Testing the suitability of a structured substance database for pharmacovigilance purposes: an exploratory study about the role of structural alerts and reactive metabolites in drug toxicity

By Helen Gatling

Supervisors: Marcel Hoefnagel and Eugene van Puijenbroek, Marjon Pasmooij, Anja van Haren, Lotte Minnema

Examiner: Ton de Boer

Abstract

Introduction

Pharmacovigilance (PV) is the discipline related to detecting, assessing, understanding, and preventing adverse reactions and other problems related to the use of medicinal products once a drug is admitted on the market. Especially idiosyncratic adverse drug reactions (IADRs) are hard to detect once a drug is marketed due to their low frequency and the lack of a clear pharmacological mechanism. It is considered possible that in idiosyncratic reactions, reactive metabolite (RM) formation occurs due to structural features of the molecule (structural alerts, SA) that makes it susceptible to RM formation. Pre-marketing, the acknowledgement of SAs is essential. However, they are hardly studied post-marketing.

A way of finding drugs that contain a SA, and therefore may pose a risk for the occurrence of related ADRs, is by using a structured substance database that has the option to be queried for molecular structure. The objective of this study was to investigate the suitability of a structured substance database for exploring ADR-SA relationships.

Methods

The GSRS database was developed by the Global Ingredient Archival System (GINAS) initiative and contains the molecular structure, classification information and metabolites of compounds used in human and animal medicinal, food, tobacco, and cosmetic products. Using GSRS, five sub-studies were performed each investigating a pre-selected SA-ADR relationship. For four sub-studies, the SA-ADR relationship was described in former studies. For the fifth sub-study, a newly discovered potential SA-ADR relationship was investigated. For each study, a GSRS search was performed, and it was extracted if a specific drug-ADR combination was reported disproportionally in VigiLyze, the global database for Individual Case Safety Reports of the WHO, and in addition the summary of product characteristics (SmPC) was checked.

Results

First it was shown that the GSRS database could indeed be queried to find relevant structures containing a number of pre-selected SAs. It was also shown that, based on a specific RM, GSRS contained sufficient information to highlight drugs that formed similar RMs or contained parts of the RM in their original structure. Thirdly, the possibilities of searching for drugs containing structural varieties within one drug class were investigated and this showed the mediating effects of a substituent on the occurrence of ADRs. This analysis showed the potential usability of the GSRS for future PV research.

Additionally, it was demonstrated that GSRS could be used to find drugs with diverse therapeutic indications containing a pre-selected SA. Looking at the SmPCs and reporting rate for the results,

signs of the involvement of this SA in the development of Steven-Johnson syndrome and methemoglobinemia were displayed.

Finally, a possible relationship between a SA that was discovered by looking at the reason behind the retraction of two fluoroquinolones and the ADR QT prolongation was described. The drugs containing the SA showed, with one exception, a high reporting rate for QT prolongation, whereas drugs that did not contain the SA did not.

Implications

This study shows that the GSRS database is a suitable tool for identifying drugs containing a SA. It can help PV research by finding structurally related drugs in pre- and postmarketing stages.

Samenvatting

Introductie

Farmacovigilantie is de discipline gerelateerd aan de detectie, beoordeling, het begrip en voorkomen van bijwerkingen en andere problemen gerelateerd aan het gebruik van medicatie na markttoelating. Vooral idiosyncratische *adverse drug reactions (IADRs)* zijn moeilijk te detecteren als een geneesmiddel op de markt komt door de lage frequentie en gebrek aan duidelijk farmacologisch mechanisme. De mogelijkheid bestaat dat in idiosyncratische reacties, reactieve metaboliet (RM) formatie plaatsvindt door structuurelementen van moleculen (*structural alerts, SA*) die het molecuul vatbaar maken voor RM formatie. Pre-marketing is rekening houden met SAs essentieel. Echter wordt er nauwelijks onderzoek gedaan naar mogelijke SAs post-marketing.

Methode

De GSRS-database is ontwikkeld door het *Global Ingredient Archival System (GINAS)* initiatief en bevat de molecuulstructuur, classificatie informatie en metabolieten van stoffen die gebruikt worden in humane en veterinaire geneesmiddelproducten, voedsel, tabak en cosmetica. Vijf sub-studies werden met behulp van GSRS uitgevoerd die een vooraf geselecteerde SA onderzochten. Voor vier sub-studies was de SA-ADR relatie beschreven in eerder onderzoek. In de vijfde sub-studie werd een nieuw ontdekte potentiële SA-ADR relatie onderzocht. Voor elke studie werd een GSRS zoekopdracht uitgevoerd en het werd geëxtraheerd of een geneesmiddel-ADR combinatie disproportioneel werd gemeld. Hiervoor werd gebruikt gemaakt van VigiLyze, een wereldwijde database met individuele *case safety reports*, en de samenvatting van productkenmerken werd bekeken.

Resultaten

Ten eerste werd aangetoond dat de GSRS-database gebruikt kon worden om relevante structuren te vinden die pre-geselecteerde SAs bevatten. Het werd ook aangetoond dat, gebaseerd op een specifiek RM, GSRS genoeg informatie bevat on geneesmiddelen te vinden die vergelijkbare RMs vormen of een deel van de RM bevatten. Hiernaast werden de mogelijkheden om geneesmiddelen te vinden die verschillende structuurelementen bevatten binnen een klasse onderzocht. Dit toonde de mediërende effecten aan van zijgroepen op het gemeld worden van een mogelijke bijwerking. Deze analyse toonde de potentie voor toekomstig farmacovigilantie onderzoek.

Het werd aangetoond dat GSRS gebruikt kon worden om geneesmiddelen met diverse therapeutische indicaties te vinden die een pre-geselecteerde SA bevatten. Kijkend naar de meldfrequentie voor de resulterende geneesmiddelen werd er een mogelijke invloed van de SA bij ontwikkeling van Steven-Johnson syndroom en methemoglobinemie gevonden.

Als laatste werd een mogelijke relatie tussen een SA, die ontdekt werd bij het kijken naar de reden van terugtrekking van twee fluorchinolonen, en de ADR QT-verlenging gevonden. De geneesmiddelen met de SA toonden een hoge meldfrequentie. Dit in contrast met de geneesmiddelen die de SA niet bevatten.

Implicaties

Dit onderzoek laat zien dat de GSRS-database een geschikte tool is voor de identificatie van geneesmiddelen die een SA bevatten. Het kan farmacovigilantie onderzoek helpen bij het vinden van geneesmiddelen die een bepaalde structuur bevatten, pre- en postmarketing.

Contents

Introduction

The objective of pharmacovigilance is to detect, assess, understand, and prevent adverse reactions and other problems related to the use of medicinal products (1). In the Netherlands, pharmacovigilance centre Lareb oversees collecting, registering, and analysing of incoming adverse drug reaction (ADR) reports from patients and healthcare professionals. Lareb will evaluate the signals and discuss relevant signals with the Medicines Evaluation Board (MEB), which is the authority that issues marketing authorisations for medicinal products. For patients and prescribers, it is important to know if there are specific safety issues related to a medicinal product. To continuously evaluate the balance between the safety and effectivity of medicinal products, the MEB assesses Lareb's findings which, next to ADR reports, include information from pharmacovigilance centres in other countries, follow-up studies about the drug in question and scientific literature published.

If the MEB concludes that the ADR occurred despite correct use of the drug and is not caused by off-label use, incorrect prescribing, or any other reason, measures can be taken to improve the safe use of the product. Based on the investigation, it can be decided to add the ADR to the information leaflet, change its delivery status (for example from over the counter to prescription), send a warning to prescribers and dispensers and lastly, to take the drug off the market entirely (2). Direct healthcare professional communications are letters that the marketing authorisation holder may use to inform healthcare professionals of new safety information, like a suspension, shortage, or restriction of indication about a drug and possible actions to take (3). Some drugs are placed under extra supervision. This can be recognized by a black triangle in the information leaflet, as shown in figure 1. In the US, a boxed warning (BW) (formerly called a black box warning) can be assigned by the Food and Drug Administration (FDA) to bring an ADR to patients' and healthcare professionals' attention (4).

Figure 1: Statement on the patient information leaflet from the Dutch medicine evaluation board that the drug is placed under extra supervision.

Taking drugs off the market is the most impactful step taken by the MEB or FDA as a result of a new ADR that was discovered post-marketing. 16 (2.9%) of 548 approved drugs in 1975-1999 were retracted from the market (4). A more recent study showed that 118 of 740 (15.9%) approved drugs in 1980-2009 were retracted from the market. It seems that more drugs get retracted. This may be due to drug development generating drugs with increased systemic exposure, which is beneficial for reaching the target tissue, but has exposure to other tissues as its own side-effect. The greater number of retractions can also be caused by better pharmacovigilance monitoring or other reasons currently unknown (5).

This system of constant safety-monitoring is in place because during clinical trials, not all ADRs will be detected. The ones that are most often found in this early stage are type A. Type A reactions are a consequence of the pharmacological mechanism of a drug.

Especially ADRs that occur infrequently or only in patient groups that are not well represented in clinical trials are most often found post-marketing. Amongst others, elderly people, children, and pregnant women are regularly excluded. In phase IV (post-marketing), when drugs become available to all patients, several other ADRs can be discovered. Ones that only occur in above mentioned underrepresented patient groups, ADRs with a long latency time, an unexpected mechanism or ADRs that take place extremely rarely may present itself for the first time in phase IV (2,6). Idiosyncratic

adverse drug reactions (IADR), also called type B reactions, are a type of rare drug reactions that cannot be explained by the pharmacological mechanism of a drug. Most of the time, they are unpredictable, and they can be life-threatening. Examples of IADRs are liver injury, (severe) skin rashes and agranulocytosis (4). IADRs are typical adverse reactions that are discovered in phase IV.

Some IADR only appear after more than a year of use, and in some cases the onset of an IADR even occurs after the drug has been discontinued (7). In general trials for small molecule drugs, patients are not regularly monitored after trials have ended. So even if the patient who would exhibit a certain IADR was included in a trial, for some IADRs it would still not mean that they were detected because of their long latency time. Additional ways of examining drug safety are thus necessary to identify these IADRs and to determine their characteristics. Additionally, a well-defined method of moving from one medicine with an ADR to ones that may be suspected of causing this same ADR has not been established. It can however be useful to not limit ADR-drug relations to individual drugs but to look at more drugs at once. One way to examine IADRs and their possible connection to other drugs is to look at structural alerts (SA). These are structural features of a molecule that are suspected of either causing an ADR or forming a reactive metabolite (RM) that causes an ADR. The mechanism of toxicity can be very broad, depending on the SA or RM. Some RM are electron deficient molecules or free radicals that cause direct damage to cells, proteins or DNA. Others can induce an immunogenic reaction (8). The SA/RM concept will be discussed in more detail later, using examples from earlier research about this subject.

This research aims to explore established and hypothesized SA-ADR relationships from literature using a structured database containing detailed information on active substances used in medicinal products, including their molecular structure. The main goal is to find out what the possibilities are to use this substance database with molecular structure-search capability for pharmacovigilance purposes. The database was used to find structures related to SAs found in literature. Summaries of product characteristics (SmPCs) and VigiLyze, a signal detection and signal management tool from the world health organisation's (WHO) 'VigiBase' spontaneous reports database were used to connect ADRs to found structures.

Pharmacovigilance and PV databases

Over the past decades, several ways of keeping track of drug safety have emerged. This all started in 1938, with the foundation of the Federal Food, Drug and Cosmetic Act. This is a series of laws that allowed the FDA to regulate the safety and quality of drugs entering the market, and of drugs applying for market authorization. The reason these laws were instated was because the use of sulphanilamide elixir, which contained the highly toxic diethyl glycol, resulting in more than 100 deaths. These laws allowed a new organization to be established that foresaw proof of safety before market approval and allowed inspection of drug production. In Europe, these governmental bodies were not well established until the sixties, with the institution Directive 65/65/EEC and the WHO program for international drug monitoring.

VigiBase was established in 1967 and since 1978, the database is managed by the Uppsala Monitoring Centre (UMC). It contains over thirty million reported potential side effects of medicinal products. This makes it the largest individual case safety report (ICSR) database in the world. It contains data from most other databases like this, including those from China and the EU. (9,10). The EU database, EudraVigilance, has the advantage of containing more detailed information about the ADRs. This can be useful when the content of the cases needs to be studied. However, VigiLyze contains a larger number of cases, as cases from across the globe are included.

VigiLyze is linked to the classification systems WHODrug, MedDRA, WHO ICD and WHO-ART.

PV databases can be used to research a possible ADR-drug relationship. However, it is inherent in spontaneous ADR reports that not all the reported adverse events were related to the drug for which this adverse event was reported. This means that in all PV databases, a share of ADR reports cannot be attributed to the drug for which the ADR was reported. Additionally, the database is subject to different forms of bias. The most frequent types of bias and other issues surrounding spontaneous report databases are discussed below.

Underreporting, which is an incorrectly low number of reports for an ADR, can occur in spontaneous report databases. There are many reasons for low reporting of certain ADRs. Examples are lack of time, lack of understanding the need for reporting, and the phenomenon that healthcare professionals may only report ADRs that they classify as severe (11).

The second form of bias discussed is notoriety bias. An example of notoriety bias is one with the warning for the increased risk of suicide with selective serotonin reuptake inhibitors (SSRIs). This risk is well-established and has been reported since the earliest uses of these kinds of drugs, but it re-emerged in 2003 with the broadcast of a television program in the UK. A study showed that before this broadcast, 0.5% of all SSRI reports were of suicide. After the broadcast this percentage went up to 2.5%. With the newer SSRI escitalopram, these reports turned out to be a relatively large share of overall reports because this SSRI was on the market for a shorter time than the others. With the older SSRIs, the signals after the broadcast were 'diluted' because of the high number of reports already in the database for these drugs. This shows that certain events that draw attention to a certain drug-ADR relationship can cause disproportionate reporting that may be unrelated to a causal relationship between the ADR and the drug (12).

Another type of bias is competition bias, which can arise when drugs or drug classes are strongly associated with an ADR. When a new drug is also responsible for generation of signals for this ADR, a disproportionate reporting signal may come in at a later time than if background signals of this ADR were not so frequent. Competition bias is also called the masking effect. There are automated methods to minimize this effect (13).

Protopathic bias leads to a false conclusion on the causal relationship between exposure and outcome. This may be because a drug gets initiated in response to a symptom of an undiagnosed disease. When the disease is diagnosed, the conclusion may be drawn that it is caused by the drug, when the use of the drug is actually an early marker of disease. The drug can also be discontinued before presentation of a symptom of a not yet diagnosed disease. One can then draw the conclusion that exposure to the drug is protective against the disease (14).

A major issue with databases containing spontaneous reports is lack of information. This is inherent of large databases, the number of records that one can use to look at differences in ADRS between drugs is an advantage. However, contextual background may be needed to confirm a relationship. Precise documentation of individual cases, preferably by healthcare professionals, is needed for this. Every individual record has a completeness score, and it is stated who submitted the report. This makes it easier to estimate the quality of the report.

Some factors are not obvious and are hard to correct for. For example, when a drug is the last therapeutic choice, and a patient was thus unresponsive to multiple other drugs, an ADR may be caused by a cumulative effect of these multiple drugs (9). This, and other factors that contribute to the foregoing bias may not be clear from the ICSR. The questions in the reporting system try to minimize these forms of bias as much as possible, but some reports do not get filled out completely leaving some information as missing.

Goals and objectives

As stated before, the goal of this research was to investigate the suitability of a structured substance database for exploring ADR-SA relationships. Multiple sub-studies with each containing either a well-established or hypothesized SA-ADR relationship, and a literature search were performed.

The literature search aimed at collecting and elucidating former research about SA-ADR relationships. In this overview of available literature, different methods of doing research on this topic are discussed, as well as known statistics about SA-ADR relationships. From this search resulted suitable cases of SAs to use in the sub-studies.

In total, five sub-studies were performed. Three sub-studies focused on identifying known SAs or RMs and finding related structured using the structured database. For the results, PV data was extracted from VigiLyze and SmPCs.

The first study was performed to explore the sensitivity and specificity of the database by using a reference study that investigated the relationship between drugs with a SA and an ADR and compared the resulting drugs from a structure search with those from the reference study. The objective of the second sub-study was to test what the possibilities are regarding structure searches for RMs. A secondary goal was to identify structures related to a hypothesized SA, and compare their extent of hepatoxicity to other drugs within their drug class that do not form this RM. The third sub-study explored the relationship between substituents of molecules and their mediating effect on occurrence of an ADR. The objective of this study was to find out to what extend the database could be used to identify various possible ADR-mediating structures within one drug class.

Two sub-studies focused on SAs that were hypothesized to cause an ADR. The objective of these two studies was primarily to explore the possibilities of the database in finding structures related to a hypothesized SA. The secondary objective was to investigate the relationship between the SA and the ADR. In one sub-study, the SA was hypothesized in earlier research. In the other, the SA was hypothesized in one of the sub-studies.

This report provides a detailed description of these sub-studies to evaluate the suitability of the database to identify drugs containing SAs, by exploring SA-ADR relationships. The five sub-studies included:

- Study 1: determining the sensitivity and specificity of the database by querying a known SA with known results
- Study 2: exploring the possibilities of using the database to search for a RM, to find if other drugs form this RM too
- Study 3: using the database to find drugs with varying substituents within one drug class that may affect occurrence of a certain ADR
- Study 4: entering a hypothesized SA in the database to find drugs that contain this SA and subsequently to see if they display the concurrent ADR
- Study 5: using a possible SA discovered in study 3 for investigating the mediating effects of this SA on display of an ADR within a drug class

Method

All five sub-studies have their own method section. Some methods were used in all five studies.

