STUDIA UNIVERSITATIS BABEŞ-BOLYAI, CHEMIA, LIV, 4, 2009

NEW WAYS FOR OLD STRUCTURES

FLORIN DAN IRIMIE[a], CSABA PAIZS[a], MONICA TOSA[a], PAULA PODEA[a]

ABSTRACT. Classical heterocyclic structures of furan, benzofuran, dibenzofuran, phenothiazine and benzothiazole type, previously synthesized and studied by Professor Fărcăşan, were derivatized using cell- or enzyme-assisted regio-, chemo- and stereoselective techniques, providing compounds extremely difficult or even impossible to produce by traditional chemical methods. The present report underlines the high potential of biocatalysis to perform selective enantiomer- and enantiotope-transformations with remarkable conversion rates and purities.

Keywords: heterocycles, bezofuran, (furyl)benzothiazole, regioselectivity, chemoselectivity, enantiomer-selectivity, enantiotop-selectivity

INTRODUCTION

Heterocyclic systems of type benzofuran, benzothiazole, (furyl)benzothiazole, dibenzofuran are found as building-blocks for compounds with specific biological activities, as antimicrobial and antifungal agents [1 - 2] cytotoxic compounds [3], antidiabetics [4 - 5] etc.

These basic five membered heterocycles were prepared and functionalised thanks to the pioneering works of Professor Farcasanu research group in the period of 1950 – 1980' [6-14]. Thus, during the initial heterosynthesis state of art, really nice achievements had been accomplished using traditional means of synthesis and analysis.

Over the years, the above heterocyclic structures were much developed, as a need for diversifying their applicability area, for example by increasing the selectivity of biological impact of the products.

Nowadays, the insertion of the initial heterocyclic systems in new compounds with high structural complexity became possible, by means of highly selective techniques, with increased efficiency and compatibility with the environment. Among these techniques, enzymatic and cellular biocatalysis detaches through their advantages concerning activity and selectivity, which impose biocatalysis as a powerful tool with a reliable future.

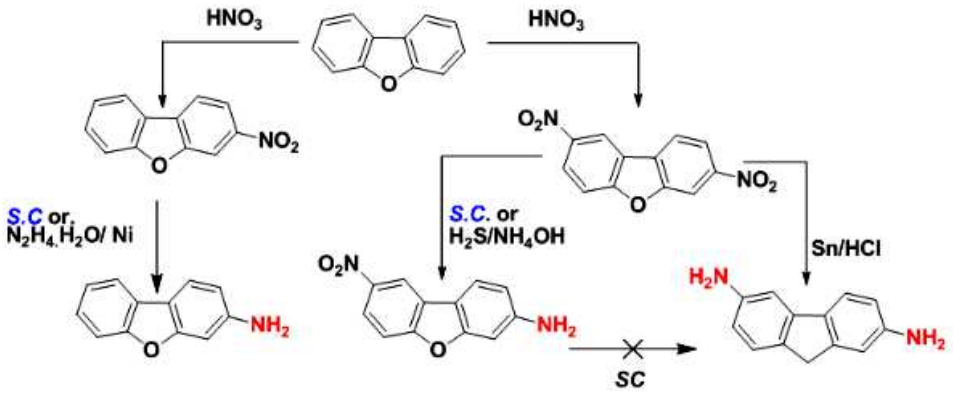
[a] *"Babes-Bolyai" University, Department of Biochemistry and Biochemical Engineering, 11 Arany Jànos st., 400 028 Cluj-Napoca, ROMANIA, irimie@chem.ubbcluj.ro*

The present dedicated mini-review is intended to highlight these recent advances in highly functionalised five membered heterocyclic chemistry, in a biochemical approach.

1. Regioselective processes

Regioselectivity is an undeniable advantage of enzymatic activity, making possible the discrimination and modification of chemically identical structures, located in different environments. For example, hydrolases express most evidently this property, being used for manipulation of polyfunctional bio compounds as carbohydrates [15], nucleosides [16], steroids [17]. Thus, Michael additions [18], hydroxylations [19 - 20] and several reduction reactions [21 - 22] are good examples of enzymatic regioselective processes.

