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Synthesis and anti-phytopathogenic activity of 8-hydroxyquinoline derivatives†

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Phytopathogenic fungi have become a serious threat to the quality of agricultural products, food security and human health globally, necessitating the need to discover new antifungal agents with *de novo* chemical scaffolds and high efficiency. A series of 8-hydroxyquinoline derivatives were designed and synthesized, and their antifungal activity was evaluated against five phytopathogenic fungi. *In vitro* assays revealed that most of the tested compounds remarkably impacted the five target fungi and their inhibitory activities were better than that of the positive control azoxystrobin. Compound **2**, in particular, exhibited the highest potency among all the tested compounds, with an EC_{50} of 0.0021, 0.0016, 0.0124, 0.0059 and 0.0120 mM respectively against *B. cinerea*, *S. sclerotiorum*, *F. graminearum*, *F. oxysporum* and *M. oryzae*, followed by compound **5c**. The morphological observations of optical microscopy and scanning electron microscopy revealed that compounds **2** and **5c** caused mycelial abnormalities of *S. sclerotiorum*. Futhermore, the results of *in vivo* antifungal activity of compounds **2** and **5c** against *S. sclerotiorum* showed that **5c** possessed stronger protective and curative activity than that of **2**, and the curative effects of **5c** at 40 and 80 μg mL^{-1} (84.18% and 95.44%) were better than those of azoxystrobin (77.32% and 83.59%). Therefore, compounds **2** and **5c** are expected to be novel lead structures for the development of new fungicides.

1. Introduction

Phytopathogenic fungi are often the main culprits in causing immense decrease in yield and quality of agricultural products, leading to serious losses in global agricultural and horticultural production, hence posing a great threat to global food security.[1,2] More importantly, many phytopathogenic fungi can produce mycotoxins that are pernicious to human and animal health.[1] Therefore, various antifungal agents have been discovered, developed and used for a long time to guarantee wholesome crops, increases in crop yields and economic benefits.[3,4] However, excessive use and misuse of many traditional antifungal agents have led to heightened resistance in target phytopathogenic fungi, residual toxicity, and even environmental pollution in recent decades.[5–7] The above concerns have called for discovery and development of novel antifungal compounds with lower application dose, higher efficiency and selectivity, unique mode of action and environmental compatibility.[3,8]

N-Heterocycle plays a key role in drug design.[9] Quinoline and its derivatives from natural products or synthetic biologically active sources are indispensable heterocyclic compounds endowed with a broad spectrum of pharmacological properties.[10–13] Amidst quinoline core compounds, 8-hydroxyquinoline (HQ) has become a privileged scaffold for the design and synthesis of novel drug candidates due to its broad biological activities,[14–17] such as cytotoxic,[18–20] antifungal,[20,21] antibacterial,[22,23] antifilarial,[24] and anti-HIV.[25] The mode of action of HQ is related to many factors, according to reports, chelation with metal ions appears to be crucial because metal ions are cofactors for many physiologically active enzymes.[17,26,27]

Highly destructive phytopathogenic fungi *S. sclerotiorum*, *B. cinerea*, *F. graminearum*, *F. oxysporum* and *M. oryzae* have garnered considerable research attention owing to their typical pathogenic characteristics. In this investigation, we chose HQ as a primer molecule and introduced the nitro group into the HQ scaffold (Fig. 1). Motivated by compound **2** displaying superb antifungal activity than HQ and the positive control azoxystrobin against the five target phytopathogens tested, we further structurally derivatized compound **2**. Interestingly, preliminary work showed that the 2-position modification of compound **2** resulted in a dramatic decrease in antifungal activity, whereas the 7-position modification of compound **2** with identical groups led to improved or comparable antifungal activity with HQ (ESI†). Thus,

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a series of HQ derivatives were synthesized to investigate their antifungal potential and structural activity relationships (SAR). Furthermore, optical microscope and scanning electron microscopy observations and effects of 8-hydroxyquinoline derivatives against *S. sclerotiorum in vivo* were performed to evaluate the antifungal properties of these compounds.

2. Results and discussion

2.1. Chemistry

The starting material 8-hydroxyquinoline (**1**) employed in the preparation of compound **2** was obtained from Sun Chemical Technology (Shanghai). Stirring the starting material **1** in concentrated HCl with $NaNO_2$ aqueous solution at 0 °C, the precipitates formed were filtered, and added to a mixture of concentrated HNO_3 and water (v/v = 3 : 2) at 17 °C to be transformed into **2** (Fig. 1).[28] Compounds **4a–4o** and **5a–5q** were conveniently assembled in a one-step synthesis according to classic Mannich reaction (Fig. 1). Compounds **4a–4o** were prepared by refluxing **2** with formaldehyde and corresponding aliphatic amine in ethanol.[29] Compounds **5a–5p** were synthesized by refluxing compound **2** with formaldehyde and appropriate aromatic piperazine in pyridine.[23] All the synthesized compounds were purified by recrystallization from absolute ethanol. Structures of all the synthesized compounds were supported by spectral data including ^1H NMR, ^{13}C NMR, and HRMS as reported in Experimental section.

2.2. Antifungal activity

The target compounds were evaluated for their antifungal activity against five economically important phytopathogenic fungi *B. cinerea*, *S. sclerotiorum*, *F. graminearum*, *F. oxysporum* and *M. oryzae*. The preliminary antifungal activity screening of the target compounds was determined at 25 and 50 μg mL^{-1}

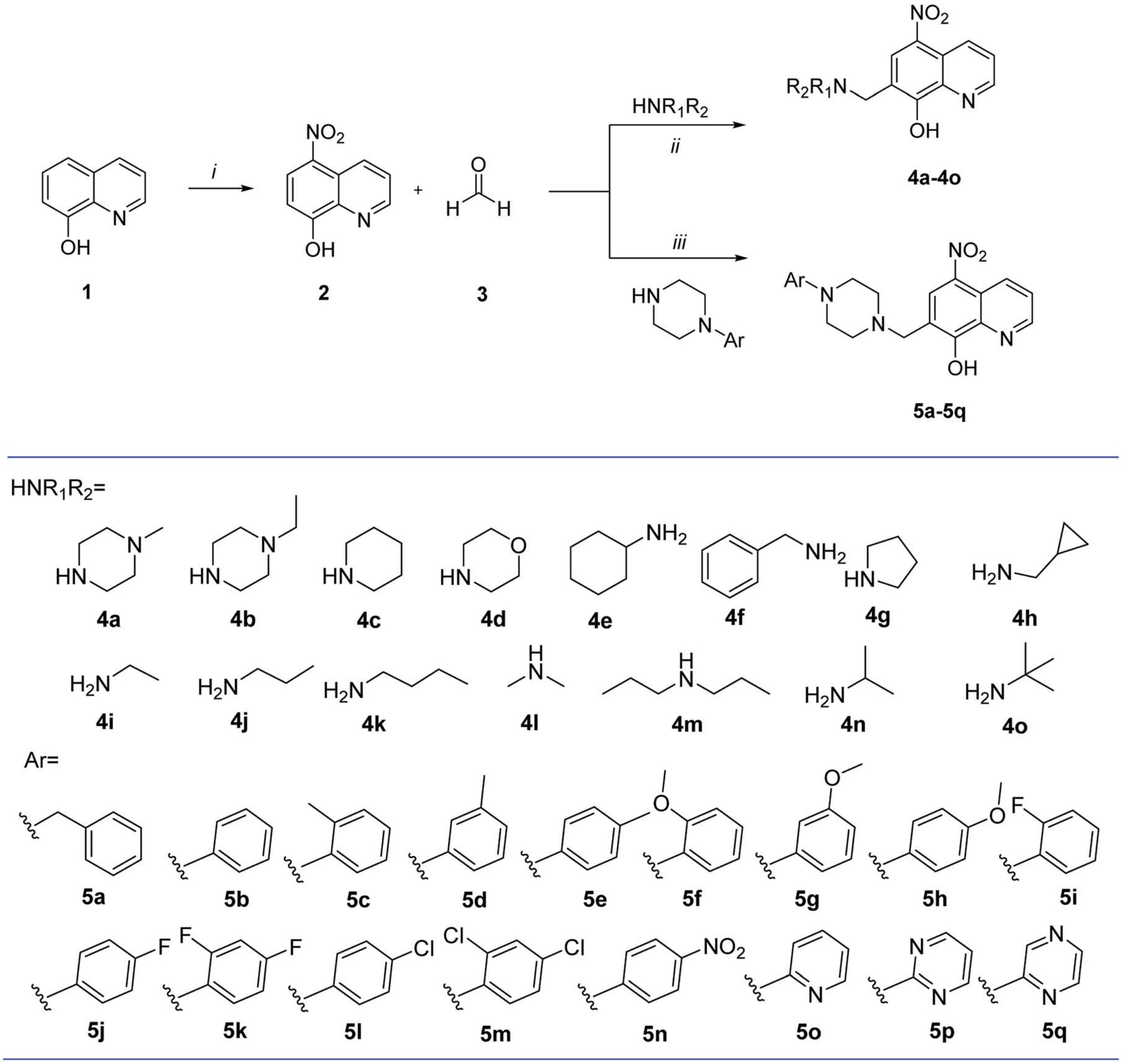

Fig. 1 Reagents and conditions: (i) $NaNO_2$, concentrated HCl, 0 °C, 1 h; HNO_3 : H_2O (3 : 2), 17 °C, 75 min; (ii) EtOH, reflux, 24 h; (iii) pyridine, 50 °C, 30 min.

