Full Paper

Synthesis and Characterization of Thiol Containing Furoxan Derivatives as Coligands for the Preparation of Potential Bioreductive Radiopharmaceuticals

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The synthesis and characterization of thiol-containing 1,2,5-oxadiazole *N*-oxide (TONO) derivatives and their use as monodentate coligands for the preparation of ^{99m}Tc complexes is presented. 3-Mercaptomethyl-4-phenyl-1,2,5-oxadiazol N_2-oxide and 3-(4-mercaptophenylmethylidenhydrazinocarbonyloxymethyl)-4-phenyl-1,2,5-oxadiazol N_2-oxide were successfully synthesized and combined with the tridentate ligand *N*,*N*-bis(2-mercaptoethyl)-*N*′,*N*′-diethylethylenediamine (BMEDA) to prepare "3+1 mixed ligand" technetium complexes. The ^{99m}Tc complexes were obtained in high yield and radiochemical purity using low concentration of ligand and coligand. An alternative procedure using a xantate and a disulphide precursor of 3-mercaptomethyl-4-phenyl-1,2,5-oxadiazol N_2-oxide yielded the same complex. Biological evaluation of the potentiality of the ^{99m}Tc complexes as bioreductive radiopharmaceuticals was performed in normal CD1 mice and in mice bearing induced sarcoma. Tumour uptake was moderate but tumour/soft tissue ratio was favourable. Although these results are encouraging, further development is still necessary in order to achieve higher tumour uptake and lower gastrointestinal activity.

Keywords: 1,2,5-Oxadiazole *N*-oxide derivatives / Bioreductive ^{99m}Tc radiopharmaceuticals / Solid tumour imaging

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Introduction

Many metallic elements play a crucial role in living systems. Electron-deficient metal ions interact with a variety of electron-rich molecules involved in essential biological functions such as proteins, DNA, etc. [1]. A natural consequence of this implication has been the development of medicinal inorganic chemistry, a relatively new field dealing with the application of coordination chemistry for medicinal purposes [2]. One important area related to this new discipline is radiopharmacy, the science devoted to the development of radioactive tracers for human diagnosis and therapy. Most of these tracers called radiopharmaceuticals are coordination compounds containing radioactive isotopes of Tc, Re, Sm, Lu, Ho, among others [3]. Diagnostic radiopharmaceuticals are based on gamma-emitters whose radiation readily escapes from the body, permitting external detection and measurement. The pattern of distribution of radiation in the body allows the physician to make a diagnostic evaluation of both morphology and function [4]. This is a unique feature, since other imaging modalities such as computed tomography or magnetic resonance give only anatomical information. ^{99m}Tc is the preferred radio-

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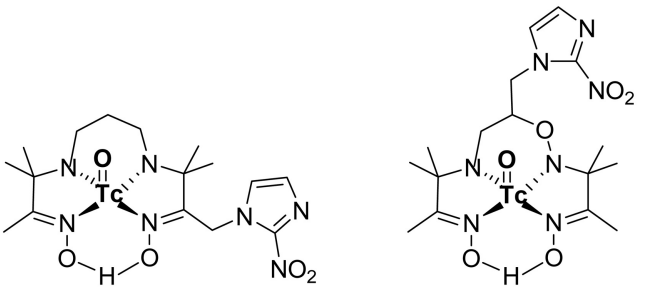
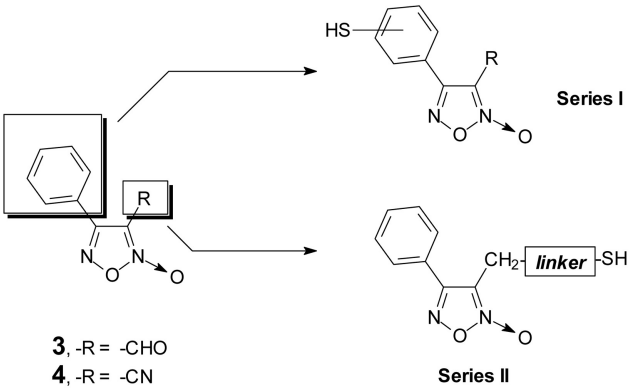
nuclide for diagnosis, due to its ideal nuclear properties for imaging ($t_{1/2}$ = 6 h, Eγ = 140 keV). As a transition element, its chemistry is dominated by the formation of coordination compounds. Technetium radiopharmaceuticals have the metal bound to a transporting moiety that delivers the radioactivity to a specific site in the body determined by the properties of the transporter [5]. Current research is directed towards compounds that imitate bioactive substrates and can be used to evaluate biochemical functions *in vivo* in a non-invasive way [6, 7]. The design of a ligand for the preparation of novel ^{99m}Tc-radiopharmaceuticals involves the combination of the active part of a biomolecule (pharmacophore) with appropriate chelating groups to bind the metal. Technetium chelate should be separated from the pharmacophore in order to prevent interference with the biological activity [8]. Proposal of a synthetic strategy for such a ligand is a crucial step in the development of new compounds.

