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Synthesis and evaluation of methylsulfonylnitrobenzamides (MSNBAs) as inhibitors of the thyroid hormone receptor–coactivator interaction

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ABSTRACT

We previously identified the methylsulfonylnitrobenzoates (MSNBs) that block the interaction of the thyroid hormone receptor with its obligate transcriptional coactivators and prevent thyroid hormone signaling. As part of our lead optimization work we demonstrated that sulfonylnitrophenylthiazoles (SNPTs), which replace the ester linkage of MSNBs with a thiazole, also inhibited coactivator binding to TR. Here we report that replacement of the ester with an amide (methylsulfonylnitrobenzamides, MSNBA) also provides active TR antagonists.

The thyroid hormone receptors (TRs), which regulate development, growth, and metabolism, belong to the nuclear hormone receptor (NR) superfamily.[1,2] The activity of TRs is induced in vivo by thyroid hormone (T3).[3] TRs contain three functional domains: an amino terminal transcription activation domain (AF-1), a central DNA binding domain (DBD), and a carboxyl terminal ligand binding domain (LBD) that includes a T3-inducible coactivator binding domain, AF-2.[4] In the absence of T3, TRs associate with corepressors and cause suppression of basal transcription at thyroid response elements (TREs). Upon binding of T3, TRs undergo a conformational change that releases corepressors and recruits coactivators, such as the p160 steroid receptor coactivators (SRC), to activate gene transcription from the TRE.[5,6] Members of SRC family include SRC1 (NcoA1), SRC2 (GRIP1/TIF2), and SRC3 (AIB1/TRAM1/RAC3/ACTR).[7] These coactivators have variable numbers of a conserved LXXLL motif, called an NR box that mediates binding to TRs.[8,9] The NR boxes interact with the AF-2 region of the TR LBD.[10]

We have previously reported two scaffolds, β-aminoketones and methylsulfonylnitrobenzoates (MSNBs), that act as antagonists of coactivator binding to TRs by competing with NR boxes for binding to the receptor. While the two families have different structures they have a similar mode of action, irreversibly modifying Cys298 within the AF-2 domain of TR.[11] Unfortunately these compounds suffered from multiple liabilities in vivo, including cardiac ion channel activity of β-aminoketones and intrinsic chemical instability of both β-aminoketones and MSBNs. Therefore we have sought to develop new scaffolds. Recently we reported that sulfonylnitrophenylthiazoles (SNPTs), which replace the ester-linkage of MSNB with a bioisosteric thiazole ring (Fig. 1), retain their TR antagonism.[12] In this Letter, we explored the replacement of the ester with an amide linkage.

The synthesis of methylsulfonylnitrobenzamide (MSNBA) analogs was accomplished using a parallel chemistry approach (Scheme 1) with two diversification steps (Fig. 2, building blocks X and Y). First, five amino-carboxylic esters (building block X), containing one to three methylenes and capped with either a primary or secondary amine, were introduced to provide a terminal carboxylate. Second, 24 amines (building block Y) were employed to convert the acid to a set of amides. The amines in the building block Y set were chosen to systematically vary the size, electrostatics, and hydrophobicity at this position. The methylsulfonyl moiety remained fixed.

Commercially available chloronitrobenzoic ester **4** was converted to compound **5** by treatment with sodium methanethiolate. Oxidation with *m*-CPBA gave the sulfonyl compound **6**. Finally hydrolysis gave the common intermediate carboxylic acid **7**. Compound **7** was then converted to amides **8** by reaction with one of the five amino-carboxylic esters (building block X) using PyBOP. After hydrolysis of compounds **8** with lithium hydroxide, the resulting carboxylic acids **9** were reacted with the 24 selected amines (building block Y), PyBOP, and diisopropylethylamine at room temperature to give the final MSNBAs **3** {X, Y}. Nomenclature

