Bioorganic & Medicinal Chemistry Letters 20 (2010) 2968–2973

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

The discovery and structure–activity relationships of pyrano[3,4-*b*]indole based inhibitors of hepatitis C virus NS5B polymerase

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ARTICLE INFO

Article history:
Received 2 February 2010
Accepted 1 March 2010
Available online 6 March 2010

Keywords:
HCV NS5B polymerase
Pyranoindoles

ABSTRACT

We describe the structure–activity relationship of the C1-group of pyrano[3,4-*b*]indole based inhibitors of HCV NS5B polymerase. Further exploration of the allosteric binding site led to the discovery of the significantly more potent compound **12**.

Hepatitis C virus (HCV) is a serious disease which often leads to chronic hepatitis, cirrhosis, and hepatocellular carcinoma.[1] These complications are responsible for about 10,000–12,000 deaths/year in the U.S.[1] The necessity for liver transplantation in the U.S. is most often the result of chronic HCV infection.[1b] Recent worldwide estimates indicate that approximately 1–3% of the total population may be infected with HCV.[2]

Current therapies for HCV involve a combination of ribavirin and interferon-α.[3] This treatment regimen causes unfavorable side effects which often results in poor patient compliance. Furthermore, only about 40% of patients infected with HCV achieve a sustained medical benefit.[3a,4,5] Recently, pegylated forms of interferon-α (Pegasys™ and PEG-INTRON™) which provide improved patient tolerabilities and response rates of over 50% have been approved.[3] Clearly there is a medical need for additional HCV antiviral agents.

Hepatitis C belongs to the *Flaviviridae* family of positive-single stranded RNA viruses.[6] The HCV genome encodes a 3000 amino acid polyprotein which is processed into structural and non-structural proteins.[6] One of the non-structural proteins that is essential for viral replication is the NS5B RNA-dependent RNA polymerase.[7,8] The HCV NS5B is an attractive target owing to the success of other antiviral agents that inhibit viral polymerases.[9] One such class is the non-nucleoside reverse transcriptase inhibitors (NNRTI's) that target HIV RT. This communication will describe our optimization efforts and structure–activity relationships on the novel pyranoindole series of allosteric HCV NS5B enzyme inhibitors.

Several thumb domain sites of HCV NS5B have been identified.[8] The compounds depicted in Scheme 1 occupy the same allosteric binding site (Thumb Pocket II) of HCV NS5B with their acidic functionalities participating in hydrogen bonding to the Ser^{476} and Tyr^{477} residues.

Another critical feature of these ligands is the ability to occupy a hydrophobic 'dimple' region defined by residues Leu^{419}, Trp^{528}, Tyr^{477}, and Arg^{422}. Gopalsamy has reported a series of tetrahydropyrano[3,4-*b*]indole-containing compounds exemplified by structure **1**.[10] Compound **1** displayed potent inhibitory activity against the HCV NS5B Δ^{21} polymerase (IC_{50} = 0.3 μM). Co-crystallographic analysis has determined that the hydrophobic 'dimple' on the protein is occupied by the *n*-propyl group of these pyranoindole-based inhibitors (see Fig. 1).

It has been previously shown that *n*-propyl substitution is greatly preferred over an ethyl group at this position.[10] However, no other variation of substituents at this position has been described. We were therefore interested in further examining the

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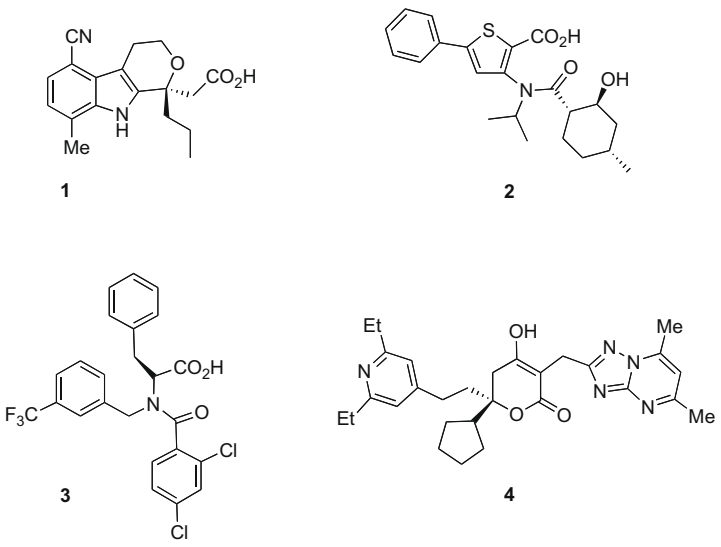
doi:10.1016/j.bmcl.2010.03.002



Scheme 1. Reported allosteric thumb pocket II HCV NS5B inhibitors.