Table 1 Antifungal activity of 8-hydroxyquinoline derivatives at 50, 25 μg mL^{-1}

		Average inhibition rate ± SD (%) ($n = 3$)				
Compd.[a]	Conc[b] (μg mL^{-1})	*B. C.*[c]	*S. S.*[c]	*F. G.*[c]	*F. O.*[c]	*M. O.*[c]
HQ	50	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	73.01 ± 0.33	100.00 ± 0.00
	25	100.00 ± 0.00	100.00 ± 0.00	77.19 ± 0.31	36.29 ± 0.67	100.00 ± 0.00
2	50	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
	25	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
4a	50	97.92 ± 0.72	100.00 ± 0.00	0.00 ± 0.00	36.00 ± 0.74	31.56 ± 0.72
	25	85.39 ± 0.65	100.00 ± 0.00	0.00 ± 0.00	24.89 ± 0.72	11.11 ± 0.72
4b	50	100.00 ± 0.00	100.00 ± 0.00	85.00 ± 0.01	93.78 ± 0.72	100.00 ± 0.00
	25	98.33 ± 0.72	86.04 ± 0.97	78.75 ± 0.01	79.56 ± 0.72	74.44 ± 0.95
4c	50	100.00 ± 0.00	100.00 ± 0.00	0.00 ± 0.00	25.78 ± 0.72	35.56 ± 0.72
	25	93.00 ± 0.41	97.92 ± 0.72	0.00 ± 0.00	16.44 ± 0.72	17.56 ± 0.95
4d	50	100.00 ± 0.00	100.00 ± 0.00	12.92 ± 0.01	28.44 ± 0.44	27.11 ± 0.34
	25	85.08 ± 0.49	89.80 ± 0.49	0.00 ± 0.00	15.33 ± 0.63	12.44 ± 0.72
4e	50	26.31 ± 0.19	0.00 ± 0.00	47.50 ± 0.02	31.25 ± 0.02	27.11 ± 0.07
	25	29.71 ± 0.16	0.00 ± 0.00	19.17 ± 0.02	14.89 ± 0.95	11.56 ± 0.72
4f	50	100.00 ± 0.00	100.00 ± 0.00	0.00 ± 0.00	33.73 ± 0.25	40.44 ± 0.72
	25	97.24 ± 0.01	97.92 ± 0.72	0.00 ± 0.00	16.67 ± 0.62	33.78 ± 0.72
4g	50	100.00 ± 0.00	100.00 ± 0.00	0.00 ± 0.00	20.00 ± 0.36	18.67 ± 0.17
	25	100.00 ± 0.00	100.00 ± 0.00	0.00 ± 0.00	13.76 ± 0.74	0.00 ± 0.00
4h	50	100.00 ± 0.00	100.00 ± 0.00	0.00 ± 0.00	18.22 ± 0.60	29.78 ± 0.15
	25	100.00 ± 0.00	100.00 ± 0.00	0.00 ± 0.00	4.89 ± 0.72	11.11 ± 0.72
4i	50	100.00 ± 0.00	100.00 ± 0.00	10.42 ± 0.01	43.11 ± 0.91	32.44 ± 0.60
	25	100.00 ± 0.00	100.00 ± 0.00	0.00 ± 0.00	19.99 ± 0.01	20.89 ± 0.72
4j	50	100.00 ± 0.00	100.00 ± 0.00	18.33 ± 0.01	44.89 ± 0.72	28.89 ± 0.91
	25	99.58 ± 0.72	100.00 ± 0.00	0.00 ± 0.00	26.00 ± 0.62	16.22 ± 0.36
4k	50	100.00 ± 0.00	100.00 ± 0.00	00.00 ± 0.00	32.89 ± 0.72	27.56 ± 0.60
	25	97.88 ± 0.31	100.00 ± 0.00	0.00 ± 0.00	23.02 ± 0.80	17.78 ± 0.72
4l	50	100.00 ± 0.00	100.00 ± 0.00	93.33 ± 0.01	78.67 ± 0.05	34.67 ± 0.25
	25	100.00 ± 0.00	100.00 ± 0.00	0.00 ± 0.00	43.56 ± 0.72	20.53 ± 0.66
4m	50	99.58 ± 0.72	100.00 ± 0.00	21.67 ± 0.03	75.56 ± 0.91	47.56 ± 0.60
	25	77.14 ± 0.74	97.92 ± 0.72	12.50 ± 0.03	52.67 ± 0.62	41.96 ± 0.63
4n	50	100.00 ± 0.00	100.00 ± 0.00	28.75 ± 0.03	36.00 ± 0.25	36.89 ± 0.72
	25	98.33 ± 0.72	100.00 ± 0.00	3.33 ± 0.03	19.56 ± 0.72	25.78 ± 0.72
4o	50	100.00 ± 0.00	100.00 ± 0.00	0.00 ± 0.00	32.89 ± 0.72	33.78 ± 0.91
	25	98.33 ± 0.72	100.00 ± 0.00	0.00 ± 0.00	18.22 ± 0.72	27.08 ± 0.75
5a	50	52.33 ± 0.86	0.00 ± 0.00	9.58 ± 0.03	20.89 ± 0.44	48.89 ± 0.73
	25	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	38.22 ± 0.72
5b	50	99.11 ± 0.09	99.14 ± 0.07	97.92 ± 0.01	100.00 ± 0.00	100.00 ± 0.00
	25	97.92 ± 0.18	97.25 ± 0.77	93.79 ± 0.01	100.00 ± 0.00	100.00 ± 0.00
5c	50	100.00 ± 0.00	100.00 ± 0.00	95.42 ± 0.01	100.00 ± 0.00	100.00 ± 0.00
	25	100.00 ± 0.00	100.00 ± 0.00	92.79 ± 0.01	100.00 ± 0.00	98.17 ± 0.48
5d	50	100.00 ± 0.00	100.00 ± 0.00	94.17 ± 0.03	100.00 ± 0.00	100.00 ± 0.00
	25	100.00 ± 0.00	100.00 ± 0.00	85.21 ± 0.01	100.00 ± 0.00	100.00 ± 0.00
5e	50	100.00 ± 0.00	100.00 ± 0.00	16.67 ± 0.02	44.89 ± 0.60	66.22 ± 0.61
	25	100.00 ± 0.00	100.00 ± 0.00	7.08 ± 0.01	34.09 ± 0.64	59.11 ± 0.72
5f	50	100.00 ± 0.00	100.00 ± 0.00	99.17 ± 0.01	100.00 ± 0.00	84.00 ± 0.03
	25	100.00 ± 0.00	100.00 ± 0.00	88.24 ± 0.01	80.34 ± 0.82	80.69 ± 0.73
5g	50	100.00 ± 0.00	100.00 ± 0.00	98.75 ± 0.01	99.56 ± 0.72	100.00 ± 0.00
	25	100.00 ± 0.00	100.00 ± 0.00	91.27 ± 0.00	75.96 ± 0.69	98.88 ± 0.60
5h	50	97.85 ± 0.08	100.00 ± 0.00	98.33 ± 0.01	100.00 ± 0.00	100.00 ± 0.00
	25	90.68 ± 0.73	91.06 ± 0.39	88.86 ± 0.02	95.94 ± 0.16	100.00 ± 0.00
5i	50	97.92 ± 0.72	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
	25	97.91 ± 0.39	100.00 ± 0.00	89.50 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
5j	50	100.00 ± 0.00	100.00 ± 0.00	99.17 ± 0.01	100.00 ± 0.00	100.00 ± 0.00
	25	100.00 ± 0.00	97.92 ± 0.72	80.87 ± 0.02	100.00 ± 0.00	100.00 ± 0.00
5k	50	100.00 ± 0.00	100.00 ± 0.00	97.85 ± 0.05	60.00 ± 0.17	44.89 ± 0.44
	25	98.33 ± 0.72	100.00 ± 0.00	57.08 ± 0.01	32.89 ± 0.72	31.16 ± 0.69
5l	50	100.00 ± 0.00	100.00 ± 0.00	93.75 ± 0.01	100.00 ± 0.70	99.13 ± 0.41
	25	95.78 ± 0.12	100.00 ± 0.00	91.21 ± 0.00	97.67 ± 0.93	94.92 ± 0.65
5m	50	83.11 ± 0.70	93.03 ± 0.80	34.58 ± 0.03	86.67 ± 0.17	83.11 ± 0.72
	25	86.46 ± 0.53	91.63 ± 0.53	23.33 ± 0.02	79.16 ± 0.79	76.67 ± 0.63
5n	50	88.66 ± 0.39	100.00 ± 0.00	48.33 ± 0.02	73.75 ± 0.50	63.56 ± 0.91

Table 1 (*Contd.*)

Compd.[a]	Conc[b] (μg mL^{-1})	Average inhibition rate ± SD (%) ($n = 3$)				
		B. C.[c]	*S. S.*[c]	*F. G.*[c]	*F. O.*[c]	*M. O.*[c]
	25	81.50 ± 0.36	97.36 ± 0.92	24.17 ± 0.05	0.00 ± 0.00	55.11 ± 0.72
5o	50	87.00 ± 0.00	90.55 ± 0.00	39.17 ± 0.01	37.78 ± 0.72	32.89 ± 0.52
	25	85.82 ± 0.81	77.30 ± 0.23	4.17 ± 0.02	28.75 ± 0.72	23.33 ± 0.62
5p	50	100.00 ± 0.00	100.00 ± 0.00	73.75 ± 0.01	54.67 ± 0.25	31.56 ± 0.71
	25	100.00 ± 0.00	100.00 ± 0.00	44.17 ± 0.02	41.62 ± 0.83	23.33 ± 0.62
5q	50	100.00 ± 0.00	100.00 ± 0.00	11.25 ± 0.03	47.56 ± 0.72	34.22 ± 0.60
	25	98.33 ± 0.72	98.13 ± 0.63	0.00 ± 0.00	43.56 ± 0.72	20.44 ± 0.72
ASB[d]	50	47.88 ± 0.43	45.57 ± 0.29	61.85 ± 0.06	36.42 ± 0.43	33.31 ± 0.25
	25	27.00 ± 0.23	30.43 ± 0.98	57.32 ± 0.28	31.87 ± 0.61	29.49 ± 0.70

[a] Compd.: compound. [b] Conc: concentration. [c] *B. C.*: *Botrytis cinerea*. *S. S.*: *Sclerotinia sclerotiorum*. *F. G.*: *Fusarium graminearum*. *F. O.*: *Fusarium oxysporum*. f. sp. *vasinfectum*. *M. O.*: *Magnaporthe oryzae*. [d] ASB: azoxystrobin.

respectively, and the test results were shown in Table 1. Satisfactorily, compound **2** exhibited the most potent antifungal activity against the five test strains, and the inhibition rate reached 100% for each strain at 25 μg mL^{-1}. Additionally, excluding **4e** and **5a**, most of the tested compounds presented the better antifungal activity against *B. cinerea* and *S. sclerotiorum* than that of the positive control azoxystrobin. Most of the compounds **5a–5q** (**5b**, **5c**, **5d**, **5f**, **5g**, **5h**, **5i**, **5j**, **5l**) demonstrated moderate to remarkable antifungal activity against *F. graminearum*, *F. oxysporum* and *M. oryzae*, with inhibitory rates ranging from 76% to 100% (25 μg mL^{-1}). However, compounds **4a–4o** showed weak activity against the three fungi. To further explore the antifungal potential of the synthesized compounds, the most active compounds (whose inhibition rates >50% at 25 μg mL^{-1}) in Table 1 were selected to determine their EC$_{50}$ values against the five fungal strains.

Results of antifungal evaluation (Table 2) indicated that 31 out of the 33 tested compounds showed moderate to strong inhibitory activity against *B. cinerea* with EC$_{50}$ values of 0.0021–0.0827 mM, which were higher than the positive control azoxystrobin (EC$_{50}$ = 0.3551 mM), and 19 of compounds with EC$_{50}$ values of 0.0021–0.0330 mM demonstrated superior activity than HQ (EC$_{50}$ = 0.0331 mM). The antifungal activity of 31 out of the 33 tested compounds against *S. sclerotiorum* was better than azoxystrobin (EC$_{50}$ = 0.1629 mM), with EC$_{50}$ values between 0.0016 and 0.0636 mM. Compound **2** showed the greatest activity, with EC$_{50}$ value of 0.0016 mM, followed by **5c** (EC$_{50}$ = 0.0030 mM) respectively. 10 of the tested compounds showed higher activity against *F. graminearum*, with EC$_{50}$ values of 0.0124–0.0211 mM, than HQ (EC$_{50}$ = 0.0931 mM) and azoxystrobin (EC$_{50}$ = 0.0229 mM) respectively. Furthermore, 10 out of 33 of the tested compounds exhibited stronger activity against *F. oxysporum* than HQ (EC$_{50}$ = 0.1840 mM) and azoxystrobin (EC$_{50}$ = 0.1265 mM) respectively, with EC$_{50}$ values of 0.0059–0.0365 mM. Similarly, antifungal activity of 10 of the synthesized compounds against *M. oryzae* was better than HQ (EC$_{50}$ = 0.0964 mM) and azoxystrobin (EC$_{50}$ > 10.0000 mM) respectively, with EC$_{50}$ values of 0.0120–0.0159 mM. The above results revealed that compound **2** was the most effective compound, followed by compound **5c**.

