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ACCEPTED MANUSCRIPT

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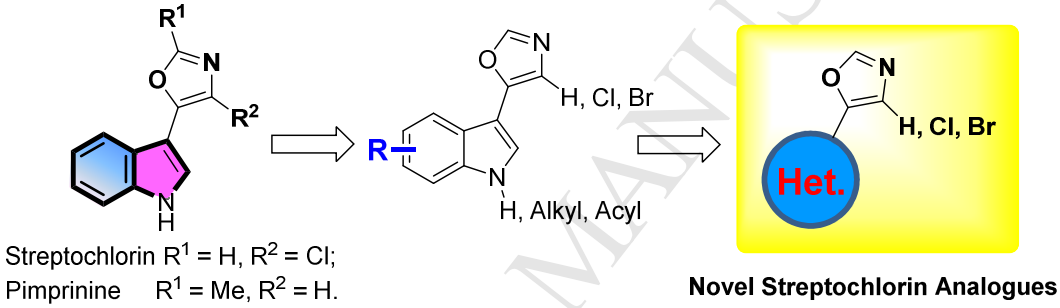

A series of streptochlorin analogues in which indole replaced by other heterocycles has been synthesized and evaluated for their antifungal activity, the SAR demonstrated indole is essential for the activity.

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Synthesis and antifungal activity of novel indole-replaced streptochlorin analogues

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Abstract:

Based on examples of the successful applications in drug discovery of bioisosterism, a series of streptochlorin analogues in which indole has been replaced by other heterocycles has been designed and synthesized, as a continuation of our studies aimed at the discovery of novel streptochlorin analogues with improved antifungal activity. Biological testing showed that most of the indole-replaced streptochlorin analogues were inactive, though compound **6f** had a broad spectrum of antifungal activity with significant activity against *Alternaria solani*. The SAR study demonstrated that indole ring is an essential moiety for the antifungal activity of streptochlorin analogues, promoting the idea of indole ring as a framework that might be exploited in the future.

Keywords: Streptochlorin analogues; active heterocycles; synthesis; antifungal activity; SAR.

1. Introduction

Streptochlorin is an indole alkaloid isolated from marine *Streptomyces sp*[1, 2], belonging to the family of naturally occurring 5-(3-indolyl)oxazoles, it's reported to possess a broad spectrum of biological activity, such as anticancer, antiangiogenic, and antiallergic effects[3-5].

Streptochlorin also exhibits selective cytotoxicity against several cancer cell lines, potently inhibits TNF-α-induced NF-κB activation and it is a potent suppressor of angiogenesis *in vitro*. Members of this family, including streptochlorin, pimprinine and other natural products, have also been claimed to demonstrate a variety of biological activities, including antiviral[6], antibiotic[7], anticonvulsant[8], and pesticidal activity[9]. Biological activity screening conducted at Syngenta showed that streptochlorin and pimprinine demonstrated good antifungal activity against *Pythium dissimile*, *Botrytis cinerea*, *Zymoseptoria tritici*, *Pyricularia oryzae*, *Fusarium culmorum* and *Rhizoctonia solani*[10], but these two compounds lack potency, rarely extending to the further study.

In order to find new lead candidates from natural products for further structural optimization, we have described various structural modifications to streptochlorin in our previous study, including different substituents at the indole ring and the nitrogen of the indole ring, replacing the Cl on the oxazole ring by Br or H[10, 11], and replacing the oxazole ring by oxadiazole[12]. In a continuation of our studies aimed at the discovery of novel streptochlorin analogues though step-by-step optimization, we now describe this study which has focused on replacement of the indole ring of streptochlorin with other active heterocycles (**Figure 1**).