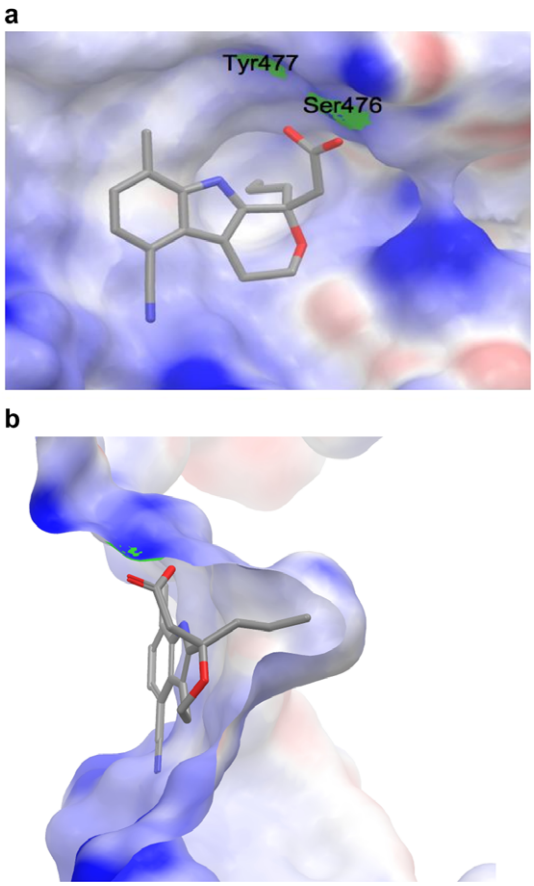


Figure 1. (a) Crystal structure of NS5B with tetrahydro[3,4-*b*]indole inhibitor. Electrostatic potential representation: Red-negative charge; Blue-positive charge; White-non-polar; Ser^{476} & Tyr^{477} highlighted in Green. (b) Crystal structure of NS5B with tetrahydro[3,4-*b*]indole inhibitor-side view indicating R1 group in hydrophobic 'dimple'.

structure–activity relationship at the C1-position of these pyranoindole inhibitors.[10] The desired pyranoindole compounds **8** were readily assembled by the Lewis acid-catalyzed cyclization of the substituted tryptophol **5** with a variety of β-ketoesters **6** (Scheme 2).[10–14] It is important to note that the compounds depicted in Table 1 were prepared as racemic mixtures. Previous studies have determined that the *R*-isomer retains all of the desired biological activity.[10a,15,16]

The SAR of the alkyl-containing C1 substituents indicates tolerance for several functionalities including small, branched alkyl groups. Compound **8h** (R = *sec*-butyl), prepared as a mixture of four stereoisomers, was found to be the most potent analog in this series. Subsequent investigation determined that all the biological activity resided in the (*S*)-*sec*-butyl isomer (vide infra).[17]

Several analogs were prepared which incorporated heteroatoms into this C1 substituent (Table 2) however all of these analogs exhibited poor enzyme inhibition.

Despite several of these 5,8-dichloro-indole analogs displaying modest activity in the HCV replicon assay (data not shown), many of these analogs also demonstrated general cellular toxicity.[10] We have observed that the 5-cyano-8-methyl-indole-containing analogs exhibited greater separation of replicon activity and cytotoxicity and therefore we selected this template for further optimization (Scheme 3).[10,18]

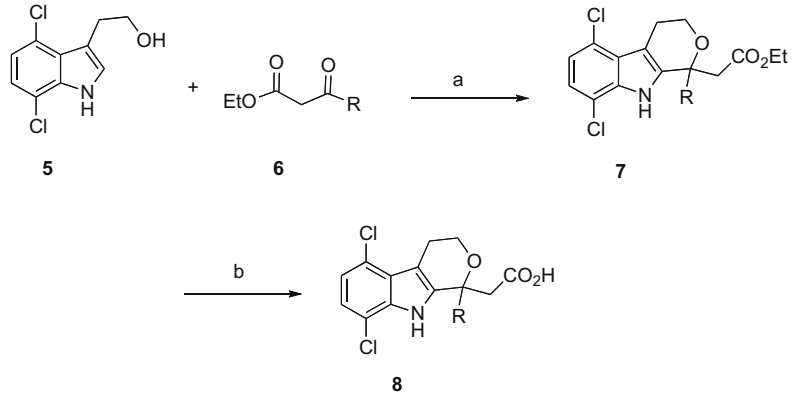
The most active compounds from this series are listed in Table 3. It was found that the (*S*)-*sec*-butyl containing group was greatly preferred over the (*R*)-isomer (cf. **11** vs **10**). Compound **11** also showed a twofold improvement in replicon cellular activity compared to the *n*-propyl containing compound **1**. The cellular activity was further enhanced when the (*S*)-*sec*-butyl substitution was incorporated onto the ethoxy-pyrazole containing scaffold (**12** vs **13**). Compound **12** has recently been reported to have in vivo activity in the chimeric mouse model of hepatitis C infection.[19,20]

While exploring the SAR around the C1 group which occupies the hydrophobic 'dimple' which occupies the C1-groups, we were also interested in the hydrogen bond interactions of the acetic acid group with the Ser^{476} and Tyr^{477} protein residues.

