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Isatin 1,2,3-triazoles as potent inhibitors against caspase-3

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ABSTRACT

Sixteen disubstituted 1,2,3-triazoles were prepared using the Huisgen cycloaddition reaction and evaluated as inhibitors against caspase-3. The two most potent inhibitors were found to be (*S*)-1-((1-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)-1*H*-1,2,3-triazol-4-yl)methyl)-5-((2-(methoxymethyl)pyrrolidin-1-yl)sulfonyl) indoline-2,3-dione (**7f**) and (*S*)-1-((1-benzyl-1*H*-1,2,3-triazol-5-yl)methyl)-5-((2-(methoxymethyl)pyrrolidin-1-yl)sulfonyl)indoline-2,3-dione (**8g**) with IC_{50}-values of 17 and 9 nM, respectively. Lineweaver–Burk plots revealed that these two triazoles show competitive inhibitory mechanism against caspase-3.

Apoptosis, or programmed cell death, plays an essential role in all multi-cellular organisms. Dysregulation of apoptosis is related to many human diseases, including cancer, diabetes and neurodegenerative diseases.[1] Several members from the caspase family are central in this process, among which caspase-3 is the key effector caspase involved in both intrinsic and extrinsic apoptotic pathways.[2] Caspase-3 inhibitors with high potency and selectivity may be useful remedies against stroke, hepatitis, brain injury and neurodegenerative diseases.

Caspases are cysteine proteases that cleave their substrate after an aspartate residue. In convention, the N-terminal residue of the scissile bond in the substrate is named P_1, the one to the C-terminal residue is named P'_1, and other residues are numbered consecutively (Fig. 1).

The corresponding binding pockets of the enzyme for these residues are named S_1, S'_1 and so on. For all caspases, P_1 to P_4 and P'_1 residues contribute to substrate recognition. For caspase-3 the sequence Asp-Glu-Ala/Val-Asp (DEAD/DEVD) in the substrate is favored for P_4 to P_1 positions. For the P'_1 position, residues with small, or polar but uncharged side chains are favored (Gly, Ala, Thr, Ser and Asn), while residues with bulky or charged side chains are not.[3]

Active caspase-3 is a homodimer of two heterodimer subunits, with one active site on each subunit. Peptide based caspase-3 inhibitors have been designed by adding an alkylating functional group to such enzyme-recognition sequences. These inhibitors bind to the substrate recognition pocket of the caspase followed by reaction with the catalytic cysteine residue in the enzyme leading to blocked protease activity. The presence of alkylating functionalities such as aldehydes or ketones results in reversible inhibitors, while α-haloketones and acyloxymethyl substituted ketones give rise to irreversible inhibitors. Although being useful in research, peptide based caspase inhibitors in general are less suitable for drug development because of low cell permeability and lack of specificity among the different caspases, as well as non-specific toxicity. Hence, developing non-peptide based caspase inhibitors remains an interesting research topic in medicinal chemistry.[4]

Isatin derivatives, such as 5-nitroisatin (**1**) and (*S*)-5-pyrrolidinyl-1-ylsulfonyl)isatins (**2a, 2b**), were first reported by Lee et al. as caspase-3 and -7 inhibitors.[5] These compounds were discovered after high-throughput screening. It is presumed that the thiol group in Cys-285 in caspase-3 forms a reversible covalent bond with the carbonyl group of the isatin moiety leading to inhibition of enzyme activity. Podichetty et al. synthesized fluorinated derivatives of isatin and evaluated their inhibition potency against several caspases.[6] Chu et al. have explored the possibility of changing the isatin 3-carbonyl group to a Michael acceptor that resulted in several inhibitors with medium to low nM IC_{50}-values.[7] Compounds with either a pyrrolo[3,4-*c*]quinoline-1,3-dione or an isoquinoline-1,3,4-trione moiety have also been reported to inhibit caspase-3 with IC_{50}-values at low μM to nM range.[8]

Lately the Huisgen 1,3-dipolar cycloaddition reaction between terminal alkynes and azides has attracted interest in medicinal chemistry.[9] This reaction has been used in several aspects for lead discovery. For example, 1,4-disubstituted 1,2,3-triazoles have been employed as mimics for amides,[10] in combinatorial chemistry library syntheses,[11] in modifications of natural products[12] and in situ library screening.[13] Grimm's bioisosteric rule[14] suggests substitution of five-membered heterocyclic rings with triazoles.[15] Moreover, the 1,2,3-triazole moiety is also a suitable mimic of a

