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Synthesis and *in vitro* evaluation of 4-trichloromethylpyrrolo[1,2-*a*]quinoxalines as new antiplasmodial agents.

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Abstract:

Thanks to a preliminary *in vitro* screening of several CCl₃-substituted-nitrogen containing heterocycles belonging to our chemical library, the 2-trichloromethylquinoxaline scaffold appeared to be of potential interest for developing new antiplasmodial agents. Then, combining these experimental results to the antimalarial properties reported for various pyrrolo[1,2-*a*]quinoxaline derivatives, an original series of fifteen 7-substituted-4-trichloromethylpyrrolo[1,2-*a*]quinoxalines was synthesized in a 4 to 5 reaction steps pathway. All molecules were evaluated *in vitro* toward both their antiplasmodial activity on the K1 multi-resistant *P. falciparum* strain and their cytotoxicity on the HepG2 human cell line.

Thus, 3 hit molecules were identified, displaying IC₅₀ values in the micromolar range and low cytotoxicity values, reaching good selectivity indexes, in comparison with the reference drugs chloroquine and doxycycline. Structure-activity relationship studies showed that the pyrrolo[1,2-*a*]quinoxaline scaffold can support selective antiplasmodial activity when substituted at position 4 by a CCl₃ group. However, substitution at position 7 of the same scaffold is neither beneficial for cytotoxicity nor favorable for the solubility in the biological media.

Keywords: *Plasmodium falciparum*; Pyrrolo[1,2-*a*]quinoxaline; Trichloromethyl group; *In vitro* antiplasmodial activity; *In vitro* HepG2 cytotoxicity, SARs.

1. Introduction

Nowadays, malaria still remains one of the most prevailing and lethal infectious disease worldwide. The protozoa parasite responsible for this infection belongs to the *Plasmodium* genus and is transmitted to humans by the bite of the infected *Anopheles* mosquito female. Among the five *Plasmodium* species responsible for malaria, the most lethal is *P. falciparum*, causing cerebral malaria. According to the WHO, in 2012, malaria affected about 207 million people in the inter-tropical area and caused an estimated 627,000 deaths [1]. Artemisinin-based combination therapies (ACTs) are recommended by the WHO as the first-line treatment for treating malaria caused by *P. falciparum*. Unfortunately, parasite resistance to artemisinin derivatives has now been detected in 4 countries of the Greater Mekong sub-region: Cambodia, Myanmar, Thailand and Vietnam [2-6]. This emerging resistance of *P. falciparum* strains is a serious problem for the containment and the treatment of this disease as it could lead to a resurgence of more virulent levels of malaria, unless new chemical entities, displaying novel mechanisms of action, be rapidly found [7-10].

Aiming at synthesizing original molecules displaying antiparasitic properties [11-15], our research group previously reported promising results in 4-substituted-2-trichloromethylquinazoline series, showing that the trichloromethyl group was crucial for the *in vitro* activity against *Plasmodium falciparum* [16-20]. These results prompted us to investigate wider the anti-infectious potential of various aromatic nitrogen-containing

heterocyclic scaffolds bearing a trichloromethyl group, in a view to identify new antiplasmodial candidates.

2. Results and discussion

2.1. Preliminary antiplasmodial screening of various trichloromethylated heterocyclic scaffolds

An antiplasmodial screening was first made from a series of closely related compounds corresponding to aromatic 6-membered bicyclic nitrogen-containing heterocycles bearing a CCl_3 group. These compounds were selected in our internal chemical library, part of the French National Chemical Library (Chimiothèque Nationale). Thus, quinoline (series **1**), isoquinoline (series **2**), quinazoline (series **3** and **4**) and quinoxaline (series **5**) derivatives (**Figure 1**) were evaluated *in vitro* toward both their antiplasmodial activity against the K1 multi-resistant *P. falciparum* strain (determination of the IC_{50} =inhibitory concentration 50%) and their cytotoxicity (determination of the CC_{50} = cytotoxic concentration 50%) on the HepG2 human cell line.

<Insert Figure 1.>

Molecules **1a**, **2a**, **4a** and **5a** were prepared from their corresponding methylated precursors (**1b**, **2b** and **5b**, purchased from Sigma-Aldrich) by a microwave-assisted chlorination reaction using PCl_5 and POCl_3 [21, 22]. Molecule **4b** was prepared as described by Liu and co-workers by reacting acetamidine hydrochloride with 2-bromobenzylamine in the presence of CuBr as catalyst [23]. Compound **3a** was obtained by using an alternative route which was previously developed in our lab [24,25]. Thus, 4-chloroquinazoline was reacted with bromotrichloromethane in the presence of tetrakis(dimethylamino)ethylene (TDAE) instead of using hazardous chlorine gas, as described in the literature [26]. Then, all these molecules were tested *in vitro* and compared with two commercial reference-drugs: chloroquine and doxycycline. For all tested compounds, the corresponding selectivity indexes (SI) were calculated. The results are presented in **Table 1**.

<Insert Table 1.>

Among all tested compounds, only the quinoline, isoquinoline or quinoxaline derivatives bearing a CCl_3 group showed some potential *in vitro* antiplasmodial activity (respectively

compounds **1a**, **2a** and **5a**), in comparison with chloroquine or doxycycline. The quinazoline derivatives including a CCl₃ group at position 2 or 4 (compounds **4a** and **3a**) did not display significant activity. These last results indicated that the CCl₃ group was not sufficient alone for providing antiplasmodial activity in quinazoline series and suggested that the position of the CCl₃ group on the quinazoline ring could also play a key-role toward biological activity. Moreover, when comparing the activities of the CCl₃ containing compounds to their CH₃ analogs, in all tested series, it clearly appeared that the CCl₃ group was involved in the antiplasmodial activity, in accordance with the experimental results which we previously obtained in 4-substituted quinazoline series [16-19].

Although displaying antiplasmodial activity, quinoline **1a** and isoquinoline **2a** did not appear to be the most potent compounds because of their rather high IC₅₀ values (respectively 8.5 and 8 μM), in comparison with quinoxaline **5a** (IC₅₀ = 1.5 μM) and reference-drugs chloroquine (IC₅₀ = 0.6 μM) and doxycycline (IC₅₀ = 6.0 μM). Nevertheless, **5a** showed a significant *in vitro* cytotoxicity (CC₅₀ = 3.1 μM) in comparison with doxorubicine (CC₅₀ = 0.2 μM), making this compound poorly selective and unsuitable for further development. In order to validate the SARs of **5a**, in addition to its methylated precursor **5b**, three closely related analogs (**5c**, **5d** and **5e**) were also tested (**Figure 2**). Compound **5c** was prepared by a chlorination reaction of **5b** using PCl₅ and POCl₃, under milder conditions than those used to prepare **5a**, while compounds **5d** and **5e** were purchased from Aldrich.

<Insert Figure 2.>

The comparison of the results noted for the tested quinoxaline derivatives **5a-e** indicated that the quinoxaline ring, itself, did not show any antiplasmodial potential (IC₅₀ of **5e** > 50 μM) and that the CCl₃ group was the only one that could confer antiplasmodial potential to the studied series, when located at position 2 of the quinoxaline ring (**Table 1**).

Then, trying to reach a new 2-trichloromethylquinoxaline derivative presenting an improved antiplasmodial profile, we synthesized compound **5f** in a view to compare its biological profile to the one of its antiplasmodial quinazoline position isomer which we previously reported [16] (**Figure 3**). **5f** was prepared from its corresponding methylated precursor which was synthesized according to the literature [27] by a microwave-assisted chlorination reaction using PCl₅ and POCl₃.

<Insert Figure 3.>

Indeed, quinoxaline **5f** appeared to be much more selective than **5a** (SI = 17.5 *versus* 2.1, respectively), presenting a quite low antiplasmodial IC₅₀ value (0.2 μM), better than the one of its quinazoline position isomer (IC₅₀ = 8 μM). However, quinoxaline **5f** remained too cytotoxic (CC₅₀ = 3.5 μM) for allowing further investigations.

Such interesting result noted with compound **5f** prompted us to carry on the investigation of the antiplasmodial potential of the 2-trichloromethylquinoxaline scaffold, especially when considering that various pyrrolo[1,2-*a*]quinoxaline derivatives (**Figure 4**) had been reported in the literature as quite promising antimalarial compounds [28-31].