Incorporation of α-ketoesters into the synthetic process provided formate-based analogs (**18**, Scheme 4) with HCV polymerase inhibition comparable to the acetate analogs (**1**) but with poor activity against other NS5B isolates (data not shown).[14,18,21]

These formic acid analogs, however, showed poorer cellular replicon activities and high potential for efflux as exemplified by high basolateral to apical permeabilities in Caco-2 assays (Table 4).[22,23]

Co-crystallographic analysis of **1**, and related pyranoindole-based ligands, bound to HCV NS5B indicated an open region located proximal to the C1 position of **1** (Fig. 2). We reasoned that a suitable substituent tethered from the α-carbon of the acetate moiety of **1** would allow access to this region of the binding site.



Scheme 2. Reagents: (a) $BF_3{\cdot}OEt_2$, DCM; (b) NaOH, EtOH.

Table 1
HCV NS5B inhibitory activities of (±) pyranoindole analogs **8**

Compd	R	HCV pol (BK) IC_{50}, μM
8a	Methyl	27
8b	Ethyl	6.3
8c	*n*-Pr	0.37
8d	3,3,3-Trifluoropropyl	0.4
8e	*n*-Bu	0.76
8f	1-Butenyl	1.7
8g	2-Propyl	7
8h*	*rac-sec*-Butyl	0.15
8i	1-Cyanoethyl	0.3
8j	*c*-Pr	7.3
8k	*c*-Bu	0.35
8l	*c*-Pent	3.3
8m	*c*-Hex	>30
8n	*c*-Propylmethyl	1.9
8o	*c*-Pentylmethyl	9.4

* Mixture of four stereoisomers.

Table 2
HCV NS5B inhibitory activities of (±) pyranoindole analogs **8**

Compd	R	HCV pol (BK) IC_{50}, μM
8p	Methoxymethyl	>30
8q	Methylthiomethyl	7
8r	Ethoxymethyl	26
8s	Ethylthiomethyl	3.7
8t	Methoxyethyl	>24

We were encouraged when it was later disclosed that the structurally-unrelated ligand (**4, PF00868554**) projected a heteroaromatic substituent into this area of the protein.[8d] Our strategy opted to incorporate a nitrogen atom at the α-position that would allow different trajectories via amide, carbamate, and sulfonamide substitutions.

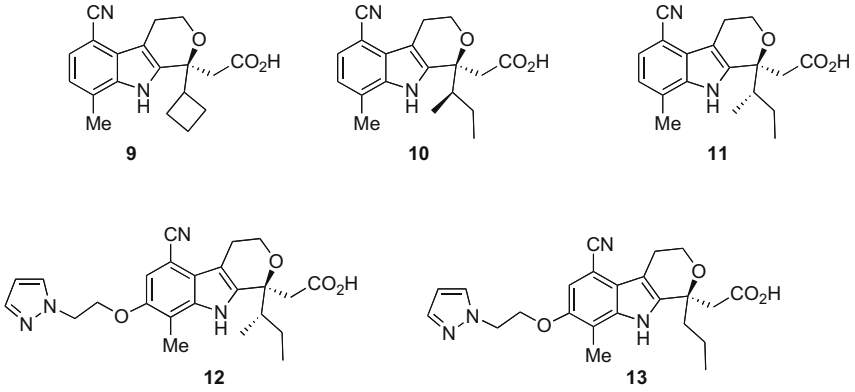
For the preparation of the amino-substituted pyranoindoles, we employed chemistry described by Maurer et al. to synthesize the ketone component (Scheme 5).[24] Opting to prepare the two diastereomers in racemic form, we treated *dl*-Cbz-serine (**19**) with an excess of *n*-propylmagnesium chloride affording the desired *n*-propyl ketone (**20**) in good yield. The key transformation was performed using 4-cyano-7-methyltryptophol **14** and 1 equiv of $BF_3{\cdot}Et_2O$ in DCM at ambient temperature to give **21** and **22** in an ~2:1 ratio by TLC, ^1H NMR analysis, and isolated yield.