For all ADR searches in VigiLyze, one or more preferred terms (PTs) were used. ‘PT’ is one of the five term-levels of the medical terminology system MedDRA. MedDRA stands for medical dictionary for

regulatory activities. A PT is one distinct descriptor or one single medical concept. A PT describes one symptom or diagnosis of a disease (15). MedDRA was used to find the most fitting PT when there were multiple synonyms for one ADR named in literature.

From VigiLyze, IC (information component) and IC025 values were extracted. In sub-study 4, the reporting odds ratio (ROR) was extracted. The IC and ROR are both measures of disproportionality. They are numeric values that say something about the actual number of reports, also indicated as cases, in a database versus the expected number for an ADR-drug combination. Both take the following factors into account for their calculation:

- The total number or reports in a database
- The number of reports for the ADR term
- The number of reports for the drug
- The number of reports for the ADR-drug combination

The difference lies in the logarithmic element that is incorporated in the IC calculation, that is not present in the ROR. This makes the IC easier to interpret when a very small or large number of reports is present for a drug-ADR combination. In VigiLyze, both the IC and ROR as the ROR025 and IC025 values are depicted. The 025 values are the lower endpoints of the 95% credibility interval for the ROR/IC. The 95% credibility interval is the Bayesian variant of the 95% confidence interval. When the 025 value for the IC is higher than 0 and for the ROR higher than 1, it is a statistical basis for signal detection by the UMC. This means that the number of reports is higher than expected.

In all five sub-studies, the total number of cases was either an exclusion criterium or drugs with a low number of cases were discussed separately. When a lower limit of cases is indicated (100 of 1000) this means the total number of cases for the included drugs, not the number of cases for the ADR-drug combination. For the ADR-drug combination, no lower limit is set, as even no ADR cases at all says something about the relationship between the drug and the ADR.

All five studies have a SA as basis. By taking SAs with identified mechanisms of RM formation and resulting toxicity from literature, it has been made sure that there is a mechanistic hypothesis as to how the SA may cause an ADR. In the last sub-study, a new SA was investigated which has not been described in literature before. Additionally, a possible ADR that is linked to a SA had to occur frequently enough to analyse in VigiLyze, without having a large background incidence. The SA-ADR combinations which met these requirements were discussed in a focus group discussion with the researcher and pharmacovigilance experts. From these discussions and the literature search, the SAs and their ADRs that are subject of the five sub-studies were chosen.

The SAs from sub-studies 1,2 and 4 originated from the literature search that is described below. The SAs used in sub-studies 3 and 5 did not stem from this literature search but from a specific search based on the focus group discussion. In this focus group discussion, suitable ADRs for exploring a relationship with a SA were discussed. Photosensitivity was noted as a potential suitable ADR. Investigation of this ADR led to the SA used in sub-study 3. Sub-study 5 was inspired by the observation of a different ADR found in sub-study 3 that only occurred in part of the related structures.

GSRS databases

GSRS stands for global substance registration system. The name GSRS is interchangeably used with ‘GINAS’. This stands for Global Ingredient Archival System. GINAS is an international project to develop a global mechanism for substance information exchange. Officially, GSRS is the software developed to support the GINAS initiative (16). GSRS is a global information system for

pharmaceutical ingredients. It contains the molecular structure, classification information and metabolites of compounds used in human and animal medicinal, food, tobacco, and cosmetic products. For all five sub-studies, this structured substance database that has the option to be queried for molecular structure was used.

GSRS is a usable, open-source database in which users can search for structures, SMILES code or simply search for the name of a molecule. SMILES codes are simplified molecular-input line-entry specifications. They describe a molecule in a simple text string. SMILES codes were generated for every SA search in each sub-study.

When a search is performed, the results can be filtered. One filter that is useful for pharmacovigilance purposes is the 'domain' filter. Here, the 'drug' filter can be applied. All chemicals, cosmetics, pesticides, and other kinds of compounds are filtered out this way. The type of moiety can be chosen by the user too. For example, active moiety and/or salts and solvates can be shown exclusively. These two filters were the main ones used in this study.

The database can be accessed through the GSRS website via https://gsrs.ncats.nih.gov/ginas/app/beta/home, but users can also run it locally using sample data. Is has been shown that with standard hardware, 100,000 records can be supported. Currently, there are 127,767 substances included in version 3.0. It is unknown if this number of records can be supported by standard hardware. For the purposes of this research, GSRS was accessed and used via the website to ensure use of the most recent dataset. The database was developed with pharmacovigilance as main goal. It may also be useful as a compound registration solution for pharmaceutical companies. A public dataset that can be downloaded was uploaded in March 2022 (16).

Literature search

Introduction

To retrieve results from a database, it needs to be queried. GSRS is not linked to a PV database. This makes the option to find SAs by searching for one ADR in the PV database and querying GSRS to search for the most frequent structural elements impossible. To look for an ADR and to manually see if the drugs that display this ADR contain a certain structural element was not realistic without computational help within the timeframe of this research. Therefore, to explore the use of the database for PV purposes, SAs that are described in literature were used to query GSRS for these SAs, and subsequently looked at the PV data of the resulting drugs. To do this, well established and hypothesized SAs had to be found. The examples identified in literature were used in the sub-studies.

The objective of this literature search is to find suitable SA/RM concepts to use for the five above mentioned sub-studies.

Method

To find literature about the SA/RM concept and ADRs, a Pubmed search for the term 'structural alert' was performed. This generated 397 results, of which 377 full text articles. The trend of publication numbers goes upward with a large spike from 2011 and on. The largest number of results was obtained for the year 2020 (35 articles). Thus, it seems like the SA concept is still emerging and gaining popularity amongst researchers. The abstract of the full text articles was scanned. The ones that focussed on finding SAs and describing RM formation or mechanism of

toxicity, or reviews of multiple SAs and their RM formation were picked. These studies were summarized, and their SA/RM concepts were used in sub-studies 1, 2 and 4.

Results

NSAIDs and hepatotoxicity

One study that takes SAs as a basis to find a relationship between the SA and an ADR was performed by Jessurun and van Puijenbroek (17). In this article, the relationship between SA's and idiosyncratic hepatotoxicity was investigated. Here, the ADR haemorrhage is chosen as comparator because it is certain that all NSAIDs cause this, as it is part of the primary pharmacology of NSAIDs. This was compared with idiosyncratic hepatotoxicity, an ADR that is hypothesized to be attributed to RM formation from certain SAs that some NSAIDs possess. Naproxen, ibuprofen, diclofenac, lumiracoxib and bromfenac were included. The first two do not possess one of the SAs, the others do. Bromfenac even possesses three SAs (figure 2). The results displayed a more disproportional reporting of hepatotoxicity for NSAIDs with more SAs. This suggested a structure-ADR relationship. This study was used as basis for **sub-study 1**, in which the sensitivity and specificity of GSRS is tested. This also allowed to explore if the database search generated all the NSAIDs that were discussed in this study.

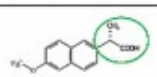
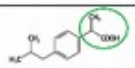
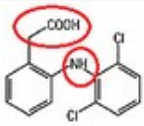
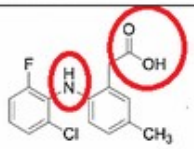
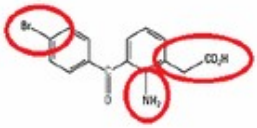
Naproxen DD: 1000 mg	**Ibuprofen** DD: 2400 mg	**Diclofenac** DD: 150 mg	**Lumiracoxib (W)** DD: 400 mg	**Bromfenac (W)** DD: 150 mg
				

Figure 2: naproxen, ibuprofen, diclofenac, lumiracoxib and bromfenac and their (lack of) structural alerts

Structural alerts for predicting drug-induced auto-immune disease

A project that used structural alerts was done for predicting drug-induced autoimmune diseases (18). Drugs were screened in literature for being auto-immune positive or negative. This screening generated 407 drugs. The 26 auto-immune related MedDRA terms were used for the full-text search. These included thrombocytopenia, hemolytic anemia, vasculitis, scleroderma and pemphigus. In parallel, a library of 171 structural alerts was created by searching for SAs in literature. Using the SMILES-SMART pattern matching function in Rdkit, which is a Python cheminformation package, the 407 drugs were screened against the SA library with 171 SAs. The reseachers used a cut-off for daily dose of > 100 mg because a high daily dose is suggested to be a contributing factor to ADRs. It was found that one structural alert was significantly associated with auto-immune positive drugs, this was a benzene ring with nitrogen-containing substituent. Especially the positive predictive value was high for the benzene ring with nitrogen-containing substituent (two N-H bonds, figure 3) and the false positive rate was 0%. However, the sensitivity was very low (6%). This SA was used in **sub-study 4.**

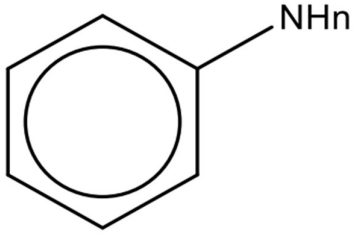


Figure 3: the structural alert that resulted from the study from Wu et al. that used structural alerts for predicting auto-immune disease.

Idiosyncratic drug toxicity and SA/RM formation – an overview

A well cited paper by Stepan et al*. (Structural alert/reactive metabolite concept as applied in medicinal chemistry to mitigate the risk of idiosyncratic drug toxicity: a perspective based on the critical examination of trends in the top 200 drugs marketed in the United States)* of a large study that looked at withdrawn drugs, ones with a BBW and the top 200 drugs marketed in the United States (8). The authors compared these three groups for presence of SAs and other toxic features. This generated a large number of discussed SAs, where for some the mechanism of toxicity was very obviously due to RM formation, and for some the involvement of their SA and possible RM formation remained unclear. This study also discussed the hepatotoxicity of the NSAIDs bromfenac and ibufenac versus ibuprofen, with a similar SA as the ones in the study from Jessurun and van Puijenbroek (8,17). One case from this study was used in **sub-study 2**.

Structural alerts in numbers

From the drugs withdrawn in 2009, 84% (26 of total 31 withdrawn drugs) possesses one or more SA which can give rise to RMs. Eighteen of these 26 undergo metabolic activation, this includes RM formation but can also be also covalent binding to target organ tissue. For five of 26 drugs with an SA, there is evidence of other toxic features being present. An example of a toxic feature is the inhibition of the bile salt export pump (BSEP). BSEP inhibition can lead to liver disease through accumulation of cytotoxic bile salts into hepatocytes (19).

Sub-study 1: NSAIDs

Introduction

Ibufenac and ibuprofen are examples of drugs where the first displays toxicity and the second does not, while having minimal structural differences. The only structural difference is an alfa-methyl substituent in ibuprofen that is not present in ibufenac. IADRs association with NSAIDs with a carboxylic acid base have been linked to formation of acyl glucuronide derivates. These derivates are the major metabolites of many NSAIDs in humans and can covalently modify proteins. They do this by forming a reactive alfa-hydroxy-aldehyde intermediate which can then form a glycated conjugate when binding to a protein. The formation of this iminium species is reversible; however, formation can be followed by an Amadori rearrangement which makes the product more stable, as depicted in figure 4. It has been shown that carboxylic acids with extended alkyl substitution at the alfa-carbon exhibit lower reactivity with protein nucleophiles. This suggests that because of inherent electrostatic and steric effects these RMs are formed to a lesser extent because of these substitutions. Ibufenac shows a higher degree of acyl glucuronide formation than ibuprofen which may explain the toxicological differences. However, protein adducts of ibuprofen that seem to be derived from the acyl glucuronide have been detected in human plasma (19).

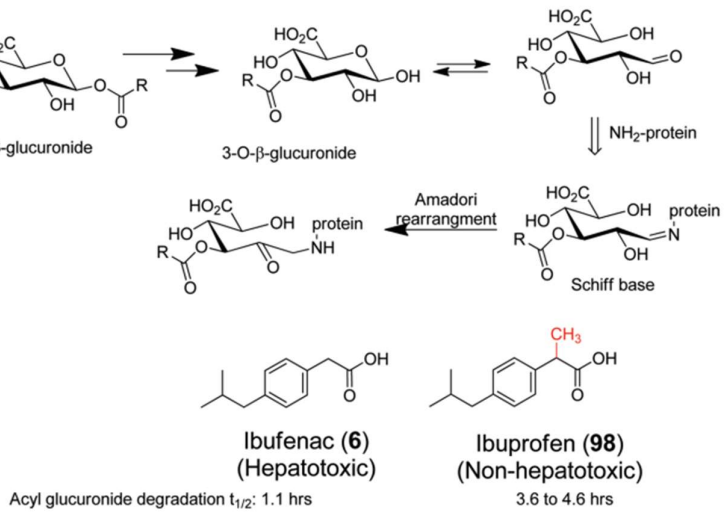


Figure 4: Mechanism of acyl glucuronide adduction to proteins, structural difference between ibufenac and ibuprofen

Arylacetic acid, 2-arylpropionic acid, and anthranilic acid derivatives are structural elements of certain NSAIDs that are suspected to cause RM formation and concurrent liver toxicity. Ibufenac has been retracted from the market since 1968 but structurally similar NSAIDs are still on the market or have been retracted relatively recently. These are lumiracoxib, bromfenac and diclofenac. These three were used in the study of Jessurun and van Puijenbroek (17).

The results of this study were used to test the GSRS database. It is indicated as 'reference article' because the studied drugs, PTs and methods were the reference for this study. The goal of this study is to explore the possibilities of using the GSRS database to find structures that possess SAs without giving results that do not possess these SAs. The objective was to investigate if, in the reference study, the GSRS database could have been used to find the structurally similar NSAIDs that cause toxicity by searching for the studied SAs. A secondary objective is to confirm the involvement of the SA in hepatotoxicity seen in NSAIDs by extracting the PV data for the resulting drugs from VigiLyze.

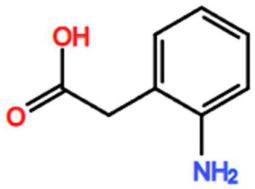


Figure 5: Structural alert for hepatoxicity of non-steroidal anti-inflammatory drugs

Method

The SA that was discussed in the reference study was entered in GSRS. The structure depicted in figure 5 was searched for, with corresponding SMILES code NC1=C(CC(O)=O)C=CC=C1. This does not cover the bromine atom that was one of the SAs (17).

No explicit hydrogen atoms were added so to each atom that is drawn, any other atom can be attached.

The resulting drugs were displayed in a table with the SA highlighted in their molecular structure. Differences between the search results and the drugs in the reference article were highlighted and their display of the ADRs that were discussed in the reference article was investigated by extracting their IC025 values from VigiLyze.

For drugs resulting from the GSRS search that were not included in the reference study it was evaluated whether these display the same liver toxicity as the drugs from the reference study. This

was done by entering the following PTs in VigiLyze: *hepatic failure*, *hepatic function abnormal*, *hepatic necrosis*, and *hepatitis*. These PTs were used in the reference article. The amount of reports of liver toxicity of the resulting drugs was compared to the level of reported liver toxicity described in the reference article to confirm the involvement of the SA.

Search results

The GSRS search resulted in ten records after filtering for the domain 'drug'. Before this filter, 48 structures were given back. The results are displayed in table 1. The drugs that resulted from this search but were not mentioned in the article are amfenac, robenacoxib and aceclofenac. These are highlighted. All the drugs that were discussed in the reference article were found using GSRS.

Aceclofenac does not have the exact similar structure of the other NSAIDs that were found in this search. It was included because an explicit hydrogen of the OH-group of the carboxylic acid group was not drawn. Including this explicit hydrogen atom only gives back lumiracoxib, amfenac, bromfenac, robenacoxib and diclofenac. Another advantage of searching with this explicit hydrogen atom is that the salt forms of the drugs are filtered out. This may also be achieved by filtering on 'active moiety'.

PV results

The drugs from the reference study (diclofenac, lumiracoxib, bromfenac) were found using GSRS and their PV data could be compared to the data in the reference study. Additionally, amfenac, robenacoxib and aceclofenac were found.

Robenacoxib is only available for veterinary use and consequently does not have any VigiLyze reports.

Amfenac is the active metabolite of the prodrug nepafenac and is in some Asian countries available as oral formulation. Nepafenac is only used as an ocular formulation. Its systemic exposure after topical ocular administration is very low so it is unlikely that amfenac, its metabolite, will reach plasma levels high enough to generate RMs and cause an ADR (20). Nepafenac does not have any cases for the four PTs which supports this notion. Amfenac only has reports for its oral formulation, so this drug was included in the VigiLyze data extraction.

It must be noted that amfenac only has 45 reports in VigiLyze, the only PT that had cases for amfenac was *hepatic function abnormal*. The other three were not reported and therefore were not depicted. Therefore, there are no data points for amfenac for the PTs *hepatic failure, hepatic necrosis, and hepatitis* in figure 6.

Aceclofenac did not contain the exact SA. To see if the lack of presence of the exact SA prevents the ADR from occurring and being reported, aceclofenac was included in the VigiLyze data extraction. The drug has a total of 12,436 cases.

Figure 6 shows the results of the VigiLyze data extraction. The trends in IC025 values of diclofenac, lumiracoxib and bromfenac are comparable to those in the reference study with diclofenac having lowest values and then ascending in lumiracoxib and bromfenac. The IC025 values of aceclofenac are all negative except for hepatitis, of which the value is 0.3. Amfenacs only IC025 value is negative.