<Insert Figure 4.>

Taking advantage of the synthetic know-how of our research teams in the fields of both trichloromethylated heterocycles [15-21] and pyrrolo[1,2-*a*]quinoxalines [32] preparation, we studied the synthesis and antiplasmodial properties of a series of original molecules, based on the quinoxaline ring. These molecules, corresponding to 4-(trichloromethyl)pyrrolo[1,2-*a*]quinoxaline derivatives, were a combination between the pyrrolo[1,2-*a*]quinoxaline scaffold and the trichloromethyl group (**Figure 5**), aiming at identifying new antiplasmodial quinoxalines with improved biological profile, being less cytotoxic than **5f**.

<Insert Figure 5.>

2.1. Synthesis of the 4-(trichloromethyl)pyrrolo[1,2-*a*]quinoxaline series

The 7-substituted-4-(trichloromethyl)pyrrolo[1,2-*a*]quinoxalines derivatives were synthesized from various commercially available 4-substituted-2-nitroanilines **6a-i** (**Scheme 1**). The Clauson-Kaas reaction between anilines and 2,5-dimethoxytetrahydrofuran (2,5-DMTHF), in refluxing glacial AcOH, provided the pyrrolic derivatives **7a-i** in 63-86% yields. The nitro group was then reduced, either by BiCl₃/NaBH₄ or tin (II) chloride in refluxing ethanol, affording the expected 1-(2-amino-4-substituted-phenyl)pyrroles **8a-i** in moderate to good yields (47-90%). Subsequent acylation of the aniline moiety with trichloroacetyl chloride furnished the corresponding trichloroacetamides **9a-i** in 46-88% yields. These final intermediates were cyclized under Bischler-Napieralski conditions, leading to the expected 7-substituted-4-(trichloromethyl)pyrrolo[1,2-*a*]quinoxalines **10a-i** in 20-69% yields. The

carboxylic acid derivative **10j** was prepared in 44% yield from methylic ester **10i** by an acidic hydrolysis in concentrated HCl. Amidoxime **10k** was obtained in 74% yield by reacting nitrile derivative **10h** with hydroxylamine hydrochloride in a refluxing EtOH/H₂O mixture. Compounds **11a**, **11f** and **11h** were obtained, respectively, by reducing the trichloromethyl group of **10a**, **10f** and **10h** with iron in refluxing glacial AcOH. Compound **12a** [33] was prepared by trifluoroacetylation of aniline **8a** in the presence of trifluoroacetic acid, trichloroacetonitrile and triphenylphosphine [34] followed by a Bischler-Napieralski cyclisation.

<Insert Scheme1.>

2.2. Biological evaluation of the 7-substituted-4-(trichloromethyl)pyrrolo[1,2-*a*]quinoxaline derivatives

Thus, a series of fifteen 4-trichloromethylpyrrolo[1,2-*a*]quinoxaline derivatives was then tested *in vitro* for both its *in vitro* cytotoxicity (human HepG2 cell line) and its antiplasmodial activity (K1 *P. falciparum* multi-resistant strain). The results (**Table 2**) were compared to the ones obtained with chloroquine and doxycycline, chosen as antimalarial reference-drugs and doxorubicin, chosen as a reference cytotoxic agent.

<Insert Table2.>

Among the eleven tested compounds bearing a trichloromethyl group, seven exerted a significant antiplasmodial activity in comparison with the 2 antimalarial drug references, presenting IC₅₀ values in the micromolar range (1.2 to 2.4 μM). Out of these seven hit compounds, four molecules displayed a limited cytotoxicity toward the HepG2 human cell line, with CC₅₀ values ranging from >15 to 53 μM, in comparison with reference-drug doxorubicin (CC₅₀ = 0.2 μM). Thus, three of these molecules reached a SI superior to 10 μM: **10a**, **10f** and **10h**. Indeed, hit compound **10a** demonstrated the same antiplasmodial activity as starting compound **5a** (IC₅₀ = 1.5 μM) but showed a greatly improved cytotoxicity (CC₅₀ = 41.2 μM), making its selectivity (SI = 27.5) intermediate between the one of chloroquine (SI = 50) and the one of doxycycline (SI = 3.3). It could then be concluded that the attachment of a pyrrole moiety to the quinoxaline ring clearly improved the antiplasmodial profile of the 2-trichloromethylquinoxaline scaffold. Due to a lack of solubility in the biological media, compounds **10d** and **10i** could not be tested.

Concerning the influence of the nature of the substituents at position 7 of the pyrrolo[1,2-*a*]quinoxaline scaffold, it appeared that halogen substituents have a negative influence on the cytotoxicity profile without improving the antiplasmodial activity. Moreover, the carboxylic acid derivative **10j** was not active. Globally, no substituent at position 7 of the chemical scaffold was required for providing antiplasmodial activity; some substituents (bromine, iodine, methyl carboxylate or hydroxyamidine) could even play a negative role toward either cytotoxicity or solubility in the biological media.

In order to study the influence of the trichloromethyl group at position 4 of the pyrroloquinoxaline scaffold, 4-methylsubstituted analogs **11a**, **11f** and **11h** of respective hit compounds **10a**, **10f** and **10h** were prepared and evaluated. The results clearly showed that these 4-methylpyrrolo[1,2-*a*]quinoxaline analogs do not show any activity against *P. falciparum*. Moreover, the 4-CF₃ substituted analog **12a** of hit compound **10a** was not active neither, demonstrating that the CCl₃ group is very specifically required for providing antiplasmodial activity in 4-trichloromethylpyrrolo[1,2-*a*]quinoxaline series.

Conclusion

From a preliminary screening, highlighting that the 2-trichloromethylquinoxaline scaffold displayed antiplasmodial potential, a new series of 7-substituted-4-trichloromethylpyrrolo[1,2-*a*]quinoxaline derivatives was synthesized in four to five reaction steps. Thus, three hit compounds were identified, presenting *in vitro* IC₅₀ values in the micromolar range ($1.5 \leq K1 P. falciparum IC_{50} \leq 2.4 \mu M$), good cytotoxicity profiles ($17 \leq HepG2CC_{50} \leq 53 \mu M$) and promising selectivity indexes ($11.3 \leq SI \leq 27.5$), in comparison with the antimalarial drug references chloroquine and doxycycline. Structure-activity relationships showed that the CCl₃ group was mandatory for providing antiplasmodial activity to the pyrrolo[1,2-*a*]quinoxaline scaffold of the studied series. They also pointed out that the attachment of a pyrrole moiety to the quinoxaline ring improved the cytotoxicity profile without disturbing the anti-infectious activity. It finally showed that the substitution of the 4-trichloromethylpyrrolo[1,2-*a*]quinoxaline scaffold at position 7 was neither necessary nor beneficial, as regards of both cytotoxicity or solubility in the biological media. Complementary pharmacomodulation works will be carried out to study the influence of the substitution of the same scaffold at other positions toward antiplasmodial activity.

3. Experimental

3.1. Chemistry

Commercial reagents were used as received without additional purification. Melting points were determined on a Kofler bench and are uncorrected. Elemental analysis and HRMS were carried out at the Spectropole, Faculté des Sciences et Techniques de Saint-Jérôme, Marseille, France. NMR spectra were recorded on a Bruker ARX 200 spectrometer at the Faculté de Pharmacie de Marseille ($^1\text{H-NMR}$: 200 MHz, $^{13}\text{C-NMR}$: 50 MHz) or on a JEOL Lambda 400 Spectrometer at the Centre d'Etudes et de Recherche sur le Médicament de Normandie ($^1\text{H-NMR}$: 400 MHz, $^{13}\text{C-NMR}$: 100 MHz). NMR references were the following: ^1H : CHCl_3 δ = 7.26, $\text{DMSO-}d_6$ δ = 2.50 and ^{13}C : CHCl_3 δ = 76.9, $\text{DMSO-}d_6$ δ = 39.5. Solvents were dried by conventional methods. The following adsorbent was used for column chromatography: silica gel 60 (Merck, particle size 0.063–0.200 mm, 70–230 mesh ASTM). TLC was performed on 5 cm \times 10 cm aluminium plates coated with silica gel 60F-254 (Merck) in an appropriate eluent. Visualization was made with ultraviolet light (234 nm). HRMS spectra were recorded on QStar Elite (Applied Biosystems SCIEX) spectrometer. PEG was the matrix for HRMS. The experimental exact mass was given for the ion which has the maximum isotopic abundance. Purity of synthesized compounds was checked with LC-MS analyses which were realized at the Faculté de Pharmacie de Marseille with a Thermo Scientific Accela High Speed LC System[®] coupled with a single quadrupole mass spectrometer Thermo MSQ Plus[®]. The RP-HPLC column used is a Thermo Hypersil Gold[®] 50 \times 2.1 mm (C18 bounded), with particles of 1.9 μm diameter. The volume of sample injected on the column was 1 μL . The chromatographic analysis, total duration of 8 min, is made with the gradient of following solvents: t = 0 min, water/methanol 50/50; $0 < t < 4$ min, linear increase in the proportion of water to a ratio water/methanol 95/5; $4 < t < 6$ min, water/methanol 95/5; $6 < t < 7$ min, linear decrease in the proportion of water to return to a ratio 50/50 water/methanol; $6 < t < 7$ min, water/methanol 50/50. The water used was buffered with 5 mM ammonium acetate. The retention times (t_{R}) of the molecules analyzed are indicated in min. The preparation of compounds **1a** [21, 35], **3a** [24, 26], **4b** [22], **5a** [21], **11a** [37,38] and **12a** [33] was achieved as described in the literature. Compounds **1b**, **2b**, **5b**, **5d** and **5e** were purchased from Sigma-Aldrich.