Table 2 EC$_{50}$ of series 8-hydroxyquinoline derivatives against five phytopathogenic fungi (mM)

Compd.[a]	EC$_{50}$				
	B. C.[b]	*S. S.*[b]	*F. G.*[b]	*F. O.*[b]	*M. O.*[b]
HQ	0.0331	0.0181	0.0931	0.1840	0.0964
2	0.0021	0.0016	0.0124	0.0059	0.0120
4a	0.0827	0.0424	—	—	—
4b	0.0165	0.0355	—	—	—
4c	0.0537	0.0490	—	—	—
4d	0.0536	0.0636	—	—	—
4f	0.0317	0.0468	—	—	—
4g	0.0444	0.0443	—	—	—
4h	0.0298	0.0434	—	—	—
4i	0.0496	0.0483	—	—	—
4j	0.0356	0.0215	—	—	—
4k	0.0277	0.0432	—	—	—
4l	0.0222	0.0234	—	—	—
4m	0.0790	0.0593	—	—	—
4n	0.0285	0.0211	—	—	—
4o	0.0330	0.0361	—	—	—
5b	0.0386	0.0362	0.0190	0.0226	0.0156
5c	0.0124	0.0030	0.0140	0.0146	0.0140
5d	0.0150	0.0205	0.0167	0.0208	0.0150
5e	0.0349	0.0343	—	—	—
5f	0.0328	0.0320	0.0192	0.0348	0.0313
5g	0.0172	0.0195	0.0228	0.0365	0.0154
5h	0.0623	0.0463	0.0193	0.0233	0.0142
5i	0.0307	0.0184	0.0183	0.0200	0.0156
5j	0.0359	0.0458	0.0211	0.0206	0.0159
5k	0.0348	0.0306	—	—	—
5l	0.0233	0.0145	0.0211	0.0233	0.0144
5m	0.0232	0.0417	—	—	—
5n	0.0125	0.0190	—	—	—
5o	0.0325	0.0419	—	—	—
5p	0.0192	0.0328	—	—	—
5q	0.0241	0.0324	—	—	—
ASB[c]	0.3551	0.1629	0.0229	0.1265	>10.0000

[a] Compd.: compound. [b] *B. C.*: *Botrytis cinerea*. *S. S.*: *Sclerotinia sclerotiorum*. *F. G.*: *Fusarium graminearum*. *F. O.*: *Fusarium oxysporum*. f. sp. *vasinfectum*. *M. O.*: *Magnaporthe oryzae*. [c] ASB: azoxystrobin.

2.3. Structure–activity relationships

Different substituent groups were introduced into the compound **2** scaffold to synthesize different analogs of **2** and

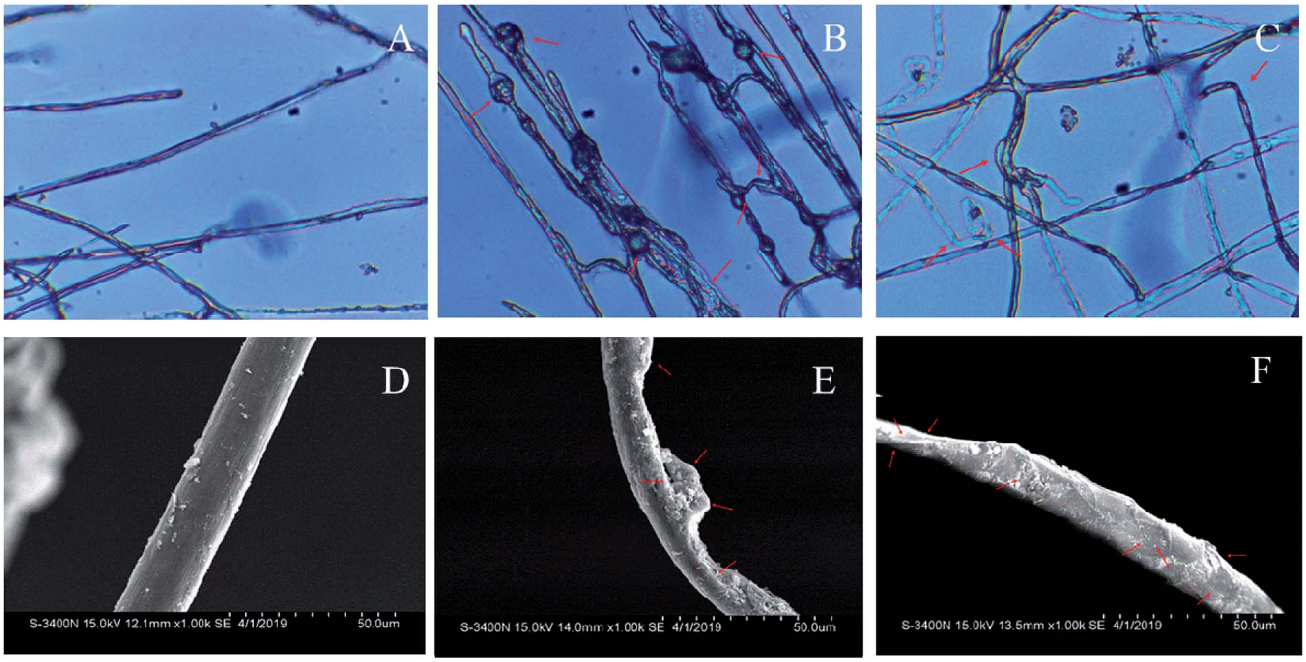

Fig. 2 Optical microscope and scanning electron micrographs of the hyphae of *S. sclerotiorum* grown on PDA medium with DMSO or compounds **2**, **5c** at 25 °C. Optical microscope: (A) untreated control, 0.5% DMSO, ×400; (B) compound **2** at 0.0016 mM (EC_{50}) treatment, ×400; (C) compound **5c** at 0.0030 mM (EC_{50}) treatment, ×400; scanning electron microscopy: (D) untreated control, 0.5% DMSO, ×1500; (E) after 72 h compound **2** at 0.0016 mM (EC_{50}) treatment, ×1500; (F) after 72 h compound **5c** at 0.0030 mM (EC_{50}) treatment, ×1500.

their structure activity relationships (SAR) were investigated. The values indicated in Table 2, it was inferred that the presence of tertiary or secondary amine on the synthesized compound was essential for the antifungal activity of the tested compounds **4a–4o** against *B. cinerea* and *S. sclerotiorum*. When NHR_1R_2 was directly substituted with aliphatic ring, the corresponding target compound displayed a very poor antifungal activity as exemplified by **4e**. In contrast, when the NHR_1R_2 was replaced by substituted phenylpiperazines, a spike in antifungal activity was observed for the corresponding phenylpiperazine derivatives (**5b–5q**). It was therefore extrapolated that, the presence of substituted phenylpiperazine derivatives played a pivotal role in their impressive antifungal activity. However, benzylpiperazine 8-hydroxyquinoline derivative **5a** showed weak activity against *B. cinerea* and *S. sclerotiorum*. Through analysis of the EC_{50} data in Table 2, revealed that unlike compounds **4a–4o**, most of the tested compounds **5a–5q** bearing aromatic piperazine substituent groups showed remarkable inhibitory activity against *F. graminearum*, *F. oxysporum* and *M. oryzae* respectively. From these observations, it was inferred that when the aromatic ring of aromatic piperazine on the target compound was benzene ring, the target compound demonstrated great antifungal activity. However, the presence of other aromatic rings, such as pyridine, pyrimidine and pyrazine on the target compounds **5o**, **5p** and **5q** respectively, led to no antifungal activity. On the other hand, it was telling that the number of substituent groups

Table 3 Protective and curative activity of compounds **2** and **5c** *in vivo*

		Curative effect		Protective effect	
Compd.[a]	Concentration (μg mL^{-1})	Lesion length (mm ± SD)	Control efficacy (%)	Lesion length (mm ± SD)	Control efficacy (%)
2	80	6.17 ± 0.38	87.91	5.87 ± 0.72	90.93
	40	8.86 ± 0.74	60.05	7.08 ± 0.92	78.29
	20	12.80 ± 0.95	19.15	9.27 ± 0.55	55.39
5c	80	5.44 ± 0.40	95.44	5.85 ± 0.69	91.09
	40	6.53 ± 0.65	84.18	7.06 ± 0.92	78.42
	20	12.71 ± 0.96	20.14	8.66 ± 0.98	61.68
ASB[b]	80	6.58 ± 0.98	83.59	5.83 ± 0.52	91.32
	40	7.19 ± 0.59	77.32	5.86 ± 0.52	91.02
	20	9.54 ± 0.94	52.94	6.78 ± 0.54	81.35
Control	—	18.04 ± 0.76	—	20.53 ± 0.75	—

[a] Compd.: compound. [b] ASB: azoxystrobin.

(mono- *versus* di-) on the phenyl ring also impacted antifungal activity of the target compounds against the various fungi tested. Premised on the above observation, direct pairwise comparisons of antifungal activity of the tested compounds (**5j** *versus* **5k**, **5l** *versus* **5m**) against the three fungi were analyzed in terms of their EC_{50} values. It was discovered that the mono-substituted compounds **5j** and **5l** exhibited better antifungal activity than their 2,4-dihalogenated counterparts **5k** and **5m** respectively. This observation showed that the number of substituents on the phenyl ring played a key role in inhibitory activity of the tested compounds. In order to determine the optimum position for mono-substitution on the phenyl ring, comparisons of compounds **5c** *versus* **5d**; **5f** *versus* **5g**; **5i** *versus* **5j** were examined, and it was found that substitution at the *ortho* position (compounds **5c**, **5i** and **5f**) conferred greater antifungal activity than substitution at the *meta* or *para* position (**5d**, **5j** and **5g**). Interestingly, the tested compounds bearing electron-donating groups such as methyl and methoxy on the aromatic piperazine (**5c**, **5d**, **5g** and **5h**), exhibited remarkable antifungal activity against the three fungi. In addition, the introduction of halogen atoms such as F and Cl on phenylpiperazine augmented antifungal activity compounds **5i**, **5j** and **5l** (EC_{50} values were <0.0250 mM) against the three fungi.

