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Synthesis, transformations and biological evaluation of 5-chloro-8-hydroxyquinoline hybrids

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ABSTRACT

Taking in consideration of previous studies in the field of biological relevance and the chelating properties of derivatives bearing the hydroxyquinoline skeleton, establishing cationic centre by direct amidation as well as preparation compound with amino acid character was proposed. In order to test anticancer activity of the synthesised compounds, the applying of preliminary biological screening systems was planned. This concept is demonstrated in the context of the synthesis and transformation of a bifunctional glycine-type precursor substituted with 5-chloro-8-hydroxyquinoline to hybrids with amino acid and amine feature. Stabilization of the precursor and formed amide composed of *N,N*-dimethylethylamine via partially aromatic *ortho*-quinone methide intermediate was tested with different cyclic imines in [4 + 2] cycloaddition. In these [4 + 2] cycloaddition reactions, ^{1}H NMR analysis of the crude reaction mixture proved the formation of a single product in all cases. The relative configuration of the two newly formed stereogenic centres was determined by 2D-NMR technique. Compared to the precursor, all new derivatives exhibited less potent anticancer activity. Based on biological evaluations, out of the synthesized compounds the derivative with amino acid feature and some amidated derivatives showed toxic activity against the Colo 205 cell line.

1. Introduction

8-Hydroxyquinolines (HQs) have considered to be privileged scaffolds for new drug candidates that have been widely explored for biological effects (Oliveri and Vecchio, 2016; Gupta et al., 2021; Rbaa et al., 2018; Suwanjang et al., 2016; Olaleye et al., 2011; Shaw et al., 2010). The mode of action of HQ has been studied and the interaction with metal ions proved to be necessary for antibacterial (Albert et al., 1954) as well as antiproliferative activity (Zhai et al., 2010). Oliveri et al. reported the synthesis of new galactose conjugates of HQ derivatives and the effect of copper(II) ions on their biological activities (Oliveri et al., 2015). According to the structure–activity relationships of copper HQ complexes, a correlation between the lipophilic nature of the ligands and the cytotoxic activity was established (Tardito et al., 2012). In the area of neurodegenerative diseases, Budimir et al. focused on getting a better understanding of coordination properties of clioquinol and its influence on the dissolution of Aβ aggregates through efficient metal exchange processes (Budimir et al., 2011). The relationship between the thermodynamic parameters and the biological activity of HQs (Pape et al., 2018), the impact of the solution stability and redox activity of iron(III) and copper(II) complexes (Pape et al., 2021), acid-base properties and metal-chelating ability (Pape et al., 2022) of Mannich bases derived from 8-hydroxyquinoline on multidrug resistance (MDR)-selective toxicity were studied. The bioactivities of HQs originate from multiple mechanisms, which function in restoring metal homeostasis and promoting protective effects (Prachayasittikul et al., 2013). Based on a recent paper, by integrating 8HQ considered as an ideal scaffold for metal ion chelation with a ruthenium(II) polypyridyl moiety, two Ru (II)-8HQ metal complexes with dual antitumor mechanisms, including Jumonji C domain-containing demethylase (JMJD) inhibitory and photodynamic therapy (PDT) activities, were designed and synthesized (Ma et al., 2022). In addition, platinum(II) coordination compounds using 5-substituted-8-hydroxyquinoline derivatives as ligands were designed and synthesized, the cytotoxic activity of prepared compounds

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were screened in both healthy cell line and cancer cell lines. By the research the potential of the platinum(II) 5-substituted-8-hydroxyquinoline coordination compounds for the new platinum-resistant cancer therapy was detectable (Yang et al., 2023). According to a previous study, 8-HQ and its derivatives can serve as metal-binding pharmacophores for metalloenzyme inhibition. The coordination chemistry of the resulting metal-binding isosteres is explored using a bioinorganic model complex. The bioactivity of the metal-binding isosteres was monitored by measuring enzymatic activity against several metalloenzymes (Seo et al., 2022).

The Mannich reaction is considered to be one of the most frequently applied multicomponent reactions in organic chemistry (Szatmári and Fülöp, 2013). The modified Mannich-type synthetic pathway was applied to prepare bifunctional precursors that were further transformed via [4 + 2] cycloaddition to naphth[1,3]oxazino-benzazepines and -thienopyridines (I. Szatmári et al., 2017) as well as to non-racemic naphth[1,3]oxazino[3,2-*a*]quinoxalinones (I. Szatmári et al., 2017). Subsequently, highly functionalised aminonaphthol derivatives (Szatmári et al., 2019) or aminophenanthrol-type Mannich bases (Belasri et al., 2020) with different cyclic imines were examined. In recent investigations, new glycine derivatives substituted with 2- and 1-naphthol have been synthesised and transformed with different dienophiles via [4 + 2] cycloaddition to new α-amino acid esters. As a result of our previous work, the synthesis of a glycine-type precursor bearing 5-chloro-8-hydroxyquinoline and its transformations to diarylmethane derivatives with indole and 7-azaindole were achieved. Regarding the biological results, inhibition of the efflux pump system in *Staphylococcus aureus* strains (Hegedűs et al., 2022) and a potent toxic activity against Colo205 and Colo320 cell lines (Hegedűs et al., 2024) were observed. The 8-hydroxyquinoline scaffold has been applied to synthesize potent antibacterial (McGowan et al., 2020; Cherdtrakulkiat et al., 2016; Enquist et al., 2012) and anticancer (Ruankham et al., 2021; Kubanik et al., 2015) derivatives. Recent studies highlight the importance of derivatives of 8-HQ as possible chemotherapeutic agents and as possible leads towards the development of new drugs to treat various diseases, including cancer. In the framework of a review Saadeh et al. presented different synthetic strategies for pharmaceutically important chemicals that incorporate the 8-HQ moiety with different pharmacological properties, including anticancer and antibacterial activities (Saadeh et al., 2020). 8-Hydroxyquinoline derivatives as antibacterial and anticancer agents were presented in Fig. 1 (Pape et al., 2022; Saadeh et al., 2020).

In the consequence of biological relevance and the chelating properties of studied compounds bearing the HQ skeleton, we aimed to establish cationic centre by direct amidation of a Mannich base consisting of the 5-chloro-8-hydroxyquinoline skeleton, morpholine and ethyl ester motif. Our further aim was to test the transformation possibility of the Mannich base as well as its amidated derivatives in [4 + 2] cycloaddition via partially aromatic *ortho*-quinone methide intermediate. Along with investigation of scope and limitations of these reactions, the aim of the current study was to test the antibacterial and anticancer activity of the synthesized compounds by preliminary biological screening systems.

