhibitors, Bioorg. Med. Chem. 25 (2017) 1666–1671.

[23] A. S. El-Azab, A. A.-M. Abdel-Aziz, R. R. Ayyad, M. Ceruso, C.T. Supuran, Inhibition of carbonic anhydrase isoforms I, II, IV, VII and XII with carboxylates and sulfonamides incorporating phthalimidephthalic anhydride scaffolds, Bioorg. Med. Chem. 24 (2016) 20–25.

[24] A.M. Alanazi, A. S. El-Azab, I. A. Al-Suwaidan, K. E. H. ElTahir, Y. A. Asiri, N. I. Abdel-Aziz, A. A.-M. Abdel-Aziz, Structure-based design of phthalimide derivatives as potential cyclooxygenase-2 (COX-2) inhibitors: Anti-inflammatory and analgesic activities, Eur. J. Med. Chem 92 (2015) 115-123.

[25] A. A.-M. Abdel-Aziz, A. S. El-Azab, M. Ceruso, C.T. Supuran, Carbonic anhydrase inhibitory activity of sulfonamides and carboxylic acids incorporating cyclic imide scaffolds, Bioorg. Med. Chem. Lett. 24 (2014) 5185–5189.

[26] I. A. Al-Suwaidan, A. M. Alanazi, A. S. El-Azab, A. A.-M Abdel-Aziz, Molecular design, synthesis and biological evaluation of cyclic imides bearing benzenesulfonamide fragment as potential COX-2 inhibitors, Bioorg. Med. Chem. Lett. 23 (2013) 2601–2605.

[27] A. S. El-Azab, A. M. Alanazi, N. I. Abdel-Aziz, I. A. Al-Suwaidan, M. A. A. El-Sayed, M. A. ElSherbeny, A. A.-M. Abdel-Aziz, Synthesis, molecular modeling study, preliminary antibacterial,and antitumore valuation of N-substituted naphthalimides and their structural analogues, Med. Chem. Res. 22 (2013) 2360-2375.

[28] A. A.-M. Abdel-Aziz, A. S. El-Azab, S. M. Attia, A. M. Al-Obaid, H. I. Al-Omar El-Subbagh, Synthesis and biological evaluation of some novel cyclic-imides as hypoglycaemic, anti-hyperlipidemic agents. European Journal of Medicinal Chemistry 46 (2011), 4324-4329.

[29] A. S. El-Azab, S. G. Abdel-Hamide, M. M. Sayed-Ahmed, G. S. Hassan, T. M. El-Hadiyah, O. A. Al-Shabanah, O. A. Al-Deeb, H. I. El-Subbagh, Novel 4(3H)-quinazolinone analogs: synthesis and anticonvulsant activity. Med. Chem. Res, 22 (2013) 2815–2827.

[30] F. Carta , D. Vullo, A. Maresca, A. Scozzafava A, C.T. Supuran. New chemotypes acting as isozyme-selective carbonic anhydrase inhibitors with low affinity for the offtarget cytosolic isoform II. Bioorg. Med. Chem. Lett. 22 (2012) 2182-2185.

[31] T. Tuccinardi, S. Bertini, C. Granchi, G. Ortore, M. Macchia, F. Minutolo, A. Martinelli, C.T. Supuran, Salicylaldoxime derivatives as new leads for the development of carbonic anhydrase inhibitors. Bioorg Med Chem. 21 (2013) 1511-1515.

[32] 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.

[33] ClinicalTrials.gov https://clinicaltrials.gov/ct2/ show/study/NCT02215850?term=SLC_0111&rank =1

[34] A. Nocentini, D. Moi, G. Balboni, V. Onnis, C.T. Supuran, Discovery of thiazolin-4-one-based aro matic sulfamates as a new class of carbonic anhydrase isoforms I, II, IV, and IX inhibitors. Bioorg. Che m. 77 (2018) 293-299.

[35] Y. Lu, Y. Liu, Z. Xu, H. Li, H. Liu, W. Zhu. Halogen bonding for rational drug design and new drug discovery. Expert Opin. Drug Discov. 7 (2012) 375-383.

