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Design, synthesis, and anti-HCV activity of thiourea compounds

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ABSTRACT

A series of thiourea derivatives were synthesized and their antiviral activity was evaluated in a cell-based HCV subgenomic replicon assay. SAR studies revealed that the chain length and the position of the alkyl linker largely influenced the in vitro anti-HCV activity of this class of potent antiviral agents. Among this series of compounds synthesized, the thiourea derivative with a six-carbon alkyl linker at the *meta*-position of the central phenyl ring (**10**) was identified as the most potent anti-HCV inhibitor (EC_{50} = 0.047 μM) with a selectivity index of 596.

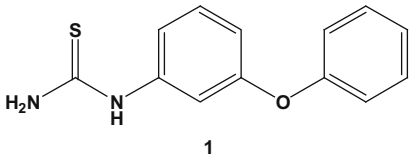
Hepatitis C virus (HCV) is a blood-borne virus that was identified in 1989.[1] Six genotypes with several subtypes within each genotype have been described for this member of the Flaviviridae family.[2] It is estimated that more than 170 million people worldwide are infected by HCV.[3] Most of those with acute hepatitis C develop a chronic infection. Chronic hepatitis C is often asymptomatic but may lead to liver cirrhosis and hepatocellular carcinoma (HCC) within the next decade.[4] Liver failure related to HCV infection is, in many countries, the leading cause of liver transplantation.[5] There is currently no vaccine or a direct antiviral agent for HCV.[6] The standard of care for treating HCV is a combination of pegylated interferon and ribavirin. However, this therapy in patients with genotype 1 is not very successful and associated with serious side effects.[7] Therefore, there is an urgent need to discover and develop new anti-HCV agents that are more effective and better tolerated by patients.

Recently, an antiviral screening program was initiated to search for new HCV inhibitors via HCV replicon assay[8] in our laboratory. Interestingly, a thiourea compound **1** was identified as a potential anti-HCV agent, the structure of which is illustrated in Figure 1.[9] This compound was found to possess antiviral activity against HCV with an EC_{50} of 0.494 μM. Therefore many structurally related thiourea compounds were synthesized and evaluated for their anti-HCV activity. Details of this investigation will be described herein.

In this Letter, we described the synthesis of antiviral thiourea compounds via the key nucleophilic substitution of phenol or aniline with phenylalkyl bromide, compound **22** and ditosylate **56** (Schemes 1–3). All the resulting compounds were then submitted for anti-HCV testing as well as cytotoxicity evaluation in the Ava5 cell lines. Preliminary structure-activity relationships against HCV are reported (Tables 1 and 2).

The thiourea compounds **1** and **5–20** were prepared by the method summarized in Scheme 1. Nucleophilic substitution of phenol (or aniline) **2** with phenylalkyl bromide using potassium carbonate as a base in 1-methyl-2-pyrrolidine (NMP) at 90 °C for 3 h afforded compound **3**. Subsequent nitro reduction of compound **3** in the presence of tin(II) chloride in ethanol at 70 °C overnight gave the aniline **4** in excellent yields. The aniline **4** was treated with 1,1′-(thiocarbonyl)diimidazole (TCDI) in dichloromethane at room temperature followed by reaction with 25% ammonia solution or the appropriate primary amines to give the desired compounds **1** and **5–20**.

Compounds **25–53** were synthesized by the method summarized in Scheme 2. In the presence of potassium carbonate, alcohol **21** can undergo nucleophilic substitution with dibromo compound to give compound **22**. Subsequent O-alkylation of 3-nitrophenol with compound **22** in the presence of potassium carbonate in NMP at 90 °C gave compound **23**. A solution of compound **23** in ethyl acetate is hydrogenated in the presence of 10% Pd/C with hydrogen filled in a balloon at room temperature overnight to give the aniline **24** in excellent yields. The aniline **24** was treated with 1,1′-(thiocarbonyl)diimidazole (TCDI) in dichloromethane at room



1

EC_{50} = 0.494 μM

Figure 1. Structure of thiourea compound **1**.

