ANTICANCER RESEARCH *44*: xxx-xxx (2024)
doi:10.21873/anticanres.11xxx

Cyclic Amines Coupled to Indole Derivatives With Improved Efflux Pump Inhibiting Activity in Bacteria and Cancer Cells

DÓRA HEGEDŰS[1], NIKOLETTA SZEMERÉDI[2], MAJA GÁBOR[2], JUDIT SAS[1], KHADIJA BELASRI[1], ISTVÁN SZATMÁRI[1,3] and GABRIELLA SPENGLER[2]

[1]*Institute of Pharmaceutical Chemistry, University of Szeged, Szeged, Hungary;*
[2]*Department of Medical Microbiology, Albert Szent-Györgyi Health Center and Albert Szent-Györgyi Medical School, University of Szeged, Szeged, Hungary;*
[3]*HUN-REN–SZTE Stereochemistry Research Group, University of Szeged, Szeged, Hungary*

Abstract. *Background/Aim: Indole skeleton has become a significant tool in the field of anticancer and antibacterial therapeutic strategies. The modified aza-Friedel–Crafts reaction by direct coupling of different cyclic imines and indole derivatives has been explored. To investigate the scope and limitations of the reaction and observe the effect of structural modifications, our aim was to resynthesize selected compounds as well as prepare new derivatives starting from 6,7-dimethoxy-3,4-dihydroisoquinoline, (4aR,8aR)-4a,5,6,7,8,8a-hexahydroquinoxalin-2(1H)-one and 7-azaindole. Our further aim was the systematic biological evaluation of selected C-3-coupled indole and azaindole derivatives in favour of having a preliminary overview about the structure–activity relationships. Materials and Methods: The synthesis and resynthesis of selected compounds were accomplished by extension of aza-Friedel–Crafts reaction. The products have been tested on bacteria and cancer cells. Results: The most significant efflux pump inhibiting EPI activity was observed in the case of 6,7-dihydrothieno[3,2-c]pyridine coupled indole derivative. The reaction of 6,7-dimethoxy-3,4-dihydroisoquinoline with 7-azaindole resulted in the most potent biofilm inhibitor product. Applying indole and 4,9-dihydro-3H-β-carboline, 6,7-dihydrothieno[3,2-c]pyridine led to the formation of a product with the highest anticancer activity. 6,7-Dimethoxy-3,4-dihydroisoquinoline skeleton and indole as an electron-rich aromatic compound have been found to be effective in the inhibition of ABCB1. Conclusion: The compounds presented in the study were investigated regarding different aspects of antibacterial and anticancer activities. Accordingly, some compounds were found to have antibacterial effect on Escherichia coli and Staphylococcus aureus strains, certain C-3-coupled derivatives showed toxicity on sensitive and ABCB1 efflux pump expressing colon adenocarcinoma and a normal, non-cancerous fibroblast cell lines.*

Correspondence to: Gabriella Spengler, Department of Medical Microbiology, Albert Szent-Györgyi Health Center and Albert Szent-Györgyi Medical School, University of Szeged, Semmelweis utca 6, 6725 Szeged, Hungary. Tel: +36 62545115, e-mail: spengler.gabriella@med.u-szeged.hu; István Szatmári, Institute of Pharmaceutical Chemistry, University of Szeged, Eötvös u. 6, H-6720 Szeged, Hungary. Tel: +36 62545563, e-mail: szatmari.istvan@szte.hu

Key Words: aza-Friedel–Crafts reaction, indole skeleton, modified Mannich reaction, efflux pump inhibitor (EPI), anti-biofilm, P-glycoprotein (ABCB1) inhibition.

The Mannich reaction is one of the most important basic reaction types in organic chemistry for C–C and C–N bond formation (1-5). A special variation of this latter reaction (modified Mannich reaction: *m*MR) uses benzaldehyde rather than formaldehyde, ammonia instead of secondary amine and replacing the C–H acid by an electron-rich aromatic compound, such as 1- or 2-naphthol (6) or nitrogen-containing naphthol analogues leading to chelating compounds with improved antiproliferative activity (7). Mechanistically, the modified *aza*-Friedel–Crafts reaction can be interpreted as a special *m*MR, where electron-rich aromatic compounds such as naphthols and their *N*-containing analogues are reacted with a wide range of cyclic imines to furnish aminonaphthols (8-11), aminoquinolinols (9) or aminoisoquinolinols (9, 12).

In previous studies the scope and limitations of the modified *aza*-Friedel–Crafts reaction by direct coupling of different cyclic imines and indole derivatives as electron-rich aromatic compounds have been examined (13-15). The reaction of indole and indole-2-carboxylic acid with 3,4-dihydroisoquinolines, 4,6-dihydro-3*H*benzo[*c*]azepine and 6,7-dihydrothieno[2,3-*c*]pyridine resulted in the formation of 3-substituted indole derivatives (13). The *aza*-Friedel–Crafts alkylation of electron-rich aromatic compounds was then extended by using other cyclic imine substrates, such as 4,9-dihydro-3*H*-β-carboline and 6-methoxy-4,9-dihydro-3*H*-β-

carboline (14). Our research group also examined the solvent-free coupling of 7-, 4-, 5- and 6-azaindoles with partially saturated cyclic amines (3,4-dihydroisoquinoline, 6,7-dihydrothieno[3,2-*c*]pyridine, 3,4-dihydro-β-carboline and 4,5-dihydro-3*H*-benz[*c*]azepine) leading to the formation of new C-3-substituted azaindole derivatives (15).

Based on previous studies an indole derivative could act on the expression level of efflux pump genes (*norA*), indole analogues can also potentiate the activity of antibiotics and destroy biofilms (16). Furthermore, other indole derivatives have been patented to be effective in combination with a known antibiotic in a synergistic manner, furthermore, they are believed to exert this effect by the inhibition of a bacterial efflux pump(s) [Indole derivatives as efflux pump inhibitors, patent (17)]. There is an extensive research on indole containing derivatives as MDR reversing agents and EPIs, there are derivatives effective against the ABCB1 efflux pump of tumor cells (18).

Eudistomin U and isoeudistomin U are important derivatives of β-carboline, tryptophan-derived metabolites. A recent paper pointed out that these compounds and related 2-substituted 1,2,3,4-tetrahydroeudistomins U showed antibacterial, antimalarial, and anticancer activities. The antibacterial activity of eudistomin U was assessed on *Streptococcus pyogenes, Mycobacterium smegmatis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* strains. In the case of dimethoxy derivative of eudistomin U antileukemic activity was reported. Moreover, 2-alkylated compounds of 1,2,3,4-tetrahydroeudistomin U possessed antitumor activity on *Pyricularia oryzae* Cavara cancer cells (19). Studies have shown that aryl hydrocarbon receptor agonist indole-3-carbinol and 3,3'-diindolylmethane, indole[3,2-*b*]carbazole are anti-carcinogenic derivatives (20). Baez-Gonzalez *et al*. established the effects of indole-3-carbinol on the proliferation, migration, invasion of different cancer cell lines by affecting cell growth, apoptosis, mobility, and invasive properties of cancer cells (21). Recently, the immune-regulatory, anti-inflammatory, and antioxidant effect of diindolylmethane against ulcerative colitis was proven by modulating the expression of lncRNAs and miRNAs (22). Furthermore, imidazo[4,5-*f*]quinoline, imidazo[4,5-*f*]quinoxaline, and imidazo[4,5-*b*]-pyridine as azaindole containing heterocycles are suggested to act as lactoperoxidase substrates (23).

Based on the widespread biological activity of the indole skeleton or their application as pharmaceuticals (16-25), our present aim was to perform a systematic biological examination of 3-isoquinolyl-, 3-thieno[3,2-*c*]pyridyl-, 3-β-carbolinyl- and 3-benz[*c*]azepinyl-indole and -azaindole derivatives. In favour of drawing the inference, we decided to resynthesize the selected derivatives and to extend the synthetic pathway to the preparation of new precursors starting from 6,7-dimethoxy-3,4-dihydroisoquinoline as well as (4*aR*,8*aR*)-4*a*,5,6,7,8,8*a*-hexahydroquinoxalin-2(1*H*)-one as cyclic imine and 7-azaindole to have a preliminary overview about the structure–activity relationship for C-3-coupled indole and azaindole derivatives.

