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Synthesis of Four SIRT1 Activators Based on an Imidazo[1,2-b]thiazole Structure, *in vitro* Derived Metabolites and Deuterated Analogs

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Abstract: The enzyme sirtuin 1 (SIRT1) is a major target for the treatment of various metabolic disorders. Herein, a practical synthesis of imidazo[1,2-b]thiazole derivatives, one of the most comprehensively studied class of synthetic SIRT1 activators, is presented. The synthesized SIRT1 activators, the *in vitro*-identified metabolite of SRT1720, and the eightfold deuterated analytical standards were obtained through a six-step protocol yielding model compounds with a conserved core structure and two variable moieties. A multiplicity of potential SIRT1 activators and metabolites can be prepared with substituents enabling the modification of biological effects.

Keywords: Isotope labeling, metabolite, SIRT1 activator, SRT1720, SRT2104.

The enzyme sirtuin 1 (SIRT1) is an important metabolic key regulator and therefore a promising target for the treatment of metabolic disorders such as type II *diabetes mellitus* [1, 2]. Since the naturally occurring polyphenol resveratrol was discovered as a SIRT1 activator in 2003 [3] several new artificial SIRT1 activators were developed based on a common template exhibiting different substituents, some of which are exemplified in Fig. (**1**) [4].

In this context, SIRT1 activators based on an imidazo[1,2-b]thiazole nucleus represent the best studied class of SIRT1 activators including SRT1720, SRT1460 and SRT2104, which were tested in several *in vitro* and *in vivo* studies within the last seven years [5-7]. Even though the activation of the enzyme SIRT1 by these low molecular mass compounds was controversially discussed [8-12], a variety of SIRT1 activators is at present undergoing preclinical and clinical studies (SRT2104 [13, 14], SRT2379 [15, 16] and SRT3025) concerning their utility in the treatment of metabolic, inflammatory and cardiovascular diseases. In addition to SIRT1 activators, the synthesis of metabolites and deuterated analogs of known activators (e.g. SRT1720) for mass spectrometric characterization and quantification is of great interest [17, 18]. Therefore an alternative, efficient and simple route to synthesize SIRT1 activators (Fig. **2**) based on an imidazo [1,2-b] thiazole structure (related to SRT1720 and SRT2104) (Fig. **1**) is presented. Four SIRT1 activators and an *in vitro* observed hydroxy metabolite of

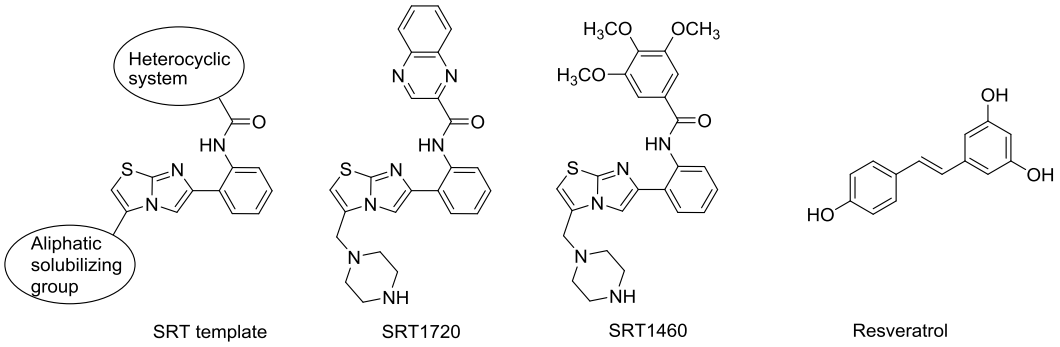

Fig. (1). Selected SIRT1 activators known from literature.

SRT1720 (**1b, 2a, 3a, 4a, 5a**; Fig. **2**) as well as the eightfold deuterated standards (**6a**), were synthesized by the procedure described in the following [19].

