Bioorganic & Medicinal Chemistry Letters 19 (2009) 4134–4138

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Design and synthesis of indole, 2,3-dihydro-indole, and 3,4-dihydro-2*H*-quinoline-1-carbothioic acid amide derivatives as novel HCV inhibitors

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ARTICLE INFO

Article history:
Received 17 February 2009
Revised 19 May 2009
Accepted 1 June 2009
Available online 6 June 2009

Keyword:
KCV inhibitors

ABSTRACT

An efficient synthetic methodology to provide indole, 2,3-dihydro-indole, and 3,4-dihydro-2*H*-quinoline-1-carbothioic acid amide derivatives is described. These conformationally restricted heterobicyclic scaffolds were evaluated as a novel class of HCV inhibitors. Introduction of an acyl group at the NH_2 of the thiourea moiety has been found to enhance inhibitory activity. The chain length and the position of the alkyl group on the indoline aromatic ring markedly influenced anti-HCV activity. The indoline scaffold was more potent than the corresponding indole and tetrahydroquinoline scaffolds and analogue **31** displayed excellent activity (EC_{50} = 510 nM) against HCV without significant cytotoxicity (CC_{50} >50 μM).

Hepatitis C virus (HCV) is a positive-strand RNA virus that was first identified in 1989.[1] Six major genotypes and many subtypes have been described for this member of the Flaviviridae family.[2] An estimated more than 170 million people worldwide are chronically infected with HCV.[3] Most people with chronic hepatitis C have no symptoms but may lead to liver cirrhosis and hepatocellular carcinoma (HCC) within the next decade.[4] HCV infection is, in many countries, the leading reason for liver transplants.[5] There is currently no vaccine or effective therapy against HCV.[6] The standard of care for HCV infection is a combination therapy of injected pegylated interferon plus oral ribavirin. However, this therapy in patients with genotype 1 has limited efficacy and suffers from serious side effects.[7] Therefore, there is an urgent need for the development of more effective and better tolerated anti-HCV agents.

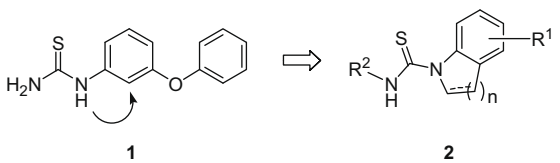
Recently, from a high throughput screening of our in-house compound collection by using a cell-based HCV replicon assay,[8] we identified an interesting thiourea compound **1**[9] (Fig. 1) with an EC_{50} of 0.49 μM. While the preliminary structure–activity relationship was explored retaining the aryl thiourea moiety, we were interested in expanding our optimization efforts to heterobicyclic scaffolds **2** like indole, indoline, and tetrahydroquinoline. Figure 1 is a schematic representation of our design strategy.

Tough the mechanism of this class of compounds is not yet fully understood, they show significant activity in a cell-based HCV replicon assay. Recently, it was reported that the structurally related acylthiourea compound ACH-806[15] showed potent anti-HCV activity with a unique mechanism of action. It selectively binds to the HCV NS4A protein,[15] resulting in altered protein composition and inactivation of the replicase complex. Therefore, it is possible that our compounds **1** and **2** also target at the HCV NS4A protein. Further mechanism studies on this class of compounds are currently under active investigation and will be reported in due courses.

In this Letter, we report the synthesis of these scaffolds and explore the structure–activity relationship requirements for these new scaffolds.

The tetrahydroquinoline **7** was generated form commercially available 1-naphthol **3** (Scheme 1). Nucleophilic substitution with 1,5-dibromopentane afforded compound **4**. Subsequent O-alkylation of 5-hydroxyquinoline with compound **4** in the presence of potassium carbonate in NMP (1-methyl-2-pyrrolidine) at 90 °C gave compound **5**. Selective reduction of quinoline **5** with sodium borohydride–nickelous chloride ($NaBH_4$–$NiCl_2$) system[10] in methanol provided the tetrahydroquinoline **6**. The desired compound **7** was then obtained by treatment of **6** with potassium thiocyanate[11] in ethanol.

Indolines **12** and **14** were synthesized by the method summarized in Scheme 2. In the presence of potassium carbonate, 4-hydroxyindole **8** can undergo nucleophilic substitution with 1,5-dibromopentane to give compound **9**. Subsequent O-alkylation



1 2

Figure 1. From aryl thiourea **1** to heterobicyclic scaffold **2**.

