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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and evaluation of cyclic nitrone derivatives as potential anti-cancer agents

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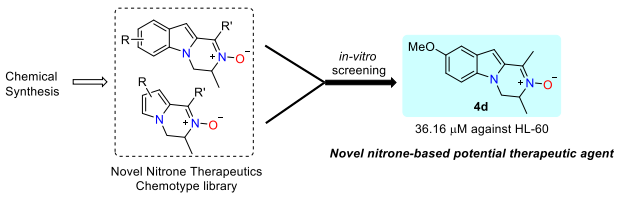
Abstract

Nitrones have been found to exhibit attractive biological values as immuno spin trapping agents. However, successful clinical cases of nitrone therapeutics are still lacking. Herein we report the synthesis and antiproliferative activity of a series of structurally diverse nitrone derivatives against a panel of 5 cancer cell lines, based on which indole- and pyrrole-fused were further evaluated by analogue preparation and in-vitro screening. Analogues with moderate to good potency were identified. This study shows the promise for further pursuit of nitrone-type small molecules in chemotherapy.

Graphical Abstract



Keywords Cyclic nitrone · Metal catalysis · Anti-proliferative activity · In-vitro screening

Introduction

Nitrone derivatives are not only highly versatile synthetic intermediate, most notably for their [3 + 2] dipolar cycloaddition reactions [1, 2], but also useful radical trap in electron paramagnetic resonance (EPR) studies of organic radicals [3] and the biology system (Fig. 1) [4]. In the biomedical field, their fascinating radical trapping capability has endowed nitrone derivatives promising therapeutic agents in the eradication of reactive oxygen species (ROS), which are related to many pathological conditions, including neurodegenerative diseases, cancer, stroke, diabetes, et al. [5–8]. However, despite this attractive property, commonly applied nitrone-based therapeutic agents or probes are far from being diverse. Novelli et al. first demonstrated the therapeutic application of nitrone by showing that phenyl *tert*-butyl nitrone (PBN) can protect rats from lethal trauma and circulatory shock [9, 10]. Since then, PBN analogues have been widely explored, and the 2,4-disulfonylphenyl PBN derivative, NXY-059, was found to be safe in humans and efficacious in the treatment of acute ischemic stroke [11]. Although it did not pass clinical phase III, its highly attractive safety profile incites intensive interest in further development. NXY-059 was found to be very effective in the treatment of several preclinical glioma models [12, 13], and shows an effect in the treatment of neurogenerative diseases in combination with

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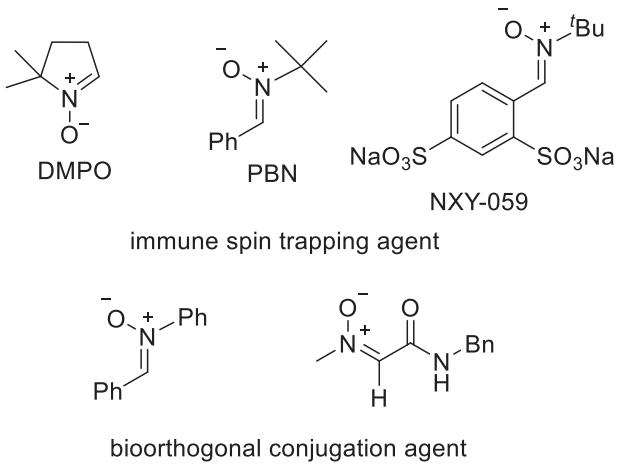


Fig. 1 Nitrone-type small molecules as functional entities due to their superb radical trapping properties

neurotropic compounds [14]. Floyd et al. found that PBN was promising in the well-known dietary choline-deficiency rat liver cancer model [15], and was highly effective against hepatocarcinoma and glioma models [16] by oral delivery. The effects were attributed to suppression of oxidative damage by ROS while causing very little toxicity. In addition, PBN was also shown to decrease iNOS activity [17], making it potentially useful in brain tumors, where increasing levels of iNOS are found. In the past two decades, there is increasingly well recognized that ROS plays a key role in the sustainment of cancer and as a potential target for cancer chemotherapy [18–22]. As ROS and oxidative stress can induce cancer, and transformed cells seem to generate more ROS compared to their normal counterparts, applying ROS modulators such as nitrones as chemotherapy agents may be a highly promising strategy. Despite these facts, however, the identification of novel nitrone-based therapeutic agents remains highly underdeveloped owing to the lack of synthetically available nitrone subtypes. Especially, in view of the low toxicity profile of these compounds, development of novel nitrone-type agents would not only be highly desirable for the capability to bring up novel therapeutic subtypes, but also provides new avenues for the elucidation of the mechanism of action of these compounds.

