

Structure-Activity Relationships and Drug Allergy

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Abstract: Structure-activity relationships (SARs) refer to the relation between chemical structure and pharmacologic activity for a series of compounds. Since the pioneering work of Crum-Brown and Fraser in 1868, they have been increasingly used in the pharmaceutical, chemical and cosmetic industries, especially for drug and chemical design purposes. Structure-activity relationships may be based on various techniques, ranging from considerations of similarity or diversity of molecules to mathematical relationships linking chemical structures to measured activities, the latter being referred to as quantitative SAR or QSAR. This review aims at briefly reviewing the history of SARs and highlighting their interest in delayed and immediate drug allergy using selected examples from the literature. Studies of SAR are commonly conducted in the area of contact dermatitis, a delayed hypersensitivity reaction, to determine the allergenic potential of a given compound without animal testing. In immediate, immunoglobulin E-mediated drug hypersensitivity, this kind of approach remains rather confidential. It has been mainly applied to neuromuscular blocking drugs (muscle relaxants) and betalactam antibiotics (penicillins, cephalosporins). This review shows that SARs can prove useful to (i) predict the allergenic potential of a chemical or a drug, (ii) help identify putative antigenic determinants for each patient or small group of patients sharing the same cross-reactivity pattern, and (iii) predict the likelihood of adverse reactions to related molecules and select safe alternatives.

Keywords: Contact dermatitis, drug allergy, drug hypersensitivity, SAR, structure-activity relationships.

INTRODUCTION

Structure-activity relationship (SAR) describes the relation between chemical structure and pharmacologic activity for a series of compounds [1]. These relationships can either be qualitative or quantitative. Qualitative SAR (qSAR) are derived from non-continuous (*i.e.*, presence or absence of a property or activity of interest) data. Quantitative SAR (QSAR) are mathematical relationships linking chemical structure and pharmacological activity in a quantitative manner for a series of compounds [1]; they constitute a subset of the more general term SPC (Structure-Property Correlations). Three-dimensional QSAR (3D-QSAR) refer to methods spatially relating the three-dimensional structure of modelled compounds to other ones [2-4]; other SAR approaches encompass Hologram QSAR (HQSAR), Inverse-QSAR (I-QSAR) and binary QSAR [5-8].

Structure-activity relationships have been extensively used in the pharmaceutical, chemical and cosmetic industries,

especially for drug and chemical design purposes. They have also proven useful for risk assessment in toxicology and environmental science. After succinctly reviewing the history of SAR and QSAR, the potential interest of these methods in delayed and immediate drug allergy will be discussed in the light of selected, non-exhaustive examples from the literature.

BRIEF HISTORY OF SAR AND QSAR

Early Years and Pioneering Discoveries

In their pioneering work published in 1868, British scientists Alexander Crum-Brown and Thomas Fraser made investigations on the connection between chemical constitution and physiological action [9]. In the following decades, Richet established a relationship between the toxicity and the physicochemical properties of a series of polar organic compounds such as ethers, alcohols and ketones, stating that “the more soluble they are, the less toxic they are” [10]. Meyer [11] and Overton [12] independently described a linear correlation between lipophilicity (oil-water partition coefficient) and biological (narcotic) effects (lipoid theory of narcosis).

In 1937, Hammett [13] introduced a method to delineate substituent effects (*i.e.* electron-donating or –withdrawing properties) on organic reaction mechanisms. Taft [14] further

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improved Hammett's model by proposing the first steric parameter (E_s). In 1962, Hansch *et al.* published their pioneering study on the correlation of biological activity of plant growth regulators, namely mono-ring-substituted phenoxyacetic acids, with Hammett substituent constants and hydrophobicity [15]. Two years later, the same group introduced a new parameter, π , the relative hydrophobicity of a substituent, which can be defined as follows:

$$\pi_x = \log P_x - \log P_H$$

where π_x represents the relative hydrophobicity of a substituent, and P_x and P_H the partition coefficients of a derived and the parent molecule, respectively [16]. This method is now referred to as traditional, Hansch-Fujita or two-dimensional QSAR (2D-QSAR) [17,18]. In 1964, Free and Wilson suggested the so-called "*de novo* approach" which postulated that each substituent gives an additive and constant effect to the biological activity regardless of the other substituents in the rest of the molecule [19,20]:

$$BA = \sum a_i x_i + u$$

where BA represents the biological activity, u the average contribution of the parent molecule, a_i the contribution of each structural feature and x_i the presence ($x_i = 1$) or absence ($x_i = 0$) of a particular structural moiety.

A few years later, topological indices describing the whole molecular structure (also referred to as 2D-descriptors) were introduced. The coining of the term "topological indices" goes back in the early 1970s (Hosoya, [21]), although initial work on numerical constitutional descriptors (*i.e.*, numerical descriptors dependent on the constitution of a molecule, such as molecular weight, number of atoms / rings / bonds,...etc.) was started almost 25 years earlier (Wiener, [22]). These first-generation topological indices were integer numbers based on integer local vertex invariants, and they essentially focused on the physical properties of saturated hydrocarbons (alkanes). Along with other seminal works, topological indices such as the Randić index (molecular branching, [23]), the Balaban index [24], Simon's minimum topological difference (MTD) [25] and Kier and Hall's molecular connectivity approach [26-28] gave the background for the modern QSAR / 3D-QSAR era.

Advent of the Modern QSAR / 3D-QSAR Era

Three-dimensional features were first implemented in the late 1970s, when statistical techniques and computer technology improvements were eventually taking place. Among many other techniques, Dynamic lattice-oriented molecular modelling system (DYLOMMS, 1981), and then Comparative Molecular Field Analysis (CoMFA, developed between 1983 and 1987) were two of the earliest 3D-QSAR approaches. The latter uses statistical correlation techniques for the analysis of the quantitative relationship between the biological activity of a set of compounds with a specified alignment, and their three-dimensional electronic and steric properties; hydrophobicity and hydrogen bonding properties may also be incorporated into the analysis [1,29].

The notions of pharmacophore (or pharmacophoric pattern) and bioisostere are also key to the development of

rational drug design and 3D-QSAR in the pharmaceutical industry [30]; the International Union of Pure and Applied Chemistry defines the pharmacophore as the ensemble of steric and electronic features that is necessary to ensure the optimal supramolecular interactions with a specific biological target structure and to trigger (or to block) its biological response [1].

The creation of captopril (Capoten[®], Squibb), the first oral angiotensin converting enzyme (ACE) inhibitor [31-36], and saquinavir (Invirase[®], Hoffmann La Roche), the first Human Immunodeficiency Virus (HIV) protease inhibitor [37-41], were two milestones in QSAR / 3D-QSAR based drug design.

