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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 ERβ (IC_{50}(ERβ)=0.2–2.7μM), but no interaction with ERα. Growth of ERβ-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, ERα and/or ERβ, 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 RAD1901[12], 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. 17β-estradiol (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 ERα or ERβ, 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 triaryl olefins to tamoxifen prompted us to investigate the functional activity (e.g., antagonistic and/or agonistic) of the compounds on human ERα and ERβ. 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 ERα/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.

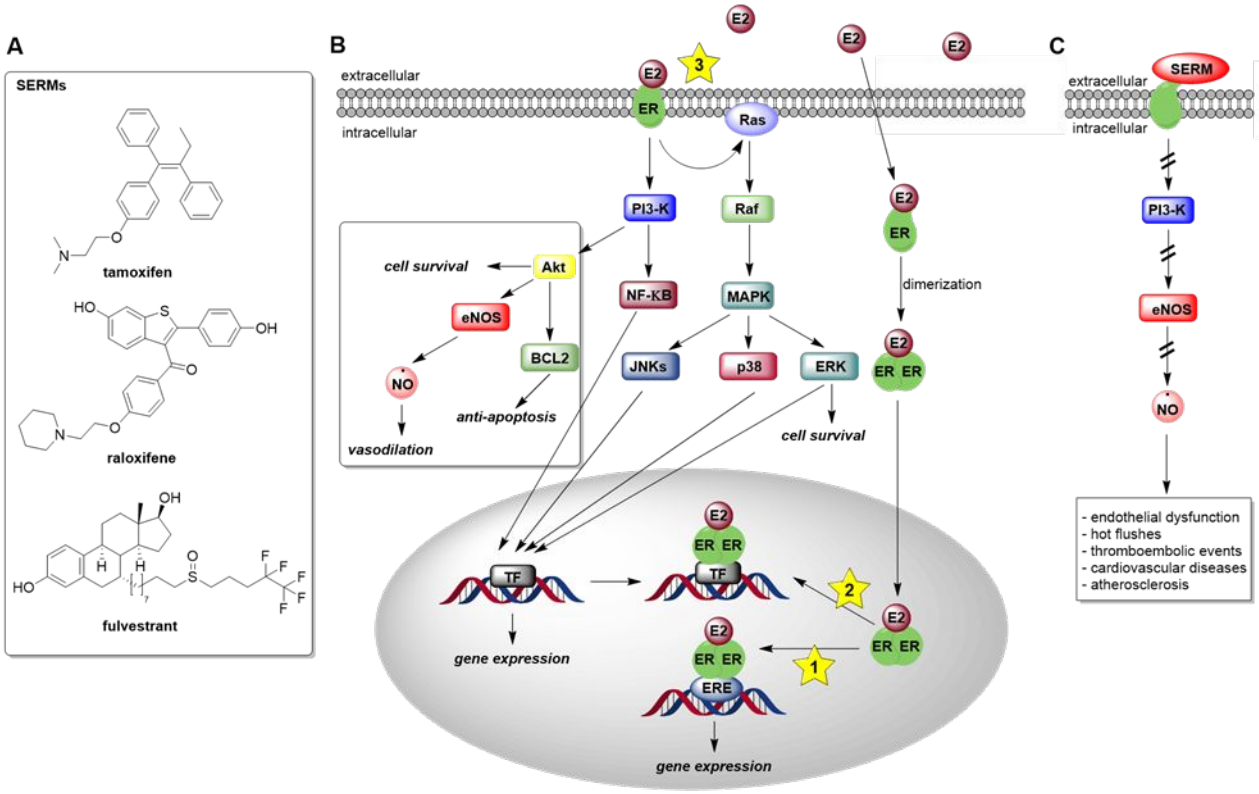


Fig. 1: Structure and modulation of the estrogen signaling by selective estrogen receptor modulators (SERMs). (**A**) Chemical structure of the SERMs tamoxifen, raloxifene, and fulvestrant. (**B**) Showed the most important intracellular signaling pathways of 17β-estradiol (E2) that could be influenced 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(4-fluorophenyl)-5-(4-(methylsulfonyl)phenyl) alkenyls (**4b**,**d**, **5b**,**d**) with and without a NO-releasing moiety 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 (**1a**, 86%) and 5-chloro-1-(4-(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 (**2a**, 76%) and 5-chloro-1-(4-(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_N2). 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.

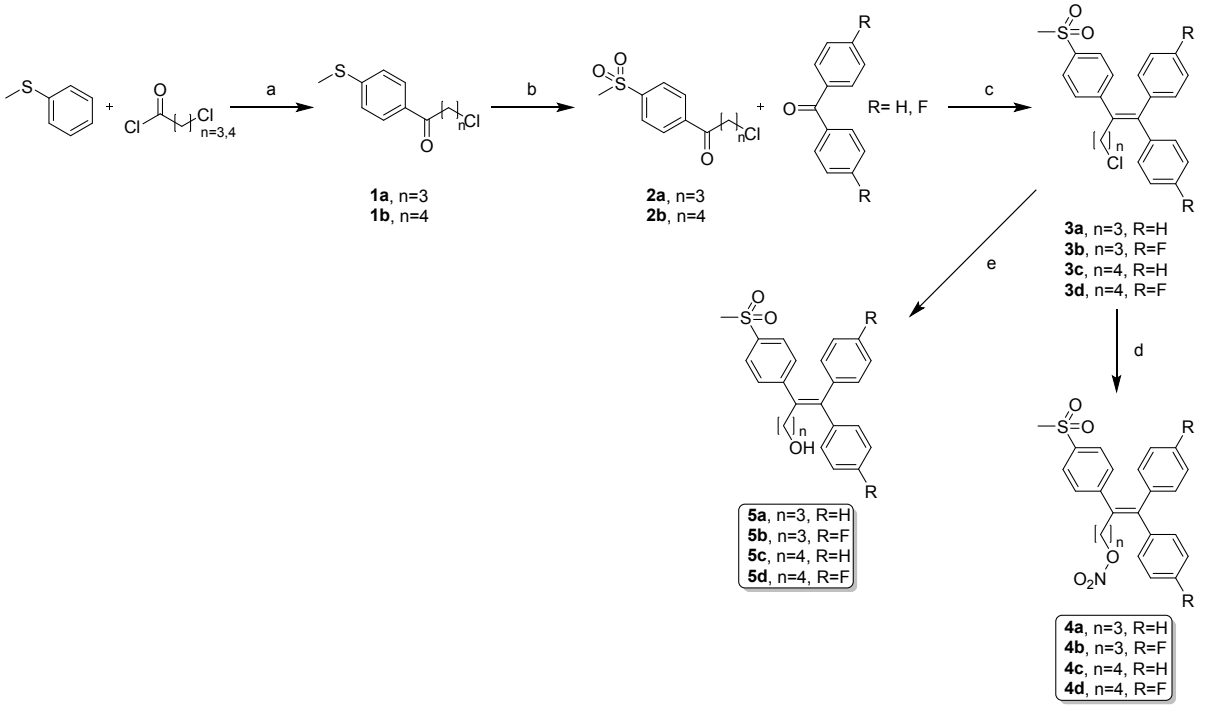


Fig. 2: Synthetic pathway of NO-releasing SERMs (4) and reference SERMs (5). Reagents and conditions: (**a**) $AlCl_3$, $CHCl_3$, argon, 2.5 h rt, (**b**) oxone® (potassium peroxymonosulphate), MeOH/THF (1/1, v/v), 31 h, (**c**) Zn, $TiCl_4$, reflux 4.5 h, (**d**) $AgNO_3$, MeCN, 24 h, 80°C, (**e**) $CuSO_4$ x 5 H_2O, DMSO/H_2O (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 $CuSO_4$ x 5 H_2O 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-1,1-diyl)bis(fluorobenzene) (**8**) was obtained in 32% yield.

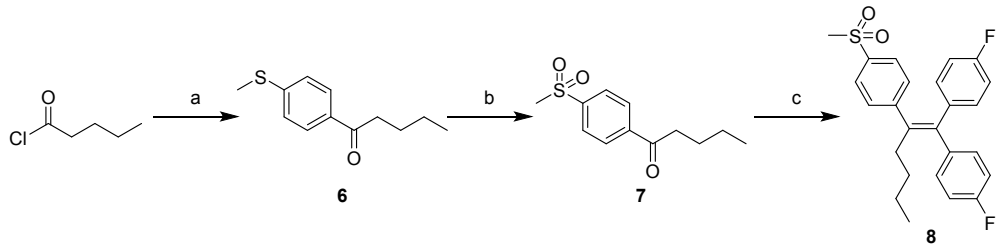


Fig. 3: Synthetic pathway of SERM 8. Reagents and conditions: (**a**) thioanisole, $AlCl_3$, $CHCl_3$, 1.5 h rt (**b**), oxone® (potassium peroxymonosulphate), MeOH/THF (1/1, v/v), 15 h, (**c**) 4,4´-difluorobenzophenone, Zn, $TiCl_4$, reflux 4.5 h, reflux.

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)

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 4OHT.[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.

ERα activity was not affected by the novel compounds up to a concentration of 5 µM (Tab. 1). In contrast, all compounds, except **4b**, showed promising antagonistic activity on ERβ ($IC_{50(NO\text{-}SERMs\ \mathbf{4})}$: 0.2 - 2.7 µM; $IC_{50(SERMs\ \mathbf{5})}$: 0.18 - 3.0 µM). The fluorinated derivatives with a butyl side chain **4d** (IC_{50}(ERβ): 0.20 µM) and **5d** (IC_{50}(ERβ): 0.18 µM) were the most potent, selective ERβ antagonists (**4d**: S.I.(ERβ) > 25.00; 5**d**: S.I.(ERβ) > 27.78). The introduction of the nitroester group seems to have only a slight influence on ERβ affinity. These data and the findings from Chen et *al.* indicate that the methylsulfonyl group is responsible for ERβ selectivity.[35] 2-(4-(methylsulfonyl)phenyl)hex-1-ene-1,1-diyl)dibenzene, previously described by Uddin et al., also showed a high ERβ selectivity whereas the corresponding NH-sulfoximine and *N*-cyano sulfoximine derivatives displayed an affinity for both estrogen receptors.[26, 35] Because of the ERβ selectivity of the novel compounds the additional NO-release is more important to reduce cardiovascular events. Studies on knockdown mice have demonstrated that ERβ 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 ERβ 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.

compound	IC_{50} [µM]		SI	NO-release [%][c]							
						plasma			L-cysteine		
	ERα[a]	ERβ[a]	ERβ[b]	PBS	oxidant	$nitrite_{free}$	NO_{total}	S-nitroso-thiol	$nitrite_{free}$	NO_{total}	S-nitroso-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	*n.d.*	2.5±1.4	0.2±0.1	1.5±1.5	1.3	0.4±0.4	3.8±2.6	3.4
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	-	-	-	*n.d.*	0.2±0.1	1.4±0.3	3.9±1.5	2.5	0.8±0.1	4.4±1.5	3.6
5a	> 5	1.5	> 3.34	-	-	-	-	-	-	-	-
5b	> 5	3.0	> 1.67	-	-	-	-	-	-	-	-
5c	> 5	1.4	> 3.57	-	-	-	-	-	-	-	-
5d	> 5	0.18	> 27.78	-	-	-	-	-	-	-	-
tamoxifen citrat	1.7[d]	0.005[d]	377.8	-	-	-	-	-	-	-	-

n.d. not detectable.

