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Novel Benzthiodiazepinones as Antiherpetic Agents: SAR Improvement of Therapeutic Index by Alterations of the Seven-Membered Ring

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Abstract—A series of novel benzthiodiazepinones was studied as antiherpetic agents. Significant improvements in potency and therapeutic index in a viral replication assay were realized over the starting molecule. The role of stereospecific substitution on the diazepine ring and optimal nitrogen substitution were investigated.

PD 146626, **1** (Table 1), was identified from a high-throughput screening in a cellular assay as an antiherpetic agent. Extensive studies on the mechanism of action suggest the compound targets a novel cellular function that is critical for the expression of HSV-1 immediate early genes but not host genes.[1] PD 146626 was not optimal for lead development as it possessed a low therapeutic index, was found to be extensively metabolized in vivo, and was not active orally in a murine herpetic model. This communication describes the structure–activity relationships (SARs) developed to address the issue of separation of viral replication inhibition from cellular toxicity, leading to a 3-log improvement in therapeutic index (TI).

The general route to the synthesis of PD 146626 and related analogues is given in Scheme 1. Synthesis of oxygen (**2**) and sulfur substitutions in the seven-membered ring has been published previously.[2] Initial investigations on the nature of the heteroatom in the seven-membered ring led us to focus on substituted nitrogen in this position (Table 1). New methodology was needed to improve yields and synthetic flexibility for these analogues. Thus, direct introduction of nitrogen to the 3-position of the benzothiophene was efficiently accomplished by oxidation of the sulfur prior to nucleophilic attack with the diamine. Reduction of the sulfoxide (trimethylsilyliodide generated in situ) allowed base catalyzed cyclization of the seven-membered ring analogues **3**.

Selective alkylation of the two nitrogens was accomplished by first derivatizing the amide nitrogen with a silyl protecting group, followed by anion formation on the second nitrogen and reaction with the appropriate electrophile. Deprotection then afforded target compounds **4**.

Substitution on the carbons of the seven-membered ring required synthesis of the mono-protected diamine from the appropriate amino acid (Scheme 2). Either BOC or CBZ could be used as the first protecting group, and then the alternative protecting group selected if the alternative stereochemistry was desired. These diamines were then used in the synthetic Scheme with appropriate deprotection prior to cyclization.

The viral replication inhibition assay used for these studies has been described in detail elsewhere.[1] Briefly, HSV-1-lacZ replication inhibition assays were done in triplicate and EC_{50}'s determined from logarithmic plots

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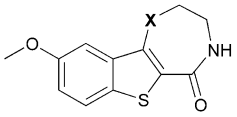
Table 1. Nature of heteroatom in seven-membered ring

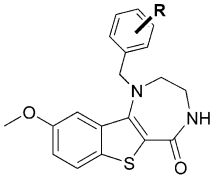


Example	X	EC_{50} (μM)	TC_{50} (μM)	TI
1 (PD146626)	S	0.125	7.6	60
5	S(O)	2.55	25.8	10
2	O	0.21	13.1	60
3a	NH	0.13	4.5	35
4a	*N*-CH_3	0.44	12.5	28
4b	*N*-Allyl	0.001	1.75	1750
4c	*N*-Benzyl	0.005	8.0	1600

of compound concentration versus mean percent inhibition. Toxicity was determined separately in uninfected cells treated with drug and assayed for their ability to generate XTT formazan. Table 1 illustrates the improvement seen in TI by changing the heteroatom of the seven-membered ring from sulfur to substituted nitrogen. This 2-log improvement was seen with most small alkyl substitutions (propyl, allyl, *i*-butyl, and *i*-pentyl), and with benzyl.

We next investigated the effect of substitution on the benzyl ring (Table 2). The highest TI values were observed with *meta*-substitutions. Compound **4k** was the most potent compound of this series, with a subnanamolar EC_{50} against viral replication. Since the cellular toxicity remained above micromolar this compound had the greatest therapeutic index as well.