Materials and Methods

Biological assays. Cell cultures. The human colon adenocarcinoma cell lines, Colo 205 (ATCC-CCL-222) doxorubicin-sensitive and Colo 320/MDR-LRP (ATCC-CCL-220.1) resistant to doxorubicin expressing ABCB1, were purchased from LGC Promochem (Teddington, UK). The cells were cultured in RPMI-1640 medium as described previously (26). The MRC-5 (ATCC CCL-171) human embryonic lung fibroblast cell line (LGC Promochem) was cultured in EMEM medium as described earlier (26).

Assay for cytotoxic effect. A total of 1×10^4 of human colonic adenocarcinoma cells or adherent human embryonic lung fibroblast cells were prepared in the appropriate culture medium. The effects of increasing concentrations of the compounds on cell growth were tested in 96-well flat-bottomed microtiter plates starting with 100 μM. Culture plates were incubated at 37˚C for 24 h; at the end of the incubation period, 20 μL of MTT (thiazolyl blue tetrazolium bromide) solution (from a 5 mg/ml stock solution) were added to each well. After incubation at 37˚C for 4 h, 100 μl of sodium dodecyl sulfate (SDS) solution (10% SDS in 0.01 M HCl) was added to each well and the plates were further incubated at 37˚C overnight. Cell growth was determined by measuring the optical density (OD) at 540 nm (ref. 630 nm) with Multiscan EX ELISA reader (Thermo Labsystems, Cheshire, WA, USA). Inhibition of cell growth was expressed as IC_{50} values, defined as the inhibitory dose that reduces the growth of the cells exposed to the tested compounds by 50%. IC_{50} values and the SD of triplicate experiments were calculated by using GraphPad Prism software version 5.00 for Windows with nonlinear regression curve fit (GraphPad Software, San Diego, CA, USA; www.graphpad.com).

Rhodamine 123 accumulation assay. The cell numbers of the human colon adenocarcinoma cell lines were adjusted to 2×10^6 cells/ml, re-suspended in serum-free RPMI 1640 medium and distributed in 0.5 mL aliquots into Eppendorf centrifuge tubes. The tested compounds were added at 2 or 20 μM concentrations, and the samples were incubated for 10 min at room temperature. The positive control was as follows: tariquidar was applied at 0.2 μM or verapamil was used at 20 μM. DMSO at 2% v/v was used as solvent control. The accumulation of the ABCB1 substrate rhodamine 123 was carried out as described previously (26).

Bacterial strains. As Gram-negative strain the wild-type *Escherichia coli* K-12 AG100 [argE3 thi-1 rpsL xyl mtl Δ(gal-uvrB) supE44], expressing the AcrAB-TolC efflux pump (EP) at its basal level and its *acrAB-tolC*-deleted mutant strain *E. coli* AG100A (a kind gift from Hiroshi Nikaido, Department of Molecular and Cell Biology and Chemistry, University of California, Berkeley, CA, USA) were used.

As Gram-positive strains, methicillin-susceptible reference *Staphylococcus aureus* American Type Culture Collection (ATCC) 25923 strain and the methicillin resistant *S. aureus* MRSA 272123 clinical strain (kindly provided by Prof. Dr. Leonard Amaral, Institute of Hygiene and Tropical Medicine, Lisbon, Portugal) were used in the experiments.

Antibacterial assay. The antibacterial activity was assessed through the minimum inhibitory concentration (MIC) of the compounds. This was determined by the microdilution method, in a 96-well plate, according to the Clinical and Laboratory Standard Institute (CLSI) guidelines. The MIC was determined by visual inspection. DMSO was used as a solvent for the compounds and was used in subinhibitory concentrations (2% v/v) (27).

Real-time ethidium bromide accumulation assay. The impact of compounds on EB accumulation was determined by the automated EB method using a CLARIOstar Plus plate reader (BMG Labtech, UK) according to a former study (28). The compounds were applied at MIC/2 concentration and added to the samples containing a non-toxic concentration of EB (2 μg/ml). Then, 50 μl of the EB solution containing the compound was transferred into 96-well black microtiter plate (Greiner Bio-One Hungary Kft, Mosonmagyaróvár, Hungary), and 50 μl of bacterial suspension (OD 600 0.6) was added to each well. Reserpine (RES) was applied at 25 μM as a positive control on *S. aureus* strains, carbonyl cyanide and *m*-chlorophenylhydrazone (CCCP) were applied at 50 μM as a positive control on *E. coli* strains. From the real-time data, the relative fluorescence index (RFI) of the last time point (minute 60) was calculated (28).

Inhibition of biofilm formation. Compounds were tested for their ability to decrease the formation of biofilm. The strains used were the Gram-negative *E. coli* K-12 AG100, *E. coli* AG100A lacking the AcrAB-TolC system and the Gram-positive *S. aureus* ATCC 25923 strain, *S. aureus* MRSA 272123 clinical isolate. The detection of the biofilm formation was possible with the use of the dye crystal violet (CV; 0.1% (v/v)), compounds were applied at MIC/2. Carbonyl cyanide m-chlorophenylhydrazone (CCCP, 50 μM) and thioridazine (TZ, 50 μg/ml) were used as positive controls.

The biofilm formation was determined by measuring the OD 600 using a Multiscan EX ELISA plate reader (Thermo Labsystems). The anti-biofilm effect of the compounds was expressed in the percentage (%) of decrease of biofilm formation.

Preparation protocols for the synthesis of the new derivatives. Melting points were determined on a Hinotek X-4 melting point apparatus. Merck Kieselgel 60F_{254} plates were applied for TLC. Microwave reactions were carried out with a CEM Discover SP microwave reactor.

^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 or $CDCl_3$ solutions in 5-mm tubes at room temperature (RT), on a Bruker DRX-500 spectrometer (Bruker Biospin, Karlsruhe, Baden Wurttemberg, Germany) at 500 (^{1}H) and 125 (^{13}C) MHz, with the deuterium signal of the solvent as the lock and TMS as the internal standard (^{1}H, ^{13}C). All spectra (^{1}H, ^{13}C) were acquired and processed with the standard BRUKER software.

The HRMS flow injection analysis was performed with Thermo Scientific Q Exactive Plus hybrid quadrupole-Orbitrap (Thermo Fisher Scientific, Waltham, MA, USA) mass spectrometer coupled to a Waters Acquity I-Class UPLC™ (Waters, Manchester, UK).

Optical rotation values were measured on a Jasco P-2000 Polarimeter.

Starting imines: 3,4-dihydroisoquinoline (13); 6,7-dimethoxy-3,4-dihydroisoquinoline (13); 6,7-dihydrothieno[3,2-*c*]pyridine (29); 4,6-dihydro-3*H*-benzo[*c*]azepine (30, 31); 3,4-dihydro-β-carboline (32); (4*aR*,8*aR*)-4*a*,5,6,7,8,8*a*-hexahydroquinoxalin-2(1*H*)-one (33) were synthesized according to literature methods. Compounds **1**, **2** (13); **3-6** (15)**; 7-9** (13); **10** (14); **11a**, **11b** (34); **13-15** (15) were resynthesized based on the previously published data as mentioned in part of Synthesis.

3-(6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinolin-1-yl)-7-azaindole (12). A mixture of 7-azaindole (**17**) (54 mg, 1.0 mmol) and cyclic imine (60 mg, 1.0 mmol) was placed into a 10 ml pressurized reaction vial. The reaction mixture was heated at 100˚C for 150 min followed by heating at 110˚C for 60 min in a CEM Discover SP MW reactor. The crude reaction mixture was purified by column chromatography (CH_3OH:EtOAc, 4:1). The desired product was isolated by crystallization with Et_2O (10 mL), recrystallized from *i*Pr_2O (5 ml) and EtOH (0.5 ml); 107 mg, (75%); beige crystals; m.p. 196-198˚C; ^{1}H-NMR (DMSO): 2.62-2.67 (m, 1H), 2.78-2.91 (m, 2H), 3.02-3.08 (m, 1H), 3.44 (s, 3H), 3.73 (s, 3H), 5.19 (s, 1H), 6.38 (s, 1H), 6.71 (s, 1H), 6.93 (dd, 1H, J_1=4.7 Hz, J_2=7.9 Hz), 7.17 (s, 1H), 7.71 (d, 1H, *J*=8.0 Hz), 8.14 (d, 1H, *J*=4.8 Hz), 11.35 (s, 1H). ^{13}C NMR (DMSO): 29.4; 42.0; 53.9; 55.9; 56.0; 111.5; 112.6; 115.2; 116.8; 118.1; 119.0; 125.0; 127.9; 128.5; 131.1; 142.8; 147.0; 147.7; 149.4. HRMS calcd for [M+H$^+$] *m*/*z*=308.13935, found *m*/*z*=308.13775.