PA Piloty´ s acid (*N*-hydroxybenzenesulfonamide).

[a] 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 IC_{50} value was calculated by non- linear fitting (Software: Origin, logistic fit).

[b] ERβ selectivity index *in vitro* (IC_{50}(ERα)/IC_{50}(ERβ)).

[c] 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).

***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 $^{\bullet}NO$ 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*.

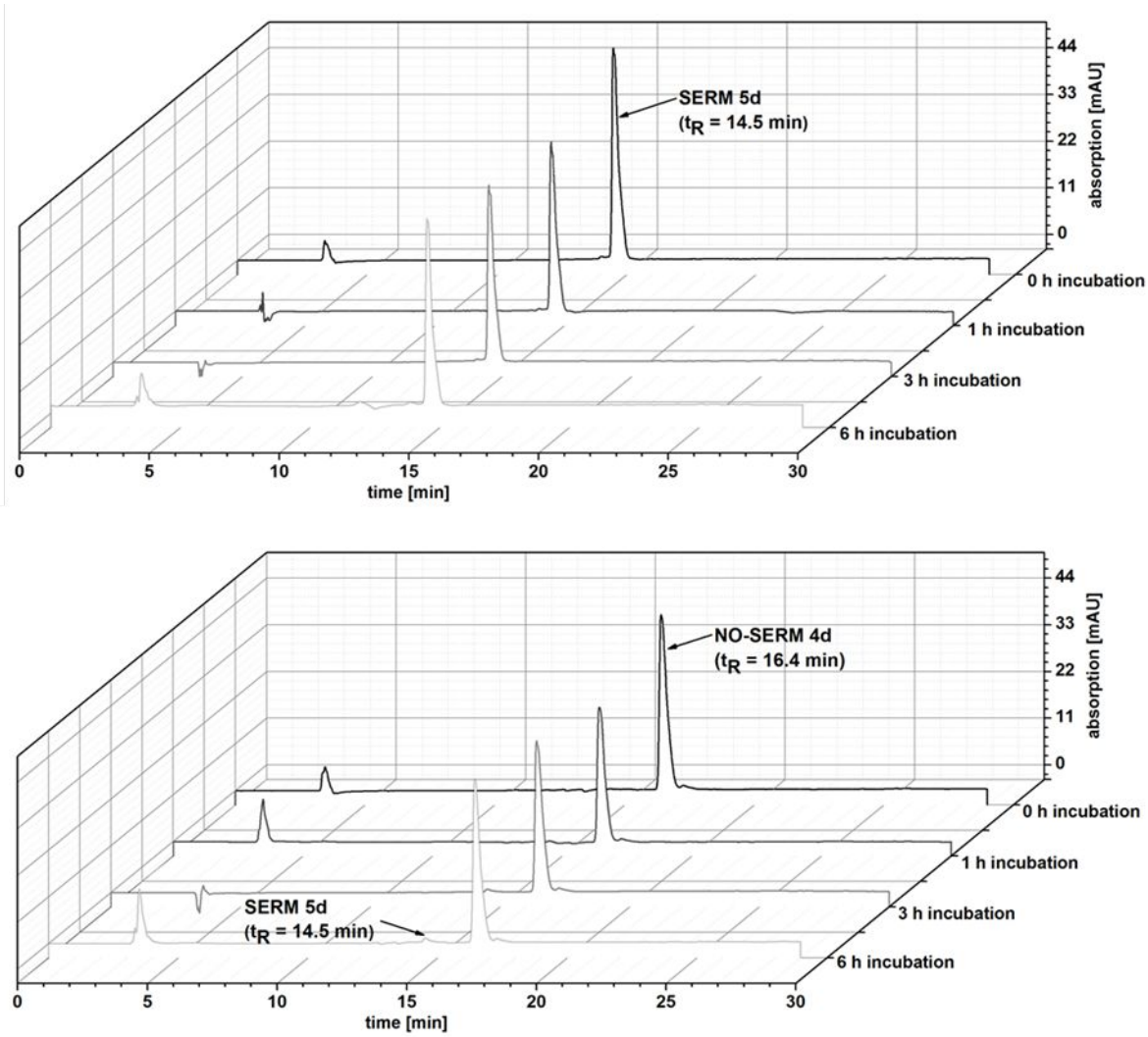
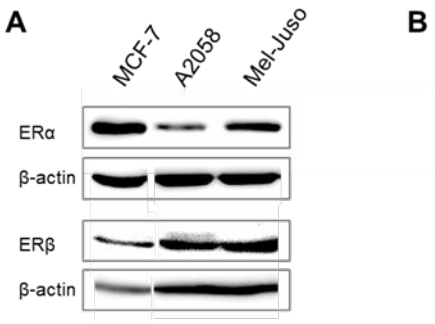


Fig. 4: Stability of SERM 5d and NO-SERM 4d in human blood. Stability was analyzed by HPLC after zero up to six hours incubation in human whole blood at 37°C. After incubation, proteins were separated by using supersol-precipitating agent (1:2, v/v) and the clear solution were analyzed using the following conditions. Agilent 1100; Zorbrax 300SB-C18 (250 x 9.4 mm; 4 µm); Zorbrax 300SB-C18 (4.6 x 12.5 mm, 5 µm); 254 nm; A: H_2O + 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.

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 NO-release 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-buffered-saline (PBS, pH 7.5) to analyze the spontaneous NO-release, (**b**) PBS containing human plasma to measure enzyme-mediated mechanisms, (**c**) PBS with 50 µM $K_3[Fe(CN)_6]$, 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 ± 0.2%), whereby for the other compounds no NO-release 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 NO-release. 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 ERα/β-positive MCF-7 cells, we used MDA-MB-231 with no detectable expression of ERα. 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 ERβ protein synthesis. Protein synthesis of ERα 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 ERβ agonists could be a useful addition in the treatment regime of breast cancer that at the moment only aimed at ERα inhibition, with routinely use of TAM (ER antagonist).[38]



B

NO-SERMs*	viability 50 µM [%]	SERMs	EC_{50} [µM]	viability 50 µM [%]
A2058				
4a	117,2 ± 13.0	**5a**	*n.E.d.*	63.0 ± 3.6*
4b	106.8 ± 4.4	**5b**	27.5	47.6 ± 10.5**
4c	119.8 ± 12.4	**5c**	*n.E.d.*	105.5 ± 9.5
4d	105.7 ± 11.6	**5d**	39.9	17.0 ± 6.0**
		8	*n.E.d.*	116.8 ± 3.4
		tamoxifen	12.5	1.8 ± 1.1**
Mel-Juso				
4a	96.5 ± 8.5	**5a**	*n.E.d.*	52.7 ± 6.4**
4b	91.5 ± 8.0	**5b**	36.3	31.4 ± 2.2**
4c	87.1 ± 8.5	**5c**	*n.E.d.*	68.6 ± 7.0**
4d	101.1 ± 4.6	**5d**	34.9	20.5 ± 5.2**
		8	*n.E.d.*	75.4 ± 5.2
		tamoxifen	14.6	1.0 ± 0.5**

* no EC_{50} value could be determined for the NO-SERMs **4** in a concentration range of 0 - 50 µM

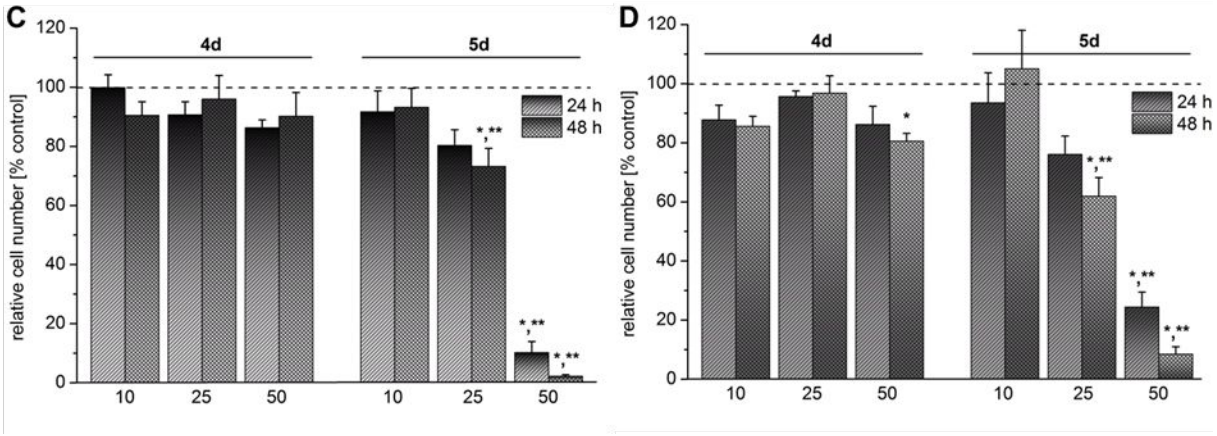


Fig. 5: ERα and ERβ expression of two melanoma cell lines and the influence of NO-SERMs 4 or SERMs 5 on cell viability and proliferation of both cell lines. (**A**) ERα and ERβ expression in A2058 and Mel-Juso human melanoma cells was determined by Western blotting. Human breast cancer cells (MCF-7) 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 ERβ 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 ERα/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 ERα, 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 ERβ selectivity resulted in a comparable response behavior of both breast cancer cell lines independent of their different ERα 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 ERα/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 (EC_{50}) of SERMs **5** was in a micromolar range (25.7 - 38.5 µM). **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 µM 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 µM SERM **5d** significantly decreased the number of proliferating cells in both cell lines. In contrast, 50 µM 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.

