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Review Article

From ICI to AstraZeneca: the development of synthetic isotope chemistry at Alderley Park, UK†

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Abstract: Synthetic isotope chemistry has played a key role in the discovery and development of candidate drugs for over 40 years at Alderley Park, UK. This review highlights the range of isotopically labelled compounds that have been synthesized and the applications for which they were used. The continually increasing demand for isotopically labelled compounds demonstrates their valuable contribution to the pharmaceutical industry. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: isotopic labelling; synthesis; pharmaceuticals

Introduction

The development of penicillin in the post-war period heralded the birth of modern drug therapy. Along with other large chemical companies Imperial Chemical Industries (ICI) expanded its medicinal chemistry group (part of Dyestuffs Division, located at Blackley, Manchester) to form a separate Pharmaceuticals Division. A green field site at Alderley Park was developed and new research laboratories were formally opened in October 1957.

Thus, the 50th anniversary of the Journal of Radiolabelled Compounds and Radiopharmaceuticals in 2007 coincides with the 50th anniversary of pharmaceutical research and development at Alderley Park. In these personal reflections, I have concentrated on the synthesis of isotopically labelled compounds at Alderley Park to support the discovery, development and launch of new drugs. Thus, I shall not cover our work in support of veterinary products or the work carried out separately at the Central Toxicology Laboratory on this site to support ICI Plant Protection Division, Zeneca Agrochemicals and now Syngenta Agrochemicals businesses.

^{14}C and ^{3}H labels for investigative drug metabolism and receptor-binding applications

The earliest radiochemical syntheses for ICI Pharmaceuticals Division were carried out in temporary facilities at Blackley but with the move to Alderley Park, a new purpose-built laboratory was established by John Burns and David White. The laboratory was designed for the safe handling of non-volatile ^{14}C, ^{3}H and ^{35}S compounds. There were no vacuum line facilities, hence more advanced ^{14}C intermediates and primary tritiations were purchased from external suppliers. The radiochemical synthesis team was supplemented by synthetic chemists from Medicinal Chemistry seconded as part of their career development.

The regular synthesis of ^{14}C, ^{3}H and occasionally ^{35}S compounds for drug metabolism and pharmacokinetic applications began in 1964. The β-adrenergic blocking agent propranolol (Inderal®) (Figure 1) was labelled with ^{14}C from 1-[1-^{14}C]-naphthol and with ^{3}H by non-specific catalytic exchange with tritiated acetic acid.[1] This work illustrates some of the techniques that were available at that time for labelling and analysis. Thus, during the analysis of ^{14}C propranolol hydrochloride by thin-layer chromatography (TLC) (in the days before HPLC!) on silica plates in a weakly acidic eluent, double spot formation was observed. This was attributed to propranolol hydrochloride forming base–hydrochloride and base–acetate ion pairs. It is interesting to also note that a special fluorographic technique was used for

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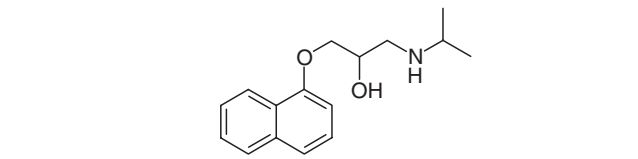
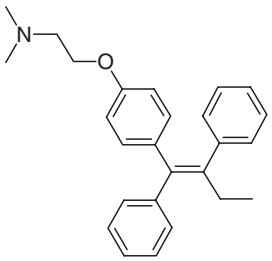


Propranolol



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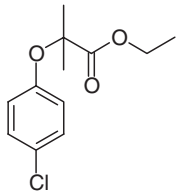
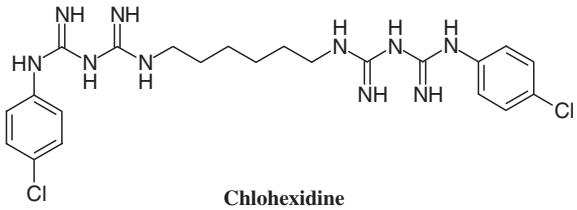
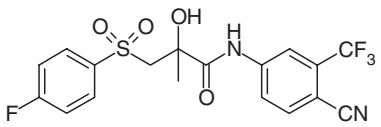


Clofibrate



Chlohexidine



Bicalutamide

pyroGlu.His.Trp.Ser.Tyr.D-Ser(^{t}Bu).Leu.Arg.Pro.Azgly.NH_2

Goserelin

Figure 1 Structures of representative development compounds isotopically labeled at Alderley Park (1).

autoradiographic studies of TLC plates used for the analysis of ^{3}H propranolol.[2]

Organizationally, the team remained closely aligned with Pharmacokinetics and Drug Metabolism as part of a larger toxicology function. The 1970s were an exciting time for the science of drug metabolism and David Foulkes and David Case from Alderley Park were instrumental in setting up the Drug Metabolism Discussion Group, a UK industry-wide group of scientists involved in this work.