An area of special interest in radiopharmacy is the development of suitable tracers for imaging hypoxic tissue. Oncology would highly benefit from agents that effectively target hypoxic cell populations of solid tumours, due to their increased radioresistance and diffusion limitations that hinder the treatment [9]. Bioreductive compounds, which are selectively reduced in hypoxic tissue to reactive intermediates that bind to intracellular molecules, have been utilized for the development of potential radiodiagnostic markers of tumour hypoxia. 2-Nitroimidazole has been the preferred bioreductive pharmacophore and propylene amine oxime (PnAO) the chelator for technetium attachment. Figure 1 shows two examples: BMS-181321 ([^{99m}Tc]oxo[[3,3,9,9-tetramethyl-1-(2-nitro-1*H*-imidazol-1-yl)-4,8-diazaundecane-2,10-dionedioximato]-(3)-*N*,*N*′,*N*″,*N*‴] technetium(V)) and BRU59-21 ([^{99m}Tc]oxo-[[3,3,9,9-tetramethyl-6-[(2-nitro-1*H*-imidazol-1-yl)methyl]-



BMS-181321 **BRU59-21**

Figure 1. Proposed ^{99m}Tc bioreductive radiopharmaceuticals bearing 2-nitroimidazoles as pharmacophore.



Scheme 1. Synthesis of thiol-containing 1,2,5-oxadiazole *N*-oxide (TONO) derivatives.

5-oxa-4,8-diazadioximato]-(3)-*N*,*N*′,*N*″,*N*‴] technetium (V)) [10].

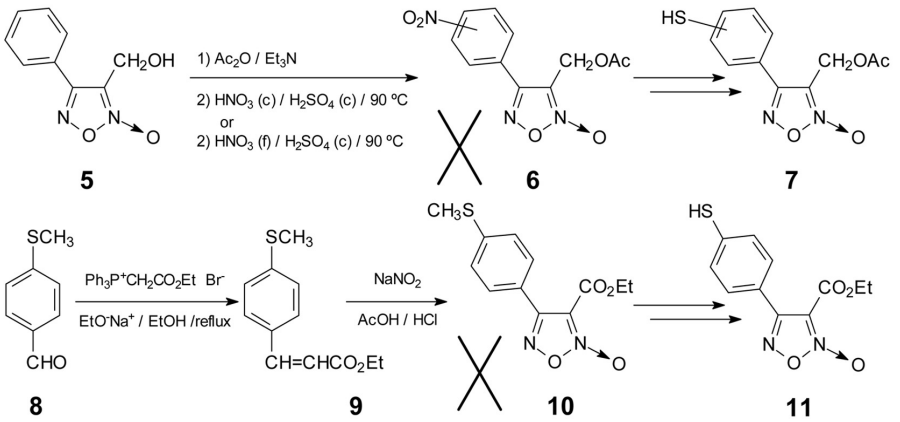
With the aim to develop new potential ^{99m}Tc-radiopharmaceuticals for imaging hypoxia, we have selected the *N*-oxide functional group as the bioreductive pharmacophore [11]. In the past years, we have described different approaches in the development of new *N*-oxide containing heterocycles as hypoxic selective cytotoxic agents [12], among them 1,2,5-oxadiazole *N*-oxide scaffold has been extensively studied [13, 14, 15]. The "3+1" mixed ligand approach, based on the simultaneous coordination of a tridentate ligand and a monodentate coligand to the metal, was chosen to attach the pharmacophore to the radionuclide [16]. The diaminodithiol *N*,*N*-*bis*(2-mercaptoethyl)-*N*′,*N*′-diethylethylenediamine (BMEDA) binds to the $[Tc(V)O]^{+3}$ core leaving an open coordination position that can be occupied by a monodentate thiol containing the pharmacophore [17].

Herein, we describe the synthesis and characterisation of thiol-containing 1,2,5-oxadiazole *N*-oxide (TONO) derivatives (Scheme 1) and their use as monodentate coligands for the preparation of ^{99m}Tc complexes. In order to assess their potentiality as hypoxia imaging radiopharmaceuticals a preliminary evaluation in normal and tumour bearing mice is also presented.