2. Results and discussions

2.1. Synthesis

In our previous paper, we described the preparation of a glycine-type precursor starting from 5-chloro-8-hydroxyquinoline, morpholine and ethyl glyoxylate via modified Mannich reaction. Mannich base **1** showed potent toxic activity against the sensitive and resistant cell lines and no toxicity on normal fibroblast cells (Hegedűs et al., 2024). In the present contribution, we decided to concentrate on the application of ethyl 2-(5-chloro-8-hydroxyquinolin-7-yl)-2-morpholinoacetate (**1**) as a precursor for the synthesis of derivative **2** with amino acid feature (Scheme 1). In our first experiment, the reaction was performed in 2 % hydrochloric acid solution at room temperature, monitoring the conversion of

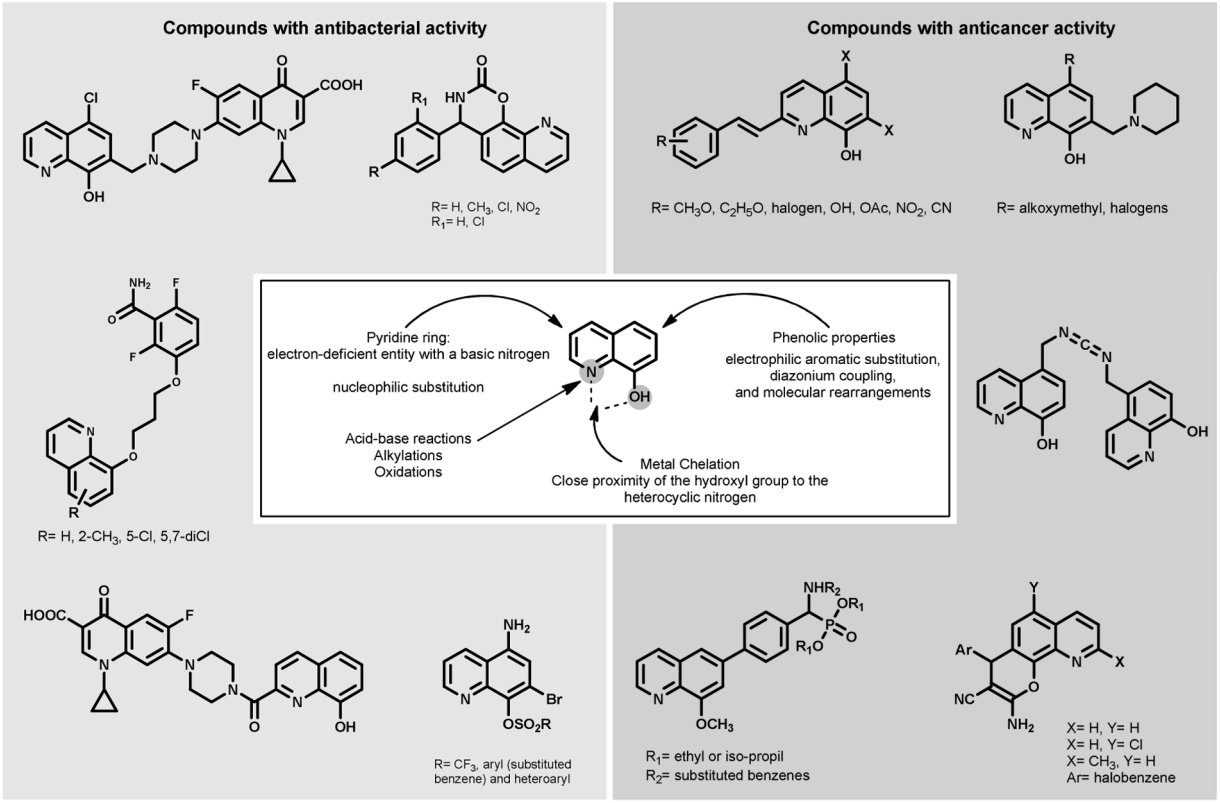
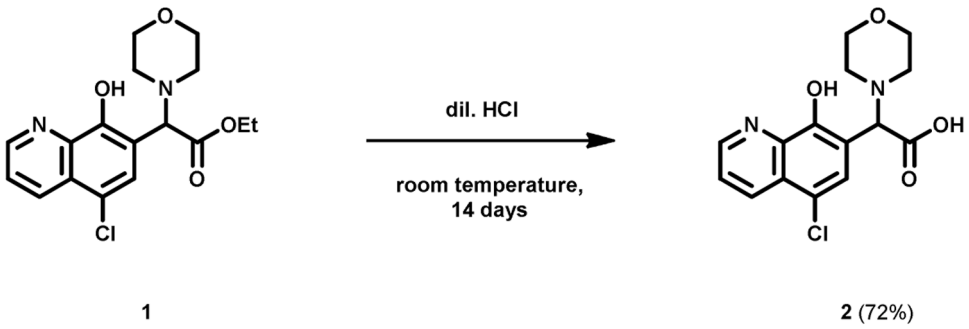


Fig. 1. Reaction sites and various antibacterial-, anticancer applications of 8-hydroxyquinoline.



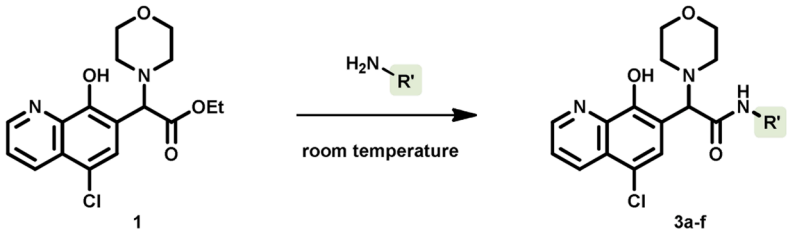
1 **2** (72%)

Scheme 1. The hydrolysis of ethyl 2-(5-chloro-8-hydroxyquinolin-7-yl)-2-morpholinoacetate (**1**).

the starting material by Thin-Layer Chromatography (TLC) analysis. After a reaction time of 14 days, TLC revealed the formation of a single product and the lack of starting material. Based on ^{1}H NMR analysis of the product, the formation of a single product has been assumed. Finally, the desired product **2** was isolated as yellow crystals in a yield of 72 %. For the purpose of reducing the reaction time, an increased concentration of the hydrochloric acid solution and a higher temperature (80 °C, oil bath) were tested. On the basis of TLC and ^{1}H NMR analysis, we found that the synthesis under these reaction conditions resulted in the formation of a multi-spot reaction mixture.

