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Synthesis and evaluation of ^{18}F-labeled tertiary benzenesulfonamides for imaging carbonic anhydrase IX expression in tumours with positron emission tomography

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ABSTRACT

Three tertiary benzenesulfonamide inhibitors **4a–c** were radiolabeled with ^{18}F and evaluated for imaging carbonic anhydrase IX (CA IX) expression with positron emission tomography. All three inhibitors exhibit <10 nM affinity for CA IX with no measurable affinity for CA II. Despite good affinity/selectivity to CA IX and excellent stability in plasma, uptake of [^{18}F]**4a–c** in CA IX-expressing HT-29 tumours was low without significant contrast. [^{18}F]**4a**,**b** were excreted rapidly, while [^{18}F]**4c** exhibited significant in vivo defluorination leading to high bone uptake. Due to minimal uptake in HT-29 tumours compared to normal organs/tissues, ^{18}F-labeled benzenesulfonamides [^{18}F]**4a–c** are not suitable as CA IX imaging agents.

Carbonic anhydrases (CAs) are a class of zinc metalloenzymes found in most living organisms.[1] Most CAs are efficient catalysts for the reversible hydration of carbon dioxide to bicarbonate ion and proton ($H_2O + CO_2 \leftrightarrow HCO_3^- + H^+$).[2,3] To date, 15 human CA isoenzymes have been identified,[1] with expression of CA IX being strongly associated with cancer progression. CA IX is not expressed in normal tissues except in the gastrointestinal mucosa,[4] but is highly expressed in malignancies such as gliomas, cervical, bladder, ovary, head and neck, lung, breast, and colon cancers.[5–14] Under hypoxic conditions CA IX is up-regulated by HIF1α, and transports HCO_3^- into the cell to maintain pH homeostasis.[15] The remaining extracellular H^+ acidifies the tumour microenvironment, activates metalloproteinases, induces angiogenesis, and facilitates invasion and metastasis.[16–19] Clinically, the expression of CA IX is associated with resistance to chemo- and radiation therapy,[20–22] increased recurrence and reduced survival.[23,24] As CA IX is expressed on the extracellular surface, it is an attractive and accessible imaging biomarker for hypoxic tumours.

Since its development in 1986,[25] anti-CA IX monoclonal antibody (mAb) cG250 have been labeled with different radioisotopes including ^{131}I, ^{111}In, ^{124}I, and ^{89}Zr to annotate CA IX expression in tumours.[26–29] In imaging clinical trials, cG250 demonstrated great sensitivity and accuracy for the diagnosis of clear cell renal cell carcinoma—a cancer subtype that commonly exhibits high constitutive expression of CA IX due to a mutation in the von Hippel–Lindau tumour suppressor gene.[30] However, imaging hypoxia-associated CA IX expression with mAbs is less likely to succeed. Hypoxic niches within tumours are less accessible for mAbs due to low perfusion rates caused by aberrant vasculature and increased interstitial pressure. Furthermore, Dubois et al. reported that anti-CA IX mAbs could not distinguish active CA IX in hypoxic cells from inactive CA IX in aerobic cells.[31] mAb binding was observed after hypoxic cells were re-oxygenated.[31] In contrast, the binding of sulfonamide inhibitors to CA IX occurred only under hypoxia exposure, and co-localized with the binding of anti-CA IX mAbs.[31] These findings suggest that CA IX-targeting probes derived from sulfonamide inhibitors will be more suitable for discriminating between aerobic and hypoxic regions.

Although several attempts have been made to develop radiolabeled small CA IX sulfonamide inhibitors for imaging, so far, none of them has been reported to successfully visualize CA IX-expressing tumours in preclinical/clinical settings. Apte et al.[32] reported the preparation of an ^{18}F-labeled sulfonamide derivative (Fig. 1A), but no biological evaluation data were presented. Akurathi et al.[33,34] reported the synthesis of ^{99m}Tc-labeled sulfonamides