We recently engaged in the development of novel methodologies for the preparation of nitrones, and documented several metal-catalyzed routes for their efficient preparation [23–25]. Given the high promise of nitrone and the available compound library produced from these studies, we initiated a program aimed at evaluating in-vitro anti-proliferative screening processes against cancer cell lines. Herein we would like to report our preliminary results in the identification of two types of *N*-heterocycle fused nitrones as potential anti-cancer agents.

Results and discussion

Initially, a few representative nitrone compounds of different structural subtypes were synthesized according to a mild Cu- and Pd-catalyzed intramolecular Cope-type amination protocol from alkenyl oxime was first evaluated [16, 17]. The antiproliferative effects of the compounds were initially performed at 40 μM concentration against a panel of 5 cancer cell lines, including HL-60 (leukemia), A549 (lung cancer), SMMC-7721 (liver cancer), MCF-7 (breast cancer), as well as SW480 (colon cancer), by MTS assay, as summarized in Fig. 2.

As can be seen from Fig. 2, among the five type of nitrone-type compounds, the pyrrole- and indole-fused nitrone derivatives **4a** and **5a** showed promising anti-proliferative activities, with **4a** being selective against A549 cell line, and **5a** showing selectivity against HL-60 cell line, respectively. While compounds **1** and **2** also showed some activities against specific cell lines (MCF-7 for **1a** and SW-480 for **1b**, **1c**, and **2**), these compounds were not considered further as their activities against other cell lines were much less pronounced. Based on this observation, a panel of pyrrole- and indole-fused nitrone, namely, 3,4-dihydropyrrolo[1,2-*a*]pyrazine 2-oxide derivatives and 3,4-dihydropyrazino[1,2-a]indole 2-oxide derivatives were synthesized and evaluated.

The pyrrole-fused nitrone derivatives were prepared by a three-step sequence from the corresponding pyrrole-2-aldehyde or 2-acetylpyrrole, involving *N*-allylation, oxime formation, and Pd-catalyzed intramolecular Cope-type hydroamination (Scheme 1) [The structure and naming of the oxime starting materials is provided in Fig. S1 of the supplementary material]. The last step constituents a highly effective neutral annulation protocol for the assembly of these 6-membered cyclic nitrones, tolerating a variety of substituents at 3-, 4-, and 5-position, including even Bromo and iodine atoms, which are typically labile under Pd catalysis.

Initial screening at 40 μM drug concentration was executed and the results are summarized in Fig. 3. From the data some interesting points can be inferred: (1) like **5a**, a majority of compounds displayed selective inhibition against HL-60 cell line. (2) **5d** unexpectedly showed selectivity towards A549 cell line, suggesting that 4-position on the pyrrole ring may be further exploited for selective purposes. The relatively good efficacy against A549 displayed by **5b**, **5d**, and **5f** may be attributed to the electron-withdrawing property of the substituents at 4- and 5-positions. (3) These compounds also generally demonstrate activity against SMMC-7721 cell line, but their activities against MCF-7 and SW480 remain low. Among these analogues, 5-iodo analogue **5l** displayed the highest activity against SW480 cell line among these series of compounds (31.5%) [The structure and naming of the

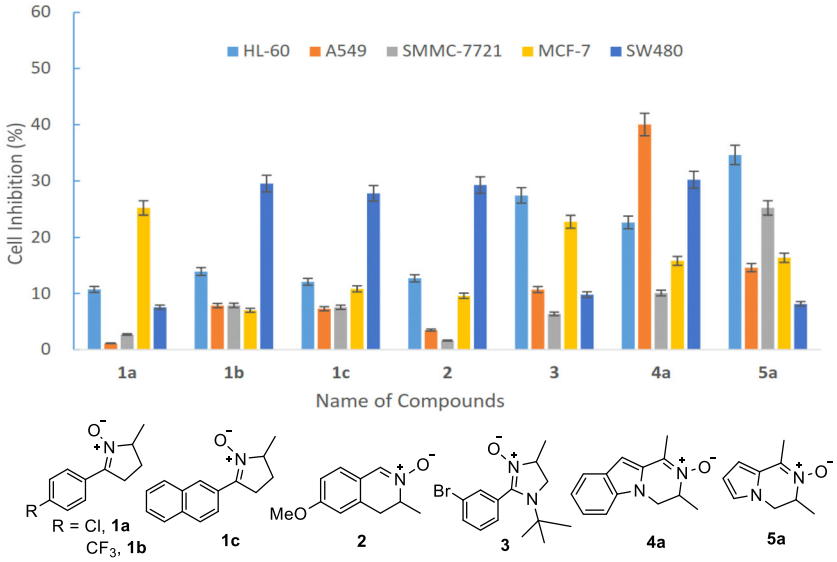
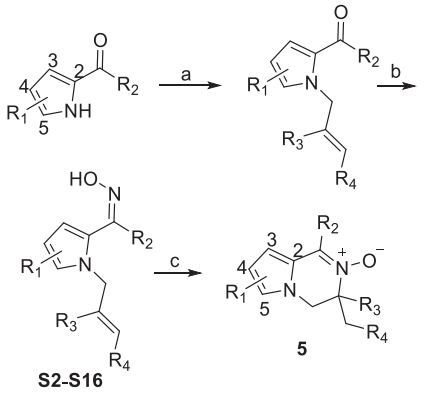


