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Application of partially aromatic *ortho*-quionone-methides for the synthesis of novel naphthoxazines with improved antibacterial activity☆

Dóra Hegedűs [a,b], Nikoletta Szemerédi [c], Gabriella Spengler [c], István Szatmári [a,b,*]

[a] Institute of Pharmaceutical Chemistry and Research Group for Stereochemistry, Hungarian Academy of Sciences, University of Szeged, H-6720, Szeged, Eötvös u. 6, Hungary
[b] Institute of Pharmaceutical Chemistry, University of Szeged, Interdisciplinary Excellence Center, H-6720, Szeged, Eötvös u. 6, Hungary
[c] Department of Medical Microbiology, Albert Szent-Györgyi Health Center and Albert Szent-Györgyi Medical School, University of Szeged, Semmelweis utca 6, 6725, Szeged, Hungary

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ABSTRACT

Starting from naphthols and morpholine and using ethyl glyoxylate as the aldehyde component in the modified Mannich reaction, new aminonaphthol derivatives substituted with 2- and 1-naphthol were synthesised. The stabilization of precursor bifunctional compounds via partially aromatic *ortho*-quinone methide intermediate was tested with different cyclic imines in [4 + 2] cycloaddition. Based on ^{1}H NMR analysis, in the case of new α-amino acid esters the formation of a single product has been assumed. The NOE spectrum proved that the relative configuration of the newly formed stereogenic centres was *trans*. The compounds have also been tested in bacteria in order to reduce or reverse antibiotic resistance. Compounds **13** and **14** could inhibit the efflux pump system in susceptible and methicillin-resistant *Staphylococcus aureus* strains.

1. Introduction

The Mannich reaction is a widely used three-component reaction in organic synthesis [1,2]. A particular variation of this C–C bond forming reaction is the modified Mannich reaction. In our case, 2- and 1-naphthol were applied as electron-rich aromatic compounds in order to provide the necessary activated C–H bond [3–5].

Arylglycine derivates are used as important building blocks in the synthesis of complex and biologically active molecules [6]. They are regarded as an important non-proteinogenic class of α-amino acids. Recent studies proved that glycopeptide antibiotics, such as vancomycin [7,8] and monocyclic β-lactam antibiotics (nocardicins [9]), are natural sources of arylglycines. On the other hand, synthetic arylglicine derivates could serve as the side-chain moiety of semisynthetic penicillins and cephalosporins. As another pharmacological property, it was found that some derivatives selectively modulate the activity of metabotropic glutamate receptors [10].

According to previous studies, an unexpected transformation between 1-α-aminobenzyl-2-naphthol and 3,4-dihydroisoquinoline enabled the synthesis of naphth[1,2-*e*] [1,3]oxazino[2,3-*a*] isoquinolines under microwave (MW) irradiation [11]. The reaction was then extended to 2-aminoalkyl-1-naphthols [12] and other C=N dienophiles (cyclic imines) allowing the synthesis of new naphthoxazino-isoquinoline, -benzazepine and -thienopyridine derivatives [13]. The synthesis of new nonracemic naphth [1,3]oxazino[3,2-*a*]quinoxalinones starting from enantiomeric (4a*S*,8a*S*)-4a,5,6,7,8,8a-hexahydro-2-quinoxalinone and 1-aminoalkyl-2-naphthols or 2-aminoalkyl-1-naphthols has already been published [14,15].

Recently the reactivity of highly functionalised aminonaphthol [16] or aminophenanthrol derivatives [17] with different cyclic imines was also tested in [4 + 2] cycloaddition. The reaction was found to be both regio- and diastereoselective. Furthermore, starting from aminodiol, the formation of naphthoxazines was observed, while new quinazolines were formed when applying diaminonaphthols as precursors [16].

In previous studies the formation and transformation of aromatic *ortho*-quinone methides (*o*-QM) via [4 + 2] cycloaddition has been examined [15]. To the best of our knowledge, the stabilization of partially aromatic *o*-QMs with cyclic imines has not been studied. Therefore, our aim was to prepare bifunctional glycine-type precursors and investigate their behaviour in [4 + 2] cycloaddition. Furthermore,

☆ This article is dedicated to the memory of Prof. Dr. Ferenc Fülöp, former research group leader and head of institute.

* Corresponding author. Institute of Pharmaceutical Chemistry and Research Group for Stereochemistry, Hungarian Academy of Sciences, University of Szeged, H-6720, Szeged, Eötvös u. 6, Hungary.
E-mail address: szatmari.istvan@szte.hu (I. Szatmári).

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the relative configurations of the newly generated stereogenic centres by using NMR spectroscopy were also planned to be determined.

Bacterial efflux pumps are membrane transporters and they have the capacity to regulate the internal environment by removing harmful agents (e.g. antibiotics, toxic bile salts), metabolites as well as cell–cell communication (quorum sensing) signal molecules. In addition, they are directly and indirectly related to other virulence determinants, thereby contributing to bacterial pathogenesis. Efflux mechanisms are widely recognised as key components of multidrug resistance [18]. The antimicrobial resistance is an alarming problem and it can lead to the reduction in effectiveness of a medication to cure infectious diseases [19]. The inhibition of efflux mechanisms [20] is a promising approach to increase the intracellular drug concentration and to restore the activity of drugs against the resistant strains. In addition, it minimizes further development of resistant strains.