STRUCTURE-ACTIVITY RELATIONSHIPS IN DRUG ALLERGY

Structure-Activity Relationships in Delayed Drug Hypersensitivity

Applications of SARs in Contact Dermatitis

Contact allergy is caused by a wide range of chemicals (including dyestuffs, fragrances, natural products, pharmaceutical excipients and drugs) after prolonged or repeated contact with the skin, and it is regarded as a delayed hypersensitivity reaction (type IV hypersensitivity), which is mediated by antigen-specific T-lymphocytes. SAR is commonly used in the area of contact dermatitis [42,43]. The need for alternative, non-animal tests for the assessment of either pharmacological or toxicological activity of new chemical entities has led to an increased interest in SARs [43,44]; a ban on *in vivo* testing of cosmetic and toiletry ingredients is planned to come into force in 3 years [45]. Current assessment of potential skin sensitization hazard relies on *in vivo* methods such as the murine Local Lymph Node Assay (LLNA) [46,47]; although *in vitro* methods exist, they still lack sensitivity so that weak sensitizers may not be identified.

From a historical point of view, Landsteiner and Jacobs were the first to describe a correlation between chemical reactivity and skin sensitization (1936) [48]; they proposed the interaction of a hapten (*i.e.* a chemically reactive small compound of low molecular weight (< 1000 Da) that bind to proteins or peptides and modify them covalently) with a cutaneous protein as a prerequisite for skin sensitization. Based on this suggestion, Dupuis and Benezra elaborated on the so called electrophilic theory of skin sensitization five decades later, which gave birth to the modern era of SAR in contact allergy [49]. In this theory, the sensitizing chemical behaves as an electrophile, and skin proteins as nucleophiles. The first QSAR model was developed by Roberts and Williams in 1982 and consisted of correlations between sensitizing capacity and physicochemical parameters for a series of alkylating agents [50]. The Relative Alkylation Index (RAI) has been the basis for many mechanism-based QSARs. It is used to quantify the degree of carrier protein alkylation and correlate it with the sensitizing potential; the general equation is as follows:

$$RAI = \log D + a \log k + b \log P$$

where RAI denotes the Relative Alkylation Index, D the molar dose of sensitizer, P the octanol / water coefficient and

k the alkylation rate or relative alkylation rate constant for the reaction of the sensitizer with a model nucleophile; a and b are experimental constants valid for a series of compounds reacting according to the same mechanism. One of the main shortcomings of this method is that it addresses only structurally related chemicals reacting according to the same mechanism. Statistical, global QSARs have been developed empirically by application of various statistical methods to sets of biological data and structural, topological or geometrical information descriptors [51-54]. However, these methods often lack a well-characterized underlying mechanism.

In parallel to QSAR models, computer-based 'Expert Systems' have been developed for the prediction of the allergenic potential of putative allergens. Examples of such expert systems are DEREK for Windows (Deductive Estimation of Risk from Existing Knowledge), TOPKAT (Toxicity Prediction by Komputer Assisted Technology), CASE / MultiCASE (Computer Automated Structure Evaluation) and TIMES-SS (Tissue Metabolism Simulator for Skin Sensitization). DEREK is a commercial expert rulebase system that correlates structural alerts for protein reactivity of chemicals with their skin sensitization potential [55,56]; apart from skin sensitization prediction, DEREK may also be used for the assessment of mutagenicity and carcinogenicity. The rules are being constantly upgraded, and users are also invited to provide their own rules. TOPKAT is a statistical system based on linear free energy

relationships coupled to statistical regression analysis; it was developed using guinea pig maximization tests data. CASE / MultiCASE is another example of statistical system; it splits molecules into small structural fragments that are associated with specific toxicity. It can be used to predict other toxicological endpoints, such as mutagenicity and carcinogenicity. TIME-SS in an hybrid expert system between knowledge-based and statistical systems; it estimates skin metabolism and the interaction of parent compound and metabolites with skin proteins.

The determination of cross-reactivity patterns among different steroids in patients with corticosteroid contact allergy is perhaps the most famous example of SAR analysis in delayed drug hypersensitivity [57,58]. Coopman *et al.* identified 4 main structural groups for the cross-reactivity pattern of corticosteroids using literature-based and experimental patch-test data [57]; the authors found substitutions on the D ring or the C₂₀ / C₂₁ side chain to have an impact on the association of patch-test positive results, whereas other structural features such as the presence of a double-bond in the A ring or fluoride substitutions on the B ring were devoid of influence (Fig. 1a). Corticosteroids were eventually classified as follows (Fig. 1b): group A consists of hydrocortisone, toxicortol pivalate, and related compounds; group B comprises triamcinolone acetonide, desonide, amcinonide, budesonide and related compounds; group C is made up of betamethasone, dexamethasone and related compounds; group D includes ester derivatives such