Fig. 2 Antiproliferative activities of nitrones **1–5** incubated at 40 μM against a panel of 5 cancer cell lines

Scheme 1 Synthesis of 3,4-dihydropyrrolo[1,2-*a*]pyrazine 2-oxide derivatives



Conditions: a) RBr, NaOH, DMSO, 30 °C; b) NH_2OH•HCl, CH_3CO_2Na, MeOH, 78 °C; c) 10 mol% $PdCl_2(CH_3CN)_2$, CH_2Cl_2, 100 °C (sealed).

Compound	R_1	R_2	R_3	R_4	yield/%
5b	4,5-Br_2	Me	H	H	51
5c	5-NO_2	Me	H	H	93
5d	4-NO_2	Me	H	H	81
5e	4-Br	H	H	H	49
5f	4,5-I_2	Me	H	H	87
5g	H	H	Me	H	26
5h	5-CF_3	Me	H	H	88
5i	3,5-Me_2-4-CO_2Me	H	H	H	35
5j	H	H	H	H	51
5k	H	Et	H	H	71
5l	4-I	Me	H	H	65
5m	H	Me	Me	H	64
5n	3,5-Me_2	H	H	H	26
5o	H	Me	H	Ph	26
5p	5-Br	Me	H	H	40

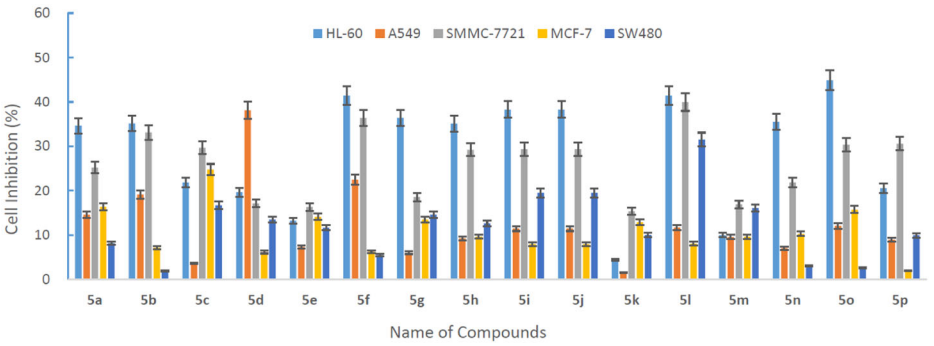
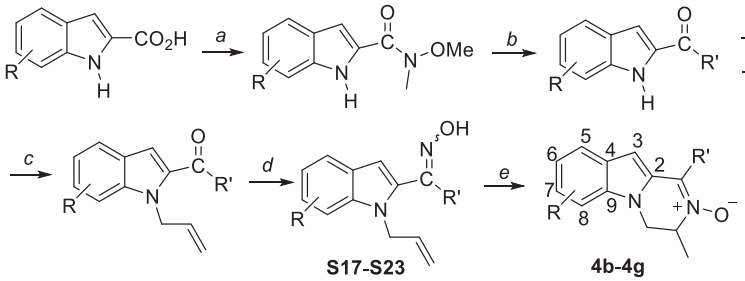


Fig. 3 In vitro antiproliferative activities of **5a–5p** incubated at 40 μM against a panel of 5 cancer cell lines

oxime starting materials is provided in Fig. S1 of the supplementary material].

Furthermore, several indole-fused nitrones **4b–e** were also synthesized and evaluated. These compounds were obtained from the corresponding carboxylic acids, which were converted to Weinreb amides, followed by methyl lithium addition to afford the corresponding methyl ketones. Then, following a similar sequence as above, the corresponding nitrones were obtained in high yields (Scheme 2).

The preliminary antiproliferative effects of these compounds at 40 μM against the five cancer cell lines are shown in Fig. 4. It can be inferred from Fig. 4 that compared to the



Compd	R	R'	yield/%
4b	6-Cl	Me	94
4c	6-Me	Me	81
4d	6-OMe	Me	91
4e	7-OMe	Me	87
4f	3-Br-6OMe	Me	65
4g	H	H	45

Condition: a) MeNHOMe•HCl, DCC, Et_3N, DCM; b) MeLi, THF, -78 °C; c) RBr, NaOH, DMSO, 30 °C; d) NH_2OH•HCl, CH_3CO_2Na, MeOH, 78 °C; e) 10 mol% $PdCl_2(CH_3CN)_2$, DCM, 100 °C (sealed)