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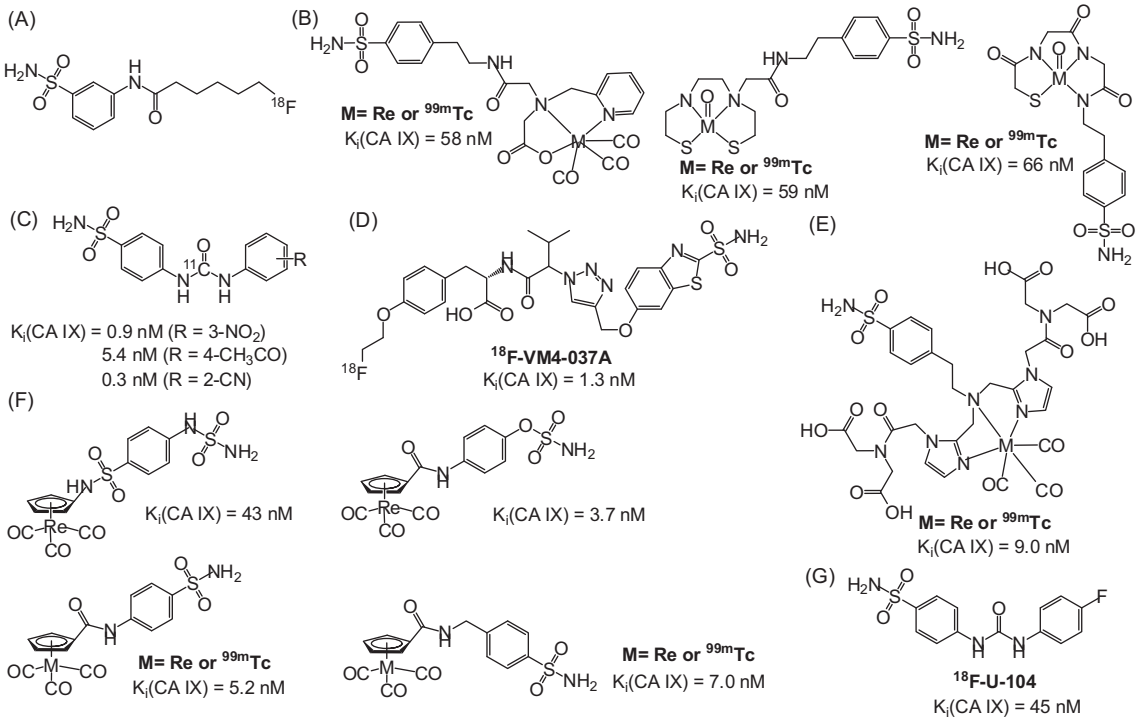
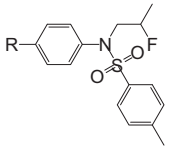


Figure 1. Structures of reported CA IX radiotracers derived from sulfonamide-based small molecule inhibitors.