In context, dibenzofuran was used as a substrate in the research group of Professor Fărcăşan. Thus, by nitration, 3-nitro-, and 3,8-dinitrobenzofuran were obtained. Reduction of 3-nitrodibenzofuran to 3-aminodibenzofuran and that of 3,8-dinitrobenzofuran to 3-amino-8-nitrodibenzofuran, in the presence of baker's yeast was accomplished by our group in 1997 (Scheme 1) [23].



Scheme 1

The chemoselective processes involve the selective transformation of just one functional group from two or more functionally related groups which may be present i) on different substrates (substrate chemoselectivity - chemospecificity) or ii) on the same substrate (product chemoselectivity or merely chemoselectivity) [24].

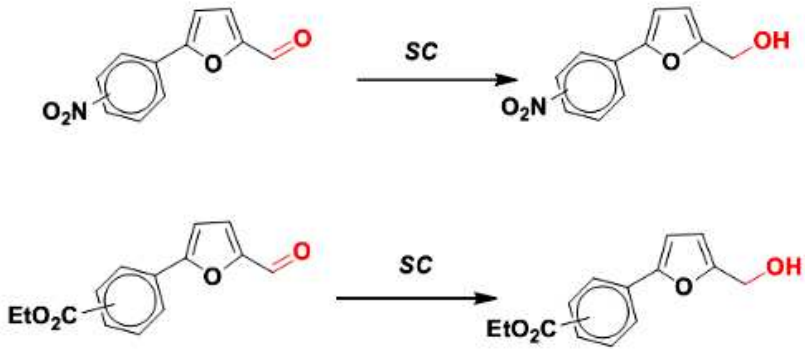
For example, Baker's yeast can selectively reduce the aldehyde group attached to a heterocyclic skeleton, such as (phenyl)furan, which has functional groups potentially vulnerable to the enzymatic equipment of yeast, nitro for reduction and ethoxycarbonyl for hydrolysis [23, 24] (Scheme 2).

2. Stereoselective processes

Stereoselectivity may be one of the major advantages of biocatalysis.

Through processes assisted by stereoselective biocatalysts, compounds with high enantiopurity can be produced. Enantiopurity is a necessary condition for bioactive compouds, because their two stereoisomers have different biological properties. At the same time, the impure nature of the unwanted enantiomer affects the economy of the synthesis. The preference of biocatalyst for a certain surface or enantiotope group in the substrate's structure conducts to the preferential forming of a certain enantiomeric product. This kind of preference defines *an enantiotope-selective process* and creates, from a symmetrical structure, a chiral one [24]. Through the preference of the biocatalyst for one of the two enantiomers of a racemic mixture, a converted enantiomer is obtained, while the other one, the unmodified enantiomer, remains in the medium. This process we entitled as *enantiomer-selective*.

Several illustrative examples of us will be discusssed hereafter.



Scheme 2

2.1. Enantiotope selective-processes

These processes allow desymmetrization of prochiral substrates, which posess either enantiotope faces or enantiotope groups, resulting in enantiopure compounds according to the selectivity of the biocatalyst.

Our group transformed 2-substituted (methyl, acetoxymethyl) benzothiazole keto-derivatives into the corresponding secondary alcohols by enantiotope-selective reduction of their prochiral carbonyl group. The biocatalyst used was Baker's yeast (*Saccharomyces Cerevisiae, SC*) which contains a complex dehydrogenase equipment, able to catalyze redox processes. Due to the presence of several dehydrogenases, some of them with opposite selectivity, the latter can be modulated by using certain additives, or by modifying the operating conditions, fermentative or non-fermentative, in order to optimise the working system.

There is a general rule, known as that of Prelog, which states that in the reduction of a prochiral ketone with a biocatalyst, a secondary alcohol with (*S*) configuration is formed. This rule can be explained by mean of the topology of the catalytic site of a dehydrogenase present in the yeast (Figure 1).