3.1.1. 1-(Trichloromethyl)isoquinoline (2a)

A 100-mL round-bottomed flask equipped with a drying tube-condenser and a magnetic stir bar was charged with 1-methylisoquinoline (500 mg, 3.49 mmol), PCl_5 (4.40 g, 20.94 mmol) and a minimal amount of POCl_3 to allow the stirring of the mixture. The reaction mixture was heated during 1 h under microwave heating (800 W, 110 $^\circ\text{C}$). After the reaction mixture was

cooled with an ice bath, it was carefully poured into 50 mL of ice and was made alkaline (pH 8) by addition of NaHCO₃. Aqueous layer was extracted three times with CH₂Cl₂, and then dried with anhydrous sodium sulphate and finally concentrated under vacuum. Purification by chromatography on silica gel (Petroleum Ether/ CH₂Cl₂) gave the expected 1-(trichloromethyl)isoquinoline.

Yield 24%. Yellow powder. Mp 66°C. ¹H NMR (200 MHz, CDCl₃) δ = 8.88 (dd, *J* = 1.8; 7.4 Hz, 1H, H-3), 8.53 (d, *J* = 5.4 Hz, 1H, H-8), 7.94 (dd, *J* = 2.2; 7.2 Hz, 1H, H-5), 7.67-7.80 (m, 3H, H-4, H-6 and H-7). ¹³C NMR (50 MHz, CDCl₃) δ = 153.9, 139.0, 138.1, 130.3, 127.9, 127.4, 127.1, 124.3, 123.3, 98.2. Anal. Calcd for C₁₀H₆Cl₃N: C, 48.72; H, 2.45; N, 5.68. Found: C, 48.32; H, 2.41; N, 5.64.

3.1.2. 2-(Trichloromethyl)quinazoline (4a)

A 100-mL round-bottomed flask equipped with a drying tube-condenser and a magnetic stir bar was charged with 2-methylquinazoline [23] (200 mg, 1.4 mmol), PCl₅ (870 mg, 4.2 mmol) and a minimal amount of POCl₃ to allow the stirring of the mixture. The reaction mixture was heated during 15 min under microwave irradiation (800 W, 150 °C). After the reaction mixture was cooled with an ice bath, it was carefully poured into 50 mL of ice and was made alkaline (pH 8) by addition of NaOH. Aqueous layer was extracted twice with CH₂Cl₂, the organic layers were washed twice with brine and then dried with anhydrous sodium sulphate and finally concentrated under vacuum. Purification by chromatography on silica gel (Petroleum Ether/ CH₂Cl₂) gave the expected 2-(trichloromethyl)quinazoline.

Yield 30%. Off-white powder. Mp 116°C. ¹H NMR (200 MHz, CDCl₃) δ = 9.57 (s, 1H, H-4), 8.22 (d, *J* = 9.1 Hz, 1H, H-8), 8.01-8.08 (m, 2H, H-5 and H-7), 7.81 (dt, *J* = 1.2; 6.7 Hz, 1H, H-6). ¹³C NMR (50 MHz, CDCl₃) δ = 161.8, 161.3, 149.3, 135.3, 129.7, 129.3, 127.2, 123.8, 97.0. HRMS (ESI): *m/z* [M + H]⁺ calcd for [C₉H₆Cl₃N₂]⁺: 246.9596; found: 246.9599.

3.1.3. 2-(Dichloromethyl)quinoxaline (5c)

A 100-mL round-bottomed flask equipped with a drying tube-condenser and a magnetic stir bar was charged with 2-methylquinoxaline (2.0 g, 13.87 mmol), POCl₃ (2.59 mL, 27.74 mmol) and PCl₅ (5.78 g, 27.74 mmol). The mixture was refluxed for 12 h. After the reaction mixture was cooled with an ice bath, the mixture was carefully poured into 200 mL of ice. The mixture was made alkaline by addition of NaHCO₃. The resulting aqueous solution was extracted three times with CHCl₃, and then the organic layer was dried with anhydrous

sodium sulphate and was concentrated under vacuum. Purification by chromatography on silica gel (CHCl_3) gave the expected 2-(dichloromethyl)quinazoline.

Yield 13%. Off-white powder. Mp 117°C . ^1H NMR (200 MHz, CDCl_3) $\delta = 9.33$ (s, 1H, H-4), 8.18-8.05 (m, 2H, H-5 and H-8), 7.87-7.78 (m, 2H, H-6 and H-7), 6.88 (s, 1H, CHCl_2). ^{13}C NMR (50 MHz, CD_2Cl_2) $\delta = 151.9, 143.3, 142.5, 140.1, 131.2, 131.0, 129.5, 129.3, 70.2$. Anal. Calcd for $\text{C}_9\text{H}_6\text{Cl}_2\text{N}_2$: C, 50.73; H, 2.84; N, 13.15. Found: C, 50.66; H, 2.99; N, 12.92.

3.1.4. 2-Phenyl-3-(trichloromethyl)quinoxaline (5f)

A 50-mL round-bottomed flask equipped with a drying tube-condenser and a magnetic stir bar was charged with 2-methyl-3-phenylquinoxaline [27] (441 mg, 2.0 mmol), POCl_3 (1.86 mL, 20.0 mmol) and PCl_5 (2.08 g, 10.0 mmol). The mixture was refluxed for 1h30. After the reaction mixture was cooled with an ice bath, the mixture was carefully poured into 200 mL of ice. The mixture was extracted three times with EtOAc. Then the organic layer was dried with anhydrous sodium sulphate and was concentrated under vacuum. Purification by chromatography on silica gel (Petroleum Ether/EtOAc) gave the expected 2-phenyl-3-(trichloromethyl)quinoxaline.

Yield 26%. White powder. Mp 125°C . ^1H NMR (200 MHz, CDCl_3) $\delta = 8.31$ -8.15 (m, 2H, H-2'), 7.95-7.83 (m, 2H, H-5 and H-8), 7.64 (dd, $J = 2.9; 6.8$ Hz, 2H, H-6 and H-7), 7.54- 7.44 (m, 3H, H-3' and H-4'). ^{13}C NMR (50 MHz, CDCl_3) $\delta = 152.6, 149.7, 141.6, 139.6, 138.6, 132.3, 131.3, 129.8, 129.8, 129.0, 129.0, 127.9, 97.1$. HRMS (ESI): m/z $[\text{M} + \text{Na}]^+$ calcd for $[\text{C}_{15}\text{H}_9\text{Cl}_3\text{N}_2\text{Na}]^+$: 344.9723; found: 344.9723.

3.1.5. Representative procedure for the preparation of 1-(4-substituted-2-nitrophenyl)-1H-pyrrole (7a-i)

A 100-mL round-bottomed single-neck flask equipped with a condenser and a magnetic stir bar was charged with appropriated 4-substituted-2-nitroaniline (25.0 mmol), 2,5-dimethoxytetrahydrofuran (25.0 mmol) and 40 mL of glacial acetic acid. The flask was refluxed until disappearance of starting material (ca. 2 h). After the reaction mixture was cooled to rt, removal of the volatiles under reduced pressure gave the crude product which was purified by column-chromatography (Petroleum Ether/EtOAc = 9:1 then 7:3) to give the desired 1-(4-substituted-2-nitrophenyl)-1H-pyrrole.