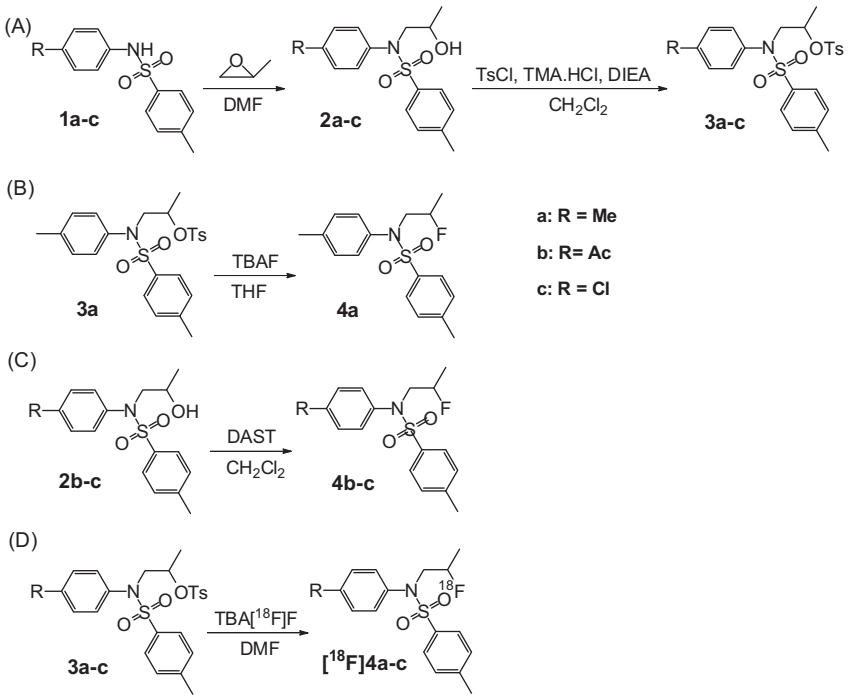
(Fig. 1B), but minimal uptake (up to 0.5%ID/g at 0.5 h post-injection (pi)) of these tracers was obtained in HT-29 tumour xenografts. Asakawa et al.[35] reported C-11 labeling of three potent benzenesulfonamides (Fig. 1C) for CA IX imaging, but no biological evaluation data were presented either. Doss et al. reported[37] that in a clinical study VM4-037 (Fig. 1D), a potent ^{18}F-labeled CA IX inhibitor developed by Siemens,[36] showed intense uptake in kidneys and liver, which reduces lesion visibility in these two organs. Lu et al.[38] recently reported the preparation of several Re/^{99m}Tc-labeled benzenesulfonamides. Among them, the ^{99m}Tc-labeled compound shown in Figure 1E exhibited good affinity (IC_{50} = 9 nM) to CA IX-expressing HeLa cells, but no biological evaluation was presented. Recently, Can et al.[39] reported four sulfonamides labeled with Re/^{99m}Tc via a cyclopentadienyl group (Fig. 1F). These compounds showed good affinity to CA IX, but no biological evaluation was presented. Previously we reported the preparation of an ^{18}F-labeled CA IX inhibitor U-104 (Fig. 1G).[40] Imaging study in mice showed low uptake in HT-29 tumour xenografts (0.83 ± 0.06%ID/g) but very high in blood (13.92 ± 3.07%ID/g) at 1 h pi. The high blood retention is likely due to the binding of U-104 to CA II (K_i = 95 nM, Table 1) which is abundantly expressed in erythrocytes.[41,42] Therefore, for this study we ^{18}F-labeled three selective tertiary sulfonamide CA IX inhibitors **4a–c** (Table 1), and evaluated their potential for imaging CA IX expression in tumours with positron emission tomography. Compounds **4a–c** were among the first identified benzenesulfonamides that are selective nanomolar CA IX inhibitors with no measurable affinity for the off target CA II.[43]

The synthesis of standards **4a–c** and their respective radiolabeling tosylate precursors **3a–c** are illustrated in Scheme 1. The starting sulfonamides **1a–c** were prepared according to published procedures.[43] Coupling of sulfonamides **1a–c** with excess propylene oxide in DMF in the presence of K_2CO_3 as base afforded the desired secondary alcohols **2a–c** in excellent yield (92–99%). Initially, we attempted to prepare mesylate precursors for radiolabeling. However, we found out that it was difficult to separate the desired mesylates from their respective alcohol reactants **2a–c** by flash column chromatography (data not shown). Therefore, tosylate precursors were prepared instead as they could be easily separated from respective alcohol reactants **2a–c** due to the additional benzene on their structures. By using diisopropylethylamine as the base and trimethylamine hydrochloride as the catalyst,[45] the reaction of alcohols **2a–c** with *p*-toluenesulfonyl chloride proceeded smoothly in CH_2Cl_2, and provided the desired tosylate precursors **3a–c** in 77–91% yield. Previously, standards **4a–c** were prepared by the reaction of HF/SBF_5 with their respective *N*-allylic intermediates.[43] However, due to safety concern with HF, different strategies for the preparation of standards **4a–c** were investigated. For the preparation of **4a**, tosylate **3a** was refluxed in THF in the presence of excess tetrabutylammonium fluoride (TBAF). However, the yield (6.5%) of desired product **4a** was low due to the formation of elimination by-product. Therefore, standards **4b**,**c** were prepared by directly converting alcohols **2b**,**c** to fluorides using diethylaminosulfur trifluoride (DAST), and better yields (23–44%) of **4b**,**c** were obtained.

Table 1
Reported inhibition constants (K_i, nM) of acetazolamide,[44] HS680,[47] U-104,[44] and tertiary sulfonamides **4a–c**[43] to human CA isoenzymes I, II, IX and XII



Compounds	hCA I	hCA II	hCA IX	hCA XII
Acetazolamide	250	12	25	5.7
HS680	–	248	7.5	35
U-104	5080	95	45	4.5
4a (R = Me)	73.1	Not active	9.3	33.6
4b (R = Ac)	89.1	Not active	9.6	83.8
4c (R = Cl)	77.3	Not active	9.1	100



Scheme 1. Syntheses of (A) precursors **3a–c** and (B and C) standards **4a–c** of tertiary sulfonamide CA IX inhibitors, and (D) their ^{18}F-labeled analogs [^{18}F]**4a–c**.

