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Originally published:

May 2019

Journal of Medicinal Chemistry 62(2019), 6525-6539

DOI: https://doi.org/10.1021/acs.jmedchem.9b00171

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Journal:	Journal of Medicinal Chemistry
Manuscript ID	jm-2019-00171j.R2
Manuscript Type:	Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Bechmann, Nicole; Helmholtz-Zentrum Dresden-Rossendorf, Institute of Radiopharmaceutical Cancer Research, Department of Radiopharmaceutical and Chemical Biology Kniess, Torsten; Helmholtz-Zentrum Dresden-Rossendorf, Department of Radiopharmaceutical and Chemical Biology Pietzsch, Jens; Helmholtz-Zentrum Dresden-Rossendorf, Institute of Radiopharmaceutical Cancer Research, Department of Radiopharmaceutical and Chemical Biology

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Nitric oxide-releasing selective estrogen receptor modulators (NO-SERMs): a bifunctional approach to improve the therapeutic index

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Abstract

When using selective estrogen receptor modulators (SERMs) in cancer therapy adverse effects such as endothelial dysfunction have to be considered. Estrogens and, consequently, SERMs regulate the synthesis of vasoactive nitric oxide (*NO). We hypothesized that a bifunctional approach combining the antagonistic action of SERMs with a targeted NO-release could diminish vascular side effects. We synthesized a series of NO-releasing SERMs (NO-SERMs) and the corresponding SERMs (after NO-release) derived from a triaryl olefin lead. Compounds showed antagonistic activity for ER β (IC₅₀(ER β)=0.2–2.7 μ M), but no interaction with ER α . Growth of ER β -positive breast cancer and melanoma cells was significantly decreased by treatment with SERM **5d**. This anti-proliferative effect was diminished by the additional release of *****NO by the corresponding NO-SERM **4d**. Moreover, targeted release of *****NO by **4d** counteracted the anti-proliferative effect of **5d** in normal vascular tissue cells. Summarizing, the therapeutic index of SERMs might be improved by this bifunctional approach.

Introduction

Estrogen signaling pathways regulate diverse physiological functions, but are also key players in osteoporosis, neurodegenerative and cardiovascular diseases, and in the progression of certain cancers, like breast, ovarian, colorectal, prostate, and endometrial cancer.¹⁻⁴ Estrogen receptors (ERs) are ligand-dependent transcription factors that can be modulated by selective estrogen receptor modulators (SERMs), like tamoxifen (TAM) and raloxifene (Fig. 1A).⁵⁻⁶ SERMs are able to interact with both subtypes of the receptor, ER α and/or ER β , acting as estrogen agonists or antagonists, in a tissue-specific manner.⁷ For example, on the one hand, tamoxifen acts as an estrogen antagonist, which is currently be used for prevention and treatment of breast cancer, on the other hand, agonistic action of tamoxifen increases the incidence of endometrial cancer.8-9 Raloxifene is currently used for the treatment of osteoporosis in postmenopausal women, because of its property to preserve bone mineral density without uterotropic side effects.¹⁰ Daily treatment with lasofoxifene reduced the risk of nonvertebral and vertebral fractures, ER-positive breast cancer, coronary heart disease, and stroke in postmenopausal women with osteoporosis, but enhanced the risk of venous thromboembolic events.¹¹ In contrast to SERMs that modulate receptor conformation and regulate coactivators and corepressors for recruitment of the receptor, selective ER degraders (SERDs), like fulvestrant (Fig. 1A) or RAD1901¹², act via binding and degrading the receptor.¹³ A third strategy to block the estrogen action, e.g., in tumor cells, is the inhibition of estrogen biosynthesis by aromatase inhibitors, like letrozole and exemestane.¹⁴⁻¹⁵ All three estrogen blocking strategies are associated with postmenopausal symptoms. For example, the treatment with SERMs is accompanied by hot flushes, but more important, with long-term effects, such as endothelial dysfunction, thromboembolic and cardiovascular disease. 17βestradiol (E2) exerts direct and indirect effects on the cardiovascular system via mechanisms involving genomic and non-genomic pathways (Fig. 1B), whereby, e.g., the vascular homeostasis is maintained.¹⁶ Antagonistic action of SERMs is responsible for the vascular side effects,

especially by inhibition of the PI3-K/Akt/endothelial NO-synthase (eNOS)-signaling (Fig. 1C) and the resulting imbalance in nitric oxide (*NO) homeostasis.¹⁷ Hayashi et al. could demonstrate that treatment of human umbilical vein endothelial cells (HUVECs) with E2 enhanced the release of the potent vasodilator *NO via an increased expression of eNOS. Furthermore, the associated cardioprotective effect of E2 is repealed via the antagonistic activity of SERMs.¹⁸⁻¹⁹

Major challenge in the development of novel SERMs is the improvement of the therapeutic index to prevent vascular side effects in particular. In contrast to the design of novel SERMs with higher selectivity towards ER α or ER β , we describe another potential approach to improve the therapeutic index of SERMs, combining the selectivity of SERM action with a targeted release of NO through the introduction of a NO-releasing moiety. Our strategy aims to compensate the reduced endogenous NO-release by the SERM-mediated inhibition of the PI3-K/Akt/eNOS signaling cascade. Therapeutic agents with an additional NO-releasing moiety gain increasing attention with regard to the different actions of 'NO as vasorelaxant, neurotransmitter and free radical.²⁰ This approach is currently being intensively investigated, for example in the development of novel selective cyclooxygenase-2 inhibitors (coxibs). The origin of this study was the development of such NO-releasing coxibs (NO-coxibs), which also are considered as potent bifunctional radiosensitizers.²¹⁻²² By using two lead structures suggested for coxib/NO-coxib development, (pyrazolyl)benzenesulfonamides^{21, 23} and acyclic triaryl olefins ²⁴⁻²⁸, soon it became obvious that the selected triaryl olefins do not fulfil the requirements for potent COX-2 inhibition (supporting information). However, the structural similarity of the novel triary lolefins to tamoxifen prompted us to investigate the functional activity (e.g., antagonistic and/or agonistic) of the compounds on human ER α and ER β . Furthermore, we indroduced a NO-releasing moiety to the triaryl olefine lead structure. First studies on the potential benefit of this bifunctional approach were performed on ER α/β -positive human melanoma and breast cancer cell lines. Investigations on human umbilical vein endothelial cells allowed us to draw first conclusions regarding the impact

of an additional NO-release on the side effect profile of SERMs on normal tissue cells of the vascular system.



Fig. 1: Structure and modulation of the estrogen signaling by selective estrogen receptor modulators (**SERMs**). (**A**) Chemical structure of the SERMs tamoxifen, raloxifene, and fulvestrant. (**B**) Showed the most important intracellular signaling pathways of 17β-estradiol (E2) that could be influenced by SERMs. Signaling pathway **1** (yellow star): canonical estrogen pathway, the ligand-activated estrogen receptor (ER) binds specifically to the estrogen responsive element (ERE) in the promoter region of the targeted gene. Signaling pathway **2**: non-ERE-depending estrogen pathway: the ligand-activated estrogen receptor interacts with other transcription factors (TF), like NF-κB, and, activates gene expression in ERE-independent manner. Signaling pathway **3**: non-genomic estrogen pathway, ERs that are localized in cell membrane activate PI3-K/MAPK-signaling and regulate for example vasodilation by an activation of the endothelial nitric oxide ('NO) synthase (eNOS). Moreover, a ligand-independent mechanism by the activation of different growth factors was described (not shown). (**C**) In particular, the influence of SERMs on the NO-signaling is coupled with several side effects, like endothelial dysfunction. PI3-K: Phosphatidylinositol-4,5-bisphosphate 3-kinase; Akt: Protein kinase B; MAPK: mitogen-activated protein kinase; NF-κB: Nuclear factor kappa B; BCL2: B-cell lymphoma 2; ERK: extracellular-signal-regulated kinase; JNK: c-Jun N-terminal kinase.

Results

Synthesis of NO-releasing triaryl olefins. Starting from a triaryl olefin lead structure (3) a group of 5-(4-(methylsulfonyl)phenyl)-6-6-diphenylalkenyls (4a,c, 5a,c) and 6,6'-bis(4fluorophenyl)-5-(4-(methylsulfonyl)phenyl) alkenyls (4b,d, 5b,d) with and without a NOreleasing molety were synthesized by applying the reaction sequence illustrated in Figure 2. Building of the triaryl olefin lead structure started with a Friedel-Crafts acylation of thioanisole with 4-chlorobutanolychloride or 5-chloropentanoylchloride to yield 4-chloro-1-(4-(methylthio)phenyl)butan-1-one 86%) 5-chloro-1-(4-(1a, and (methylthio)phenyl)pentan-1-one (1b, 87%), respectively. A similar synthesis strategy was applied for 5-chloro-1-phenylpentan-1-one.²⁹ Subsequent oxidation of the ketones 1a-b with oxone® (potassium peroxymonosulfate) generated 4-chloro-1-(4-(methylsulfonyl)phenyl)butan-1-one 76%) 5-chloro-1-(4-(2a, and (methylsulfonyl)phenyl)pentan-1-one (2b, 92%) for subsequent McMurry olefination. Reductive cross-coupling reaction of 2a-b with benzophenone or 4.4'difluorobenzophenone formed the chloroalkyl substituted triaryl olefin derivatives 3a-d in 40-75% yield. The desired NO-SERMs (4a-d) were obtained by nucleophilic substitution of the chloroalkyl derivatives **3a-d** with silver nitrate in acetonitrile ($S_N 2$). Due to the moderate nucleophilic power of the nitrate that resulted from the delocalization of the negative charge in the anion and the comparatively poor chloro-leaving group, the reaction mixture had to be heated at 80°C for 24 h. Introduction of the nitroester functionality was confirmed by high-resolution mass spectrometry (HRMS) and infrared spectroscopy (Supporting information). Additionally, asymmetric (1640 – 1620 cm⁻¹) and symmetric (1285 – 1270 cm⁻¹) stretching vibration for the introduced nitroester group could be observed.

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Fig. 2: Synthetic pathway of NO-releasing SERMs (4) and reference SERMs (5). Reagents and conditions: (a) AlCl₃, CHCl₃, argon, 2.5 h rt, (b) oxone[®] (potassium peroxymonosulphate), MeOH/THF (1/1, v/v), 31 h, (c) Zn, TiCl₄, reflux 4.5 h, (d) AgNO₃, MeCN, 24 h, 80°C, (e) CuSO₄ x 5 H₂O, DMSO/H₂O (1/3, v/v), 130°C, 48 h.

Synthesis of corresponding hydroxyalkyl triaryl olefins. From literature, it's well known that organic nitrates release 'NO after a 3-electron-transmission to leave a hydroxyl rest via an enzymatic or thiol dependent mechanism.³⁰ The corresponding hydroxyl substituted triaryl olefin derivatives **5a-d** were synthesized as reference compounds. To introduce the hydroxyl group on the chlorinated side chain of the triaryl olefin lead, a synthesis strategy previously described by Abdellatif et *al.* was used.²⁷ Each of the chloroalkyl derivatives **3a-d** was heated (130°C) in presence of CuSO₄ x 5 H₂O in a mixture of DMSO/water (1/3, v/v) for 48 h and the reference compounds after NO-release **5a-d** were obtained in 52-74% yield. In further experiments, it could be demonstrated that the reaction follows a nucleophilic substitution (S_N2) mechanism with the hydroxyl ions from water, whereby the presence of sulfate or copper rather disturbs the reaction (Fig.

S1). A direct synthesis of the hydroxyalkyl compounds by using the hydroxy-1-(4-(methylsulfonyl)phenyl)alkan-1-ones was not feasible (intensively described in the supporting information, Fig. S2-S4). To compare the impact of the fluorine substituents on the benzene rings and the nitroester- and the hydroxyl-substituents on the alkenyl chain, a fluorinated reference compound **8** was synthesized by following the reaction sequence illustrated in Figure 3, previously described by Uddin et al.²⁶ Ketone **6** was oxidized to 1-(4-(methylsulfonyl)phenyl)pentan-1-one (**7**) and after McMurry olefination with 4,4'difluorobenzophenone, 4,4'-(2-(4-(methylsulfonyl)phenyl)hex-1-ene-1,1diyl)bis(fluorobenzene) (**8**) was obtained in 32% yield.



Fig. 3: Synthetic pathway of SERM 8. Reagents and conditions: (**a**) thioanisole, AlCl₃, CHCl₃, 1.5 h rt (**b**), oxone[®] (potassium peroxymonosulphate), MeOH/THF (1/1, v/v), 15 h, (**c**) 4,4′-difluorobenzophenone, Zn, TiCl₄, reflux 4.5 h, reflux.

ER interactions of compounds 4 and 5. The estrogen receptor binding affinity of the novel NO-SERMs **4** and the corresponding SERMs **5** after NO-release was evaluated by using human receptor reporter assays. The cell-based luminescence assay allows the screening of test compounds to quantify the influence of the functional activity of the receptors both in agonist and antagonist manner. However, preliminary experiments showed that the agonist assay mode is not applicable for triaryl olefin derivatives. This is also the case for the partial agonist TAM as it was confirmed by the manufacturer. *In vivo*, TAM is easily converted by the liver cytochrome P450 into 4-hydroxytamoxifen (4OHT)

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that binds to ERs within the equal binding pocket like E2.³¹⁻³² The resulting nuclear complex decreases DNA synthesis and inhibits the action of E2.³² Furthermore, the complex also recruits co-repressor proteins, that contribute to the antagonistic action of 40HT.³³ It could be demonstrated that the agonistic effect of tamoxifen depends on the cell type, ER subtype, and ERE-promoter context.³⁴ Thus, the cell-based ER reporter assay is not useable for the analysis of the agonistic action of triaryl olefins.

ER α activity was not affected by the novel compounds up to a concentration of 5 μ M (Tab. 1). In contrast, all compounds, except **4b**, showed promising antagonistic activity on ER β (IC_{50(NO-SERMs 4)}: 0.2 - 2.7 µM; IC_{50(SERMs 5)}: 0.18 - 3.0 µM). The fluorinated derivatives with a butyl side chain 4d (IC₅₀(ER β): 0.20 μ M) and 5d (IC₅₀(ER β): 0.18 μ M) were the most potent, selective ER β antagonists (4d: S.I.(ER β) > 25.00; 5d: S.I.(ER β) > 27.78). The introduction of the nitroester group seems to have only a slight influence on ER β affinity. These data and the findings from Chen et al. indicate that the methylsulfonyl group is selectivity.³⁵ responsible for ERβ 2-(4-(methylsulfonyl)phenyl)hex-1-ene-1,1dividibenzene, previously described by Uddin et al., also showed a high ERß selectivity whereas the corresponding NH-sulfoximine and N-cyano sulfoximine derivatives displayed an affinity for both estrogen receptors.^{26, 35} Because of the ERß selectivity of the novel compounds the additional NO-release is more important to reduce cardiovascular events. Studies on knockdown mice have demonstrated that ERß is required for E2-mediated vasodilatation and protection from age-related hypertension.³⁶⁻³⁷ Hence, the diminished vasodilatation that might be associated with antagonistic activity on ER^β of the novel compounds, might possibly be compensated by the release of the vasodilator 'NO.