3.1.5.1. 1-(4-Iodo-2-nitrophenyl)-1H-pyrrole (7e)

Yield 82%. Brown powder. Mp 70°C. ^1H NMR (400 MHz, CDCl_3) δ = 8.15 (d, J = 1.7 Hz, 1H, H-3'), 7.96 (ddd, J = 0.7; 2.0; 8.6 Hz, 1H, H-5'), 7.21 (d, J = 8.2 Hz, 1H, H-6'), 6.76 (t, J = 1.7 Hz, 2H, H-2), 6.37 (t, J = 2.0 Hz, 2H, H-3). ^{13}C NMR (100 MHz, CDCl_3) δ = 145.5, 142.2, 134.0, 133.6, 129.3, 121.3, 111.7, 90.6. HRMS (ESI): m/z $[\text{M} + \text{H}]^+$ calcd for $[\text{C}_{10}\text{H}_8\text{N}_2\text{O}_2\text{I}]^+$: 314.9625; found: 314.9626.

3.1.5.2. Methyl 3-nitro-4-(1H-pyrrol-1-yl)benzoate (7i)

Yield 79%. Yellow powder. Mp 118°C. ^1H NMR (200 MHz, CDCl_3) δ = 8.47 (d, J = 1.5 Hz, 1H, H-2), 8.28 (dd, J = 1.7; 8.4 Hz, 1H, H-6), 7.54 (d, J = 8.4 Hz, 1H, H-5), 6.81 (t, J = 2.1 Hz, 2H, H-2'), 6.39 (t, J = 2.1 Hz, 2H, H-3'), 3.98 (s, 3H, CH_3). ^{13}C NMR (50 MHz, CDCl_3) δ = 164.5, 144.4, 137.4, 134.0, 129.2, 127.3, 126.5, 121.0, 112.2, 53.0. HRMS (ESI): m/z $[\text{M} + \text{H}]^+$ calcd for $[\text{C}_{12}\text{H}_{11}\text{N}_2\text{O}_4]^+$: 247.0713; found: 247.0714.

3.1.6. Representative procedure for the preparation of 5-substituted-2-(1H-pyrrol-1-yl)aniline

A 250-mL round-bottomed single-neck flask equipped with a condenser and a magnetic stir bar was charged with appropriated 1-(4-substituted-2-nitrophenyl)-1H-pyrrole (15.0 mmol), tin (II) chloride dihydrate (16.92 g, 75.0 mmol) and 60 mL of ethanol. The flask was refluxed until disappearance of starting material (ca. 3 h). After the reaction mixture was cooled to rt, the mixture was quenched with a saturated solution of sodium carbonate. The white precipitate was filtered through a pad of Celite[®] and was thoroughly washed with ethanol. Then, removal of the volatiles under reduced pressure gave the crude 5-substituted-2-(1H-pyrrol-1-yl)aniline which was engaged in the next reaction step without any further purification

3.1.6.1. 5-Iodo-2-(1H-pyrrol-1-yl)aniline (8e)

Yield 71%. Orange powder. Mp 80°C. ^1H NMR (400 MHz, CDCl_3) δ = 7.15 (d, J = 1.7 Hz, 1H, H-6), 7.09 (dd, J = 2.0; 8.1 Hz, 1H, H-4), 6.85 (d, J = 8.0 Hz, 1H, H-3), 6.80 (t, J = 1.7 Hz, 2H, H-2'), 6.34 (t, J = 2.0 Hz, 2H, H-3'), 3.75 (s, NH_2). ^{13}C NMR (100 MHz, CDCl_3) δ = 143.5, 128.7, 127.4, 127.2, 124.7, 121.6, 109.9, 93.4. HRMS (ESI): m/z $[\text{M} + \text{H}]^+$ calcd for $[\text{C}_{10}\text{H}_{10}\text{N}_2\text{I}]^+$: 284.9883; found: 284.9884.

3.1.6.2. Methyl 3-amino-4-(1H-pyrrol-1-yl)benzoate (8i)

Yield 52%. Brown powder. Mp 80°C. ^1H NMR (200 MHz, CDCl_3) δ = 7.52-7.39 (m, 2H, H-2 and H-6), 7.18 (d, J = 8.0 Hz, 1H, H-5), 6.87 (t, J = 2.1 Hz, 2H, H-2'), 6.36 (t, J = 2.1 Hz, 2H,

H-3'), 3.91 (s, 3H, CH₃). ¹³C NMR (50 MHz, CDCl₃) δ =166.8, 141.7, 131.1, 130.0, 126.8, 121.4, 119.8, 117.4, 110.1, 52.3. HRMS (ESI): *m/z* [M + H]⁺ calcd for [C₁₂H₁₁N₂O₂]⁺: 215.0826; found: 215.0825.

3.1.7. Representative procedure for the preparation of 2,2,2-trichloro-*N*-[5-substituted-2-(1*H*-pyrrol-1-yl)phenyl]acetamide (9a-i)

A 100-mL round-bottomed flask equipped with a condenser, an Ar inlet and a magnetic stir bar was charged with appropriated 5-substituted-2-(1*H*-pyrrol-1-yl)aniline (15.0 mmol), 25 mL of anhydrous dioxane, triethylamine (3.14 mL, 22.5 mmol). The mixture was cooled with a ice bath and trichloroacetyl chloride was added dropwise (1.67 mL, 15.0 mmol). The flask was refluxed until disappearance of starting material (ca. 4 h). After the reaction mixture was cooled to rt, removal of the volatiles under reduced pressure gave the crude product which was dissolved in EtOAc. The organic layer was washed with a aqueous solution of sodium bicarbonate and brine, then the organic layer was dried with anhydrous sodium sulphate and was concentrated under vacuum. Purification by chromatography on silica gel (Petroleum Ether/EtOAc) gave the expected 2,2,2-trichloro-*N*-[5-substituted-2-(1*H*-pyrrol-1-yl)phenyl]acetamide.

3.1.7.1. *N*-[2-(1*H*-Pyrrol-1-yl)phenyl]-2,2,2-trichloroacetamide (9a)

Yield 65%. Off-white powder. Mp 79°C. ¹H NMR (200 MHz, CDCl₃) δ = 8.41 (m, 1H, H-6), 8.37 (bs, NH), 7.48 (dt, *J* = 1.8; 8.4 Hz, 1H, H-5), 7.37 (dt, *J* = 1.6; 7.8 Hz, 1H, H-3), 7.28-7.24 (m, 1H, H-4), 6.82 (t, *J* = 2.2 Hz, 2H, H-2 pyrrole), 6.43 (t, *J* = 2.2 Hz, 2H, H-3 pyrrole). ¹³C NMR (50 MHz, CDCl₃) δ =159.3, 132.4, 131.8, 129.2, 127.3, 125.9, 122.1, 120.8, 111.2, 92.6. HRMS (ESI): *m/z* [M + H]⁺ calcd for [C₁₂H₁₀N₂OCl₃]⁺: 302.9853; found: 302.9852.

3.1.7.2. 2,2,2-Trichloro-*N*-[5-fluoro-2-(1*H*-pyrrol-1-yl)phenyl]acetamide (9b)

Yield 65%. Brown powder. Mp 64°C. ¹H NMR (400 MHz, CDCl₃) δ = 8.34 (bs, NH), 6.85 (dd, *J* = 2.7; 10.2 Hz, 1H, H-6), 7.36 (dd, *J* = 5.6; 8.8 Hz, 1H, H-3), 6.98 (td, *J* = 2.9; 7.8 Hz, 1H, H-4), 6.78 (t, *J* = 2.0 Hz, 2H, H-2 pyrrole), 6.43 (t, *J* = 2.0 Hz, 2H, H-3 pyrrole). ¹³C NMR (100 MHz, CDCl₃) δ =162.4 (d, *J* = 266.8 Hz), 159.3, 134.1 (d, *J* = 12.3 Hz), 128.6 (d, *J* = 9.9 Hz), 127.5, 122.3, 112.5 (d, *J* = 23.0 Hz), 111.5, 108.1 (d, *J* = 29.6 Hz), 92.3. HRMS (ESI): *m/z* [M + H]⁺ calcd for [C₁₂H₉N₂OFCl₃]⁺: 320.9759; found: 320.9762.

3.1.7.3. 2,2,2-Trichloro-*N*-[5-chloro-2-(1*H*-pyrrol-1-yl)phenyl]acetamide (9c)

Yield 63%. Yellow powder. Mp 98°C. ¹H NMR (400 MHz, CDCl₃) δ = 8.43 (d, *J* = 2.2 Hz, 1H, H-6), 8.29 (bs, NH), 7.26 (d, *J* = 8.6 Hz, 1H, H-3), 7.20 (dd, *J* = 2.2; 8.6 Hz, 1H, H-4), 6.73 (t, *J* = 1.7 Hz, 2H, H-2 pyrrole), 6.38 (t, *J* = 1.7 Hz, 2H, H-3 pyrrole). ¹³C NMR (100 MHz, CDCl₃) δ = 159.3, 135.1, 133.4, 130.0, 128.2, 125.9, 122.1, 120.8, 111.6, 92.3. HRMS (ESI): *m/z* [M - H]⁻ calcd for [C₁₂H₇N₂OCl₄]⁻: 336.9289; found: 336.9288.