A

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

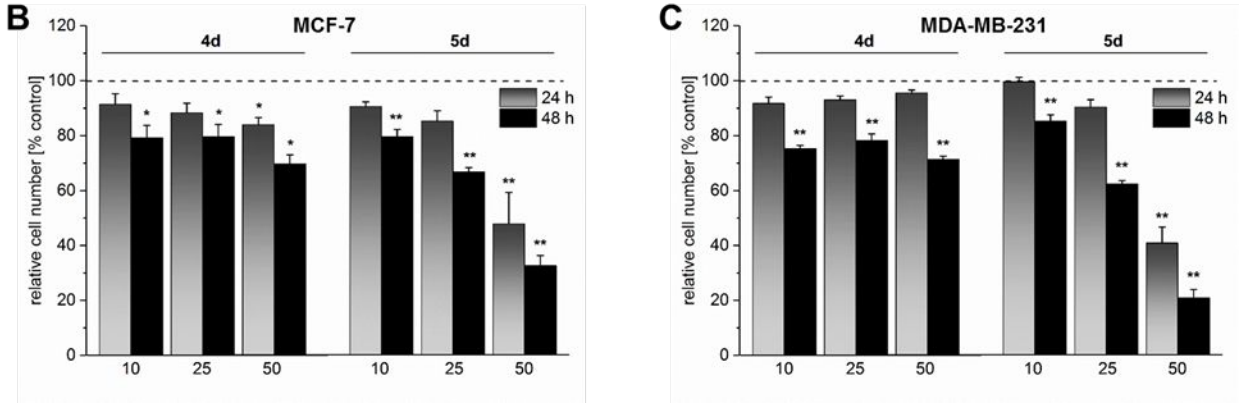
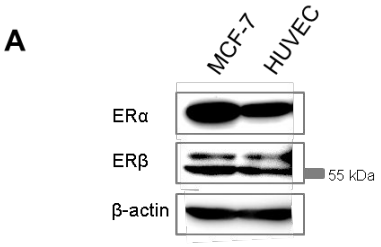


Fig. 6: Influence of NO-SERMs 4 or SERMs 5 on cell viability and proliferation of breast cancer cell lines. (**A**) Influence of the NO-SERMs **4** and SERMs **5** on cell viability was investigated. Additional release of •NO preserved viability of MCF-7 cells. Three independent experiments were performed (n =9). Mean ± 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, ** $p < 0.01$.

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

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 the basal NO-release of HUVECs is a non-genomic effect of E2 [39] via the activation of Akt signaling[40].



B

	EC_{50} [µM]	viability 50 µM [%]
HUVEC		
4d	*n.E.d.*	91.1 ± 4.0
5d	19.6	*b.l.d.*

Fig. 7: ERα and ERβ expression and the influence of NO-SERM 4d or SERM 5d on viability of HUVEC. (**A**) ERα and ERβ expression in HUVECs as determined by Western blotting. 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 **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 EC_{50}-value could be detected; *b.l.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-κB), the activation of NF-κB 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 μM) slightly affected the COX-2 expression level, while treatment with the ERβ 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 anti-estrogens was previously described and document the molecular complexity of estrogen receptor signaling.[43]

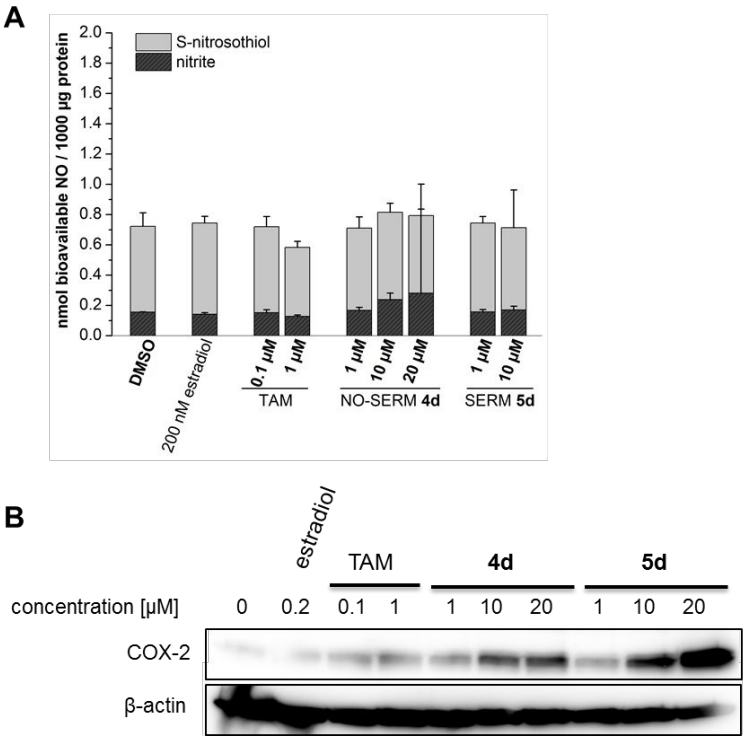


Fig. 8: Influence of NO-SERM 4d or SERM 5d on (A) NO-bioavailability and (B) the inflammatory marker COX-2 of HUVECs. (**A**) The influence of SERMs and the novel NO-SERM **4d** on the bioavailable •NO was measured as sum of nitrite and *S*-nitrosothiols in cell culture supernatants. Therefore, cells 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

group of the compounds seems to be responsible for the high ERβ 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 (SO_2NHOH) 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 ERβ-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 ERβ 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 ERβ in tumor progression, metastasis and therapy resistance, in particular towards radiotherapy, is largely unknown. By our novel ERβ-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 ≥ 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. ^{1}H NMR, ^{13}C NMR and ^{19}F 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 ^{1}H and ^{13}C, for ^{19}F $CFCl_3$ 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 $CHCl_3$, 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 $CHCl_3$, the combined organic layer was dried over Na_2SO_4 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 $AlCl_3$ (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); ^{1}H NMR (400 MHz, CD_3CN) δ (ppm) 2.17–2.08 (m, 2H, $CH_{2\text{-}\beta}$), 2.53 (s, 3H, CH_3), 3.12 (t, ^3J=7.1 Hz, 2H, $CH_{2\text{-}\alpha}$), 3.68 (t, ^3J=6.6 Hz, 2H, $CH_{2\text{-}\gamma}$), 7.33 (d, ^3J=8.7 Hz, 2H, $CH_{aromatic}$), 7.89 (d, ^3J=8.8 Hz, 2H, $CH_{aromatic}$); ^{13}C NMR (101 MHz, CD_3CN) δ (ppm) 14.82 (CH_3), 27.98 ($CH_{2\text{-}\beta}$), 36.05 ($CH_{2\text{-}\alpha}$), 45.75 ($CH_{2\text{-}\gamma}$), 125.87, 129.36 (2 x $CH_{aromatic}$), 134.22, 146.89 (2 x $C_{aromatic}$), 199.02 (C_{ketone}); $C_{11}H_{13}ClOS$ [$M_{monoisotopic}$] requires 228, ESI-MS (ES+) *m/z*: 229 [M(^{35}Cl)+H]$^+$, 231 [M(^{37}Cl)+H]$^+$; (ES-) *m/z*: 227 [M(^{35}Cl)-H]$^-$; HRMS (ESI+) *m/z* calcd for $C_{11}H_{13}ClOS$ [M+Na$^+$,^{35}Cl] 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 $AlCl_3$ (39.7 mmol). yellow solid; yield: 86.7%; mp 76-80°C, Rf 0.62 (ethyl acetate/petroleum ether; 1/1; v/v); ^{1}H NMR (400 MHz, CD_3CN) δ (ppm) 1.87–1.74 (m, 4H, $CH_{2\text{-}\beta}$, $CH_{2\text{-}\gamma}$), 2.53 (s, 3H, CH_3), 3.03–2.96 (m, 2H, $CH_{2\text{-}\alpha}$), 3.63 (t, ^3J=6.4 Hz, 2H, $CH_{2\text{-}\delta}$), 7.33 (d, ^3J=8.7 Hz, 2H, $CH_{aromatic}$), 7.88 (d, ^3J=8.7 Hz, 2H, $CH_{aromatic}$); ^{13}C NMR (101 MHz, CD_3CN) δ (ppm) 14.83 (CH_3), 22.30 ($CH_{2\text{-}\gamma}$), 32.84 ($CH_{2\text{-}\beta}$), 38.06 ($CH_{2\text{-}\alpha}$), 46.04 ($CH_{2\text{-}\ \delta}$), 125.87, 129.38, (2 x $CH_{aromatic}$), 134.38, 146.68, (2 x $C_{aromatic}$), 198.76 (C_{ketone}); $C_{12}H_{15}ClOS$ [$M_{monoisotopic}$] requires 242, ESI-MS (ES+) *m/z*: 243 [M(^{35}Cl)+H]$^+$, 245 [M(^{37}Cl)+H]$^+$, HRMS (ESI+) *m/z* calcd for $C_{12}H_{15}ClOS$ [M+Na$^+$,^{35}Cl] 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 Na_2SO_4. 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 (2a). 2.56 g 4-chloro-1-(4-(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); ^{1}H NMR (400 MHz, CD_3CN) δ (ppm) 2.21–2.12 (m, 2H, $CH_{2\text{-}\beta}$), 3.10 (s, 3H, CH_3), 3.22 (t, ^{3}J=7.0 Hz, 2H, $CH_{2\text{-}\alpha}$), 3.70 (t, ^{3}J=6.6 Hz, 2H, $CH_{2\text{-}\gamma}$), 8.03 (d, ^{3}J=8.7 Hz, 2H, $CH_{aromatic}$), 8.16 (d, ^{3}J=8.7 Hz, 2H, $CH_{aromatic}$); ^{13}C NMR (101 MHz, CD_3CN) δ (ppm) 27.64 ($CH_{2\text{-}\beta}$), 36.88 ($CH_{2\text{-}\alpha}$), 44.48 ($CH_{2\text{-}\gamma}$), 45.55 (CH_3), 128.60, 129.76 (2 x $CH_{aromatic}$), 141.74, 145.43 (2 x $C_{aromatic}$), 199.44 (C_{ketone}). $C_{11}H_{13}ClO_3S$ [$M_{monoisotopic}$] requires 260, ESI-MS (ESI+) *m/z*: 261 [M(^{35}Cl)+H]$^+$, 263 [M(^{37}Cl)+H]$^+$, 283 [M(^{35}Cl)+Na]$^+$, 285 [M(^{35}Cl)+H]$^+$; (ES-) *m/z*: 260 [M(^{35}Cl)-H]$^-$; HRMS (ESI+) *m/z* calcd for $C_{11}H_{13}ClO_3S$ [M+Na$^+$,^{35}Cl] 283.01716, found 283.01656; Anal. Calcd for $C_{11}H_{13}ClO_3S$: 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); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 2.02–1.83 (m, 4H, $CH_{2\text{-}\beta}$, $CH_{2\text{-}\gamma}$), 3.16–3.00 (m, 5H, CH_3, $CH_{2\text{-}\alpha}$), 3.60 (t, ^{3}J=6.1 Hz, 2H, $CH_{2\text{-}\delta}$), 8.06 (d, ^{3}J=8.5 Hz, 2H, $CH_{aromatic}$), 8.13 (d, ^{3}J=8.5 Hz, 2H, $CH_{aromatic}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 21.38 ($CH_{2\text{-}\gamma}$), 31.99 ($CH_{2\text{-}\beta}$), 38.25 ($CH_{2\text{-}\alpha}$), 44.48 ($CH_{2\text{-}\delta}$), 44.72 (CH_3), 128.01, 129.01 (2 x $CH_{aromatic}$), 140.88, 144.33 (2 x $C_{aromatic}$), 198.38 (C_{ketone}). $C_{12}H_{15}ClO_3S$ [$M_{monoisotopic}$] requires 274, ESI-MS (ES+) *m/z*: 275 [M(^{35}Cl)+H]$^+$, 277 [M(^{37}Cl)+H]+, 297 [M(^{35}Cl)+Na]$^+$, 299 [M(^{37}Cl)+Na]$^+$, (ES-) *m/z*: 274 [M(^{35}Cl)-H]$^-$; HRMS (ESI+) *m/z* calcd for $C_{12}H_{15}ClO_3S$ [M+Na$^+$,^{35}Cl] 297.03281, found 297.03241, calcd for [2M+Na$^+$, ^{35}Cl] 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 Na_2SO_4 and the solvent was removed under vacuum. The raw product was purified by column chromatography (EtOAc/petroleum ether).