To have an overview about the possibilities of structural modifications, a series of amidated derivatives has been systematically designed (Scheme 2). The reaction between Mannich base **1** and benzylamine was first tested. The reaction was performed under solvent-free conditions at room temperature. In this case, 9 days proved to be optimal, and **3a** was isolated in a yield of 60 %. Extensive data from the literature suggest that chelating ability is an important factor modulating anticancer activities of 8-hydroxyquinoline-derived Mannich bases (Pape et al., 2018; Pape et al., 2021; Pape et al., 2022). Thus, we focused our attention on the introduction of a cationic centre by reacting precursor **1** with *N, N*-dimethylethylenediamine. Compound **3b** was isolated by crystallization with a mixture of Et_2O and EtOH after 7 days at room temperature. Afterwards, we extended the series of reactants bearing a cationic centre. Accordingly, 3-dimethylamino-1-propylamine was tested, wherein the tertiary nitrogen is connected via a longer chain compared with *N,N*-dimethylethylenediamine. Applying solvent-free conditions and a long reaction time (9 days), the TLC showed the formation of a new compound. Since the target compound could not be isolated by crystallization, extraction with EtOAc was applied to isolate **3c**. In this case the isolated yield was found to be 63 %. The reaction between Mannich base **1** and *N*-(2-aminoethylpyrrolidine) resulted in a product with a cationic centre enclosed in a five-membered ring (compound **3d**). The possibility of extending the reaction was tested by applying 1-(2-aminoethyl)-piperidine with a larger ring compared to *N*-(2-aminoethylpyrrolidine). In the case of compounds **3d** and **3e**, the reaction mixtures were treated at room temperature in solvent-free conditions. The progress of the reactions was monitored by TLC that showed the formation of a new spot next to that of the starting materials. Since the yields were not satisfactory, the reactions were carried out using EtOH as solvent. After a reaction time of 9 days (derivative **3d** and derivative **3e**) at room temperature, the addition of EtOH led to the formation of desired compounds in a relatively higher amount. Finally, in order to identify the correlation between presented structural modifications and activity, the introduction of an additional heteroatom in the case of **3f** was achieved. Accordingly, glycine-type precursor **1** was reacted with 2-morpholinoethylamine in solvent-free conditions at room temperature. In general terms, when the amidation reactions were repeated at a higher reaction temperature in order to reduce reaction time, the formation of a multi-spot reaction mixture was monitored by TLC.

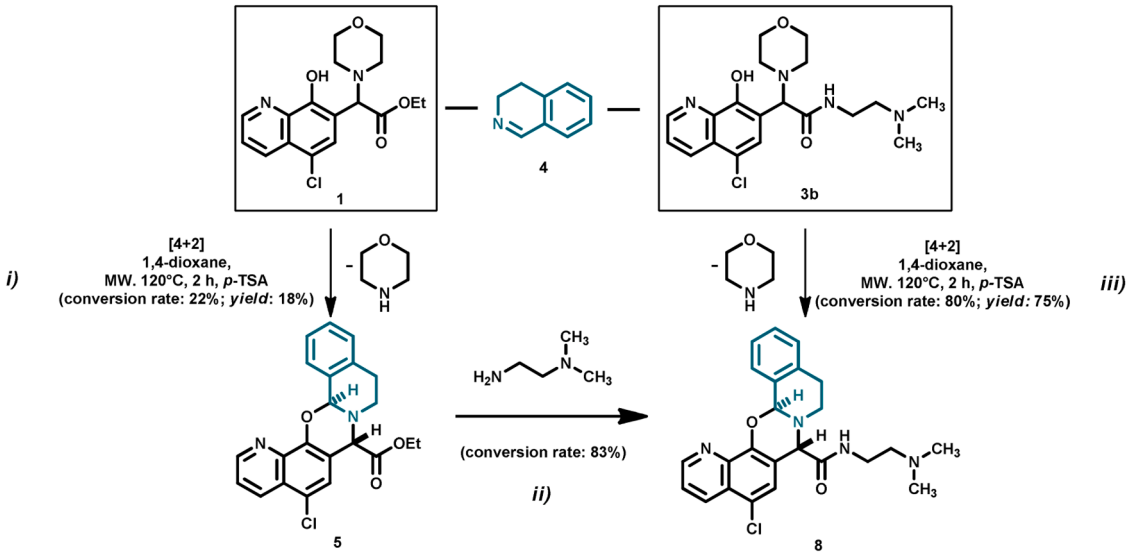
Regarding our previous findings in the field of the transformation of partially aromatic *ortho*-quinone methides (*o*-QM) via [4 + 2] cycloaddition (Hegedűs et al., 2022), our present aim was to study the behaviour of glycine-type bifunctional precursor **1** interpreted as a 1-naphthol analogue in [4 + 2] cycloaddition with selected cyclic imines. At first, **1** was reacted with 3,4-dihydroisoquinoline (**4**) (Szatmári et al., 2013) at 120 °C for 2 h under microwave (MW) irradiation in 1,4-dioxane (Scheme 3). Although longer reaction times and a higher temperature were applied, TLC and ^{1}H NMR spectrum of the crude reaction mixture proved the low conversion of starting materials. According to literature data, *p*-toluenesulfonic acid (*p*-TSA) an acid catalyst was used frequently



1 **3a-f**

R':						
	3a (60%)	**3b** (70%)	**3c** (63%)	**3d** (65%)	**3e** (63%)	**3f** (62%)

Scheme 2. The direct amidation of Mannich base **1** furnishing cationic centre.

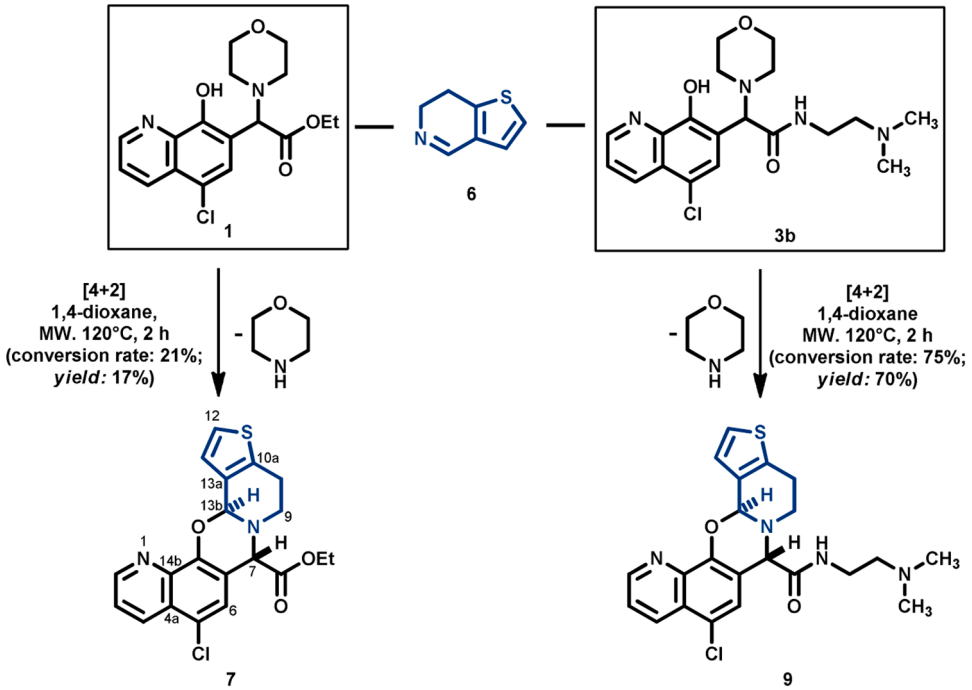


Scheme 3. [4 + 2] Cycloaddition starting from precursors **1** or **3b** and 3,4-dihydroisoquinoline (**4**).

in the modified Mannich reaction (Csütörtöki et al., 2011). Therefore, next the reaction was carried out in the presence of *p*-TSA at 120 °C under MW irradiation in 1,4-dioxane, monitoring the conversion of the reactants by TLC and ^{1}H NMR analysis. After a reaction time of 2 h, the desired product **5** was isolated in a relatively higher amount.