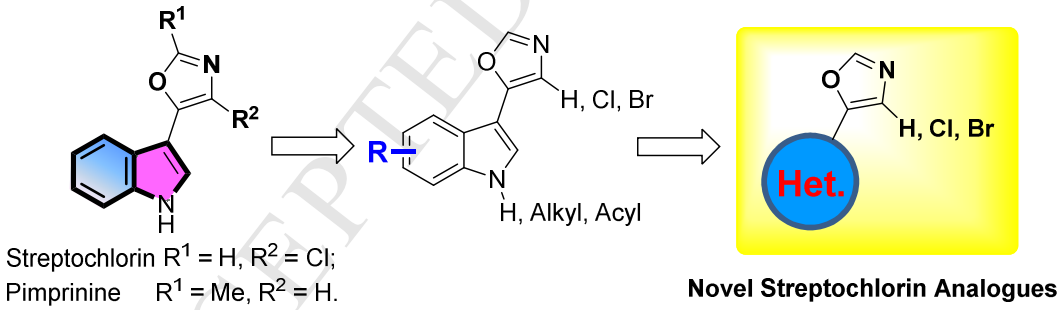


Figure 1. Design strategies of target molecules

As good bioisosteres of indole, other heterocycles such as benzofuran, benzothiophene, piperonal, naphthalene and quinoline exist widely in commercial drugs and agricultural chemicals[13-15], displaying a broad spectrum of biological activities (**Figure 2**). For example, **Vilazodone**, containing both an indole and benzofuran ring, is approved for the treatment of major depressive disorder and is a selective serotonin reuptake inhibitor (SSRI)[16]. **Dronedarone**, approved by the FDA in 2009, is a benzofuran-containing antiarrhythmic drug developed by Sanofi-Aventis[17]. **Raloxifene**, a benzothiophene-containing drug developed by Eli Lilly and

marketed as Evista, is used in the prevention of osteoporosis in postmenopausal women as an oral selective estrogen receptor modulator (SERM)[18]. **Oxolinic acid**, a piperonyl antibiotic developed in Japan, shows high bioavailability and is widely used in aquaculture for the treatment of a variety of systemic bacterial infections of fish[19]. **Tadalafil**, listed in the Top 100 pharmaceutical drugs by US retail sales under the name Cialis in 2012 and 2013 [20], contains both indole and piperonyl ring in its structure and is a PDE5 inhibitor for the treatment of men's erectile dysfunction (ED). **Berberine**, an isoquinoline-containing natural alkaloid revered and with a long history in traditional Chinese medicine, was used orally for various parasitic and fungal infections[21]. **Quinine** occurs naturally in the bark of the cinchona tree and was the first effective western treatment for malaria caused by *Plasmodium falciparum*[22].