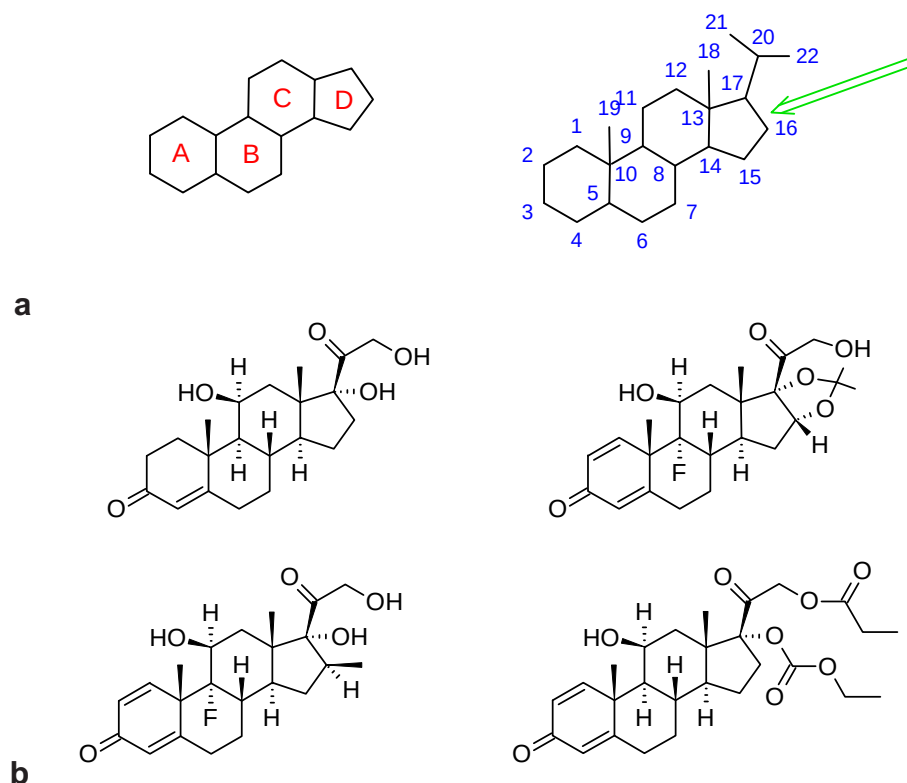


Fig. (1). (a). Chemical structure of the cyclopentanoperhydrophenanthrene moiety, which constitutes the skeleton of all steroids (left); chemical structure and numbering of steroids (right). The arrow denotes the proposed epitope in patients presenting contact dermatitis to corticosteroids. (b). Chemical structures of a few steroids (left to right): hydrocortisone (group A), triamcinolone acetonide (group B), betamethasone (group C), and prednicarbate (group D).

as hydrocortisone-17-butyrate, prednicarbate or clobetasone-17-butyrate.

Lepoittevin and co-workers later elaborated on these findings and performed statistical calculations on additional case reports as well as conformational analysis on selected corticosteroids [58]; groups A, B and D were found to be homogenous in both volume and shape, explaining why cross-reactions between corticosteroids belonging to different groups are unlikely to be observed. Clinical cross-reactivity between budesonide (B) and some molecules of the D group (*e.g.* hydrocortisone-17-butyrate) were explained on the basis of conformation analysis results. Specific chemical moieties on the D ring are associated with each group of corticosteroids and seem to be significant epitopes in corticosteroid-allergic patients (Fig. 1a).

Finally, it has to be noticed that many compounds are prohaptens, *i.e.* they are not allergenic by themselves, but may become activated by means of various bioactivation pathways (*e.g.* hydroxylations, epoxidations, oxidations or conjugations); an extensive review of this subject has been given by Karlberg *et al.* [44]. Sulfamethoxazole [59] and hydrocortisone [60] are well-studied examples of drugs that behave as prohaptens.

Other Applications of SARs in Delayed Drug Hypersensitivity

An experimental SAR study was undertaken to gain information on antigenic determinants of sulfamethoxazole recognized by T-cells in an *in vivo* animal model [61]. The results provide evidence that induction of a cellular immune response in the rat may depend on drug metabolism (*e.g.* generation of nitroso sulfamethoxazole) and cell surface haptation.

Barbaud *et al.* [62] studied cross-reactivity between pristnamycin and other synergists (virginiamycin and dalfopristin-quinupristin) in patients with cutaneous adverse drug reactions to pristnamycin. Cross-reactions between pristnamycin and virginiamycin and between pristnamycin and dalfopristin – quinupristin were found in 9 out of 22 cases and in 7 out of 8 cases, respectively. These high rates of cross-reactivity were explained according to similarities in their chemical structures (Fig. 2).

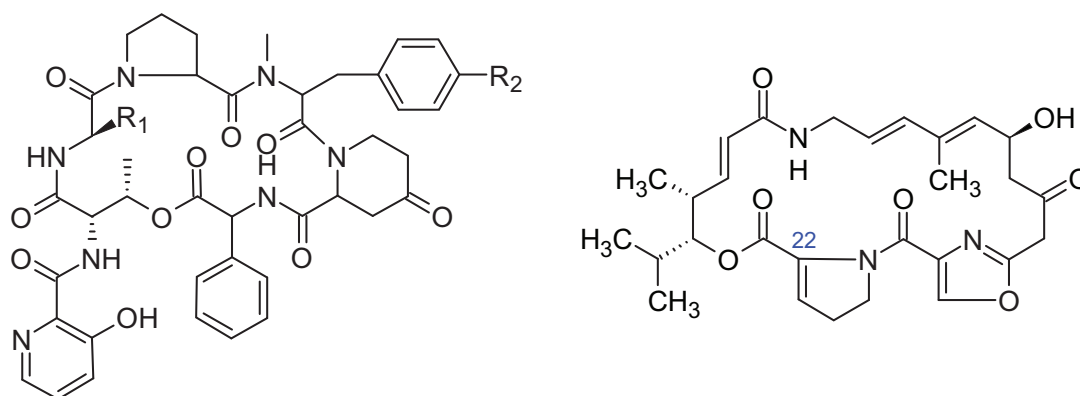


Fig. (2). Chemical structures of group I (left, pristnamycin analogues) and group II (right, virginiamycin analogues) synergists. Synergists differ by their R_1 and R_2 substituents (group I) and the presence of a double bond in position 22 (group II).

Structure-Activity Relationships in Immediate Drug Hypersensitivity

Beta-Lactam Antibiotics

Beta-lactams (*viz.* penicillins, cephalosporins, monobactams, carbapenems, beta-lactamase inhibitors) are the most widely used antibiotics worldwide, and they account for the majority of immediate drug hypersensitivity reactions in nowadays clinical practice [63,64]. Immunoglobulin E-mediated penicillin sensitization is supposed to proceed after conjugation with carrier proteins, although the exact details pertaining to this mechanism are yet lacking; this led to the classic concept of ‘major’ and ‘minor’ allergenic determinants of penicillin, depending on the nature of ring modifications and the point of linkage to the carrier protein [65]. The term ‘major’ refers to the fact that the vast majority (95 per cent) of penicillin molecules that bind covalently to proteins yield penicilloyl derivatives; other conjugates belong to so-called ‘minor’ determinants, which encompass penicillenate, penicilloic acid, penicillanyl, penamaldate, penaldate, D-penicillamine and penicoyl. In cephalosporins, there is evidence that the unstable cephalosporoyl-protein conjugate undergoes further reactions to yield numerous breakdown products, which have not been fully isolated and characterized so far. Skin testing with the major (*i.e.* the benzylpenicillin-polylysine conjugate, commonly referred to as PPL) and minor determinants (such as the minor determinant mixture or MDM) still represent the first-line method for diagnosing immediate hypersensitivity reactions to beta-lactams in both Europe [66] and America [67].

