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Database extraction of metabolite information of drug candidates: Analysis of 27 AstraZeneca compounds with human ADME data

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List of nonstandard abbreviation:

ACDLabs: Advanced Chemistry Development Laboratories

ADME: Absorpion, Distribution, Metabolism, Excretion

HLM: Human Liver Microsomes

KCN: Potassium Cyanide

LC-MS/MS: Liquid Chromatography coupled to Tandem Mass Spectrometry

LM: Liver Microsomes

Met-ID : Metabolite Identification

MIST: Safety Testing of Drug Metabolites

MS/MS: Tandem Mass Spectrometry

OATs: Organic Anion Transporters

UPLC- QToF: Ultra-Performance Liquid Chromatography coupled to Quadrupole time of Flight Mass Spectrometry

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Abstract

As part of the drug discovery and development process, it is important to understand the human metabolism of a candidate drug prior to clinical studies. Pre-clinical *in vitro* and *in vivo* experiments across species are conducted to build knowledge concerning human circulating metabolites in preparation for clinical studies and therefore, the quality of these experiments is critical. Within AstraZeneca, all metabolite identification (Met-ID) information is stored in a global database using ACDLabs software. In this study, the Met-ID information derived from *in vitro* and *in vivo* studies for 27 AstraZeneca drug candidates that underwent human ADME studies was extracted from the database. The retrospective analysis showed that 81% of human circulating metabolites were previously observed in pre-clinical *in vitro* and/or *in vivo* experiments. A detailed analysis was carried out to understand which human circulating metabolites were not captured in the pre-clinical experiments. Metabolites observed in human hepatocytes and rat plasma, but not seen in circulation in humans (extraneous metabolites) were also investigated.The majority of human specific circulating metabolites derive from multi-step biotransformation reactions that may not be observed in *in vitro* studies within the limited time frame cryopreserved hepatocytes are active. Factors leading to the formation of extraneous metabolites in pre-clinical studies seemed to be related to species differences with respect to transporter activity, secondary metabolism and enzyme kinetics. This retrospective analysis assesses the predictive value of Met-ID experiments and improves our ability to discriminate between metabolites expected to circulate in humans and irrelevant metabolites seen in pre-clinical studies.

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1. Introduction

A typical drug discovery and development campaign includes Met-ID studies on a large number of compounds with the aim of optimizing metabolic clearance, designing away from reactive metabolite formation, creating awareness of active metabolites and most importantly building confidence with respect to which human circulating metabolites to expect in clinical studies.(Isin et al., 2012, Stepan et al., 2013) This knowledge will support the planning and execution of appropriate safety studies, e.g., selection of toxicology animal species prior to clinical trials. Without a streamlined way of capturing this structural information, metabolite knowledge generated on the many hundreds of project compounds studied is easily lost, as is the ability for collective learning. In order to remedy this issue, we have established a company-wide metabolite database with the aim of capturing information on metabolites generated at all stages of the drug discovery and development process from both *in vitro* and *in vivo* experiments carried out at all AstraZeneca sites.

Before taking drug candidates to the clinic to assess safety, tolerability, pharmacokinetic properties and efficacy, it is essential to build confidence in the safety of the new chemical entity using a host of *in silico* as well as *in vitro* and *in vivo* preclinical models. In addition to the safety of the parent drug candidate, it is equally important to evaluate the safety of the expected human metabolites.(Frederick and Obach, 2010, Nedderman et al., 2011) Prior to exposing human volunteers or patients to a drug candidate, metabolite identification studies *in vitro* and in animal models constitute the basis as surrogates for safety testing of expected human metabolites.(Luffer-Atlas, 2012) These approaches have the limitations inherent to the *in vitro* models (e.g., hepatocytes neglecting extrahepatic metabolism, viability of cells), as well as limitations due to differences in biotransformation and drug disposition between the various species used in the safety studies and hence may be of questionable predictive value. From a biotransformation perspective, the output from these and other investigational studies is a starting point in building an understanding of the metabolic routes in humans and ultimately an understanding of the full disposition of the drug candidate of interest. It is also an important part of the preparations for the first clinical studies where the drug candidate will

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be administered to man for the first time ("first time in man studies"). While urine and plasma samples should both be collected for metabolite investigations in these first clinical studies, the focus is on metabolites in systemic circulation for comparison with those metabolites circulating in the animal species used in the early preclinical safety studies. Plasma samples from these preclinical safety and the first human studies are analyzed for comparison of metabolite profiles and their relative exposure to each species.(Gao and Obach, 2011, Ma and Chowdhury, 2011) Early identification of significantly different metabolite profiles between species, may give time to proactively mitigate and preferably avoid resource-consuming problem solving studies. Such studies may involve, but are definitely not limited to, synthesis of the metabolite(s) and dosing to animals to reach adequate exposure, or even identifying new animal species that will form the human metabolite to adequate exposure levels and repeating the in vivo toxicology program.(Guengerich, 2009, Leclercq et al., 2009, Smith and Obach, 2009, Smith and Obach, 2006)

In this present report we describe an in-house database, where *in vitro* and *in vivo* animal and human biotransformation data on compounds from early as well as late stage drug development projects have been captured and stored over a period of close to one decade. This database currently contains over 12,500 biotransformation schemes for >5000 compounds. We present here our first investigations on how this data can form a knowledge base from which we can build an understanding of the quality of *in vitro* and pre-clinical systems for the prediction of circulating metabolites in humans.

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[Materials and Methods]

2. Metabolite identification studies

Met-ID information was generated from the analysis of AstraZeneca proprietary compounds in *in vitro* incubations in liver microsomes (LM) and hepatocytes from human, rat, dog, mouse and rabbit. Similarly *in vitro* incubations from reactive metabolite trapping experiments in human LM (HLM) with GSH, KCN and methoxylamine and from arachlor 1254 induced rat liver S9 fraction experiments was generated. Met-ID data from *in vivo* samples including plasma, urine, bile and faeces from dosed rat, dog, mouse, rabbit, cynomolgous monkey, marmoset and plasma, urine and faeces from human were likewise generated.

The analysis of *in vitro* and *in vivo* Met-ID experiments is typically performed by LC-MS/MS on Waters UPLC-Synapt QToF (Waters, UK) or ThermoFinnigan Orbitrap instrumentation.(Ekdahl et al., 2013) MS/MS spectra of parent compound and metabolites are acquired and analyzed using a combination of vendor software (e.g. MetaboLynx, Waters, UK) and interpretation carried out by biotransformation experts. Structures of both parent and metabolites are drawn as a biotransformation scheme in ACDLabs Spectrus DB Enterprise software (Advanced Chemistry Development, Toronto, Canada) with reaction arrows linking metabolites to parent molecule. Metabolite numbering, quantification (by MS response or radioactivity) of each metabolite and biotransformation type are also captured at this stage. The scheme is then uploaded to the database along with a standardized form capturing globally agreed experimental metadata fields e.g. responsible scientist, test system, project code, date etc.

PDF reports of the new database record are also generated and shared with drug discovery teams via upload to the corporate drug discovery database IBIS (Figure 1).

2.1 ACD database

The ability of the software to draw and capture Markush structures(Markush, 1924, Cosgrove et al., 2012) to cover vagueness in structural assignments is a useful feature which facilitates reporting of metabolites in cases where the site of metabolism cannot be narrowed down to a

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single atom.(Yerin and Peirson, 2012) The common platform nature of the database, allowed biotransformation scientists globally across the AstraZeneca organisation to capture metabolite structural information in the form of biotransformation schemes and experimental metadata.

The database currently contains over 12,500 schemes and approximately 50,000 metabolic reactions on proprietary AstraZeneca compounds of which only a small percentage is in the public domain.

2.1.1 Searching AstraZeneca Metabolite Database

The database can be searched in two ways, by text or by structure searching.

Experimental metadata e.g. incubation time, compound and project identifiers and responsible scientist, is captured as text fields alongside the biotransformation scheme These are accessible within the database by a simple text search function.