For example, reduction of (2-oxo-2-hetaryl)ethyl acetates **3a-d** and (2-hydroxy-1-hetaryl)ethanones **4a-d** (Scheme 3) was performed with opposite enantiotope preference affording, after optimization, excellent yields (92-94%) and good enantiomeric excesses (89-99%), for each enantiomer of the hetarylethane-1,2-diols **5a-d** [25]. One can note the fast hydrolysis of the intermediates (*R*)-(2-hydroxy-2-hetaryl)ethyl acetates, post slow reduction step, by the enzymatic equipment of Baker's yeast. Similar results we obtained in the case of 1-(benzofuran-2-yl)ethanols and ethane-1,2-diols [26]

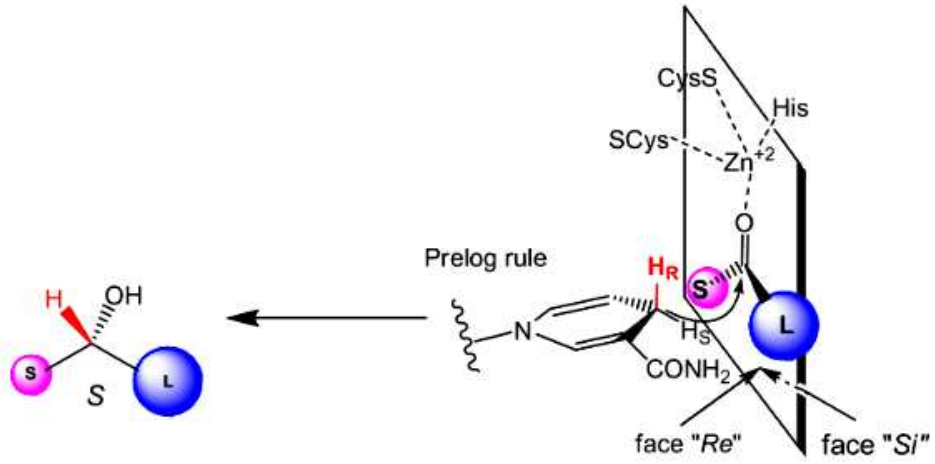
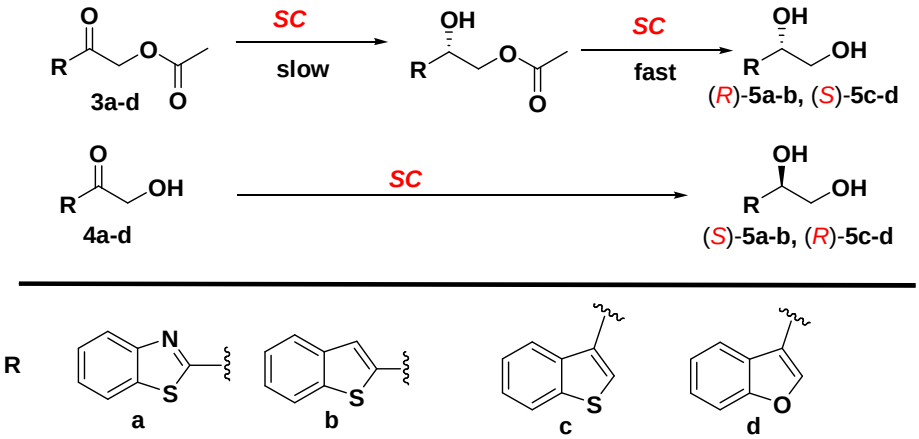