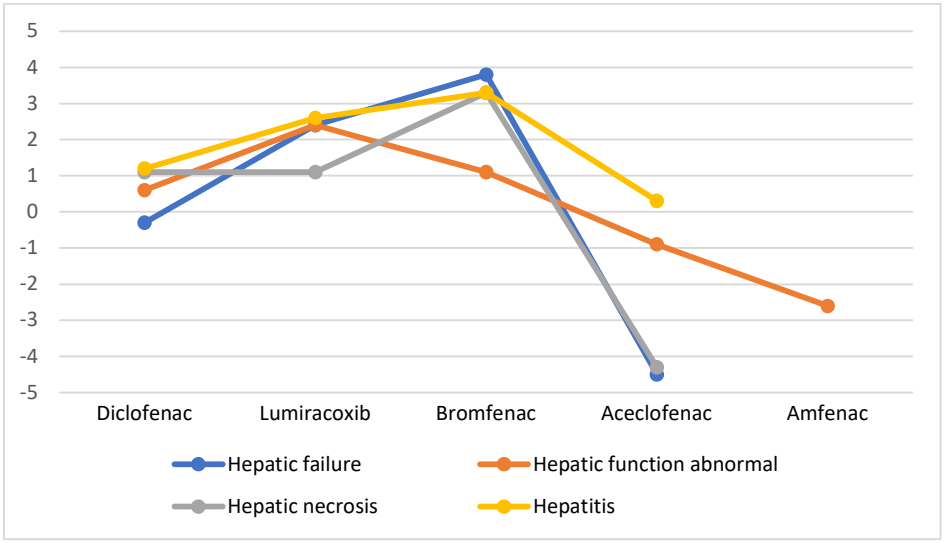
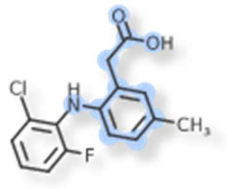
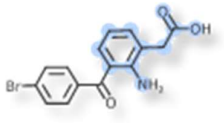
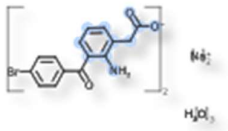
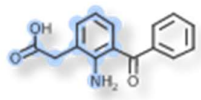
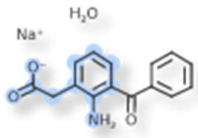
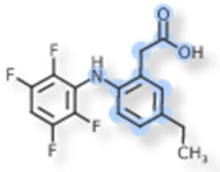
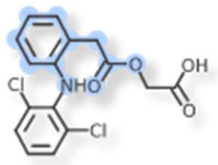
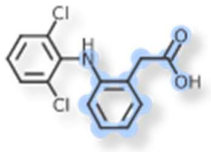
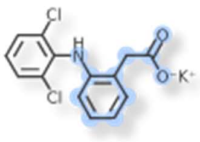
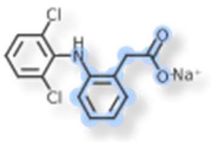


Figure 6: NSAIDs and their information component 95% confidence interval lower limit value for the four researched preferred terms. IC = information component.

Table 1: Search results from the structural alert query and their molecular structure extracted from the GSRS database, with the queried structural alert highlighted in blue. Discrepancies between reference article and search results highlighted in grey.

Lumiracoxib 	Bromfenac 
Bromfenac sodium 	Amfenac 
Amfenac sodium 	Robenacoxib 
Aceclofenac 	Diclofenac 
Diclofenac potassium 	Diclofenac sodium 

Conclusion and discussion

The results of this search prove that the GSRS database can be queried to find all relevant structures and, in this case, did not give back false positive results. The first search contained aceclofenac, which did not exactly contain the SA of interest. However, this was not a false positive because it fell within the searching criteria that were set. When the search was specified better to the SA, aceclofenac was not returned by GSRS. GSRS is a suitable system to use when looking for structurally similar molecules containing a SA. When the IC025 values for the hepatotoxicity PTs in VigiLyze are compared to those in reference study, the drugs containing the SA still show a similar level of reported hepatotoxicity. Amfenac could not be used as an additional SA-containing drug to confirm these results as the number of reports was too low. The lack of hepatotoxicity of aceclofenac was confirmatory of the involvement of the SA, as only hepatitis had a small positive IC025 value, and the other PTs did not.

The possibility of filtering on 'active moiety' in GSRS filters out the salt or solvate forms of drugs. It may be the case that a drug is only entered in GSRS as a salt or solvate form because it only exists as such. They will be filtered out when the 'active moiety' filter is applied. When this is the case, results that may be relevant will be missed. However, it is unknown if there are compounds that are only entered as salt or solvate.

In this one case, the sufficient sensitivity of the database search was demonstrated. The specificity was acceptable too. However, amfenac and robenacoxib were two results that were rightfully not included in the reference study as amfenac is rarely used and robenacoxib is only for veterinary use. The upside of GSRS is that it has a wide variety of compounds included, the downside is that this may cause a cluttering of the database. Especially when in future research a search is performed with a less specific SA. Additional filter options would be a possible solution for this problem. The wide variety of compounds means that drugs were included in GSRS from a very early stage of investigation. It is useful to have these included, as toxicity caused by certain SA containing drugs in early stages can be studied. The option to filter would make research about drugs in specific investigational or clinical stages easier.

Sub-study 2: COMT inhibitors

Introduction

The COMT inhibitors tolcapone and entacapone inhibit the enzyme catechol-O-methyltransferase. Inhibition of this enzyme slows the metabolism of the dopamine precursor levodopa to its metabolite 3-O-methyldopa. This prolongs the time levodopa is in circulation which in turn causes a prolonged dopaminergic effect. A prolonged dopaminergic effect reduces the symptoms of Parkinson's disease like tremor and rigidity (21).

The COMT inhibitors entacapone and tolcapone are currently on the market. Tolcapone was retracted from the market in 1998 because of the risk of sometimes fatal hepatitis and neuroleptic malignant syndrome. Its suspension was lifted in 2004 under certain conditions. It currently has a boxed warning in the US, and in the EU liver function tests are required during use of this drug. Additionally, some contra indications are to be kept in mind and only prescribers with experience with treatment of late-stage Parkinson's disease are to prescribe Tolcapone (22,23).

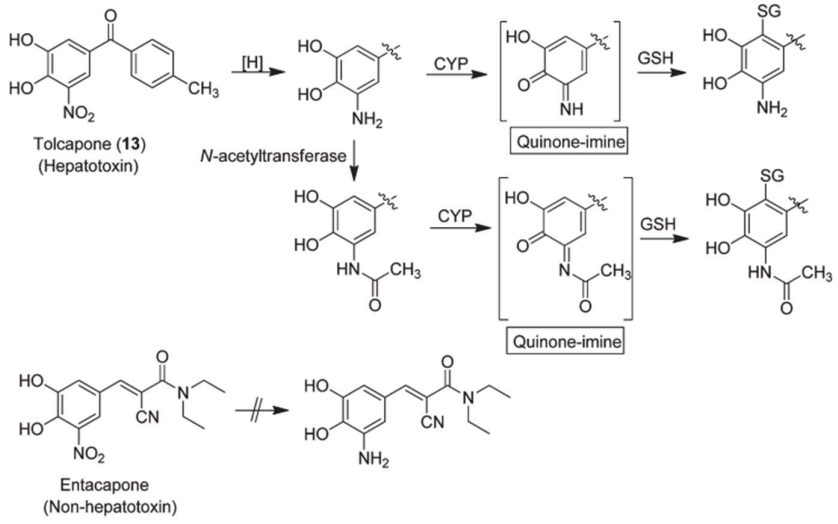
In contrast, entacapone has no reports of severe liver toxicity, only abnormal hepatic function and hepatitis are mentioned in the SmPC (24). Entacapone is the first drug of choice when a COMT inhibitor is indicated. Tolcapone is only used when entacapone does not work well enough or if a patient is intolerant for entacapone (25).

RM formation has been proposed as an explanation for this difference in toxicity. Figure 7 shows the metabolites formed: aniline and N-acetylaniline derivates (8). These undergo oxidation to a quinone-imine species that can be trapped by GSH in liver cells. This does not happen with entacapone.

The shared structure that these two RMs share is depicted in figure 8 with the following SMILES code: SMILES code NC1=C(O)C(O)=CC=C1. This is the general SA that belongs to the RMs of tolcapone and can be used to search for related structures. Only if the metabolites of the parent drugs are included in the database, it can be tested if this RM gets formed from other drugs. If not all metabolites are present in the database, it may be interesting to see if a parent drug containing this structure displays the same toxicity as a metabolite. The primary goal of this search is to test what the possibilities are regarding structure searches for RMs in GSRS. A secondary goal is to identify structures related to the hypothesized SA of tolcapone, and compare their extent of hepatoxicity to other drugs within their drug class that do not form this RM.

Method

The first search that was performed was for the SMILES code NC1=C(O)C(O)=CC=C1. When the results were metabolites, the corresponding drug was extracted from the database. A second search using the structural element that tolcapone and entacapone share was performed to possibly identify more (investigational) COMT inhibitors. For these records, their metabolites and possible display of hepatotoxicity were studied. This way, COMT inhibitors that do and do not form the hypothesized RMs were compared in terms of hepatotoxicity. The hepatotoxicity of the resulting drugs and related metabolites was compared using results of clinical trials that investigated hepatotoxicity of the compounds and, where possible, VigiLyze reports.



[a] Rationalization for the improved safety of structural analogue entacapone through metabolic differences.

Figure 7: Metabolism of tolcapone to reactive metabolites and the metabolite of entacapone

Results

Entering the SA structure (figure 8, SMILES code NC1=C(O)C(O)=CC=C1) in the GSRS database the search gave three results in total, of which two were the aniline and N-acetylaniline derivate metabolites of tolcapone. No results for entacapone were obtained and the third result was a metabolite of the drug nebicapone.

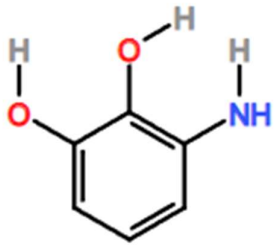


Figure 8:The structural alert for the reactive metabolite of tolcapone which was entered in GSRS

Nebicapone is a COMT inhibitor currently in development. In a phase II study, clinically relevant transaminase elevations were reported in 4 of 46 (8,7%) patients who received 150 mg nebicapone. This was not seen in patients receiving 50 and 100 mg. In phase II and III studies of tolcapone, 1-3% of patients reported abnormal liver enzyme levels (26). Nebicapone is not entered in VigiLyze.

Entering the moiety that tolcapone and entacapone share in GSRS (figure 9, SMILES code OC1=C(O)C(=CC=C1)[N+]([O-])=O), gives back 37 results, of which 5 drugs. These are nitecapone, opicapone, entacapone, tolcapone and nebicapone.

Nitecapone has been described in literature but has not been brought on the market. Nitecapone is more structurally similar to entacapone than to tolcapone. No metabolites of nitecapone are entered in GSRS. Nitecapone is not entered in VigiLyze. No studies on clinicaltrials.gov were found for nitecapone, in PubMeb, no articles were found about nitecapone that mentioned liver function.

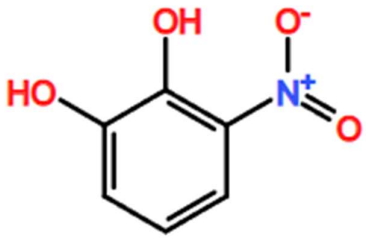


Figure 9: the shared structure of tolcapone and entacapone, which was used to find structurally related drugs

Opicapone (figure 10) is a new COMT inhibitor that was approved by the MEB in the Netherlands (27). Two metabolites of opicapone are entered in GSRS. One des n-oxide and one sulphate metabolite. No metabolites that are similar to the hypothesized RMs are present in the record of opicapone in GSRS. The new Dutch guidelines on Parkinson's disease mention that opicapone is not superior to entacapone (25). With generic formulations of entacapone available at a lower price and only a non-inferiority study, there is a low chance that opicapone will ever be marketed in the Netherlands. In VigiLyze, opicapone has 1,083 cases in total. This number is unlikely to grow significantly if it only stays therapeutically relevant for a small group of patients.

For the high-level group term (HLGT) 'Hepatic and hepatobiliary disorders', no PT was significant for opicapone. Additionally, in the SmPC there is no mention of liver related ADRs in section 4.8.

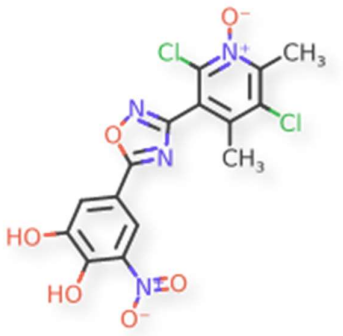


Figure 10: molecular structure of opicapone

For entacapone, as noted before, abnormal hepatic function and hepatitis are mentioned in the SmPC. For the HLGT 'Hepatic and hepatobiliary disorders' the PTs 'jaundice' and 'hepatic function abnormal' have a significant IC025 (0,3 and 0,4, respectively). Entacapone has a total of 2,042 cases in VigiLyze. The phase II study that investigated nebicapone included one trial arm with entacapone. None of the 50 patients who used 200 mg entacapone had abnormal transaminases. Additionally, entacapone may be used up to ten times a day, making the maximum daily dosage 2 grams (29). This is significantly higher than the dose of tolcapone which is 300 mg, and 600 mg in exceptional situations (23).

Tolcapones SmPC mentions hepatocellular damage, in rare cases with a fatal outcome. Additionally, it has a warning in section 4.4 for liver damage which describes the contra-indications and precautions around hepatotoxicity (30). In VigiLyze, the PTs jaundice, hepatic necrosis, hepatocellular injury, hepatic failure, hepatic function abnormal, hyperbilirubinaemia, cholestatic liver injury and hepatitis fulminant have a significant IC025, ranging from 0.6 to 2.0.

Conclusion and discussion

This case shows that in addition to searching for drugs that may form a RM, the RM in question can also be used to perform a structure search for other drugs or for metabolites of drugs. In this case, it was shown that the GSRS database could be used to find that one of the hypothesized RMs is formed by nebicapone.

The hepatotoxicity of the two drugs that formed the RM seemed to differ from the ones that do not. This is shown by the difference in hepatoxicity in SmPCs, VigiLyze and clinical studies. Nebicapone is not yet on the market, if a larger phase 3 study is performed there will be more information about the hepatotoxicity. The fact that like entacapone, opicapone does not seem to cause liver toxicity supports the involvement of the hypothesized RMs.

One limitation of this study is that the number of results for both searches were low. If drugs of other classes were found, it would have been possible to distinguish if the hepatoxicity arises in drugs with other mechanisms of action. Another limitation is the limited information about nitecapone. If more information was available about its toxicity profile, a better comparison could be made between toxicity in drugs with RM formation and without.

Sub-study 3: Fluoroquinolones

Photosensitivity reactions are abnormal cutaneous responses to ultraviolet (UV) radiation or visible light. They can be induced by exogenous agents that contain a structural element that can cause the agent to get in an excited state (31). This means that this ADR is strongly linked to molecular structure.

Photosensitivity is an ADR that occurs in varying drug classes, including diuretics, NSAIDs, and antibacterial drugs (32). There are two ways a molecule can cause photosensitivity. One is direct. This happens after a molecule a is converted to an excited state by UV radiation. This excited molecule then photo binds to endogenous molecules. The other mechanism begins with the molecule in the excited state too. This indirect mechanism requires further energy transfer or free radical generation and concurrent formation of superoxides or singlet oxygen molecules. These reactive oxygen species can oxidate DNA or a drug.

Both mechanisms require molecules to get in an excited state. This can only occur when structural elements are present that cause the drug to absorb UV/VIS within the range of natural sunlight (290 – 700 nm) (33). To find structural elements that cause photosensitivity, a literature search was performed. The aim of this search was to find articles describing a possible mechanism in which a drug or structural element can lead to photosensitivity reactions, for example by generation of photo toxicants.

In literature, multiple structures were identified that were suspected of causing photosensitivity. The search strategy for this ADR was aimed at exploring possible SAs for any photosensitivity -causing drugs. In Google Scholar, 'drug induced photosensitivity' was searched for. From this first search, relevant articles were selected, and related articles were found in their sources. Many of the SAs related to photosensitivity that were described were specific to one drug class. This made it difficult to establish if photosensitivity is caused by the structure in question, or as a result of the pharmacological mechanism of the drug class. When no drugs with other mechanisms of action are found, both can be the case. One example of this kind of structure is discussed below.

3a: Fluoroquinolones and phototoxicity

In multiple articles, the photo lability of fluoroquinolones is described (32,34–39). Fluoroquinolones are a class of antibiotics. They belong to the quinolones, a class that can be divided in four generations. The first generation is the only generation in which the molecules are not fluorized. In the Netherlands, no first-generation quinolones are on the market. This means that only fluoroquinolones are available. However, the first-generation quinolones: nalidixic acid and cinoxacin, were once widely used (40).

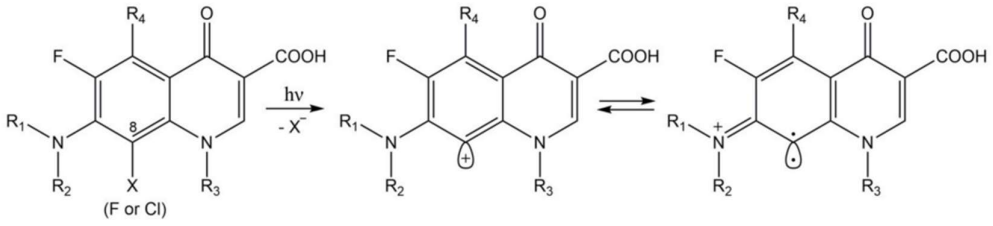


Figure 11: formation of phototoxic products from the basic fluoroquinolone structure

Phototoxicity is seen in all generations of quinolones. The majority of the reactions are phototoxic reactions, although photo allergy was reported sometimes as well (38). Both 'photosensitivity allergic reaction' and 'phototoxicity' are lowest level terms (LLT) that fall under the PT 'photosensitivity reaction'. This PT was used to search for the photosensitivity reactions that the quinolones display.