3-[(1R,4R,6R)-3-oxo-2,5-diazabicyclo[4.4.0]dec-4-yl]-7-azaindole (16a) and 3-[(1R,4S,6R)-3-oxo-2,5-diazabicyclo[4.4.0]dec-4-yl]-7azaindole (16b). (4*aR*,8*aR*)-4*a*,5,6,7,8,8*a*-hexahydroquinoxalin-2(1*H*)-one (152 mg, 1 mmol) and 7-azaindole (**17**; 118 mg, 1 mmol) were dissolved in CH_2Cl_2 (10 ml) in a 35-mL pressurized reaction vial. The reaction mixture was heated under MW irradiation for 2 h at 100˚C. Following the removal of the solvent, the residue was purified (the diastereomers separated) by column chromatography (CH_3OH:$CHCl_3$, 1:6). The desired products (**16a**, **16b**) were crystallised from Et_2O (10 ml).

16a. 104 mg, (38.5%); beige solid, m.p. 173-175˚C. $[\alpha]^{20}_D$ -131.9 (c 0.25, CH_2Cl_2). ^{1}H-NMR ($CDCl_3$): 1.30-1.38 (m, 2H), 1.67-1.79 (m, 5H), 1.83-1.89 (m, 1H), 2.62-2.72 (m, 1H), 3.11-3.20 (m, 1H), 5.08 (s, 1H), 6.62 (s, 1H), 7.09 (dd, 1H, J_1=4.7 Hz, J_2=7.9 Hz), 7.32 (s, 1H), 8.18 (d, 1H, *J*=7.9 Hz), 8.30 (d, 1H, *J*=4.7 Hz), 10.17 (s, 1H). ^{13}C NMR ($CDCl_3$): 23.9; 24.8; 30.8; 31.7; 53.3; 55.9; 59.0; 113.9; 115.9; 119.2; 123.5; 128.3; 143.0; 149.0; 171.0. HRMS calcd for [M + H$^+$] *m*/*z*=271.15534, found *m*/*z*=271.15386.

16b. 26 mg, (9.5%); beige solid, m.p. 180-182˚C. $[\alpha]^{20}_D$ –116.3 (c 0.25, CH_2Cl_2). ^{1}H-NMR ($CDCl_3$): 1.36-1.45 (m, 4H), 1.79-1.91 (m, 4H), 2.69-2.79 (m, 1H), 3.24-3.33 (m, 1H), 4.95 (s, 1H), 5.87 (s, 1H), 7.08 (dd, 1H, J_1=4.7 Hz, J_2=7.9 Hz), 7.40 (s, 1H), 8.07 (d, 1H, *J*=8.0 Hz), 8.25-8.32 (m, 1H), 8.71 (s, 1H). ^{13}C NMR ($CDCl_3$): 23.8; 24.6; 29.3; 31.5; 55.8; 57.9; 59.0; 116.0; 118.8; 124.3; 128.7; 143.1; 148.8; 158.9; 170.5. HRMS calcd for [M+H$^+$] *m*/*z*=271.15534, found *m*/*z*=271.15382.

Results and Discussion

Synthesis. To examine the effect of the indole skeleton in primary biological screening (Figure 1), the isoquinoline part was stabilized and the C-1 coupled (relative to tetrahydroisoquinoline core) indole moiety was varied among indole, indole-2-carboxylic acid, and 4-, 5- and 6-azaindoles (Figure 2). The synthesis of 3-(1,2,3,4-tetrahydroisoquinolin-1-

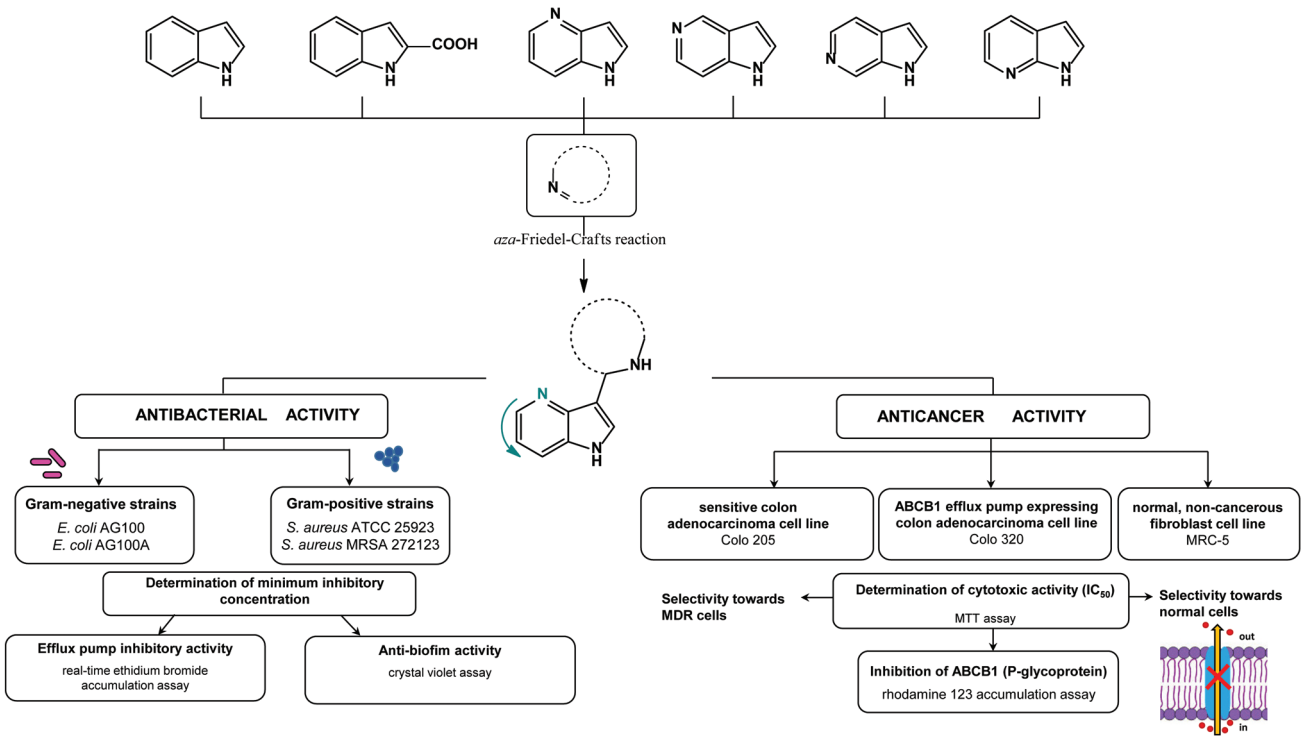


Figure 1. *Systematic biological evaluation of selected C-3-coupled indole and azaindole derivatives.*

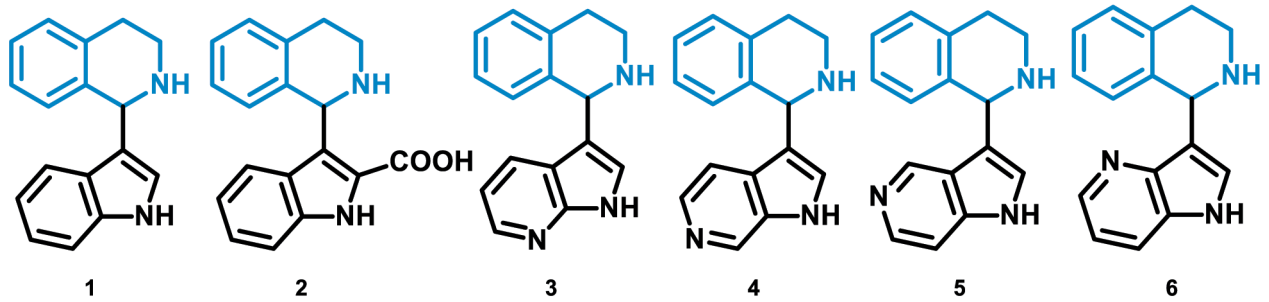


Figure 2. *C-1 substituted 1,2,3,4-tetrahydroisoquinolines.*

yl)indole (**1**) and 3-(1,2,3,4-tetrahydroisoquinolin-1-yl)indole-2-carboxylic acid (**2**) was accelerated under microwave irradiation as described earlier by our research group (13). To investigate the role of the heteroatom (nitrogen) on the activity, 3-(1,2,3,4-tetrahydroisoquinolin-1-yl)azaindoles (**3-6**) have also been resynthesized (15).