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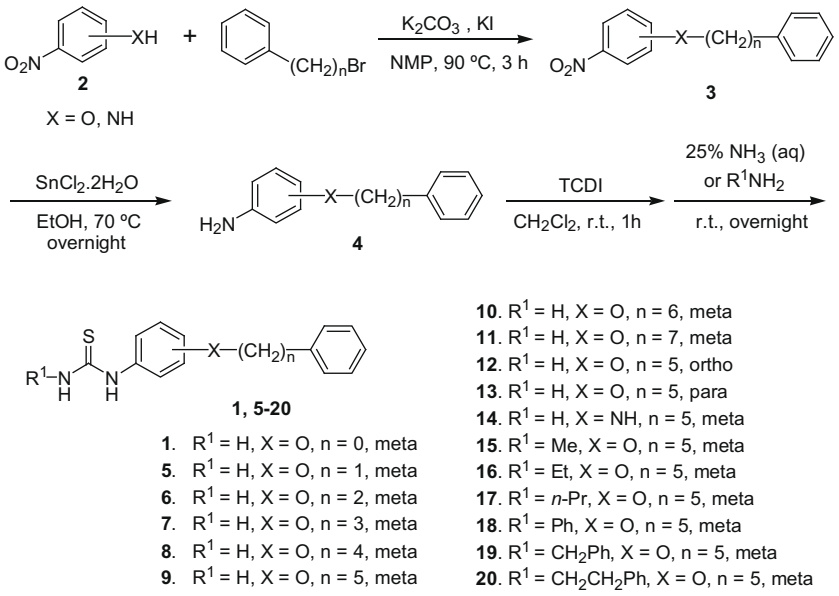
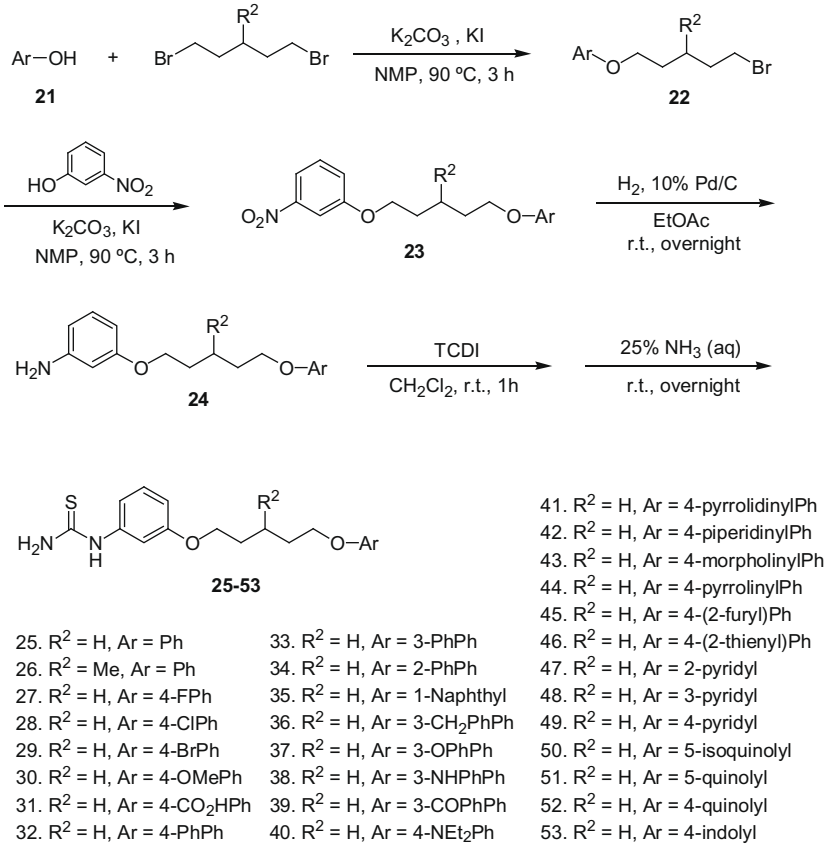


