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Synthesis and evaluation of novel potent HCV NS5A inhibitors

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Abstract

Judicious modifications to the structure of the previously reported HCV NS5A inhibitor**1**, resulted in more potent anti-HCV compounds with similar and in some cases improved toxicity profiles. The synthesis of nineteen new NS5A inhibitors is reported along with their ability to block HCV replication in an HCV 1b replicon system. For the most potent compounds chemical stability, stability in liver microsomes and inhibition of relevantCYP450 enzymes is also presented.

Keywords

NS5A; Antiviral; HCV

Hepatitis C virus (HCV) is the leading cause of chronic liver disease, liver transplantation and is the most common blood-borne infection in developed countries.[1] The prevalence of HCV infection ranges from 1–5% in most developed countries and is estimated to be 5-fold greater than HIV infection.[1] Until recently, the only approved drugs for the treatment of HCV were pegylated interferon-α (IFN) and ribavirin (RBV), both of which are poorly tolerated and have limited efficacy, with less than 50% response rates among individuals infected with the most common virus genotype (1b).[2] The FDA approval, in May 2011, of the protease inhibitors (PI) boceprevir (Victrelis) and telaprevir (Incivek) which created a lot of excitement however, their real impact on standard of care (SOC) remains unclear as sustained virologic response (SVR) for genotype 1 HCV is still only about 70 to 80% when administered with IFN and RBV.[3]

Efforts toward improved HCV treatment include the development of antiviral agents that inhibit HCV via new targets such as the viral NS5A protein. Despite significant gaps in the understanding of the role of NS5A in the virus replication cycle, it is known that it plays an integral role in a number of host and/or pathogen events such as RNA replication, assembly of viral particles and host immune response.[4] NS5A phosphokinase is phosphorylated by cellular protein kinases and the phosphorylation sites are conserved among the various HCV genotypes.[5,6]Recently, the NS5A inhibitor BMS-790052 (Figure 1) was discovered and displayed *in vitro* potency ((EC_{50}) of ≤ 50 pM)[7] that translated to rapid viral load decline (≈

3.3 $\log_{10}$) after a single 100 mg dose with no signs of toxicity.[8] While compound **1** has established NS5A as a valid target for effective clinical treatment of HCV, it also provided a foundation for the design of second-generation inhibitors that could be more resistant to selection of mutant virus with reduced potential cytotoxic liabilities. This lead us to dissect the symmetrical inhibitor **1** into four major structural zones: **zone I** (biphenyl linker zone) has already been studied extensively by other research groups; **zone II** (imidazole portion) despite claims of small modifications no significant exploration of this area has been disclosed; **zone III** (proline moiety), minor modifications such as fluorination or cyclopropanation have been reported; **zone IV** (amino acid and its "cap") has been extensively studied.[8,9]

In consideration of novelty and retention of potency, we initially chose to focus on both zones II and III. Indeed, the lack of significant exploration of zone II along with the ease of halogenation of the imidazole ring, which would allow for the efficient preparation of more complex structures using transition metal catalyzed cross coupling reactions, made zone II ideal for investigation. Similarly, zone III has not been extensively explored while readily available proline analogs would allow for extensive modifications from well known organic transformations. Herein we wish to report our preliminary structure activity relationship (SAR) efforts for zones II and III.

The biphenyl core of the targeted compounds was prepared according to general Scheme 1. Diketone **1** was first converted to the α, α′-dibromodiketone using Br_2 in CH_2Cl_2 then coupled with *N*-Boc protected proline derivatives **A**[10] in the presence of DIPEA. Refluxing the corresponding ester intermediates with ammonium acetate in toluene allowed for the formation of the imidazole ring and lead to the versatile Boc protected intermediates **3a**-**c** in 63% to 75%yields.

Exploration of 4-substituted imidazole derivatives, as shown in Scheme 2, marked the beginning of our synthetic efforts and initial SAR studies. Bromination of the 4-position of the imidazoles of **3a** was accomplished using *N*-bromosuccinimide (NBS) in dichloromethane (52% yield). After removal of Boc protection with 6N HCl, the resulting amine was coupled with *N*-methylcarbamate protected valine using *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) and *N*-hydroxybenzotriazole (HOBt) in the presence of *N*, *N*-diisopropylethylamine (DIPEA). The palladium catalyzed cross coupling reaction of **5a** under Stille conditions[11] resulted in 4-aryl and 4-heteroaryl compounds **6**-**8** in moderate yields providing the first in this zone II series of aryl substituted imidazoles.