As illustrated in Scheme 1D, the preparation of [^{18}F]**4a–c** was performed via aliphatic nucleophilic substitution reactions between tosylate precursors **3a–c** and TBA[^{18}F]F in DMF at 125 °C. After HPLC purification, [^{18}F]**4a–c** were obtained in 7.1–43% decay-corrected yields with >99% radiochemical purity, and >740 GBq/μmole specific activity at the end of synthesis (~100 min). The stability of [^{18}F]**4a–c** was determined in mouse plasma, and no detectable metabolites of [^{18}F]**4a–c** were observed by HPLC analysis after 2 h incubation at 37 °C. The log$D_{7.4}$ (D: distribution coefficient) values of [^{18}F]**4a–c** were 3.45 ± 0.04, 3.05 ± 0.01, and 3.51 ± 0.27, respectively, which were measured using the traditional shake flask method. These values were used to assess if [^{18}F]**4a–c** could cross cell membrane freely, and potentially bind to intracellular off targets CA I and II which are expressed in erythrocytes.[41,42] Since the molecular weights (MW) and log$D_{7.4}$ values of **4a–c** are in the range of 321–349 Daltons, and 3.05–3.51, respectively, they are likely to enter cells freely according to the Lipinski's rule of five (MW < 500 and log$D_{7.4}$ < 5).[46]

To evaluate the potential of [^{18}F]**4a–c** for imaging CA IX expression, biodistribution and PET imaging studies were performed in mice bearing HT-29 human colorectal tumour xenografts. HT-29 tumour model is commonly used in literature for evaluating CA IX-targeting tracers as it constitutively expresses CA IX.[33,34,40,47] As shown in Table 2, fast blood clearance of [^{18}F]**4a–c** was observed with only 0.58 ± 0.49, 0.37 ± 0.11, and 0.98 ± 0.36%ID/g, respectively, retained in blood at 1 h pi. These numbers are substantially lower than the previously reported 13.92 ± 3.07%ID/g obtained with ^{18}F-labeled U-104[40] indicating no significant binding of [^{18}F]**4a–c** to the intracellular off target CA II. However, the uptake of [^{18}F]**4a–c** in HT-29 tumours did not improve as only 0.51 ± 0.45, 0.59 ± 0.29, and 0.98 ± 0.48%ID/g, respectively, were observed at 1 h pi. The majority of [^{18}F]**4a**,**b** radioactivity was excreted via the hepatobiliary pathway reflecting the lipophilic nature of these two radiotracers. For [^{18}F]**4c** very high bone uptake (12.61 ± 5.18%ID/g at 1 h pi) was observed which indicated massive defluorination of [^{18}F]**4c** in vivo. However, it is unclear at this stage why it was [^{18}F]**4c** but not [^{18}F]**4b** that resulted in massive in vivo defluorination. Based on the structures of **4a–c**, the 4-acetyl group in **4b** is the most electron-withdrawing group followed by the 4-chloro group in **4c**, and then the 4-methyl group in **4a**. Presumably, the 4-acetyl group would make the nitrogen of sulfonamide **4b** more electron deficient, and the adjacent fluoro a better leaving group, resulting in a higher degree of in vivo defluorination. Although bone uptake of [^{18}F]**4b** was higher than [^{18}F]**4a** (2.72 ± 0.57 vs. 0.91 ± 0.70%ID/g at 1 h pi), these numbers were much lower than 12.61 ± 5.18%ID/g obtained by using [^{18}F]**4c** suggesting other factors might contribute to in vivo defluorination of these compounds. Since the uptake of [^{18}F]**4a–c** in HT-29 tumours was not significantly higher than the uptake values in the surrounding tissues (blood, fat, muscle and bone), no clear tumour visualization was obtained from the PET images (Fig. 2).

