Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 11, pp. 1279-1284, 1996

0960-894X/96 $15.00 + 0.00

PII: S0960-894X(96)00216-8

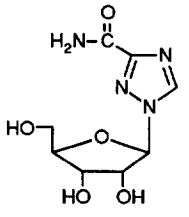
DESIGN, SYNTHESIS AND ANTIPROLIFERATIVE ACTIVITY OF METHYL 4-IODO-1-β-D-RIBOFURANOSYL-PYRAZOLE-3-CARBOXYLATE AND RELATED COMPOUNDS

Stefano Manfredini,* Rita Bazzanini, Pier Giovanni Baraldi, Daniele Simoni,§ Silvia Vertuani, Alessandra Pani,# Elisabetta Pinna,◊ Franca Scintu◊ Donatella Lichino# and Paolo La Colla.#◊

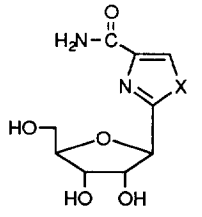
Dipartimento di Scienze Farmaceutiche, Università di Ferrara, Via Fossato di Mortara 17-19; I-44100 Ferrara. Fax: +39.532.29.1296, E-mail: mv9@dns.unife.it. §*Istituto Farmacochimico, Università di Palermo,* #*Dipartimento di Biologia Sperimentale, Università di Cagliari,* ◊*Sardinian Antiviral Research Consortium, Cagliari. Italy.*

Abstract: In a SAR study on azole-related nucleosides we have designed some pyrazole-nucleoside analogs characterised, for the first time, by a carboxylic ester moiety. 4-Iodo-1-β-D-ribofuranosyl-pyrazole-3-carboxylate showed a wide spectrum of antiproliferative activity and a particularly low cytotoxicity against resting PBL, being, unlike the other azole nucleosides, more active than the corresponding primary amide.

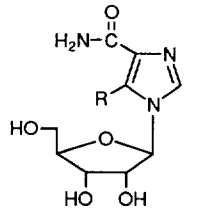
Azole nucleosides are a class of antimetabolites structurally related to 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide (AICAR), the 5'-monophosphate of which is a key intermediate in purine biosynthesis. Important members of this class are bredinin, pyrazofurin and ribavirin and its analogs endowed with immunosuppressive, antitumor and antiviral activity, respectively. Ribavirin,[1] a broad spectrum antiviral agent which also displays antitumor activity in mice,[2] has attracted considerable attention because of the peculiar mechanism of action, i.e. the reduction of GTP pool as a consequence of inosine monophosphate dehydrogenase (IMPDH) inhibition.[3]



Ribavirin



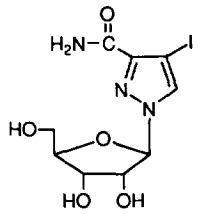
X = S: *Tiazofurin*
X = Se: *Selenazofurin*
X = O: *Oxazofurin*



R = -C≡C-H: *EICAR*
R = F: *FICAR*
R = OH: *Bredinin*

We have recently described a series of 4,5-substituted pyrazole-3-carboxamide derivatives that may be formally considered as 4-deaza-analogs of ribavirin.[4] Among them, compound **1** showed selective antiproliferative activity *in vitro* against T-cell lines with IC_{50} values ranging from 4 to 12 μM, thus proving of interest for possible clinical applications against T-cell leukemias and lymphomas and as an immunosuppressive

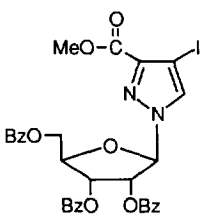
agent.[5] While SAR studies on known azole nucleosides, leading to important information on the structural requirements for their biological activity have been extensively reported, simple pyrazole nucleosides which may be considered as deaza-analogs of ribavirin have been hitherto scarcely studied.[6]