The stabilization of Mannich base **1** via partially aromatic *ortho*-quinone methide intermediate in [4 + 2] cycloaddition was next examined with 6,7-dihydrothieno[3,2-*c*]pyridine (**6**) (Herz and Tsai, 1955). The synthesis of derivative **7** was achieved applying 120 °C under MW irradiation for 2 h in 1,4-dioxane (Scheme 4). Based on our preliminary findings regarding the application of *p*-TSA, we aimed to study the effect of this catalyst on the conversion. In this case, however, the presence of *p*-TSA did not result in higher conversion of the initial compounds. From a practical point of view, it must be underlined that conversions were maximised at around 20 % in spite of applying longer reaction times and a higher temperature in the case of reactions starting from glycine-type precursor **1**. This observation is in agreement with our previous findings (Hegedűs et al., 2022), indicating that the formation of derivatives **5** and **7** seemed to proceed under equilibrium conditions.

To investigate the scope and limitations of the reaction, we targeted to perform the [4 + 2] cycloaddition by reacting amidated product **3b** as precursor with 3,4-dihydroisoquinoline (**4**). The crude reaction mixture was purified by column chromatography. Target derivative **8** was isolated by crystallisation with Et_2O. At this stage, we decided to test different pathways of **8** synthesis starting either via oxazine **5** (Scheme 3, route *ii*) or amidated product **3b** (route *iii*). In our experiments, 3,4-dihydroisoquinoline (**4**) was selected as a representative cyclic imine. Based on route *ii*, according to the ^{1}H NMR spectrum of the crude reaction mixtures, the reaction between cycloaddition product **5** and *N,N*-dimethylenediamine resulted in the same product that was observed in



Scheme 4. [4 + 2] Cycloaddition starting from precursors **1** or **3b** and 6,7-dihydrothieno[3,2-*c*]pyridine (**6**).

the case of route *iii* in [4 + 2] cycloaddition of derivative **3b** Considering that the transformation rate in the case of route *i* and *ii* was determined as 15 %, route *iii* was selected to synthesise target derivative **8**. The differences in the stability of the initial glycine-type precursor **1** or the amidated product **3b** and the *ortho*-quinone methide intermediate probably contributed to the rate of transformation. Along route *iii*, our further aim was to test 6,7-dihydrothieno[3,2-*c*]pyridine (**6**) as dienophile in [4 + 2] cycloaddition starting from precursor **3b** (Scheme 4). The reaction was therefore carried out with MW treatment, after 2 h at 120 °C applying the method used for the synthesis of compound **8** with the addition of Et_2O leading to the isolation of **9** in a yield of 70 %. Referring to [4 + 2] cycloaddition reactions, ^1H NMR analysis of the crude reaction mixture proved the formation of a single product in all cases. The relative configuration of the newly formed two stereogenic centres was determined by 2D-NMR technique. Accordingly, the relatively weak cross peak on the NOE spectrum of **7** confirmed that the arrangement of H-7 and H-13b is *trans*.

Since the conversions were found to be low in the case of products **5** and **7** in [4 + 2] cycloaddition, we focused our efforts to investigate the effect of the quantity of *p*-TSA on the transformation of starting materials. Technically, the reactions were monitored by TLC and NMR analysis of the crude reaction mixtures after 120 min applying 0; 0.25; 0.50; 0.75 and 1.00 equivalents of *p*-TSA. It can be concluded that the conversion, in most cases, improved on further increasing the equivalent of *p*-TSA (compound **5**). Note, however, that this was maximised at 22 % (Fig. 2). A comparison of the reaction of 3,4-dihydroisoquinoline (**4**) with **1** and **3b**, allows the conclusion that stabilization of amidated precursor **3b** via a partially aromatic *ortho*-quinone methide intermediate is more preferable (conversion rate: 80 %) compared to the reaction starting from Mannich base **1** regarding the [4 + 2] cycloaddition. In the case of derivative **7**, the addition of *p*-TSA did not result in the improvement of conversion rate.

2.2. Biological evaluations

2.2.1. Determination of the minimum inhibitory concentration (MIC)

The investigation of the antibacterial activity showed that none of the tested compounds were effective against the Gram-positive and Gram-negative bacterial strains included in the experiment.

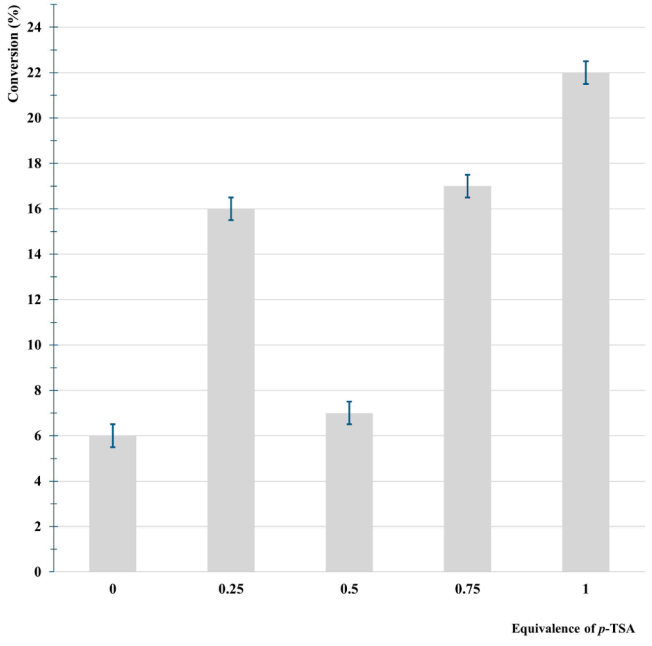


Fig. 2. The effect of equivalence of *p*-TSA on the conversion rate in the case of compound **5** (error bars: ±0.5 %).