Figure 1. Topology of catalytic site of alcohol dehydrogenase from Baker`s yeast



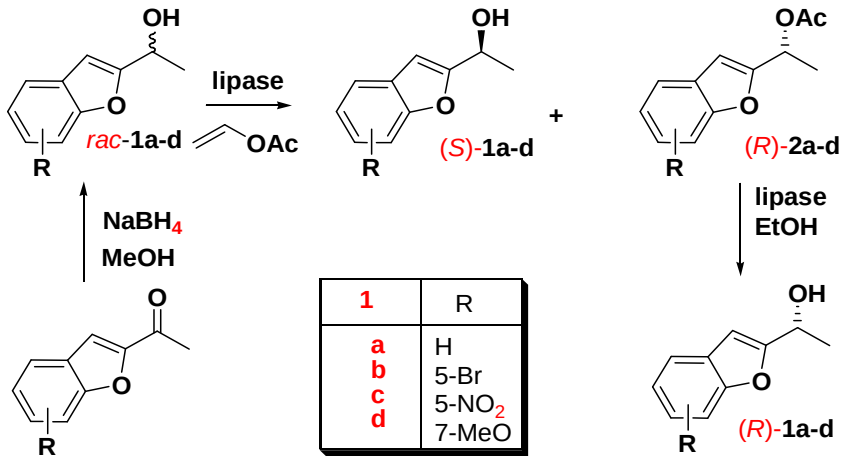
Scheme 3

2.2. Enantiomer selective processes

If biocatalysis assisted *enantiotope-selective processes* can be applied to prochiral substrates, *enantiomer-selective processes* are used to transform enantiomeric mixtures, most frequently racemic, in their nature. *Enantiomer-selectivity* implies that one enantiomer is converted faster than the other one. The reaction products will have different values of a certain separation property (solubility, melting point, etc.). So, the untransformed enantiomer can be easier separated from the reacted one. This system defines a kinetic resolution.

2.2.1. Kinetic resolution

An illustrative example of kinetic resolution is that of substituted 1-(benzofuran-2-yl)ethanols racemates (*rac*-**1a-d**, Scheme 4). They were synthesized upon unselective reduction of certain substituted 1-(benzofuran-2-yl)ethanones [27].

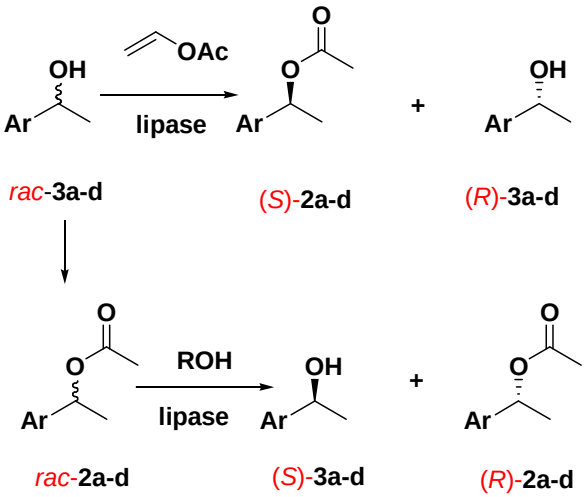


1	R
a	H
b	5-Br
c	5-NO_2
d	7-MeO

Scheme 4

The enantiopreference of enzyme for certain configuration can also be exploited for synthetic purposes [28].

Thus, starting from a racemic mixture, if enzyme is (*S*) selective, it will mediate only the acylation of a the (*S*) hetarylethanol, leaving the (*R*) alcohol unaffected (Scheme 5, the nature of heterocycles is the same as in Scheme 3). If the same enzyme will catalyze the hydrolysis of a racemic mixture of *O*-acylated hetarylethanols, such as *rac*-**2a-d**, the product will be the (*S*) alcohol, and the (*R*) ester remains untransformed. So, using racemates, *via* *O*-acylation, one can isolate the (*R*) alcohol, and by hydrolysis the (*S*) enantiomer becomes accessible. To conclude, by exploiting the conservation of enantiopreference of a lipase, one can obtain any of the two enantiomers, up to desire, from their racemic mixture, by mean a selective acylation or unselective acylation followed by a selective hydrolysis



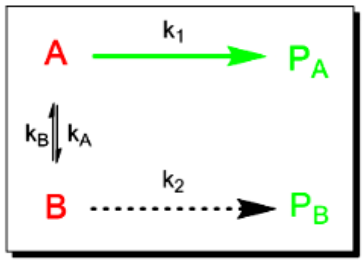
Scheme 5

However, the main drawback of kinetic resolution remains the maximum conversion as 50% calculated with respect to the racemic mixture.