However, it has to be mentioned that this hapten-carrier model does not take into account the chemical and structural diversity of beta-lactam side chains [68]. The latter, as well as the beta-lactam ring, have been investigated as a putative link to cross-allergenicity between drugs and among drug classes [69,70]; innumerable studies have reported comparative cross-reactivity on the basis of skin test results [71-76]. Clinicians are often unaware of the fact that chemical structure of cephalosporin side chains is not correlated with the antimicrobial classification (*i.e.* first, second, third or fourth ‘generation’ cephalosporins), and that cross-reactivity between cephalosporins belonging to different ‘generations’ or between cephalosporins and penicillins may occur

[74,75]. Hasdenteufel *et al.* recently reported a case of an immediate allergic reaction to cefuroxime axetil and described a SAR-based approach based on skin tests to predict the likelihood of adverse reactions to related antibiotics in a given patient [74]. In this study, all beta-lactam antibiotics that yielded positive results carried either a methoxyimino group (cefuroxime, cefotaxime, cefepime,

ceftriaxone) or a methyl-substituted isoxazole moiety (oxacillin), which is a bioisostere of the former. The patient was not sensitized to other compounds lacking this group or carrying moieties of greater steric hindrance (*i.e.* the alkoxyimino group: ceftazidime) (Fig. 3a and 3b).

The alkoxyimino group, as well as the methyl-substituted isoxazole group, were introduced in the side chains of beta-

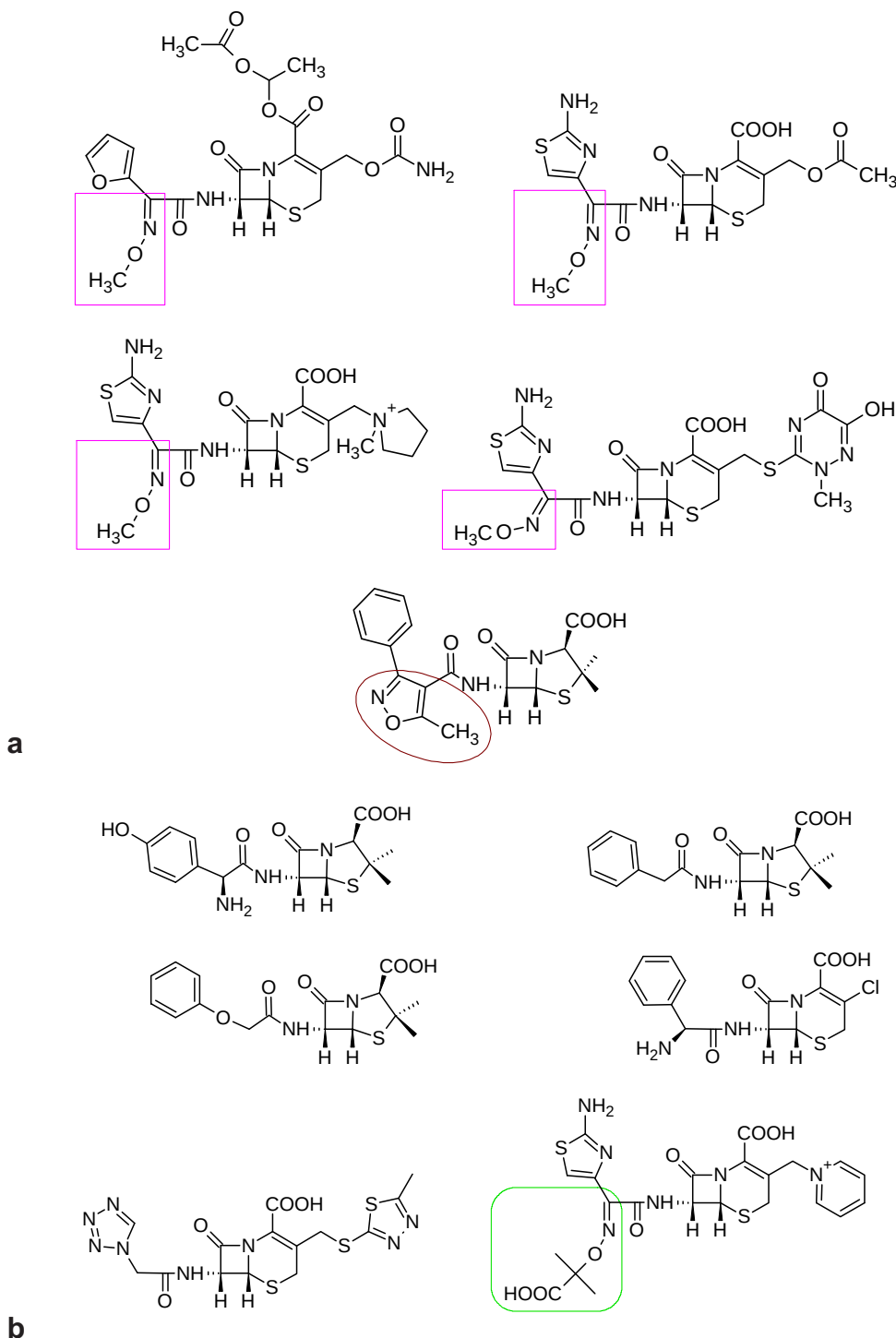


Fig. (3). (a). Chemical structures of beta-lactam antibiotics yielding positive results in skin tests (*i.e.*, cefuroxime axetil, cefotaxime, cefepime, ceftriaxone, and oxacillin (from left to right)). Boxes highlight the methoxyimino group common to each molecule; the methyl-substituted isoxazole moiety is circled [74]. (b). Chemical structures of beta-lactam antibiotics yielding negative results in skin tests. The box depicts the alkoxyimino moiety in ceftazidime [74].

lactam antibiotics to strengthen their resistance towards beta-lactamase productive strains; these chemical moieties aim at protecting the beta-lactam ring by providing a kind of 'shielding' (i.e. steric hindrance) that prevents it from being hydrolyzed by beta-lactamases [77-79]. Furthermore, this concept of steric shielding might explain why the methoxyimino group and its bioisostere are more exposed than the rest of the molecule and thus could favour sensitization in this patient. Clinical validation of this SAR study was achieved using a rechallenge test with amoxicillin, which was indeed well tolerated up to a cumulated dose of 1g administered over 2h intravenously.

To date, few *in vitro* immunochemical studies have used SAR-based methods in the determination of the specificity of IgE antibodies in the case of penicillin [80-82] or cephalosporin immediate hypersensitivity [82-85]. Briefly, 2 main methodologies have been used for this purpose (Fig. 4), i.e. (i) coupling of the beta-lactam of interest with a carrier protein (e.g. human serum albumin) without ring opening, followed by inhibition experiments [68] and (ii) synthesis of chemical species mimicking hapten structures that are supposed to be coupled to proteins *in vivo* [84]. In the latter, it has to be mentioned that structural integrity of the initial beta-lactam is not preserved.