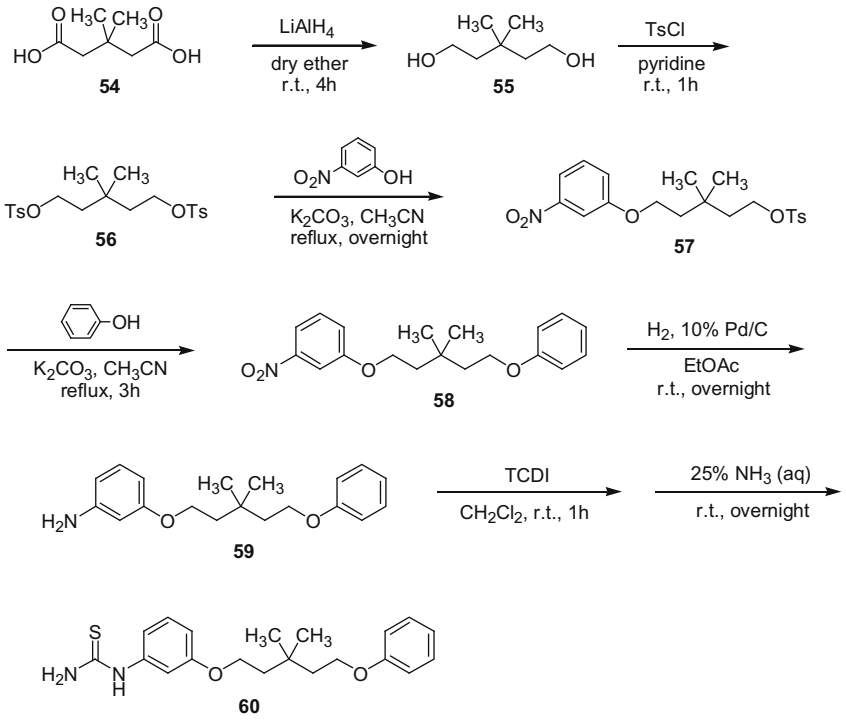
Scheme 1. General synthetic route to thiourea compounds **1** and **5**–**20**.



Scheme 2. General synthetic route to thiourea compounds **25**–**53**.

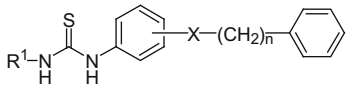
temperature followed by reaction with 25% ammonia solution gave the target compounds **25–53**.

Compound **60** was synthesized by the method summarized in Scheme 3. Reduction of the glutaric acid **54** with lithium aluminum hydride in dry ether gave the diol **55**, which was then reacted with *p*-toluenesulfonyl chloride in pyridine to give the corresponding ditosylate **56**. Subsequent nucleophilic substitution of 3-nitrophenol with 2 equiv of the ditosylate **56** using potassium carbonate as a base at refluxing acetonitrile gave compound **57**, which was coupled with phenol in the presence of potassium carbonate in acetonitrile to give the compound **58**. A solution of compound **58** in ethyl acetate is hydrogenated in the presence of 10% Pd/C with hydrogen filled in a balloon at room temperature overnight to give the aniline **59** in excellent yields. The aniline **59** was treated with



Scheme 3. Synthesis of thiourea compound **60**.

Table 1
Anti-HCV activity and cytotoxicity for compounds **1** and **5–20**



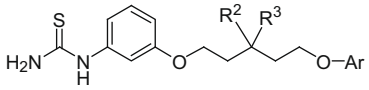
Compound	R^1	X	Isomer	*n*	1b EC_{50} (μM)[a]	CC_{50} (μM)[a]	SI[b]
1	H	O	*Meta*	0	0.494	>50	>101
5	H	O	*Meta*	1	0.561	>50	>89
6	H	O	*Meta*	2	0.335	>50	>149
7	H	O	*Meta*	3	0.231	>50	>216
8	H	O	*Meta*	4	0.159	>50	>314
9	H	O	*Meta*	5	0.048	22	458
10	H	O	*Meta*	6	0.047	28	596
11	H	O	*Meta*	7	0.097	23	237
12	H	O	*Ortho*	5	1.012	31	31
13	H	O	*Para*	5	0.197	10	51
14	H	NH	*Meta*	5	0.143	>50	>350
15	Me	O	*Meta*	5	0.822	>50	>61
16	Et	O	*Meta*	5	0.685	>50	>73
17	*n*-Pr	O	*Meta*	5	0.708	32	45
18	Ph	O	*Meta*	5	31.497	35	1
19	CH_2Ph	O	*Meta*	5	7.238	32	4
20	CH_2CH_2Ph	O	*Meta*	5	13.388	33	2

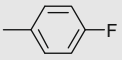
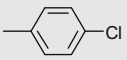
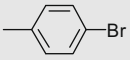
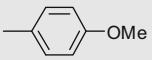
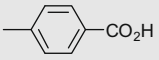
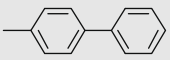
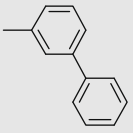
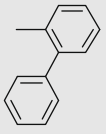
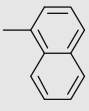
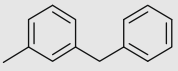
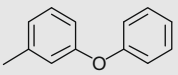
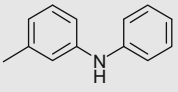
[a] Mean of triplicate well values. All experiments were performed at least twice.
[b] In vitro selectivity index (CC_{50}/EC_{50}).

