Introduction & Aims

Organic syntheses design and execution urges the development of novel solutions towards improved efficiency in terms of higher yields and conversions, better selectivities, less number of synthetic steps applied, lower costs and easy reaction performance. The synthesis of organic compounds are typically accomplished in a step-by-step fashion, moreover, each products of the individual steps are often subjected to complex purification procedures, then the isolated intermediates are used as further starting materials of the subsequent synthetic step. The synthetic design based on domino approaches can serve as a useful alternative for the above mentioned problems. Domino reactions are defined as one-pot processes of two or more bond-forming reactions under identical conditions, in which the subsequent transformation takes place at the functionalities obtained in the former transformation. Multicomponent reactions (MCRs) are considered to be a subclass of domino reactions, whereby more than two reactants combine in a one-pot sequential manner to give complex products with excellent chemo-, regio-, and stereoselectivities. In addition, the isolation of the intermediates contrary to the conventional synthetic methodologies is not necessary. Importantly, a high level of structural diversity can be achieved using MCR approaches by varying the components arbitrarily.

We aimed to develop specific synthetic routes towards novel pharmacophore *O*-, and *N*,*N*-heterocyclic scaffolds, moreover modified curcumin derivatives based on one-pot multicomponent and/or domino synthetic strategies.

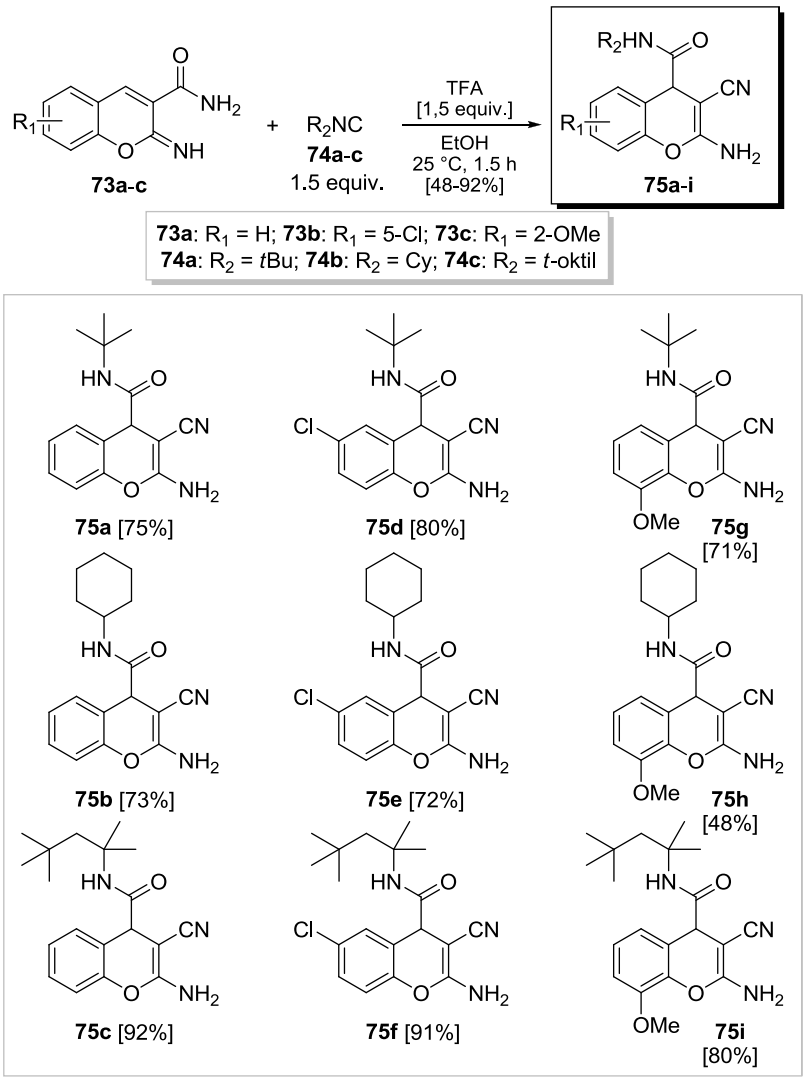
Materials & Methods