Exact, similarity or substructure searches can be performed and are accessed *via* the Chemsketch drawing module. Parent or metabolite structures or substructures are drawn in Chemsketch or simply imported from the database and suitably modified. The desired structure is selected and searched against the entire database.

For the purposes of this work, Met-ID information on AstraZeneca compounds that underwent radiolabelled studies in humans and for which plasma metabolite profiles were available was extracted using the text-search option. The list was refined further by carrying out text searching for compounds having human hepatocytes and rat plasma Met-ID information (Figure 2). Subsequently, the substructure search option was used to determine in which matrices each metabolite was observed.

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3. Results

3.1 Database extraction

As a result of the database extraction effort, a list of 27 candidate drugs that underwent radiolabelled cross-species *in vitro*, pre-clinical *in vivo*, and human ADME studies was compiled. For each compound, the database provided a list of records corresponding to the various biotransformation reactions reported to occur in different matrices (e.g. hepatocytes, liver microsomes, induced rat liver S9 fraction, urine, plasma, faeces, bile) of different species (e.g. human, rat, dog, monkey, mouse, rabbit). An example of an ACD database record containing Met-ID information obtained from the analysis of male rat plasma is given (Supplemental Figure 1). Prior to the descriptive and structural analysis, a unique metabolite scheme, including all the metabolites observed during these Met-ID studies, was built on the extracted data for each of the 27 compounds. One such summary scheme is shown in Figure 3. Moreover, tables listing all metabolites versus matrices were created for each compound to facilitate the various analyses and as an example the table created for AZD1 is given (Supplemental Table 1).

3.2 Descriptive analysis

The aim of the first part of the work was to evaluate the quality of our *in vitro* and *in vivo* pre-clinical experiments in terms of human metabolite prediction (Figure 4). All of the 27 AstraZeneca compounds underwent Met-ID studies in human and rat hepatocytes, and *in vivo* in rat and human using radiolabelled compound. Depending on the drug project, some compounds were also tested either *in vitro* and/or *in vivo* in other species, e.g., cynomolgus, marmoset, mouse or dog. However, as these species were not uniformly used as a test system for the whole subset of candidate drugs reported in this work, these data were included only in the descriptive analysis.

From the Met-ID studies on the 27 AstraZeneca candidate drugs, a total of 564 metabolite structures were identified including those with Markush labelling. Of the 564 metabolite

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structures, 234 were observed in human *in vivo* and/or *in vitro* samples and 144 of these were found in systemic circulation (i.e. observed in plasma). Of the 144 human circulating metabolites, 117 (81%) were observed before the human ADME study in at least one other test system. The actual number of previously observed human circulating metabolites varied however between candidate drugs (Figure 5) and in 16 out of the 27 cases it would have been possible to predict all human circulating metabolites based on Met-ID experiments prior to going into man.

A descriptive analysis was performed in order to assess the frequency at which human excreted metabolites (i.e. observed in urine and/or faeces samples) were observed in pre-clinical *in vitro* or *in vivo* studies prior to going into man. The analysis showed that 73% of the excreted human metabolites (157/216) were observed in at least one study type prior to the human ADME studies.

An evaluation of the predictive value of the different *in vitro* and *in vivo* systems used pre-clinically was carried out for human circulating metabolites. The Venn diagram in Figure 6 shows that by performing *in vitro* experiments only (all studied test species and systems), 46% (33% human hepatocytes only) of human circulating metabolites were observed. On the other hand, by performing *in vivo* studies only (all studied test species and matrices), 77% of circulating metabolites in man were observed.

3.3 Human circulating metabolites not observed in pre-clinical studies

For the studied 27 candidate drugs, only 27 circulating human metabolites (out of 144) were seen for the first time in man, whereas the remainder was previously observed in *in vitro* and/or *in vivo* pre-clinical studies (Figure 6). The biotransformation routes leading to these 27 previously unobserved metabolites were scrutinized to understand the underlying reasons for this lack of predictability. An analysis of the metabolic schemes indicates that the metabolites missed were primarily derived from multi-step reactions and evidence for the pathways leading to these metabolites had already been captured in *in vitro* experiments by the identification of intermediate or precursor metabolites. In several cases, these multi-step

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pathways resulted in the formation of a conjugated metabolite but examples of oxidative multi-step biotransformations were also identified. The initial enzymatic *O*-dealkylation of AZD24 is followed by phosphate hydrolysis leading to M24-a (Figure 7), was observed in human hepatocytes as well as in rat plasma, urine. However, metabolite M24-b, derived from the sulphonation of the intermediate phenol (M24-a) was observed for the first time in human plasma.

Another example of a previously unobserved human circulating metabolite involves AZD8.(Guo et al., 2015) A hydroxylated metabolite M8-a (Figure 8) was observed in human plasma and urine, mouse and rat plasma and human hepatocytes. The metabolite M8-b derives from the reaction of the drug (and/or M8-b) with endogenous CO_2 to form a carbamic acid intermediary metabolite which is subsequently conjugated with glucuronic acid. This resulting *N*-carbamoyl glucuronide M8-b was captured in human plasma only and hence unobserved prior to human studies.(Schaefer, 2006)

Only one compound (AZD10) had a metabolite found for the first time in human plasma where no precursors were captured in *in vitro* systems. As shown in Figure 9, the hydrolytic ring opening of the candidate drug AZD10, gives the previously unobserved metabolite M10-a.

3.4 Extraneous metabolites

The analysis of the 27 AstraZeneca compounds indicates that 58% of the metabolites observed during the pre-clinical Met-ID studies (*in vitro* and/or *in vivo*) are not relevant to what is observed subsequently in human circulation. In other words, during pre-clinical metabolite identification work, 330 of the metabolites formed and characterized, are not found in circulation in humans.

A deeper structural analysis of the metabolites observed in human hepatocytes and rat plasma but not identified in human plasma was performed and the results are shown in Table 1. Human hepatocytes and rat plasma were studied since these matrices were considered the most relevant to the retrospective analysis objective of this work. In particular, human

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hepatocytes allowed an *in vivo/in vitro* intra-species comparison to be made and rat plasma enabled the cross-species comparison. Rat was chosen over other species as it was used in radiolabelled Met-ID studies for all of the subset of compounds analysed.

3.5 Structural analysis of extraneous metabolites captured in human hepatocytes

Several metabolites observed in human cryopreserved hepatocytes but not in human plasma appear to be further metabolized in humans and hence these extraneous metabolites are intermediates or precursors of what is observed in *in vivo* studies. An example is depicted in Figure 10 where M7-a is a metabolite observed in *in vitro* systems only (human and rat hepatocytes). The metabolites formed *via* further metabolism of M7-a (*N*-oxidation to M7-b, oxidation to carboxylic acid M7-c, glucuronidation to M7-d) are not observed in hepatocytes but are captured in human urine (M7-b,-c,-d) and human plasma (M7-b).

Interestingly, in several examples metabolites seen in human hepatocytes but not in human plasma (or urine) are formed *via* amide dealkylation of tertiary amides. Examples for this biotransformation pathway are the secondary amide metabolites M9-a**,** M9-b derived from AZ9 (Figure 11A) and M13-a derived from AZD13 (Figure 11B) which were formed in human hepatocytes but were not found in human plasma or urine.

3.6 Structural analysis of extraneous metabolites captured in rat plasma

Metabolites captured in rat plasma but not observed in human plasma were investigated to understand if certain biotransformations are species specific.

Of the metabolites seen in rat plasma but not in human plasma, a relatively large number are organic anions. However, while not observed in plasma some of these metabolites are found in human urine. The majority of metabolites captured in rat plasma, but not circulating in human, were formed *via* biotransformations occurring on alkyl substituents of heterocyclic aromatic rings. No evidence for these pathways or further metabolism was found in human neither *in vitro n*or *in vivo*. An example is the *C*-methyl-hydroxylation of 2-methyl-pyrazine ring of AZD10 leading to the formation of M10-b (Figure 12). M10-c is a metabolite derived

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from further metabolism of M10-b. The proposed pathways involve the hydrolysis of the amide to carboxylic acid and a decarboxylation of the carboxylic acid to form M10-c. Neither M10-b nor M10-c were seen in human matrices.