The alternative synthesis employed a six-step reaction protocol utilizing intermediates (**7, 9, 10a-b**, Scheme **1**)

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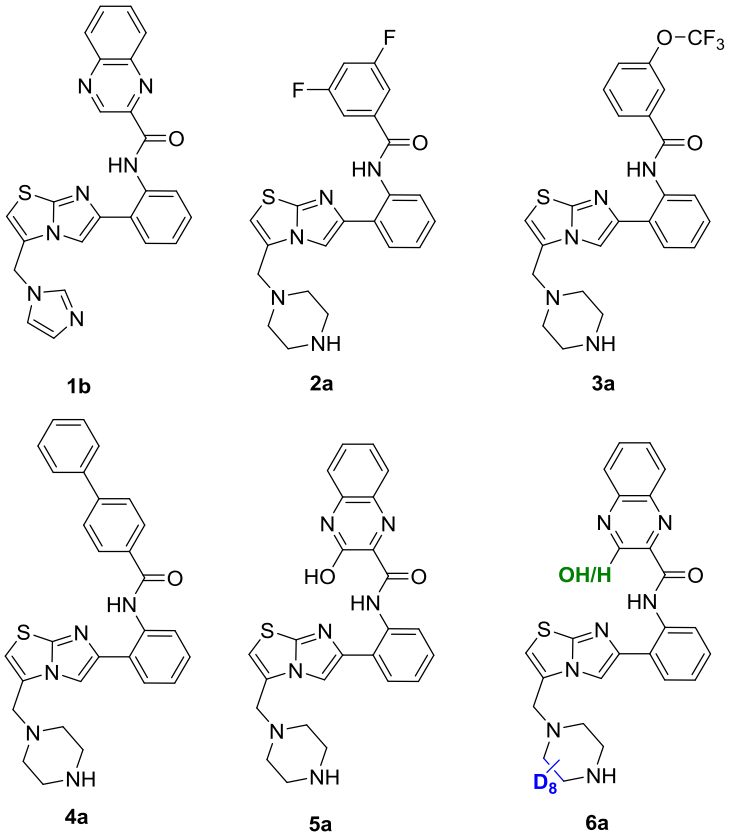


Fig. (2). Synthesized SIRT1 activator (**1b-4a**), SRT1720 *in vitro* hydroxy metabolite (**5a**) and eightfold deuterated analytical standards (SRT1720/*in vitro* SRT1720 metabolite) (**6a**).