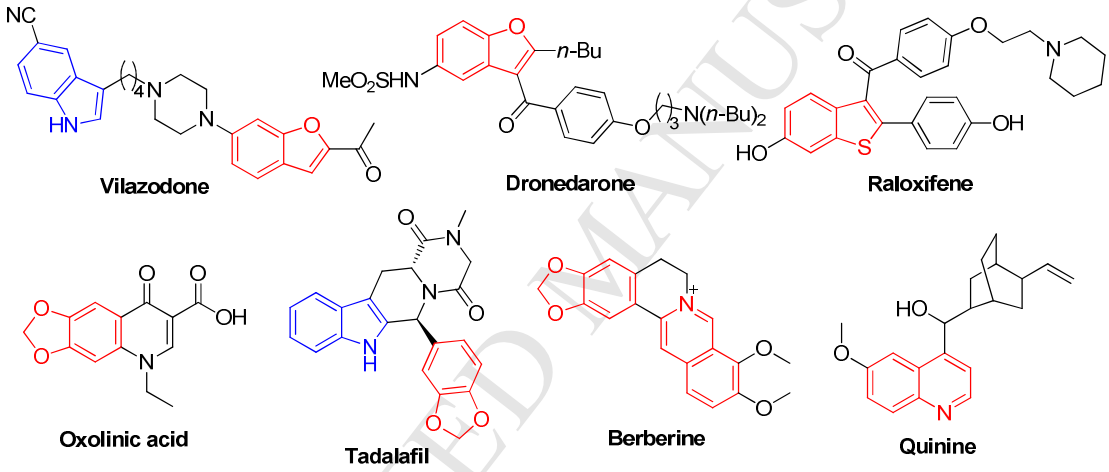


Figure 2.Structures of drugs and agrochemicals containing active heterocycles

Replacement of an indole ring with other active heterocycles was proved to be an effective protocol for structural optimization. There are several examples (**Figure 3**), such as indole-containing compound **1**, which has been evaluated for anticancer activity against a panel of 60 human cancer cell lines, it showed potent anti-leukemic activity against leukemia MV4-11 cell lines with an LD_{50} value of 44 nM. The synthesized compound **2** in which indole is replaced by benzothiophene showed improved activity with an LD_{50} value of 18 nM[23]. **Fluvastin 3**, the first synthetic indole-containing statin launched by Sandoz pharmaceutical company, opened up the opportunity of discovering more potent synthetic statins including **Pitavastatin 4**, the quinolone-containing blood cholesterol-lowering drug[24].

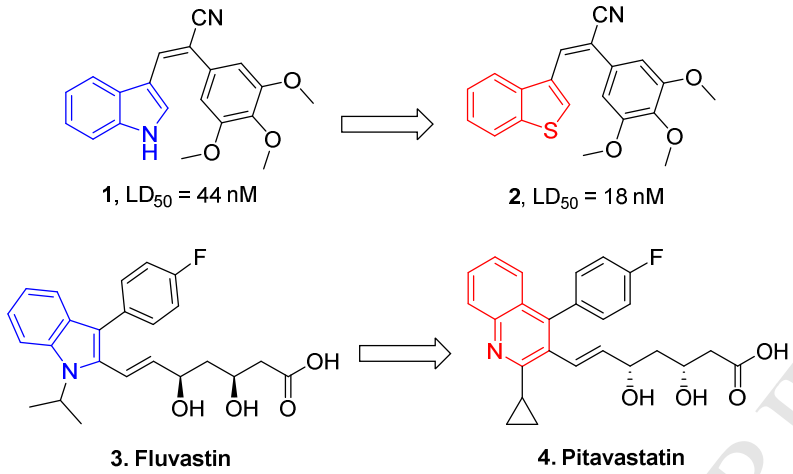


Figure 3. Structural optimization–replacement of indole by alternative heterocycles