2.2.2. Cytotoxicity assay

Compared to the precursor compound **1** (Colo 205 IC_{50}: 2.05 μM, Colo 320 IC_{50}: 1.72 μM) (Hegedűs et al., 2024), all new derivatives exhibited less potent anticancer activity. Compounds **2, 3e** and **3a** exerted potent anticancer effect on the Colo 205 cell line (IC_{50} of 16.19, 22.12 and 26.37 μM, respectively). In contrast, the resistant cell line was less sensitive (IC_{50} of 22.18, 43.41 and 39.35 μM, respectively). Regarding the other derivatives, the IC_{50} values were near 40 μM or more on both cell lines (Table 1). Taking into account that **2, 3e** and **3a** showed potent anticancer effect on sensitive Colo 205 and resistant Colo 320 adenocarcinoma cell lines, for these derivatives selectivity indexes (SI) were calculated (Table 3). Based on the obtained results, the synthesized compounds can be considered selective, as they were not toxic to normal CCD-19Lu cell line cells (Table 2). Compounds with SI ⟨ 1 are defined as non-selective, whereas SI ⟩ 6 means that the compounds are strong selective towards cancer cell line. It is worth mentioning that derivative **2** exerted strong selective effect on the normal cell line compared to the Colo 205 cancer cell line (Table 3, SI > 6.18). Since the IC_{50} of the compounds against the normal cell line were >100 μM (Table 2), in this case we did not get an accurate SI value (the SI value has to be calculated by dividing the IC_{50} against the normal cell by the IC_{50} against the cancer cell). Therefore, the division was carried out by 100 μM. Doxorubicin was applied as a positive control, and DMSO was the solvent control. In our research the selectivity of the compounds towards cancer cells (when the activity is compared to normal cells) was investigated.

In point of correlation between structure and biological activity, in favour of improved cytotoxic activity, in the case of compound **2** the presence of 5-chloro-8-hydroxyquinoline skeleton with amino acid character through water solubility is relevant. Considering the biological evaluations, the direct amidation of a Mannich base consisting of the 5-chloro-8-hydroxyquinoline skeleton with benzylamine found to be beneficial in terms of anticancer activity. With respect to the applied amine, 1-(2-aminoethyl)-piperidine bearing a cationic centre has been interpreted to be a beneficial structural element.

Based on recent investigations, chelating ability is an important factor modulating anticancer activities of 8-hydroxyquinoline-derived Mannich bases (Pape et al., 2018; Pape et al., 2021; Pape et al., 2022). In accordance with literature data, our intention was to improve cytotoxic activity by introduction of cationic centre establishing polar side chain (applied amines: *N,N*-dimethylethylenediamine, 3-dimethylamino-1-propylamine, *N*-(2-aminoethylpyrrolidine), 1-(2-aminoethyl)-piperidine, 2-morpholinoethyl-amine). Regarding the biological examinations, the compounds bearing a cationic centre and possessing polar side chain did not exerted potent anticancer effect except for derivative **3e**. Interesting to note that in the case of compound **3a -** of wich the aromatic ring is connected via the sp^3 C atom and contains an apolar side chain - potent cytotoxic activity was observed, therefore no clear

Table 1
Cytotoxic activity of the hydroxyquinoline hybrids on sensitive Colo 205 and resistant Colo 320 adenocarcinoma cell lines. DOX: doxorubicin; CIS: cisplatin.

Cpds.	Colo 205		Cpds.	Colo 320	
	IC_{50} (μM)	SD ±		IC_{50} (μM)	SD ±
2	**16.19**	0.30	**2**	**22.18**	0.50
3a	26.37	1.30	**3a**	39.35	1.48
3b	61.19	2.03	**3b**	94.72	0.59
3c	92.91	1.67	**3c**	>100	–
3d	63.00	0.11	**3d**	63.00	0.11
3e	22.12	2.43	**3e**	43.41	1.53
3f	44.45	3.94	**3f**	61.87	0.18
5	45.55	0.61	**5**	79.24	0.07
7	47.71	0.45	**7**	>100	–
8	>100	–	**8**	>100	–
9	45.86	1.01	**9**	39.23	2.43
DOX	3.53	0.33	**DOX**		0.02
CIS	22.46	1.24	**CIS**	37.06	0.76

Table 2
Cytotoxic effect of the compounds on normal CCD-19Lu cell line cells based on the IC_{50} values. SD: standard deviation. Doxorubicin (DOX) was used as a positive control.

Cpds.	CCD-19Lu	
	IC_{50} (μM)	SD ±
2	>100	–
3a	>100	–
3e	>100	–
DOX	>8.62	–

Table 3
Selectivity indexes (SI) of the compounds **2, 3a** and **3e**.

Cpds.	CCD-19Lu /Colo205 SI	CCD-19Lu /Colo320
2	> 6.18	> 4.5
3a	> 3.79	> 2.54
3e	> 4.52	> 2.3

relationship was apparent between the establishment of a cationic centre and the anticancer effect of the synthesized compounds.

Based on a recent study related to copper and iron binding properties on the anticancer activity of 8-hydroxyquinoline derivatives (Pape et al., 2018), as part of a future work, correlation analysis of the anticancer activity and the metal binding properties would offer possibility to better understand the mechanism of action of compounds consisting 8-hydroxyquinolines prepared as result of our research work.

3. Conclusions

The synthesis of 2-(5-chloro-8-hydroxyquinolin-7-yl)-2-morpholinoacetic acid (**2**) with amino acid feature via hydrolysis of precursor bearing 8-hydroxyquinoline skeleton and ethyl ester was achieved. In order to have an overview about the possibilities of structural modifications and establish cationic centre, a series of amidated derivatives of Mannich base consisting 5-chloro-8-hydroxyquinoline skeleton has been systematically designed. The stabilization of precursor and formed amide **3b** consisting of *N,N*-dimethylethylamine via partially aromatic *ortho*-quinone methide intermediate was tested with different cyclic imines in [4 + 2] cycloaddition. Referring to [4 + 2] cycloaddition reactions, ^{1}H NMR analysis of the crude reaction mixture proved the formation of a single product in all cases. The relative configuration of the newly formed two stereogenic centres was determined by 2D-NMR technique. Accordingly, the relatively weak cross peak on the NOE spectrum confirmed that the arrangement of H-7 and H-13b is *trans*. According to biological evaluations, all new derivatives exhibited less potent anticancer activity compared to the precursor **1**. Based on obtained results, **2, 3e** and **3a** exerted potent anticancer effect on the Colo 205 cell line (IC_{50} of 16.19, 22.12 and 26.37 μM). Considering the correlation between structure and biological activity, it can be concluded that the presence of 5-chloro-8-hydroxyquinoline skeleton with amino acid character through water solubility is beneficial. In addition, the direct amidation of a Mannich base consisting of the 5-chloro-8-hydroxyquinoline skeleton with benzylamine, 1-(2-aminoethyl)-piperidine found to be relevant in terms of anticancer activity. In favour of improvement anticancer activity, future study in the field of direct amidation of Mannich base bearing 5-chloro-8-hydroxyquinoline skeleton with benzylamine-type derivative ((*S*)-(-)-*α*-methylbenzylamine) would lead to a comprehensive overview about the correlation between structural modification (introduction of asymmetric C atom) and biological activity.

4. Experimental section

4.1. Biological assays

4.1.1. Bacterial strains

Among the Gram-positive strains *Staphylococcus aureus* American Type Culture Collection (ATCC) 25923 strain was used as methicillin-susceptible reference; the clinical isolate *S. aureus* MRSA 272123 and the methicillin and oxacillin-resistant *S. aureus* MRSA ATCC 43300 strains were investigated. *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were used among the Gram-negative strains.

