Application of linear and multicomponent reaction pathways towards the construction of pharmacophore libraries, from vessel to the main goal

Thesis of Ph.D. dissertation

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Szeged

2021

„A kutatás vagy fejlesztés célratörő tanulás és kreatív munka összehangolt egysége.”

(Simonyi Károly)

1. Introduction and aims

The main ambition of each medicinal chemist is the successful access towards a drug candidate by incorporating the use of suitable organic chemistry techniques and conscious construction of a diverse and high-membered chemical library supported by relevant Structure–Activity Relationship (SAR).

In regard to diseases possessing multifactorial pathophysiological background (existing on some types of tumor and neurodegenerative disease), the phenotypical approach is an optimal solution for the research instead of high-throughput screening (HTS) or other mechanistic attempts. The priority of this execution includes the verification of effectiveness of the hit compounds based on the preliminary *in vitro* study followed by *in vivo* investigation. After sufficient information in hand, the background/mechanism of their efficacy is revealed by the identification of possible target docking sites. Through a hit-to-lead drug discovery, the morphological/structural modification of selected hit compounds affords the lead compound, which could turn into a drug candidate. However, a suitable aspirant should have metabolic stability, less non-desired side effects as well as feasible bioavailability.

Our aims can be outlined as the design and synthesis of small-membered heterocyclic libraries with antitumor features exploiting linear (S_N, S_NAr) and multicomponent reaction routes (Gewald-3CR and Betti-3CR).

The research conception is based on the following three pillars:

1. Synthesis of piperidine-based heterocycles involving 1,2,3,4-tetrahydro(iso)quinoline, 2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole, and 4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine ring systems modified with sulfonamide moieties, as well as morphological optimalization followed by SAR utilizing linear and multicomponent reactions (S_N reactions and Gewald-3CR).
2. Improvement of conventional S_NAr and purification protocol to observe the less-favored but more bioefficient phthalimide regioisomers in the assemblies of perfluorinated isoindolin-1,3-diones and primary/secondary amines.
3. Utilization of Betti-3CR to construct a feasible C–7 (*ortho*)-substituted 8-hydroxiquinoline (8-HQ) library.

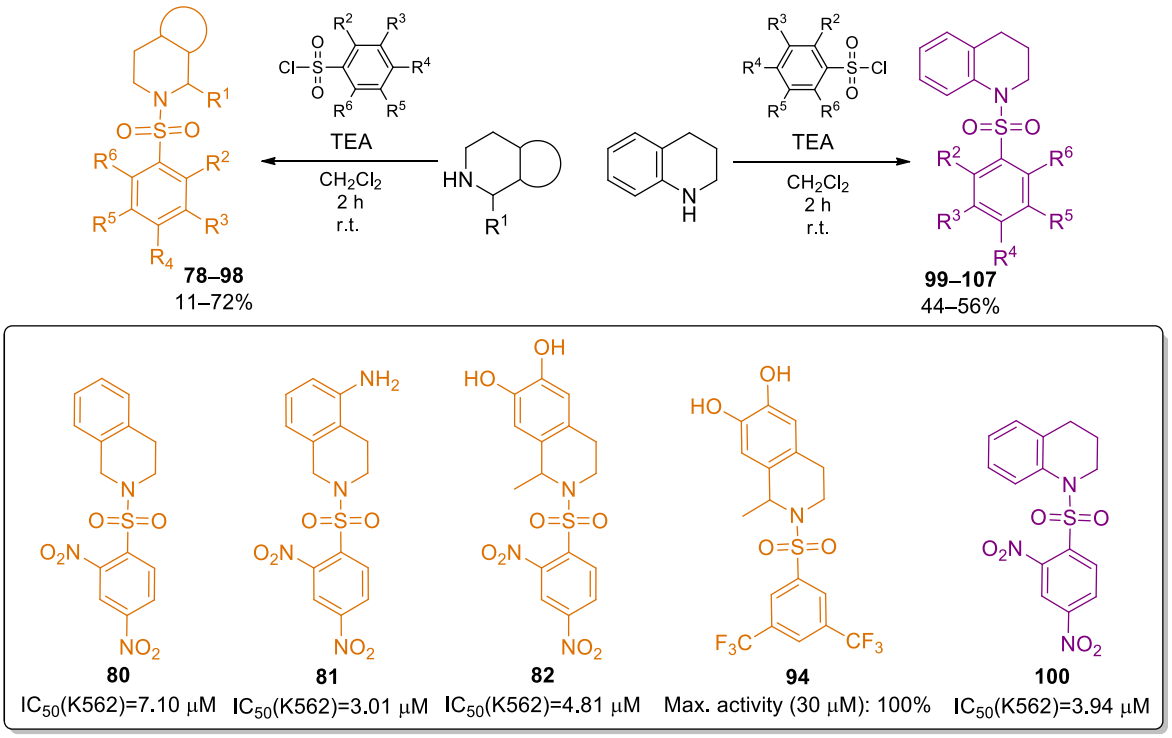
2. Materials and methods

In the course of the synthetic work, the majority of the reactions were performed in the millimolar scale. Transformations were monitored by either thin-layer chromatography or