Baldo and co-workers found heterogeneous patterns of cross-reactivity in patients with penicillin allergy [80]; some patients had an epitope presumably located on the R₁ side chain, whereas others showed recognition of the thiazolidine ring. The same group also studied the recognition pattern of IgE antibodies from 3 sera of flucloxacillin-allergic patients [81]. Using various isoxazolylpenicillin derivatives and benzylpenicillin, quantitative hapten inhibition methods demonstrated that the 5-methyl-3-phenyl-4-isoxazolyl side chain may be the allergenic determinant in all three patients; one of the 3 sera also showed recognition of benzylpenicillin, so that an additional common structural feature to all penicillins (e.g. the penam moiety or part of it) may as well be recognized by this patient. One year later, Baldo and co-workers applied the same methodology to 12 sera from cefaclor-allergic patients [83]; as was the case with sera from patients suffering from penicillin allergy [80],

inhibition results revealed multiple and heterogeneous recognition patterns: R₂ dihydrothiazine ring substituents, along with chemical features on the R₁ side chain, may account for these findings. In 2002, the same research group investigated the chemical features that could account for cross-reactivity between benzylpenicillin and cephalothin, a 7-(2-heteroaryl-acetamido)-cepheme derivative by means of quantitative inhibition studies [82]; the authors concluded that the methylene group, present in the side chains of both compounds, was the presumed main IgE combining site. However, another explanation could be the bioisosterism of the side chains of benzylpenicillin and cephalothin (Fig. 5); benzene and thiophene indeed constitute a typical example of classic ring bioisosterism.

Using a comparable methodology, Kim *et al.* studied serum samples of 3 cefaclor-allergic patients [85]. Hapten inhibition testing revealed 2 distinct recognition patterns, the first comprising the R₁ side chain and the second possibly encompassing the whole cefaclor molecule.

In 2003, Sanchez-Sancho *et al.* described the multistep synthesis and subsequent physicochemical characterization of a series of 6 α N-acyl-L-alanylbutanamides, which were proposed as putative cephalosporin haptenic structures [84] (Fig. 6).

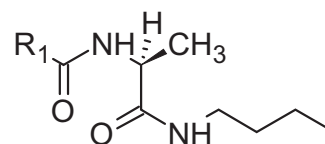


Fig. (6). Generic chemical structure of putative cephalosporin haptenic structures synthesized by Sanchez-Sancho *et al.* [84]. R₁ represents the side chain of each cephalosporin derivative.

The major shortcoming of this approach may be the non-consideration of the R₂ side chain, which is supposed to disappear during the breakdown process of the cephalosporoyl intermediate. Radio Allergosorbent Test (RAST) inhibition studies were performed with these compounds on sera from 4 patients with allergic reactions to cephalosporins (ceftriaxone and cefuroxime); results suggested the

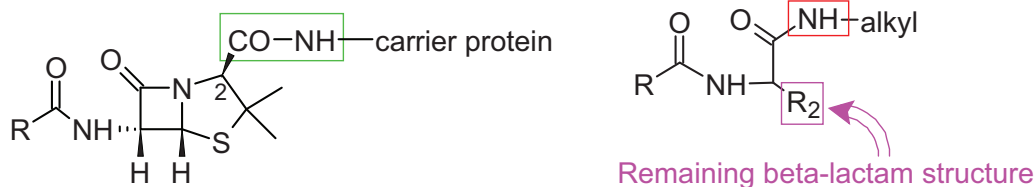


Fig. (4). Two beta-lactam coupling strategies for *in vitro* immunochemical studies: a) coupling via the 2'-carboxyl function on the thiazolidine (penicillins, left) or dihydrothiazine (cephalosporins) ring; (b) azetidin-2-one (beta-lactam) ring opening through nucleophilic attack (cephalosporins, right).

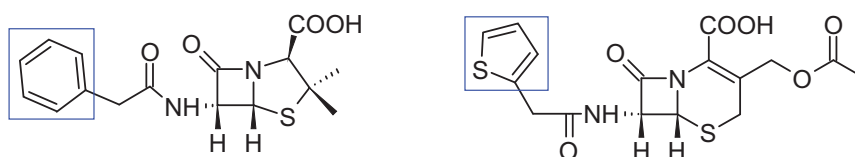


Fig. (5). Chemical structures of benzylpenicillin (penicillin G, left) and cephalotin (right). Moieties included in the boxes are classic ring bioisosteres.

involvement of either the corresponding R_1 side chain or its heteroaryl-methoxyimino bioisostere in the recognition pattern of 3 patients and of additional, yet hypothetical structural features for the remaining case. Inhibition results were found to be in good agreement with both skin tests and RAST results. These findings were later confirmed by a study involving 24 patients with immediate hypersensitivity reactions to cephalosporins and RAST-positive to at least 1 cephalosporin; RAST and RAST inhibition studies demonstrated a predominant role of the R_1 side chain in the pathogenesis of the reaction [86].

Sulfonamide Drugs (Antibiotics and Non-Antibiotics) and Other Antibacterial Agents

SAR studies have been applied to the determination of the IgE-binding sites of sulfamethoxazole in three patients [87]. Inhibition experiments with different compounds of the sulphonamide group demonstrated the involvement of the 5-methyl-3-isoxazolyl group.

Verdel and co-workers conducted a case-control study on the possible relationship between chemical structure of sulfonamide drugs (antibiotics or not) and their potential for eliciting allergic events in patients with diabetes mellitus using the General Practice Research Database [88]. Sulfonamides were shared out among three subgroups according to the presence of absence of a N_1 substituent and/or an arylamine on N_4 (Fig. 7). They did not find any major difference between the 3 subgroups.

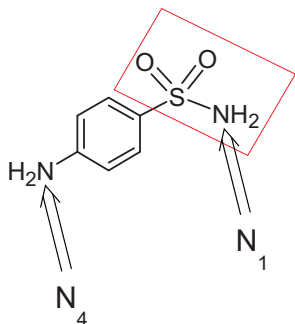


Fig. (7). Chemical structure of sulfanilamide. The sulphonamide moiety is included in the box.

Smal *et al.* carried out immunoassays on the sera of two patients who had anaphylaxis following trimethoprim administration [89]. Each patient exhibited a different pattern of epitope recognition: the first sera reacted towards the 3,4-dimethoxybenzyl group, and the second one towards both the trimethoxybenzyl and diaminopyridine rings of trimethoprim (Fig. 8). The authors concluded that more than one antigenic epitope might be involved in immediate hypersensitivity reactions to trimethoprim.

