Bioorganic & Medicinal Chemistry Letters 19 (2009) 5652–5656

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Non-nucleoside inhibitors of HCV polymerase NS5B. Part 4: Structure-based design, synthesis, and biological evaluation of benzo[d]isothiazole-1,1-dioxides

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ARTICLE INFO

Article history:
Received 7 June 2009
Revised 4 August 2009
Accepted 5 August 2009
Available online 8 August 2009

Keywords:
HCV
Polymerase
NS5B
Diels–Alder
DMPK

ABSTRACT

A series of benzo[d]isothiazole-1,1-dioxides were designed and evaluated as inhibitors of HCV polymerase NS5B. Structure-based design led to the incorporation of a high affinity methyl sulfonamide group. Structure–activity relationship (SAR) studies of this series revealed analogues with submicromolar potencies in the HCV replicon assay and moderate pharmacokinetic properties. SAR studies combined with structure based drug design focused on the sulfonamide region led to a novel and potent cyclic analogue.

Hepatitis C virus (HCV) is the major causative agent of blood-borne non-A, non-B hepatitis, and it is estimated to infect over 170 million people worldwide.[1] Chronic HCV infections have been associated with liver fibrosis, liver cirrhosis, heptacellular carcinoma, and other forms of liver dysfunction.[2,3] Given the widespread impact of this disease, there is a substantial medical need for new anti-HCV agents to complement current therapies.[4] In this article we report on our efforts to develop orally bioavailable, non-nucleoside inhibitors of the HCV RNA-dependent RNA polymerase.

In the previous Letter, we described the synthesis and SAR studies of a new class of benzothiazine-substituted tetramic acids as non-nucleoside inhibitors of HCV NS5B polymerase.[3] While these compounds (**1**; Fig. 1) were found to be potent inhibitors of HCV polymerase both in enzymatic and replicon assays, they displayed low oral bioavailability and high clearance (Fig. 1). These findings were attributed to the metabolic instability of the benzothiazine ring, which also intervened in an undesired intramolecular hydrogen bond that contributed to their in poor permeability.

Herein, we report a novel series of inhibitors of HCV polymerase in which the benzothiazine fragment is replaced by a benzo[d]isothiazole-1,1-dioxide.[5,6] Our initial SAR studies on the tetramic acid portion, which were conducted in parallel with the studies on the benzothiazine-substituted tetramic acids, demonstrated that the optimal substitutions on the pyrrolidinone ring are *tert*-butyl and *p*-fluorobenzyl groups.[7] An early lead compound (**2**, Fig. 1) exhibited reasonable potency against the genotype 1 NS5B enzyme (IC_{50} = 0.069 μM) and HCV subgenomic replicon (EC_{50} = 0.648 μM). Compound **2** provided a good starting point for further optimization. Our early studies showed that the addition of a methyl sulfonamide group to the 7-position of the benzothiazine ring dramatically improved the potency in that series.[3] Installation of the methyl sulfonamide group at the 7-position of the benzo[d]isothiazole-1,1-dioxides (compound **3**, Fig. 1) provided an excellent boost in enzyme activity (IC_{50} = 0.005 μM) but showed poor replicon inhibition potency (EC_{50} = 4.924 μM).

An atom extension of the methyl sulfonamide group (compound **5**, Fig. 1) retained good potency against the enzyme (IC_{50} = 0.003 μM), and showed dramatically improved replicon potency (EC_{50} = 0.1 μM). To test the optimal substitution on the

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doi:10.1016/j.bmcl.2009.08.022