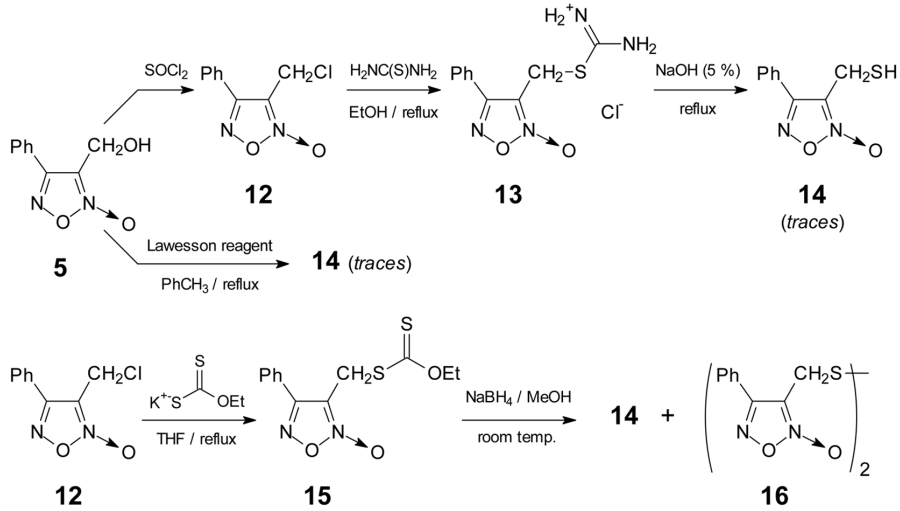
Results

Chemistry

Using the approach shown in Scheme 1, we designed two different series of TONO derivatives. For series-I derivatives, the procedures depicted in Scheme 2 were assayed in order to prepare compounds **7** and **11**. However, nitration of compound **5**, in different conditions, did not afford the desired product **6**, precursor of TONO **7**. On the



Scheme 2. Procedures tested to prepare compounds **7** and **11**.

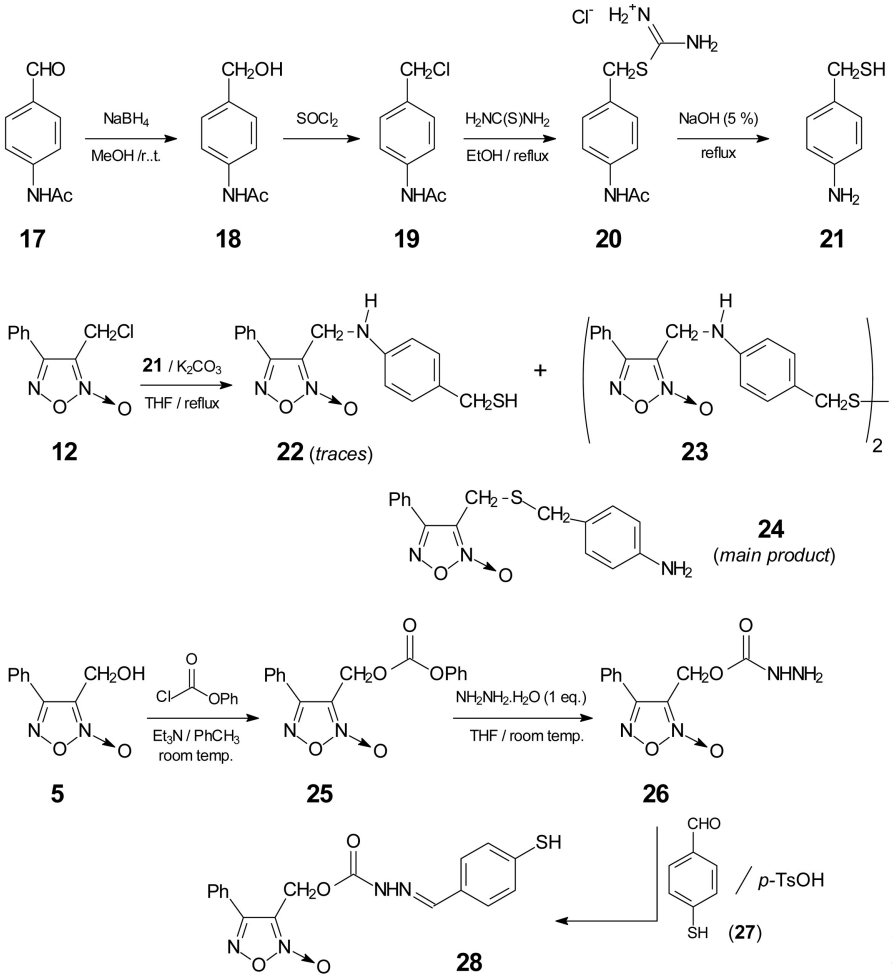


Scheme 3. Synthetic routes of thiol **14.**

other hand, we assayed the preparation of series-I derivatives from phenylalkene bearing a thiol moiety. Thus, we prepared via Wittig procedure compound **9** which was unable to react with $NaNO_2$ in acid medium to yield the furoxan heterocycle [18].