Introduction of thiophene, furan or phenyl at the 4-position of the imidazole lead to a noticeable decrease of potency toward HCV replication (EC_{50} of 100, 26 and 200 pM respectively) when compared to **1**. Evaluation of the brominated intermediate **5a** for anti-replication activity *versus* HCV proved surprising as it displayed an EC_{50} of 5.9 pM and EC_{90} of 14 pM; thus compound **5a** was equipotent to BMS-790052 at the EC_{50} and EC_{90}. This unexpected result prompted us to investigate the influence of other halogens at the 4-position of the imidazole ring. Iodine and chlorine were thus introduced on the imidazole ring of compound **3a** using *N*-iodosuccinimide (NIS) and *N*-chlorosuccinimide (NCS) respectively while fluorine was introduced by careful reaction with *N*-fluorobenzenesulfonimide (NFSI) and $NaHCO_3$. It is noteworthy that this fluorination reaction leads to the formation of both the difluorinated derivative **4d** and the monofluorinated derivative **4e**. As above, simple Boc deprotection under acidic conditions followed by the coupling of *N*-methylcarbamate protected valine in the presence of HOBt and DIPEA resulted in the dihalogeneated compounds **5b**-**d** and monofluoro derivative **5e**. Remarkably the bis-iodo, bis-chloro and bis-fluoro compounds **5b**, **5c** and **5d** exhibited an

EC_{50} of 6.4 ± 3.0, 5.5 ± 1.9 and 6.8 ± 3.3 pM respectively which, as in the case of the bis-bromo compound, are equipotent to the reference compound **1**. The EC_{90} values for these three were all close to 10 pM representing a modest improvement relative to the reference compound **1**. In addition, all of the bis-halogen compounds **5c** and **5d** exhibited increased toxicity in primary human lymphocytes, although they were non-toxic at concentrations up to 100 μM in Vero cells. These results suggest that a small modification in this region (Zone II) such as introduction of a halogen is well tolerated but introduction of larger groups such as thiophene, furane and phenyl are detrimental to the HCV replicon activity.

Zone III modifications (Scheme 3) included introduction of various triazoles using copper catalyzed alkyne azide cycloadditions.[12] Azido intermediates **3b** and **3c** were first deprotected under acidic conditions then coupled with *N*-methylcarbamate protected valine in presence of HOBt and DIPEA to give compounds **9**-**10** in respectively 64 and 59% yields. Finally, treatment of **9**-**10** with various alkenes in the presence of $CuSO_4$ and sodium ascorbate afforded the 1,4-triazolo derivatives **11**-**14** in good yields. Like for Zone II, introduction of bulky modifications such as 1,4-substituted triazoles on the proline moiety (Zone III) considerably decreased potencies. On the other hand, introduction of a small group such an azido group seems to be well tolerated. Indeed, (4-*R*)-azido compound **10** with an EC_{50} = 9.5 pM appeared as potent as reference compound **1** without displaying any signs of toxicity up to 100 μM in PBM, CEM and Vero cells. Interestingly, (4-*S*)-azido compound **9** appeared slightly less active (EC_{50} = 23 pM) and showed toxicities in PBM and CEM cells (IC_{50} = 20.3 and 18.4 μM respectively).

Based on these results, a combination of our best modifications in both zone II and III was also studied. Thus, (4-*S*)- and (4-*azido compounds 9 and 10 were halogenated at the R*)-azido compounds **9** and **10** were halogenated at the 4-position of the imidazoles rings by using either NBS or NCS in CH_2Cl_2 (Scheme 4) and all the synthesized compounds displayed an $EC_{50} \leq 10$ pM. Interestingly, compound **17** showed an EC_{50} of 2.6 pM with no toxicity toward Vero cells up to 100 μM.