Tab. 1: Estrogen receptor antagonistic activity and NO-release of NO-releasing SERMs and the corresponding SERMs.

NO-release [%]c IC₅₀ [µM] SI plasma L-cysteine compound ERβ^b ERα^a **ER**^{β^a} PBS oxidant S-nitroso-S-nitrosonitrite_{free} NO_{total} nitrite_{free} NO_{total} thiol thiol 4a > 5 2.7 > 1.85 n.d 1.5±0.5 0.2±0.2 3.8±0.5 3.6 0.7±0.3 2.0±1.3 1.3 4b > 5 > 5 1.00 2.2±0.2 0.1±0.1 0.7±0.2 2.8±1.5 2.1 0.1±0.1 2.0±1.3 1.9 4c > 5 1.3 > 3.85 0.1±0.1 1.1±0.7 0.2±0.2 3.3±1.7 3.1 0.9±0.5 0.5±0.6 0.4 4d > 5 0.20 > 25.00 2.5±1.4 0.2±0.1 1.5±1.5 1.3 0.4±0.4 3.8±2.6 3.4 n.d. PA -33.5±1.3 48.3±4.2 0.3±0.2 4.3±1.4 4.0 0.3±0.3 3.6±1.3 3.3 -NO-aspirin 0.2±0.1 1.4±0.3 3.9±1.5 2.5 0.8±0.1 4.4±1.5 3.6 n.d. > 5 1.5 > 3.34 5a 5b > 5 30 > 1 67 5c > 5 1.4 > 3.57 5d > 5 > 27.78 0.18 tamoxifen 1.7^d 0.005^d 377.8 citrat

n.d. not detectable.

PA Piloty' s acid (N-hydroxybenzenesulfonamide).

 Values are means of two determinations using a cell based human estrogen receptor alpha or beta reporter assay (Human Estrogen Receptor Beta Reporter Assay System, #IB00411 or Human Estrogen Receptor Alpha Reporter Assay System, #IB00401; Indigo Biosciences; PA; USA).

The IC₅₀ value was calculated by non-linear fitting (Software: Origin, logistic fit).

^b ER β selectivity index *in vitro* (IC₅₀(ER α)/IC₅₀(ER β)).

NO measured by nitrite using the reaction of nitrite with 2,3-diaminonaphthalen (DAN) to the high fluorescence 2,3-naphthotriazol (NAT) under acid conditions. Percent of NO-released based on the theoretical maximum release of 1 mol of NO/mol of the test compound. Three to four independent experiments were performed, each in duplicate (n = 6–8). Mean ± SEM.

^d analyzed by the manufacturer of the cell based human estrogen receptor alpha and beta reporter assay (Indigo Bioscience, Technical Manual).

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In vitro stability of 4d and 5d. Exemplarily, *in vitro* stability of the most potent NO-SERM 4d and the corresponding SERM 5d was analyzed after incubation in human whole blood (Fig. 4). SERM 5d is stable over a period of > 6 h, no metabolites could be detected by HPLC after 6 h incubation time. The measurable decrease of 5d between 0 min and 1 h incubation, suggests a binding of 5d (logP_{calculated}: 4.85) on lipophilic components of the blood. In opposite, a 6 h incubation of NO-SERM 4d in whole blood resulted in the release of 'NO and formation of the corresponding SERM 5d, as expected. The low ratio of 5d indicates a slow NO-release by 4d. Other metabolites could not be detected, but there is also a loss of 4d (logP_{calculated}: 6.21) engendered with the high lipophilicity after 1 h incubation time. These results suggest that the novel NO-SERMs and the corresponding SERMs are stable under physiological conditions, as human whole blood. This underlines suitability for further applications *in vitro* and *in vivo*.



Fig. 4: Stability of SERM 5d and NO-SERM 4d in human blood. Stability was analyzed by HPLC after zero up to six hours incubation in human whole blood at 37°C. After incubation, proteins were separated by using supersol-precipitating agent (1:2, v/v) and the clear solution were analyzed using the following conditions. Agilent 1100; Zorbrax 300SB-C18 (250 x 9.4 mm; 4 μ m); Zorbrax 300SB-C18 (4.6 x 12.5 mm, 5 μ m); 254 nm; A: H₂O + 0.1% TFA B: MeCN + 0.1% TFA, 1-5 min 85% A, 10 min 70% B, 25-26 min 95% B, 29-30 min 85% A; flow rate 3.0 mL/min at 50°C.

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Evaluation of the NO-release by NO-SERMs. In presence of oxygen, the free radical nitric oxide is immediately oxidized to nitrite and further to nitrate. The exogenous NOrelease by the novel NO-SERMs in vitro was determined by a fluorescence-based nitrite assay.²¹ For quantification the reaction of nitrite with 2,3-diaminonaphthalene (DAN) to the highly fluorescent 2,3-naphthotriazole (NAT) under acidic conditions was utilized. Mercury(II)chloride was added to the samples to detect nitric oxide that was stabilized as bioactive S-nitrosothiols in presence of free thiol groups. The NO-release by the novel NO-SERMs was determined after incubation at 37°C for 90 min in (a) phosphate-bufferedsaline (PBS, pH 7.5) to analyze the spontaneous NO-release, (b) PBS containing human plasma to measure enzyme-mediated mechanisms, (c) PBS with 50 μ M K₃[Fe(CN)₆], as oxidizing agent, or (d) PBS in presence of 5 mM L-cysteine to detect the release by free thiol groups. The results were summarized in table 1. The NO-release by the novel organic nitrates **4a-d** is moderate, as expected. Interestingly, **4b** showed a comparatively high spontaneous release of 'NO $(2.2 \pm 0.2\%)$, whereby for the other compounds no NOrelease after incubation in PBS could be observed. After incubation under oxidizing conditions, an inverse effect was found. For 4b no NO-release was detected, whereas 4a and 4c-d showed a NO-release of 1.1 - 2.5%. For all compounds a moderate NO-release under enzymatic and thiol dependent conditions could be observed (0.5 - 3.8%). A similar NO-release was verifiable for the organic nitrate, NO-aspirin. A moderate NO-release was aimed to ensure a good manageability of the NO-SERMs, and to prevent side effects associated with high exogenous 'NO concentration.

Anti-tumorigenic activity of the novel compounds in dependence of their NOrelease. For analyzing the influence of the novel compounds on tumor cell proliferation, different breast adenocarcinoma (MCF-7 and MDA-MD-231) and human melanoma

(A2058 and Mel-Juso) cell lines were selected as model. Their dissimilar basal COX-2 protein expression was the initial intent for using the melanoma cell lines.²² The melanoma model was retained in this investigation because it allows a direct comparison of both NO-SERMs and NO-coxibs, particularly, with regard to the additional NO-release. However, the stronger emphasis on SERM development led to the introduction of a suitable breast adenocarcinoma model. Beside the ERa/ β -positive MCF-7 cells, we used MDA-MB-231 with no detectable expression of ERa. ER protein expression of the melanoma cell lines was analyzed by Western blot analysis in comparison to the MCF-7 cells (Fig. 5A). All cell lines showed a comparable ER β protein synthesis. Protein synthesis of ERa was lower in A2058 and Mel-Juso cells than in MCF7 cells. Blocking of estrogen-dependent cell proliferation is utilized as a common treatment option for several types of ER-positive breast cancer. Helguero and coworkers suggested that application of ER β agonists could be a useful addition in the treatment regime of breast cancer that at the moment only aimed at ER α inhibition, with routinely use of TAM (ER antagonist).³⁸



Fig. 5: ERα and ERβ expression of two melanoma cell lines and the influence of NO-SERMS 4 or SERMS 5 on cell viability and proliferation of both cell lines. (A) ERα and ERβ expression in A2058 and Mel-Juso human melanoma cells was determined by Western blotting. Human breast cancer cells (MCF-7) here were used as positive control. Representative sections of the immunochemical Western blot analysis of ERα, ERβ and β-actin used as housekeeping protein are shown (three independent experiments). (B) The influence of NO-SERMS 4 and SERMS 5 on cell viability was investigated. Interestingly, the additional NO-release preserved the cell viability. Three to five independent experiments were performed (n = 9 - 15). Mean ± SEM, ANOVA and Bonferroni post hoc test comparison vs. DMSO control, * p < 0.05 or, ** p < 0.01. Moreover, the influence of the corresponding compounds 4d and 5d on the number of proliferating cells on (C) A2058 and (D) Mel-Juso cells was analyzed. The proliferation assays confirmed the results of the viability assays that the additional release of *NO reduces the anti-tumorigenic activity of SERMs in melanoma cells. Three independent experiments were performed (n = -5-6). Mean ± SEM, ANOVA and Bonferroni *post hoc* test comparison vs. DMSO control, * p < 0.05 or, ** p < 0.01.

In order to investigate the anti-proliferative activity of our novel ER β antagonists on breast cancer cells, we performed cell viability and proliferation assays for the most potent NO-SERM **4d** and the corresponding SERM **5d**. Viability of the ER α/β -positive MCF-7 cells was reduced significantly after 24 h treatment with 50 µM **4d** or **5d** (Fig. 6A). The additional release of *****NO diminished this effect and preserved the viability of the cells. In comparison, viability of MDA-MB-231 cells, naturally lacking ER α , was not affected by the treatment with **4d** and **5d**. In both breast cancer cell lines the number of proliferating cells (proliferation assay) was significantly decreased after 48 h treatment with SERM **5d** in a concentration dependent manner. Interestingly, the additional release of *****NO maintained the number of proliferating cells of both cell lines. The high ER β selectivity resulted in a comparable response behavior of both breast cancer cell lines independent of their different ER α expression pattern. However, the concentration of SERM **5d** needed to reduce the number of proliferating cells significantly is relative high.

The impact of the additional NO-release was evaluated more in detail by the use of two ER α/β -positive melanoma cell lines to obtain results that could be compared with our previous study on NO-coxibs.²¹⁻²² Viability of both ER-positive melanoma cell lines was significantly diminished by treatment with the novel SERMs **5a-d** (Fig. 5B). The half-maximal effective concentration (EC₅₀) of SERMs **5** was in a micromolar range (25.7 - 38.5 μ M). **5c** was the only compound that showed no significant influence on cell viability. Remarkably, the additional NO-release resulted in a preservation of cell viability. Treatment with the NO-SERMs **4a-d** up to a concentration of 50 μ M showed no detectable influence on cell viability. Exemplary, for the most potent NO-SERM **4d** and the corresponding SERM **5d** the impact on the number of proliferating cells was analyzed (Fig. 5C-D). A concentration of 25 μ M SERM **5d** significantly decreased the number of proliferating cells in both cell lines. In contrast, 50 μ M NO-SERM **4d** showed no influence

on the number of proliferating cells. In summary, the additional NO-release reduces the influence on cell proliferation on both ER-positive melanoma cells. These preliminary results indicate that by the additional NO-release the tolerability of SERMs could be enhanced but their anti-tumorigenic activity is decreased.

4	NO-SERMs	viability 50 µM [%]	SERMs	viability 50 µM [%]		
	MCF-7					
	4d	82.8 ± 1.8 ^{*,#}	5d	72.2 ± 3.8*		
	MDA-MB-231					
	4d	91.3 ± 8.4	5d	86.4 ± 15.0		



Fig. 6: Influence of NO-SERMs 4 or SERMs 5 on cell viability and proliferation of breast cancer cell lines. (A) Influence of the NO-SERMs 4 and SERMs 5 on cell viability was investigated. Additional release of 'NO preserved viability of MCF-7 cells. Three independent experiments were performed (n =9). Mean \pm SEM, ANOVA and Bonferroni post hoc test comparison vs. DMSO control, * p < 0.05 or, ** p < 0.01. Furthermore, the influence of the corresponding compounds 4d and 5d on the number of proliferating cells on (B) MCF-7 and (C) MDA-MB-231 cells was analyzed. In both breast cancer cell lines, the number of proliferating cells was preserved by the additional release of 'NO. Four independent experiments were performed (n = 8). Mean \pm SEM, ANOVA and Bonferroni *post hoc* test comparison vs. DMSO control, * p < 0.05 or, ** p < 0.01.

Impact of the additional NO-release on normal vascular tissue cells. Taking into account our working hypothesis, we evaluated the benefit of the additional NO-release on vascular cells. Therefore, human umbilical vein endothelial cells, HUVECs, were used. ER

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protein expression of HUVECs was analyzed by Western blotting (Fig. 7A). ERβ protein synthesis was comparable with the expression of the MCF-7 cells, used as control. Moreover, HUVECs also showed a comparatively high ERα protein expression that is only slightly lower than in the MCF-7 cells. To confirm the protective influence of the additional NO-release on normal vascular tissue cells viability assays were performed (Fig. 7B). 24 h treatment with SERM **5d** resulted in a significant decrease of HUVECs viability. Moreover, treatment with 50 µM **5d** led to the complete loss of cell viability. As expected, HUVECs are more sensitive for the treatment with SERM **5d** than the breast cancer and the melanoma cell lines. In contrast, treatment with NO-SERM **4d** had no measurable influence on the cell viability up to a concentration of 50 µM. These data indicate that additional release of 'NO does protect endothelial cell viability. Caulin-Glaser et al. showed that the 17β-estradiol dependent regulation of Akt signaling⁴⁰.