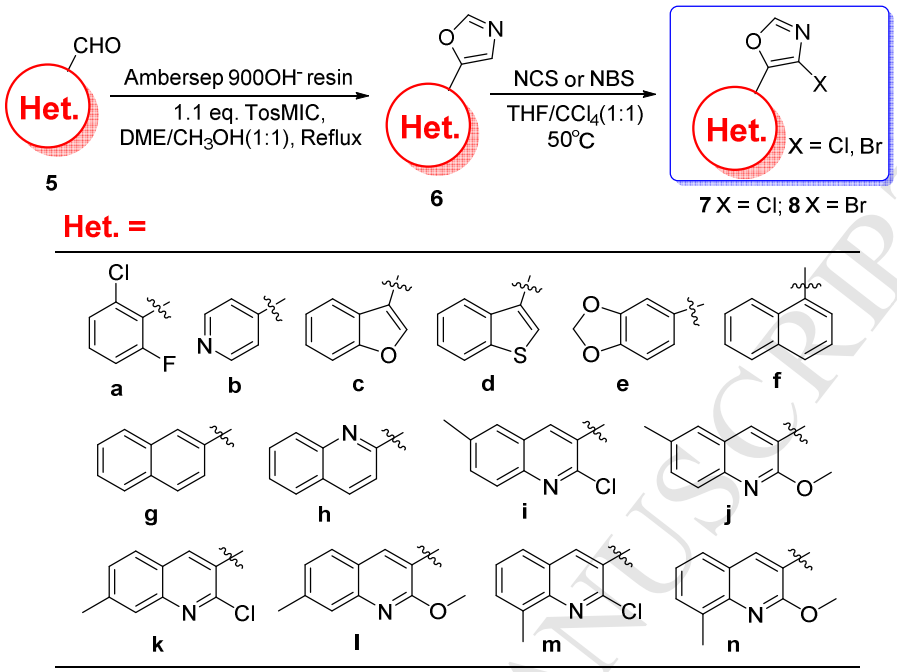
2. Materials and methods

2.1. Chemistry

All chemicals were purchased from commercial sources (e.g., Crystal Chemicals, Alfa Aesar Co.) and used without further purification unless otherwise stated. All solvents and liquid reagents were dried by standard methods in advance and distilled before use. Melting points were taken on a Büchi B-545 melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a VARIAN Mercury-Plus 600 spectrometer in $CDCl_3$ or DMSO-$d_6$with TMS as the internal reference. ^{13}C NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 on a Varian Mercury 400/600 (100/150 MHz) spectrometer and chemical shifts (δ) were given in ppm relative to the center line of a triplet at 77.0 ppm of $CDCl_3$or 39.5 ppm of DMSO-d_6. Mass spectra were determined using a Trace MS 2000 organic mass spectrometry (EI-MS) or a ThermoFisher Mass platform DSQ II by electrospray ionization (ESI-MS), and the signals were given in *m/z*. Reaction yields were not optimized.

2-Chloro-6-fluorobenzaldehyde (**5a**), isonicotinaldehyde (**5b**), benzo[*b*]thiophene-3-carbaldehyde (**5d**), benzo[*d*][1,3]dioxole-5-carbaldehyde (**5e**), 1-naphthaldehyde (**5f**), 2-naphthaldehyde (**5g**) and quinoline-2-carbaldehyde (**5h**) were purchased from commercial sources. Benzofuran-3-carbaldehyde (**5c**) were synthesized by using the reported method[25], and 2-chloro-6-methylquinoline-3-carbaldehyde (**5i**), 2-methoxy-6-methylquinoline-3-carbaldehyde (**5j**), 2-chloro-7-methylquinoline-3-carbaldehyde (**5k**), 2-methoxy-7-methylquinoline-3-carbaldehyde (**5l**),

2-chloro-8-methylquinoline-3-carbaldehyde (**5m**), 2-methoxy-8-methylquinoline-3-carbaldehyde (**5n**) were obtained through the reported methods[26].



Scheme 1: Synthetic routes for the target compounds

2.1.1. Preparation of heterocycle-5-oxazole 6 (Scheme 1)

A stirred mixture of heterocycle-carboxaldehyde **5** (1.0 mmol) and *p*-toluene-sulfonylmethyl isocyanide (TosMIC) (1.1 mmol, 0.21 g) in 1:1 DME/ MeOH (15 mL, both anhydrous) was refluxed with Ambersep® 900(OH) ion exchange resin (2.0 g, exchange capacity 1.18 meq/mL) for 2 h. The reaction mixture was filtered, the resin was washed by MeOH twice (2×5 mL), and the combined filtrates were concentrated under reduced pressure to give the crude product, which was purified by column chromatography on silica gel (eluent: petroleum ether/acetone = 4:1) to afford the pure compound **6**.

2.1.1.1. *5-(2-chloro-6-fluorophenyl)oxazole (**6a**)*: a white solid, yield 74%, mp, 93-95℃. ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.07–7.16 (*m*, 1H), 7.28–7.37 (*m*, 2H), 7.48 (*s*, 1H), 8.06 (*s*, 1H). ESI-MS: *m/z* 160.8 (MH^+). Anal.Calcd. for C_9H_5ClFNO: C, 54.71; H, 2.55; N, 7.09; Found: C, 54.66; H, 2.60; N, 7.12.

*2.1.1.2. 5-(pyridin-4-yl)oxazole (**6b**)*: a white solid, yield 99%, mp, 78-80℃.^{1}H NMR (600 MHz, $CDCl_3$) δ 7.54 (*d*, *J* = 4.8 Hz, 2H), 7.59 (*s*, 1H), 8.02 (*s*, 1H), 8.69 (*d*, *J* = 4.8 Hz, 2H). Anal.Calcd. for $C_8H_6N_2O$: C, 65.75; H, 4.14; N, 19.17; Found: C, 65.82; H, 4.20; N, 19.11.