HPLC analyses. Products were purified by column chromatography (silica gel 40–63 or 60–200 μm), flash chromatography (Teledyne Isco CombiFlash® R_f, Redisept™ silica column) or utilizing simple filtration and/or recrystallization. The molecular structures of products were determined by one- and two-dimensional techniques combined with mass spectrometric measurements. Commercially available reagents used in my study were purchased from Sigma-Aldrich, Alfa Aesar, AK Scientific, Fluorochem, Combi-Blocks, Apollo Scientific, and Molar and they were used without any further purification. The biological assays were performed by Avidin's co-workers. The establishment of IC_{50} values and SAR were carried out in collaboration using the GraphPad Prism 4 software.

3. Results*

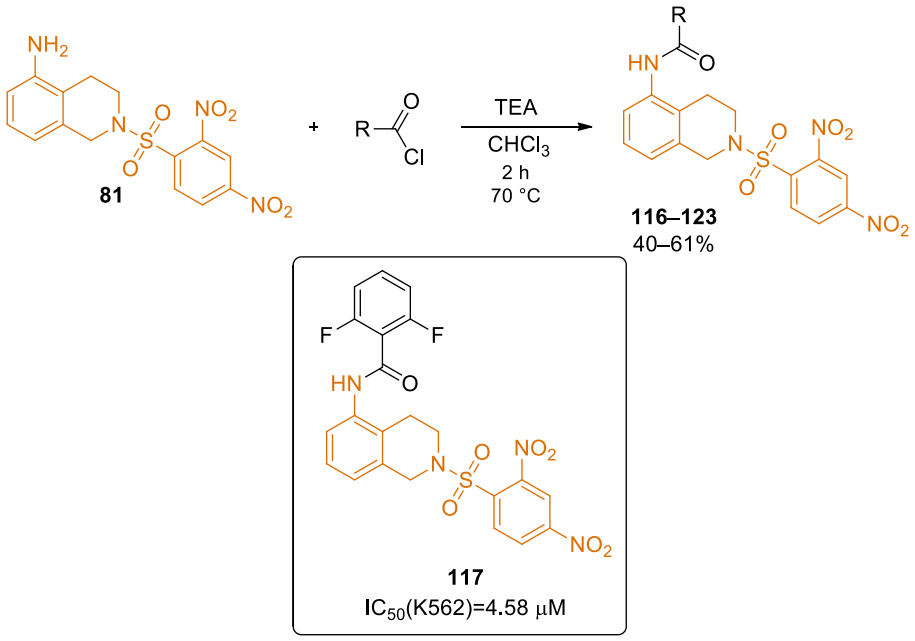
3.1. At the first attempt, a cytotoxic sulfonamide library involving piperidine fused heterocycles was created. Following a nucleophilic substitution pathway, sulfonamides **78**–**107** were prepared including 1,2,3,4-tetrahydroquinoline, 1,2,3,4-tetrahydroisoquinoline as well as 2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole frameworks. All 30 derivatives were synthesized under the same reaction conditions involving the use of TEA in DCM at room temperature for 2 hours and isolation after column chromatography giving the pure products in yields of 11–72%. The *in vitro* MTS assay against K562 tumor cell line showed moderate cytotoxic activities (Scheme 1).



Scheme 1.

* The numbering of compounds in the thesis are identical to those in the dissertation.

To enhance the potency, a further, modified treatment of selected compound **81** was carried out (Scheme 2). By the acylation of its free amino function at the C–5 position (reaction conditions: TEA, $CHCl_3$, 70 °C, 2 h), 8 analogues have been prepared in yields of 40–61% after simple filtration. Unfortunately, the formed **116**–**123** amides showed diminished efficiency in comparison with that of parent compound **81** (IC_{50}=4.58–(>30) μM).