Some of the extraneous metabolites reported to be present in rat plasma, suggest that biotransformations differ between species. An example for a reversible metabolic pathway as seen in rat and human plasma is shown in Figure 13. M7-e derived from compound AZD7 is observed *in vivo* in rat but not in humans. On the other hand, M7-f is observed in human plasma and has been reported to form in rat hepatocytes but not observed in rat plasma.

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4. Discussion

The AstraZeneca Metabolite database provides a central repository for all metabolite structural information on AstraZeneca compounds. It is a structure and text searchable resource which allows global sharing of metabolite data across AstraZeneca sites, therapeutic areas and projects and has secured that metabolite knowledge accumulated over many years is permanently accessible. The database contains metabolite identification data generated across the entire value chain from early discovery to product life cycle management. The majority of the data contained is from compounds synthesized during the discovery phase with human liver microsomal or hepatocyte Met-ID data, but a subset of development compounds contains a more comprehensive package of data, including Met-ID from human ADME studies.

The database differs from commercially available drug metabolite databases (BIOVIA Metabolite Database, BIOVIA, San Diego, U.S.) since it is built on studies of proprietary drugs and drug-like compounds combined with strictly defined experimental protocols and analytical methodologies.

The descriptive analysis of 27 AstraZeneca candidate drugs showed that *in vitro* and *in vivo* pre-clinical experiments were able to generate 81% of the human circulating metabolites and 73% of human excreted metabolites captured in the human ADME studies. *In vitro* experiments in human hepatocytes alone were able to predict 33% of human circulating metabolites. Inclusion of data from other *in vitro* systems in addition to human hepatocytes increases the predictability to 46%. This number is comparable to the outcome of the analysis done by Anderson *et al.* where it was shown that *in vitro* data predicted the major human circulating metabolites for 41% of the studied drug candidates (Anderson et al., 2009) whereas in another study human hepatocytes have been reported to predict complete circulating metabolite profiles for 65% of the studied compounds.(Dalvie et al., 2009) This observation is not surprising considering the caveats associated with *in vitro* systems (see below and (Dalvie et al., 2009)(De Graaf et al., 2002)), in particular the limited incubation times due to the decaying metabolic activity which leads to the inability of predicting

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secondary metabolites.(Dalvie et al., 2008) Micropatterned hepatocyte coculture systems have been reported to have considerably improved ability to predict major human metabolites over more conventional *in vitro* human systems such as microsomes and hepatocytes.(Wang et al., 2010) That these types of systems can show enhanced metabolic capability over primary hepatocyte suspensions was demonstrated by the incubation of a set of 27 drug compounds for 7 days in a micropatterned coculture of hepatocytes. It was found that of the circulating human metabolites exceeding 10% of total circulating drug-related material, 75% were found in the *in vitro* coculture system in comparison to 53% found in human hepatocyte suspensions. Recently various other *in vitro* approaches to maintain metabolic capability for extended periods of time have been explored including 3D cell cultures such as spheroids,(Godoy et al., 2013) liver bioreactor systems,(Darnell et al., 2012) stem cell derived hepatocytes(Ulvestad et al., 2013) as well as different technical approaches such as the relay method.(Ballard et al., 2014) Even though these approaches show promise that further improved *in vitro* systems will predict a greater number of human metabolites, in particular, secondary or tertiary metabolites, whether the metabolites would be captured as excreted or in circulation in humans, would remain uncertain.

In vitro experiments as a whole (i.e. human hepatocytes and LM, rat hepatocytes, LM and liver S9 fraction, dog, mouse, rabbit, monkey, marmoset LM and hepatocytes), gave very little information in addition to what was observed *in vivo.* Less than 4% of human circulating metabolites were unique to *in vitro* experiments. Based on this analysis, non-human *in vivo* systems may be perceived as a better system than human hepatocytes in predicting human circulating metabolites. However, the issue around extraneous metabolites (*see below*) observed in these *in vivo* systems has to be taken into account for a proper comparison.

An analysis of human circulating metabolites not captured in pre-clinical experiments/studies was carried out and the results provided a plausible explanation for why some metabolites were seen for the first time in human circulation. Interestingly, only one metabolite (Figure 11) was observed for the first time in man and no reaction type or molecular motif consistently leading to a unique human metabolite was observed. Six of the metabolites

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classified as previously unseen are vague Markush structures. Due to the broadness of the Markush labelling, identification of the exact biotransformation that occurred is not possible and therefore uncertainty remains as far as their elucidation is concerned.

In 13 cases out of the 27 previously unobserved human circulating metabolites, evidence of biotransformation pathways seen in human plasma, is observed during *in vitro* cross-species experiments. Therefore, due to the limited viability of cryo-preserved hepatocytes, the metabolism found *in vivo* in humans is more extensive and thus the final metabolite is not captured in the *in vitro* systems. Thus, if metabolic pathways instead of specific human circulating metabolites are considered, the predictability of human hepatocytes increases to 87 % which is similar to what has been reported by Dalvie *et al*. with respect to metabolic pathways.(Dalvie et al., 2009) When also LM, hepatocytes, microsomes and S9 fractions of other species (i.e. rat, mouse, dog, rabbit) are included, the predictability of *in vitro* systems goes up to 92% with regard to biotransformation pathways. This assessment emphasizes the importance of the critical evaluation of observed metabolites by the biotransformation scientists in an attempt to predict and later on elucidate complete metabolic pathways in humans. Other examples (4 out of 27) of previously unseen metabolites suggest that species specificity with respect to glucuronidation may play a role as to why human circulating metabolites are not seen in other species. Since conjugated metabolites are generally considered to be non-toxic, less concern has been expressed by the U.S. Food and Drug Administration over their presence in circulation.(U.S. Food and Drug Administration, 2008)

The number of human circulating metabolites predicted from human hepatocytes is similar to the number predicted from rat plasma. The number of extraneous metabolites in the two matrices is also comparable (Table 1). However, structural analysis reveals that extraneous metabolites found in human hepatocytes often tend to be a precursor or an intermediate of a human circulating metabolite (5 metabolites), or are seen as excreted metabolites (15 metabolites). This latter figure could be greater (20 metabolites) if metabolites that could be excreted *via* bile and for which metabolite profile in faeces were not analysed, were considered. This could be the case, e.g., for metabolites deriving from amide dealkylation,

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and glucuronides and other polar metabolites that once formed in the liver may be directly excreted *via* the bile, or rapidly excreted *via* the urine without accumulation in plasma to detectable concentrations.(Loi et al., 2013)

Overall, these analyses showed that *in vitro* experiments in human hepatocytes generate a few misleading metabolites due to specific reactions taking place in human *in vitro* systems and 5 metabolites only appear to be irrelevant to human in vivo metabolites. One of these 5 irrelevant metabolites, underwent formylation biotransformation and this might be the result of an artifact of the in vitro experiment itself – i.e. high concentration of formic acid. The other 4 metabolites found uniquely in human hepatocytes are metabolites of an AstraZeneca marketed drug and might reflect a more extensive Met-ID work done on this drug.

When metabolites found in human hepatocytes are interpreted with the aim of predicting human circulating metabolites, two pieces of evidence need to be considered: a) the metabolites may be further metabolized *in vivo* in humans, and b) finding metabolites in hepatocytes does not indicate whether or not they will enter the circulation.

On the other hand, extraneous metabolites in rat plasma are generally irrelevant to metabolites seen in human plasma (25 metabolites) or excreted (8 metabolites) suggesting that the prediction of human metabolites from *in vivo* studies may be misleading.

Misleading metabolites in rat plasma are mainly organic anions, which are formed in human *in vitro* systems but not further metabolized in humans in comparison to rats. Therefore, whether a metabolite will be found in circulation may not only depend on its formation *per se*, but also on the species-specific expression of drug transporters such as the organic anion transporters (OATs). Species differences with respect to location on the apical or basolateral membranes of some human OATs and rat Oats (OAT2) are reported in literature.(Burckhardt, 2012) Organic anion transporters such as Oat5 have been demonstrated in rat kidney but not in humans and its role is either to uptake xenobiotics from the primary urine into the cells or to release organic anions into the lumen.