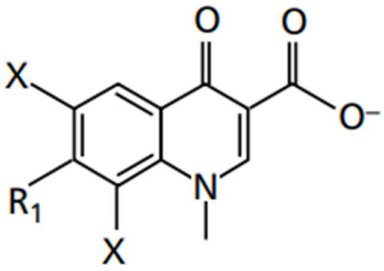


Figure 12: structural alert for phototoxicity generated by the DEREK system

Phototoxicity reactions caused by the fluoroquinolones are attributed to the formation of an aryl cation. This structure possesses a carbene character and is therefore very reactive. It can react with oxygen to produce quinone-imine and hydrogen peroxide. Hydrogen peroxide plays a role in hydroxyl radical formation, singlet oxygen generation does not occur (41). The fluor atom that is depicted in the left structure in figure 11 is not the only structural element necessary for the mechanism of toxicity, as quinolones without this group, like nalidixic aced, display phototoxicity too. This makes the structure depicted in figure 12 more likely to be the minimum requirement for the reaction to take place. This quinolone SA was generated in an earlier project about SAs, using the DEREK system. DEREK stands for "Deductive Estimation of Risk from Existing Knowledge" (42). Where one or two X groups are F, Cl, Br or I and R1 is N, O or S.

For the first-generation quinolones, two photoproducts were found that were involved in toxicity. One is decarboxylated and one is a dimer. It is unknown if these products play a role in photosensitivity reactions in the skin (43). A mechanism or RM formation like in figure 11 cannot be produced. It is however well known that the first-generation quinolones cause photosensitivity.

Using the above stated mechanism from literature and the knowledge that the fluoroquinolones that contain the discussed structure display photosensitivity, it can be investigated if there are other drug classes that possess this structure and see if they display this ADR too. This will answer the following question: what is the relationship between the ADR 'photosensitivity reaction' and the quinolone SA?

Method

To see if the photosensitivity reaction that quinolones display has something to do with the structure, the hypothesized SA was entered in GSRS to find quinolones and drugs outside the class of quinolones. This makes it possible to confirm that the ADR is related to the structure, like the mechanistic hypothesis.

The following SMILES code was used to search for related structures in GSRS: OC(=O)C(=CN(C)C1=CC2)C(C(=C(C=2)[H])1)=O. The requirements of the X and R1 groups were checked manually, as the structure search does not have an 'or' function and there are many possible combinations of the R1 and two X groups.

The first-generation quinolones do not contain the quinolone nucleus. Their basic structure is built up around the naphthyridone nucleus, like in nalidixic acid (figure 13). The difference between the quinolone and naphthyridone nucleus is just one nitrogen atom. Because it is well established that the first generation quinolones cause photosensitivity reactions, this nucleus was searched for in GSRS too, with the following SMILES code: OC(=O)C1=CNC2=C(C=CC=N2)C1=O (38).

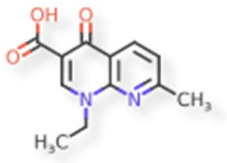


Figure 13: molecular structure of nalidixic acid

Results were grouped in 'quinolones' and 'non-quinolones'. Of the non-quinolones, the IC025 and IC values for the PT photosensitivity were extracted from VigiLyze. Where possible, the SmPC was checked for presence of the PT. This could be a Dutch, English (United Kingdom), German or French SmPC. As there are many quinolones, investigational and on the market, the results were for the largest part expected to be quinolones. Therefore, one quinolone was randomly matched to every non-quinolone result. Its IC and IC025 value for photosensitivity and presence of photosensitivity in its SmPC were compared to the non-quinolone. When this quinolone was not entered in VigiLyze, the next one was chosen until one was found with a total of > 100 cases in VigiLyze.

Results

When the quinolone structure was entered in GSRS, 192 results were returned. After applying the 'drug' filter, 64 records remained. In GSRS, it is possible to filter on ATC code. This would have made it easier to identify the class of the 64 structures. However, when all possible ATC codes are selected, less than 64 records remain. This could either be because of the filtering out of salt forms, not all records having an ATC code attached, or because some records have multiple ATC codes. If this function would be optimized, one could simply filter out all drugs with ATC code J01M (QUINOLONE ANTIBACTERIALS, ATC level 3). Because it is unclear if the records are filtered correctly, the 64 records were checked by hand.

Quinolone search

Of the 64 records, 56 were fluoroquinolones, 6 were quinolones, and the remaining two were cadazolid and elvitegravir. Cadazolid does not exactly meet the requirements for the structural alert, as the atom attached to C-8 is hydrogen and R1 is a fluor atom. Elvitegravir also does not match the requirements, as the two X groups are oxygen and hydrogen and R1 is a carbon atom.

Cadazolid

Cadazolid only has one case in VigiLyze, photosensitivity was not reported. It is an experimental drug, which explains the low number of cases. The matched quinolone was prulifloxacin. This is a quinolone that is on the market in some Asian and European countries. It has 530 cases in VigiLyze in total and an IC025 and IC value for photosensitivity reaction of 0.2 and 1.9, respectively. No SmPC was available.

Elvitegravir

Elvitegravir has 176 cases. This number may be relatively low because elvitegravir is used almost exclusively in combination with other drugs against HIV (44). The combination emtricitabine/ tenofovirdisoproxil/elvitegravir/cobicistat for example, has 14,035 cases. This combination has an IC025 value of -3.3 with 5 observed cases. For elvitegravir, there are no cases of photosensitivity. Only an SmPC for the combination is available and herein, photosensitivity is not mentioned (45).

The matched quinolone was norfloxacin. This fluoroquinolone has a total of 15,393 cases. The IC and IC025 values for photosensitivity reaction are 2.0 and 2.3, respectively. In the SmPC, photosensitivity is mentioned with a frequency of ≥ 1/1000 to < 1/100 (46).

Naphthyridone search

When the naphthyridone structure is entered in GSRS, 44 results are given back. After applying the 'drug' filter, 15 records remain. Just one of these 15 results is not a quinolone antibiotic.

Vosaroxin

Vosaroxin is an investigational anticancer quinolone derivative. Just like the quinolone antibiotics, this inhibits topoisomerase II (47). This one result thus does not have a different pharmacology. It does have a dissimilar patient group. This may elude a possible relationship between patient

characteristics and the ADR. However, vosaroxin has only 8 cases in VigiLyze. This number is too small to make conclusions about possible relationships between the drug and the ADR. In these 8 cases, photosensitivity was not reported.

Trovafloxacin was the matched quinolone. This drug was withdrawn from the market due to the risk of hepatotoxicity. It has 3,927 cases in VigiLyze. The IC and IC025 values for photosensitivity reaction are 0.6 and 1.4, respectively. A SmPC was not available.

Conclusion and discussion

No other drug classes were found that contained the exact SA that is present in the fluoroquinolones. The only drug that was found which had more than 10 records in VigiLyze was elvitegravir. For this drug, no signs of phototoxicity are present. Neither in its SmPC nor in VigiLyze. This confirms the SA that was found in literature. The lack of phototoxicity of elvitegravir points to a possible involvement of the substituents of the SA.

All the comparators from the quinolone group did show a significant IC025 value for phototoxicity, which confirms that the quinolones display this ADR. Regarding the SA-ADR relationship, it is not clear whether this ADR is caused by the structure in question or the pharmacology of the fluoroquinolones. The lack of toxicity in elvitegravir may either be explained by the different substituents or by the distinct pharmacology.

3b: ADR-mediating effects of substituents

Another possible way to see if the occurrence of the ADR is related to the SA is to see if within the drug class there are drugs that do not contain the SA or parts of the SA. This way, certain elements of a structure can be linked to exacerbating or diminishing a hypothesized reaction mechanism. In literature, a difference in photosensitivity reactions within the class of fluoroquinolones is attributed to the kind of C-8 moiety that is present. Halogenation at the C-8 position has shown to be related to a greater display of photosensitivity than a methoxy group (48). This is confirmed in an in vivo study by comparing the phototoxicity of moxifloxacin (with a methoxy group) with lomefloxacin (with a halogenated C-8 group) in healthy volunteers. Moxifloxacin failed to show phototoxicity, in contrast to lomefloxacin (49). An exception to this trend is the halogenated fluoroquinolone delafloxacin. This new drug failed to exhibit photosensitivity reactions in clinical trials. This is attributed to the large and heavily substituted N atom, as shown in figure 14 (50).

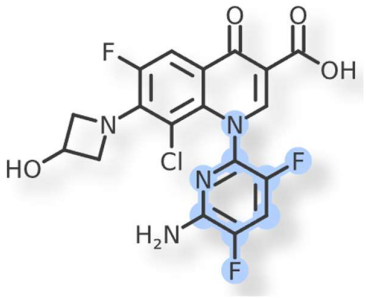


Figure 14: molecular structure of delafloxacin with the substituent that is hypothesized to diminish reactive metabolite formation highlighted in blue

In addition to ADR mediation, the modification of the quinolone nucleus is linked to the alteration of the antimicrobial activity and the pharmacokinetic profile. For example, the addition of a fluorine atom at C-6 increased DNA gyrase inhibitory activity, a second fluorine at C-8 resulted in a better absorption and longer half-life and a methoxy group at C-8 targets specifically both topoisomerase II and IV, which may decrease resistance (39).

In various documents like SmPCs, the occurrence of photosensitivity reactions in fluoroquinolones with a methoxy group at C-8 is still mentioned as possible side-effect with the disclaimer that this is seen in other quinolones. To confirm these various studies and comparisons, GSRS can be used to identify both the quinolones on the market and investigational quinolones. When investigational quinolones with different substituents are considered, it is possible to compare if certain structural

elements are a predicting factor for getting through the different phases of (pre)clinical research. VigiLyze can then be used to relate these search results from GSRS to the number of ADR reports.

The following research question can be formulated using this information: what is the difference in occurrence of photosensitivity reactions between quinolones with various C-8 moieties? With as secondary research question: What is the difference in authorization status between the different C-8 moiety groups?

Method

To answer the research questions, quinolones are searched for using GSRS. The results were grouped by their C-8 moiety. All results were searched for in VigiLyze. Their IC0,025 values were compared between groups. A secondary analysis was done using only the results with > 1000 records, as their IC may be more reliable than drugs with a small number of reports. Additionally, for each record it was noted what the pharmaceutical use status is of the molecule. This could be either 'in use', 'retracted', 'investigational' or 'investigation terminated'. It was then compared between C-8 groups what the share of the different status categories is of the total amount of records in each group.

Search strategy

To identify as many quinolones as possible, GSRS was used. In GSRS, it is possible to filter on ATC code. However, when a single record is opened, many more code systems are attached. When a structure is entered, there can be filtered if the code system is entered for the results but there cannot be filtered within all code systems. This can only be done for the ATC code system.

One record can be classified using multiple code systems, this can be viewed in the *Codes – Classifications* section of the record. Some are only classified using ATC classification, others have multiple. The often-used fluoroquinolone ciprofloxacin even has 27 classification trees in its record, using 8 different code systems. One drug can have multiple classification trees per code system depending on, for example, indication or dosage form. Selecting the following classification tree Pharmacologic Substance[C1909] → Anti-Infective Agent[C254] → Antibiotic[C258] → **Quinolone Antibiotic** from the NCI Thesaurus code system yielded the most results. This may be because this system classifies biomedical concepts, so molecules receive a code early in their development process (51). This was suitable for this search, as it was intended to find investigational quinolones too. The results for this categorization were used to create the different C-8 groups with a status label for each individual record.

Results

Using the Quinolone antibiotic label from the NCI Thesaurus code system, 92 records resulted that belong to this class. After filtering for 'drug', 86 remained. After filtering for 'active moiety', 61 records remained. These 61 records where categorized. The 23 salts or solvates were checked to see if the active moiety for each of these records was present too. Prulifloxacin was the only drug that was filtered out completely.

These 61 individual quinolones had six different types of C-8 moieties. The distribution of the moieties and status is depicted in table 2.

The largest part of the quinolones has no group attached to the C-8 atom, this is indicated by CH. The CO group can carry different groups attached to the O-atom. This can be an O carrying a CH3 group like in moxifloxacin, or an O atom carrying a cyclic structure like in marbofloxacin.

The N group are the naphthyridones like nalidixic acid. In this group, a relatively large share is retracted. Two of these are nalidixic acid and pipemidic acid. These were suspended by the CHMP for their impactful side-effects on muscles, tendons, joints, and the nervous system (52). Trovafloxacin and alatrofloxacin were retracted due to cases of hepatotoxicity (53). Of the four drugs with a terminated investigation, gemifloxacin may be available in countries outside of the EU, but the EMA has not granted it market authorization due to concerns about effectivity and genotoxicity (54). Of ecenofloxacin, piromidic acid and esafloxacin it is unclear why the investigation was terminated.

There is one fluoroquinolone with an S atom attached to C-8. This is rufloxacin. In Italy, this drug was authorized first in 1992 (55).

The number of investigational and investigation terminated drugs relative to the total number of drugs in the group does not seem to vary between groups.

Table 2: number of results from the GSRS search in each category for each of the possible C-8 moieties

	CH (n = 29)	**CO (n = 9)**	**CCL (n = 3)**	**CF (n = 5)**	**N (n = 12)**	**S (n = 1)**
In use	5	5	2	2	2	1
In use (vet.)	6	2	1	1	0	0
Investigational	4	1	1	1	2	0
Investigation terminated	6	1	0	0	4	0
Investigation terminated (vet.)	1	0	0	0	0	0
Retracted	4	0	0	1	4	0
Retracted (vet.)	3	0	0	0	0	0

In table 3, the IC values for photosensitivity reaction are depicted for the drugs with more than 1000 cases in VigiLyze. Especially lomefloxacin has a high IC value.

The O-containing drug moxifloxacine has a negative IC025 value and photosensitivity is thus less frequently reported than expected. Garenoxacin has a total of 1,142 cases but no reports of photosensitivity reaction. Ofloxacin, also an O-containing fluoroquinolone, does have a positive IC025 value. This means that photosensitivity is reported more frequently than expected.

The N-containing quinolones have varying IC values for photosensitivity. Trovafloxacin was only on the market for one year but had a significant number of reports submitted in this one year (56). Gemifloxacin has a low IC value too. It has a limited availability. The CHMP rejected the application for market authorization of the drug in 2009 (57). Recent reports stem mainly from Korea.

Table 3: IC025, IC values and relevant comments for results from the GSRS search with more than 1000 cases in VigiLyze, sorted by C-8 moiety category. IC = information component.

Name	C-8	Status	IC025	IC	Comments
Lomefloxacin	CF	IU	6.6	6.7	
Pefloxacin	CH	IU	4.2	4.6	
Temafloxacin	CH	R	1.9	2.6	R due to allergy and hemolytic anemia
Norfloxacin	CH	IU	2	2.3	
Ciprofloxacin	CH	IU	1.6	1.7	
Ofloxacin	CH3O	IU	1.7	2	
Moxifloxacin	CH3O	IU	-0.2	0.1	
Garenoxacin	CH3O	IU	-	-	Only used in Asian countries
Nalidixic acid	N	R	4.9	5.1	Marketing authorization suspended by CHMP
Enoxacin	N	IU/R	4.4	4.8	Marketing authorization repealed in France
Trovafloxacin	N	R	0.6	1.4	Retracted due to hepatotoxicity
Gemifloxacin	N	IT	0.1	1.2	CHMP doubted effectivity and toxicity level

In table 4, the IC values for photosensitivity reaction are depicted for the drugs with less than 1000 cases in VigiLyze. The number of cases is added to the table too. Besifloxacin is a drug with a halogenated C-8 group. The only formulation for besifloxacin is ophthalmic (58).

Sparfloxacin has been retracted from the market for QT prolongation and because of phototoxicity. The strong association with photosensitivity is confirmed again by the high IC value for the PT. Fleroxacin displays this too but not as strongly as sparfloxacin. Again, the O-containing drug displays a low IC value.

Table 4: IC025, IC values, number of cases and relevant comments for results from the GSRS search with less than 1000 cases in VigiLyze, sorted by C-8 moiety category. IC = information component.

Name	C-8	Status	IC	IC	No. Cases	Comments
Besifloxacin	CCL	IU	-0.6	1.5	563	Only in ophthalmic formulation
Sparfloxacin	CF	R	7.5	7.7	533	R due to QT prolongation and phototoxicity
Fleroxacin	CF	IU	3.9	4.5	482	
Grepafloxacin	CH	R	4.7	5.3	202	R due to QT prolongation
Cinoxacin	CH	R	1.3	2.7	384	Marketing authorisation suspended by CHMP
Oxolinic acid	CH	IU (vet.)	-0.6	2	85	First used in humans, now veterinary
Pazufloxacin	CH3O	IU	-3.5	0.3	567	
Pipemidic acid	N	R	2.1	3	919	Marketing authorisation suspended by CHMP
Tosufloxacin	N	IU	-3.7	0.1	712	
Rufloxacin	S	IU	-0.9	1.7	201	

There were 9 quinolones that did not have more than 1000 cases in VigiLyze and also no cases of photosensitivity reaction. 6 of these had less than 75 cases. This makes it not unusual that an ADR like photosensitivity does not get reported. 3 drugs had more than 150 cases, but photosensitivity was not reported once. These were delafloxacin (CCL), flumequine (CH), and alatrofloxacin (N).