2.4. Effects of compounds 2 and 5c on hyphal morphology of *S. sclerotiorum*

The hyphae morphology of *S. sclerotiorum* treated with EC_{50} of the two most effective compounds **2** and **5c** were observed by optical microscope (Fig. 2A, B and C) and scanning electron microscopy (Fig. 2D, E and F), respectively. Satisfactorily, the two experiments yielded consistent results. The mycelia of the control group displayed a normal morphology with smooth, linear, regular, homogeneous and a constant diameter (Fig. 2A and D). However, mycelial morphology of the two tested compounds treated showed significant alteration. Hyphae of *S. sclerotiorum* treated with the EC_{50} of compound **2** were abnormal, with distinct bulges (Fig. 2B), correspondingly,

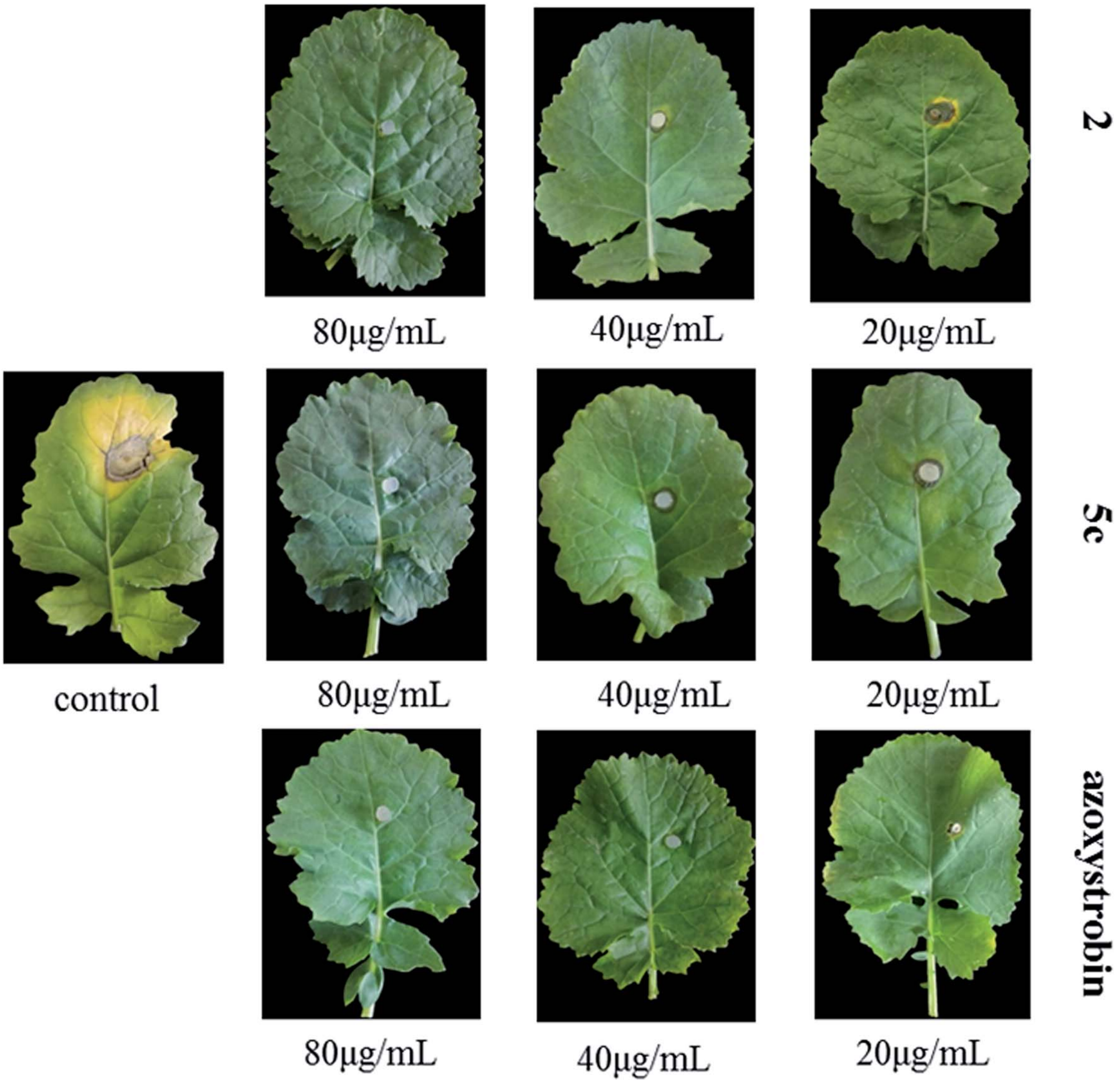

Fig. 3 *In vivo* protective efficacy of compounds 2, 5c and azoxystrobin against *S. sclerotiorum* on rape leaves.

scanning electron microscope observation results showed that hyphae become clear swelling or shrinking (Fig. 2E); the hyphae treated with the EC_{50} of compound **5c** appeared obviously contort and wrinkle in the observation of optical microscope (Fig. 2C), further than that, scanning electron microscope observation results showed that hyphae appeared shriveled and collapsed (Fig. 2F). From the above observations, it was inferred that the two compounds destroyed the cell membrane and wall of *S. sclerotiorum*, culminating in the death of hyphae.

2.5. Effects of compounds 2 and 5c against *S. sclerotiorum in vivo*

The results of pot experiments showed that compounds **2** and **5c** exhibited moderate to excellent curative and protective effects *in vivo* (Table 3). Three conclusions were deduced from the data in Table 3: firstly, the curative and protective effects of the two compounds exhibited concentration-dependent properties; secondly, compound **5c** possessed stronger protective and curative activity than that of **2**; thirdly, the curative effects of compounds **5c** and **2** at 80 μg mL^{-1} (95.44%, 87.91%) were better than the control azoxystrobin (83.59%) and the protective effects of compounds **5c** and **2** at 80 μg mL^{-1} (91.09%, 90.93%) were close to that of azoxystrobin (91.32%). Underivatized compound **2** exhibited better activity *in vitro*, but the compound **5c** possessed superior activity *in vivo*, it was therefore extrapolated that the introduction of 1-(2-methylphenyl)piperazine significantly improved the absorbability of compound **2** scaffold in plants. From pictures in Fig. 3, it was deduced that compounds **2** and **5c** demonstrated no obvious phytotoxicity on oilseed rape leaves at a high concentration (80 μg mL^{-1}), which were benign to the oilseed rape.

2.6. Antifungal and antibacterial spectrum of compound 2

Compound **2** possessed the highest antifungal activity *in vitro* among all of 8-hydroxyquinoline derivatives, making it a promising lead compound for the development of novel antifungal agents. Hence, the antifungal spectrum of this compound was investigated and the results revealed that compound **2** showed impressive antifungal activity against many deleterious fungal pathogens, including *Rhizoctonia solani*, *Mycosphaerlla melonis*, *Phyllosticta zeae*, *Colletotrichum gossypii*, *Phytophthora capsici* and *Pythium aphanidermatum*. When it came to *M. melonis*, *C. gossypii*, *P. capsici* and *P. aphanidermatum*, compound **2** revealed excellent antifungal activity with EC_{50} of 0.0081, 0.0068, 0.0019 and 0.0043 mmol, respectively (Table 4). Additionally, the inhibitory activity of compound **2** against eight agricultural pathogenic bacteria (Table 5) was explored. The data in Table 5 indicated that compound **2** exhibited inhibitory effects against the plant pathogenic bacteria tested, and the activity against *Acidovorax avenae* subsp. *citrulli*, *Pseudomanas solanacearum*, *Xanthomonas oryzae* pv. *oryzae* and *Xanthomonas oryzae* pv. *oryzicola* was higher than that of the control streptomycin sulfate. The above results showed that compound **2** has a broad spectrum of antifungal and antibacterial activities.

Table 4 The antifungal spectrum of compound **2** against six plant pathogenic fungi

Fungi	EC_{50} (mM)	95% Cl
R. solani	0.0149	0.0120–0.0185
M. melonis	0.0081	0.0059–0.0111
P. zeae	0.0127	0.0077–0.0208
C. gossypii	0.0068	0.0058–0.0081
P. capsici	0.0019	0.0016–0.0023
P. aphanidermatum	0.0043	0.0034–0.0055

Table 5 The antibacterial spectrum of compound **2** against eight plant pathogenic bacteria

	MIC (mM)	
Bacterium	Compound **2**	Streptomycin sulfate
Acidovorax avenae subsp. *citrulli*	0.0263	0.0412
Agrobacterium tumefaciens	0.1578	0.1372
Erwinia carotovora	0.1578	0.1372
Pseudomonas syringae pv. *actinidiae*	0.1578	0.0069
Pseudomonas syringae pv. *lachrymans*	0.1578	0.0206
Pseudomanas solanacearum	0.3155	—
Xanthomonas oryzae pv. *oryzae*	0.2104	—
Xanthomonas oryzae pv. *oryzicola*	0.2104	—

3. Conclusion

A series of 8-hydroxyquinoline derivatives were designed, synthesized and their antifungal activity was evaluated against five phytopathogenic fungi. Most of the tested compounds exhibited stronger antifungal activity against the five fungi than the primer molecule HQ. Especially, compound **2** demonstrated the best antifungal activity *in vitro* against *B. cinerea*, *S. sclerotiorum*, *F. graminearum*, *F. oxysporum* and *M. oryzae* with EC_{50} values of 0.0021, 0.0016, 0.0124, 0.0059 and 0.0120 mM respectively, followed by **5c**. Moreover, compound **5c** exhibited better protective and curative activity than that of compound **2** *in vivo*, and the curative effects of compounds **5c** and **2** at 80 μg mL^{-1} (95.44%, 87.91%) respectively were better than the positive control azoxystrobin (83.59%), compounds **2** and **5c** effectively controlled the disease development in *S. sclerotiorum* infected oilseed rape *in vivo*, indicating great potential of these two compounds to control fungal diseases. The obvious teratogenic effect of compounds **2** and **5c** on hyphal morphology of *S. sclerotiorum* will provide valuable insights into understanding the antifungal mechanism of 8-hydroxyquinoline derivatives. Additionally, compound **2** also displayed remarkable activity against eight agricultural pathogenic bacteria.

4. Experimental section

4.1. General methods

All reactions were performed using commercially available regents without further purification. Thin-layer chromatography (TLC) was employed to monitor all reactions and column chromatography was performed with silica gel (Qingdao Haiyang Chemical Co., Ltd., Qingdao, China). Melting points (mp) were determined using glass capillary tubes on a WRS-2U melting point apparatus (Shanghai Precision Instrument Co., Ltd., Shanghai, China) and were uncorrected. Mass spectrometry was performed using ESI mode on a Bruker Daltonics APEXII49e spectrometer (Bruker Daltonics Inc., Billerica, MA, US). Nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR) spectra were recorded at 400 and 100 MHZ on a Bruker AM-400 (Bruker Company, Billerica, MA, and US.) spectrometer with TMS as an internal standard.