Next, we selected compounds **7-11**, with the indole skeleton connected to varied partially saturated cyclic amines, to investigate the influence of the cationic centre (amine) on the biological activity (Figure 3). The selected derivatives were synthesized based on the research protocol published previously by direct aza-Friedel–Crafts reaction of indole with 6,7-dimethoxy-3,4-dihydroisoquinoline, 6,7-dihydrothieno[3,2-*c*]pyridine, 4,6-dihydro-3*H*benzo[*c*]azepine and 4,9-dihydro-3*H*-β-carboline (13, 14). Microwave irradiation, as the optimal reaction condition, has been used that led to the formation of the desired **7-10** products in good yields. Recently, the applicability of (4*aR*,8*aR*)-4*a*,5,6,7,8,8*a*-hexahydroquinoxalin-2(1*H*)-one as chiral imine in the modified aza-Friedel–Crafts reaction has been tested by Iwanejko *et al.* (34). Compounds **11a** and **11b** were synthesized followed by isolation (separation) by column chromatography and determining the

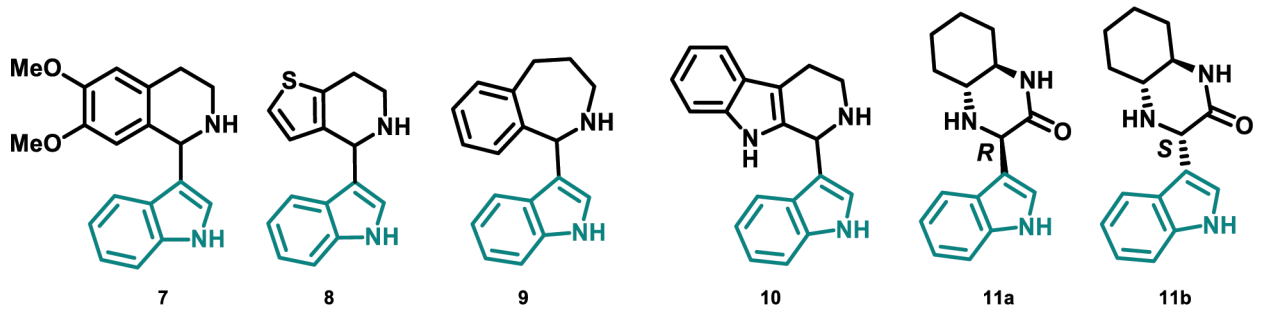


Figure 3. *C-3-substituted indole derivatives*.

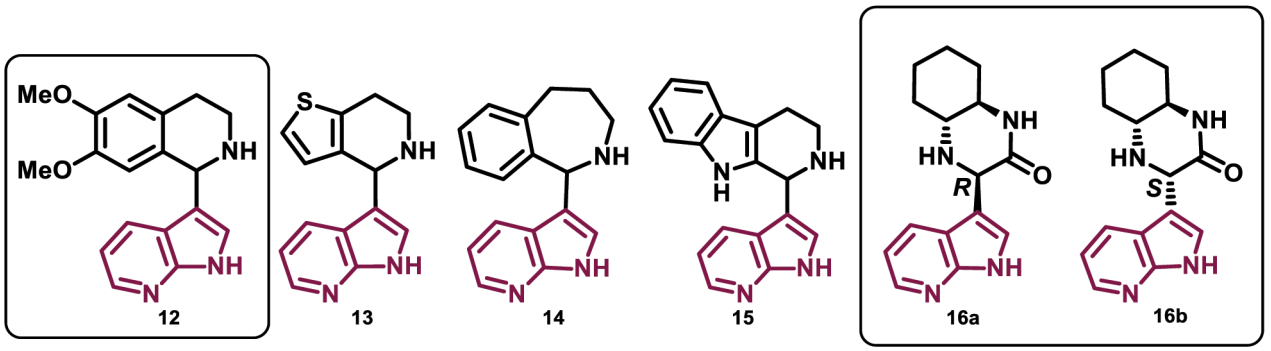


Figure 4. *C-3-substituted 7-azaindole derivatives*.

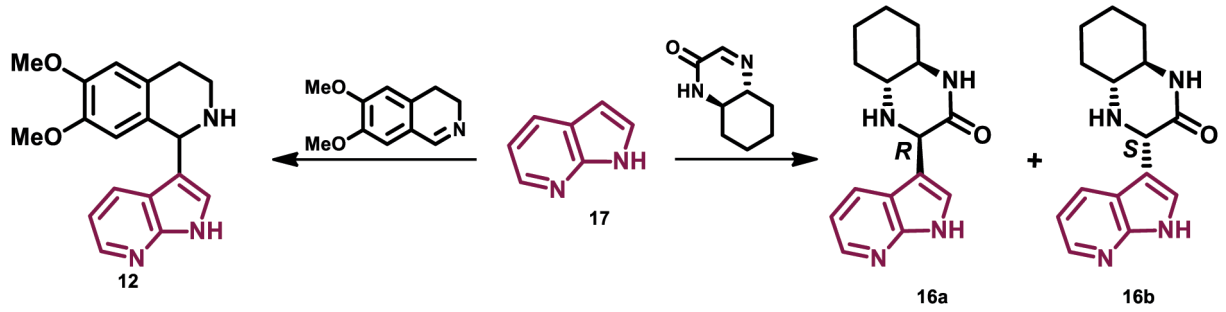


Figure 5. *Synthesis of new derivatives starting from 7-azaindole as an electron-rich aromatic compound*.

absolute configuration of the newly generated asymmetric centre. This allowed to identify the diastoremers by comparing the published characteristic data of **11a** and **11b** with those measured (melting points, ^{1}H-NMR chemical shifts and rotation values) (34).

Regarding the biological activity of the azaindole skeleton, 7-azaindole derivatives have been found to be a hinge-binding element in kinase inhibition (35). Mechanistically, because of its structural characteristics, 7-azaindole is able to form bidentate hydrogen bonds with the hinge region of kinase. Vemurafenib has been described as 7-azaindole-based kinase drug for the treatment of melanoma (36). Accordingly, our next selected set of compounds was designed with the 7-azaindole skeleton connected to partially saturated cyclic amines. The direct coupling of 7-azaindole with cyclic imines, such as 6,7-dihydrothieno[3,2-*c*]pyridine, 3,4-dihydro-β-carboline and 4,5-dihydro-3*H*-benz[*c*]azepine, was performed utilizing the solvent-free method previously described leading to the formation of compounds **13-15** (Figure 4) (15).

Table I. *Reaction conditions for the synthesis of 3-[(1R,4R,6R)-3-oxo-2,5-diazabicyclo[4.4.0]dec-4-yl]-7-azaindole (**16a**) and 3-[(1R,4S,6R)-3-oxo-2,5-diazabicyclo[4.4.0]dec-4-yl]-7azaindole (**16b**).*

Entry	Time (h)	Heating technique	Temperature (˚C)	Conversion[a] (%)	d.r.
1	23	Classical heating (oil bath)	Room temperature	11	**16a:16b** 5:1
2	1.5 then 1.5	MW	40 then 50	9	**16a:16b** 5:1
3	1.5	MW	80	12	**16a:16b** 5:1
4	2	MW	100	57	**16a:16b** 5:1

[a]Determined from crude NMR spectra.

To obtain an overview about the influence of the dimethoxy-substituents tetrahydoisoquinoline skeleton on activity, 3-(6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-1-yl)-7-azaindole (**12**) as new precursor was also synthesized starting from 6,7-dimethoxy-3,4-dihydroisoquinoline as cyclic imine and 7-azaindole (**17**). The reaction was performed under solvent-free conditions by applying microwave irradiation (2.5 h, 100˚C; 1 h, 110˚C) as the optimal condition (Figure 5).