Reactions were carried out in 1-40 millimole scale, and products were purified either by means of column chromatography or simple filtration and recrystallization. All new compounds were characterized by melting points, IR and NMR (^{1}H and ^{13}C) spectroscopy, and mass spectrometry (ESI). The model compounds were characterized by using one- or two-dimensional NMR spectroscopies (^{1}H/^{13}C NMR, COSY, NOESY, HSQC, HMBC). The microwave-assisted reactions were performed in a CEM Discover^{TM} Microwave System. All chemicals and solvents were of commercial grade (Sigma-Aldrich, Alfa Aesar, AK Scientific) and used without further purification. The *in vitro* anti-proliferative activity of the synthesized curcumin analogs was tested by Avidin Ltd.

Results*

1. Synthesis of novel six-membered fused heterocycles[1]

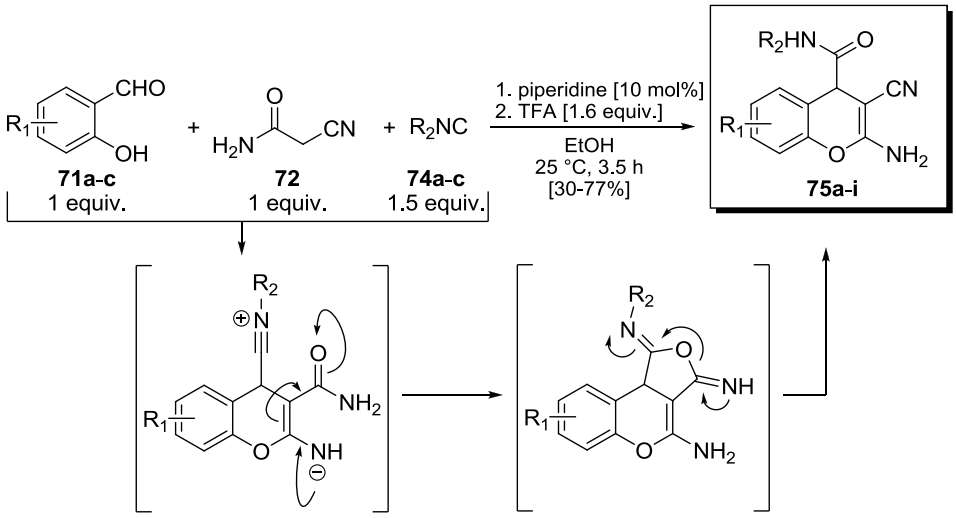
1.1. In the initial stage of the work we investigated whether 2-imino-chromene-3-carboxamides **73a-c**, possessing iminolacton, activated alkene, as well as 1,3-azadiene motifs can be subjected to a specific 1,4-conjugate addition/*O*-trapping rearrangement sequence with isocyanides **74a-c**. A model reaction was chosen in order to adjust the reaction parameters by testing both Lewis- and Brønsted acids and examining the solvent effect. Upon tuning the reaction conditions (1.5 equivalents of TFA and isocyanide each, EtOH, RT), the synthesis of nine novel 2-amino-3-cyano-4-carboxamide-4*H*-chromene frameworks **75a-i** was completed in moderate to good yields (48-92%) by means of three isocyanide and three 2-iminochromene building blocks (Scheme 1.).



Scheme 1.

* Compound numbering is identical to that applied in the thesis.

1.2. Besides the development of a novel isocyanide-based approach, the 2-amino-3-cyano-4*H*-chromene-4-carboxamide derivatives **75a-i** were also synthesized *via* a TFA promoted one-pot three-component reaction of salicylaldehydes **71a-c**, cyanoacetamide (**72**) and isocyanides **74a-c** with overall yields up to 77% (Scheme 2.).