Fig. 7: ER α and ER β expression and the influence of NO-SERM 4d or SERM 5d on viability of HUVEC. (A) ER α and ER β expression in HUVECs as determined by Western blotting. Representative sections of the immunochemical Western blot analysis of ER α , ER β and β -actin used as housekeeping protein are shown (three independent experiments). (B) The influence of NO-SERMs 4d and SERM 5d on cell viability was investigated. The additional NO-release resulted in a preservation of the cell viability in comparison to the strong decrease of cell viability by the treatment with SERM 5d. Three to five independent experiments were performed (n = 3 - 15). *n.E.d.*: no EC₅₀-value could be detected; *b.I.d.*: below limit of detection. The influence of E2 in comparison to the treatment with NO-SERM 4d on bioavailability of NO in HUVECs was analyzed (Fig. 8A) via a fluorescence-based assay. Treatment with 200 nM E2 showed no detectable impact on NO-bioavailability. Furthermore, the influence of the NO-SERM 4d and SERM 5d on COX-2 protein expression was investigated as marker for inflammatory stress. Since the ER/PI3K pathway is also associated with a regulation of the transcription factor nuclear factor-kappa B (NF-kB), the activation of NFκB resulted in an increase in COX-2 transcription.⁴¹ Twenty-four hours treatment with 200 nM E2 showed no influence on COX-2 expression (Fig. 8B). TAM (up to 1 μ M) slightly affected the COX-2 expression level, while treatment with the ERß selective SERM 5d resulted in a significant up-regulation of COX-2 in a concentration dependent manner. Importantly, this effect was diminished by the additional release of 'NO caused by NO-SERM 4d. Tamura et al. demonstrated on primary human uterine microvascular endothelial cells (HUMEC) that various levels of E2 significantly increased COX-2 expression via estrogen receptors.⁴² This effect could not be observed in the utilized HUVECs. A tissue-specific regulation of COX-2 expression via estrogens and antiestrogens was previously described and document the molecular complexity of estrogen receptor signaling.43



Fig. 8: Influence of NO-SERM 4d or SERM 5d on (A) NO-bioavailability and (B) the inflammatory marker COX-2 of HUVECs. (A) The influence of SERMs and the novel NO-SERM 4d on the bioavailable 'NO was measured as sum of nitrite and S-nitrosothiols in cell culture supernatants. Therefore, cells were treated with different concentrations of SERM 5d, NO-SERM 4d, tamoxifen (TAM) or 17β -estradiol and the amount of nitrite and S-nitrosothiol after 24 h treatment was analyzed in HUVECs. Three independent experiments were performed (n = 6). Mean ± SEM, ANOVA and Bonferroni *post hoc* test comparison vs. DMSO control were performed. (B) The influence of the ER-modulation on the inflammatory marker COX-2 was determined by Western blotting. Representative sections of the four independent experiments were shown.

Discussion and Conclusion

Starting from the hypothesis, that a bifunctional approach combining the antagonistic action of SERMs with a targeted exogenous release of 'NO could diminish vascular side effects associated with treatment with this commonly used drugs, we synthesized a series of novel NO-SERMs basing on a triaryl olefin lead bearing a nitroester functionality. NO-SERMs as well as the corresponding SERMs showed an antagonistic activity towards ERβ, whereby the methylsulfonyl

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group of the compounds seems to be responsible for the high ER β selectivity. The novel SERMs with a NO-releasing moiety underlie a moderate NO-release, this was aimed to prevent side effects associated with high *NO concentration, and to ensure a good manageability of the novel compounds. Similar results were obtained for NO-coxibs basing on a (pyrazolyl)benzenesulfonamide lead.²¹ Using for example a sulfohydroxamic acid (SO₂NHOH) substituent on a triaryl olefin lead resulted in an undesirable increased spontaneous NO-release (6 - 14%) that was enhanced by the addition of oxidizing agents (4 - 26%).⁴⁴

For the first time, the present study could demonstrate that the targeted release of 'NO diminished the anti-proliferative activity of SERMs on normal vascular tissue cells as well as on different ER β -positive breast cancer and melanoma cells. In order to evaluate these *in vitro* findings, several facts have to be taken into account. After the release of 'NO in the vascular system or in the tumor, the corresponding SERM with its increased anti-tumor activity becomes available, and would logically affect the ERβ-positive tumor. Further, it can be assumed that the NO-release occurs faster in vivo than under in vitro conditions. On the other hand, NO-release can be considered as therapeutically useful, since in contrast to the effect as gasotransmitter and vasodilator in the musculature of the vascular system, here the damaging effect of the free radical is the major impact. In this context, NO-releasing compounds are considered as potential radiosensitizers or chemosensitizers.^{22, 45-46} By following this concept, coxibs and NO-coxibs can be used for a comparative analysis. Data achieved by us demonstrate that the effect of 'NO is dependent on the underlying inhibitory mechanism of the lead structure.²² The additional release of 'NO by coxibs resulted in an increase of the anti-tumorigenic activity of these compounds, while the effect is reduced in SERMs. We could demonstrate that the bifunctional NO-coxibs are more potent radiosensitizers than the corresponding

conventional coxibs.²² The radiation response of different tumor entities is regulated by several factors. In this context, the role of ERβ is largely unknown. For further studies, we hypothesized that a manipulation of the ER-signaling, by SERMs in particular, provides a promising strategy to sensitize radioresistant ER-positive tumor cells for radiotherapy.⁴⁷ Perhaps, this effect is enhanced by the additional release of 'NO. The negligible cytotoxicity of the novel NO-SERMs in this regard is a positive aspect, because an ideal radiosensitizer shows a significant impact on tumor cells without cytotoxic side effects.⁴⁸⁻⁴⁹ However, confirmation of this concept requires further investigations.

In conclusion, a group of novel triaryl olefins with or without a NO-releasing moiety was synthesized, via McMurry olefination and identified as potential ER β selective SERMs. To the best of our knowledge, our data showed for the first time that a targeted release of *NO could diminish the anti-proliferative and pro-inflammatory effect of SERMs in normal vascular tissue and thereby could possibly improve the therapeutic index of this extensively used drug class. The high affinity and selectivity towards ER expressing cells should also contribute to reinforced effect on ER-positive tumor cells. The role of ER β in tumor progression, metastasis and therapy resistance, in particular towards radiotherapy, is largely unknown. By our novel ER β -selective SERMs with or without a NO-releasing moiety, useful tools to investigate these therapy relevant questions are available.

Experimental Section

Synthetic procedures. If not indicated otherwise, all reagents and solvents were of highest purity available from VWR International GmbH (Darmstadt, Germany), Sigma-Aldrich GmbH (Taufkirchen, Germany), Merck KGaA (Darmstadt, Germany), Thermo Fisher Scientific Inc. (Bonn, Germany) or Carl Roth GmbH & Co. KG (Karlsruhe, Germany).

Column chromatography was performed using silica gel with a mesh size of 40-63 µm. Semi-preparative HPLC by using the HPLC system ProStar from Varian was performed for further purification. The purity of the nitroester and hydroxyalkyl reference compounds was determined by analytical HPLC (Varian ProStar, microsorb 60, C18, 250 x 21.4 mm; acetonitrile/water + 0.1% TFA, 5/5, v/v). The purity of the novel compounds were \geq 95%. For determination of R_f-values by thin-layer chromatography (TLC) silica gel F-254 aluminum plates (Merck KGaA, Darmstadt, Germany) were used. Melting points were recorded by a Galen III (Cambridge Instruments, Cambridge, UK) melting point apparatus and are uncorrected. ¹H NMR, ¹³C NMR and ¹⁹F NMR were recorded on Agilent DD2-400 OneNMR probe (Agilent Technologies, Santa Clara, CA, USA). NMR spectra were referenced to the residual solvent shifts for ¹H and ¹³C, for ¹⁹F CFCl₃ as internal standard was used. Infrared (IR) spectroscopy was performed by FTIR spectrometer iS5 (Thermo Fisher Scientific, Rockfort, USA) and the spectra were recorded by ATR (attenuated total reflection). Low-resolution mass spectra (MS) were recorded by MS/MS Xevo TQ-S (Water GmbH, Eschborn, Germany) using electrospray (ES) ionization. High-resolution mass spectra were obtained by ESI-TOF-MS (Impact II, Bruker Daltonics) also using ES ionization. Elemental analysis was performed for C, H, N and S with the elemental analyzer EA3000 from EuroVector (Italia) and was within ± 0.5% of the theoretical values. All spectra are shown in the supporting information.

General procedure for synthesis of compound (1a-b). To a stirring suspension of $AlCl_3$ (1.3 eq.) in CHCl₃, 1.2 eq. chloroalkanolychloride and 1 eq. thioanisole were added under argon atmosphere at 0°C. After 2.5 h stirring at room temperature the reaction mixture was added to water. The organic layer was separated and washed three times with water. The combined aqueous solution was extracted three times with CHCl₃, the combined organic layer was dried over Na₂SO₄ and the solvent was removed under vacuum.

4-chloro-1-(4-(methylthio)phenyl)butan-1-one (1a). 4 ml 4-chlorobutanolychloride (35.7 mmol) was stirred with 3.4 mL thioanisole (28.9 mmol) in presence of 5.28 g AlCl₃ (39.6 mmol). After reconditioning **1a** was obtained as white solid (86.1% yield). mp 53-58°C (lit. 73-75°C ⁵⁰); Rf 0.60 (ethyl acetate/petroleum ether; 1/1; v/v); ¹H NMR (400 MHz, CD₃CN) δ (ppm) 2.17–2.08 (m, 2H, CH_{2-β}), 2.53 (s, 3H, CH₃), 3.12 (t, ³*J*=7.1 Hz, 2H, CH_{2-α}), 3.68 (t, ³*J*=6.6 Hz, 2H, CH_{2-γ}), 7.33 (d, ³*J*=8.7 Hz, 2H, CH_{aromatic}), 7.89 (d, ³*J*=8.8 Hz, 2H, CH_{aromatic}); ¹³C NMR (101 MHz, CD₃CN) δ (ppm) 14.82 (CH₃), 27.98 (CH_{2-β}), 36.05 (CH_{2-α}), 45.75 (CH_{2-γ}), 125.87, 129.36 (2 x CH_{aromatic}), 134.22, 146.89 (2 x C_{aromatic}), 199.02 (C_{ketone}); C₁₁H₁₃ClOS [M_{monoisotopic}] requires 228, ESI-MS (ES+) *m/z*: 229 [M(³⁵Cl)+H]⁺, 231 [M(³⁷Cl)+H]⁺; (ES-) *m/z*: 227 [M(³⁵Cl)-H]⁻; HRMS (ESI+) *m/z* calcd for C₁₁H₁₃ClOS [M+Na⁺,³⁵Cl] 251.02733, found 251.02694.

5-chloro-1-(4-(methylthio)phenyl)pentan-1-one (1b). 5.58 mL 5-chloropentanoylchloride (49.9 mmol) and 4.14 mL thioanisole (35.2 mmol) were stirred in presence of 5.29 g AlCl₃ (39.7 mmol). yellow solid; yield: 86.7%; mp 76-80°C, Rf 0.62 (ethyl acetate/petroleum ether; 1/1; v/v); ¹H NMR (400 MHz, CD₃CN) δ (ppm) 1.87–1.74 (m, 4H, CH_{2-β}, CH_{2-γ}), 2.53 (s, 3H, CH₃), 3.03–2.96 (m, 2H, CH_{2-α}), 3.63 (t, ³*J*=6.4 Hz, 2H, CH_{2-δ}), 7.33 (d, ³*J*=8.7 Hz, 2H, CH_{aromatic}), 7.88 (d, ³*J*=8.7 Hz, 2H, CH_{aromatic}); ¹³C NMR (101 MHz, CD₃CN) δ (ppm) 14.83 (CH₃), 22.30 (CH_{2-γ}), 32.84 (CH_{2-β}), 38.06 (CH_{2-α}), 46.04 (CH_{2-δ}), 125.87, 129.38, (2 x CH_{aromatic}), 134.38, 146.68, (2 x C_{aromatic}), 198.76 (C_{ketone}); C₁₂H₁₅CIOS [M_{monoisotopic}] requires 242, ESI-MS (ES+) *m/z*: 243 [M(³⁵CI)+H]⁺, 245 [M(³⁷CI)+H]⁺, HRMS (ESI+) *m/z* calcd for C₁₂H₁₅CIOS [M+Na⁺,³⁵CI] 265.04298, found 265.04226.

General procedure for synthesis of compound (2a-b). To an ice cooled solution of 1 (1 eq.) in THF/MeOH (1/1; v/v) an aqueous solution of oxone[®] (4 eq.) was added. The reaction mixture was stirred at room temperature for 31 h. After removal of the solvent EtOAc and water were added. The aqueous fraction was extracted three times with EtOAc and the combined organic fraction was washed with water, followed by drying with Na₂SO₄. The

vacuum dried row product was purified by column chromatography (ethyl acetate / petroleum ether, 1/1, v/v).

4-chloro-1-(4-(methylsulfonyl)phenyl)butan-1-one 2.56 (2a). 4-chloro-1-(4g (methylthio)phenyl)butan-1-one (1a, 11.2 mmol) an aqueous solution of oxone (14.45 g, 43.8 mmol) was added. After purification 2a was obtained as white solid (75.8% yield). mp 88-93°C; Rf 0.37 (ethyl acetate/petroleum ether; 1/1; v/v); ¹H NMR (400 MHz, CD₃CN) δ (ppm) 2.21–2.12 $(m, 2H, CH_{2-\beta}), 3.10$ $(s, 3H, CH_3), 3.22$ $(t, {}^{3}J=7.0 Hz, 2H, CH_{2-\gamma}), 3.70$ $(t, {}^{3}J=6.6 Hz, 2H, CH_{2-\gamma}), 3.70$ 8.03 (d, ³*J*=8.7 Hz, 2H, CH_{aromatic}), 8.16 (d, ³*J*=8.7 Hz, 2H, CH_{aromatic}); ¹³C NMR (101 MHz, CD₃CN) δ (ppm) 27.64 (CH_{2-β}), 36.88 (CH_{2-g}), 44.48 (CH_{2-ν}), 45.55 (CH₃), 128.60, 129.76 (2 x CH_{aromatic}), 141.74, 145.43 (2 x C_{aromatic}), 199.44 (C_{ketone}). C₁₁H₁₃CIO₃S [M_{monoisotopic}] requires 260, ESI-MS (ESI+) *m/z*: 261 [M(³⁵CI)+H]⁺, 263 [M(³⁷CI)+H]⁺, 283 [M(³⁵CI)+Na]⁺, 285 [M(³⁵CI)+H]⁺; (ES-) *m/z*: 260 [M(³⁵Cl)-H]⁻; HRMS (ESI+) *m/z* calcd for C₁₁H₁₃ClO₃S [M+Na⁺,³⁵Cl] 283.01716, found 283.01656; Anal. Calcd for C₁₁H₁₃ClO₃S: C: 50.67, H: 5.03, S: 12.30. Found: C: 50.80, H: 5.01, S: 11.90.