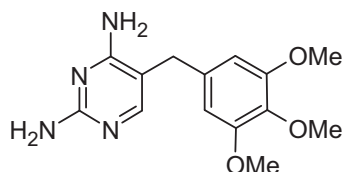


Fig. (8). Chemical structure of trimethoprim.

Neuromuscular Blocking Agents (NMBAs)

Neuromuscular blocking agents, also referred to as muscle relaxants, are known to cause immediate, potentially life-threatening hypersensitivity reactions. Depending on the country, the frequency of such reactions varies between 1:200000 and 1:5000 [90]. In the early 1980s, initial immunochemical investigations by Baldo and Fisher demonstrated the putative involvement of substituted ammonium groups in the pathogenesis of the reaction using several NMBAs and other compounds endowed with either a tertiary amine or a quaternary ammonium group [91,92]. However, the allergenic determinant is probably not unique since various patterns of cross-reactivity exist; neighbouring groups might as well be recognized by patients [93]. The dependency of histamine release in allergic patients on the length and flexibility of the chain linking the 2 ammonium groups was studied by Didier *et al.* [94]. The optimal length for allergic histamine release was found to be greater than or equal to 6 Å; in contrast, chains smaller than 4 Å did not yield significant histamine release.

Their divalency has been proposed as an explanation for mediator release without requiring protein binding [94,95]. Interestingly, this divalency is also responsible for their pharmacologic (*i.e.* neuromuscular blocking) properties (Fig. 9).

The combination of SARs responsible for both pharmacological and immunological activity is unusual. There is variability in the cross-sensitivity between NMBAs in both the results of RIA testing and the clinical expression which may relate to the distance between substituted ammonium ions, the flexibility of the molecule, and the involvement of adjacent molecules. The exposed single quaternary ammonium ion on the morphine molecule allows it to bind to anti-NMBA IgE providing a better diagnostic test for NMBA allergy than most individual RIAs and facilitated the hypothesis that codeine variants may have a role in sensitisation.

Depending on their country of origin, up to 75 % of patients that experienced immediate allergy to NMBAs had never been exposed to these drugs. In their comprehensive review, Baldo, Fisher and Pham have discussed the possible origin of antibodies to NMBAs, which remains so far unclear [96]. Among other explanations, environmental agents, such as cosmetics or disinfectants, and exposition to other ammonium-containing drugs may be responsible for sensitization to NMBAs. Given the fact that anaphylactic reactions to NMBAs are around 6 times more frequent in Norway than in Sweden, and that pholcodine (a cough suppressant structurally related to morphine) is freely accessible in Norway but not in Sweden, the 'pholcodine hypothesis' has been postulated by Florvaag and co-workers [97-100] (Fig. 10).

Recently, a multicentre study was undertaken to validate this assumption in 9 high or low pholcodine-consuming countries: Sweden, Denmark, United States of America, Germany, The Netherlands, Finland, Norway, United Kingdom, France) [101]. Results were rather controversial, indicating at least a partial correlation between pholcodine

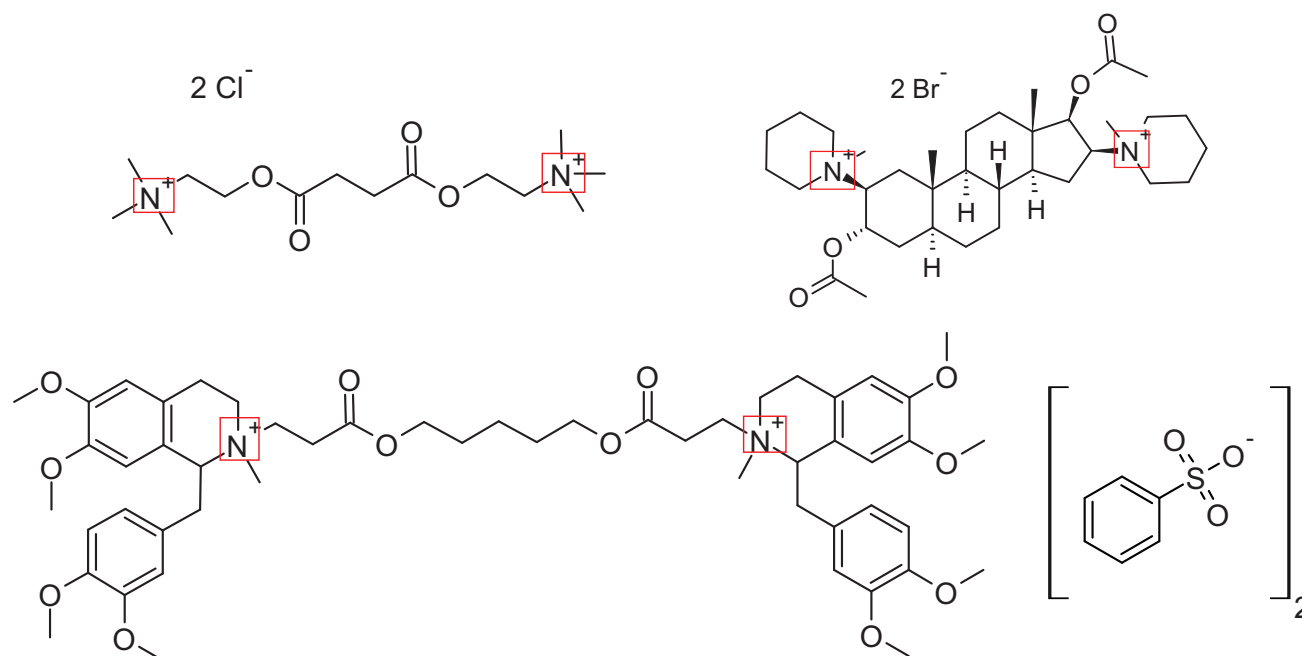


Fig. (9). Examples of each NMBA subtype (left to right): suxamethonium chloride (depolarizing, non-competitive NMBA), pancuronium bromide (aminosteroid, non-depolarizing, competitive NMBA), and atracurium besilate (benzylisoquinolinium, non-depolarizing, competitive NMBA). The boxes indicate the nitrogen atoms of each ammonium group.