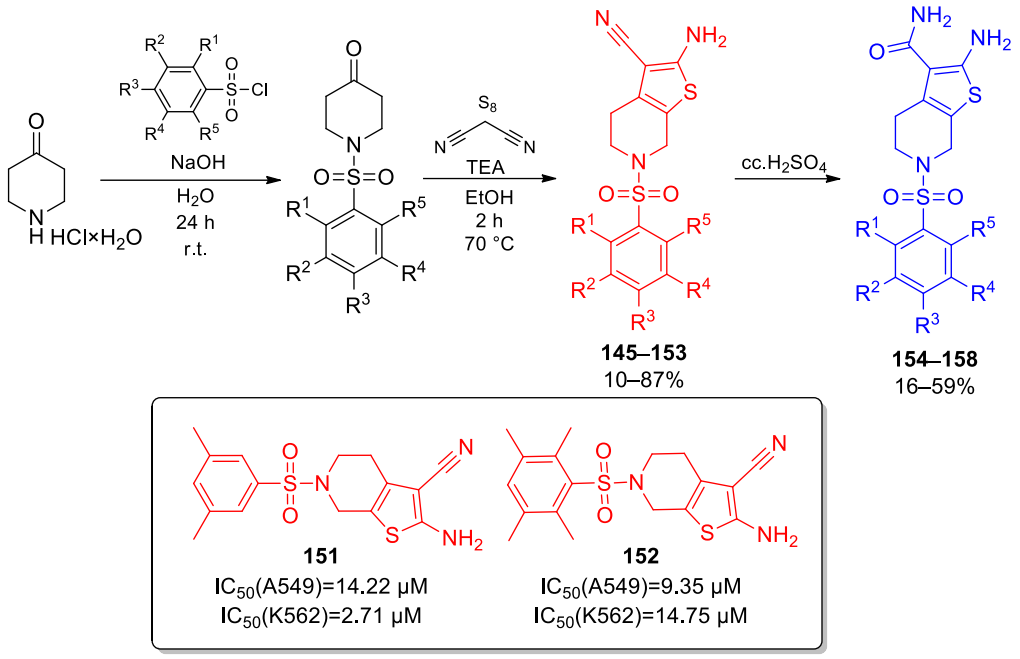


Scheme 2.

A full characterization of sulfonamide **81** (as hit compound) was accomplished including complete *in vitro* and *in vivo* investigations. Unfortunately, owing to its insufficient solubility and absorption feature as well as its powerful toxicity against human cells and fibroblast, further development was terminated.

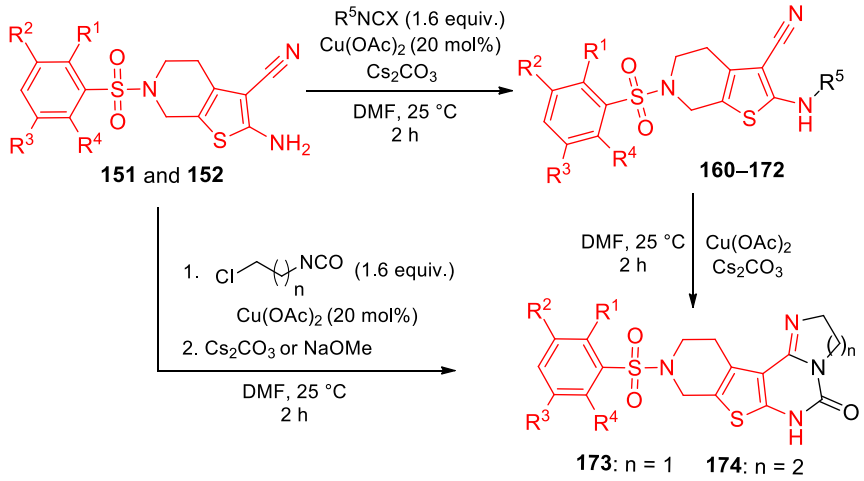
3.2. In the second effort of sulfonamide research, the molecular library was extended for 4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine based analogues. First, the synthesis of *N*-protected 4-piperidone precursors **134**–**142** was performed in isolated yields of 10–69%. Afterwards, they were exploited through Gewald-3CR to obtain 4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-based carbonitriles **145**–**153** in 10–84% yields. For other representative examples, five carboxamides were furnished by simple hydrolysis of the corresponding aminonitriles (Scheme 3).

Of 14 compounds, only carbonitriles **151** and **152** exhibited moderate cytotoxic activities (**151**, IC_{50}(A549)=14.22 μM, IC_{50}(K562)=2.71 μM; **152**, IC_{50}(A549)=9.35 μM, IC_{50}(K562)=14.75 μM).



