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ORIGINAL PAPER

Synthesis, antimicrobial and antioxidant activity of novel 1-oxo-1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazine-8-carboxylic acids, esters, and amides thereof

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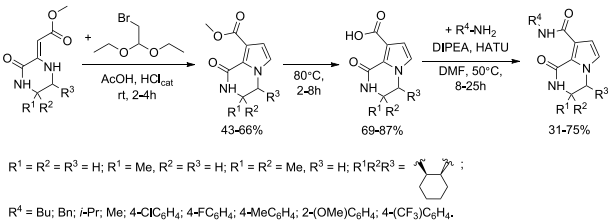
Abstract

A preparatively convenient and efficient method is proposed for the synthesis of novel 1-oxo-1,2,3,4-tetrahydropyrrolo[1,2-*a*]-pyrazine-8-carboxylic acids, based on the reaction of (3-oxopiperazine-2-ylidene)ethanoates with 2-bromo-1,1-diethoxyethane and accomplished through the stage of intermediate methyl 1-oxo-1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazine-8-carboxylates, which were also isolated as individual compounds. A method of directly transforming 1-oxo-1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazine-8-carboxylic acids into 1-oxo-*N*-(alkyl)aryl-1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazine-8-carboxamides via the former's interaction with aliphatic and aromatic amines in the presence of DIPEA and HATU was developed, with yields of 31–78%. A reliable structural determination of all the synthesized compounds has been performed by elemental analysis and a number of spectroscopic methods (^{1}H and ^{13}C NMR, HPLC/MS) as well as by X-ray diffraction analysis. Biological screening of all types of synthesized compounds revealed their moderate antibacterial and antifungal activity. The antioxidant effect level of the most active carboxamides was in the range of 59.3–74.5%, as compared to ascorbic acid (97.3%).

Graphical abstract



Keywords Heterocycles · Annelation · X-ray structure determination · NMR spectroscopy

Introduction

Tetrahydropyrrolo[1,2-*a*]pyrazinones are heterocyclic systems, the nucleus of which is part of a wide array of natural bioactive products isolated from fungi, plants, or marine sponges [1]. Striking representatives of such natural compounds are bromopyrrole alkaloids: mukanadin C [2] and longamide A [3] were moderate inhibitors of GSK-3, DYRK1A, CK-1 and may have future development potential for treating various diseases [4, 5], longamide B [6] being a powerful trypanocidal and antileishmanial agent [7]. In turn, hanishin demonstrates cytotoxicity against human

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non-small-cell-lung carcinoma NSCLC-N6 [8], stylisine D is an effective inhibitor of the Gram-negative bacteria biofilm formation [9] (Fig. 1).

The most important synthetic derivatives of tetrahydropyrrolo[1,2-*a*]pyrazine are represented by ranirestat, a potent aldose reductase inhibitor used to treat diabetic neuropathy [10]. In addition, compounds with tetrahydropyrrolo[1,2-*a*]pyrazine skeleton act as antagonists of melanin-concentrating hormone (MCH-R1) and can be used in anti-obesity therapy [11], are highly selective ERK1/2 inhibitors [12], inhibitors of PIM-kinases with low nanomolar activity and selectivity against a large panel of kinases [13], noncompetitive antagonists of mGluR1 [14], and inhibitors of HIV-1 replication [15] (Fig. 2).

Thus, the pronounced biological and pharmacological effects of both natural and synthetic compounds with the tetrahydropyrrolo[1,2-*a*]pyrazinone fragment are an incentive to obtain their new derivatives, which may prove to be very promising for further biomedical research. That is why the search for preparatively convenient approaches to tetrahydropyrrolo[1,2-*a*]pyrazines with synthetically potent functional groups, which can be advantageously used for targeted structural modification, remains relevant. Since the carboxyl function fully meets these requirements, the subject of our study was previously unknown derivatives of pyrrolo[1,2-*a*]pyrazine-8-carboxylic acids.

Results and discussion

The analysis of the available literature showed that 1-oxo-1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazine-8-carboxylic acids remain obscure compounds, although a small number of their esters with substituents at positions 6 and 7 of the bicyclic system have been described. These were synthesized in a three-component reaction of ethane-1,2-diamine with esters of acetylenedicarboxylic acid and a limited number of 1,2-bielectrophilic reagents: ethyl bromopyruvate [16, 17], nitrostyrenes [18], and glyoxals [19].

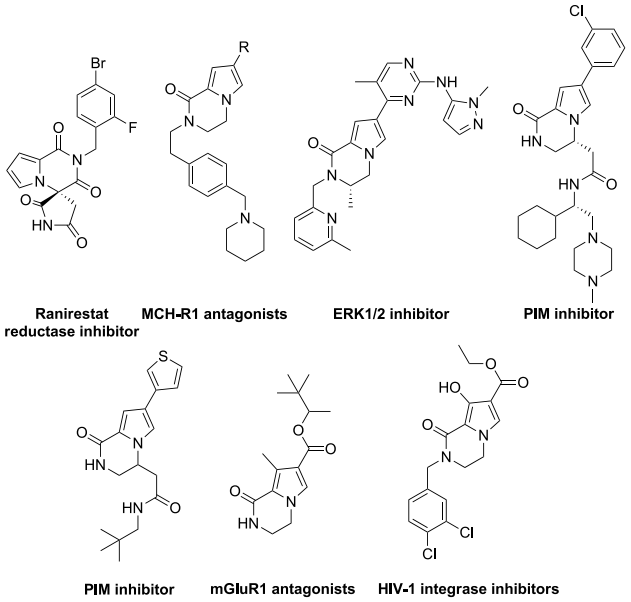


Fig. 2 Synthetic bioactive compounds with the tetrahydropyrrolo[1,2-*a*]pyrazinone fragment

It seemed reasonable for us to investigate the behavior of other available reagents in such cyclocondensation: chloroacetaldehyde and bromoacetaldehyde diethyl acetal, which have been used to form a pyrrole cycle based on enaminones and their synthetic analogues [20–23], thus making it possible to obtain pyrrolo[1,2-*a*]pyrazine-8-carboxylate derivatives, unsubstituted at positions 6 and 7.

The model three-component reactions of ethane-1,2-diamine (**1a**), dimethyl acetylenedicarboxylate (**2**), and chloroacetaldehyde (**3a**) or bromoacetaldehyde diethyl acetal (**3b**) showed that their heating in acetonitrile [16] or acetic acid did not lead to the expected result, which is most likely due to competitive condensation of the aldehyde or acetal groups with ethylenediamine. That is why the realization of the set goal required to implement a sequential transformation with isolation of intermediate

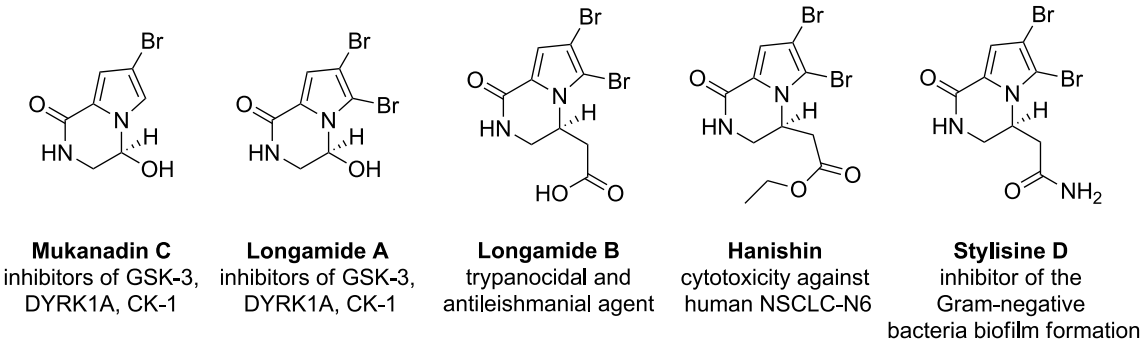
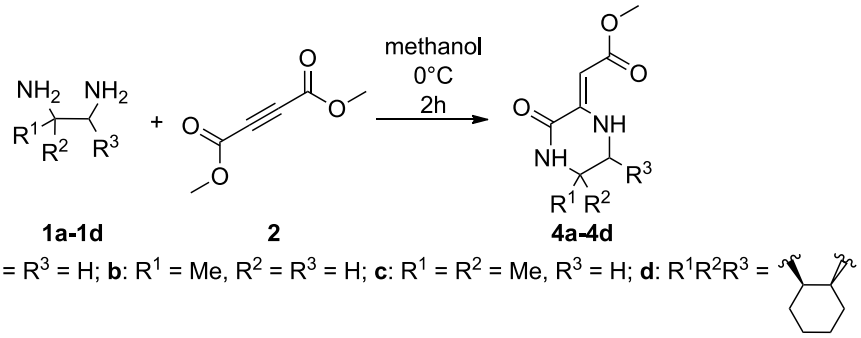


Fig. 1 Tetrahydropyrrolo[1,2-*a*]pyrazinones of natural origin

Scheme 1



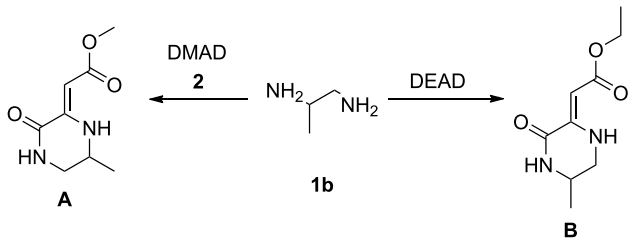
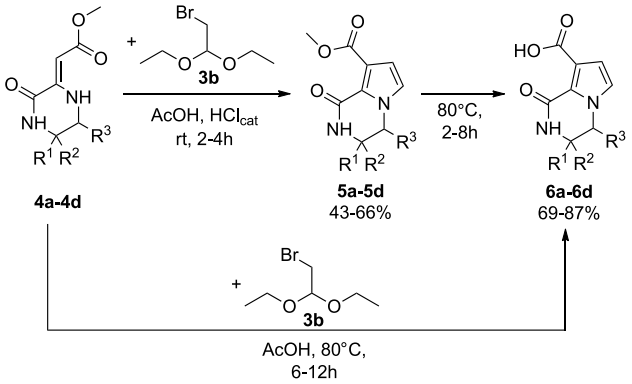


Fig. 3 Compounds **A** and **B** [25–27]

Scheme 2



products–(3-oxopiperazine-2-ylidene)ethanoates. For this purpose, in the first step, various 1,2-diamines—ethane-1,2-diamine (**1a**), propane-1,2-diamine (**1b**), 2-methylpropane-1,2-diamine (**1c**), and *trans*-cyclohexane-1,2-diamine (**1d**)–were reacted with dimethyl acetylenedicarboxylate (DMAD, **2**) in methanol at 0 °C. In the case of symmetrical diamines **1a**, **1d**, (3-oxopiperazine-2-ylidene)ethanoates **4a**, **4d** were isolated with high yields (see Scheme 1), their physicochemical constants matching those described in the literature [24–26].

At the same time, the literature data concerning the interaction of nonsymmetric propane-1,2-diamine (**1b**) with acetylenedicarboxylates are ambiguous. For example, the authors of [27, 28] proposed the structure of methyl (6-methyl-3-oxopiperazine-2-ylidene)ethanoate (**A**) as the reaction product of diamine **1b** with dimethyl acetylenedicarboxylate (DMAD). However, the work [29] asserts that the product of a similar reaction with diethyl acetylenedicarboxylate (DEAD) is ethyl (5-methyl-3-oxopiperazine-2-ylidene)ethanoate (**B**) (Fig. 3).