Scheme 2 Synthetic scheme for the indole-fused nitrones **4**

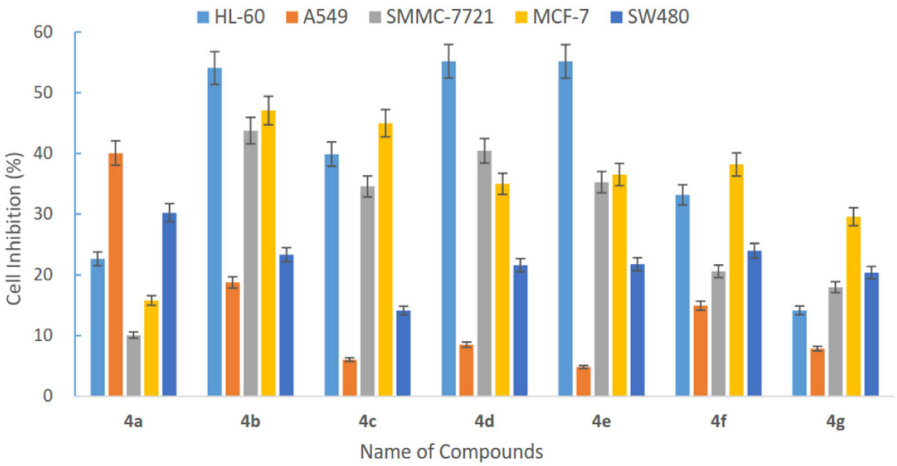


Fig. 4 In vitro anti-proliferation of indole-based nitrone analogues **4a**–**4g** at 40 μM against 5 cancer cell lines

activity of **4a**, introduction of 6- and 7-substituents most significantly improved the activities against HL-60 cells. Appreciable improvement in the activities against SMMC-7721 was observed for **4b**–**4e**, but not for **4f** and **4g**, which is in line with the results against HL-60 cell line. Similar patterns were also found for the activities against MCF-7. Compared to **4a**, substituted analogues **4b**–**4g** showed even worse activity against A549 and SW-480 cell lines. These observations on the cell line specificity suggest a particular mechanism of action that targets more effectively against HL-60, SMMC-7721, and MCF-7 but not for A549 and SW480 cell lines, on which further studies are required. In contrast, the comparatively inferior overall activities displayed by **4f** and **4g** suggest that functionalization at the 3-position of indole may be detrimental, and that the nitrone-substitution R' is beneficial to the anti-proliferative effects.

After obtaining preliminary in vitro single-dose data, we subsequently selected several compounds that displayed the highest activities in the initial screening for the determination of IC_{50} values. The results were summarized in Table 1. As can be seen, while unfortunately, **5d** and **5l** were found to be ineffective in the dose-dependent activity test, showing that their activities may vary depending on stability reasons, compound **4b** was found to be moderately active against SMMC-7721 cell line; and **4d** was found to be a good candidate against leukemia model HL-60 cell line, demonstrating an IC_{50} of 36.16 μM, showing that these compounds hold promise for further applications in anti-cancer pharmaceutics, and selectivity against different cell lines may be realized by substitution modulation. [There seem to be some discrepancy of activity between single dose and dose-dependent activities of the tested compounds; we suspect that this may arise from the mediocre stability of these compounds in solution under aerobic conditions].

Conclusion

In summary, we have prepared two types of novel nitrone-type compounds in moderate to good yields. Some of the new compounds exhibited anticancer activities against the human tumor cell lines HL-60, A549, SMMC-7721, MCF-7, and SW-480 in the 40 μM initial screening, based on which an indole-fused nitrone analogue, **4d**, with good anti-proliferative activities against HL-60 cell line, is identified. The data support the conclusion that 3,4-dihydropyrazino

Table 1 IC_{50} values determined of selected compounds

Cmpd.	IC_{50}/μM				
	HL-60	A549	SMMC-7721	MCF-7	SW-480
5d	>200	>200	>200	>200	>200
5l	>200	>200	>200	>200	>200
4b	62.35 ± 1.11	170.02 ± 3.50	105.66 ± 15.54	>200	>200
4d	36.16 ± 0.89	>200	>200	>200	>200
DPP	4.96 ± 0.28	25.33 ± 0.73	11.13 ± 1.22	18.30 ± 1.33	21.74 ± 0.30
Taxol	<0.008	<0.008	0.134 ± 0.011	<0.008	<0.008

[1,2-a]indole 2-oxide derivatives are promising novel scaffold for anti-tumor drug development. We anticipate that these promising results may incite more extensive interest in the development of nitrone-based therapeutic agents. Further improvement upon these discoveries is undergoing in our laboratory.

