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PAPER

Fluorine- and rhenium-containing geldanamycin derivatives as leads for the development of molecular probes for imaging Hsp90†

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Heat shock protein 90 (Hsp90) is an ATP-dependent molecular chaperone responsible for protein quality control in cells. Hsp90 has been shown to be overexpressed in many human cancers. This has prompted extensive research on Hsp90 inhibitors as novel anticancer agents and, more recently, the development of molecular probes for imaging Hsp90 expression *in vivo*. This work describes the development of various fluorine-containing and rhenium-containing geldanamycin derivatives as leads for the development of corresponding ^{18}F-labeled and ^{99m}Tc-labeled PET and SPECT probes for molecular imaging of Hsp90 expression. All compounds were evaluated in an *in vitro* ATPase activity assay using Hsp90 isoform Hsp82p. Fluorobenzoylated geldanamycin derivative **5** displayed comparable inhibitory potency like parent compound geldanamycin.

Introduction

Heat shock protein 90 (Hsp90) is an ATP-dependent molecular chaperone which belongs to the ATPase/kinase protein superfamily. Hsp90 contains a Bergerat ATP-binding fold responsible for the regulation of signalling protein function and protein quality control, including protein folding and protein degradation.[1] Over the last 15 years, Hsp90 has gained considerable attention as a highly promising anticancer drug target. Hsp90 is largely in a latent state in normal cells but becomes overexpressed in cancer cells.[2] Hsp90 plays a central role in the conformational maturation, the controlling of function, trafficking and turnover of many oncogenic proteins also referred to as client proteins, which are involved in the genesis and progression of cancer. Hsp90 influences the activity and stability of oncogenic client proteins that function as key regulators in cellular growth, differentiation, and apoptotic pathways. Such important oncogenic client proteins include p53, Raf-1, ErbB2, Akt, Bcr-Abl, MEK-1, HIF-1α and cdk4. More than 100 of the known Hsp90 client proteins regulate multiple signal transduction pathways that are aberrantly activated in many human cancers and connected to the hallmarks of cancer like cell signalling, proliferation, survival and immortalization, invasion and metastasis, and angiogenesis.[1d,e,2a,3] Therefore, combinatorial inhibition of several oncogenic pathways in cancer cells should give a powerful anticancer effect and limit the development of drug resistance. The potential value of Hsp90 as an anticancer target is further enhanced by the fundamental structural difference of the Hsp90 protein complex in cancer cells compared to normal cells. In cancer cells, Hsp90 is present in an activated multi-chaperone complex which binds significantly more tightly to Hsp90 inhibitors than Hsp90 in normal cells.[4] Moreover, Hsp90 is constitutively expressed at 2- to 10-fold higher levels in cancer cells compared to normal cells.[2] Another factor in the cancer selectivity of Hsp90 inhibitors is the stress response. Malignant cells are likely to become dependent on Hsp90 due to the stressful conditions present in tumors like deregulated oncogenic signaling, hypoxia, acidosis, and nutrient deprivation.[4b]

Considerable progress has been made in understanding the three-dimensional molecular structure of Hsp90, which led to insights of the mechanism of Hsp90 action provided by X-ray crystallography and electron microscopy.[5]

The recognition of Hsp90 as an important drug target for the development of anticancer drugs has led to the design, synthesis and evaluation of numerous Hsp90 inhibitors. Several very good review articles summarize the structural diversity of Hsp90 inhibitors.[6]

Inhibitors of the ATP-pocket of Hsp90 can be classified into natural product-based compounds like geldanamycin (GA), 17-AAG, 17-DMAG and radicicol, and synthetic drugs based on purines (PUH71), pyrazoles (VER-49009), and isoxazoles (NVP-AUY922). The structures of prominent Hsp90 inhibitors are given in Fig. 1.

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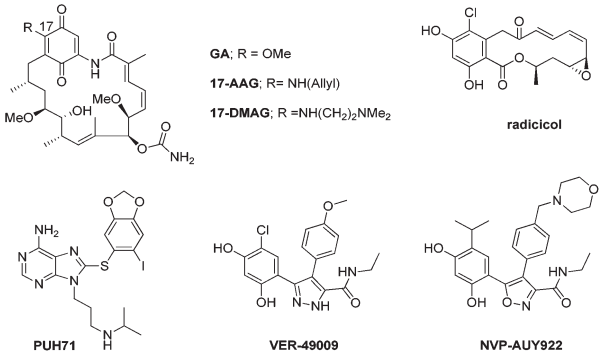

Fig. 1 Structures of prominent Hsp90 inhibitors.

About 10 Hsp90 inhibitors, both natural product-based and synthetic drugs, have reached advanced clinical trials.[7]

Geldanamycin (GA), a naturally occurring benzoquinone ansamycin, was among the first Hsp90 inhibitors to study Hsp90 function. GA was originally isolated from the broth of *Streptomyces hygroscopicus* in the 1970s and characterized as an antibiotic. The antitumor activity of GA was reported in the early 1990s. GA binds to the N-terminal ATP-binding site of Hsp90 locking the protein into the "intermediate complex" which results in the ubiquitin-mediated proteolytic degradation of client proteins.[3,8] However, early clinical studies revealed some undesirable properties of GA as an anti-cancer agent such as hepatotoxicity, cellular instability, and poor solubility.

This led to the development of a number of GA derivatives with improved toxicity and solubility profile, better bioavailability and increased metabolic stability. Among those GA derivatives, 17-allylamino-17-demethoxy-geldanamycin (17-AAG) and 17-(2-dimethylaminoethyl)-17-demethoxygeldanamycin (17-DMAG) are currently in different stages of various clinical trials for cancer treatment. The chemical structures of GA and GA derivatives 17-AAG and 17-DMAG are displayed in Fig. 1. Structure–activity analyses of modified GA derivatives demonstrated that position 17 on the quinone moiety of GA is particularly tolerant to modifications without substantial loss of biological activity. The tolerance of the 17 position for diverse substitution patterns is consistent with observed structures of GA–Hsp90 complexes showing the 17 position of GA exposed to the less sterically demanding outside. Consequently, numerous reports in the literature have described the synthesis and evaluation of GA derivatives modified in position 17 of the molecule.[9]

Current medicinal chemistry is increasingly implementing molecular imaging techniques for the characterization and evaluation of novel molecular targets, and the assessment of the pharmacologic profile of novel drugs.[10] Among the plethora of molecular imaging techniques, especially radionuclide-based imaging methodologies positron emission tomography (PET) and single photon emission computed tomography (SPECT) allow the exact elucidation of biological and physiological processes at the cellular and molecular level *in vivo*.[11] The development of molecular probes for PET and SPECT targeting Hsp90 *in vivo* would allow assessment of functional HSP90 expression in cancer. Molecular probes for non-invasive imaging of Hsp90 expression would have significant impact on early tumor diagnosis, monitoring of disease progression, and evaluation of therapeutic interventions. Only a few reports have been published in the literature dealing with the synthesis and evaluation of radiolabeled Hsp90 inhibitors. First reports describe labeling of GA and GA-derivatives with radioiodine isotopes I-131 and I-125.[12] Another series of reports deals with the radiolabeling and radiopharmacological evaluation of radioiodinated purine derivatives [^{124}I]PUH71, [^{124}I]PUDZ13, and [^{124}I]PUDZ13.[13]