The commercial fungicide azoxystrobin (analytical grade, 98% purity) provided by Jiangsu Balling Agrochemical Co., Ltd. Jiangying, China was used as a positive control *in vitro* experiment. And the commercial bactericide streptomycin sulfate (analytical grade, 98% purity) (J&K) was used as a positive control in minimal inhibitory concentration (MIC) test of compound **2** against the eight phytopathogenic bacterial.

Botrytis cinerea, *Sclerotinia sclerotiorum*, *Fusarium graminearum*, *Fusarium oxysporum* f. sp. *vasinfectum*, *Magnaporthe oryzae*, *Rhizoctonia solani*, *Mycosphaerlla melonis*, *Phyllosticta zeae*, *Colletotrichum gossypii*, *Phytophthora capsici* and *Pythium aphanidermatum* were provided by the Institute of Plant Protection, Gansu Academy of Agricultural Science, and Lanzhou, China. *Acidovorax avenae* subsp. *citrulli*, *Agrobacterium tumefaciens*, *Erwinia carotovora*, *Pseudomonas syringae* pv. *actinidiae*, *Pseudomonas syringae* pv. *lachrymans*, *Pseudomanas solanacearum*, *Xanthomonas oryzae* pv. *oryzae* and *Xanthomonas oryzae* pv. *oryzicola*, which were obtained from Shenyang Research Institute of Chemical Industry, and Shenyang, China.

4.2. Synthesis and characterization of compounds

4.2.1. The preparation of compound 2. 8-Hydroxyquinoline (1.0 mmol) was dissolved in suitable concentrated HCl in a 50 mL flask, the mixture was cooled to 0 °C, followed by dropwise addition of $NaNO_2$ (1.5 mmol) aqueous solution into it. The yellow precipitate was filtered and washed with cold water to give 8-hydroxy-5-nitrosoquinoline. After vacuum drying, 8-hydroxy-5-nitrosoquinoline powder was added to a mixture of concentrated HNO_3 and water (v/v = 3 : 2) at 17 °C. The nitrosoquinoline was rapidly converted to the insoluble nitro compound. The mixture was diluted with water after 75 min stirring, cooled to 0 °C and made alkaline with sodium acetate. The product was washed with water and recrystallized from ethanol. Yellow solid; yield 65%; mp 226–228 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 7.17 (d, J = 8.8 Hz, 1H, ArH), 7.86 (dd, J = 8.8, 4.0 Hz, 1H, ArH), 8.52 (d, J = 8.8 Hz, 1H, ArH), 8.99 (d, J = 4.0 Hz, 1H, ArH), 9.18 (d, J = 8.8 Hz, 1H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 110.45, 122.97, 125.67, 129.51, 132.84, 135.51, 137.72, 149.62, 161.09. HRMS calcd for $C_9H_6N_2O_3$, $[M + H]^+$, 190.0378; found 190.2645.

4.2.2. General procedure for preparation of target compounds 4a–4o. In a 50 mL flask, compound **2** (1 mmol) was mixed with formaldehyde (4.5 mmol) in 20 mL dry ethanol, the desired amine (1.1 mmol) was dropped into this solution. The mixture was refluxed at 80 °C for 24 h and the precipitate formed was filtered. The crude product was purified by recrystallization from 1 : 1 EtOH–H_2O to yield the final product.

*4.2.2.1. 7-((4-Methylpiperazin-1-yl)methyl)-5-nitroquinolin-8-ol (**4a**).* Yellow solid; yield 66%; mp 206–208 °C; ^{1}H NMR (400 MHz, chloroform-d, δ ppm): 2.36 (s, 3H, CH_3), 2.62–2.77 (m, 8H, piperazine), 3.98 (s, 2H, CH_2), 7.66 (dd, J = 8.9, 4.1 Hz, 1H, ArH), 8.42 (s, 1H, ArH), 8.96 (d, J = 4.0 Hz, 1H, ArH), 9.28 (d, J = 8.8 Hz, 1H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 42.34, 48.45, 48.45, 50.52, 53.47, 53.47, 114.57, 117.46, 123.96, 126.39, 132.88, 135.11, 136.69, 147.99, 158.85. HRMS calcd for $C_{15}H_{18}N_4O_3$, $[M + H]^+$, 303.1412; found 303.2014.

*4.2.2.2. 7-((4-Ethylpiperazin-1-yl)methyl)-5-nitroquinolin-8-ol (**4b**).* Yellow solid; yield 90%; mp 187–189 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 1.06 (t, J = 7.1 Hz, 3H, CH_3), 2.74–2.84 (m, 8H, $4CH_{2piperazine}$), 3.08 (s, 2H, CH_2), 3.91 (s, 2H, CH_2), 7.61 (dd, J = 8.9, 4.1 Hz, 1H, ArH), 8.52 (s, 1H, ArH), 8.69 (d, J = 4.0 Hz, 1H, ArH), 9.32 (d, J = 8.8 Hz, 1H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 9.42, 49.64, 49.64, 49.64, 50.98, 53.84, 53.84, 115.29, 118.22, 123.04, 126.02, 131.71, 134.20, 137.06, 148.71, 159.17. HRMS calcd for $C_{16}H_{20}N_4O_3$, $[M + H]^+$, 317.1569; found 317.2228.

*4.2.2.3. 5-Nitro-7-(piperidin-1-ylmethyl)quinolin-8-ol (**4c**).* Yellow solid; yield 90%; mp 206–207 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 1.53–1.72 (m, 2H, $CH_{2piperidine}$), 1.75 (t, J = 5.8 Hz, 4H, $2CH_{2piperidine}$), 3.13 (t, J = 4.8 Hz, 4H, $2CH_{2piperidine}$), 4.17 (s, 2H, CH_2), 7.55 (dd, J = 8.7, 4.1 Hz, 1H, ArH), 8.58 (s, 1H, ArH), 8.60 (d, J = 3.0 Hz, 1H, ArH), 9.31 (d, J = 8.8, 1H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 21.55, 22.96, 22.96, 52.48, 52.48, 53.69, 112.13, 124.11, 126.50, 132.48, 133.33, 135.06, 136.74, 148.08, 162.83. HRMS calcd for $C_{15}H_{17}N_3O_3$, $[M + H]^+$, 288.1303; found 288.2205.

*4.2.2.4. 7-(Morpholinomethyl)-5-nitroquinolin-8-ol (**4d**).* Yellow solid; yield 89%; mp 219–220 °C; ^{1}H NMR (400 MHz, chloroform-d, δ ppm): 2.71 (t, J = 3.4 Hz, 4H, $2CH_{2morpholine}$), 3.83 (t, J = 4.7 Hz, 4H, $2CH_{2morpholine}$), 3.97 (s, 2H, CH_2), 7.69 (dd, J = 8.9, 4.2 Hz, 1H, ArH), 8.52 (s, 1H, ArH), 8.96 (d, J = 4.0 Hz, 1H, ArH), 9.28 (d, J = 8.9 Hz, 1H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 51.58, 51.58, 53.99, 63.74, 63.74, 117.71, 124.27, 126.56, 132.30, 133.46, 135.27, 136.67, 147.94, 163.05. HRMS calcd for $C_{14}H_{15}N_3O_4$, $[M + H]^+$, 290.1096; found 290.1586.

*4.2.2.5. 7-((Cyclohexylamino)methyl)-5-nitroquinolin-8-ol (**4e**).* Yellow solid; yield 85%; mp 206–207 °C; ^{1}H NMR (400 MHz, chloroform-d, δ ppm): 2.53–1.31 (m, 10H, $5CH_{2cyclohexane}$), 3.13 (s, 1H, CH), 4.05 (s, 2H, CH_2), 7.55 (dd, J = 8.7, 4.1 Hz, 1H, ArH), 7.92 (s, 1H, ArH), 8.62 (d, J = 3.0 Hz, 1H, ArH), 9.28 (d, J = 8.9 Hz, 1H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 24.39, 24.39, 25.17, 29.12, 29.12, 42.08, 56.95, 117.46, 126.32, 126.32, 131.94, 132.83, 134.76, 136.60, 148.50, 161.60. HRMS calcd for $C_{16}H_{19}N_3O_3$, $[M + H]^+$, 302.1460; found 302.2065.

*4.2.2.6. 7-((Benzylamino)methyl)-5-nitroquinolin-8-ol (**4f**).* Yellow solid; yield 50%; mp 204–207 °C; ^{1}H NMR (400 MHz, chloroform-d, δ ppm): 4.07 (s, 4H, $2CH_2$), 4.80 (s, NH), 7.46–7.33 (m, 5H, ArH), 8.57 (dd, J = 8.9, 4.1 Hz, 1H, ArH), 8.88 (s, 1H, ArH), 9.24 (d, J = 8.8 Hz, 1H, ArH), 9.32 (d, J = 8.8 Hz, 1H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 53.82, 59.41, 114.24, 117.12, 119.99, 124.29, 126.30, 129.09, 129.45, 130.49, 131.50, 132.57, 134.50, 136.88, 145.63, 148.35, 158.69. HRMS calcd for $C_{17}H_{15}N_3O_3$, $[M + H]^+$, 310.1147; found 310.1745.

*4.2.2.7. 7-(Pyrrolidin-1-ylmethyl)-5-nitroquinolin-8-ol (**4g**).* Yellow-green solid; yield 80%; mp 218–218 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 1.73 (t, $J = 5.9$ Hz, 4H, $2CH_{2\text{pyrrole}}$), 3.11 (t, $J = 5.5$ Hz, 4H, $2CH_{2\text{pyrrole}}$), 4.17 (s, 2H, CH_2), 7.56 (dd, $J = 8.7$, 4.1 Hz, 1H, ArH), 8.59 (s, 1H, ArH), 8.61 (d, $J = 4.0$ Hz, 1H, ArH), 9.33 (d, $J = 8.7$ Hz, 1H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 22.96, 22.96, 51.59, 53.73, 53.73, 113.65, 124.07, 126.47, 132.43, 132.43, 135.17, 136.65, 148.08, 162.40. HRMS calcd for $C_{14}H_{15}N_3O_3$, $[M + H]^+$, 274.1147; found 274.1811.

*4.2.2.8. 7-(((Cyclopropylmethyl)amino)methyl)-5-nitroquinolin-8-ol (**4h**).* Yellow solid; yield 44%; mp 219–219 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 0.35 (d, $J = 5.6$ Hz, 2H, $CH_{2\text{cyclopropnane}}$), 0.57 (d, $J = 7.6$ Hz, 2H, $CH_{2\text{cyclopropnane}}$), 1.13 (s, 1H, $CH_{\text{cyclopropnane}}$), 2.87 (d, $J = 7.4$ Hz, 2H, CH_2), 4.13 (s, 2H, CH_2), 7.54 (dd, $J = 8.7$, 4.1 Hz, 1H, ArH), 8.55 (s, 1H, ArH), 8.62 (d, $J = 3.0$ Hz, 1H, ArH), 9.34 (d, $J = 8.7$ Hz, 1H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 4.42, 4.42, 7.54, 44.80, 51.83, 114.46, 116.21, 123.99, 126.16, 132.14, 134.51, 137.28, 148.21, 158.64.