For series-II derivatives, we designed TONO with and without linker moiety (see Scheme 1). In the first case, derivative **14** synthesis was assayed using different methodologies (Scheme 3) [19]. The pathway from alcohol **5**, via the chloride **12** [14] and the salt **13**, produced the desired product in very low yield because the last step (hydrolysis in basic medium) produced a complex mixture of products. The direct transformation of alcohol **5** to thiol **14** using Lawesson's reagent yielded the desired product (in low yield remaining **5**). Finally, thiol **14** was prepared via the xantate **15**, followed by the reduction in mild conditions, as shown in Scheme 3. In these conditions **14** was isolated chromatographically together with the dimmer **16**. Stability of derivative **14** was not extensively studied, but it was necessary to store it under nitrogen atmosphere and at −20°C in order to avoid decomposition. For the synthesis of "3+1" Tc complexes (see below) freshly prepared derivative **14** was used.

For series-II derivatives with different linker moieties (Scheme 1), two different approaches were assayed (Scheme 4). Starting from chloride **12**, achievement of derivative **22** was intended via a nucleophilic substitution process involving the reactant **21**, prepared from aldehyde **17**. Because the sulphur is the best nucleophile in the reaction media, derivative **24** was the main product of reaction. However, this product is not adequate for coordination with Tc in our "3+1" approach. Derivative **22** and dimmer **23** were generated as marginal products in the assayed conditions. So, we decided to use another approach in order to generate a TONO with phenyl linker. Consequently, the synthetic procedures shown in Scheme 4 were chosen to prepare derivative **28** [15]. This compound was obtained in good yields from alcohol **5**, via the carbonate **25** and the carbazate **26,** which reacted with aldehyde **27**, obtained from aldehyde **8** [20]. All new



Scheme 4. Synthetic procedures for derivatives **22** and **28** [12c].

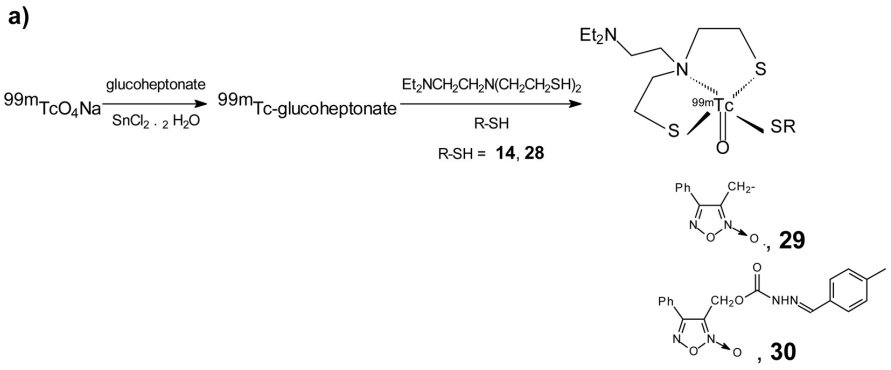
1,2,5-oxadiazole *N*-oxide derivatives were characterised by IR, MS, and one- and two-dimensional ^{1}H-NMR, ^{13}C-NMR experiments, and their purity was established by TLC and microanalysis.

The TONO derivatives **14** and **28** were used as monodentate coligands together with the tridentate ligand BMEDA in the preparation of "3+1" ^{99m}Tc mixed ligand complexes. Both compounds were prepared by ligand-exchange reaction using ^{99m}Tc-glucoheptonate as precursor together with ligand and coligand in a ratio of 1 : 1, as shown in Scheme 5. According to previous experience and literature data about "3+1 mixed ligand" technetium complexes bearing BMEDA as tridentate ligand, we expect the formation of neutral and lipophilic complexes due to the ionisation of the sulphur groups of ligand and coligand [16, 21]. Consequently, isolation of the mixed ligand complexes from the reaction mixture was achieved upon extraction by dichloromethane. Complexes **29** and **30** were obtained in high yield (>85%, determined by CH_2Cl_2 extraction) and with high radiochemical purity (>90%, determined by HPLC), using low amount (2×10^{-5} mol) of the coligand that carries the pharmacophore group. The radioactivity recovery from the column after injection of complexes was monitored by means of an on-line solid scintillation detector coupled to the HPLC system and found to be quantitative.

An alternative route for the preparation of ^{99m} Tc complexes is shown in Scheme 5b. Xantate **15** and dimmer **16** were used as precursors of compound **14** and reacted with ^{99m}Tc-glucoheptonate in the presence of BMEDA to achieve complex **29**. Although the thiol groups are necessary for the complexation, the presence of an excess of the strong reducing agent $SnCl_2$ produced enough amount of compound **14** to achieve the desired final product as shown by HPLC analysis.