The short-lived positron emitter fluorine-18 (^{18}F, $t_{1/2}$ = 109.8 min) and the gamma emitter technetium-99m (^{99m}Tc, $t_{1/2}$ = 6.0 h) are among the most widely used radioisotopes for the preparation of molecular probes for PET-imaging (^{18}F) and SPECT-imaging (^{99m}Tc). Fluorine-18 chemistry is mostly based on organic chemistry based on no-carrier-added (n.c.a.) [^{18}F]-fluoride,[14] whereas radiochemistry with radiometal technetium-99m exploits various coordination chemistry tools for the formation of ^{99m}Tc-based radiotracers.[15] This work describes the design, synthesis and evaluation of a series of fluorine- and rhenium-containing geldanamycin derivatives as leads for the development of ^{18}F-labeled and ^{99m}Tc-labeled geldanamycin derivatives as PET and SPECT probes, respectively, for molecular imaging of Hsp90 expression.

Results and discussion

Synthesis of fluorine- and rhenium-containing GA derivatives

The design of fluorine- and rhenium-containing GA derivatives was based on three different synthesis routes taking into account the special radiochemistry requirements involving n.c.a. [^{18}F] fluoride and ^{99m}Tc, and organic chemistry of benzoquinone GA. GA is a member of the ansamycin natural product family which displays a methoxy quinone moiety and the ansa ring as key structural motifs. Chemistry of GA is especially controlled by the methoxy quinone motif. The methoxy quinone moiety in GA undergoes rapid Michael addition with primary amines followed by an elimination reaction to furnish the corresponding vinylogous amine products. Thus, the principal design and synthesis of fluorine- and rhenium-containing GA derivatives as target compounds was based on the treatment of GA with various primary amines to give (1) respective fluorine-containing GA derivatives directly as the first synthesis route or a primary amine group-containing GA intermediate, which was further converted into the final (2) fluorine- and (3) rhenium-containing compounds using bioconjugation chemistry with prosthetic groups or coordination chemistry as the second and third synthesis route, respectively.

The first set of compounds was prepared through reaction of fluorine-containing primary amines with GA to afford compounds **1**, **2** and **3**. Fluorine-containing amines like 2-fluoroethylamine, 4-fluorobenzylamine and 2,2,2,-trifluoroethylamine represent suitable building blocks for the radiosynthesis of corresponding compounds labeled with the short-lived positron emitter ^{18}F. Radiolabeled 2-[^{18}F]fluoroethylamine[16] and 4-[^{18}F]-fluorobenzylamine[17] as well as *N*-dibenzyl protected 2,2,2,-tri-[^{18}F]fluoroethylamine[18] have been used for the radiosynthesis of various ^{18}F-labeled compounds. The synthesis of fluorine-containing GA derivatives **1**, **2** and **3** is given in Scheme 1.

Treatment of GA with an excess of the respective primary amine in DMSO afforded corresponding vinylogous amine

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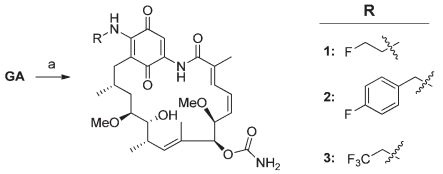


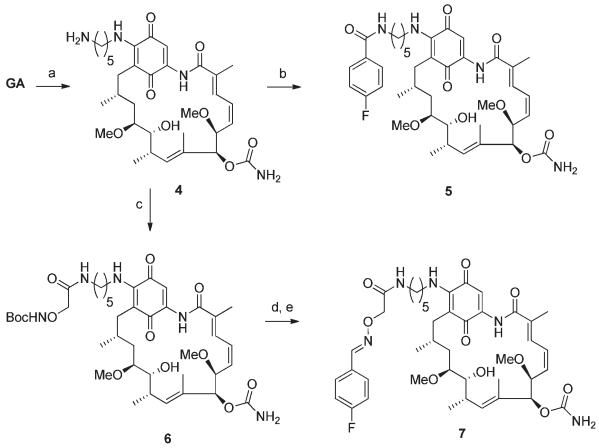
Scheme 1 Reagents and conditions for compounds **1**, **2** and **3**: (a) DMSO, R-NH_2, r.t., 2 h.

products **1** and **2** in high chemical yields of 89%, whereas compound **3** could be isolated in only 38% yield. The obtained lower yield of compound **3** can be attributed to the lower nucleophilicity of the primary amine group in 2,2,2,-trifluoroethylamine. Formation of compounds **1**, **2** and **3** could easily be monitored through the colour change of the reaction mixture from yellow to purple after the addition of the primary amines to GA in DMSO. The purple colour is indicative of the vinylogous amine motif in compounds **1**, **2** and **3**.

A second set of reactions was based on the attachment of various fluorine-containing prosthetic groups to GA. The use of ^{18}F-labeled prosthetic groups is a frequently employed approach in ^{18}F chemistry for the labelling of peptides and protein with ^{18}F under mild conditions. The used prosthetic groups differ in the complexity of their radiosynthesis, labeling yield, and efficiency and chemoselectivity of conjugation to biomacromolecules. Among the arsenal of available ^{18}F-labeled prosthetic groups, acylation agent succinimidyl-4-[^{18}F]fluorobenzoate ([^{18}F]SFB) and oxime-forming agent 4-[^{18}F]fluorobenzaldehyde ([^{18}F]FBA) are widely and frequently used in ^{18}F chemistry for bioconjugation to peptides and proteins.[19] SFB can be attached to primary amine groups *via* an acylation reaction with the active ester moiety, whereas FBA undergoes "click chemistry"-like reactions with amino-oxy compounds to form the corresponding oximes.

The application of prosthetic groups SFB and FBA for bioconjugation reactions to GA requires the presence of a primary amine group in the case of SFB, and an amino-oxy group in the case of FBA. For this purpose, GA was converted into 5-aminopentyl group-containing GA derivative **4**, which was used as a starting material for the synthesis of fluoro-benzoylated GA compound **5** and fluorobenzylidene-oxime **7**.

