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Discovery of thiophene-2-carboxylic acids as potent inhibitors of HCV NS5B polymerase and HCV subgenomic RNA replication. Part 1: Sulfonamides

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Abstract—The discovery of a novel class of HCV NS5B polymerase inhibitors, 3-arylsulfonylamino-5-phenyl-thiophene-2-carboxylic acids is described. SAR studies have yielded several potent inhibitors of HCV polymerase as well as of HCV subgenomic RNA replication in Huh-7 cells.

In 1989, Hepatitis C virus (HCV) was identified as the pathogen responsible for the majority of the cases of non-A non-B hepatitis. This positive strand RNA virus of the Flaviviridae family chronically infects approximately 3% of the world population or 175 million individuals causing severe liver malfunction and morbidity. To date, there is no prophylactic vaccine and the recommended treatments, interferon α2b or a pegylated conjugate (PEG-Intron®) alone or in combination with ribavirin provide sustained viral suppression only in a fraction of the cases. Furthermore, severe side effects often limit compliance to the therapy. As a result, intensive efforts have been focused on the development of an efficacious and well-tolerated low molecular weight anti-HCV agent. The major obstacle towards this goal has been the lack of a cell culture system and/or a convenient small animal model to propagate the virus.[1] A major breakthrough was recently made by Bartenschlager and co-workers who engineered a Huh-7 human hepatoma cell line that supports replication of subgenomic HCV RNA replicons (replicon cell line).[2] These replicons encode all the non-structural proteins (NS3, NS4A, NS4B, NS5A and NS5B) of HCV and thus provide a useful surrogate antiviral model for evaluating compounds which show activity against any of these functional targets of HCV.

The HCV NS5B RNA dependent RNA polymerase[3] is one of the most studied target because it has been shown that a functional polymerase is essential for infectivity in chimpanzees.[4] Recently, a group from GlaxoSmithKline Pharmaceuticals reported the anti HCV NS5B polymerase activity of several benzo-1,2,4-thiadiazine analogues which also inhibited the replication of HCV replicons.[5] An inhibitor from Japan Tobacco Inc. is also reported to be undergoing Phase II clinical trials.[6] We have recently described the identification and SAR studies of a novel class of HCV polymerase inhibitors which are characterized by a *N,N*-disubstituted phenylalanine moiety **A**.[7] Crystal structures of inhibitor–NS5B HCV polymerase complexes were also generated and this led to the identification of an allosteric binding site located about 35 Å from the active site.[8]

Compound libraries were screened against HCV NS5B polymerase using the assay described previously;[7] selected compounds were then screened in a HCV replicon

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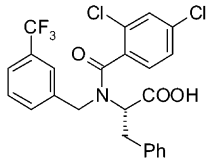
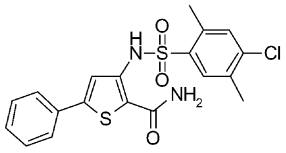
assay using a Huh-7 derived cell line (5.2 cells) which carries a replicon containing the luciferase reporter gene.[9] RNA replication can thus be conveniently measured by determining the level of luciferase activity. The concentration of compound required to suppress RNA replication by 50% was expressed as IC_{50}. Since cytotoxic compounds are detrimental to cell viability and hence HCV RNA replication, cytotoxicity was determined by measuring the incorporation of ^{3}H-thymidine in Huh-7 cells. The concentration required to suppress ^{3}H-thymidine uptake by 50% was expressed as CC_{50} and the Selectivity Index (SI) was calculated as the ratio of CC_{50} over IC_{50}. Our screening efforts led to the identification of a novel class of low molecular weight inhibitors based on a 5-phenyl-thiophene scaffold **1** which bears a carboxamide and a sulfonamide at the 2- and 3-positions respectively.



HCV NS5B IC_{50} 1.8 μM

A

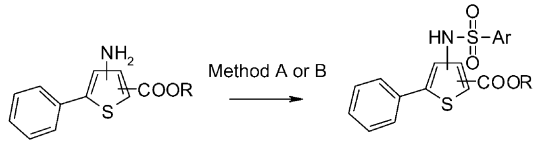


HCV NS5B IC_{50} 14 μM

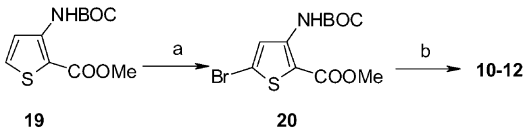
1

Preliminary SAR data showed the 2-carboxy analogue **2** to be 3-fold more potent than the corresponding carboxamide **1**. Furthermore, both the methyl carboxylate and 5-desphenyl derivatives of **2** were inactive against NS5B polymerase. It is interesting to note that in our previously described series, a carboxylic acid moiety was essential for polymerase inhibition.[7] Taking these findings into consideration, a systematic modification of the 3- and 5-positions was undertaken. In addition, the importance of the relative position of the three functionalities on the thiophene nucleus was also studied.