Conclusion and discussion

The relationship between the ADR photosensitivity and the C-8 moiety of quinolones was shown in the results of this study. The quinolones with halogenated moieties had higher IC values in VigiLyze than the ones with a methoxy group, or other groups containing an O atom. The exception for this were delafloxacin, which was expected, and besifloxacin. For besifloxacin the lack of toxicity can be

explained by the route of administration. The ophthalmic formulation is likely to have very limited systemic bioavailability so not a significant part may reach the skin to cause toxicity. The numbers of investigational drugs and drugs of which the investigation was terminated was very low. This may be because the fluoroquinolones are a relatively old drug class and innovation has halted. Although a large difference was expected, e.g. with many investigational drugs in the O-group and less in the halogenated groups, this was not observed.

The GSRS database made it possible to identify many fluoroquinolones at once. However, in literature one other quinolone is described that did not make it through clinical trials due to phototoxicity. This is BAY y 3118. This leaves the question if there are others that were not identified by the used search strategy. BAY y 3118 is entered In GSRS but has no code system attached, which makes it only possible to find the molecule using a direct search or a structure search. However, when a structure search was performed, older quinolones like pipemidic acid may not have been found. This molecule, amongst others, contains the naphthyridone structure but has an extra N atom in the ring. An OR/AND function per atom had made it possible to search for quinolones with different structures.

Prulifloxacin is the pro-drug of ulifloxacin. It was found that prulifloxacin did not appear in the results when the 'active moiety' filter was applied. It is not entered in a salt or solvate form. A salt or solvate form can be recognized by terms as 'hydrochloride' and 'hydrate'. When applying the 'salt or solvate' filter, prulifloxacin also does not appear. This means that there are other filter options for moiety types that are not displayed in GSRS. Prulifloxacin would probably be in a 'pro-drug' category, but this filter option is not available.

The confirmation of the results found in literature says something about the accuracy of VigiLyze. Because it is a spontaneous report database, a causal relationship between the reported ADR and the drug is not guaranteed for every case. When the SmPC says that moxifloxacin may cause photosensitivity reactions, but clinical research does not confirm this, in this case VigiLyze may be a source of real-world data to support the clinical data available. When eventually the ADR is not linked to moxifloxacin, it can be removed from the SmPC.

While categorizing the quinolones that resulted from the search, two were found whose market authorization was retracted due to QT prolongation. When comparing these two structures, a ring structure consisting of three C-atoms attached to the N-atom of the basic quinolone structure was noted as similarity between these molecules. This could be a potential SA for the occurrence of QT prolongation. To further explore this observation, this structural element was chosen as basis for sub-study 5.

Sub-study 4: Possible SA-ADR relationship: N4-arylamine moiety and SJS, TEN and methemoglobinemia

Introduction

The sulphonamides are a class of antibiotics that are known for displaying a wide variety of ADRs. They are composed of three structural elements: a sulphonamide, a N4-arylamine and a N1-heterocycle. These displayed in yellow, blue and red respectively in figure 15.

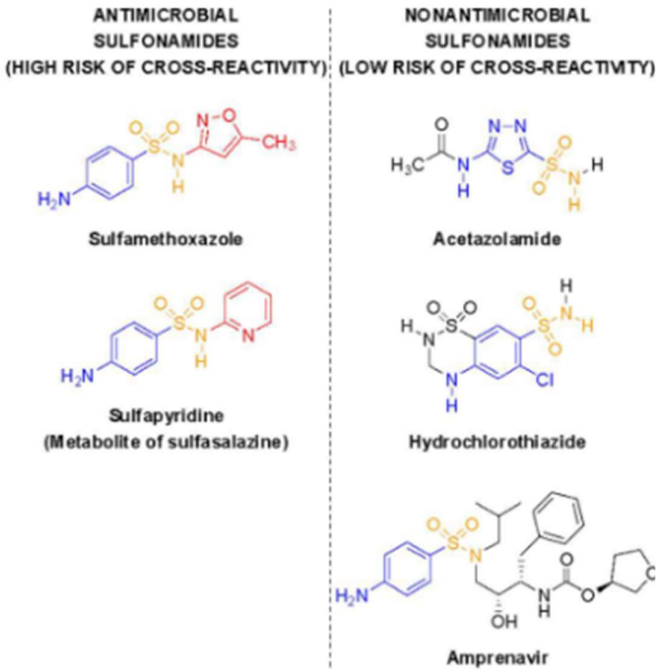


Figure 15: structural elements of sulfonamide antimicrobials and nonantimicrobials. Sulfonamide in yellow, N1-heterocycle in red and N4-arylamine in blue.

None of sulphonamides without an antimicrobial effect have an N-containing ring attached to the N1 nitrogen atom of the sulphonamide group. This structural element is required for antimicrobial activity (59). A structural element that multiple drugs do possess is the N4-arylalamine that is responsible for metabolization to a hydroxylamine. This hydroxylamine metabolite may be the RM that is involved in the occurrence of certain ADRs. In literature, methemoglobinemia and Steven-Johnson syndrome (SJS) and Toxic epidermal necrolysis (TEN) are hypothesized to be related to formation of this RM (60).

Type 1 hypersensitivity reactions arise immediately after re-administration of a drug and they involve immunoglobulin (Ig) E. An individual needs to be sensitized before a type 1 reaction occurs. Symptoms of a type 1 reaction can be mild like nasal allergic rhinitis or atopic diseases but a systemic reaction like anaphylaxis is the most severe type 1 reaction. The result of a true type 1 reaction to a sulphonamide antibiotic is the presence of serum IgE antibodies against the parent drug. These antibodies bind exclusively to the N1 heterocycle. No reactivity with the sulphonamide or N4 arylamine group was detected (60). This means that a relationship between structure and ADR may

exist, but only for the sulphonamide antibiotics and their concurrent type 1 hypersensitivity reaction.

10-20% of the sulphonamide antibiotic sulfamethoxazole gets metabolized by CYP2C9 to sulfamethoxazole hydroxylamine, as shown in figure 16. This metabolize auto-oxidates to a nitrosulphonamide metabolite that can be further acetylated by NAT2, after which it gets excreted in urine, or the nitrosulphonamide reacts with glutathione and gets reduced back to the original hydroxylamine metabolite. This cycling reaction occurs NADPH dependent in multiple cell types, including erythrocytes. The superoxide radicals that are produced during this repeated redox cycling process may be the cause of sulphonamide-antibiotic associated methemoglobinemia. This ADR is not immune related but a consequence of direct cytotoxicity of this RM.

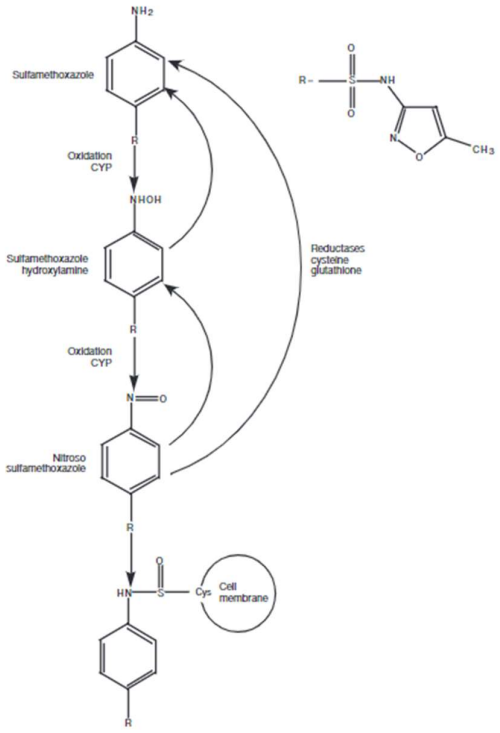


Figure 16: The metabolism of sulfamethoxazole to its reactive metabolite.

This metabolite may thus be directly cytotoxic, but it is described as a potent immunogen too. It may bind covalently to T-cells or native proteins and induce type 2, 3 or 4 reactions. Examples are maculopapular rash, SJS and TEN. SJS and TEN are hypothesized to be type 4 reactions. Type 4 reactions are known as delayed hypersensitivity and involve T-cell mediated reactions, a cellular response involving cytokine release. Activated immune cells then cause tissue damage (61). Typical to type 4 reactions is that they generally take place about 24-72 hours after exposure to the allergen (62).

Additionally, CYP2C9, the enzyme that can catalyse the reaction of the hydroxylamine metabolite formation (figure 16) is not only expressed in the liver and gastro-intestinal tract, but also in the skin. This would suggest that it would potentially be a local effect (60).

Using the information above about this suspected SA, the GSRS database can be used to find the drugs that possess this SA. The results of this search can be used to determine a possible relationship between the SA and one or more ADRs. Therefore, the goal of this sub-study is to investigate what the relationship is between the N4-arylamine moiety and the ADRs SJS, TEN and methemoglobinemia.

Method

First, the GSRS database was queried to find structures that possess the N4-arylamine. The following SMILES code describes the N4-arylamine moiety: C(=CC=C1N([H])[H])C=C1. The NH2 group was set as a requirement, so no other groups or atoms than hydrogens could be attached to the N atom. On the ring, no requirements were set. The presence of one or more extra groups on the six-ringed structure was allowed. Substituents may have a mediating effect on the proposed reaction mechanism. However, due to the explorative nature of this research, it was decided to not exclude any substituents.

Inclusion and exclusion criteria

Only drugs that were found in VigiLyze were included. For every drug found in VigiLyze, the number of cases was checked. When this was less than 1000, the drug was excluded. When no Dutch, French, German or English (UK) SmPC was available, the drug was excluded (figure 18). The drugs that did have > 1000 records in VigiLyze but had no SmPC were discussed separately, as toxicity may be a reason for retraction from the market. For the remaining records, duplicates were removed.

For the drugs which resulted from the SA search, which are indicated as drugs with N4A (N4-arylamine), a therapeutic alternative was found without the N4A SA. These therapeutic alternatives served as controls. To do this, the KNMP kennisbank and Farmacotherapeutisch Kompas were used (63,64). These are two Dutch drug databases that contain drugs on the Dutch market and for these drugs, information about indications and guidelines on drug choices. When needed, the guideline database from the Dutch medical specialists federation was used (65).

A potential control drug that is prescribed in combination with an N4A containing drug was excluded. To be included, the control drugs must have a Dutch, French, German of English (UK) SmPC. The indication for which the N4A drugs and the controls are used had to overlap. Controls with less than 1000 cases in VigiLyze were excluded if there was one available with more than 1000 cases. If there was no control drug available with > 1000 cases, the therapeutic alternative with the most records in VigiLyze was chosen. When multiple therapeutic alternatives with > 1000 cases in VigiLyze were found, the one with the most overlapping indications with the N4A containing drug was chosen.

Analysis

To see if methemoglobinemia, SJS and TEN take place more often in drugs with the SA, the IC025 values from VigiLyze for the corresponding PTs, and the SmPCs of both groups were compared. The IC025 values were depicted in a forest plot. The SmPCs were searched for presence for the ADR PTs, either in section 4.4 (Special warnings and precautions for use) or 4.8 (Undesirable effects). The number and percentages of drugs containing the PTs in the SmPC were presented in a table.

Search results and included drugs

The GSRS search yielded 2,074 results. 230 results were left after filtering on the criterium ‘drug’. These 230 records were manually checked. After applying the in -and exclusion criteria, 19 drugs remained that were eligible for inclusion. The one drug with the ‘Other’ reason for exclusion was chlorhexidine phosphanilate. The SA was not present in the drug but in the phosphanilate moiety. This form of chlorhexidine is not used therapeutically and is thus excluded. The four drugs with no SmPC are discussed below.

Afloqualone

Afloqualone is a centrally acting muscle relaxant. It is not on the market in the EU or the US, only in Japan and Korea. Photosensitization is an important ADR of afloqualone (66). Afloqualone has 2,543 ADR reports. The IC025 for SJS is -5.3, TEN and methaemoglobinaemia were not reported.

Mosapride

Mosapride is a serotonin 5-hydroxytryptamine receptor antagonist. It promotes motility across the GI tract. It is on the market in Japan and in some other Asian countries but not in Europe or the US (67). Most common ADRs that were reported in VigiLyze are somnolence, GI symptoms like diarrhea, rash and dizziness. Mosapride has 12,949 cases in VigiLyze. The IC025 value for SJS is -1.8 and for TEN -1.1. Methaemoglobinaemia was not reported.

Nomifensine

Nomifensine is a norepinephrine-dopamine reuptake inhibitor (NDRI). It came on the market in the 1960s and was retracted in 1986 by the manufacturers. The reason for the withdrawal were numerous case reports of hemolytic anemia. Multiple cases of hepatotoxicity were reported too. The suspected mechanism was immunologic (68). Nomifensine has 1,753 cases in VigiLyze. The IC025 for SJS is -4.9, TEN and methaemoglobinaemia were not reported.

Procainamide

Procainamide is still used in the Netherlands. However, no standardized products are currently on the market. It is a class 1A anti-arrhythmic (69). N-Acetylprocainamide (acecainide) is the main metabolite and is active. A second metabolite is desethyl-N-acetyl-procaïnamide. Procainamide (figure 17) is metabolized by NAT2 (70). In long term users, a clinical presentation like one of lupus erythematodes is seen, which is a typical type 3 hypersensitivity reaction, especially in slow acetylators. Blood dyscrasias like thrombocytopenia were also reported relatively often (69). One study described methemoglobinemia (71). Procainamide has 3,090 cases in VigiLyze. The IC025 for SJS is -1.9 and for TEN -2.5. Methaemoglobinaemia was not reported.

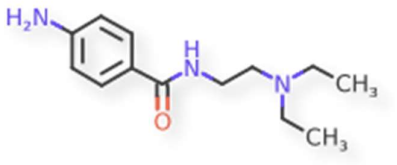


Figure 17: molecular structure of procainamide

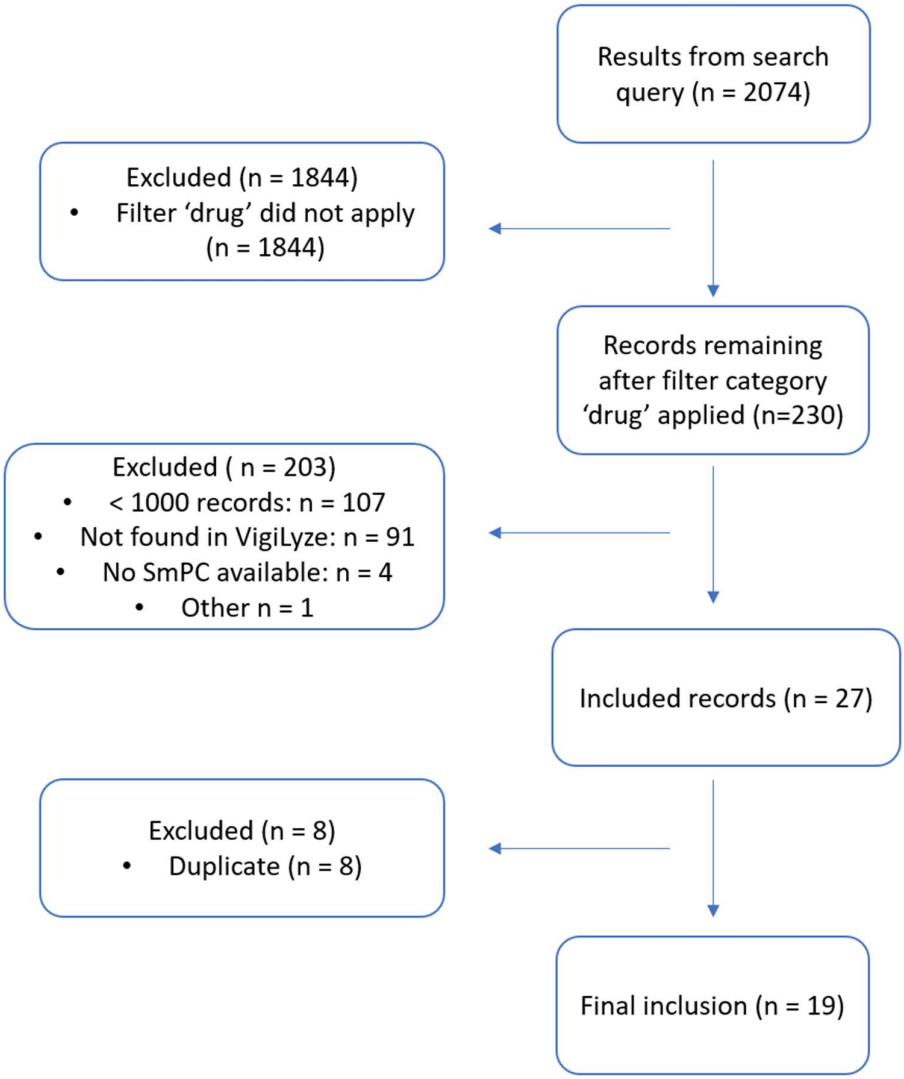


Figure 18: flow chart of inclusion and exclusion of the drugs resulting from the search.

Methemoglobinemia results

When *Methaemoglobinaemia* (PT) is entered in VigiLyze, just 2557 cases in total were found. Only 15 drugs had more than 25 observed cases. The only drugs that had more than 100 cases were dapsone, benzocaine, lidocaine and prilocaine. This low number of reports must be kept in mind while interpreting the results.

Comparison of ICs in VigiLyze

In table 5, the IC025 values and number of cases of N4A and control drugs are depicted. This table was made instead of a forest plot, as it is easier to include the number of reports. From the control group, lidocaine is the only drug with a significant IC025 value. For the N4A group, sulfadiazine, metoclopramide, dapsone, sulfamethoxazole and mesalazine have a significant IC025 value.

