Syntheses of Radioiodinated Pyrimidine-2,4,6-Triones as Potential Agents for Non-Invasive Imaging of Matrix Metalloproteinases

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Abstract: Dysregulated expression or activation of matrix metalloproteinases (MMPs) is observed in many kinds of live-threatening diseases. Therefore, MMP imaging for example with radiolabelled MMP inhibitors (MMPIs) potentially represents a valuable tool for clinical diagnostics using non-invasive single photon emission computed tomography (SPECT) or positron emission tomography (PET) imaging. This work includes the organic chemical syntheses and in vitro evaluation of five iodinated barbiturate based MMPIs and the selection of derivative 9 for radiosyntheses of isotopologues [123I]9 potentially useful for MMP SPECT imaging and [124I]9 for MMP PET imaging.

Keywords: pyrimidine-2,4,6-triones; radioiodination; matrix metalloproteinase inhibitor; fluorometric in vitro inhibition assays

1. Introduction

The in vivo molecular imaging of locally upregulated and activated matrix metalloproteinases (MMPs) that are observed in pathologies such as cardiovascular diseases, inflammation or cancer remains a substantive clinical issue [1]. Current targeting strategies for noninvasive imaging of MMPs should not only account for high binding affinity and specificity towards the enzyme but also for drug-target residence time [2], subgroup selectivity, sensitivity, target-to-background ratio as well as in vivo stability [3]. Maybe insufficient consideration of these parameters caused that most of the preclinical studies of the past 20 years with radiolabeled MMPIs were either disappointing or remained at a preliminary stage [4,5]. In addition an inadequate validation of the animal models regarding their level of MMP expression leads to challenging data [6].

Our own approaches towards the development of radiolabelled and fluorescent-dye conjugated MMP tracers focused on two different classes of non-peptidic MMPIs, on the one hand hydroxamate-based inhibitors (i.e., derivatives of CGS 27023A and CGS 25966 [7] with a broad-spectrum inhibitory profile) and, on the other hand, pyrimidine-2,4,6-trione-based inhibitors (i.e., barbiturates, derivatives of RO-2653 [8–11] with sub-group selectivity for the gelatinases A (MMP-2) and B (MMP-9), neutrophil collagenase (MMP-8) and the membrane-bound MMPs MT-1-MMP (MMP-14) and MT-3-MMP (MMP-16)). Initially, in 2005 we suggested in the latter project a first radiolabelled barbiturate-based MMPI, compound 12 (see Table 2) labelled with the radionuclide iodine-125 (125I), for first in vitro and...
ex vivo applications [12]. In 2008 we developed for the first time the barbiturate-based near-infrared fluorescent photo probe Cy5.5-AF443 that was suitable for in vitro and in vivo imaging of the gelatinases MMP-2 and MMP-9 [13,14]. In 2010 we published the radiosynthesis and evaluation of the first fluorine-18 (18F) labelled barbiturate-based MMPI [15] and two years later, of several more hydrophilic radiofluorinated analogues with improved biodistribution behavior [16]. Moreover, a gallium-68 (68Ga) labelled version was introduced by our group in 2012 [17]. Favorable MMP binding affinities for our barbiturate-based tracers were indeed measured by in vitro assays and in vivo biodistribution studies using wt-mice. However, in animal models with increased MMP expression mentioned barbiturate-based tracers did not meet the expectations. Anyhow in vivo MMP imaging was feasible and specific with our hitherto most encouraging optical imaging probe Cy5.5-AF443 suggesting the assumption that improved imaging performance of the photoprobe Cy5.5-AF443 compared to the barbiturate radiotracers is caused by the cyanine dye substituent with the four hydrophilic sulfonic acid moieties. Actually, these structural characteristics change the physicochemical properties and accordingly the essential biodistribution pattern influenced i.a. by the excretion routes (renal or hepatobiliary), plasma-protein binding, binding to non-target-organs and/or off-target interactions with other proteins. In summary, adopting or transferring features from optical tracers to radiotracers could support their development, an aspect that was recently reviewed by Faust et al. [18]. Therefore, the aim of this work was the synthesis of radioiodinated barbiturate-based MMPI tracers with increased hydrophilicity for potential SPECT/PET imaging. Radionuclides iodine-123 and iodine-124 were used for the radiosyntheses and applied on our target molecule 9, which is ca. 3 log units more hydrophilic as compared to our initial preclinical research tracer [125I]12 (see Table 2) [12]. To achieve increased hydrophilicity two different chemical modifications of the C5 phenoxyphenyl moiety in 12 that occupies the SI’ enzyme pocket were realized. Moreover the commercially available radionuclides iodine-123 (for SPECT) and iodine-124 (for PET) exhibit prolonged half-lives t½ compared to the most common γ-emitter for SPECT technetium-99m (t½ 13.2 h vs. 6.0 h) and β+ -emitter for PET fluorine-18 (t½ 4.2 d vs. 110 min) allowing long-term studies with the corresponding 123/I-124I-labelled barbiturate-based tracer in the next steps.