Interestingly, the diastereoselection of this cyclization is under thermodynamic control since independent treatment of either pure **21** or **22** under the $BF_3{\cdot}Et_2O$ conditions reproduced **21** and **22** in an ~2:1 ratio suggesting the intermediacy of carbocationic species **23** (Fig. 3).

The assignment of relative stereochemistry was established by the synthetic intersection of **22** beginning from L-*N*-phenylsulfonylserine (not shown) and later confirmed by single crystal X-ray analysis (Supplementary data).

Having secured a reliable synthetic route to the racemates (**21** and **22**), we next sought to functionalize the amino groups within each isomer series. For illustrative purposes, the methanol group in **21** (or **22**) was oxidized in two steps ((i) IBX; (ii) $NaClO_2$) and esterified. The Cbz group was removed under standard conditions (H_2, Pd/C, Scheme 6).

An initial set of simple amides and sulfonamides were prepared to identify the stereochemical preference at the α-position. In vitro inhibition of HCV NS5B was evaluated using the truncated BB7Δ[21]



Scheme 3. 5-Cyano-8-methylpyranoindoles.

Table 3
HCV NS5B inhibitory and cellular activities of pyranoindoles

Compd	HCV pol (BK) IC_{50}, μM	HCV pol (BB7) IC_{50}, μM	HCV NS5A EC_{50}, μM	HCV RNA, μM	GAPDH μM
1	0.3	0.4	13.1	4.8	>160
9	0.4	1.1	29.7	nd	nd
(±) 10	3.2	4.9	nd	nd	nd
11	0.13	0.26	2.2	2.1	>10
12	0.009	0.002	0.12	0.02	>20
13	0.012	0.005	0.88	0.14	>90

(genotype 1b) NS5B enzyme and cellular activity was evaluated using a replicon-containing cell line (Huh7-BB7, genotype 1b, Apath, LLC, St. Louis, MO); inhibition of viral replication was monitored using an ELISA assay (HCV NS5A).[10c] Replicon results were qualified with a counter assay for general cytotoxicity (BrdU incorporation).

Table 5 lists the results from a small set of analogs prepared beginning with the major diastereomer *rac*-**21**. In general, each of the amino substituents were tolerated but only the acetate analog (**26a**) exhibited NS5B inhibition comparable to the lead **1**.

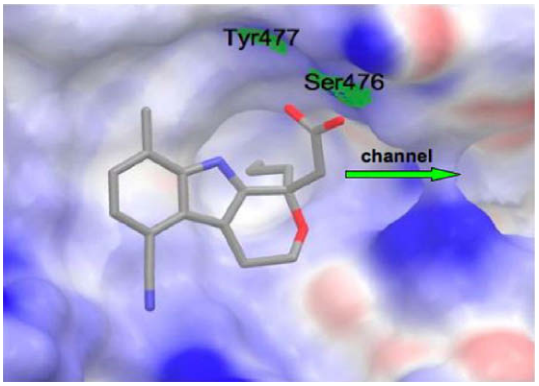
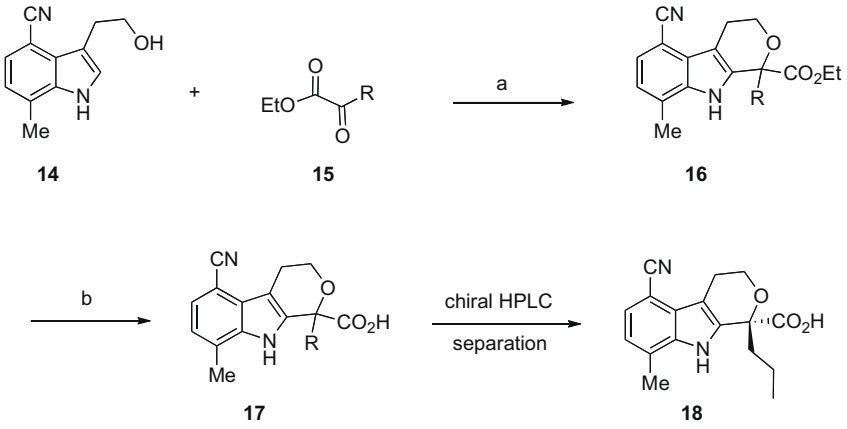


