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Synthesis and activity of benzimidazole-1,3-dioxide inhibitors of separase

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ABSTRACT

Due to the oncogenic activity of cohesin protease, separase in human cancer cells, modulation of separase enzymatic activity could constitute a new therapeutic strategy for targeting resistant, separase-overexpressing aneuploid tumors. Herein, we report the synthesis, structural information, and structure–activity relationship (SAR) of separase inhibitors based on modification of the lead molecule 2,2-dimethyl-5-nitro-2*H*-benzimidazole-1,3-dioxide, named Sepin-1, (**1**) identified from a high-throughput-screen. Replacement of $-NO_2$ at C5 with other functional groups reduce the inhibitory activity in separase enzymatic assay. Substitution of the two methyl groups with other alkyl chains at the C2 moderately improves the effects on the inhibitory activity of those compounds. Modifications on 2*H*-benzimidazole-1,3-dioxide or the skeleton have variable effect on inhibition of separase enzymatic activity. Density-functional theory (DFT) calculations suggest there may be a correlation between the charges on the oxide moieties on these compounds and their activity in inhibiting separase enzyme.

In recent years, breast cancer (BC) has become one of the most common types of cancer in humans, both in new cases and cancer death rates in females.[1] Separase, an enzyme that cleaves the chromosomal cohesin complex during mitosis, is overexpressed and mislocalized in a number of human tumors.[2] Overexpression of separase has been found in more than 60% of BC, 50% of triple-negative BC, and 65% of Luminal-B BC tumors.[2–4] Separase overexpression in animal models induces aneuploid mammary tumors. Since the structure of human separase has not been solved and structural information of a fungal separase has only recently become available,[5] high throughput screening was used to search for potential separase inhibitors.[6] The screening of 14,400-compound library provided 5 compounds with good activity, in which 2,2-dimethyl-5-nitro-2*H*-benzimidazole-1,3-dioxide (**1** Fig. 1) exhibited the highest activity toward inhibiting separase with a half-maximal inhibitory concentration (IC_{50}) of 15 μM.[6] In the original paper this compound was named Sepin-1.[6] Further studies showed that the benzimidazole-1,3-dioxide **1** inhibits the growth of separase-overexpressing human triple-negative BC xenografts in mice in a dose-dependent manner, and has no appreciable effect on such tumors with low-separase expression, suggesting that the specificity and efficacy of this compound in targeting tumors is related to separase overexpression. Targeting separase by **1** also results in high levels of apoptosis. These results suggest that inhibition of separase represents a new line of therapy to treat breast and other tumors overexpressing separase. In this contribution, we report the synthesis, structural information, biological activity, and structure–activity relationship (SAR) study of separase inhibitors based on structural modification of Sepin-1 (**1**). The study was designed to determine the sites on the molecule most relevant for activity and through their modification develop more active analogs. Three regions of **1** scaffold were modified and the resulting molecules tested for activity; the substituents at the C5 position, the heterocycle attached to the benzene ring, and the C2 position of carbon between the two nitrogen atoms (Fig. 1).

The structure of Sepin-1 (1) is not typically what one would consider to be 'drug like'. The molecule is small, highly polar, possess a nitro group and may be redox active. However, this report is not the first time these molecules have been found to have selective and interesting biological activity. Boiani et al. has previously reported that derivatives of the 2*H*-benzimidazole-1,3-dioxide scaffold are effective anti-trypanosomatid agents. With the tested versions possessing no toxicity in BALB/c mice with dosing of 30 mg/kg/day for 10 days, with the animals observed for 60 days.[7]

The general route to the benzimidazole derivatives starts with the acid catalyzed cyclization of nitroaniline (**2**) to give benzofuroxans (**3**). Reaction with an alcohol in the presence of sulfuric

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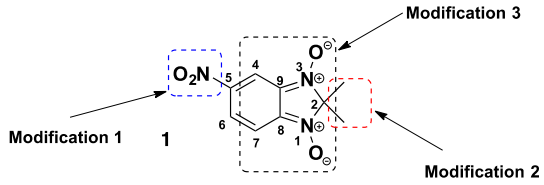
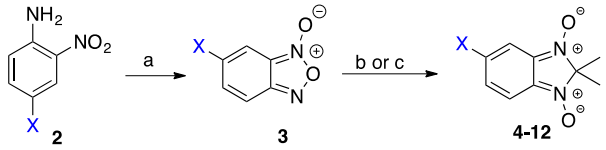


Figure 1. Structure of **1** with domains marked for structure activity study.



Scheme 1. Synthesis of derivatives at C5 position (a) $NaNO_2$, H_2SO_4, AcOH, NaN_3, H_2O, 0–80 °C, 2 h or KOH, EtOH, NaOCl 8%. (b) *i*PrOH, H_2SO_4, RT, 2 h. (c) *i*$PrNO_2$, pyridine, THF, RT, 18 h, (**10**) followed by treatment of **9** with *n*-butyl amine, EtOH, RT, overnight, (**12**) followed by treatment of **11** with $PhB(OH)_2$, (Si*P*r)Pd(allyl)Cl, *t*-BuONa, 1,4-dioxane, 60 °C, 3 h.

acid then provides the 2*H*-benzimidazole-1,3-dioxides derivatives (**4**–**12**).[6,7] Versions where the nitro group was replaced by other functionality, including carboxylic acid, ester, fluorine, bromine, and ethoxyl groups, were synthesized by starting with the appropriate nitroaniline (**2**). The bromine-substituted compound (**11**) was used for further modification by the Suzuki coupling reaction. Additionally, the product bearing fluorine (**9**) was reacted with different nucleophiles such as primary amines, allowing for modification at the C5 position on the 2*H*-benzimidazole-1,3-dioxides skeleton. Scheme 1 outlines the synthetic route and the chemical structures of derivatives with the nitro group replaced by other groups to give compounds **4**–**12** (Table 1).