HRMS calcd for $C_{14}H_{15}N_3O_3$, $[M + H]^+$, 274.1147; found 274.1638.

*4.2.2.9. 7-((Ethylamino)methyl)-5-nitroquinolin-8-ol (**4i**).* Yellow solid; yield 90%; mp 210–214 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 1.25 (d, $J = 9.1$ Hz, 3H, CH_3), 1.74 (s, 2H, CH_2), 4.31 (s, 2H, CH_2), 7.78 (dd, $J = 8.7$, 4.1 Hz, 1H, ArH), 8.62 (s, 1H, ArH), 8.95 (d, $J = 4.0$ Hz, 1H, ArH), 9.21 (d, $J = 9.6$ Hz, 1H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 11.41, 42.52, 44.36, 114.33, 123.68, 126.31, 131.89, 132.70, 134.68, 136.72, 148.49, 161.81. HRMS calcd for $C_{12}H_{13}N_3O_3$, $[M + H]^+$, 248.0990; found 248.1824.

*4.2.2.10. 7-((Propylamino)methyl)-5-nitroquinolin-8-ol (**4j**).* Yellow solid; yield 90%; mp 204–205 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 0.91 (d, $J = 7.7$ Hz, 3H, CH_3), 1.83–1.57 (m, 2H, CH_2), 2.89 (d, $J = 8.2$ Hz, 2H, CH_2), 4.08 (s, 2H, CH_2), 7.54 (dd, $J = 8.7$, 4.1 Hz, 1H, ArH), 8.55 (s, 1H, ArH), 8.61 (d, $J = 4.0$ Hz, 1H, ArH), 9.34 (d, $J = 8.8$ Hz, 1H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 11.38, 19.45, 44.76, 48.85, 114.26, 123.68, 126.34, 131.96, 132.77, 134.76, 136.66, 148.47, 161.75. HRMS calcd for $C_{13}H_{15}N_3O_3$, $[M + H]^+$, 262.1147; found 262.1171.

*4.2.2.11. 7-((Butylamino)methyl)-5-nitroquinolin-8-ol (**4k**).* Yellow solid; yield 90%; mp 194–196 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 0.89 (t, $J = 7.4$ Hz, 3H, CH_3), 1.30–1.37 (m, 2H, CH_2), 1.64 (s, 2H, CH_2), 2.94 (t, $J = 7.8$ Hz, 2H, CH_2), 4.08 (s, 2H, CH_2), 7.55 (dd, $J = 8.7$, 4.1 Hz, 1H, ArH), 8.55 (s, 1H, ArH), 8.61 (d, $J = 4.0$ Hz, 1H, ArH), 9.35 (d, $J = 8.7$ Hz, 1H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 13.96, 19.72, 27.91, 44.79, 47.05, 114.29, 123.69, 126.34, 131.96, 132.74, 134.77, 136.65, 148.46, 161.75. HRMS calcd for $C_{14}H_{17}N_3O_3$, $[M + H]^+$, 276.1303; found 276.1975.

*4.2.2.12. 7-((Dimethylamino)methyl)-5-nitroquinolin-8-ol (**4l**).* Yellow solid; yield 75%; mp 210–212 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.70 (s, 6H, $2CH_3$), 4.15 (s, 2H, CH_2), 7.57 (d, $J = 4.1$ Hz, 1H, ArH), 8.58 (s, 1H, ArH), 8.64 (d, $J = 3.4$ Hz, 1H, ArH), 9.38 (d, $J = 8.7$, 1H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 42.67, 42.67, 54.60, 112.72, 124.26, 126.52, 132.24, 133.04, 135.31, 136.62, 147.91, 162.91. HRMS calcd for $C_{12}H_{13}N_3O_3$, $[M + H]^+$, 248.0990; found 248.1636.

*4.2.2.13. 7-((Dipropylamino)methyl)-5-nitroquinolin-8-ol (**4m**).* Yellow solid; yield 14%; mp 156–158 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 0.88 (t, $J = 3.2$ Hz, 6H, $2CH_3$), 1.72 (t, $J = 12.0$ Hz, 4H, $2CH_2$), 3.07–2.91 (m, 4H, $2CH_2$), 4.25 (s, 2H, CH_2), 7.58 (dd, $J = 9.0$, 4.1 Hz, 1H, ArH), 8.58 (s, 1H, ArH), 8.65 (d, $J = 4.0$ Hz, 1H, ArH), 9.29 (d, $J = 8.9$ Hz, 1H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 11.47, 11.47, 17.35, 17.35, 54.16, 54.16, 54.78, 114.45, 124.94, 126.44, 132.47, 134.05, 136.59, 141.92, 146.53, 150.06. HRMS calcd for $C_{16}H_{21}N_3O_3$, $[M + H]^+$, 304.1616; found 204.2135.

*4.2.2.14. 7-((Isopropylamino)methyl)-5-nitroquinolin-8-ol (**4n**).* Yellow solid; yield 90%; mp 207–211 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 1.67–0.88 (m, 6H, $2CH_3$), 3.60 (s, 1H, CH), 4.08 (s, 2H, CH_2), 4.57 (s, 1H, NH), 7.56 (s, 1H, ArH), 8.62 (d, $J = 17.7$ Hz, 2H, ArH), 9.37 (d, $J = 9.3$ Hz, 1H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 19.12, 19.12, 42.30, 50.52, 117.18, 123.62, 126.30, 131.85, 132.90, 134.73, 136.62, 148.54, 161.49. HRMS calcd for $C_{13}H_{15}N_3O_3$, $[M + H]^+$, 262.1147; found 262.1806.

*4.2.2.15. 7-((tert-Butylamino)methyl)-5-nitroquinolin-8-ol (**4o**).* Yellow solid; yield 90%; mp 215–216 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 1.44 (s, 9H, $3CH_3$), 4.01 (s, 2H, CH_2), 7.44 (dd, $J = 8.7$, 4.1 Hz, 1H, ArH), 8.46 (s, 1H, ArH), 8.56 (d, $J = 4.2$ Hz, 1H, ArH), 9.23 (d, $J = 9.2$ Hz, 1H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 25.62, 25.62, 25.6, 55.31, 57.38, 114.22, 117.10, 123.62, 126.33, 131.90, 134.63, 136.69, 148.69, 158.67. HRMS calcd for $C_{14}H_{17}N_3O_3$, $[M + H]^+$, 276.1303; found 276.1962.

4.2.3. General procedure for preparation of target compounds 5a–5q. The formaldehyde (3.75 mmol) and corresponding aromatic piperazine (1 mmol) were added to a 50 mL round-bottom flask, the mixture was stirred at 0 °C to give a white precipitate. The precipitate was added to the compound **2** (1 mmol) dissolved in pyridine at 50 °C, a yellow precipitate was formed after a few minutes. After 30–40 minutes, the precipitate was filtered through a Buchner funnel. The crude product was purified by recrystallization from 2 : 1 EtOH–H_2O to yield the final product.

*4.2.3.1. 7-((4-Benzylpiperazin-1-yl)methyl)-5-nitroquinolin-8-ol (**5a**).* Yellow solid; yield 50%; mp 204–207 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.62 (t, $J = 4.8$ Hz, 4H, $2CH_{2\text{piperazine}}$), 2.99 (t, $J = 4.4$ Hz, 4H, $2CH_{2\text{piperazine}}$), 3.57 (s, 2H, CH_2), 4.07 (s, 2H, CH_2), 7.53–7.09 (m, 5H, ArH), 7.65 (dd, $J = 8.7$, 4.2 Hz, 1H, ArH), 8.57 (s, 1H, ArH), 8.73 (d, $J = 4.1$ Hz, 1H, ArH), 9.25 (d, $J = 8.3$ Hz, 1H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 49.44, 49.69, 53.68, 59.41, 115.33, 118.26, 123.26, 126.11, 129.21, 129.21, 129.60, 131.25, 131.25, 132.01, 133.29, 134.38, 136.96, 148.54, 158.80. HRMS calcd for $C_{21}H_{22}N_4O_3$, $[M + H]^+$, 379.1725; found 379.2229.

*4.2.3.2. 7-((4-Phenylpiperazin-1-yl)methyl)-5-nitroquinolin-8-ol (**5b**).* Yellow solid; yield 21%; mp 197–199 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.98 (t, $J = 4.8$ Hz, 4H, $2CH_{2\text{piperazine}}$), 3.29 (t, $J = 4.8$ Hz, 4H, $2CH_{2\text{piperazine}}$), 4.06 (s, 2H, CH_2), 6.81 (t, $J = 7.2$ Hz, 1H, ArH), 6.95 (d, $J = 8.2$ Hz, 2H, ArH), 7.23 (t, $J =$

7.8 Hz, 2H, ArH), 7.73 (dd, $J = 8.7$, 4.1 Hz, 1H, ArH), 8.64 (s, 1H, ArH), 8.84 (d, $J = 4.7$ Hz, 1H, ArH), 9.26 (d, $J = 8.7$ Hz, 1H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 7.52, 7.52, 44.47, 44.47, 51.90, 114.35, 114.35, 114.98, 117.90, 123.62, 126.32, 131.87, 131.87, 132.81, 134.76, 136.61, 148.46, 158.61, 158.96, 161.58. HRMS calcd for $C_{20}H_{20}N_4O_3$, $[M + H]^+$, 365.1569; found 365.2229.

*4.2.3.3. 7-((4-(o-Tolyl)piperazin-1-yl)methyl)-5-nitroquinolin-8-ol (**5c**).* Yellow solid; yield 44%; mp 188–189 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.25 (s, 3H, CH_3), 3.15–2.99 (m, 8H, $4CH_{2\text{piperazine}}$), 4.14 (s, 2H, CH), 7.03–6.96 (m, 2H, ArH), 7.19–7.12 (m, 2H, ArH), 7.66 (dd, $J = 8.8$, 4.2 Hz, 1H, ArH), 8.62 (s, 1H, ArH), 8.76 (d, $J = 4.1$ Hz, 1H, ArH), 9.24 (d, $J = 8.8$ Hz, 1H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 17.88, 48.76, 48.76, 52.14, 52.14, 53.61, 111.99, 114.83, 117.73, 119.42, 124.26, 126.55, 127.16, 131.45, 132.54, 133.51, 135.23, 136.72, 148.00, 150.16, 163.00. HRMS calcd for $C_{21}H_{22}N_4O_3$, $[M + H]^+$, 379.1725; found 379.1820.