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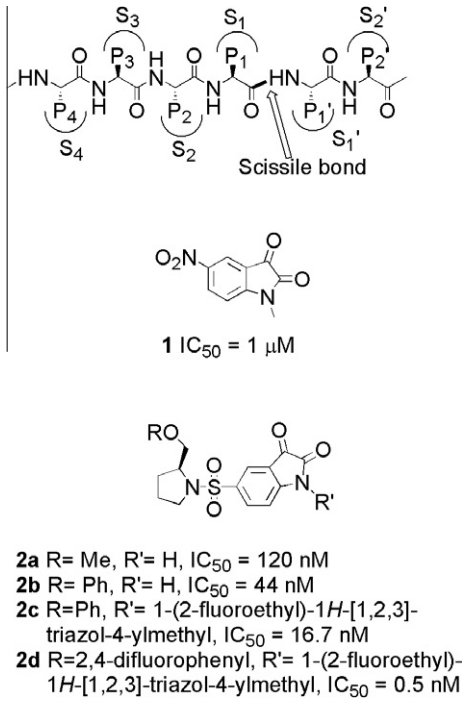


Figure 1. The sequence of caspase-3 substrates and examples of known caspase-3 inhibitors.

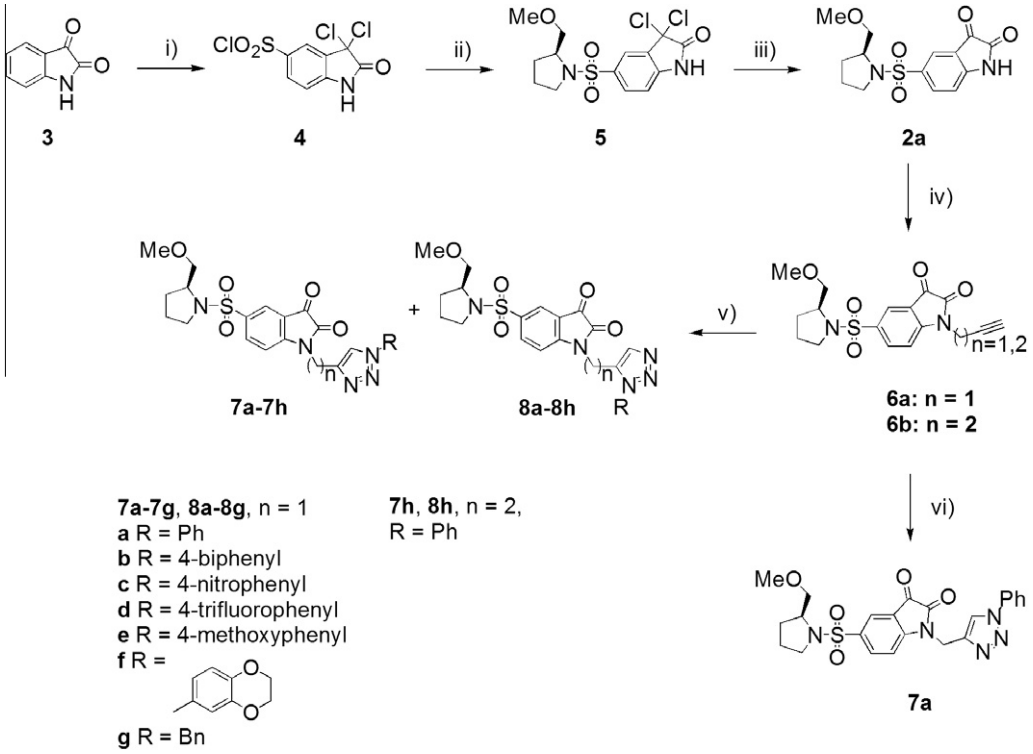
peptide bond due to topological and electronic similarities.[16] This feature becomes especially attractive when developing inhibitors of different proteases.[17] Ng et al. reported in 2008 several 1,4-disubstituted 1,2,3-triazoles as caspase-3 and -7 inhibitors with activity in the low micromolar range.[18] Recently Aboagye and co-workers have used this reaction to prepare ^{18}F-labeled isatin derivatives for positron emission tomography studies of apoptosis, and two triazole derivatives showed high inhibition potency with IC_{50}-values of 16.7 and 0.5 nM, respectively (Fig. 1, compounds **2c** and **2d**).[19] Hence, we became interested in preparing disubstituted 1,2,3-triazole derivatives of **2a** as potentially more potent inhibitors against capsase-3.