Thus, we carried out a more detailed study of nonsymmetric diamines **1b**, **1c** reaction with DMAD under the conditions used for diamines **1a**, **1d**. It was found that in the case of propane-1,2-diamine, the reaction yielded a mixture of two regioisomers **4b** and **A** in a ratio of 7:3, from which the major product **4b** was isolated with yield of 64%. Its ^{1}H–^{1}H COSY NMR analysis confirmed the formation of methyl (5-methyl-3-oxopiperazine-2-ylidene)ethanoate (**4b**). A previously undescribed similar interaction involving 2-methylpropane-1,2-diamine (**1c**) proceeds with a rather high regioselectivity to form the 5,5-dimethyl derivative **4c**, the structure of which was also confirmed by ^{1}H–^{1}H COSY NMR spectroscopy (see supplementary).

To evaluate the efficiency of the compounds **3a** and **3b** as bielectrophilic cyclizing reagents, their interaction with the model piperazinone **4a** was studied. It was found that in the acetic acid solution in the presence of a catalytic amount of HCl for 2 h at room temperature, the product of both reactions is methyl 1-oxo-1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazine-8-carboxylate (**5a**), although its yield in the case of chloroacetaldehyde reaches 31% and in the case of bromoacetaldehyde diethyl acetal–43%. Therefore, it was the latter that was used under the above conditions to synthesize a series of pyrrolo[1,2-*a*]pyrazinecarboxylates **5b**–**5d**, which were isolated with yields of 49–66%. It is characteristic that further heating of the reaction mixture containing esters **5a**–**5d** for 2–8 h in acetic acid (method *a*) or cyclization of piperazinones **4a**–**4d** under the same conditions (method *b*) leads to the formation of 1-oxo-1,2,3,4-tetrahydropyrrolo[1,2-*a*] pyrazine-8-carboxylic acids **6a**–**6d** with yields of 69–87% (see Scheme 2, Table 1).

The achieved result clearly proves that the hydrolysis of esters **5b**–**5d** to acids **6a**–**6d** is catalyzed by HBr, which is a by-product of the cyclocondensation of pyrazinones **4a**–**4d** with bromoacetaldehyde diethyl acetal. The structures of the synthesized acids **6a**–**6d** were confirmed by elemental analysis, HPLC/MS, ^{1}H

Table 1 Synthesis of methyl (3-oxopiperazine-2-ylidene)ethanoates **4a**–**4d**, methyl 1-oxo-1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazine-8-carboxylates **5a**–**5d**, and 1-oxo-1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazine-8-carboxylic acids **6a–6d**

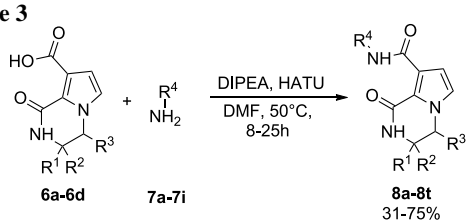
Product	R^1	R^2	R^3	Time/h	*t*/°C	Yield/%
4b	H	Me	H	2	0	64
4c	Me	Me	H	2	0	91
5a	H	H	H	2	rt	43
5b	H	Me	H	3	rt	49
5c	Me	Me	H	3	rt	58
5d	*trans*-cyclohexane-1,2-diamine			4	rt	66
6a	H	H	H	12	80	69
6b	H	Me	H	8	80	72
6c	Me	Me	H	8	80	79
6d	*trans*-cyclohexane-1,2-diamine			6	80	87

and ^{13}C NMR spectroscopy. The dihydropyrrolo[1,2-*a*]-pyrazinone ring formation is most clearly evidenced by the ^1H NMR doublet signals of the H^6 and H^7 protons for **5a**–**5c** in the range of 6.71–6.98 ($^3J_{HH}$ = 2.7–2.3 Hz, C^6H), 6.44–6.66 ($^3J_{HH}$ = 2.5–2.6 Hz, C^7H) ppm and for **6a**–**6c** in the range of 7.17–7.21 ($^3J_{HH}$ = 2.4–2.7 Hz, C^6H), 6.67–6.68 ($^3J_{HH}$ = 2.5–2.7 Hz, C^7H) ppm and the doublet signals of the H^1 and H^2 protons for **5d** at 6.80 ($^3J_{HH}$ = 3.0 Hz, C^1H), 6.65 ($^3J_{HH}$ = 3.0 Hz, C^2H) ppm, for **6d** at 6.96 ($^3J_{HH}$ = 2.7 Hz, C^1H), 6.94 ($^3J_{HH}$ = 2.7 Hz, C^2H) ppm, which agree with the spectral characteristics of structural analogues [16, 19]. The structure of *trans*-4-oxo-4,5,5*a*,6,7,8,9,9*a*-octahydropyrrolo[1,2-*a*]quinoxaline-3-carboxylic acid (**6d**) was established by its X-ray structural study.

It is known that amide bond is a key structural unit of peptides, many synthetic and natural products, and functional materials [30–33]. It is also an important element of a large number of drug candidates and pharmaceuticals [34, 35], which makes amide synthesis a fundamental process in modern medicinal chemistry. Given the potent pharmacological potential of the pyrrolo[1,2-*a*]pyrazinone nucleus, it seemed reasonable to carry out its amide functionalization using the acids **6a**–**6d** as basic substrates for the design of potentially bioactive amides.

The preparatively simple method of converting the acids **6a**–**6d** into the corresponding chloroanhydrides by subjecting them to various chlorinating reagents did not lead to the expected success, which is probably due to the presence of a chlorination-prone C(O)NH group in their structure. Other methods of activating the carboxyl group using reagents such as CDI and TBTU also proved ineffective. A positive result was obtained by using such a coupling reagent as HATU (*N*-[(dimethylamino)-(3*H*-[1,2,3]triazolo[4,5-*b*]pyridin-3-yloxy)methylidene]-*N*-methylmethanaminium hexafluorophosphate) [36, 37]. It is demonstrated that tetrahydropyrrolo[1,2-*a*]pyrazine-8-carboxylic acids **6a**–**6d** react with aliphatic (**7a**–**7d**) and aromatic (**7e**–**7i**) amines in the presence of DIPEA and

Scheme 3



R^4 = Bu; Bn; *i*-Pr; Me; 4-ClC_6H_4; 4-FC_6H_4; 4-MeC_6H_4; 2-$(OMe)C_6H_4$; 4-$(CF_3)C_6H_4$.

HATU in DMF at 50 °C for 8–25 h to form a series of carboxamides **8a**–**8t** with yields of 31–78% (see Scheme 3, Table 2).

The structures of the synthesized compounds **4a**–**4d**, **5a**–**5d**, **6a**–**6d**, **8a**–**8t** have been confirmed by elemental analysis, HPLC/MS, ^1H and ^{13}C NMR spectroscopy. In order to accurately establish the structure of *trans*-4-oxo-4,5,5*a*,6,7,8,9,9*a*-octahydropyrrolo[1,2-*a*]quinoxaline-3-carboxylic acid (**6d**) in the crystalline state, its X-ray structural study was performed (Fig. 4).

The X-ray diffraction study has shown that the tricyclic fragment of compound **6d** has an angular structure (Fig. 4). The six-membered partially saturated cycles are disordered over two positions (A and B) with equal populations. The heterocycle adopts a half-chair conformation in both conformers (the puckering parameters [38] are: S = 0.72, Θ = 31.84°, Ψ = 27.55° in conformer A or S = 0.75, Θ = 34.57°, Ψ = 27.63° in conformer B). Atoms C1 and C6 deviate from the mean plane of the remaining atoms of this cycle on 0.36 Å and −0.33 Å in conformer A or −0.38 Å and 0.36 Å in conformer B. The cyclohexane ring adopts a chair conformation in conformers A and B (the puckering parameters are: S = 1.14, Θ = 3.33°, Ψ = 5.92° in conformer A or S = 1.12, Θ = 1.87°, Ψ = 17.71° in conformer B). Atoms C2 and C5 deviate from the mean plane of the remaining atoms of this cycle on -0.71 Å and 0.63 Å in conformer A or 0.69 Å and −0.64 Å in conformer B.

An intramolecular hydrogen bond O3–H…O1 (the H…O distance is 1.72 Å, O–H…O bond angle is 162°)

Table 2 Synthesis of *N*-substituted-1-oxo-1,2,3,4-tetrahydropyrrolo[1,2-*a*]-pyrazine-8-carboxamides **8a**–**8o** and *trans*–*N*-substituted-4-oxo-4,5,5a,6,7,8,9,9a-octahydropyrrolo[1,2-*a*]-quinoxaline-3-carboxamides **8p**–**8t**

Product	R^1	R^2	R^3	R^4	Time/h	*t*/°C	Yield/%
8a	H	H	H	Bn	15	40	38
8b	H	H	H	Bu	25	40	33
8c	H	H	H	4-FC_6H_4	8	40	60
8d	H	H	H	2-(OMe)C_6H_4	8	40	44
8e	H	H	H	4-(CF_3)C_6H_4	10	40	71
8f	Me	H	H	Bu	25	40	31
g	Me	H	H	4-ClC_6H_4	8	40	74
8h	Me	H	H	Me	25	40	30
8i	Me	H	H	*i*-Pr	25	40	59
8j	Me	H	H	4-(CF_3)C_6H_4	10	40	78
8k	Me	Me	H	4-FC_6H_4	8	40	68
8l	Me	Me	H	4-MeC_6H_4	8	40	70
8m	Me	Me	H	2-(OMe)C_6H_4	8	40	54
8n	Me	Me	H	*i*-Pr	25	40	64
8o	Me	Me	H	4-(CF_3)C_6H_4	10	40	75
8p	*trans*-cyclohexane-1,2-diamine			Bn	15	40	40
8q	*trans*-cyclohexane-1,2-diamine			4-ClC_6H_4	8	40	74
8r	*trans*-cyclohexane-1,2-diamine			Me	25	40	32
8s	*trans*-cyclohexane-1,2-diamine			4-MeC_6H_4	8	40	69
8t	*trans*-cyclohexane-1,2-diamine			4-(CF_3)C_6H_4	10	40	73

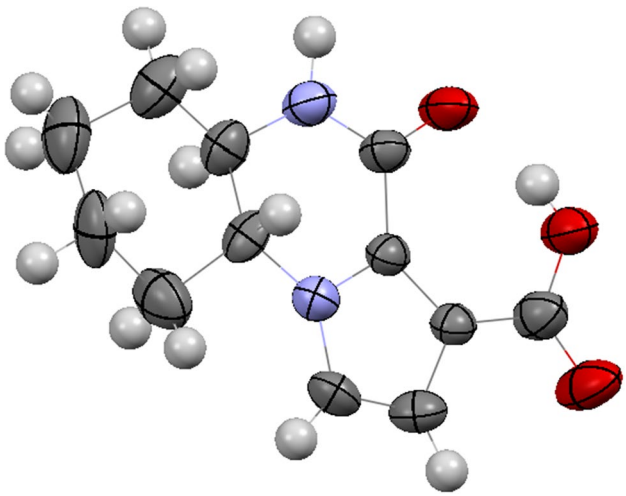


Fig. 4 Molecular structure of compound **6d** according to X-ray diffraction data. For clarity, only one conformer is presented. Thermal ellipsoids are shown at a 50% probability level

has been found between the carboxyl and the carbonyl groups, resulting in elongation of the C11–O1 bond up to 1.237(7) Å as compared to its mean values [39] 1.210 Å, while the C12–O3 (1.318(9) Å) is typical for the carboxyl group (mean value is 1.305 Å).