2.2.2. Dynamic kinetic resolutions (DKR)

DKR are alternatives to kinetic resolutions, which eliminate the main disadvantage of kinetic resolutions: the limitation of conversion to 50%. Indeed the enatiopreference for one enantiomer leaves the other one unmodified, hence creating problems of i) separation (even if they are not as important as in the case of separation of enantiomers) and ii) in finding an applicability for the "non-preferred" enantiomer.

The DKR mechanism is simple and useful in systems for which one can find a reaction of racemization involving the starting enantiomers **A** and **B** (Scheme 6), [29 - 30].

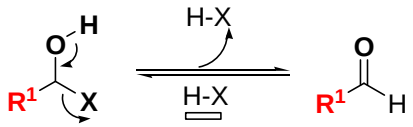


Scheme 6

If **A** is the preferred enantiomer by the biocatalyst, its concentration will be decreasing faster than that of **B**. Similarly, the concentration of product $\mathbf{P_A}$ will be increasing faster than the concentration of $\mathbf{P_B}$, these being the conditions for a successful kinetic resolution.

In contrast, if the system allows racemization of mixture of **A** and **B**, as **A** is consumed, a part of **B** is converted into **A**, to reestablish the equilibrium.

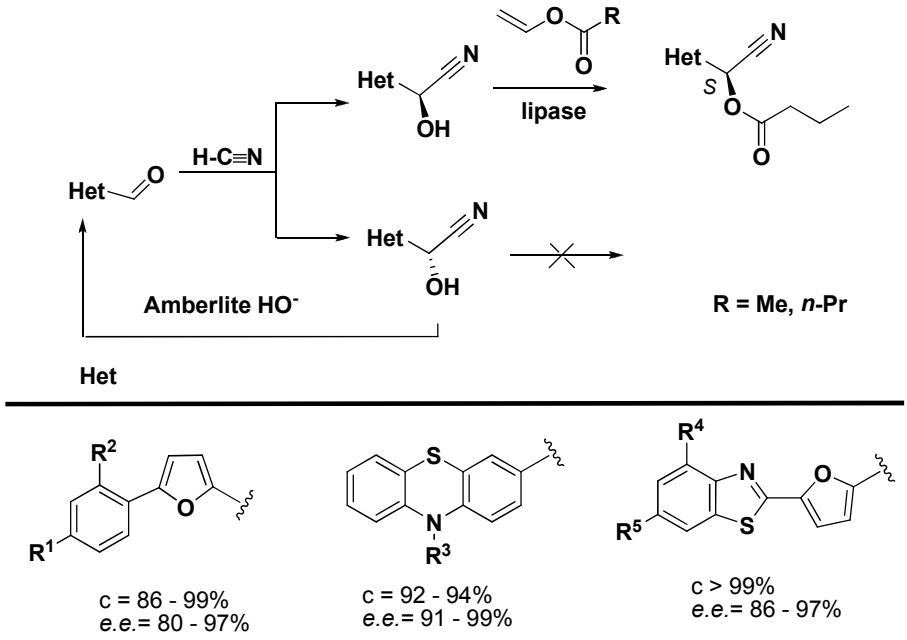
To setup an efficient DKR system as good selectivity, it is necessary that direct reaction(s), **A → P_A** and **B → P_B**, be irreversible. That is, the racemization rate should be greater than direct transformation rate, $k_{rac} > \max(k_1,k_2)$. Side reactions and product racemization should be avoided and generally, the **DKR** methodology is limited, so far, to compounds having a single stereocenter.



Scheme 7

The key feature of a DKR system is the racemization system. One of the most valuable systems are those in which, by sequence elimination – addition, such the one described in Scheme 7, the stereogenic center is firstly suppressed then recreated in a totally unselective manner. Another strategy can be used when, at the stereogenic center, one can find a "mobile proton" activated by an electron withdrawing group.