Among all the molecules synthesized, the most promising compounds **5a**-**d** were further investigated. First of all, their stability at pH 3 and 7 in phosphate buffer was evaluated. After 48 h at 20°C, LC/MS analysis at time 0, 1, 8, 24 and 48 h revealed no evidence of degradation of these compounds. Next stability in human liver microsomes was established (Table 2). Compounds **5a**-**d** (1 μM) were incubated with human liver microsomes in potassium phosphate buffer and 1 mg/mL microsomal protein. Samples were collected at 5, 15, 30 and 60 min, analyzed by LC-MS/MS and half-life ($t_{1/2}$) and intrinsic clearance (CL_{int}) for each compound were calculated based on the elimination rate constant (k). Interestingly, compounds **5b** and **5d** show higher metabolic stability and therefore lower clearance ($CL_{int} < 8.6$) compared with BMS-790052.

Drug-drug interactions have become an important issue in modern health care. It is now apparent that many drug-drug interactions can be explained by alterations in the metabolic lenzymes that are present in the liver and other extra-hepatic tissues. Many of the major pharmacokinetic interactions between drugs are due to hepatic cytochrome P450 (CYP450) enzymes being affected by one or more of the drugs present in a given individual. Furthermore, drug interactions can be a result of inhibition or induction of CYP450 enzymes. The potential drug-drug interaction liabilities of these compounds were investigated by a CYP450 reversible inhibition assay. Compounds **5b** and **5d** did not appear to inhibit the major CYP450 enzymes at the IC_{90} level and had a favorable CYP profile, which is suggestive of no potential drug-drug interactions (Table 3). Due to the large therapeutic window, the inhibition observed for 3A4 (in the micromolar range) is expected not to be clinically relevant at therapeutic doses.

Initial resistant virus selection studies using **5b** and BMS-790052 were performed and mutations identified by population sequencing. Mutations observed for BMS-790052 included L31V, which was previously reported,[13] in addition to several unreported mutations, which we will disclose in full detail in subsequent publications. Compound **5b** selected for several different mutations including Y93H, which was previously reported as an NS5A resistance mutation.[13]

In conclusion, extremely potent new NS5A inhibitors (picomolar activity) with a wide therapeutic window (> 10^4) are disclosed. Among all the compounds prepared **5b** and **5d** exhibited improved liver microsome stability compared to reference compound BMS-790052 and no inhibition of major human CYP enzymes was exhibited at therapeutically relevant concentrations. In addition, these two halogenated compounds displayed improved stability toward human liver microsomes *versus* BMS-790052 while maintaining comparable anti-HCV replication activity. Finally, further mutation selection studies are currently being investigated in our laboratory and will be the subject of future publications.