Biodistribution studies

In order to assess the potentiality of our approach for the design of radiopharmaceuticals, a preliminary evaluation in normal and tumour-bearing mice was performed.



Scheme 5. Preparation of ^{99m} Tc complexes **29** and **30**.

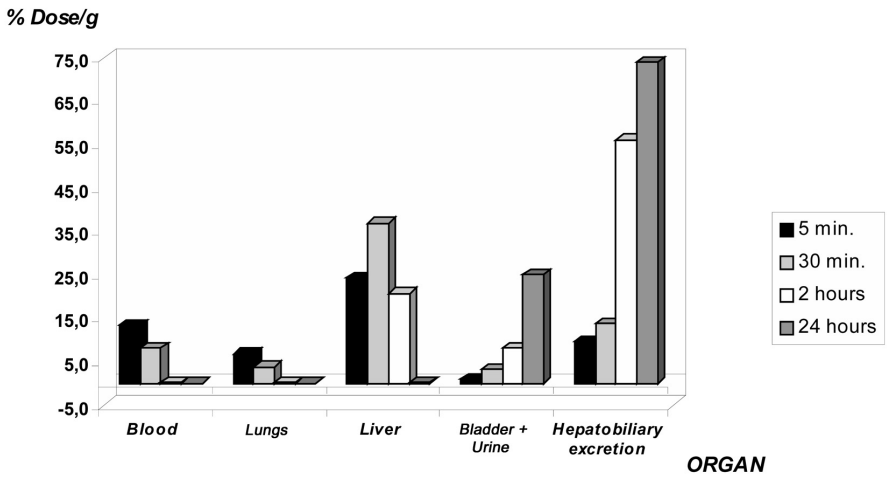
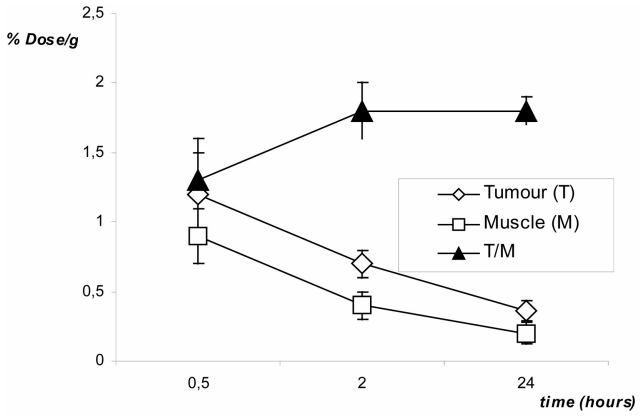


Figure 2. Biodistribution of complex **29** in normal CD1 mice.

Normal biodistribution of ^{99m} Tc complex **29** as a function of time is shown in Figure 2. The compound showed high initial blood, lung, and liver uptake (13.3%, 6.8%, and 24.4%, respectively). This behaviour is similar to the previously reported "3+1" complexes obtained with the same ligand [16, 21]. Blood and lungs clearance was quite fast (% injection dose in blood and lungs 0.36% and 0.18%, respectively at 2 h post-injection), while liver activity remained high for a longer period (% injection dose 20.7% at 2 h post-injection). The radioactivity from the novel technetium complex was excreted mainly through the hepatobiliary system (56% at 2 h post-injection). On the other hand, urinary excretion was low (8.2% in urine at 2 h post-injection). Depuration from blood and soft tissues, after 24 h, was almost complete and excretion after this period above 98%. Stomach and thyroid values were within acceptable levels (approximately 0.4% and 0.1%, respectively at 2 h post-injection) demonstrating no *in vivo* reoxidation.

Behaviour of ^{99m}Tc complex **29** in mice bearing induced sarcoma as a function of time is shown in Figure 3. Initial tumour uptake was moderate (1.2%/g at 30 min post-injection) but clearance was rather slow (0.7%/g and 0.35%/g after 2 and 24 h, respectively). Tumour to muscle ratio was favourable at all time points and increased with time (1.3, 1.8, and 1.8 at 30 min, 2 h, and 24 h post-injection, respectively) due to soft tissue depuration.

Overall biodistribution and tumour uptake of complex **30** was studied at one time point (12 h) for comparison. *In vivo* behaviour was analogous to complex **29**, with low blood, lung, and liver activity (0.2%, 0.7%, and 3.6%, respectively) and very high hepatobiliary elimination (above 80%). Tumour uptake was also moderate (0.4%/g) and tumour/muscle ratio was about 2. Due to these simi-



% DOSE / g	0.5 h	2 h.	24 h
TUMOUR (T)	1.2 ± 0.3	0.7 ± 0.1	0.35 ± 0.06
MUSCLE (M)	0.9 ± 0.2	0.4 ± 0.1	0.20 ± 0.08
T/M	1.3 ± 0.3	1.8 ± 0.2	1.8 ± 0.1

Figure 3. Tumour uptake and retention of complex **29** in CD1 mice bearing induced sarcoma.

larities in *in vivo* behaviour no other time points were studied.