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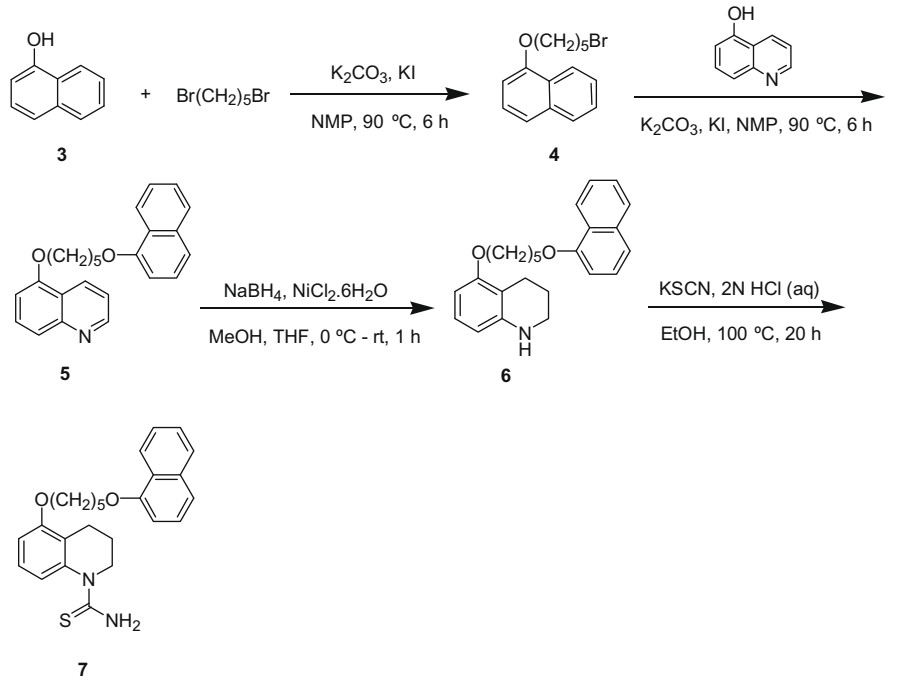
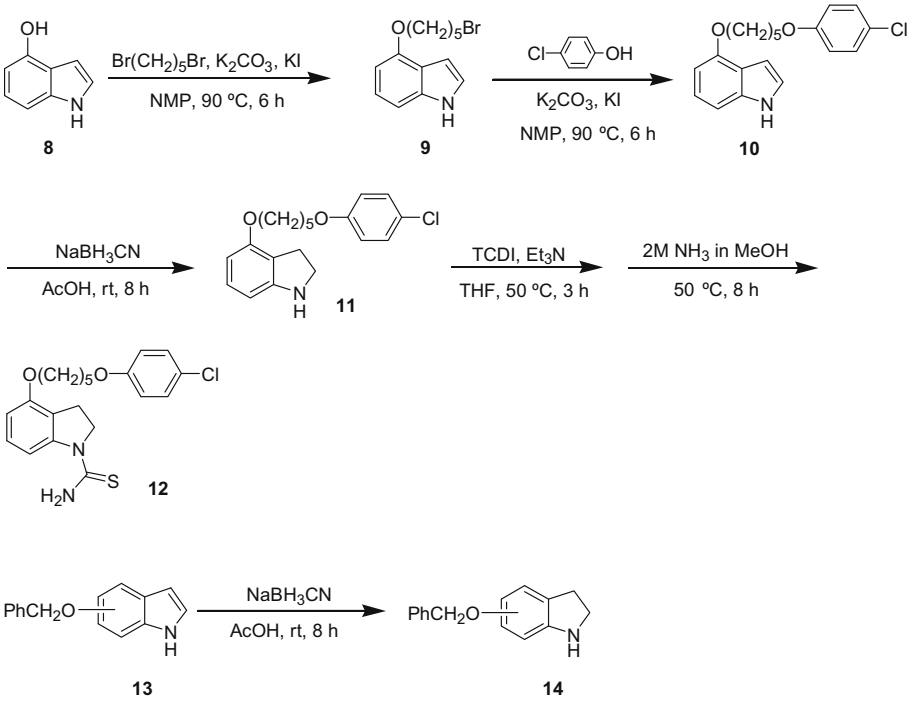
doi:10.1016/j.bmcl.2009.06.009



Scheme 1. Synthesis of tetrahydroquinoline 7.