Due to the large number of human CA isoenzymes and their highly conserved catalytic domain, it is very challenging to design potent inhibitors specifically targeting an individual CA isoform.[1]

Table 2
Biodistribution data (%ID/g at 1 h pi; N = 4) of [^{18}F]**4a–c** in NSG mice bearing HT-29 human colorectal tumour xenografts

Tissues/organs	Radiotracers		
	[^{18}F]**4a**	[^{18}F]**4b**	[^{18}F]**4c**
Blood	0.58 ± 0.49	0.37 ± 0.11	0.98 ± 0.36
Fat	0.76 ± 0.55	1.48 ± 0.53	0.94 ± 0.51
Intestine	7.47 ± 7.46	16.7 ± 5.85	2.64 ± 0.26
Stomach	1.35 ± 2.21	1.25 ± 0.98	0.40 ± 0.16
Spleen	0.68 ± 0.53	0.82 ± 0.23	0.81 ± 0.35
Liver	9.77 ± 8.43	3.35 ± 1.72	3.57 ± 1.80
Pancreas	0.81 ± 0.83	0.75 ± 0.26	0.61 ± 0.20
Kidney	1.68 ± 1.66	2.03 ± 0.65	2.34 ± 1.04
Lungs	1.22 ± 0.90	1.46 ± 1.07	1.51 ± 0.42
Heart	0.59 ± 0.46	0.48 ± 0.11	0.93 ± 0.38
Muscle	0.52 ± 0.47	0.51 ± 0.18	0.92 ± 0.50
Bone	0.91 ± 0.70	2.72 ± 0.57	12.61 ± 5.18
Brain	0.32 ± 0.28	0.20 ± 0.06	0.33 ± 0.05
Tumour	0.51 ± 0.45	0.59 ± 0.29	0.98 ± 0.48

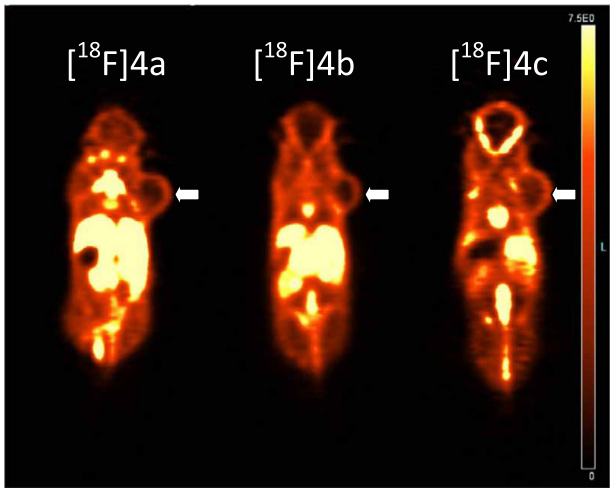


Figure 2. Representative PET images of [^{18}F]**4a**–**c** at 1 h pi in NSG mice bearing CA IX-expressing HT-29 human colorectal tumour xenografts. White arrows point to HT-29 tumours.

Compounds **4a**–**c** were explored for imaging CA IX expression in HT-29 tumours because they were reported to have nanomolar binding affinity for CA IX, and most importantly to be the first sulfonamides to show no interaction with CA II in CO_2 hydration assays (Table 2). The binding of **4a**–**c** to CA XII could be beneficial as CA XII, a transmembrane protein, is also up-regulated in hypoxic tumours.[48] Cytosolic CA I and II are expressed in large quantities in erythrocytes[41,42] and the binding of CA IX-targeting tracers to CA I and II would reduce imaging contrast. Previously, we observed slow blood clearance of ^{18}F-labeled U-104 presumably due to binding with CA II (K_i = 95 nM, Table 2).[40] In this study, we did not observe much retention of [^{18}F]**4a**–**c** in blood at 1 h pi despite their suitable physical characteristics (neutral, lipophilic, and with MW < 500 Daltons) to cross cell membrane, and good binding affinity to CA I (K_i = 73.1–89.1 nM, Table 2). Possible explanations for the low tumour uptake and blood retention of [^{18}F]**4a**–**c** could be due to relatively fast metabolism and/or blood clearance of these tracers, and in the case of [^{18}F]**4c**, rapid in vivo defluorination.