A common trend was observed during the analysis of extraneous metabolites in rat plasma. In three cases, biotransformations occurring on substituents of heterocyclic aromatic rings

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lead to metabolites that are observed in rat only. Different substrate specificity between otherwise similar drug metabolizing enzymes in rats and humans might provide explanations for these observations. Therefore, in contrast to what was observed for extraneous metabolites found in human hepatocytes, extraneous metabolites found in rat plasma are not related to human metabolites.

5. Summary

The value of a corporate metabolite database was highlighted through the analysis of all metabolites generated during Discovery and Development for a set of 27 candidate drugs. The analysis showed that human hepatocytes alone were only able to predict 33% of the human circulating metabolites. However, the majority of metabolites missed were further transformations of the metabolites seen in hepatocytes. If instead the criteria was the indication of metabolic pathways, the predictability of human hepatocytes to human plasma increased to 87 %. This indicates that the advances now being made in human hepatic in vitro systems with improved viability and longer incubation times, should considerably improve our ability to predict which metabolites will be systemically exposed to humans.

Rat plasma was similar to human hepatocytes in the number of correctly predicted human circulating metabolites. However, the considerably larger number of metabolites irrelevant to human metabolism seen in rat plasma, make this a much less useful tool than human hepatocytes.

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Authorship contributions

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Wrote or contributed to the writing of the manuscript: Iegre, Hayes, Thompson, Weidolf, Isin

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Figure legends

Figure 1. Biotransformation data capture and dataflow showing publishing of Met-ID data into IBIS via the Global Metabolite database.

Figure 2. A graphic illustration of the filters applied to the search to obtained the subset analysed. The first three filters were applied using the text search option while the manual re-organization part was done together with a substructure search to identify the matrices in which the same metabolites were observed

Figure 3. Comprehensive metabolic scheme for compound AZD1. The manually-constructed scheme includes all the metabolites observed in all the matrices on which Met-ID experiments were performed.

Figure 4. Definition of the terms used in this paper to describe the experiments performed at different stages of the drug discovery and development process.

Figure 5. Bar chart comparison of the number of human circulating metabolites (in red) with the ones previously observed in pre-clinical studies (in blue).

Figure 6. This analysis includes all the *in vitro* data available (hepatocytes and/or microsomes and/or S9 fractions from human, rat, dog, monkey, rabbit, mouse, common marmoset) and *in vivo* data (plasma, urine, feces, bile from human, rat, dog, monkey, mouse, marmoset) for the 27 compounds. The numbers indicate the quantity of observed metabolites.

Figure 7. Multi-step biotransformation of AZD24 observed in human plasma resulting in the formation of the previously unobserved sulfo-conjugated metabolite M24-b.

Figure 8. M8-b shows a previously unobserved human metabolite deriving from multi-step metabolism of AZD8.

Figure 9. Metabolism of a methylpyrazinone moiety leading to a previously unobserved human circulating metabolite.

Figure 10. Example of a metabolite (M7-a) observed in human hepatocytes but not observed in human *in vivo.* In the latter, products of further metabolism of M7-a were captured.

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Figure 11. Examples of metabolites deriving from amide *N*-dealkylation found in human hepatocytes but not in human plasma.

Figure 12. Metabolism occurring on a pyrazinone moiety leading to metabolites observed in rat plasma but not in human plasma.

Figure 13. AZD7 underwent metabolism showing the redox reversibility between rat and human.

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Tables

Table 1. The table lists the number of human circulating metabolites and the number of extraneous metabolites observed in the different matrices. The extraneous metabolites are sub-divided into three classes: not observed but evidence of the same pathways captured in human plasma; not observed in plasma but seen as excreted; and true misleading metabolites. The first two are the extraneous metabolites that are actually precursors or intermediates to the final metabolites captured in human plasma. The last are metabolites irrelevant to the human circulating metabolites.

Matrix	Human circulating metabolites observed	Metabolites not seen in human as circulating and observed in the matrices listed here (extraneous)		
		Not observed but evidence of the same pathways captured in human plasma	Not observed in plasma but excreted	Irrelevant metabolites
Human hepatocytes	47	5	15	13 (5)[(a)]
Rat plasma	46	0	8	25(19)[(b)]

[(a)] The number of irrelevant metabolites is 13 but might be smaller (down to 5) if we consider that 3 of these metabolites are broad Markush structures and 5 might be excreted via bile and therefore faeces which is not always analysed (compounds AZD3, AZD13, AZD14 do not have faeces Met-ID data available in the Database).

[(b)] The number of irrelevant metabolites observed in rat plasma is 25 but might be smaller (down to 19) if we consider the vagueness around 6 Markush structures.

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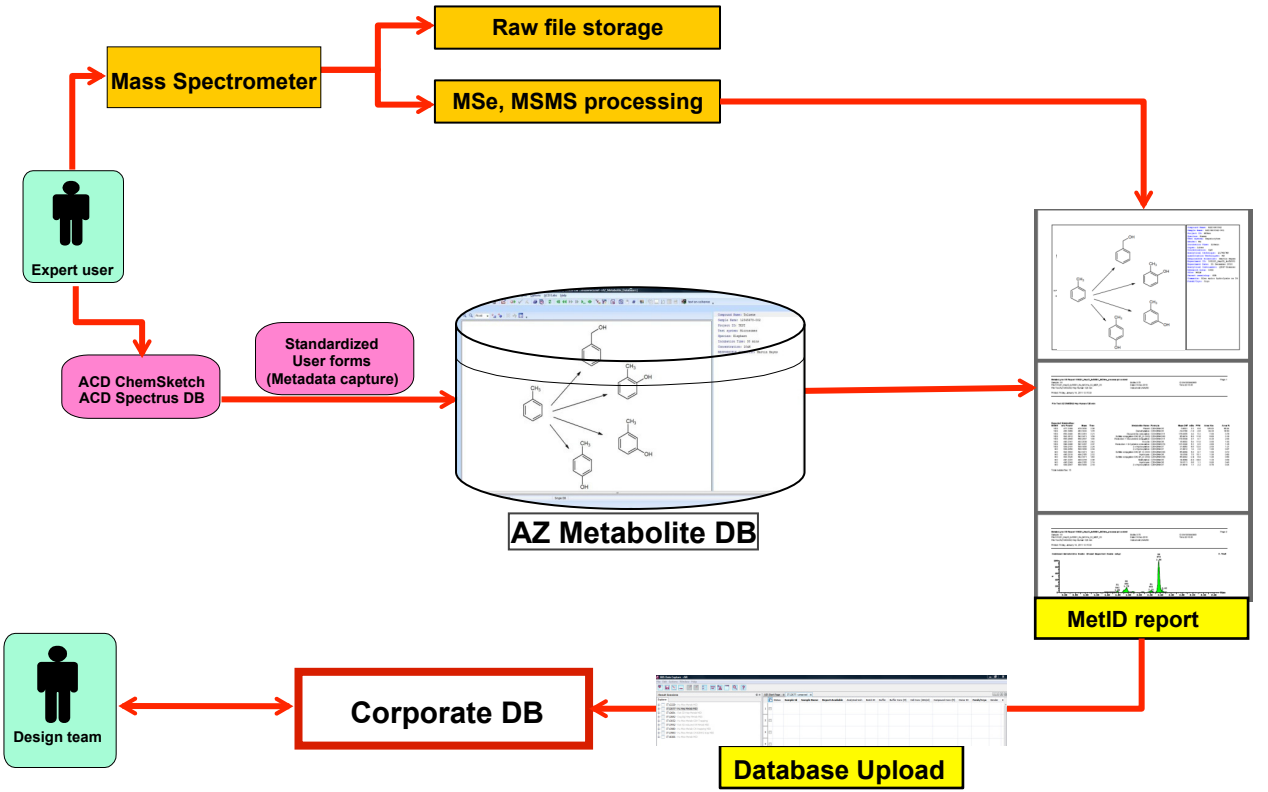
Raw file storage
Mass Spectrometer
MSe, MSMS processing
Expert user
Standardized
User forms
(Metadata capture)
ACD ChemSketch
ACD Spectrus DB
AZ Metabolite DB
MetID report
Design team
Corporate DB
Database Upload