3.1.7.4. N-[5-Bromo-2-(1H-pyrrol-1-yl)phenyl]-2,2,2-trichloroacetamide (9d)

Yield 63%. Beige powder. Mp 90°C. ¹H NMR (400 MHz, CDCl₃) δ = 8.57 (d, *J* = 2.0 Hz, 1H, H-6), 8.29 (bs, NH), 7.37 (dd, *J* = 2.2; 8.6 Hz, 1H, H-3), 7.20 (d, *J* = 8.6 Hz, 1H, H-4), 6.73 (t, *J* = 1.7 Hz, 2H, H-2 pyrrole), 6.38 (t, *J* = 1.7 Hz, 2H, H-3 pyrrole). ¹³C NMR (100 MHz, CDCl₃) δ = 159.3, 133.5, 130.6, 128.9, 128.5, 123.7, 122.8, 122.0, 111.7, 92.3. HRMS (ESI): *m/z* [M + H]⁺ calcd for [C₁₂H₉N₂OBrCl₃]⁺: 382.8934; found: 382.8933.

3.1.7.5. 2,2,2-Trichloro-N-[5-iodo-2-(1H-pyrrol-1-yl)phenyl]acetamide (9e)

Yield 69%. Brown powder. Mp 90°C. ¹H NMR (400 MHz, CDCl₃) δ = 8.79 (d, *J* = 1.7 Hz, 1H, H-6), 8.32 (bs, NH), 7.63 (dd, *J* = 1.9; 8.3 Hz, 1H, H-4), 7.10 (d, *J* = 8.3 Hz, 1H, H-3), 6.79 (t, *J* = 1.9 Hz, 2H, H-2 pyrrole), 6.43 (t, *J* = 1.9 Hz, 2H, H-3 pyrrole). ¹³C NMR (100 MHz, CDCl₃) δ = 159.3, 135.1, 133.3, 131.3, 129.5, 128.6, 122.0, 111.6, 93.8, 82.3. HRMS (ESI): *m/z* [M + H]⁺ calcd for [C₁₂H₉N₂OICl₃]⁺: 428.8820; found: 428.8818.

3.1.7.6. 2,2,2-Trichloro-N-(5-methyl-2-(1H-pyrrol-1-yl)phenyl)acetamide (9f)

Yield 46%. Amber oil. ¹H NMR (200 MHz, CDCl₃) δ = 8.31 (bs, NH), 8.23 (s, 1H, H-6), 7.26 (d, *J* = 8.0 Hz, 1H, H-3), 7.09 (d, *J* = 8.0 Hz, 1H, H-4), 6.79 (t, *J* = 2.1 Hz, 2H, H-2 pyrrole), 6.41 (t, *J* = 2.1 Hz, 2H, H-3 pyrrole), 2.46 (s, 3H, CH₃). ¹³C NMR (50 MHz, CDCl₃) δ = 159.2, 139.5, 132.0, 129.3, 126.9, 126.5, 122.2, 121.2, 110.9, 92.6, 21.6. HRMS (ESI): *m/z* [M + H]⁺ calcd for [C₁₃H₁₁N₂OCl₃]⁺: 317.0010; found: 317.0013.

3.1.7.7. 2,2,2-Trichloro-N-(5-methoxy-2-(1H-pyrrol-1-yl)phenyl)acetamide (9g)

Yield 88%. Yellow powder. Mp 67°C. ¹H NMR (200 MHz, CDCl₃) δ = 8.29 (bs, NH), 8.04 (d, *J* = 2.8 Hz, 1H, H-6), 7.28 (d, *J* = 9.3 Hz, 1H, H-3), 6.79 (dd, *J* = 2.2; 8.2 Hz, 1H, H-4), 6.76 (t, *J* = 2.1 Hz, 2H, H-2 pyrrole), 6.40 (t, *J* = 2.1 Hz, 2H, H-3 pyrrole), 3.88 (s, 3H, OCH₃). ¹³C NMR (50 MHz, CDCl₃) δ = 159.9, 159.2, 133.5, 128.0, 124.3, 122.4, 111.6,

110.8, 105.1, 92.4, 55.8. HRMS (ESI): m/z $[M + H]^+$ calcd for $[C_{13}H_{12}N_2O_2Cl_3]^+$: 332.9959; found: 332.9958.

3.1.7.8. 2,2,2-Trichloro-*N*-(5-cyano-2-(1*H*-pyrrol-1-yl)phenyl)acetamide (9h)

Yield 77%. Yellow powder. Mp 129°C. 1H NMR (200 MHz, $CDCl_3$) δ = 8.75 (d, J = 1.6 Hz, 1H, H-6), 8.54 (bs, NH), 7.59 (dd, J = 1.7; 8.2 Hz, 1H, H-4), 7.48 (d, J = 8.1 Hz, 1H, H-3), 6.84 (t, J = 2.1 Hz, 2H, H-2 pyrrole), 6.48 (t, J = 2.1 Hz, 2H, H-3 pyrrole). ^{13}C NMR (50 MHz, $CDCl_3$) δ = 159.5, 135.1, 132.8, 129.6, 127.9, 124.5, 121.7, 117.6, 112.8, 112.4, 92.1. HRMS (ESI): m/z $[M + H]^+$ calcd for $[C_{13}H_9N_3OCl_3]^+$: 327.9806; found: 327.9802.

3.1.7.9. Methyl 4-(1*H*-pyrrol-1-yl)-3-(2,2,2-trichloroacetamido)benzoate (9i)

Yield 77%. Amber oil. 1H NMR (200 MHz, $CDCl_3$) δ = 9.00 (d, J = 1.8 Hz, 1H, H-6), 8.46 (bs, NH), 7.98 (dd, J = 1.8; 8.2 Hz, 1H, H-4), 7.44 (d, J = 8.2 Hz, 1H, H-3), 6.85 (t, J = 2.1 Hz, 2H, H-2 pyrrole), 6.45 (t, J = 2.1 Hz, 2H, H-3 pyrrole), 3.96 (s, 3H, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ = 165.9, 159.4, 135.5, 131.8, 130.7, 127.5, 127.0, 112.5, 121.8, 111.8, 92.4, 52.7. HRMS (ESI): m/z $[M + H]^+$ calcd for $[C_{14}H_{12}N_2O_3Cl_3]^+$: 360.9908; found: 360.9904.

3.1.8. Representative procedure for the preparation of 7-substituted-4-(trichloromethyl)pyrrolo[1,2-*a*]quinoxaline (10a-i)

A 100-mL round-bottomed flask equipped with a condenser, an Ar inlet and a magnetic stir bar was charged with appropriated 2,2,2-trichloro-*N*-[5-substituted-2-(1*H*-pyrrol-1-yl)phenyl]acetamide (5.0 mmol), 15 mL of freshly distilled $POCl_3$ and anhydrous pyridine (1 mL). The mixture was refluxed until disappearance of starting material (ca. 4 h). After the reaction mixture was cooled with an ice bath, it was carefully poured over 200 mL of ice. The mixture was made alkaline with concentrated ammonia to pH 10. The alkaline aqueous phase was extracted three times with CH_2Cl_2 . The combined organic layers were washed with 10% hydrochloric acid and then by water. Then the organic layer was dried with anhydrous sodium sulphate and was concentrated under vacuum. Purification by chromatography on silica gel (Petroleum Ether/EtOAc) gave the expected 7-substituted-4-(trichloromethyl)pyrrolo[1,2-*a*]quinoxaline.

3.1.8.1. 4-(Trichloromethyl)pyrrolo[1,2-*a*]quinoxaline (10a)

Yield 57%. Beige powder. Mp 202°C. 1H NMR (400 MHz, $CDCl_3$) δ = 8.10 (dd, J = 1.4; 8.3 Hz, 1H, H-6), 8.02 (dd, J = 1.0; 2.7 Hz, 1H, H-1), 7.90 (dd, J = 1.2; 8.3 Hz, 1H, H-9), 7.63

(dt, $J = 1.2$; 8.3 Hz, 1H, H-8), 7.50 (dt, $J = 0.7$; 7.3 Hz, 1H, H-7), 7.40 (dd, $J = 0.7$; 4.2 Hz, 1H, H-3), 6.98 (dd, $J = 2.7$; 4.2 Hz, 1H, H-2). ^{13}C NMR (100 MHz, CDCl_3) $\delta = 150.1, 133.5, 131.5, 130.0, 127.8, 125.8, 120.6, 115.2, 114.2, 113.8, 110.4, 96.3$. Anal. Calcd for $\text{C}_{12}\text{H}_7\text{Cl}_3\text{N}_2$: C, 50.47; H, 2.47; N, 9.81. Found: C, 50.35; H, 2.37; N, 9.67.