1,1′-(thiocarbonyl)diimidazole (TCDI) in dichloromethane at room temperature followed by reaction with 25% ammonia solution gave the target compound **60**.

The thiourea compounds described herein were tested in an HCV subgenomic replicon assay with a SEcreted Alkaline Phosphatase (SEAP) reporter. Compounds **1**, **5–53** and **60** were submitted for anti-HCV testing as well as cytotoxicity evaluation in the Ava5 cell lines. As shown in Table 1, compared to the lead compound **1**, several thiourea compounds with various tether lengths (**5–11**) were synthesized in order to determine the optimal spacing between the oxygen atom and the distal phenyl group. Interestingly, the chain length was found to be of considerable importance for the activity of compounds. Compound **5** with a chain length of one carbon was found to be less active compared to lead compound **1**. On the other hand compounds **6–10** with a chain length of two, three, four, five and six carbons increased the potency about 1.5- to 10-fold with respect to compound **1**. However, going to longer seven-carbon homologue (**11**) was not favorable for activity. These significant results demonstrated that the five and six-carbon homologues (**9**, **10**) in this series were more active against HCV (EC_{50} = 0.048 and 0.047 μM, respectively) than their corresponding longer or shorter compounds. These observations provide remarkable evidence that the hydrophobic interaction and conformational flexibility of the alkyl linker influence anti-

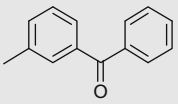
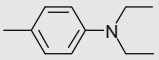
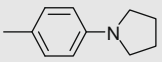
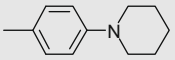
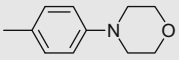
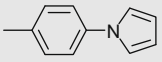
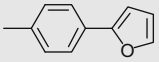
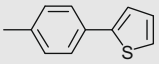
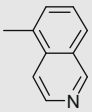
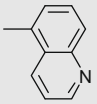
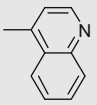
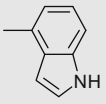
Table 2
Anti-HCV activity and cytotoxicity for compounds **25–53** and **60**



Compound	R^2	R^3	Ar	1b EC_{50} (μM)[a]	CC_{50} (μM)[a]	SI[b]
25	H	H		0.059	>50	>847
26	Me	H		0.104	>50	>481
60	Me	Me		0.105	13	124
27	H	H		0.072	5	69
28	H	H		0.072	7	97
29	H	H		0.072	19	264
30	H	H		0.218	>50	>229
31	H	H		>50	>50	>1
32	H	H		0.191	>50	>262
33	H	H		0.214	13	61
34	H	H		0.097	13	134
35	H	H		0.058	14	241
36	H	H		0.058	13	224
37	H	H		0.107	18	168
38	H	H		0.177	11	62

(*continued on next page*)

Table 2 (*continued*)

Compound	R^2	R^3	Ar	1b EC_{50} (μM)[a]	CC_{50} (μM)[a]	SI[b]
39	H	H		0.218	13	60
40	H	H		0.594	>50	>84
41	H	H		0.913	16	18
42	H	H		0.407	12	29
43	H	H		0.485	>50	>103
44	H	H		0.255	>50	>196
45	H	H		0.136	11	81
46	H	H		0.121	>50	>413
47	H	H		0.279	>50	>179
48	H	H		1.134	>50	>44
49	H	H		2.992	>50	>17
50	H	H		0.481	26	54
51	H	H		0.250	15	60
52	H	H		0.411	24	58
53	H	H		0.355	>50	>141

[a] Mean of triplicate well values. All experiments were performed at least twice.