The aim of this study was the investigation of the antibacterial and efflux pump inhibiting activity of the derivatives. For this reason, new antibacterial agents interfering with virulence factors (e.g. efflux pumps) contributing to multidrug resistance (MDR) could provide an alternative to overcome MDR in bacteria.

2. Results and discussions

2.1. Synthesis

To start our investigation on the formation of partially aromatic *ortho*-quinone methides (*o*-QM) via [4 + 2] cycloaddition, 2-naphthol-substituted glycine precursor **3** was synthesised. Accordingly, 2-naphthol (**1**) and morpholine (**2**) were reacted in the presence of ethyl glyoxylate as an aldehyde component. In one of our previous works, it was found, that hydroxynaphthyl-substituted glycine derivatives can be generated from 2- or 1-naphthol, benzyl carbamate and glyoxylic acid via the modified Mannich reaction. We pointed out that in the presence of *p*-toluenesulfonic acid the reaction yield increased. Furthermore, the absolute configuration of the enantiomers was determined by circular dichroism (CD) analysis supported by TDDFT calculations [21]. Moreover, in the case of glycine ester analogues substituted with 2- or 1-naphthol, the separation of enantiomers was achieved via HPLC measurements and a systematic influence was observed between the character of the ester function and HPLC parameters [22]. In order to form functionalised aminonaphthol derivatives, morpholine (**2**) as stable cyclic secondary amine was selected as substrate in the reactions. The crude reaction mixtures formed under microwave irradiation in 30 min at 80 °C and in 30 min at 100 °C in toluene were examined by ^{1}H NMR measurements. It is interesting to note that using conventional heating, the formation of the desired product **3** could not be detected (Scheme 1).

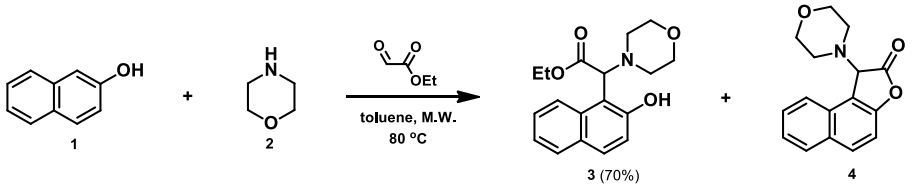
After optimisation of reaction conditions, the reaction was repeated and TLC proved the presence of two main products; therefore, column chromatographic purification was required. After separation of the desired glycine precursor **3** from side-product **4**, we aimed to investigate the reaction mechanism. A detailed NMR spectroscopic and mass spectrometry analysis of product **4** indicated the formation of a lactone via intramolecular loss of ethanol. Based on literature data (melting point, NMR spectroscopy), the structure of **4** was verified [23]. It is worth mentioning that Matuszczak published the reaction of 2-naphthol and *N*-acetyl-α-hydroxyglycinate furnishing lactone **4** by using acetic anhydride [24]. In contrast, in our case the formation of lactone was achieved in situ in toluene under microwave irradiation.

Afterwards, with aminonaphthol derivative **3** in hand, its reaction with different cyclic imines via [4 + 2] cycloaddition was tested (Scheme 2). Mechanistically, during the reaction via loss of morpholine **2**, the presence of partially aromatic *ortho*-quinone methide intermediate has been proved and it was transformed with different dienophiles to new α-amino acid esters. First, we wanted to investigate the reaction of bifunctional precursor **3** with 3,4-dihydroisoquinoline (**5**) [25] in [4 + 2] cycloaddition. The reaction was performed first at 80 °C under microwave irradiation for 60 min in 1,4-dioxane. The progress of the synthesis was monitored by TLC that showed the formation of a new spot next to those of the starting materials. By using higher temperatures and longer reactions (100 °C, 300 min), the desired product was isolated in a relatively higher amount. Based on ^{1}H NMR analysis of the crude reaction mixture, the formation of a single product has been assumed. In order to determine the relative configuration of the newly formed two stereogenic centres, 2D-NMR technique was applied. The relatively weak cross peak on the NOE spectrum of **8** proved that the arrangement of H-7a and H-15 is *trans*.