3.1.8.2. 7-Fluoro-4-(trichloromethyl)pyrrolo[1,2-*a*]quinoxaline (10b)

Yield 56%. Yellow powder. Mp 192°C. ^1H NMR (400 MHz, CDCl_3) $\delta = 7.97$ (dd, $J = 1.2$; 2.7 Hz, 1H, H-1), 7.85 (dd, $J = 4.9$; 9.0 Hz, 1H, H-6), 7.77 (dd, $J = 2.9$; 9.0 Hz, 1H, H-9), 7.41-7.34 (m, 2H, H-3 and H-8), 6.97 (dd, $J = 2.7$; 4.1 Hz, 1H, H-2). ^{13}C NMR (100 MHz, CDCl_3) $\delta = 160.0$ (d, $J = 244.3$ Hz), 151.1, 134.6 (d, $J = 11.5$ Hz), 124.4, 120.4, 117.9 (d, $J = 23.9$ Hz), 116.6 (d, $J = 23.0$ Hz), 115.4, 115.0 (d, $J = 9.1$ Hz), 114.4, 110.7, 96.2. Anal. Calcd for $\text{C}_{12}\text{H}_6\text{Cl}_3\text{FN}_2$: C, 47.48; H, 1.99; N, 9.23. Found: C, 47.81; H, 1.76; N, 9.10.

3.1.8.3. 7-Chloro-4-(trichloromethyl)pyrrolo[1,2-*a*]quinoxaline (10c)

Yield 52%. Yellow-green powder. Mp 132°C. ^1H NMR (400 MHz, CDCl_3) $\delta = 8.09$ (d, $J = 2.2$ Hz, 1H, H-6), 7.97 (dd, $J = 1.2$; 2.9 Hz, 1H, H-1), 7.81 (d, $J = 8.8$ Hz, 1H, H-9), 7.57 (dd, $J = 2.4$; 8.8 Hz, 1H, H-8), 7.41 (dd, $J = 1.0$; 4.2 Hz, 1H, H-3), 6.98 (dd, $J = 2.7$; 4.2 Hz, 1H, H-2). ^{13}C NMR (100 MHz, CDCl_3) $\delta = 151.1, 134.4, 131.0, 130.8, 130.0, 126.4, 120.5, 115.5, 115.0, 114.6, 110.9, 95.9$. Anal. Calcd for $\text{C}_{12}\text{H}_6\text{Cl}_4\text{N}_2$: C, 45.04; H, 1.89; N, 8.75. Found: C, 45.44; H, 1.89; N, 8.75.

3.1.8.4. 7-Bromo-4-(trichloromethyl)pyrrolo[1,2-*a*]quinoxaline (10d)

Yield 59%. Yellow-green powder. Mp 156°C. ^1H NMR (400 MHz, CDCl_3) $\delta = 8.27$ (d, $J = 2.2$ Hz, 1H, H-6), 7.99 (dd, $J = 1.0$; 2.7 Hz, 1H, H-1), 7.77 (d, $J = 8.8$ Hz, 1H, H-9), 7.72 (dd, $J = 2.2$; 8.8 Hz, 1H, H-8), 7.41 (dd, $J = 1.0$; 4.2 Hz, 1H, H-3), 6.99 (dd, $J = 2.7$; 4.2 Hz, 1H, H-2). ^{13}C NMR (100 MHz, CDCl_3) $\delta = 151.0, 134.7, 133.9, 132.9, 126.8, 120.6, 118.3, 115.5, 115.2, 114.7, 111.0, 95.9$. Anal. Calcd for $\text{C}_{12}\text{H}_6\text{BrCl}_3\text{N}_2$: C, 39.55; H, 1.66; N, 7.69. Found: C, 39.39; H, 1.74; N, 7.61.

3.1.8.5. 7-Iodo-4-(trichloromethyl)pyrrolo[1,2-*a*]quinoxaline (10e)

Yield 56%. Brown powder. Mp 170°C. ^1H NMR (400 MHz, CDCl_3) $\delta = 8.45$ (d, $J = 2.0$ Hz, 1H, H-6), 7.97-7.95 (m, 1H, H-1), 7.87 (dd, $J = 1.7$; 8.6 Hz, 1H, H-8), 7.62 (d, $J = 8.8$ Hz, 1H, H-9), 7.40 (d, $J = 4.2$ Hz, 1H, H-3), 6.98 (dd, $J = 2.7$; 4.0 Hz, 1H, H-2). ^{13}C NMR (100 MHz, CDCl_3) $\delta = 150.8, 139.9, 138.3, 134.6, 127.4, 120.5, 115.4, 115.3, 114.5, 110.9, 95.9, 88.6$. Anal. Calcd for $\text{C}_{12}\text{H}_6\text{ICl}_3\text{N}_2$: C, 35.03; H, 1.47; N, 6.81. Found: C, 34.69; H, 1.49; N, 6.69.

3.1.8.6. 7-Methyl-4-(trichloromethyl)pyrrolo[1,2-*a*]quinoxaline (10f)

Yield 20%. Yellow powder. Mp 136°C. ¹H NMR (200 MHz, CDCl₃) δ = 7.97 (dd, *J* = 1.2; 2.7 Hz, 1H, H-1), 7.90 (s, 1H, H-6), 7.78 (d, *J* = 8.4 Hz, 1H, H-9), 7.44 (dd, *J* = 1.6; 8.4 Hz, 1H, H-8), 7.37 (dd, *J* = 1.2 ;4.2 Hz, 1H, H-3), 6.94 (dd, *J* = 2.8; 4.2 Hz, 1H, H-2), 2.51 (s, 3H, CH₃). ¹³C NMR (50 MHz, CDCl₃) δ =150.0, 135.7, 133.5, 131.2, 131.1, 125.6, 120.5, 114.9, 113.9, 113.5, 110.0, 96.6, 21.1. Anal. Calcd for C₁₃H₉Cl₃N₂: C, 52.12; H, 3.03; N, 9.35. Found: C, 52.18; H, 2.76; N, 9.60.

3.1.8.7. 7-Methoxy-4-(trichloromethyl)pyrrolo[1,2-*a*]quinoxaline (10g)

Yield 60%. Brown powder. Mp 108°C. ¹H NMR (200 MHz, CDCl₃) δ = 7.93 (dd, *J* = 1.1; 2.6 Hz, 1H, H-1), 7.79 (d, *J* = 9.1 Hz, 1H, H-9), 7.52 (d, *J* = 2.8 Hz, 1H, H-6), 7.36 (dd, *J* = 1.1; 4.2 Hz, 1H, H-3), 7.23 (dd, *J* = 2.5;8.8 Hz, 1H, H-8), 6.93 (dd, *J* = 2.7; 4.2 Hz, 1H, H-2), 3.94 (s, 3H, CH₃). ¹³C NMR (50 MHz, CDCl₃) δ =157.5, 150.4, 134.6, 122.0, 120.4, 119.6, 114.8, 114.7, 113.9, 112.1, 109.9, 96.5, 56.0. Anal. Calcd for C₁₃H₉Cl₃N₂O: C, 49.48; H, 2.87; N, 8.88. Found: C, 49.72; H, 3.22; N, 8.98.

3.1.8.8. 4-(Trichloromethyl)pyrrolo[1,2-*a*]quinoxaline-7-carbonitrile (10h)

Yield 60%. Beige powder. Mp 177°C. ¹H NMR (200 MHz, CDCl₃) δ = 8.40 (d, *J* = 1.6 Hz, 1H, H-1), 8.04 (d, *J* = 2.6 Hz, 1H, H-6), 7.97 (d, *J* = 8.6 Hz, 1H, H-9), 7.84 (dd, *J* = 1.6; 8.6 Hz, 1H, H-8), 7.47 (d, *J* = 4.2 Hz, 1H, H-3), 7.08-7.05 (m, 1H, H-2). ¹³C NMR (50 MHz, CDCl₃) δ =151.8, 135.9, 133.5, 132.3, 130.5, 120.7, 117.9, 116.2, 115.7, 115.1, 112.0, 109.3, 95.8. Anal. Calcd for C₁₃H₆Cl₃N₃: C, 50.28; H, 1.95; N, 13.53. Found: C, 50.63; H, 2.02; N, 13.66.