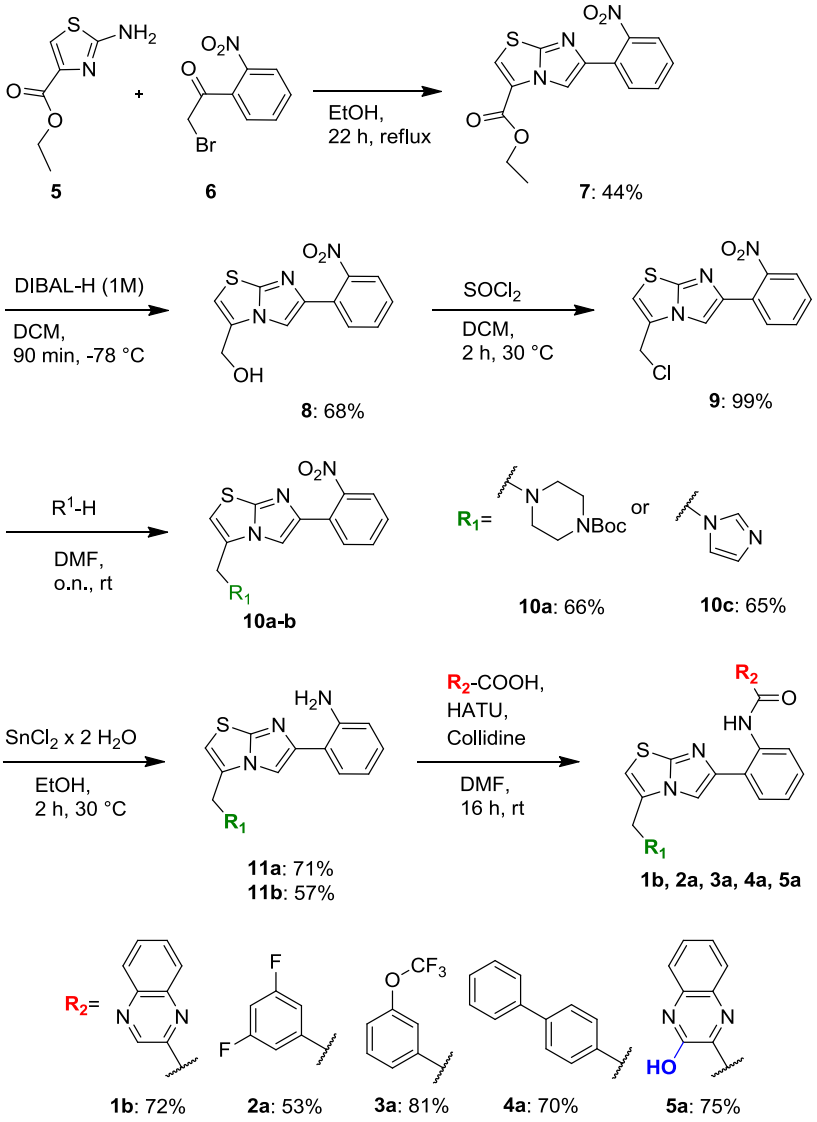
similar to earlier reports [2, 20, 21]. New aspects particularly concern the reaction steps 2, 5 and 6 and the optimization of reaction parameters at various occasions resulting in improved yields as well as an accelerated and more facile synthesis. Four model activators were prepared, differing at two sites (R_1 and R_2) with the substituent R_1 being a piperazine (**2a-4a**), eightfold deuterated piperazine (**6a**), or imidazole (**1b**) moiety, and the substituent R_2 a quinoxaline (**1b**), 3,5-difluorophenyl (**2a**), trifluoromethoxyphenyl (**3a**), biphenyl (**4a**), or a 3-hydroxy quinoxaline (**5a and 6a**) residue (Scheme **1**). The first three reaction steps, which were identical for all four model activators, were dedicated to the preparation of the imidazo[1,2-b]thiazole core structure (**9**), which was subsequently extended by consecutively attaching the first (step 4, **10a-b**) and the second substituent (step six, **1b, 2a, 3a, 4a**).

The commercially available ethyl-2-amino-4-thiazol-carboxylate (**5**) was condensed with bromo-2'-nitro acetophenone (**6**) under reflux in ethanol. In the literature, several examples for this condensation reaction are given, differing in solvent, reaction time, and purification approach with a wide range in yield (20-86%) depending on the choice of reactants [21-25]. For the purpose of this study, ethanol and a reflux time of 22 h resulted in superior yield and purity of the product after crystallization compared to e.g. butanone or acetone (Table **1**).

The obtained ester (**7**) was reduced with DIBAL-H (diisobutyl-aluminium hydride, 1.0 M in methylene chloride, DCM). Compared to earlier procedures necessitating a two-step reaction [2, 21] including the preparation of the respective carboxylic acid of **7** followed by activation with isobutyl chloroformate for the subsequent reduction with $NaBH_4$, [20, 25] the use of DIBAL-H allowed the considerably faster (90 min) one-step procedure affording the desired alcohol (**8**) with yields (68%) comparable to the earlier approaches. The mixture was cooled to -78 °C and DIBAL-H in DCM (1.0 M) was added within 30 minutes under argon atmosphere. After purification, the alcohol (**8**) was activated by thionyl chloride for inserting the substituent R_1. Here, similar to established procedures, the alcohol (**8**) was dissolved in DCM and thionyl chloride was added slowly over 30 minutes. After filtration, the desired chloride (**9**) was obtained without further purification (yield: 99%). Introducing the substituent R_1 (boc-piperazine/ eightfold deuterated boc-piperazine (**10a**) or imidazole (1**0b**)) was accomplished by dissolving the chloride (**9**) with the

Table 1. Comparison of employed solvents in reaction step one with regard to reaction time, reaction temperature and yield.