All the sulfonamides and amides described in this study were prepared by the reaction of commercially available acid or sulfonyl chlorides[10] with a 3-amino thiophene derivative in the presence of base (Scheme 1). Substituted 5-phenyl thiophene analogues **10**–**12** were obtained under Suzuki coupling conditions from the corresponding 5-bromothiophene **20** (Scheme 2). This compound was conveniently prepared from LDA mediated 5-proton abstraction from **19** followed by quenching with 1,2-dibromotetrafluoroethane[11] to give the desired 5-bromo derivative **20** in 92% yield (Scheme 2). This approach represents a highly efficient way of effecting a lithium anion mediated bromination in the presence of a methyl ester without the added complication of cross-condensation from the anion. The adjacent bulky BOC protecting group probably sterically hinders the methyl ester carbonyl from nucleophilic attack. Regioisomer **13** was prepared from the corresponding commercially available 2-amino thiophene derivative. The synthesis of regioisomer **14** is depicted in Scheme 3. The 4-amino thiophene derivative **22** used in the preparation of regioisomer **14** was obtained from nitration[12] of 5-bromothiophene **21** followed by Suzuki coupling with phenylboronic acid and reduction with tin(II) chloride. In contrast direct nitration of commercial 5-phenyl-2-methyl carboxylate resulted in exclusive nitration of the phenyl ring. Urea **17** and carbamate **18** were prepared from the corresponding isocyanate and chloroformate, respectively.



Scheme 1. Preparation of **2**–**9** and **13**. Reagents: compounds **2**, **6**, **13**: Method A (R = Me) (i) sulfonyl chloride, pyridine; (ii) LiOH, THF/MeOH/H_2O (3:2:1), rt; then HCl (1 N). Compounds **3**–**5**, **7**–**9**: Method B (R = H) sulfonyl chloride, Na_2CO_3 dioxane/H_2O (1:1); then HCl (1 N).

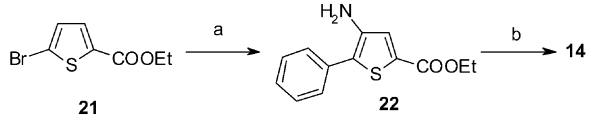


Scheme 2. Preparation of **10**–**12**. Reagents: (a) LDA, THF, −78 °C then $(CF_2Br)_2$, 92%; (b) (i) arylboronic acid, $Pd(PPh_3)_4$, toluene/MeOH, Na_2CO_3 (aq), 65 °C; (ii) TFA/CH_2Cl_2 (1:1), rt; (iii) LiOH, THF, MeOH, H_2O (3:2:1), rt, then HCl (1 N); (iv) Method B.

The compounds described in this study were evaluated for inhibition of HCV NS5B polymerase and selected compounds were also evaluated for inhibition of HCV subgenomic RNA replication in the replicon cell line. A systematic study of the importance of the substituents on the phenyl ring of the sulfonamide was undertaken and the results are depicted in Table 1.

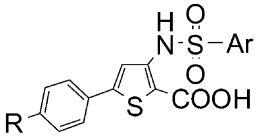
From the in vitro polymerase inhibition of compounds **4**–**7**, it became apparent that single substitution at the *ortho*, *meta* or *para* position does not provide any potency enhancement compared to the trisubstituted phenyl sulfonamide **2**. No improvement was also observed for the 2,5-dimethyl substituted analogue **3**. However, the 2,4-disubstituted analogues provided a 5-fold enhancement in potency; the 4-chloro-2-methyl, analogue **8** and the 2,4-dimethyl derivative **9** had IC_{50}'s of 0.75 and 1 μM, respectively. Polymerase inhibition was also enhanced by introduction of an acyl or methylsulfonyl unit on the *para* position of the 5-phenyl ring. The *ortho* methyl substituted analogues **10** and **12** had an IC_{50} of approximately 1.5 μM, whereas combining the acyl unit with the 2,4-dimethyl derivative gave the most potent compound (**11**, IC_{50} 0.39 μM) of this study.

Most of the compounds described in Table 1 were deemed too toxic to be able to differentiate between



Scheme 3. Preparation of **14**. Reagents: (a) (i) NO_2BF_4, CH_3CN, 75%; (ii) $PhB(OH)_2$, $Pd(PPh_3)_4$, toluene/MeOH, Na_2CO_3 (aq) 65 °C, 75%; (iii) $SnCl_2{\cdot}2H_2O$, AcOEt, 57%. (b) (i) LiOH, THF, MeOH, H_2O (3:2:1), rt, 93%, then HCl (1 N); (ii) Method B, 48%.