2. Results and Discussion

2.1. Chemistry

Phenyl barbiturates 5a–d and 6 were prepared as outlined in Scheme 1 by four- (compounds 5a–c), five- (compound 5d) or six-step (compound 6) sequences, respectively.

Scheme 1. Syntheses of phenyl barbiturates 5a–d and 6. Reagents and yields: (a) NaH, dimethyl carbonate, dioxane, 82% (2a), 73% (2b); (b) urea, NaOEt, EtOH, 29% (3a), 91% (3b); (c) NBS, dibenzoyl peroxide, CCl4, 51% (4a); Br2, HBr, H2O, 89% (4b); (d) N-(2-hydroxyethyl)-piperazine or 3-(piperazin-1-yl)-propionic acid, MeOH, 53% (5a), 18% (5b), 28% (5c); (e) H2, Pd/C, MeOH, 82% (5d); (f) NaI, NaOCl, NaOH, MeOH, 12% (6).
In detail, 4-iodophenyl acetic acid methylester (1a), synthesized by the esterification of commercial 4-iodophenyl acetic acid using MeOH/H2SO4 was methoxycarbonylated to give the corresponding malonic ester 2a. 4-Benzoyloxyphenyl malonic acid dimethyl ester (2b) [19] was obtained from methyl 2-((4-benzyloxy)phenyl)acetate (1b) [20] analogous to 2a by the literature procedure. Malonic esters 2a and 2b were cyclized with urea using sodium ethoxide as a base to yield the 5-phenylbarbituric acid derivatives 3a and 3b, which subsequently were brominated with N-bromosuccinimide (NBS) (compound 3a) or bromine/HBr (compound 3b), resulting in the 5-bromo-5-phenyl barbituric acids 4a and 4b. These were reacted with either of the commercially available piperazines N-(2-hydroxyethyl)-piperazine or 3-(piperazin-1-yl)-propionic acid in MeOH to yield the barbituric acid derivatives 5a–c in overall yields of 6% (5a), 2% (5b) and 17% (5c). Cleavage of the benzyloxy group in 5c was achieved by catalytic hydrogenation (H2, Pd/C, MeOH) resulting in the hydroxyl compound 5d in 14% overall yield. Iodination of 5d using sodium iodide, sodium hypochlorite and sodium hydroxide in methanol yielded the ortho iodination product 6 in 2% overall yield.

To obtain carboxy derivative 9 and fluoro derivative 10 (Scheme 2) the bromo intermediate 7, whose synthesis was described previously [12], was reacted either with commercially available 3-(piperazin-1-yl)-propionic acid or piperazine 8 [15]. The yields were 83% for 9 and 8% for 10 (Scheme 2).

![Scheme 2. Syntheses of compounds 9 and 10. Reagents and yields: (a) MeOH, 83% (9), 8% (10).]

2.2. Enzyme Assays and clogD Values

The MMP inhibition potencies of the barbituric acid derivatives 5a, 5b, 6, 9 and 10 were measured in fluorometric in vitro inhibition assays as described previously [21]. The IC50-values of 5a, 5b and 6 were determined for gelatinases MMP-2 and MMP-9 (Table 1), the IC50-values of 9 and 10 for MMP-2, MMP-8, MMP-9, MMP-13 and MMP-14 (only 9) (Table 2). The results are depicted in Tables 1 and 2. The tables also contain the calculated logP/logD values (clogP/clogD) of the synthesized barbituric acids derivatives to indicate the changes of lipophilicities caused by the structural modifications.

Replacement of the phenoxypyhenyl moiety in 12 (see Table 1) by a phenyl group resulted in compounds 5a–d and 6. As expected the lipophilicities of iodinated 5a–b and 6 are significantly reduced, with clogD values ranging between −1.34 and 1.26 compared to 12 with a clogD value of 3.53. On the other hand the IC50 values for MMP-2 and -9 of these compounds are generally increased compared to the derivatives with a phenoxypyphenyl residue (Table 2). This is also expected because the phenoxypyphenyl core is optimized for the deep and narrow S1′ pocket of the target MMPs [8]. While 5b and 6 possess IC50 values for MMP-2 and -9 in the micromolar range (0.67–1.6 μM, Table 1), 5a is at least a potent MMP-2 inhibitor with an IC50 value of 10 nM.
Table 1. IC₅₀ values of phenyl barbiturates 5a, 5b and 6.

| Cpd. | R₁  | R₂  | R₃         | IC₅₀ [nM] |
|------|-----|-----|------------|-----------|
|      |     |     | MMP-2      | MMP-9     |
| 5a   | -I  | -H  | 10 ± 3     | 550 ± 180 |
| 5b   | -I  | -H  | 1200 ± 340 | 1600 ± 600|
| 6    | -OH | -I  | 670 ± 210  | 1300 ± 320|

Values are the mean ± standard deviation (SD) of three assays.