Acknowledgments

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References and notes

1. Liang, JT.; Hoofnagle, JH. Hepatitis C, Biomedical Research Reports. Academic Press; London, UK: 2000. p. 185-203.
2. Bobeck DR, Schinazi RF, Coats SJ. Antivir Ther. 2010; 15:935. [PubMed: 21041908]
3. Sheridan C. Nature Biotech. 2011; 29:553.
4. Schinazi RF, Bassit L, Gavegnano CJ. Viral Hepatitis. 2010; 17:77.
5. Katze MG, Kwieciszewski B, Goodlett DR, Blakely CM, Neddermann P, Tan SL, Aebersold R. Virology. 2000; 278501
6. Kim J, Lee D, Choe J. Biochem Biophys Res Commun. 1999; 257777
7. Fridell RA, Qiu D, Wang C, Valera L, Gao M. Antimicrob Agents Chemother. 2010; 54:3641. [PubMed: 20585111]
8. Gao M, Nettles RE, Belema M, Snyder LB, Nguyen VN, Fridell RA, Serrano-Wu MH, Langley DR, Sun JH, O'Boyle DR 2nd, Lemm JA, Wang C, Knipe JO, Chien C, Colono RJ, Grasela DM, Meanwell NA, Hamann LG. Nature. 2010; 465:96. [PubMed: 20410884]
9. (a) Sun, JH.; Gao, M.; O'Boyle, DR., II; Lemm, JA.; Roberts, SB.; Belema, M.; Meanwell, NA. PCT Int Appl. WO 2012009394 A2 20120119. 2012. (b) Lopez, OD.; St Laurent, DR.; Goodrich, J.; Romine, JLee; Serrano-Wu, M.; Yang, FK.; Kakarla, R.; Yang, XJ.; Qiu, YP.; Snyder, LB. US Pat Appl Publ. US 20110294819 A1 20111201. 2011. (c) Belema, M.; Romine, JL.; Nguyen, VN.; Wang, G.; Lopez, OD.; St Laurent, DR.; Chen, Q.; Bender, JA.; Bender, JA.; Yang, Z.; Hewawasam, P.; Xu, NN.; Meanwell, NA.; Easter, JA.; Su, BN.; Smith, MJ. US Pat Appl Publ. US 20110237636 A1 20110929. 2011. d) Belema, M.; Hewawasam, P. US Pat Appl Publ. US 20110237636 A1 20110929. 2011. (d) Bender, JA.; Hewawasam, P.; Kadow, JF.; Lopez, OD.; Meanwell, NA.; Nguyen, VN.; Romine, JL.; Snyder, LB.; St Laurent, DR.; Wang, G.; Xu, NN.; Belema, M. PCT Int Appl. WO 2010117635 A1 20101014. 2010. (e) Belema, M.; Nguyen, VN.; Serrano-Wu, M.; St Laurent, DR.; Qiu, YP.; Ding, M.; Meanwell, NA.; Snyder, LB. US Pat Appl Publ. US 20100080772 A1 20100401. 2010. (f) Bachand, C.; Belema, M.; Deon, DH.; Good, AC.; Goodrich, J.; Hamann, LG.; James, CA.; Langley, DR.; Lavoie, R.; Lopez, OD.; Martel, A.; Meanwell, NA.; Nguyen, VN.; Romine, JL.; Ruediger, EH.; Snyder, LB.; St Lauurent, DR.; Yang, FK.; Wang, G. PCT Int Appl. WO 2008144380 A1 20081127. 2008. (g) Milbank, JBJ.; Tran, TD.; Wakenhut, F. PCT Int Appl. WO 2011154871 A1 20111215. 2011. (h) Vandyck, K.; Verschueren, WG.; Raboisson, PJMB. PCT Int Appl. WO 2012013643 A1 20120202. 2012. (i) Li, LP.; Zhong,

M. PCT Int Appl. WO 2011156543 A2 20111215. 2011. (j) Qiu, YL.; Wang, C.; Ying, L.; Peng, XW.; Or, YS. PCT Int Appl. WO 2010091413 A1 20100812. 2010. (k) Li, LP.; Zhong, M. PCT Int Appl. WO 2011150243 A1 20111201. 2011. (l) Kim, S.; Gao, Q.; Yang, FK. US Pat Appl Publ. US 20100158862 A1 20100624. 2010. (m) Bachand, C.; Belema, M.; Deon, DH.; Good, AC.; Godrich, J.; James, CA.; Lavoie, R.; Lopez, OD.; Martel, A.; Meanwell, NA.; Nguyen, VN.; Romine, JL.; Ruediger, EH.; Snyder, LB.; St Laurent, DR.; Yang, FK.; Langley, DR.; Wang, G.; Hamann, LG. PCT Int Appl. WO 2009102318 A1 20090820. 2009. (n) Bachand, C.; Belema, M.; Deon, DH.; Good, AC.; Goodrich, J.; James, CA.; Lavoie, R.; Lopez, OD.; Martel, A.; Meanwell, NA.; Nguyen, VN.; Romine, JL.; Ruediger, EH.; Snyder, LB.; St Laurent, DR.; Yang, FK.; Langley, DR.; Hamann, LG. PCT Int Appl. WO 2008021936 A2 20080221. 2008. (o) Belema, M.; Romine, JL.; Nguyen, VN.; Wang, G.; Lopez, OD.; St Laurent, DR.; Chen, Q.; Bender, JA.; Yang, Z.; Hewawasam, P.; Xu, NN.; Meanwell, NA.; Easter, JA.; Su, BN.; Smith, MJ. PCT Int Appl. WO 2011075439 A1 20110623. 2011. (p) Corte, JR.; Fang, T.; Decicco, CP.; Pinto, DJP.; Rossi, KA.; Hu, ZL.; Jeon, Y.; Quan, ML.; Smallheer, JM.; Wang, YF.; Yang, W. PCT Int Appl. WO 2011100401 A1 20110818. 2011.

