Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 5345–5349

Substituted 5-benzyl-2-phenyl-5*H*-imidazo[4,5-*c*]pyridines: A new class of pestivirus inhibitors

Gerhard Puerstinger,[a,*] Jan Paeshuyse,[b] Piet Herdewijn,[b] Jef Rozenski,[b] Erik De Clercq[b] and Johan Neyts[b]

[a]*Institut für Pharmazie, Abteilung Pharmazeutische Chemie, Universität Innsbruck, Innrain 52a, A-6020 Innsbruck, Austria*
[b]*Rega Institute for Medical Research, Katholieke Universiteit Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium*

Received 7 June 2006; revised 23 July 2006; accepted 25 July 2006
Available online 9 August 2006

Abstract—A novel class of inhibitors of pestiviruses (5-substituted 2-phenyl-5*H*-imidazo[4,5-*c*]pyridines) is described. Modification of the substituent in position 5 resulted in analogues with high activity ($EC_{50} < 100$ nM) and selectivity (SI > 1000) against the pestivirus BVDV (bovine viral diarrhea virus).

The bovine viral diarrhea virus (BVDV) belongs together with viruses such as the classical swine fever virus (CSFV) and the border disease virus (BDV) to the genus Pestivirus that is classified along with the genus Hepacivirus and Flavivirus in the family of the Flaviviridae.[1]

Pestiviruses cause important diseases of livestock such as bovine viral diarrhea in cattle,[2] classical swine fever in pigs,[3] and border disease in sheep. Regardless of the availability of vaccines against BVDV and CSFV, and the implementation of eradication or control programs,[4,5] both viruses remain an agronomical burden. An alternative approach to combat BVDV and CSFV infections could be the use of antiviral agents that specifically inhibit the replication of the virus. Although likely not suited to treat large herds, it may be important to have selective anti-pestivirus compounds at hand. Possible uses for anti-pestivirus drugs could be (i) to treat valuable animals in zoologic collections, (ii) to treat expensive animals in breeding programs and in vitro embryo production,[6] (iii) to cure established cell lines from contaminating pestiviruses,[7,8] or (iv) as a means to rapidly control outbreaks of classical swine fever. Current control measures in case of an CSF outbreak consist of massive culling of healthy animals in farms surrounding the infected farm. Although emergency vaccination could be considered, it takes about 2 weeks before the animals have mounted a protective immune response. BVDV is also considered to be a surrogate virus for hepatitis C virus (HCV),[9] although adequate HCV replicon and HCV cell culture systems are now available.[10–12]

Worldwide 170 million people are chronic carriers of HCV. This virus is a major cause of cirrhosis and primary hepatocellular carcinoma, and the main reason for liver transplantations among adults in Western countries.[13] The current standard therapy for hepatitis C, that is, the combination of pegylated interferon-α and the nucleoside analogue ribavirin, is only effective in about 50–60% of patients that suffer from chronic HCV infection, and is associated with important side-effects.[14] Consequently, there is an urgent need for highly effective and selective inhibitors of HCV replication.

In the course of a screening effort dedicated to the search for new classes of inhibitors of BVDV (as a surrogate for HCV) compound **1** was found to elicit antiviral activity.[15] It was obtained as the main product from the reaction of 2-(2,6-difluorophenyl)-1(3)*H*-imidazo-[4,5-*c*]pyridine with 2,6-difluorobenzyl bromide in 65% yield together with the isomers **2** and **3** (15% yield each; Fig. 1). The crude product mixture was separated by column chromatography (silica gel; eluent: dichloromethane/methanol = 12:1). Alternatively, pure **1** can be

Keywords: Imidazo[4,5-*c*]pyridines; Pestivirus inhibitors; BVDV; HCV; Antiviral.
* Corresponding author. Tel.: +43 512 507 5260; fax: +43 512 507 2940; e-mail: Gerhard.Puerstinger@uibk.ac.at