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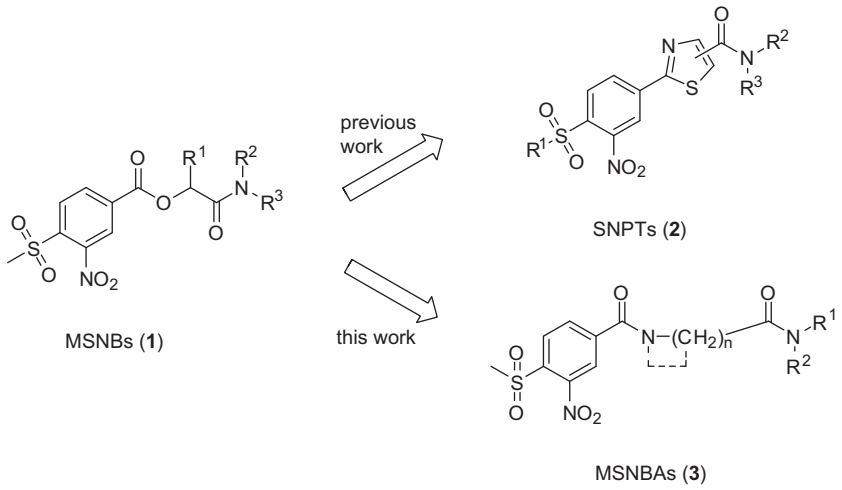
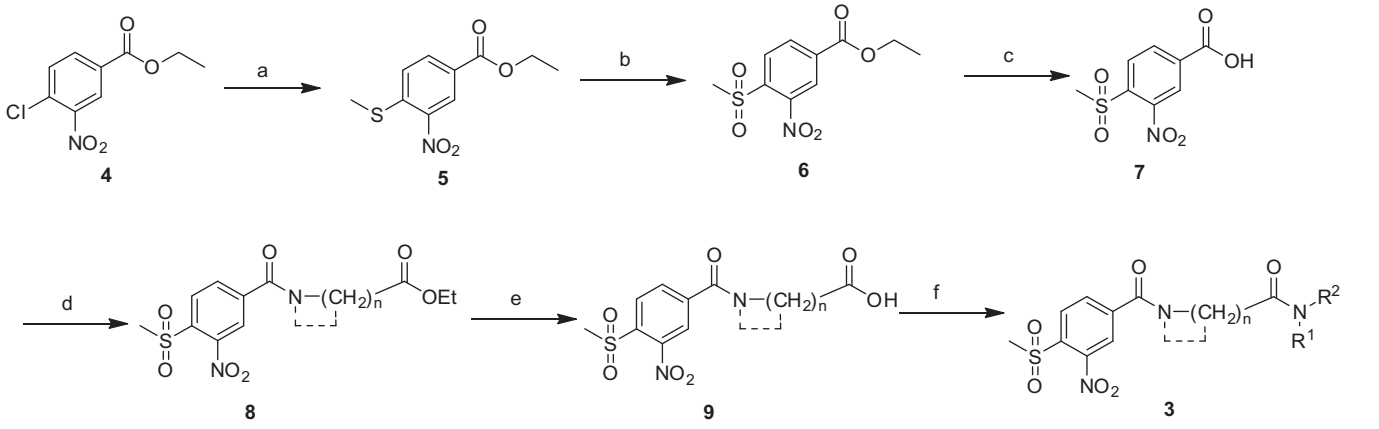


Figure 1. Structural modification of MSNBs (**1**) to SNPTs (**2**) and MSNBAs (**3**).



Scheme 1. Synthesis of MSNBAs. Reagents and conditions: (a) NaSMe, tetrahydrofuran, 50 °C, 94%; (b) *m*-chloroperoxybenzoic acid, dichloromethane, rt, 84%; (c) LiOH, tetrahydrofuran, rt, 94%; (d) amino-carboxylic esters (building block X), benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate, diisopropylethylamine, dimethylformamide, rt, 49–98%; (e) LiOH, tetrahydrofuran, rt, 62–84%; (f) amines (building block Y), benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate, diisopropylethylamine, dimethylformamide, rt, 10–99%.

follows the Chemset convention. All reaction mixtures were concentrated in vacuo. The phosphine oxide byproduct generated from the phosphonium coupling reagent was removed by filtration through silica. The resulting crude products were purified directly by automated reverse phase preparative high pressure liquid chromatography (HPLC). Ninety-five targeted MSNBAs were successfully isolated from 120 attempted reactions. All products were of >95% purity and had the correct identity as established by HPLC/MS/ELSD. The compounds were dissolved in DMSO to give standard 10 mM stock solutions for all further (Fig. 2) studies.

The MSNBAs were evaluated for their ability to antagonize the TR-coactivator interaction using a fluorescence polarization (FP) assay measuring the interaction of TRβ-LBD and Texas Red-labeled SRC2-2 peptide (Tx-SRC2-2).[13,14] The specificity of the MSNBA series was examined by comparing the antagonism of TRβ–SRC2-2 with that of the vitamin D receptor (VDR) with SRC2-3.[15,16] We also measured the cytotoxicity of the MSNBA series in HepG2, a hepatocellular carcinoma derived cell line. Compounds were serially diluted using a threefold scheme from a 10,000 μM top concentration in DMSO. Serially diluted MSNBAs were transferred to test solutions by 100 H pins (V&P Scientific). All assays were run in triplicate and the FP assays for TR-COA and VDR-COA antagonism were replicated twice, for a total of 6 replicates; the data are reported as average values across all assays as EC_{50} value with standard deviations.

The observed EC_{50} values for each assay are shown as a heatmap in Figure 3 and the data from active compounds is summarized in Table 1. Five out 95 MSNBAs were active (<20 μM EC_{50}), while most of MSNBAs exhibited no inhibition of the TRβ–SRC2-2 interaction. Among the actives, four compounds contained the methylene linker (**3**{1,y}) between the two amide moieties (EC_{50}'s: **3**{1,1} = 16, **3**{1,11} = 5.8, **3**{1,12} = 6.6, and **3**{1,18} = 3.6 μM). Interestingly, these four actives have bulky and highly hydrophobic secondary amines: adamantylamine, *N*-methylaniline, *N*-propylaniline, and dibenzylamine, respectively. In contrast, compounds **3**{4,y}, bearing an *N*-methyl group on the 'left' amide, were inactive (especially, **3**{4,1}, {4,12}, {4,13}, and {4, 18} with EC_{50}'s >60 μM). Likewise, the ethyl **3**{2,y}, propyl **3**{3,y}, and piperidinyl **3**{5,y} linkers gave no inhibitory activity. Remarkably, compound **3**{3,1} showed moderate activity (EC_{50} = 11 μM for TRβ) although it has propyl linker. The same trend was followed for TRα-COA antagonism. The only exceptions were compounds **3**{1,13} and **3**{1,18}, which exhibited slightly more potency against TRα. We examined the NR specificity of the MSNBA series by testing VDR-coactivator interactions in FP assay. Similar to the TRs, the VDR has a strongly activated cysteine residue (Cys 284) in the ligand binding