Table 1
Inhibition data expressed as IC_{50}-values

Compd	Caspase-3 inhibition IC_{50},[a] nM
7a	21
7b	23
7c	21
7d	18
7e	44
7f	17
7g	30
7h	243
8a	103
8b	136
8c	213
8d	267
8e	70
8f	136
8g	9
8h	207

[a] Values are means of two independent experiments, each carried out in duplicates.

Synthesis of the alkynes was achieved using the literature procedures with minor modifications.[5b,20] In brief, compound **3** was treated with chlorosulfonic acid to yield the *gem*-dichloro derivative **4**. Reacting **4** with (*S*)-2-(methoxymethyl)pyrrolidine followed by acidic hydrolysis of the *gem*-dichloro moiety afforded 5-(*S*)-(2-methoxymethyl)pyrrolidinylsulfonyl isatin **2a**. Alkylation of the amide nitrogen using 3-bromopropyne or 4-bromobutyne in the



Scheme 1. Synthesis of triazole compounds. Reagents and conditions: (i) $ClSO_3H$, 10 equiv (86%, crude); (ii) (*S*)-2-methoxylmethyl-pyrrolidine; (iii) AcOH–H_2O 1:1 (46% yield over two steps); (iv) Cs_2CO_3, acetonitrile, $HCC(CH_2)_nBr$ (**6a**, *n* = 1, 76%; **6b**, n = 2, 60%); (v) R-N_3, MeOH, Δ; (vi) Ph-N_3, $CuSO_4$, sodium ascorbate, MeOH, rt.

presence of Cs_2CO_3 yielded alkynes **6a** and **6b** in 30% and 24% overall yields, respectively. Azides were prepared using a literature procedure.[21] The disubstituted triazoles **7a–7g** or **8a–8g** were obtained either by thermal[9a] or by copper(I)-catalyzed[9b,c] cycloaddition reactions (Scheme 1). The 1,4- and 1,5-disubstituted triazole isomers obtained from the thermal reaction were separated by chromatography. The products were characterized by spectral data (see Supplementary data).

The enzyme inhibition assay was carried out as previously reported with fluorogenic substrate Ac-DEVD-AFC[22] and the IC_{50}-values of all synthesized triazoles were determined and compiled in Table 1.

Compared to lead compound **2a** (IC_{50} = 120 nM)[5b] the 1,4-disubstituted triazole **7a** was approximately 5-fold more potent (IC_{50} = 21 nM). Changing the phenyl group to a bulkier biphenyl group had no effect on the potency (**7b**, IC_{50} = 23 nM). Adding an electron-withdrawing nitro (**7c**, IC_{50} = 21 nM) or trifluoromethyl (**7d**, IC_{50} = 18 nM) group in the *para*-position of the phenyl C-ring gave little effect on the potency, either. However, introducing an electron-donating methoxy group in the *para*-position of the C-ring decreased the potency 2-fold (**7e**, IC_{50} = 44 nM). Replacing the phenyl C-ring with a benzodioxin moiety as in **7f**, the inhibition potency (IC_{50} = 17 nM) was observed at the same level as **7a** (IC_{50} = 21 nM). The 1,5-disubstituted 1,2,3-triazoles **8a–8f** were all less potent than the corresponding 1,4-regioisomers **7a–7f** (Table 1). Interestingly, the 1,5-disubstituted triazole **8g** derived from benzyl azide exhibited more than 2-fold higher potency (IC_{50} = 9 nM) than **7a**. However, the 1,4-regioisomer **7g** (IC_{50} = 30 nM) was less potent than **8g**. Apparently, the substitution pattern in the 1,2,3-triazole ring affects the binding affinity towards caspase-3. The potency seems to be higher for 1,4-disubstiuted 1,2,3-triazoles prepared from phenyl azides, but for the 1,5-disubstituted 1,2,3-triazole **8g** prepared from benzyl azide a 3-fold higher potency than the 1,4-isomer was observed. Moreover, for compounds **7h** and **8h** with two methylene groups between the triazole ring and the isatin moiety, 10- to 12-fold lower potency as the compound **7a** was observed. In addition, the potencies were lower than most other new inhibitors reported herein (Table 1). These results indicate that the 1,2,3-triazole ring provides additional affinity to the enzyme when it is connected to the isatin nitrogen via one methylene group, not with two.