5-chloro-2-(4-(methylsulfonyl)phenyl)pent-1-en-1,1-diyl)dibenzene (3a). To the stirring complex of 1.06 mL $TiCl_4$ (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); ^{1}H NMR (400 MHz, CD_3CN) δ (ppm) 1.80–1.68 (m, 2H, $CH_{2\text{-}\beta}$), 2.62–2.55 (m, 2H, $CH_{2\text{-}\alpha}$), 3.00 (s, 3H, CH_3), 3.46 (t, ^3J=6.6 Hz, 2H, $CH_{2\text{-}\gamma}$), 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, ^3J=8.6 Hz, 2H, H-2,6 CH_3SO_2Phe). ^{13}C NMR (101 MHz, CD_3CN) δ (ppm) 32.29 ($CH_{2\text{-}\alpha}$), 33.69 ($CH_{2\text{-}\beta}$), 44.42 (CH_3), 45.83 ($CH_{2\text{-}\gamma}$), 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. $C_{24}H_{23}ClO_2S$ [$M_{monoisotopic}$] 410, MS (ES+) *m/z*: 411 [M(^{35}Cl)+H]$^+$, 413 [M(^{37}Cl)+Na]$^+$, 433 [M(^{35}Cl)+Na]$^+$, 435 [M(^{37}Cl)+Na]$^+$; HRMS (ESI+) *m/z* calcd for $C_{24}H_{23}ClO_2S$ [M+Na$^+$,^{35}Cl] 433.10050, found 433.09975, calcd for [2M+Na$^+$,^{35}Cl] 843.21123, found 843.21068; IR (ATR): ν

= 1589 (m, olefin), 1149 (s, SO_2) cm^{-1}; Anal. calcd for $C_{24}H_{23}ClO_2S$: 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 $TiCl_4$ (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: H_2O/MECN + 0.1% TFA; 1/1; v/v); ^{1}H-NMR (400 MHz, CD_3CN) δ (ppm) 1.78–1.69 (m, 2H, $CH_{2\text{-}\beta}$), 2.60–2.55 (m, 2H, $CH_{2\text{-}\alpha}$), 3.01 (s, 3H, CH_3), 3.46 (t, ^3J=6.4 Hz, 2H, $CH_{2\text{-}\gamma}$), 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=$^3J_{H,F}$= 8.9 Hz, 2H, H–3,5 FPhe), 7.30 (dd, ^3J=8.8 Hz, $^4J_{H,F}$=5.5 Hz, 2H, H–2,6 FPhe), 7.38 (d, ^3J=8.6 Hz, 2H, H–3,5 CH_3SO_2Phe), 7.73 (d, ^3J=8.6 Hz, 2H, H–2,6 CH_3SO_2Phe). ^{13}C NMR (101 MHz, CD_3CN) δ (ppm) 32.19 ($CH_{2\text{-}\alpha}$), 33.71 ($CH_{2\text{-}\beta}$), 44.50 (CH_3), 45.82 ($CH_{2\text{-}\gamma}$), 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, $^1J_{C,F}$=244.4 Hz, C–1 FPhe), 162.88 (d, $^1J_{C,F}$=244.2 Hz, C–1 FPhe). ^{19}F NMR (376 MHz, CD_3CN) δ (ppm) -114.40 – -114.51 (m), -113.97 – -114.09 (m). $C_{24}H_{21}ClF_2O_2S$ [$M_{monoisotopic}$] requires 446, ESI-MS (ES+) *m/z*: 447 [M(^{35}Cl)+H]$^+$, 449 [M(^{37}Cl)+H]$^+$, 469 [M(^{35}Cl)+Na]$^+$, 471 [M(^{37}Cl)+Na]$^+$; HRMS (ESI+) *m/z* calcd for $C_{24}H_{21}ClF_2O_2S$ [M+Na$^+$,^{35}Cl] 469.08165, found 469.08061, calcd for [2M+Na$^+$,^{35}Cl] 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 $TiCl_4$ (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); ^{1}H NMR (400 MHz, CD_3CN) δ (ppm) 1.47–1.37 (m, 2H, $CH_{2\text{-}\beta}$), 1.71–1.59 (m, 2H, $CH_{2\text{-}\gamma}$), 2.50–2.43 (m, 2H, $CH_{2\text{-}\alpha}$), 3.00 (s, 3H, CH_3), 3.44 (t, ^{3}J=6.6 Hz, 2H, $CH_{2\text{-}\delta}$), 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 CH_3SO_2Phe). ^{13}C NMR (101 MHz, CD_3CN) δ (ppm) 26.40 ($CH_{2\text{-}\gamma}$), 33.03 ($CH_{2\text{-}\beta}$), 35.30 ($CH_{2\text{-}\alpha}$), 44.44 (CH_3), 45.72 ($CH_{2\text{-}\delta}$), 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. $C_{25}H_{25}ClO_2S$ [$M_{monoisotopic}$] requires 424, ESI-MS (ES+) *m/z*: 447 [M(^{35}Cl)+Na]$^{+}$, 449 [M(^{37}Cl)+Na]$^{+}$, HRMS (ESI+) *m/z* calcd for $C_{25}H_{25}ClO_2S$ [M+NH_4^{+},^{35}Cl] 442.16498, found 442.16020, calcd for [M+Na^{+},^{35}Cl] 447.11615, found 447.11560, calcd for [2M+Na^{+},^{35}Cl] 871.24253, found 871.24198; IR (ATR): ν = 1591 (m, olefin), 1147 (s, SO_2) cm^{-1}; Anal. calcd for $C_{25}H_{25}ClO_2S$: 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); ^{1}H NMR (400 MHz, CD_3CN) δ (ppm) 1.47–1.35 (m, 2H, $CH_{2\text{-}\beta}$), 1.70–1.58 (m, 2H, $CH_{2\text{-}\gamma}$), 2.52–2.42 (m, 2H, $CH_{2\text{-}\alpha}$), 3.01 (s, 3H, CH_3), 3.48–3.41 (m, 2H, $CH_{2\text{-}\delta}$), 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 CH_3SO_2Phe), 7.72 (d, ^{3}J=8.6 Hz, 2H, H–2,6 CH_3SO_2Phe). ^{13}C NMR (101 MHz, CD_3CN) δ (ppm) 26.32 ($CH_{2\text{-}\gamma}$), 33.02 ($CH_{2\text{-}\beta}$), 35.34 ($CH_{2\text{-}\alpha}$), 44.50 (CH_3), 45.72 ($CH_{2\text{-}\delta}$), 115.45 (d, $^{2}J_{C,F}$=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). $C_{25}H_{23}ClF_2O_2S$ [$M_{monoisotopic}$] requires 460, ESI-MS (ES+) *m/z*: 483 [M(^{35}Cl)+Na]$^{+}$; 485 [M(^{37}Cl)+Na]$^{+}$, (ES-) *m/z*: 459 [M(^{35}Cl)-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); ^{1}H NMR (400 MHz, CD_3CN) δ (ppm) 1.75–1.65 (m, 2H, $CH_{2\text{-}\beta}$), 2.59–2.52 (m, 2H, $CH_{2\text{-}\alpha}$), 3.00 (s, 3H, CH_3), 4.35 (t, ^{3}J=6.5 Hz, 2H, $CH_{2\text{-}\gamma}$), 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 CH_3SO_2Phe). ^{13}C NMR (101 MHz, CD_3CN) δ (ppm) 26.33 ($CH_{2\text{-}\beta}$), 32.31 ($CH_{2\text{-}\alpha}$), 44.44 (CH_3), 74.11 ($CH_{2\text{-}\gamma}$), 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. $C_{24}H_{23}NO_5S$ [$M_{monoisotopic}$] requires 437, ESI-MS (ES+) *m/z*: 460 [M+Na]$^{+}$, 476 [M+K]$^{+}$; HRMS (ESI+) *m/z* calcd for $C_{24}H_{23}NO_5S$ [M+Na]$^{+}$ 460.11946, found 460.11830, calcd for [2M+Na]$^{+}$ 897.24915, found 897.24861; IR (ATR): ν = 1618, 1283 (s, O-NO_2), 1599 (m, olefin),