10. Webb TR, Eigenbrot CJ. Org Chem. 1991; 56:3009.
11. Shrestha S, Bhattarai BR, Lee KH, Cho H. Bioorg Med Chem. 2007; 15:6535. [PubMed: 17692525]
12. For reviews on the CuAAC see(a) Hein JE, Fokin VV. Chem Soc Rev. 2010; 39:1302. [PubMed: 20309487] (b) Aragao-Leoneti V, Campo VL, Gomes AS, Field RA, Carvalho I. Tetrahedron. 2010; 66:9475.(c) Amblard F, Cho JH, Schinazi RF. Chem Rev. 2009; 109:4207. [PubMed: 19737023]
13. Lemm J, O'Boyle D II, Liu M, Nower PT, Colonno R, Deshpande MS, Snyder LB, Martin SW, St Laurent DR, Serrano-Wu MH, Romine JL, Meanwell NA. Virol. 2010; 84:482.

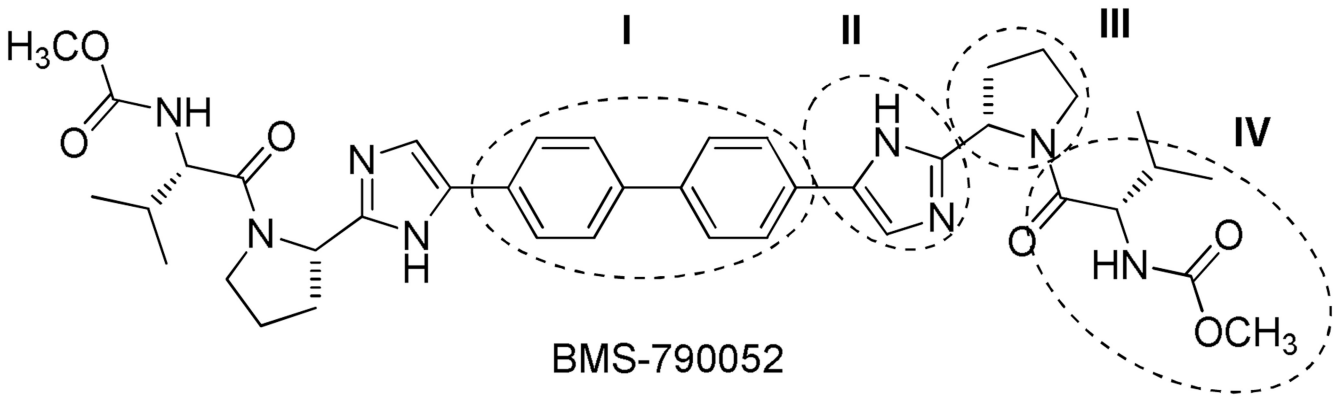
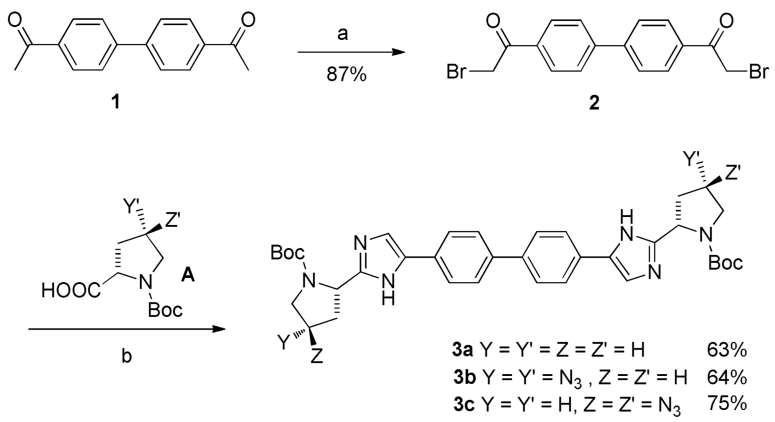
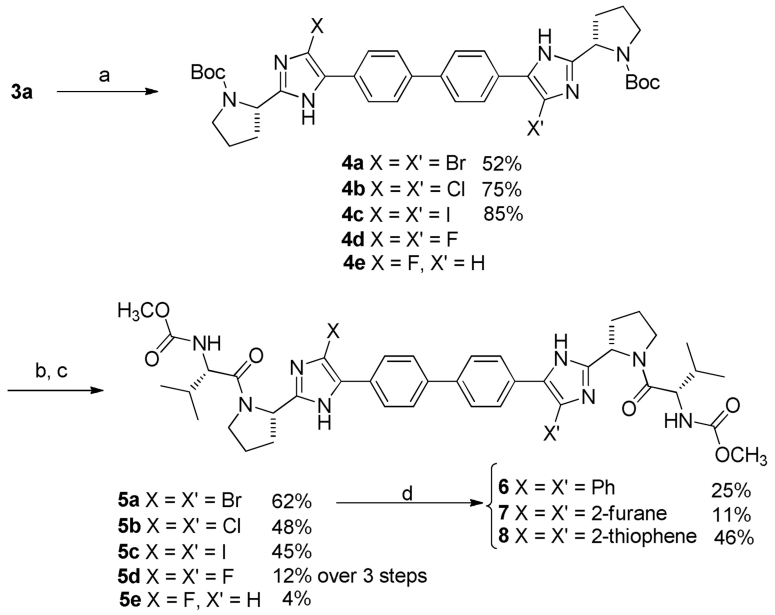