For instance, by applying the DKR mthodology, we were able to obtain α-(hetaryl)cyanomethyl acylates from the corresponding cyanohydrins (Scheme 8) in high enatiopure forms [31-33] with good conversions.

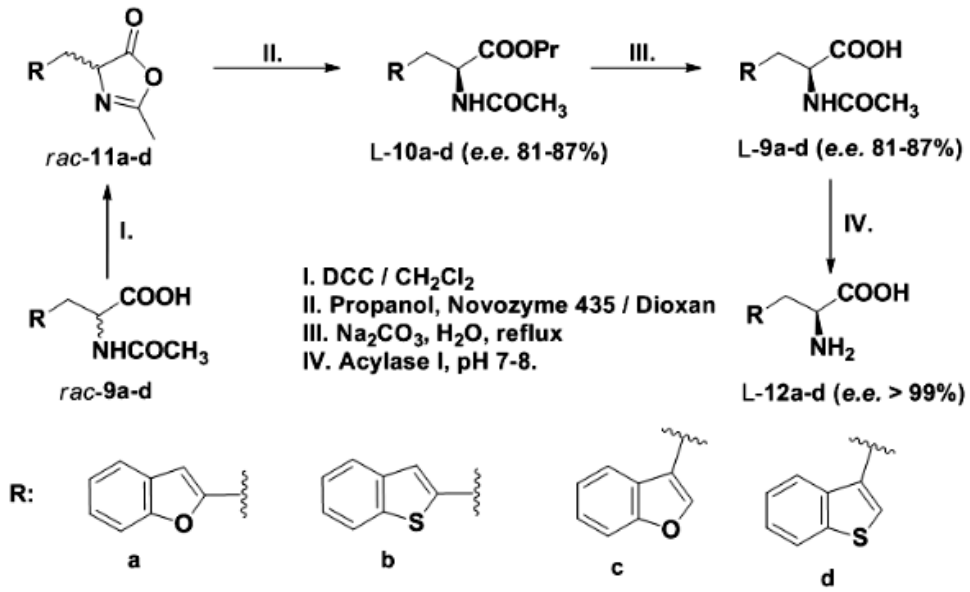


Scheme 8

The racemization step consisted of elimination – addition of hydrogen cyanide in the presence of a basic resin, Amberlite IRA-904.

The DKR we also used for the preparation of some hetaryl L-alanines of high enantiopurity [34] in a chemical and enzymatic sequence (Scheme 9). The racemization step in this DKR system had 4-(hetaryl)methyl-2-methyloxazol-5(4*H*)-ones, *rac*-**11a-d**, as a substrates [34]. We demonstrated that, due to the low pKa of their C-4 proton combined with a high reactivity against different nucleophiles, 2-substituted oxazol-5(4*H*)-ones are excellent substrates to undergo enzymatic dynamic kinetic resolution [35].

The oxazolone ring opening was performed through lipase-mediated alcoholysis. The L-hetaryl aminoacid was released after the basic hydrolysis of the ester bond (**III**, Scheme 9) followed by the enzymatic hydrolysis of the amidic bond. Consequently, this last enzymatic *N*-deprotection made a second chiral selection, promoting a final enantiopurity almost absolute. The global conversion of chemoenzymatic synthesis of enantiopure L-benzofuranyl- and L-benzo[*b*]thiophenyl alanines ranged between 76-85%. It was by far greater than the maximal theoretical conversion of enzymatic kinetic resolution assisted by Acylase I (50%).