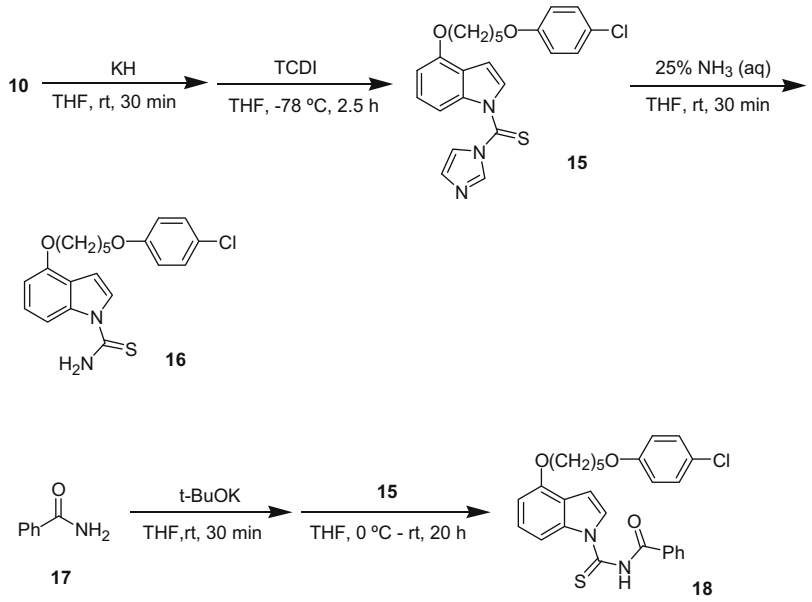


Scheme 2. Synthesis of indolines **12** and **14**.

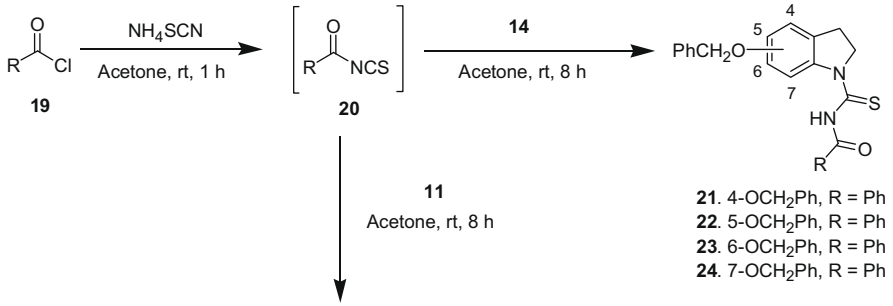
of 4-chlorophenol with compound **9** in the presence of potassium carbonate in NMP at 90 °C afforded compound **10**. Selective reduction of indole **10** and commercially available indole **13** with sodium cyanoborohydride ($NaBH_3CN$) in acetic acid[12] gave indolines **11** and **14**, respectively. Treatment of **11** with 1,1′-(thiocarbonyl)diimidazole (TCDI) in THF at 50 °C followed by reaction with 2 M ammonia in methanol provided the target compound **12**.

Indoles **16** and **18** were prepared according to the chemistry in Scheme 3. The compound **10** was treated with KH[13] in THF at room temperature for 30 min followed by reaction with TCDI at −78 °C afforded compound **15**. The desired compound **16** was then obtained by treatment of **15** with 25% ammonia solution in THF at room temperature. The benzamide **17** was treated with *t*-BuOK in THF at room temperature for 30 min followed by reaction with **15** in THF gave the target compound **18**.

Indolines **21**–**51** were prepared from commercially available acyl chloride **19**, which was converted to acyl isothiocyanate **20** upon reaction with ammonium thiocyanate (Scheme 4). Subsequent



Scheme 3. Synthesis of indoles **16** and **18**.