Table 1. NS5B Polymerase and replicon inhibition[16]



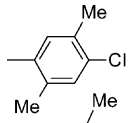
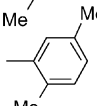
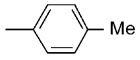
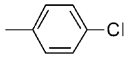
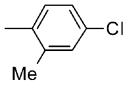
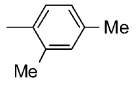
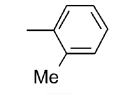
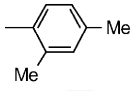
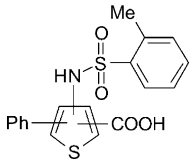
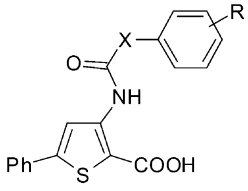
	R	Ar	HCV NS5B polymerase IC_{50} (μM)	Replicon IC_{50} (μM)	Huh-7 CC_{50} (μM)
1			14		
2	H		5.4	14	80
3	H		13	11	24
4	H		5.2	24	76
5	H		7.4	100	112
6	H		4.6	33	79
7	H		4.1	77	92
8	H		0.75	13	54
9	H		1	5	57
10			1.4	12	147
11			0.39	8	100
12	$MeSO_2$		1.8	>100	>200

Table 2. HCV polymerase activity of regiosomers[16]



Compd	Position			HCV NS5B polymerase
	COOH	$NHSO_2Ar$	Ph	IC_{50} (μM)
13	3	2	5	20
14	2	4	5	>50

Table 3. Replacement of sulfonyl with a carbonyl moiety[16]



Compd	X	R	HCV NS5B polymerase IC_{50} (μM)
15	—	2-Me	14
16	—	4-Me	12
17	NH	4-Cl	>50
18	O	4-Cl	13

anti-HCV activity and cytotoxicity; analogues **2–8** had selectivity indices (SI) of less than 5. However, compounds with 2,4-disubstituted phenylsulfonamides and/or those with acyl moieties on 5-phenyl gave anti-HCV replicon IC_{50}'s of 5–13 μM with SI's of about 12. The lack of inhibition of HCV replication with methylsulfonyl analogue **12** was surprising considering the level of HCV polymerase inhibition (IC_{50} 1.8 μM). This discrepancy may be attributed to conformational differences that exist between the polymerase/homopolymeric template/primer complex in the in vitro assay and the cellular environment in the replicon assay where, in addition to viral RNA template, the polymerase enzyme is likely associated with viral and cellular proteins. A recent report has provided evidence for modulation of HCV polymerase activity by NS3 and NS4B.[13] In addition, the inability of these compounds to achieve sufficient intra-cellular concentration for inhibition cannot be ignored at this point.

Regioisomers **13–14** were also less active (Table 2) than the series of compounds depicted in Table 1. Other 3-amino derivatives such as amide, urea and carbamate (**15–18**) were less active (Table 3). The 2,3,5 arrangement of substituents on the thiophene scaffold appears to be necessary for activity but whether this requirement is related to the angles of bisection remains to be determined.[14]

In summary, a novel class of HCV NS5B polymerase inhibitors has been identified and modification of the lead has resulted in a series of potent polymerase inhibitors and some of which also inhibited HCV subgenomic RNA replication. However, it is not clear at this point why in some cases, in vitro inhibition of HCV NS5B polymerase fails to translate into inhibition of replicon RNA replication. Further studies in optimizing this series of compounds are reported in the following publication.[15]

Acknowledgements

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References and notes

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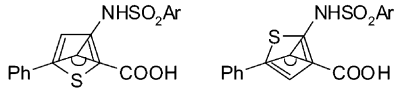

4 θ = 155° **13** θ = 138°

Regioisomers **4** and **13** were built with the MOE program. (Chemical Computing Group Inc. MOE program, 2002.) The conformers were minimized using the MMFF94s force field using a gradient of 0.01 kcal/mol Å. The resulting minimized structures were imported into ISIS-Draw (version 2.4; MDL, San Leandro, CA, USA) as MDLMOL files. A centroid was set on the thiophene ring and two 3-D objects were constructed as lines connecting the centroid to the individual two- (or three-) and five-carbon atoms. The bisection angles for each regioisomer were measured from creating 3-D geometric angles and are reported as (180-measured angle = θ). The use of bisection angles was also reported in: Wai, J. S.; Egbertson, M. S.; Payne, L. S.; Fisher, T. E.; Embrey, M. W.; Tran, L. O.; Melamed, J. Y.; Langford, H. M.; Guare, J. P., Jr.; Zhuang, L.; Grey, V. E.; Vacca, J. P.; Holloway, M. K.; Naylor-Olsen, A. M.; Hazuda, D. J.; Felock, P. J.; Wolfe, A. L.; Stillmock, K. A.; Schleif, W. A.; Gabryelski, L. J.; Young, S. D. *J. Med. Chem.* **2000**, *43*, 4923.
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16. IC_{50}'s values were determined singly from dose-response curves using 11 concentrations for each compound. Curves were fitted to data points using nonlinear regression analysis and IC_{50}'s values were interpolated from the resulting curves using GraphPad Prism software, version 2.0 (GraphPad Software, Inc., San Diego, CA, USA). A positive control was included as an internal standard in each set of experiments, and results were considered accurate only when the IC_{50} value of the positive control was within 0.45 ± 0.16 μM. Similarly, IC_{50}'s in replicons were determined using 12 concentrations in duplicate and data were considered accurate when IC_{50} of a positive control was within 8.9 ± 2.4 μM. CC_{50}'s in Huh-7 cells were determined from 5 concentrations in quadruplicate and data were taken when CC_{50} of a positive control was within 62 ± 10 μM.