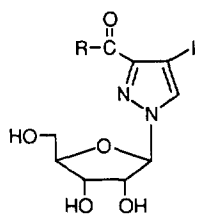
1

We initiated our study by a careful examination of the SAR on the simple pyrazole nucleoside **1** and addressed our attention on the role of the primary amide. It is well known that the activity of ribavirin is related to steric and hydrogen-bonding requirements at the primary amide at C3-position. While the presence of isosteric thio-carboxamide or carboxamidine groups is compatible with the biological activity, the presence of the corresponding N-methyl-carboxamide is not. Therefore, we decided to focus our efforts on structural modifications of the C3-amide moiety of compound **1**. We also investigated the C4-position, which is of interest because it represents the position usually occupied by a nitrogen in ribavirin, bredinin, EICAR and FICAR.[7]

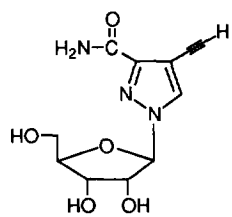
In this communication we wish to report our findings on methyl 4-iodo-1-β-D-ribofuranosyl-pyrazole-3-carboxylate (**4**) which, when compared to **1**, showed increased potency and wider spectrum of antiproliferative activity. Moreover, the effects of the replacement of the primary amide at C-3 with substituted amides, a carboxylic acid group or a butyl ester, together with the introduction of an ethynyl moiety at C4, as in EICAR,[8] will be discussed.



2



3a-c: R = NHMe; NMe_2; NHBut;
4: R = OMe; **6** = OH; **7**: R = OBut



5

Substituted amides **3a-c** were simply prepared by reaction of methyl 1-(β-D-2',3',5'-tri-O-benzoyl-ribofuranosyl)-4-iodo-pyrazole-3-carboxylate (**2**) with the corresponding amines in alcoholic solution, with concomitant removal of the benzoyl protecting groups. Synthesis of iodo- (**4**) and ethynyl-pyrazole nucleosides **5** also started from the above cited protected intermediate: a treatment with sodium methoxide in methanol afforded the deprotected methyl carboxylate derivative **4** in 75% yield. The ethynyl derivative **5** was obtained in good yield (82%) by coupling reaction between **2** and (trimethylsilyl)acetylene with a catalytic amount of bis(triphenylphosphine)palladium dichloride in triethylamine,[9] followed by treatment with methanolic ammonia. The carboxylic acid derivative **6** was simply obtained by standard hydrolysis of **2**. The corresponding butyl ester

7 was prepared under standard conditions from **4** which was protected as 2',3',5'-tri-O-terbutyldimethylsilylderivative. Final treatment with ammonium fluoride (NH_4F)[10] in methanol, gave the expected compound **7**.

All derivatives were tested for antiviral activity against HSV-1, HSV-2, Vaccinia, HRV-14, Coxsackie B2 and Vescicular Stomatitis Virus, but none of them was found active at concentrations as high as 300 μM (data not shown). More interesting results were obtained in assays for antiproliferative activity. Compounds were tested against a panel of cell lines, mostly derived from human leukemias, lymphomas (Table 1) and solid tumors (Table 2). Doxorubicin and ribavirin were used as reference drugs, the former because of proven efficacy against various neoplasias, including haematological malignancies, the latter because of structural similarity to the test compounds. While ribavirin showed only marginal antiproliferative activity, doxorubicin confirmed itself as a potent and broad spectrum antitumor agent, with IC_{50} values ranging from 0.05 to 1.7 μM.

Table 1. Effect of selected compounds on the proliferation of lymphoblastoid cell lines.

Compound	IC_{50} (μM)[a]							
	L1210	RAJI	SB	WIL2-NS	CEM	MOLT-4	MT-4	C8166
1	>300	>300	>300	>300	2.4	9.1	2.1	4.0
4	2.4	0.2	0.9	1.6	1.5	0.9	1.6	0.2
5	>300	>300	>300	>300	>300	>300	>300	22
6	>300	>300	>300	>300	>300	>300	>300	>300
7	ND	17	21	23	19	16	25	15
8	ND	ND	2.8	3.5	2.6	3	2.3	ND
9	>300	>300	>300	>300	>300	>300	>300	ND
10	>300	>300	>300	>300	>300	>300	>300	ND
Ribavirin	16.6	56	>100	>100	19	5.7	23	>100
Doxorubicin	0.9	0.1	0.05	0.2	0.08	0.05	0.05	0.05