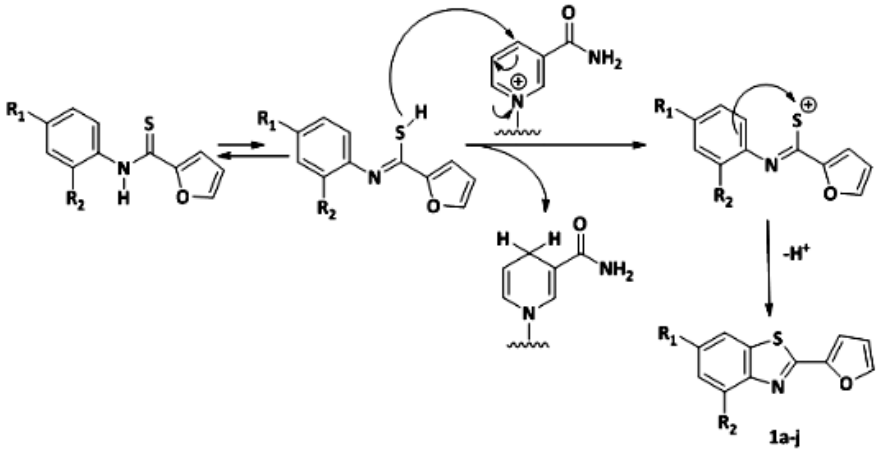


Scheme 9

3. Green processes

One of the most important features of a biocatalyst is its compatibility with the environment, face to the aggressivity of certain chemocatalysts and substrates. An illustrative example is provided by the cyclization of thiofuranilides assisted by *Saccharomyces Cerevisiae* (Scheme 10) [36].

The mechanism of cyclization involves the hydride ion transfer from thioenolic form to the NAD^+ cofactor. The chemical oxidant conventionally used is potassium dichromate. The conversions of the chemical *vs.* biochemical protocol are comparable [36].



Scheme 10

CONCLUSIONS

Biotransformations are versatile, environmentally friendly, selective ways for synthesis of organic compounds, particularly chiral ones with high enantiopurity, being able to replace toxic and environmental aggressive catalysts or reagents.

ACKNOWLEDGMENTS

We kindly wish to express here our gratitude to Professor Valer Fărcăşan, for his helpful advices and discussions along the years.

REFERENCES

1. F. Messina, M. Botta, F. Corelli, A. Paladino. *Tetrahedron: Asymmetry,* **2000**, *11*: 4895.
2. B.F. Abdel-Wahab, H.A. Abdel-Aziz, E.M. Ahmed. *European Journal of Medicinal Chemistry,* **2009**, *44*: 2632.
3. K. Kaniwa, T. Ohtsuki, Y. Yamamoto, M. Ishibashi. *Tetrahedron Letters,* **2006**, *47*: 1505.
4. L. Di Nunno, C. Franchini, A. Scilimati, M.S. Sinicropi, P. Tortorella. *Tetrahedron: Asymmetry,* **2000**, *11*: 1571.
5. H. Moreno-Díaz, R. Villalobos-Molina, R. Ortiz-Andrade, D. Díaz-Coutiño, J.L. Medina-Franco, S.P. Webster, M. Binnie, S. Estrada-Soto, M. Ibarra-Barajas, I. León-Rivera, G. Navarrete-Vázquez. *Bioorganic & Medicinal Chemistry Letters,* **2008**, *18*: 2871.