The modification of functionality at the C2 position of the 2*H*-benzimidazole-1,3-dioxides was obtained by using different secondary alcohols in the reaction with benzofuroxans **3** while keeping the composition of other positions, such as nitro group, the same as in **1**. Because this step in the reaction mechanism involves a carbocation, the products obtained can result from rearrangement. Due to intermediacy of a carbocation and the harsh conditions requiring sulfuric acid the scope of alcohol substrates is limited. The installation of functional groups, including alkenes, alkynes, alcohols and amines into this position, has been challenging. Six different compounds (**13**–**18**, Scheme 2, Table 2) that have alkyl chains, spiro-derivatives, and aryl chains have been synthesized and evaluated to determine the effect of this position on the biological activity of the 2*H*-benzimidazole-1,3-dioxide derivatives.

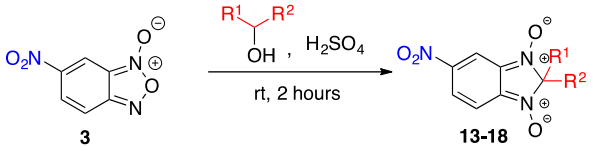
Further modification of **1** was performed in a series of individual reactions. Compounds **20** and **21** were generated from their corresponding benzofuroxan using sulfuric acid and *iso*-propanol. Structure **23** was formed by the bromination of **1** at the position *meta*- to the nitro group. The quinoxaline-1,4-dioxide (**25**) was synthesized from benzofuroxan (**24**) bearing methyl ester group and evaluated for its ability to inhibit separase due to its potential bioactivity.[8,9] The precursor to **1**, 5-nitrobenzofuroxan (**3**) and its reduced form **22** were also assayed to determine their inhibition of separase enzyme.

As mentioned earlier, **1** exhibits inhibitory activity toward separase with an IC_{50} of 15 μM. To determine what structural features are important for separase inhibition, the derivatives were evaluated for their ability to inhibit separase relative to the parent compound **1**.

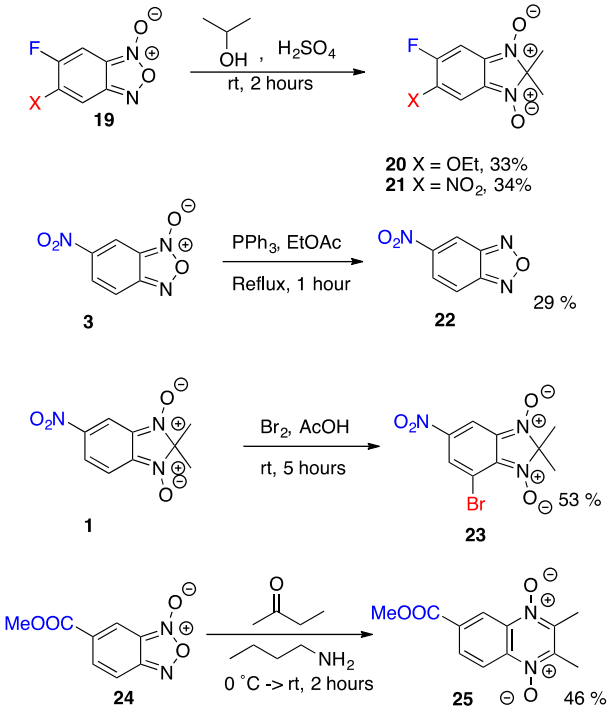
Replacement of the nitro group at the C5 position by other functional groups significantly reduces the inhibitory activity of the derivatives compared to the lead compound **1**. Since, due to their

Table 1
Yield of derivatives at the nitro group

Compound	X	Yield (%) from **3**
1	NO_2	74
4	COOH	31
5	$COOCH_3$	56
6	OEt	38
7	CF_3	49
8	SO_2CF_3	42
9	F	77
10	$NH(C_4H_9)$	70
11	Br	71
12	C_6H_5	31



Scheme 2. Synthesis of derivatives at C2 position.



Scheme 3. Other derivatives of scaffold.

reduction to nitroso functionality, aromatic nitro groups are often problematic in drug development and biological assays, the first goal was to replace that group with a pharmacophore equivalent. Unfortunately all versions in which the nitro group has been replaced, including versions with groups that may be considered pharmacophore equivalents for a nitro group, (**4**–**12**) were less active (Fig. 2A and B). The derivative with a (trifluoromethyl) sulfonyl group substituted for nitro (**8**), inhibits 65.6% separase enzyme at 100 μM concentration, was the most active among those molecules.

As shown in Table 3 the inhibitory activity toward separase seems to increase with the strength of the electron withdrawing group (–NO_2 > –SO_2CF_3 > –COOH > –F > –Br > –C_6H_5 > –$COOCH_3$ > –$NH(C_4H_9)$>–CF_3 > –OEt). Derivatives that have functional groups