Treatment of GA with a 10-fold molar excess of 1,5-diaminopentane in CH_2Cl_2 afforded 5-aminopentyl-substituted GA derivative **4** in almost quantitative yield after purification using column chromatography. Amine **4** was treated with a 1.4 molar excess of SFB to provide fluorobenzoylated compound **5** in 59% yield after purification. In a second acylation reaction, amine **4** was treated with (Boc-aminooxy)acetic acid in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCl) as a carboxyl group activating agent in CH_2Cl_2 to form the corresponding amide **6** in very good chemical yields of 89%. Removal of the Boc-protecting group in amide **6** with HCl and subsequent reaction of the free amino-oxy intermediate with FBA according to a click chemistry reaction gave fluorobenzylidene-oxime **7** in a high yield of 83% for both steps. Fluorobenzylidene-oxime **7** was formed in the *E*-configuration exclusively.



Scheme 2 Reagents and conditions for compounds **5** and **7**: (a) CH_2Cl_2, NH_2–$(CH_2)_5$–NH_2, r.t., 2 h; (b) SFB; (c) (Boc-aminooxy)acetic acid, EDCl; (d) HCl; (e) FBA.

The synthesis of fluorobenzoylated GA derivative **5** and fluorobenzylidene-oxime **7** according to the prosthetic group approach is depicted in Scheme 2.

The third synthesis route was directed to the preparation of a rhenium-tricarbonyl complex with GA. Rhenium is used as a surrogate for radioactive technetium. The coordination chemistry of rhenium and technetium is similar, and radiopharmacological profiles of ^{99m}Tc- and radio-rhenium complexes are comparable *in vivo*, except for some differences in lipophilicity. Therefore, we set up the synthesis of a GA derivative containing a rhenium-complex moiety as a surrogate for a ^{99m}Tc-labeled compound.

As a member of the group 7 of the Periodic Table, technetium and rhenium are typical transition metal elements with rich and diverse coordination chemistry. The chemistry of technetium and rhenium is well explored, and numerous excellent books, monographs and reviews have been published to address aspects of technetium and rhenium coordination chemistry extensively.[20]

Recently, organometallic Tc-tricarbonyl complexes have brought new interest in the design of novel ^{99m}Tc radiopharmaceuticals. The facile synthesis route to the $\{^{99m}Tc(CO)_3\}^+$ core, its water solubility, and the robust chemical character and kinetic inertness of the $\{^{99m}Tc(CO)_3\}^+$ core offer important advantages for the preparation of radiopharmaceuticals. Various bifunctional chelators for the $\{^{99m}Tc(CO)_3\}^+$ core have been developed. Single amino acid chelates (SAAC) based on pyridyl, imidazole, and/or carboxylate derivatized amino acids were shown to be particularly useful for tripodal chelation of the $\{^{99m}Tc(CO)_3\}^+$ core.[21]

We performed the synthesis of SAAC **12** containing a pyridine nitrogen/amine nitrogen/carboxylate set of donor groups for efficient tripodal chelation of a $\{Re(CO)_3\}^+$ core as a surrogate for radioactive $\{^{99m}Tc(CO)_3\}^+$. SAAC **13** contains an additional primary amine group required for the Michael reaction with the methoxy quinone motif in GA, and an ethylene glycol chain to lower the lipophilicity of the quite lipophilic $\{Re(CO)_3\}^+$ core. The complete multi-step synthesis route for the preparation of SAAC **13** and its attachment to GA to afford compound **14** is displayed in Scheme 3.

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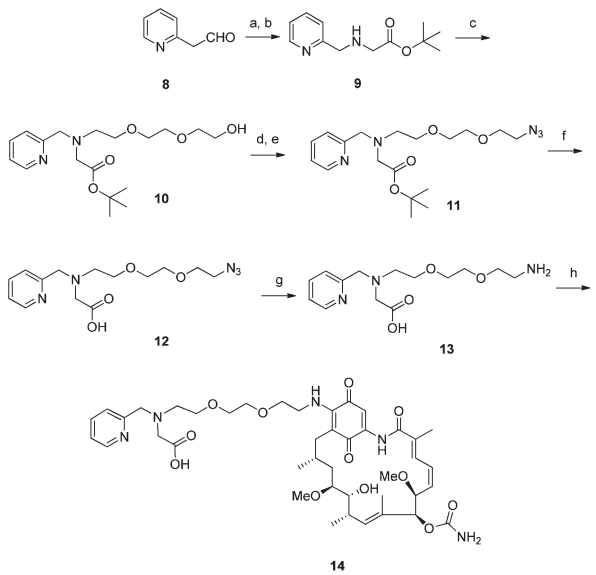


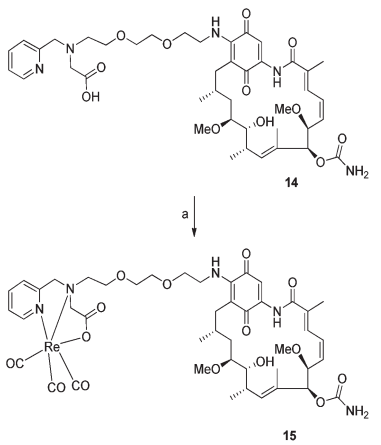
Scheme 3 Reagents and conditions for compound **12**: (a) glycine-*tert*-butyl ester, toluene, reflux, 2 h; (b) $NaBH_4$, MeOH, r.t., 1 h; (c) 2-(2-(2-iodoethoxy)ethoxy)ethanol, $NaCO_3$, acetone–water, reflux, 5 h; (d) MsCl, TEA, THF, 0 °C to r.t., overnight; (e) NaN_3, CH_3CN, reflux, 4 h; (f) TFA, CH_2Cl_2, r.t., overnight; (g) 10% Pd/C, 1.8 bar H_2, MeOH, r.t., 2 h; (h) GA, compound **13**, DMSO, r.t., 4 h.

The multi-step reaction sequence commenced with a reductive amination reaction using commercially available pyridine-2-carbaldehyde and glycine-*tert*-butyl ester, which afforded amine **9** in 56% yield. Secondary amine **9** was alkylated with 2-(2-(2-iodoethoxy)-ethoxy)ethanol in 25% isolated yield to afford compound **10**. 2-(2-(2-Iodoethoxy)ethoxy)ethanol was prepared according to the literature procedure reported by Ishow *et al.*[22] Compound **10** was converted into azide **11** through treatment with MsCl followed by the reaction with an excess of sodium azide. Azide **11** was prepared in 80% yield for both steps. Removal of the *tert*-butyl group in compound **11** with TFA afforded compound **12** in 82% yield. Final reduction of the azide group in compound **12** using Pd/C and H_2 gave tripodal chelator **13** in 76% yield after purification with column chromatography. Chelator **13** was further attached to GA according to an established Michael reaction protocol to afford compound **14** in 88% isolated yield.