In vitro and *in vivo* drug metabolism studies became increasingly more sophisticated and the synthesis of several isotopomers of a development candidate became routine in order to fully define the metabolism of the compound. During the 1970s, in addition to radiotracers, stable labelled compounds were synthesized to aid metabolite identification by mass spectrometry in studies where $^{14}C/^{13}C/^{12}C$ mixtures were dosed. Dual-labelling studies using $^{14}C/^{3}H$ mixtures were also used to elucidate complex metabolism pathways using the minimum number of experiments.

There was also increased interest in the use of ^{3}H-labelled compounds for receptor binding studies to investigate mechanisms of action. With the anti-oestrogen tamoxifen (Nolvadex®) (Figure 1), initial exchange and specific labelling methods produced only low specific activity products (4.7 and 401 mCi/mmol, respectively). However, the dehalogenation of a suitable dibromo-precursor with tritium gas followed by dehydration and chromatographic isolation of the required

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trans-isomer gave ^{3}H tamoxifen at a specific activity of 19.6 Ci/mmol. This specific activity was sufficient to allow quantitative measurement of changes at receptor binding sites in selected target organs.[3]

The ready availability of radiolabelled compounds was also used to generate comprehensive retrospective metabolism packages for compounds, such as the anti-hyperlipidemic agent clofibrate (Atromid-S®)[4] and the anti-bacterial chlorhexidine (Hibitane®)[5] (Figure 1), that had been on sale since the 1960s.

I joined the ICI team in 1977, following early training in radiochemistry at The Radiochemical Centre, Amersham when Tony Evans was Head of Department, then took over as Head of Radiochemistry when John Burns retired in 1985. The team continued to report through the Drug Kinetics/Safety Of Medicines organization. We continued to synthesize ^{14}C- and ^{3}H-labelled compounds for ADME and mechanistic studies with increasing numbers of drug development candidates including the anti-androgen bicalutamide (Casodex®) (Figure 1), now used for the treatment of prostate cancer.[6] The development of much more potent compounds required the use of higher specific activity tracers. Thus, the development of the luteinizing hormone-releasing hormone analogue goserelin (Zoladex®) (Figure 1) for the treatment of prostate cancer required extensive use of [3,5-$^{3}H_2$-Tyr^{5}]-labelled compound, prepared by catalytic dehalogenation of the corresponding diiodo-analogue, at a specific activity of 43 Ci/mmol. The fate of a key unnatural amino acid in the molecule was investigated using [D-3-^{14}C-Ser(^{t}Bu)^{6}]-labelled compound, prepared from Fmoc-D-[3-^{14}C]Ser(^{t}Bu) by solid-phase synthesis, at a specific activity of 54 mCi/mmol.[7]

^{125}I and stable isotope labels to support bioanalysis

During this period, radioimmunoassay became the method of choice for pre-clinical and clinical bioanalysis and specialist method developers (Brian Law, Mike Warwick and Ron Moore) carried out the synthesis of the ^{125}I tracers in our laboratories as part of the development of the full assay. Additionally, ^{125}I peptides were synthesized for discovery support applications. The rapid increase in labelling work culminated in the construction of a new radiosynthesis laboratory with additional facilities for ^{125}I labelling in 1989. The comprehensive labelling programme (^{14}C, ^{3}H, ^{2}H, ^{125}I) for the pure oestrogen receptor antagonist, fulvestrant (Faslodex®)[8] and the multi-^{14}C labelling approach used for fully elucidating the metabolic pathways of the selective aromatase inhibitor, anastrozole (Arimidex®)[9] (Figure 2) illustrate the vital role isotopic labelling plays in the full development of a drug candidate through to launch.

In 1993, ICI demerged three of its businesses, including pharmaceuticals to form a separate company Zeneca and this provided additional focus to our work. Around this time, as well as providing labelled compounds for Alderley Park projects, the team was also supporting both discovery and development requirements for labelled compounds for our US site in Wilmington, Delaware.