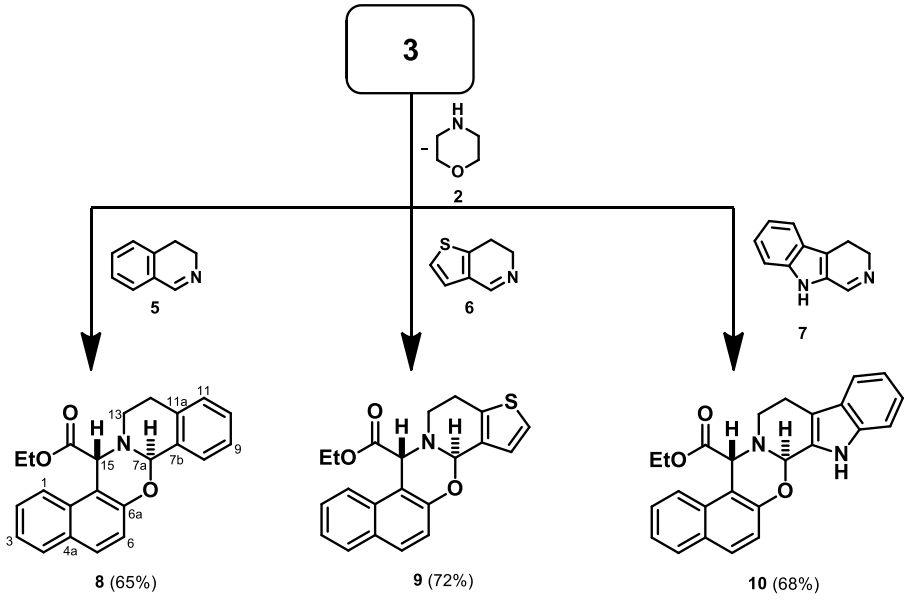
Then we focused on testing the possibility to extend the reaction by using 6,7-dihydrothieno[3,2-*c*]pyridine (**6**) [26] and 3,4-dihydro-*β*-carboline (**7**) [27] as initial cyclic imine dienophiles. The [4 + 2] cycloaddition reactions were performed at 80 °C and 100 °C in 1, 4-dioxane. The formation of the desired naphthoxazines was monitored by both TLC and the NMR analysis of the crude products. This latter method proved that all reactions led to the formation of a single product. In order to separate the desired polycycles from unreacted starting materials, column chromatographic purification was required. Finally, the target compounds were isolated by crystallisation. In the case of [4 + 2] cycloaddition, 100 °C and reaction times of 280–360 min were found to be the optimal reaction conditions (Table 1). By using 2D-NMR technique (NOE measurements) the relative configurations of the newly formed stereogenic centres was proved to be *trans*. Comparing the reactivity of cyclic imines (3,4-dihydroisoquinoline (**5**), 6,7-dihydrothieno [3,2-c]pyridine (**6**), and 3,4-dihydro-*β*-carboline (**7**), the reaction of **6** gave compound **9** isolated as a pure product in a relatively good yield of 72%.

Next, in order to investigate the scope and limitation of the [4 + 2] cycloaddition we aimed to study the reaction by using 1-naphthol-substituted glycine compound **12** as precursor. The synthesis was achieved by reacting 1-naphthol (**11**) as an electron-rich aromatic compound and morpholine (**2**) with ethyl glyoxylate as the aldehyde component (Scheme 3). According to TLC, after 1 h in toluene at 80 °C and 40 min at 100 °C under MW irradiation, desired product **12** was formed. The NMR investigation of the crude product proved the presence of initial compounds; therefore, column chromatographic purification was applied.

By extending the modified Mannich reaction, the stabilization of aminonaphthol derivative **12** via partially aromatic *ortho*-quinone methide intermediate was tested with different cyclic imines in [4 + 2] cycloaddition (Scheme 4). Accordingly, 1-naphthol-substituted glycine precursor **12** was reacted with 3,4-dihydroisoquinoline (**5**) at 100 °C under MW irradiation in 1,4-dioxane. After a reaction time of 80 min, the formation of two new spots in TLC were observed. According to the

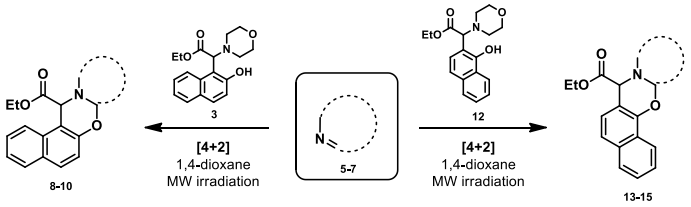


Scheme 1. Synthesis of ethyl 2-(2-hydroxynaphthalen-1-yl)-2-morpholinoacetate (**3**).



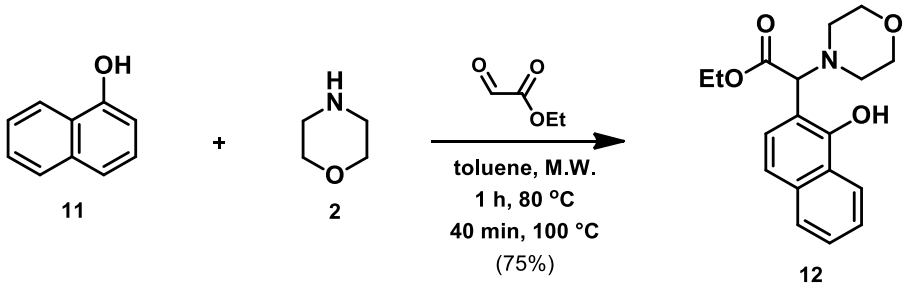
Scheme 2. Cycloaddition reactions starting from precursor **3**.

Table 1
Reaction conditions for preparation of naphthoxazino derivates via [4 + 2] cycloaddition (8–10, 13–15).