In the crystal phase, the **6d** molecules are bound by the intermolecular hydrogen bond N1–H…O2 (symmetry operation is 0.5 + x, 1.5−y, −0.5 + z; the H…O distance is 2.01 Å, the N–H…O bond angle is 170°).

Study of antimicrobial and antioxidant activity

The developed convenient synthetic approaches to pyrrolo[1,2-*a*]pyrazine-8-carboxylic acids, their carboxamides, and esters have created good conditions for conducting their biological screening. Taking into account the previously discovered antimicrobial [40–43] and antioxidant [44–47] properties of condensed pyrroles and pyrazines, it seemed reasonable to test the compounds **5a**–**5d**, **6a**–**6d**, and **8a**–**8t**, which are combinations of structurally modified pyrrole and pyrazine cycles, for these activity types.

Antibacterial and antifungal activity in vitro of the synthesized compounds **5a**–**5d**, **6a**–**6d**, and **8a**–**8t** was determined using the two-fold serial dilutions technique with the reference strains of various Gram-negative (*Escherichia coli* ATCC 25922, *Proteus vulgaris* ATCC 4636, *Pseudomonas aeruginosa* ATCC 27853), Gram-positive (*Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 6783, *Bacillus cereus* ATCC 10702) bacteria and fungi (*Candida albicans* ATCC 885/653, *Aspergillus niger* K9, *Aspergillus amstelodami* K12). DMSO was used as a diluent and negative control, the antibacterial agent decasan [48]—as a positive control for antibacterial activity, and clotrimazole [49], a fungicidal agent–as a positive control for antifungal activity. The antibacterial and antifungal activity was assessed by the values of minimum inhibitory (MIC) and

Table 3 Antibacterial activity of the esters **5a**–**5d**, acids **6a**–**6d**, and amides **8a**–**8t**

Compound	Gram-positive						Gram-negative					
	S. aureus ATCC 25923		*E. faecalis* ATCC 6783		*B. cereus* ATCC 10702		*E. coli* ATCC 25922		*P. vulgaris* ATCC 4636		*P. aeruginosa* ATCC 27853	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
5a	62.5	125	62.5	62.5	62.5	62.5	62.5	62.5	62.5	125	62.5	62.5
5b	62.5	125	62.5	62.5	62.5	62.5	62.5	125	62.5	125	62.5	62.5
5c	62.5	125	62.5	62.5	62.5	125	62.5	62.5	62.5	62.5	62.5	62.5
5d	62.5	125	62.5	62.5	62.5	125	62.5	62.5	62.5	62.5	62.5	62.5
6a	62.5	62.5	125	250	62.5	62.5	62.5	62.5	125	250	62.5	62.5
6b	62.5	125	62.5	125	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5
6c	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	125	62.5	62.5
6d	62.5	125	62.5	62.5	62.5	62.5	125	125	62.5	62.5	62.5	62.5
8a	31.25	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5
8b	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5
8c	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5
8d	62.5	62.5	62.5	62.5	62.5	62.5	31.25	31.25	62.5	62.5	62.5	62.5
8e	62.5	62.5	62.5	62.5	31.25	62.5	62.5	62.5	62.5	62.5	31.25	62.5
8f	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	31.25	62.5
8g	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5
8h	62.5	62.5	62.5	62.5	31.25	62.5	62.5	62.5	62.5	62.5	31.25	62.5
8i	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5
8j	31.25	31.25	62.5	62.5	31.25	62.5	31.25	31.25	62.5	62.5	31.25	62.5
8k	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5
8l	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5
8m	62.5	62.5	62.5	62.5	62.5	62.5	31.25	31.25	62.5	62.5	62.5	62.5
8n	31.25	31.25	62.5	62.5	31.25	62.5	31.25	31.25	62.5	62.5	31.25	62.5
8o	62.5	62.5	62.5	62.5	62.5	62.5	31.25	31.25	62.5	62.5	31.25	62.5
8p	31.25	31.25	62.5	62.5	31.25	62.5	31.25	31.25	62.5	62.5	31.25	62.5
8q	62.5	62.5	62.5	62.5	31.25	31.25	62.5	62.5	62.5	62.5	62.5	62.5
8r	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	31.25	62.5
8s	62.5	62.5	62.5	62.5	31.25	31.25	62.5	62.5	62.5	62.5	62.5	62.5
8t	62.5	62.5	62.5	62.5	62.5	62.5	31.25	31.25	62.5	62.5	31.25	62.5
DMSO*	+	+	+	+	+	+	+	+	+	+	+	+
C**	0.48	0.97	3.9	7.81	0.97	1.95	1.95	3.9	7.81	15.625	31.25	31.25

*Proliferation of bacteria detected

**Decasan (a solution consisting of 0.2 mg/cm^3 of decamethoxin) made by "Yuria-Pharm" was used as the control

minimal bactericidal (MBC) or fungicidal (MFC) concentrations. Analysis of the antimicrobial screening data (see Tables 3, 4) showed that the minimum inhibitory concentration of the tested compounds against the bacteria and the fungi varied from 31.25 to 125 μg/cm^3, the minimum bactericidal concentration varied from 31.25 to 250 μg/cm^3, and the minimum fungicidal concentration from 31.25 to 125 μg/cm^3 which attests the presence of antimicrobial activity in this class of compounds. From analyzing the antibacterial activity results, it can be concluded that some amides of the 1-oxo-1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazine-8-carboxylic acids (MIC = 31.25–62.5 μg/cm^3) display twice the activity of the corresponding acids and esters (MIC = 62.5–125 μg/cm^3). In particular, the minimum inhibitory concentration is 31.25 μg/cm^3 for the amides **8a**, **8j**, **8n**, **8p** with *Staphylococcus aureus* ATCC 2592, **8e**, **8h**, **8j**, **8n**, **8p**, **8q**, **8s** with *Bacillus cereus* ATCC 10702, **8d**, **8j**, **8m**-**8p**, **8t** with *Escherichia coli* ATCC 25922, **8e**, **8f**, **8h**, **8k**, **8n**-**8p**, **8r**, **8t** with *Pseudomonas aeruginosa* ATCC 27853. For the antifungal activity, the best results were obtained with the pyrroloquinoxaline derivatives **5d**, **6d**, and **8q** with the fungal strain *Aspergillus niger* K9 (MIC = 31.25 μg/cm^3).

Table 4 Antifungal activity of the esters **5a**–**5d**, acids **6a**–**6d**, and amides **8a**–**8t**

Compound	*Candida albicans* ATCC 885/653		*Aspergillus niger* K9		*Aspergillus amstelodami* K12	
	MIC	MFC	MIC	MFC	MIC	MFC
5a	62.5	125	62.5	62.5	62.5	62.5
5b	62.5	125	62.5	62.5	62.5	62.5
5c	62.5	62.5	62.5	62.5	62.5	62.5
5d	62.5	62.5	31.25	62.5	62.5	62.5
6a	62.5	62.5	62.5	62.5	62.5	62.5
6b	62.5	62.5	62.5	62.5	62.5	62.5
6c	62.5	62.5	62.5	62.5	62.5	62.5
6d	62.5	125	31.25	62.5	62.5	62.5
8a	62.5	62.5	62.5	62.5	62.5	62.5
8b	62.5	125	62.5	62.5	62.5	62.5
8c	62.5	125	62.5	125	125	125
8d	62.5	62.5	62.5	125	62.5	125
8e	125	125	125	125	62.5	62.5
8f	62.5	62.5	62.5	62.5	62.5	62.5
8g	62.5	62.5	62.5	125	62.5	125
8h	62.5	125	62.5	125	62.5	62.5
8i	62.5	62.5	62.5	62.5	62.5	125
8j	62.5	62.5	62.5	62.5	62.5	62.5
8k	62.5	125	62.5	125	62.5	125
8l	62.5	125	62.5	125	125	125
8m	62.5	62.5	62.5	125	62.5	125
8n	62.5	62.5	62.5	62.5	125	62.5
8o	62.5	125	62.5	62.5	62.5	62.5
8p	62.5	62.5	62.5	62.5	125	62.5
8q	125	125	31.25	62.5	125	125
8r	62.5	62.5	62.5	62.5	62.5	62.5
8s	125	125	62.5	125	125	125
8t	125	125	62.5	62.5	62.5	62.5
DMSO*	+		+		+	
C**	0.97	1.95	0.48	0.48	1.95	1.95

*Proliferation of fungi detected

**Clotrimazole (a solution consisting of 10 mg/cm^3 of clotrimazole) made by PJSC SIC "Borshchahivskiy CPP" was used as the control

Antioxidant action of the synthesized compounds

The antioxidant activity assessment of the synthesized compounds was carried out using the DPPH radical scavenging assay according to the method described in [50]. 1 cm^3 of DPPH solution (0.08 mg/cm^3) was added to each of the methanol solutions of test compounds, as well as to a standard solution of ascorbic acid used as reference, and left at room temperature in a dark place for 1 h. The absorption value was determined with a UV-1800 spectrophotometer (Shimadzu, Japan) at 517 nm wavelength relative to the control. Each sample was analyzed in three repeats. The inhibition percentage was calculated relative to the blank sample:

$$\mathrm{I}\% = \frac{\left(A_{\mathrm{blank}} - \left(A_{\mathrm{sample+DPPH}} - A_{\mathrm{sample}}\right)\right)}{A_{\mathrm{blank}}} \cdot 100\% \quad (1)$$

wherein A_{blank} is the absorption of the control reaction (including all reagents except the tested compounds); $A_{\mathrm{sample+DPPH}}$ is the absorption of the tested compounds after 60 min of incubation with the DPPH solution; A_{sample} is the absorption of the tested compounds without the DPPH solution.