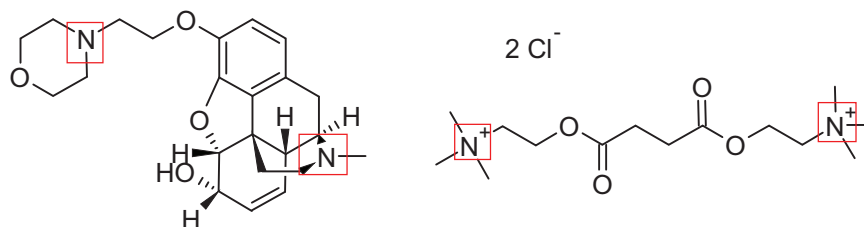


Fig. (10). Chemical structures of pholcodine (left) and suxamethonium chloride (right). Both molecules are endowed with two nitrogen atoms (divalency).

consumption and prevalence of IgE sensitization to pholcodine, morphine and suxamethonium; correlation with suxamethonium could only be reached when data from the Netherlands and the USA were excluded from the analysis. Furthermore, some findings (especially in the USA, where high prevalence of IgE antibodies to pholcodine and suxamethonium were found despite the unavailability of pholcodine-based drugs on the market) indicate that other environmental factors may be involved. In 2007, pholcodine was withdrawn from the Norwegian market by its manufacturer; studies to assess the effect of this event on the prevalence of IgE sensitization are still in progress, but there seems to be evidence that both prevalence of IgE sensitization and frequency of NMBA anaphylaxis are decreasing in Scandinavia [101].

Morphine and Other Related Analgesics

Morphine and other related analgesics are known to be potent histamine-releasing drugs [102]; Harle *et al.* analyzed SAR using hapten inhibition studies in the serum of a patient that experienced anaphylaxis following the administration of papaveratum-hyoscine [103]. Extensive cross-reactivity was found with codeine, and, to a lesser extent, nalorphine

(Fig. 11a); cross-reactivity was significantly weaker with mepiridine, methadone and all other tested opioids. Based on these results and on the drugs' respective chemical structures, the authors concluded that the cyclohexenyl ring (C ring) with a hydroxyl located at C₆ and a methyl substituent on the nitrogen atom (D ring) could constitute allergenic epitopes of morphine in this patient (Fig. 11b).

Insulin and Insulin Analogues

Structure-activity relationships of insulin have been extensively investigated since its initial discovery by Frederick Banting and Charles Best in 1921. Human insulin is composed of two peptide chains referred to as A chain (21 amino acids) and B chain (30 amino acids); these chains are linked by two intercatenar disulfide bonds, and there is an additional disulfide bond within the A chain. The C-terminal octapeptide of the B-chain is known to have a function in the self-association behaviour of insulin, the last five residues being not essential for *in vitro* bioactivity [104]. Indeed, this region has been thoroughly studied and has led to the emergence of both fast-acting and long-acting insulin analogues.

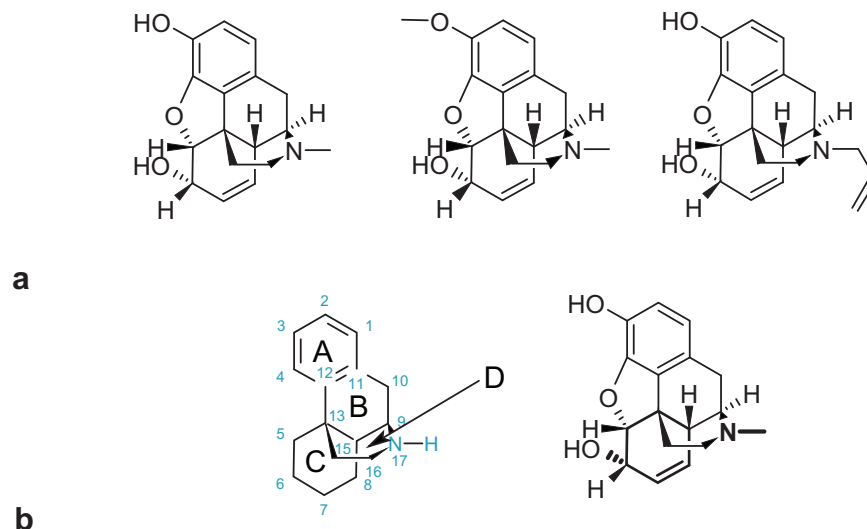


Fig. (11). (a). Chemical structures of morphine, codeine and naltrexone. (b). Chemical structure and numbering of morphinane (basic structure of morphine, left); **A**, **B**, and **C** represent the phenanthrene moiety, **C** and **D** constitute the isoquinoline moiety. Putative morphine allergenic epitopes for the patient are drawn in bold (right) [103].

The introduction of human recombinant insulin has significantly reduced the frequency of insulin allergy as compared to former non-human insulins [105,106]. The reasons that may explain immunogenicity of subcutaneous injected human insulin remain so far unclear. Among other hypotheses, it has been postulated that chemical alterations of the molecule (*i.e.* aggregation during storage or after injection) as well as process-related parameters could be responsible for this phenomenon [107,108]. Some authors have pointed out the importance of residue B30 (Thr) as one of the immunogenic determinants of human insulin [109,110]. Luyasu and co-workers recently reported an anaphylactic shock cause by human insulin and protamin in a 50-year-old type 2 diabetic patient; cross-reactivity pattern between the different insulin derivatives was assessed using basophile activation tests. Modifications and/or substitutions on the B28 and B29 residues (insulin aspart, lispro, and detemir) were postulated to decrease basophil activation in the patient [111]. In the case of allergy to insulin detemir, a long-acting, basal insulin analogue, Sola-Gazagnes *et al.* postulated that the myristoyl moiety might be involved in the pathogenesis of the reaction, possibly through non-specific interaction with the cell membrane components of dendritic cells or lymphocytes [112].

H₂-Receptor Antagonists

Histamine-2-receptor antagonists, along with proton pump inhibitors, are drugs suppressing gastric acid secretion. Morisset *et al.* [113] described a case of immediate hypersensitivity to ranitidine proved by history, skin and leukocyte histamine release tests; treatment with nizatidine provoked the same symptoms. Nizatidine yielded negative prick-test, but intradermal test could not be performed because this drug was not available in injectable form. Prick-tests and IDT were negative for cimetidine and famotidine. The latter was chosen for an oral challenge test which was performed without any subsequent adverse reaction. This pattern of cross-reactivity was explained using a SAR approach; famotidine is endowed with a guanidyl moiety on the thiazol ring, which allows hydrogen linkage to take place

and possibly freezes the conformation. The connecting aliphatic chain is shorter than that of both ranitidine and nizatidine; lastly, the polar moiety is an amidine group instead of guanidine in the aforementioned compounds.