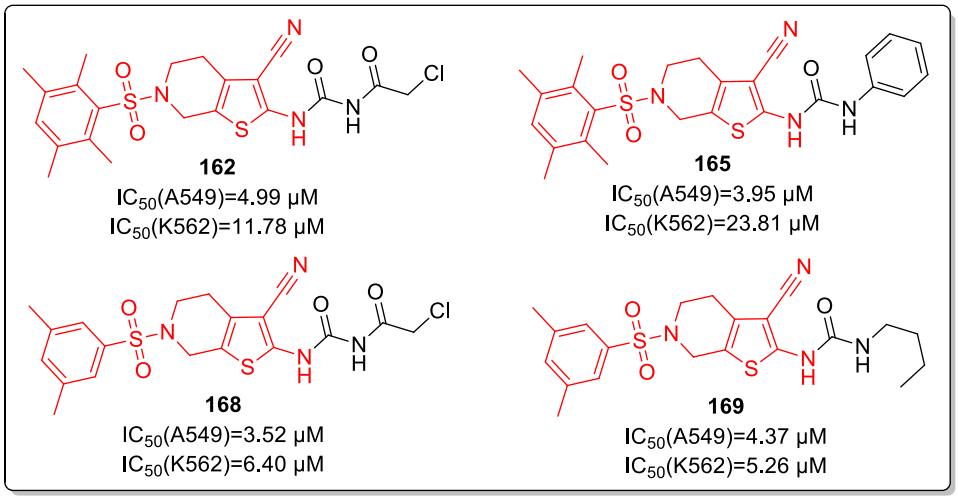
Scheme 3.

To increase their antitumor capabilities, compounds **151** and **152** were functionalized at the C–2 position by utilizing iso(thio)cyanates in the presence of $Cu(OAc)_2$ as Lewis acid catalyst in dry DMF. Upon completion of the reactions, the corresponding **160**–**172** iso(thio)carbamates were isolated in yields of 17–69% after column chromatography and recrystallization. In addition, tetracycles **173** and **174** were prepared via either sequential one-pot or two-step domino strategy by exploiting halogenated alkyl (2-chloroethyl and 3-chloropropyl) isocyanate reactants followed by an intramolecular sequence induced by NaOMe. The corresponding representative products were isolated in yields of 54% (**173**) and 61% (**174**) (Scheme 4).



Scheme 4.

The structural modification on C–2 did not lead to significant improvement of efficiency for the tested **160**–**174** analogues in comparison with parent architectures **151** and **152** (A549: **168** proved to be the most potent with IC_{50}=3.52 μM; for K562: compound **169**, IC_{50}=5.26 μM, Scheme 5). Moreover, further research was aborted owing to their general toxic feature and low potential as well as side-effect profile.



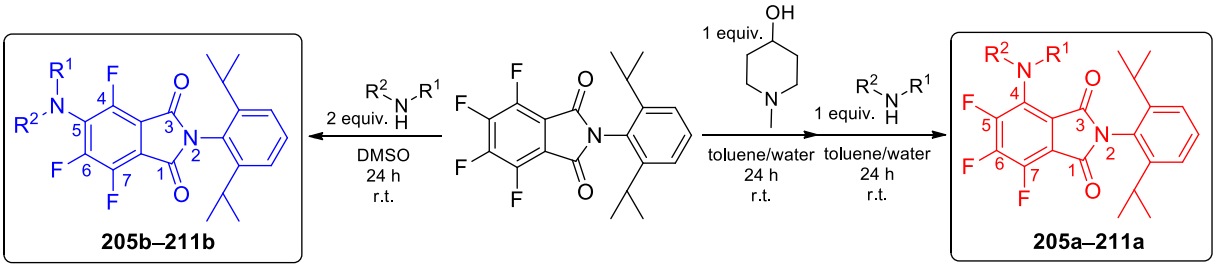
Scheme 5.

3.3. Next, the improvement of a preliminary phthalimide research was accomplished by means of the development of an efficient S_NAr method for the regioselective synthesis of C-4 substituted isoindolone-1,3-dione. Initially, a diverse and high-membered chemical library containing a series of C–4 and C–5 NHAr-substituted phthalimides was created for cytotoxic evaluation by assembling 2-(2,6-diisopropylphenyl)-4,5,6,7-tetrafluoroisoindolin-1,3-dione as precursor and primary/secondary amines via S_NAr. Because of the formation of various regioisomeric mixtures in all assemblies, an individual rapid separation technique had to be developed by means of the Teledyne Isco CombiFlash® Rf flash chromatography system.

For the construction of the initial molecular library, the conventional reaction route involving two equivalents of the corresponding amines was followed (DCM solvent, ambient temperature, 24 h) affording the formation of inactive **b** compounds (C–5 NHAr-substituted phthalimides) as the major isomer. Taking into consideration the difficult isolation of bioactive C–4 NHAr-substituted **a** isomer compounds, the less-favored regioisomeric outcome, and the biological results, development of the **a** regioselective method was mandatory. For the access to the desired condition, seven amines were selected from the

original group and the solvents, additives, concentrations as well as temperature were altered. Conversions were monitored after calibration with HPLC.