6. C. Bodea, V. Farcasan, I. Oprean. *Studii si Cercetari de Chimie,* **1965**, *13*: 1157.
7. C. Bodea, V. Farcasan, I. Oprean. *Revue Roumaine de Chimie,* **1966**, *11*: 1117.
8. C. Bodea, V. Farcasan, T. Panea. *Revue Roumaine de Chimie,* **1966**, *11*: 239.
9. V. Farcasan, F. Paiu. *Studia Universitatis Babes-Bolyai, Chemia,* **1966**: 107.
10. V. Farcasan, I. Mester. *Studia Universitatis Babes-Bolyai, Chemia,* **1967**, *12*: 69.
11. C. Bodea, V. Farcasan, T. Panea. *Revue Roumaine de Chimie,* **1968**, *13*: 971.
12. V. Farcasan, I. Balazs. *Studia Universitatis Babes-Bolyai, Chemia,* **1968**, *13*: 123.
13. V. Farcasan, F. Paiu. *Studia Universitatis Babes-Bolyai, Chemia,* **1968**, *13*: 103.
14. V. Farcasan, S. Florea. *Journal fuer Praktische Chemie (Leipzig),* **1970**, *312*: 1007.
15. A. Xia, Q. Wu, B. Liu, X. Lin. *Enzyme and Microbial Technology,* **2008**, *42*: 414.
16. N. Li, M.-H. Zong, D. Ma. *Tetrahedron,* **2009**, *65*: 1063.
17. M.M. Cruz Silva, S. Riva, M.L. Sá e Melo. *Tetrahedron,* **2005**, *61*: 3065.
18. J.-L. Wang, J.-M. Xu, Q. Wu, D.-S. Lv, X.-F. Lin. *Tetrahedron,* **2009**, *65*: 2531.
19. M. Rahman, C. Hayes Sutter, G.L. Emmert, T.R. Sutter. *Toxicology and Applied Pharmacology,* **2006**, *216*: 469.
20. C. Roh, S.-H. Seo, K.-Y. Choi, M. Cha, B.P. Pandey, J.-H. Kim, J.-S. Park, D.H. Kim, I.S. Chang, B.-G. Kim. *Journal of Bioscience and Bioengineering,* **2009**, *108*: 41.
21. C.L. Davey, L.W. Powell, N.J. Turner, A. Wells. *Tetrahedron Letters,* **1994**, *35*: 7867.
22. K. Nakamura, S.-i. Kondo, Y. Kawai, K. Hida, K. Kitano, A. Ohno. *Tetrahedron: Asymmetry,* **1996**, *7*: 409.
23. F.-D. Irimie, C. Paizs, M.-I. Tosa, C. Afloroaei, V. Miclaus. *Heterocyclic Communications,* **1997**, *3*: 549.
24. F.D. Irimie, C. Paisz, M. Tosa. "Biotransformari in sinteza organica, aspecte fundamentale". Napoca Star, **2006**
25. P.V. Podea, C. Paizs, M.I. Tosa, F.D. Irimie. *Tetrahedron Asymmetry,* **2008**, *19*: 1959.
26. C. Paizs, M. Tosa, C. Majdik, P. Moldovan, L. Novak, P. Kolonits, A. Marcovici, F.-D. Irimie, L. Poppe. *Tetrahedron Asymmetry,* **2003**, *14*: 1495.
27. C. Paizs, M. Tosa, V. Bodai, G. Szakacs, I. Kmecz, B. Simandi, C. Majdik, L. Novak, F.-D. Irimie, L. Poppe. *Tetrahedron Asymmetry,* **2003**, *14*: 1943.
28. M. Tosa, S. Pilbak, P. Moldovan, C. Paizs, G. Szatzker, G. Szakacs, L. Novak, F.-D. Irimie, L. Poppe. *Tetrahedron Asymmetry,* **2008**, *19*: 1844.
29. H. Pellissier. *Tetrahedron,* **2008**, *64*: 1563.
30. A. Kamal, M.A. Azhar, T. Krishnaji, M.S. Malik, S. Azeeza. *Coordination Chemistry Reviews,* **2008**, *252*: 569.
31. C. Paizs, P. Taehtinen, M. Tosa, C. Majdik, F.-D. Irimie, L.T. Kanerva. *Tetrahedron,* **2004**, *60*: 10533.
32. C. Paizs, M. Tosa, C. Majdik, P. Tahtinen, F.D. Irimie, L.T. Kanerva. *Tetrahedron Asymmetry,* **2003**, *14*: 619.
33. C. Paizs, P. Tahtinen, K. Lundell, L. Poppe, F.-D. Irimie, L.T. Kanerva. *Tetrahedron Asymmetry,* **2003**, *14*: 1895.
34. P.V. Podea, M.I. Tosa, C. Paizs, F.D. Irimie. *Tetrahedron Asymmetry,* **2008**, *19*: 500.
35. S.A. Brown, M.-C. Parker, N.J. Turner. *Tetrahedron: Asymmetry,* **2000**, *11*: 1687.
36. C. Paisz, C. Majdik, M. Tosa, R. Misca, F.D. Irimie. *Romanian Biotechnological Letters,* **2001**, *6*: 325.