5-chloro-1-(4-(methylsulfonyl)phenyl)pentan-1-one (2b). 9.89 g 5-chloro-1-(4-(methylthio)phenyl)pentan-1-one (1b, 40.7 mmol) and 50.11 g oxone[®] (163.0 mmol) were stirred as previously described. After purification **2b** was obtained as white solid in 92.3% yield. mp 99-102°C; Rf 0.46 (ethyl acetate/petroleum ether; 1/1; v/v); ¹H NMR (400 MHz, CDCl₃) δ (ppm) 2.02–1.83 (m, 4H, CH_{2-β}, CH_{2-γ}), 3.16–3.00 (m, 5H, CH₃, CH_{2-α}), 3.60 (t, ³*J*=6.1 Hz, 2H, CH_{2-δ}), 8.06 (d, ³*J*=8.5 Hz, 2H, CH_{aromatic}), 8.13 (d, ³*J*=8.5 Hz, 2H, CH_{aromatic}); ¹³C NMR (101 MHz, CDCl₃) δ (ppm) 21.38 (CH_{2-γ}), 31.99 (CH_{2-β}), 38.25 (CH_{2-α}), 44.48 (CH_{2-δ}), 44.72 (CH₃), 128.01, 129.01 (2 x CH_{aromatic}), 140.88, 144.33 (2 x C_{aromatic}), 198.38 (C_{ketone}). C₁₂H₁₅ClO₃S [M_{monoisotopic}] requires 274, ESI-MS (ES+) *m/z*: 275 [M(³⁵Cl)+H]⁺, 277 [M(³⁷Cl)+H]+, 297 [M(³⁵Cl)+Na]⁺, 299 [M(³⁷Cl)+Na]⁺, (ES-) *m/z*: 274 [M(³⁵Cl)-H]⁻; HRMS (ESI+) *m/z* calcd for C₁₂H₁₅ClO₃S [M+Na⁺,³⁵Cl] 297.03281, found 297.03241, calcd for [2M+Na⁺, ³⁵Cl] 571.07585, found 571.07531.

General procedure for synthesis of compound (3a-d). To a stirred suspension of zinc powder (8 eq.) in dry THF under argon atmosphere at -10°C titan tetrachloride (4 eq.) was added dropwise. The suspension was headed under reflux for 2 h and after cooling the suspension to 0°C a solution of 2 (1 eq.) and 4,4'-difluoro-benzophenone or benzophenone (1 eq.) in dry THF was added. After 2.5 h heating under reflux the reaction mixture was tempered to 25°C and poured into a 10% aqueous potassium carbonate solution. The mixture was stirred for 5 min and the insoluble material was removed by vacuum filtration. After separation of the organic fraction the aqueous fraction was extracted with EtOAc three times. The combined organic fraction was dried over Na₂SO₄ and the solvent was removed under vacuum. The raw product was purified by column chromatography (EtOAc/petroleum ether).

5-chloro-2-(4-(methylsulfonyl)phenyl)phenyl)phen-1-en-1,1-diyl)dibenzene (**3a**). To the stirring complex of 1.06 mL TiCl₄ (1.8 mmol) and 0.99 g Zinc powder (15.1 mmol) in 1.1 mL THF a suspension of 4-chloro-1-(4-(methylsulfonyl)phenyl)butan-1-one (**2a**, 0.50 g, 1.9 mmol) and benzophenone (0.35 g, 1.9 mmol) in 38 mL THF was added. Upon purification by column chromatography (ethyl acetate/petroleum ether; 2/3; v/v) **3a** was obtained as white solid with 63.3% yield. mp: 129-133°C; Rf 0.55 (EtOAc/petroleum ether, 1/1, v/v); ¹H NMR (400 MHz, CD₃CN) δ (ppm) 1.80–1.68 (m, 2H, CH_{2-β}), 2.62–2.55 (m, 2H, CH_{2-α}), 3.00 (s, 3H, CH₃), 3.46 (t, ³*J*=6.6 Hz, 2H, CH_{2-γ}), 6.97–6.91 (m, 2H, CH_{aromatic}), 7.10–7.03 (m, 3H, CH_{aromatic}), 7.35–7.28 (m, 3H, CH_{aromatic}), 7.44–7.38 (m, 4H, CH_{aromatic}), 7.71 (d, ³*J*=8.6 Hz, 2H, H-2,6 CH₃SO₂Phe). ¹³C NMR (101 MHz, CD₃CN) δ (ppm) 32.29 (CH_{2-α}), 33.69 (CH_{2-β}), 44.42 (CH₃), 45.83 (CH_{2-γ}), 127.37, 127.66, 128.08, 128.68, 129.40, 129.89, 131.15, 131.53, 138.92, 139.77, 142.90, 143.27, 143.47, 148.90. C₂₄H₂₃ClO₂S [M_{monoisotopic}] 410, MS (ES+) *m/z*: 411 [M(³⁵Cl)+H]^{*}, 413 [M(³⁷Cl)+Na]^{*}, 435 [M(³⁷Cl)+Na]^{*}; HRMS (ESI+) *m/z* calcd for C₂₄H₂₃ClO₂S [M+Na^{*},³⁵Cl] 433.10050, found 433.09975, calcd for [2M+Na^{*},³⁵Cl] 843.21123, found 843.21068; IR (ATR): v

= 1589 (m, olefin), 1149 (s, SO₂) cm⁻¹; Anal. calcd for C₂₄H₂₃ClO₂S: C 70.14, H 5.64, S: 7.80, found: C 70.33, H 5.71, S 7.34.

4,4'-(5-chloro-2-(4-(methylsulfonyl)phenyl)pent-1-en-1,1-diyl)bis(fluorobenzene) (3b). To the stirring complex of 2.2 mL TiCl₄ (19.7 mmol) and 2.00 g Zinc powder (30.6 mmol) in 36 mL THF a suspension of 1.00 g 4-chloro-1-(4-(methylsulfonyl)phenyl)butan-1-one (2a, 3.8 mmol) and 0.85 g 4,4'-difluorobenzophenone (3.9 mmol) in 76 mL THF was added. After purification by column chromatography (ethyl acetate/petroleum ether; 1/3; v/v) 3b was obtained as white solid with 56.3% yield. mp: 140-142°C; Rf 0.49 (EtOAc/petroleum ether, 1/1, v/v); purity: 99.0% (analyzed: H₂O/MECN + 0.1% TFA; 1/1; v/v); ¹H-NMR (400 MHz, CD₃CN) δ (ppm) 1.78–1.69 (m, 2H, CH₂₋₈), 2.60–2.55 (m, 2H, CH₂₋₇), 3.01 (s, 3H, CH₃), 3.46 (t, ${}^{3}J$ =6.4 Hz, 2H, CH₂₋₇), 6.81 (t, ³J=³J_{H F}=9.0 Hz, 2H, H–3,5 FPhe), 6.93 (dd, ³J=8.9 Hz, ⁴J_{H F}=5.6 Hz, 2H, H–2,6 FPhe), 7.15 (t, ³J=³J_{HF}= 8.9 Hz, 2H, H–3,5 FPhe), 7.30 (dd, ³J=8.8 Hz, ⁴J_{HF}=5.5 Hz, 2H, H–2,6 FPhe), 7.38 (d, ³J=8.6 Hz, 2H, H–3,5 CH₃SO₂Phe), 7.73 (d, ³J=8.6 Hz, 2H, H–2,6 CH₃SO₂Phe). ¹³C NMR (101 MHz, CD₃CN) δ (ppm) 32.19 (CH_{2-α}), 33.71 (CH_{2-β}), 44.50 (CH₃), 45.82 (CH_{2-ν}), 115.48 (d, ²J_{C F}=21.6 Hz, C–3,5 FPhe), 116.21 (d, ²J_{C F}=21.6 Hz, C–3,5 FPhe), 127.83, 131.50, 131.95 (d, ³J_{C,F}=8.1 Hz, C–2,6 FPhe), 133.11 (d, ³J_{C,F}=8.1 Hz, C–2,6 FPhe), 139.38 (d, ⁴J_{C,F}=3.3 Hz, C–4 FPhe), 139.46 (d, ⁴J_{C,F}=3.4 Hz, C–4 FPhe), 139.94, 139.97, 140.70, 148.62, 162.25 (d, ¹*J*_{C.F}=244.4 Hz, C–1 FPhe), 162.88 (d, ¹*J*_{C.F}=244.2 Hz, C–1 FPhe). ¹⁹F NMR (376 MHz, CD₃CN) δ (ppm) -114.40 - -114.51 (m), -113.97 - -114.09 (m). C₂₄H₂₁CIF₂O₂S [M_{monoisotopic}] requires 446, ESI-MS (ES+) m/z: 447 [M(³⁵Cl)+H]⁺, 449 [M(³⁷Cl)+H]⁺, 469 [M(³⁵Cl)+Na]⁺, 471 [M(³⁷Cl)+Na]⁺; HRMS (ESI+) *m/z* calcd for C₂₄H₂₁CIF₂O₂S [M+Na⁺, ³⁵CI] 469.08165, found 469.08061, calcd for [2M+Na⁺,³⁵Cl] 915.17353, found 915.17299.

6-chloro-2-(4-(methylsulfonyl)phenyl)hex-1-en-1,1-diyl)dibenzene (3c). To the stirring complex of 3.9 mL TiCl₄ (35.6 mmol) and 3.50 g Zinc powder (53.5 mmol) in 63 mL THF a suspension of 1.85 g 5-chloro-1-(4-(methylsulfonyl)phenyl)pentan-1-one (**2b**, 6.7 mmol) and 1.25

g benzophenone (6.8 mmol) in 133 mL THF was added. Upon purification by column chromatography (ethyl acetate/petroleum ether; 1/4; v/v) **3c** was obtained as white solid with 39.4% yield. mp: 96-100°C; Rf 0.60 (EtOAc/petroleum ether, 1/1, v/v); ¹H NMR (400 MHz, CD₃CN) $\bar{\delta}$ (ppm) 1.47–1.37 (m, 2H, CH_{2- β}), 1.71–1.59 (m, 2H, CH_{2- γ}), 2.50–2.43 (m, 2H, CH_{2- α}), 3.00 (s, 3H, CH₃), 3.44 (t, ³*J*=6.6 Hz, 2H, CH_{2- δ}), 6.97–6.92 (m, 2H, CH_{aromatic}), 7.10–7.03 (m, 3H, CH_{aromatic}), 7.34–7.27 (m, 3H, CH_{aromatic}), 7.44–7.36 (m, 4H, CH_{aromatic}), 7.70 (d, ³*J*=8.2 Hz, 2H, H-2,6 CH₃SO₂Phe). ¹³C NMR (101 MHz, CD₃CN) $\bar{\delta}$ (ppm) 26.40 (CH_{2- γ}), 33.03 (CH_{2- β}), 35.30 (CH_{2- α}), 44.44 (CH₃), 45.72 (CH_{2- δ}), 127.32, 127.59, 128.03, 128.67, 129.38, 129.92, 131.20, 131.53, 139.67, 139.94, 142.35, 143.37, 143.64, 149.18. C₂₅H₂₅CIO₂S [M_{monoisotopic}] requires 424, ESI-MS (ES+) *m/z*: 447 [M(³⁵CI)+Na]⁺, 449 [M(³⁷CI)+Na]⁺, HRMS (ESI+) *m/z* calcd for C₂₅H₂₅CIO₂S [M+NH₄⁺,³⁵CI] 442.16498, found 442.16020, calcd for [M+Na⁺,³⁵CI] 447.11615, found 447.11560, calcd for [2M+Na⁺,³⁵CI] 871.24253, found 871.24198; IR (ATR): v = 1591 (m, olefin), 1147 (s, SO₂) cm⁻¹; Anal. calcd for C₂₅H₂₅CIO₂S: C 70.65, H 5.93, S: 7.55, found: C 70.76, H 5.99, S 7.22.

4,4'-(6-chloro-2-(4-(methylsulfonyl)phenyl)hex-1-en-1,1-diyl)bis(fluorobenzene) (3d). To a stirring suspension of 1.12 mL TiCl4 (8.0 mmol) and 1.05 g Zinc powder (16.3 mmol) in 19 mL THF a suspension of 0.56 g 5-chloro-1-(4-(methylsulfonyl)phenyl)pentan-1-one (**2b**, 2.0 mmol) and 0.44 g 4,4'-difluorobenzophenone (2.0 mmol) in 40 mL THF was added. Upon purification by column chromatography (ethyl acetate/petroleum ether; 1/1; v/v) **3d** was obtained as colorless oil with 75.2% yield. mp: 99-105°C; Rf 0.56 (EtOAc/petroleum ether, 1/1, v/v); ¹H NMR (400 MHz, CD₃CN) δ (ppm) 1.47–1.35 (m, 2H, CH_{2- β}), 1.70–1.58 (m, 2H, CH_{2- γ}), 2.52–2.42 (m, 2H, CH_{2- α}), 3.01 (s, 3H, CH₃), 3.48–3.41 (m, 2H, CH_{2- δ}), 6.81 (t, ³*J*=³*J*_{H,F}=9.0 Hz, 2H, H–3,5 FPhe), 6.92 (dd, ³*J*=8.9 Hz, ⁴*J*_{H,F}=5.6 Hz, 2H, H–2,6 FPhe), 7.15 (t, ³*J*=³*J*_{H,F}=8.9 Hz, 2H, H–3,5 FPhe), 7.29 (dd, ³*J*=8.8 Hz, ⁴*J*_{H,F}=5.5 Hz, 2H, H–2,6 FPhe), 7.38 (d, ³*J*=8.6 Hz, 2H, H–3,5 CH₃SO₂Phe), 7.72 (d, ³*J*=8.6 Hz, 2H, H–2,6 CH₃SO₂Phe). ¹³C NMR (101 MHz, CD₃CN) δ (ppm) 26.32 (CH_{2- γ}), 33.02 (CH_{2- β}), 35.34 (CH_{2- α}), 44.50 (CH₃), 45.72 (CH_{2- δ}), 115.45 (d, ²*J*_{C-}=21.6 Hz, C–3,5 FPhe), 116.03

(d, ${}^{2}J_{C,F}$ =21.5 Hz, C–3,5 FPhe), 127.75, 131.49, 131.94 (d, ${}^{3}J_{C,F}$ =8.2 Hz, C–2,6 FPhe), 133.15 (d, ${}^{3}J_{C,F}$ =8.1 Hz, C–2,6 FPhe), 139.47 (d, ${}^{4}J_{C,F}$ =3.2 Hz, C–4 FPhe), 139.61 (d, ${}^{4}J_{C,F}$ =3.5 Hz, C–4 FPhe), 139.85, 140.11, 140.95, 148.90, 162.21 (d, ${}^{1}J_{C,F}$ =244.6 Hz, C–1 FPhe), 162.85 (d, ${}^{1}J_{C,F}$ =244.2 Hz, C–1 FPhe). C₂₅H₂₃CIF₂O₂S [M_{monoisotopic}] requires 460, ESI-MS (ES+) *m/z*: 483 [M(35 CI)+Na]⁺; 485 [M(37 CI)+Na]⁺, (ES-) *m/z*: 459 [M(35 CI)-H]⁻.