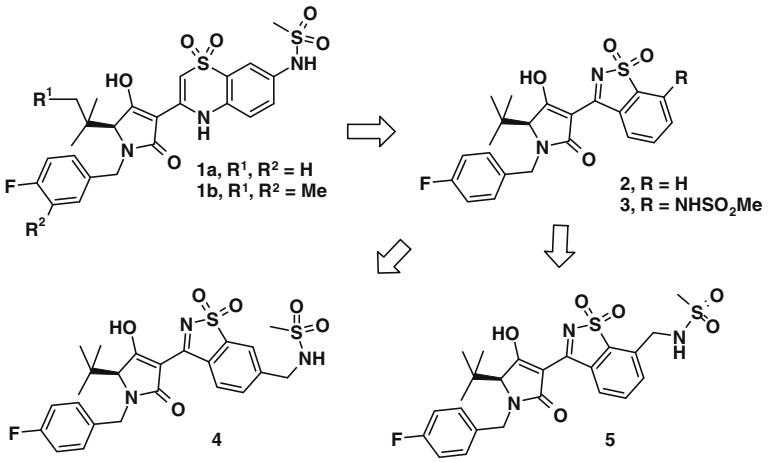


Figure 1. Previous lead compounds **1a**, **1b** and scaffold hopping strategy.

benzo[d]isothiazole ring, the extended methyl sulfonamide group was also installed at the 6-position (compound **4**, Fig. 1). Substitution at this position was tolerated but did not provide a similar boost in replicon potency (IC_{50} = 0.043 μM, EC_{50} = 3.844 μM).

X-ray analysis of compounds **1b** and **5** demonstrates the optimal location of the methyl sulfonamide group.[8] The inclusion of a CH_2 spacer between the benzo[d]isothiazole-1,1-dioxide ring an the sulfonamide moiety allows the functionality to appropriately access the far right side of the palm binding pocket. As shown in Figure 2, the sulfonamide oxygen atoms interact directly with Asn291 and with Ser288 via a conserved water molecule. In contrast to the benzothiazine series, the sulfonamide NH does not interact strongly with Asp318. Thus, we decided to explore sulfonamide analogues were this NH was substituted or replaced by C in hopes of improving cell permeation and replicon inhibition potency.[4]

A series of benzo[d]isothiazole-1,1-dioxides were evaluated for in vitro potency against the NS5B enzyme[9] and in an HCV subgenomic replicon (GT-1b)[10,11] cell based assay in the presence of 5% fetal bovine serum (FBS). The effect of protein binding on the inhibitors potency was evaluated in the HCV subgenomic replicon cell based assay where 40% human serum (HS) is added. The results of these assays are shown in Table 1. Replacement of the sulfonamide NH by a methylene linker had a mild detrimental effect in replicon potency (compare **5** and **7**). These compounds were likely protein bound as the antiviral potency was reduced by addition of HS. In order to increase the polarity of the molecules, the sulfonamide or sulfone group was exchanged by a sulfamide. Relative to **5**, sulfamides **10**, **11** and **12** showed similar levels of replicon potency and as predicted the serum shift was modestly improved. Interestingly, the introduction of a single methyl group on the NH sulfamide was very well tolerated (compound **12**). This modification also reduced serum shift so it was incorporated into our initial sulfonamide, leading to compounds **13**[6] and **14**. Cyclization of the methyl sulfonamide onto the nitrogen (compound **15**) did not provide an improvement in potency.

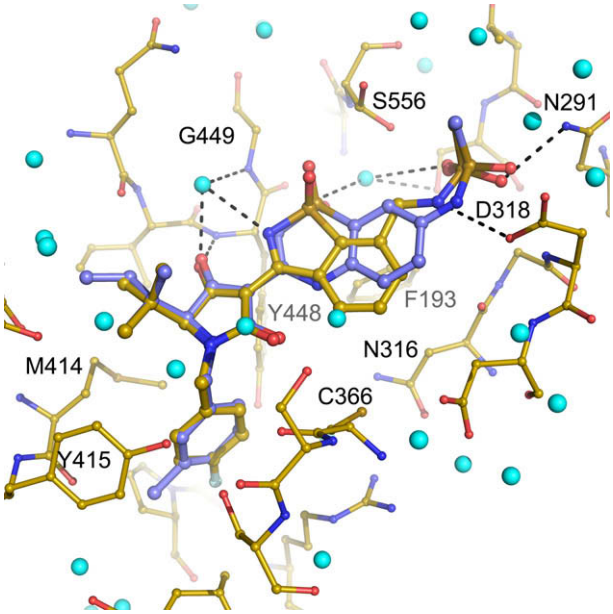


Figure 2. X-ray crystal structure overlap of compounds **1b** and **5** bound to the palm site of CΔ21 NS5B polymerase.