Discussion

A series of new furoxan derivatives bearing thiol moieties were synthesized and assayed as coligands for the preparation of technetium "3+1 mixed ligand" complexes with potential application as bioreductive radiopharmaceuticals.

Preparation of phenylthio derivatives of 'series I' (Scheme 1) was attempted by different procedures but unfortunately without success. Derivatives of 'series II' (Scheme 1) were successfully produced, in this sense two different linkers were used to join the thiol moiety with the furoxan system. Derivative **14** was developed from chloride **12** (Scheme 3) and derivative **28** from alcohol **5** (Scheme 4).

These compounds were used together with equimolecular amounts of the well known ligand BMEDA to prepare two novel ^{99m}Tc compounds, whose proposed structure based on the ligand system used and the existing literature data is shown in Scheme 5. The ^{99m}Tc complexes were obtained in high yield and radiochemical purity using low concentration of ligand and coligand.

The xantate **15** and disulphide **16** were able to produce, in the reductive medium of the reaction, complex **29** using ^{99m}Tc-glucoheptonate as precursor.

In vivo behaviour for complexes **29** and **30** was the expected for this kind of compounds. Tumour uptake was moderate but tumour/soft tissue ratio was favourable. Although these results are encouraging, further development is still necessary in order to achieve higher tumour uptake and lower gastrointestinal activity.

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Experimental

Chemistry

All starting materials were commercially available research-grade chemicals and used without further purification. All solvents were dried and distilled prior to use. All the TONO syntheses were carried out in nitrogen atmosphere. The typical work-up included washing with brine and drying the organic layer with sodium sulphate before concentration. Melting points were determined using a Leitz Microscope Heating Stage Model 350 (Leitz, Wetzlar, Germany) apparatus and are uncorrected. Elemental analyses were obtained from vacuum-dried samples (over phosphorous pentoxide at 3–4 mm Hg, 24 h at room temperature), performed on a Fisons EA 1108 CHNS-O analyzer (Fisons, Valencia, CA, USA), and were within (±0.4% of theoretical values. ^{1}H-NMR, ^{13}C-NMR spectra and HETCOR experiments were recorded on a Bruker DPX-400 (at 400 MHz and 100 MHz (Bruker, Rheinstetten, Germany)) instrument, with tetramethylsilane as the internal reference and in the indicated solvent. Mass spectra were recorded on a Shimadzu GC-MS QP 1100 EX (Shimadzu, Kyoto, Japan) instrument using electron impact ionization at 70 eV. The compounds **5**, **12**, **27**, BMEDA and ^{99m}Tc-glucoheptonate were prepared as previously reported [14, 20, 21, 22]. [^{99m}Tc] $NaTcO_4$ was obtained from an Elumatic III generator (Cis-Bio-international). High performance liquid chromatography (HPLC) analysis was performed on a LC-10 AS Shimadzu Liquid Chromatography System coupled to both SPD-M10A, Shimadzu photodiode array detector (UV trace for ligands) and a Parken 3" × 3" NaI (Tl) crystal scintillation detector (γ trace for ^{99m}Tc). Separations were achieved on a reverse phase μ Bondapack C18 column (3.9 × 300 mm), eluted with a binary gradient system at a 1.0 mL/min flow rate. Mobile phase A was phosphate buffer pH 7.4 with 2% triethylamine while mobile phase B was methanol. The elution profile was: 0 min 0% B followed by a linear gradient to 100% B in 7 min; this composition was held for another 15 min. Activity measurements were performed either in a Capintec CRC-5R dose calibrator or in a scintillation counter, using a 3" × 3" NaI (Tl) crystal detector associated to an ORTEC monochanel analyzer.