HCV activity of these thiourea compounds. The position of the alkyl linker at the central phenyl ring of five-carbon homologues (**9**, **12**, **13**) impacted anti-HCV activity. The activity increased from *ortho*-substitution (**12**) to *para*-substitution (**13**), and to *meta*-substitution (**9**), the latter being the most preferred pattern for sterically demanding substituents. Replacing the oxygen linker (**9**) with a

NH unit (**14**) resulted in a threefold decrease in activity, perhaps caused by a conformational change of the side chain. Substitution at the NH_2 of the thiourea moiety with methyl (**15**), ethyl (**16**), *n*-propyl (**17**), phenyl (**18**), benzyl (**19**) and phenethyl (**20**) groups showed a 14- to 656-fold loss in activity compared to the corresponding compound **9**. The results of antiviral testing indicate that a free NH_2 is required for maintaining activity.

In this study, we observed that the thiourea compound **10**, in terms of potency and selectivity index (SI = 596), appear to be the most promising candidate for further development as an anti-HCV agent. On the basis of the skeleton of compound **10**, several thiourea compounds **25**–**53** and **60** were thus synthesized (Schemes 2 and 3). The results are shown in Table 2 and are compared to the compound **10**. The simple replacement of a carbon atom by oxygen (**25**) in the alkyl linker of compound **10** showed a much better selectivity index (SI > 847) in the replicon assay with an EC_{50} of 0.059 μM and no cytotoxicity up to 50 μM. Introduction of one (**26**) or two (**60**) methyl groups at the 3-position of the alkyl linker resulted in a 1.7-fold decrease in activity compared to compound **25**. This effect might be due to their drastically conformational change and steric requirement of the alkyl linker of these thiourea compounds. Introduction of a substituent (F, Cl, Br, OMe) at the *para*-position of the distal phenyl ring of compound **25**, such as compounds **27–30**, resulted in a 1.2- to 3.7-fold decrease in activity. It is also interesting to note that the methoxy analogue **30** was considerably less active than the halo analogues **27–29**. On the other hand, the carboxylic acid analogue **31** showed a total loss of activity. Among the biphenyl analogues (**32–34**), the *ortho*-biphenyl analogue **34** showed better activity (EC_{50} = 0.097 μM) than the *para*- and the *meta*-analogues (**32**, **33**). On a direct comparison of compound **25** with compounds **35** and **36**, where the phenyl ring (**25**) was replaced with naphthalene (**35**) and diphenylmethane (**36**), an equivalent potency was observed (EC_{50} = 0.058 μM for **35** and **36**).

Replacing the methylene group (**36**) of the diphenylmethane moiety by an oxygen atom (**37**), amino group (**38**) and carbonyl group (**39**) resulted in a slight loss of activity, indicating that methylene linker is optimal for activity. Additionally, replacement of the phenyl group (**32**) in the *para*-position of biphenyl moiety with diethyl amino (**40**), pyrrolidinyl (**41**), piperidinyl (**42**), morpholinyl (**43**) and pyrrolyl (**44**) groups caused a loss of activity. This effect might be due to their distinct differences of hydrophobic properties. A comparison of the furan-containing compound (**45**) with thiophene analogue (**46**) reveals some interesting differences. The thienyl compound **46** exhibited 1.1 times more potent anti-HCV activity (EC_{50} = 0.121 μM), 5 times higher selective index (SI > 413) than the furyl compound **45**. Among the pyridyl analogues (**47–49**), the 2-pyridyl analogue **47** was more active than 3- and 4-pyridyl analogues (**48**, **49**) without significant cytotoxicity (CC_{50} > 50 μM). Replacement of the naphthyl group of compound **35** with isoquinolyl (**50**), quinolyl (**51**, **52**) and indolyl (**53**) groups resulted in a 4.3- to 8.3-fold decrease in activity. The significant results demonstrated that the endocyclic nitrogen of the pyridyl, isoquinolyl and quinolyl moieties was not tolerated (compare **25** to **47–49** and **35** to **50–52**). This unexpected biological result is not fully understood and is worthy of further study.

In conclusion, from the compound screening using a cell-based HCV subgenomic replicon assay, we identified a lead compound **1** with an EC_{50} of 0.494 μM. We explored the structure-activity relationship for this class of inhibitors. A free NH_2 group of the thiourea moiety is critical for activity. The activities of this series of compounds were very sensitive to the variation in the chain length of the alkyl linker. Attempts to replace the oxygen linker with nitrogen caused a loss of activity. On the basis of these biological results, compound **10** with a chain length of six carbons was found to exhibit the most potent antiviral activity against HCV (EC_{50} = 0.047 μM). Further SAR studies and mechanistic studies on this class of antiviral compounds are currently under active investigation and will be reported in due course.

Acknowledgements

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References and notes

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