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.07.081

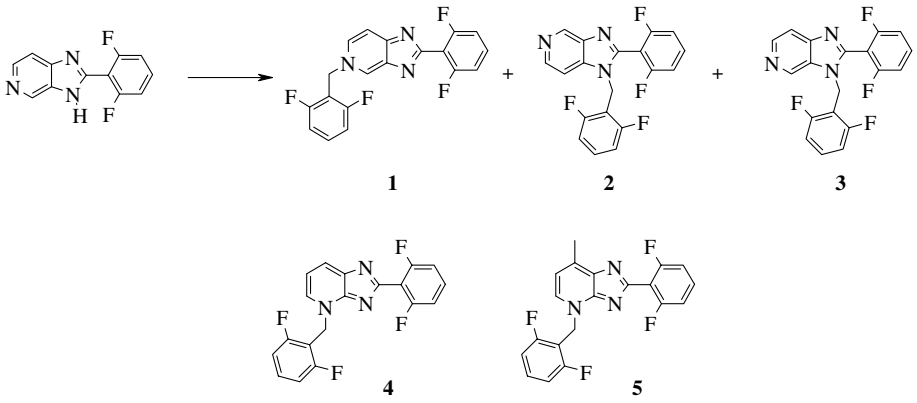


Figure 1. Synthesis of compounds **1**, **2**, and **3**. Structures of compounds **4** and **5**.

obtained by recrystallization of the crude isomer mixture from a mixture of diisopropyl ether and ethyl acetate. The assignment of the structures of the three products was achieved by one-dimensional NOE difference spectroscopy (irradiation at the resonance frequencies of the CH_2 linkers). Compounds **2** and **3** showed reduced anti-BVDV (NADL) activities in MDBK cells, as did the imidazo[4,5-*b*]pyridine analogues **4** and **5** (Table 1). Therefore, compound **1** was selected as the lead compound.

In a first attempt to understand the structural requirements for the antiviral activity, the defluorinated analogues **6**, **7** and **8** were prepared (Fig. 2). The biological results showed that removal of the 2 fluorines on the 2-phenyl results in more active antiviral compounds (Table 2).

To further investigate the influence of substituents on the benzyl group on the anti-BVDV activity, a set of 26 analogues was prepared (compounds **9**–**35**, see Table 3).

Table 1. Anti-BVDV activity and cytotoxicity for compounds **1**–**5**

Compound	EC_{50}[a] (μM)	CC_{50}[a] (μM)	S.I.[b]
1	1.5 ± 0.7	>100	>65
2	16.20	>100	>6
3	⩾48	99 ± 2	⩽2
4	⩾32	>100	⩽3
5	⩾88	>100	⩽1.1

[a] Values are means of six independent experiments ± standard deviation.
[b] In vitro selectivity index (CC_{50}/EC_{50}).

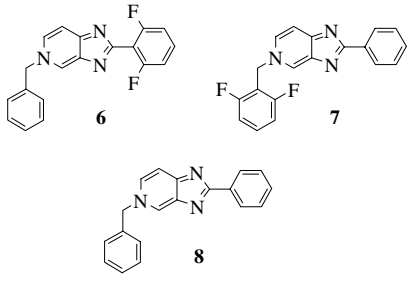


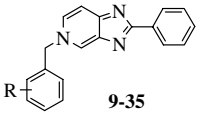
Figure 2. Structures of compounds **6**, **7**, and **8**.

Table 2. Anti-BVDV activity and cytotoxicity for compounds **6**–**8**

Compound	EC_{50}[a] (μM)	CC_{50}[a] (μM)	S.I.[b]
6	4 ± 1	>100	>25
7	0.23 ± 0.02	>100	>435
8	0.25 ± 0.05	>100	>400

[a] Values are means of four independent experiments ± standard deviation.
[b] In vitro selectivity index (CC_{50}/EC_{50}).