3.1.8.9. Methyl 4-(trichloromethyl)pyrrolo[1,2-*a*]quinoxaline-7-carboxylate (10i)

Yield 69%. Beige powder. Mp 224°C. ¹H NMR (200 MHz, CDCl₃) δ = 8.79 (d, *J* = 1.6 Hz, 1H, H-6), 8.39-8.21 (m, 1H, H-8), 8.05 (d, *J* = 1.1 Hz, 1H, H-1), 7.94 (dd, *J* = 1.5; 8.7 Hz, 1H, H-9), 7.44 (d, *J* = 1.5 Hz, 1H, H-3), 7.02 (d, *J* = 4.2 Hz, 1H, H-2), 3.99 (s, 3H, CH₃). ¹³C NMR (50 MHz, CDCl₃) δ =166.1, 150.9, 133.5, 133.2, 130.7, 127.6, 127.5, 120.8, 115.8, 115.1, 114.0, 11.3, 96.1, 52.6. Anal. Calcd for C₁₄H₉Cl₃N₂O₂: C, 48.94; H, 2.64; N, 8.15. Found: C, 49.15; H, 2.63; N, 8.09.

3.1.9. 4-(Trichloromethyl)pyrrolo[1,2-*a*]quinoxaline-7-carboxylic acid (10j)

A 100-mL round-bottomed flask equipped with a condenser and a magnetic stir bar was charged with methyl 4-(trichloromethyl)pyrrolo[1,2-*a*]quinoxaline-7-carboxylate (500 mg, 1.45 mmol) and 15 mL of conc. HCl. The mixture was refluxed until disappearance of starting material (ca. 5 h). After the reaction mixture was cooled to rt, the mixture was extracted five times with CH₂Cl₂. The combined organic layers were washed twice with water. Then the organic layer was dried with anhydrous sodium sulphate and was concentrated under vacuum. Trituration of the residue in Et₂O afforded the expected compound after filtration.

Yield 44%. Yellow powder. Mp >260°C. ¹H NMR (200 MHz, DMSO-*d*₆) δ = 8.70 (d, *J* = 1.8 Hz, 1H, H-6), 8.50-8.45 (m, 2H, H-1 and H-9), 8.22 (dd, *J* = 1.6; 8.7 Hz, 1H, H-8), 7.36 (d, *J* = 3.8 Hz, 1H, H-3), 7.18-7.05 (m, 1H, H-2). ¹³C NMR (50 MHz, DMSO-*d*₆) δ = 166.3, 149.8, 132.0, 131.7, 130.6, 130.1, 128.3, 119.4, 118.3, 115.5, 115.2, 110.8, 95.8. Anal. Calcd for C₁₃H₇Cl₃N₂O₂: C, 47.38; H, 2.14; N, 8.50. Found: C, 47.52; H, 2.21; N, 8.47.

3.1.10. *N'*-Hydroxy-4-(trichloromethyl)pyrrolo[1,2-*a*]quinoxaline-7-carboximidamide (10k)

A 50-mL round-bottomed flask equipped with a condenser and a magnetic stir bar was charged with 4-(trichloromethyl)pyrrolo[1,2-*a*]quinoxaline-7-carbonitrile (311 mg, 1.0 mmol), hydroxylamine hydrochloride (70 mg, 1.0 mmol) and 5 mL of a mixture of EtOH/H₂O 7/3. The mixture was refluxed until disappearance of starting material (ca. 24 h). After the reaction mixture was cooled to rt and the volatiles were removed, the mixture was extracted five times with CH₂Cl₂. The combined organic layers were washed twice with water. Then, the organic layer was dried with anhydrous sodium sulphate and was concentrated under vacuum. Purification by chromatography on silica gel (Petroleum Ether/EtOAc 7/3 then 5/5) gave the expected product.

Yield 74%. Yellow powder. Mp 114°C. ¹H NMR (200 MHz, DMSO-*d*₆) δ = 9.92 (s, 1H, OH), 8.68 (m, 1 H, H-6), 8.42 (d, *J* = 8.8 Hz, 1H, H-9), 8.35 (d, *J* = 1.6 Hz, 1H, H-1), 8.10 (dd, *J* = 1.6; 8.8 Hz, 1H, H-8), 7.36 (dd, *J* = 1.0; 4.2 Hz, 1H, H-3), 7.12 (dd, *J* = 2.7; 4.2 Hz, 1H, H-2), 6.23 (sl, 2H). ¹³C NMR (50 MHz, DMSO-*d*₆) δ = 151.3, 149.6, 132.2, 129.4, 128.0, 127.8, 127.6, 119.3, 117.9, 115.1, 114.9, 110.4, 96.0. HRMS (ESI): *m/z* [M + H]⁺ calcd for [C₁₃H₁₀N₄OCl₃]⁺: 342.9915; found: 342.9920.

3.1.11. Representative procedure for the preparation of 4-methyl-7-substituted-pyrrolo[1,2-*a*]quinoxaline (11f,h)

A small reactor equipped with a magnetic stir bar was charged with the appropriate 7-substituted 4-(trichloromethyl)pyrrolo[1,2-*a*]quinoxaline (1.1 mmol) and iron fine powder (880 mg, 15.8 mmol) in 15 mL of glacial AcOH. The mixture was heated at reflux for 1 h. After cooling at rt, the mixture was filtered through a pad of Celite[®] and thoroughly washed with CHCl₃. Volatiles were removed under vacuum then the mixture was made alkaline with a saturated solution of Na₂CO₃. The aqueous layer was extracted with CHCl₃, and then the organic layer was dried with anhydrous sodium sulphate and was concentrated under vacuum. Purification by chromatography on silica gel (CHCl₃/acetone) gave the expected product.

3.1.11.1. 4,7-Dimethylpyrrolo[1,2-*a*]quinoxaline (11f)

Yield 56%. Beige powder. Mp 140°C. ¹H NMR (200 MHz, CDCl₃) δ = 7.85 (dd, *J* = 1.3, 2.6 Hz, 1H, H-1), 7.72 (s, 1H, H-6), 7.69 (d, *J* = 8.4 Hz, 1H, H-9), 7.27 (dd, *J* = 2.2, 7.6 Hz, 1H, H-8), 6.90-6.80 (m, 2H, H-2 and H-3), 2.73 (s, 3H, CH₃), 2.47 (s, 3H, CH₃). ¹³C NMR (50 MHz, CDCl₃) δ = 153.5, 135.3, 135.2, 128.8, 128.3, 126.1, 125.2, 114.5, 113.6, 113.5, 106.9, 21.8, 21.2. Anal. Calcd for C₁₃H₁₂N₂: C, 79.56; H, 6.16; N, 14.27. Found: C, 79.89; H, 2.18; N, 13.99.

3.1.11.2. 4-Methylpyrrolo[1,2-*a*]quinoxaline-7-carbonitrile (11h)

Yield 57%. White powder. Mp 140°C. ¹H NMR (200 MHz, CDCl₃) δ = 8.17 (d, *J* = 1.6 Hz, 1H, H-1), 7.97-7.77 (m, 2H, H-6 and H-9), 7.68 (dd, *J* = 1.7; 8.5 Hz, 1H, H-8), 7.01-6.87 (m, 2H, H-2 and H-3), 2.73 (s, 3H, CH₃). ¹³C NMR (50 MHz, CDCl₃) δ = 155.9, 135.9, 133.8, 130.3, 129.8, 126.4, 118.6, 115.4, 115.2, 114.9, 108.6, 108.4, 22.1. HRMS (ESI): *m/z* [M + H]⁺ calcd for [C₁₃H₁₀N₃]⁺: 208.0869; found: 208.0871.