Tricarbonylrhenium moieties are readily available as stable $[NEt_4]_2[ReBr_3(CO)_3]$ complexes, which can be prepared in multigram quantities starting from commercially available $NH_4[ReO_4]$.[23] Formation of tricarbonylrhenium(I) complex **15** through reaction of equimolar amounts of $[NEt_4]_2[ReBr_3(CO)_3]$ with tripodal ligand **14** is displayed in Scheme 4. The two nitrogen donor groups and the carboxylate group in compound **14** are capable of replacing all three bromine atoms in complex $[NEt_4]_2[ReBr_3(CO)_3]$ to afford neutral tricarbonylrhenium(I) complex **15** in 87% yield.

Biological evaluation of novel GA derivatives using an *in vitro* ATPase assay

The *Saccharomyces cerevisiae* Hsp90 isoform Hsp82 was used to assay APTase activity.[24] The observed ATPase activity was



Scheme 4 Reagents and conditions for compound **15**: (a) $[NEt_4]_2[ReBr_3(CO)_3]$, CH_2Cl_2, 1.5 h.

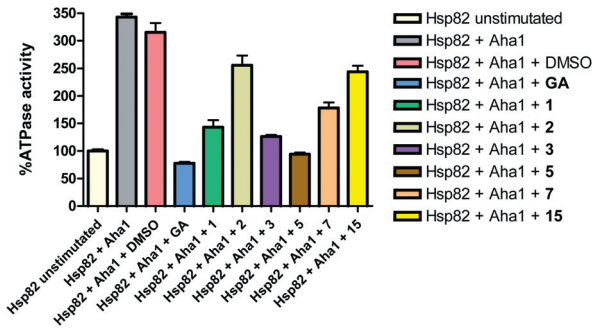


Fig. 2 ATPase activity assay using yeast Hsp90 isoform Hsp82p. ATPase rates are expressed as a percentage of the unstimulated rate of Hsp82p.

shown to be specific for Hsp90. The ATP-binding site in Hsp90 as demonstrated by various structural and biochemical studies is also the binding site for GA. GA binds to the ATP-binding site of yeast Hsp90 with a K_D of 0.5 μM, making GA a potent inhibitor of ATP binding in Hsp90 protein.

In the absence of client proteins, Hsp90 maintains inherent ATPase activity which is significantly reduced by Hsp90 inhibitors. Inhibition of inherent ATPase activity of Hsp90 was used to evaluate fluorine-containing compounds **1**, **2**, **3**, **5**, **7** and rhenium-tricarbonyl-containing compound **15** in an *in vitro* ATPase activity assay. Hsp90-specific inhibitor GA was used as a reference in the assay. Co-chaperone Aha1 was used as an activator of ATPase activity. Aha1 is known to stimulate ATPase activity upon binding to Hsp90.[25] All compounds were used in an inhibitor concentration of 1.0 μM. The results of the ATPase assay using Hsp90 isoform Hsp82p are displayed in Fig. 2.

The activating effect of co-chaperone Aha1 towards ATPase activity of Hsp90 is clearly demonstrated. ATPase activity of Hsp90 was increased 3.5-fold upon activation with co-chaperone Aha1 when compared with unstimulated Hsp90. No effect upon reduction of ATPase activity was observed in the control experiment with DMSO, whereas treatment of Aha1 activated Hsp90 with 1.0 μM of inhibitor GA led to a significant 80% reduction of ATPase activity. This is in agreement with the known high

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inhibitory potency of GA towards the ATP-binding site of Hsp90.

Within the series of fluorine-containing GA derivatives, only fluorobenzoylated compound **5** displayed comparable ATPase inhibitory potency as found for parent compound GA. Compound **5** led to a 75% reduction of ATPase activity at 1 μM concentration of the inhibitor. Tricarbonyl-rhenium complex **15** and fluorobenzyl-containing GA derivative **2** were the least active compounds in the ATPase assay at this inhibitor concentration. Treatment of Aha1 activated Hsp90 with both compounds at a concentration of 1.0 μM resulted in a 25–35% reduction of ATPase activity. Fluorine-containing compounds **1**, **3**, and **7** reduced ATPase activity by 60%, 65%, and 50%, respectively.

These findings are in agreement with various structure–activity relationship studies of 17-aminogeldanamycin derivatives.[9] The ATP-binding site of Hsp90 accommodates small lipophilic substituents in position 17 of the GA scaffold as demonstrated for fluoroethyl and trifluoroethyl group-containing compounds **1** and **3**. Larger groups directly attached to the GA scaffold like a fluorobenzyl group in compound **2** significantly reduced inhibitory potency compared to parent compound GA. Low inhibitory potencies were also found for fluorobenzylideneoxime **7** and, more profound, for tricarbonyl-rhenium complex **15**. Both compounds contain a linker to separate the fluorine- and tricarbonyl-rhenium-containing motifs from the GA scaffold. An adverse effect was observed for fluorobenzoylated compound **5**, which exhibited comparable Hsp90 ATPase inhibitory potency as GA. This clearly favors compound **5** as a lead for the development of an ^{18}F-labeled molecular probe.

Summary and conclusions

The synthesis of a series of fluorine- and rhenium-containing GA derivatives as leads for the development of respective ^{18}F- and ^{99m}Tc-labeled molecular probes for Hsp90 imaging has been accomplished. All compounds were prepared based on the treatment of GA with primary amines to furnish corresponding vinylogous amine products. Design and synthesis of fluorine- and rhenium-containing GA derivatives as described in this work was directed to the special requirements encountered for the preparation of respective molecular probes labeled with ^{18}F and ^{99m}Tc. All described synthesis routes are easily applicable to radiosyntheses with short-lived positron-emitter ^{18}F and gamma-emitter ^{99m}Tc. This especially counts for fluorobenzoylated compound **5**, which displayed the highest Hsp90 ATPase inhibitory potency comparable to that of GA. This makes compound **5** a promising lead for the development of an ^{18}F-labeled molecular probe for Hsp90 imaging *in vivo*.

Radiosynthesis and radiopharmacological evaluation of ^{18}F-labeled compound **5** employing prosthetic group [^{18}F]SFB as a radiolabel is currently in progress.

Experimental

General methods

^{1}H-NMR and ^{13}C-NMR and ^{19}F-spectra were recorded on a Varian Inova-400 at 400 MHz, 100 MHz and 376 MHz, respectively. Chemical shifts (δ) were determined relative to the solvent and converted to the TMS scale. Mass spectra were recorded using a Micromass ZabSpec Hybrid Sector-TOF by positive mode electrospray ionization. Flash chromatography was conducted using MERCK silica gel (mesh size 230–400 ASTM). Thin-layer chromatography (TLC) was performed on Merck silica gel F-254 aluminum plates, with visualization under UV light (254 nm). HPLC analyses were performed with a Beckman HPLC system on a semi-preparative Luna C18 column (100 Å, 10 μm, 250 × 10 mm) using gradient elution at a flow rate of 3 mL min^{-1} and UV detection at 254 nm. The elution started with an acetonitrile–water gradient from 15/85 to 50/50 (v/v) for 8 min, followed by an 8 min gradient elution from 50/50 to 70/30 (v/v) and a final 14 min elution at 70/30 (v/v).