*Table 5: IC values and number of observed cases for methaemoglobinemia in VigiLyze for the N4A and control group. IC = information component, N4A = N4-arylamine. *Sulfamethoxazole is not used separately from trimethoprim and therefore has few reports (1.824), Cotrimoxazole has 13.744 records. Therefore, cotrimoxazole is displayed in the table. Trimethoprim has 11365 records. The IC0,25 of trimethoprim for Methaemoglobinaemia is -0,1 with 4 cases.*

Drug (N4A SA present)	IC025	Number of observed cases	Drug (control)	IC025	Number of observed cases
Ambroxol	-4.5	1	Acetylcysetine	-0,7	4
Sulfadiazine	1,6	6	Spiramycine	-	0
Nepafenac	-	0	Brinzolamide	-	0
Amisulpride	-	0	Haloperidol	-3,1	2
Lenalidomide	-4,8	3	Thalidomide	-	0
Metoclopramide	2,6	50	Droperidol	-	0
Benzocaine	9.2	383	Lidocaine	5.8	207
Bromfenac	-	0	Ketorolac	-4,9	1
Dapsone	9.0	548	Clofazimine	-3,0	1
Cisapride	-1.6	2	Domperidone	-2,0	2
Daptomycin	-	0	Benzylpenicillin	-	0
Dactinomycin	-	0	Etoposide	-5,6	1
Pomalidomide	-	0	Bortezomib	-	0
Sulfamethoxazole*	2.6	87	Nitofurantoine	-1,0	4
Mesalazine	1.2	10	Entocort	-	0
Fosamprenavir	-2.5	1	Ritonavir	-2.3	2
Bromhexine	0	-	Dornase alfa	-	0
Darunavir	-3.4	1	Atazanavir	-3.6	1
Prucalopride	0	-	Linaclotide	-	0

Comparison of SmPCs

For lidocaine, the occurrence of methemoglobinemia varies across different SmPCs. Lidocaine has 96 different Dutch SmPCs due to its over-the-counter availability, fixed-dose combinations, and many forms of administration in both generic and brand-name formulations. For the different formulations for injection or infusion, methemoglobinemia is mentioned in some of the SmPCs, but not all. The section in which the term in mentioned varies too. In some SmPCs it is present in section 4.4 (Special warnings and precautions for use), in others it is under 4.8 (undesirable effects) or even 4.9 (overdose). In SmPCs of topical, rectal, and ocular formulations, methemoglobinemia does not seem to be mentioned. As it is mentioned in at least one SmPC, it has been counted.

In the N4A group, methemoglobinemia was mentioned in the SmPC of benzocaine, dapsone, metoclopramide and cotrimoxazole. Sulfadiazine and mesalazine thus had a significant IC025 value but methemoglobinemia is not mentioned in their SmPC.

For methaemoglobinaemia, the ROR was not depicted. This because the number of cases for many of the drugs were very low. The ROR is not corrected for very high or low numbers of reports and thus is not a good outcome measure for this ADR.

Table 6: number and percentage of positive IC values for methemoglobinemia and the presence of methemoglobinemia in the SmPC of the control and N4A containing drugs. IC = information component, N4A = N4-arylamine.

Group (control/N4A)	Outcome measure (IC, or SmPC)	Significant 95% CI lower bound (%)
N4A containing	IC	6/19 (32%)
Control	IC	1/19 (5%)
N4A containing	SmPC	4/19 (21%)
Control	SmPC	1/19 (5%)

Steven-Johnson syndrome and toxic epidermal necrolysis results

SJS and TEN are severe cutaneous reactions. They involve necrosis and detachment of the epidermis. SJS and TEN are distinguished by what percentage of body surface is affected by blisters and erosions. When less than 10% of the body surface suffers skin detachment, it is classified as SJS. When this is more than 30%, the diagnosis TEN is made. When 10-30% of the body is affected, the term SJS/TEN overlap syndrome is diagnosed. About two to seven cases per million people per year of SJS, TEN and SJS/TEN overlap occur. SJS is more common and occurs about three times more often than TEN.

Most cases of SJS/TEN can be attributed to a drug. Approximately 25-33% cannot be clearly contributed to one. Infections, with for example mycoplasma pneumoniae, are the second common trigger. Other causes are only hypothesized, not confirmed (72). Some HLA genes are associated with severe cutaneous reactions, of which most are dominant in Asian populations. However, no genome-wide association studies have identified highly penetrant genetic risk factors for development of SJS/TEN across drugs (72).

Looking further at Steven-Johnson syndrome (PT) in VigiLyze, it is stated that 47,572 cases match this filter, for Toxic epidermal necrolysis (PT) this number is 18,100. For SJS-TEN overlap (PT), only 53 cases are identified for all drugs in the database. Based on this information, SJS and TEN were chosen as ADRs and SJS-TEN overlap was not included due to the low number of records in the database.

Comparison of ICs in VigiLyze

In the first figure (figure 19), the IC values with 95% credibility intervals (CI) of the N4A drugs and their controls are depicted for SJS. In the second figure (figure 20), the IC values with 95% CI for the N4A drugs and their controls are depicted for TEN. The CI for the control compounds are generally smaller. This is because these are therapeutic alternatives that are used often in daily practice. This is not the case for all records that possess the structural element.

For SJS, 7 of 19 N4A drugs had a significant IC025, for the control compounds this number was 5 out of 19. For TEN, 9 of 19 N4A drugs had a significant IC025, for the control compounds this was 6 of 19.

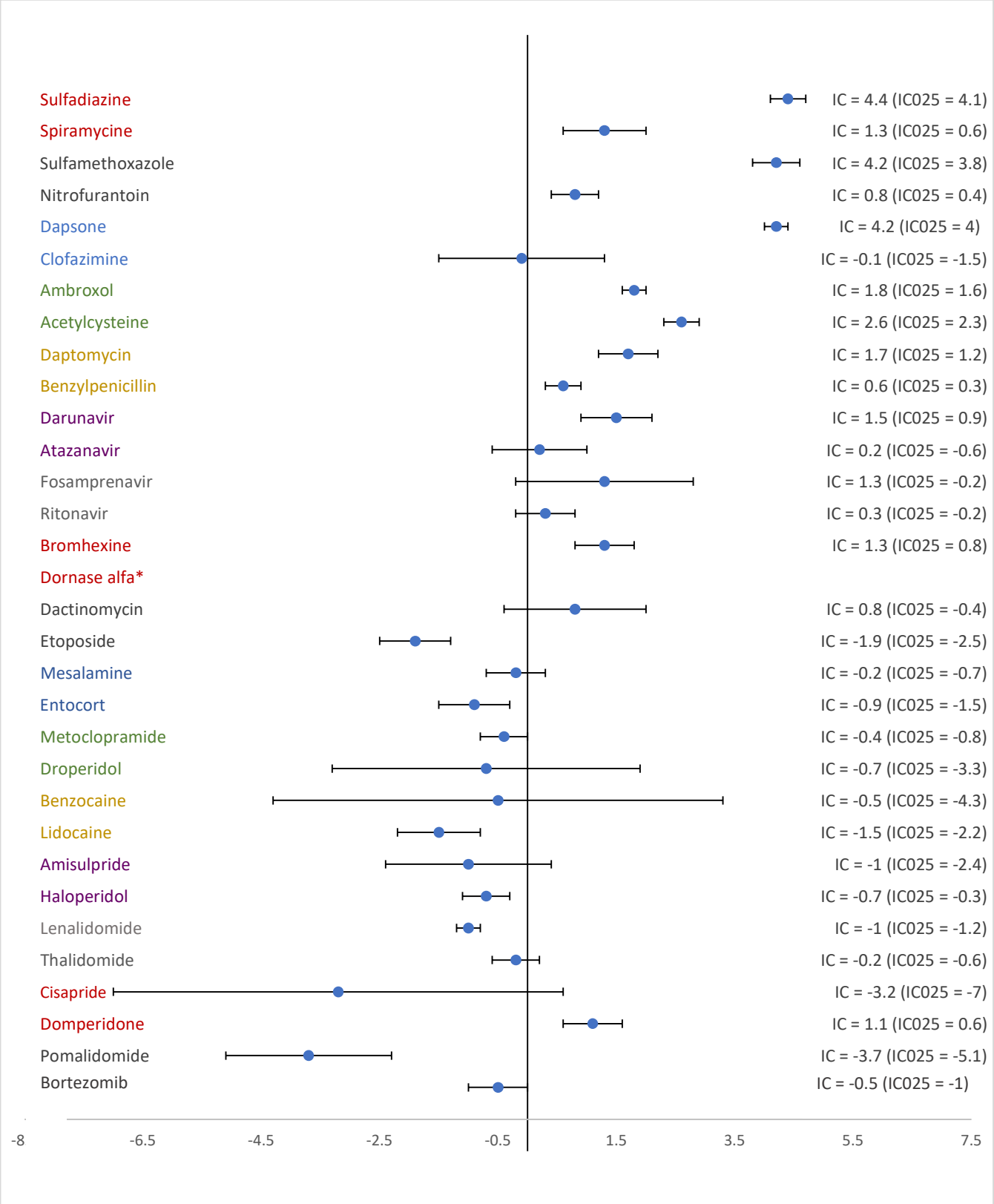


*Figure 19: *: IC values for Steven-Johnson syndrome and their 95% confidence intervals. Alternately one N4A and then one control compound. Two consecutive compounds with one colour represents one control-N4A pair. Dornase alfa had 0 reports of SJS and thus no IC value. IC = information component.*

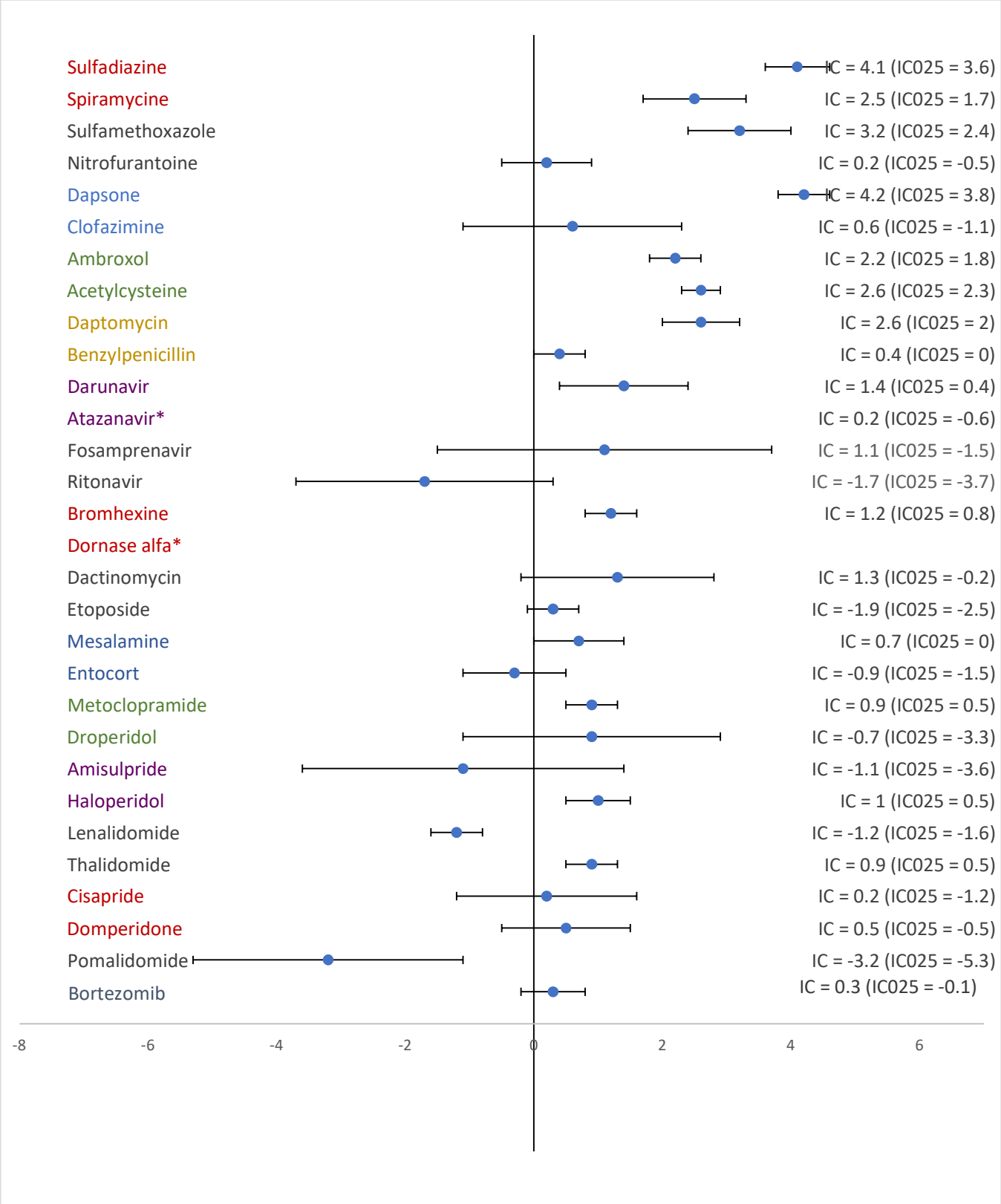


*Figure 20: *: IC values for toxic epidermal necrolysis and their 95% confidence intervals. Alternately one N4A and then one control compound. Two consecutive compounds with one colour represents one control-N4A pair. Atazanavir and dornase alfa had 0 reports of TEN and thus no IC value*

Comparison of SmPCs

For the N4A group, 11 of 19 (58%) had SJS, and 10 of 19 (53%) had TEN mentioned in their SmPC. For the control group these numbers were 9 of 19 (47%) and 5 of 19 (26%), respectively. These percentages show that SJS and TEN were both more often present in SmPCs of the N4A group than of the control group. The results of both outcome measures (IC and SmPC) are presented in table 7 for SJS and table 8 for TEN. The results for the ROR are depicted too. This outcome measure shows different results than the IC. This can be explained by the low quantity of cases reported for some of the N4A or control groups. The IC is adjusted for this, the ROR is not.

Table 7: percentages of positive values for the SJS IC for the N4A containing and control group, the percentages of occurence of TEN in the SmPC of the N4A and control group. (N4A = N4-arylamine, SJS Steven-Johnson syndrome, IC = information component, SmPC = summary of product characteristics

Group (control/N4A)	Outcome measure (IC, ROR or SmPC)	Significant 95% CI lower bound (%)
N4A containing	IC	7/19 (37%)
Control	IC	5/19 (26%)
N4A containing	SmPC	11/19 (58%)
Control	SmPC	9/19 (47%)
N4A containing	ROR	8/19 (42%)
Control	ROR	5/19 (26%)

Table 8: percentages of positive values for the TEN IC for the N4A and control group, the percentages of occurrence of TEN in the SmPC of the N4A and control group. (N4A = N4-arylamine TEN = toxic epidermal necrolysis, IC = information component, SmPC = summary of product characteristics

Group (control/N4A containing)	Outcome measure (IC, ROR or SmPC)	Significant 95% CI lower bound (%)
N4A containing	IC	9/19 (47%)
Control	IC	6/19 (32%)
N4A containing	SmPC	10/19 (53%)
Control	SmPC	5/19 (26%)
N4A containing	ROR	10/19 (53%)
Control	ROR	9/19 (47%)

In table 9, the drugs in the N4A group and their controls are displayed. There were no cases in which the SmPC of the control did mention SJS or TEN and the N4A drug did not. The combinations with discrepancies are highlighted.

Table 9: occurrence of SJS and TEN in the SmPC of the N4A and control group (N4A = N4-arylamine, SJS = Steven-Johnson syndrome, TEN = toxic epidermal necrolysis, SmPC = summary of product characteristics)

Drug (N4A)	SJS in SmPC	TEN in SmPC	Control drug	SJS in SmPC	TEN in SmPC
Ambroxol	Yes	Yes	Acetylcysteine	Yes	No
Amisulpride	No	No	Haloperidol	No	No
Benzocaine	No	No	Lidocaine	No	No
Bromfenac	No	No	Ketorolac	No	No
Bromhexine	Yes	Yes	Dornase alfa	No	No
Chlorhexidine	No	No	Benzalkonium	No	No
Cisapride	No	No	Domperidone	No	No

Dactinomycin	Yes	Yes	Etoposide	Yes	Yes
Dapsone	No	No	Clofazimine	No	No
Daptomycin	Yes	Yes	Benzylpenicillin	Yes	Yes
Darunavir	Yes	Yes	Atazanavir	Yes	No
Fosamprenavir	Yes	No	Ritonavir	Yes	No
Lenalidomide	Yes	Yes	Thalidomide	Yes	Yes
Mesalazine	Yes	Yes	Entocort	No	No
Metoclopramide	No	No	Droperidol	No	No
Nepafenac	No	No	Brinzolamide	No	No
Pomalidomide	Yes	Yes	Bortezomib	Yes	Yes
Prucalopride	No	No	Linaclotide	No	No
Sulfadiazine	Yes	Yes	Spiramycin	Yes	Yes
Sulfamethoxazole	Yes	Yes	Nitrofurantoin	Yes	No

Conclusion and discussion

By comparing the SmPCs and IC values for methemoglobinemia between the N4A group and control group, this study showed that there may be a role of the N4-arylaniline moiety in the occurrence of this ADR. However, methemoglobinemia is very rare and more cases are needed to confirm this. For SJS and TEN, there was a difference in the number of significant IC025 values between groups. More N4A containing drugs had a significant IC025 value than the controls. However, this difference is too small to draw a firm conclusion that the N4-arylamine moiety plays a role in the development of these ADRs based on this outcome measure. Comparing the SmPCs for TEN, the difference (26% vs. 53%) is notable. This large difference is not seen for SJS. If this is due to the SA or if there are other influencing factors may be investigated in future research, seeing the difference between SJS and TEN.