3.2 Biology

3.2.1 *In vitro* Antiplasmodial evaluation

In this study, a K1 culture-adapted *P. falciparum* strain resistant to chloroquine, pyrimethamine and proguanil was used in an *in vitro* culture. Maintenance in continuous culture was done as described previously by Trager and Jensen [38]. Cultures were maintained in fresh A+ human erythrocytes at 2.5% hematocrit in complete medium (RPMI 1640 with 25 mM HEPES, 25 mM NaHCO₃, 10% of A+ human serum) at 37 °C under reduced O₂ atmosphere (gas mixture 14% O₂, 6% CO₂, and 80% N₂). Parasitaemia was maintained daily between 1% and 6%. The *P. falciparum* drug susceptibility test was carried out by comparing quantities of DNA in treated and control cultures of parasite in human

erythrocytes according to a SYBR Green I fluorescence-based method [39] using a 96-well fluorescence plate reader. Compounds, previously dissolved in DMSO (final concentration less than 0.5% v/v) were incubated in a total assay volume of 200 μ L (RPMI, 4% hematocrit and 1% parasitaemia) for 72 h in a humidified atmosphere (14% O₂ and 6% CO₂) at 37 °C, in 96-well flat bottom plates. Duplicate assays were performed for each sample. After incubation, 125 μ L supernatant was discarded and cells were washed twice with 125 μ L 1X PBS. 15 μ L re-suspended cells were transferred to 96-well flat bottom nonsterile black plates (Greiner Bio-one) already containing 15 μ L of the SYBR Green I lysis buffer (2X SYBR Green I, 20 mM Tris base pH 7.5, 20 mM EDTA, 0.008% w/v saponin, 0.08% w/v Triton X-100). Negative control, treated by solvents (DMSO or H₂O) and positive controls (chloroquine and doxycycline) were added to each set of experiments. Plates were incubated for 15 min at 37 °C and then read on a TECAN Infinite F-200 spectrophotometer with excitation and emission wavelengths at 485 and 535 nm, respectively. The concentrations of compounds required to induce a 50% decrease of parasite growth (IC₅₀ K1) were calculated from three independent experiments.

3.2.2 *In vitro* Cytotoxicity evaluation

HepG2 cell line was maintained at 37 °C, 6% CO₂, 14% O₂, 80% N₂, with 90% humidity in RPMI supplemented with 10% foetal bovine serum, 1% L-glutamine (200 mM) and penicillin (100 U/mL) / streptomycin (100 μ g/mL) (complete RPMI medium). The evaluation of the tested molecules cytotoxicity on the HepG2 (hepatocarcinoma cell line purchased from ATCC, ref HB-8065) cell line was performed according to the method of Mosmann [40] with slight modifications. Briefly, 5.10³ cells in 100 μ L of complete medium were inoculated into each well of 96-well plates and incubated at 37 °C in a humidified 6% CO₂. After 24 h incubation, 100 μ L of medium with various product concentrations dissolved in DMSO (final concentration less than 0.5% v/v) were added and the plates were incubated for 72 h at 37 °C. Triplicate assays were performed for each sample. Each plate-well was then microscope-examined for detecting possible precipitate formation before the medium was aspirated from the wells. 100 μ L of MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) solution (0.5 mg/mL in medium without FCS) were then added to each well. Cells were incubated for 2 h at 37 °C. After this time, the MTT solution was removed and DMSO (100 μ L) was added to dissolve the resulting blue formazan crystals. Plates were shaken vigorously (700 rpm) for 10 min. The absorbance was measured at 570 nm with 630 nm as reference wavelength using a BIO-TEK ELx808 Absorbance Microplate Reader. DMSO was

used as blank and doxorubicin (purchased from Sigma Aldrich) as positive control. Cell viability was calculated as percentage of control (cells incubated without compound). The 50% cytotoxic concentration (CC₅₀) was determined from the dose–response curve by using the TableCurve software 2D v.5.0. CC₅₀ values represent the mean value calculated from three independent experiments.

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Figures captions:

Figure 1. Structures of the heterocyclic compounds which were selected from our chemical library for the preliminary *in vitro* antiplasmodial screening

Figure 2. Structures of **5a** and its 2-substituted analogs in quinoxaline series

Figure 3. Structures of quinoxaline **5f** and its position isomer in 2-trichloromethylquinazoline series

Figure 4. Structures of some previously described antimalarial pyrrolo[1,2-*a*]quinoxalines **A-F**

Figure 5. Rational for the synthesis of new 4-trichloromethylpyrrolo[1,2-*a*]quinoxaline derivatives as potential antiplasmodial agents

Figures:

Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.

Schemes:

Scheme 1. Synthesis of 7-substituted-pyrrolo[1,2-*a*]quinoxaline series.

Reagents and conditions : (i) 2,5-DMTHF, AcOH, reflux, 2 h; (ii) SnCl₂.2H₂O, EtOH, reflux, 3 h or BiCl₃, NaBH₄, EtOH, overnight; (iii) ClCOCCl₃, NEt₃, dioxane, reflux, 4 h; (iv) POCl₃, pyridine, reflux, 4 h; (v) conc. HCl, reflux, 5 h; (vi) NH₂OH.HCl, EtOH/H₂O, reflux, 24 h; (vii) Fe, AcOH, reflux, 1 h; (viii) PPh₃, Et₃N, TFA, CCl₃CN, CH₃CN, rt, overnight.

Tables:

Table 1. *In vitro* antiplasmodial and cytotoxicity evaluation of compounds **1-5**.

Entry	Molecule	HepG2 CC ₅₀ (μ M)	K1 <i>P.falciparum</i> IC ₅₀ (μ M)	Selectivity Index ^c
1	1a	>125 ^d	8.5	>14.7
2	1b	>500 ^d	>50 ^d	-
3	2a	180	8	22.5
4	2b	179	>50 ^d	<3.6
5	3a	>500 ^d	>50 ^d	-
6	4a	61	17.6	3.5
7	4b	350	>50 ^d	<7
8	5a	3.1	1.5	2.1
9	5b	>500 ^d	>50 ^d	-
10	5c	19.3	12.8	1.5
11	5d	>500 ^d	>50 ^d	-
12	5e	>500 ^d	>50 ^d	-
13	5f	3.5	0.2	17.5
	Doxorubicine ^a	0.2	-	-
	Chloroquine ^b	30	0.6	50
	Doxycycline ^b	20	6.0	3.3

^aDoxorubicine was used as a cytotoxic reference-drug; ^bChloroquinine and doxycycline were used as antimalarial reference-drugs, ^cSelectivity indexes were calculated according to the formula : SI = HepG2 CC₅₀ / K1 IC₅₀. ^dNo activity was observed at the highest concentration tested.

Table 2. *In vitro* antiplasmodial and cytotoxicity evaluation of the 4-trichloromethylpyrrolo-[1,2-*a*]quinoxaline series

Entry	Molecule	R ₁ -	R ₂ -	HepG2CC ₅₀ (μM)	PfK1 IC ₅₀ (μM)	SI ^c
1	10a	-CCl ₃	-H	41.2	1.5	27.5
2	10b	-CCl ₃	-F	>15 ^{d*}	1.7	>8.8
3	10c	-CCl ₃	-Cl	9.8	1.6	6.1
4	10d	-CCl ₃	-Br	nd*	nd*	-
5	10e	-CCl ₃	-I	6.6	1.2	5.5
6	10f	-CCl ₃	-CH ₃	53	2.4	22.1
7	10g	-CCl ₃	-OCH ₃	35	4.2	8.3
8	10h	-CCl ₃	-CN	17	1.5	11.3
9	10i	-CCl ₃	-COOCH ₃	nd*	nd*	-
10	10j	-CCl ₃	-COOH	106	22	<10.6
11	10k	-CCl ₃	-C(NH ₂)=NOH	>15 ^{d*}	1.75	>8.6
12	11a	-CH ₃	-H	120	>50 ^d	<2.4
13	11f	-CH ₃	-CH ₃	57	>50 ^d	<4.7
14	11h	-CH ₃	-CN	>31.5 ^d	>50 ^d	-
15	12a	-CF ₃	-H	160	>50 ^d	<3.2
	Doxorubicine ^a			0.2	-	-
	Chloroquine ^b			30	0.6	50
	Doxycycline ^b			20	6.0	3.3

^aDoxorubicine was used as a cytotoxic reference-drug; ^bChloroquinine and doxycycline were used as antimalarial reference-drugs; ^cSelectivity index (SI) was calculated according to the formula: $SI = CC_{50}/IC_{50}$; ^dNo activity was observed at the highest concentration tested; *Not determined (nd) because of lack of solubility in the culture media.

Graphical Abstract:

Highlights:

- ▶ Antiplasmodial screening of CCl₃-substituted-nitrogen containing heterocycles was made.
- ▶ 2-Trichloromethylquinoxaline scaffold was the most interesting but was too cytotoxic.
- ▶ Original 4-trichloromethylpyrrolo[1,2-*a*]quinoxalines analogs were prepared.
- ▶ 3 Compounds displayed an IC₅₀ < 2.4 μM *in vitro* on K1 *P. falciparum*.
- ▶ Cytotoxicity was assessed in parallel on the human HepG2 cell line showing good selectivity.