Although no small sulfonamide-based radiotracers have been reported to visualize CA IX-expressing tumours in preclinical imaging studies, a successful optical imaging probe HS680 is already commercially available. HS680, a conjugate of acetazolamide and the near-infrared fluorochrome VivoTag 680 developed by Perkin-Elmer, showed impressive 10% injected dose accumulation in HT-29 tumour xenografts in mice at 24 h pi.[47] Acetazolamide is a promiscuous CA inhibitor, and therefore the derivatives of acetazolamide including HS680 are expected to have good binding affinity to most CAs including the intracellular isoenzymes I and II (Table 2). However, the conjugation of acetazolamide with the highly charged and bulky (MW > 1000 Daltons) VivoTag 680 prevents it from crossing cell membrane and binding to intracellular off targets. The success of HS680 could be a valuable lesson for the future design of radiotracers for imaging CA IX expression with positron emission tomography or single photon emission computed tomography. Instead of radiolabeling CA IX-specific inhibitors which may be difficult to synthesize, the design of cell-impermeable sulfonamide-based radiotracers represents an easier and quicker solution. This could be achieved, for example, by conjugating the CA-targeting sulfonamide moiety with a polyaminocarboxylate chelator for labeling with radiometals such as ^{68}Ga, ^{64}Cu, or ^{111}In for imaging. The radiometal–polyaminocarboxylate-chelator complexes are generally highly hydrophilic and charged, and would prevent the radiolabeled sulfonamide conjugates from entering cells. Another promising alternative is the use of a multivalent design. This approach combines several CA-targeting sulfonamides into one single molecule, which could potentially enhance binding affinity to CA IX and afford cell impermeability by accumulation of MW.

In conclusion, we successfully synthesized and radiolabeled tertiary benzenesulfonamides **4a**–**c**, and evaluated their potential as CA IX imaging agents. Despite their good affinity and selectivity for CA IX, imaging and biodistribution data showed only minimal tumour uptake in xenografted mice relative to normal tissues. Therefore, [^{18}F]**4a**–**c** are not suitable for CA IX targeted molecular imaging.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.05.021.