1148 (s, SO_2) cm^{-1}; Anal. calcd for $C_{24}H_{23}NO_5S$: 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); ^{1}H NMR (400 MHz, CD_3CN) δ (ppm) 1.74–1.65 (m, 2H, $CH_{2\text{-}\beta}$), 2.56–2.50 (m, 2H, $CH_{2\text{-}\alpha}$), 3.01 (s, 3H, CH_3), 4.35 (t, ^3J=6.4 Hz, 2H, $CH_{2\text{-}\gamma}$), 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, ^3J=$^3J_{H,F}$=8.9 Hz, 2H, H–3,5 FPhe), 7.29 (dd, ^3J=8.9 Hz, $^4J_{H,F}$=5.5 Hz, 2H, H–2,6 FPhe), 7.39 (d, ^3J=8.6 Hz, 2H, H–3,5 CH_3SO_2Phe), 7.73 (d, ^3J=8.6 Hz, 2H, H–2,6 CH_3SO_2Phe). ^{13}C NMR (101 MHz, CD_3CN) δ (ppm) 26.26 ($CH_{2\text{-}\alpha}$), 32.31 ($CH_{2\text{-}\beta}$), 44.47 (CH_3), 74.05 ($CH_{2\text{-}\gamma}$), 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). ^{19}F NMR (376 MHz, CD_3CN) δ (ppm) -117.38 (tt, $^3J_{H,F}$=8.9 Hz, $^4J_{H,F}$=5.7 Hz), -116.67 (tt, $^3J_{H,F}$=8.9 Hz, $^4J_{H,F}$=5.5 Hz). $C_{24}H_{21}F_2NO_5S$ [$M_{monoisotopic}$] requires 473, ESI-MS (ES+) *m/z*: 474 $[M+H]^+$; HRMS (ESI+) *m/z* calcd for $C_{24}H_{21}F_2NO_5S$ $[M+Na]^+$ 496.10062, found 496.09963, calcd for $[2M+Na]^+$ 969.21147, found 969.21092; IR (ATR): ν = 1602, 1280 (s, O-NO_2), 1220 (s, C-F), 1148 (s, SO_2) cm^{-1}; Anal. calcd for $C_{24}H_{21}F_2NO_5S$: 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); ^{1}H NMR (400 MHz, CD_3CN): δ (ppm) 1.42–1.34 (m, 2H, $CH_{2\text{-}\beta}$), 1.66–1.55 (m, 2H, $CH_{2\text{-}\alpha}$), 2.53–2.44 (m, 2H, $CH_{2\text{-}\gamma}$), 3.00 (s, 3H, CH_3), 4.32 (t, ^{3}J=6.5 Hz, 2H, $CH_{2\text{-}\delta}$), 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, ^{3}J=8.3 Hz, 2H, H–2,6 CH_3SO_2Phe). ^{13}C NMR (101 MHz, CD_3CN) δ (ppm) 25.21 ($CH_{2\text{-}\beta}$), 26.95 ($CH_{2\text{-}\gamma}$), 35.45 ($CH_{2\text{-}\alpha}$), 44.43 (CH_3), 74.27 ($CH_{2\text{-}\delta}$), 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. $C_{25}H_{25}NO_5S$ [$M_{monoisotopic}$] 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 $C_{25}H_{25}NO_5S$ $[M+NH_4]^+$ 469.18394, found 469.17917, calcd for $[M+Na]^+$ 474.13511, found 474.13464; IR (ATR): ν = 1621, 1277 (s, O-NO_2), 1592 (m, olefin), 1148 (s, SO_2) cm^{-1}; Anal. calcd for $C_{25}H_{25}NO_5S$: 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). A suspension of 0.26 g 4,4´-(6-chloro-2-(4-(methylsulfonyl)phenyl)hex-1-en-1,1-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); ^{1}H NMR (400 MHz, CD_3CN) δ (ppm) 1.41–1.32 (m, 2H, $CH_{2\text{-}\beta}$), 1.65–1.55 (m, 2H, $CH_{2\text{-}\gamma}$), 2.50–2.43 (m, 2H, $CH_{2\text{-}\alpha}$), 3.01 (s, 3H, CH_3), 4.33 (t, ^{3}J=6.5 Hz, 2H, $CH_{2\text{-}\delta}$), 6.81 (t, J=$^{3}J_{H,F}$= 9.0 Hz, 2H, H–3,5 FPhe), 6.93 (dd, ^{3}J=8.9 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=8.8 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 CH_3SO_2Phe), 7.72 (d, ^{3}J=8.6 Hz, 2H, H–2,6 CH_3SO_2Phe). ^{13}C NMR (101 MHz, CD_3CN) δ (ppm) 25.13 ($CH_{2-\beta}$), 26.95 ($CH_{2-\gamma}$), 35.47 ($CH_{2-\alpha}$), 44.45 (CH_3), 74.25 ($CH_{2-\delta}$), 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, CD_3CN) δ (ppm) -117.65 – -117.74 (m), -117.15 – -117.27 (m). $C_{25}H_{23}F_2NO_5S$ [$M_{monoisotopic}$] requires 487, ESI-MS (ES+) *m/z*: 510 $[M+Na]^+$; (ES-) m/z: 486 $[M-H]^-$; HRMS (ESI+) *m/z* calcd for $C_{25}H_{23}F_2NO_5S$ $[M+Na]^+$ 510.11627, found 510.11578, calcd for $[2M+Na]^+$ 997.24277, found 997.24222; IR (ATR): ν = 1622, 1279 (s, O-NO_2), 1591 (m, olefin), 1214 (s, C-F), 1148 (s, SO_2) cm^{-1}; Anal. calcd for $C_{25}H_{23}F_2NO_5S$: 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 5-chloro-2-(4-(methylsulfonyl)phenyl)pent-1-en-1,1-diyl)dibenzene (**3a**; 0.7 mmol) and 0.18 g $CuSO_4$ x 5 H_2O (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); ^{1}H NMR (400 MHz, CD_3CN) δ (ppm) 1.50–1.40 (m, 2H, $CH_{2-\alpha}$), 2.40 (t, ^{3}J=5.1 Hz, 1H, OH), 2.53–2.46 (m, 2H, $CH_{2-\beta}$), 3.00 (s, 3H, CH_3),

3.39–3.31 (m, 2H, $CH_{2\text{-}\gamma}$), 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, ^3J=8.5 Hz, 2H, H–2,6 CH_3SO_2Phe). ^{13}C NMR (101 MHz, CD_3CN) δ (ppm) 32.5 ($CH_{2\text{-}\alpha}$), 32.9 ($CH_{2\text{-}\beta}$), 44.4 (CH_3), 47.0 ($CH_{2\text{-}\gamma}$), 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. $C_{24}H_{24}O_3S$ [$M_{monoisotopic}$] requires 392, ESI-MS (ES+) *m/z*: 393 $[M+H]^+$, 415 $[M+Na]^+$; HRMS (ESI+) *m/z* calcd for $C_{24}H_{24}O_3S$ $[M+Na]^+$ 415.13439, found 415.13358, calcd for $[2M+Na]^+$ 807.27901, found 807.27845; IR (ATR): ν = 3509 (s, OH), 1601 (m, olefin), 1146 (s, SO_2) cm^{-1}; Anal. calcd for $C_{24}H_{24}O_3S$: 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 $CuSO_4$ x 5 H_2O (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 → 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); ^{1}H NMR (400 MHz, CD_3CN) δ (ppm) 1.50–1.40 (m, 2H, $CH_{2\text{-}\beta}$), 2.42 (t, ^3J=5.3 Hz, 1H, OH), 2.51–2.45 (m, 2H, $CH_{2\text{-}\alpha}$), 3.01 (s, 3H, CH_3), 3.36 (dd, ^3J=11.7 Hz, ^3J=6.3 Hz, 2H, $CH_{2\text{-}\gamma}$), 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, ^3J=$^3J_{H,F}$=8.9 Hz, 2H, H–3,5 FPhe), 7.30 (dd, ^3J=8.9 Hz, $^4J_{H,F}$=5.6 Hz, 2H, H–2,6 FPhe), 7.37 (d, ^3J=8.6 Hz, 2H, H–3,5 CH_3SO_2Phe), 7.72 (d, ^3J =8.6 Hz, 2H, H–2,6 CH_3SO_2Phe). ^{13}C NMR (101 MHz, CD_3CN) δ (ppm) 32.3 ($CH_{2\text{-}\beta}$), 33.0 ($CH_{2\text{-}\alpha}$), 44.5 (CH_3), 62.2 ($CH_{2\text{-}\gamma}$), 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, $^1J_{C,F}$=244.4 Hz, C–1 FPhe). ^{19}F NMR (376 MHz, CD_3CN) δ -117.4 (tt, $^3J_{H,F}$=8.9 Hz, $^4J_{H,F}$=5.5 Hz), -116.9 (tt, $^3J_{H,F}$=8.9 Hz, $^4J_{H,F}$=5.5 Hz). $C_{24}H_{22}F_2O_3S$ [$M_{monoisotopic}$] requires 428, ESI-MS (ES+/-)

m/z: not ionizable; HRMS (ESI+) *m/z* calcd for $C_{24}H_{22}F_2O_3S$ $[M+NH_4]^+$ 446.16437, found 446.15953, calcd for $[M+Na]^+$ 451.11554, found 451.11516; IR (ATR): ν = 3538 (s,OH), 1591 (m, olefin), 1145 (s, SO_2) 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 $CuSO_4$ x 5 H_2O (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); ^{1}H NMR (400 MHz, CD_3CN) δ (ppm) 1.42–1.27 (m, 4H, $CH_{2\text{-}\beta}$, $_{CH2\text{-}\gamma}$), 2.36 (t, ^3J=5.3 Hz, 1H, OH), 2.49–2.42 (m, 2H, $CH_{2\text{-}\alpha}$), 3.00 (s, 3H, CH_3), 3.37–3.31 (m, 2H, $CH_{2\text{-}\delta}$), 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 CH_3SO_2Phe). ^{13}C NMR (101 MHz, CD_3CN) δ (ppm) 25.8 ($CH_{2\text{-}\beta}$), 33.4 ($CH_{2\text{-}\alpha}$), 36.0 ($CH_{2\text{-}\gamma}$), 44.5 (CH_3), 62.2 ($CH_{2\text{-}\delta}$), 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. $C_{25}H_{26}O_3S$ $[M_{monoisotopic}]$ 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 $C_{25}H_{26}O_3S$ $[M+H]^+$ 407.16817, found 407.16754, calcd for $[M+NH_4]^+$ 424.19887, found, 424.19409, calcd for $[M+Na]^+$ 429.15004, found 429.14949; IR (ATR): ν = 3507 (s, OH), 1591 (m, olefin), 1144 (s, SO_2) cm^{-1}; Anal. calcd for $C_{25}H_{26}O_3S$: 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´-(6-chloro-2-(4-(methylsulfonyl)phenyl)hex-1-en-1,1-diyl)bis(fluorobenzene) (**3d**; 2.3 mmol) and 0.59 g $CuSO_4$ x 5 H_2O (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);

