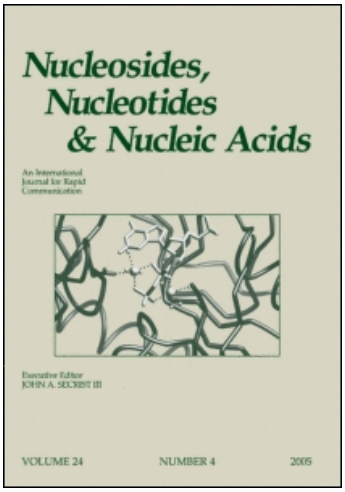

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[^{3}H]R75231; A New Radiolabelled Nucleoside Transporter Probe Related to Mioflazine and Lidoflazine

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

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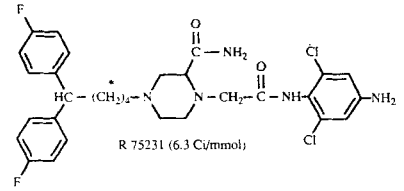
The transport of nucleosides and analogues across the plasma membrane of animal cells is mediated by nucleoside-specific transport proteins, by means of facilitated diffusion. Development of highly potent tritiated transport inhibitors allowed ligand binding studies, performed at various tissues. [^{3}H]Nitrobenzylthioinosine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



R 75231 (6.3 Ci/mmol)

Recently we reported on a new class of highly potent transport inhibitors, all substituted piperazines related to lidoflazine and mioflazine, displacing specific [^{3}H]NBI binding (k_d= 0.65 ± 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, [^{3}H]R75231.

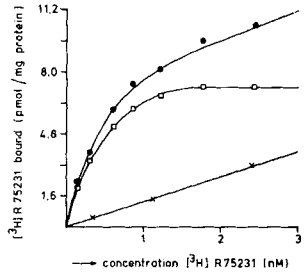


Figure 1. *Binding of [^{3}H]R75231 to a membrane preparation of calf lung tissue. Specific binding (○) is defined as the difference between binding in presence (×) and in absence (●) of 3 μM dipyridamole. Results shown are from a typical experiment.*

Saturation studies revealed the specific binding of [^{3}H]R75231 to a membrane preparation of calf lung tissue to be saturable and reversible, displaying high affinity with a K_d= 0.32 ± 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.

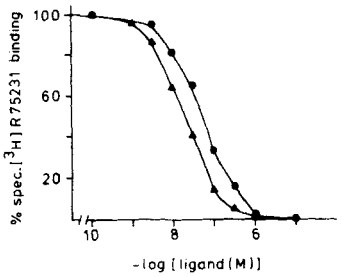


Figure 2. *Dose-dependent displacement of specific [³H]R75231 binding from a membrane preparation of calf lung tissue by NBI (▲) and dipyridamole (●). Results shown are from a typical experiment.*

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

The pseudo-Hill coefficients equalling unity for both [³H]NBI and [³H]R75231 displacement indicates the specific [³H]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 [³H]R75231 binding to a membrane preparation of calf lung tissue. Listed are K_i-values and pseudo Hill coefficients (±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 [³H]R75231 and [³H]NBI binding (dilazep > hexobendine > dipyridamole), [³H]R75231 is a new probe for the nucleoside transporter binding site displaying a higher affinity than [³H]NBI.

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

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