General procedure for synthesis of compound (4a-d). To a solution of **3** (1 eq.) in acetonitrile silver nitrate (3 eq.) was added and the reaction mixture was heated for 24 hours at 80°C. The resulting silver salt was filtered off and the solvent was evaporated at reduced pressure. The dry reaction mixture was suspended in EtOAc, washed three times with water and dried with Na_2SO_4 . The raw product was purified by column chromatography (EtOAc/ petroleum ether).

4-(4-(methylsulfonyl)phenyl)-5,5-diphenylpent-4-en-1-yl nitrate (4a). 21 g 5-chloro-2-(4-(methylsulfonyl)phenyl)pent-1-en-1,1-diyl)dibenzene (**3a**, 0.5 mmol) and 0.27 g silver nitrate (1.6 mmol) were heated in 3.5 mL acetonitrile. After purification with column chromatography (ethyl acetate/petroleum ether; 2/3; v/v) and semi-preparative HPLC (acetonitrile/water + 0.1% TFA; 7/3; v/v; 5 mL/min) **4a** was obtained as white solid. yield: 32.6%; purity: 98.6% (water/acetonitrile + 0.1% TFA; 1/1; v/v); mp: 109-111°C; Rf 0.61 (EtOAc/petroleum ether, 1/1, v/v); ¹H NMR (400 MHz, CD₃CN) δ (ppm) 1.75–1.65 (m, 2H, CH_{2-β}), 2.59–2.52 (m, 2H, CH_{2-α}), 3.00 (s, 3H, CH₃), 4.35 (t, ³*J*=6.5 Hz, 2H, CH_{2-γ}), 6.97–6.93 (m, 2H, CH_{aromatic}), 7.10–7.04 (m, 3H, CH_{aromatic}), 7.36–7.26 (m, 3H, CH_{aromatic}), 7.44–7.37 (m, 4H, CH_{aromatic}), 7.74 (d, ³*J*=8.2 Hz, 2H, H–2,6 CH₃SO₂Phe). ¹³C NMR (101 MHz, CD₃CN) δ (ppm) 26.33 (CH_{2-β}), 32.31 (CH_{2-α}), 44.44 (CH₃), 74.11 (CH_{2-γ}), 127.44, 127.70, 128.15, 128.71, 129.47, 129.75, 131.11, 131.57, 138.70, 139.86, 142.13, 143.15, 143.44, 148.66. C₂₄H₂₃NO₅S [M_{monoisotopic}] requires 437, ESI-MS (ES+) *m/z*: 460 [M+Na]⁺, 476 [M+K]⁺; HRMS (ESI+) *m/z* calcd for C₂₄H₂₃NO₅S [M+Na]⁺ 460.11946, found 460.11830, calcd for [2M+Na]⁺ 897.24915, found 897.24861; IR (ATR): v = 1618, 1283 (s, O-NO₂), 1599 (m, olefin), 1148 (s, SO₂) cm⁻¹; Anal. calcd for C₂₄H₂₃NO₅S: C 65.89, H 5.30, S: 7.33, found: C 65.82, H 5.29, S 6.96.

5,5'-bis-(4-fluorophenyl)-4-(4-methylsulfonyl)phenyl)pent-4-en-1-yl nitrate (4b). For the synthesis of 5,5'-bis-(4-fluorophenyl)-4-(4-methylsulfonyl)phenyl)pent-4-en-1-yl nitrate (4b) 0.15 g 5-chloro-2-(4-(methylsulfonyl)phenyl)pent-1-en-1.1-diyl)dibenzene (3a, 0.3 mmol) was heated in presence of 0.21 g silver nitrate (1.3 mmol) in 2.1 mL acetonitrile. After purification by column chromatography (ethyl acetate/petroleum ether; 1/1; v/v) and semi-preparative HPLC (water/acetonitrile + 0.1% TFA; 0-7 min: 1/1, 17-25 min: 3/7, 35-40 min: 1/1; v/v; 7 mL/min) 4b was obtained as white solid. yield: 54.5%, purity: 99.3% (water/ acetonitril + 0.1% TFA; 1/1; v/v) mp: 122-126°C; Rf 0.51 (EtOAc/petroleum ether, 1/1, v/v); ¹H NMR (400 MHz, CD₃CN) δ (ppm) 1.74–1.65 (m, 2H, CH₂₋₈), 2.56–2.50 (m, 2H, CH_{2-a}), 3.01 (s, 3H, CH₃), 4.35 (t, ³J=6.4 Hz, 2H, CH_{2-v}), 6.82 (t, J=³J_{H,F}=9.0 Hz, 2H, H–3,5 FPhe), 6.93 (dd, ³J=9.0 Hz, ⁴J_{H,F}=5.6 Hz, 2H, H–2,6 FPhe), 7.15 (t, ³*J*=³*J*_{H.F}=8.9 Hz, 2H, H–3,5 FPhe), 7.29 (dd, ³*J*=8.9 Hz, ⁴*J*_{H.F}=5.5 Hz, 2H, H–2,6 FPhe), 7.39 (d, ³J=8.6 Hz, 2H, H–3,5 CH₃SO₂Phe), 7.73 (d, ³J=8.6 Hz, 2H, H–2,6 CH₃SO₂Phe). ¹³C NMR (101 MHz, CD₃CN) δ (ppm) 26.26 (CH_{2-a}), 32.31 (CH_{2-b}), 44.47 (CH₃), 74.05 (CH_{2-v}), 115.47 (d, ²J_{C,F}=21.6 Hz, C–3,5 FPhe), 116.25 (d, ²J_{C,F}=21.7 Hz, C–3,5 FPhe), 127.84, 131.50, 131.79 (d, ³J_{C,F}=8.1 Hz, C–2,6 FPhe), 133.04 (d, ³J_{C,F}=8.0 Hz, C–2,6 FPhe), 139.22 (d, ⁴J_{C,F}=3.3 Hz, C-4 FPhe), 139.39 (d, ⁴J_{C.F}=3.5 Hz, C-4 FPhe), 139.69, 140.01, 140.88, 148.35, 162.25 (d, ¹J_{C F}=244.7 Hz, C–1 FPhe), 162.88 (d, ¹J_{C F}=244.5 Hz, C–1 FPhe). ¹⁹F NMR (376 MHz, CD₃CN) δ (ppm) -117.38 (tt, ${}^{3}J_{H,F}$ =8.9 Hz, ${}^{4}J_{H,F}$ =5.7 Hz), -116.67 (tt, ${}^{3}J_{H,F}$ =8.9 Hz, ${}^{4}J_{H,F}$ =5.5 Hz). C₂₄H₂₁F₂NO₅S [M_{monoisotopic}] requires 473, ESI-MS (ES+) *m/z*: 474 [M+H]⁺; HRMS (ESI+) *m/z* calcd for C₂₄H₂₁F₂NO₅S [M+Na]⁺ 496.10062, found 496.09963, calcd for [2M+Na]⁺ 969.21147, found 969.21092; IR (ATR): v = 1602, 1280 (s, O-NO₂), 1220 (s, C-F), 1148 (s, SO₂) cm⁻¹; Anal. calcd for C₂₄H₂₁F₂NO₅S: C 60.88, H 4.47, S: 6.77, found: C 60.23, H 4.36, S 6.62.

5-(4-(methylsulfonyl)phenyl)-6,6-diphenylhex-5-en-1-yl nitrate (4c). 0.20 g 6-chloro-2-(4-(methylsulfonyl)phenyl)hex-1-en-1,1-diyl)dibenzene (3c, 0.5 mmol) and 0.25 g silver nitrate (1.5 mmol) were stirred at 80°C in 3.1 mL acetonitrile. After purification by column chromatography (ethyl acetate/petroleum ether; 2/3; v/v) 5-(4-(methylsulfonyl)phenyl)-6-6-diphenylhex-5-en-1-yl nitrate (4c) was obtained in 61.8 % yield. beige solid; purity: 97.3% (water/acetonitrile + 0.1% TFA; 1/1; v/v); mp 101-107°C; Rf 0.31 (EtOAc/petroleum ether, 1/1, v/v); ¹H NMR (400 MHz, CD₃CN): δ (ppm) 1.42–1.34 (m, 2H, CH_{2-β}), 1.66–1.55 (m, 2H, CH_{2-α}), 2.53–2.44 (m, 2H, CH_{2-ν}), 3.00 (s, 3H, CH₃), 4.32 (t, ³J=6.5 Hz, 2H, CH₂₋₅), 6.96–6.92 (m, 2H, CH_{aromatic}), 7.09–7.03 (m, 3H, CH_{aromatic}), 7.34–7.26 (m, 3H, CH_{aromatic}), 7.43–7.37 (m, 4H, CH_{aromatic}), 7.70 (d, ³J=8.3 Hz, 2H, H– 2,6 CH₃SO₂Phe). ¹³C NMR (101 MHz, CD₃CN) δ (ppm) 25.21 (CH_{2-β}), 26.95 (CH_{2-ν}), 35.45 (CH₂₋ _a), 44.43 (CH₃), 74.27 (CH_{2-δ}), 127.33, 127.61, 128.04, 128.67, 129.38, 129.85, 131.16, 131.51, 139.69, 139.74, 142.48, 143.30, 143.62, 149.04. C25H25NO5S [Mmonoisotopic] requires 451, ESI-MS (ES+) m/z: 452 [M+H]⁺, 474 [M+Na]⁺, (ES-) m/z: 451 [M-H]⁻; HRMS (ESI+) m/z calcd for C₂₅H₂₅NO₅S [M+NH₄]⁺ 469.18394, found 469.17917, calcd for [M+Na]⁺ 474.13511, found 474.13464; IR (ATR): v = 1621, 1277 (s, O-NO₂), 1592 (m, olefin), 1148 (s, SO₂) cm⁻¹; Anal. calcd for C₂₅H₂₅NO₅S: C 66.50, H 5.58, S: 7.10, found: C 66.15, H 5.64, S 6.75.

6,6'-bis(4-fluorophenyl)-5-(4-(methylsulfonyl)phenyl)hex-5-en-1-yl nitrate (4d). А of 4,4'-(6-chloro-2-(4-(methylsulfonyl)phenyl)hex-1-en-1,1suspension 0.26 g diyl)bis(fluorobenzene) (3d, 0.6 mmol) and 0.25 g silver nitrate (1.5 mmol) in 3.1 mL acetonitrile was stirred at 80°C. The purification was performed by column chromatography (ethyl acetate/petroleum ether; 2/3; v/v) and 4d was obtained as white solid with 71.9% yield. purity: 98.1% (water/acetonitrile + 0.1% TFA; 1/1; v/v); mp: 109-113°C; Rf 0.45 (EtOAc/petroleum ether, 1/1, v/v); ¹H NMR (400 MHz, CD₃CN) δ (ppm) 1.41–1.32 (m, 2H, CH_{2-β}), 1.65–1.55 (m, 2H, CH₂₋ _v), 2.50–2.43 (m, 2H, CH_{2- α}), 3.01 (s, 3H, CH₃), 4.33 (t, ³*J*=6.5 Hz, 2H, CH_{2- δ}), 6.81 (t, *J*=³*J*_{H,F}= 9.0 Hz, 2H, H–3,5 FPhe), 6.93 (dd, ³J=8.9 Hz, ⁴J_{H F}=5.6 Hz, 2H, H–2,6 FPhe), 7.14 (t, ³J=³J_{H F}=8.9 Hz, 2H, H–3,5 FPhe), 7.29 (dd, 3 J=8.8 Hz, 4 J_{H,F}=5.5 Hz, 2H, H–2,6 FPhe), 7.37 (d, 3 J=8.5 Hz, 2H, H–3,5 CH₃SO₂Phe), 7.72 (d, 3 J=8.6 Hz, 2H, H–2,6 CH₃SO₂Phe). 13 C NMR (101 MHz, CD₃CN) δ (ppm) 25.13 (CH_{2-β}), 26.95 (CH_{2-γ}), 35.47 (CH_{2-α}), 44.45 (CH₃), 74.25 (CH_{2-δ}), 115.41 (d, 2 J_{C,F}=21.6 Hz, C–3,5 FPhe), 116.15 (d, 2 J_{C,F}=21.6 Hz, C–3,5 FPhe), 127.73, 131.43, 131.84 (d, 3 J_{C,F}=8.2 Hz, C–2,6 FPhe), 133.07 (d, 3 J_{C,F}=8.1 Hz, C–2,6 FPhe), 139.36 (d, 4 J_{C,F}=3.1 Hz, C–4 FPhe), 139.56 (d, 4 J_{C,F}=3.2 Hz, C–4 FPhe), 139.83, 140.22, 140.72, 148.72, 162.18 (d, 1 J_{C,F}=244.1 Hz, C–1 FPhe), 162.81 (d, 1 J_{C,F}=244.1 Hz, C–1 FPhe). 19 F NMR (376 MHz, CD₃CN) δ (ppm) -117.65 – 117.74 (m), -117.15 – -117.27 (m). C₂₅H₂₃F₂NO₅S [M_{monoisotopic}] requires 487, ESI-MS (ES+) *m*/*z*: 510 [M+Na]⁺; (ES-) m/z: 486 [M-H]⁻; HRMS (ESI+) *m*/*z* calcd for C₂₆H₂₃F₂NO₅S [M+Na]⁺ 510.11627, found 510.11578, calcd for [2M+Na]⁺ 997.24277, found 997.24222; IR (ATR): v = 1622, 1279 (s, O-NO₂), 1591 (m, olefin), 1214 (s, C-F), 1148 (s, SO₂) cm⁻¹; Anal. calcd for C₂₅H₂₃F₂NO₅S: C 61.59, H 4.76, S: 6.58, found: C 62.28, H 4.78, S 6.39.

General procedure for synthesis of compound (5a-d). A suspension of 1 eq. **3** and 1 eq. copper(II)sulfate in water/DMSO (1/3; v/v) was stirred at 130°C under reflux for 48 h. After cooling to room temperature, the reaction mixture was extracted three times with EtOAc and the combined organic fraction was washed three times with water. Purification was performed by semi-preparative HPLC or by column chromatography (EtOAc/ petroleum ether) respectively.