mp: 128-133°C; Rf=0.04 (EtOAc/petroleum ether, 1/1, v/v); ^{1}H NMR (600 MHz, CD_3CN): δ (ppm) 1.35–1.29 (m, 2H, $CH_{2\text{-}\beta}$), 1.40–1.35 (m, 2H, $CH_{2\text{-}\gamma}$), 2.39 (s, 1H, OH), 2.46–2.42 (m, 2H, $CH_{2\text{-}\alpha}$), 3.01 (s, 3H, CH_3), 3.36–3.32 (m, 2H, $CH_{2\text{-}\delta}$), 6.80 (t, J=$^{3}J_{H,F}$=8.9 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), 7.14 (t, ^{3}J=$^{3}J_{H,F}$=8.9 Hz, 2H, H–3,5 FPhe), 7.29 (dd, ^{3}J=8.9 Hz, $^{4}J_{H,F}$=5.6 Hz, 2H, H–2,6 FPhe), 7.37 (d, ^{3}J=8.5 Hz, 2H, H–3,5 CH_3SO_2Phe), 7.72 (d, ^{3}J=8.5 Hz, 2H, H–2,6 CH_3SO_2Phe). ^{13}C NMR (151 MHz, CD_3CN): δ (ppm) 25.7 ($CH_{2\text{-}\beta}$), 33.3 ($CH_{2\text{-}\alpha}$), 36.0 ($CH_{2\text{-}\gamma}$), 44.5 (CH_3), 62.1 ($CH_{2\text{-}\delta}$), 115.4 (d, $^{2}J_{C,F}$=21.5 Hz, C–3,5 FPhe), 116.1 (d, $^{2}J_{C,F}$=21.5 Hz, C–3,5 FPhe), 127.7, 131.5, 132.0 (d, $^{3}J_{C,F}$=8.2 Hz, C–2,6 FPhe), 133.2 (d, $^{3}J_{C,F}$=8.2 Hz, C–2,6 FPhe), 139.6 (d, $^{4}J_{C,F}$=3.4 Hz, C–4 FPhe), 139.7 (d, $^{4}J_{C,F}$=3.4 Hz, C–4 FPhe), 139.7, 139.8, 141.5, 149.1, 162.2 (d, $^{1}J_{C,F}$=244.0 Hz, C–1 FPhe), 162.8 (d, $^{1}J_{C,F}$=244.0 Hz, C–1 FPhe). IR (ATR): ν = 3513 (s, OH), 1601 (m, olefin), 1221 (s, C-F), 1144 (s, SO_2) cm^{-1}; $C_{25}H_{24}F_2O_3S$ [$M_{monoisotopic}$] requires 442, MS (ESI +) *m/z*: 443 [M+H]$^{+}$; 465 [M+Na]$^{+}$; 885 [2M+H]$^{+}$; HRMS (ESI+) *m/z* calcd for $C_{25}H_{24}F_2O_3S$ [M+NH_4]$^{+}$ 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 $C_{25}H_{24}F_2O_3S$: 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 Na_2SO_4. 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); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 0.95 (t, ^{3}J=7.4 Hz, 3H, CH_3), 1.44–1.36 (m, 2H, $CH_{2\text{-}\gamma}$), 1.76–1.66 (m, 2H, $CH_{2\text{-}\beta}$), 2.52 (s, 3H, CH_3SO_2), 2.92 (t, ^{3}J=7.4 Hz, 2H, $CH_{2\text{-}\alpha}$), 7.26 (d, ^{3}J=8.5 Hz, 2H, H-3,5), 7.87 (d, ^{3}J=8.5 Hz, 2H, H-2,6 CH_3SPhe). $C_{12}H_{16}OS$ [$M_{monoisotopic}$] requires 208, ESI-

MS (ES+): *m/z* = 209 $[M+H]^+$; HRMS (ESI+) *m/z* calcd for $C_{12}H_{16}OS$ $[M+Na^+]$ 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); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 0.96 (t, ^3J=7.3 Hz, 3H, CH_3), 1.36–1.48 (m, 2H, $CH_{2\text{-}\gamma}$), 1.67–1.82 (m, 2H, $CH_{2\text{-}\beta}$), 2.97–3.03 (m, 2H, $CH_{2\text{-}\alpha}$), 3.08 (s, 3H, CH_3SO_2), 8.04 (d, ^3J=8.6 Hz, 2H, H-3,5 CH_3SO_2Phe), 8.12 (d, ^3J=8.6 Hz, 2H, H-2,6 CH_3SO_2Phe). ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 14.04 (CH_3), 22.50 ($CH_{2\text{-}\gamma}$), 26.28 ($CH_{2\text{-}\beta}$), 38.95 ($CH_{2\text{-}\alpha}$), 44.48 (CH_3SO_2), 76.84, 77.16, 77.48, 127.92, 129.02, 141.16, 144.11, 199.30 (CO). $C_{12}H_{16}O_3S$ [$M_{monoisotopic}$] requires 240, ESI-MS (ES+) *m/z*: 241 $[M+H]^+$; HRMS (ESI+) *m/z* calcd for $C_{12}H_{16}O_3S$ $[M+Na^+]$ 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

organic fraction the aqueous fraction was extracted three times with EtOAc (20 mL). The combined organic fraction was dried over Na_2SO_4 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); ^{1}H NMR (400 MHz, CD_3CN) δ (ppm) 0.76 (t, ^3J=7.1 Hz, 3H, CH_3), 1.32–1.16 (m, 4H, $CH_{2\text{-}\beta}$, $CH_{2\text{-}\gamma}$), 2.46–2.40 (m, 2H, $CH_{2\text{-}\alpha}$), 3.01 (s, 3H, CH_3SO_2), 6.80 (t, ^3J=$^3J_{H,F}$=8.9 Hz, 2H, H-3,5 FPhe), 6.92 (dd, ^3J=8.8 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=8.7, $^4J_{H,F}$=5.5 Hz, 2H, H-2,6 FPhe), 7.36 (d, ^3J=8.4 Hz, 2H, H-3,5 CH_3SO_2Phe), 7.71 (d, ^3J=8.4 Hz, 2H, H-2,6 CH_3SO_2Phe). ^{13}C NMR (151 MHz, CD_3CN) δ (ppm) 14.03 (CH_3), 23.31 ($CH_{2\text{-}\gamma}$), 31.36 ($CH_{2\text{-}\alpha}$), 35.97 ($CH_{2\text{-}\beta}$), 44.51 (CH_3SO_2), 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). $C_{25}H_{24}F_2O_2S$ [$M_{monoisotopic}$] requires 426, ESI-MS (ES+) *m/z*: 427 $[M+H]^+$; HRMS (ESI+) *m/z* calcd for $C_{25}H_{24}F_2O_2S$ $[M+H]^+$ 427.15441, found 427.15378, calcd for $[M+NH_4]^+$ 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): ν = 2963, 2872 (m, CH_3), 1592 (m, olefin), 1218 (s, C-F), 1148 (s, SO_2) cm^{-1}; Anal. calcd for $C_{25}H_{24}F_2O_2S$: C: 70.40, H: 5.67, S: 7.52, found: C: 70.57, H: 5.59, S: 7.38.

Cell-based ERα and ERβ assays. To analyze the functional activity (i.e., antagonistic and/or agonistic) of the novel compounds on human ERα and ERβ 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 ERα or ERβ 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 IC_{50} 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 CO_2. 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 CO_2 incubator (5% CO_2). 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 ERα, ERβ 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

blocking solution (5% skimmed milk powder + 2% bovine serum albumin) followed by the incubation with the primary antibody anti-ERα (1:500; monoclonal rabbit, abcam plc, Cambridge, UK, CHIP Grade, ab108398), anti-ERβ(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 7×10^4 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.

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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 ^{1}H 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 ERβ inhibition by NO-SERMs **4** (Figure S5); Dose-response curves of the ERβ 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); ^{1}H- and ^{13}C-NMR-spectra 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); ^{1}H- and ^{13}C-NMR-spectra 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); ^{1}H- and ^{13}C-NMR-spectra 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); ^{1}H- and ^{13}C-NMR-spectra of 5-chloro-1-(4-(methylsulfonyl)phenyl)pentan-1-one (**2b**) (Figure S14); Section of the HSMR spectra of 5-chloro-2-(4-(methylsulfonyl)phenyl)pent-1-en-1,1-diyl)bibenzene (**3a**) (Figure S15); ^{1}H-, and ^{13}C-NMR-spectra 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); ^{1}H- and ^{13}C-spectra of 4,4´-(5-chloro-2-(4-(methylsulfonyl)phenyl)pent-1-en-1,1-diyl)bis(fluorobenzene) (**3b**) (Figure S19); ^{19}F-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); ^{1}H-NMR-spectra of 6-chloro-2-(4-(methylsulfonyl)phenyl)hex-1-en-1,1-diyl)dibenzene (**3c**) (Figure S22); ^{13}C-NMR-spectra of 6-chloro-2-(4-(methylsulfonyl)phenyl)hex-1-en-1,1-diyl)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-

fluorophenyl)-4-(4-methylsulfonyl)phenyl)pent-4-en-1-ol (**5b**) (Figure S46); IR-spectra of 5,5´-bis(4-fluorophenyl)-4-(4-methylsulfonyl)phenyl)pent-4-en-1-ol (**5b**) (Figure S47); Section of the HRMS spectra of 5-(4-(methylsulfonyl)phenyl)-6,6-diphenylhex-5-en-1-ol (**5c**) (Figure S48); ^{1}H- and ^{13}C-NMR-spectra of 5-(4-(methylsulfonyl)phenyl)-6,6-diphenylhex-5-en-1-ol (**5c**) (Figure S49); IR-spectra of 5-(4-(methylsulfonyl)phenyl)-6,6-diphenylhex-5-en-1-ol (**5c**) (Figure S50); Section of the HRMS spectra of 6,6´-bis(4-fluorophenyl)-5-(4-(methylsulfonyl)phenyl)hex-5-en-1-ol (**5d**) (Figure S51); ^{1}H-NMR-spectra of 6,6´-bis(4-fluorophenyl)-5-(4-(methylsulfonyl)phenyl)hex-5-en-1-ol (**5d**) (Figure S52); ^{13}C- and ^{19}F-NMR-spectra of 6,6´-bis(4-fluorophenyl)-5-(4-(methylsulfonyl)phenyl)hex-5-en-1-ol (**5d**) (Figure S53); IR-spectra of 6,6´-bis(4-fluorophenyl)-5-(4-(methylsulfonyl)phenyl)hex-5-en-1-ol (**5d**) (Figure S54); . Characterization of reference compound **8**; HRMS spectra of 1-(4-(methylthio)phenyl)pentan-1-one (**6**) (Figure S55); ^{1}H-spectra of 1-(4-(methylthio)phenyl)pentan-1-one (**6**) (Figure S56); HRMS spectra of 1-(4-(methylsulfonyl)phenyl)pentan-1-one (**7**) (Figure S57); ^{1}H- and ^{13}C-NMR-spectra of 1-(4-(methylsulfonyl)phenyl)pentan-1-one (**7**) (Figure S58); Section of the HRMS spectra of 4,4'-(2-(4-(methylsulfonyl)phenyl)hex-1-ene-1,1-diyl)bis(fluoro-benzene) (8) (Figure S59); ^{1}H- and ^{13}C-NMR-spectra of 4,4'-(2-(4-(methylsulfonyl)phenyl)hex-1-ene-1,1-diyl)bis(fluorobenzene) (**8**) (Figure S60); IR-spectra of 4,4'-(2-(4-(methylsulfonyl)phenyl)hex-1-ene-1,1-diyl)bis(fluorobenzene) (**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