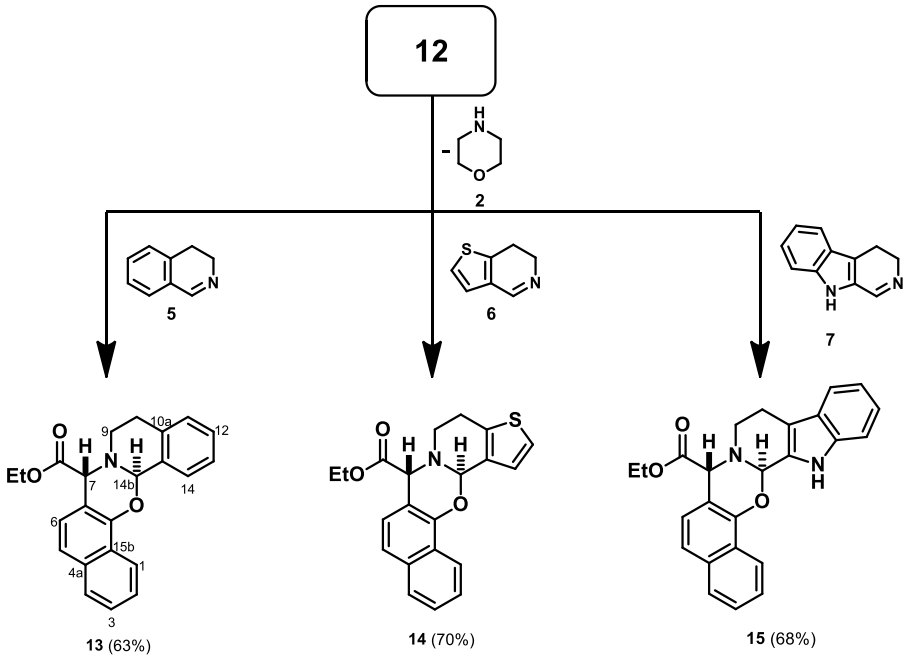


Product	Cyclic imine	Time (min)	Temperature (°C)	Conversion[a] (%)	Yield[b] (%)
8	**5**	60	80	65	
		300	**100**	**73**	**65**
9	**6**	60	80	70	
		280	**100**	**77**	**72**
10	**7**	80	80	67	
		360	**100**	**75**	**68**
13	**5**	20	80	55	
		80	**80**	**70**	**63**
14	**6**	60	100	68	
		80	**100**	**78**	**70**
15	**7**	20	80	65	
		40	100	72	
		260	**120**	**75**	**68**

[a] Determined from crude NMR spectra.
[b] Isolated yields. Work-up was performed in the case of each product from its reaction at the highest conversion.



Scheme 3. Synthesis of ethyl 2-(1-hydroxynaphthalen-2-yl)-2-morpholinoacetate (**12**).



Scheme 4. Cycloaddition reactions starting from precursor **12**.

^{1}H NMR spectrum of the crude reaction mixture, the decomposition of the desired naphthoxazino derivative **13** was proved. The separation of product **13** from the side product was achieved by using column chromatographic purification. Next, we focused on avoiding the formation of the side product by controlling reaction conditions. Therefore, the reaction was repeated at lower temperature (80 °C). The progress of synthesis was monitored in every 20 min by TLC showing the formation of a single product after 80 min. After optimising reaction conditions, the crude reaction mixture was purified by column chromatography. Based on preceeding experiments, we wanted to investigate the behaviour of other dienophiles in [4 + 2] cycloaddition, namely 6,7-dihydrothieno[3,2-*c*]pyridine (**6**) and 3,4-dihydro-*β*-carboline (**7**) (Table 1). TLC and crude product NMR analysis, in each case, indicated the formation of a single product. In the case of newly formed stereogenic centres via [4 + 2] cycloaddition, NOE measurements proved that the relative configuration is *trans*. In conclusion, 80–120 °C and reaction times of 80–260 min were found to be the optimal reaction conditions. Purification of crude mixtures were achieved by column chromatography.

As observed in our study, although longer reaction times and a higher temperature accelerated the [4 + 2] cycloaddition reactions, conversions were maximised at around 78%. Based on our results, the transformation of new *α*-amino acid esters seemed to proceed under equilibrium condititons. Accordingly, morpholine, because of its nucleophilic property, is able to stabilize *ortho*-quinone methide to recover the initial aminonaphthol derivative thereby inhibiting [4 + 2] cycloaddition.

2.2. Biological evaluations

In order to investigate the biological properties of newly synthesised 2- and 1-naphthol-substituted glycine derivatives and α-amino acid esters, antibacterial and efflux pump inhibiting activities were examined. According to the data no significant antibacterial effect was observed for most of the compounds on either Gram-negative or Gram-positive strains. However, compound **15** exhibited antibacterial effect on the reference *S. aureus* ATCC 25923 strain (Table 2). The activity of the compounds on efflux pump inhibition can be assessed by real-time fluorimetry applying a fluorochrome (e.g. ethidium bromide), a substrate of the bacterial efflux pumps. Since the compounds, with the exception of **15**, had no antibacterial activity, they were tested at 50 and 100 μM concentrations. In the real-time ethidium bromide accumulation assay, compounds with an efflux pump inhibitory (EPI) effect have a higher relative fluorescence index (RFI) compared to that of the untreated control. The derivatives were not effective on the Gram-negative *Escherichia coli* AG100 strains (Table 3). However, they had potent EPI activity on the susceptible and resistant *S. aureus* strains. In the case of the susceptible reference *S. aureus* ATCC 25923, **3** and **13** were effective EPIs at 100 μM. In addition, **3**, **13** and **14** showed EPI activity at 50 μM. Since **15** had antibacterial activity on this *S. aureus* strain, the compound was applied at MIC/2 concentration and presented an RFI of 0.26 (Table 4).

With respect to the resistant *S. aureus* MRSA ATCC 43300, **13** and **14** were effective at both concentrations tested (Table 5).

Table 2
Antibacterial activity (minimum inhibitory concentration, MIC in μM) of the derivatives on Gram-negative and Gram-positive bacterial strains.