Table 2. Structures and IC₅₀ values of phenoxyphenyl barbiturates 9–12.

| Cpd. | R₁  | R₂         | IC₅₀ [nM] |
|------|-----|------------|-----------|
|      |     | MMP-2      | MMP-8     | MMP-9     | MMP-13    | clogP ⁺ | clogD ⁺ |
| 9ключение b | -I  | N       | 29 ± 2    | 1170 ± 80 | 1.3 ± 0.2 | 362 ± 24 | 1.40  | 0.92  |
| 10   | -I  | OH        | 23 ± 5    | 146 ± 48  | 17 ± 5    | 28 ± 11  | 3.13  | 2.99  |
| 12   | -I  | COOH      | 7 ± 1     | n. d.     | 2 ± 0.2   | n. d.    | 3.68  | 3.53  |
| 13ключение c | -H  | OH        | 23 ± 9    | 138 ± 12  | 7 ± 2     | 645 ± 17 | 3.17  | 2.88  |

Values are the mean ± SD of three assays.

b IC₅₀ (MMP-14) = 49 ± 8 nM. c IC₅₀ (MMP-1) > 50 μM, IC₅₀ (MMP-3) = 760 μM. d experimental logD = 2.15 ± 0.02.

For comparison reasons the IC₅₀ values of compound 12 [12] and 13 [15] from previous work are also shown.

The second hydrophilic modification included the substitution of the hydroxy group with a carboxy group in the piperazine residue of 12 to yield carboxylic acid 9 resulting in a clogD shift of 2.6 units (approximately 400 fold increased water solubility, Table 2) from 3.53 towards 0.92. Despite this modification the high MMP-2- and -9-inhibition potency of 12 was only marginally influenced (Table 2, 12: IC₅₀ (MMP-2) = 7 nM, IC₅₀ (MMP-9) = 2 nM. 9: IC₅₀ (MMP-2) = 29 nM, IC₅₀ (MMP-9) = 1.3 nM). Moreover, in vitro data showed, that 9 was also a nanomolar inhibitor of collagenase 3 (MMP-13) and MMP-14 (MT-1 MMP), but only a micromolar inhibitor of neutrophil collagenase (MMP-8) (9: IC₅₀ (MMP-14) = 49 nM (Table 2, footnote b), IC₅₀ (MMP-8) = 1170 nM, IC₅₀ (MMP-13) = 362 nM). In summary, compound 9 confirms the results from Grams et al. [8] that 5,5-disubstituted barbiturates with a para-substituted phenoxyphenyl unit represent potent inhibitors for MMP-2, -9 and -14. Additionally, as shown by comparison of the IC₅₀ values of 9 and 13 with 10 the elongation of the substituent of the piperazine residue with fluorinated tri-ethylenglycol resulted in a
decrease of selectivity for unknown reasons. From this selection of iodinated barbiturate derivatives carboxylic acid 9 was chosen for radiochemical synthesis of the radioiodinated isotopologues $^{[123/124]}$I9 (see Section 2.3) because this compound possesses the most favourable characteristics indicated by clogD and IC$_{50}$-values.

2.3. Radiochemistry

Palladium-catalyzed cross-coupling reaction (Stille coupling) of non-radioactive reference compound 9 with tributyltin hydride (or hexabutylditin) (yield: 28%) [20] provided the stannyl precursor 11 (Scheme 3).

![Scheme 3. Precursor synthesis of 11 and radiosynthesis of the barbituric acid-based MMP-targeted model tracer $^{[123/124]}$I9. Reagents and yields: (a) Bu$_3$SnH or Bu$_4$Sn$_2$ (2.0 eq.), PdCl$_2$(PPh$_3$)$_2$ or PdCl$_2$ (3 mol%), KOAc (3.0 eq.), N-methyl-pyrrolidone, 28%; (b) $^{[123/124]}$INaI, chloroamine-T hydrate, 0.1 M K$_2$HPO$_4$.](image-url)

Subsequent radioiododestannylation of 11 with no-carrier-added (n. c. a.) $^{[123/124]}$INaI and chloroamine-T led to the radioligands $^{[123/124]}$I9. The decay-corrected radiochemical yields were 28 ± 7% ($n = 6$) for $^{[123]}$I9 and 44 ± 6% ($n = 3$) for $^{[124]}$I9 at the end of synthesis (EOS) and the radiochemical purities were 95 ± 3% for $^{[123]}$I9 and 93 ± 5% for $^{[124]}$I9 (as determined by radio-HPLC). The molar activities are 0.2–6.3 GBq/µmol and 0.4–14.0 GBq/µmol, respectively. The identities of $^{[123/124]}$I9 were proven by HPLC (coinjection and coelution with reference compound 9).