Experimental

Unless otherwise noted, all reactions were carried out with distilled and dried solvents a nitrogen atmosphere. All commercial chemicals were used without further purification. Tetrahydrofuran and toluene was distilled from calcium hydride. All $Pd(CH_3CN)_2Cl_2$, NaO*t*-Bu, $NH_2OH{\cdot}HCl$, ketones and aldehydes were purchased from commercial sources. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker 400 spectrometer at 22 °C. Chemical shifts (δ) were expresses in part per million (ppm) relative to internal standards (0 ppm (TMS) for ^1H NMR and 77.0 ppm ($CDCl_3$) for ^{13}C NMR). High resolution mass spectra were recorded on a Bruker microTOF spectrometer. Flash chromatography was performed on silica gel 60 particle size 300–400 mesh ASTM, purchase from Taizhou, China. Cells were and obtained from Shanghai National Collection of Authenticated Cell Cultures (HL-60, A549, MCF-7, and SW480) or Beijing Bei Na Biotechnology Institute (SMMC-7721) and maintained in DMEM or RPMI1640 (Gibco) supplemented with 10% FBS.

MTS cell anti-proliferation assay

40 μM screening: cells were grown to confluence, seeded (3000–15,000 cells/well, 100 μL total media) in clear, flat-bottom 96-well plates and allowed to attach overnight. Compounds at 40 μM in DMSO (final volume: 200 μL) was added. Three blank cells were reserved for blank control (1% DMSO). Cells were returned to the incubator for an additional 48 h. After 48 h, for attaching cells media was removed, and MTS (20 μL/cell) and media (100 μL/cell) were added. For non-attaching cells, 100 μL of supernatant was removed and 20 μL of MTS is added. Cells are then cultivated for a further 2–4 h before being tested by absorption by a microplate reader (MULTISKAN FC) at 492 nm. Cells incubated in 1% DMSO were used as 100% proliferation (i.e., DMSO = 100% growth) and the relative growth for each compound was compared to 1% DMSO. DPP and Taxol were used as positive control. IC_{50} values of DPP and Taxol were calculated by Reed and Muench method from two separate experiments performed in triplicate.

IC_{50} value determination: cells were grown and seeded as above. Compounds of five different concentrations (200, 100, 50, 25, and 12.5 μM), along with positive controls (DPP and Taxol) were added to the cell plates, and the Cells were returned to the incubator for an additional 48 h. After 48 h, for attaching cells media was removed, and MTS (20 μL/cell) and media (100 μL/cell) were added. For non-attaching cells, 100 μL of supernatant was removed and 20 μL of MTS is added. Cells are then cultivated for a further 2–4 h before being tested by absorption by a microplate reader (MULTISKAN FC) at 492 nm. Cells incubated in 1% DMSO were used as 100% proliferation (i.e., DMSO = 100% growth) and the relative growth for each compound was compared to 1% DMSO. IC_{50} values were calculated by Reed and Muench method from two separate experiments performed in triplicate. DPP and Taxol were used as positive control.

Synthesis of nitrones: to a solution of oximes (0.2 mmol, 1.0 equiv.) in 2 ml anhydrous DCM was added $PdCl_2(CH_3CN)_2$ (0.02 mmol, 10 mol%). The resulting solution was heated to 100 °C for 20 h. The solvent was removed in vacuo and the residue was purified by flash chromatograph to afford nitrones. Compounds **1a** [24], **1b** [24], **1c** [24], **2** [24], **3** [24], **4a** [25], **4g** [25], **5a** [25], **5j** [25], **5k** [25], **5l** [25], **5m** [24], **5n** [25], **5o** [24] were synthesized following previously reported procedures.

8-Chloro-1,3-dimethyl-3,4-dihydropyrazino[1,2-a]indole 2-oxide **(4b)**: following the general produce, the reaction of oxime **S18** (0.2 mmol, 49.7 mg), $PdCl_2(CH_3CN)_2$ (0.02 mmol, 5.2 mg) afforded product **(4b)** (46.8 mg, 94 % yield) as a colorless oil. ^1H NMR (400 MHz, $CDCl_3$) δ 7.56 (d, $J = 1.6$ Hz, 1H), 7.21 (dd, $J = 8.7$, 1.8 Hz, 1H), 7.15 (d, $J = 8.7$ Hz, 1H), 6.58 (s, 1H), 4.37 (dd, $J = 15.9$, 4.9 Hz, 2H), 4.11 (dd, $J = 12.1$, 3.2 Hz, 1H), 2.41 (s, 3H), 1.57 (d,

$J = 6.7$ Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 135.76 (s), 135.55 (s), 131.78 (s), 129.74 (s), 126.34 (s), 124.13 (s), 120.74 (s), 109.74 (s), 101.30 (s), 63.57 (s), 45.45 (s), 16.94 (s), 13.45 (s). HRMS(ESI) calcd for $C_{13}H_{14}ClN_2O^+$ ([M+H]$^+$): 249.0789, found 249.0786.