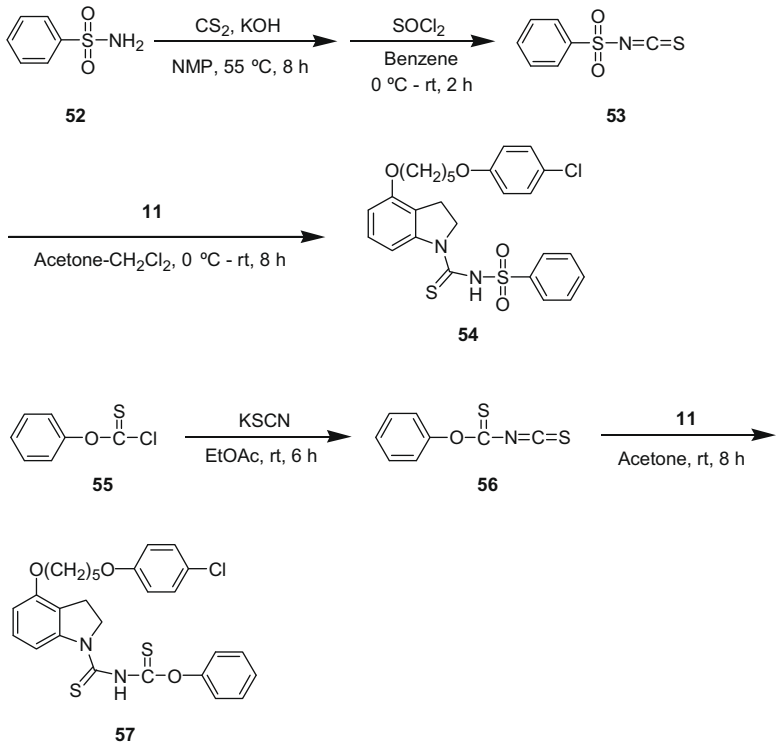
25. R = Ph
26. R = PhO
27. R = 2-NO_2-Ph
28. R = 3-NO_2-Ph
29. R = 4-NO_2-Ph
30. R = 3-CN-Ph
31. R = 4-CN-Ph
32. R = 4-F-Ph
33. R = 4-Cl-Ph
34. R = 4-Br-Ph
35. R = 4-CF_3-Ph
36. R = 4-Me-Ph
37. R = 4-MeO-Ph
38. R = 4-*t*-Bu-Ph
39. R = 4-$N(CH_3)_2$-Ph
40. R = CH_2CH_2Ph
41. R = $CH_2CH_2CH_3$
42. R = Cyclopentyl
43. R = Cyclohexyl
44. R = 2-Naphthyl
45. R = 2-Furyl
46. R = 3-Furyl
47. R = 2-Thienyl
48. R = 3-Thienyl
49. R = 3-Pyridyl
50. R = 5-Isoxazolyl
51. R = 2-Benzothienyl

Scheme 4. General synthetic route to compounds **21**–**51**.

addition of compounds **11** and **14** to the resulting acyl isothiocyanate **20**, without isolation, gave the desired compounds **21**–**51**.

Other indolines **54** and **57** were synthesized as shown in Scheme 5. The benzenesulfonyl isothiocyanate **53** was prepared[14] by coupling the starting benzenesulfonamide **52** with carbon disulfide in the presence of KOH and subsequent addition of thionyl chloride to the resulting compound. Compound **11** was then coupled with **53** to give the target compound **54**. The desired compound **57** was obtained through the reaction of *o*-phenyl chlorothionoformate **55** with potassium thiocyanate and subsequent coupling of **11** with the resulting compound **56** in acetone.

The heterobicyclic compounds described herein were tested for anti-HCV activity using an in vitro assay system that is suitable for monitoring anti-HCV activities of compounds. This system is composed of a human hepatocarcinoma cell line (Huh-7) supporting multiplication of a HCV replicon. We examined compounds' ability to inhibit the replication of subgenomic HCV RNA in a HCV replicon cell system.[8] All compounds were also tested for their cytotoxicity



Scheme 5. Synthesis of indolines **54** and **57**.

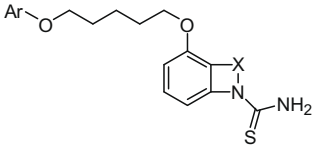
toward human Huh-7 cells. As shown in Figure 1, the aryl thiourea moiety of **1** was converted to a conformationally restricted heterobicyclic moiety **2**. Initial leads for the heterobicyclic series are disclosed in Table 1. Conformational restriction of **1** to give bicyclic analogues (**7**, **12**, **16**) led to a significant decrease in activity. The most potent heterobicycle was indoline **12**, which showed an EC_{50} of 4.71 μM and did not exhibit cytotoxicity up to 50 μM. Dehydrogenation (**16**) or increase in ring size (**7**) decreased anti-HCV activity by fivefold or more compared to **12**.