All chemicals were obtained from commercial suppliers (reagent grade) and used without further purification. Geldanamycin was purchased in multi-gram quantities from LC Laboratories.

2-(2-(2-Iodoethoxy)ethoxy)ethanol,[22] succinimidyl-4-fluorobenzoate (SFB),[26] and $[NEt_4]_2[ReBr_3(CO)_3]$[24] were prepared according to literature procedures.

Chemical syntheses

17-(2-Fluoroethylamino)-17-demethoxy-geldanamycin 1. GA (50 mg, 89.18 μmol), 2-fluoroethylamine hydrochloride (88.6 mg, 0.89 mmol) and triethylamine (124 μL, 0.89 mmol) were stirred in DMSO (1 mL) for 2 h at room temperature. The reaction mixture was poured into 1 N HCl and extracted with $CHCl_3$. After drying with Na_2SO_4 and evaporation of the solvent under reduced pressure the residue was purified by flash chromatography (10% MeOH–$CHCl_3$) to give 47 mg (89%) of the desired compound. HPLC analysis: t_R = 17.25 min. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.99 (m, 6H, 2× CH_3), 1.80 (s, 3H, CH_3), 2.02 (s, 3H, CH_3), 2.31 (m, 1H), 2.72 (m, 2H), 3.27 (s, 3H, OCH_3), 3.36 (s, 3H, OCH_3), 3.46 (m, 1H), 3.57 (d, J = 8.6 Hz, 1H), 3.87 (m, 2H), 4.31 (d, J = 10.1 Hz, 1H), 4.65 (dt, J = 46.9 Hz, J = 4.7 Hz, 2H), 5.19 (s, 1H), 5.85 (d, J = 10.9 Hz, 1H), 5.87 (dd, J = 9.4 Hz, J = 3.1 Hz, 1H), 6.39 (t, J = 5.5 Hz, 1H), 6.58 (t, J = 11.7 Hz, 1H), 6.94 (d, J = 11.7 Hz, 1H), 7.30 (s, 1H), 9.11 (s, 1H); ^{19}F-NMR (376 MHz, $CDCl_3$): δ −225.9 (m). LR-MS m/z (ESI): 614.3 [M + Na]$^+$. HR-MS m/z (ESI) $C_{30}H_{42}FN_3O_8Na$ ([M + Na$^+$]) calcd 614.2848, found 614.2838.

17-(4-Fluorobenzylamino)-17-demethoxy-geldanamycin 2. GA (54 mg, 96.3 μmol) was dissolved in DMSO (1.0 mL). 4-Fluorobenzylamine (55 μL, 482 μmol) was added and the mixture was stirred at room temperature for 30 min. The reaction mixture was diluted with 1 N HCl and extracted with $CHCl_3$. After drying with Na_2SO_4 and evaporation of the solvent under reduced pressure the residue was purified by flash chromatography (10% MeOH–$CHCl_3$) to give 56 mg (89%) of the desired compound. HPLC analysis: t_R = 22.92 min. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.01 (m, 6H, 2× CH_3), 1.80 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 2.43 (m, 1H), 2.68 (d, J = 13.3 Hz, 1H), 2.75 (m, 1H), 3.28 (s, 3H, OCH_3), 3.37 (s, 3H, OCH_3), 3.45 (m, 1H), 3.58 (m, 1H), 4.15 (bs, 1H), 4.32 (d, J = 9.4 Hz, 1H), 4.66 (ddd, J = 57.0 Hz, J = 14.1 Hz, J = 5.5 Hz, 2H), 5.19 (s, 1H), 5.86 (d, J = 10.2 Hz, 1H), 5.87 (dd, J = 9.4 Hz, J = 3.1 Hz, 1H), 6.38 (t, J = 5.5 Hz, 1H), 6.59 (t, J = 11.7 Hz, 1H), 6.96 (d, J = 11.7 Hz,

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1H), 7.09 (t, J = 8.6 Hz, 2H), 7.24–7.28 (m, 2H), 7.31 (s, 1H), 9.15 (s, 1H); ^{19}F-NMR (376 MHz, $CDCl_3$): δ −113.2 (m). LR-MS m/z (ESI): 676.3 $[M + Na]^+$. HR-MS m/z (ESI): $C_{35}H_{44}FN_3O_8Na$ ($[M + Na]^+$) calcd 676.3005, found 676.2991.

17-(2,2,2-Trifluoroethylamino)-17-demethoxy-geldanamycin 3. GA (100 mg, 178 μmol) was dissolved in DMSO (1.0 mL). 2,2,2-Trifluoroethylamine (72 μL, 901 μmol) was added and the mixture was stirred at room temperature for 30 min. The reaction mixture was diluted with 1 N HCl and extracted with $CHCl_3$. After drying with Na_2SO_4 and evaporation of the solvent under reduced pressure the residue was purified by flash chromatography (10% MeOH–$CHCl_3$) to give 42 mg (38%) of the desired compound. HPLC analysis: t_R = 18.68 min. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.99 (m, 6H, 2× CH_3), 1.79 (s, 3H, CH_3), 2.02 (s, 3H, CH_3), 2.19 (m, 1H), 2.74 (m, 1H), 3.27 (s, 3H, OCH_3), 3.36 (s, 3H, OCH_3), 3.43 (m, 1H), 3.57 (m, 1H), 4.13 (m, 1H), 4.30 (m, 2H), 5.19 (s, 1H), 5.83 (d, J = 10.6 Hz, 1H), 5.87 (d, J = 10.6 Hz, 1H), 5.97 (t, J = 7.0 Hz, 1H), 6.57 (t, J = 11.7 Hz, 1H), 6.94 (d, J = 11.1 Hz, 1H), 7.26 (s, 1H), 7.31 (s, 1H), 9.15 (s, 1H); ^{19}F-NMR (376 MHz, $CDCl_3$): δ −72.0 (t, J = 8.5 Hz). LR-MS m/z (ESI): 650.3 $[M + Na]^+$. HR-MS m/z (ESI) $C_{30}H_{40}F_3N_3O_8Na$ ($[M + Na]^+$) calcd 650.2660, found 650.2647.