*2.1.1.3. 5-(benzofuran-3-yl)oxazole (**6c**)*: a white solid, yield 46%, mp, 99-101℃.^{1}H NMR (600 MHz, $CDCl_3$) δ 7.33–7.46 (*m*, 2H), 7.57 (*d*, *J* = 8.4 Hz, 1H), 7.81 (*d*, *J* = 7.8 Hz, 1H), 7.99 (*d*, *J* = 13.8 Hz, 1H). EI-MS: *m/z* (%) 185 (M^+, 100), 156 (15), 130 (42), 102(28). Anal.Calcd. for $C_{11}H_7NO_2$: C, 71.35; H, 3.81; N, 7.56; Found: C, 71.44; H, 3.78; N, 7.47.

*2.1.1.4. 5-(benzo[b]thiophen-3-yl)oxazole (**6d**)*: a white solid, yield 28%, mp, 109-111℃. ^{1}H NMR (600 MHz, $CDCl_3$): δ: 7.34 (*s*, 1H), 7.36-7.40 (*m*, 2H), 7.56 (*s*, 1H), 7.79-7.80 (*d*, *J* = 7.8 Hz, 2H), 7. 83-7.84(*d*, *J* = 7.8Hz, 1H), 7.93(s, 1H). EI-MS: *m/z* (%) 201 (M^+, 100), 173 (14), 146 (31). Anal.Calcd. for C11H7NOS: C, 65.65; H, 3.51; N, 6.96; Found: C, 65.56; H, 3.35; N, 6.96.

*2.1.1.5. 5-(benzo[d][1,3]dioxol-5-yl)oxazole (**6e**)*: a white solid, yield 75%, mp, 70-72℃.^{1}H NMR (600 MHz, $CDCl_3$) δ 6.02 (*s*, 2H), 6.87 (*d*, *J* = 8.4 Hz, 1H), 7.12 (*s*, 1H), 7.18 (*d*, *J* = 8.4 Hz, 1H), 7.22 (*s*, 1H), 7.88 (*s*, 1H). Anal.Calcd. for $C_{10}H_7NO_3$: C, 63.49; H, 3.73; N, 7.40; Found: C, 63.24; H, 3.77; N, 7.20.

*2.1.1.6. 5-(naphthalen-1-yl)oxazole (**6f**)*: a white solid, yield 59%, ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.46 (*s*, 1H) , 7.56 (*m*, 3H), 7.74 (*d*, *J* = 7.2 Hz, 1H), , 7.92 (*dd*, *J* = 7.2, 4.2 Hz, 2H), 8.08 (*s*, 1H), 8.25 (*d*, *J* = 8.4 Hz, 1H). Anal.Calcd. for $C_{13}H_9NO$: C, 79.98; H, 4.65; N, 7.17; Found: C, 80.03; H, 4.58; N, 7.25.

*2.1.1.7. 5-(naphthalen-2-yl)oxazole (**6g**)*: a white crystal, yield 71%, mp, 102-104℃. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.48 (*s*, 1H), 7.52 (*m*, 2H), 7.73 (*d*, *J* = 8.4 Hz, 1H), 7.85 (*d*, *J* = 7.8 Hz, 1H), 7.89 (*d*, *J* = 7.8 Hz, 2H), 7.99 (*s*, 1H), 8.15 (*s*, 1H). EI-MS: *m/z* (%) 195 (M^+, 100), 167 (27), 140 (44), 127 (30). Anal.Calcd. for $C_{13}H_9NO$: C, 79.98; H, 4.65; N, 7.17; Found: C, 79.75; H, 4.88; N, 7.07.

*2.1.1.8. 5-(quinolin-2-yl)oxazole (**6h**)*: a white crystal, yield 69%, mp, 60-62℃. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.56 (*t*, *J* = 7.4 Hz, 1H), 7.72–7.81 (*m*, 2H), 7.83 (*d*, *J* = 7.8 Hz, 1H), 7.87 (*s*, 1H), 8.07 (*s*, 1H), 8.15 (*d*, *J* = 8.4 Hz, 1H), 8.24 (*d*, *J* = 8.4 Hz, 1H). EI-MS: *m/z* (%) 196 (M^+, 100), 168(78), 140(30), 128 (23). Anal.Calcd. for $C_{12}H_8N_2O$: C, 73.46; H, 4.11; N, 14.28; Found: C, 73.30; H, 4.29; N, 14.07.

