

Recent patents in Allergy/Immunology: New pyrazinones for the diagnosis of allergies to aminocephalosporins

Angela Martin-Serrano^{1,2} | Ezequiel Perez-Inestrosa^{2,3} | Cristobalina Mayorga^{1,2,4} |
María José Torres^{1,2,4,5} | María Isabel Montañez^{1,2}

¹Allergy Research Group, Instituto de Investigación Biomédica de Málaga-IBIMA, Málaga, Spain

²Nanostructures for Diagnosing and Treatment of Allergic Diseases Laboratory, Andalusian Center for Nanomedicine and Biotechnology-BIONAND, Málaga, Spain

³Departamento de Química Orgánica, Universidad de Málaga-IBIMA, Málaga, Spain

⁴Allergy Unit, Hospital Regional Universitario de Málaga, Málaga, Spain

⁵Departamento de Medicina, Facultad de Medicina, Universidad de Málaga, Málaga, Spain

Correspondence: María Isabel Montañez, Andalusian Center for Nanomedicine and Biotechnology-BIONAND. Parque Tecnológico de Andalucía, 29590 Málaga, Spain.

Email: maribel.montanez@ibima.eu

Funding information

Andalusian Regional Ministry of Economy and Knowledge, Grant/Award Number: CTS-06603; Instituto de Salud Carlos III, Grant/Award Number: PI12/02529, PI15/01206, CP15/00103 and PI17/01237; Premio UNICAJA a la innovación en biomedicina y salud, Grant/Award Number: Conv. 2017; Andalusian Regional Ministry of Health, Grant/Award Number: PI-0699-2011 and PI-0179-2014

1 | DESCRIPTION OF INVENTION AND POTENTIAL APPLICATIONS

Cephalosporins are, after penicillins, the most important β -lactam inducers of immediate allergic reactions. Their antigenic determinants are not completely identified due to difficulties for studying the structure of cephalosporin-protein conjugates, hampering the existence of *in vitro* tests with enough sensitivity to detect cephalosporin allergy.¹ Therefore, the only diagnostic standardized options are skin tests and drug provocation tests, which are risky, especially in cases of severe reactions. This forces physicians to select alternative β -lactams that, although safe for penicillin/cephalosporin-allergic patients, could be less effective and pose problems related to costs and antibacterial resistance. Therefore, an accurate *in vitro* diagnosis is demanded not only for cephalosporin-allergic reactions, but also for those induced by cross-reactive penicillins with similar or identical R¹ side chain.

Our patent covers pyrazinone derivatives as novel potential antigenic determinants of α -aminocephalosporins to diagnose β -lactam allergies.² A straightforward synthetic protocol for precisely defined

pyrazinone structures corresponding to cefaclor and cefadroxil was developed, and they were used for quantification of specific IgE antibodies to such cephalosporins in immunoassays.²⁻⁴

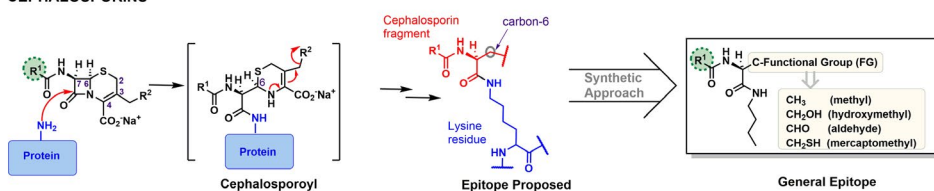
The pyrazinone determinant was designed according to our degradation hypothesis for α -aminocephalosporins after their protein conjugation,³ briefly summarized in Figure 1B. Haptenation occurs through nucleophilic opening of the β -lactam ring by amino groups of lysines via amide formation (Figure 1A). The resulting cephalosporoyl structure is unstable, which fragments the dihydrotriazine portion, arising multiple possible degradation structures. One likely structure exhibits the aldehyde functional group in the carbon 6, whose reactivity against the amino group of the R¹ side chain of α -aminocephalosporins provokes an intramolecular cyclization. The resulting six-member cycle oxidizes forming a stable pyrazinone. The synthetic pathway for patented compounds followed the Ugi/Deprotect/Cyclize strategy (Figure S1), affording target pyrazinone (1 and 2) with yields ranging from 25% to 99%.²⁻⁴