3. Materials and Methods

3.1. General Methods. Chemistry

All chemicals, reagents and solvents for the synthesis of the compounds were of analytical grade, purchased from commercial sources and used without further purification, unless otherwise specified. Melting points were determined in capillary tubes on a SMP3 capillary melting point apparatus (Stuart Scientific, Staffordshire, UK) and are uncorrected. $^1$H-NMR, $^{13}$C-NMR and $^{19}$F-NMR spectra were recorded on ARX 300 and/or AMX 400 spectrometers (Bruker, Karlsruhe, Germany). CDCl$_3$ contained tetramethylsilane (TMS) as an internal standard. Mass spectra were obtained on a MAT 212 (EI = 70 eV) spectrometer (Varian Medical Systems, Palo Alto, CA, USA) and a Bruker MALDI-TOF-MS Reflex IV instrument (matrix: DHB). Exact mass analyses were conducted on a Quattro LC (Waters, Milford, MA, USA) and a Bruker MicroTof apparatus. Elemental analyses were realized by a Vario EL III analyzer (Elementar Analysensysteme Comp.„ Hanau, Germany). All aforementioned spectroscopic and analytical investigations were done by staff members of the Institute of Organic Chemistry, University of Münster, Germany. All purifications of compounds and determinations of purity by HPLC were performed by using a gradient RP-HPLC system (Knauer, Berlin, Germany) equipped with two K-1800 pumps, an S-2500 UV detector and RP-HPLC Nucleosil Eurosphere 100-10 C-18 columns for analytical (250 mm × 4.6 mm) purposes. The following eluents were used (unless specified otherwise): eluent A: water (0.1% TFA), eluent B: acetonitrile (0.1% TFA). The following conditions were used (unless specified otherwise): Gradient from 90% A to 20% A over 30 min, constant 20% A over 5 min and from 20% A to 90% A over 5 min, at a flow rate of 1.5 mL/min, detection at λ = 254 nm.
3.2. General Procedure for 4-Iodophenyl Malonic Acid Dimethyl Ester (2a) and 4-Benzylxylophnyl Malonic Acid Dimethyl Ester (2b)

A suspension of 4.00 g NaH (166 mmol, 6.66 g of a 60% suspension in paraffin; the paraffin was removed by repeated washings with petroleum benzene) and 48.0 g (533 mmol) dimethyl carbonate in 80 mL absolute dioxane was heated to 100–120 °C and a solution of 23.00 g (83.3 mmol) 4-Iodophenyl acetic acid methylester (1a, prepared by the esterification of 4-iodophenyl acetic acid using MeOH/H2SO4) in absolute dioxane (125 mL) was added dropwise over a period of 1 h. Refluxing was continued for 3 h and the reaction mixture was allowed to come to room temperature overnight. The mixture was poured onto ice water and subsequently extracted with methylene chloride (3 ×). The combined organic layers were washed with water (1 ×), brine (1 ×), dried (Na2SO4) and concentrated. The crude 2a was used in the next step without further purification. Yield: 22.8 g (68.2 mmol, 82%). 1H-NMR (300 MHz, DMSO-d6): δ [ppm]: 7.90 (d, 3J = 8.3 Hz, 2 H, HAryl), 7.35 (s, 1 H, CH), 4.92 (s, 1 H, CH). 13C-NMR (75.5 MHz, DMSO-d6): δ [ppm]: 179.66, 170.80, 137.81, 133.18, 127.65, 95.76, 75.99. MS (EI): m/e (intensity %): 430 (M+), 282 (45), 244 (45), 196 (62), 129 (29), 89 (38), 43 (39). Anal. Calcd for C10H7I3O4: C 29.37, H 1.48, N 6.85. Found: C 29.96, H 1.48, N 6.80.

3.3. General Procedure for 5-(4-Iodophenyl)-pyrimidine-2,4,6-trione (3a) and 5-(4-Benzylxylophenyl)-pyrimidine-2,4,6-trione (3b)

Under an argon atmosphere 2 eq. of sodium were dissolved in ethanol (0.35 mL/mmol Na) and 1.7 eq. of urea were added. A solution of malonic ester 2a or 2b in ethanol (2.2 mL/mmol) was added dropwise and the reaction mixture was heated to reflux for 6 h. After cooling to room temperature, the mixture was poured onto ice water and adjusted to pH 2 using dilute hydrochloric acid. The precipitate was collected by suction and dried in vacuo. Yield: 91%. Mp 233–236 °C. 1H-NMR (300 MHz, DMSO-d6): δ [ppm]: 11.34 (s, 1 H, OH), 7.4–6.94 (m, 9 H, HAryl). Anal. Calcd for C10H7I3O4: C 29.37, H 1.48, N 6.85. Found: C 29.96, H 1.48, N 6.80.