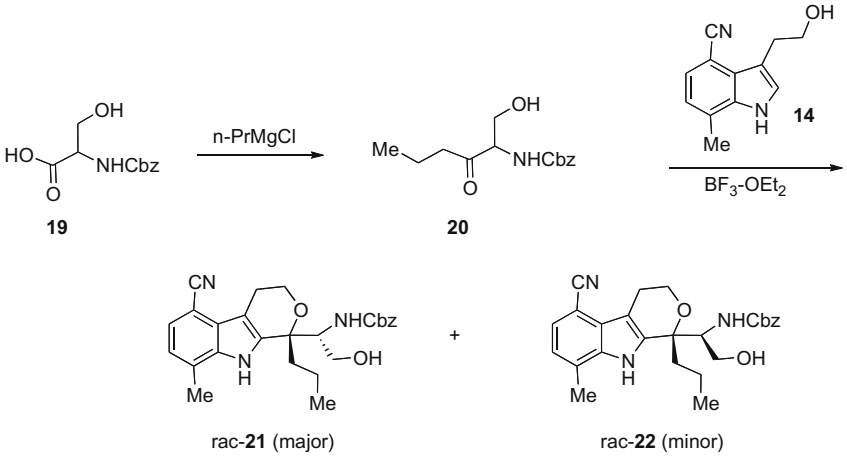
Figure 2. Crystal structure of NS5B with tetrahydro[3,4-*b*]-indole.



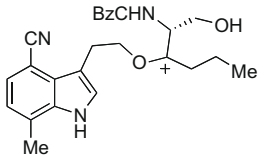
Scheme 4. Reagents: (a) $BF_3{\cdot}OEt_2$, DCM; (b) NaOH, EtOH.

Table 4
Formic acid versus acetate analogs

Compd	HCV pol (BK) IC_{50}, μM	HCV pol (BB7) IC_{50}, μM	HCV RNA EC_{50}, μM	GAPDH RNA CC_{50}, μM	P_{app} (A–B) cm/s (10^{-6})	P_{app} (B–A) cm/s (10^{-6})	P_{app}(B–A)//P_{app}(A–B) cm/s (10^{-6})
1	0.3	0.4	4.8	>30	24	2.2	0.09
12	0.009	0.002	0.02	>20	nt	nt	nt
18	0.7	3.4	12	>30	3.2	24.2	7.5



Scheme 5. Preparation of amino-alcohol intermediates.



23

Figure 3. Putative intermediate **23** in acid-mediated cyclization.

An analogous set of analogs was prepared beginning with the minor isomer *rac*-**22** (Table 6). It is apparent that these analogs were much less active than those listed in Table 5 suggesting that the *S*-configuration is preferred at the α-position. None of these amino-substituted analogs displayed activity in the whole cell assay of HCV replication suggesting that, in addition to their modest anti-polymerase activity, these analogs suffered from either high protein binding and/or poor membrane transport properties.

A final set of carbamate analogs were prepared beginning with the more active diastereomer *rac*-**21** (Table 7). The racemic Cbz-capped amine (**24**, Scheme 6) was the most active analog in the enzymatic (IC_{50} = 0.72 μM) and cellular assays (EC_{50} = 35 μM). Unfortunately, inhibition of replication in the cellular assay does not appear to be specific for HCV since incorporation of bromodeoxyuridine (BrdU) into cellular DNA was also inhibited at comparable levels. From a ligand design perspective, however, the enzyme inhibition may suggest that the carbamate moiety projects the phenyl ring of the Cbz group into the targeted sub-site in a fashion analogous to **4**.[8d]

We have described the SAR of the C1-position of tetrahydropyrano[3,4-*b*]indole based inhibitors of HCV NS5B polymerase. Through structure-based design, we have identified and detailed the preparation and biological activities of novel series containing either formic acid functionalities or α-amino-substituted-acetates. From these investigations on the pyranoindole template, a potent analog **12** was realized which was significantly more potent than