The *in vitro* specific recognition of pyrazinone derivatives in the context of allergy to α -aminocephalosporin was found in their immunological evaluation through competitive immunoassays

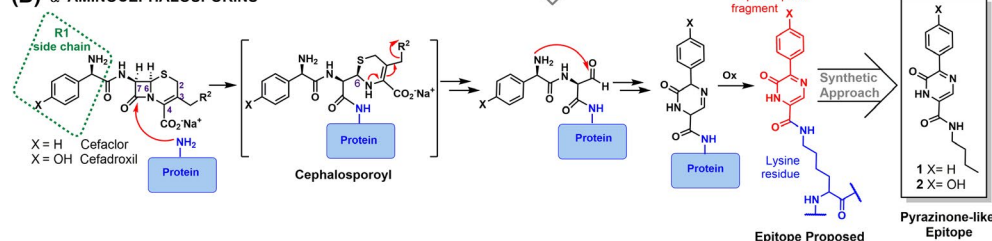
María José Torres and María Isabel Montañez are the authors with the same contribution.

© 2020 EAACI and John Wiley and Sons A/S. Published by John Wiley and Sons Ltd.

(A) CEPHALOSPORINS



(B) α-AMINOCEPHALOSPORINS



(C) Synthetic Determinants

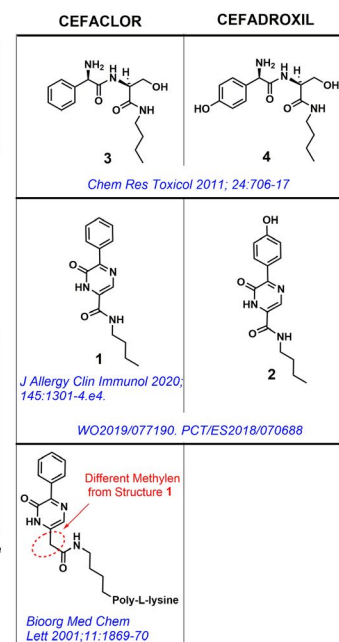


FIGURE 1 Cephalosporin epitope formation pathways and synthetic antigenic determinants. (A) Our hypothesis of how cephalosporins (general) haptenate proteins, and which are our proposed synthetic determinants; (B) hypothesized aminocephalosporin haptentation pathway, focusing on the reactivity of the additional amino group in R¹, and the resulting candidate target determinant, pyrazinone; (C) structures of the synthetic antigenic determinants reported for cefaclor and cefadroxil