We then focused on the modifications of the indoline series. As seen from Table 2, introduction of a benzoyl group (**25**) at the NH_2 of the thiourea moiety resulted in a sixfold increase in activity compared to the parent compound (**12**). Making the same change to indole **16** to give **18** also showed an increase in activity. Replacement of the benzoyl group of compound **25** with phenoxycarbonyl (**26**), phenylsulfonyl (**54**), and phenoxythiocarbonyl (**57**) groups resulted in a complete loss of activity, indicating the importance of the benzoyl group. Compound **25** has an EC_{50} of 0.79 μM in the 1b replicon assay, and a CC_{50} of greater than 50 μM. This compound was chosen for further study as it was identified to be the most active compound thus far.

Several indolines with benzyl and alkyl substituents at the different positions of the phenyl ring are described in Table 3. The position of the benzyl group at the phenyl ring of indoline moiety (**21**–**24**) impacted anti-HCV activity. The activity increased from C-7 substitution (**24**) to C-6 substitution (**23**), C-5 substitution (**22**), and to C-4 substitution (**21**), the latter being the most preferred pattern for sterically demanding substituents.

The benzoyl substituent of the indoline scaffold was diversified to further improve the anti-HCV activity (Table 4). We have introduced substituents (**27**–**39**) into the phenyl ring of the benzoyl moiety of compound **25**. The 3- and 4-nitro analogues (**28**, **29**) were more active than the 2-nitro analogue (**27**). Interestingly, the 3- and 4-cyano analogues (**30**, **31**) exhibited potent inhibitory activities with EC_{50}s of 0.57 and 0.51 μM, respectively. In particu-

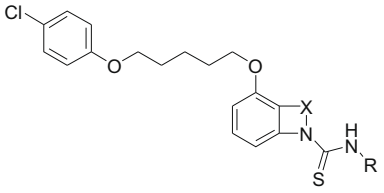
Table 1
Anti-HCV activity and cytotoxicity for heterobicyclic scaffolds



Compound	X	Ar	1b EC_{50}[a] (μM)	CC_{50}[a] (μM)	SI[b]
7	$-(CH_2)_3-$	1-Naphthyl	>50	>50	>1
12	$-(CH_2)_2-$	4-Cl-Ph	4.71 ± 0.26	>50	>11
16	–CH=CH–	4-Cl-Ph	24.54 ± 1.32	24.71 ± 1.18	1

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

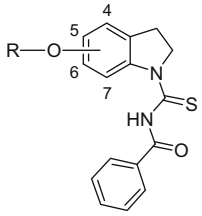
Table 2
Anti-HCV activity and cytotoxicity for heterobicyclic scaffolds



Compound	X	R	1b EC_{50}[a] (μM)	CC_{50}[a] (μM)	SI[b]
12	$-(CH_2)_2-$	H	4.71 ± 0.26	>50	>11
18	–CH=CH–	PhCO	4.06 ± 0.83	29.51 ± 2.73	7
25	$-(CH_2)_2-$	PhCO	0.79 ± 0.14	>50	>63
26	$-(CH_2)_2-$	PhO–CO	>50	>50	>1
54	$-(CH_2)_2-$	$PhSO_2$	>50	>50	>1
57	$-(CH_2)_2-$	PhO–CS	>50	>50	>1

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

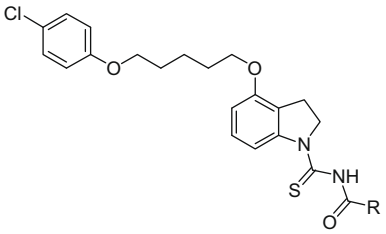
Table 3
Anti-HCV activity and cytotoxicity for indolines **21**–**24**



Compound	R	1b EC_{50}[a] (μM)	CC_{50}[a] (μM)	SI[b]
21	4-CH_2Ph	3.18 ± 0.36	>50	>16
22	5-CH_2Ph	4.81 ± 0.27	45.21 ± 2.63	9
23	6-CH_2Ph	5.05 ± 0.93	43.80 ± 1.48	9
24	7-CH_2Ph	>50	>50	>1