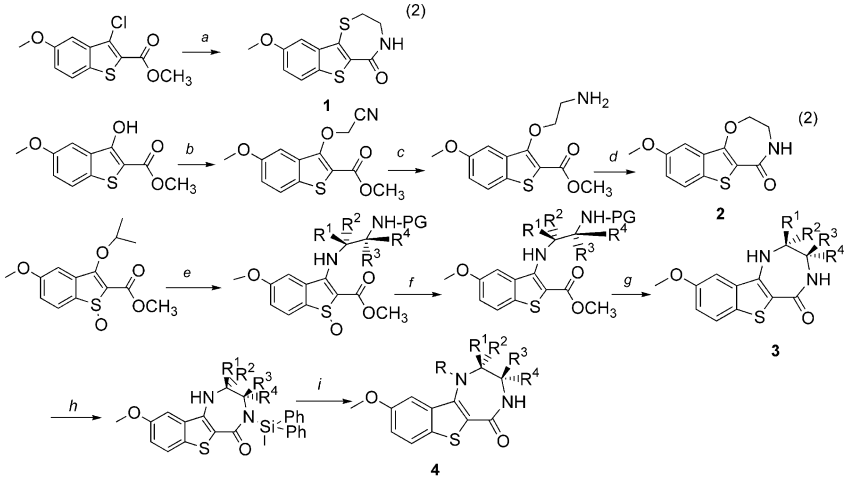
Table 2. Benzyl ring substitution



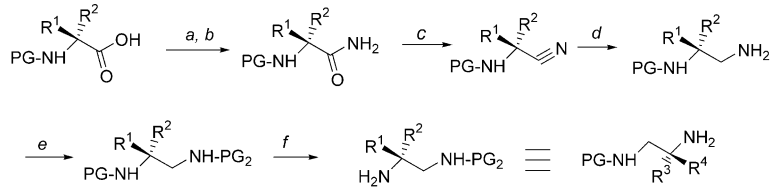
Compd	R	EC_{50} (μM)	TC_{50} (μM)	TI
4c	H	0.005	8.0	1600
4d	*p*-Cl	0.040	12.0	300
4e	*p*-CH_3	0.030	11.0	360
4f	*p*-OCH_3	0.012	10.0	830
4g	*p*-NO_2	0.050	25.0	500
4h	*m*-Cl	0.005	20.0	4000
4i	*m*-CH_3	0.005	12.0	2400
4j	*m*-NO_2	0.004	25.0	6250
4k	*m*-OCH_3	0.0004	10	25,000
4l	*o*-Cl	0.008	12.0	1500
4m	*o*-CH_3	0.021	25.0	1200

Finally, we looked at substitution on carbons of the seven-membered ring (Table 3). This was done to hinder sites of metabolism seen with some of these analogues (to be discussed in a separate publication), as well as to investigate whether there was an influence on TI. In the case of stereoisomers adjacent to the amide nitrogen, the only significant improvement was seen with the (*S*) analogue **3h**. When the stereocenter was adjacent to the other nitrogen there was a preference for the *S*-configuration (**3j** and **3l**).

Combination of carbon substitution and nitrogen substitution led to compounds less potent than the original

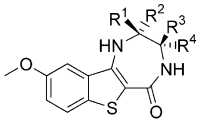


Scheme 1. Synthesis of target compounds. (a) 2-Aminoethanethiol/DBU; (b) $ClCH_2CN$/KO-*t*-Bu/DMSO; (c) $BH_3.Me_2S$; (d) NaOMe; (e) mono-protected 1,2-diamino-ethane where protecting group = BOC or CBZ/base (Scheme 2); (f) TMS-Cl/NaI; (g) deprotection/NaOMe; (h) base/Cl-Si-$(Ph)_2Me$; (i) base/R-X/deprotection.



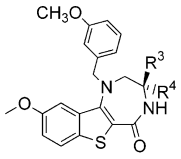
Scheme 2. Synthesis of diamines. (a) CDI; (b) NH_3 or NH_4HCO_3; (c) cyanuric chloride; (d) H_2/Raney nickel; (e) alternative protecting group; (f) deprotection PG. Intermediates and final diamines checked for chiral integrity by chiral HPLC.

Table 3. Carbon- and nitrogen-substitutions on seven-membered ring



Compd	R^1	R^2	R^3	R^4	EC_{50} (μM)	TC_{50} (μM)	TI
3a	H	H	H	H	0.13	4.5	35
3b (S)	H	H	H	CH_3	0.17	6.0	35
3c (R)	H	H	CH_3	H	0.02	1.0	50
3d	H	H	H	Bz	0.28	1.9	7
3e	H	H	Bz	H	0.34	10.0	29
3f	H	H	H	*i*Pr	0.06	4.0	67
3g	H	H	*i*Pr	H	0.09	3.3	37
3h	H	H	H	*i*Bu	0.02	2.0	100
3i	H	H	*i*Bu	H	0.39	7.0	18
3j (S)	CH_3	H	H	H	0.05	2.0	40
3k (R)	H	CH_3	H	H	0.13	7.0	54
3l	Bz	H	H	H	0.01	1.1	110
3m	H	Bz	H	H	0.09	1.4	16

Table 4.



Compd	R^3	R^4	EC_{50} (μM)	TC_{50} (μM)	TI
4k	H	H	0.0004	10.0	25,000
4n	H	CH_3	0.02	11.0	550
4o	CH_3	H	0.03	3.4	170
4p	H	Bz	0.09	6.0	67
4q	Bz	H	0.45	4.0	9

nitrogen-only substituted analogue (Table 4). Thus the compounds **4n** through **4q** showed no advantage in TI over the analogous **4k**. In each case the (*S*) isomer was somewhat better than the corresponding (*R*) isomer in terms of TI. The analogues with R^1 or R^2 substituents could not be alkylated selectively on the desired nitrogen.

The results of these studies show the dramatic improvement in TI achieved by alteration of the seven-membered ring of compound **1**. The antiviral effect seen with these compounds is not due to direct interaction with the virus itself, rather to an interference with cellular machinery utilized by the virus in its replication. Thus the importance of a large therapeutic index is critical. The exquisite selectivity of these compounds for halting the virus without harming the cell as seen with compound **4k** offers an alternative therapeutic approach to the treatment of herpetic infections. Unfortunately metabolism issues with selected analogues precluded in vivo activity, and the SAR to address these problems is the subject of a forthcoming communication.

Acknowledgements

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References and Notes

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