Figure 1

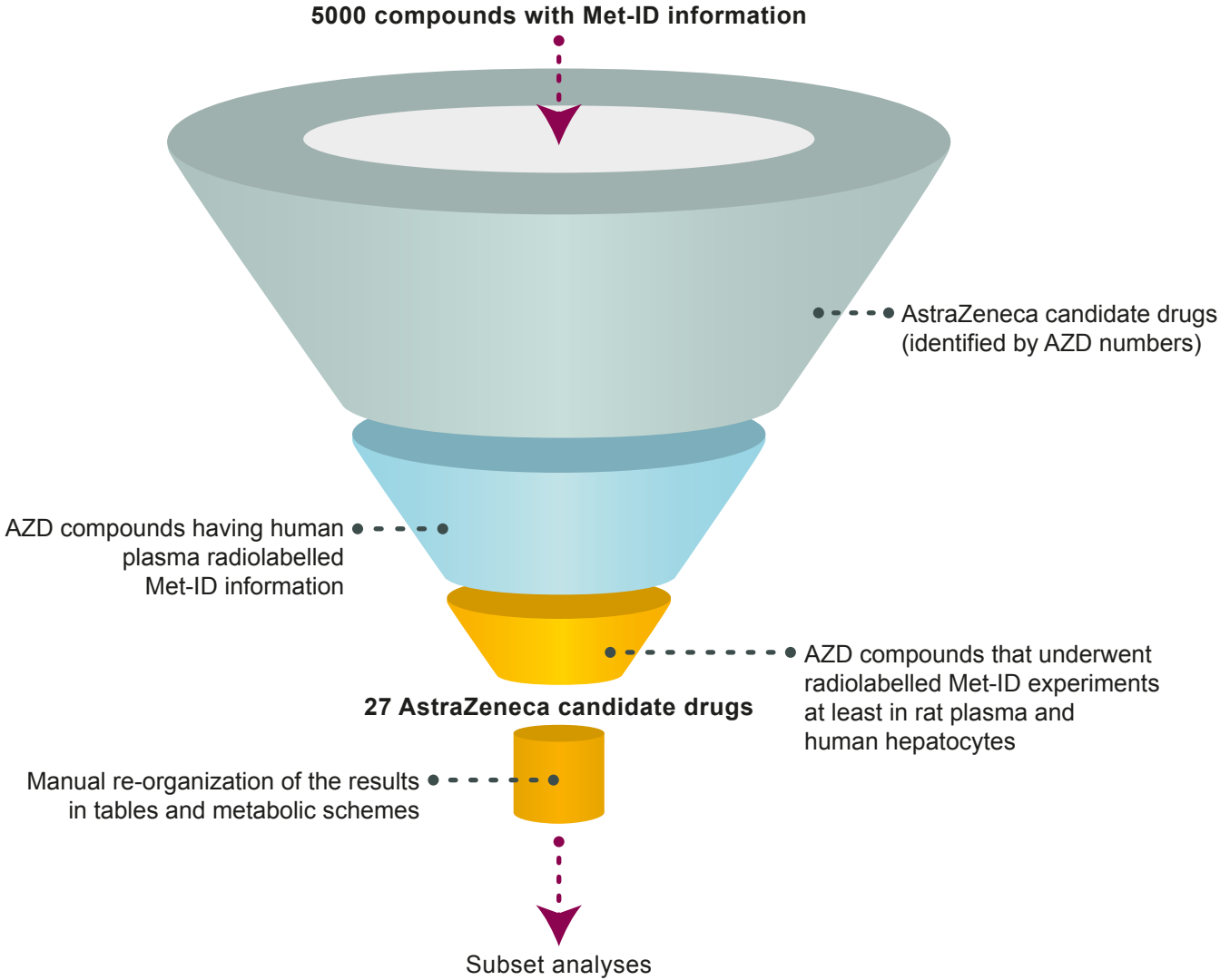
5000 compounds with Met-ID information
AstraZeneca candidate drugs
(identified by AZD numbers)
AZD compounds having human
plasma radiolabelled
Met-ID information
AZD compounds that underwent
radiolabelled Met-ID experiments
at least in rat plasma and
human hepatocytes
27 AstraZeneca candidate drugs
Manual re-organization of the results
in tables and metabolic schemes
Subset analyses