Solvent	t/[h]	Temp.	Yield/[%]
Butanone	48	reflux	10
Acetone	20	reflux	28
Ethanol	22	reflux	44



Scheme 1. Six-step synthesis of four SIRT1 activators and the SRT1720 *in vitro* hydroxy metabolite.

corresponding amine in DMF. The reaction mixture was stirred overnight to obtain the coupling products boc-piperazine (**10a**), yield: 66%, and imidazole (**10b**), yield: 65%. The reduction of the nitro function enabling the subsequent insertion of the second substituent R_2 was achieved by means of $SnCl_2$. This option proved more efficient than sodium hydrosulfide hydrate [21] (reaction time: 24 h) or catalytic hydration with 10% Pd/C [2] (reaction time: 60 h) as comparable yields were obtained within a considerably shorter reaction (reaction time: 30 min). Educts were dissolved in ethanol and sodium acetate (to buffer the pH of the reaction mixture and thus exclude any boc-deprotection), $SnCl_2$, potassium carbonate, and sodium iodide were added. The mixture was stirred at 78 °C for 30 minutes to give the resulting amine (**11a, b**) (yield: boc-piperazine: 71%; imidazole: 57%) after purification by column chromatography. The second substituent R_2 (quinoxaline (**1b**), 3,5-difluorophenyl (**2a**), trifluoromethoxy phenyl (**3a**), biphenyl (**4a**) or 3-hydroxy quinoxaline (**5a**)) was introduced by using the respective carboxylic acids. In accordance with common peptide syntheses, the educts were dissolved in DMF followed by the addition of HATU (O-(7-azabenzotriazol-1-yl) -N,N,N',N'-tetramethyl-uroniumhexa fluorophosphate) and collidine at 0°C and the mixture was stirred overnight at room temperature. After purification by column chromatography, the boc-piperazine was deprotected by stirring in trifluoroacetic acid to obtain the desired SIRT1 activators (**1b, 2a, 3a, 4a**) in good yields (53%-81%). All synthesized SIRT1 activators were characterized by high resolution/high accuracy mass spectrometry, ^1H and ^{13}C NMR spectroscopy (see supplemental information).

Conclusively, an efficient and convenient synthetic route for SIRT1 activators with an imidazo[1,2-b]thiazole-based structure was presented. The approach followed earlier protocols concerning the required intermediates but optimized conditions regarding reaction time/steps and employed educts were used. Moreover, analytical data characterizing the obtained model substances (HRMS; ^1H and ^{13}C NMR) of three potential SIRT1 activators (**1b, 2a, 4a**), the metabolite of SRT1720 (**5a**) and deuterated standards (**6a**) were provided, facilitating the production and

characterization of structurally related compounds. The imidazo[1,2-b]thiazole-based SIRT1 activators have received considerable attention by their ability of activation *in vitro*, effect on metabolism *in vivo* animal experiments and the clinical tested SRT2104 [13, 14] which is a promising therapeutic candidate in the near future. The four model activators, metabolite and deuterated standards (**1b, 2a, 3a** and **4a**) were prepared to demonstrate the applicability of the modified synthesis for medicinal and analytical research purposes. The suggested route of synthesis allows a rapid variation of both substituents R_1 and R_2 and provided good yields for each of the six synthesis steps.

CONFLICT OF INTEREST

The authors declare no competing financial interest.

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SUPPLEMENTARY MATERIAL

Synthetic procedure and compound characterization data (HRMS, ^{1}H NMR and ^{13}C NMR data).

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