For SJS/TEN to occur, hypothetically, the formation of the suspected RM must take place. The metabolism pathway of some N4A containing drugs may be the reason why a large difference in the occurrence of SJS and TEN is not seen. If for example a drug is excreted in unchanged form, the hypothesized RM will not be generated, and the ADR will thus not be displayed. There also may be other characteristics of the molecules other than the SA that influence RM formation to such an extent that not, or not enough RM's are generated to cause SJS or TEN. Examples of these characteristics may be steric effects, charge of the molecule or different (large) substituents.

An ADR that is suspected to be caused by this RM and may have enough reports to detect a difference, if there is one, may be able to provide information about a possible ADR-SA relationship. A specific ADR with a low background incidence is easier to detect than one that occurs in many drugs or one that can present spontaneously or as a result of an underlying condition. Methemoglobinemia is an extremely rare ADR, with only 2,557 cases in VigiLyze. With IC values based on a small number of reports, there is a low certainty of the actual causal relationship between the drug and the ADR. The IC values of mesalazine and sulfadiazine were positive, but based on only 10 and 6 reports, respectively. SJS and TEN had more cases, in total 65,672. In only 1 of 8 N4A drugs with a significant IC, this value was based on 5 observed cases. The others ranged from 32 to 176 cases.

The SmPCs showed a difference in the occurrence of TEN between the N4A and control group. This may be due to an actual difference, it can for example be the case that the N4-arylamine moiety

mediates the development of SJS to TEN. This may explain the difference in the SmPC for TEN but not for SJS. However, this same result is not seen in the IC025 values for TEN.

The SmPC should be the most reliable source of information about unwanted effects, as a causal relationship between an ADR and the drug is evaluated before entry into the SmPC. Despite that, a study about quality and consistency of SmPCs showed that between manufacturers contra-indications are identical in only 20% of German SmPCs. This study also showed that 40% of German SmPCs have at least one pair of ADRs that may be conflicting (73). This may be due to ADRs being added as precaution without a confirmed causal relationship, which is more often seen in older drugs.

More research is needed to confirm these findings about methemoglobinemia. The findings about SJS and TEN need to be looked at further for the possible involvement of substituents, metabolism, or other mediating effects. The results from the GSRS search may be investigated further. It can be the case that some drugs did not have 1000 cases in VigiLyze due to being retracted from the market or due to not making it through clinical trials. It can be investigated what the reasons are that these drugs are retracted or, if available, why research has been terminated. This may confirm the possible relationship between the SA and methemoglobinemia and TEN, and possibly show a relationship with SJS too.

Sub-study 5: fluoroquinolones and QT prolongation

Introduction

A wide variety of drugs may cause cardiac ADRs, depending on their mechanism of action and off-target effects. Long QT syndrome is a well-studied cardiac ADR, caused by a disorder of myocardial repolarization. It can be recognized by a prolonged QT interval on the electrocardiogram. When QT prolongation occurs, there is an increased risk of torsade de pointes (TdP), a life-threatening cardiac arrythmia. In a retrospective study, all hospital admissions over a six-month time period were checked for severe QT prolongation, defined as a QTc > 500 milliseconds. Of all 41,649 patients, 293 (0.7%) had a QTc > 500 ms and 18 (6%) of those patients experienced syncope or a life-threatening arrhythmia. This shows that QT prolongation occurs more frequently than TdP. Use of medication is a common cause of a prolonged QT interval and TdP. Fluoroquinolones, especially moxifloxacin, are often listed as one of the drug classes that cause these ADRs (74).

All fluoroquinolones have QT prolongation mentioned in their SmPC. However, there are differences in frequency and some studies doubt any involvement of some fluoroquinolones in this ADR at all (50). This raises the question as to why there is such a difference in display of QT prolongation and TdP within this drug class. To investigate factors that contribute to the difference in QT prolongation seen in fluoroquinolones, a literature search was performed. Pubmed and Google scholar were searched for factors influencing QT prolongation in fluoroquinolones.

One structural alert was found that may be related to QT prolongation. This is an amine or methyl moiety at the C-5 position (34). However, only one drug with this moiety of each exists. Sparfloxacin has an amine, and grepafloxacin a methyl moiety.

No structural modifications have been associated with the cardiovascular effects of CYPP450 mediated metabolism. The fluoroquinolones do not inhibit the CYP enzymes that are associated with most drug interactions: 2A4, 2C9 and 2C19. Only ciprofloxacin inhibits 1A2, this is not associated with QT prolongation. The peak plasma concentrations that are reached can be a contribution to a difference in QT prolongating effects. However, both oral and IV formulations of the quinolones reach a very similar peak concentration. Thus, this is an unlikely contributor to a possible difference (75).

In sub-study 3, sparfloxacin and grepafloxacin were observed to both have been retracted from the market due to QT prolongation. This raised the question if the cyclopropane R1 group they both contain has a relationship with this ADR. What is known about the effects of the R1 group is that it seems to be involved in genetic toxicity via inhibition of mammalian cellular topoisomerase II. Substitutions at the 7 and 8 positions have an additive effect. Genotoxic effects occur only at very high concentrations, a carcinogenic potential has not been discovered (34).

So, no links between an R1 cyclopropane substituent and QT prolongation have been made before. The goal of this study is to explore the possible relationship between this structural element and QT prolongation, by looking at spontaneous reports of this ADR.

Method

GSRS was used to first find the quinolones with the SA and a second search for quinolones without the SA was performed. Both groups were entered in VigiLyze. As all the fluoroquinolones contain QT prolongation in their SmPC, these were not compared. The IC and IC025 values were extracted from VigiLyze and displayed in a table. Additionally, the number of cases in total for all included

fluoroquinolones was extracted. There is a large variation in number of cases due to some only being in use in specific countries.

The SMILES code that was used for the SA is C(=CC(=C1C(=O)C2C(O)=O)N(C=2)C(C3)C3)C(=C1)F. The SMILES code that was used as a reference was C(=CC(=C1C(=O)C2C(O)=O)NC=2)C(=C1)F. The drugs resulting from the SA search were included in the second search, but there was no option to exclude one specific structure from a search, so these were sorted out manually.

The PT *electrocardiogram QT prolonged* was used to search in VigiLyze, as this describes the ADR QT prolongation best of the Meddra PTs. The PT 'torsade de pointes' was included too, as this may in some patients be the clinical result of QT prolongation. Only the fluoroquinolones which were entered in VigiLyze and had more than 1000 records were included.

Electrocardiogram QT prolonged has 28,145 cases and torsade de pointes has 7,021 cases.

Results

From the search, 26 non-SA drugs and 19 SA drugs resulted. From the 26 non-SA drugs, 14 were found in VigiLyze and 7 of these had > 1000 records. From the 19 SA drugs, 9 were found in VigiLyze and 3 of these had > 1000 records.

One table was made for QT prolongation (table 10) and one for torsade de pointes (table 11). The Nadifloxacin and temafloxacin did not have an IC value for both PTs because there were no cases for both terms. Pefloxacin did have an IC value for QT prolongation but not for torsade de pointes.

Table 10: IC values for 'electrocardiogram QT prolonged' and total number of cases for drugs without the SA (left) and with (right). IC = information component, SA = structural alert

Drug (no SA)	No. Records	IC025	IC	Drug (SA)	No. Records	IC025	IC
Nadifloxacin	1,163			Ciprofloxacin	127,772	1.6	1.7
Norfloxacin	15,394	-1	-0.1	Gatifloxacin	5,253	4	4.3
Levofloxacin	178,057	2.2	2.3	Moxifloxacin	50,291	4	4.1
Lomefloxacin	2,972	-4.9	-1.1				
Ofloxacin	26,834	-1.1	-0.4				
Pefloxacin	2,302	-4.6	-0.8				
Temafloxacin	1,604						

The only IC value that is significant for the non-SA group is levofloxacin. Of the SA group, all IC values are significant. This is the case for both QT prolongation and torsade de pointes.

Table 11: IC values for 'torsade de pointes' and total number of cases for drugs without the SA (left) and with (right). IC = information component, SA = structural alert

Drug (no SA)	No. Records	IC025	IC	Drug (SA)	No. Records	IC025	IC
Nadifloxacin	1,163			Ciprofloxacin	127,772	2.5	2.7
Norfloxacin	15,394	-3.2	-0.6	Gatifloxacin	5,253	4.6	5
Levofloxacin	178,057	2.6	2.7	Moxifloxacin	50,291	4.1	4.3
Lomefloxacin	2,972	-1.5	1.1				
Ofloxacin	26,834	-1	0.2				
Pefloxacin	2,302						
Temafloxacin	1,604						

Conclusion and discussion

A difference between the IC values in both groups is observed, with levofloxacin as the exception. There may be a SA-ADR relationship, but this cannot be made sure, as both groups are quite small and levofloxacin does not possess the SA but does display the ADR. To further investigate this possible relationship, all drugs that resulted from the GSRS searches can be looked into. For some of these, cardiotoxicity studies may have been performed. It may also be interesting to search for a mechanism behind the QT prolongating effects of levofloxacin to see why this was the only non-SA drug for which QT prolongation and TdP were reported disproportionally.

It is not always possible to find out why the investigation of a drug candidate has stopped. However, it may be interesting to see if investigational drugs in the SA group displayed cardiotoxicity that halted research and compare that to the cardiotoxicity of the investigational fluoroquinolones in the non-SA group. This may generate some useful additional information because QT prolongation and TdP are serious ADRs that can be reasons to stop investigation.

Discussion

In this report, it was investigated if the GSRS database is a suitable tool for discovering ADR-SA relationships. This was done by performing five sub-studies, each testing a pre-selected SA. The database was used for structure searches and in one sub-study the classification systems that are attached to drugs were utilized to find drugs of the same class. For the resulting drugs, it was extracted from VigiLyze if the ADR was reported more often than expected. In part of the sub-studies, the SmPC was checked. This way, SA-ADR relationships were investigated. And the database was tested.

Although the GSRS database was found suitable for performing this research it was still hampered by several issues that can be addressed in the following way:

- Filter options are present in the database, but for some filters it is unclear for the user if all results are filtered correctly. An example of this phenomenon is seen with the ATC code filter. If all possible filter options are selected, the total number of records that are displayed is less than the number of records that resulted from the search. This is also seen in the 'moiety type' filter.
- It would have been very useful to sort records by their developmental stage. For instance, if a drug is currently in development, on the market or retracted from the market. This would have made it easier to leave the developmental drugs out in a study about phase IV safety.
- For the structure searches, an AND/OR option per atom would have been very useful. This can be used when for example a substituent of a molecule may be any halogen atom.
- When a user that accessed the GSRS database from the website performs a search, the results cannot be exported. This is only possible when the user has an account, that needs to be requested to the GSRS staff. This makes sorting results and checking for duplicates a time-consuming process, as all results need to be exported manually.
- Additional to the exportation, performing VigiLyze searches for all results is time-consuming too. Especially when there is a large number of results, the step to check if the results are entered in VigiLyze takes up a large share of time. This would be easier if the GSRS database was linked to VigiLyze or another spontaneous reports database. This is not easy to facilitate due to limited access of the general public to these types of databases, but it would be the most impactful step towards facilitating this method of doing research in daily practice.

Overall conclusion

It was shown that the GSRS database performs well in structure searches. The structure search option is basic but has some useful features like adding explicit hydrogen atoms. This function draws all hydrogen atoms and hereby sets the requirement that no other atoms can be attached, as shown in figure 21. All resulting drugs from the five sub-studies were found using GSRS. Especially sub-study 4, in which one structural alert was queried that was present in a wide variety of drug classes, could not have been performed this well by searching SA-containing drugs manually. The database showed promise for RM searches too, as in sub-study 2 it

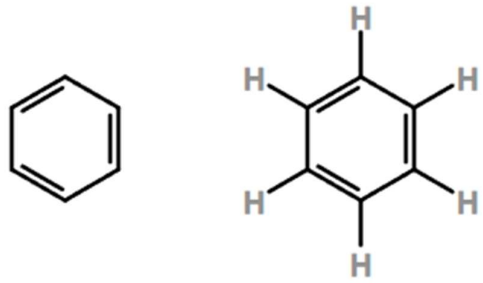


Figure 21: visual representation of the explicit hydrogen function. In the left structure, any groups may be attached to the ring. In the right structure, this may only be the drawn hydrogen atoms.

was shown that for the COMT inhibitors, a hypothesized RM was entered as metabolite. The GSRS database could be used to find the drug from which this metabolite originated.

Future developments and suggestions for future research

When it is known that a certain metabolite or part of a metabolite can cause toxicity, it may be very useful to find if other drugs generate this metabolite too or if parent drugs exist that contain this structure. This was done in sub-study 2, but it will be interesting to see if a less specific SA of a RM can be found, as the RM discussed in sub-study 2 was formed exclusively by COMT-inhibitors. Thus, the metabolites that are included in GSRS have potential for use in future research. For some drugs, impurities were added too. This was not touched upon in this study but may be an interesting topic for further SA-ADR or other PV research.

The GSRS database includes drugs that are in (pre-)clinical development, on the market and retracted from the market. This brings opportunities for varying types of research. Unfortunately, the reason that drug investigation is halted is not always known. However, the fact that GSRS contains all these drugs is a unique feature that can be used to study reasons for halting research or market retraction of a wide variety of drugs.

More structured databases containing information regarding drug products and molecular structure search options are in the making. The European Substance Registration System (EU-SRS) will be a database with scientific descriptions of substances which are used in medicinal products that are available in the EU. For identification of these substances, ISO IDMP standards are complied with. This EU-SRS database uses the Global Substance Registration System (GSRS) software that was developed by the FDA and The National Center for Advancing Translational Sciences (NCATS at the National Institutes of Health (NIH) (76). This database could be used for the same type of pharmacovigilance research performed here. When the selectivity of created records will be higher, the relevance of search results will increase.

References

1. European Medicines Agency | Pharmacovigilance: Overview [Internet]. [cited 2022 Jun 20]. Available from: https://www.ema.europa.eu/en/human-regulatory/overview/pharmacovigilance-overview

2. College ter Beoordeling van Geneesmiddelen | Bijwerking melden [Internet]. [cited 2022 Feb 18]. Available from: https://www.cbg-meb.nl/onderwerpen/medicijninformatie-bijwerkingen-melden

3. European Medicines Agency | Direct healthcare professional communications [Internet]. [cited 2022 Jun 20]. Available from: https://www.ema.europa.eu/en/human-regulatory/post-authorisation/pharmacovigilance/direct-healthcare-professional-communications

4. Lasser KE, Allen PD, Woolhandler SJ, Himmelstein DU, Wolfe SM, Bor DH. Timing of New Black Box Warnings and Withdrawals for Prescription Medications. JAMA [Internet]. 2002 May 1 [cited 2022 Feb 18];287(17):2215–20. Available from: https://jamanetwork-com.proxy.library.uu.nl/journals/jama/fullarticle/194879

5. Kalgutkar AS, Didiuk MT. Structural alerts, reactive metabolites, and protein covalent binding: How reliable are these attributes as predictors of drug toxicity? Chemistry and Biodiversity. 2009 Nov;6(11):2115–37.

6. Ge-Bu | Geneesmiddelen en bijwerkingen [Internet]. [cited 2022 Feb 18]. Available from: https://www.ge-bu.nl/artikel/geneesmiddelen-en-bijwerkingen

7. Uetrecht J, Naisbitt DJ. Idiosyncratic Adverse Drug Reactions: Current Concepts. Pharmacological Reviews [Internet]. 2013 [cited 2022 Feb 25];65(2):779. Available from: /pmc/articles/PMC3639727/

8. Stepan AF, Walker DP, Bauman J, Price DA, Baillie TA, Kalgutkar AS, et al. Structural alert/reactive metabolite concept as applied in medicinal chemistry to mitigate the risk of idiosyncratic drug toxicity: a perspective based on the critical examination of trends in the top 200 drugs marketed in the United States. Chem Res Toxicol [Internet]. 2011 Sep 19 [cited 2022 Feb 15];24(9):1345–410. Available from: https://pubmed.ncbi.nlm.nih.gov/21702456/

9. Bihan K, Lebrun-Vignes B, Funck-Brentano C, Salem JE. Uses of pharmacovigilance databases: An overview. Therapie. 2020;

10. Uppsala Monitoring Centre | UMC [Internet]. [cited 2022 Mar 16]. Available from: https://who-umc.org/vigibase/

11. Gahr M, Eller J, Connemann BJ, Schönfeldt-Lecuona C. Underreporting of adverse drug reactions: Results from a survey among physicians. European Psychiatry. 2017 Apr 1;41(S1):S369.

12. Pariente A, Daveluy A, Laribière-Bénard A, Miremont-Salame G, Begaud B, Moore N. Effect of date of drug marketing on disproportionality measures in pharmacovigilance: The example of suicide with SSRIs using data from the UK MHRA. Drug Safety [Internet]. 2009 Nov 20 [cited 2022 Mar 16];32(5):441–7. Available from: https://link-springer-com.proxy.library.uu.nl/article/10.2165/00002018-200932050-00007

13. Arnaud M, Salvo F, Ahmed I, Robinson P, Moore N, Bégaud B, et al. A Method for the Minimization of Competition Bias in Signal Detection from Spontaneous Reporting Databases.