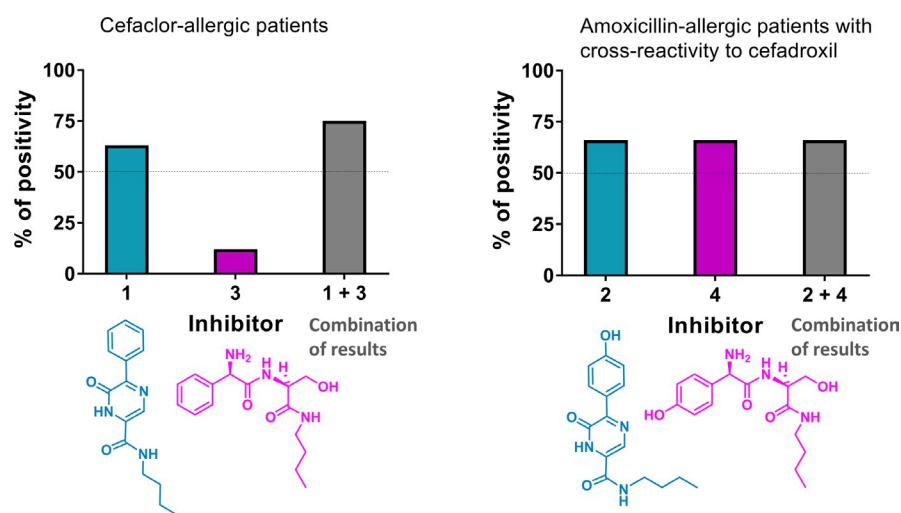


FIGURE 2 Immunological evaluation results with synthetic determinants obtained by competitive RAST inhibition. Comparisons among inhibitors: molecules 1 and 3 for cefaclor-allergic patients, molecules 2 and 4 for amoxicillin-allergic patients with cross-reactivity to cefadroxil and the combination of results, in percentages, of positive inhibition cases at 100 mM concentration

in patients allergic to these cephalosporins. Structure-activity relationship (SAR) studies were carried out for evaluating the recognition of different structures (previously described synthetic determinants (3 and 4) and novel pyrazinones (1 and 2) (Figure 1C)) through radioallergosorbent test (RAST) inhibition. The pyrazinone 1 was recognized in 63% of patients with cefaclor immediate reactions,³ whereas the pyrazinone 2 was recognized in 66% of amoxicillin-allergic patients with cross-reactivity to cefadroxil (Figure 2).² Cefadroxil is the most likely cephalosporin to cross-react with amoxicillin, since they share the same R¹ side chain. Moreover, no recognition of pyrazinone 2 was observed in

cefadroxil-tolerant patients with amoxicillin immediate reactions.⁴ The latter result could be explained because the generated pyrazinone 2 is exclusively derived from cefadroxil, and amoxicillin would not structurally be transformed in pyrazinone. According to these results, we hypothesize that pyrazinone 1 may be recognized by IgE specific to cefaclor or cefprozil (cephalosporins with equal R¹) but to a lesser extent by IgE specific to ampicillin (penicillin containing the same R¹). Further studies are still needed to evaluate whether this in vitro tool would represent a safe alternative to in vivo tests for determining cross-reactivity, since negative skin test is not enough for predicting tolerance.⁵

Based on the outstandingly observed specific IgE recognition from patients with α -aminocephalosporin immediate reactions,^{3,4} these pyrazinone structures could be potentially involved in the sensitization of aminocephalosporin-allergic reactions; therefore, we claimed the use of these structures to diagnose patients with β -lactam immediate reactions, especially for α -aminocephalosporins.²

2 | PATH TAKEN TO THE INVENTION

Structural studies to identify the drug epitope linked to the protein are difficult to perform.⁶ Our first attempt consisted of employing a simple amino compound, butylamine, as emulator of lysine residues in β -lactam haptentation.⁷ Butylamine-molecule simplicity was not enough to elucidate the chemical structures in the mixture of compounds resultant from cephalosporin conjugation, in which the presence of the R¹ was the only structural information available (by nuclear magnetic resonance) regarding epitopes.⁸ Using such series of monomeric cephalosporin-butylamine conjugates, immunochemical inhibition studies confirmed the importance of this side chain in specific IgE recognition.⁷ Further studies have involved more precise epitope structure elucidation through synthesis and immunological evaluation of well-defined proposed antigenic determinants for cephalosporins, which included the β -lactam fragment opened by butylamine, chosen as nucleophile to emulate the protein lysines (Figure 1A,B).^{8,9}

We tested the immunoreactivity of model synthetic determinants of several cephalosporins, consisting of R¹ and some portion of the dihydrothiazine ring with varied functional groups at the carbon 6, concluding that this determinant type defined reaction specificity, so that the strongest recognition occurred when the R¹ of a synthetic inhibitor matched that of the culprit cephalosporin. Additional cross-reactivity occurred with structurally related R¹. Moreover, the functional group in the carbon 6 refined IgE molecular recognition, being improved with hydroxymethyl and aldehyde functionalities compared with methyl group, whereas mercaptomethyl group showed no recognition.⁹ The immunological evaluation of such structures (3 and 4) with amino-R¹ side chain and hydroxymethyl functional group at carbon 6 in cefaclor-allergic patients, and in cefadroxil-allergic patients, indicated potential application for evaluating reactivities, although with different extent of recognition, 12% vs 66%, respectively (Figure 2).^{2,3}