Figure 1.
Chemical structure of BMS-790052



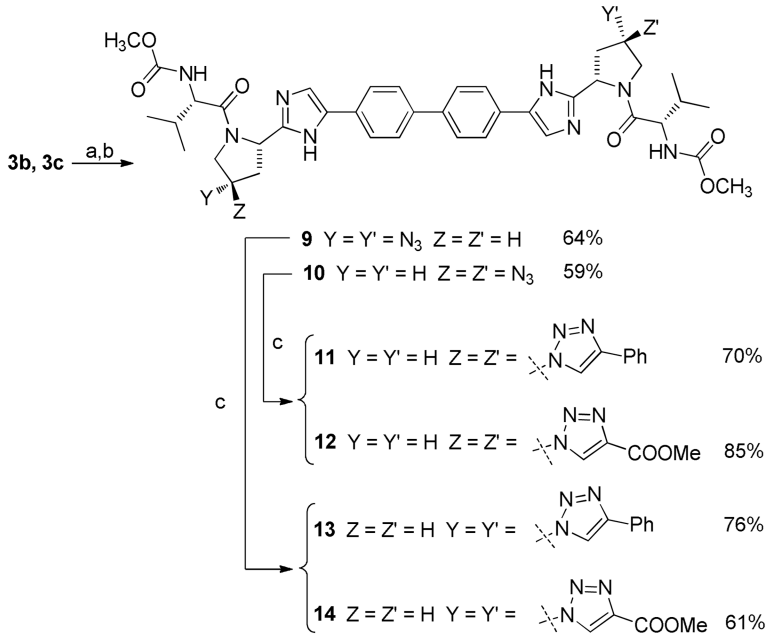
Scheme 1.

Reagents and conditions: (a) Br_2, CH_2Cl_2, rt, overnight; (b) (i) **A**, DIPEA, rt, 5 h; (ii) NH_4OAc, toluene, 100-110 °C, 15-20 h.



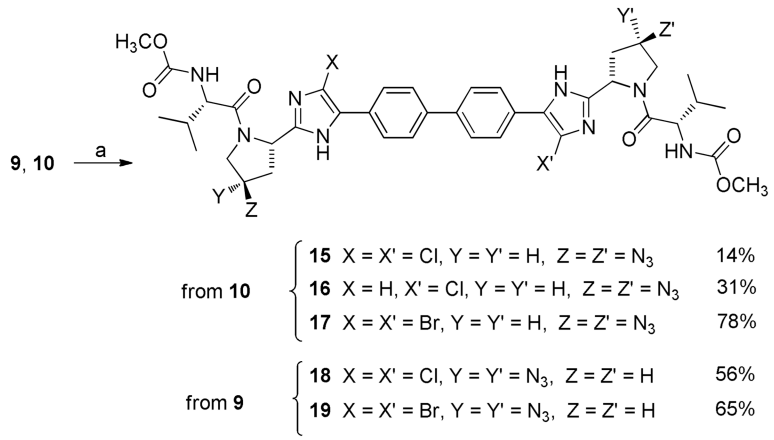
Scheme 2.