As a further structural modification, we wanted to observe the effect of saturated nonracemic cyclic amine coupled with 7-azaindole. Accordingly, 7-azaindole (**17**) and 1.0 equivalent of (4*aR*,8*aR*)-4*a*,5,6,7,8,8*a*-hexahydroquinoxalin-2(1*H*)-one as chiral imine were reacted at room temperature in dichloromethane. After a relatively long reaction (23 h), the NMR spectrum of the crude reaction mixture proved the appearance of both possible products **16a** and **16b**. In this case, the ratio of **16a**:**16b** was found to be 5:1. Since the conversion was not satisfactory, the synthesis was repeated by using a two-step microwave reaction (1.5 h, 40˚C; 1.5 h, 50˚C). A further increase of the temperature (100˚C) under microwave conditions resulted in the formation of **16a** and **16b** in a conversion of 57%. Before the work-up procedure, the diastereomeric ratio of **16a**:**16b** wase also measured and found to be 5:1 again. It means that the diastereoselectivity of the reaction is not influenced by the reaction temperature (Table I). The desired products **16a** and **16b** were isolated by column chromatography and the configurations of the newly created stereogenic centres (Figure 5) were determined by comparing the measured characteristic data (melting points, ^{1}H-NMR chemical shifts and rotation values) with the published data found for the analogues **11a** and **11b** (Figure 3) (34).

Biological evaluation. Regarding the antibacterial activity (Table II), the compounds were tested on Gram-negative *Escherichia coli* and Gram-positive methicillin susceptible and resistant *Staphylococcus aureus* strains. Two derivatives exerted a moderate antibacterial effect on the reference *S. aureus* strains: **14** and **10** had an MIC of 50 μM. The other derivatives had an MIC of 100 μM or >100 μM on the tested bacterial strains.

The efflux pump inhibiting (EPI) properties of the compounds were assessed on Gram-negative and Gram-positive bacterial strains, such as AcrAB-TolC pump expressing *E. coli* AG100, its AcrAB-TolC-deleted mutant strain *E. coli* AG100A and methicillin susceptible *S. aureus* ATCC 25923 reference strain, methicillin resistant *S. aureus* MRSA 272123 clinical strains, respectively. The EPI activity was compared to the positive control and in the case of the *E. coli* strains, an RFI value above 0.30 was considered as an effective efflux pump inhibitor (the positive control CCCP had an RFI of 0.74 on the pump expressing and pump deleted strains). Compound **3** was effective on *E. coli* AG100 (RFI: 0.33) and caused higher EB accumulation in the mutant *E. coli* strain (RFI: 0.52). Similar activity was obtained on *E. coli* AG100 in the presence of compounds **4** (RFI: 0.47) and **6** (RFI: 0.37). Furthermore, the compounds were also more active on the mutant strain indicating that AcrAB-TolC is crucial in the defense mechanisms of bacteria against toxic compounds. Compounds **7** and **9** were potent EPIs at 50 μM with RFIs of 0.33 and 0.36 on *E. coli* AG100, respectively. Furthermore, their activity was near the same on the mutant *E. coli* strain, because they have more targets in the bacterial cells, *e.g.* membrane destabilizing activity without affecting efflux pumps. The most potent EPI of the AcrAB-TolC system was **8** with RFI of 0.56 on the *E. coli* AG100 strain at 50 μM, and lower activity was observed on the pump mutant strain (RFI: 0.43). On the Gram-positive strains the positive control was reserpine: it was more active on the methicillin susceptible reference strain (RFI: 0.32) than on the MRSA clinical isolate (RFI: 0.23). None of the compounds had EPI properties on the MRSA strain. On the methicillin susceptible strain, the most potent EPIs at 50 μM are the following: **8** (RFI: 0.68), **9** (RFI: 0.30), **12** (RFI: 0.30), **7** (RFI: 0.17). Considering the antibacterial evaluations, the indole skeleton connected with 6,7-dihydrothieno[3,2-*c*]pyridine as a partially saturated cyclic amine has been found to be the most effective (Table II, Entry 8).

Using the bacterial strains mentioned above, the anti-biofilm activity of the derivatives was determined using crystal violet staining. No biofilm inhibition was observed

Table II. *Antibacterial effectivity of synthesized compounds*.

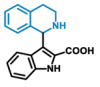
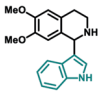
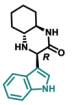
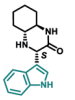
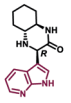
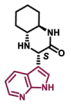
			Antibacterial activity								
			Anti-biofilm activity E. coli AG100[a]					Efflux pump inhibition			
Entry	Product	Structure	*S. aureus* MRSA 272123[b]					RFI			
			cc. µM, µg/mL	AV.	SD	comp. OD	Inh. %	cc. µM	*E. coli* AG100	*E. coli* AG100A	*S. aureus* ATCC 25923	*S. aureus* MRSA 272123
1	**1**		50	0.96[a]	0.42[a]	0.81[a]	–151.92[a]	50	0.20	0.52	–0.18	–0.03
			100	0.71[b]	0.06[b]	0.55[b]	–42.08[b]					
				0.67[a]	0.09[a]	0.52[a]	–62.92[a]					
				0.89[b]	0.02[b]	0.74[b]	–90.25[b]					
2	**2**		50	0.75[a]	0.28[a]	0.60[a]	–88.57[a]	50	–0.01	–0.01	–0.14	0.01
			100	0.76[b]	0.12[b]	0.61[b]	–56.75[b]					
				0.60[a]	0.10[a]	0.45[a]	–40.16[a]					
				0.78[b]	0.11[b]	0.63[b]	–62.19[b]					
3	**3**		50	0.65[a]	0.24[a]	0.50[a]	–56.12[a]	50	0.33	0.52	–0.22	0.09
			100	0.68[b]	0.07[b]	0.53[b]	–36.37[b]					
				0.54[a]	0.09[a]	0.39[a]	–20.45[a]					
				0.70[b]	0.25[b]	0.54[b]	–39.38[b]					
4	**4**		50	0.64[a]	0.27[a]	0.49[a]	–54.32[a]	50	0.47	0.53	–0.09	0.10
			100	0.64[b]	0.11[b]	0.48[b]	–24.38[b]					
				0.72[a]	0.06[a]	0.57[a]	–77.10[a]					
				0.58[b]	0.08[b]	0.42[b]	–8.41[b]					
5	**5**		50	0.48[a]	0.08[a]	0.33[a]	–4.03[a]	50	0.04	0.42	–0.17	0.00
			100	0.71[b]	0.05[b]	0.56[b]	–43.49[b]					
				0.48[a]	0.14[a]	0.32[a]	–1.37[a]					
				0.53[b]	0.07[b]	0.37[b]	3.95[b]					
6	**6**		50	0.82[a]	0.32[a]	0.67[a]	–109.61[a]	50	0.37	0.66	–0.14	0.02
			100	0.65[b]	0.11[b]	0.49[b]	–26.68[b]					
				0.56[a]	0.02[a]	0.40[a]	–26.63[a]					
				0.67[b]	0.13[b]	0.51[b]	–31.40[b]					
7	**7**		50	0.84[a]	0.33[a]	0.69[a]	–115.71[a]	50	0.33	0.34	0.17	0.04
			100	0.94[b]	0.03[b]	0.78[b]	–100.97[b]	100	0.44	0.40	0.38	0.11
				0.51[a]	0.11[a]	0.36[a]	–12.66[a]					
				0.50[b]	0.14[b]	0.35[b]	10.12[b]					
8	**8**		50	1.03[a]	0.35[a]	0.88[a]	–174.29[a]	50	0.56	0.43	0.68	0.07
			100	0.75[b]	0.12[b]	0.59[b]	–52.76[b]	100	0.71	0.85	-	0.17
				0.63[a]	0.13[a]	0.48[a]	–49.78[a]					
				0.89[b]	0.24[b]	0.74[b]	–89.75[b]					
9	**9**		50	1.08[a]	0.24[a]	0.93[a]	–189.93[a]	50	0.36	0.36	0.30	–0.09
			100	0.55[b]	0.03[b]	0.40[b]	–2.23[b]	100	0.33	0.26	-	0.03
				0.67[a]	0.11[a]	0.52[a]	–63.44[a]					
				0.50[b]	0.03[b]	0.35[b]	9.87[b]					
10	**10**		50	0.45[a]	0.03[a]	0.29[a]	8.02[a]	25	-	-	0.16	-
			100	0.62[b]	0.08[b]	0.46[b]	–18.53[b]	50	0.24	0.22	-	0.01
				0.48[a]	0.05[a]	0.33[a]	–2.78[a]	100	0.25	0.36	-	–0.10
				0.43[b]	0.08[b]	0.28[b]	29.17[b]					
11	**11a**		50	0.36[a]	0.03[a]	0.21[a]	34.30[a]	50	0.20	0.18	0.14	–0.06
			100	0.75[b]	0.06[b]	0.60[b]	–53.96[b]	100	0.11	–0.07	0.24	–0.08
				0.47[a]	0.06[a]	0.32[a]	0.04[a]					
				0.65[b]	0.06[b]	0.50[b]	–27.41[b]					
12	**11b**		50	0.43[a]	0.03[a]	0.28[a]	13.80[a]	50	0.17	–0.10	0.05	–0.02
			100	0.74[b]	0.05[b]	0.58[b]	–49.75[b]	100	0.12	–0.02	0.14	0.01
				0.37[a]	0.04[a]	0.22[a]	31.45[a]					
				0.84[b]	0.04[b]	0.68[b]	–75.58[b]					