4-(4-(methylsulfonyl)phenyl)-5,5-diphenylpent-4-en-1-ol (5a). A suspension of 0.30 g 5chloro-2-(4-(methylsulfonyl)phenyl)pent-1-en-1,1-diyl)dibenzene (**3a**; 0.7 mmol) and 0.18 g CuSO₄ x 5 H₂O (0.7 mmol) in 2.8 mL water/DMSO (1/3; v/v) was stirred for 48 h at 130°C under reflux. After purification by column chromatography (ethyl acetate/petroleum ether; 1/1; v/v) **5a** was obtained as white solid. yield: 66.0%; purity: 99.2% (water/acetonitrile + 0.1% TFA; 1/1; v/v); mp 144-148°C: Rf 0.07 (EtOAc/petroleum ether, 1/1, v/v); ¹H NMR (400 MHz, CD₃CN) δ (ppm) 1.50–1.40 (m, 2H, CH_{2-α}), 2.40 (t, ³*J*=5.1 Hz, 1H, OH), 2.53–2.46 (m, 2H, CH_{2-β}), 3.00 (s, 3H, CH₃),

3.39–3.31 (m, 2H, CH_{2-v}), 6.95–6.90 (m, 2H, CH_{aromatic}), 7.09–7.02 (m, 3H, CH_{aromatic}), 7.34–7.27 (m, 3H, CH_{aromatic}), 7.42–7.36 (m, 4H, CH_{aromatic}), 7.70 (d, ³*J*=8.5 Hz, 2H, H–2,6 CH₃SO₂Phe). ¹³C NMR (101 MHz, CD₃CN) δ (ppm) 32.5 (CH_{2- α}), 32.9 (CH_{2- β}), 44.4 (CH₃), 47.0 (CH_{2- γ}), 118.3, 127.3, 127.6, 128.0, 128.6, 129.3, 130.0, 131.2, 131.5, 139.6, 140.2, 142.1, 143.5, 143.7, 149.4. C₂₄H₂₄O₃S [M_{monoisotopic}] requires 392, ESI-MS (ES+) *m/z*: 393 [M+H]⁺, 415 [M+Na]⁺; HRMS (ESI+) *m/z* calcd for C₂₄H₂₄O₃S [M+Na]⁺ 415.13439, found 415.13358, calcd for [2M+Na]⁺ 807.27901, found 807.27845; IR (ATR): v = 3509 (s, OH), 1601 (m, olefin), 1146 (s, SO₂) cm⁻¹; Anal. calcd for C₂₄H₂₄O₃S: C 73.44, H 6.16, S: 8.17, found: C 73.26, H 6.14, S 7.80.

5,5'-bis(4-fluorophenyl)-4-(4-methylsulfonyl)phenyl)pent-4-en-1-ol (5b). A suspension of 0.15 g 4,4'-(5-chloro-2-(4-(methylsulfonyl)phenyl)pent-1-en-1,1-diyl)-bis(fluoro-benzene) (3b; 0.3 mmol) and 0.08 g CuSO₄ x 5 H₂O (0.3 mmol) in 1.0 mL water/DMSO (1/3; v/v) was stirred for 48 h at 130°C under reflux. After purification by column chromatography (ethyl acetate/petroleum ether; $1/1 \rightarrow 7/3$; v/v) **5b** was obtained as beige solid. yield: 73.5%; purity: 98.5% (water/acetonitrile + 0.1% TFA; 1/1; v/v); mp 165-169°C: Rf 0.08 (EtOAc/petroleum ether, 1/1, v/v); ¹H NMR (400 MHz, CD₃CN) δ (ppm) 1.50–1.40 (m, 2H, CH₂₋₈), 2.42 (t, ³*J*=5.3 Hz, 1H, OH), 2.51-2.45 (m, 2H, CH_{2- α}), 3.01 (s, 3H, CH₃), 3.36 (dd, ³J=11.7 Hz, ³J=6.3 Hz, 2H, CH_{2- α}), 6.81 (t, J=³J_{H,F}=8.9 Hz, 2H, H–3.5 FPhe), 6.92 (dd, ³J=8.9 Hz, ⁴J_{H,F}=5.6 Hz, 2H, H–2,6 FPhe), 7.14 (t, ³*J*=³*J*_{H,F}=8.9 Hz, 2H, H–3,5 FPhe), 7.30 (dd, ³*J*=8.9 Hz, ⁴*J*_{H,F}=5.6 Hz, 2H, H–2,6 FPhe), 7.37 (d, ³J=8.6 Hz, 2H, H–3,5 CH₃SO₂Phe), 7.72 (d, ³J =8.6 Hz, 2H, H–2,6 CH₃SO₂Phe). ¹³C NMR (101 MHz, CD₃CN) δ (ppm) 32.3 (CH_{2-β}), 33.0 (CH_{2-q}), 44.5 (CH₃), 62.2 (CH_{2-ν}), 115.4 (d, ²J_{C,F}=21.7 Hz, C–3,5 FPhe), 116.1 (d, ²J_{C,F}=21.7 Hz, C–3,5 FPhe), 127.7, 131.5, 131.8 (d, ³J_{C,F}=8.3 Hz, C– 2,6 FPhe), 133.1 (d, ³J_C_F=8.3 Hz, C–2,6 FPhe), 139.6 (d, ⁴J_C_F=3.3 Hz, C–4 FPhe), 139.7 (d, ⁴J_{C.F}=3.3 Hz, C–4 FPhe), 139.8, 139.8, 141.1, 149.1, 162.2 (d, ¹J_{C.F}=244.4 Hz, C–1 FPhe), 162.8 (d, ¹*J*_{C,F}=244.4 Hz, C–1 FPhe). ¹⁹F NMR (376 MHz, CD₃CN) δ -117.4 (tt, ³*J*_{H,F}=8.9 Hz, ⁴*J*_{H,F}=5.5 Hz), -116.9 (tt, ³J_{H,F}=8.9 Hz, ⁴J_{H,F}=5.5 Hz). C₂₄H₂₂F₂O₃S [M_{monoisotopic}] requires 428, ESI-MS (ES+/-)

m/z: not ionizable; HRMS (ESI+) *m/z* calcd for $C_{24}H_{22}F_2O_3S$ [M+NH₄]⁺ 446.16437, found 446.15953, calcd for [M+Na]⁺ 451.11554, found 451.11516; IR (ATR): v = 3538 (s,OH), 1591 (m, olefin), 1145 (s, SO₂) cm⁻¹; Anal. calcd for $C_{24}H_{22}F_2O_3S$: C 67.27, H 5.18, S: 7.48, found: C 67.48, H 5.40, S 6.94.

5-(4-(methylsulfonyl)phenyl)-6,6-diphenylhex-5-en-1-ol (5c). A suspension of 0.31 g 6-chloro-2-(4-(methylsulfonyl)phenyl)hex-1-en-1,1-diyl)dibenzene (3c; 0.7 mmol) and 0.18 g CuSO₄ x 5 H₂O (0.7 mmol) in 2.4 mL water/DMSO (1/3; v/v) was stirred for 48 h at 130°C under reflux. After purification by column chromatography (ethyl acetate/petroleum ether; 1/1; v/v) 5c was obtained as beige solid. yield: 65.8%; purity: 98.8% (water/acetonitrile + 0.1% TFA; 1/1; v/v); mp 137-140°C: Rf 0.21 (EtOAc/petroleum ether, 1/1, v/v); ¹H NMR (400 MHz, CD₃CN) δ (ppm) 1.42–1.27 (m, 4H, $CH_{2-\beta}$, $CH_{2-\gamma}$), 2.36 (t, ${}^{3}J$ =5.3 Hz, 1H, OH), 2.49–2.42 (m, 2H, $CH_{2-\gamma}$), 3.00 (s, 3H, CH_{3}), 3.37-3.31 (m, 2H, CH₂₋₅), 6.96-6.90 (m, 2H, CH_{aromatic}), 7.10-7.02 (m, 3H, CH_{aromatic}), 7.34-7.27 (m, 3H, CH_{aromatic}), 7.44–7.36 (m, 4H, CH_{aromatic}), 7.70 (d, ³J=8.6 Hz, 2H, H-2,6 CH₃SO₂Phe). ¹³C NMR (101 MHz, CD₃CN) δ (ppm) 25.8 (CH_{2-β}), 33.4 (CH_{2-α}), 36.0 (CH_{2-ν}), 44.5 (CH₃), 62.2 (CH₂₋ δ), 118.3, 127.3, 127.6, 128.0, 128.7, 129.4, 130.0, 131.3, 131.5, 139.6, 140.5, 142.0, 143.5, 143.8, 149.5. C₂₅H₂₆O₃S [M_{monoisotopic}] requires 406, ESI-MS (ES+) *m/z*: 407 [M+H]⁺, 429 [M+Na]⁺, ESI-MS (ES-) *m/z*: 405 [M-H]; HRMS (ESI+) *m/z* calcd for C₂₅H₂₆O₃S [M+H]⁺ 407.16817, found 407.16754, calcd for [M+NH₄]⁺ 424.19887, found, 424.19409, calcd for [M+Na]⁺ 429.15004, found 429.14949; IR (ATR): v = 3507 (s, OH), 1591 (m, olefin), 1144 (s, SO₂) cm⁻¹; Anal. calcd for C₂₅H₂₆O₃S: C 73.86, H 6.45, S: 7.89, found: C 73.88, H 6.50, S 7.50.

6,6^{$^{-}}$ **bis(4-fluorophenyl)-5-(4-(methylsulfonyl)phenyl)hex-5-en-1-ol (5d).** $1.08 g 4,4<math>^{-}$ (6-chloro-2-(4-(methylsulfonyl)phenyl)hex-1-en-1,1-diyl)bis(fluorobenzene) (**3d**; 2.3 mmol) and 0.59 g CuSO₄ x 5 H₂O (2.3 mmol) in 2.4 mL water/DMSO (1/3; v/v) was stirred for 48 h at 130°C under reflux. After purification by column chromatography (ethyl acetate/petroleum ether; 1/1; v/v) **5d** was obtained as white solid. yield: 52.0%; purity: 98.3% (water/acetonitrile + 0.1% TFA; 1/1; v/v);</sup>

 mp: 128-133°C; Rf=0.04 (EtOAc/petroleum ether, 1/1, v/v); ¹H NMR (600 MHz, CD₃CN): δ (ppm) 1.35–1.29 (m, 2H, CH_{2-β}), 1.40–1.35 (m, 2H, CH_{2-γ}), 2.39 (s, 1H, OH), 2.46–2.42 (m, 2H, CH_{2-α}), 3.01 (s, 3H, CH₃), 3.36–3.32 (m, 2H, CH_{2-δ}), 6.80 (t, $J^{=3}J_{H,F}$ =8.9 Hz, 2H, H–3,5 FPhe), 6.92 (dd, ³J=8.9 Hz, ⁴J_{H,F}=5.6 Hz, 2H, H–2,6 FPhe), 7.14 (t, ³J=³J_{H,F}=8.9 Hz, 2H, H–3,5 FPhe), 7.29 (dd, ³J=8.9 Hz, ⁴J_{H,F}=5.6 Hz, 2H, H–2,6 FPhe), 7.37 (d, ³J=8.5 Hz, 2H, H–3,5 CH₃SO₂Phe), 7.72 (d, ³J=8.5 Hz, 2H, H–2,6 CH₃SO₂Phe). ¹³C NMR (151 MHz, CD₃CN): δ (ppm) 25.7 (CH_{2-β}), 33.3 (CH₂₋ α), 36.0 (CH_{2-γ}), 44.5 (CH₃), 62.1 (CH_{2-δ}), 115.4 (d, ²J_{C,F}=21.5 Hz, C–3,5 FPhe), 116.1 (d, ²J_{C,F}=21.5 Hz, C–3,5 FPhe), 127.7, 131.5, 132.0 (d, ³J_{C,F}=8.2 Hz, C–2,6 FPhe), 133.2 (d, ³J_{C,F}=8.2 Hz, C–2,6 FPhe), 139.6 (d, ⁴J_{C,F}=3.4 Hz, C–4 FPhe), 139.7 (d, ⁴J_{C,F}=3.4 Hz, C–4 FPhe), 139.7, 139.8, 141.5, 149.1, 162.2 (d, ¹J_{C,F}=244.0 Hz, C–1 FPhe), 162.8 (d, ¹J_{C,F}=244.0 Hz, C–1 FPhe). IR (ATR): v = 3513 (s, OH), 1601 (m, olefin), 1221 (s, C-F), 1144 (s, SO₂) cm⁻¹; C₂₅H₂₄F₂O₃S [M_{monolsotopic}] requires 442, MS (ESI +) *m*/z: 443 [M+H]⁺; 465 [M+Na]⁺; 885 [2M+H]⁺; HRMS (ESI+) *m*/z calcd for C₂₅H₂₄F₂O₃S [M+NH₄]* 460.18002, found 460.17525, calcd for [M+Na*] 465.13119, found 465.13035, calcd for [2M+Na]* 907.27261, found 907.27206; Anal. calcd for C₂₅H₂₄F₂O₃S: C 67.85, H 5.47, S: 7.25, found: C 68.01, H 5.42, S 7.14.

1-(4-(methylthio)phenyl)pentan-1-one (6). To ice cooled suspension of 3.52 g aluminum chloride (26.4 mmol) in 20 mL chloroform 3.4 mL valeroyl chloride (28.8 mmol) and 2.6 mL thioanisole (22.2 mmol) were added and stirred for 1.5 h at room temperature. The reaction mixture was added to ice cooled water, the organic layer was separated and extracted with 30 mL ethyl acetate. The combined organic layer was washed with 10 mL water and dried with Na₂SO₄. After reduction of the solvent under reduced pressure 1-(4-(methylthio)phenyl)pentan-1-one (**6**) was quantitative obtained as beige solid. mp 45-47°C; Rf 0.61 (EtOAc/petroleum ether, 1/1, v/v); ¹H NMR (400 MHz, CDCl₃) δ (ppm) 0.95 (t, ³*J*=7.4 Hz, 3H, CH₃), 1.44–1.36 (m, 2H, CH₂₋), 1.76–1.66 (m, 2H, CH_{2- β}), 2.52 (s, 3H, CH₃SO₂), 2.92 (t, ³*J*=7.4 Hz, 2H, CH_{2- α}), 7.26 (d, ³*J*=8.5 Hz, 2H, H-3,5), 7.87 (d, ³*J*=8.5 Hz, 2H, H-2,6 CH₃SPhe). C₁₂H₁₆OS [M_{monoistonic}] requires 208, ESI-

MS (ES+): $m/z = 209 [M+H]^+$; HRMS (ESI+) m/z calcd for C₁₂H₁₆OS [M+Na⁺] 231.08196, found 231.08162.