4.1.2. Cell lines

The anticancer activity of the compounds was investigated on the doxorubicin-sensitive Colo 205 (ATCC—CCL-222) human colonic adenocarcinoma cell line and the doxorubicin resistant Colo 320/MDR-LRP human colonic adenocarcinoma cell line expressing P-gp (MDR1)-LRP (ATCC—CCL-220.1) (LGC Promochem, Teddington. UK). Their culture conditions are the following: Colo 205 (ATCC—CCL-222) and Colo 320/MDR-LRP (MDR1)-LRP (ATCC—CCL-220.1 human colon adenocarcinoma cell lines were cultured in RPMI 1640 medium supplemented with 10 % heat-inactivated fetal bovine serum, 2 mM L-glutamine, 1 mM Na-pyruvate and 10 mM Hepes. The cell lines were incubated at 37 °C in a 5 % CO_2, 95 % air atmosphere.

4.1.3. Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentrations (MICs) of the compounds were assessed following the guidelines established by the Clinical and Laboratory Standards Institute (CLSI) (Weinstein, 2020). The MIC values of the tested compounds were evaluated through visual inspection. Dimethyl sulfoxide (DMSO) served as a negative control to confirm the absence of any antibacterial activity.

4.1.4. Cytotoxicity assay

The impact of increasing concentrations of the compounds on cell growth was evaluated using 96-well flat-bottom microtiter plates. Two-fold serial dilutions of the compounds (ranging from 0.19 to 100 μM) were prepared in the plate, and then colonic adenocarcinoma cells (10^4 cells in 100 μL of RPMI 1640 medium) were added into each well, except for the medium control wells.

The culture plates were incubated at 37 °C for 24 h. Following this incubation, 20 μL of MTT solution (5 mg/mL stock) was added to each well, and the plates were incubated for an additional 4 h at 37 °C. Afterwards, 100 μL of a 10 % SDS solution in 0.01 M HCl was added to each well, and the plates were incubated overnight at 37 °C.

Cell growth was assessed by measuring the optical density (OD) at 540 nm (reference 630 nm) using a Multiscan EX ELISA reader (Thermo Labsystems, Cheshire, WA, USA). Growth inhibition was quantified as IC_{50} values, which represent the concentration required to inhibit 50 % of cell growth relative to untreated controls. IC_{50} values and standard deviations from triplicate experiments were calculated using GraphPad Prism version 5.00 for Windows, employing non-linear regression analysis (GraphPad Software, San Diego, CA, USA). Doxorubicin (from a 2 mg/mL stock, Teva Pharmaceuticals) served as the positive control. The solvent DMSO was confirmed to have no impact on cell growth at the tested concentrations.

4.2. 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.

Starting compound ethyl 2-(5-chloro-8-hydroxyquinolin-7-yl)-2-morpholinoacetate (**1**) (Hegedűs et al., 2024) and dienophiles 3,4-dihydroisoquinoline (**4**) (Szatmári et al., 2013), 6,7-dihydrothieno[3,2-*c*]

pyridine (**6**) (Herz and Tsai, 1955) were synthesised according to literature methods.

^{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 internal standard (^{1}H, ^{13}C). All spectra (^{1}H, ^{13}C and NOESY) 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).

*4.2.1. 2-(5-Chloro-8-hydroxyquinolin-7-yl)-2-morpholinoacetic acid (**2**)*

A mixture of ethyl 2-(5-chloro-8-hydroxyquinolin-7-yl)-2-morpholinoacetate (**1**; 50.0 mg, 0.14 mmol) and hydrochloric acid solution (2 %, 17.5 mL) was stirred in a 10 mL pressurised reaction vial for 14 days at room temperature. The solvent was removed under reduced pressure. The desired product was isolated by crystallisation with Et_2O (10 mL); 32.9 mg, (72 %); yellow crystals; m.p. 146–149 °C; ^{1}H NMR (D_2O): 2.98–3.57 (m, 4H), 3.89–4.00 (m, 4H), 5.28 (s, 1H), 7.69 (s, 1H), 7.81–7.86 (m, 1H), 8.78 (d, 1H, J = 8.46 Hz), 8.95 (s, 1H). ^{13}C NMR (MeOD): 63.3; 65.6; 110.1; 121.1; 124.1; 126.3; 127.6; 130.7; 132.9; 138.9; 149.9; 153.1. HRMS calcd for $[M + H]^+$ m/z = 323.07931, found m/z = 323.07889.

*4.2.2. General procedure for the synthesis of amidated derivatives 3a–f starting from ethyl 2-(5-chloro-8-hydroxyquinolin-7-yl)-2-morpholinoacetate (**1**)*

Mannich base **1** (50.0 mg, 0.14 mmol) and different amount of amines were placed in a 10 mL pressurised reaction vial and stirred under solvent-free conditions (in the case of compounds **3d-e**, EtOH was added to the reaction mixture as a next step) at room temperature.

*4.2.3. N-Benzyl-2-(5-chloro-8-hydroxyquinolin-7-yl)-2-morpholinoacetamide (**3a**)*

Applied amine: benzylamine (76.3 mg, 0.71 mmol), reaction time: 9 days. The product was crystallised from Et_2O (2 mL); 35.0 mg, (60 %); beige crystals; m. p. 165–170 °C; ^{1}H NMR ($CDCl_3$): 2.50–2.64 (m, 4H), 3.70 (t, 4H, J = 4.53 Hz), 4.47 (dd, 1H, J_1=5.82 Hz, J_2=14.77 Hz), 4.57 (dd, 1H, J_1=6.13 Hz, J_2=14.93 Hz), 4.69 (s, 1H), 7.27–7.36 (m, 5H), 7.48 (brs, 1H), 7.53–7.60 (m, 2H), 8.51 (d, 1H, J = 8.43 Hz), 8.84 (s, 1H). ^{13}C NMR ($CDCl_3$): 43.5; 67.0; 68.4, 116.8; 120.7; 122.8; 126.0; 127.6; 127.7; 127.8; 128.8; 133.4; 138.3; 138.7; 148.6; 150.2; 170.0. HRMS calcd for $[M + H]^+$ m/z = 412.14225, found m/z = 412.14194.

*4.2.4. 2-(5-Chloro-8-hydroxyquinolin-7-yl)-N-(2-(dimethylamino)ethyl)-2-morpholinoacetamide (**3b**)*

Applied amine: *N,N*-dimethylethylenediamine (62.8 mg, 0.71 mmol), reaction time: 7 days. The product was crystallised from a mixture of Et_2O (0.5 mL) and EtOH (1.5 mL); 38.9 mg, (70 %); beige crystals; m. p. 161–163 °C; ^{1}H NMR ($CDCl_3$): 2.27 (s, 6H), 2.45 (t, 2H, J = 6.03 Hz), 2.52–2.64 (m, 4H), 3.34–3.47 (m, 2H), 3.74 (t, 4H, J = 4.50 Hz), 4.65 (s, 1H), 7.54–7.58 (m, 2H), 7.62 (brs, 1H), 8.49 (d, 1H, J = 8.53 Hz), 8.83 (s, 1H). ^{13}C NMR ($CDCl_3$): 36.7; 45.1; 58.0; 67.1; 68.2; 117.2; 120.6; 122.7; 126.0; 127.7; 133.3; 138.9; 148.6; 150.4; 170.2. HRMS calcd for $[M + H]^+$ m/z = 393.16879, found m/z = 393.16833.