Figure 2

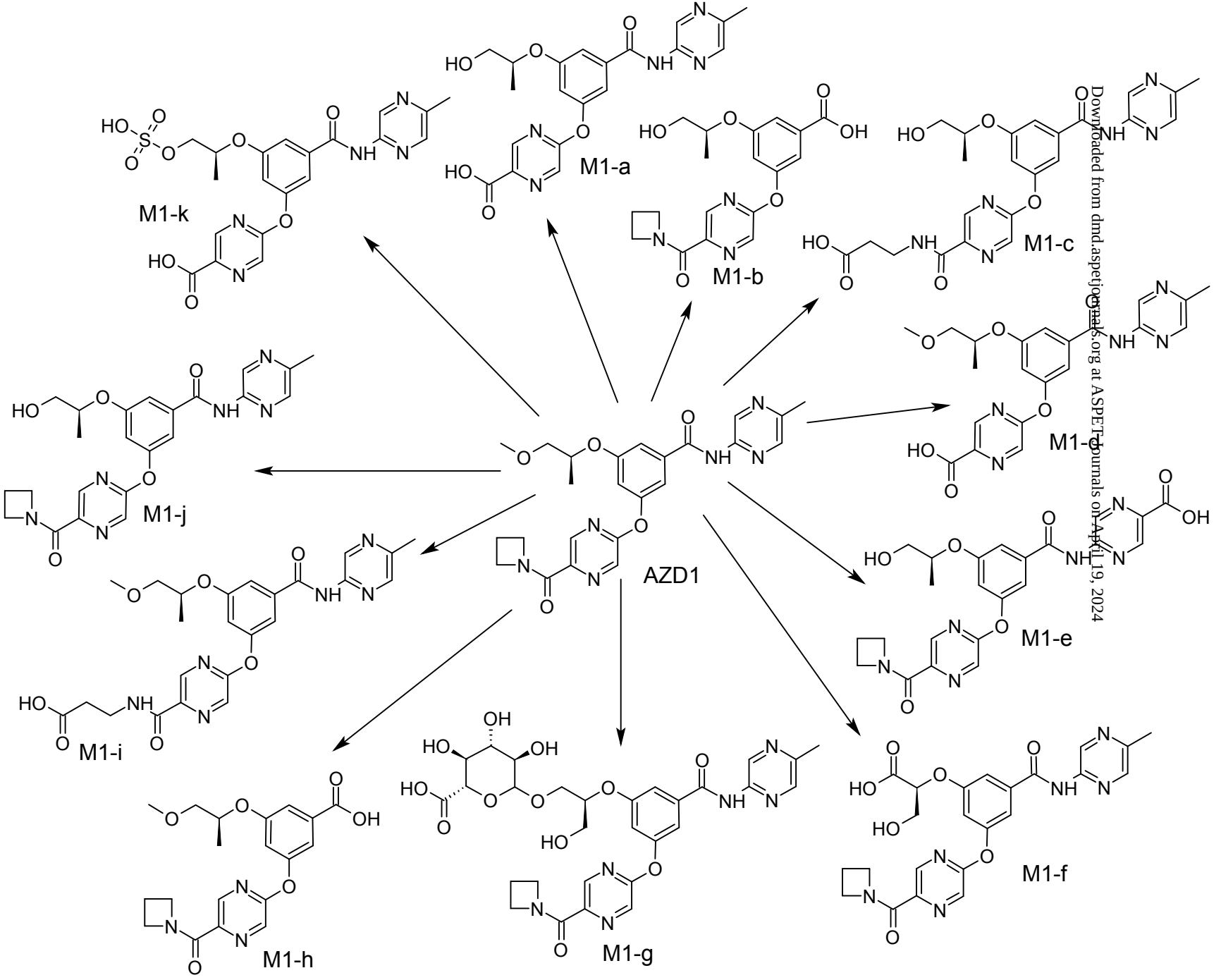

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Figure 3

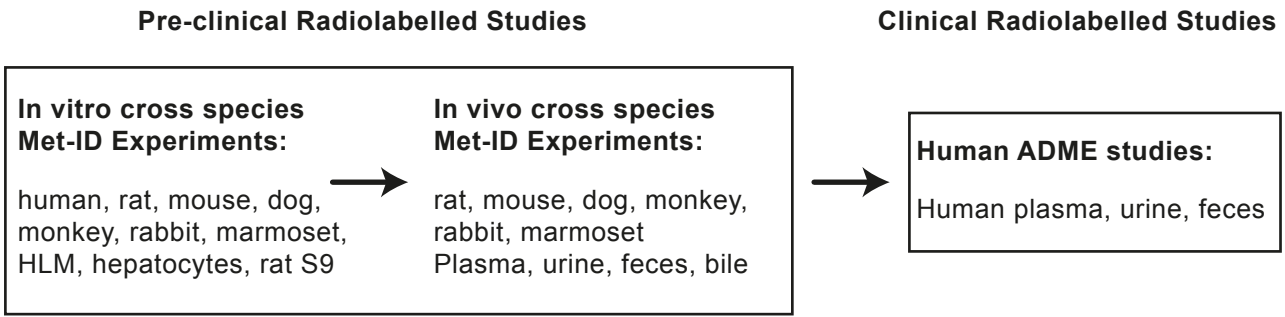
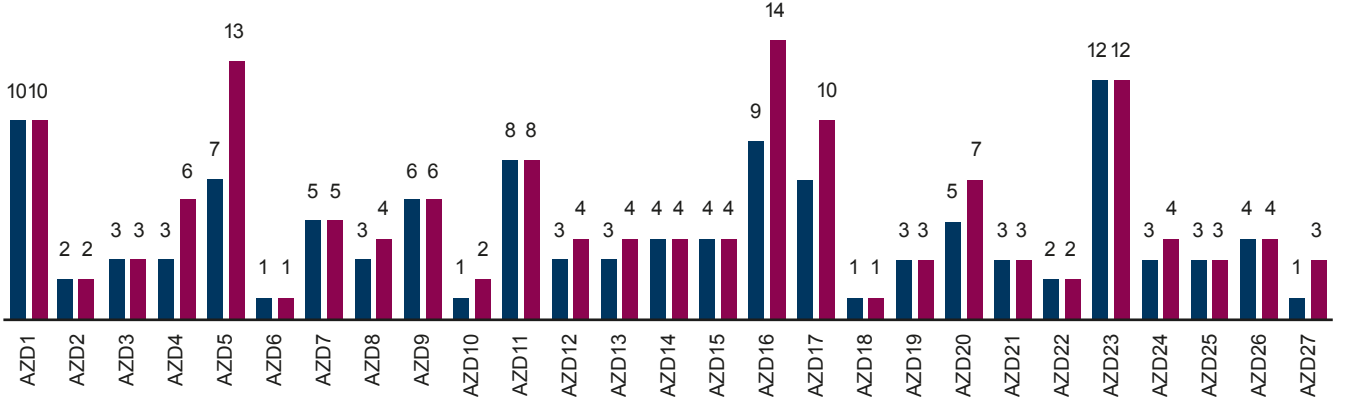

Pre-clinical Radiolabelled Studies
Clinical Radiolabelled Studies
In vitro cross species Met-ID Experiments:
human, rat, mouse, dog, monkey, rabbit, marmoset, HLM, hepatocytes, rat S9
In vivo cross species Met-ID Experiments:
rat, mouse, dog, monkey, rabbit, marmoset Plasma, urine, feces, bile
Human ADME studies:
Human plasma, urine, feces

Figure 4


10 10
2 2
3 3
3 6
7 13
1 1
5 5
3 4
6 6
1 2
8 8
3 4
3 4
4 4
4 4
9 14
10
1 1
3 3
5 7
3 3
2 2
12 12
3 4
3 3
4 4
1 3
AZD1
AZD2
AZD3
AZD4
AZD5
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AZD27

Figure 5

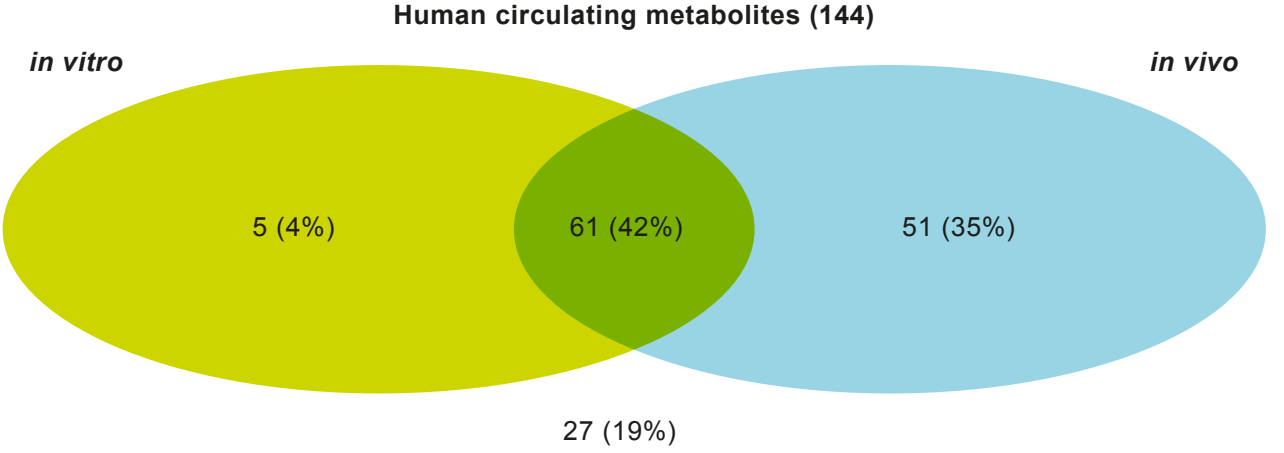
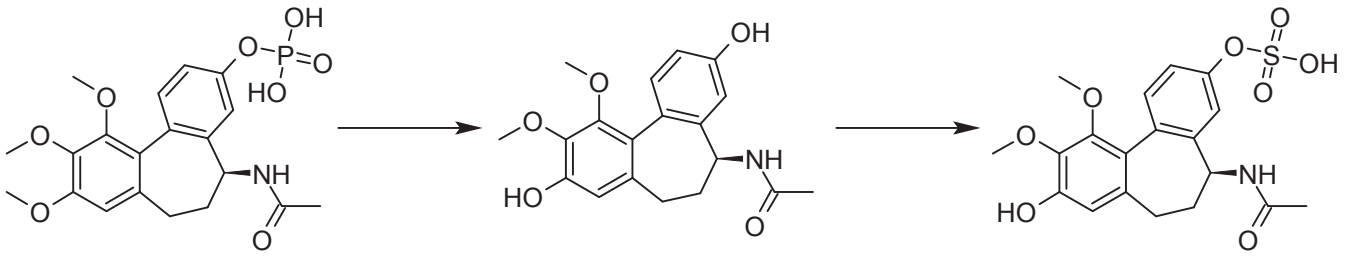

Human circulating metabolites (144)
in vitro
in vivo
5 (4%)
61 (42%)
51 (35%)
27 (19%)