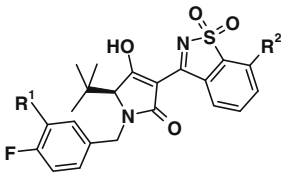
In vivo PK data were obtained for our most potent compounds (**11**, **6** and **14**, Table 2) following administration of a single oral dose to rats or monkeys. Sulfonamide **11** displayed poor exposure in rats due to low oral bioavailability and rapid clearance. This data was supported by our in vitro data: poor Caco-2 permeability and low rat liver microsomal stability. Compound **6** showed low clearance in vivo and modest levels of exposure and oral bioavailability in rat and monkey. The N-methylated sulfonamide **14** also showed encouraging levels of exposure. In general, we observed improved oral exposure in analogues bearing the 3-methyl-4-fluorobenzyl lipophilic group.

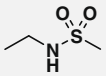
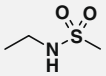
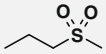
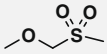
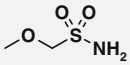
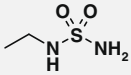
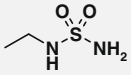
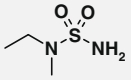
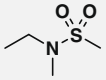
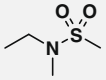
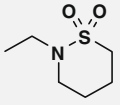
Compound **6** showed promising levels of exposure but poor replicon activity in presence of 40% human serum. It is estimated that the minimum efficacious concentration of an HCV inhibitor should be greater than the replicon EC_{90} in presence of 40% HS.[12] In order to enhance the potency of our benzo[d]isothiazole-1,1-dioxides series, we turned our attention to the binding conformation and interactions of sulfonamide **5**.

Molecular orbital calculations with solvent corrections,[13] suggested the bound sulfonamide side chain conformation of **5** is ca. 2.1 kcal/mol less favored relative to the low energy conformation containing an internal hydrogen bond to the SO_2 of the benzo[d]isothiazole ring. Interestingly, the small molecule X-ray structure of *N*-benzyl methane-sulfonamide (CSD entry KOYVID0001) displays a very similar overall conformation to the analogous side chain portion of **5** in our bound X-ray structure (Fig. 2), which further suggests the system is not highly strained.

We hypothesized that added potency could be gained by locking the sulfonamide group in the X-ray bound conformation by connecting the sulfonamide nitrogen and the C6 position of the

Table 1
Enzyme and replicon data for benzo[d]isothiazole 1,1-dioxide derivatives **5**–**15**



Compds	R^1	R^2	NS5B IC_{50}[a] (μM)	Replicon EC_{50} (μM) [a,b]	
				5% FBS	40% HS (FS) [c]
5	H		0.003	0.1	3.728 (37.3)
6	Me		0.004	0.082	2.573 (31.3)
7	H		0.004	0.282	4.796 (17)
8	H		0.009	0.235	4.662 (19.8)
9	H		0.006	0.228	2.896 (12.7)
10	H		0.003	0.105	1.299 (12.4)
11	Me		0.003	0.076	1.166 (15.3)
12	H		0.001	0.272	1.272 (5.9)
13	Cl		0.01	0.108	3.038 (28.1)
14	Me		0.005	0.129	1.067 (8.27)
15	H		0.012	0.202	4.66 (23.1)

[a] IC_{50} and EC_{50} values for inhibition calculated from two independent determinations.
[b] CC_{50} > 50 M for all analogues.
[c] IC_{50} fold-shift values (40% human serum/5% FBS) shown in parentheses.

benzo[d]isothiazole ring with an ethylene linker (Fig. 3). Since substitution at these positions was tolerated (recall compound **4**), we embarked on the synthesis of fused ring analogue **16**.

The synthesis of fused ring analogue **16** began with the construction of the benzo[d]isothiazole-1,1-dioxide core. This tricyclic system was readily assembled by a Diels–Alder reaction of diene **19** and known dienophile **20** (Scheme 1).[14] Olefination of ketone **17** followed by standard manipulations led to aldehyde **18**. Condensation of α,β-unsaturated aldehyde **18** with dimethyl amine provided diene **19**, which upon treatment with a stoichiometric amount of dienophile **20** resulted in the formation of the desired benzo[d]isothiazole 1,1-dioxide core **21** as well as compound **22**.