*4.2.5. 2-(5-Chloro-8-hydroxyquinolin-7-yl)-N-(3-(dimethylamino) propyl)-2-morpholinoacetamide (**3c**)*

Applied amine: 3-dimethylamino-1-propylamine (72.8 mg, 0.71 mmol), reaction time: 9 days. The solid residue was dissolved in EtOAc (10 mL) and extracted with water (3 × 10 mL). The aqueous extracts were combined and made alkaline with 10 % Na_2CO_3 solution and extracted with EtOAc (3 × 10 mL). The organic extracts were combined, dried (Na_2SO_4) and concentrated under reduced pressure; 36.2 mg, (63 %); oil; ^{1}H NMR ($CDCl_3$): 1.65–1.71 (m, 2H), 2.24 (s, 6H), 2.34- 2.39 (m, 2H), 2.51–2.64 (m, 4H), 3.36–3.42 (m, 2H), 3.75 (t, 4H, J = 4.59 Hz), 4.55 (s, 1H), 7.53–7.59 (m, 2H), 8.02 (brs, 1H), 8.49 (d, 1H, J = 8.42 Hz), 8.84 (s, 1H). ^{13}C NMR ($CDCl_3$): 26.3; 39.0; 45.6; 58.5; 67.1; 69.0; 117.4; 120.6; 122.7; 126.0; 127.6; 133.2; 138.9; 148.7; 150.4; 169.9. HRMS calcd for $[M + H]+$ m/z = 407.18444, found m/z = 407.18425.

*4.2.6. 2-(5-Chloro-8-hydroxyquinolin-7-yl)-2-morpholino-N-(2-(pyrrolidin-1-yl)ethyl)acetamide (**3d**)*

Applied amine: *N*-(2-aminoethylpyrrolidine) (65.1 mg, 0.57 mmol), reaction time: 5 days (under solvent-free conditions). EtOH (1 mL) was added to the reaction mixture and stirred for 4 days at room temperature. The product was crystallised from *i*Pr_2O (2 mL); 38.6 mg, (65 %); beige crystals; m. p. 178–180 °C; ^{1}H NMR ($CDCl_3$): 1.75–1.81 (m, 4H), 2.48–2.66 (m, 10H), 3.36–3.49 (m, 2H), 3.73 (t, 4H, J = 4.32 Hz), 4.65 (s, 1H), 7.53–7.58 (m, 2H), 7.65 (brs, 1H), 8.49 (d, 1H, J = 8.42 Hz), 8.83 (s, 1H). ^{13}C NMR ($CDCl_3$): 23.6; 38.0; 53.8; 54.5; 67.2; 68.2; 117.2; 120.5; 122.7; 126.0; 127.8; 133.3; 138.8; 148.6; 150.4; 170.1. HRMS calcd for $[M + H]^+$ m/z = 419.18444, found m/z = 419.18395.

*4.2.7. 2-(5-Chloro-8-hydroxyquinolin-7-yl)-2-morpholino-N-(2-(piperidin-1-yl)ethyl)acetamide (**3e**)*

Applied amine: 1-(2-aminoethyl)-piperidine (73.0 mg, 0.57 mmol), reaction time: 5 days (under solvent-free conditions). EtOH (1 mL) was added to the reaction mixture and stirred for 4 days at room temperature. The product was crystallised from a mixture of Et_2O (0.5 mL) and EtOH (1.5 mL); 38.5 mg, (63 %); beige crystals; m. p. 153–158 °C; ^{1}H NMR ($CDCl_3$): 1.43–1.49 (m, 2H), 1.56–1.61 (m, 4H), 2.35–2.49 (m, 6H), 2.54–2.65 (m, 4H), 3.36–3.44 (m, 2H), 3.73–3.79 (m, 4H), 4.66 (s, 1H), 7.51–7.57 (m, 2H), 7.69 (brs, 1H), 8.49 (d, 1H, J = 8.59 Hz), 8.83 (s, 1H). ^{13}C NMR ($CDCl_3$): 24.4; 26.2; 36.0; 54.3; 57.3; 67.3; 68.2; 117.3; 120.5; 122.7; 126.0; 127.8; 133.3; 138.8; 148.6; 150.4; 169.9. HRMS calcd for $[M + H]^+$ m/z = 433.20010, found m/z = 433.19952.

*4.2.8. 2-(5-Chloro-8-hydroxyquinolin-7-yl)-2-morpholino-N-(2-morpholinoethyl)acetamide (**3f**)*

Applied amine: 2-morpholinoethyl-amine (74.2 mg, 0.57 mmol), reaction time: 9 days. The product was crystallised from a mixture of Et_2O (0.5 mL) and EtOH (1.5 mL); 38.3 mg, (62 %); beige crystals; m. p. 168–172 °C; ^{1}H NMR ($CDCl_3$): 2.43–2.53 (m, 6H), 2.54–2.65 (m, 4H), 3.38–3.49 (m, 2H), 3.69 (t, 4H, J = 4,29 Hz), 3.76 (t, 4H, J = 4.23 Hz), 4.65 (s, 1H), 7.50–7.62 (m, 3H), 8.50 (d, 1H, J = 8.28 Hz), 8.84 (s, 1H). ^{13}C NMR ($CDCl_3$): 35.5; 53.4; 57.2; 67.1; 67.2; 68.3; 117.1; 120.6; 122.8; 126.0; 127.6; 133.3; 138.8; 148.7; 150.3; 170.0. HRMS calcd for $[M + H]^+$ m/z = 435.17936, found m/z = 435.17891.

*4.2.9. General procedure for the synthesis of compounds 5 and 8 via [4 + 2] cycloaddition starting from 3,4-dihydroisoquinoline (**4**)*

3,4-Dihydroisoquinoline (**4**; 20.0 mg, 0.15 mmol) and glycine type precursor **1** (53.6 mg, 0.15 mmol) or amidated precursor **3b** (60.0 mg, 0.15 mmol) in the presence of *p*-TSA (26.3 mg, 0,15 mmol) in 1,4-dioxane (10 mL) were placed in a 35-mL pressurised reaction vial and heated at 120 °C for 2 h in a CEM SP microwave reactor. The solvent was removed under reduced pressure.