E2, 17β-estradiol

EC, effective concentration

ER, estrogen receptor

ERE, estrogen responsive element

ERK, extracellular-signal-regulated kinase

HUVECs, human umbilical vein endothelial cells

HRMS, high-resolution mass spectrometry

IC, inhibitory concentration

JNK, c-Jun N-terminal kinase

MAPK, mitogen-activated protein kinase

NAT, 2,3-naphthotriazole

NF-κB, nuclear factor kappa B

•NO, nitric oxide

NO-SERM, nitric oxide releasing selective estrogen receptor modulator

4OHT, 4-hydroxytamoxifen

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

SERD, selective ER degrader

SERM, selective estrogen receptor modulator

S.I., selectivity index

TAM, tamoxifen

TF, transcription factors

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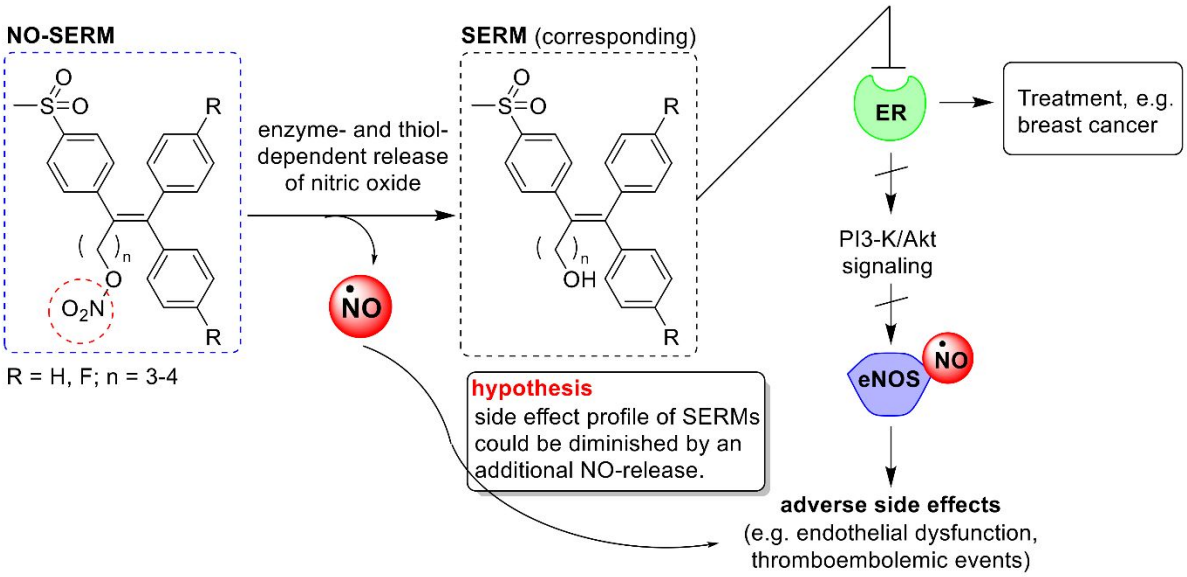

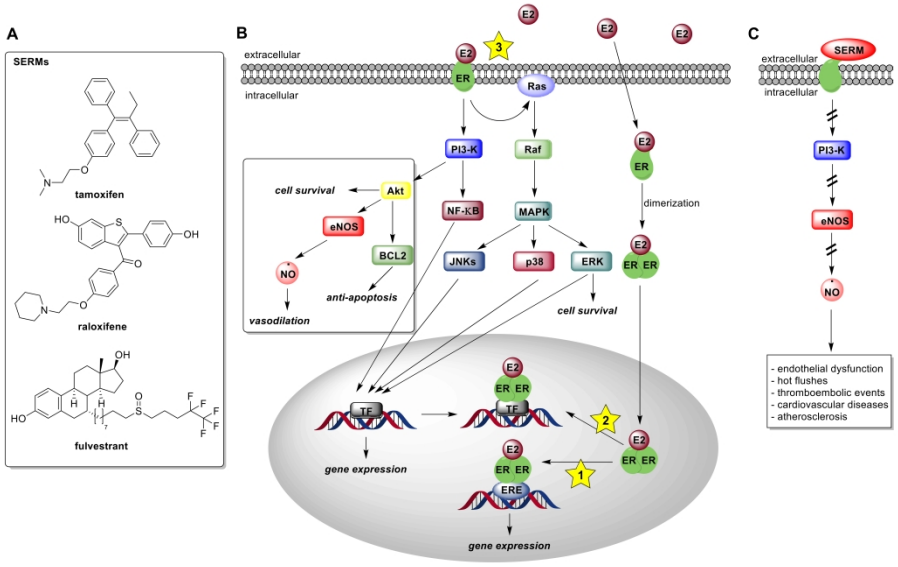

Fig. 1: Structure and modulation of the estrogen signaling by selective estrogen receptor modulators (SERMs). (A) Chemical structure of the SERMs tamoxifen, raloxifene, and fulvestrant. (B) Showed the most important intracellular signaling pathways of 17β-estradiol (E2) that could be influenced by SERMs. 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.

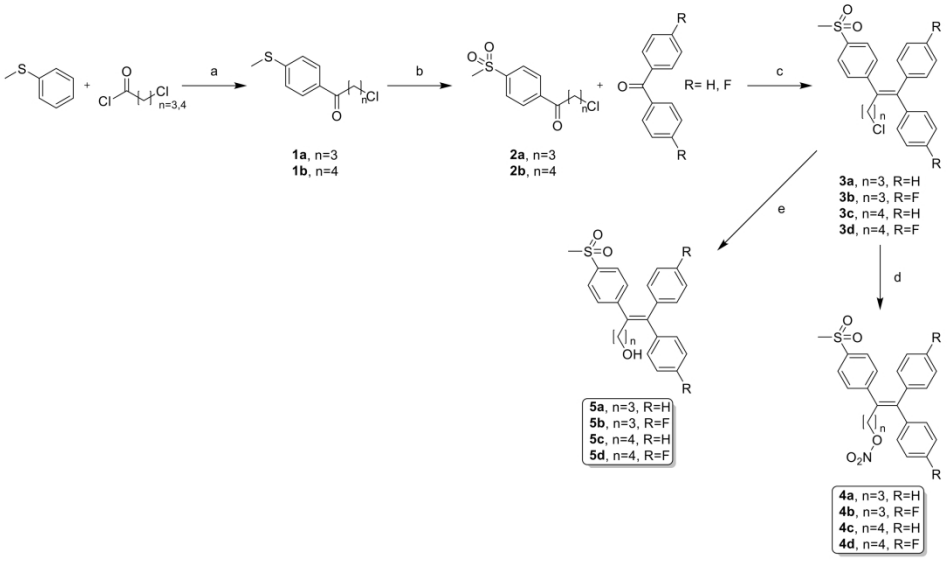

Fig. 2: Synthetic pathway of NO-releasing SERMs (4) and reference SERMs (5). Reagents and conditions: (a) AlCl3, CHCl3, argon, 2.5 h rt, (b) oxone® (potassium peroxymonosulphate), MeOH/THF (1/1, v/v), 31 h, (c) Zn, TiCl4, reflux 4.5 h, (d) AgNO3, MeCN, 24 h, 80°C, (e) CuSO4 x 5 H2O, DMSO/H2O (1/3, v/v),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

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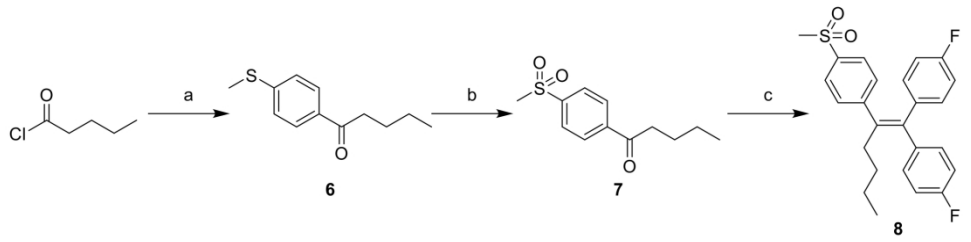

Fig. 3: Synthetic pathway of SERM 8. Reagents and conditions: (a) thioanisole, AlCl3, CHCl3, 1.5 h rt (b), oxone® (potassium peroxymonosulphate), MeOH/THF (1/1, v/v), 15 h, (c) 4,4´-difluorobenzophenone, Zn, TiCl4, reflux 4.5 h, reflux.

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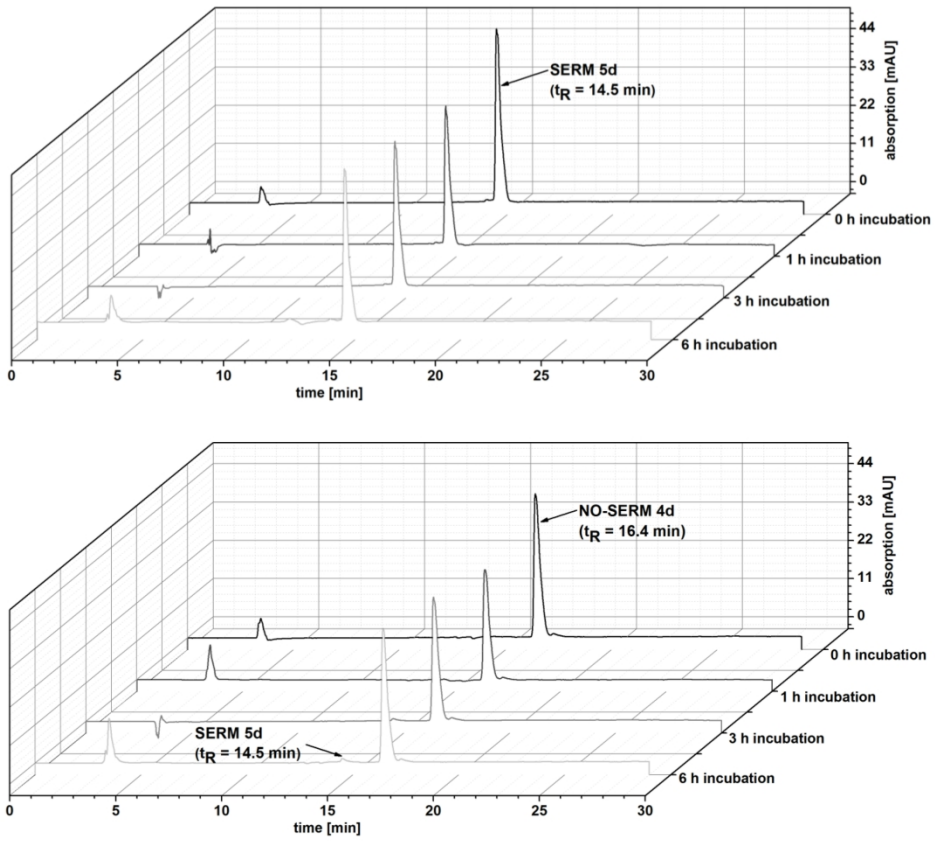