[36] M.Z. Hernandes, S.M. Cavalcanti, D.R. Moreira, W.F. de Azevedo J, A.C. Leite. Halogen atoms in the modern medicinal chemistry: hints for the drug design. Curr. Drug Targets. 11 (2010) 303-314.

[37] R.G. Khalifah, The carbon dioxide hydration activity of carbonic anhydrase. J. Biol. Chem. 246 (19 71) 2561−2573.

[38] 1. Phoebe F. Lamie, John N. Philoppes, Ahmed O. El-Gendy, Lucie Rarova, Jiri Gruz Design, Synt hesis and Evaluation of Novel Phthalimide Derivatives as in Vitro Anti-Microbial, Anti-Oxidant and An ti-Inflammatory Agents Molecules. 20 (2015) 16620-16642.

[39] A. Nocentini, S. Bua, S. Del Prete, Y.E. Heravi, A.A. Saboury, A. Karioti, A.R. Bilia, C. Capasso, P. Gratteri, C.T. Supuran. Natural Polyphenols Selectively Inhibit β-Carbonic Anhydrase from the Dand ruff-Producing Fungus Malassezia globosa: Activity and Modeling Studies, Chem. Med. Chem. 2018. d oi: 10.1002/cmdc.201800015.

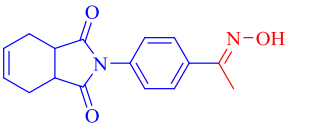
[40] S.U. Mahmood, A. Saeed, S. Bua, A. Nocentini, P. Gratteri, C.T. Supuran, Synthesis, biological eva luation and computational studies of novel iminothiazolidinone benzenesulfonamides as potent carbonic anhydrase II and IX inhibitors. Bioorg Chem. 77 (2018) 381-386.

[41] J. Leitans, A. Kazaks, A. Balode, J. Ivanova, R. Zalubovskis, C.T. Supuran, K. Tars. Efficient Expr ession and Crystallization System of Cancer-Associated Carbonic Anhydrase Isoform IX. J. Med. Chem. 58 (2015) 9004-9009.

Highlights

- A new chemotype for CA inhibition was identified: isoindoline-1,3-dione-based oximes.
- Isoindoline-1,3-dione benzenesulfonamides were also evaluated as CAIs.
- Remarkable CA IX selectivity was found within both series
- Promising anti-cancer leads/tools were reported.

## Graphical abstract



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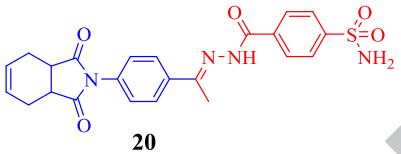

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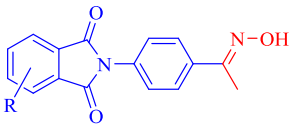

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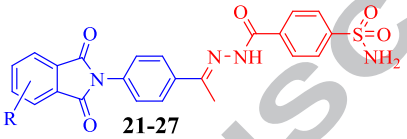

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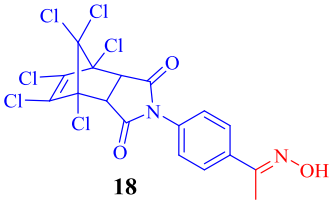
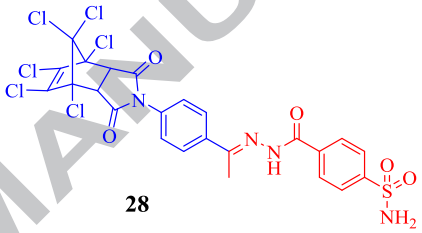


**18**

$K_I$ (hCA I) = 9667->100000 nM
$K_I$ (hCA II) = 5582-97840 nM
$K_I$ (hCA IV) = 3360-46530 nM
$K_I$ (hCA IX) = 240.1-19900 nM



**28**

$K_I$ (hCA I) = 5551-63000 nM
$K_I$ (hCA II) = 369.1-834.9 nM
$K_I$ (hCA IV) = 406.1-24410 nM
$K_I$ (hCA IX) = 17.3-164.3 nM