The synthesized carboxylates **5a**–**5d**, carboxylic acids **6a**–**6d**, and carboxamides **8a**–**8t** were evaluated for their ability to inhibit the DPPH (1,1-diphenyl-2-picrylhydrazyl) radicals [50]. The assessment of DPPH radical scavenging

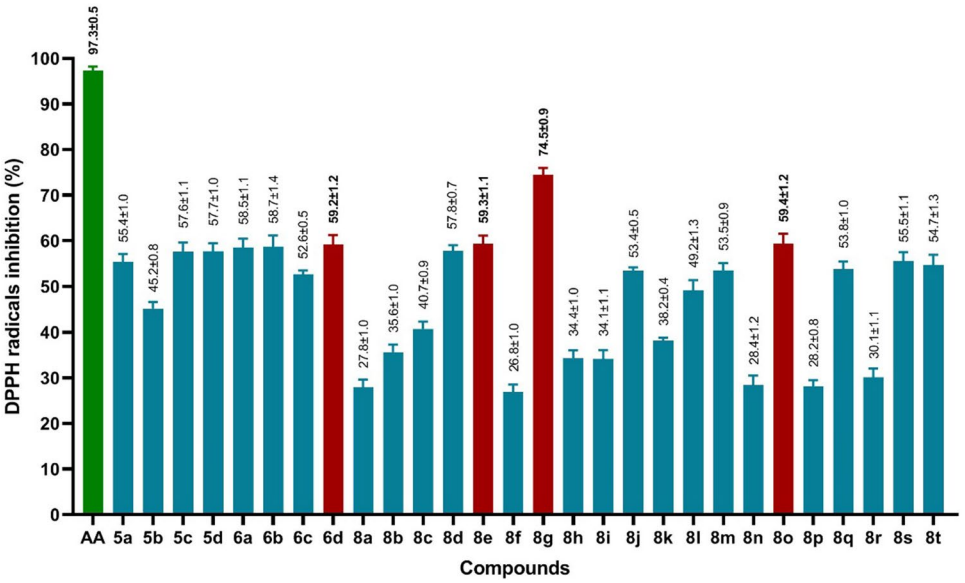


Fig. 5 The DPPH radical scavenging by the derivatives **5a**–**5d**, **6a**–**6d**, and **8a**–**8t** at 5 μM concentration. Ascorbic acid (AA) was employed as a positive control (green). The highest activity was observed for compounds **6d**, **8e**, **8g**, and **8o** (red)

activity was done at 5 μM concentration (a methanol solution, measurement after 60 min). This approach allows for quick identification of potential hit compounds while saving time and the compounds' quantities. Ascorbic acid was used as a standard compound. The results of the radical scavenging activity screening for the **5a**–**5d**, **6a**–**6d**, and **8a**–**8t** compounds are shown in Fig. 5.

So, the carboxylates **5a**–**5d** are characterized by a moderate antioxidant effect with a DPPH radical scavenging level of 45.2–57.7%. While the acids **6a**–**6d** demonstrate a higher level of radical scavenging in comparison with the corresponding esters, in the range of 52.6–59.2%. Most likely, the stronger antioxidant activity of the **6a**–**6d** compounds occurs because of the presence of an active hydrogen atom of the carboxyl group.

In turn, the carboxamides **8a**–**8t** demonstrated a broad distribution of radical scavenging values, from 26.8 to 74.5%, which is probably due to the varying nature of the substituents in the amide fragment. For instance, the *N*-alkyl-substituted derivatives **8a**, **8b**, **8f**, **8h**, **8i**, **8n**, **8p**, and **8r** are characterized by a lower radical scavenging level in comparison with the *N*-aryl derivatives **8c**–**8e**, **8g**, **8j**–**8m**, **8o**, **8q**, and **8s**–**8t**. This resulted in the identification of the hit compounds **6d**, **8e**, **8g**, and **8o**, which can be used for in-depth pharmacological research and potential synthetic antioxidant design.

Conclusion

To summarize, we have shown that the interaction of (3-oxopiperazine-2-ylidene)ethanoates and 2-bromo-1,1-diethoxyethane is a convenient method for the synthesis of the novel 1-oxo-1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazine-8-carboxylate derivatives, which, under the conditions of HBr catalysis, have a tendency to hydrolyze into the corresponding acids. For the latter, a method of direct amidation with alkyl- and arylamines in the presence of DIPEA and HATU in DMF was developed, which allowed to obtain a focused library of 1-oxo-*N*-(alkyl)aryl-1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazine-8-carboxamides. The results of testing the synthesized compounds against Gram-positive and Gram-negative bacteria and fungi showed that they are characterized by moderate activity, although the majority of carboxamides display a twice stronger antibacterial effect as compared to acids and esters. Amongst the carboxamides, the antioxidant properties screening revealed compounds with maximum DPPH scavenging levels in the range of 59.3–74.5% in comparison with ascorbic acid (97.3%).

Experimental

All commercially available chemicals were purchased from Sigma-Aldrich Chemicals (Steinheim, Germany), Merck Chemicals (Darmstadt, Germany), Enamine Ltd (Kyiv, Ukraine). Melting points were determined on a Kofler bench. ^{1}H NMR spectra were acquired on a Varian VXR-400 spectrometer (400 MHz) in $CDCl_3$ solution (for compounds **5a**, **5d**, **6d**, **8a**, **8b**, **8f**, **8i**) and in DMSO-d_6 solution (for compounds **4b**, **4c**, **5b**, **5c**, **6a**–**6c**, **8c**–**8e**, **8g**, **8h**, **8j**–**8t**) with TMS as an internal standard. ^{13}C NMR spectra were acquired on a Varian Mercury 300 spectrometer (76 MHz), Bruker Avance DRX-500 spectrometer (125 MHz), and a Agilent 600 MHz spectrometer (150 MHz) in $CDCl_3$ solution (for compounds **5a**, **8a**, **8b**, **8f**) and in DMSO-d_6 solution (for compounds **4b**, **4c**, **5b**–**5d**, **6a**–**6d**, **8c**–**8e**, **8g**–**8s**, **8t**) with TMS as an internal standard. ^{19}F NMR spectra were

acquired on a Varian Mercury-400 spectrometer (376 MHz) in $CDCl_3$ solution (for compounds **8c**, **8k**) and in DMSO-d_6 solution (for compounds **8e**, **8j**, **8o**, **8t**). Mass spectra were recorded on an Agilent LC/MSD SL instrument; column Zorbax SB-C18, 4.6 × 15 mm, 1.8 µm (PN 82(c) 75–932); solvent DMSO, at atmospheric pressure, electrospray ionization. Merck 60 (40–63 µ) silica gel was used for column chromatography.

Synthesis of methyl (3-oxopiperazin-2-ylidene)-ethanoates 4a-4c and methyl *trans*-(3-oxooctahydroquinoxalin-2(1*H*)-ylidene)-ethanoate (4d) (general method)

To a cooled (0 °C) solution of DMAD (29.9 mmol) in 40 cm^3 MeOH, a solution of diamines **1a**–**1d** (29.9 mmol) in 20 cm^3 MeOH was added. The resulting mixture was stirred at 0 ℃ for 2 h. After the reaction was completed, the insoluble materials were filtered off, washed with cold MeOH (2 × 2 cm^3), hexane (2 × 4 cm^3), and dried under reduced pressure. For the compounds **4a** [24] and **4d** [25, 26], the ^{1}H NMR and ^{13}C NMR spectra were found to be identical with the ones described in Refs. [24–26].

Methyl (5-methyl-3-oxopiperazin-2-ylidene)ethanoate (4b, $C_8H_{12}N_2O_3$) Yield: 3.54 g (64%); m.p.: 189–190 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.49 (s, NH), 8.32 (s, NH), 5.21 (s, =CH), 3.65–3.59 (m, 1H), 3.57 (s, OCH_3), 3.40 (dt, $^2J_{HH}$ = 13.0, $^3J_{HH}$ = 4.0 Hz, 1H), 3.02–2.95 (m, 1H), 1.10 (d, $^3J_{HH}$ = 6.5 Hz, C^5CH_3) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 18.14 ($C^5\underline{C}H_3$), 44.99 (C^6), 45.56 ($\underline{C}^5CH_3$), 50.15 (OCH_3), 82.93 (=CH), 149.36 (C^2), 159.52 (C^3), 169.47 (O=$\underline{C}$–OCH_3) ppm; MS (70 eV): *m*/*z* = 185 ($[M+H]^+$).

Methyl (5,5-dimethyl-3-oxopiperazin-2-ylidene)ethanoate (4c, $C_9H_{14}N_2O_3$) Yield: 5.40 g (91%); m.p.: 165–166 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.50 (s, NH), 8.33 (s, NH), 5.22 (s, =CH), 3.57 (s, OCH_3), 3.18 (d, $^3J_{HH}$ = 2.7 Hz, C^6H_2), 1.18 (s, $C^5(CH_3)_2$) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 26.04 ($C^5(\underline{C}H_3)_2$), 49.53 (C^5), 50.13 (OCH_3), 50.47 (C^6), 82.94 (=CH), 148.82 (C^2), 159.02 (C^3), 169.43 (O=$\underline{C}$–OCH_3) ppm; MS (70 eV): *m*/*z* = 199 ($[M+H]^+$).

Synthesis of methyl 1-oxo-1,2,3,4-tetrahydropyrrolo-[1,2-*a*]pyrazine-8-carboxylates 5a-5c and methyl *trans*-4-oxo-4,5,5a,6,7,8,9,9a-octahydropyrrolo[1,2-*a*]quinoxaline-3-carboxylate (5d) (general method)

To a solution of 2.7 mmol of methyl (3-oxopiperazine-2-ylidene)ethanoates **4a**–**4c** or methyl *trans*-[3-oxooctahydroquinoxalin-2(*1H*)-ylidene]ethanoate (**4d**) in 5 cm^3 AcOH, 0.53 g bromoacetaldehyde diethyl acetal (2.7 mmol) and a few drops of an HCl solution in 1,4-dioxane were added. The resulting mixture was stirred at room temperature for 2–4 h. After the reaction was completed, sodium acetate was added to the obtained mixture until pH 7, and it was dried under reduced pressure. The formed precipitate was dissolved in 50 cm^3 ethyl acetate, washed with saturated aqueous $NaHCO_3$ (5 cm^3), with H_2O (2 × 5 cm^3), and brine (2 × 5 cm^3), the organic phase was dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel, eluent $CHCl_3$–MeOH, 10:1 (for compounds **5a**, **5c**), and EtOAc (for compounds **5b**, **5d**).

Methyl 1-oxo-1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazine-8-carboxylate (5a, $C_9H_{10}N_2O_3$) Yield: 0.21 g (43%); m.p.: 122–123 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.79 (s, NH), 6.71 (d, $^3J_{HH}$ = 2.6 Hz, C^6H), 6.66 (d, $^3J_{HH}$ = 2.6 Hz, C^7H), 4.13 (dd, $^3J_{HH}$ = 6.9, 4.7 Hz, C^4H_2), 3.87 (s, CH_3), 3.68 (td, $^3J_{HH}$ = 6.5, 5.9, 3.7 Hz, C^3H_2) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 39.93 (C^3), 44.91 (C^4), 51.79 (O–CH_3), 112.71 (C^7), 119.65 (C^8), 122.30 (C^6), 124.35 (C^{8a}), 159.87, 164.35 (C^1=O + O=$\underline{C}$–OCH_3) ppm; MS (70 eV): *m*/*z* = 195 ($[M+H]^+$).

Methyl 3-methyl-1-oxo-1,2,3,4-tetrahydropyrrolo[1,2-*a*]-pyrazine-8-carboxylate (5b, $C_{10}H_{12}N_2O_3$) Yield: 0.27 g (49%); m.p.: 109–110 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.92 (s, NH), 6.98 (d, $^3J_{HH}$ = 2.3 Hz, C^6H), 6.44 (d, $^3J_{HH}$ = 2.5 Hz, C^7H), 4.19 (dd, $^2J_{HH}$ = 11.6, $^3J_{HH}$ = 2.3 Hz, C^4H), 3.87–3.72 (m, $C^3H + C^4H$), 3.68 (s, OCH_3), 1.17 (d, $^3J_{HH}$ = 6.1 Hz, C^3CH_3) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 17.75 ($C^3\underline{C}H_3$), 45.93 (C^3), 50.08 (C^4), 51.23 (OCH_3), 110.97 (C^7), 117.92 (C^8), 122.60 (C^6), 123.77 (C^{8a}), 157.63 (C^1=O), 164.34 (O=$\underline{C}$–OCH_3) ppm; MS (70 eV): *m*/*z* = 209 ($[M+H]^+$).