[a] Inhibitory concentration 50. Compound concentration required to reduce the number of viable cells by 50%. Data represent mean values for three separate experiments; variability among triplicate samples was less than 10%. ND = not determined; L1210, murine leukemia; Raji, Burkitt lymphoma; CCRF-SB, human lymphoblastic B-leukemia; WIL-2-NS, variant not secerning Ig of WIL-2 B-cells; CCRF-CEM, human lymphoblastic T-leukemia; MOLT-4, human lymphoblastic T-leukemia; MT4 and C8166, T-cells expressing the TAT gene of HTLV-1.

Among test compounds, substituted amides **3a-c** as well as the carboxylic acid **6** and the 4-ethynyl derivatives **5** resulted inactive (the latter resulting only partially active on C8166 cell lines); antiproliferative activity appeared when the primary amide was substituted by methyl and butyl esters (**4** and **7**). In particular methyl 4-iodo-1-β-D-ribofuranosyl-3-pyrazole carboxylate (**4**) showed the best activity, superior in both potency and spectrum to that of **1**. This different specificity for T-cells, shown by **1** and **4**, may account for differences in the mechanism of action. The butyl ester **7**, which displayed an increment of lipophilicity of about one LOGP unit when compared to **4**,[11] showed antiproliferative activity comparable to **1**. Interestingly, the absence of the glycosylic moiety completely abolished the antiproliferative activity (IC_{50} > 300 μM, data not shown).

The cytotoxicity of **4** was then tested against normal human PBL (proliferating and resting) in comparison with that of doxorubicin and tiazofurin. Interestingly, **4** proved non-cytotoxic for resting PBL (CC_{50} > 500 μM). However, when evaluated against PHA-stimulated PBL (PBL_{PHA}), **4** was cytotoxic (CC_{50} = 2.6 μM) at concentrations comparable to those active against lymphoblastoid cell lines. As clearly appear from the data, the

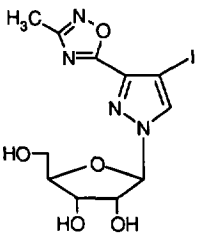
prospective advantages of **4** are evident when it is compared to doxorubicin and tiazofurin: the former proved equally cytotoxic to resting and stimulated PBL (CC_{50} = 0.03 μM) at concentrations comparable to those active against tumor cell lines; the latter resulted comparable to **4** on PBL_{PHA} but more cytotoxic on resting PBL (CC_{50} > 100 μM). To the best of our knowledge, this is the first time that an azole nucleoside analog featured with a carboxylic ester moiety shows such a promising pattern of activity; it is in fact well documented and generally accepted that the C3-primary amide is an essential prerequisite for the biological activity of ribavirin and related nucleosides.[12]

Table 2. Effect of selected compounds on the proliferation of solid tumor-derived and embryonal cell lines.

Compound	IC_{50} (μM)[a]			
	HT-29	ACHN	CHO-K1	HELL-299
1	>300	>300	>300	>300
4	2.8	3.6	7.9	4
5	>300	287	>300	235
6	>300	>300	>300	>300
7	25	ND	37	40
8	4.8	6.2	8.5	7.9
9	>300	>300	>300	>300
10	>300	>300	>300	>300
Ribavirin	ND	>100	50	45
Doxorubicin	1.1	0.7	1.2	1.7

[a] Inhibitory concentration 50. Compound concentration required to reduce the number of viable cells by 50%. Data represent mean values for three separate experiments; variability among triplicate samples was less than 10%. ND = not determined. HT-29 human colon adenocarcinoma; ACHN, human kidney adenocarcinoma; CHO-K1, hamster ovarian cancer; HELL-299, human diploid embryonal lung.