3.4. 5-Bromo-5-(4-iodophenyl)-pyrimidine-2,4,6-trione (4a)

A suspension of 3a (6.51 g, 19.7 mmol), N-bromosuccinimide (4.20 g, 23.6 mmol, 1.2 eq.) and a catalytic amount of dinitrobenzene in carbon tetrachloride (400 mL) was heated to reflux for a period of 3 h. After cooling to room temperature the mixture was concentrated, the residue was treated with water and extracted with ethyl acetate (3 ×). The combined extracts were washed with brine, dried (Na2SO4) and the solvent was evaporated. The residue was stirred in CHCl3 for 2 h to give a colorless solid. Yield: 4.10 g (10.0 mmol, 51%). Mp 183–186 °C. 1H-NMR (300 MHz, DMSO-d6): δ [ppm]: 11.47 (s, broad, s), 10.94 (s, broad, s), 8.64 (s, broad, s), 7.71 (d, 2 H, HAryl), 7.15 (d, 2 H, HAryl). 13C-NMR (75.5 MHz, DMSO-d6): δ [ppm]: 179.66, 170.80, 137.81, 133.18, 127.65, 95.76, 75.99. MS (EI): m/e (intensity %): 410 (M+), 408 (M+), 396 (M+), 329 (100), 282 (45), 244 (45), 196 (62), 129 (29), 89 (38). Anal. Calcd for C10H6BrI3O2: C 29.37, H 1.48, N 6.85. Found: C 29.96, H 1.48, N 6.80.

3.5. 5-(4-Benzylxylophenyl)-5-bromo-pyrimidine-2,4,6-trione (4b)

A suspension of the 3b (5.00 g, 16.1 mmol) in water (48 mL) was cooled to 0–5 °C and 48% HBr (3.25 mL, 28.4 mmol) and bromine (1.32 mL, 25.8 mmol) were added dropwise. After stirring for 4–5 h at 0–10 °C the precipitate was collected by filtration and dried in vacuo. Yield: 5.59 g (14.4 mmol, 89%). Mp 145–147 °C. 1H-NMR (300 MHz, DMSO-d6): δ [ppm]: 11.44 (broad, s), 7.43–7.01 (m, 9 H, HAryl), 5.08 (s, 2 H, CH2). 13C-NMR (75.5 MHz, DMSO-d6): δ [ppm]: 171.34, 159.00, 150.21, 137.18, 130.86...
128.79, 128.03, 127.99, 116.88, 115.30, 76.28, 69.70. MS (ESI-EM) m/e: 410.9951 (M + Na)+ calcd for 
C17H13BrN2O4Na 410.9956. Anal. Calcd for C17H13BrN2O4·0.3 H2O: C 52.10, H 3.42, N 7.15. Found: 
C 51.79, H 3.12, N 6.96.

3.6. General Procedure for Compounds 5a–5c

A solution of 4a or 4b in methanol (2–4 mL/mmol) was treated with 2 eq. of N-(2-hydroxyethyl)-
piperazine (in case of 5a and 5c) or 3-(piperazin-1-yl)-propionic acid (in case of 5b) and stirred for 2 d 
at room temperature. The colorless precipitate was collected by suction and dried in vacuo.

3.6.1. 5-[4-(2-Hydroxyethyl)piperazin-1-yl]-5-(4-iodophenyl)pyrimidine-2,4,6-trione (5a)

Yield: 53%. Mp 168–172 °C. 1H-NMR (400 MHz, DMSO-d6): δ [ppm]: 7.72 (d, 3J = 8.7 Hz, 2 H, 
HAr1), 7.16 (d, 3J = 8.7 Hz, 2 H, HAr2), 7.61 (d, 3J = 8.7 Hz, 2 H, HAr3), 3.43–2.31 (m, 12 H, CH2). 
13C-NMR (75.5 MHz, DMSO-d6): δ [ppm]: 169.36, 151.07, 138.72, 131.17, 130.13, 95.72, 85.71, 59.68, 58.20, 53.59, 47.24. MS (ESI-EM) 
m/e: 459.0518 (M + H)+ calcd for C16H20IN4O4 459.0524. Anal. Calcd for C16H19IN4O4·0.3 H2O: C 40.35, 
H 4.23, N 11.76. Found: C 40.93, H 4.54, N 11.47.

3.6.2. 5-[4-(2-Carboxyethyl)piperazin-1-yl]-5-(4-iodophenyl)pyrimidine-2,4,6-trione (5b)

Yield: 18%. Mp 139–142 °C. 1H-NMR (300 MHz, DMSO-d6): δ [ppm]: 9.42 (broad, s), 8.00 (d, 
3J = 8.7 Hz, 2 H, HAr1), 7.61 (d, 3J = 8.7 Hz, 2 H, HAr2), 7.30–3.21 (m, 14 H, CH2). 
13C-NMR (100 MHz, DMSO-d6): δ [ppm]: 173.74, 164.25, 151.49, 138.89, 135.07, 131.70, 86.35, 
85.64, 53.23, 49.47, 43.38, 32.01. MS (ESI-EM) m/e: 487.0453 (M + H)+ calcd for C17H19IN4O5 487.0473. 
Anal. Calcd for C17H19IN4O5·1.8 H2O: C 39.88, H 4.04, N 10.80. Found: C 39.59, H 4.23, N 10.48.