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

Table 4
Anti-HCV activity and cytotoxicity for indolines **25** and **27**–**51**



Compound	R	1b EC_{50}[a] (μM)	CC_{50}[a] (μM)	SI[b]
25	Ph	0.79 ± 0.14	>50	>63
27	2-NO_2-Ph	1.65 ± 0.33	>50	>30
28	3-NO_2-Ph	0.58 ± 0.12	12.13 ± 1.18	21
29	4-NO_2-Ph	0.72 ± 0.21	13.62 ± 1.45	19
30	3-CN-Ph	0.57 ± 0.09	9.75 ± 0.86	17
31	4-CN-Ph	0.51 ± 0.15	>50	>98
32	4-F-Ph	0.95 ± 0.28	31.11 ± 2.56	33
33	4-Cl-Ph	0.69 ± 0.18	21.71 ± 1.43	31
34	4-Br-Ph	0.88 ± 0.16	29.08 ± 1.78	33
35	4-CF_3-Ph	1.23 ± 0.43	32.69 ± 1.67	27
36	4-Me-Ph	1.94 ± 0.19	>50	>26
37	4-MeO-Ph	2.02 ± 0.61	34.42 ± 1.48	17
38	4-*t*-Bu-Ph	1.95 ± 0.53	>50	>26
39	4-$N(CH_3)_2$-Ph	42.37 ± 1.83	>50	>1
40	CH_2CH_2Ph	13.36 ± 0.79	>50	>4
41	$CH_2CH_2CH_3$	31.58 ± 1.65	>50	>2
42	Cyclopentyl	25.39 ± 1.23	>50	>2
43	Cyclohexyl	42.47 ± 2.57	>50	>1
44	2-Naphthyl	2.16 ± 0.56	>50	>23
45	2-Furyl	0.58 ± 0.15	4.62 ± 0.63	8
46	3-Furyl	1.72 ± 0.23	20.06 ± 1.35	12
47	2-Thienyl	0.66 ± 0.08	10.66 ± 0.95	16
48	3-Thienyl	1.02 ± 0.18	>50	>49
49	3-Pyridyl	2.37 ± 0.24	11.1 ± 0.86	5
50	5-Isoxazolyl	2.06 ± 0.17	37.08 ± 1.83	18
51	2-Benzothienyl	1.1 ± 0.07	>50	>45

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

lar, compound **31** proved to be more potent than the unsubstituted compound **25** with a selectivity index of greater than 98. All other 4-substituted analogues (**32**–**39**) showed similar or slightly reduced activity, with the exception of the 4-dimethylamino analogue **39**, which resulted in a considerable loss of activity. Introduction of an ethylene linker (**40**) between the carbonyl group and the phenyl ring showed a decrease in potency. Replacing the phenyl ring (**25**) of the benzoyl moiety by an *n*-propyl (**41**) or a cycloalkyl group (**42**, **43**) dramatically decreased the activity. These findings indicate that the aromatic ring seems to play an important role in activity. Replacement of the phenyl ring (**25**) by a naphthyl (**44**) or a heterocycle (**45**–**51**) resulted in slightly improved or reduced activity. The 2-furyl (**45**) and 2-thienyl (**47**) analogues were more active than their 3-furyl (**46**) and 3-thienyl (**48**) isomers, but were also somewhat more cytotoxic. The most potent inhibitor **31** from this SAR study was selected for further development of anti-HCV agents.

In conclusion, we have developed an efficient synthetic methodology to provide indole-1-carbothioic acid amide, 2,3-dihydro-indole-1-carbothioic acid amide, and 3,4-dihydro-2*H*-quinoline-1-carbothioic acid amide that could be useful for preparing conformationally restricted novel HCV inhibitors. Incorporation of an aromatic acyl group at the NH_2 of the thiourea moiety has been found to enhance inhibitory activity. Substitution at the 4-position of the indoline was preferred over the 5, 6, and 7-positions. In addition, the chain length of the alkyl group also influences anti-HCV activity. The indoline scaffold is clearly favored over the corresponding indole and tetrahydroquinoline scaffolds and is a novel chemotype for further exploration of anti-HCV drugs.

Acknowledgment

We gratefully acknowledge the financial support of the National Health Research Institutes in Taiwan (ROC).

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