4.2.10. (7S,14bS*)-ethyl-5-chloro-7,9,10,14b-tetrahydroisoquinolino [1′,2′:2,3][1,3]oxazino[5,6-h]-quinoline-7-carboxylate (**5**)*

Following the purifying by column chromatography (CH_2Cl_2:EtOAc: EtOH, 40:7:3), the product was crystallised from Et_2O (5 mL); 11.1 mg, (18 %); beige crystals; m. p.163–166 °C; ^{1}H NMR ($CDCl_3$): 1.33 (t, 3H, J = 7.10 Hz), 2.81–2.94 (m, 2H), 3.22–3.32 (m, 1H), 3.35–3.43 (m, 1H), 4.28 (q, 2H, J = 7.10 Hz), 4.57 (s, 1H), 6.33 (s, 1H), 7.14–7.20 (m, 1H), 7.28–7.33 (m, 2H), 7.49–7.55 (m, 1H), 7.58–7.65 (m, 2H), 8.51 (d, 1H, J = 8.53 Hz), 8.95 (s, 1H). ^{13}C NMR ($CDCl_3$): 14.2; 29.0; 44.9; 61.8; 63.6; 85.3; 114.5; 121.8; 122.3; 125.9; 126.5; 128.5; 129.1; 129.7; 131.9;

132.9; 134.8; 140.4; 149.2; 150.2; 157.6; 170.5. HRMS calcd for $[M + H]^+$ $m/z = 395.11570$, found $m/z = 395.11500$.

4.2.11. (7S*,14bS*)-5-chloro-N-(2-(dimethylamino)ethyl)-7,9,10,14b-tetrahydroisoquinolino-[1′,2′:2,3][1,3]oxazino[5,6-h]quinoline-7-carboxamide (8)

The crude reaction mixture was purified by column chromatography (CH_2Cl_2:MeOH, 4:1) and the desired product was isolated by crystallisation with Et_2O (10 mL); 50.2 mg, (75 %); brown crytals; m. p. 132–140 °C; ^{1}H NMR (CDCl3): 2.52–2.61 (m, 6H), 2.74–2.81 (m, 2H), 3.13–3.17 (m, 2H), 3.20–3.27 (m, 1H), 3.60–3.75 (m, 2H), 3.94–4.04 (m, 1H), 5.17 (s, 1H), 5.90 (s, 1H), 7.18–7.22 (m, 1H), 7.32–7.37 (m, 2H), 7.43–7.45 (m, 1H), 7.50–7.60 (m, 2H), 7.69 (brs, 1H), 8.51 (d, 1H, $J = 8.39$ Hz), 8.85 (s, 1H). ^{13}C NMR (DMSO): 24.0; 40.3; 46.2; 60.1; 73.0; 119.0; 123.6; 125.9; 126.9; 128.2; 128.4; 128.6; 129.6; 132.8; 133.0; 134.3; 137.1; 139.3; 149.5; 152.0; 171.4. HRMS calcd for $[M + H]^+$ $m/z = 437.17388$, found $m/z = 437.17404$.

4.2.12. General procedure for the synthesis of compounds 7 and 9 via [4 + 2] cycloaddition starting from 6,7-dihydrothieno[3,2-c]pyridine (6)

6,7-Dihydrothieno[3,2-*c*]pyridine (**6**; 20.0 mg, 0.15 mmol) and glycine type precursor **1** (51.2 mg, 0.15 mmol) or amidated precursor **3b** (57.37 mg, 0.15 mmol) were dissolved in 1,4-dioxane (10 mL) in a 35 mL pressurised reaction vial. The reaction mixture was heated at 120 °C for 2 h in a CEM SP microwave reactor. The solvent was removed in *vacuo*.

4.2.13. (7S*,13bS*)-ethyl-5-chloro-7,9,10,13b-tetrahydrothieno [3′′,2′′:3′,4′]pyrido[2′,1′:2,3]-oxazino[5,6-h]quinoline-7-carboxylate (7)

The mixture was purified by column chromatography (CH_2Cl_2: EtOAc:EtOH, 40:7:3) and the desired product was isolated by crystallisation with Et_2O (5 mL); 10.0 mg, (17 %); beige crystals; m. p. 192–201 °C; ^{1}H NMR (DMSO): 1.22–1.26 (m, 3H), 2.87–2.96 (m, 1H), 3.00–3.13 (m, 3H), 4.20 (q, 2H, $J = 7.07$ Hz), 5.06 (s, 1H), 6.11 (s, 1H), 7.24 (d, 1H, $J = 5.33$ Hz), 7.47 (d, 1H, $J = 5.09$ Hz), 7.65 (s, 1H), 7.68–7.74 (m, 1H), 8.49 (d, 1H, $J = 8.76$ Hz), 8.91 (s, 1H). ^{13}C NMR (DMSO): 14.6; 25.5; 44.8; 61.8; 61.9; 81.6; 115.3; 120.4; 123.6; 124.5; 126.0; 127.1; 127.2; 132.7; 132.9; 138.5; 139.8; 148.5; 150.5; 170.8. HRMS calcd for $[M + H]^+$ $m/z = 401.07212$, found $m/z = 401.07170$.

4.2.14. (7S*,13bS*)-5-chloro-N-(2-(dimethylamino)ethyl)-7,9,10,13b-tetrahydrothieno-[3′′,2′′:3′,4′]pyrido[2′,1′:2,3][1,3]oxazino[5,6-h] quinoline-7-carboxamide (9)

Following the purifying by column chromatography (CH_2Cl_2:MeOH, 3:2), the product was crystallised from Et_2O (10 mL); 45.2 mg, (70 %); brown crystals; m. p. 160–165 °C; ^{1}H NMR ($CDCl_3$): 2.41 (s, 6H), 2.67–2.77 (m, 3H), 3.08–3.16 (m, 1H), 3.22–3.48 (m, 2H), 3.39–3.50 (m, 1H), 3.61–3.80 (m, 1H), 5.18 (s, 1H), 5.84 (s, 1H), 7.07 (d, 1H, $J = 5.19$ Hz), 7.22 (d, 1H, $J = 5.23$ Hz), 7.53–7.58 (m, 1H), 7.68 (s, 1H), 8.51 (d, 1H, $J = 8.65$ Hz), 8.85 (s, 1H). ^{13}C NMR ($CDCl_3$): 29.7; 39.2; 44.2; 45.5; 56.3; 58.4; 71.5; 120.9; 122.6; 123.8; 124.6; 126.2; 127.6; 132.4; 133.3; 136.7; 138.9; 148.6; 148.8; 151.0; 172.2. HRMS calcd for $[M + H]^+$ $m/z = 443.13030$, found $m/z = 443.13044$.

CRediT authorship contribution statement

Dóra Hegedűs: Writing – original draft, Visualization, Investigation. **Nikoletta Szemerédi:** Writing – original draft, Investigation. **Dorka Gubó:** Investigation. **Gabriella Spengler:** Writing – review & editing, Writing – original draft, Conceptualization. **István Szatmári:** Writing – review & editing, Writing – original draft, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ejps.2025.107084.

Data availability

Data will be made available on request.

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