Table 5
Bioactivities of amides and sulfonamides derived from *rac*-**21**

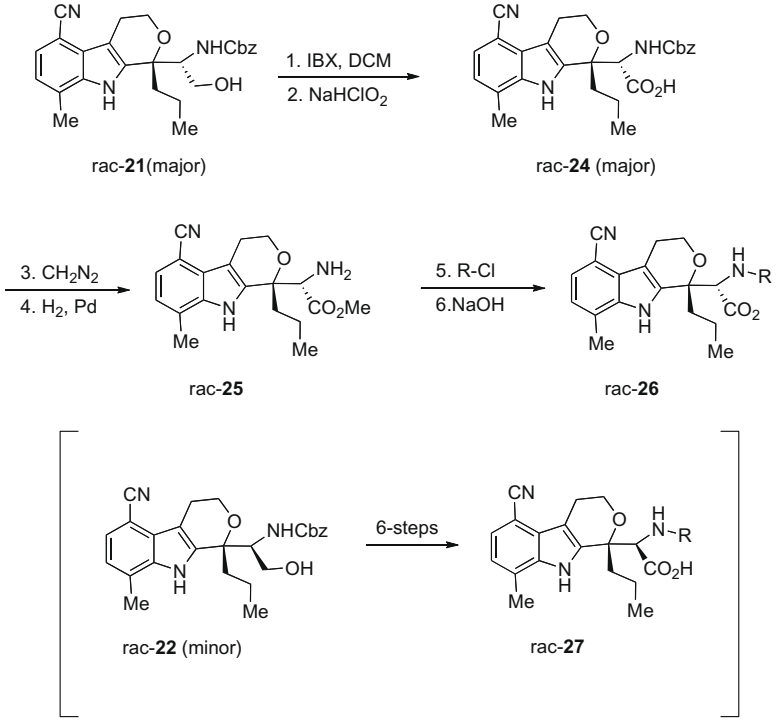
Compd	R	HCV pol (BB7) IC_{50}, μM	HCV NS5A EC_{50}, μM	BrdU CC_{50}, μM
1	–	0.4	13	>100
26a	$-C(O)CH_3$	1.7	>100	>100
26b	$-C(O)(CH_2)_3CH_3$	5	>100	>100
26c	–C(O)Ph	4.5	>100	>100
26d	$-SO_2Me$	6	>100	>100
26e	$-SO_2(CH_2)_3CH_3$	3.6	>100	>100
26f	$-SO_2Ph$	4	>100	>100

Table 6
Bioactivities of amides and sulfonamides derived from *rac*-**22**

Compd	R	HCV pol (BB7) IC_{50}, μM	HCV NS5A EC_{50}, μM	BrdU CC_{50}, μM
27a	$-C(O)CH_3$	17	>100	>100
27b	$-C(O)(CH_2)_3CH_3$	>30	>100	>100
27c	–C(O)Ph	>30	>100	>100
27d	$-SO_2Me$	13	>100	>100
27e	$-SO_2(CH_2)_3CH_3$	22	>100	>100
27f	$-SO_2Ph$	16	>100	>100

Table 7
Bioactivities of carbamates derived from *rac*-**21**

Compd	R	HCV pol (BB7) IC_{50}, μM	HCV NS5A EC_{50}, μM	BrdU CC_{50}, μM
26g	–C(O)OMe	5.6	>100	>100
26h	–C(O)OEt	7.2	>100	>100
26i	–C(O)OAllyl	5.9	>100	>100
26i	$-C(O)O^iPr$	11	>100	>100
26k	$-C(O)O^iBu$	9.5	>100	>100
26l	$-C(O)O^tBu$	18	>100	>100
24	–C(O)OBn	0.72	35	45



Scheme 6. Preparation of rac-amino-ester intermediate.

HCV-371 (**1**) and showed in vivo activity in chimeric mouse model of HCV infection.[19]

Acknowledgments

We wish to thank our collaborators at Wyeth for their assistance. We also wish to thank Victor Young (University of Minnesota) for solving the crystal structure of *rac*-**22**.

Supplementary data

Supplementary data (structure derived from single crystal X-ray analysis of **22**) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.03.002.