Given the importance of IgE recognition of determinants with the aldehyde functionality at carbon 6, we considered intramolecular rearrangement with primary amino groups present at R¹ of cephalosporins. This immunochemical consideration was supported by a Venemalm study,¹⁰ in which the aminolysis of α -aminocephalosporins resulted in a pyrazinone derivative, although isolated with low yields. In the same study, for evaluating IgE recognition from cefaclor-allergic patients, a pyrazinone analog was synthesized for coupling to the ImmunoCAP solid phase that could determine pyrazinone-specific IgE antibodies in 60% of cefaclor-allergic patients.¹⁰

This analog is structurally different from this isolated pyrazinone: presenting an additional methylene moiety between the carrier and the pyrazinone ring.¹⁰ Since small differences in the chemical structures could have important implications in immunological recognition, we synthesized the precise cyclized structures (1 and 2) to evaluate their role in RAST inhibition studies, showing that 63%–66% aminocephalosporin-allergic patients were positive to their corresponding pyrazinone (1 or 2).^{2–4}

Achieving the structures 1 and 2 is not trivial; we previously tried other five different synthetic routes, involving each at least 8–10 time-consuming reaction steps, to reach the desired pyrazinone without success. Finally, the Ugi/Deprotect/Cyclize strategy enabled the efficient versatile synthesis of target molecules (Figure S1).^{2,3}

3 | CONCLUSIONS

Studies in sera from α -aminocephalosporin-allergic patients indicate a role for both pyrazinone structures (1 and 2) in specific IgE recognition. These pyrazinones are potential epitopes derived exclusively from α -aminocephalosporins. These structures are useful for in vitro diagnosis of patients suffering suspected allergic reactions to cephalosporins or penicillins containing α -amino-R¹ side chain, demonstrating to be promising tools for confirming cefaclor immediate reactions and determining in vitro cross-reactivity to cefadroxil in amoxicillin-allergic patients. Moreover, their evaluation as skin test reagents should be useful to further understand their impact in clinical practice.

ACKNOWLEDGEMENTS

The present study has been supported by the Institute of Health 'Carlos III' (ISCIII) of MINECO (grants cofunded by ERDF: 'Una manera de hacer Europa': Grant Nos. PI12/02529, PI15/01206, CP15/00103, PI17/01237 and PI18/00095; RETIC ARADyAL: RD16/0006/0001; RD16/0006/0012; and EuroNanoMed Program: AC19/00082); Spanish Ministerio de Economía, Industria y Competitividad (CTQ 2016-75870-P); Ministerio de Ciencia y Educación (PID 2019-104293GB-I00), Ministerio de Ciencia e Innovación [Proyectos de I+D+I Programación Conjunta Internacional, EuroNanoMed 2019 (PCI 2019-111825-2)]; Andalusian Regional Ministry of Economy and Knowledge, Junta de Andalucía (grants cofunded by ERDF: 'Andalucía se mueve con Europa': Grant No. CTS-06603 and UMA18-FEDERJA-007); Andalusian Regional Ministry of Health (Grant Nos. PI-0699-2011, PI-0179-2014 and PE-0172-2018, cofunded by ERDF); and 'Premio UNICAJA a la innovación en biomedicina y salud'. MIM holds a 'Miguel Servet I' (Grant No. CP15/00103). CM holds a 'Nicolas Monardes' by Andalusian Regional Ministry Health (Grant C-0044-2012 SAS2013). We thank Ms Claudia Corazza for her help with the English version of the manuscript.

CONFLICT OF INTEREST

The authors declare no other relevant conflicts of interest.