Fig. 4: Stability of SERM 5d and NO-SERM 4d in human blood. Stability was analyzed by HPLC after zero up to six hours incubation in human whole blood at 37°C. After incubation, proteins were separated by using supersol-precipitating agent (1:2, v/v) and the clear solution were analyzed using the following conditions. Agilent 1100; Zorbrax 300SB-C18 (250 x 9.4 mm; 4 µm); Zorbrax 300SB-C18 (4.6 x 12.5 mm, 5 µm); 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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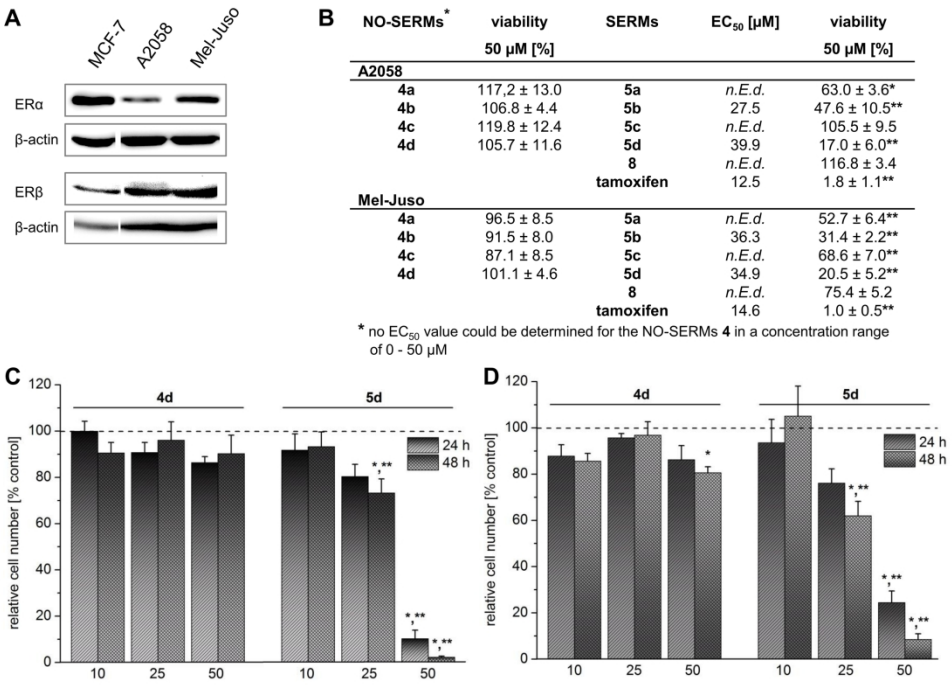

NO-SERMs*	viability 50 µM [%]	SERMs	EC_{50} [µM]	viability 50 µM [%]
A2058				
4a	117,2 ± 13.0	**5a**	*n.E.d.*	63.0 ± 3.6*
4b	106.8 ± 4.4	**5b**	27.5	47.6 ± 10.5**
4c	119.8 ± 12.4	**5c**	*n.E.d.*	105.5 ± 9.5
4d	105.7 ± 11.6	**5d**	39.9	17.0 ± 6.0**
		8	*n.E.d.*	116.8 ± 3.4
		tamoxifen	12.5	1.8 ± 1.1**
Mel-Juso				
4a	96.5 ± 8.5	**5a**	*n.E.d.*	52.7 ± 6.4**
4b	91.5 ± 8.0	**5b**	36.3	31.4 ± 2.2**
4c	87.1 ± 8.5	**5c**	*n.E.d.*	68.6 ± 7.0**
4d	101.1 ± 4.6	**5d**	34.9	20.5 ± 5.2**
		8	*n.E.d.*	75.4 ± 5.2
		tamoxifen	14.6	1.0 ± 0.5**

* no EC_{50} value could be determined for the NO-SERMs **4** in a concentration range of 0 - 50 µM

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, ** $p < 0.01$.

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A

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

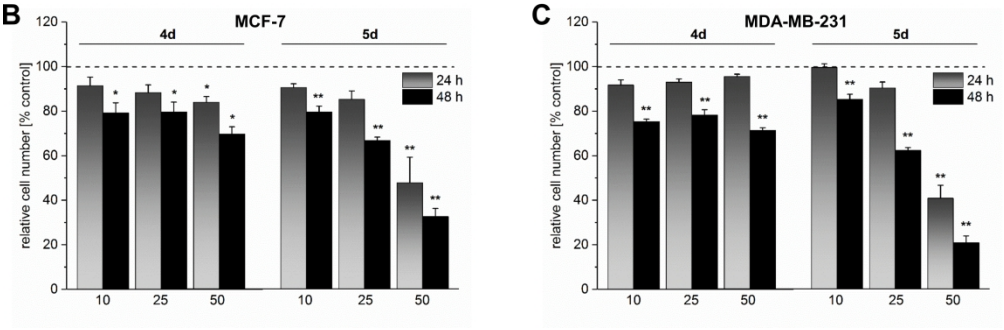

Fig. 6: Influence of NO-SERMs 4 or SERMs 5 on cell viability and proliferation of breast cancer cell lines. (A) Influence of the NO-SERMs 4 and SERMs 5 on cell viability was investigated. Additional release of □NO preserved viability of MCF-7 cells. Three independent experiments were performed (n =9). Mean ± 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, ** $p < 0.01$.

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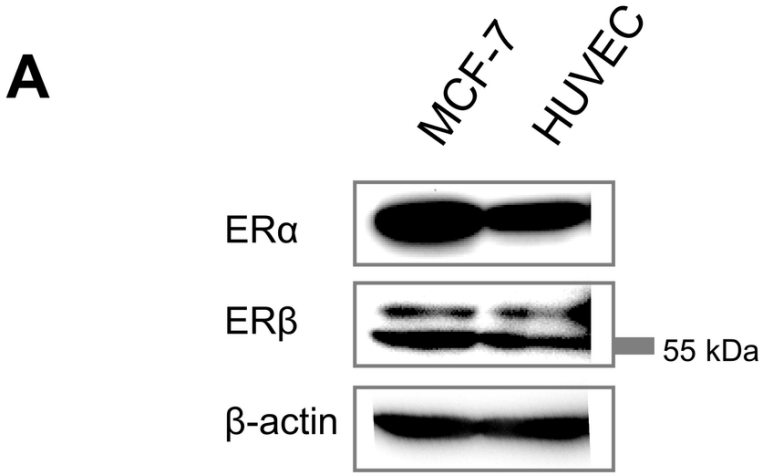

B

	EC_{50} [µM]	viability 50 µM [%]
HUVEC		
4d	*n.E.d.*	91.1 ± 4.0
5d	19.6	*b.l.d.*

Fig. 7: ERα and ERβ expression and the influence of NO-SERM 4d or SERM 5d on viability of HUVEC. (A) ERα and ERβ expression in HUVECs as determined by Western blotting. 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 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.l.d.: below limit of detection.

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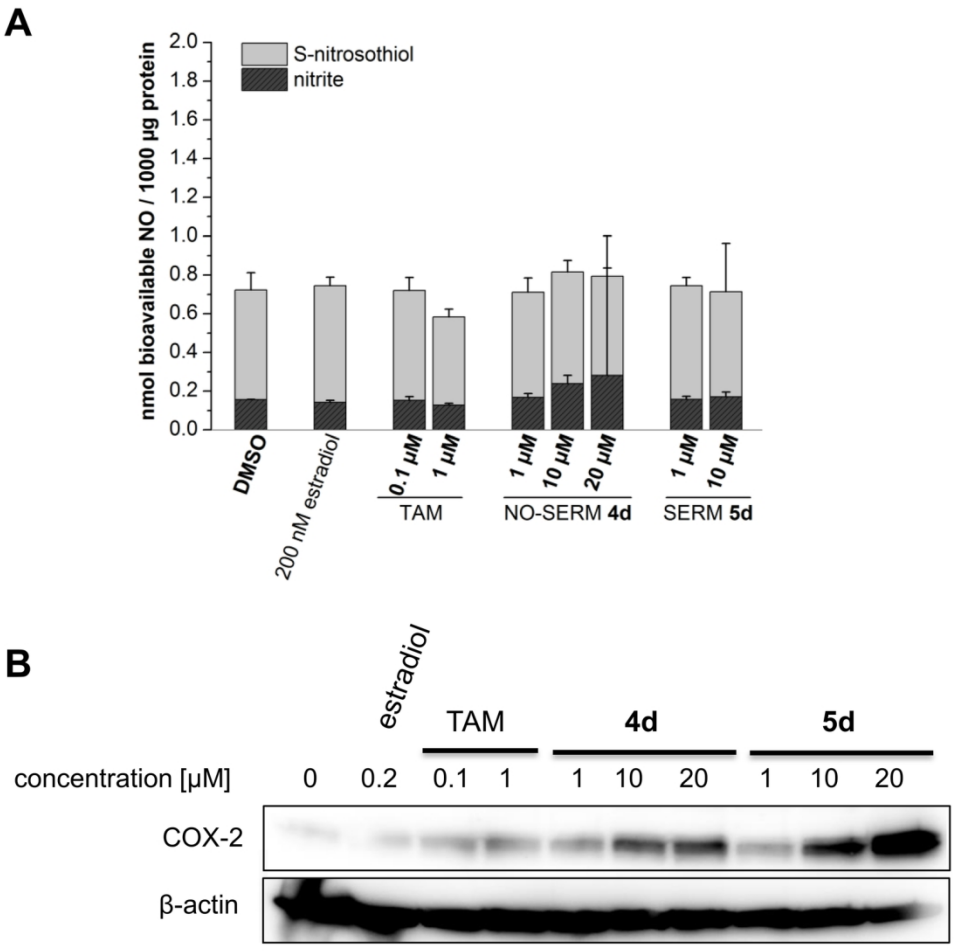

Fig. 8: Influence of NO-SERM 4d or SERM 5d on (A) NO-bioavailability and (B) the inflammatory marker COX-2 of HUVECs. (A) The influence of SERMs and the novel NO-SERM 4d on the bioavailable □NO was measured as sum of nitrite and S-nitrosothiols in cell culture supernatants. Therefore, cells were treated 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.

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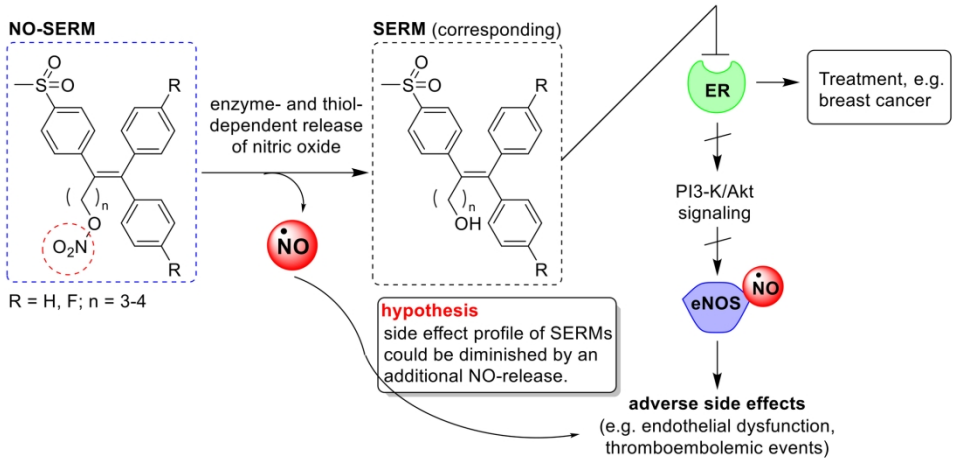

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