In the late 1990s, rapid developments in liquid chromatography–mass spectrometry technology revolutionized pre-clinical and clinical bioanalysis and mass spectrometry-based assays became the methods of choice. Initially analogues of the analyte were used as internal standards, although it soon became clear that the use of stable labelled forms of the analyte as internal standards produced more robust high-sensitivity assays. In favourable cases, the required mass increases can be readily introduced through commercially available polydeuterated precursors. This is illustrated in the synthesis of a [d_8]-labelled analogue of the epidermal growth factor receptor (EGFR) inhibitor gefitinib (Iressa®)[10] (Figure 2) used for the treatment for non-small cell lung cancer. However, the morpholino side chain has an important influence on the chromatographic behaviour of the compound and an isotope effect is seen that must be taken into account in the assay. Reducing levels of unlabelled compound in the stable labelled internal standard became an increasingly important consideration. The chromatographic, isotopic abundance and potential lability issues with ^{2}H gradually led to a preference for incorporating ^{13}C and ^{15}N isotopes to provide the desired mass increase. However, this often resulted in more complex chemistry and longer lead times. The requirement to have stable labelled versions of all drug development candidates available at nomination produced a large increase in workload and a subsequent increase in size of the team from four to six staff. Although, radioimmunoassays did continue for some time, these were largely maintained by outsourcing the regular supply of ^{125}I tracer and no new assays were developed.

Improved ^{3}H labelling procedures to aid problem solving in drug discovery

The merger of Zeneca with Astra in 1999 to form AstraZeneca brought together five Radiochemistry teams (a total of 15 staff) on different sites in the UK and Sweden. The Astra teams were based in Medicinal Chemistry rather than in Development DMPK as we were at Alderley Park and, in many cases, provided

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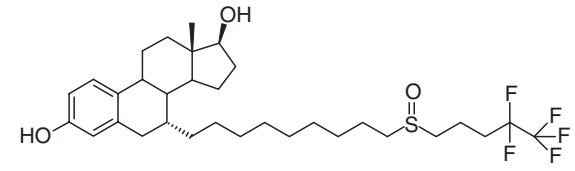
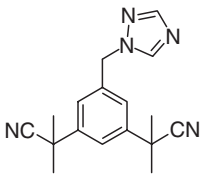
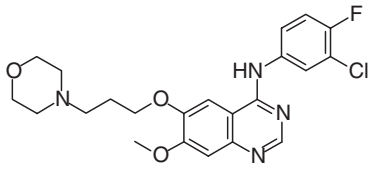


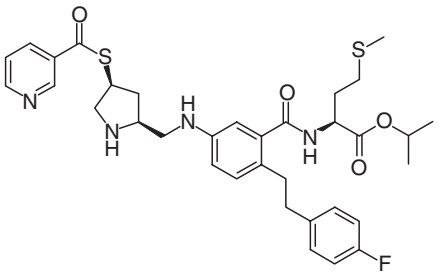
Fulvestrant



Anastrozole



Gefitinib



AZD3409

Figure 2 Structures of representative development compounds isotopically labeled at Alderley Park (2).

more Discovery support with all groups having their own tritium labelling capability. Tritium labelling to support drug discovery was facilitated by (i) the ready availability of manifold systems that allow safe handling of tritium gas (and tritiated reagents) in the laboratory with control and recycling of radioactive waste, (ii) improved rapid labelling chemistries[11] and (iii) advances in the resolution and sensitivity of ^{3}H NMR spectroscopy[12] and mass spectrometry to specify the position and degree of incorporation of labels. With time, in line with business operations, the teams have concentrated on providing local support to particular Research and Therapeutic Areas.

At Alderley Park, we established the same tritium labelling capability in 2003 and are providing increased earlier labelling together with development support for Oncology and Infection projects from Alderley Park, Gatehouse Park (Boston, US) and Bangalore (India). In particular, the desire to minimize the risk associated with reactive metabolites and the impact of the Draft Guidance for Industry on Safety Testing of Drug Metabolites from the US Food and Drug Administration[13] has led to earlier reactive metabolite screening and metabolite identification studies where the availability of labelled compound can be of real benefit. In order to understand the metabolic basis for idiosyncratic adverse reactions in man where reactive intermediates have been implicated, my team has provided radiosynthesis and metabolite synthesis effort to support the AstraZeneca reactive metabolite strategy through joint collaborations with Liverpool University.[14–17] During the development of pro-drugs, the requirement for both radio- and stable labelled versions of both the pro-drug, drug and major metabolites has greatly increased the size of the labelling package for the project. This is illustrated with recent programme for the peptidomimetic farnesyl transferase (FAR) inhibitor, AZD3409 (Figure 3), where the double pro-drug is rapidly broken down to thiol ester and thiol acid metabolites.[18] In this case, the synthesis of novel unlabelled metabolites was also carried out by the Isotope Chemistry team.