	Staphylococcus aureus ATCC 25923	*Staphylococcus aureus* MRSA ATCC 43300	*Escherichia coli* AG100
3	>100	>100	>100
8	>100	>100	>100
9	>100	>100	>100
10	>100	>100	>100
12	>100	>100	>100
13	>100	>100	>100
14	>100	>100	>100
15	**6.25**	>100	>100

3. Conclusions

New glycine derivatives substituted with 2- and 1-naphthol were synthesised by the reaction of 2- and 1-naphthol, morpholine and ethyl glyoxylate as the aldehyde component by using a modified Mannich-type synthetic pathway. To investigate the scope and limitation of the [4 + 2] cycloaddition, the reaction of aminonaphthol derivatives was tested with different cyclic imines (3,4-dihydroisoquinoline, 6,7-dihydrothieno[3,2-*c*]pyridine, 3,4-dihydro-*β*-carboline). During the reaction

Table 3
Efflux pump inhibiting activity of the derivatives on *E. coli* AG100 strain.

RFI		*Escherichia coli* AG100	
Concentration	**Mean**	**SD**	**RFI**
50 μM			
3	44310.00	4952.07	0.29
8	35837.67	1168.24	0.05
9	34078.33	913.06	0.00
10	34706.00	2021.98	0.01
12	7485.67	430.73	−0.78
13	37156.33	730.23	0.09
14	34561.67	412.53	0.01
15	33567.67	1248.85	−0.02
100 μM	**Mean**	**SD**	**RFI**
3	36685.33	2500.46	0.07
8	36732.00	1686.71	0.07
9	34921.33	2842.67	0.02
10	36337.67	1379.55	0.06
12	6561.67	368.16	−0.81
13	36999.33	1041.58	0.08
14	35945.67	1447.65	0.05
15	32220.33	944.63	−0.06
	Mean	**SD**	**RFI**
DMSO	34228.33	1342.39	0.00
CCCP	72448.33	624.50	**1.12**

Table 4
Efflux pump inhibiting activity of the derivatives on *S. aureus* ATCC 25923 strain.

RFI		*S.aureus* ATCC 25923	
Concentration	Mean	SD	RFI
50 μM			
3	58168.67	865.83	**1.10**
8	31759.00	1778.37	0.15
9	29306.00	1165.51	0.06
10	27892.33	884.07	0.01
12	11677.33	1308.70	−0.58
13	44414.33	946.94	**0.61**
14	36826.67	2499.36	0.33
100 μM	**Mean**	**SD**	**RFI**
3	51721.00	905.77	**0.87**
8	31231.67	894.65	0.13
9	33816.67	3255.64	0.22
10	29131.67	2453.80	0.05
12	7624.33	1372.54	−0.72
13	42864.00	2360.31	**0.55**
14	77859.67	5158.78	**1.82**
3.125 μM	**Mean**	**SD**	**RFI**
15	34824.33	1646.59	0.26
	Mean	SD	RFI
DMSO	27636.67	1089.18	0.00
Reserpine	54056.00	829.47	**0.96**

via loss of morpholine, the presence of partially aromatic *ortho*-quionone methide intermediates has been proved and they were transformed with dienophiles to new α-amino acid esters. ^{1}H NMR confirmed that [4 + 2] cycloaddition resulted in the formation of a single product in each case. According to 2D-NMR analysis, the relatively weak cross peak verified that the relative configurations of the newly formed stereogenic centres was *trans*. In order to separate the required naphthoxazino derivatives, column chromatographic purification was applied.

Regarding the biological results, it can be concluded that in the case of some compounds antibacterial effect was observed on the reference *S. aureus* ATCC 25923 strain. With respect to EPI activity, certain derivatives were effective in the case of the susceptible reference *S. aureus* ATCC 25923 strain. Referring to the resistant *S. aureus* MRSA ATCC 43300, some of the naphthoxazino derivatives were effective at both concentrations tested.

Table 5
Efflux pump inhibiting activity of the derivatives on *S.aureus* MRSA ATCC 43300 strain.

	Staphylococcus aureus MRSA ATCC 43300		
Concentration	Mean	SD	RFI
50 μM			
3	87762.67	3187.90	0.12
8	92734.00	528.62	0.19
9	88184.67	666.65	0.13
10	76072.00	1171.02	−0.03
12	56126.33	299.38	−0.28
13	111300.67	1782.32	**0.43**
14	101450.33	1316.67	**0.30**
15	84683.00	455.64	0.09
100 μM	**Mean**	**SD**	**RFI**
3	84280.00	1539.30	0.08
8	93822.67	4081.38	0.20
9	88655.67	1118.00	0.14
10	78009.00	1574.59	0.00
12	18886.00	648.23	−0.76
13	114226.00	744.67	**0.46**
14	103410.00	1139.71	**0.33**
15	90479.33	1534.61	0.16
	Mean	SD	RFI
DMSO	78039.33	1254.261	
Reserpine	101532.7	3878.731	**0.30**

4. Experimental section

4.1. Biological assays

4.1.1. Reagents and media

DMSO (Sigma-Aldrich, St Louis, MO, USA), phosphate-buffered saline (PBS; pH 7.4), Mueller Hinton (MH) broth, tryptic soy broth (TSB), tryptic soy agar (TSA), Luria-Bertani broth (LBB), Luria-Bertani agar (LBA), reserpine, CCCP (carbonyl cyanide 3-chlorophenylhydrazone), ethidium bromide (EB). All reagents were purchased from Sigma.

4.1.2. Bacterial strains

The wild-type *Escherichia coli* K-12 AG100 Gram-negative strain [argE3 thi-1 rpsL xyl mtl Δ(gal-uvrB) supE44], expressing the AcrAB-TolC efflux pump (EP) at its basal level was used. As Gram-positive strains, *Staphylococcus aureus* American Type Culture Collection (ATCC) 25923 as methicillin-susceptible reference strain, and the methicillin and oxacillin-resistant *S. aureus* MRSA ATCC 43300 strain were investigated in this study.