Drug Safety 2015 39:3 [Internet]. 2015 Dec 29 [cited 2022 Mar 16];39(3):251–60. Available from: https://link.springer.com/article/10.1007/s40264-015-0375-8

14. Quantifying Health | Protopathic Bias: Simple Explanation + Examples [Internet]. [cited 2022 Mar 16]. Available from: https://quantifyinghealth.com/protopathic-bias/

15. MedDRA | MedDRA Hierarchy [Internet]. [cited 2022 Jun 20]. Available from: https://www.meddra.org/how-to-use/basics/hierarchy

16. GINAS | Welcome to ginas [Internet]. [cited 2022 Mar 1]. Available from: https://gsrs.ncats.nih.gov/#/gsrs/faq

17. Jessurun N, van Puijenbroek E. Relationship between structural alerts in NSAIDs and idiosyncratic hepatotoxicity: an analysis of spontaneous report data from the WHO database. Drug Saf [Internet]. 2015 May 1 [cited 2022 Feb 15];38(5):511–5. Available from: https://pubmed.ncbi.nlm.nih.gov/25787329/

18. Wu Y, Zhu J, Fu P, Tong W, Hong H, Chen M. Machine Learning for Predicting Risk of Drug-Induced Autoimmune Diseases by Structural Alerts and Daily Dose. Int J Environ Res Public Health [Internet]. 2021 Jul 1 [cited 2022 Apr 1];18(13). Available from: https://pubmed.ncbi.nlm.nih.gov/34281077/

19. Stepan AF, Walker DP, Bauman J, Price DA, Baillie TA, Kalgutkar AS, et al. Structural alert/reactive metabolite concept as applied in medicinal chemistry to mitigate the risk of idiosyncratic drug toxicity: A perspective based on the critical examination of trends in the top 200 drugs marketed in the United States. Chemical Research in Toxicology [Internet]. 2011 Sep 19 [cited 2022 Apr 1];24(9):1345–410. Available from: https://pubs.acs.org/doi/abs/10.1021/tx200168d

20. EMC | Nevanac 1mg/ml eye drops, suspension - Summary of Product Characteristics (SmPC) [Internet]. [cited 2022 Jun 22]. Available from: https://www.medicines.org.uk/emc/product/6549/smpc#gref

21. KNMP Kennisbank | PARKINSONMIDDELEN [Internet]. [cited 2022 Apr 13]. Available from: https://kennisbank-knmp-nl.proxy.library.uu.nl/article/Informatorium_Medicamentorum/G566.html#G566

22. Stocchi F, de Pandis MF. Utility of tolcapone in fluctuating Parkinson's disease. Clinical Interventions in Aging [Internet]. 2006 [cited 2022 Feb 15];1(4):317. Available from: /pmc/articles/PMC2699640/

23. KNMP Kennisbank | Tolcapon [Internet]. [cited 2022 Apr 13]. Available from: https://kennisbank-knmp-nl.proxy.library.uu.nl/article/Informatorium_Medicamentorum/S2693.html

24. College ter Beoordeling van Geneesmiddelen| Geneesmiddeleninformatiebank | Comtan, 200 mg omhulde tabletten [Internet]. [cited 2022 Apr 13]. Available from: https://www.geneesmiddeleninformatiebank.nl/ords/f?p=111:3::SEARCH:::P0_DOMAIN,P0_LANG,P3_RVG1:H,NL,70202

25. Richtlijnendatabase | Richtlijn | Medicamenteuze behandeling van motorische klachten en beginnende responsfluctuaties bij de ziekte van Parkinson [Internet]. [cited 2022 Apr 13]. Available from:

https://richtlijnendatabase.nl/richtlijn/ziekte_van_parkinson/medicamenteuze_behandeling_van_motorische_klachten_bij_de_ziekte_van_parkinson/medicamenteuze_behandeling_van_motorische_klachten_en_beginnende_responsfluctuaties_bij_de_ziekte_van_parkinson.html?query=opicapon#considerations

26. Ferreira JJ, Rascol O, Poewe W, Sampaio C, Rocha JF, Nunes T, et al. A double-blind, randomized, placebo and active-controlled study of nebicapone for the treatment of motor fluctuations in Parkinson's disease. CNS Neurosci Ther [Internet]. 2010 Dec [cited 2022 May 23];16(6):337–47. Available from: https://pubmed.ncbi.nlm.nih.gov/20653695/

27. College ter Beoordeling van Geneesmiddelen | Geneesmiddeleninformatiebank | Ongentys 25 mg harde capsules [Internet]. [cited 2022 May 24]. Available from: https://www.geneesmiddeleninformatiebank.nl/ords/f?p=111:3::SEARCH:::P0_DOMAIN,P0_LANG,P3_RVG1:H,NL,116748

28. College ter Beoordeling van Geneesmiddelen | Geneesmiddeleninformatiebank | Ongentys 50 mg harde capsules [Internet]. [cited 2022 Apr 13]. Available from: https://www.geneesmiddeleninformatiebank.nl/ords/f?p=111:3::SEARCH:::P0_DOMAIN,P0_LANG,P3_RVG1:H,NL,116748

29. KNMP Kennisbank | Entacapon [Internet]. [cited 2022 May 24]. Available from: https://kennisbank-knmp-nl.proxy.library.uu.nl/article/Informatorium_Medicamentorum/S2692.html

30. College ter Beoordeling van Geneesmiddelen | Geneesmiddeleninformatiebank | Tasmar 200 mg, filmomhulde tabletten [Internet]. [cited 2022 May 24]. Available from: https://www.geneesmiddeleninformatiebank.nl/ords/f?p=111:3::SEARCH:::P0_DOMAIN,P0_LANG,P3_RVG1:H,NL,70108

31. UpToDate | Photosensitivity disorders (photodermatoses): Clinical manifestations, diagnosis, and treatment [Internet]. [cited 2022 Jun 27]. Available from: https://www-uptodate-com.proxy.library.uu.nl/contents/photosensitivity-disorders-photodermatoses-clinical-manifestations-diagnosis-and-treatment?search=photosensitivity&source=search_result&selectedTitle=1~150&usage_type=default&display_rank=1#H2261559764

32. Moore DE. Drug-induced cutaneous photosensitivity: incidence, mechanism, prevention and management. Drug Saf [Internet]. 2002 [cited 2022 Jun 20];25(5):345–72. Available from: https://pubmed.ncbi.nlm.nih.gov/12020173/

33. Kim K, Park H, Lim KM. Phototoxicity: Its Mechanism and Animal Alternative Test Methods. Toxicological Research [Internet]. 2015 [cited 2022 May 2];31(2):97–104. Available from: http://dx.

34. Mandell L, Tillotson G. Safety of fluoroquinolones: An update. The Canadian Journal of Infectious Diseases [Internet]. 2002 [cited 2022 Jun 17];13(1):54. Available from: /pmc/articles/PMC2094848/

35. Domagala JM. Structure-activity and structure-side-effect relationships for the quinolone antibacterials. J Antimicrob Chemother [Internet]. 1994 Apr [cited 2022 Jun 14];33(4):685–706. Available from: https://pubmed.ncbi.nlm.nih.gov/8056688/

36. Rubinstein E. History of Quinolones and Their Side Effects. Chemotherapy [Internet]. 2001 [cited 2022 May 23];47(Suppl. 3):3–8. Available from: https://www.karger.com/Article/FullText/57838

37. Fluoroquinolone antimicrobials: singlet oxygen, superoxide and phototoxicity - PubMed [Internet]. [cited 2022 May 16]. Available from: https://pubmed.ncbi.nlm.nih.gov/9559584/

38. Vassileva SG, Mateev G, Parish LC. Antimicrobial Photosensitive Reactions. Archives of Internal Medicine [Internet]. 1998 Oct 12 [cited 2022 May 16];158(18):1993–2000. Available from: https://jamanetwork.com/journals/jamainternalmedicine/fullarticle/209976

39. Andriole VT. The Quinolones: Past, Present, and Future. Clinical Infectious Diseases [Internet]. 2005 Jul 15 [cited 2022 May 16];41(Supplement_2):S113–9. Available from: https://academic.oup.com/cid/article/41/Supplement_2/S113/307164

40. Farmacotherapeutisch Kompas | fluorochinolonen [Internet]. [cited 2022 Jun 13]. Available from: https://www.farmacotherapeutischkompas.nl/bladeren/groepsteksten/fluorochinolonen

41. Kowalska J, Rok J, Rzepka Z, Wrześniok D. Drug-Induced Photosensitivity—From Light and Chemistry to Biological Reactions and Clinical Symptoms. Pharmaceuticals [Internet]. 2021 Aug 1 [cited 2022 May 2];14(8):723. Available from: /pmc/articles/PMC8401619/

42. Barratt MD. Structure-activity relationships and prediction of the phototoxicity and phototoxic potential of new drugs. ATLA Alternatives to Laboratory Animals [Internet]. 2004 Apr 9 [cited 2022 May 6];32(5):511–24. Available from: https://journals-sagepub-com.proxy.library.uu.nl/doi/10.1177/026119290403200506?url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org&rfr_dat=cr_pub++0pubmed

43. Fernández E, Cárdenas AM. The mechanism of photohaemolysis by photoproducts of nalidixic acid. J Photochem Photobiol B [Internet]. 1990 [cited 2022 Jun 13];4(3):329–33. Available from: https://pubmed.ncbi.nlm.nih.gov/2107294/

44. KNMP Kennisbank | Elvitegravir [Internet]. [cited 2022 Jun 13]. Available from: https://kennisbank-knmp-nl.proxy.library.uu.nl/article/Informatorium_Medicamentorum/S5115.html

45. College ter Beoordeling van Geneesmiddelen | Geneesmiddeleninformatiebank | Stribild 150 mg/150 mg/200 mg/245 mg filmomhulde tabletten [Internet]. [cited 2022 Jun 13]. Available from: https://www.geneesmiddeleninformatiebank.nl/ords/f?p=111:3::SEARCH:::P0_DOMAIN,P0_LANG,P3_RVG1:H,NL,111342

46. College ter Beoordeling van Geneesmiddelen | Geneesmiddeleninformatiebank | Norfloxacine CF 400 mg, omhulde tabletten [Internet]. [cited 2022 Jun 13]. Available from: https://www.geneesmiddeleninformatiebank.nl/ords/f?p=111:3::SEARCH:::P0_DOMAIN,P0_LANG,P3_RVG1:H,NL,24265

47. Vosaroxin - Wikipedia [Internet]. [cited 2022 May 16]. Available from: https://en.wikipedia.org/wiki/Vosaroxin

48. Mandell L, Tillotson G. Safety of fluoroquinolones: An update. The Canadian Journal of Infectious Diseases [Internet]. 2002 [cited 2022 May 13];13(1):54. Available from: /pmc/articles/PMC2094848/

49. Man I, Murphy J, Ferguson J. Fluoroquinolone phototoxicity: a comparison of moxifloxacin and lomefloxacin in normal volunteers. J Antimicrob Chemother [Internet]. 1999 [cited 2022 May 13];43 Suppl B(SUPPL. B):77–82. Available from: https://pubmed.ncbi.nlm.nih.gov/10382879/

50. Lodise T, Corey R, Hooper D, Cammarata S. Safety of Delafloxacin: Focus on Adverse Events of Special Interest. Open Forum Infectious Diseases [Internet]. 2018 Oct 1 [cited 2022 May 13];5(10). Available from: /pmc/articles/PMC6189306/

51. NCI Thesaurus [Internet]. [cited 2022 May 13]. Available from: https://ncithesaurus.nci.nih.gov/ncitbrowser/

52. European Medicines Agency | Summary of the EMA public hearing on quinolone and fluoroquinolone antibiotics. 2018; [internet]. [cited 2022 Jun 29]. Available from: https://www.ema.europa.eu/en/documents/report/summary-ema-public-hearing-quinolone-fluoroquinolone-antibiotics_en.pdf

53. European Medicines Agency | Public Statement on Trovan / Trovan IV / Turvel / Turvel IV (Trovafloxacin/Alatrofloxacin) recommendation to suspend the marketing authorisation in the European Union [Internet]. [cited 2022 Jun 15]. Available from: https://www.ema.europa.eu/en/news/public-statement-trovan-trovan-iv-turvel-turvel-iv-trovafloxacinalatrofloxacin-recommendation

54. European Medicines Agency | Factive: Withdrawn application [Internet]. [cited 2022 Jun 15]. Available from: https://www.ema.europa.eu/en/medicines/human/withdrawn-applications/factive

55. AIFA Medicines Database | Medication [Internet]. [cited 2022 Jun 14]. Available from: https://farmaci.agenziafarmaco.gov.it/bancadatifarmaci/farmaco?farmaco=028048

56. Pannu HK, Gottlieb L, Fishman EK. Acute liver failure due to trovafloxacin: CT findings. Emergency Radiology 2001 8:2 [Internet]. 2001 [cited 2022 Jun 14];8(2):108–10. Available from: https://link.springer.com/article/10.1007/PL00011876

57. European Medicines Agency | CHMP. Factive; INN: Gemifloxacin. [cited 2022 Jun 14]; Available from: http://www.emea.europa.eu

58. Bausch & Lomb: Bausch & Lomb Receives FDA Approval of Besivance™, New Topical Ophthalmic Antibacterial for the Treatment of Bacterial Conjunctivitis (“Pink Eye”) [Internet]. [cited 2022 Jun 14]. Available from: https://web.archive.org/web/20090601075545/http://www.bausch.com/en_US/corporate/corpcomm/news/besivance_approval.aspx

59. Giles A, Foushee J, Lantz E, Gumina G. Sulfonamide Allergies. Pharmacy: Journal of Pharmacy Education and Practice [Internet]. 2019 Sep 11 [cited 2022 Feb 9];7(3):132. Available from: /pmc/articles/PMC6789825/

60. Brackett CC, Singh H, Block JH. Likelihood and mechanisms of cross-allergenicity between sulfonamide antibiotics and other drugs containing a sulfonamide functional group.

Pharmacotherapy [Internet]. 2004 Jul [cited 2022 Mar 8];24(7):856–70. Available from: https://pubmed.ncbi.nlm.nih.gov/15303450/

61. Type I Hypersensitivity Reaction - PubMed [Internet]. [cited 2022 Mar 9]. Available from: https://pubmed.ncbi.nlm.nih.gov/32809396/

62. Mak TW, Saunders ME. Allergy and Hypersensitivity. The Immune Response. 2006 Jan 1;923–62.

63. Farmacotherapeutisch Kompas [Internet]. [cited 2022 Jun 22]. Available from: https://www.farmacotherapeutischkompas.nl/

64. KNMP Kennisbank | Home [Internet]. [cited 2022 Jun 22]. Available from: https://kennisbank-knmp-nl.proxy.library.uu.nl/

65. Richtlijnendatabase [Internet]. [cited 2022 Jun 22]. Available from: https://richtlijnendatabase.nl/

66. Kitami Y, Takei H, Nagai Y, Fujita K, Miyama S. Coma and seizure caused by an afloqualone overdose. Pediatrics International [Internet]. 2019 Feb 1 [cited 2022 Apr 12];61(2):212–3. Available from: https://onlinelibrary.wiley.com/doi/full/10.1111/ped.13777

67. Hallerbäck BI, Bommelaer G, Bredberg E, Campbell M, Hellblom M, Lauritsen K, et al. Dose finding study of mosapride in functional dyspepsia: a placebo-controlled, randomized study. Aliment Pharmacol Ther [Internet]. 2002 [cited 2022 Apr 8];16(5):959–67. Available from: https://pubmed.ncbi.nlm.nih.gov/11966505/

68. CSM Update: Withdrawal of nomifensine. British Medical Journal (Clinical research ed) [Internet]. 1986 Jul 5 [cited 2022 Apr 12];293(6538):41. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1340782/

69. KNMP Kennisbank | Procaïnamide [Internet]. [cited 2022 Apr 8]. Available from: https://kennisbank-knmp-nl.proxy.library.uu.nl/article/Informatorium_Medicamentorum/S501.html

70. Okumura K, Kita T, Chikazawa S, Komada F, Iwakawa S, Tanigawara Y. Genotyping of N-acetylation polymorphism and correlation with procainamide metabolism. Clin Pharmacol Ther [Internet]. 1997 May [cited 2022 Apr 8];61(5):509–17. Available from: https://pubmed.ncbi.nlm.nih.gov/9164413/

71. Roberts SM, Adams LE, Donovan-Brand R, Budinsky R, Skoulis NP, Zimmer H, et al. Procainamide hydroxylamine lymphocyte toxicity—I. Evidence for participation by hemoglobin. International Journal of Immunopharmacology. 1989 Jan 1;11(4):419–27.

72. UpToDate | Stevens-Johnson syndrome and toxic epidermal necrolysis: Pathogenesis, clinical manifestations, and diagnosis [Internet]. [cited 2022 Mar 15]. Available from: https://www-uptodate-com.proxy.library.uu.nl/contents/stevens-johnson-syndrome-and-toxic-epidermal-necrolysis-pathogenesis-clinical-manifestations-and-diagnosis?search=maculopapular%20eruption&topicRef=15762&source=see_link

73. Pfistermeister B, Saß A, Criegee-Rieck M, Bürkle T, Fromm MF, Maas R. Inconsistencies and misleading information in officially approved prescribing information from three major drug markets. Clin Pharmacol Ther [Internet]. 2014 Sep 3 [cited 2022 Jun 8];96(5):616–24. Available from: https://pubmed.ncbi.nlm.nih.gov/25062063/

74. UpToDate | Acquired long QT syndrome: Definitions, causes, and pathophysiology [Internet]. [cited 2022 Jun 23]. Available from: https://www-uptodate-com.proxy.library.uu.nl/contents/acquired-long-qt-syndrome-definitions-causes-and-pathophysiology?search=qt%20prolongation&source=search_result&selectedTitle=1~150&usage_type=default&display_rank=1

75. Briasoulis A, Agarwal V, Pierce WJ. QT Prolongation and Torsade de Pointes Induced by Fluoroquinolones: Infrequent Side Effects from Commonly Used Medications. Cardiology [Internet]. 2011 Dec [cited 2022 Jun 17];120(2):103–10. Available from: https://www.karger.com/Article/FullText/334441

76. UNICOM | First reference documents of the EU-SRS – the European Substance Registration System are now available! [Internet]. [cited 2022 Mar 1]. Available from: https://unicom-project.eu/5835-2/