*2.1.1.9. 5-(2-chloro-6-methylquinolin-3-yl)oxazole (**6i**)*: a white solid, yield 59%, mp, 161-163℃. ^{1}H NMR (600 MHz, DMSO-d_6): *δ* 2.49(*s*, 3H), 7.59-7.61 (*d*, *J* = 8.4 Hz, 1H), 7.81 (*m*, 2H), 7.91(*s*, 1H), 8.60(*s*, 1H), 8.69(*s*, 1H). ^{13}C NMR (150 MHz, DMSO-d_6): *δ* 21.2, 120.4, 126.3, 126.9, 127.2, 133.7, 135.8, 137.0, 137.8, 139.2, 144.4, 145.9, 152.8. EI-MS: *m/z* (%) 240 (M^+, 100), 211 (91), 184 (66). Anal.Calcd. for $C_{13}H_9ClN_2O$: C, 63.81; H, 3.71; N, 11.45; Found: C, 64.04; H, 3.88; N,

11.39.

2.1.1.10. 5-(2-methoxy-6-methylquinolin-3-yl)oxazole ***(6j)***: a white solid, yield 29%, mp, 102-104℃. ^{1}H NMR (600 MHz, DMSO-d_6): δ 2.51(*s*, 3H), 4.12 (*s*, 3H), 7.51-7.52 (*d*, *J* = 7.2 Hz, 1H), 7.68-7.72 (*m*, 3H), 8.43 (*s*, 1H), 8.59(*s*, 1H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 20.9, 53.7, 112.2, 124.4, 126.2, 126.5, 127.1, 132.2, 133.0, 134.0, 143.1, 145.8, 152.1, 156.6. EI-MS: *m/z* (%) 240 (M^+, 100), 211 (91), 184 (66). Anal.Calcd. for $C_{14}H_{12}N_2O_2$: C, 69.99; H, 5.03; N, 11.66; Found: C, 70.14; H, 5.01; N, 11.60.

2.1.1.11. 5-(2-chloro-7-methylquinolin-3-yl)oxazole ***(6k)***: a white solid, yield 34%, mp, 154-156℃. ^{1}H NMR (600 MHz, $CDCl_3$): δ 2.58(*s*, 3H), 7.44-7.45(*d*, *J* = 7.8 Hz, 1H), 7.78-7.81(*m*, 2H), 7.90(*s*, 1H), 8.04(*s*, 1H),8.53 (*s*, 1H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 21.4, 102.7, 119.7, 124.5, 126.6, 128.1、130.1, 136.4, 140.6, 145.2, 146.0, 152.7, 153.2. EI-MS: *m/z* (%) 244(M^+, 100), 181(17), 154 (29). Anal.Calcd. for $C_{13}H_9ClN_2O$: C, 63.81; H, 3.71; N, 11.45; Found: C, 63.72; H, 3.94; N, 11.26.

2.1.1.12. 5-(2-methoxy-7-methylquinolin-3-yl)oxazole ***(6l)***: a white solid, yield 25%, mp, 138-140℃. ^{1}H NMR (600 MHz, $CDCl_3$): δ 2.54 (*s*, 3H), 4.22 (*s*, 3H), 7.26 (*s*, 1H), 7.68-7.69 (*m*,3H), 7.97 (*s*, 1H), 8.38 (*s*, 1H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 21.4, 53.7, 111.5, 122.4, 125.8, 126.2，126.7，127.9,，133.4, 140.4, 145.0，145.9, 151.9，157.1. EI-MS: *m/z* (%) 240 (M^+, 100), 211 (77), 184 (65). Anal.Calcd. for $C_{14}H_{12}N_2O_2$: C, 69.99; H, 5.03; N, 11.66; Found: C, 69.90; H, 5.23; N, 11.52.