Figure 6



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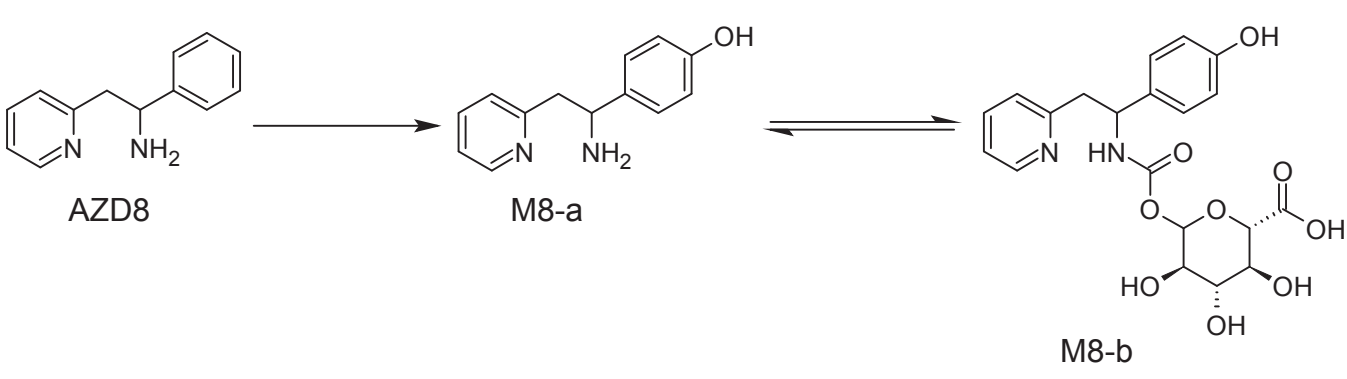

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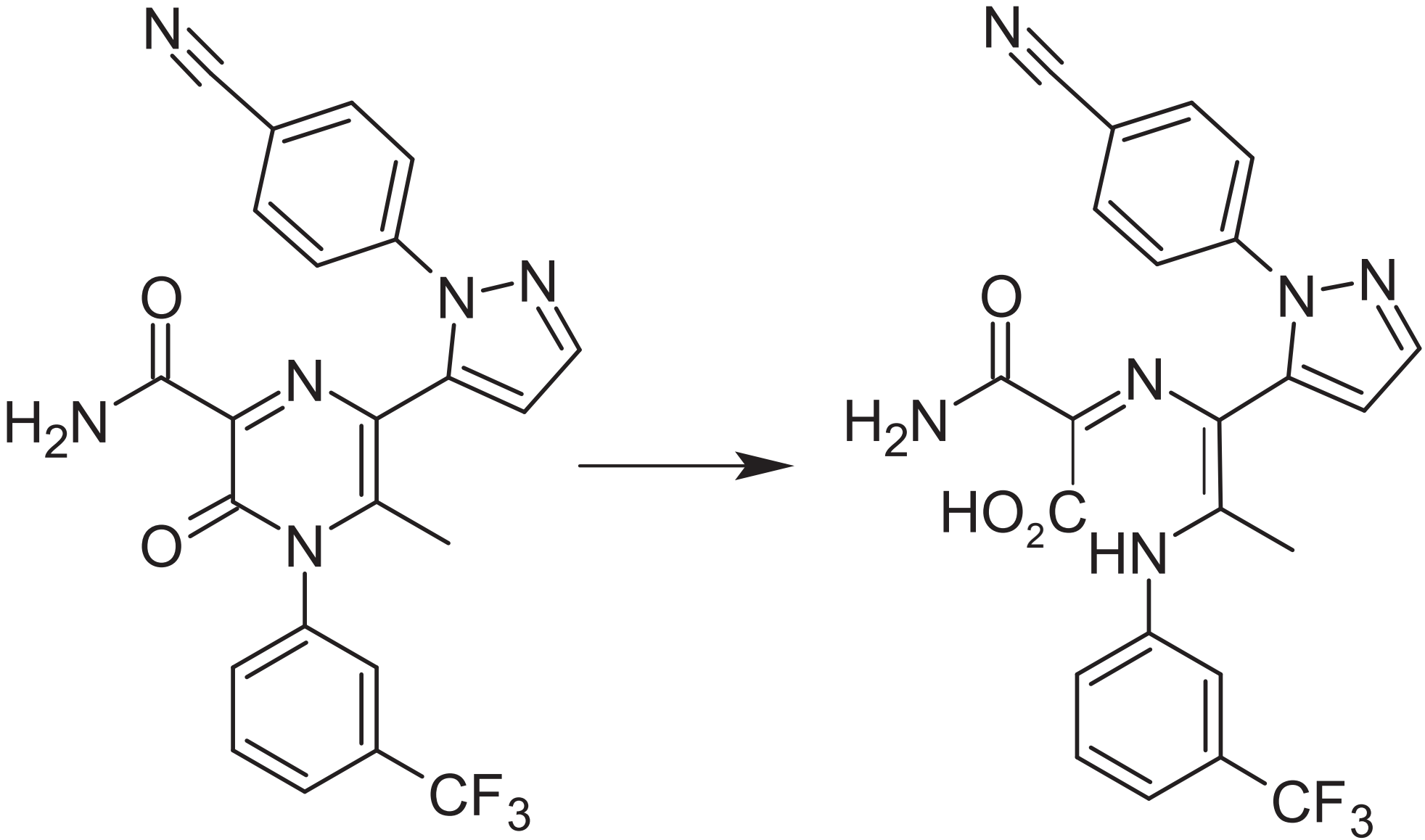