The number of AstraZeneca teams has increased to six with the re-establishment of a team in Wilmington by Dick Heys and the size of teams on some sites has increased to reflect Research and Therapy Area

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demand (25 staff by the end of 2006). The large increase in projects being progressed by the business, together with increased demand for early radiolabelling to support problem-solving activities in Discovery, has impacted all teams and this has resulted in higher levels of outsourcing to key suppliers. Facilities have been continually updated culminating at Alderley Park with the opening of a larger, more flexible, facility for the synthesis of radiolabelled, stable labelled and unlabelled compounds to support drug projects in November 2006.

An important advance during this period was the increasing use of microwave-enhanced conditions for labelling reactions. Although the utility of this approach was initially investigated through external collaborations with the University of Surrey and Amersham Biosciences,[19,20] these procedures are now being used regularly in house.

Human radiolabelled studies; present and future

Radiolabelled metabolism studies in volunteers and patients (for some Oncology products) are a key element of the DMPK package for a new development product. The availability of a clinical pharmacology unit at Alderley Park has provided a focus for these studies over 30 years that continues today within AstraZeneca. The manufacture of radiolabelled drug substance (API) for these studies presents a particular challenge to the radiochemist in terms of synthesis, stability and analysis. The quality standards for this work are the subject of active debate within the pharmaceutical industry following the implementation of the European Clinical Trials Directive 2001/20/EC that came into force in the UK in May 2004 although it is clear that the formulated product for dosing (IMP) must be manufactured in accordance with the appropriate principles of cGMP.

Within AstraZeneca, studies are conducted with ^{14}C- and ^{3}H-labelled compounds, typically early in the Phase 2 clinical programme. For many years, oral dosing using milled powders mixed with lactose in gelatine capsules was widely used. However, the specific surface area of the labelled compound was crucial to the successful outcome of the study for compounds with low bioavailability. Today compounds are usually dosed in oral solution or in parenteral formulations. The discovery of unique human metabolites is not unusual and it is important to investigate the implications of such findings as soon as possible.

To date microdosing techniques have not yet been widely used at Alderley Park. Accelerator mass spectrometry (AMS) has been used to measure low levels of radiolabelled drug and metabolites in tissues and biological fluids, but has not been used in 'first in human' studies to provide initial pharmacokinetics and metabolism information in volunteers. Similarly, within Oncology, positron emission tomography (PET) using radiolabelled AstraZeneca compounds has not been a focus of attention.

Conclusions

Synthetic isotope chemistry has played a key role in the discovery and development of candidate drugs for over 40 years at Alderley Park. The demand for ^{14}C- and ^{3}H-labelled compounds is higher than ever before despite the advances in non-radioactive technologies and the use of a combination of the local in-house team and external suppliers has worked well to meet the demand. The pressure to reduce attrition rates and development times for new drugs has led to the increasing use of tritium labelling compounds for problem-solving activities during drug discovery. The provision of stable isotope-labelled internal standards for use in mass spectrometry-based assays has made a major contribution to a revolution in pre-clinical and clinical bioanalysis. The wider use of microwave-enhanced isotopic labelling reactions offers significant productivity advantages. For the future, microdosing techniques (such as AMS and PET) may offer some real benefits in particular therapy areas.

Acknowledgements

In memory of Professor John Jones who was a constant source of enthusiasm, encouragement and guidance. Thanks to all past and present members of Radiochemistry and Isotope Chemistry teams at Alderley Park for their commitment and technical skills and in setting such high standards of delivery and safety. In particular, I would like to recognize the key contributions of (the late) John Burns, David White, Julie Bergin, Helen Booth, David Killick, Clare King, Clare Silcock, Nick Bushby, Angela Jordan and Ryan Bragg. In addition, we are indebted to the suppliers of isotopically labelled starting materials, intermediates and final products, without whom we would not have been able to conduct our work and respond so flexibly to project demands. I would also like to thank AstraZeneca and International Isotope Society radiochemistry colleagues for many valuable discussions and the DMPK function for their long-term support in developing the synthetic isotope chemistry capability at Alderley Park.

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