17-(5-Aminopentylamino)-17-demethoxy-geldanamycin 4. GA (100 mg, 178 μmol) was dissolved in CH_2Cl_2 (10.0 mL). 1,5-Diaminopentane (210 μL, 1.78 mmol) was added and the mixture was stirred at room temperature for 1 h. The purple reaction mixture was washed thoroughly several times with saturated $CaCl_2$ solution, followed by water and brine. The organic layer was dried, and the solvent was evaporated to give 106 mg (95%) of compound **4** as a purple solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.01 (m, 6H, 2× CH_3), 1.48 (m), 1.57 (m), 1.70(m), 1.79 (s, 3H, CH_3), 2.02 (s, 3H, CH_3), 2.68 (d, J = 13.3 Hz, 1H), 2.73 (t, J = 6.5 Hz, 2H), 3.27 (s, 3H, OCH_3), 3.36 (s, 3H, OCH_3), 3.46 (m, 1H), 3.56 (m, 1H), 4.31 (d, J = 9.4 Hz, 1H), 5.18 (s, 1H), 5.29 (s, 1H), 5.84 (d, J = 10.2 Hz, 1H), 5.87 (d, J = 9.4 Hz, 1H), 6.29 (t, J = 5.7 Hz, 1H), 6.58 (t, J = 11.7 Hz, 1H), 6.95 (d, J = 11.7 Hz, 1H), 7.27 (t, J = 8.6 Hz, 2H), 9.18 (s, 1H). m/z (ESI) $C_{33}H_{50}N_4O_8Na$ ($[M + Na]^+$) calcd 653.3526, found 653.3521.

17-(4-Fluorobenzamido)pentyl)amino)-17-demethoxy-geldanamycin 5. *N*-Succinimidyl-4-fluorobenzoate (6.25 mg, 26.0 μmol) dissolved in 500 μL of dry CH_2Cl_2 was added to a solution of compound **4** (12.5 mg, 18.9 μmol) in 500 μL of dry CH_2Cl_2. The reaction mixture was stirred for 2 h at 25 °C. The mixture was then evaporated to dryness and purified by column chromatography (10% MeOH–$CHCl_3$) to afford 8.5 mg of compound **5** (59% yield). HPLC analysis: t_R = 18.33 min. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.96 (d, J = 6.6 Hz, 3H), 1.00 (d, J = 7.0 Hz, 3H), 1.48–1.53 (m, 3H), 1.65–1.80 (m, 12H), 2.12 (s, 3H), 2.39 (dd, J = 10.7 Hz, J = 13.8 Hz, 1H), 2.67 (d, J = 13.4 Hz, 1H), 2.39 (dd, J = 10.7 Hz, J = 13.8 Hz, 1H), 2.73 (M, 1H), 3.27 (s, 3H, OCH_3), 3.36 (s, 3H, OCH_3), 3.45–3.50 (m, 4H), 3.56–3.58 (m, 2H), 4.30 (d, J = 5.0 Hz, 1H), 5.19 (s, 1H), 5.85 (d, J = 10.5 Hz, 1H), 5.89 (d, J = 12.0 Hz, 1H), 6.09 (t, J = 3 Hz, 1H), 6.25 (s, 1H), 6.58 (t, J = 11.5 Hz, 1H), 6.95 (d, J = 11.4 Hz, 1H), 7.09–7.13 (m, 2H), 7.29 (s, 1H), 7.75–7.79 (m, 2H), 9.17 (s, 1H); ^{19}F-NMR (376 MHz, $CDCl_3$): δ −108.2 (m). LR-MS m/z (ESI): 775.4 $[M + Na]^+$. HR-MS m/z (ESI) $C_{40}H_{53}N_4O_9Na$ ($[M + Na]^+$) calcd 775.3689, found 775.3678.

17-(*tert*-Butyl 2-(((5-aminopentyl)-2-oxoethoxycarbamate))-amino)-17-demethoxy-geldanamycin 6. 17-(5-Amino-pentyl-amino)-17-demethoxy-geldanamycin **4** (60 mg, 95 μmol), (Boc-aminooxy)acetic acid (18.2 mg, 95 μmol), and EDCl (22.8 mg, 119 μmol) were stirred in 1.0 mL of dry CH_2Cl_2 at room temperature for 1 h. The reaction mixture was diluted with CH_2Cl_2 (25 mL) and extracted with 0.1 N HCl. The organic layer was dried and the solvent was evaporated. The residue was purified with flash chromatography (10% MeOH–$CHCl_3$) to afford 68 mg (89%) of compound **6**. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.96 (d, J = 6.6 Hz, 3H), 1.00 (d, J = 7.0 Hz, 3H), 1.23 (t, J = 7.0 Hz, 2H), 1.47 (m, 9H), 1.61–1.78 (m, 8H), 1.80 (m, 4H), 2.12 (s, 3H), 2.40 (m, 1H), 2.66 (d, J = 13.4 Hz, 1H), 2.72 (m, 1H), 3.26 (s, 3H, OCH_3), 3.36 (s, 3H, OCH_3), 3.41–3.49 (m, 4H), 3.52–3.58 (m, 2H), 4.32 (m, 1H), 5.17 (s, 1H), 5.83 (d, J = 10.5 Hz, 1H), 5.87 (d, J = 12.0 Hz, 1H), 6.27 (m, 1H), 6.58 (t, J = 11.5 Hz, 1H), 6.94 (d, J = 11.4 Hz, 1H), 7.26 (s, 1H), 7.70 (m, 1H), 8.22 (bs, 1H), 9.18 (s, 1H). m/z (ESI) $C_{40}H_{61}N_5O_{12}Na$ ($[M + Na]^+$) calcd 826.4214, found 826.4208.

17-(2-(((4-Fluorobenzylidene)amino)oxy)acetamido)pentyl-amino-17-demethoxy-geldanamycin 7. Compound **6** (60 mg, 74.7 μmol) was dissolved in 4.0 N HCl in dioxane. The reaction mixture was stirred at room temperature for 1 h. After removal of the solvent under reduced pressure, the residue was re-dissolved in DMF. 4-Fluorobenzaldehyde was added, and the reaction mixture was stirred for 2 h at room temperature. The reaction mixture was diluted with 1.0 N HCl, and the mixture was extracted with CH_2Cl_2. The solvent was evaporated and the residue purified with flash chromatography (10% MeOH–$CHCl_3$) to afford 48 mg (80%) of compound **7**. HPLC analysis: t_R = 18.25 min. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.96 (d, J = 6.6 Hz, 3H), 1.00 (d, J = 7.0 Hz, 3H), 1.24 (t, J = 7.0 Hz, 2H), 1.41–1.74 (m, 8H), 1.79 (m, 4H), 2.02 (s, 3H), 2.17 (m, 1H), 2.42 (m, 1H), 2.66 (d, J = 13.4 Hz, 1H), 2.72 (m, 1H), 3.26 (s, 3H, OCH_3), 3.36 (s, 3H, OCH_3), 3.42–3.49 (m, 4H), 3.52–3.58 (m, 2H), 4.30 (d, J = 9.4 Hz, 1H), 5.18 (s, 1H), 5.29 (s, 1H), 5.83 (d, J = 10.5 Hz, 1H), 5.87 (d, J = 12.0 Hz, 1H), 6.29 (m, 1H), 6.58 (t, J = 11.5 Hz, 1H), 6.94 (d, J = 11.4 Hz, 1H), 7.26 (m, 1H), 9.18 (s, 1H); ^{19}F-NMR (376 MHz, $CDCl_3$): δ −110.9 (m). LR-MS m/z (ESI): 882.4 $[M + Na]^+$. HR-MS m/z (ESI) $C_{42}H_{56}FN_5O_{10}Na$ ($[M + Na]^+$) calcd 832.3909, found 832.3905.