Table II. *Continued*

Table II. *Continued*

			Antibacterial activity								
			Anti-biofilm activity E. coli AG100[a]					Efflux pump inhibition			
Entry	Product	Structure	*S. aureus* MRSA 272123[b]					RFI			
			cc. μM, μg/mL	AV.	SD	comp. OD	Inh. %	cc. μM	*E. coli* AG100	*E. coli* AG100A	*S. aureus* ATCC 25923	*S. aureus* MRSA 272123
13	**12**		50	0.45[a]	0.11[a]	0.30[a]	6.53[a]	50	0.27	0.17	0.30	0.02
			100	0.73[b]	0.07[b]	0.57[b]	–47.18[b]	100	0.26	0.40	0.16	0.02
				0.35[a]	0.05[a]	0.20[a]	38.34[a]					
				0.47[b]	0.08[b]	0.31[b]	19.13[b]					
14	**13**		50	0.49[a]	0.03[a]	0.34[a]	–6.06[a]	50	0.06	0.29	–0.10	–0.01
			100	0.53[b]	0.01[b]	0.38[b]	2.49[b]					
				0.43[a]	0.02[a]	0.28[a]	12.06[a]					
				0.59[b]	0.14[b]	0.44[b]	–12.95[b]					
15	**14**		50	0.48[a]	0.03[a]	0.33[a]	–3.17[a]	50	0.12	0.57	–0.16	0.06
			100	0.49[b]	0.07[b]	0.33[b]	13.90[b]					
				0.45[a]	0.04[a]	0.30[a]	5.83[a]					
				-	-	-	-					
16	**15**		50	0.57[a]	0.10[a]	0.42[a]	–32.34[a]	50	0.01	0.30	–0.25	–0.02
			100	0.41[b]	0.28[b]	0.26[b]	33.38[b]					
				0.41[a]	0.03[a]	0.26[a]	19.98[a]					
				0.55[b]	0.15[b]	0.39[b]	–1.29[b]					
17	**16a**		50	0.46[a]	0.10[a]	0.31[a]	3.71[a]	50	0.17	0.04	–0.01	0.01
			100	0.46[b]	0.01[b]	0.30[b]	21.96[b]	100	0.06	–0.03	0.33	0.02
				0.41[a]	0.06[a]	0.26[a]	18.73[a]					
				0.61[b]	0.02[b]	0.46[b]	–17.93[b]					
18	**16b**		50	0.51[a]	0.09[a]	0.36[a]	–11.22[a]	50	0.17	0.30	0.10	–0.02
			100	0.52[b]	0.26[b]	0.37[b]	4.71[b]	100	–0.01	0.14	0.12	–0.05
				0.42[a]	0.03[a]	0.27[a]	14.82[a]					
				0.57[b]	0.12[b]	0.41[b]	–5.75[b]					
		CCCP							0.74	0.74	-	-
		Reserpine (RES)									0.32	0.23

[a]Determined anti-biofilm activity on *E. coli* AG100 strain. [b]Determined anti-biofilm activity on *S. aureus* MRSA 272123 strain.

on the pump-deleted mutant *E. coli* AG100A and on the methicillin susceptible *S. aureus* ATCC 25923 strains. Three derivatives could inhibit the biofilm formation of *E. coli* AG100 with inhibition degree of nearly 30%: **12** (inhibition: 38.34%), **11b** (inhibition: 31.45%) at 100 μM and **11a** (inhibition: 34.30%) at 50 μM. Four derivatives could inhibit the biofilm formation of the MRSA strain: the most potent one was **15** demonstrating an inhibition of 33.38% at 50 μM, then **10** with an inhibition of 29.17% at 100 μM. Furthermore, **16a** (an inhibition of 21.96% at 50 μM) and the weakest activity of 19.13% was detected in the presence of **12** at 100 μM. In point of correlation between structure and biological activity, 7-azaindole as an electron-rich aromatic compound could be identified as a significant moiety (Table II, Entry 13, 16).

The cytotoxic activity of the derivatives was determined using sensitive (Colo 205) and ABCB1 efflux pump expressing (Colo 320) colon adenocarcinoma cell lines and a normal, non-cancerous fibroblast cell line MRC-5. The most potent derivatives were **10**, **1**, **8**, **3** and **9**. The highest anticancer activity was measured in the presence of **8** and **10** on Colo 205 cells (IC_{50}: 21.81 and 24.71 μM, respectively) and Colo 320 cells (IC_{50}: 12.94 and 13.55 μM, respectively). However, compound **10** showed moderate toxicity on normal cells too (IC_{50}: 22.86 μM), whereas compound **8** had no toxic effect on normal cells. The IC_{50} values of **1** and **8** were between 10 and 30 μM on the colon adenocarcinoma cells and, fortunately, these derivatives were not toxic on normal MRC-5 fibroblasts (IC_{50} >100 μM). In the case of **3** and **9**, the derivatives were more effective on the resistant cell line (IC_{50} of 53.52 and 41.06 μM, respectively) compared to the sensitive one (IC_{50} of 64.35 and 97.85 μM, respectively). In addition, they were not toxic on normal fibroblasts (IC_{50} >100 μM for both compounds). Derivative **7** was more toxic

Table III. *Cytotoxic applicability of prepared derivatives*.

Entry	Product	Structure	Cytotoxic activity										
			Colo 205		Colo 320		MRC-5		Inhibition of P-glycoprotein				
			IC_{50} μM	SD±	IC_{50} μM	SD±	IC_{50} μM	SD±	cc. μM	FSC	SSC	FL-1	FAR
1	**1**		22.37	2.04	30.76	0.86	>100	-	2 20	2,010 2,032	1,041 1,026	3.34 6.49	0.86 1.66
2	**2**		>100	-	53.58	1.52	>100	-	2 20	2,016 2,016	1,041 1,024	3.74 2.62	0.96 0.67
3	**3**		64.35	2.17	53.52	0.90	>100	-	2 20	1,621 1,594	896 917	9.64 9.75	1.47 1.48
4	**4**		>100	-	>100	-	98.80	2.17	2 20	1,999 1,963	1,045 1,027	3.61 3.26	0.92 0.83
5	**5**		>100	-	>100	-	>100	-	2 20	2,033 2,054	1,042 1,038	3.93 2.84	1.01 0.73
6	**6**		>100	-	>100	-	>100	-	2 20	1,633 1,623	901 896	6.51 6.58	0.99 1.00
7	**7**		47.38	0.97	60.38	1.61	>100	-	2 20	1,612 1,591	876 883	15.40 26.604	2.3 4.04
8	**8**		21.81	1.33	12.94	0.26	>100	-	2 20	1,615 1,618	865 910	6.48 33.80	0.99 5.14
9	**9**		97.85	0.61	41.06	1.20	>100	-	2 20	1,609 1,596	888 897	7.26 17.10	1.10 2.60
10	**10**		24.71	0.10	13.55	0.98	22.86	0.57	2 20	1,643 1,627	1,030 992	4.13 13.00	0.72 2.26
11	**11a**		>100	-	>100	-	>100	-	2 20	1,655 1,629	1,028 1,041	3.79 3.07	0.66 0.53
12	**11b**		>100	-	>100	-	>100	-	2 20	1,670 1,636	1,014 1,043	3.39 3.27	0.59 0.57
13	**12**		>100	-	>100	-	>100	-	2 20	1,669 1,668	1,040 1,036	3.38 2.96	0.59 0.52
14	**13**		>100	-	>100	-	>100	-	2 20	1,974 2,007	1,009 1,058	6.86 7.46	1.76 1.91
15	**14**		79.56	4.20	68.47	6.06	>100	-	2 20	2,014 2,006	1,024 1,017	4.79 12.10	1.23 3.10
16	**15**		66.79	1.52	54.23	1.70	>100	-	2 20	2,025 2,023	1,011 1,022	5.68 6.38	1.45 1.63
17	**16a**		>100	-	>100	-	>100	-	2 20	1,652 1,639	1,016 1,040	4.44 3.55	0.77 0.62
18	**16b**		>100	-	>100	-	>100	-	2 20	1,660 1,662	1,033 1,048	2.68 2.51	0.47 0.44

on the sensitive cells (IC_{50}: 47.38 μM) than on the resistant ones (IC_{50}: 60.38 μM). Compounds **14** and **15** showed mild cytotoxic activity on cancer cells (IC_{50}: 50-80 μM) and they did not affect the viability of normal cells (IC_{50} >100 μM). To conclude, in favour of improved cytotoxic activity, the presence of the indole skeleton and 6,7-dihydrothieno[3,2-*c*]pyridine as well as 3,4-dihydro-*β*-carboline as cyclic imine is relevant (Table III, Entry 8, 10).