References and notes

1. Alterio, V.; Di Fiore, A.; D'Ambrosio, K.; Supuran, C. T.; De Simone, G. *Chem. Rev.* **2012**, *112*, 4421.
2. Supuran, C. T. *Nat. Rev. Drug Disc.* **2008**, *7*, 168.
3. Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3467.
4. Oosterwijk, E.; Ruiter, D. J.; Hoedemaeker, P. J.; Pauwels, E. K. J.; Jonas, U.; Zwartendijk, J.; Warnaar, S. O. *Int. J. Cancer* **1986**, *38*, 489.
5. Said, H. M.; Hagemann, C.; Staab, A.; Stojic, J.; Kuehnel, S.; Vince, G. H.; Flentje, M.; Roosen, K. *Radiother. Oncol.* **2007**, *83*, 398.
6. Ord, J. J.; Agrawal, S.; Thamboo, T. P.; Roberts, I.; Campo, L.; Turley, H.; Han, C.; Fawcett, D. W.; Kulkarni, R. P.; Cranston, D.; Harris, A. L. *J. Urol.* **2007**, *178*, 677.
7. Swietach, P.; Vaughan-Jones, R. D.; Harris, A. L. *Cancer Metastasis Rev.* **2007**, *26*, 299.
8. Hutchison, G. J.; Valentine, H. R.; Loncaster, J. A.; Davidson, S. E.; Hunter, R. D.; Roberts, S. A.; Harris, A. L.; Stratford, I. J.; Price, P. M.; West, C. M. L. *Clin. Cancer Res.* **2004**, *10*, 8405.
9. Koukourakis, M. I.; Giatromanolaki, A.; Sivridis, E.; Pastorek, J.; Karapantzos, I.; Gatter, K. C.; Harris, A. L. *Int. J. Radiat. Oncol. Biol. Phys.* **2004**, *59*, 67.
10. Potter, C. P. S.; Harris, A. L. *Br. J. Cancer* **2003**, *89*, 2.
11. Swinson, D. E. B.; Jones, J. L.; Richardson, D.; Wykoff, C.; Turley, H.; Pastorek, J.; Taub, N.; Harris, A. L.; O'Byrne, K. J. *J. Clin. Oncol.* **2003**, *21*, 473.
12. Trastour, C.; Benizri, E.; Ettore, F.; Ramaioli, A.; Chamorey, E.; Pouyssegur, J.; Berra, E. *Int. J. Cancer* **2007**, *120*, 1451.
13. Hussain, S. A.; Ganesan, R.; Reynolds, G.; Gross, L.; Stevens, A.; Pastorek, J.; Murray, P. G.; Perunovic, B.; Anwar, M. S.; Billingham, L.; James, N. D.; Spooner, D.; Poole, C. J.; Rea, D. W.; Palmer, D. H. *Br. J. Cancer* **2007**, *96*, 104.
14. Dorai, T.; Sawczuk, I.; Pastorek, J.; Wiernik, P. H.; Dutcher, J. P. *Cancer Invest.* **2006**, *24*, 754.
15. Swietach, P.; Hulikova, A.; Vaughan-Jones, R. D.; Harris, A. L. *Oncogene* **2010**, *29*, 6509.
16. Svastova, E.; Hulikova, A.; Rafajova, M.; Zatovicova, M.; Gibadulinova, A.; Casini, A.; Cecchi, A.; Scozzafava, A.; Supuran, C. T.; Pastorek, J.; Pastorekova, S. *FEBS Lett.* **2004**, *577*, 439.
17. Swietach, P.; Vaughan-Jones, R. D.; Harris, A. L. *Cancer Metastasis Rev.* **2007**, *26*, 299.
18. Kroemer, G.; Pouyssegur, J. *Cancer Cell* **2008**, *13*, 472.
19. Stock, C.; Schwab, A. *Pflugers Arch.* **2009**, *458*, 981.
20. Brennan, D. J.; Jirstrom, K.; Kronblad, A.; Millikan, R. C.; Landberg, G.; Duffy, M. J.; Ryden, L.; Gallagher, W. M.; O'Brien, S. L. *Clin. Cancer Res.* **2006**, *12*, 6421.
21. Chia, S. K.; Wykoff, C. C.; Watson, P. H.; Han, C.; Leek, R. D.; Pastorek, J.; Gatter, K. C.; Ratcliffe, P.; Harris, A. L. *J. Clin. Oncol.* **2001**, *19*, 3660.
22. Span, P. N.; Bussink, J.; Manders, P.; Beex, L. V.; Sweep, C. G. *Br. J. Cancer* **2003**, *89*, 271.
23. Colpaert, C. G.; Vermeulen, P. B.; Fox, S. B.; Harris, A. L.; Dirix, L. Y.; Van Marck, E. A. *Breast Cancer Res. Treat.* **2003**, *81*, 137.
24. Kilbride, K. E.; Lee, M. C.; Nees, A. V.; Cimmino, V. M.; Diehl, K. M.; Sabel, M. S.; Hayes, D. F.; Schott, A. F.; Kleer, C. G.; Chang, A. E.; Newman, L. A. *Ann. Surg. Oncol.* **2008**, *15*, 3252.
25. Oosterwijk, E.; Ruiter, D. J.; Hoedemaeker, P. J.; Pauwels, E. K. J.; Jonas, U.; Zwartendijk, J.; Warnaar, S. O. *Int. J. Cancer* **1986**, *38*, 489.