[(Pyridin-2-ylmethyl)-amino]-acetic acid *tert*-butyl ester 9. Pyridine-2-carbaldehyde **8** (0.84 mL, 8.83 mmol) and glycine-*tert*-butyl ester (1.0 g, 8.83 mmol) were refluxed in toluene (10 mL) for 2 h. The solvent was evaporated and the residue was dissolved in MeOH (10 mL). $NaBH_4$ (333 mg, 8.8 mmol) was added and the mixture was stirred at room temperature for 1 h. After the addition of acetone (2 mL) and stirring for an additional 30 min, the solvent was evaporated and the residue was purified by column chromatography (10% MeOH–$CHCl_3$) to give 1.1 g (56%) of the desired product **9** as an oil. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 1.46 (s, 9H, CH_3), 3.34 (s, 2H, CH_2), 3.93 (s, 2H, CH_2), 7.15 (ddd, J = 7.7 Hz, J = 4.8 Hz, J = 0.7 Hz, 1H, Ar-H), 7.33 (d, J = 7.7 Hz, 1H, Ar-H), 7.63 (td, J = 7.7 Hz, J = 1.8 Hz, 1H, Ar-H), 8.55 (m, 1H, Ar-H); ^{13}C-NMR

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($CDCl_3$, 100 MHz): δ 28.10, 51.26, 54.63, 81.15, 121.95, 122.07, 136.44, 149.33, 159.34, 171.45; LR-MS (ESI) 223 $[M - H]^+$.

({2-[2-(2-Hydroxy-ethoxy)-ethoxy]-ethyl}-pyridin-2-yl-methyl-amino)-acetic acid *tert*-butyl ester 10. A mixture of [(pyridin-2-ylmethyl)-amino]-acetic acid *tert*-butyl ester **9** (150 mg, 0.67 mmol), 2-[2-(2-iodo-ethoxy)-ethoxy]-ethanol (348 mg, 1.35 mmol) and Na_2CO_3 (300 mg) was refluxed in acetone (3 mL) and water (1 mL) for 5 h. The solvent was reduced and the residue was purified by column chromatography (10% MeOH–$CHCl_3$) to give 60 mg (25%) of compound WUE16 as a pale yellow oil. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 1.45 (s, 9H, CH_3), 2.93 (t, J = 5.7 Hz, 2H, CH_2), 3.38 (s, 2H, CH_2), 3.55–3.63 (m, 8H), 3.71 (t, J = 4.5 Hz, 2H, CH_2), 4.00 (s, CH_2), 7.13 (ddd, J = 7.7 Hz, J = 4.8 Hz, J = 1.0 Hz, 1H, Ar-H), 7.52 (d, J = 7.7 Hz, 1H, Ar-H), 7.64 (td, J = 7.7 Hz, J = 1.8 Hz, 1H, Ar-H), 8.50 (m, 1H, Ar-H); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 28.16, 53.28, 56.28, 60.43, 61.64, 69.70, 70.23, 70.30, 72.63, 80.97, 121.95, 122.96, 136.58, 148.95, 159.62, 170.88; LR-MS (ESI) 355 $[M - H]^+$.

({2-[2-(2-Azido-ethoxy)-ethoxy]-ethyl}-pyridin-2-ylmethyl-amino)-acetic acid *tert*-butyl ester 11. A mixture of ({2-[2-(2-hydroxy-ethoxy)-ethoxy]-ethyl}-pyridin-2-ylmethyl-amino)-acetic acid *tert*-butyl ester **10** (1.56 g, 4.4 mmol) and triethylamine (1.23 mL, 8.8 mmol) in THF (50 mL) was cooled to 0 °C. MsCl (681 µL, 8.8 mmol) was added dropwise while stirring. The mixture was slowly warmed up to room temperature and stirred overnight. After the addition of water (100 mL) the mixture was extracted with EtOAc. The organic layer was flushed through a short silica-gel plug. After evaporation of the solvent the mesylate was obtained as a brown-yellow oil (1.68 g), which was used without further purification in the next step. The mesylate was dissolved in acetonitrile (50 mL). After the addition of sodium azide (1.43 g, 22 mmol) the mixture was refluxed for 4 h. Water (100 mL) was added and the mixture was extracted with EtOAc. The organic layer was flushed through a short silica-gel plug. After evaporation of the solvent the desired azide **11** was obtained as a brown-yellow oil, which was sufficiently pure. Yield: 1.34 g (80%) for two steps. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 1.46 (s, 9H, CH_3), 2.93 (t, J = 5.7 Hz, 2H, CH_2), 3.36 (t, J = 5.5 Hz, 2H, CH_2), 3.41 (s, 2H, CH_2), 3.56–3.68 (m, 8H), 3.99 (s, CH_2), 7.14 (ddd, J = 7.7 Hz, J = 4.8 Hz, J = 1.0 Hz, 1H, Ar-H), 7.54 (d, J = 7.7 Hz, 1H, Ar-H), 7.64 (td, J = 7.7 Hz, J = 1.8 Hz, 1H, Ar-H), 8.52 (m, 1H, Ar-H); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 28.16, 50.60, 53.33, 56.56, 60.62, 69.95, 70.02, 70.29, 70.58, 80.83, 121.87, 122.90, 136.39, 148.97, 159.78, 170.81; LR-MS (ESI) 380 $[M - H]^+$.

({2-[2-(2-Azido-ethoxy)-ethoxy]-ethyl}-pyridin-2-ylmethyl-amino)-acetic acid 12. ({2-[2-(2-Azido-ethoxy)-ethoxy]-ethyl}-pyridin-2-ylmethyl-amino)-acetic acid *tert*-butyl ester (1.3 g, 3.5 mmol) was stirred overnight in methylene chloride (10 mL) and FTA (1 mL) at room temperature. The solvent was reduced and the residue was purified by column chromatography (15% MeOH–$CHCl_3$ containing 1% HOAc) to give 930 mg (82%) of compound **12** as a yellow-brown oil. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 3.00 (t, J = 5.3 Hz, 2H, CH_2), 3.39 (t, J = 5.0 Hz, 2H, CH_2), 3.57 (s, 2H, 2H, CH_2), 3.58–3.67 (m, 8H), 4.18 (s, 2H, CH_2), 7.28 (m, 1H, Ar-H), 7.30 (s, 1H, Ar-H), 7.73 (td, J = 7.7 Hz, J = 1.8 Hz, 1H, Ar-H), 8.58 (m, 1H, Ar-H); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 20.84, 50.62, 54.53, 58.30, 60.34, 68.89, 70.02, 70.41, 70.50, 123.01, 137.66, 148.51, 157.19, 176.08; LR-MS (ESI) 324 $[M - H]^+$.