*O-Ethyl S-[(2-oxide-4-phenyl-1,2,5-oxadiazole-3-yl)-methyl]xantate **15***

A mixture of 3-chloromethyl-4-phenyl-1,2,5-oxadiazole N^2-oxide, **12** (1 eq.), potassium ethylxantate (1 eq.) and THF as solvent was

stirred at reflux until the chloride reactant was not present (Al_2O_3, 5% EtOAc in petroleum ether). The resulting precipitate, KCl, was filtered and washed with THF. The resulting organic layer was evaporated *in vacuo* and the residue was purified by chromatography. Yellow-white needles (60%). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.39 (t, 3H, *J* = 7.1 Hz, −C*H*$_3$), 4.45 (s, 2H, Ar-C*H*$_2$), 4.63 (q, 2H, *J* = 7.1 Hz, O−C*H*$_2$), 7.58 (m, 3H, Ph), 7.69 (m, 2H, Ph). ^{13}C-NMR (HMQC, HMBC) ($CDCl_3$, 100 MHz) δ (ppm): 14.05 (−*C*H$_3$), 28.62 (Ar-*C*H$_2$), 71.36 (O−*C*H$_2$), 112.11 (−*C*=N$^+$−O$^-$), 126.69, 128.15, 129.76, 131.68 (Ph−*C*), 156.84 (−*C*=N), 210.96 (−*C*=S). MS *m/z* (rel. int.%): 296 (M$^+$, 30.0%), 280 (8.0). mp (°C) 56.0−57.0. Anal. Calcd. for $C_{12}H_{12}N_2O_3S_2$: C, 48.6; H, 4.1; N, 9.5; S, 21.6. Found: C, 48.5; H, 4.0; N, 9.1; S, 21.2.

3-Mercaptomethyl-4-phenyl-1,2,5-oxadiazol N$_2$-oxide ***14***

Derivative **15** (1 eq.) dissolved in THF was cooled at 0°C. Then, three portions of $NaBH_4$ (1 eq.) were added over the vigorously stirred solution. The mixture was stirred at room temperature until the xantate reactant was not present any more (SiO_2, 5% EtOAc in petroleum ether). The mixture of reaction was evaporated *in vacuo* and the residue was purified by chromatography (SiO_2, petroleum ether : EtOAc (0 to 10%)). In the chromatographic process, thiol **14** was mainly converted into derivative **16**.

14: Brown oil (5%). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 2.50 (brs, 1H, SH), 4.15 (s, 2H, Ar-C*H*$_2$), 7.55 (m, 3H, Ph), 7.70 (m, 2H, Ph). ^{13}C-NMR (HMQC, HMBC) ($CDCl_3$, 100 MHz) δ (ppm): 22.00 (Ar-*C*H$_2$), 110.00 (−*C*=N$^+$−O$^-$), 126.75, 128.50, 129.80, 132.00 (Ph−*C*), 155.90 (−*C*=N). MS *m/z* (rel. int.%): 208 (M$^+$, 5.0), 192 (1.5), 178 (0.5).

16: Yellow oil (75%). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 4.20 (s, 2H, Ar-C*H*$_2$), 7.60 (m, 3H, Ph), 7.68 (m, 2H, Ph). ^{13}C-NMR (HMQC, HMBC) ($CDCl_3$, 100 MHz) δ (ppm): 30.00 (Ar-*C*H$_2$), 111.00 (−*C*=N$^+$−O$^-$), 126.60, 128.55, 129.70, 131.90 (Ph−*C*), 156.00 (−*C*=N). MS *m/z* (rel. int.%): 414 (M$^+$, 35.0), 398 (2.0), 382 (1.0), 358 (0.5). Anal. Calcd. for $C_{18}H_{14}N_4O_4S_2$: C, 52.2; H, 3.4; N, 13.5; S, 15.5. Found: C, 51.8; H, 3.1; N, 13.1; S, 15.1.

Phenyl (4-phenyl-2-oxide-1,2,5-oxadiazole-3-yl)methyl carbonate ***25***

Phenyl chloroformate (1 eq.) was added to a stirred and cooled (0°C) solution of the alcohol **5** (1 eq.) and triethylamine (1 eq.), using toluene as solvent. After addition, the mixture was stirred at room temperature for 45 min. After the work-up the solvent was evaporated to give product **25** which were pure enough to be used in the next preparation without further purification. Colourless oil (95%). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 5.40 (s, 2H, Ar-C*H*$_2$), 7.00 (m, 3H, Ph), 7.20 (m, 2H, Ph), 7.60 (m, 3H, Ph), 7.72 (m, 2H, Ph). MS *m/z* (rel. int.%): 312 (M$^+$, 8.5), 296 (0.5).

O-(4-Phenyl-2-oxide-1,2,5-oxadiazole-3-yl)methyl carbazate ***26***

A mixture of **25** (1 eq.) and hydrazine monohydrate (1 eq.) in THF as solvent was stirred at room temperature until the carbonate was not present any more. The solvent was evaporated and the residue was treated with ethyl acetate and washed with aqueous NaOH (5%). The organic layer was dried with sodium sulphate and evaporated to give product **26** which were pure enough to be used in the next preparation without further purification. Yellow-brown oil (69%). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ (ppm): 5.25 (s, 2H, Ar-C*H*$_2$), 6.00 (brs, 2H, −N*H*$_2$), 7.00 (m, 3H, Ph), 7.55 (m, 3H, Ph), 7.70 (m, 2H, Ph), 8.00 (brs, 1H, NH). MS *m/z* (rel. int.%): 250 (M$^+$, 2.0), 234 (6.0).