Methyl 3,3-dimethyl-1-oxo-1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazine-8-carboxylate (5c, $C_{11}H_{14}N_2O_3$) Yield: 0.34 g (58%); m.p.: 135–136 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.92 (s, NH), 6.97 (d, $^3J_{HH}$ = 2.6 Hz, C^6H), 6.46 (d, $^3J_{HH}$ = 2.7 Hz, C^7H), 3.97 (s, C^4H_2), 3.69 (s, OCH_3), 1.19 (s, $C^3(CH_3)_2$) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 25.91 ($C^3(\underline{C}H_3)_2$), 51.23 (OCH_3), 51.27 (C^3), 54.43 (C^4), 111.06 (C^7), 117.84 (C^8), 122.78 (C^6), 123.16 (C^{8a}), 157.06 (C^1=O), 164.29 (O=$\underline{C}$–OCH_3) ppm; MS (70 eV): *m*/*z* = 223 ($[M+H]^+$).

Methyl *trans*-4-oxo-4,5,5a,6,7,8,9,9a-octahydropyrrolo[1,2-*a*]quinoxaline-3-carboxylate (5d, $C_{13}H_{16}N_2O_3$) Yield: 0.44 g (66%); m.p.: 173–174 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.80 (d, $^3J_{HH}$ = 2.6 Hz, C^1H), 6.65 (d, $^3J_{HH}$ = 2.6 Hz, C^2H), 5.83 (s, NH), 3.87 (s, CH_3), 3.71 (td, $^3J_{HH}$ = 10.9, 3.7 Hz, $C^{9a}H$), 3.44 (td, $^3J_{HH}$ = 11.2, 3.8 Hz, $C^{5a}H$), 2.52 (d, $^3J_{HH}$ = 12.6 Hz,

1H), 2.01 (d, $^3J_{HH}$ = 12.5 Hz, 2H), 1.89 (d, $^3J_{HH}$ = 12.1 Hz, 1H), 1.59–1.37 (m, 4H) ppm; ^{13}C NMR (151 MHz, DMSO-d_6): δ = 23.05, 23.35 (C^7 + C^8), 27.15, 29.03 (C^6 + C^9), 51.29 (OCH_3), 55.01 (C^{5a}), 58.30 (C^{9a}), 110.83 (C^2), 118.64 (C^3), 119.02 (C^1), 124.69 (C^{3a}), 157.76 (C^4), 164.59 (O=C–OCH_3) ppm; MS (70 eV): *m*/*z* = 249 ([M + H]$^+$).

Synthesis of 1-oxo-1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazine-8-carboxylic acids 6a-6c and *trans*-4-oxo-4,5,5a,6,7,8,9,9a-octahydropyrrolo[1,2-*a*]-quinoxaline-3-carboxylic acid (6d) (general method)

To a solution of 26.6 mmol of methyl (3-oxopiperazine-2-ylidene)ethanoates **4a**–**4c** or methyl [3-oxooctahydroquinoxalin-2(*1H*)-ylidene]ethanoate (**4d**) in 10 cm^3 AcOH, 5.24 g bromoacetaldehyde diethyl acetal (26.6 mmol) was added. The resulting mixture was stirred at 80 ℃ for 6–12 h. After the reaction was completed, the mixture was cooled and the insoluble materials were filtered off, washed with AcOH (2 × 2 cm^3), MTBE (2 × 2 cm^3), hexane (2 × 4 cm^3) and dried under reduced pressure.

1-Oxo-1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazine-8-carboxylic acid (6a, $C_8H_8N_2O_3$) Yield: 3.30 g (69%); m.p.: 274–275 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 15.36 (s, OH), 9.08 (s, NH), 7.20 (d, $^3J_{HH}$ = 2.7 Hz, C^{6}H), 6.67 (d, $^3J_{HH}$ = 2.7 Hz, C^{7}H), 4.23 (t, $^3J_{HH}$ = 6.2 Hz, C$^4H_2$), 3.66–3.61 (m, C$^3H_2$) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 39.30 (C^3), 43.28 (C^4), 113.20 (C^7), 121.27, 121.37 (C^8 + C^{8a}), 125.05 (C^6), 161.94, 162.73 (C^1=O + HO–C=O) ppm; MS (70 eV): *m*/*z* = 181 ([M + H]$^+$).

3-Methyl-1-oxo-1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazine-8-carboxylic acid (6b, $C_9H_{10}N_2O_3$) Yield: 3.72 g (72%); m.p.: 222–223 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 15.37 (s, OH), 9.14 (s, NH), 7.18 (d, $^3J_{HH}$ = 2.4 Hz, C^{6}H), 6.67 (d, $^3J_{HH}$ = 2.5 Hz, C^{7}H), 4.32 (dd, $^2J_{HH}$ = 12.5, $^3J_{HH}$ = 3.9 Hz, C^{4}H), 4.06–3.97 (m, C^{3}H), 3.92 (dd, $^2J_{HH}$ = 12.4, $^3J_{HH}$ = 9.5 Hz, C^{4}H), 1.23 (d, $^3J_{HH}$ = 6.4 Hz, CH_3) ppm; ^{13}C NMR (151 MHz, DMSO-d_6): δ = 17.56 (CH_3), 46.41 (C^3), 48.88 (C^4), 113.22 (C^7), 120.83, 121.16 (C^8 + C^{8a}), 125.00 (C^6), 161.54 (C^1=O), 162.59 (OH–C=O) ppm; MS (70 eV): *m*/*z* = 195 ([M + H]$^+$).

3,3-Dimethyl-1-oxo-1,2,3,4-tetrahydropyrrolo[1,2-*a*]-pyrazine-8-carboxylic acid (6c, $C_{10}H_{12}N_2O_3$) Yield: 4.37 g (79%); m.p.: 243–244 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 15.36 (s, OH), 9.20 (s, NH), 7.17 (d, $^3J_{HH}$ = 2.5 Hz, C^{6}H), 6.68 (d, $^3J_{HH}$ = 2.6 Hz, C^{7}H), 4.11 (s, C$^4H_2$), 1.27 (s, 2CH_3) ppm; ^{13}C NMR (151 MHz, DMSO-d_6): δ = 25.63 (2CH_3), 52.50 (C^3), 53.36 (C^4), 113.31 (C^7), 120.26, 121.28 (C^8 + C^{8a}), 125.22 (C^6), 160.89 (C^1=O), 162.57 (OH–C=O) ppm; MS (70 eV): *m*/*z* = 209 ([M + H]$^+$).

***trans*-4-Oxo-4,5,5a,6,7,8,9,9a-octahydropyrrolo[1,2-*a*]quinoxaline-3-carboxylic acid (6d, $C_{12}H_{14}N_2O_3$)** Yield: 5.44 g (87%); m.p.: 245–246 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 14.75 (s, OH), 7.07 (s, NH), 6.96 (d, $^3J_{HH}$ = 2.7 Hz, C^{1}H), 6.94 (d, $^3J_{HH}$ = 2.7 Hz, C^{2}H), 3.81 (td, $^3J_{HH}$ = 11.3, 4.0 Hz, C^{9a}H), 3.57 (td, $^3J_{HH}$ = 11.5, 3.9 Hz, C^{5a}H), 2.58 (dd, $^3J_{HH}$ = 12.0, 2.9 Hz, 1H), 2.14–2.11 (m, 1H), 2.07–2.00 (m, 1H), 1.96–1.93 (m, 1H), 1.69–1.57 (m, 2H), 1.55–1.41 (m, 2H) ppm; ^{13}C NMR (151 MHz, DMSO-d_6): δ = 22.97, 23.21 (C^7 + C^8), 26.74, 28.75 (C^6 + C^9), 55.34 (C^{5a}), 57.50 (C^{9a}), 113.40 (C^2), 121.58 (C^1), 121.75, 121.94 (C^3 + C^{3a}), 161.89 (C^4=O), 162.76 (HO–C=O) ppm; MS (70 eV): *m*/*z* = 235 ([M + H]$^+$).

X-ray diffraction study of compound 6d

The colourless crystals of compound **6d** ($C_{12}H_{14}N_2O_3$) are monoclinic. At 293 K *a* = 8.033(2) Å, *b* = 11.939(4) Å, *c* = 12.172(3) Å, β = 98.645(15)°, *V* = 1154.0(6) Å^3, M_r = 234.25, *Z* = 4, space group $P2_1/n$, d_{calc} = 1.348 g/cm^3, *m*(MoK_a) = 0.098 mm^{-1}, F(000) = 496. Intensities of 13,891 reflections (2033 independent, R_{int} = 0.025) were measured on the Bruker APEX II diffractometer (graphite monochromated MoK_α radiation, CCD detector, ω-scaning, $2\Theta_{max}$ = 50°). The structure was solved by direct method using SHELXTL package [51]. Some restrictions were applied for the bond distances within the disordered fragment (Csp^3–Csp^3 = 1.54 Å, Csp^3–N = 1.47 Å). Positions of the hydrogen atoms were located from electron density difference maps and refined using "riding" model with U_{iso} = 1.2 U_{eq} of the carrier atom. Hydrogen bond of the carboxylic group is refined isotropycally. Full-matrix least-squares refinement against F^2 in anisotropic approximation for non-hydrogen atoms using 2033 reflections was converged to wR_2 = 0.246 (R_1 = 0.119 for 1718 reflections with $F > 4\sigma(F)$, *S* = 1.114). The final atomic coordinates, and crystallographic data for molecule **6d** have been deposited to with the Cambridge Crystallographic Data Centre, 12 Union Road, CB2 1EZ, UK (fax: + 44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk) and are available on request quoting the deposition numbers CCDC 2225234).

Synthesis of N-substituted 1-oxo-1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazine-8-carboxamides 8a-8o and N-substituted *trans*-4-oxo-4,5,5a,6,7,8,9,9a-octahydropyrrolo[1,2-*a*]quinoxaline-3-carboxamides 8p–8t (general method)

To a solution of 1.28 mmol of 1-oxo-1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazine-8-carboxylic acids **6a**–**6c** or *trans*-4-oxo-4,5,5*a*,6,7,8,9,9*a*-octahydropyrrolo[1,2-*a*]quinoxaline-3-carboxylic acid (**6d**) in 3 cm^3 DMF,

1.34 mmol of corresponding amines **7a**–**7i**, 0.22 g DIPEA (1.66 mmol), and 0.58 g HATU (1.54 mmol) were added. The resulting mixture was stirred at 50 ℃ for 8–25 h. After the reaction was completed, for the compounds **8c**, **8e**, **8g**, **8j**–**8m**, **8o**, **8q**, **8s**, and **8t** the reaction mixture was cooled and water (5 cm^3) added, the insoluble materials were filtered off, washed with H_2O (2 × 5 cm^3), hexane (2 × 4 cm^3) and dried under reduced pressure. For the compounds **8a**, **8b**, **8d**, **8f**, **8h**, **8i**, **8n**, **8p**, **8r** the obtained mixture was evaporated under reduced pressure. The formed precipitate was dissolved in 50 cm^3 $CHCl_3$, washed with with H_2O (2 × 2 cm^3) and brine (2 × 2 cm^3), the organic phase was dried over Na_2SO_4 and evaporated under reduced pressure. The formed precipitate was purified by column chromatography on silica gel, eluent CH_2Cl_2–MeOH, 25:1 (for compound **8d**), $CHCl_3$–MeOH, 20:1 (for compounds **8a**, **8b**, **8f**, **8i**, **8n**, **8p**), and $CHCl_3$–MeOH, 10:1 (for compounds **8h**, **8r**).