3.6.3. 5-[4-(2-Hydroxyethyl)piperazin-1-yl]-5-(4-benzyloxyphenyl)pyrimidine-2,4,6-trione (5c)

Yield: 28%. Mp 211–213 °C. 1H-NMR (400 MHz, DMSO-d6): δ [ppm]: 7.48–7.02 (m, 9 H, HAr1), 
5.09 (s, 2 H, CH2), 3.48–3.44 (m, 2 H, CH2OH), 2.57–2.38 (m, 10 H, CH2). 
13C-NMR (100 MHz, DMSO-d6): δ [ppm]: 171.08, 159.63, 150.35, 137.67, 130.00, 129.32, 128.78, 128.28, 128.08, 115.76, 74.99, 
70.26, 61.07, 59.28, 48.10, 44.91. MS (MALDI-TOF) m/e: 461 (M + Na)+, 439 (M + H)+. Anal. Calcd for 
C23H26N4O5·1 H2O: C 60.52, H 6.18, N 12.27. Found: C 60.35, H 5.71, N 12.27.

3.7. 5-[4-(2-Hydroxy-3-iodophenyl)-1-yl]-5-(4-hydroxyphenyl)pyrimidine-2,4,6-trione (5d)

Compound 5c (1.66 g, 3.79 mmol) was dissolved in absolute methanol (150 mL), treated with 
Pd/C (10%, 250 mg) and heated to reflux for 12 h under an H2 atmosphere. After cooling to room 
temperature, the mixture was stirred overnight. The catalyst was filtered off and washed with methanol 
(80 mL). The solvent was evaporated and the solid residue was dried in vacuo. The crude product was 
taken up in a CHCl3/ethylacetate mixture (1/1, v/v) and stirred at room temperature for 2–3 h and 
finally re-isolated by suction filtration. Yield: 1.08 g (3.31 mmol, 82%). Mp 185 °C. 1H-NMR (300 MHz, 
DMSO-d6): δ [ppm]: 7.45 (d, 3J = 8.6 Hz, 2 H, HAr1), 7.03 (d, 3J = 8.6 Hz, 2 H, HAr2), 3.79 (m, 2 H, 
CH2OH), 2.99–2.69 (m, 10 H, CH2). 
13C-NMR (75.5 MHz, DMSO-d6): δ [ppm]: 170.66, 158.47, 149.82, 
131.22, 129.29, 115.94, 74.63, 59.74, 57.66, 53.73, 46.67. MS (ESI-EM) m/e: 349.1521 (M + H)+ calcd for 
C16H12IN4O5 349.1506. Anal. Calcd for C16H20IN4O5·2.2 H2O: C 49.56, H 5.77, N 14.44. Found: C 49.08, 
H 5.54, N 14.02.

3.8. 5-(4-Hydroxy-3-iodophenyl)-5-[4-(2-hydroxyethyl)piperazin-1-yl]pyrimidine-2,4,6-trione (6)

Compound 5d (500 mg, 1.44 mmol) was dissolved in methanol (10 mL) and treated with 58 mg 
(1.44 mmol) sodium hydroxide and 216 mg (1.44 mmol) sodium iodide. The solution was cooled in 
an ice bath and 824 mg (687 µL, 1.44 mmol) sodium hypochlorite (13% active chlorine) were added 
dropwise over a period of 60 min. The orange suspension was stirred in the ice bath for further 2 h 
until a nearly colorless solution had formed. The ice bath was removed and 2–3 crystals sodium
thiosulfate were added at room temperature. The solution was acidified to pH 6.8 by adding 1 M HCl and extracted with ethylacetate (3 × 30 mL). The combined extracts were washed with brine (1 × 30 mL), dried (MgSO₄) and evaporated to dryness. Yield: 80 mg (0.17 mmol; 12%). Mp 168–170 °C (decomposition). MS (ESI-EM) m/e 157.32, 149.35, 137.99, 129.10, 127.04, 115.37, 84.56, 73.25, 59.71, 58.27, 53.70, 47.11. MS (ESI-EM) m/e.

3.9. General Procedure for Compounds 9 and 10

A solution of 5-bromo-5-[4-(4-iodo-phenoxy)phenyl]pyrimidine-2,4,6-trione 7 [8,12] in methanol (ca. 5–10 mL/mmol) was treated with 2.0 eq. of the appropriate piperazine (8 or 3-(piperazin-1-yl)-propionic acid) and stirred at room temperature overnight. The colorless solids which precipitated after ca. 1 h were collected by suction and dried in vacuo. Compound 10 was further purified by silica gel column chromatography (EtOAc/MeOH (4/1, v/v) + 1% NEt₃).