1,3,8-Trimethyl-3,4-dihydropyrazino[1,2-a]indole 2-oxide **(4c)**: following the general produce, the reaction of oxime **S19** (0.2 mmol, 45.7 mg), $PdCl_2(CH_3CN)_2$ (0.02 mmol, 5.2 mg) afforded product **(4c)** (37.1 mg, 81% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (s, 1H), 7.12 (d, $J = 2.7$ Hz, 2H), 6.57 (s, 1H), 4.33 (dd, $J =$ 19.0, 6.4 Hz, 2H), 4.19–3.98 (m, 1H), 2.42 (d, $J = 5.4$ Hz, 6H), 1.56 (d, $J = 6.5$ Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 135.97 (s), 135.88 (s), 130.56 (s), 129.99 (s), 129.02 (s), 125.60 (s), 121.00 (s), 108.39 (s), 101.66 (s), 63.48 (s), 45.30 (s), 21.26 (s), 16.90 (s), 13.43 (s). HRMS(ESI) calcd for $C_{14}H_{17}N_2O^+$ ([M+H]$^+$): 229.1335, found 229.1344.

8-Methoxy-1,3-dimethyl-3,4-dihydropyrazino[1,2-a] indole 2-oxide **(4d)**: following the general produce, the reaction of oxime **S20** (0.2 mmol, 48.9 mg), $PdCl_2(CH_3CN)_2$ (0.02 mmol, 5.2 mg) afforded product **(4d)** (44.4 mg, 91% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.13 (d, $J = 8.9$ Hz, 1H), 7.04 (d, $J = 2.1$ Hz, 1H), 6.93 (dd, $J =$ 8.9, 2.2 Hz, 1H), 6.57 (s, 1H), 4.33 (dd, $J = 19.0$, 6.5 Hz, 2H), 4.07 (dd, $J = 12.2$, 3.0 Hz, 1H), 3.83 (s, 3H), 2.41 (s, 3H), 1.56 (d, $J = 6.5$ Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.73 (s), 135.90 (s), 132.82 (s), 131.04 (s), 129.23 (s), 114.52 (s), 109.54 (s), 102.70 (s), 101.72 (s), 63.46 (s), 55.73 (s), 45.48 (s), 16.90 (s), 13.42 (s). HRMS(ESI) calcd for $C_{14}H_{17}N_2O_2^+$ ([M+H]$^+$): 245.1285, found 245.1279.

7-Methoxy-1,3-dimethyl-3,4-dihydropyrazino[1,2-a] indole 2-oxide **(4e)**: following the general produce, the reaction of oxime **S21** (0.2 mmol, 48.9 mg), $PdCl_2(CH_3CN)_2$ (0.02 mmol, 5.2 mg) afforded product **(4e)** (42.7 mg, 87 % yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (d, $J = 8.7$ Hz, 1H), 6.78 (dd, $J = 8.7$, 2.0 Hz, 1H), 6.67 (s, 1H), 6.59 (s, 1H), 4.45–4.23 (m, 2H), 4.07 (dd, $J = 12.4$, 3.3 Hz, 1H), 3.86 (s, 3H), 2.39 (s, 3H), 1.56 (d, $J = 6.7$ Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.96 (s), 138.51 (s), 136.08 (s), 129.61 (s), 123.02 (s), 122.23 (s), 110.88 (s), 102.42 (s), 92.01 (s), 63.24 (s), 55.55 (s), 45.29 (s), 16.93 (s), 13.41 (s). HRMS(ESI) calcd for $C_{14}H_{17}N_2O_2^+$ ([M+H]$^+$): 245.1285, found 245.1279.

10-Bromo-8-methoxy-1,3-dimethyl-3,4-dihydropyrazino [1,2-a]indole 2-oxide **(4f)**: following the general produce, the reaction of oxime **S22** (0.2 mmol, 64.6 mg), $PdCl_2(CH_3CN)_2$ (0.02 mmol, 5.2 mg) afforded product **(4f)** (42.9 mg, 65% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.10 (d, $J = 9.4$ Hz, 1H), 6.96 (d, $J = 7.4$ Hz, 2H), 4.32 (dd, $J = 21.4$, 8.7 Hz, 2H), 4.14–3.97 (m, 1H), 3.86 (s, 3H), 2.69 (s, 3H), 1.51 (d, $J = 6.6$ Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.26 (s), 135.82 (s), 130.81 (s), 129.13 (s), 126.48 (s), 116.20 (s), 109.67 (s), 100.80 (s), 90.90 (s), 63.81 (s), 55.71 (s), 45.28 (s), 16.48 (s), 13.65 (s). HRMS(ESI) calcd for $C_{14}H_{16}BrN_2O_2^+$ ([M+H]$^+$): 323.0390, found 323.0393.