References and notes

1. (a) Cohen, J. *Science* **1999**, *285*, 26; (b) National Institutes of Health Consensus Development Conference Panel Statement: Management of Hepatitis C: 2002, 1.
2. (a) Alter, M. J.; Moran, D. K.; Nainan, O. V.; McQuillan, G. M.; Gao, F.; Moyer, L. A.; Kaslow, R. A.; Margolis, H. S. *N. Eng. J. Med.* **1999**, *341*, 556; (b) World Health Organization: Hepatitis C; Weekly Epidemiological Record **1997**, *72*, 65.
3. (a) Lauer, G. M.; Walker, B. D. *N. Eng. J. Med.* **2001**, *345*, 41; (b) Idéo, G.; Bellobuono, A. *Curr. Pharm. Des.* **2002**, *8*, 959; (c) Di Bisceglie, A. M.; McHutchison, J.; Rice, C. M. *Hepatology* **2002**, *35*, 224; (d) Youngster, S.; Wang, Y.-S.; Grace, M.; Bausch, J.; Bordens, R.; Wyss, D. F. *Curr. Pharm. Des.* **2002**, *8*, 2139.
4. For a recent medicinal chemistry perspective on HCV, See: Gordon, C. P.; Keller, P. A. *J. Med. Chem.* **2005**, *48*, 1.
5. Tan, S.-L.; Pause, A.; Shi, Y.; Sonenberg, N. *Nat. Rev. Drug Disc.* **2002**, *1*, 867.
6. (a) Bartenschlager, R.; Lohmann, V. *J. Gen. Virol.* **2000**, *81*, 1631; (b) Moradpour, D.; Brass, V.; Gosert, R.; Wölk, B.; Blum, H. E. *Trends Mol. Med.* **2002**, *8*, 476.
7. (a) Wu, J. Z.; Hong, Z. *Curr. Drug Targets* **2003**, *3*, 207; (b) Walker, M. P.; Hong, Z. *Curr. Opin. Pharmacol.* **2002**, *22*, 1; (c) Zhang, X. *Drugs* **2002**, *5*, 154; (d) Bartenschlager, R. *Antiviral Chem. Chemother.* **1997**, *8*, 281; (e) Dymock, B. W.; Jones, P. S.; Wilson, F. X. *Antiviral Chem. Chemother.* **2000**, *11*, 79; (f) De Clercq, E. *Nat. Rev. Drug Disc.* **2007**, *6*, 1001.
8. For recent reviews on HCV RNA RdRp inhibitors, see: (a) Lévêque, V. J.-P.; Wang, Q. M. *Cell. Mol. Life Sci.* **2002**, *59*, 909; (b) De Francesco, R.; Tomei, L.; Altamura, S.; Summa, V.; Migliaccio, G. *Antiviral Res.* **2003**, *58*, 1; (c) Condon, S. M.; LaPorte, M. G.; Herbertz, T. *Curr. Med. Chem.* **2005**, *4*, 99; (d) Li, H.; Tatlock, J.; Linton, A.; Gonzalez, J.; Jewell, T.; Patel, L.; Ludlum, S.; Drowns, M.; Rahavendran, S. V.; Skor, H.; Hunter, R.; Shi, S. T.; Herlihy, K. J.; Parge, H.; Hickey, M.; Yu, X.; Chau, F.; Nonomiya, J.; Lewis, C. *J. Med. Chem.* **2009**, *52*, 1255; (e) Li, H.; Tatlock, J.; Linton, A.; Gonzalez, J.; Borchardt, A.; Dragovich, P.; Jewell, T.; Prins, T.; Zhou, R.; Blazel, J.; Parge, H.; Love, R.; Hickey, M.; Doan, C.; Shi, S.; Duggal, R.; Lewis, L.; Fuhrman, S. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4834; (f) Beaulieu, P. L. *Curr. Opin. Invest. Drugs* **2007**, *8*, 614.
9. For HCV NS5B inhibitors demonstrating antiviral effects in early clinical trials, see: (a) Kwong, A. D.; McNair, L.; Jacobson, I.; George, S. *Curr. Opin. Pharmacol.* **2008**, *8*, 522; (b) Asselah, T.; Benhamou, Y.; Marcellin, P. *Liver Int.* **2009**, *29*, 57.
10. (a) Gopalsamy, A.; Lim, K.; Ciszewski, G.; Park, K.; Ellingboe, J. W.; Bloom, J.; Insaf, S.; Upeslacis, J.; Mansour, T. S.; Krishnamurthy, G.; Damarla, M.; Pyatski, Y.; Ho, D.; Howe, A. Y. M.; Orlowski, M.; Feld, B.; O'Connell, J. *J. Med. Chem.* **2004**, *47*, 6603; (b) Ciszewski, G.; Gopalsamy, A.; Lim, K.; Park, K.; Shi, M.; Bloom, J.; Chopra, R.; Agarwal, A.; Krishnamurthy, G.; Ellingboe, J. W.; Upeslacis, J.; Mansour, T.; Condon, S. M.; LaPorte, M. G.; Miller, L. E.; Burns, C. J.; Howe, A. Y. M.; Orlowski, M.; van Zeijl, M.; O'Connell, J. 'Structure–Activity Relationship of Substituted Pyranoindoles as HCV RNA Dependent RNA Polymerase Inhibitors'. 229th American Chemical Society National Conference, March 2005, San Diego, California.; (c) Howe, A. Y. M.; Bloom, J.; Baldick, C. J.; Benetatos, C. A.; Cheng, H.; Christensen, J. S.; Chunduru, S. K.; Coburn, G. A.; Feld, B.; Gopalsamy, A.; Gorczyca, W. P.; Herrmann, S.; Johann, S.; Jiang, X.; Kimberland, M. L.; Krisnamurthy, G.; Olson, M.; Orlowski, M.; Swanberg, S.; Thompson, I.; Thorn, M.; Del Vecchio, A.; Young, D. C.; van Zeijl, M.; Ellingboe, J. W.; Upeslacis, J.; Collett, M.; Mansour, T. S.; O'Connell, J. F. *Antimicrob. Agents Chemother.* **2004**, *48*, 4813.