The Diels–Alder cycloaddition of **19** and **20** is followed by a double elimination and aromatization (Scheme 2, path a). Interestingly, cycloadduct **22** was also isolated. This compound was possibly obtained by a Diels–Alder reaction between rearranged diene **26** and dienophile **20** (Scheme 2, path b). A postulated mechanism for the formation of diene **26** involves an unusual conrotatory ring closure of diene **19** followed by an acid promoted double bond isomerization and a conrotatory ring opening. The stereospecificity of the above reactions are in agreement with the optimal orbital overlap of dienes **19** and **26** (HOMO) with unsaturated amide **20** (LUMO).

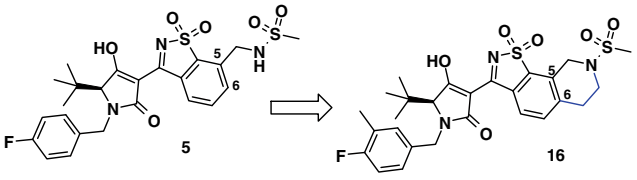
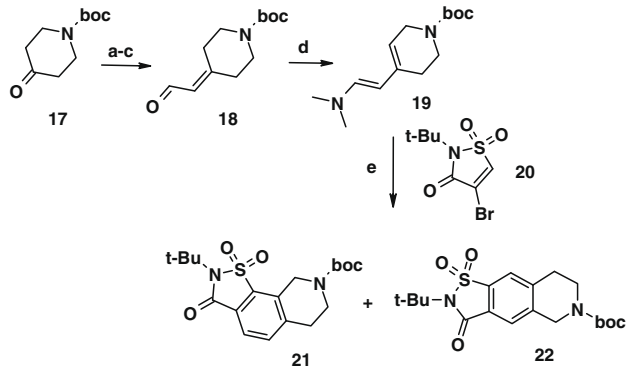


Figure 3. Rational design of constrained analogue **16** from initial lead **5**.

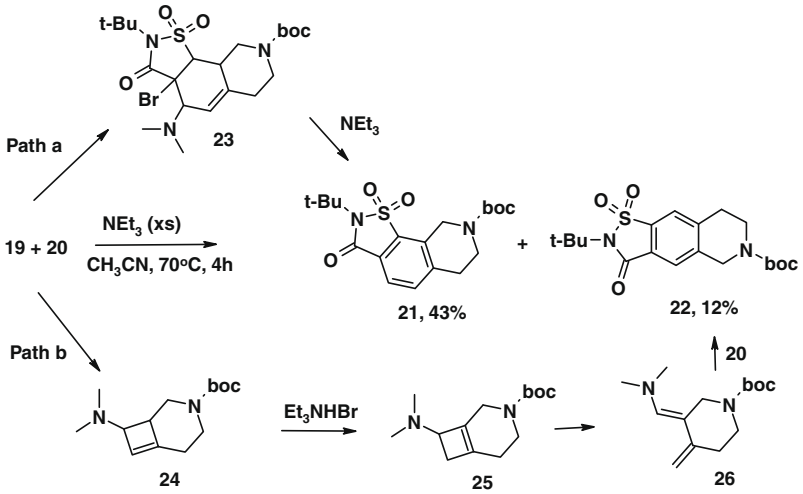


Scheme 1. Reagents and conditions: (a) $EtO_2CCH_2PO(OMe)_2$, MeONa, THF, 0 °C; (b) DIBAL, THF, −78 °C; (c) MnO_2, DCM, 25 °C, 56% (three steps); (d) $NHMe_2$, toluene, 90 °C, 100%; (e) TEA, MeCN, 70 °C, **21** (43%), **22** (12%).