FUNDING INFORMATION

The group collaborates in research grants with Diater Laboratories (Madrid, Spain). Dr Martín-Serrano, Dr Mayorga, Dr Torres and Dr Montañez report grants from ISCIII and Junta de Andalucía, during the conduct of the study and have a patent WO2019/077190. PCT/ES2018/070688 issued. Dr Perez-Inestrosa reports grants from ISCIII, Junta de Andalucía and MINECO, during the conduct of the study and has a patent WO2019/077190. PCT/ES2018/070688 issued.

ORCID

Cristobalina Mayorga  <https://orcid.org/0000-0001-8852-8077>

María José Torres  <https://orcid.org/0000-0001-5228-471X>

Maria Isabel Montañez  <https://orcid.org/0000-0001-6641-5979>

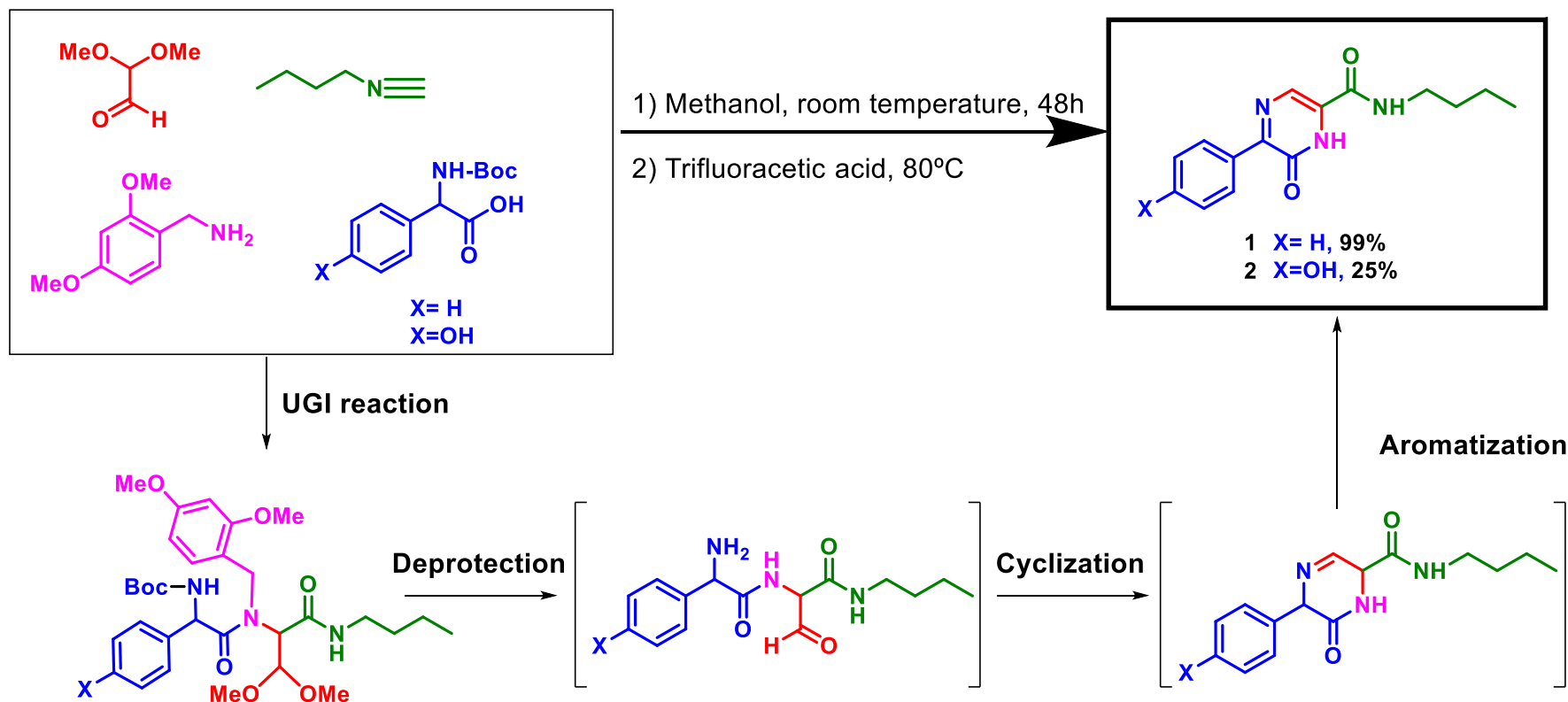
REFERENCES

1. Mayorga C, Celik G, Rouzaire P, et al. In vitro tests for Drug Hypersensitivity Reactions. An ENDA/EAACI Drug Allergy Interest Group Position Paper. *Allergy*. 2016;71:1103-1134.
2. Torres M, Montañez M, Mayorga C, Martín-Serrano A, Doña I, Perez-Inestrosa E. New pyrazinone compounds for the diagnosis of allergies to beta-lactam antibiotics. WO2019/077190. PCT/ES2018/070688. ES P201731240 (20.10.2017). WO2019/077190.
3. Martín-Serrano A, Mayorga C, Barrionuevo E, et al. Design of an antigenic determinant of cefaclor: Chemical structure-IgE recognition relationship. *J Allergy Clin Immunol*. 2020;145:1301-1304.
4. Bogas G, Mayorga C, Martín-Serrano Á, Fernández-Santamaría R, Jiménez-Sánchez IM, Ariza A, Barrionuevo E, Posadas T, Salas M, Fernández TD, Torres MJ, Montañez MI. Penicillin and cephalosporin cross-reactivity: role of side chain and synthetic cefadroxil epitopes. *Clinical and Translational Allergy*. 2020;10(1). <http://dx.doi.org/10.1186/s13601-020-00368-1>
5. Doña I, Romano A, Torres MJ. Algorithm for betalactam allergy diagnosis. *Allergy* 2019;74:1817-1819.