({2-[2-(2-Amino-ethoxy)-ethoxy]-ethyl}-pyridin-2-ylmethyl-amino)-acetic acid 13. ({2-[2-(2-Azido-ethoxy)-ethoxy]-ethyl}-pyridin-2-ylmethyl-amino)-acetic acid **12** (600 mg, 2.05 mmol) and 10% Pd/C (60 mg) were stirred in MeOH (150 mL) over H_2 (1.8 bar) for 2 h. The reaction mixture was filtered through celite to remove Pd/C. The residue was evaporated under reduced pressure to give amine **13** (450 mg, 76%) as a yellow-brown oil. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 2.66 (t, J = 4.7 Hz, 2H, CH_2), 3.17 (t, J = 4.7 Hz, 2H, CH_2), 3.19 (s, 2H, CH_2), 3.47–3.57 (m, 6H), 3.63–3.67 (m, 2H), 3.79 (s, 2H, CH_2), 3.81 (m, 2H, CH_2), 7.19–7.25 (m, 2H, Ar-H), 7.66 (td, J = 7.8 Hz, J = 1.8 Hz, 1H, Ar-H), 8.56 (m, 1H, Ar-H). LR-MS (ESI) 298.4 $[M + H]^+$.

17-({2-[2-(2-Amino-ethoxy)-ethoxy]-ethyl}-pyridin-2-yl-methyl-amino)-acetic acid-17-demethoxy-geldanamycin 14. GA (123 mg, 220 µmol) and {2-[2-(2-amino-ethoxy)-ethoxy]-ethyl}-pyridin-2-ylmethyl-amino)-acetic acid **13** (330 mg, 1.1 mmol) were stirred in DMSO (4 mL) for 4 h at room temperature. The reaction mixture was diluted with saturated $CaCl_2$-solution and extracted with $CHCl_3$. The organic layer was washed with water, dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by flash chromatography (20% MeOH–$CHCl_3$ containing 0.5% TFA) to give 160 mg (85%) of the desired compound as a purple foam. ^{1}H-NMR (400 MHz, CD_3OD): δ 0.96 (d, J = 3.9 Hz, 3H), 0.97 (d, J = 3.9 Hz, 3H), 1.72 (s, 3H, CH_3), 2.00 (s, 3H, CH_3), 2.30 (m, 1H), 2.73 (m, 1H), 3.29 (s, 3H, OCH_3), 3.33 (s, 3H, OCH_3), 3.42 (t, J = 4.7 Hz, 2H), 3.65 (m, 4H), 3.72 (m, 2H), 3.82 (t, J = 4.7 Hz, 2H), (m, 2H), 4.09 (s, 2H), 4.54 (d, J = 7.8 Hz, 1H), 4.61 (s, 2H), 5.23 (s, 1H), 5.55 (d, J = 9.4 Hz, 1H), 5.87 (m, 1H), 6.61 (t, J = 10.9 Hz, 1H), 6.99 (s, 1H), 7.13 (d, J = 10.9 Hz, 1H), 7.60 (dd, J = 7.0 Hz, J = 5.5 Hz, 1H), 7.66 (d, J = 7.8 Hz, 1H), 8.10 (td, J = 7.8 Hz, J = 1.6 Hz, 1H), 8.68 (d, J = 4.7 Hz, 1H). LR-MS (ESI positive): m/z (%) = 848.61 (100) (M + Na).

Tricarbonyl-{17-({2-[2-(2-amino-ethoxy)-ethoxy]-ethyl}-pyridin-2-yl-methyl-amino)-acetic acid-17-demethoxy-geldanamycin}rhenium(I) 15. 17-({2-[2-(2-Amino-ethoxy)-ethoxy]-ethyl}-pyridin-2-ylmethyl-amino)-acetic acid-17-demethoxy-geldanamycin **14** (20 mg, 24.2 µmol) and $[Et_4N][Re(CO)_3Br_3]$ (16.3 mg, 24.2 mmol) were stirred in CH_2Cl_2–MeOH (1 : 1) (4 mL) for 2 h at room temperature. The solvent was removed under reduced pressure and the residue was purified by flash chromatography (10% MeOH–$CHCl_3$) to give 23 mg (87%) of the rhenium complex as a purple glass. HPLC analysis: t_R = 16.26 min. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.96 (d, J = 6.5 Hz, 3H), 0.99 (d, J = 6.5 Hz, 3H), 1.79 (s, 3H, CH_3), 2.02 (s, 3H, CH_3), 3.27 (s, 3H, OCH_3), 3.35 (s, 3H, OCH_3), 3.43 (m, 1H), 3.48–3.57 (m, 2H), 3.70 (m, 6H), 3.83 (m, 2H), 3.89–4.00 (m, 2H), 4.31 (dd, J = 10.1 Hz, J = 3.1 Hz, 1H), 4.56–4.68 (m, 2H), 5.18 (d, J = 6.2 Hz, 1H), 5.87 (t, J = 10.9 Hz, 1H), 6.58 (t, J = 10.9 Hz, 1H), 6.75 (dt, J = 17.9 Hz, J = 5.5 Hz, 1H), 6.95 (bd, J = 10.9 Hz, 1H), 7.19 (d, J = 2.3 Hz, 1H), 7.41 (dt, J = 7.8 Hz,

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J = 4.7 Hz, 1H), 7.54 (dd, J = 10.1 Hz, J = 7.8 Hz, 1H), 7.93 (m, 1H), 8.80 (bt, J = 4.7 Hz, 1H), 9.16 (d, J = 2.3 Hz, 1H). LR-MS (ESI positive): m/z (%) = 1116.21 (60), 1118.20 (100) (M + Na). HR-MS m/z (ESI) $C_{45}H_{58}N_5O_{15}Na$[187Re] ([M + Na]$^+$) calcd 1118.3379, found 1118.3368.

Biological evaluation

ATPase activity assays were carried out using an enzyme-coupled system as previously described.[24] All reactions were carried out in 100 µL volumes in a 96-well plate and in triplicate. Average values of those triplicates are shown with error expressed as standard error of the mean. All reactions contained 0.5 µM Hsp82p, 1 µM Aha1p, 31.4 mM Hepes pH 7.2, 1 mM sodium chloride, 19.3 mM potassium acetate, 5 mM magnesium chloride, 1 mM dithiothreitol, 0.3 mM NADH, 2 mM ATP, 1 mM phosphoenol pyruvate, and 2.5 µL of pyruvate kinase–lactate dehydrogenase (Sigma) in 100 µL. Drugs were used at 10 µM final concentrations where indicated.

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