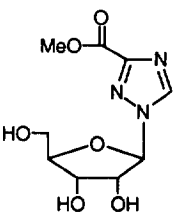
In view of these results we decided to further investigate whether the carbomethoxy function was of importance for the activity of this new compound. To this regards, a basic bioisosteric modification of a carbomethoxy ester is represented by an 1,2,4-oxadiazole ring (**8**).[13] Beside this moiety, we have also considered of interest to study the substitution of the primary amide of ribavirin and tiazofurin[14] with a methyl ester (**9** and **10**).



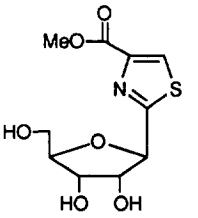
8

The 3-methyl-1,2,4-oxadiazole derivative (**8**) was prepared in a single step, from the protected methyl ester **2**, by reaction with acetamidoxime and sodium hydride. Ribavirin methyl ester (**9**) was prepared as reported;[15] tiazofurin methyl ester (**10**) was obtained starting from ethyl 2-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)thiazole-4-carboxylate[14] by treatment with sodium methoxide in methanol.

As depicted in tables 1 and 2, the oxadiazole **8** presented a pattern of activity very similar to that of **4**; both were more active against lymphoblastoid (IC_{50} = 0.2 to 3.5 μM) than solid tumor cell lines (IC_{50} = 2.8 to 8.5 μM). In our opinion this activity unequivocally confirm the needing of a methyl ester at position C3 to maximise activity. It is also noteworthy to observe that this is the first time that the oxadiazole ring has been used in the field of nucleoside analogs, further extending the applications of this group as an ester replacement.[13] On the contrary, ribavirin and tiazofurin methyl esters (**9** and **10**) resulted inactive.



9



10

In view of the capability of ribavirin and tiazofurin to potentiate the antiretroviral activity of ddI and to antagonise that of AZT,[16,17] **4** was tested against HIV-1, alone or in combination with either ddI or AZT (Table 3). Compound **4** showed no intrinsic antiviral activity. Nevertheless, it enhanced the anti-HIV-1 activity of ddI and slightly antagonised that of AZT.

Table 3. Effect of **4** on the anti-HIV-1 activity of ddI and AZT.

Compound	ddI/AZT EC_{50} (±SD)
ddI	10 ± 1.1
0.5 μM **4** + ddI	3 ± 0.4
AZT	0.002 ± 0.0003
0.5 μM **4** + AZT	0.005 ± 0.0004

[a] Effective concentration 50. Dose of compound (μM) required to achieve 50% protection of MT-4 cells against the cytopathic effect of HIV-1. Values represent the mean of at least three separate experiments.

Although combination studies indicate that **4** slightly potentiates the anti-HIV-1 activity of ddI, it cannot be concluded that, like ribavirin,[18] this new azole nucleoside targets the IMPDH. On the contrary, the evidence that the methyl esters of ribavirin and tiazofurin (**9** and **10**) were inactive suggests that IPCAR targets an enzyme other than IMPDH involved in the purine *de novo* biosynthesis (PRPP ---> PRA ---> IMP).[19]

In conclusion, from the data reported here, **4** appears to be a promising candidate for antitumor chemotherapy. In particular, the low cytotoxicity of **4** for resting PBL, associated with the potentiation of the anti-HIV-1 activity of ddI, makes this compound a potential candidate for the therapy of AIDS-associated neoplasias. Further studies are currently in progress to clarify the mechanism of action and the nature of the potentiation mechanism of the anti-HIV activity of ddI.

Acknowledgements. This work was supported by Ministero dell'Università e della Ricerca Scientifica e Tecnologica (MURST) and by Sardinian Antiviral Research Consortium (SARC) and Istituto Superiore di Sanità (Progetto AIDS 1995, n. 9304-75).