Since the difference between the sensitive and resistant colon adenocarcinoma cell lines is the expression of the MDR efflux transporter ABCB1 (P-glycoprotein), the activity of the compounds was assessed on the inhibition of ABCB1. The ABCB1 inhibitory activity was compared based on the fluorescence activity ratio (FAR) values at 2 and 20 μM. FAR values above 2 are considered as active ABCB1 modulators. Out of all derivatives, **7**, **8**, **9**, **10** and **14** showed inhibitory effect. The most potent efflux pump inhibitor (EPI) was **7** showing inhibition in both 2 and 20 μM (FAR: 2.34 and 4.04). The other compounds exerted inhibition only at 20 μM: **8** (FAR: 5.14), **14** (FAR: 3.10), **9** (FAR: 2.60) and **10** (FAR: 2.26). In the case of inhibition of P-glycoprotein, the indole moiety has been described as the most active electron-rich aromatic component. With respect to the coupled cyclic amine side, 6,7-dimethoxyisoquinoline has been interpreted to be a beneficial structural element (Table III, Entry 7).

Conclusion

A series of cyclic amines coupled with indole and azaindole derivatives has been systematically designed and their biological effects were evaluated. The investigated precursors were synthesized by the coupling of indole with cyclic imines, such as 3,4-dihydroisoquinoline, 6,7-dimethoxy-3,4-dihydro-isoquinoline, 6,7-dihydrothieno[3,2-*c*]pyridine, 3,4-dihydro-*β*-carboline, 4,5-dihydro-3*H*-benz[*c*]azepine and (4*aR*,8*aR*)-4*a*,5,6,7,8,8*a*-hexahydroquinoxalin-2(1*H*)-one. By fixing the isoquinoline part, the influence of the indole skeleton was also varied among indole-2-carboxylic acid, 4-, 5-, 6- and 7-azaindoles. To have a comprehensive analysis about the correlation between structure and biological activity, the 7-azaindole analogues of the previously mentioned C-3-substituted indoles were also resynthesized or synthesized by using the modified *aza*-Friedel–Crafts reaction. The preparation of new 7-azaindole derivatives starting from 6,7-dimethoxy-3,4-dihydroisoquinoline as cyclic imine and (4*aR*,8*aR*)-4*a*,5,6,7,8,8*a*-hexahydroquinoxalin-2(1*H*)-one as chiral cyclic imine were also achieved by using microwave conditions. The selected C-3-coupled indole and azaindole products have been tested on Gram-negative and Gram-positive strains, colon adenocarcinoma cell lines and normal, non-cancerous fibroblast cell line in order to investigate the effect of structural modification on biological activity. Regarding their biological evaluation, some compounds were found to have antibacterial effect on *E. coli* AG100, *E. coli* AG100A, *S. aureus* ATCC 25923, and *S. aureus* MRSA 272123 strains. As a result of biological investigations, certain C-3-coupled indole and azaindole derivatives showed toxicity on sensitive (Colo 205) and ABCB1 efflux pump expressing (Colo 320) colon adenocarcinoma cell lines and a normal, non-cancerous fibroblast cell line MRC-5. Antibacterial activity was observed on *S. aureus* strains in the case of 3-(2,3,4,5-tetrahydro-1*H*-benz[*c*]azepin-1-yl)-7-azaindole **(14)** and 3-(2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indol-1-yl)indole **(10)** (MIC: 50 μM). The synthesized 3-(4,5,6,7-tetrahydrothieno[3,2-*c*]pyridin-4-yl) indole **(8)** has been described as a potent efflux pump inhibitor on the *E. coli* AG100 strain. The highest anti-biofilm activity was determined in the presence of 3-(6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-1-yl)-7-azaindole **(12)** with inhibition of 38.34%. 3-(4,5,6,7-Tetrahydrothieno[3,2-*c*]pyridin-4-yl)indole **(8)** and 3-(2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indol-1-yl)indole **(10)** showed appreciable cytotoxic activity on Colo 205 cells and Colo 320 cells. Considering the inhibition of the cancer MDR efflux transporter ABCB1, 3-(6,7-dimethoxy-tetrahy-droisoquinolyl)-indole **(7)** modulated this efflux pump in 2 and 20 μM effectively. It is worth noting that the presence of basic nitrogen in the synthesized structures enable the fine-tuning of water solubility *via* salt formation. Future study in this direction would lead to a deeper understanding of the mechanism of C-3-coupled indole and 7-azaindol derivatives in biological systems.

Conflicts of Interest

The Authors declare no conflicts of interest.

Authors' Contributions

Conceptualization, I.S., G.S.; investigation, D.H., N.S., M.G., J.S., K.B.; visualization, D.H.; writing – original draft preparation, I.S., D.H., N.S., G.S.; writing – review and editing, I.S., G.S. All Authors have read and agreed to the published version of the manuscript.

Acknowledgements

The Authors thank the Hungarian Research Foundation (OTKA No. K-138871), the Ministry of Human Capacities, Hungary grant, TKP-2021-EGA-32. N. S. was supported by the ÚNKP-23-4-SZTE-347 New National Excellence Program of the Ministry for Innovation and Technology from the source of the National Research, Development and Innovation Fund. G.S. was supported by the János Bolyai Research Scholarship (BO/00158/22/5) of the Hungarian Academy of Sciences and by the ÚNKP-23-5-SZTE-677 New National Excellence Program of the Ministry for Culture and Innovation from the source of the National Research, Development and Innovation Fund.

References

1 Bur SK, Martin SF: Vinylogous Mannich reactions: selectivity and synthetic utility. Tetrahedron 57(16): 3221-3242, 2001. DOI: 10.1016/S0040-4020(01)00035-7

2 Speckamp WN, Moolenaar MJ: New developments in the chemistry of N-acyliminium ions and related intermediates. Tetrahedron 56(24): 3817-3856, 2000. DOI: 10.1016/S0040-4020(00)00159-9

3 Lian X, Lin L, Fu K, Ma B, Liu X, Feng X: A new approach to the asymmetric Mannich reaction catalyzed by chiral N,N'-dioxide-metal complexes. Chem Sci 8(2): 1238-1242, 2017. DOI: 10.1039/c6sc03902b

4 Liras S, Davoren JE, Bordner J: An approach to the skeleton of the securinega alkaloids. The total synthesis of (±)-securinine. Org Lett 3(5): 703-706, 2001. DOI: 10.1021/ol0070482

5 Ito M, Clark CW, Mortimore M, Goh JB, Martin SF: Biogenetically inspired approach to the Strychnos alkaloids. Concise syntheses of (+/–)-akuammicine and (+/–)-strychnine. J Am Chem Soc 123(33): 8003-8010, 2001. DOI: 10.1021/ja010935v

6 Szatmári I, Fülöp F: Syntheses and transformations of 1-(α-aminobenzyl)-2-naphthol derivatives. Curr Org Synth 1(2): 155-165, 2004. DOI: 10.2174/1570179043485402

7 Pape VFS, Palkó R, Tóth S, Szabó MJ, Sessler J, Dormán G, Enyedy ÉA, Soós T, Szatmári I, Szakács G: Structure-activity relationships of 8-hydroxyquinoline-derived Mannich bases with tertiary amines targeting multidrug-resistant cancer. J Med Chem 65(11): 7729-7745, 2022. DOI: 10.1021/acs.jmedchem.2c00076

8 MacLeod PD, Li Z, Feng J, Li CJ: Solvent-free direct aza-Friedel-Crafts reactions between 3,4-dihydroisoquinoline and 1- or 2-naphthols. Tetrahedron Lett 47(38): 6791-6794, 2006. DOI: 10.1016/j.tetlet.2006.07.066