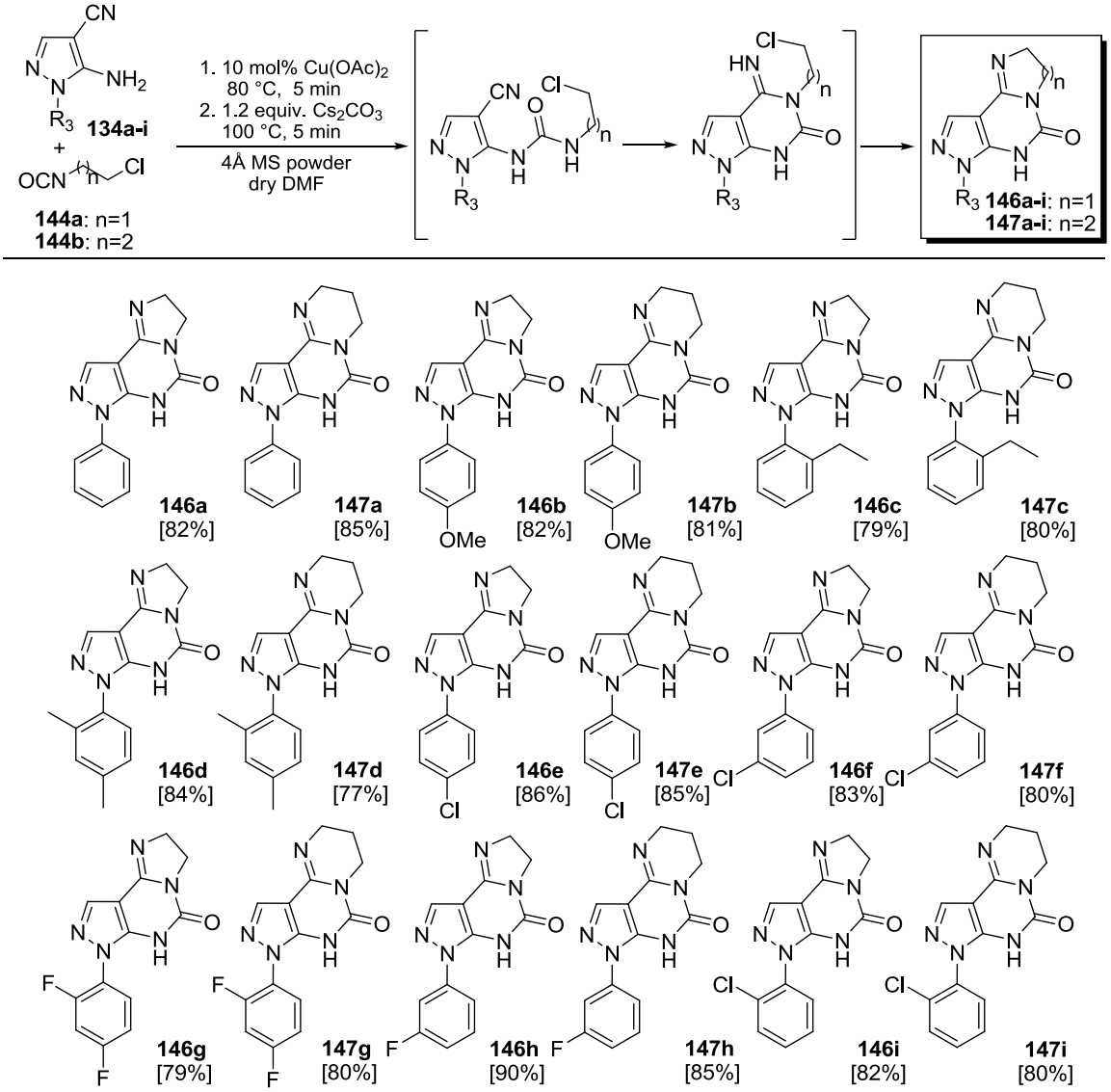
Scheme 2.