11. The tryptophol intermediate was constructed by Fischer-Indole cyclization of the corresponding hydrazone, see Ref. 10.
12. The β-ketoesters were either commercially available or made by literature procedures. See: Lokot, I. P.; Pashkovsky, F. S.; Lakhvich, F. A. *Tetrahedron* **1999**, *55*, 4783.
13. All new compounds gave satisfactory ^{1}H NMR and mass spectral analyses.
14. *The pyranoindoles depicted in* Tables 1, 2 and 4 *were prepared using the following general method*: To a solution of the substituted tryptophol in DCM (0.2 M) was added the corresponding β- (or α-) ketoester (1–1.1 equiv) followed by $BF_3{\cdot}OEt_2$ (1–1.2 equiv) at room temperature. The reactions were generally complete within 2–4 h as determined by TLC (SiO_2) analysis. The reaction mixture was diluted with DCM, washed with saturated $NaHCO_3$ and brine. The organic phase was dried over Na_2SO_4, filtered, and concentrated. The crude products were purified by chromatography (SiO_2). The resulting esters were hydrolyzed using aqueous NaOH in THF/EtOH to give the desired pyranoindoles.
15. The IC_{50}'s are an average of multiple experiments with the standard deviations generally within 10% of this value.
16. *RNA dependent RNA polymerase assay*: Plasmid containing full-length BB7 NS5B gene was licensed from APATH, LLC (St. Louis, MO). The HCV NS5B region was amplified by PCR from BB7 plasmid DNA containing HCV genotype 1b (BB7), cloned expressed, and purified by *Escherichia coli*. The RNA dependent RNA polymerase (RdRp) assay was performed in a final volume of 50 μl per reaction. Twenty microliters of the NS5B enzyme mix containing 24 nM NS5BdCT21-His, 20 mM HEPES (pH 7.5), 5 mM $MgCl_2$, 1 mM DDT, 0.05 mg/ml BSA, 0.5 μM UTP, 1 μM ATP, 0.08 μM CTP, and 0.025 μM GTP (all in final concentrations) was incubated in the presence of test compounds at varying concentrations (3 nM–30 μM) or EDTA dissolved in DMSO (10 μl) for 15 min at room temperature. Concentrations of RNA and NTP's were kept at apparent K_m levels. The final concentration of DMSO present in the reaction was 3%. The reaction mixture was initiated by adding 3 nM pOF transcribed RNA substrate, 0.4 U/μl RNasin, and 0.125 μCi [α-^{33}P]GTP (indicated are final concentrations in the 50 μl reaction mix). After 120 min at room temperature, the amount of RNA synthesized was quantified by collecting the radiolabeled product RNA on Millipore multiscreen membrane filter plates. The filters containing the reaction products were allowed to dry at room temperature and counted in a Wallac Microbeta after an addition of 50 μl of Optiphase™ scintillant. Inhibition data were analyzed using the sigmoidal dose–response (variable slope) equation in GraphPad Prism (GraphPad Software Inc., San Diego, CA). The 50% inhibitory concentration (IC_{50}) was the drug concentration that decreased the enzyme activity by 50% relative to control samples incubated without compound.
17. The chiral β-ketoester derived from (*S*)-(+)-2-methylbutyric acid was prepared to afford diastereomeric pyranoindoles which were separated using chiral chromatography.
18. Chiral HPLC separation using CHIRALPACK AD, mobile phase Heptane/IPA.
19. LaPorte, M. G.; Jackson, R. W.; Draper, T. L.; Gaboury, J. A.; Galie, K.; Herbertz, T.; Hussey, A. R.; Rippin, S. R.; Benetatos, C. A.; Chunduru, S. K.; Christensen, J. S.; Coburn, G. A.; Rizzo, C. J.; Rhodes, G.; O'Connell, J.; Howe, A. Y. M.; Mansour, T.; Collett, M. S.; Pevear, D. C.; Young, D. C.; Gao, T.; Tyrrell, D. L. J.; Kneteman, N. M.; Burns, C. J.; Condon, S. M. *ChemMedChem* **2008**, *3*, 1508.
20. At this point, the reason for the increased activities of the pyrazole containing compounds is not well understood.
21. For the preparation of α-ketoesters **15**, See: Wasserman, H. H.; Ho, W. B. *J. Org. Chem.* **1994**, *59*, 4364.
22. Hillgren, K. M.; Kato, A.; Borchardt, R. T. *Med. Res. Rev.* **1995**, *15*, 83.
23. Efflux is considered significant if: P_{app}(B–A) $\geqslant 1.0 \times 10^{-6}$ cm/s and, P_{app}(B–A)/P_{app}(A–B) $\geqslant 3.0 \times 10^{-6}$ cm/s.
24. Maurer, P. J.; Takahata, H.; Rapoport, H. *J. Am. Chem. Soc.* **1984**, *106*, 1095.