Table 3. Anti-BVDV activity and cytotoxicity for compounds **9**–**35**



Compound	R	EC_{50}[a] (μM)	CC_{50}[a] (μM)	S.I.[b]
9	2-CH_3	0.3 ± 0.1	>100	>333
10	3-CH_3	0.6 ± 0.2	>100	>167
11	4-CH_3	0.13 ± 0.02	>100	>769
12	2-OCH_3	0.27 ± 0.05	>100	>370
13	3-OCH_3	0.5 ± 0.2	>100	>200
14	4-OCH_3	0.14 ± 0.05	>100	>714
15	2-F	0.10 ± 0.01	>100	>1000
16	3-F	0.15 ± 0.07	>100	>667
17	4-F	0.12 ± 0.04	>100	>833
18	2-Cl	0.19 ± 0.04	>100	>526
19	3-Cl	0.21 ± 0.05	>100	>476
20	4-Cl	0.08 ± 0.05	>100	>1250
21	2-Br	0.28 ± 0.06	>100	>357
22	3-Br	0.28 ± 0.08	85 ± 13	304
23	4-Br	0.07 ± 0.02	83 ± 20	1186
24	2-CN	0.76 ± 0.12	>100	>132
25	3-CN	4 ± 2	>100	>25
26	4-CN	0.23 ± 0.03	>100	>435
27	2-CF_3	1.5 ± 0.3	>100	>67
28	3-CF_3	0.24 ± 0.05	98 ± 4	408
29	4-CF_3	0.22 ± 0.04	84 ± 13	455
30	4-*tert*-Butyl	0.49 ± 0.20	22 ± 1	45
31	4-Ph	0.08 ± 0.02	18 ± 3	225
32	4-I	0.12 ± 0.04	22 ± 4	183
33	3,4-Cl_2	0.11 ± 0.04	71 ± 8	645
34	4-OCF_3	0.27 ± 0.06	66 ± 7	244
35	4-COOH	>100	>100	n.a.

[a] Values are means of four independent experiments ± standard deviation.
[b] In vitro selectivity index (CC_{50}/EC_{50}).

In general, all prepared analogues showed activity with the exception of the 4-carboxy derivative **35**, but this can probably be contributed to the inability of the carboxylic acid to penetrate cell membrane rather than loss of intrinsic binding ability. With respect to the position of the substituents ortho- and para-substitution is preferred. Halogen substituents result in the best improvements of activity and selectivity, in particular chlorine and bromine in position 4 (**20** and **23**,[16,17] respectively). Also, the 2-monofluoro analogue **15** showed high activity and selectivity. Methyl and methoxy substituents were tolerated in all three positions, whereas introduction of a cyano substituent in position 3 or of a trifluoromethyl group in position 2 results in reduced activity (**25** and **27**, respectively). A bulky substituent (*tert*-butyl, Ph or I) in position 4 resulted in analogues with increased toxicity (compounds **30**, **31**, and **32**, respectively).

To further explore the structure–activity relationship of the 5-substituent in this class of compounds a set of analogues was prepared, where (the length of) the linker was modified (**36**–**39**, **47**, and **48**), where the benzyl group was replaced by alkyl or alkenyl (**41**–**46**) or where the phenyl ring of the benzyl substituent was replaced by other (heterocyclic) ring(system)s (**49**–**54**) (Table 4).

Increasing the length of the linker to 2 or 4 carbons resulted in compounds with reduced antiviral activity (**36** vs **6** and **38** vs **8**, respectively), whereas a 3-atom linker was better tolerated (**37**, **47**, and **48**). Replacing one hydrogen of the methylene linker by methyl (compound **39**) resulted in reduced activity. The alkyl or alkenyl analogues exhibited reduced activity with the cyclohexylmethyl analogue being the most active (**41**–**46**). The two naphthyl analogues showed different effects: the 1-naphthyl analogue **49** elicited excellent activity, but the 2-naphthyl analogue **50** proved almost inactive. All three pyridine analogues (**51**–**53**) had reduced activity, but replacing the phenyl by 5-chloro-2-thienyl resulted in an analogue (**54**) with high activity and selectivity. The 5-unsubstituted intermediate **40** proved only slightly active.