The tandem multicomponent reaction can be elucidated as a one-pot Knoevenagel condensation/intramolecular Pinner reaction followed by an isocyanide 1,4-conjugate addtion/intramolecular *O*-trapping rearrangement (carboxamide→carbonitrile conversion) sequence.

2. Synthesis of novel *N,N*-heterocycles *via* domino reactions[2]

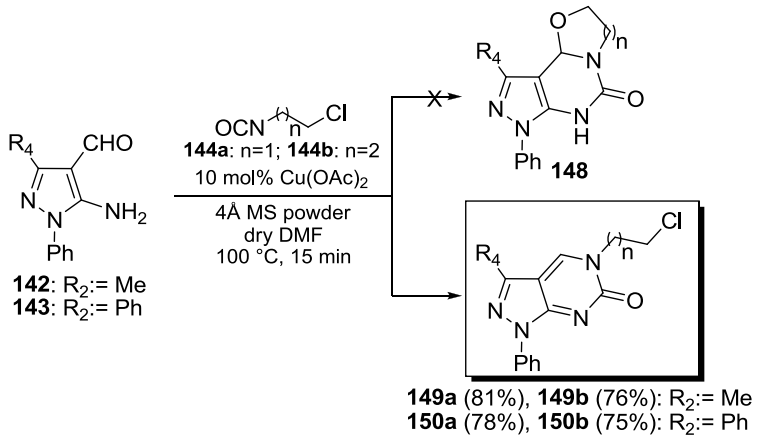
2.1. The second experimental session deals with the domino annulations of 5-amino-1-aryl-1*H*-pyrazol-4-carbonitriles **134a-i** with chloroalkyl isocyanates **144a** and **144b** (Scheme 3.). We investigated the catalytic activation of isocyanates towards amine-isocyanate coupling by Lewis acids due to the extremely low nucleophilicity of 5-aminopyrazoles observed in the course of the project. Followed by an extensive catalyst screen, the use of catalytic amount of copper(II)-acetate proved to be highly efficient. Due to the excellent conversion and short reaction time of the copper(II)-catalyzed urea formation observed, further efforts were focused on the one-pot realization of the domino process without isolating the corresponding intermediates. Moreover, the effect of various basic additives was examined in order to trigger the domino ring-closure process. The use of Cs_2CO_3 in a one-pot fashion was found to be the most efficient way to obtain pyrazole-fused imidazo[1,2-*c*]pyrimidinones. By using the well-established and rapid one-pot protocol (10 mol% of $Cu(OAc)_2$ and 1.2 equivalent of Cs_2CO_3,

10 min), an 18-membered novel pyrazolo[3,4-*d*]pyrimidine library **146a-i** and **147a-i** was successfully generated with isolated yields in the range 77-90% (Scheme 3.).



Scheme 3.