*4.2.3.4. 7-((4-(m-Tolyl)piperazin-1-yl)methyl)-5-nitroquinolin-8-ol (**5d**).* Yellow solid; yield 81%; mp 187–188 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.25 (s, 3H, CH_3), 3.03 (t, $J = 5.0$ Hz, 4H, $2CH_{2\text{piperazine}}$), 3.30 (t, $J = 5.4$ Hz, 4H, $2CH_{2\text{piperazine}}$), 4.07 (s, 2H, CH_2), 6.63 (d, $J = 7.4$ Hz, 1H, ArH), 6.77 (s, 1H, ArH), 7.10 (t, $J = 7.8$ Hz, 1H, ArH), 7.39 (d, $J = 13.4$ Hz, 1H, ArH), 7.68 (dd, $J = 8.8$, 4.1 Hz, 1H, ArH), 8.60 (s, 1H, ArH), 8.77 (d, $J = 4.1$ Hz, 1H, ArH), 9.23 (d, $J = 8.7$ Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 21.80, 46.01, 46.01, 51.26, 51.26, 53.53, 111.96, 113.60, 117.01, 121.22, 124.28, 126.55, 129.41, 132.30, 133.53, 135.28, 136.69, 138.75, 147.94, 149.95, 163.07. HRMS calcd for $C_{21}H_{22}N_4O_3$, $[M + H]^+$, 379.1725; found 379.2224.

*4.2.3.5. 7-((4-(p-Tolyl)piperazin-1-yl)methyl)-5-nitroquinolin-8-ol (**5e**).* Yellow solid; yield 51%; mp 206–206 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.20 (s, 3H, CH_3), 2.94 (t, $J = 5.3$ Hz, 4H, $2CH_{2\text{piperazine}}$), 3.21 (t, $J = 5.5$ Hz, 4H, $2CH_{2\text{piperazine}}$), 4.03 (s, 2H, CH_2), 6.85 (d, $J = 8.3$ Hz, 2H, ArH), 7.03 (d, $J = 8.3$ Hz, 2H, ArH), 7.73 (dd, $J = 8.9$, 4.2 Hz, 1H, ArH), 8.64 (s, 1H, ArH), 8.83 (s, 1H, ArH), 9.28 (d, $J = 8.9$ Hz, 1H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 20.48, 46.42, 46.42, 51.28, 51.28, 53.52, 111.96, 116.65, 116.65, 117.94, 124.28, 126.56, 129.40, 130.00, 130.00, 132.29, 133.53, 135.29, 136.70, 147.83, 163.08. HRMS calcd for $C_{21}H_{22}N_4O_3$, $[M + H]^+$, 379.1725; found 379.1804.

*4.2.3.6. 7-((4-(2-Methoxyphenyl)piperazin-1-yl)methyl)-5-nitroquinolin-8-ol (**5f**).* Yellow solid; yield 79%; mp 196–201 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 3.09–3.15 (m, 8H, $4CH_{2\text{piperazine}}$), 3.78 (s, 3H, OCH_3), 4.13 (s, 2H, CH_2), 7.15–6.76 (m, 4H, ArH), 7.68 (dd, $J = 8.8$, 4.1 Hz, 1H, ArH), 8.62 (s, 1H, ArH), 8.76 (d, $J = 5.8$ Hz, 1H, ArH), 9.26 (d, $J = 10.4$ Hz, 1H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 45.91, 45.91, 51.19, 51.19, 53.52, 55.42, 102.73, 105.58, 108.84, 111.95, 117.76, 124.29, 126.56, 130.34, 132.31, 133.53, 135.30, 136.69, 147.94, 151.26, 160.73. HRMS calcd for $C_{21}H_{22}N_4O_4$, $[M + H]^+$, 395.1675; found 395.2277.

*4.2.3.7. 7-((4-(3-Methoxyphenyl)piperazin-1-yl)methyl)-5-nitroquinolin-8-ol (**5g**).* Yellow solid; yield 79%; mp 177–178 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 3.01 (t, $J = 5.2$ Hz, 4H, $2CH_{2\text{piperazine}}$), 3.32 (t, $J = 5.1$ Hz, 4H, $2CH_{2\text{piperazine}}$), 3.71 (s, 3H, OCH_3), 4.05 (s, 2H, CH_2), 6.40 (d, $J = 8.1$ Hz, 1H, ArH), 6.47 (s, 1H, ArH), 6.53 (d, $J = 8.4$ Hz, 1H, ArH), 7.12 (t, $J = 8.2$ Hz, 1H, ArH), 7.68 (dd, $J = 8.8$, 4.2 Hz, 1H, ArH), 8.60 (s, 1H, ArH), 8.78 (d, $J = 3.9$ Hz, 1H, ArH), 9.23 (d, $J = 8.8$ Hz, 1H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 47.27, 47.27, 52.23, 52.23, 55.37, 56.20, 102.29, 105.01, 108.67, 111.95, 116.70, 124.68, 125.08, 130.17, 132.78, 135.30, 147.66, 152.12, 158.63, 159.99, 160.68. HRMS calcd for $C_{21}H_{22}N_4O_4$, $[M + H]^+$, 395.1675; found 395.2348.

*4.2.3.8. 7-((4-(4-Methoxyphenyl)piperazin-1-yl)methyl)-5-nitroquinolin-8-ol (**5h**).* Yellow solid; yield 64%; mp 172–173 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 3.00 (t, $J = 4.0$ Hz, 4H, $2CH_{2\text{piperazine}}$), 3.18 (t, $J = 4.6$ Hz, 4H, $2CH_{2\text{piperazine}}$), 3.68 (s, 3H, OCH_3), 4.07 (s, 2H, CH_2), 6.99–6.78 (m, 4H, ArH), 7.72 (dd, $J = 8.8$, 4.2 Hz, 1H, ArH), 8.63 (s, 1H, ArH), 8.81 (d, $J = 4.2$ Hz, 1H, ArH), 9.26 (d, $J = 8.8$ Hz, 1H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 47.32, 47.32, 51.46, 51.46, 53.53, 55.66, 111.96, 114.85, 114.85, 118.49, 118.49, 124.29, 126.57, 132.31, 133.52, 135.32, 136.68, 144.15, 147.94, 154.16, 163.06. HRMS calcd for $C_{21}H_{22}N_4O_4$, $[M + H]^+$, 395.1675; found 395.2326.

*4.2.3.9. 7-((4-(2-Fluorophenyl)piperazin-1-yl)methyl)-5-nitroquinolin-8-ol (**5i**).* Yellow solid; yield 68%; mp 160.0–161 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.96 (s, 4H, $2CH_{2\text{piperazine}}$), 3.16 (t, $J = 5.4$ Hz, 4H, $2CH_{2\text{piperazine}}$), 4.02 (s, 2H, CH_2), 7.39 (dd, $J = 7.5$, 5.1 Hz, 4H, ArH), 8.58 (s, 1H, ArH), 8.63 (s, 1H, ArH), 8.87 (s, 1H, ArH), 9.26 (d, $J = 9.0$ Hz, 1H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 47.55, 47.55, 51.57, 51.57, 53.65, 111.89, 114.65, 117.55, 120.11, 125.45, 126.56, 127.39, 132.34, 133.52, 135.29, 136.69, 143.29, 145.80, 147.94, 163.05. HRMS calcd for $C_{20}H_{19}FN_4O_3$, $[M + H]^+$, 383.1475; found 383.2014.

*4.2.3.10. 7-((4-(4-Fluorophenyl)piperazin-1-yl)methyl)-5-nitroquinolin-8-ol (**5j**).* Yellow solid; yield 55%; mp 168–171 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 3.02 (t, $J = 3.2$ Hz, 4H, $2CH_{2\text{piperazine}}$), 3.27 (t, 4H, $2CH_{2\text{piperazine}}$), 4.06 (s, 2H, CH_2), 6.97 (t, $J = 4.5$ Hz, 2H, ArH), 7.06 (t, $J = 8.9$ Hz, 2H, ArH), 7.70 (dd, $J = 8.8$, 4.1 Hz, 1H, ArH), 8.61 (s, 1H, ArH), 8.79 (d, $J = 2.5$ Hz, 1H), 9.22 (d, $J = 8.8$ Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 48.89, 50.97, 50.97, 53.71, 53.74, 115.09, 118.0, 118.00, 118.00, 120.92, 123.38, 126.16, 133.19, 133.19, 134.54, 136.92, 148.45, 158.88, 159.23, 161.53. HRMS calcd for $C_{20}H_{19}FN_4O_3$, $[M + H]^+$, 383.1475; found 383.2049.

*4.2.3.11. 7-((4-(2,4-Difluorophenyl)piperazin-1-yl)methyl)-5-nitroquinolin-8-ol (**5k**).* Yellow solid; yield 62%; mp 197–203 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.96–2.54 (m, 8H, $4CH_{2\text{piperazine}}$), 3.92 (s, 2H, CH_2), 7.39 (d, $J = 6.8$ Hz, 1H, ArH), 7.61 (dd, $J = 8.7$, 4.1 Hz, 1H, ArH), 7.79 (s, 1H, ArH), 8.52 (s, 1H, ArH), 8.69 (s, 1H, ArH), 9.31 (d, $J = 8.8$ Hz, 1H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 48.84, 48.84, 50.98, 50.98, 53.67, 111.95, 114.85, 117.76, 117.76, 120.67, 123.51, 126.23, 126.23, 132.31, 133.05, 134.70, 136.86, 148.36, 159.14, 161.74. HRMS calcd for $C_{20}H_{18}F_2N_4O_3$, $[M + H]^+$, 401.1381; found 401.2038.

*4.2.3.12. 7-((4-(4-Chlorophenyl)piperazin-1-yl)methyl)-5-nitroquinolin-8-ol (**5l**).* Yellow solid; yield 61%; mp 192–197 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.99 (t, $J = 5.0$ Hz, 4H, $2CH_{2\text{piperazine}}$), 3.32 (t, $J = 4.4$ Hz, 4H, $2CH_{2\text{piperazine}}$), 4.04 (s, 2H, CH_2), 6.96 (d, $J = 8.6$ Hz, 2H, ArH), 7.24 (d, $J = 8.9$ Hz, 2H, ArH), 7.71 (dd, $J = 8.8$, 4.1 Hz, 1H, ArH), 8.60 (s, 1H, ArH), 8.80 (d, $J =$

4.1 Hz, 1H, ArH), 9.21 (d, $J = 8.9$ Hz, 1H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 45.81, 45.81, 51.08, 51.08, 53.55, 111.93, 114.86, 117.94, 117.94, 124.04, 126.57, 129.28, 129.28, 132.27, 133.53, 135.34, 136.68, 147.91, 148.78, 163.10. HRMS calcd for $C_{20}H_{19}ClN_4O_3$, $[M + H]^+$, 399.1146; found 399.1839.