References and Notes

1. Streetter, D. G.; Witkowski, J. T.; Khare, G. P.; Sidwell, R. W.; Bauer, R. J.; Robins, R. K. ; Simon, L. N. *Proc. Natl. Acad. Sci. Usa* **1973**, *70*, 1174.
2. Riley, T. A.; Larson, S. B.; Avery, T. L.; Finch, R. A. ; Robins, R. K. *J. Med. Chem.* **1990**, *33*, 572
3. Goswami, B. B.; Borek, E.; Sharma, O. K.; Fujitaki, J. ; Smith, R. A. *Biochem. Biophys. Res. Commun.* **1979**, *89*, 830.
4. Manfredini, S.; Bazzanini, R.; Baraldi, P. G.; Guarneri, M.; Simoni, D.; Marongiu, M. E.; Pani, A.; Tramontano, E. ; La Colla, P. *J. Med. Chem.* **1992**, *35*, 917.
5. Parsons, W. H. *Annual Reports in Medicinal Chemistry*; Hagmann, W. K., Ed.; Academic Press: New York, **1994**, pp. 175-184.
6. Bhattacharya, B. K.; Robins, R. K. ; Revankar, G. R. *J. Heterocyclic Chem.* **1990**, *127*, 795.
7. Hobbs, J.B. *Comprehensive Medicinal Chemistry, Volume 2*. Pergamon Press, Oxford, **1990**, p 299
8. De Clercq, E.; Cools, M.; Balzarini, J.; Snoeck, R.; Andrei, G.; Hosoya, M.; Shigeta, S.; Ueda, T.; Minakawa, N. ; Matsuda, A. *Antimicrob. Agents. Chemother.* **1991**, *35*, 679.
9. Robins, M. J.; Manfredini, S.; Wood, S. G.; Wanklin, R. J.; Rennie, B. A. ; Sacks, S. L. *J. Med. Chem.* **1991**, *34*, 2275.
10. Zhang, W.; Robins, M.J. *Tetrahedron Lett.* **1992**, *33*, 1177.
11. Global lipophilicity calculated with the CLOGP3 program, Medicinal Chemistry Project, Pomona College, CLOGP-3.54.
12. Gabrielsen, B.; Phelan, M. J.; Barthel-Rosa, L.; See, C.; Huggins, J. W.; Kefauver, D. F.; Monath, T. P.; Ussey, M. A.; Chmurny, G. N.; Schubert, E. M.; Upadhya, K.; Kwong, C.; Carter, D. A.; Secrist III, J. A.; Kirsi, J. J.; Shannon, W. M.; Sidwell, R. W.; Kini, G. D. ; Robins, R. K. *J. Med. Chem.* **1992**, *35*, 3231.
13. Orlek, B. S.; Blaney, F. E.; Brown, F.; Clark, M. S. G.; Hadley, M. S.; Hatcher, J.; Riley, G. J.; Rosenberg, H. E.; Wadsworth, H. J. ; Wyman, P. *J. Med. Chem.* **1991**, *34*, 2726.
14. Srivastava, P. C.; Pickering, M. V.; Allen, L. B.; Streetter, D. G.; Campbel, M. T.; Witkowski, J. T.; Sidwell, R. W. ; Robins, R. K. *J. Med. Chem.* **1977**, *20*, 256.
15. Witkowski, J. T.; Robins, R. R.; Sidwell, R. W. ; Simon, L. N. *J. Med. Chem.* **1972**, *15*, 1150.
16. Hartman, N. R.; Ahluwalia, G. S.; Cooney, D. A.; Mitsuya, H.; Kajeyama, S.; Fridland, A.; Broder, S. ; Johns, D. G. *Mol. Pharmacol.* **1991**, *40*, 118.
17. Vogt, W. M.; Hartshorn, L. K.; Furman, A. P.; Chou, T. C.; Fyfe, A. J.; Coleman, L. A.; Crumpacker, C.; Schooley, R. T. ; Hirsch, M. S. *Science* **1987**, *235*, 1376.
18. Weber, G.; Hata, Y.; Prajda, N. *Pharm. World Sci* **1994**, *16*, 77.
19. Further biological studies are in progress to verify this hypothesis.

(*Received in Belgium* 11 *March* 1996; *accepted* 7 *May* 1996)