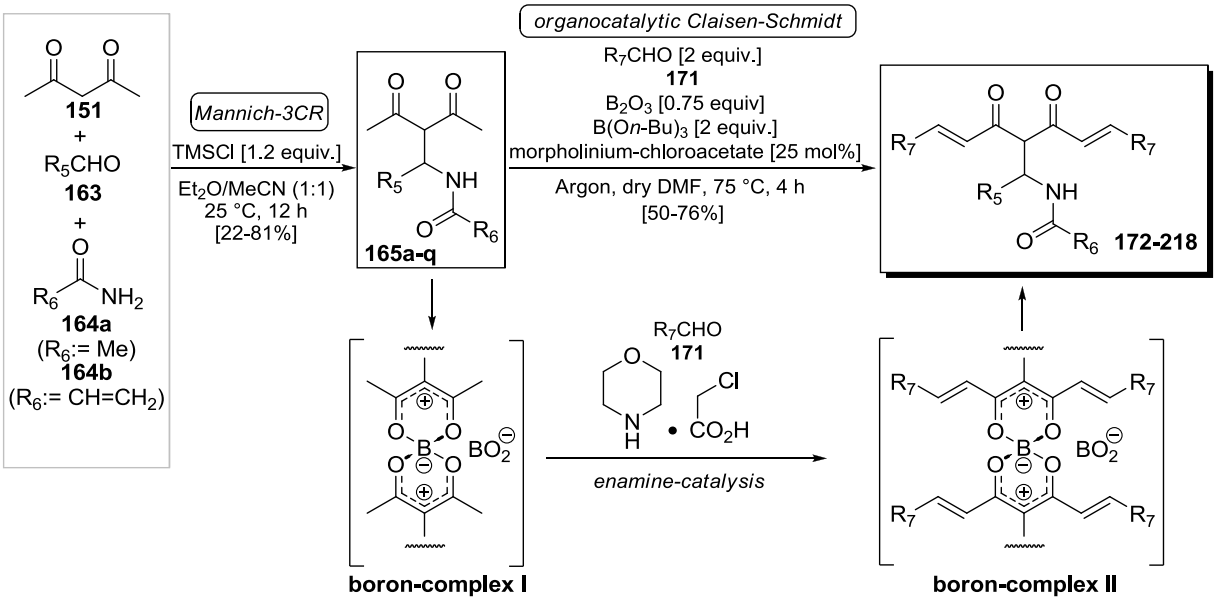
2.2. Experiments were then performed to reveal whether β-aminocarbaldehydes **142** and **143** as bifunctional building blocks can be subjected to a copper(II)-catalyzed domino transformation with chloroalkyl isocyanates **144a** and **144b**. Interestingly, the formation of bicyclic pyrazolo[3,4-*d*]pyrimidine-6(5*H*)-one species **149a**, **149b**, **150a** and **150b** were observed instead of the anticipated tricycles **148** when pyrazole-based β-aminocarbaldehydes **142** and **143** were employed. Nevertheless, the mechanisms of the copper-catalyzed amine–isocyanate coupling and the following domino processes were proposed based on an isocyanate electrophilic activation through mono- and dinuclear copper(II)-transition complexes.



Scheme 4.

3. Synthesis of novel curcumin derivatives[3 and 4]

3.1. The final experimental part describes the synthesis of 47 novel *C*-4 substituted curcumin derivatives **172-218**. In order to prove the appropriate structural diversity, the precursor β-acylamide-1,3-diones **165a-q** were synthesized *via* a modified Mannich three-component reaction in yields of 22-81% (Scheme 5.).

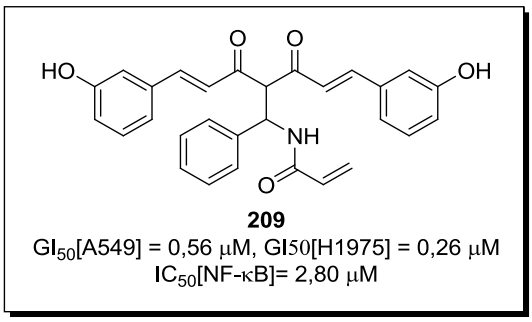


Scheme 5.

In addition, a Mannich intermediate possessing an activated terminal double bond was subjected to Heck-coupling to demonstrate further variability. Afterwards, the construction of the curcumin backbone was achieved through an improved morpholinium-chloroacetate-catalyzed double aldol condensation (Claisen-Schmidt reaction). Based on the well-established protocol and by modifying R_5, R_6 and R_7 diversity points, a series of novel curcuminoids **172-218** were synthesized in moderate to good yields (50-76%) (Scheme 5.).

The proposed mechanism includes the *in situ* formation of a spirocyclic boron-complex, the dynamic ring-opening and closure of the boron-complex, and a secondary amine salt-catalyzed multiple aldol condensation *via* enamine activation.