The compounds (as exemplified by **23** (BPIP)[16]) target the viral RNA-dependent RNA polymerase and induce the same mutation (F224S in the fingertip of the polymerase) as the structurally unrelated compound (3-[(2-dipropylamino)ethyl)thio]-5*H*-1,2,4-triazino[5,6-*b*]indole).[18]

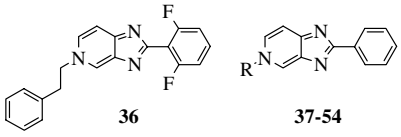
All 54 analogues were also tested against HCV in a genotype 1a subgenomic replicon system,[19] but proved inactive against this virus.

In conclusion, 5-substituted 2-phenyl-5*H*-imidazo[4,5-*c*] pyridines represent a class of highly active and selective anti-BVDV compounds with activities down to below 100 nM and antiviral selectivities >1000. These compounds lack activity against the HCV.

Acknowledgments

We thank Katrien Geerts and Geoffrey Férir for excellent technical assistance. This work was supported by

Table 4. Anti-BVDV activity and cytotoxicity for compounds **36-54**



36 **37-54**

Compound	R	EC_{50}[a] (μM)	CC_{50}[a] (μM)	S.I.[b]
36	—	57 ± 3	>100	>2
37	3-Ph–1-propyl	0.7 ± 0.2	>100	>143
38	4-Ph–1-butyl	2.1 ± 0.3	95 ± 10	45
39	(±)-1–Ph-ethyl	2.1 ± 0.6	>100	>48
40	H	10 ± 3	>100	>10
41	Ethyl	15 ± 4	>100	>7
42	3-Methyl-1-butyl	3.8 ± 0.2	>100	>26
43	2-Ethyl-1-butyl	1.9 ± 0.4	>100	>52
44	2-(Diisopropyl-amino)-ethyl	2.2 ± 0.2	>100	>45
45	3,3-Dimethylallyl	3 ± 1	>100	>33
46	Cyclohexylmethyl	0.85 ± 0.09	>100	>118
47	2-Phenoxy-ethyl	0.27 ± 0.02	>100	>370
48	(*E*)-3-Ph–allyl	0.17 ± 0.06	78 ± 18	459
49	1-Naphthylmethyl	0.06 ± 0.01	61 ± 4	1017
50	2-Naphthylmethyl	5 ± 2	67 ± 30	13
51	2-Pyridylmethyl	1.6 ± 0.1	>100	>63
52	3-Pyridylmethyl	1.7 ± 0.3	>100	>59
53	4-Pyridylmethyl	2.0 ± 0.3	>100	>50
54	5-Cl–2-thienylmethyl	0.21 ± 0.09	>100	>476

[a] Values are means of four experiments ± standard deviation.
[b] In vitro selectivity index (CC_{50}/EC_{50}).

VIRGIL, the European Network of Excellence on Antiviral Drug Resistance (Grant LSHM-CT-2004-503359 from the Priority 1 'Life Sciences, Genomics and Biotechnology'), and by a grant (S-6146-Sectie1) from the Belgian Government ('Federale Overheidsdienst Volksgezondheid, Veiligheid van de Voedselketen en Leefmilieu').

