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Antiviral 2,5-disubstituted imidazo[4,5-*c*]pyridines: Further optimization of anti-hepatitis C virus activity

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Abstract—Substituted 5-benzyl-2-phenyl-5*H*-imidazo[4,5-*c*]pyridines represent a novel class of compounds with activity against pestiviruses and the hepatitis C virus (HCV). Several series of analogues with modifications of the substituents in positions 2 and 5 were prepared. These efforts resulted in the discovery of several compounds with potent antiviral activity of which 2-(2,3-difluorophenyl)-5-[4-(trifluoromethyl)benzyl]-5*H*-imidazo[4,5-*c*]pyridine (**46**) was most potent against HCV (EC_{50} of 0.10 μM and a selectivity index of 1080).

Worldwide more than 170 million people are chronically infected with the hepatitis C virus and thus at increased risk of developing serious life-threatening liver disease (including chronic hepatitis, cirrhosis and hepatocellular carcinoma). Current standard therapy for chronic hepatitis C consists of the combination of pegylated IFN-α 2a in combination with ribavirin. However, this therapy is only effective in 50–60% of the patients and associated with serious side-effects.[1,2] There is, therefore, an urgent need for potent and non-toxic drugs for the treatment of HCV infections.

We recently reported on the identification of a class of imidazo[4,5-*c*]pyridine analogues with potent anti-pestivirus activity.[3,4] Pestiviruses belong, together with the flaviviruses and HCV, to the family of the Flaviviridae. Introduction of a fluorine atom in position 2 of the phenyl of 5-(4-bromobenzyl)-2-phenyl-5*H*-imidazo[4,5-*c*]pyridine resulted in a compound with not only anti-pesti (bovine viral diarrhoea virus (BVDV)) but also anti-HCV activity (compound **1**, Table 1).[5] To further understand the structure–activity relationships for HCV within this class of compounds and to identify analogues with improved anti-HCV activity, we have synthesized[6] several sets of substituted 5-benzyl-2-phenyl-5*H*-imidazo[4,5-*c*]pyridines.

First we have introduced additional substituents starting from the lead compound **1**. An additional fluorine on the 2-phenyl was well tolerated with respect to anti-BVDV activity,[7] but only the 2,3-difluoro analogue **2** was active against HCV in a subgenomic HCV replicon system[8] with slightly improved activity and selectivity, compared to the lead compound (compounds **2**–**5**, Table 1). The 2,3,6-trifluoro analogue **6** was less active than the lead compound **1** both against BVDV and HCV. Introduction of a chlorine in position 3 of the 2-phenyl resulted in an analogue (**7**) with excellent anti-BVDV but slightly reduced anti-HCV activity. Introducing a trifluoromethyl group in the same position resulted in reduced antiviral activity (compound **8**). Interestingly, an additional fluorine in position 2 of the 5-benzyl improved anti-BVDV activity but was detrimental for anti-HCV activity (compound **9**).

In the second series the substituent on the 5-benzyl residue was modified (Table 2). In general, these modifications did not affect the anti-BVDV activities with the exception of 4-carboxy analogue **19**. The unsubstituted analogue **10** and all three methyl-substituted compounds (**11**–**13**) proved inactive against HCV. All other analogues prepared were substituted in position 4 of the benzyl. From these the 4-isopropyl (**14**), the 4-*tert*-butyl

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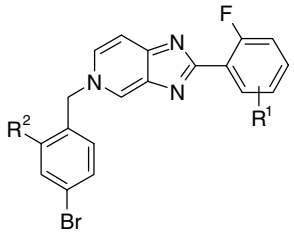
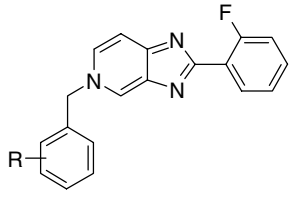
Table 1. Structure, anti-BVDV activity, anti-HCV activity and cytotoxic/cytostatic activity of compounds **1–9**