2.1.1.13. 5-(2-chloro-8-methylquinolin-3-yl)oxazole ***(6m)***: a white solid, yield 29%, mp, 147-149℃. ^{1}H NMR (600 MHz, $CDCl_3$): δ 2.65 (*s*, 3H), 7.59-7.61 (*t*, *J* = 6.6 Hz, 1H), 7.72-7.73 (*d*, *J* = 6.0 Hz, 1H), 7. 96 (*s*, 1H), 7.96 (*s*, 1H),7.99-8.00 (*d*, *J* = 7.8 Hz,1H), 8.70 (*s*, 1H), 8.82 (*s*, 1H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 17.2, 126.3, 126.9, 127.7, 131.5, 135.4, 136.1, 137.0, 139.2, 144.9, 145.4, 152.8, 177.7. EI-MS: *m/z* (%) 244 (M^+, 100), 181 (17), 154 (19). Anal.Calcd. for $C_{13}H_9ClN_2O$: C, 63.81; H, 3.71; N, 11.45; Found: C, 63.85; H, 3.60; N, 11.37.

2.1.1.14. 5-(2-methoxy-8-methylquinolin-3-yl)oxazole ***(6n)***: a white solid, yield 17%, mp, 122-124℃. ^{1}H NMR (600 MHz, DMSO-d_6): δ 2.62 (*s*, 3H), 4.14 (*s*, 3H), 7.34-7.36 (*t*, *J* = 7.2 Hz, 1H), 7.53-7.54 (*d*, *J* = 6.0 Hz, 1H), 7.70 (*s*, 1H), 7.79-7.80 (*d*, *J* = 7.2 Hz,1H), 8.48 (*s*, 1H), 8.58 (*s*, 1H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 17.1, 54.0, 111.8, 124.2, 124.3, 126.0, 126.4, 130.2, 133.8, 134.0, 143.4, 145.7, 152.0, 156.1. EI-MS: *m/z* (%) 240 (M^+, 100), 211 (14), 184 (36). Anal.Calcd. for $C_{14}H_{12}N_2O_2$: C, 69.99; H, 5.03; N, 11.66; Found: C, 69.78; H, 5.28; N, 11.47.

2.1.2. General procedure for the synthesis of target compounds 7-8 (Scheme 1)

To a stirred solution of **6** (1.0 mmol) in THF/CCl_4 (1:1, 15 mL) was added NCS or NBS (1.1 mmol) and the resulting mixture was heated at 50 °C for 8 h till full conversion of the substrates was indicated by TLC analysis, then allowed to cool. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography using petroleum ether/acetone (5:1) as eluent to give the desired compounds **7** and **8**, respectively.

*2.1.2.1. 5-(benzofuran-3-yl)-4-chlorooxazole (**7c**)*: a white crystal, yield 35%, mp, 119-121℃. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.38 (*m*, 2H), 7.47-7.51 (*m*, 1H), 7.55 (*s*, 1H), 7.88–7.95 (*m*, 1H), 8.06 (*s*, 1H). Anal.Calcd. for $C_{11}H_6ClNO_2$: C, 60.16; H, 2.75; N, 6.38; Found: C, 60.21; H, 2.80; N, 6.35.

*2.1.2.2. 5-(benzo[b]thiophen-3-yl)-4-chlorooxazole (**7d**):* a white solid, yield 61%，mp, 101-103℃. ^{1}H NMR (600 MHz, $CDCl_3$): δ: 7.43-7.45 (*t*, *J* = 7.5 Hz 1H), 7.48-7.50 (*t*, *J* = 7.8, 1H), 7.82-7.83 (*d*, *J* = 7.8 Hz, 2H), 7. 86-7.87(*d*, *J* = 7.8Hz, 1H), 7.97 (s, 1H). EI-MS: *m/z* (%) 237 (37), 235 (M^+, 100), 180 (41), 145 (50). Anal.Calcd. for $C_{11}H_6ClNOS$: C, 56.06; H, 2.57; N, 5.94; Found: C, 56.31; H, 2.30; N, 5.83.