***N*-Benzyl-1-oxo-1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazine-8-carboxamide (8a, $C_{15}H_{15}N_3O_2$)** Yield: 0.13 g (38%); m.p.: 164–165 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 10.90 (s, NH), 7.38 (d, $^3J_{HH}$ = 7.4 Hz, 2H_{Ar}), 7.30 (t, $^3J_{HH}$ = 7.5 Hz, 2H_{Ar}), 7.22 (t, $^3J_{HH}$ = 7.3 Hz, H_{Ar}), 7.02 (d, $^3J_{HH}$ = 2.7 Hz, C^6H), 6.76 (d, $^3J_{HH}$ = 2.8 Hz, C^7H), 6.69 (s, C^1NH), 4.62 (d, $^3J_{HH}$ = 5.3 Hz, CH_2Ph), 4.11 (t, $^3J_{HH}$ = 5.9 Hz, C^4H_2), 3.58–3.46 (m, C^3H_2) ppm; ^{13}C NMR (151 MHz, $CDCl_3$): δ = 39.92 (C^3), 43.62, 44.39 (C^4 + C−Ph), 114.08 (C^7), 120.17 (C^8), 123.39 (C^6), 126.16 (C^{8a}), 126.92 (C_{Ar}), 127.80 (2C_{Ar}), 128.50 (2C_{Ar}), 139.31 (C_{Ar}), 162.19, 162.78 (C^1=O + O = C−NHBn) ppm; MS (70 eV): *m*/*z* = 270 ([M + H]$^+$).

***N*-Butyl-1-oxo-1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazine-8-carboxamide (8b, $C_{12}H_{17}N_3O_2$)** Yield: 0.10 g (33%); m.p.: 138–139 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 10.38 (s, NH), 7.01 (d, $^3J_{HH}$ = 2.8 Hz, C^6H), 6.77 (d, $^3J_{HH}$ = 2.8 Hz, C^7H), 5.99 (s, C^1NH), 4.21–4.16 (m, C^4H_2), 3.74–3.68(m, C^3H_2), 3.44–3.38 (m, NH–C<u>H</u>$_2$–CH_2), 1.64–1.56 (m, C<u>H</u>$_2$–CH_2–CH_3), 1.48–1.38 (m, C<u>H</u>$_2$–CH_3), 0.94 (t, $^3J_{HH}$ = 7.3 Hz, CH_3) ppm; ^{13}C NMR (151 MHz, $CDCl_3$): δ = 13.92 (CH_3), 20.34 (<u>C</u>H_2–CH_3), 31.60 (<u>C</u>H_2–CH_2–CH_3), 39.23, 40.13 (NH–CH_2 + C^3), 44.49 (C^4), 113.92 (C^7), 120.04 (C^8), 123.30 (C^6), 126.58 (C^{8a}), 162.19, 162.67 (C^1=O + O=C−NHBu) ppm; MS (70 eV): *m*/*z* = 236 ([M + H]$^+$).

***N*-(4-Fluorophenyl)-1-oxo-1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazine-8-carboxamide (8c, $C_{14}H_{12}FN_3O_2$)** Yield: 0.21 g (60%); m.p.: 205–206 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.24 (s, NH), 8.58 (s, C^1−NH), 7.67 (dd, $^3J_{HH}$ = 9.0, $^4J_{HF}$ = 5.0 Hz, 2H_{Ar}), 7.20–7.13 (m, 2H_{Ar} + C^6H), 6.78 (d, $^3J_{HH}$ = 2.7 Hz, C^7H), 4.23 (t, $^3J_{HH}$ = 6.1 Hz, C^4H_2), 3.62–3.56 (m, C^3H_2) ppm; ^{13}C NMR (151 MHz, DMSO-d_6): δ = 39.19 (C^3), 43.84 (C^4), 112.52 (C^7), 115.49 (d, $^2J_{CF}$ = 22.3 Hz, CH_{Ar}), 120.23 (C^8), 120.60 (d, $^3J_{CF}$ = 7.7 Hz, CH_{Ar}), 124.13 (C^6), 124.58 (C^{8a}), 135.98 (d, $^4J_{CF}$ = 1.8 Hz, C_{Ar}), 157.84 (d, $^1J_{CF}$ = 239.4 Hz, F−C_{Ar}), 160.14, 161.58 (C^1=O + O=C−NHAr) ppm; ^{19}F NMR (376 MHz, $CDCl_3$): δ = −120.1 (CF) ppm; MS (70 eV): *m*/*z* = 274 ([M + H]$^+$).

***N*-(2-Methoxyphenyl)-1-oxo-1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazine-8-carboxamide (8d, $C_{15}H_{15}N_3O_3$)** Yield: 0.16 g (44%); m.p.: 173–174 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.25 (s, NH−Ar), 8.65 (s, NH), 7.44 (s, 1H_{Ar}), 7.23 (t, $^3J_{HH}$ = 8.1 Hz, 1H_{Ar}), 7.16 (d, $^3J_{HH}$ = 2.8 Hz, C^6H), 7.11 (d, $^3J_{HH}$ = 8.0 Hz, 1H_{Ar}), 6.78 (d, $^3J_{HH}$ = 2.2 Hz, C^7H), 6.63 (d, $^3J_{HH}$ = 8.3 Hz, 1H_{Ar}), 4.22 (t, $^3J_{HH}$ = 6.0 Hz, C^4H_2), 3.75 (s, OCH_3), 3.61–3.56 (m, C^3H_2), ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 39.18 (C^3) 43.84 (C^4), 54.95 (OCH_3), 104.76, 108.46, 111.30, 112.55, 120.19 (C^8), 124.11, 124.73 (C^{8a}), 129.66, 140.74 (C_{Ar}−NH), 159.69 (C_{Ar}−OCH_3), 160.24, 161.58 (C^1=O + O=C−NHAr) ppm; MS (70 eV): *m*/*z* = 286 ([M + H]$^+$).

1-Oxo-*N*-[4-(trifluoromethyl)phenyl]-1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazine-8-carboxamide (8e, $C_{15}H_{12}F_3N_3O_2$) Yield: 0.29 g (71%); m.p.: 259–260 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.63 (s, NH−Ar), 8.71 (s, C^1−NH), 7.86 (d, $^3J_{HH}$ = 7.5 Hz, 2H_{Ar}), 7.70 (d, $^3J_{HH}$ = 7.6 Hz, 2H_{Ar}), 7.19 (s, C^6H), 6.80 (s, C^7H), 4.27–4.21 (m, C^4H_2), 3.63–3.58 (m, C^3H_2) ppm; ^{13}C NMR (151 MHz, DMSO-d_6): δ = 39.18 (C^3), 43.85 (C^4), 112.64 (C^7), 118.86 (2CH_{Ar}), 120.48 (C^8), 122.93 (q, $^2J_{CF}$ = 31.9 Hz, C_{Ar}−CF_3), 124.18 (C^{8a}), 124.31 (C^6), 124.45 (q, $^1J_{CF}$ = 271.3 Hz, CF_3), 126.24 (q, $^3J_{CF}$ = 3.4 Hz, 2CH_{Ar}), 143.13 (C_{Ar}), 160.70, 161.56 (C^1=O + O=C−NHAr) ppm; ^{19}F NMR (376 MHz, DMSO-d_6): δ = −60.7 (CF_3) ppm; MS (70 eV): *m*/*z* = 324 ([M + H]$^+$).

***N*-Butyl-3-methyl-1-oxo-1,2,3,4-tetrahydropyrrolo[1,2-*a*]-pyrazine-8-carboxamide (8f, $C_{13}H_{19}N_3O_2$)** Yield: 0.10 g (31%); m.p.: 194–195 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 10.40 (s, NH), 6.99 (d, $^3J_{HH}$ = 2.8 Hz, C^6H), 6.74 (d, $^3J_{HH}$ = 2.7 Hz, C^7H), 6.40 (s, C^1−NH), 4.14 (dd, $^2J_{HH}$ = 12.2, $^3J_{HH}$ = 4.0 Hz, C^4<u>H</u>H), 4.07–3.98 (m, C^3H), 3.85 (dd, $^2J_{HH}$ = 12.3, $^3J_{HH}$ = 9.8 Hz, C^4H<u>H</u>), 3.44–3.38 (m, NH−C<u>H</u>$_2$), 1.63–1.55 (m, C<u>H</u>$_2$−CH_2−CH_3), 1.47–1.35 (m, C<u>H</u>$_2$−CH_3 + C^3−CH_3), 0.93 (t, $^3J_{HH}$ = 7.3 Hz, CH_2−C<u>H</u>$_3$) ppm; ^{13}C NMR (151 MHz, $CDCl_3$): δ = 13.93 (<u>C</u>H_3−CH_2), 18.29 (C^3−CH_3), 20.39 (<u>C</u>H_2−CH_3), 31.74 (<u>C</u>H_2−CH_2−CH_3), 39.26 (<u>C</u>H_2−CH_2−CH_2−CH_3), 46.95 (C^3), 50.70 (C^4), 114.08 (C^7), 119.49 (C^8), 123.35 (C^6), 126.57 (C^{8a}), 162.03, 162.62 (C^1=O + O=C−NHBu) ppm; MS (70 eV): *m*/*z* = 250 ([M + H]$^+$).

***N*-(4-Chlorophenyl)-3-methyl-1-oxo-1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazine-8-carboxamide (8g, $C_{15}H_{14}ClN_3O_2$)** Yield: 0.29 g (74%); m.p.: 199–200 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.34 (s, NH−Ar), 8.63 (s, C^1−NH), 7.69 (d, $^3J_{HH}$ = 8.4 Hz, 2H_{Ar}), 7.38 (d, $^3J_{HH}$ = 8.4 Hz, 2H_{Ar}), 7.13 (d, $^3J_{HH}$ = 2.8 Hz, C^6H), 6.79 (d, $^3J_{HH}$ = 2.8 Hz, C^7H), 4.31 (d, $^3J_{HH}$ = 10.1 Hz, C^4H), 3.94–3.89 (m, C^3H + C^4H), 1.23 (d, $^3J_{HH}$ = 5.6 Hz, CH_3) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 17.75 (CH_3), 46.10 (C^3), 49.51 (C^4), 112.65 (C^7), 119.81 (C^8), 120.47 (2CH_{Ar}), 124.26 (C^6), 124.35 (C^{8a}), 126.46 (C_{Ar}), 128.85 (2CH_{Ar}), 138.49 (C_{Ar}), 160.30, 161.25 (C^1=O + O=C−NHAr) ppm; MS (70 eV): *m*/*z* = 304, 306 ([M + H]$^+$).