4.1.3. Antibacterial activity

The minimum inhibitory concentrations (MICs) of the compounds were determined according to the Clinical and Laboratory Standard Institute guidelines (CLSI). The MIC values of the compounds were determined by visual inspection. DMSO as solvent was also assayed to ensure that it had no antibacterial effect.

4.1.4. Real-time ethidium bromide accumulation assay

The efflux pump inhibiting activity of compounds was assessed on *S. aureus* ATCC 25923 and *S. aureus* MRSA ATCC 43300 strains by real-time fluorimetry based on the intracellular accumulation of the efflux pump substrate EB using a CLARIOstar Plus plate reader (BMG Labtech, UK). Reserpine (RES) was applied in a concentration of 25 μM as a positive control on *S. aureus* strains with DMSO as solvent at 1 v/v%. Carbonyl cyanide 3-chlorophenylhydrazone (CCCP) was applied in a concentration of 50 μM as a positive control on *E . coli* AG100. The

bacterial cultures were incubated at 37 °C in a shaking incubator until they reached an optical density (OD) of 0.6 at 600 nm. The cells were then washed with phosphate-buffered saline (PBS; pH 7.4) and centrifuged at 13,000×*g* for 2 min followed by re-suspending the pellet in PBS. The compounds were added at ½ MIC (in the case where no MIC value was observed, concentrations of 50 and 100 μM were used) to PBS containing a non-toxic concentration of EB (2 μg/mL). Then, the solutions were pipetted into a 96-well black microtiter plate (Greiner Bio-One Hungary Kft, Hungary), followed by pipetting 50 μL of bacterial suspension (OD_{600} 0.6) to the wells. Then the plates were placed into the CLARIOstar plate reader, and the fluorescence was monitored at excitation and emission wavelengths of 530 nm and 600 nm, respectively, every minute for 1 h. From the real-time data, the relative fluorescence index (RFI) of the last time point (minute 60) of the EB accumulation assay was calculated according to the subsequent equation:

$$RFI = (RF_{treated} - RF_{untreated})/RF_{untreated}$$

where $RF_{treated}$ is the relative fluorescence (RF) at the last time point of EB retention curve in the presence of an inhibitor, and $RF_{untreated}$ is the RF at the last time point of the EB retention curve of the untreated control having the solvent control (DMSO).

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 dienophiles 3,4-dihydroisoquinoline (**5**) [22], 6,7-dihydrothieno[3,2-*c*]pyridine (**6**) [23] and 4,9-dihydro-*β*-carboline (**7**) [24] 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. Ethyl 2-(2-hydroxynaphthalen-1-yl)-2-morpholinoacetate (3)

A mixture of 2-naphthol (**1**) (1.0 g, 6.94 mmol), ethyl glyoxylate (2.0 g, 19.61 mmol; 50% in toluene) and morpholine (**2**) (0.8 g, 9.20 mmol) in toluene (15 mL) was placed into a 35-mL pressurised reaction vial and heated 30 min at 80 °C and 30 min at 100 °C in a CEM SP microwave reactor. The solvent was removed *in vacuo*. The crude reaction mixture was purified by column chromatography (*n*-hexane:EtOAc, 2:1). The desired product was isolated by crystallisation with diethyl ether (10 mL) and *n*-hexane (1 mL) resulting in **3**; 1.54 g, (70%); white crystals; m.p. 86–87 °C; ^{1}H NMR (DMSO): 1.01 (t, 3H, J = 7.1 Hz), 2.45–2.49 (m, 2H), 2.59–2.69 (m, 2H), 3.55–3.64 (m, 4H), 3.95–4.10 (m, 2H), 5.06 (s, 1H), 7.09 (d, 1H, J = 8.8 Hz), 7.29 (t, 1H, J = 7.4 Hz), 7.46 (t, 1H, J = 7.5 Hz), 7.77 (t, 2H, J = 9.0 Hz), 8.24 (d, 1H, J = 8.6 Hz), 10.75 (brs, 1H). ^{13}C NMR ($CDCl_3$); 13.9; 51.6; 61.4; 66.7; 68.7; 108.5; 119.4; 121.6; 122.9; 126.9; 128.6; 128.8; 130.9; 132.7; 156.3; 169.1. HRMS calcd for [M + H$^+$] m/z = 316.1543, found m/z = 316.1540.

4.2.2. General procedure for the synthesis of α-amino acid esters 8–10 via [4 + 2] cycloaddition starting from bifunctional precursor 3

Aminonaphthol derivative **3** (70.0 mg, 0.22 mmol) and cyclic imines **5**, **6**, **7** (0.22 mmol) was dissolved in 1,4-dioxane (10 mL) in a 35 mL pressurised reaction vial. The reaction mixture was heated under MW irradiation according to the conditions given in Table 1. The solvent was removed *in vacuo*.