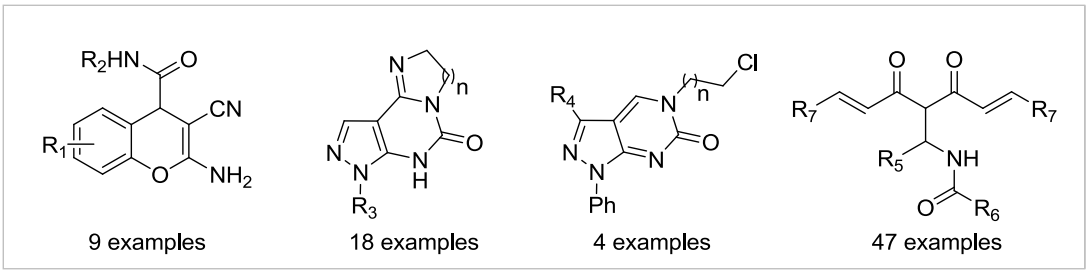
3.2. The *in vitro* anti-proliferative activity of the synthesized curcumin analogs **172**-**218** was tested by Avidin Ltd. in A549 and H1975 lung adenocarcinoma cells using viability assays. Furthermore, a strong correlation of the cytotoxic potential of the analogs **172**-**218** was found followed by the detection of TNFα-induced NF-κB inhibition *in vitro*. Structure-activity relationships were established based on the obtained *in vitro* data, and the most prominent member of the library **209** was assigned as lead compound (GI_{50}[A549] = 0.56 μM, GI_{50}[H1975] = 0.26 μM, NF-κB inhibition (IC_{50}= 2.80 μM)) for further investigations (Scheme 6.).



Scheme 6.

3.3. In addition, the scale-up trials of the lead compound in ≤40 mmol range were successfully accomplished in isolated yield of 60% and with excellent NMR purity (>98%). It should be emphasized, that only simple filtration and recrystallization was applied during the work-up procedure.

In the course of my PhD work, the synthetic elaboration of 78 novel compounds, including *O*- and *N*,*N*-heterocycles (4*H*-chromenes, pyrazolo[3,4-*d*]pyrimidinones), as well as *C*-4 modified curcumin species using one-pot multicomponent and domino synthetic strategies were accomplished (Scheme 7.).



Scheme 7.

List of publications related to the thesis:

1. **Synthesis of 2-Amino-3-cyano-4*H*-chromene-4-carboxamide Derivatives by an Isocyanide-Based Domino Conjugate Addition/O-Trapping Rearrangement Sequence**
   M. Gyuris, R. Madácsi, L. G. Puskás, G. K. Tóth, J. Wölfling, I. Kanizsai
   European Journal of Organic Chemistry, **5**, 848-851, (2011).
   IF: 3.329
2. **Synthesis of novel pyrazole-based heterocycles via a copper(II)-catalyzed domino annulation**
   M. Gyuris, L. G. Puskás, G. K. Tóth, I. Kanizsai
   Organic & Biomolecular Chemistry, **11**(37), 6320-6327, (2013).
   IF: 3.568 (2012)
3. **Új gyógyhatású vegyületek - Szubsztituált kurkumin származékok, eljárás előállításukra és ezeket tartalmazó gyógyszerkészítmények**
   (hungarian patent application)
   M. Gyuris, L. Puskás, I. Kanizsai, B. Ózsvári, L. Hackler, L. I. Nagy
   P1100532**,** 2011.09.23.
4. **Novel medicinal compounds**
   (international patent application)
   M. Gyuris, L. Puskás, I. Kanizsai, B. Ózsvári, L. Hackler, L. I. Nagy
   PCT Int. Appl. (2013), WO2013041895A1

List of other publications:

5. **Use of trifluoro phthalimides for the treatment of cancerous diseases**
   (international patent application)
   L. Puskás, I. Kanizsai, M. Gyuris, R. Madácsi, B. Ózsvári, L. Fehér, G. Fábián, K. Kitajka
   PCT Int. Appl. (2012), WO2012085608A2

6. **8-Hydroxy-quinoline derivatives**

   (international patent application)

   L. Puskás, C. Szabó, I. Kanizsai, M. Gyuris, R. Madácsi, B. Ózsvári, L. Fehér, G. Tamás

   PCT Int. Appl. (2011), WO2011148208A1

7. **Aromatic Sulfonamides Containing a Condensed Piperidine Moiety as Potential Oxidative Stress-Inducing Anticancer Agents**

   R. Madácsi, I. Kanizsai, L. Z. Fehér, M. Gyuris, B. Ózsvári, A. Erdélyi, J. Wölfling, L. G. Puskás

   Medicinal Chemistry, **9**(7), 911-919, (2013).

