

This article was downloaded by:

On: 27 January 2011

Access details: *Access Details: Free Access*

Publisher *Taylor & Francis*

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Nucleosides, Nucleotides and Nucleic Acids

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713597286>

[³H]R75231; A New Radiolabelled Nucleoside Transporter Probe Related to Mioflazine and Lidoflazine

M. Kruidering^a; A. P. Ijzerman^a; H. Van Belle^b

^a Div. of Medicinal Chemistry, Center for Bio-Pharmaceutical Sciences., RA Leiden, The Netherlands ^b Janssen Research Foundation, Beerse, Belgium

To cite this Article Kruidering, M. , Ijzerman, A. P. and Van Belle, H.(1991) '[³H]R75231; A New Radiolabelled Nucleoside Transporter Probe Related to Mioflazine and Lidoflazine', *Nucleosides, Nucleotides and Nucleic Acids*, 10: 5, 1223 — 1224

To link to this Article: DOI: 10.1080/07328319108047285

URL: <http://dx.doi.org/10.1080/07328319108047285>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.informaworld.com/terms-and-conditions-of-access.pdf>

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

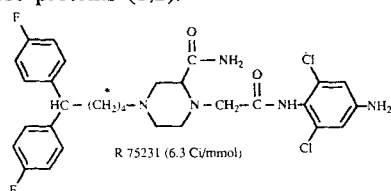
The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

[³H]R75231: A NEW RADIOLABELLED NUCLEOSIDE TRANSPORTER PROBE RELATED TO MIOFLAZINE AND LIDOFLAZINE.

M.Kruidering,* A.P. IJzerman and H Van Belle¹.

Center for Bio-Pharmaceutical Sciences, Div. of Medicinal Chemistry, P.O. Box 9502, 2300 RA Leiden, The Netherlands, and ¹Janssen Research Foundation, Beerse, Belgium.

The transport of nucleosides and analogues across the plasma membrane of animal cells is mediated by nucleoside-specific transport proteins, by means of facilitated diffusion. Development of highly potent tritiated transport inhibitors allowed ligand binding studies, performed at various tissues. [³H]Nitrobenzylthioinosine ([³H]NBI) and [³H]dipyridamole are used in this respect, yielding a wealth of information about the molecular characteristics of these proteins (1,2).



Recently we reported on a new class of highly potent transport inhibitors, all substituted piperazines related to lidoflazine and mioflazine, displacing specific [³H]NBI binding ($K_d = 0.65 \pm 0.05$ nM) from calf lung

tissue with high affinity and pseudo-Hill coefficients larger than unity (3).

Here we report on the characteristics of a radiolabelled mioflazine analogue, [³H]R75231.

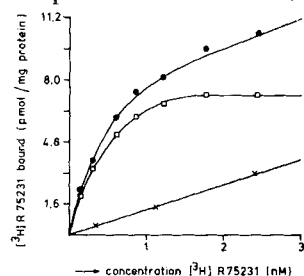


Figure 1. Binding of [³H]R75231 to a membrane preparation of calf lung tissue. Specific binding (○) is defined as the difference between binding in presence (×) and in absence (●) of 3 μM dipyridamole. Results shown are from a typical experiment.

Saturation studies revealed the specific binding of [³H]R75231 to a membrane preparation of calf lung tissue to be saturable and reversible, displaying high affinity with a $K_d = 0.32 \pm 0.06$ nM and a capacity of 6.1 ± 0.3 pmol/mg protein. Equilibrium of binding is reached within 20 minutes at 25 °C. Non-specific binding, predominantly to glass and plastic could be reduced by adding 0.1% CHAPS to the incubation medium and presoaking the Whatman GF/C filters with 0.3%

polyethylenimine.

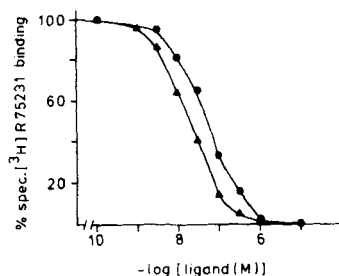


Figure 2. Dose-dependent displacement of specific $[^3\text{H}]\text{R75231}$ binding from a membrane preparation of calf lung tissue by NBI (▲) and dipyridamole (●). Results shown are from a typical experiment.

Potent displacers of $[^3\text{H}]\text{NBI}$ binding such as dipyridamole, dilazep and hexobendine are capable of displacing $[^3\text{H}]\text{R75231}$ binding with high affinity and the same potency rank order.

The pseudo-Hill coefficients equalling unity for both $[^3\text{H}]\text{NBI}$ and $[^3\text{H}]\text{R75231}$ displacement indicates the specific $[^3\text{H}]\text{R75231}$ binding does occur at the carrier protein.

The n_H and K_i values are listed in table 1.

Table 1. Displacement of specific $[^3\text{H}]\text{R75231}$ binding to a membrane preparation of calf lung tissue. Listed are K_i -values and pseudo Hill coefficients (\pm S.E.M.), obtained according to the Hill procedure.

compound	K_i (nM)	n_H
dilazep	0.65 ± 0.09	1.00 ± 0.07
mioflazine	0.89 ± 0.09	1.03 ± 0.05
R75231	1.08 ± 0.03	1.13 ± 0.03
NBI	4.8 ± 0.7	0.94 ± 0.05
hexobendine	6.2 ± 0.5	0.94 ± 0.01
dipyridamole	10.0 ± 1.0	0.89 ± 0.04

Since the potency rank order of displacers was equal for both $[^3\text{H}]\text{R75231}$ and $[^3\text{H}]\text{NBI}$ binding (dilazep > hexobendine > dipyridamole), $[^3\text{H}]\text{R75231}$ is a new probe for the nucleoside transporter binding site displaying a higher affinity than $[^3\text{H}]\text{NBI}$.

The substituted piperazines display pseudo Hill coefficients equal to unity when displacing $[^3\text{H}]\text{R75231}$ (n_H larger than unity when displacing $[^3\text{H}]\text{NBI}$) suggesting a difference in interaction of both radioligands at the nucleoside transporter binding site.

REFERENCES

- (1) Jarvis, SM, Young, JD, (1983). *Biochem.J.* **190**, 377.
- (2) Marangos, PJ, Houston, M, Montgomery, P, (1985). *Eur.J. Pharmacol.* **117**, 393.
- (3) IJzerman, AP, Thedinga, KH, Custers, AFCM, Hoos, B, Van Belle, H, (1989). *Eur.J. Pharmacol.* **172**, 273.