Contrast Agents Used in Magnetic Resonance Imaging (MRI)

Magnetic resonance imaging contrast agents are widely used and generally considered to be safe, although serious adverse events may occur. Anaphylactic shock induced by gadolinium-containing MRI contrast agents is rare: occurrence ranges from 0.004% to 0.01% [114,115]. As the free gadolinium ion (Gd^{3+}) is highly toxic (blockage of voltage-gated calcium channels, depression of the reticuloendothelial system), chelates have been designed to reduce its acute toxicity while preserving overall MRI properties [116,117]. Owing to the chemical structure of the chelate, gadolinium-containing MRI contrast agents may be divided into 2 different subtypes, *i.e.* the macrocyclic and the linear, open-chain subtypes (Fig. 12a and 12b); MRI contrast agents may also be classified as ionic or non-ionic compounds. Both molecular structure and ionicity influence the stability of MRI contrast agents [117].

Hasdenteufel *et al.* recently reported three cases of anaphylactic shock occurring after first exposure to gadoterate meglumine (Gd-DOTA) [118]. The patients were skin tested with both macrocyclic (Gd-DOTA, Gd-HP-DO3A, Gd-DO3A-butrol) and linear (Gd-DTPA, Gd-BOPTA, Gd-DTPA-BMA) gadolinium MRI contrast agents. Positive skin test results were obtained with contrast agents of the macrocyclic type, whereas all linear molecules yielded negative results. A simple SAR study revealed that all MRI contrast agents with positive skin tests share a common tetraazacyclodecane derivative ligand which might account for the sensitization of these patients (Fig. 13). The rechallenger test with a linear molecule (gadopentetate dimeglumine (Gd-DTPA); Fig. 12b) was indeed well-tolerated in two patients up to a cumulative dose of 0.1 mmol/kg administered over 15 minutes intravenously.

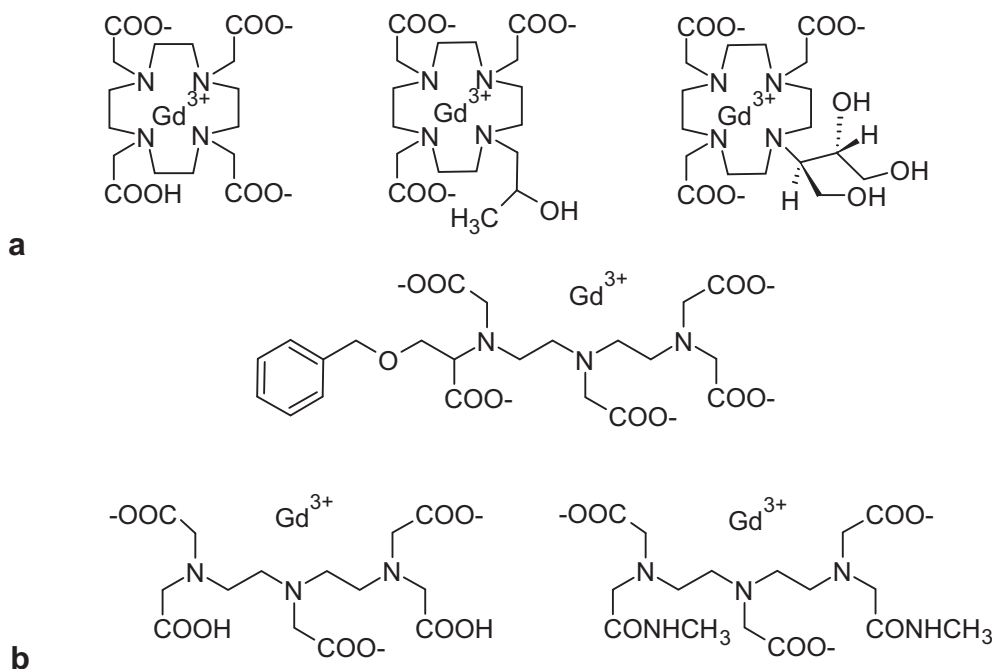


Fig. (12). (a). Chemical structures of macrocyclic MRI contrast agents (left to right): Gd-DOTA, Gd-HP-DO3A, and Gd-HP-DO3A-butrol. Coordination bonds are not shown on the drawings. (b). Chemical structures of open-chain (linear) MRI contrast agents (left to right): Gd-BOPTA, Gd-DTPA, and Gd-DTPA-BMA. Coordination bonds are not shown on the drawings.

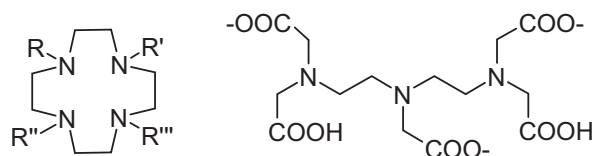


Fig. (13). Chemical structures of the tetra-azacyclododecane derivative ligand (left) and DTPA (right).

Future Prospects

Structure-activity relationships in drug allergy are still at an early stage of development. The main shortcoming of simple, qualitative SAR approaches is their dependence upon either skin or biological test results which may be falsely positive or negative for various reasons (including lack of standardization) or may be carried out with an insufficient number of molecules. Whatever the methodology, it has been shown that each patient or small subgroup of patients is endowed with its own recognition pattern, and that no general rule on drug rechallenge can be drawn without an appropriate individual allergy assessment. Structure-activity relationships should not be seen as a diagnostic tool in themselves, but rather as providing assistance in decision making for the clinician.

Taking into account the diversity and complexity of pathophysiological mechanisms underlying both immediate and delayed drug allergy, further mechanistic and experimental studies on large patient series are needed to deepen our knowledge of drug allergenic epitopes. Within this context, X-ray crystallography, SAR / QSAR / 3D-QSAR methodologies as well as computer-based modelling could prove useful.

SUMMARY

Structure-activity relationships have found widespread use in various areas of pharmacy, chemistry and toxicology. In the past 30 years, several attempts have been made to relate drug or chemical allergenicity to chemical structure, especially in the assessment of contact dermatitis and of immediate, IgE-mediated hypersensitivity reactions. In conclusion, SARs can prove useful to (i) predict the allergenic potential of a chemical or a drug, (ii) help identify putative antigenic determinants for each patient or small group of patients sharing the same cross-reactivity pattern, and (iii) predict the likelihood of adverse reactions to related molecules and select safe alternatives. Simple, qualitative SARs should be implemented in current allergological practice through synergies between clinicians and pharmacists.

CONFLICT OF INTEREST

The authors have declared that they have no conflict of interest.

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