1-(4-(methylsulfonyl)phenyl)pentan-1-one (7). To a suspension of 5.9 g oxone (9.6 mmol) in 30 mL water a suspension of an ice refrigerated suspension of 1.00 g 1-(4-(methylthio)phenyl)pentan-1-one (**6**, 4.8 mmol) in 15 mL tetrahydrofuran/methanol (1/1; v/v) was slowly added. After warming to room temperature the reaction mixture was stirred for 15 h. After drying under reduced pressure the reaction mixture was resuspended in 30 mL water and extracted with 40 mL ethyl acetate (3x). The combined organic layer was reduced under vacuum conditions and purified by column chromatography (ethyl acetate/petroleum ether; 1/3; v/v). **7** was obtained as beige solid in 86% yield. mp 78-82°C; Rf 0.40 (EtOAc/petroleum ether, 1/1, v/v); ¹H NMR (400 MHz, CDCl₃) δ (ppm) 0.96 (t, ³*J*=7.3 Hz, 3H, CH₃), 1.36–1.48 (m, 2H, CH_{2-γ}), 1.67– 1.82 (m, 2H, CH_{2-β}), 2.97–3.03 (m, 2H, CH_{2-α}), 3.08 (s, 3H, CH₃SO₂), 8.04 (d, ³*J*=8.6 Hz, 2H, H-3,5 CH₃SO₂<u>Phe</u>), 8.12 (d, ³*J*=8.6 Hz, 2H, H-2,6 CH₃SO₂<u>Phe</u>). ¹³C NMR (101 MHz, CDCl₃) δ (ppm) 14.04 (CH₃), 22.50 (CH_{2-γ}), 26.28 (CH_{2-β}), 38.95 (CH_{2-α}), 44.48 (CH₃SO₂), 76.84, 77.16, 77.48, 127.92, 129.02, 141.16, 144.11, 199.30 (CO). C₁₂H₁₆O₃S [M_{monoisotopic}] requires 240, ESI-MS (ES+) *m/z*: 241 [M+H]⁺; HRMS (ESI+) *m/z* calcd for C₁₂H₁₆O₃S [M+Na⁺] 263.07179, found 263.07150, calcd for [2M+Na]⁺ 503.15381, found 503.15325.

4,4'-(2-(4-(methylsulfonyl)phenyl)hex-1-ene-1,1-diyl)bis(fluorobenzene) (8). To a stirring suspension of 0.33 g zinc powder (5.0 mmol) in 0.7 mL dry THF under argon atmosphere at - 10°C 0.27 mL titan tetrachloride (2.5 mmol) were added dropwise. The suspension was headed under reflux for 2 h and after cooling the suspension to 0°C a solution of 7 (0.15 g, 0.6 mmol) and 0.14 g 4,4'-difluorobenzophenone (0.6 mmol) in 12 mL dry THF was added. After 2.5 h heating under reflux the reaction mixture was tempered to 25°C and poured into 20 mL 10% aqueous potassium carbonate solution. The mixture was stirred for 5 min; the insoluble material was removed by vacuum filtration and washed three times with THF (10 mL). After separation of the

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organic fraction the aqueous fraction was extracted three times with EtOAc (20 mL). The combined organic fraction was dried over Na₂SO₄ and the solvent was removed under vacuum. The mixture was purified by column chromatography (ethyl acetate/petroleum ether; 1/3; v/v) and 8 was obtained as white solid. yield: 32%; mp 108-110°C; Rf 0.40 (EtOAc/petroleum ether, 1/1, v/v); ¹H NMR (400 MHz, CD₃CN) δ (ppm) 0.76 (t, ³*J*=7.1 Hz, 3H, CH₃), 1.32–1.16 (m, 4H, CH₂₋₈, CH_{2-v}), 2.46–2.40 (m, 2H, CH_{2-α}), 3.01 (s, 3H, CH₃SO₂), 6.80 (t, ³J=³J_{H,F}=8.9 Hz, 2H, H-3,5 FPhe), 6.92 (dd, ³*J*=8.8 Hz, ⁴*J*_{H,F}=5.6 Hz, 2H, H-2,6 FPhe), 7.14 (t, ³*J*=³*J*_{H,F}=8.9 Hz, 2H, H-3,5 FPhe), 7.29 (dd, ³*J*=8.7, ⁴*J*_{H F}=5.5 Hz, 2H, H-2,6 FPhe), 7.36 (d, ³*J*=8.4 Hz, 2H, H-3,5 CH₃SO₂Phe), 7.71 (d, ³*J*=8.4 Hz, 2H, H-2,6 CH₃SO₂Phe). ¹³C NMR (151 MHz, CD₃CN) δ (ppm) 14.03 (CH₃), 23.31 (CH_{2-ν}), 31.36 (CH_{2-α}), 35.97 (CH_{2-β}), 44.51 (CH₃SO₂), 115.42 (d, ²J_{C,F}=21.6 Hz, C–3,5 FPhe), 116.09 (d, ²J_{C F}=21.6 Hz, C-3,5 FPhe), 127.71, 131.16 (d, ³J_{C F}=8.1 Hz, C-2,6 FPhe), 131.45, 133.98 (d, ³J_{C.F}=8.1 Hz, C–2,6 FPhe), 139.60 (d, ⁴J_{C.F}=4.7 Hz, C–4 FPhe), 139.73 (d, ⁴J_{C.F}=3.5 Hz, C–4 FPhe), 139.72, 139.74, 141.58, 149.22, 162.16 (d, ¹J_{C,F}=244.2 Hz, C–1 FPhe), 162.80 (d, ¹J_{C,F}=244.2 Hz, C–1 FPhe). C₂₅H₂₄F₂O₂S [M_{monoisotopic}] requires 426, ESI-MS (ES+) *m/z*: 427 $[M+H]^+$; HRMS (ESI+) m/z calcd for C₂₅H₂₄F₂O₂S [M+H]⁺ 427.15441, found 427.15378, calcd for [M+NH₄]⁺ 444.18511, found 44.18033, calcd for [M+Na]⁺ 449.13628, found 449.13565, calcd for [2M+Na]⁺ 875.28279, found 875.28224; IR (ATR): v = 2963, 2872 (m, CH₃), 1592 (m, olefin), 1218 (s, C-F), 1148 (s, SO₂) cm⁻¹; Anal. calcd for C₂₅H₂₄F₂O₂S: C: 70.40, H: 5.67, S: 7.52, found: C: 70.57, H: 5.59, S: 7.38.

Cell-based ERa and ERß assays. To analyze the functional activity (i.e., antagonistic and/or agonistic) of the novel compounds on human ERa and ERß cell-based receptor reporter assays were used ("Human Estrogen Receptor alpha reporter assay", #IB00401; "Human Estrogen Receptor beta reporter assay", #IB00411; Indigo Biosciences; PA; USA) according to manufacturer's instructions. The assay used non-human mammalian cells contrived to express human ERa or ERß and luciferase reporter gene, which is functionally

linked to a responsive promotor. The quantification of changes in luciferase activity allows the sensitive measurement of changes in ER activity by the novel compounds. The luminescence was quantified by using Synergy HT plate reader BioTek Instruments, Bad Friedrichshall, Germany. The read time was 500 mSec per well. The dose-response curves were generated by using OrginPro 9.0 software (Fig. S5 and S6). The IC₅₀ values were estimated using a non-linear logistic regression fitting approach based on the concentration of the antagonist versus % inhibition (Table 1).

Biological evaluation. As model, two human melanoma cell lines were used. The human melanoma cell Mel-Juso line was purchased from the German Collection of Microorganisms (DSMZ) and the human melanoma cell line A2058 was purchased from the American Type Culture Collection (LGC Standards). Cell culture techniques were previously described by us.²² Additionally, two human breast adenocarcinoma cell lines were used. MCF-7 cells were purchased from DSMZ and cultivated with Leibovitz's L-15 medium supplemented with 10% fetal calf serum (FCS) and 1% penicillin/streptomycin (Pen-Strep) in absence of CO₂. MDA-MB-231 cells were obtained from LGC Standards, cultivated in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FCS plus 1% Pen-Strep in a CO₂ incubator (5% CO₂). The human umbilical vein endothelial cell line HUVEC (PromoCell GmbH) was cultured in endothelial cell growth medium (PromoCell GmbH) with addition of 1% Zell Shield® (Biochrom AG). In all cases cells were cultured at 37°C and 95% humidity. For harvesting, sub-culturing and counting, cells were detached by using a DetachKit (PromoCell GmbH) according to manufacturer's instructions.

Western blot analysis. Protein synthesis of ER α , ER β and β -actin was detected by Western blot analysis. Cell detachment, cell lysis, SDS-PAGE and Western blotting were performed as described previously.²² Membranes were blocked for 1 h at room temperature in

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blocking solution (5% skimmed milk powder + 2% bovine serum albumin) followed by the incubation with the primary antibody anti-ER α (1:500; monoclonal rabbit, abcam plc, Cambridge, UK, CHIP Grade, ab108398), anti-ER β (H-150) (1:500; polyclonal rabbit, Santa Cruz Biotechnology, Heidelberg, Germany, sc-8974) or β -actin (1:1000; monoclonal mouse, Sigma-Aldrich, Steinheim, Germany, A5316). After three washing steps in TBS-T membranes were incubated with secondary antibody (1:5000; anti-rabbit IgG POD polyclonal goat, A0545, Sigma-Aldrich; anti-mouse IgG POD polyclonal rabbit, A9044, Sigma-Aldrich).

Viability assay. To determine the influence of the novel compounds on the viability of melanoma and breast cancer cell lines, the CellTiter-Blue[®] Cell Viability Assay (Promega, Germany) was used, as previously described.²² In comparison to the melanoma cell lines (8.5×10^4 cells/well) only 7 x 10⁴ cells of the HUVECs were seeded in each well of a 96-well plate.

Proliferation assay. In dependence of the particular cell line, cells were plated in a 6-well plate (A2058: 24 h 0.5×10^6 , 48 h 0.35×10^6 ; Mel-Juso: 24 h 0.6×10^6 , 48 h 0.45×10^6 cells/well). After 24 h cells were treated with different concentrations of NO-SERM **4d** and SERM **5d** (0 – 50 µM). Cells treated with DMSO were used as control. After 24 or 48 h incubation, cells were counted by using CASY cell counter (model TT, Schaerfe System, Germany).

Conflicts of interest

There are no conflicts to declare

Author Contributions

N.B and J.P designed the research study. N.B. performed the experiments and analyzed the data. N.B., T.K. and J.P. wrote and edited the manuscript. All authors read and approved the final version of the submitted manuscript.

Acknowledgements

The excellent technical assistance of Petra Jäckel, Peggy Wecke, Anne Reichstein, Mareike Barth, Johanna Pufe, Sonja Lehnert, Catharina Heinig, Aline Morgenegg, Andrea Suhr, Karin Landrock, and Bianca Kreisl is greatly acknowledged. We also thank Lena Verhülsdonk for the support in graphic design. The authors thank Prof. Evamarie Hey-Hawkins, Wilma Neumann, Ph.D., and Stefan Saretz, Ph.D., Leipzig University, Institute of Inorganic Chemistry, Germany, for performing the HRMS analysis. The published work has been funded in the frame of the intramural research initiative "Radiation-Induced Vascular Dysfunction (RIVAD)". Nicole Bechmann and Jens Pietzsch also are thankful to the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) for supporting this work within the Collaborative Research Center Transregio 205 "The Adrenal: Central Relay in Health and Disease" (CRC/TRR 205/1). Nicole Bechmann was recipient of fellowships by Europäische Sozialfonds (ESF) (2011–2014) and Graduiertenakademie TU Dresden (2015).

Supporting Information

Supporting Information is available.

Sulfate and copper dependence of the synthesis of SERMs **5**; Analysis of sulfate and copper dependence of the reaction of chlorinated derivative **3a** to SERM **5a** (Figure S1); Synthesis of hydroxy-1-(4-(methylsulfonyl)phenyl)alkan-1-one; Synthesis route 1 for the synthesis of 5-hydroxy-1-(4-(methylsulfonyl)phenyl)pentan-1-one (III) (Figure S2); Characterization of 5-hydroxy-1-(4-(methylsulfonyl)phenyl)pentan-1-one (III) by ¹H NMR and mass spectrometry

(Figure S3); Synthesis route 2 for the synthesis of 5-hydroxy-1-(4-(methylsulfonyl)phenyl)pentan-1-one (III) (Figure S4); Antagonistic activity of NO-SERMs 4 and SERMs 5; : Dose-response curves of the ER β inhibition by NO-SERMs 4 (Figure S5); Dose-response curves of the ER β inhibition by SERMs 5 (Figure S6); COX inhibitory activity of NO-SERMs 4 and the corresponding SERMs 5; In vitro COX-1 and COX-2 inhibition data from the triaryl olefins 4, 5 and 6 (Table S1); Characterization of the novel NO-SERMs 4 and corresponding SERMs 5: Section of the HRMS spectra of 4-chloro-1-(4-(methylthio)phenyl)butan-1-one (1a) (Figure S7); ¹H- and ¹³C-NMRspectra of 4-chloro-1-(4-(methylthio)phenyl)butan-1-one (1a) (Figure S8); Section of the HSMR spectra of 5-chloro-1-(4-(methylthio)phenyl)pentan-1-one (1b) (Figure S9); ¹H- and ¹³C-NMRspectra of 5-chloro-1-(4-(methylthio)phenyl)pentan-1-one (1b) (Figure S10); Section of the HSMS spectra of 4-chloro-1-(4-(methylsulfonyl)phenyl)butan-1-one (2a) (Figure S11); ¹H- and ¹³C-NMRspectra of 4-chloro-1-(4-(methylsulfonyl)phenyl)butan-1-one (2a) (Figure S12); Section of the HSMR spectra of 5-chloro-1-(4-(methylsulfonyl)phenyl)pentan-1-one (2b) (Figure S13); ¹H- and ¹³C-NMR-spectra of 5-chloro-1-(4-(methylsulfonyl)phenyl)pentan-1-one (2b) (Figure S14); HSMR spectra of 5-chloro-2-(4-(methylsulfonyl)phenyl)pent-1-en-1,1-Section of the S15); ¹H-, and ¹³C-NMR-spectra divl)bibenzene (**3a**) (Figure of 5-chloro-2-(4-(methylsulfonyl)phenyl)pent-1-en-1,1-diyl)bibenzene (3a) (Figure S16); IR-spectra of 5-chloro-2-(4-(methylsulfonyl)phenyl)pent-1-en-1,1-diyl)bibenzene (3a) (Figure S17); Section of the HSMR spectra of 4,4'-(5-chloro-2-(4-(methylsulfonyl)phenyl)pent-1-en-1,1-diyl)bis-(fluorobenzene) (3b) (Figure S18); ¹H- and ¹³C-spectra of 4,4'-(5-chloro-2-(4-(methylsulfonyl)phenyl)pent-1-en-1,1divl)bis(fluorobenzene) (**3b**) (Figure S19); ¹⁹F-spectra of 4,4'-(5-chloro-2-(4-(methylsulfonyl)phenyl)pent-1-en-1,1-diyl)bis(fluorobenzene) (3b) (Figure S20); Section of the HRMS spectra of 6-chloro-2-(4-(methylsulfonyl)phenyl)hex-1-en-1,1-diyl)dibenzene (3c) (Figure S21); ¹H-NMR-spectra of 6-chloro-2-(4-(methylsulfonyl)phenyl)hex-1-en-1,1-diyl)dibenzene (3c) (Figure S22): ¹³C-NMR-spectra of 6-chloro-2-(4-(methylsulfonyl)phenyl)hex-1-en-1,1diyl)dibenzene (3c) (Figure S23); IR-spectra of 6-chloro-2-(4-(methylsulfonyl)phenyl)hex-1-en-