Compound	R^1	R^2	BVDV			HCV		
			EC_{50}[a] (μM)	CC_{50}[a] (μM)	SI[b]	EC_{50}[a] (μM)	CC_{50}[a] (μM)	SI[b]
1	H	H	0.12 ± 0.01	>33	>275	3.0 ± 1.6	136 ± 42	45
2	3-F	H	0.24 ± 0.12	>104	>433	0.60 ± 0.2	>55 ± 27	>92
3	4-F	H	0.50 ± 0.28	>104	>208	45 ± 22	65	1.4
4	5-F	H	0.08 ± 0.04	>104	>1300	75	115	1.5
5	6-F	H	0.48 ± 0.27	>104	>217	75	80	1.07
6	3,6-F_2	H	0.53 ± 0.10	>104	>196	69	77	1.1
7	3-Cl	H	0.12 ± 0.04	>104	>867	1.2 ± 1.3	36 ± 1.7	30
8	3-CF_3	H	6 ± 2	>104	>17	47 ± 11	>125	>2.38
9	H	F	0.050 ± 0.02	>104	>2080	>52	52	n.a.

[a] Data (for active compounds) are mean values ± standard deviation for 4–6 independent experiments.
[b] In vitro selectivity index (CC_{50}/EC_{50}).

Table 2. Structure, anti-BVDV activity, anti-HCV activity and cytotoxic/cytostatic activity of compounds **10–28**



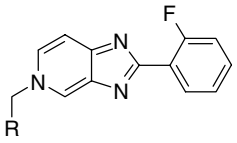
Compound	R	BVDV			HCV		
		EC_{50}[a] (μM)	CC_{50}[a] (μM)	SI[b]	EC_{50}[a] (μM)	CC_{50}[a] (μM)	SI[b]
10	H	0.29 ± 0.09	>52	>179	>162	162	n.a.
11	2-CH_3	0.29 ± 0.08	>104	>359	95	101	1.06
12	3-CH_3	0.25 ± 0.11	>104	>416	>85	85	n.a.
13	4-CH_3	0.20 ± 0.10	>104	>520	>91	91	n.a.
14	4-Isopropyl	0.36 ± 0.17	75	208	0.080 ± 0.009	10 ± 6	125
15	4-*tert*-Butyl	0.68 ± 0.07	61	89	0.30 ± 0.2	16	53
16	4-Ph	0.10 ± 0.04	40	400	0.60 ± 0.08	6	10
17	4-CF_3	0.25 ± 0.09	>104	>416	0.20 ± 0.1	43 ± 30	215
18	4-CN	0.27 ± 0.10	>104	>385	>152	>152	n.a.
19	4-COOH	21 ± 4	>104	>5	>144	>144	n.a.
20	4-CH_2OH	0.69 ± 0.14	>104	>151	>150	>150	n.a.
21	4-CH_2OCH_3	0.67 ± 0.24	>104	>155	>144	>144	n.a.
22	4-F	0.25 ± 0.14	>104	>416	>121	121	n.a.
23	4-OCH_3	0.11 ± 0.04	>104	>945	84	129	1.53
24	4-OCF_3	0.18 ± 0.08	83	454	0.20 ± 0.03	26 ± 1.5	130
25	4-Benzyloxy	0.40 ± 0.13	>104	>260	1.0 ± 0.8	24 ± 2	24
26	4-SCF_3	0.52 ± 0.08	>104	>200	0.30 ± 0.2	40 ± 10	133
27	4-NO_2	0.16 ± 0.08	>104	>650	115	>144	>1.25
28	4-NH_2	0.60 ± 0.20	>104	>173	107	148	1.38

[a] Data (for active compounds) are mean values ± standard deviation for 4–6 independent experiments.
[b] In vitro selectivity index (CC_{50}/EC_{50}).

(**15**), the 4-trifluoromethoxy (**24**) and the 4-benzyloxy analogue (**25**) had the best antiviral activity. The 4-phenyl analogue (**16**) was active but also more toxic with a selectivity index of only 9. All other 4-substituted analogues proved more or less inactive.