After setting the optimal conditions, a sequential, one-pot *ortho*-selective (C–4) S_NAr methodology was successfully developed through the involvement of intermediates triggered by 1,3-aminoalcohol. Application of one equivalent of 1-methyl-4-hydroxypiperidine additive and the corresponding amine (toluene/water 1:1, ambient temperature, 48 h) afforded good to excellent regioselective outcomes concerning the formation of the **a** regioisomers (HPLC conversion data: 79–91%, HPLC yields: 86–100%, isolated yields: 38–63%). On the other hand, the **b** regioselective method was also introduced by means of two equivalents of amine, DMSO solvent at room temperature affording full conversions and HPLC yields up to 97% (Scheme 6).

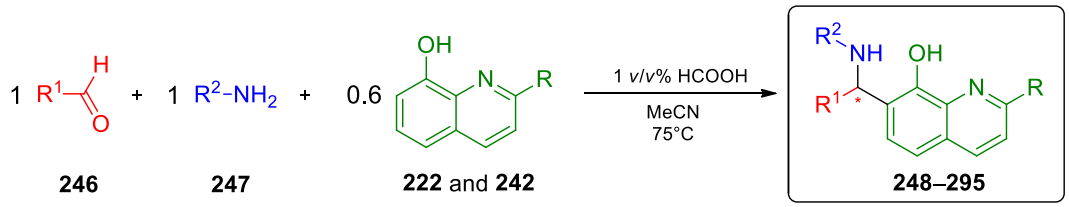


Scheme 6.

The prepared derivatives **205a**–**211a** have shown significant cytotoxic effect in several tumor cell cultures. In addition, they exhibited much higher efficiency than the other regioisomers as shown by the corresponding IC_{50} values: C–4 (**a** isomers) IC_{50}(K562) 1.09–(>100), IC_{50}(HepG2) 2.56–(>100), IC_{50}(HT168) 2.14–(>100); C–5 (**b** isomers) IC_{50}(K562) 39.99–(>100), IC_{50}(HepG2) 6.06–(>100), IC_{50}(HT168) 28.47–(>100).

3.4. Following our antitumor research strategy, first a 15-membered 8-HQ-based heterocyclic library was created by exploiting Betti-3CR. The preliminary biological assessment (investigation of cytoxic activities against HL-60 and K-562) revealed no antitumor activities. However, cytoprotective investigation of **248**–**262** on U251 MG glioblast assay showed significant protection in the submicromolar range (IC_{50}=0.106–0.687 μM).

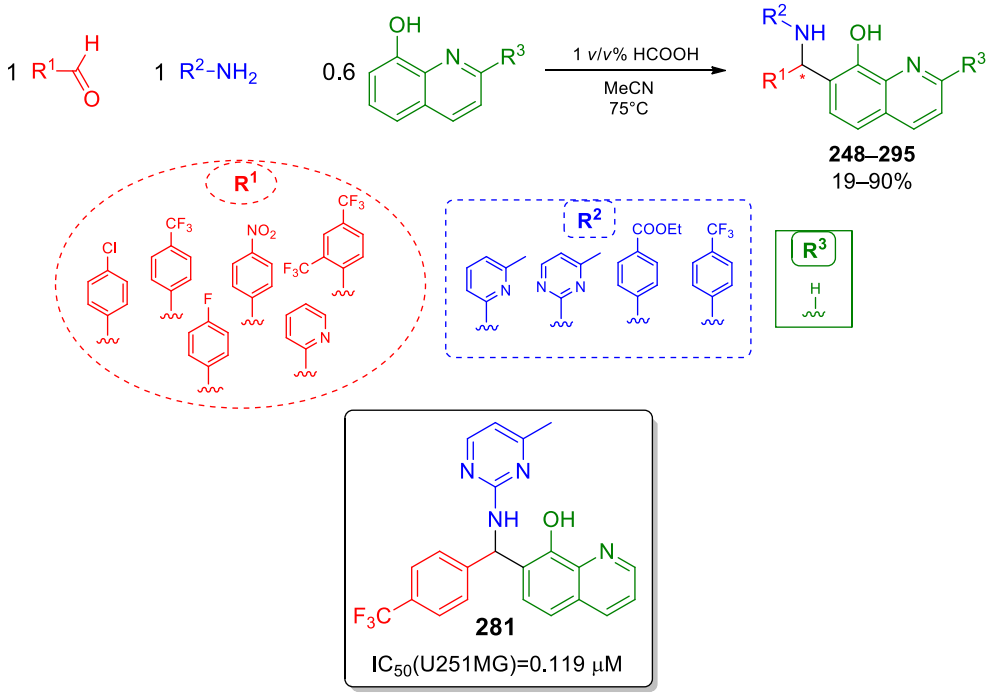
In further research and development, the extension of the 8HQ-based heterocyclic library was achieved. The application of a slightly modified Betti-3CR involving 1 mmol of aldehyde, 1 equivalent of amine, 0.6 equivalent of 8-HQ, 1 *v*/*v* % HCOOH in MeCN solvent at 75 °C furnished a 48-membered library in isolated yields of 12–90% (Scheme 7).