For the most potent triazoles **7f** and **8g**, the inhibition kinetics was measured (Fig. 2).[23] These compounds were determined to be competitive inhibitors, the same mechanism as that has been observed for other isatin inhibitors, including **2a**.[5b] Thus the K_i-values for **7f** and **8g** were determined to be 14.5 ± 0.4 nM and 10.5 ± 3.4 nM respectively, as calculated from inhibition kinetics data.[24]

Various substitutions at P_1' position have been explored for several peptide based caspase inhibitors,[25] and structure–activity relationship studies on isatinsulfonamide compounds have been carried out by several groups also.[5b,7a] Substitution groups containing aromatic rings were found to be preferable in many of these explorations. Our work has shown that introduction of a 1,2,3-triazole ring at the nitrogen atom of the isatin moiety results in significantly enhanced inhibitory activity. Aulabaugh et al. reported that sulfonyl isatin inhibitors showed a different mode of interaction with caspase-3 compared to peptide based inhibitors.[26] In the report on isoquinolin-1,3,4-trione inhibitors by Du et al., it was proposed that caspase-3 was inactivated by reactive oxygen species generated by the inhibitor.[8d] All these results indicate that the inhibition of caspase-3 by isatin sulfonamide derivatives or analogs may involve different modes or mechanisms. Our results would provide the basis for further development of other inhibitors against caspase-3, as well as experimental basis for further molecular modeling studies. These efforts will be reported in due time.

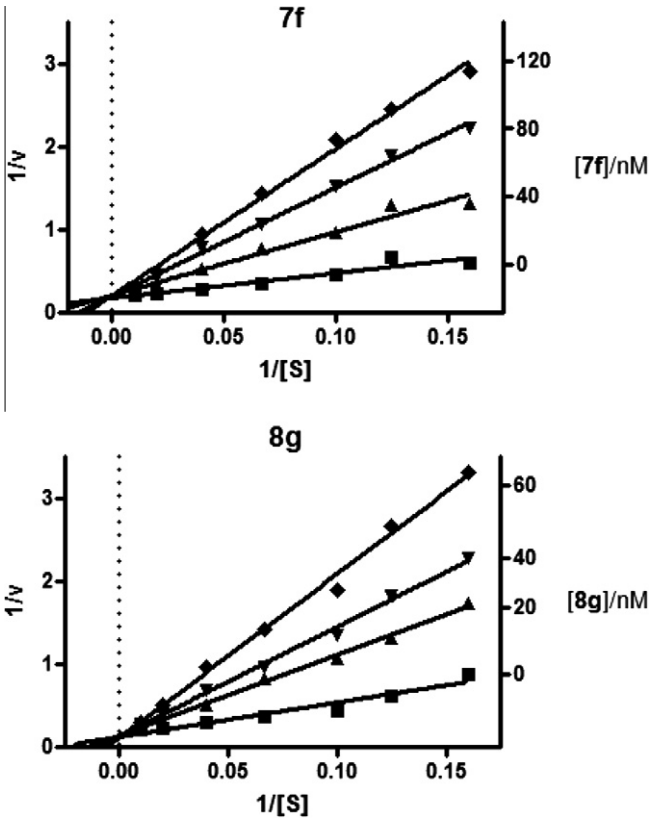


Figure 2. Lineweaver–Burk plot of triazoles **7f** and **8g**.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.01.110.

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22. Enzyme assay was performed at 25 °C on a PerkinElmer VICTOR plate reader with the excitation and emission wavelengths at 405 and 530 nm, respectively. The assay were run in 200 μL volumes and contained the following: 25 mM K^+ HEPES (pH 7.5), 50 mM KCl, 0.1% CHAPS, 5 mM β-mercaptoethanol, 1 mM EDTA, 10% sucrose and 10 μM Ac-DEVD-AFC. The final concentration of caspase-3 was 0.25 nM (calculated as heterodimer), and the final concentration of DMSO was 5%. Compounds tested were diluted in DMSO prior to the assay.
23. The inhibition kinetics for compounds **7f** and **8g** against caspase-3 were determined using buffer conditions described above. The concentration of Ac-DEVD-AFC was varied from 6.25 to 100 μM, and the concentration of the inhibitor was varied from 0 to 120 nM and from 0 to 60 nM for compounds **7f** and **8g**, respectively. The maximum slope of the curve of AFC fluorescence was used as the initial rate.
24. K_m (without inhibitor) and K'_m (with inhibitor) values were calculated by fitting the raw v versus $[S]$ data into $v = V_{max}[S]/(K_m + S)$, using BioDataFit (http://www.changbioscience.com/stat/ec50.html). K_i-values were calculated using the equation for competitive inhibitors, $K'_m = K_m(1 + [I]/K_i)$. We are grateful to an anonymous referee for helpful suggestions on how to perform these calculations.
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