1,1-diyl)dibenzene (3c) (Figure S24); ¹ H- and ¹³ C-spectra of 4,4'-(6-chloro-2-(4-
(methylsulfonyl)phenyl)hex-1-en-1,1-diyl)bis(fluorobenzene) (3d) (Figure S25); Section of the
HRMS spectra of 4-(4-(methylsulfonyl)phenyl)-5-5-diphenylpent-4-en-1-yl nitrate (4a) (Figure
S26); ¹ H- and ¹³ C-spectra of 4-(4-(methylsulfonyl)phenyl)-5-5-diphenylpent-4-en-1-yl nitrate (4a)
(Figure S27); IR-spectra of 4-(4-(methylsulfonyl)phenyl)-5-5-diphenylpent-4-en-1-yl nitrate (4a)
(Figure S28); Section of the HRMS spectra of 5,5'-bis-(4-fluorophenyl)-4-(4-
methylsulfonyl)phenyl)pent-4-en-1-yl nitrate (4b) (Figure S29); ¹ H- and ¹³ C-NMR -spectra of 5,5'-
bis-(4-fluorophenyl)-4-(4-methylsulfonyl)phenyl)pent-4-en-1-yl nitrate (4b) (Figure S30); ¹⁹ F-NMR
-spectra of 5,5'-bis-(4-fluorophenyl)-4-(4-methylsulfonyl)phenyl)pent-4-en-1-yl nitrate (4b)
(Figure S31); IR-spectra of 5,5'-bis-(4-fluorophenyl)-4-(4-methylsulfonyl)phenyl)pent-4-en-1-yl
nitrate (4b) (Figure S32); Section of the HRMS spectra of 5-(4-(methylsulfonyl)phenyl)-6-6-
diphenylhex-5-en-1-yl nitrate (4c) (Figure S33); ¹ H- and ¹³ C-NMR-spectra of 5-(4-
(methylsulfonyl)phenyl)-6-6-diphenylhex-5-en-1-yl nitrate (4c) (Figure S34); IR-spectra of 5-(4-
(methylsulfonyl)phenyl)-6-6-diphenylhex-5-en-1-yl nitrate (4c) (Figure S35); Section of the HRMS
spectra of 6,6'-bis(4-fluorophenyl)-5-(4-(methylsulfonyl)phenyl)hex-5-en-1-yl nitrate (4d) (Figure
S36); ¹ H-NMR -spectra of 6,6'-bis(4-fluorophenyl)-5-(4-(methylsulfonyl)phenyl)hex-5-en-1-yl
nitrate (4d) (Figure S37); ¹³ C- and ¹⁹ F-NMR-spectra of 6,6´-bis(4-fluorophenyl)-5-(4-
(methylsulfonyl)phenyl)hex-5-en-1-yl nitrate (4d) (Figure S38); IR-spectra of 6,6'-bis(4-
fluorophenyl)-5-(4-(methylsulfonyl)phenyl)hex-5-en-1-yl nitrate (4d) (Figure S39); Section of the
HRMS spectra of 4-(4-(methylsulfonyl)phenyl)-5,5-diphenylpent-4-en-1-ol (5a) (Figure S40); ¹ H-
NMR-spectra of 4-(4-(methylsulfonyl)phenyl)-5,5-diphenylpent-4-en-1-ol (5a) (Figure S41); ¹³ C-
NMR-spectra of 4-(4-(methylsulfonyl)phenyl)-5,5-diphenylpent-4-en-1-ol (5a) (Figure S42); IR-
spectra of 4-(4-(methylsulfonyl)phenyl)-5,5-diphenylpent-4-en-1-ol (5a) (Figure S43); Section of
the HRMS spectra of 5,5'-bis(4-fluorophenyl)-4-(4-methylsulfonyl)phenyl)pent-4-en-1-ol (5b)
(Figure S44); ¹ H- and ¹³ C-NMR-spectra of 5,5'-bis(4-fluorophenyl)-4-(4-
methylsulfonyl)phenyl)pent-4-en-1-ol (5b) (Figure S45); ¹⁹ F-NMR-spectra of 5,5'-bis(4-

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fluoro	ophenyl)-4	-(4-methyls	sulfonyl)phen	yl)pent-	4-en-1-ol	(5b) (F	igure S	46); IR-spe	ectra of 5	5,5´-
bis(4	-fluorophe	nyl)-4-(4-m	ethylsulfonyl)phenyl))pent-4-en	-1-ol (5	b) (Figu	re S47); S	ection of	the
HRM	S spectra	of 5-(4-(m	ethylsulfonyl)phenyl)	-6,6-diphe	enylhex-	5-en-1-c	ol (5c) (Figu	ure S48);	¹ H-
and	¹³ C-NMR-	spectra of	5-(4-(methy	/Isulfony	/l)phenyl)-	6,6-diph	enylhex	-5-en-1-ol	(5c) (Fig	gure
S49)	; IR-spect	ra of 5-(4-	(methylsulfo	nyl)pher	nyl)-6,6-dij	phenylh	ex-5-en-	1-ol (5c) (Figure S	50);
Secti	on of the I	HRMS spee	ctra of 6,6´-b	s(4-fluo	rophenyl)-	-5-(4-(m	ethylsulf	onyl)pheny	l)hex-5-e	n-1-
ol	(5d)	(Figure	S51);	¹ H-NMF	R-spectra	of	6,6′-	bis(4-fluoro	phenyl)-5	5-(4-
(metł	nylsulfonyl)phenyl)he	x-5-en-1-ol (5d) (Fig	ure S52);	¹³ C- and	ց ¹⁹ F-NN	IR-spectra	of 6,6´-bi	s(4-
fluoro	ophenyl)-5	-(4-(methy	sulfonyl)phe	nyl)hex-	5-en-1-ol	(5d) (F	igure S	53); IR-spe	ectra of 6	6,6′-
bis(4	-fluorophe	nyl)-5-(4-(r	nethylsulfony	l)pheny	I)hex-5-en	-1-ol	(5d)	(Figure	S54);	
Char	acterizatio	on of refere	nce compou	nd 8 ; HI	RMS spec	tra of 1	-(4-(met	hylthio)phe	nyl)penta	n-1-
one (6) (Figure	S55); ¹ H-s	pectra of 1-(4	-(methy	lthio)phen	yl)penta	in-1-one	(6) (Figure	S56); HF	RMS
spect	tra of 1-(4	-(methylsul	fonyl)phenyl)	pentan-	1-one (7)	(Figure	S57); ¹ ŀ	H- and ¹³ C-	NMR-spe	ctra
of 1-	(4-(methyl	sulfonyl)ph	enyl)pentan-	1-one (7	7) (Figure	S58); S	Section of	of the HRM	IS spectr	a of
4,4'-(2-(4-(meth	nylsulfonyl)	phenyl)hex-1	-ene-1, ⁻	1-diyl)bis(f	luoro-be	enzene)	(8) (Figur	re S59);	¹ H-
and 1	¹³ C-NMR-s	spectra of 4	4,4'-(2-(4-(me	ethylsulf	onyl)phen	yl)hex-1	-ene-1,^	l-diyl)bis(flu	lorobenze	ene)
(8)	(Figure	S60);	IR-spectra	of	4,4'-(2-(4-(meth	ylsulfon	/l)phenyl)he	ex-1-ene-	1,1-
diyl)b	ois(fluorob	enzene) (8)) (Figure S61).						

All molecular formula strings are provided as CVS file.

Abbreviations

Akt, protein kinase B

BCL2, B-cell lymphoma 2

DAN, 2,3-diaminonaphthalene

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E2, 17β-estradiol

EC, effective concentration

IC, inhibitory concentration

JNK, c-Jun N-terminal kinase

NAT, 2,3-naphthotriazole

•NO, nitric oxide

NF-kB, nuclear factor kappa B

4OHT, 4-hydroxytamoxifen

SERD, selective ER degrader

S.I., selectivity index

TF, transcription factors

TAM, tamoxifen

ERE, estrogen responsive element

ERK, extracellular-signal-regulated kinase

HRMS, high-resolution mass spectrometry

MAPK, mitogen-activated protein kinase

NO-SERM, nitric oxide releasing selective estrogen receptor modulator

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PI3-K, phosphatidylinositol-4,5-bisphosphate 3-kinase

SERM, selective estrogen receptor modulator

HUVECs, human umbilical vein endothelial cells

ER, estrogen receptor

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Fig. 1: Structure and modulation of the estrogen signaling by selective estrogen receptor modulators (SERMs). (A) Chemical structure of the SERMs tamoxifen, raloxifene, and fulvestrant. (B) Showed the most important intracellular signaling pathways of 17β-estradiol (E2) that could be influenced by SERMs.
Signaling pathway 1 (yellow star): canonical estrogen pathway, the ligand-activated estrogen receptor (ER) binds specifically to the estrogen responsive element (ERE) in the promoter region of the targeted gene. Signaling pathway 2: non-ERE-depending estrogen pathway: the ligand-activated estrogen receptor interacts with other transcription factors (TF), like NF-κB, and, activates gene expression in ERE-independent manner. Signaling pathway 3: non-genomic estrogen pathway, ERs that are localized in cell membrane activate PI3-K/MAPK-signaling and regulate for example vasodilation by an activation of the endothelial nitric oxide (□NO) synthase (eNOS). Moreover, a ligand-independent mechanism by the activation of different growth factors was described (not shown). (C) In particular, the influence of SERMs on the NO-signaling is coupled with several side effects, like endothelial dysfunction. PI3-K:
Phosphatidylinositol-4,5-bisphosphate 3-kinase; Akt: Protein kinase B; MAPK: mitogen-activated protein kinase; NF-κB: Nuclear factor kappa B; BCL2: B-cell lymphoma 2; ERK: extracellular-signal-regulated kinase; JNK: c-Jun N-terminal kinase.



Fig. 2: Synthetic pathway of NO-releasing SERMs (4) and reference SERMs (5). Reagents and conditions: (a) AlCl3, CHCl3, argon, 2.5 h rt, (b) oxone® (potassium peroxymonosulphate), MeOH/THF (1/1, v/v), 31 h, (c) Zn, TiCl4, reflux 4.5 h, (d) AgNO3, MeCN, 24 h, 80°C, (e) CuSO4 x 5 H2O, DMSO/H2O (1/3, v/v), 130°C, 48 h.

256x156mm (300 x 300 DPI)

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Fig. 4: Stability of SERM 5d and NO-SERM 4d in human blood. Stability was analyzed by HPLC after zero up to six hours incubation in human whole blood at 37°C. After incubation, proteins were separated by using supersol-precipitating agent (1:2, v/v) and the clear solution were analyzed using the following conditions. Agilent 1100; Zorbrax 300SB-C18 (250 x 9.4 mm; 4 μ m); Zorbrax 300SB-C18 (4.6 x 12.5 mm, 5 μ m); 254 nm; A: H2O + 0.1% TFA B: MeCN + 0.1% TFA, 1-5 min 85% A, 10 min 70% B, 25-26 min 95% B, 29-30 min 85% A; flow rate 3.0 mL/min at 50°C.

190x170mm (300 x 300 DPI)



Fig. 5: ERa and ER β expression of two melanoma cell lines and the influence of NO-SERMs 4 or SERMs 5 on cell viability and proliferation of both cell lines. (A) ERa and ER β expression in A2058 and Mel-Juso human melanoma cells was determined by Western blotting. Human breast cancer cells (MCF-7) here were used as positive control. Representative sections of the immunochemical Western blot analysis of ERa, ER β and β -actin used as housekeeping protein are shown (three independent experiments). (B) The influence of NO-SERMs 4 and SERMs 5 on cell viability was investigated. Interestingly, the additional NO-release preserved the cell viability. Three to five independent experiments were performed (n = 9 - 15). Mean ± SEM, ANOVA and Bonferroni post hoc test comparison vs. DMSO control, * p < 0.05 or, ** p < 0.01. Moreover, the influence of the corresponding compounds 4d and 5d on the number of proliferating cells on (C) A2058 and (D) Mel-Juso cells was analyzed. The proliferation assays confirmed the results of the viability assays that the additional release of \Box NO reduces the anti-tumorigenic activity of SERMs in melanoma cells. Three independent experiments were performed (n = -5-6). Mean ± SEM, ANOVA and Bonferroni post hoc test comparison vs. DMSO control, * p < 0.05 or, ** p < 0.01.

241x174mm (300 x 300 DPI)





Fig. 6: Influence of NO-SERMS 4 or SERMS 5 on cell viability and proliferation of breast cancer cell lines. (A) Influence of the NO-SERMS 4 and SERMS 5 on cell viability was investigated. Additional release of □NO preserved viability of MCF-7 cells. Three independent experiments were performed (n =9). Mean ± SEM, ANOVA and Bonferroni post hoc test comparison vs. DMSO control, * p < 0.05 or, ** p < 0.01.
Furthermore, the influence of the corresponding compounds 4d and 5d on the number of proliferating cells on (B) MCF-7 and (C) MDA-MB-231 cells was analyzed. In both breast cancer cell lines, the number of proliferating cells was preserved by the additional release of □NO. Four independent experiments were performed (n = 8). Mean ± SEM, ANOVA and Bonferroni post hoc test comparison vs. DMSO control, * p < 0.05 or, ** p < 0.01.

250x141mm (300 x 300 DPI)



92x90mm (300 x 300 DPI)