Replacing the 4-bromophenyl part of the 5-(4-bromobenzyl) substituent by 2- or 3-pyridyl (**29** and **30**) resulted in analogues without anti-HCV activity and reduced anti-BVDV activity. The 5-cyclohexylmethyl analogue **31** and the 5-(2-phenoxyethyl) analogue **32**

Table 3. Structure, anti-BVDV activity, anti-HCV activity and cytotoxic/cytostatic activity of compounds **29**–**32**



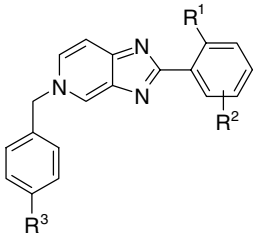
Compound	R	BVDV			HCV		
		EC_{50}[a] (μM)	CC_{50}[a] (μM)	SI[b]	EC_{50}[a] (μM)	CC_{50}[a] (μM)	SI[b]
29	2-Pyridyl	4.5 ± 2.3	>104	>23	>164	>164	n.a.
30	3-Pyridyl	2.3 ± 1.2	>104	>44	>164	>164	n.a.
31	Cyclohexyl	0.61 ± 0.05	>104	>170	84	97	1.15
32	Phenoxymethyl	0.28 ± 0.08	>104	>371	48 ± 27	96 ± 33	2

[a] Data (for active compounds) are mean values ± standard deviation for 4–6 independent experiments.
[b] In vitro selectivity index (CC_{50}/EC_{50}).

were active against BVDV but devoid of activity against HCV (Table 3).

Combining the above findings resulted in analogues with further improved activity against HCV (Table 4). In particular the combination of 2-(3-halo-2-fluorophenyl) with 5-(4-(trifluoromethoxy)benzyl) or 5-(4-(trifluoromethyl)benzyl) resulted in compounds with potent anti-HCV activity (**33**, **34**, **46** and **47**, respectively). An additional methyl or methoxy function in position 3 of the 2-phenyl resulted in compounds with reduced activity against HCV (**36**, **37**, **48** and **49**). Similar to the results obtained with fluorine substitution, an additional chlorine in position 4, 5 or 6 of the 2-(2-fluorophenyl)

Table 4. Structure, anti-BVDV activity, anti-HCV activity and cytotoxic/cytostatic activity of compounds **33**–**56**



Compound	R^1	R^2	R^3	BVDV			HCV		
				EC_{50}[a] (μM)	CC_{50}[a] (μM)	SI[b]	EC_{50}[a] (μM)	CC_{50}[a] (μM)	SI[b]
33	F	3-F	OCF_3	0.20 ± 0.02	>100	>500	0.20 ± 0.03	81	405
34	F	3-Cl	OCF_3	0.38 ± 0.19	>104	>274	0.20 ± 0.16	>119	>595
35	F	3-Br	OCF_3	0.49 ± 0.21	>104	>212	0.30 ± 0.08	28 ± 2	93
36	F	3-CH_3	OCF_3	0.32 ± 0.11	78	246	5.2 ± 0.3	40 ± 21	7.6
37	F	3-OCH_3	OCF_3	0.56 ± 0.24	>104	>186	53 ± 14	>120	>2.27
38	F	4-Cl	OCF_3	1.7 ± 0.8	>104	>62	76 ± 1.7	>119	>1.56
39	F	5-Cl	OCF_3	0.32 ± 0.12	>104	>325	7.7 ± 5.7	>119	>15
40	F	5-CH_3	OCF_3	0.29 ± 0.09	>104	>359	0.50 ± 0.15	>125	>250
41	F	5-OCH_3	OCF_3	0.27 ± 0.12	>104	>385	1.3 ± 0.6	58	44
42	F	6-Cl	OCF_3	3.9 ± 1.7	92	23	36	73	2
43	F	6-OCH_3	OCF_3	0.69 ± 0.14	91	132	43 ± 14	62	1.4
44	CF_3	H	OCF_3	5.7 ± 4.8	75	13	39 ± 4.5	48 ± 3.2	1.23
45	OCH_3	H	OCF_3	0.050 ± 0.03	26	520	11 ± 1.8	14 ± 1.7	1.27
46	F	3-F	CF_3	0.72 ± 0.19	>104	>144	0.10 ± 0.013	108	1080
47	F	3-Cl	CF_3	0.44 ± 0.22	>104	>236	0.30 ± 0.17	121	403
48	F	3-CH_3	CF_3	0.38 ± 0.15	>104	>274	16 ± 0.8	70	4.4
49	F	3-OCH_3	CF_3	0.37 ± 0.10	75	203	27	55	2
50	F	5-CH_3	CF_3	0.23 ± 0.10	>104	>452	1.3 ± 1.03	39	30
51	F	3-F	Isopropyl	0.80 ± 0.16	>104	>130	0.30 ± 0.069	33	110
52	F	3-Cl	Isopropyl	0.72 ± 0.06	>104	>144	0.70 ± 0.21	47	67
53	F	5-CH_3	Isopropyl	0.83 ± 0.08	89	107	1.6 ± 1.11	45	28
54	F	3-Cl	Cl	0.18 ± 0.14	>104	>578	4.94	30	6
55	F	3-Cl	I	0.14 ± 0.07	>104	>743	0.43 ± 0.24	22	50
56	F	3-Cl	*tert*-Butyl	0.71 ± 0.26	68	96	0.36 ± 0	25	71