3-(4-Mercaptophenylmethylidenhydrazinocarbonyloxy-methyl)-4-phenyl-1,2,5-oxadiazol N$_2$-oxide ***28***

To a stirred mixture of aldehyde **27** (1 eq.) and *p*-toluenesulfonic acid (catalytic amounts) in toluene, derivative **26** was added in three portions. The suspension was stirred at room temperature until the aldehyde was not present any more. The mixture of reaction was evaporated *in vacuo* and the residue was purified by chromatography (SiO_2, CH_2Cl_2 : MeOH (0 to 5%)). Yellow oil (50%). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 5.35 (s, 2H, Ar-C*H*$_2$), 7.20−7.80 (m, 10H, Ph+S*H*), 8.00 (s, 1H, C*H*=N), 9.00 (brs, 1H, N*H*). ^{13}C-NMR (HMQC, HMBC) ($CDCl_3$, 100 MHz) δ (ppm): 55.00 (Ar-CH_2), 109.00 (−*C*=N$^+$−O$^-$), 125.00−133.00 (Ph−*C*), 144.00 (−*C*=N), 155.40 (−*C*=N), 156.00 (−*C*=O). MS *m/z* (rel. int.%): 370 (M$^+$, 3.5), 354 (1.0). Anal. Calcd. for $C_{17}H_{14}N_4O_4S$: C, 55.1; H, 3.8; N, 15.1; S, 8.7. Found: C, 54.9; H, 3.6; N, 14.7; S, 8.4.

General procedure for the preparation of complexes ***29*** *and* ***30***

A vial containing a lyophilized mixture of 200 mg calcium glucoheptonate and 0.2 mg $SnCl_2 \times 2H_2O$ was reconstituted with 5 mL water, and 0.5 mL of this solution was mixed with 0.5 − 1.0 mL [^{99m}Tc]$NaTcO_4$ with an activity of 185 − 1850 MBq (5 − 50 mCi). The ligand BMEDA (0.02 mmol, 4.7 mg) and the coligand **14** or **28** (0.02 mmol) were added and the mixture was agitated in a vortex mixer and left to react at room temperature for 10 minutes. The lipophilic species were extracted with CH_2Cl_2 and the organic layer dried with $MgSO_4$, filtered, and analysed by HPLC. When the same procedure was performed with **15** or **16** instead of coligand **14**, formation of complex **29** was evidenced by HPLC.

*[^{99m}TcO(BMEDA)(**14**)]* ***29***

Yield: >85%. Radiochemical purity: >90% $t_{R,\,HPLC}$ = 13.5 min.

*[^{99m}TcO(BMEDA)(**28**)]* ***30***

Yield: >85%. Radiochemical purity: >90% $t_{R,\,HPLC}$ = 14.1 min.

Biodistribution studies

Animal studies were approved by the Ethics Committee of the Faculty of Chemistry from Uruguay. *Ex vivo* evaluation of ^{99m}Tc complex **29** was performed by biodistribution using either normal mice or animals bearing induced tumours.

Normal CD1 mice were purchased from the Animal Experimental Laboratory, − Faculty of Chemistry, Universidad de la República, Uruguay − (female, 25−30 g, 4 animals per group) were injected via a lateral tail vein with the HPLC purified ^{99m}Tc complex reconstituted with 30% methanol (0.1 mL, 3.7 MBq [100 μCi]). At different intervals after injection (5 min, 30 min, 2 h, and 24 h) the animals were sacrificed by neck dislocation. Whole organs and samples of blood and muscle were collected, weighed, and assayed for radioactivity. Animals were kept in a metabolic cage during the biodistribution period in order to collect total urine volume. Urine was also removed from bladder after sacrifice. The bladder, urine, and intestines were not weighed. Corrections by different sample geometry were applied when necessary. Results were expressed as % Dose/organ and % Dose/g.

Animals bearing induced tumours were obtained by subcutaneous inoculation of CCRFS-180 II murine sarcoma cells (5–6×10^6 cells/animal in 200 mL PBS) in the right limb of CD1 mice (female, 8–10 wk old, 25–30 g). After 15–20 days post-inoculation, animals developed palpable nodules and were used for biodistribution studies at 0.5 to 24 h post-injection using the previously described procedure. The tumour/muscle ratio was calculated from the corresponding percent dose/g values.

Uptake of ^{99m}Tc complex **30** in induced tumors at 12 h post-injection was also evaluated by the above described technique.

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