Reagents and conditions: (a) X = Br, I: NXS, CH_2Cl_2, rt, 30 min; X = Cl: NCS, 40 °C, 15 h; X = F, NFSI, $NaHCO_3$, CH_2Cl_2/acetone (1:1), 50 °C. (b) 6 N HCl, CH_3OH, 50 °C, 5 h; (c) HOOC-CH[$CH(CH_3)_2$]-$NHCOOCH_3$, EDC, HOBt, DIPEA, 0 °C – rt, 15 h; (d) R-$Sn(Bu)_3$, $Pd(PPh_3)_2Cl_2$, dioxane, 80 °C, overnight.



Scheme 3.

Reagents and conditions: (a) 6 N HCl, CH_3OH, 50 °C, 5 h (b) HOOC-CH[$CH(CH_3)_2$]-$NHCOOCH_3$, EDC, HOBt, DIPEA, 0 °C, rt, overnight. (c) alkyne, sodium ascorbate, $CuSO_4.5H_2O$, *t*-BuOH/H_2O (1:1), rt, overnight.



Scheme 4.

Reagents and conditions: (a) X = Br: NBS, CH_2Cl_2, rt, 0.5-1 h; X = Cl: NCS, CH_2Cl_2, 50 °C, 4-24 h.

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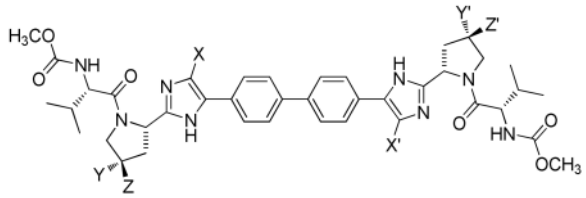
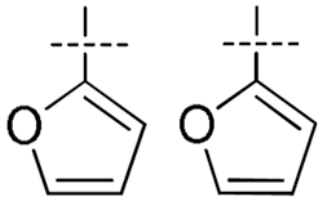
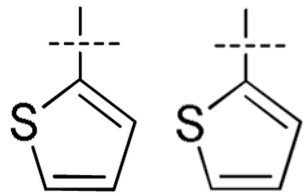
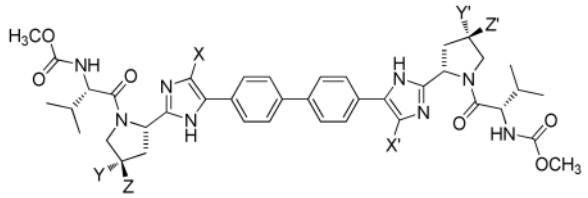
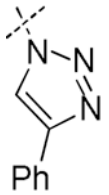
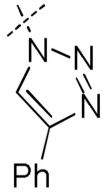
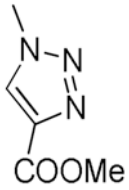
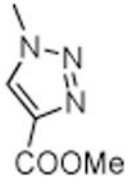
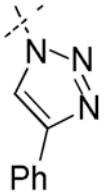
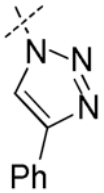
Table 1

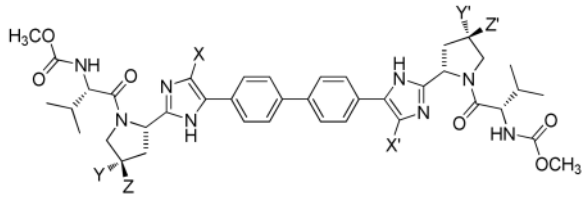
Chemical Structure, Anti-HCV Activity and Cytotoxicity of Compounds 5-19

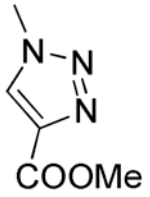
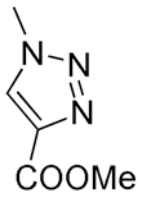