26. Brouwers, A. H.; Dorr, U.; Lang, O.; Boerman, O. C.; Oyen, W. J. G.; Steffens, M. G.; Oosterwijk, E.; Mergenthaler, H. G.; Hihl, H.; Corstens, F. H. M. *Nucl. Med. Commun.* **2002**, *23*, 229.
27. Brouwers, A. H.; Buijs, W. C.; Boerman, O. C.; Oosterwijk, E.; de Mulder, P. H.; Corstens, F. H.; Mulders, P. F. *J. Nucl. Med.* **2003**, *44*, 171P.
28. Divgi, C. R.; Pandit-Taskar, N.; Jungbluth, A. A.; Reuter, V. E.; Gönen, M.; Ruan, S.; Pierre, C.; Nagel, A.; Pryma, D. A.; Humm, J.; Larson, S. M.; Old, L. J.; Russo, P. *Lancet Oncol.* **2007**, *8*, 304.
29. Hoeben, B. A. W.; Kaanders, J. H. A. M.; Franssen, G. M.; Troost, E. G. C.; Rijken, P. F. J. W.; Oosterwijk, E.; van Dongen, G. A. M. S.; Oyen, W. J. G.; Boerman, O. C.; Bussink, J. *J. Nucl. Med.* **2010**, *51*, 1076.
30. Mandriota, S. J.; Turner, K. J.; Davies, D. R.; Murray, P. G.; Morgan, N. V.; Sowter, H. M.; Wykoff, C. C.; Maher, E. R.; Harris, A. L.; Ratcliffe, P. J.; Maxwell, P. H. *Cancer Cell* **2002**, *1*, 459.
31. Dubois, L.; Douma, K.; Supuran, C. T.; Chiu, R. K.; van Zandvoort, M. A. M. J.; Pastorekova, S.; Scozzafava, A.; Wouters, B. G.; Lambin, P. *Radiother. Oncol.* **2007**, *83*, 367.
32. Apte, S. D.; Chin, F. T.; Graves, E. E. J. *Labelled Compd. Radiopharm.* **2009**, *52*, S408.
33. Akurathi, V.; Dubois, L.; Lieuwes, N. G.; Chitneni, S. K.; Cleynhens, B. J.; Vullo, D.; Supuran, C. T.; Verbruggen, A. M.; Lambin, P.; Bormans, G. M. *Nucl. Med. Biol.* **2010**, *37*, 557.
34. Akurathi, V.; Dubois, L.; Celen, S.; Lieuwes, N. G.; Chitneni, S. K.; Cleynhens, B. J.; Innocenti, A.; Supuran, C. T.; Verbruggen, A. M.; Lambin, P.; Bormans, G. M. *Eur. J. Med. Chem.* **2014**, *71*, 374.
35. Asakawa, C.; Ogawa, M.; Kumata, K.; Fujinaga, M.; Yamasaki, T.; Xie, L.; Yui, J.; Kawamura, K.; Fukumura, T.; Zhang, M. R. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 7017.
36. Kolb, H. C.; Walsh, J. C.; Kasi, D.; Mocharla, V. P.; Wang, B.; Gangadharmath, U. B.; Duclos, B. A.; Chen, K.; Zhang, W.; Chen, G.; Padgett, H. C.; Karimi, F.; Scott, P. J. H.; Gao, Z.; Liang, Q.; Collier, T. L.; Zhao, T.; Xia, C. U.S. Patent 0,317,842, 2010.
37. Doss, M.; Alpaugh, R. K.; Yu, J. Q. *Mol. Imaging Biol.* **2010**, *12*, S124.
38. Lu, G.; Hillier, S. M.; Maresca, K. P.; Zimmerman, C. N.; Eckelman, W. C.; Joyal, J. L.; Babich, J. W. *J. Med. Chem.* **2013**, *56*, 510.
39. Can, D.; Spingler, B.; Schmutz, P.; Mendes, F.; Raposinho, P.; Fernandes, C.; Carta, F.; Innocenti, A.; Santos, I.; Supuran, C. T.; Alberto, R. *Angew. Chem., Int. Ed.* **2012**, *51*, 3354.
40. Pan, J.; Lau, J.; Mesak, F.; Hundal, N.; Pourghiasian, M.; Liu, Z.; Bénard, F.; Dedhar, S.; Supuran, C. T.; Lin, K. S. *J. Enzyme Inhib. Med. Chem.* **2014**, *29*, 249.
41. Gokce, B.; Gencer, N.; Arslan, O.; Turkoglu, S. A.; Alper, M.; Kockar, F. *J. Enzyme Inhib. Med. Chem.* **2012**, *27*, 37.
42. Senturk, M.; Gulcin, I.; Beydemir, S.; Kufrevioglu, O. I.; Supuran, C. T. *Chem. Biol. Drug Des.* **2011**, *77*, 494.
43. Metayer, B.; Mingot, A.; Vullo, D.; Supuran, C. T.; Thibaudeau, S. *Chem. Commun.* **2013**, 6015.
44. Pacchiano, F.; Carta, F.; MaDonald, P. C.; Lou, Y.; Vullo, D.; Scozzafava, A.; Dedhar, S.; Supuran, C. T. *J. Med. Chem.* **2011**, *54*, 1896.
45. Yoshida, Y.; Sakakura, Y.; Aso, N.; Okada, S.; Tanabe, Y. *Tetrahedron* **1999**, *55*, 2183.
46. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Deliv. Rev.* **1997**, *23*, 3.
47. Groves, K.; Bao, B.; Zhang, J.; Handy, E.; Kennedy, P.; Cuneo, G.; Supuran, C. T.; Yared, W.; Peterson, J. D.; Rajopadhye, M. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 653.
48. Guler, O. O.; de Sımone, G.; Supuran, C. T. *Curr. Med. Chem.* **2010**, *17*, 151.