References and notes

1. Lindenbach, B. D.; Rice, C. M. In *Fields Virology*; Knipe, D. M., Howley, P. M., Griffin, D. E., Martin, M. A., Lamb, R. A., Roizman, B., Straus, S. E., Eds.; Lippincott Williams & Wilkins: Philadelphia, 2001; pp 991–1041.
2. Houe, H. *Biologicals* **2003**, *31*, 137.
3. Edwards, S.; Fukusho, A.; Lefevre, P. C.; Lipowski, A.; Pejsak, Z.; Roehe, P.; Westergaard, J. *Vet. Microbiol.* **2000**, *73*, 103.
4. Greiser-Wilke, I.; Grummer, B.; Moennig, V. *Biologicals* **2003**, *31*, 113.
5. Stegeman, A.; Elbers, A.; de Smit, H.; Moser, H.; Smak, J.; Pluimers, F. *Vet. Microbiol.* **2000**, *73*, 183.
6. Stringfellow, D. A.; Riddell, K. P.; Givens, M. D.; Galik, P. K.; Sullivan, E.; Dykstra, C. C.; Robl, J.; Kasinathan, P. *Theriogenology* **2005**, *63*, 1004.
7. Durantel, D.; Carrouee-Durantel, S.; Branza-Nichita, N.; Dwek, R. A.; Zitzmann, N. *Antimicrob. Agents Chemother.* **2004**, *48*, 497.
8. Givens, M. D.; Stringfellow, D. A.; Dykstra, C. C.; Riddell, K. P.; Galik, P. K.; Sullivan, E.; Robl, J.; Kasinathan, P.; Kumar, A.; Boykin, D. W. *Antiviral Res.* **2004**, *64*, 113.
9. Buckwold, V. E.; Beer, B. E.; Donis, R. O. *Antiviral Res.* **2004**, *60*, 1.
10. Lindenbach, B. D.; Evans, M. J.; Syder, A. J.; Wolk, B.; Tellinghuisen, T. L.; Liu, C. C.; Maruyama, T.; Hynes, R. O.; Burton, D. R.; McKeating, J. A.; Rice, C. M. *Science* **2005**, *309*, 623.
11. Wakita, T.; Pietschmann, T.; Kato, T.; Date, T.; Miyamoto, M.; Zhao, Z.; Murthy, K.; Habermann, A.; Krausslich, H. G.; Mizokami, M.; Bartenschlager, R.; Liang, T. J. *Nat. Med.* **2005**, *11*, 791.
12. Zhong, J.; Gastaminza, P.; Cheng, G.; Kapadia, S.; Kato, T.; Burton, D. R.; Wieland, S. F.; Uprichard, S. L.; Wakita, T.; Chisari, F. V. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 9294.
13. Thomson, B. J.; Finch, R. G. *Clin. Microbiol. Infect.* **2005**, *11*, 86.
14. Lauer, G. M.; Walker, B. D. *N. Eng. J. Med.* **2001**, *345*, 41.
15. Cells and viruses. Madin-Darby bovine kidney (MDBK) cells were grown in MEM (Gibco) supplemented with 5% heat-inactivated FCS (Integro). FCS was shown to be free of BVDV-1 and BVDV-2 by RT-PCR.[20] First-passage BVDV NADL stock was generated from pNADLp15a as previously described.[21] Anti-BVDV assay for cp strains (MTS). MDBK cells were seeded at a density of 5×10^3 per well in 96-well cell culture plates (confluency 10–15%) in MEM-FCS. Following 24 h incubation at 37 °C and 5% CO_2 medium was removed and 5-fold serial dilutions of the test compounds were added in a total volume of 100 μL, after which the cp BVDV virus inoculum (MOI = 2) was added to each well. This inoculum resulted in a greater than 90% destruction of the cell monolayer after 3 days of incubation at 37 °C. Uninfected cells and cells receiving virus without compound were included in each assay plate. After 5 days, medium was removed, and 90 μL of MEM-FCS supplemented with 10 μL of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium/phenazinemethosulfate (MTS/PMS) solution (Promega, Leiden, The Netherlands) was added to each well. Following a 2 h incubation period at 37 °C, the optical density of each well was read at 490 nm in a microplate reader (signal to noise ratio = 5). The percentage CPE was calculated as follows: % CPE = $((OD_{treated})_{BVDV} - (OD_{control})_{BVDV})/((OD_{control})_{mock} - (OD_{VC})_{BVDV})$; in which $(OD_{treated})_{BVDV}$ = the OD_{490nm} of cells infected with BVDV and treated with a certain dilution of compound, $(OD_{control})_{BVDV}$ = the OD_{490nm} of cells infected with BVDV and left untreated, and $(OD_{control})_{mock}$ = the OD_{490nm} of cells mock infected and left untreated. The 50% effective concentration (EC_{50}) was defined as the concentration of compound that offered 50% protection of the cells against virus-induced cytopathic effect (CPE) and was calculated using logarithmic interpolation. Cytostatic assay. MDBK cells were seeded at a density of 5×10^3 cells per well of a 96-well plate (confluency 10–15%) in MEM-FCS; 24 h later, serial dilutions of the test compounds were added. Cells were allowed to proliferate for 3 days at 37 °C, after which the cell number was determined by means of the MTS/PMS (Promega) method (signal to noise ratio = 5). The % cell growth was calculated as follows: $(OD_{treated}/OD_{control})$; in which $(OD_{treated})$ = the OD_{490nm} of cells treated with a certain dilution of compound, $(OD_{control})$ = the OD_{490nm} of cells left untreated. The 50% cytostatic concentration (CC_{50}) was defined as the concentration that inhibited the proliferation of exponentially growing cells by 50% and was calculated using linear interpolation.
16. Paeshuyse, J.; Leyssen, P.; Mabery, E.; Boddeker, N.; Vrancken, R.; Froeyen, M.; Ansari, I. H.; Dutartre, H.; Rozenski, J.; Gil, L. H. V. G.; Letellier, C.; Lanford, R.; Canard, B.; Koenen, F.; Kerkhofs, P.; Donis, R. O.; Herdewijn, P.; Watson, J.; De Clercq, E.; Puerstinger, G.; Neyts, J. *J. Virol.* **2006**, *80*, 149.
17. All new compounds were fully characterized by NMR, MS, and HRMS spectra. Synthesis of compound **23** (BPIP): A mixture of 3,4-diaminopyridine (2.000 g), benzoic acid (1 equiv), and polyphosphoric acid (50 g) was heated at 190 °C for 3 h with stirring. Then the mixture was cooled to ambient temperature and poured into ice/water. The resulting mixture was neutralized by addition of solid Na_2CO_3. The crude product was collected by filtration, washed with water, and dried. Recrystallized from water; off-white crystals; mp: 229–230 °C; yield: 96%; ^{1}H NMR (200 MHz, DMSO-d_6) δ 8.95 (d, 1H, H4, J = 1.0 Hz), 8.31 (d, 1H, H6, J = 5.4 Hz), 8.28–8.17 (m, 2H, arom. H), 7.64–7.50 (m, 4H, arom. H). 2-Phenyl-1(3)*H*-imidazo[4,5-*c*]pyridine (0.500 g) was dissolved in dry DMF (5 mL) and the resulting solution was cooled to 0 °C. Aqueous 50% sodium hydroxide (1.5 equiv) was added and the mixture was stirred for 15 min. Then 4-bromobenzyl bromide (1.2 equiv) was added and the resulting mixture was stirred for 24 h at room temperature. Finally, water (50 mL) was added, the precipitate was collected by filtration and dried to give the crude product mixture. Recrystallized from a mixture of diisopropyl ether (10 mL) and ethyl acetate (26 mL); colorless crystals; mp: 212–214 °C; yield: 45%; ^{1}H NMR (200 MHz, DMSO-d_6) δ 9.09 (br s, 1H, H4), 8.40–8.33 (m, 2H, arom. H), 8.17 (dd, 1H, H6, J = 6.8, 1.5 Hz), 7.73 (d, 1H, H7, J = 6.8 Hz), 7.64–7.58 (AA′BB′, 2H, arom. H),

7.52–7.37 (m, 5H, arom. H), 5.64 (s, 2H, CH_2). HRMS (ESI+): *m/z* calcd for $C_{19}H_{15}BrN_3$ $(M+H)^+$ 364.0449, found 364.0457. ^{13}C NMR (50 MHz, DMSO-d_6): 171.4, 155.8, 145.5, 135.8, 135.0, 131.8, 131.4, 130.8, 130.0, 129.2, 128.3, 127.6, 121.8, 112.3, 60.3.

18. Baginski, S. G.; Pevear, D. C.; Seipel, M.; Sun, S. C.; Benetatos, C. A.; Chunduru, S. K.; Rice, C. M.; Collett, M. S. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 7981.
19. Lohmann, V.; Korner, F.; Koch, J.; Herian, U.; Theilmann, L.; Bartenschlager, R. *Science* **1999**, *285*, 110.
20. Letellier, C.; Kerkhofs, P.; Wellemans, G.; Vanopdenbosch, E. *Vet. Microbiol.* **1999**, *64*, 155.
21. Vassilev, V. B.; Donis, R. O. *Virus Res.* **2000**, *69*, 95.