R^1: phenyl, 4-CF_3-C_6H_4, 2-pyridyl, 4-F-C_6H_4, 4-NO_2-C_6H_4, 4-(*i*PrO)-C_6H_4, 4-(CF_3O)-C_6H_4, 3-CF_3-C_6H_4, 3-F-5-CF_3-C_6H_4, 3,5-diCF_3-C_6H_3, 4-F-3-CF_3-C_6H_3, 2,4-diCF_3-C_6H_3, 3,4-diF-C_6H_3, 4-SF_5-C_6H_4, 4-I-C_6H_4, 4-Br-C_6H_4
R^2: phenyl, 4-COOEt-C_6H_4, 4-COOH-C_6H_4, 2-pyridyl, isoxazole, 5-*t*Bu-isoxazole, 5-Me-isoxazole, 6-Me-C_5H_3N, pyrimidyl, 3-Me-C_5H_3N, 4-Me-C_5H_3N, 4-Me-$C_4H_2N_2$, 5-Br-C_4H_2N
R: H, Me

Scheme 7.

During the end-point assay, 9 of the 48 analogues have shown considerable IC_{50} values (**251**, **264**, **265**, **267**, **268**, **281**, **282**, **287**, **290**; 0.70–0.12 μM). For this reason, an orthogonal assay was effectuated selecting the most suitable lead compound. The prominent architecture proved to be compound **281** possessing an IC_{50} value of 0.119 μM (Scheme 8).

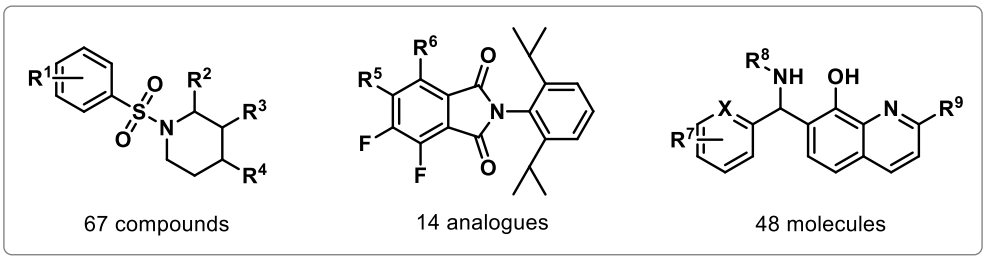


Scheme 8.

Finally, a GMP-compatible enantioselective synthetic procedure was developed by preparing the corresponding *R* or *S* enantiomers. The Human Phase Study for the eutomer (*R* enantiomer) of compound **281** is in progress after a successful Phase I/A.