6,7-Dibromo-1,3-dimethyl-3,4-dihydropyrrolo[1,2-a] pyrazine 2-oxide **(5b)**: following the general produce, the reaction of oxime **S2** (0.2 mmol, 64.4 mg), $PdCl_2(CH_3CN)_2$ (0.02 mmol, 5.2 mg) afforded product **5b** (33.0 mg, 51% yield)as a colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.40 (s, 1H), 4.44–4.13 (m, 2H), 3.98 (d, $J = 13.0$ Hz, 1H), 2.27 (s, 3H), 1.53 (d, $J = 6.7$ Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 134.42 (s), 126.89 (s), 111.22 (s), 107.17 (s), 100.93 (s), 62.93 (s), 48.38 (s), 16.24 (s), 12.98 (s). HRMS (ESI) calcd for $C_9H_{11}Br_2N_2O^+$ ([M+H]$^+$): 320.9233, found 320.9225.

1,3-Dimethyl-6-nitro-3,4-dihydropyrrolo[1,2-a] pyrazine 2-oxide **(5c)**: following the general produce, the reaction of oxime **S3** (0.2 mmol, 42.0 mg), $PdCl_2(CH_3CN)_2$ (0.02 mmol, 5.2 mg) afforded product **5c** (39.3 mg, 93% yield) as a colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (d, $J = 4.0$ Hz, 1H), 6.37 (d, $J = 4.5$ Hz, 1H), 4.83 (dd, $J =$ 14.4, 5.3 Hz, 1H), 4.74 (dd, $J = 14.4$, 4.9 Hz, 1H), 4.34 (dd, $J = 11.4$, 6.0 Hz, 1H), 2.35 (s, 3H), 1.58 (t, $J = 5.2$ Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 137.95 (s), 134.00 (s), 130.79 (s), 115.35 (s), 108.17 (s), 63.01 (s), 48.03 (s), 16.04 (s), 13.03 (s). HRMS(ESI) calcd for $C_9H_{12}N_3O_3^+$ ([M+H]$^+$): 210.0873, found 210.0879.

1,3-Dimethyl-7-nitro-3,4-dihydropyrrolo[1,2-a] pyrazine 2-oxide **(5d)**: following the general produce, the reaction of oxime **S4** (0.2 mmol, 42.0 mg), $PdCl_2(CH_3CN)_2$ (0.02 mmol, 5.2 mg) afforded product **5d** (34.3 mg, 81% yield) as a colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (s, 1H), 6.83 (s, 1H), 4.45 (dd, $J = 13.4$, 4.5 Hz, 1H), 4.35 (s, 1H), 4.02 (dd, $J = 13.3$, 3.7 Hz, 1H), 2.33 (s, 3H), 1.55 (d, $J = 6.8$ Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.17 (s), 134.05 (s), 125.83 (s), 122.65 (s), 103.24 (s), 63.15 (s), 49.02 (s), 29.54 (s), 16.19 (s), 13.04 (s). HRMS(ESI) calcd for $C_9H_{12}N_3O_3^+$ ([M+H]$^+$): 210.0873, found 210.0879.

7-Bromo-3-methyl-3,4-dihydropyrrolo[1,2-a]pyrazine 2-oxide **(5e)**: following the general produce, the reaction of oxime **S5** (0.2 mmol, 46.0 mg), $PdCl_2(CH_3CN)_2$ (0.02 mmol, 5.2 mg) afforded product **5e** (22.7 mg, 49% yield) as a colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (s, 1H), 6.72 (s, 1H), 6.27 (s, 1H), 4.31 (dd, $J = 13.1$, 4.5 Hz, 1H), 4.19 (d, $J = 6.0$ Hz, 1H), 3.90 (dd, $J = 13.1$, 4.0 Hz, 1H), 1.52 (d, $J = 6.7$ Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 125.12 (s), 124.33 (s), 122.62 (s), 111.36 (s), 98.47 (s), 63.49 (s), 48.95 (s), 16.12 (s). HRMS(ESI) calcd for $C_8H_{10}BrN_2O^+$ ([M+H]$^+$): 228.9971, found 228.9966.

6,7-Diiodo-1,3-dimethyl-3,4-dihydropyrrolo[1,2-a] pyrazine 2-oxide **(5f)**: following the general produce, the reaction of oxime **S6** (0.1 mmol, 41.6 mg), $PdCl_2(CH_3CN)_2$ (0.01 mmol, 2.6 mg) afforded product **5f** (36.0 mg, 87% yield) as a colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.37

(s, 1H), 4.32–4.13 (m, 2H), 3.96 (dd, $J = 13.0$, 3.7 Hz, 1H), 2.24 (s, 3H), 1.50 (d, $J = 6.6$ Hz, 3H); ^{13}CNMR (101 MHz, $CDCl_3$) δ 134.08 (s), 126.91 (s), 111.00 (s), 106.95 (s), 100.82 (s), 62.86 (s), 48.31 (s), 16.14 (s), 12.84 (s). HRMS (ESI) calcd for $C_9H_{11}I_2N_2O^+$ ($[M+H]^+$): 416.8955, found 416.8954.

