EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

ESRF

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office using the **Electronic Report Submission Application:**

http://193.49.43.2:8080/smis/servlet/UserUtils?start

Reports supporting requests for additional beam time

Reports can now be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** Nitroreductase – Prodrug complexes	**Experiment number**: LS-1820
Beamline: ID14-4	**Date of experiment**: from: 01/11/00 to: 03/11/00	**Date of report**:
Shifts: 2	**Local contact(s)**: Raimond Ravelli	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Prof Stephen Neidle – Institute of Cancer Research

Dr Gary Parkinson* – Institute of Cancer Research

Dr Mark Roe* – Institute of Cancer Research

Eric Johannsen* – Institute of Cancer Research

Report:

The ability of the protein, Nitroreductase (NR), from *E. coli*, to reduce nitro groups to their corresponding hydroxalamines, makes it an ideal candidate for use as a pro-drug activator in gene therapy. A wide range of compounds, based on the pro-drug, CB1954, have been synthesized to date. However, these modifications have been largely *ad hoc*, and no clear structure-activity relationship has yet been postulated. The solution of the structure of NR in our lab (*J. Med. Chem.* 2000, 43, 3624-3631), has provided us with the potential to address this problem.

As a first step to quantifying a structure-activity relationship, we collected several datasets at station ID14.4 on co-crystals of the enzyme with the pro-drugs CB1954, SN23862 and the irreversible inhibitor, dicoumarol (Fig.2a-c). These structures, all three of which diffracted to 2Å resolution, now in the final stages of refinement, have yielded valuable insight into the structure-activity relationship of these compounds. Further structural studies are necessary to determine binding modes, with absolute certainty. The effect on side-chain position and composition, in particular, needs to be addressed. These studies will include co-crystallization with other drugs having varying side-chain composition. Effort is also ongoing to obtain suitable crystals of the reduced form of the enzyme. Due to the detrimental effect reduction seems to have on these crystals, a powerful X-ray source is required.

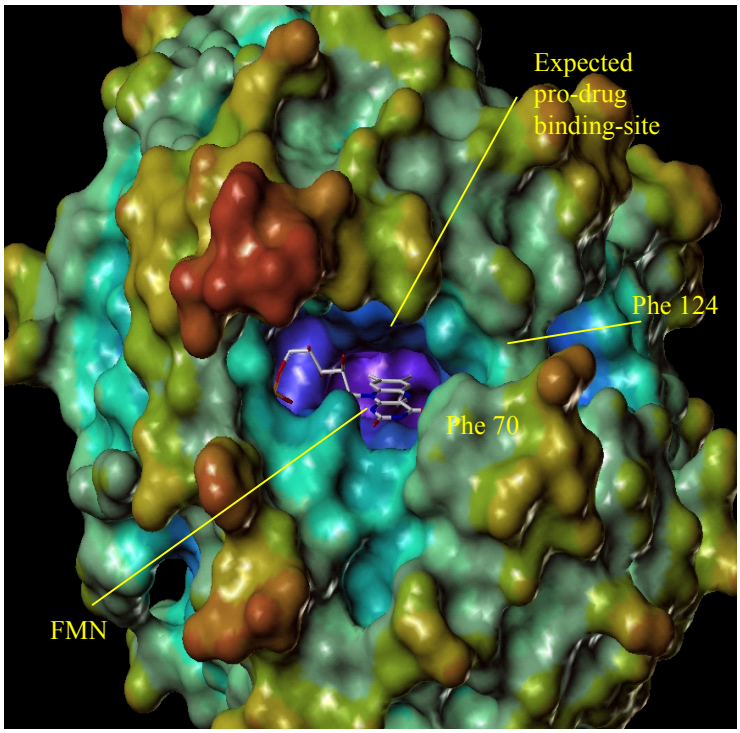
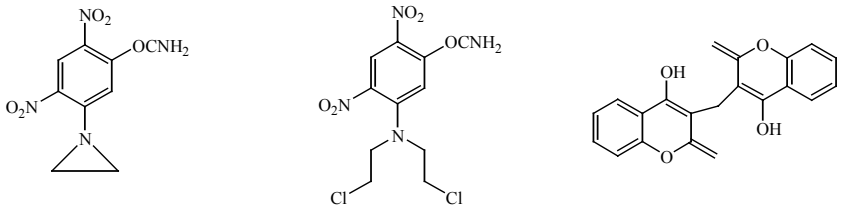


Fig.12 – Connelly solvent-accessible surface representation of native NR around the substrate binding pocket region coloured by charge. The two channels are labelled A and B. Residue Phe124 of Helix G is labelled.



CB1954 SN23862 Dicoumarol

Fig.1- Structures of two prodrugs, (a), CB1954, and (b), SN23862, as well as the irreversible inhibitor, dicoumarol.

Knowledge gained from these studies will be applied in an attempt to improve on the current range of available prodrugs for use in enzyme prodrug therapy.