***N*,3-Dimethyl-1-oxo-1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazine-8-carboxamide (8h, $C_{10}H_{13}N_3O_2$)** Yield: 0.08 g (30%); m.p.: 206–207 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.56 (s, NH), 8.28 (s, C^1−NH), 7.01 (d, $^3J_{HH}$ = 2.7 Hz, C^6H), 6.66 (d, $^3J_{HH}$ = 2.9 Hz, C^7H), 4.28–4.21 (m, C^4H), 3.91–3.80 (m, C^3H + C^4H), 2.77 (d, $^3J_{HH}$ = 4.4 Hz, NH–CH_3), 1.20 (d, $^3J_{HH}$ = 5.8 Hz, C^3–CH_3) ppm; ^{13}C NMR (151 MHz, DMSO-d_6): δ = 17.77 (CH_3−C^3), 25.49 (CH_3−NH), 46.04 (C^3), 49.55 (C^4), 112.14 (C^7), 119.70 (C^8), 123.58 (C^6), 124.29 (C^{8a}), 160.89, 162.51 (C^1=O + O=C−$NHCH_3$) ppm; MS (70 eV): *m*/*z* = 208 ([M + H]$^+$).

3-Methyl-1-oxo-*N*-(2-propyl)-1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazine-8-carboxamide (8i, $C_{12}H_{17}N_3O_2$) Yield: 0.18 g (59%); m.p.: 154–155 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 10.33 (s, NH−*i*Pr), 7.00 (d, $^3J_{HH}$ = 2.6 Hz, C^6H), 6.74 (d, $^3J_{HH}$ = 2.7 Hz, C^7H), 5.99 (s, C^1−NH), 4.26–4.18 (m, C<u>H</u>−$(CH_3)_2$), 4.15 (dd, $^2J_{HH}$ = 12.2, $^3J_{HH}$ = 3.7 Hz, C^4<u>H</u>H), 4.06–3.98 (m, C^3H), 3.85 (dd, $^2J_{HH}$ = 12.2, $^3J_{HH}$ = 9.6 Hz, C^4H<u>H</u>), 1.37 (d, $^3J_{HH}$ = 6.4 Hz, C^3−CH_3), 1.25 (dd, $^3J_{HH}$ = 6.5, 4.5 Hz, 2CH_3) ppm; ^{13}C NMR (151 MHz, DMSO-d_6): δ = 17.83 (C^3−<u>C</u>H_3), 22.55, 22.60 (N−CH(<u>C</u>H_3)$_2$), 40.29 (N−<u>C</u>H$(CH_3)_2$), 46.01 (C^3), 49.55 (C^4), 112.15 (C^7), 119.63 (C^8), 123.51 (C^6), 124.75 (C^{8a}), 160.90, 160.93 (C^1=O + O=C−NH*i*Pr) ppm; MS (70 eV): *m*/*z* = 236 ([M + H]$^+$).

3-Methyl-1-oxo-*N*-[4-(trifluoromethyl)phenyl]-1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazine-8-carboxamide (8j, $C_{16}H_{14}F_3N_3O_2$) Yield: 0.34 g (78%); m.p.: 237–238 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.63 (s, NH−Ar), 8.75 (s, C^1−NH), 7.86 (d, $^3J_{HH}$ = 8.3 Hz, 2H_{Ar}), 7.70 (d, $^3J_{HH}$ = 8.6 Hz, 2H_{Ar}), 7.16 (d, $^3J_{HH}$ = 2.2 Hz, C^6H), 6.81 (d, $^3J_{HH}$ = 2.4 Hz, C^7H), 4.32 (d, $^3J_{HH}$ = 10.6 Hz, C^4H), 3.98–3.90 (m, C^3H + C^4H), 1.23 (d, $^3J_{HH}$ = 4.1 Hz, CH_3) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 17.71 (CH_3), 46.10 (C^3), 49.51 (C^4), 112.73 (C^7), 118.84 (2CH_{Ar}), 119.99 (C^8), 122.92 (q, $^2J_{CF}$ = 31.8 Hz), 124.10 (C^{8a}), 124.33 (C^6), 124.43 (q, $^1J_{CF}$ = 271.4 Hz), 126.23 (q, $^3J_{CF}$ = 4.2 Hz), 143.11 (C_{Ar}), 160.67, 161.24 (C^1=O + O=C−NAr) ppm; ^{19}F NMR (376 MHz, DMSO-d_6): δ = −60.9 (CF_3) ppm; MS (70 eV): *m*/*z* = 338 ([M + H]$^+$).

***N*-(4-Fluorophenyl)-3,3-dimethyl-1-oxo-1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazine-8-carboxamide (8k, $C_{16}H_{16}FN_3O_2$)** Yield: 0.26 g (68%); m.p.: 213–214 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.23 (s, NH − Ar), 8.62 (s, C^1NH), 7.67 (dd, $^3J_{HH}$ = 8.8, $^4J_{HF}$ = 5.0 Hz, 2H_{Ar}), 7.16 (dd, $^3J_{HH}$ = 8.7, $^3J_{HF}$ = 8.7 Hz, 2H_{Ar}), 7.11 (d, $^3J_{HH}$ = 2.7 Hz, C^6H), 6.80 (d, $^3J_{HH}$ = 2.8 Hz, C^7H), 4.09 (s, C^4H_2), 1.27 (s, $C^3(CH_3)_2$) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 25.66 (2CH_3), 51.81, 53.98 (C^3 + C^4), 112.65 (C^7), 115.48 (d, $^2J_{CF}$ = 22.1 Hz), 119.18 (C^8), 120.60 (d, $^3J_{CF}$ = 7.7 Hz), 124.33 (C^6), 124.52 (C^{8a}), 135.93 (d, $^4J_{CF}$ = 2.2 Hz), 157.83 (d, $^1J_{CF}$ = 239.4 Hz), 160.10, 160.66 (C^1=O + (O=C−NHAr) ppm; ^{19}F NMR (376 MHz, $CDCl_3$): δ = -120.1 (CF) ppm; MS (70 eV): *m*/*z* = 302 ([M + H]$^+$).

3,3-Dimethyl-*N*-(4-methylphenyl)-1-oxo-1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazine-8-carboxamide (8l, $C_{17}H_{19}N_3O_2$) Yield: 0.27 g (70%); m.p.: 258–259 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.06 (s, NH−Ar), 8.55 (s, C^1−NH), 7.55 (d, $^3J_{HH}$ = 8.1 Hz, 2H_{Ar}), 7.13 (d, $^3J_{HH}$ = 8.2 Hz, 2H_{Ar}), 7.09 (d, $^3J_{HH}$ = 2.5 Hz, C^6H), 6.79 (d, $^3J_{HH}$ = 2.7 Hz, C^7H), 4.08 (s, C^4H_2), 2.27 (s, CH_3−Ar), 1.27 (s, $C^3(CH_3)_2$) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 20.45 (CH_3−Ar), 25.68 (C^3(<u>C</u>H_3)$_2$), 51.80, 53.99 (C^3 + C^4), 112.64 (C^7), 118.95 (2CH_{Ar}), 119.06 (C^8), 124.26 (C^6), 124.81 (C^{8a}), 129.30 (2CH_{Ar}), 131.84 (C_{Ar}), 137.04 (C_{Ar}), 159.97, 160.66 (C^1=O + (O=C−NHAr) ppm; MS (70 eV): *m*/*z* = 298 ([M + H]$^+$).

***N*-(2-Methoxyphenyl)-3,3-dimethyl-1-oxo-1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazine-8-carboxamide (8m, $C_{17}H_{19}N_3O_3$)** Yield: 0.22 g (54%); m.p.: 177–178 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.26 (s, NH−Ar), 8.74 (s, C^1NH), 7.43 (s, 1H_{Ar}), 7.23 (t, $^3J_{HH}$ = 7.8 Hz, 1H_{Ar}), 7.13–7.10 (m, C^6H + 1H_{Ar}), 6.79 (d, $^3J_{HH}$ = 2.3 Hz, C^7H), 6.63 (d, $^3J_{HH}$ = 8.2 Hz, 1H_{Ar}), 4.09 (s, C^4H_2), 3.74 (s, OCH_3), 1.26 (s, $C^3(CH_3)_2$) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 25.67 (C^3(<u>C</u>H_3)$_2$, 51.80, 53.98 (C^3 + C^4), 54.92 (OCH_3), 104.77, 108.47, 111.28, 112.70, 119.15 (C^8), 124.34, 124.69 (C^{8a}), 129.68, 140.72 (C_{Ar}−NH), 159.68(C_{Ar}−OCH_3), 160.21, 160.68 (C^1=O + O=C−NHAr) ppm; MS (70 eV): *m*/*z* = 314 ([M + H]$^+$).

3,3-Dimethyl-1-oxo-*N*-(2-propyl)-1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazine-8-carboxamide (8n, $C_{13}H_{19}N_3O_2$) Yield: 0.20 g (64%); m.p.: 201–202 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.73 (d, $^3J_{HH}$ = 6.9 Hz, N<u>H</u>−CH), 8.34 (s, C^1 − NH), 7.02 (d, $^3J_{HH}$ = 2.7 Hz, C^6H), 6.65 (d, $^3J_{HH}$ = 2.5 Hz, C^7H), 4.01 (s, C^4H_2), 3.98–3.90 (m, C<u>H</u>−$(CH_3)_2$), 1.22 (s, C^3−$(CH_3)_2$),

1.13 (d, $^{3}J_{HH}$ = 6.6 Hz, CH−(C<u>H</u>$_3$)$_2$) ppm; ^{13}C NMR (151 MHz, DMSO-d_6): δ = 22.57 (CH−(<u>C</u>H$_3$)$_2$), 25.72 (C^{3}−(<u>C</u>H$_3$)$_2$), 40.32 (<u>C</u>H−(CH$_3$)$_2$), 51.59 (C^{3}), 54.03 (C^{4}), 112.19 (C^{7}), 119.06 (C^{8}), 123.68 (C^{6}), 124.77 (C^{8a}), 160.33, 160.90 (C^{1}=O + O=C−N*i*Pr) ppm; MS (70 eV): *m*/*z* = 250 ([M + H]$^+$).

3,3-Dimethyl-1-oxo-*N*-[4-(trifluoromethyl)phenyl]-1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazine-8-carboxamide (8o, $C_{17}H_{16}F_3N_3O_2$) Yield: 0.34 g (75%); m.p.: 228–229 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.64 (s, NH−Ar), 8.79 (s, C^{1}−NH), 7.86 (d, $^{3}J_{HH}$ = 8.1 Hz, 2H$_{Ar}$), 7.70 (d, $^{3}J_{HH}$ = 8.3 Hz, 2H$_{Ar}$), 7.15 (d, $^{3}J_{HH}$ = 2.7 Hz, C^{6}H), 6.81 (d, $^{3}J_{HH}$ = 2.7 Hz, C^{7}H), 4.10 (s, C^{4}H$_2$), 1.26 (s, C^{3}(CH$_3$)$_2$) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 25.66 (2CH$_3$), 51.87, 53.97 (C^{3} + C^{4}), 112.79 (C^{7}), 118.85 (2CH$_{Ar}$), 119.42 (C^{8}), 122.93 (q, $^{2}J_{CF}$ = 31.2 Hz), 124.14 (C^{8a}), 124.42 (q, $^{1}J_{CF}$ = 270.8 Hz), 124.52 (C^{6}), 126.24 (q, $^{3}J_{CF}$ = 4.5 Hz), 143.09 (C$_{Ar}$), 160.63, 160.65 (C^{1}=O + O=C−NAr) ppm; ^{19}F NMR (376 MHz, DMSO-d_6): δ = −60.7 (CF$_3$) ppm; MS (70 eV): *m*/*z* = 352 ([M + H]$^+$).