3,3-Dimethyl-3,4-dihydropyrrolo[1,2-a] pyrazine 2-oxide **(5g)**: following the general produce, the reaction of oxime **S7** (0.2 mmol, 32.8 mg), $PdCl_2(CH_3CN)_2$ (0.02 mmol, 5.2 mg) afforded product **5g** (8.4 mg, 26% yield) as a colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (s, 1H), 6.71 (s, 1H), 6.31–6.09 (m, 2H), 4.01 (s, 2H), 1.50 (s, 7H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 126.04 (s), 124.24 (s), 123.00 (s), 110.83 (s), 109.46 (s), 66.47 (s), 54.62 (s), 23.18 (s). HRMS (ESI) calcd for $C_9H_{13}N_2O^+$ ($[M+H]^+$): 165.1022, found 165.1030.

1,3-Dimethyl-6-(trifluoromethyl)-3,4-dihydropyrrolo[1,2-a]pyrazine 2-oxide **(5h)**: following the general produce, the reaction of oxime **S8** (0.2 mmol, 46.4 mg), $PdCl_2(CH_3CN)_2$ (0.02 mmol, 5.2 mg) afforded product **5h** (41.1 mg, 88% yield) as a colorless oil. ^{1}H NMR (400 MHz, Acetone-d_6) δ 6.70 (s, 1H), 6.40 (d, $J = 2.6$ Hz, 1H), 4.50 (dd, $J = 13.2$, 3.9 Hz, 1H), 4.28 (s, 1H), 4.19 (dd, $J = 13.2$, 3.8 Hz, 1H), 2.22 (s, 3H), 1.45 (d, $J = 6.4$ Hz, 3H); ^{13}C NMR (101 MHz, Acetone) δ 133.32, 131.57, 122.45 (q, $J = 38.4$ Hz), 122.35 (q, $J = 267.7$ Hz), 113.55 (q, $J = 4.0$ Hz), 107.52, 64.03, 48.17, 16.33, 13.25. ^{19}F NMR (376 MHz, Acetone-d_6) δ -59.23 (s). HRMS (ESI) calcd for $C_{10}H_{12}F_3N_2O^+$ ($[M+H]^+$): 233.0896, found 2333.0896.

7-(Methoxycarbonyl)-3,6,8-trimethyl-3,4-dihydropyrrolo [1,2-a]pyrazine 2-oxide **(5i)**: following the general produce, the reaction of oxime **S9** (0.2 mmol, 47.3 mg), $PdCl_2(CH_3CN)_2$ (0.02 mmol, 5.2 mg) afforded product **5i** (16.5 mg, 35% yield) as a colorless oil. ^{1}H NMR (400 MHz, Acetone-d_6) δ 7.56 (s, 1H), 4.50–4.19 (m, 1H), 4.09 (dd, $J = 22.3$, 9.1 Hz, 2H), 3.76 (s, 3H), 2.49 (s, 3H), 2.22 (s, 3H), 1.42 (d, $J = 6.4$ Hz, 3H); ^{13}C NMR (101 MHz, Acetone) δ 165.86 (s), 138.49 (s), 122.76 (s), 122.13 (s), 119.66 (s), 113.58 (s), 63.67 (s), 50.48 (s), 46.22 (s), 15.81 (s), 10.97 (s), 10.50 (s). HRMS(ESI) calcd for $C_{12}H_{17}N_2O_3^+$ ($[M+H]^+$): 237.1234, found 237.1219.

6-Bromo-1,3-dimethyl-3,4-dihydropyrrolo[1,2-a] pyrazine 2-oxide **(5p)**: following the general produce, the reaction of oxime **S16** (0.1 mmol, 24.3 mg), $PdCl_2(CH_3CN)_2$ (0.01 mmol, 2.6 mg) afforded product **5p** (9.9 mg, 40% yield) as a colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.76 (s, 1H), 6.42 (s, 1H), 4.35 (d, $J = 13.1$ Hz, 1H), 4.25 (s, 1H), 3.92 (d, $J = 13.4$ Hz, 1H), 2.29 (s, 3H), 1.51 (d, $J = 6.6$ Hz, 3H). ^{13}C NMR (101 MHz, Acetone-d_6) δ 132.60 (s), 129.00 (s), 128.29 (s), 114.52 (s), 63.99 (s), 61.05 (s), 49.07 (s), 16.18 (s), 12.94 (s). HRMS (ESI) calcd for $C_9H_{12}BrN_2O^+$ ($[M+H]^+$): 243.0128, found 243.0130.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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