   IF: 1.373 (2012)

8. **Q50, an iron-chelating and zinc-complexing agent, improves cardiac function in rat models of ischemia/reperfusioninduced myocardial injury**

   S. Korkmaz, E. Barnucz, S. Loganathan, S. Li, T. Radovits, P. Hegedűs, A. Zubarevich, K. Hirschberg, A. Weymann, L. G. Puskás, B. Ózsvári, N. Faragó, I. Kanizsai, G. Fábián, M. Gyuris, B. Merkely, M. Karck, C. Szabó, G. Szabó.

   Circulation Journal, **77**(7), 1817-1826, (2013).

   IF: 3.578 (2012)

9. **Polyunsaturated fatty acids synergize with lipid droplet binding thalidomide analogs to induce oxidative stress in cancer cells**

   L. G. Puskás, L. Z. Fehér, C. Vizler, F. Ayaydin, E. Rásó, E. Molnár, I. Magyary, I. Kanizsai, M. Gyuris, R. Madácsi, G. Fábián, K. Farkas, P. Hegyi, F. Baska, B. Ózsvári, K. Kitajka

   Lipids in health and disease, **9**:56, (2010).

   IF: 2.239

10. **A cell-microelectronic sensing technique for the screening of cytoprotective compounds**

    B. Ózsvári, L. G. Puskás, L. I. Nagy, I. Kanizsai, M. Gyuris, R. Madácsi, L. Z. Fehér, D. Gerő, C. Szabó

    International Journal of Molecular Medicine, **25**(4), 525-530, (2010).

    IF: 1.814

Total impact factor: 15.901

Conferences:

- ***Lecture*: Unexpected one-pot phosphine triggered domino synthesis of novel trisubstituted dihydropyridin-2-one species**
  M. Gyuris
  5th ORCA Meeting & Training School
  Alicante, Spain, **2013**.

- ***Poster*: Synthesis of α-aminosulfonyl amide peptidomimetics via an Ugi three-component reaction**
  M. Gyuris, L. G. Puskás, G. K. Tóth, I. Kanizsai
  13th Belgian Organic Synthesis Symposium (BOSS XIII)
  Leuven, Belgium, **2012**.

- ***Poster*: Synthesis of novel pyrazole-based heterocycles via a copper(II)-catalyzed domino annulation**
  M. Gyuris, L. G. Puskás, G. K. Tóth, I. Kanizsai
  13th Tetrahedron Symposium - Challenges in Bioorganic & Organic Medicinal Chemistry
  Amsterdam, Netherlands, **2012**.

- ***Poster*: Multicentrumú imino-kromén-karboxamidok reakciója izocianidokkal**
  Gyuris M., Kanizsai I., Madácsi R., Puskás L. G., Tóth G. K., Wölfling J.
  Vegyészkonferencia és 53. Magyar Spektrokémiai Vándorgyűlés
  Hajdúszoboszló, Hungary, **2010**.

- ***Lecture*: Multicentrumú imino-kromén-karboxamidok reakciója izocianidokkal**
  Gyuris M.
  XXXIII. Kémiai Előadói Napok
  Szeged, Hungary, **2010**.

- ***Poster*: Lipid droplet interacting ER-stress inducing amino-trifluoro-phthalimides as cytotoxic agents**
  I. Kanizsai, M. Gyuris, L. Z. Fehér, B. Ózsvári, E. Molnár, G. Fábián, R. Madácsi, L. G. Puskás
  16th European Symposium on Organic Chemistry - ESOC 2009
  Prague, Czech Republic, **2009**.