***trans*-*N*-Benzyl-4-oxo-4,5,5a,6,7,8,9,9a-octahydropyrrolo[1,2-*a*]quinoxaline-3-carboxamide (8p, $C_{19}H_{21}N_3O_2$)** Yield: 0.17 g (40%); m.p.: 194–195 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.30 (t, $^{3}J_{HH}$ = 5.0 Hz, N<u>H</u>–Bn), 8.49 (s, C^{4}−NH), 7.34–7.29 (m, 4H$_{Ar}$), 7.26–7.21 (m, 1H$_{Ar}$), 7.18 (d, $^{3}J_{HH}$ = 2.5 Hz, C^{1}H), 6.72 (d, $^{3}J_{HH}$ = 2.5 Hz, C^{2}H), 4.53–4.43 (m, C<u>H</u>$_2$–Ph), 3.80–3.73 (m, C^{9a}H), 3.43–3.34 (m, C^{5a}H), 2.58 (d, $^{3}J_{HH}$ = 9.4, 1H), 1.99 (d, $^{3}J_{HH}$ = 10.7 Hz, 1H), 1.84 (d, $^{3}J_{HH}$ = 7.7 Hz, 1H), 1.74 (d, $^{3}J_{HH}$ = 10.2 Hz, 1H), 1.50–1.29 (m, 4H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 22.95, 23.30 (C^{7} + C^{8}), 26.97, 28.93 (C^{6} + C^{9}), 42.35 (CH$_2$−Bn), 54.99 (C^{5a}), 57.69 (C^{9a}), 112.50 (C^{2}), 119.97 (C^{1}), 120.86 (C^{3}), 124.76 (C^{3a}), 126.71 (C$_{Ar}$), 127.14 (2C$_{Ar}$), 128.34 (2C$_{Ar}$), 139.56 (C$_{Ar}$), 161.15, 162.06 (C^{4}=O + O=C−NHBn) ppm; MS (70 eV): *m*/*z* = 324 ([M + H]$^+$).

***trans*-*N*-(4-Chlorophenyl)-4-oxo-4,5,5a,6,7,8,9,9a-octahydropyrrolo[1,2-*a*]quinoxaline-3-carboxamide (8q, $C_{18}H_{18}ClN_3O_2$)** Yield: 0.33 g (74%); m.p.: > 300 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.45 (s, NH−Ar), 8.78 (s, C^{4}−NH), 7.69 (d, $^{3}J_{HH}$ = 8.8 Hz, 2H$_{Ar}$), 7.39 (d, $^{3}J_{HH}$ = 8.7 Hz, 2H$_{Ar}$), 7.25 (d, $^{3}J_{HH}$ = 2.9 Hz, C^{1}H), 6.81 (d, $^{3}J_{HH}$ = 2.8 Hz, C^{2}H), 3.83 (td, $^{3}J_{HH}$ = 10.4 Hz, $^{3}J_{HH}$ = 3.5 Hz, C^{9a}H), 3.45 (td, $^{3}J_{HH}$ = 11.2 Hz, $^{3}J_{HH}$ = 3.5 Hz, C^{5a}H), 2.60 (d, $^{3}J_{HH}$ = 9.0 Hz, 1H), 2.03 (d, $^{3}J_{HH}$ = 12.1 Hz, 1H), 1.85 (d, $^{3}J_{HH}$ = 10.0 Hz, 1H), 1.75 (d, $^{3}J_{HH}$ = 11.3 Hz, 1H), 1.52–1.30 (m, 4H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 22.95, 23.30 (C^{7} + C^{8}), 26.92, 28.90 (C^{6} + C^{9}), 54.99 (C^{5a}), 57.74 (C^{9a}), 112.78 (C^{2}), 120.52 (2CH$_{Ar}$), 120.57 (C^{1}), 120.78 (C^{3}), 125.00 (C^{3a}), 126.50 (C$_{Ar}$), 128.89 (2CH$_{Ar}$), 138.50(C$_{Ar}$), 160.41, 161.49 (C^{4}=O + O=<u>C</u>−NHAr) ppm; MS (70 eV): *m*/*z* = 344, 346 ([M + H]$^+$).

***trans*-*N*-Methyl-4-oxo-4,5,5a,6,7,8,9,9a-octahydropyrrolo[1,2-*a*]quinoxaline-3-carboxamide (8r, $C_{13}H_{17}N_3O_2$)** Yield: 0.10 g (32%); m.p.: 247–248 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.62 (s, NH), 8.33 (s, C^{4}−NH), 7.12 (d, $^{3}J_{HH}$ = 2.8 Hz, C^{1}H), 6.69 (d, $^{3}J_{HH}$ = 2.8 Hz, C^{2}H), 3.75 (td, $^{3}J_{HH}$ = 10.8, $^{3}J_{HH}$ = 4.2 Hz, C^{9a}H), 3.37 (td, $^{3}J_{HH}$ = 11.1, $^{3}J_{HH}$ = 4.0 Hz, C^{5a}H), 2.77 (d, $^{3}J_{HH}$ = 4.5 Hz, CH$_3$), 2.57 (d, $^{3}J_{HH}$ = 7.0 Hz, 1H), 2.02 (d, $^{3}J_{HH}$ = 8.2 Hz, 1H), 1.85 (d, $^{3}J_{HH}$ = 6.9 Hz, 1H), 1.75 (d, $^{3}J_{HH}$ = 10.9 Hz, 1H), 1.53–1.30 (m, 4H) ppm; ^{13}C NMR (151 MHz, DMSO-d_6): δ = 22.92, 23.29 (C^{7} + C^{8}), 25.51 (CH$_3$), 26.94, 28.91 (C^{6} + C^{9}), 54.99 (C^{5a}), 57.65 (C^{9a}), 112.26 (C^{2}), 119.79 (C^{1}), 120.67, 124.94 (C^{3} + C^{3a}), 161.08, 162.59 (C^{4}=O + O=<u>C</u>−NHMe) ppm; MS (70 eV): *m*/*z* = 248 ([M + H]$^+$).

***trans*-*N*-(4-Methylphenyl)-4-oxo-4,5,5a,6,7,8,9,9a-octahydropyrrolo[1,2-*a*]quinoxaline-3-carboxamide (8s, $C_{19}H_{21}N_3O_2$)** Yield: 0.28 g (69%); m.p.: > 300 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.20 (s, NH − Ar), 8.72 (s, C^{4}−NH), 7.55 (d, $^{3}J_{HH}$ = 8.0 Hz, 2H$_{Ar}$), 7.24 (s, C^{1}H), 7.13 (d, $^{3}J_{HH}$ = 8.0 Hz, 2H$_{Ar}$), 6.79 (s, C^{2}H), 3.88–3.77 (m, 1H), 3.50–3.40 (m, 1H), 2.61–2.59 (m, 1H), 2.26 (s, CH$_3$), 2.04–2.01 (m, 1H), 1.86–1.84 (m, 1H), 1.77–1.74 (m, 1H), 1.53–1.33 (m, 4H) ppm; ^{13}C NMR (151 MHz, DMSO-d_6): δ = 20.45 (CH$_3$−Ar), 22.92, 23.28 (C^{7} + C^{8}), 26.91, 28.90 (C^{6} + C^{9}), 54.97 (C^{5a}), 57.71 (C^{9a}), 112.71 (C^{2}), 118.96 (2CH$_{Ar}$), 120.34 (C^{1}), 120.59 (C^{3}), 125.44 (C^{3a}), 129.29 (2CH$_{Ar}$), 131.84 (C$_{Ar}$), 137.07 (C$_{Ar}$), 160.05, 161.48 (C^{4}=O + O=C−NHAr) ppm; MS (70 eV): *m*/*z* = 324 ([M + H]$^+$).

***trans*-4-Oxo-*N*-[4-(trifluoromethyl)phenyl]-4,5,5a,6,7,8,9,9a-octahydropyrrolo[1,2-*a*]quinoxaline-3-carboxamide (8t, $C_{19}H_{18}F_3N_3O_2$)** Yield: 0.35 g (73%); m.p.: > 300 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.70 (s, NH−Ar), 8.83 (s, NH), 7.86 (d, $^{3}J_{HH}$ = 8.4 Hz, 2H$_{Ar}$), 7.70 (d, $^{3}J_{HH}$ = 8.4 Hz, 2H$_{Ar}$), 7.28 (d, $^{3}J_{HH}$ = 2.9 Hz, C^{1}H), 6.83 (d, $^{3}J_{HH}$ = 2.8 Hz, C^{2}H), 3.85 (td, $^{3}J_{HH}$ = 10.9 Hz, $^{3}J_{HH}$ = 3.0 Hz, C^{9a}H), 3.47 (td, $^{3}J_{HH}$ = 11.4 Hz, $^{3}J_{HH}$ = 2.8 Hz, C^{5a}H), 2.61 (d, $^{3}J_{HH}$ = 8.3 Hz, 1H), 2.04 (d, $^{3}J_{HH}$ = 10.4 Hz, 1H), 1.86 (d, $^{3}J_{HH}$ = 8.8 Hz, 1H), 1.76 (d, $^{3}J_{HH}$ = 11.6 Hz, 1H), 1.54–1.31 (m, 4H) ppm; ^{13}C NMR (76 MHz, DMSO-d_6): δ = 22.84, 23.19 (C^{7} + C^{8}), 26.83, 28.81 (C^{6} + C^{9}), 54.94 (C^{5a}), 57.70 (C^{9a}), 112.76 (C^{2}), 118.82 (2CH$_{Ar}$), 120.47 (C^{1}), 120.88 (C^{3}), 122.89 (q, $^{2}J_{CF}$ = 31.8 Hz), 124.33 (q, $^{1}J_{CF}$ = 271.1 Hz), 124.69 (C^{3a}), 126.15 (2CH$_{Ar}$), 143.03 (C$_{Ar}$), 160.67, 161.39 (C^{4}=O + O=C−NHAr) ppm; ^{19}F NMR (376 MHz, DMSO-d_6): δ = −60.7 (CF$_3$) ppm; MS (70 eV): *m*/*z* = 378 ([M + H]$^+$).

Study of antimicrobial activity

The antimicrobial activity of the synthesized compounds was investigated by the method of nutrient broth microdilution as recommended by EUCAST (European Committee on antimicrobial susceptibility testing) [52]. According to this method, the minimal inhibitory concentration (MIC) was determined as the concentration of every synthesized compound required to suppress the proliferation of the given microbial culture in the multihole microplate. The stock 1000 μg/cm^3 solution was prepared by dissolving the required amount of a compound in dimethylsulfoxide (DMSO). Further, diluted solutions with the concentrations from 500 to 3.9 μg/cm^3 (or from 500 to 0.48 μg/cm^3 in the case of controls) were used to find the MIC values. The sensitivity of every microbial culture to every concentration of the synthesized compounds was tested three times. In addition, the control experiments were carried out to check the proliferation of microbes in the clean broth, in the same broth with an admixture of DMSO, and in the broth with DMSO and the control dugs (decasan and clotrimazole) (Tables 3, 4). The control clear broth remained sterile and transparent (no proliferation of the microbial cultures), while some proliferation of the cultures has been registered in the case of a mixture of DMSO and the broth.

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Data availability We declare that the data supporting the findings of this study are available within the paper and its Supplementary Information files. Should any raw data files be needed in another format, they are available from the corresponding author upon reasonable request.

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