4.2.3. (7aR*,15R*)-Ethyl-7a,12,13,14-tetrahydronaphth[1,2-e] [1,3] oxazino[2,3-a]isoquinoline-15-carboxylate (8)

The mixture was purified by column chromatography (*n*-hexane: EtOAc, 5:1) and the desired product was isolated by crystallisation with diethyl ether (10 mL) and *n*-hexane (1 mL); 51.34 mg, (65%); white crystals; m.p. 146–147 °C; ^{1}H NMR ($CDCl_3$): 1.28 (t, 3H, J = 7.0 Hz), 2.81–2.99 (m, 2H), 3.20–3.41 (m, 2H), 4.16–4.32 (m, 2H), 4.92 (s, 1H), 6.31 (s, 1H), 7.08 (d, 1H, J = 8.9 Hz), 7.20 (d, 1H, J = 7.0 Hz), 7.28–7.38 (m, 3H), 7.45–7.52 (m, 2H), 7.66–7.80 (m, 3H). ^{13}C NMR ($CDCl_3$); 14.1; 29.3; 45.7; 61.5; 62.8; 83.0; 108.4; 119.1; 121.5; 123.4; 126.3; 126.9; 128.6; 128.8; 128.9; 129.0; 129.1; 129.5; 132.0; 132.7; 134.8; 152.1; 171.4. HRMS calcd for [M + H$^+$] m/z = 360.1594, found m/z = 360.1591.

4.2.4. (7aR*,14R*)-Ethyl-7a,11,12,13-tetrahydronaphth[1,2-e] [1,3] oxazino[2,3-a]thieno[3,2-c]pyridine-14-carboxylate (9)

Following the purifying by column chromatography (*n*-hexane: dichloromethane:EtOAc, 4:3:3), the product was crystallised from diethyl ether (10 mL) and *n*-hexane (1 mL); 57.67 mg, (72%); white crystals; m.p. 117–118 °C; ^{1}H NMR ($CDCl_3$): 1.28 (t, 3H, J = 7.12 Hz), 2.89–2.96 (m, 1H), 3.00–3.08 (m, 1H), 3.16–3.25 (m, 1H), 3.37–3.45 (m, 1H), 4.16–4.32 (m, 2H), 4.96 (s, 1H), 6.32 (s, 1H), 7.09 (d, 1H, J = 9.1 Hz), 7.14 (d, 1H, J = 5.22 Hz), 7.21 (d, 1H, J = 5.30 Hz), 7.36 (t, 1H, J = 7.41 Hz), 7.49 (t, 1H, J = 7.56 Hz), 7.68–7.81 (m, 3H). ^{13}C NMR ($CDCl_3$); 14.2; 26.0; 46.4; 61.6; 62.3; 79.9; 108.5; 119.0; 121.4; 123.5; 123.8; 126.0; 127.0; 128.9; 129.1; 129.6; 131.8; 132.9; 137.9; 151.8; 171.5. HRMS calcd for [M + H$^+$] m/z = 366.1158, found m/z = 366.1156.

4.2.5. (7aR*,16R*)-Ethyl-7a,13,14,15-tetrahydronaphth[1,2-e]oxazino [2,3-a]-β-carboline-16-carboxylate (10)

The crude reaction mixture was purified by column chromatography (*n*-hexane:EtOAc, 2:1) and the desired product was isolated by crystallisation with diethyl ether (10 mL) and *n*-hexane (1 mL); 59.70 mg, (68%); beige crystals; m.p. 200–203 °C; ^{1}H NMR ($CDCl_3$): 1.27–1.31 (m, 3H), 2.86–2.94 (m, 1H), 3.05–3.14 (m, 2H), 3.40–3.49 (m, 1H), 4.17–4.33 (m, 2H), 5.02 (s, 1H), 6.52 (s, 1H), 7.05–7.09 (d, 1H, J = 9.0 Hz), 7.13 (t, 1H, J = 7.36 Hz), 7.20–7.24 (m, 1H), 7.34–7.42 (m, 2H), 7.50 (t, 1H, J = 7.9 Hz), 7.55 (d, 1H, J = 7.9 Hz), 7.71 (d, 1H, J = 9.0 Hz), 7.79 (t, 2H, J = 8.5 Hz), 8.16 (brs, 1H). ^{13}C NMR ($CDCl_3$); 14.2; 22.1; 47.1; 61.5; 62.1; 78.8; 108.9; 111.4; 111.4; 118.8; 119.0; 119.8; 121.5; 122.9; 123.6; 126.3; 127.0; 128.8; 129.2; 129.7; 129.8; 131.9; 136.8; 151.4; 171.5. HRMS calcd for [M + H$^+$] m/z = 399.1703, found m/z = 399.1709.

4.2.6. Ethyl 2-(1-hydroxynaphthalen-2-yl)-2-morpholinoacetate (12)

1-Naphthol (**11**) (1.0 g, 6.94 mmol), ethyl glyoxylate (2.0 g, 19.61 mmol; 50% in toluene) and morpholine (**2**) (0.8 g, 9.20 mmol) in toluene were heated in a 35 mL pressurised reaction vial at 80 °C for 1 h and at 100 °C for 40 min under MW irradiation. Following the removal of the solvent, the residue was purified by column chromatography (*n*-hexane: EtOAc, 2:1); 1.65 g, (75%); brown oil; ^{1}H NMR (DMSO): 1.11 (t, 3H, J = 7.1 Hz), 2.47–2.50 (m, 2H), 2.54–2.64 (m, 2H), 3.56–3.70 (m, 4H), 4.01–4.16 (m, 2H), 4.52 (s, 1H), 7.28 (d, 1H, J = 8.6 Hz), 7.38 (d, 1H, J = 8.4 Hz), 7.45–7.53 (m, 2H), 7.82 (d, 1H, J = 7.63 Hz), 8.15 (d, 1H, J = 7.80 Hz), 10.75 (brs, 1H). ^{13}C NMR ($CDCl_3$); 14.0; 51.3; 61.4; 66.8; 74.1; 110.9; 119.1; 122.6; 125.0; 125.1; 126.7; 126.8; 127.2; 134.5; 153.3; 169.4. HRMS calcd for [M + H$^+$] m/z = 316.1543, found m/z = 316.1542.