9 Heydenreich M, Koch A, Klod S, Szatmári I, Fülöp F, Kleinpeter E: Synthesis and conformational analysis of naphth[1',2':5,6][1,3]oxazino[3,2-c][1,3]benzoxazine and naphth[1',2':5,6][1,3]oxazino[3,4-c][1,3]benzoxazine derivatives. Tetrahedron 62(48): 11081-11089, 2006. DOI: 10.1016/j.tet.2006.09.037

10 Heydenreich M, Koch A, Szatmári I, Fülöp F, Kleinpeter E: Synthesis and conformational analysis of naphth[1,2-e][1,3]oxazino[4,3-a][1,3]isoquinoline and naphth[2,1-e][1,3]oxazino[4,3-a]isoquinoline derivatives. Tetrahedron 64(30-31): 7378-7385, 2008. DOI: 10.1016/j.tet.2008.05.025

11 Szatmári I, Barta P, Tóth G, Balázs A, Halász J, Fülöp F: Synthesis and conformational behaviour of enantiomeric naphthoxazinoquinoxalinone derivatives. European J Org Chem 2017(37): 5537-5545, 2017. DOI: 10.1002/ejoc.201700699

12 Barta P, Szatmári I, Fülöp F, Heydenreich M, Koch A, Kleinpeter E: Synthesis and stereochemistry of new naphth[1,3]oxazino[3,2-a]benzazepine and naphth[1,3]oxazino[3,2-e]thienopyridine derivatives. Tetrahedron 72(19): 2402-2410, 2016. DOI: 10.1016/j.tet.2016.03.058

13 Szatmári I, Sas J, Fülöp F: Catalyst-free coupling of indole derivatives with 3,4-dihydroisoquinoline and related compounds. Tetrahedron Lett 54(37): 5069-5071, 2013. DOI: 10.1016/j.tetlet.2013.07.039

14 Sas J, Szatmári I, Fülöp F: One-Pot α-Arylation of β-carboline with indole and naphthol derivatives. Curr Org Synth 13(4): 611-616, 2016. DOI: 10.2174/1570179413666151218201331

15 Belasri K, Fülöp F, Szatmári I: Solvent-free C-3 coupling of azaindoles with cyclic imines. Molecules 24(19): 3578, 2019. DOI: 10.3390/molecules24193578

16 Chandal N, Tambat R, Kalia R, Kumar G, Mahey N, Jachak S, Nandanwar H: Efflux pump inhibitory potential of indole derivatives as an arsenal against norA over-expressing Staphylococcus aureus. Microbiol Spectr 11(5): e0487622, 2023. DOI: 10.1128/spectrum.04876-22

17 Zhang Y, Lavoie EJ, Yuan Y, Parhi A, Sun Y: Indole derivatives as efflux pump inhibitors. 2018. Patent WO2018165611A1. Available at: https://patents.google.com/patent/WO2018165611A1/en [Last accessed on January 29, 2024]

18 Puri S, Stefan K, Khan SL, Pahnke J, Stefan SM, Juvale K: Indole derivatives as new structural class of potent and antiproliferative inhibitors of monocarboxylate transporter 1 (MCT1; SLC16A1). J Med Chem 66(1): 657-676, 2023. DOI: 10.1021/acs.jmedchem.2c01612

19 Kolodina AA, Serdyuk OV: Eudistomin U, isoeudistomin U, and related indole compounds: synthesis and biological activity. Heterocycles 96(7): 1171-1196, 2018. DOI: 10.3987/rev-18-882

20 Amare DE, Bovee TFH, Mulder PPJ, Hamers A, Hoogenboom RLAP: Acid condensation products of indole-3-carbinol and their in-vitro (anti)estrogenic, (anti)androgenic and aryl hydrocarbon receptor activities. Arab J Chem 13(9): 7199-7211, 2020. DOI: 10.1016/j.arabjc.2020.08.002

21 Baez-Gonzalez AS, Carrazco-Carrillo JA, Figueroa-Gonzalez G, Quintas-Granados LI, Padilla-Benavides T, Reyes-Hernandez OD: Functional effect of indole-3 carbinol in the viability and invasive properties of cultured cancer cells. Biochem Biophys Rep 35: 101492, 2023. DOI: 10.1016/j.bbrep.2023.101492

22 El-Boghdady NA, El-Hakk SA, Abd-Elmawla MA: The lncRNAs UCA1 and CRNDE target miR-145/TLR4/NF-κB/TNF-α axis in acetic acid-induced ulcerative colitis model: The beneficial role of 3,3-Diindolylmethane. Int Immunopharmacol 121: 110541, 2023. DOI: 10.1016/j.intimp.2023.110541

23 Sheikh IA, Jiffri EH, Kamal MA, Ashraf GM, Beg MA: Lactoperoxidase, an antimicrobial milk protein, as a potential activator of carcinogenic heterocyclic amines in breast cancer. Anticancer Res 37(11): 6415-6420, 2017. DOI: 10.21873/anticanres.12095

24 Sundberg RJ: Indoles. London, UK, Academic Press, 1996.

25 Gribble GW: Heterocyclic Scaffolds II: Reactions and applications of indoles. Berlin, Heidelberg, Springer, pp. 26, 2010.

26 Rácz B, Kincses A, Laczi K, Rákhely G, Domínguez-Álvarez E, Spengler G: Reversal of multidrug resistance by symmetrical selenoesters in colon adenocarcinoma cells. Pharmaceutics 15(2): 610, 2023. DOI: 10.3390/pharmaceutics15020610

27 Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 11th ed. Wayne, PA, USA, Clinical and Laboratory Standards Institute, 2018. Available at: https://clsi.org/media/1928/m07ed11_sample.pdf [Last accessed on January 29, 2024]

28 Szemerédi N, Kincses A, Rehorova K, Hoang L, Salardón-Jiménez N, Sevilla-Hernández C, Viktorová J, Domínguez-Álvarez E, Spengler G: Ketone- and cyano-selenoesters to overcome efflux pump, quorum-sensing, and biofilm-mediated resistance. Antibiotics (Basel) 9(12): 896, 2020. DOI: 10.3390/antibiotics9120896

29 Herz W, Tsai L: Sulfur analogs of isoquinolines. IV. The Pictet-Gams reaction and attempts to prepare analogs of papaverine[1,2]. J Am Chem Soc 77(13): 3529-3533, 1955. DOI: 10.1021/ja01618a031

30 Meyers AI, Hutchings RH: The asymmetric synthesis of 1-alkyl-2,3,4,5-tetrahydro-benzazepines and benzo[β]-1-azabicyclo[5,3,1] decanes. Tetrahedron 49(9): 1807-1820, 1993. DOI: 10.1016/S0040-4020(01)80537-8

31 Jakubec P, Helliwell M, Dixon DJ: Cyclic imine nitro-Mannich/lactamization cascades: a direct stereoselective synthesis of multicyclic piperidinone derivatives. Org Lett 10(19): 4267-4270, 2008. DOI: 10.1021/ol801666w

32 Chen Z, Hu G, Li D, Chen J, Li Y, Zhou H, Xie Y: Synthesis and vasodilator effects of rutaecarpine analogues which might be involved transient receptor potential vanilloid subfamily, member 1 (TRPV1). Bioorg Med Chem 17(6): 2351-2359, 2009. DOI: 10.1016/j.bmc.2009.02.015

33 Wojaczyńska E, Bąkowicz J, Dorsz M, Skarżewski J: Monoimine derived from trans-1,2-diaminocyclohexane and ethyl glyoxylate: an intermediate in aza-Diels-Alder and Mannich reactions. J Org Chem 78(6): 2808-2811, 2013. DOI: 10.1021/jo302820m

34 Iwanejko J, Wojaczyńska E, Wojaczyński J, Bąkowicz J: Stereoselective preparation of chiral compounds in Mannich-type reactions of a bicyclic imine and phenols or indole. Tetrahedron Lett 55(49): 6619-6622, 2014. DOI: 10.1016/j.tetlet.2014.10.081

35 Irie T, Sawa M: 7-Azaindole: a versatile scaffold for developing kinase inhibitors. Chem Pharm Bull 66(1): 29-36, 2018. DOI: 10.1248/cpb.c17-00380

36 Bollag G, Tsai J, Zhang J, Zhang C, Ibrahim P, Nolop K, Hirth P: Vemurafenib: the first drug approved for BRAF-mutant cancer. Nature 11(11): 873-886, 2012. DOI: 10.1038/nrd3847

Received December 20, 2023
Revised January 23, 2024
Accepted January 29, 2024