Table 2
Mean pharmacokinetic parameter values for selected compounds **11**, **6** and **14** following single dose administration to rats and monkeys

Compds	Caco-2 A to B (ER)[a]	Species	LM[b] (μL/min/mg)	AUC (h ng/mL)	$T_{1/2}$ (h)	Cl (mL/kg/min)	*F*(%)
11	0.29 (6.7)	Rat[c]	239	102	4.7	48	8.2
6	0.09 (31)	Rat[c]	431	2750	4.18	8.4	17
		Monkey[d]	23	1230	1.75	10	11
14	0.21 (4.3)	Rat[c]	939	582	3.37	34.4	24

[a] Caco-2 assay run for 21 days. ER = (B-to-A)/(A-to-B).
[b] LM: in vitro liver microsomal data.
[c] Rat PK study: dose iv = 2 mg/Kg, dose po = 5 mg/Kg.
[d] Cynomolgus monkey PK study: dose iv = 2 mg/Kg, dose po = 5 mg/Kg.



Scheme 2. Proposed mechanism for the formation of tetrahydroisoquinolines **21** and **22**.

The Diels–Alder adduct **21** was elaborated to the desired benzo[d]isothiazole-1,1-dioxide **27** by first switching the *t*-butoxycarbonyl group to the desired methyl sulfonamide (Scheme 3). Amide deprotection gave **27**, which was then activated to provide imidate **28**. Final coupling of this imidate with the sodium salt derived from tetramic acid **29** led to the desired piperidine fused benzo[d]isothiazole-1,1-dioxide **16**.

Compound **16** displayed NS5B inhibition activity of 0.005 μM. We speculate that less than ideal interactions with D318 may be partially offsetting the anticipated benefit of restricting the sulfonamide side chain conformation. Regardless, for this series fused analogue **16** exhibited the most potent activity (0.026 μM) in our replicon assay. However, this compound showed a high serum shift (4.938 μM). In order to confirm our initial hypothesis, compound **16** was soaked into CΔ21 NS5B for X-ray analysis.[15] The observed binding conformation of cyclic sulfonamide **16** was close to the predicted ground state conformation and the sulfonamide group position was almost identical to the corresponding acyclic analogue (Fig. 4).

In conclusion, we have reported on the structure activity evaluation of a novel benzo[d]isothiazole-1,1-dioxides series of non-nucleoside inhibitors of HCV NS5B polymerase. The incorporation of the extended methyl sulfonamide group led to potent inhibitors in our standard and serum shift replicon assays. The most relevant compounds in this series contained a 3-methyl-4-fluorobenzyl group. Pharmacokinetic data obtained from selected compounds indicated only moderate levels of exposure. Structure-based design and molecular modeling were employed to guide the discovery and optimization of the methyl sulfonamide group. As a result, the novel cyclic sulfonamide analogue **16** was shown to be the most potent analogue in our replicon assay. The novel synthesis of compound **16** also highlights an efficient DA approach for the synthesis of complex polyheterocyclic systems. Although compound **16** exhibited good potency in both the biochemical NS5B and the replicon cell-based assays, the reduced potency in presence of 40% HS limited the development of this compound as drug candidate.