*4.2.3.13. 7-((4-(2,4-Dichlorophenyl)piperazin-1-yl)methyl)-5-nitroquinolin-8-ol (**5m**).* Yellow solid; yield 83%, mp 165–165 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.84 (t, $J = 4.0$ Hz, 4H, $2CH_{2\text{piperazine}}$), 3.31 (t, $J = 4.2$ Hz, 4H, $2CH_{2\text{piperazine}}$), 3.95 (s, 2H, CH_2), 6.94 (d, $J = 4.5$ Hz, 1H, ArH), 7.15 (d, $J = 4.8$ Hz, 1H, ArH), 7.39 (s, 1H, ArH), 7.77 (dd, $J = 8.8$, 4.1 Hz, 1H, ArH), 8.60 (s, 1H, ArH), 8.86 (d, $J = 2.5$ Hz, 1H, ArH), 9.25 (d, $J = 9.0$ Hz, 1H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 45.34, 45.34, 50.87, 50.87, 53.59, 111.90, 116.31, 117.45, 121.28, 124.33, 126.58, 127.40, 131.11, 132.12, 133.55, 135.36, 136.67, 147.90, 149.64, 158.66. HRMS calcd for $C_{20}H_{18}Cl_2N_4O_3$, $[M + H]^+$, 434.0726; found 434.3588.

*4.2.3.14. 5-Nitro-7-((4-(4-nitrophenyl)piperazin-1-yl)methyl)quinolin-8-ol (**5n**).* Yellow solid; yield 73%; mp 176–176 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.81 (t, $J = 5.1$ Hz, 4H, $2CH_{2\text{piperazine}}$), 3.57 (t, $J = 5.0$ Hz, 4H, $2CH_{2\text{piperazine}}$), 3.95 (s, 2H, CH_2), 7.05 (d, $J = 9.5$ Hz, 2H, ArH), 7.39 (dd, $J = 7.7$, 5.7 Hz, 1H, ArH), 7.95–7.67 (m, 2H, ArH), 8.64 (s, 1H, ArH), 8.92 (d, $J = 10.5$ Hz, 1H, ArH), 9.22 (d, $J = 9.2$ Hz, 1H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 44.18, 44.18, 50.77, 50.77, 53.65, 112.96, 113.94, 113.94, 124.31, 126.10, 126.10, 128.39, 132.26, 133.52, 135.30, 136.71, 138.44, 147.91, 154.14, 163.13. HRMS calcd for $C_{20}H_{19}N_5O_5$, $[M + H]^+$, 410.1420; found 410.2100.

*4.2.3.15. 5-Nitro-7-((4-(pyridin-2-yl)piperazin-1-yl)methyl)quinolin-8-ol (**5o**).* Yellow solid; yield 88%; mp 190–191 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.97 (t, $J = 5.1$ Hz, 4H, $2CH_{2\text{piperazine}}$), 3.68 (t, $J = 4.8$ Hz, 4H, $2CH_{2\text{piperazine}}$), 4.05 (s, 2H, CH_2), 6.68 (dd, $J = 7.2$, 4.8 Hz, 1H, ArH), 6.87 (d, $J = 8.6$ Hz, 1H, ArH), 7.39 (t, 7.36, 1H, ArH), 7.70 (dd, $J = 8.8$, 4.2 Hz, 1H, ArH), 7.79 (t, $J = 6.7$ Hz, 1H, ArH), 8.61 (s, 1H, ArH), 8.79 (d, $J = 4.0$ Hz, 1H, ArH), 9.22 (d, $J = 8.7$ Hz, 1H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 42.64, 42.64, 50.87, 50.87, 53.64, 109.30, 111.9, 114.53, 117.81, 127.14, 132.33, 133.48, 135.27, 136.70, 139.67, 143.84, 146.01, 147.94, 163.05. HRMS calcd for $C_{19}H_{19}N_5O_3$, $[M + H]^+$, 366.1521; found 366.2084.

*4.2.3.16. 5-Nitro-7-((4-(pyrimidin-2-yl)piperazin-1-yl)methyl)quinolin-8-ol (**5p**).* Yellow solid; yield 94%; mp 191–192 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.95 (t, $J = 5.2$ Hz, 4H, $2CH_{2\text{piperazine}}$), 3.92 (t, $J = 5.2$ Hz, 4H, $2CH_{2\text{piperazine}}$), 4.02 (s, 2H, CH_2), 6.68 (t, $J = 4.7$ Hz, 1H, ArH), 7.39 (t, $J = 4.7$ Hz, 1H, ArH), 7.69 (dd, $J = 8.6$, 4.0 Hz, 1H, ArH), 8.39 (d, $J = 4.7$ Hz, 1H, ArH), 8.58 (s, 1H, ArH), 8.79 (d, $J = 4.1$ Hz, 1H, ArH), 9.19 (d, $J = 8.8$ Hz, 1H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 40.55, 40.55, 51.01, 51.01, 53.68, 111.93, 124.24, 127.28, 132.38, 133.44, 135.21, 136.70, 143.51, 145.45, 147.97, 158.60, 158.60, 161.09. HRMS calcd for $C_{18}H_{18}N_6O_3$, $[M + H]^+$, 367.1474; found 367.2326.

*4.2.3.17. 5-Nitro-7-((4-(pyrazin-2-yl)piperazin-1-yl)methyl)quinolin-8-ol (**5q**).* Yellow solid; yield 53%; mp 206–207 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.89 (t, $J = 5.2$ Hz, 4H, $2CH_{2\text{piperazine}}$), 3.72 (t, $J = 5.2$ Hz, 4H, $2CH_{2\text{piperazine}}$), 3.99 (s, 2H, CH_2), 7.39 (dd, $J = 7.6$, 5.7 Hz, 1H, ArH), 7.87 (d, $J = 2.6$ Hz, 1H, ArH), 8.16–8.05 (m, 1H, ArH), 8.35 (d, $J = 2.3$ Hz, 1H, ArH), 8.62 (s, 1H, ArH), 8.84 (d, $J = 2.5$ Hz, 1H, ArH), 9.21 (d, $J = 8.8$ Hz, 1H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 41.74, 41.74, 50.77, 50.77, 53.69, 111.48, 114.33, 117.21, 127.65, 132.11, 133.48, 135.24, 141.99, 142.64, 146.65, 147.92, 154.21, 158.75. HRMS calcd for $C_{18}H_{18}N_6O_3$, $[M + H]^+$, 367.1474; found 367.2580.

4.3. Antifungal activity

The *in vitro* antifungal activity against *B. cinerea*, *S. sclerotiorum*, *F. graminearum*, *F. oxysporum*, *M. oryzae*, *R. solani*, *M. melonis*, *P. zeae*, *C. gossypii*, *P. capsici* and *P. aphanidermatum* were assayed by mycelium linear growth rate method as previously reported.[1] The strains were removed from their storage tubes and grown on potato dextrose agar (PDA) mediums for one week at 25 °C to allow the mycelia growth to be used for antifungal assays. The tested compounds were dissolved in DMSO and water containing Tween-80, and then added to the PDA to obtain mediums with different drug concentrations. The final concentration of DMSO was 0.5% because it had been shown to have no significant effect on the growth of the tested fungi. Azoxystrobin with different concentrations in the PDA mediums containing 0.5% DMSO (v/v) and 0.5% DMSO in the PDA medium were used as positive control and blank control respectively. A 5 mm diameter disc of fungus cut from subcultured Petri dishes was placed at the center of Petri dishes which contained PDA mediums with different drug concentrations. The diameter of mycelia was measured when the fungi in the blank control completely covered the Petri dish. The inhibition percentages were calculated using the formula:[30] $I\ (\%) = ([[(C - d) - (T - d)]])/((C - d)) \times 100$, where d is diameter of the cut fungus (5 mm), I is the inhibition (%), and C and T are the average colony diameters of the mycelium of the blank control and treatment respectively.

After the preliminary antifungal activity screening, compounds with better activity were selected to further determine their medium effective concentrations (EC$_{50}$) according to the same methods described above. A series of PDA mediums containing 50, 25, 10, 5, 2.5 μg mL^{-1} respectively of the tested compounds were prepared. Azoxystrobin was used as a positive control and 0.5% DMSO as a blank control respectively. Each test was performed in triplicate.

4.4. Effects of compounds 2 and 5c on hyphal morphology of *S. sclerotiorum*

A mycelial disk (5 mm diameter) was taken from the periphery of the colony grown on PDA mediums containing EC$_{50}$ (0.0016 mM) of compound **2** and EC$_{50}$ (0.0030 mM) of compound **5c** respectively. The samples were inoculated to microscope slides on the first day, and observed the mycelial morphology by optical microscope (Motic AE31E) on the third day, respectively. Scanning electron microscopy observations on the hyphae of *S. sclerotiorum* were conducted according to the method of previous studies.[32] A mycelial disk (5 mm diameter) was taken from the periphery of the colony grown on PDA mediums containing EC$_{50}$ (0.0016 mM) of compound **2** and EC$_{50}$ (0.0030 mM) of compound **5c** respectively. Samples were fixed in 2.5% glutaraldehyde for 24 h at room temperature, and were washed for 15 min with 0.1 mol L^{-1} phosphate buffer for three times, followed another 1 h fixation in 1% OsO_4 solution. The specimens were dehydrated in a grated ethanol series (20%, 50%, 80% and 100% respectively, 5 min for each alcohol dilution). After drying at critical point and gold coating, SEM observations were carried out with a scanning electron microscope (Hitachi, S-3400N, Japan) at an accelerating voltage of 15.0 kV.

4.5. Effects of compounds 2 and 5c against *S. sclerotiorum in vivo*

The control efficacy (protective and curative activity) of compounds **2** and **5c** against *S. sclerotiorum* in leaves of oilseed rape was assessed with pot experiments according to the method described by Yan *et al.*[33] Firstly, 30 day-old oilseed rape leaves were washed with distilled water. For curative effect assay, the mycelial plugs were inoculated to the leaves on the first day, on the second day, the compounds **2** and **5c** solutions as well as the positive control azoxystrobin with different concentrations (20, 40 and 80 μg mL^{-1}) respectively (containing 0.1% Tween 80 as surfactant) were sprayed on the leaves. Plants sprayed with water (plus 0.1% Tween 80) were used as a negative control. Then the plants were placed in a greenhouse at 25 °C with 100% relative humidity. After 3 days, the lesion diameter was measured and the curative efficacy of compounds **2** and **5c** was calculated according to the following formula: (diameter of lesion in negative control − diameter of lesion in the treatment)/diameter of lesion in negative control. There were three replicates for each treatment, and the experiment was repeated at least twice. For protection assay, the mycelial plugs were inoculated to the leaves for one day after the leaves were sprayed with test sample solutions. The rest of the steps were the same as the above.

4.6. Minimal inhibitory concentration (MIC) test of compound 2 against plant pathogenic bacteria

MIC values were determined by the broth microdilution method in 96-well microtiter plates.[31] Dilutions of compound **2**, ranging from 1 to 1000 μg mL^{-1} were incubated with corresponding bacterial suspensions adjusted to 5×10^5 CFU mL^{-1} in Mueller–Hinton Broth (MHB). Streptomycin sulfate was used as a positive control, and the vehicle was used as a negative control. The microtiter plates were incubated at 37 °C, after 24 h of incubation, readings were performed by visual reading and optical-density (OD 595 nm) determination in a BioTek microplate reader (Highland Park, Winooski, USA). The MIC value was defined as the lowest compound concentration that prevented bacterial growth after a 24 h incubation. MIC values were determined by three independent replicates.

Conflicts of interest

The authors state no conflict of interest.

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