[a] Data (for active compounds) are mean values ± standard deviation for 4–6 independent experiments.
[b] In vitro selectivity index (CC_{50}/EC_{50}).

substituent resulted in analogues with reduced anti-HCV activity (**38**, **39** and **42**). Introduction of a methyl group in position 5 of the 2-phenyl was tolerated in view of anti-HCV activity (**40**, **50**), whereas a methoxy group in the same position resulted in reduced activity against this virus (**41**). Analogues with an isopropyl group in position 4 of the benzyl (**51**–**53**) were less active than the corresponding 4-trifluoromethoxy or 4-trifluoromethyl analogues, as were the analogues with chlorine, iodine or *tert*-butyl (**54**–**56**). Substituting the fluorine in position 2 of the phenyl in compound **24** by trifluoromethyl or methoxy resulted in analogues with reduced activity (**44** and **45**, respectively). Again, most of the modifications were well tolerated with respect to anti-BVDV activity.

This class of compounds was shown to target HCV polymerase. The exact molecular mechanism by which these compounds inhibit HCV replication will be published elsewhere. We reported earlier on the fact that the anti-pestivirus activity of this class of compounds can be explained by an interaction at the tip of the fingerdomain of the pestivirus polymerase.[4]

In summary, we have prepared several sets of analogues of the lead compound **1** with modifications both at the 2-phenyl and 5-benzyl position. Whereas many modifications were well tolerated with respect to anti-BVDV activity, only few modifications resulted in analogues with anti-HCV activity. Combination of these findings allowed us to synthesize analogues (e.g., **34** and **46**) with potent and selective activity on HCV replication.

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6. All compounds were synthesized as described before[3,5] by a two-step route, starting from 3,4-diaminopyridine and a substituted benzoic acid, followed by alkylation of the intermediate substituted 2-phenyl-imidazo[4,5-*c*]pyridine. Synthesis of and analytical data for compound **46**: prepared from 2-(2,3-difluorophenyl)-1(3)*H*-imidazo[4,5-*c*]pyridine and 4-(trifluoromethyl)benzyl bromide; recrystallized from a mixture of diisopropyl ether and ethyl acetate to give a colourless powder; yield: 58%; mp 209 °C; ^{1}H NMR (200 MHz, DMSO-d_6): δ 9.27 (d, 1H, imidazopyridine-H4, J = 1.4 Hz), 8.25 (dd, 1H, imidazopyridine-H6, J = 6.8, 1.4 Hz), 8.17–8.09 (m, 1H, phenyl-H6), 7.86–7.76 (m, 3H, arom. H), 7.66–7.60 (AA′**BB**′, 2H, benzyl-H), 7.55–7.42 (m, 1H, phenyl-H), 7.36–7.24 (m, 1H, phenyl-H), 5.82 (s, 2H, CH_2); MS (CI): 390.1 [M^+ +1, 44%]. Anal. calcd for $C_{20}H_{12}F_5N_3$: C, 61.70; H, 3.11; N, 10.79. Found: C, 61.63; H, 3.24; N, 10.72.
7. For a detailed procedure regarding the anti-BVDV screening, see Ref. 4.
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