6. Perez-Inestrosa E, Suau R, Montañez MI, et al. Cephalosporin chemical reactivity and its immunological implications. *Curr Opin Allergy Clin Immunol*. 2005;5:323-330.
7. Antunez C, Blanca-Lopez N, Torres MJ, et al. Immediate allergic reactions to cephalosporins: Evaluation of cross-reactivity with a panel of penicillins and cephalosporins. *J Allergy Clin Immunol*. 2006;117:404-410.
8. Sanchez-Sancho F, Perez-Inestrosa E, Suau R, et al. Synthesis, characterization and immunochemical evaluation of cephalosporin antigenic determinants. *J Mol Recognit*. 2003;16:148-156.
9. Montañez MI, Mayorga C, Torres MJ, Ariza A, Blanca M, Perez-Inestrosa E. Synthetic Approach to Gain Insight into Antigenic Determinants of Cephalosporins. In Vitro Studies of Chemical Structure-IgE Molecular Recognition Relationships. *Chem Res Toxicol*. 2011;24:706-717.
10. Venemalm L. Pyrazinone conjugates as potential cephalosporin allergens. *Bioorg Med Chem Lett*. 2001;11:1869-1870.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

Synthetic Methodology



Supplementary Figure 1. Synthetic methodology follow to achieve hypothesised pyrazinone-type determinants derived from cefaclor (**1**) and cefadroxil (**2**). The protocol involves the Ugi/Deprotect/Cyclize strategy,^{1,2} shows efficiency and versatility for different side chains R¹.

References:

1. Azuaje J, El Maatougui A, Pérez-Rubio JM, Coelho A, Fernández F, Sotelo E. Multicomponent Assembly of Diverse Pyrazin-2(1H)-one Chemotypes. *J Org Chem*. 2013;78(9):4402-9.
2. Martín-Serrano A, Mayorga C, Barrionuevo E, Pérez N, Romano A, Moreno E, Ariza A, Pérez-Inestrosa E, Torres MJ, Montañez MI. Design of an antigenic determinant of cefaclor: Chemical structure–IgE recognition relationship. *J Allergy Clin Immunol*. 2020;145(4):1301-4.e4.