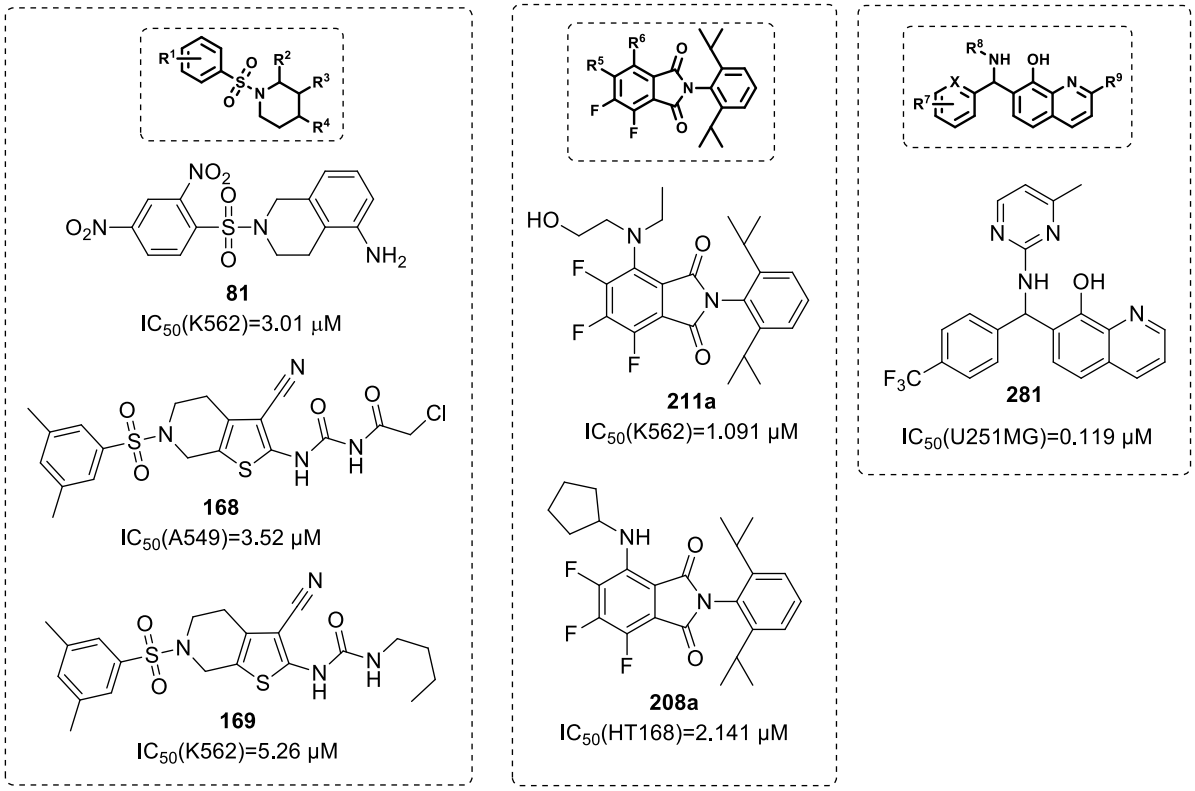
4. Summary

As a summary of my laboratory work, 129 piperidine-based condensed heterocyclic sulfonamides, *N*-aryl-substituted 1,3-isoindoline-1,3-diones as well as 8-HQ-based Betti products were synthesized via S_N and S_NAr reactions as well as applying Gewald-3CR and Betti-3CR (Scheme 9).



Scheme 9.

The most active compounds from the tested four heterocyclic groups are depicted in Scheme 10.



Scheme 10.

Scientific publications forming the basis of the theses

1. Puskás LG, Fehér LZ, Vizler C, Ayaydin F, Rásó E, Molnár E, Magyary I, Kanizsai I, Gyuris M, **Madácsi R**, Fábián G, Farkas K, Hegyi P, Baska F, Ozsvári B, Kitajka K. Polyunsaturated fatty acids synergize with lipid droplet binding thalidomide analogs to induce oxidative stress in cancer cells; *Lipids Health Dis.* **2010**, *9*, 56.

   IF: 2.239

2. **Madácsi R**, Kanizsai I, Fehér LZ, Gyuris M, Ozsvári B, Erdélyi A, Wölfling J, Puskás LG. Aromatic sulfonamides containing a condensed piperidine moiety as potential oxidative stress-inducing anticancer agents; *Med. Chem.* **2013**, *9*, 911–919.

   IF: 1.373

3. **Madácsi R**, Gyuris M, Kanizsai I, Sipos P, Wölfling J, Puskás LG. 4-Amino-5,6,7-trifluor-flátimidek előállítása, *in vitro* és *in vivo* biológiai vizsgálata; *Magy. Kém. Foly.* **2014**, *120*, 95–101.

   IF: -

4. **Madácsi R**, Gyuris M, Wölfling J, Puskás LG, Kanizsai I. Highly regioselective 4-hydroxy-1-methylpiperidine mediated aromatic nucleophilic substitution on a perfluorinated phthalimide core; *J. Fluorine Chem.* **2018**, *212*, 38−44.

   IF: 2.055

5. Kanizsai I, **Madácsi R**, Hackler L Jr, Gyuris M, Szebeni GJ, Huzián O, Puskás LG. Synthesis and Cytoprotective Characterization of 8-Hydroxyquinoline Betti Products; *Molecules* **2018**, *23*, 1934.

   IF: 3.060

6. **Madácsi R**, Traj P, Hackler L Jr., Nagy LI, Kari B, Puskás LG, Kanizsai I. Synthesis and biological evaluation of 4,5,6,7-tetrahydrothieno [2,3-c]pyridine-based β-aminonitriles and their derivatives; β-amino carboxamides, (thio)ureas and tetracycles; *J. Heterocyclic Chem.* **2020**, *57*, 635–652.

   IF: 1.484 (2019)

Patent forming the basis of the theses

1. Puskás LG, Szabó C, Kanizsai I, Gyuris M, **Madácsi R**, Ózsvári B, Fehér L, Tamás G. 8-hydroxy-quinoline derivatives; WO Patent, **2011**, WO 2,011,148,208.

IF:-

Sum of IF: 10.211

Other published publications:

1. Zsigmond Á, **Madácsi R**, Notheisz F, Mernyák E, Joó F. Selective hydrogenations of steroids catalyzed by heterogenized Ru complexes; *React. Kinet. Catal. Lett.* **2006**, *87*, 297–304.