4.2.7. General procedure for the synthesis of α-amino acid esters via [4 + 2] cycloaddition starting from precursor bifunctional compound 12 (13,14,15)

1-Naphthol-substituted glycine derivative **12** (70.0 mg, 0.22 mmol) and 3,4-dihydroisoquinoline (**5**) (0.22 mmol) or 6,7-dihydrothieno[3,2-*c*]pyridine (**6**) (0.31 mmol) or 3,4-dihydro-*β*-carboline (**7**) (0.22 mmol) in 1,4-dioxane (10 mL) were placed in a 35-mL pressurised reaction vial and heated in a CEM SP microwave reactor under the conditions given in Table 1. The solvent was removed under reduced pressure.

4.2.8. (7R,14bR*)-Ethyl-8,9,10,14b-tetrahydronaphth[2,1-e] [1,3] oxazino[2,3-a]isoquinoline-7-carboxylate (13)*

Following column chromatography purification (toluene:methanol, 40:1), the eluent was removed *in vacuo*; 49.90 mg, (63%); oil; ^{1}H NMR ($CDCl_3$): 1.31–1.35 (m, 3H), 2.83–2.89 (m, 1H), 2.89–2.95 (m, 1H), 3.20–3.29 (m, 1H), 3.31–3.38 (m, 1H), 4.21–4.28 (m, 2H), 4.58 (s, 1H), 6.33 (s, 1H), 7.18–7.23 (m, 1H), 7.32–7.37 (m, 2H), 7.37–7.40 (m, 2H), 7.41–7.48 (m, 2H), 7.53–7.58 (m, 1H), 7.75 (d, 1H, $J = 7.91$ Hz), 8.16 (d, 1H, $J = 8.40$ Hz). ^{13}C NMR ($CDCl_3$); 14.2; 29.3; 45.0; 61.3; 63.7; 84.2; 119.8; 122.0; 125.3; 125.4; 126.2; 126.5; 127.4; 128.6; 128.8; 129.2; 132.9; 133.8; 134.2; 135.0; 139.2; 152.5; 162.9. HRMS calcd for $[M + H^+]$ $m/z = 360.1594$, found $m/z = 360.1592$.

4.2.9. (7R,13bR*)-Ethyl-8,9,10,13b-tetrahydronaphth[2,1-e] [1,3] oxazino[2,3-a]thieno[3,2-c]pyridine-7-carboxylate (14)*

The crude reaction mixture was purified by column chromatography (*n*-hexane:EtOAc, 4:1), the eluent was removed under reduced pressure; 56.21 mg, (70%); oil; ^{1}H NMR ($CDCl_3$): 1.30 (t, 3H, $J = 7.15$ Hz), 2.89–2.96 (m, 1H), 2.98–3.05 (m, 1H), 3.14–3.23 (m, 1H), 3.33–3.41 (m, 1H), 4.20–4.28 (m, 2H), 4.62 (s, 1H), 6.33 (s, 1H), 7.18–7.24 (m, 2H), 7.35–7.40 (m, 2H), 7.41–7.49 (m, 2H), 7.75 (d, 1H, $J = 7.55$ Hz), 8.18 (d, 1H, $J = 8.09$ Hz). ^{13}C NMR ($CDCl_3$); 14.2; 26.0; 45.9; 61.4; 63.3; 81.2; 110.3; 119.9; 121.9; 123.4; 125.0; 125.1; 125.4; 126.2; 126.5; 127.4; 133.2; 133.8; 137.9; 149.1; 171.3. HRMS calcd for $[M + H^+]$ $m/z = 366.1158$, found $m/z = 366.1155$.

4.2.10. (7R,15bR*)-Ethyl-8,9,10,15b-tetrahydronaphth[2,1-e]oxazino [2,3-a]-β-carboline-7-carboxylate (15)*

The mixture was purified by column chromatography (*n*-hexane: EtOAc, 2:1), the eluent was removed *in vacuo*; 59.70 mg, (68%); oil; ^{1}H NMR ($CDCl_3$): 1.30 (t, 3H, $J = 7.12$ Hz), 2.85–2.94 (m, 1H), 3.03–3.17 (m, 2H), 3.36–3.46 (m, 1H), 4.20–4.30 (m, 2H), 4.68 (s, 1H), 6.54 (s, 1H), 7.14 (t, 1H, $J = 7.3$ Hz), 7.34–7.50 (m, 6H), 7.57 (d, 1H, $J = 7.86$ Hz), 7.76 (d, 1H, $J = 7.91$ Hz), 8.17 (d, 1H, $J = 7.8$ Hz), 8.27 (brs, 1H). ^{13}C NMR ($CDCl_3$); 14.2; 22.1; 46.5; 61.4; 63.1; 80.1; 111.4; 119.1; 119.8; 120.1; 121.7; 122.9; 125.0; 125.1; 125.5; 126.4; 126.6; 127.5; 130.1; 133.8; 136.8; 148.7; 149.5; 171.4; 174.6. HRMS calcd for $[M + H^+]$ $m/z = 399.1703$, found $m/z = 399.1702$.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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