AZD10

M10-a

Figure 9

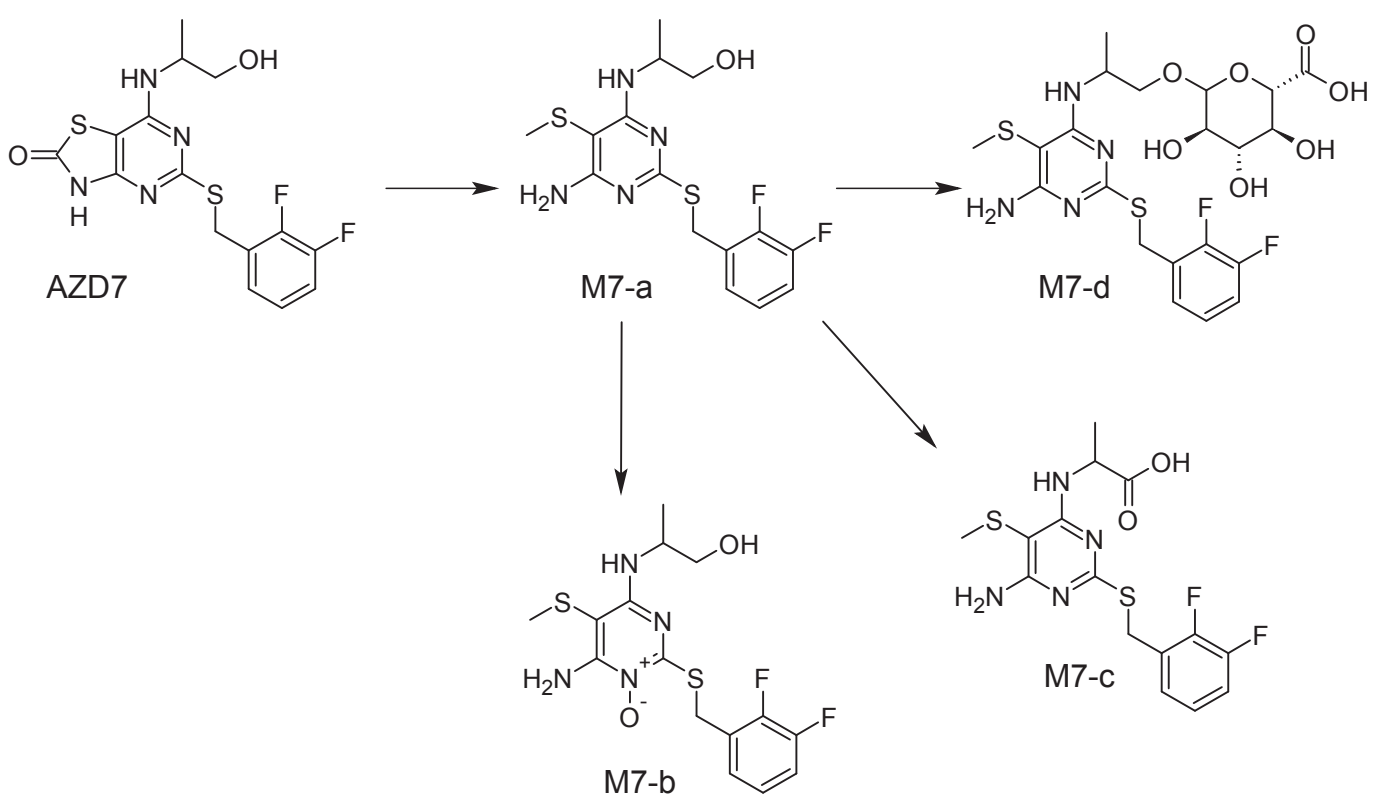
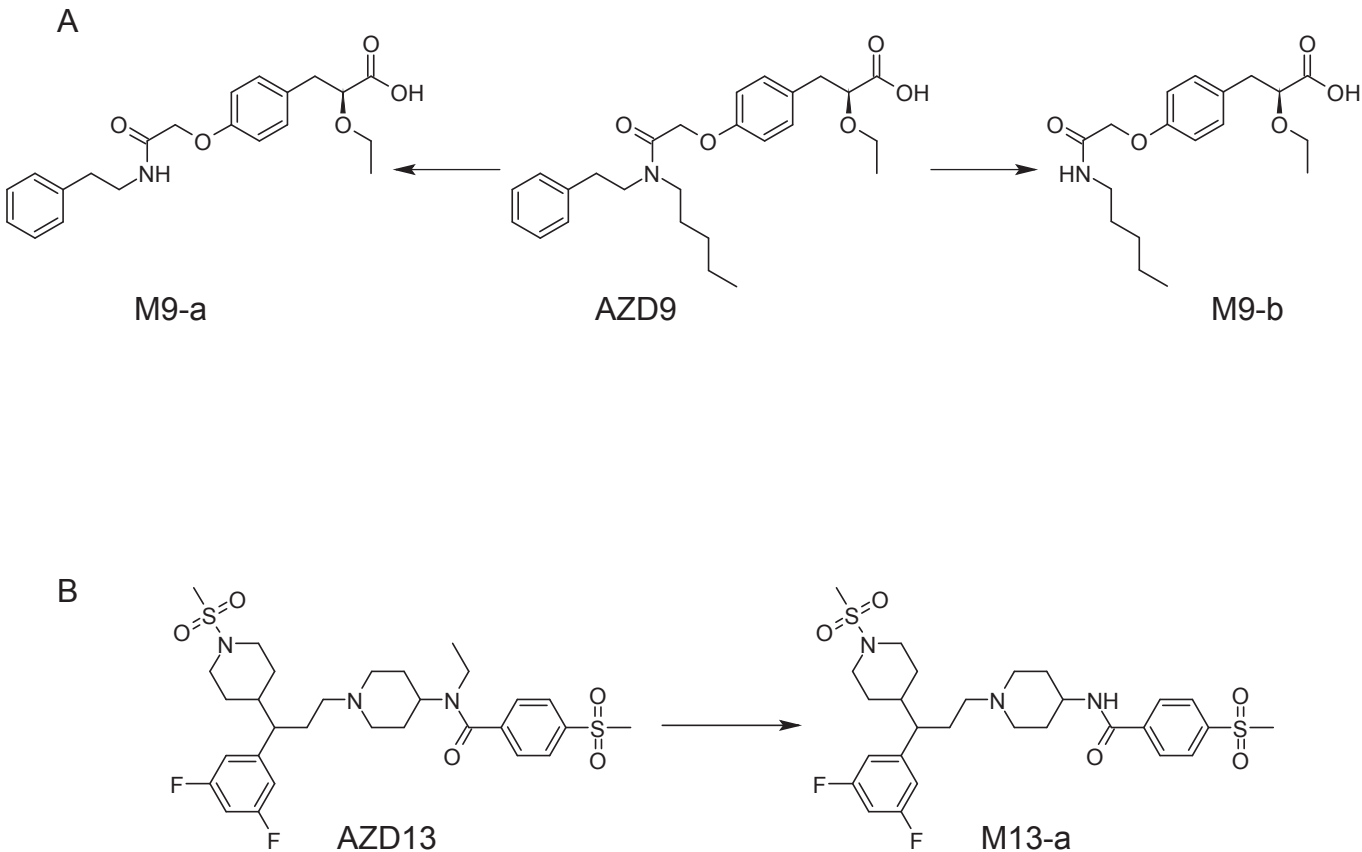


Figure 10



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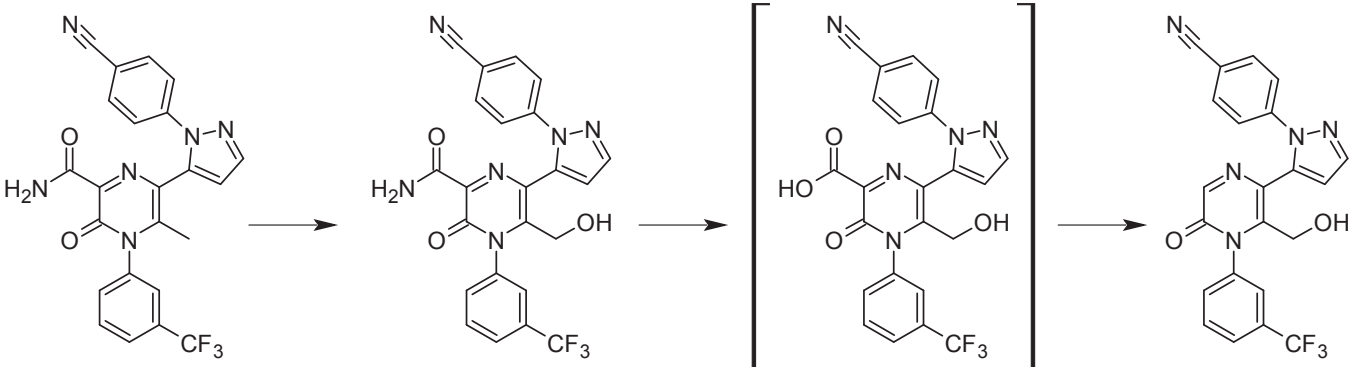

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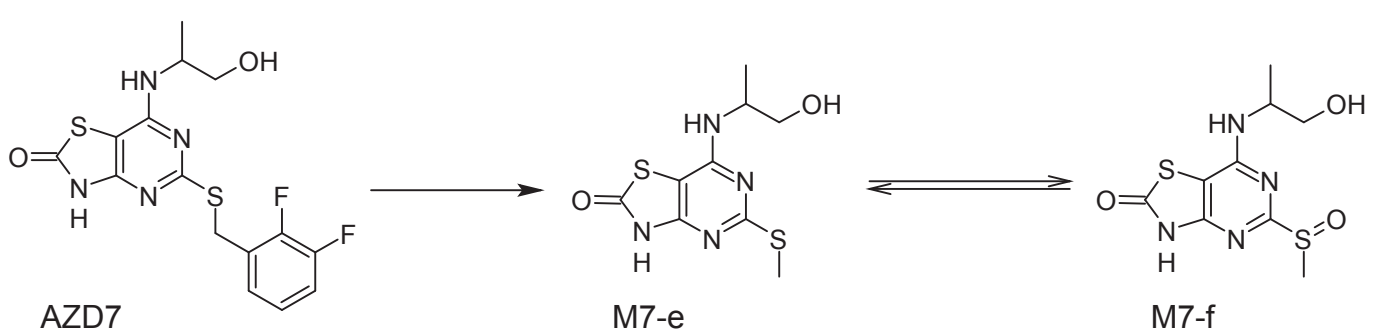

Figure 13