Cmpd	X	X′	Y	Y′	Z	Z′	HCV Replicon (pM)		Cytotoxicity, CC_{50} (μM)*		
							EC_{50}	EC_{90}	PBM	CEM	Vero
BMS-790052	H	H	H	H	H	H	4.2 ± 1.5	20 ± 7.5	19	9.6	21
5b	Cl	Cl	H	H	H	H	6.4 ± 3.0	11.4 ± 4.8	2.8	12	≥ 100
5a	Br	Br	H	H	H	H	5.9 ± 3.9	14 ± 8.3	2.9	5.7	84
5c	I	I	H	H	H	H	5.5 ± 1.9	11 ± 2.2	4.7	35	> 100
5d	F	F	H	H	H	H	6.8 ± 3.3	21.2 ± 11.0	19	3.0	> 100
5e	H	F	H	H	H	H	14.4 ±4.0	52.5 ±11.1	16	2.8	83
6	Ph	Ph	H	H	H	H	200	700	> 100	37.4	> 100
7			H	H	H	H	26	97	49.2	2.9	>100
8			H	H	H	H	100	300	44.6	> 100	> 100
9	H	H	N_3	N_3	H	H	23	35.2	20.3	18.4	> 100



Cmpd	X	X′	Y	Y′	Z	Z′	HCV Replicon (pM)		Cytotoxicity, CC_{50} (μM)*		
							EC_{50}	EC_{90}	PBM	CEM	Vero
10	H	H	H	H	N_3	N_3	18.4 ± 12.6	54.2 ± 34.1	> 100	≥ 100	> 100
11	H	H	H	H			2700	9200	> 100	> 100	> 100
12	H	H	H	H			11.8	42.8	6.6	10.7	> 100
13	H	H			H	H	757 10^2	> 10^4	96	> 100	> 100



Cmpd	X	X′	Y	Y′	Z	Z′	HCV Replicon (pM)		Cytotoxicity, CC_{50} (μM)*		
							EC_{50}	EC_{90}	PBM	CEM	Vero
14	H	H			H	H	> 10^4	> 10^4	> 100	> 100	> 100
15	Cl	Cl	H	H	N_3	N_3	4.7	9.6	7.8	3.8	> 100
16	Cl	H	H	H	N_3	N_3	6.3	29.5	9.7	31	> 100
17	Br	Br	H	H	N_3	N_3	2.6	21.6	19.8	10.8	> 100
18	Cl	Cl	N_3	N_3	H	H	11.2	74.6	3.3	4.0	98.1
19	Br	Br	N_3	N_3	H	H	8.3	55.3	3.4	4.5	> 100

*All compounds had a CC_{50} of > 1 μM in Huh-7 cells

Table 2

Half-life and intrinsic clearance values in human liver microsomes of compounds 5a-d and BMS-790052

Cmpd	Elimination rate constant (k)	$t_{1/2}$ (min)	CL_{int} (μL/min/mg protein)
5a	0.01	67.3	10.3
5b	0.0058	119.5	5.8
5c	0.018	38.5	18.0
5d	0.0056	123.8	5.6
BMS-790052	0.012	56.3	12.3

Table 3

Cytochrome P450 inhibition data for compounds 5b, 5d and BMS-790052[a]

	CYP1A2 (μM)		CYP3A4 (μM)		CYP2D6 (μM)		CYP2C9 (μM)	
Cmpd	**IC_{50}**	**IC_{90}**	**IC_{50}**	**IC_{90}**	**IC_{50}**	**IC_{90}**	**IC_{50}**	**IC_{90}**
5b	> 100	> 100	13.8 ± 11.2	> 100	> 100	> 100	81.6 ± 26.1	> 100
5d	> 100	> 100	3.1 ± 2.3	> 100	> 100	> 100	> 100	> 100
BMS-790052	> 100	> 100	7.2 ± 1.7	> 100	> 100	> 100	59.5 ± 13.6	> 100

[a]Mean ± SD of at least two independent assays; IC_{50} = 50% inhibitory concentration. The following positive controls were used (IC_{50}, μM): α -naphthoflavone for 1A2 (> 93% at 3 μM); ketoconazole for 3A4 (> 93% at 10 μM); PH-053 (proprietary) for 2D6 (> 70% at 100 μM); sulfaphenazole for 2C9 (> 90% at 10 μM).