3.9.1. 5-[4-(2-Carboxylethyl)piperazin-1-yl]-5-[4-(4-iodophenoxy)phenyl]pyrimidine-2,4,6-trione (9)

Yield: 83%. Mp: 236–242 °C (decomposition). MS (ESI-EM) m/e 475.1529. The purity of 9 was determined by analytical gradient HPLC to be > 95% (system and conditions see Section 3.1). The retention times tᵣ were: tᵣ(9) = 25.92 ± 0.36 min (n = 3).

3.10. 5-[4-(2-Carboxylethyl)piperazin-1-yl]-5-[4-(4-(tributylstannyl)phenoxy)phenyl]pyrimidine-2,4,6-trione (11)

PdCl₂ (PMePh₂)₂ (18 mg, 30 μmol, 3 mol%) or PdCl₂ (18 mg, 100 μmol, 10 mol%), KOAc (294 mg, 3.00 mmol, previously dried for several hours at 100 °C in vacuo before use) and N-methyl-pyrrolidinone (NMP, 15 mL) were mixed under an argon atmosphere [20]. Compound 9 (578 mg, 1.00 mmol) and tributyltin hydride (582 mg, 2.00 mmol) or hexabutylditin (870 mg, 1.50 mmol) were added and the mixture was heated to 110 °C for 10–15 h. The progress of the reaction was monitored by HPLC (system and conditions see Section 3.1). The retention times tᵣ were: tᵣ(9) = 34.15 ± 1.38 min (n = 10), tᵣ(11) = 40.83 ± 1.17 min (n = 6). When the conversion was complete, the hot black reaction mixture was filtered through a pad of Celite by suction and the filter cake was washed with a small amount of NMP. The orange filtrate was stored at −30 °C overnight. The beige solid which precipitated upon cooling was isolated by suction. When there was no precipitation, the volatile components of the filtrate were removed by short-path distillation. The solid residue was taken up in hot methanol and insoluble components were filtered off. The solvent was removed in vacuo and the residue was treated with acetone. Yield: 208 mg (0.28 mmol, 28%). The purity of the product was confirmed by analytical gradient HPLC (system and conditions see Section 3.1). The purity of 11 was determined to be > 95% (system and conditions see Section 3.1) by HPLC, tᵣ = 20.88 ± 0.56 min (n = 3).
was >95% as determined by analytical gradient HPLC. Mp: 285–286 °C (decomposition). 1H-NMR (400 MHz, DMSO-d6): δ [ppm]: 7.89–7.12 (m, 8 H, HAr), 3.56–1.94 (m, 12 H, CH2), 1.77–1.67 (m, 6 H, CH2), 1.56–1.44 (m, 6 H, CH3), 1.25–1.20 (m, 6 H, CH2), 1.06 (t, J = 8.3 Hz, 9 H, CH3). MS (ESI-EM) m/e: 585.1847 (M – C7H13N2O2)⁺ calcd for C25H37N2O4Sn 585.1775.

3.11. General Methods. Radiochemistry

N. c. a. [124I]NaI was provided by Department of Nuclear Medicine, University Hospital Essen, University Duisburg-Essen, Germany. Typical radioactivities used for the radiiodination were 134 ± 48 MBq [124I]NaI in 50 ± 18 µl in 0.01 N NaOH. N. c. a. [123I]NaI was purchased from GE Healthcare Buchler GmbH & Co KG (Braunschweig, Germany). Typical radioactivities used for the radiiodination were 136 ± 63 MBq [123I]NaI in 10 ± 3 µl in 0.05 N NaOH. Separation, purification and analyses of the radiochemical yields and the radiochemical purities of all radioiodinated compounds were performed by a gradient radio-HPLC chromatograph system (HPLC A) using a Knauer K-500 and a Latek P 402 pump, a Knauer K-2000 UV-detector (λ = 254 nm) a Crismatec NaI(Tl) Scintibloc 51 SP51 γ-detector and a RP-HPLC Nucleosil column 100-5 C18 (250 mm × 4.6 mm), a corresponding precolumn (20 mm × 4.0 mm). Sample injection was carried out using a Rheodyne injector block (type 7125 incl. 200 µL loop). The recorded data were processed by the NINA version 4.9 software (GE Medical Systems-Functional Imaging GmbH, Münster, Germany). Separation and purification of the radiosynthesized compounds were—unless specified otherwise—performed by radio-HPLC (HPLC A, see above) using a Nucleosil 100-10 C18 column (250 mm × 8 mm). The recorded data were processed by the NINA version 4.9 software. The radiochemical purities and the specific activities of the radiolabelled product were determined using a radiochemical gradient HPLC chromatograph system (HPLC B) composed of a Sykam S1021 pump, a Knauer K-2501 UV-detector (λ = 254 nm), a Crismatec NaI(Tl) Scintibloc 51 SP51 γ-detector, a Nucleosil 100-3 C18 column (200 mm × 3 mm), a VICI injector block (type C1 incl. 20 µL loop) and the GINA Star version 4.07 radiochromatography software (raytest Isotopenmeßgeräte GmbH, Straubenhardt, Germany). Radio-TLC was analyzed using a miniGITA TLC-Scanner (raytest Isotopenmeßgeräte GmbH, Straubenhardt, Germany).