IF: 0.490

2. Sipos P, Berkesi O, Tombácz E, Katona H, **Madácsi R**. The nature of interactions between iron(III) and the structure of the Fe(III)-chitosan "complex"; *Acta Pharm. Hung.* **2007**; *77*, 165–175.

IF: 0.190

3. McAuley G, Schrag M, Sipos P, Sun SW, Obenaus A, Neelavalli J, Haacke EM, Holshouser B, **Madácsi R**, Kirsch W. Quantification of punctate iron sources using magnetic resonance phase; *Magn. Reson. Med.* **2010**, *63*, 106–115.

IF: 3.267

4. Ozsvári B, Puskás LG, Nagy LI, Kanizsai I, Gyuris M**, Madácsi R**, Fehér LZ, Gerö D, Szabó C. A cell-microelectronic sensing technique for the screening of cytoprotective compounds; *Int. J. Mol. Med.* **2010**, *25*, 525–530.

IF: 1.814

5. Gyuris M, **Madácsi R**, Puskás LG, Tóth GK, Wölfling J, Kanizsai I. Synthesis of 2-Amino-3-cyano-4H-chromene-4-carboxamide Derivatives by an Isocyanide-Based

Domino Conjugate Addition/O-Trapping Rearrangement Sequence; *Eur. J. Org. Chem.* **2011**, *46*, 848–851.

IF: 3.329

6. Nagy LI, Molnár E, Kanizsai I, **Madácsi R**, Ózsvári B, Fehér LZ, Fábián G, Marton A, Vizler C, Ayaydin F, Kitajka K, Hackler LJr, Mátés L, Deák F, Kiss I, Puskás LG. Lipid droplet binding thalidomide analogs activate endoplasmic reticulum stress and suppress hepatocellular carcinoma in a chemically induced transgenic mouse model; *Lipids Health Dis.* **2013**, *12*, 175.

IF: 2.015

7. Hackler L Jr, Gyuris M, Huzián O, Alföldi R, Szebeni GJ, **Madácsi R**, Knapp L, Kanizsai I, Puskás LG. Enantioselective Synthesis of 8-Hydroxyquinoline Derivative, Q134 as a Hypoxic Adaptation Inducing Agent; *Molecules*, **2019**, *24*, 4269.

IF: 3.267

Patent:

1. Puskás LG, Kanizsai I, Gyuris M, **Madácsi R**, Ózsvári B, Fehér LZ, Fábián G, Kitajka K. Use of trifluoro phthalimides for the treatment of cancerous diseases; WO patent, **2012**, WO 2,012,085,608.

IF: -

Conference presentations:

1. Gyuris M, **Madácsi R**, Kanizsai I. Ac915/Ac929 anyagok és analógjaik szintézise, tisztítása és analitikája, nehézségek, további lehetőségek; Jedlik Ányos pályázati tudományos ülés, Szeged, **2010**.
2. **Madácsi R.** 4,5,6,7-tetrahidrotieno[2,3-*c*]piridinek előállítása és citotoxikus hatásának vizsgálata; Heterociklusos és Elemorganikus Munkabizottság, Balatonszemes, **2012**.

Conference poster:

1. Kanizsai I, Gyuris M, Fehér LZ, Ózsvári B, Molnár E, Fábián G, **Madácsi R**, Puskás LG. Lipid droplet interacting ER-stress inducing amino-trifluoro-phthalimides as cytotoxic agents; ESOC, Prága, **2009**.
2. Gyuris M, Kanizsai I, **Madácsi R**, Puskás LG, Tóth G, Wölfling J. Multicentrumú imino-kromén-karboxamidok reakciója izocianidokkal; MKE Vegyészkonferencia és 53. Spektrokémiai Vándorgyűlés, Hajdúszoboszló, **2010**.
3. Gyuris M, Demjén A, **Madácsi R**, Puskás LG, Wölfling J.,Kanizsai I. Imidazo[1,2-b]pirazol származékok előállítása GBU reakcióval; MKE 1. Nemzeti Konferencia, Sopron, **2011**.
4. **Madácsi R**, Wölfling J, Puskás LG., Kanizsai I. Investigation of S_NAr reactions of 2-(2,6-diisopropylphenyl)-4,5,6,7 tetrafluoroisoindoline-1,3-dione with amines; ICOS 20, Budapest, **2014**.
5. **Madácsi R**, Huzián O., Hackler LJr., Puskás LG., Kanizsai I. 8-hidroxi-kinolin származékok szintézise, a lombiktól a cél felé; MKE Vegyészkonferencia, Hajdúszoboszló, **2017**.