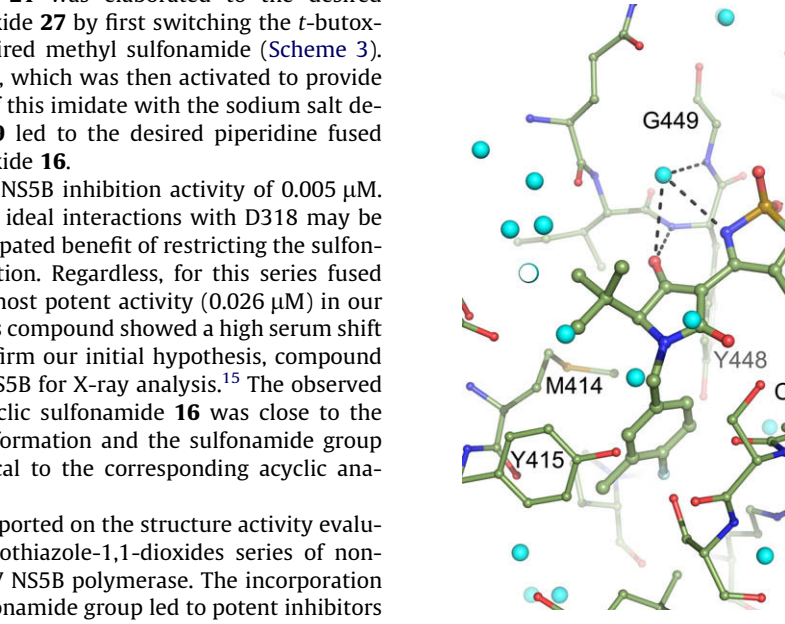
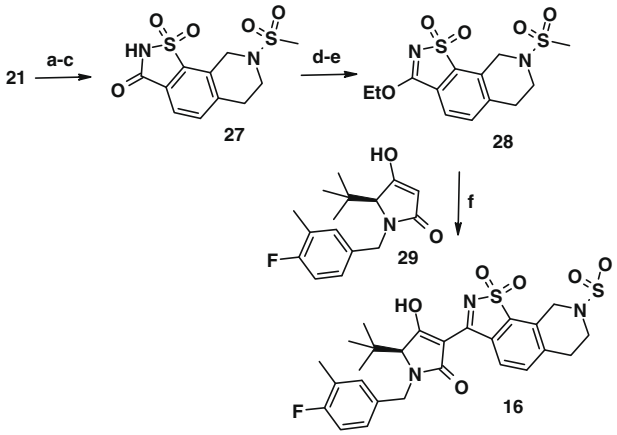


Figure 4. X-ray crystal structure of compound **16** bound to the palm site of CΔ21 NS5B polymerase.



Scheme 3. Reagents and conditions: (a) TFA, DCM, 25 °C, 100%; (b) MsCl, Py, DCM, 25 °C; (c) BCl_3, DCM, 25 °C; (d) $SOCl_2$, DMF; (e) EtOH, 81% (four steps); (f) NaH, THF, 45%.

Acknowledgments

The authors thank Drs. Eric Sjogren, Hans Maag, and John Josey for their support and helpful discussion during the course of this work.

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11. A representative number of benzo[d]isothiazole-1,1-dioxides analogues (**5**, **6**, **11**, **14**) were also evaluated in a GT-1a replicon assay. This class of compounds had a minimal loss in inhibitory activity of two to eightfold relative to our standard GT-1b replicon assay.
12. (a) Kneteman, N. M.; Howe, A. Y. M.; Gao, T.; Lewis, J.; Pevear, D.; Lund, G.; Douglas, D.; Mercer, D. F.; Tyrrell, D. L. J.; Immermann, F.; Chaudhary, I.; Speth, J.; Villano, S. A.; O'Connell, J.; Collett, M. *Hepatology* **2009**, *49*, 745; (b) Chandra, P.; Raible, D.; Harper, D.; Speth, J.; Villano, S.; Bichier, G.; Presented at the Digestive Disease Week, Los Angeles, CA, May 21–24, 2006.
13. GAMESS Version 24 March 2007 (R6). Schmidt, M. W.; Baldridge, K. K.; Boatz, J. A.; Elbert, S. T.; Gordon, M. S.; Jensen, J. J.; Koseki, S.; Matsunaga, N.; Nguyen, K. A.; Su, S.; Windus, T. L.; Dupuis, M.; Montgomery, J. A. *J. Comput. Chem.* **1993**, *14*, 1347. All geometries and energetics were computed at the 6-311++G(d,p) level of theory. Aqueous solvent corrections were applied via AMSOL5.0 with the SM2.1 model. Cramer, C. J.; Hawkins, G. D.; Lynch, G. C.; Giesen, D. J.; Rossi, I.; Storer, J. W.; Truhlar, D. G.; Liotard, D. A. (1995) AMSOL version 5.0, QCPE Program 606.
14. Burri, K. F. *Helv. Chim. Acta* **1989**, *72*, 1416.
15. Atomic coordinates of the structure **16** bound to CΔ21 NS5B polymerase were deposited with the RCSB Protein Data Bank (PDB) under the accession code **3H5S**. The crystal structure was also determined following previously described procedures as noted in Ref.[8]