3.12. 5-[4-(2-Carboxyethyl)piperazin-1-yl]-5-[4-(4-[123/124I]-iodophenoxy)phenyl]pyrimidine-2,4,6-trione ([123/124I]9)

In a conical glass vial 89 µg (0.12 µmol) stannyl precursor 11 in 39 µl MeOH was added to n. c. a. [123I]Na or [124I]Na. The radiosynthesis was started by addition of 34 mg (0.14 µmol) chloroamine-T hydrate in 39 µl 0.1 M K2HPO4 buffer (pH 7.34). The mixture was vortexed for 10 s and allowed to stand for 5 min at room temperature. To quench the reaction 50 µL of a 10% Na2SO3 solution (in water for injection) was added and the mixture was vortexed again for 10 s. After 10 min the quenched reaction solution was injected onto the HPLC column (see Section 3.11. HPLC A) to isolate the fraction of the radiolabelled product [124I]9 (HPLC conditions: eluent A: CH3CN/H2O/TFA 950/50/0.1, v/v/v; gradient: from 92% B to 40% B within 45 min, 5 min constant at 40% B, and from 40% B to 92% B within 5 min at a flow rate of 2.0 mL/min, λ = 254 nm; tR (stannyl precursor 11) = 40.80 ± 0.81 min (n = 3), tR ([124I]9) = 32.75 ± 2.13 min (n = 3). The product fraction was evaporated to dryness and redissolved in 0.9% NaCl (200 µL). The decay-corrected radiochemical yield (after evaporation) was 28 ± 7% (n = 6) for [123I]9 and 44 ± 6% (n = 3) for [124I]9, respectively. An aliquot (50 µL) was taken for analytical radio-HPLC. The radiochemical purities were 95 ± 3% and 93 ± 5% for [123I]9 and [124I]9, and the molar activities were 0.2–6.3 GBq/µmol and 0.4–14.0 GBq/µmol, respectively. The identity of [123I]9 and [124I]9 was proven by HPLC using the non-radioactive analog 9.

3.13. In Vitro Enzyme Inhibition Assays

The synthetic fluorometric substrate (7-methoxycoumarin-4-yl) acetyl pro-Leu-Gly-Leu-(3-(2,4-dinitrophenyl)-l-2,3-diamino-propionyl)-Ala-Arg-NH2 (R & D Systems, Minneapolis, MN, USA)
was used to assay activated MMP-2, MMP-8, MMP-9 and MMP-13 as described previously [21]. The inhibitions of human active MMP-2, MMP-8, MMP-9 MMP-13 and MMP-14 (only 9) by the barbituric acid derivatives 9 and 10, of human active MMP-2 and MMP-9 by 5a, 5b and 6 were assayed by preincubating MMP-2, MMP-3, MMP-8, MMP-9, MMP-13 or MMP-14 (each at 2 nM) and inhibitor compounds at varying concentrations (10 pM–1 mM) in 50 mM Tris·HCl, pH 7.5, containing 0.2 M NaCl, 5 mM CaCl₂, 20 µM ZnSO₄ and 0.05% Brij 35 at 37 °C for 30 min. An aliquot of substrate (10 µL of a 50 µM solution) was then added to 90 µL of the preincubated MMP/inhibitor mixture, and the fluorescence was determined at 37 °C by following product release with time. The fluorescence changes were monitored using a Fusion Universal Microplate Analyzer (Packard Bioscience, Boston, MA, USA) with excitation and emission wavelengths set to 330 and 390 nm, respectively. Reaction rates were measured from the initial 10 min of the reaction profile where product release was linear with time and plotted as a function of inhibitor dose. From the resulting inhibition curves, the IC₅₀ values for each inhibitor were calculated by non-linear regression analysis, performed using the Grace 5.1.8 software (Linux).

4. Conclusions

Starting from the lipophilic preclinical research tracer [125]I₁₂ (clogD 3.53, Table 2), that was already developed by our group [12], we intended to develop a more hydrophilic radioiodinated barbiturate-based MMP-targeted tracer for the potential non-invasive visualization of activated MMPs in vivo. This was achieved by the substitution of the hydroxy group of 12 by a carboxy group yielding derivative 9 with a hydrophilic shift compared to 12 (clogD 0.92 vs. 3.53). Similar to 12 carboxylic acid 9 represents a very potent inhibitor of MMP-2 and -9 (IC₅₀ (MMP-2) = 29 nM, IC₅₀ (MMP-9) = 1.3 nM). Therefore radioiodinated analogues [123/124]I₉ were successfully synthesized for further in vivo evaluations with SPECT and PET.

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