*2.1.2.3. 5-(benzo[d][1,3]dioxol-5-yl)-4-chlorooxazole (**7e**)*: a white crystal, yield 53%, ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.03 (*s*, 2H), 6.91 (*d*, *J* = 8.4 Hz, 1H), 7.35 (*s*, 1H), 7.40 (*d*, *J* = 8.4 Hz, 1H), 7.79 (*s*, 1H). ^{13}C NMR (600 MHz, DMSO-d_6): *δ*: 102.2, 105.6, 109.5, 120.1, 120.2, 123.0, 143.8, 148.4, 148.5, 150.9. Anal.Calcd. for $C_{10}H_6ClNO_3$: C, 53.71; H, 2.70; N, 6.26; Found: C, 53.99; H, 2.88; N, 6.15.

*2.1.2.4. 5-(benzofuran-3-yl)-4-bromooxazole (**8c**)*: a white solid, yield 61%, mp, 122-124℃. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.31–7.40 (*m*, 2H), 7.50 (*d*, *J* = 7.2 Hz, 1H), 7.63 (*s*, 1H), 7.92 (*d*, *J* = 7.2 Hz, 1H), 8.06 (*s*, 1H). Anal.Calcd. for $C_{11}H_6ClNO_2$: C, 50.03; H, 2.29; N, 5.30; Found: C, 49.98; H, 2.32; N, 5.35.

*2.1.2.5. 5-(benzo[b]thiophen-3-yl)-4-bromooxazole (**8d**)*: a white solid, yield 39%, mp, 98-100℃. ^{1}H NMR (600 MHz, $CDCl_3$): *δ*: 7.42-7.45 (*t*, *J* = 7.2 Hz 1H), 7.48-7.50 (*t*, *J* = 7.8 Hz, 1H), 7.82-7.83 (*d*, *J* = 7.8 Hz, 1H), 7. 85-7.86(*d*, *J* = 8.4 Hz, 1H), 7.94(*s*, 1H),7.98(*s*, 1H). ^{13}C NMR (600 MHz, DMSO-d_6): *δ*: 105.1, 122.9, 123.0, 124.9, 125.2, 126.1, 126.5, 136.5, 137.9, 144.2, 152.3. EI-MS: *m/z* (%) 281(M^+, 100), 280 (100), 172 (26), 145 (71). Anal.Calcd. for $C_{11}H_6BrNOS$: C, 47.16; H, 2.16; N, 5.00; S, 11.45; Found: C, 47.09; H, 2.22; N, 4.91; S, 11.64.

*2.1.2.6. 5-(benzo[d][1,3]dioxol-5-yl)-4-bromooxazole (**8e**)*: a white crystal, yield 31%, ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.02 (*s*, 2H), 6.87 (*d*, *J* = 8.4 Hz, 1H), 7.17 (*d*, *J* = 8.4 Hz, 1H), 7.22 (*s*, 1H),

7.87 (*s*, 1H). Anal.Calcd. for $C_{10}H_6BrNO_3$: C, 44.81; H, 2.26; N, 5.23; Found: C, 44.87; H, 2.35; N, 5.32.

*2.1.2.7. 4-bromo-5-(naphthalen-2-yl)oxazole (**8g**)*: a white solid, yield 25%, ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.41 (*s*, 1H), 7.57–7.49 (*m*, 3H), 7.65 (*d*, *J* = 7.8 Hz, 1H), 7.84 (*d*, *J* = 7.2 Hz, 1H), 7.88 (*d*, *J* = 8.4 Hz, 1H), 8.09 (*s*, 1H). Anal.Calcd. for $C_{10}H_6BrNO_3$: C, 56.96; H, 2.94; N, 5.11; Found: C, 57.05; H, 2.98; N, 5.21.

2.1.3. X-ray Diffraction Analysis

The crystallographic data have been deposited with the Cambridge Crystallographic Data Centre (CCDC) as supplementary publication number: 933865 (compound **6g**), 930417 (compound **7e**), shown in **Figure 4**. Copies of the data can be obtained free of charge via http://www.ccdc.cam.ac.uk/, or on application to CCDC, 12 Union Road, Cambridge CB12 1EZ, UK (Fax +44 1223 336033 or E-mail: deposit@ccdc.cam.ac.uk).

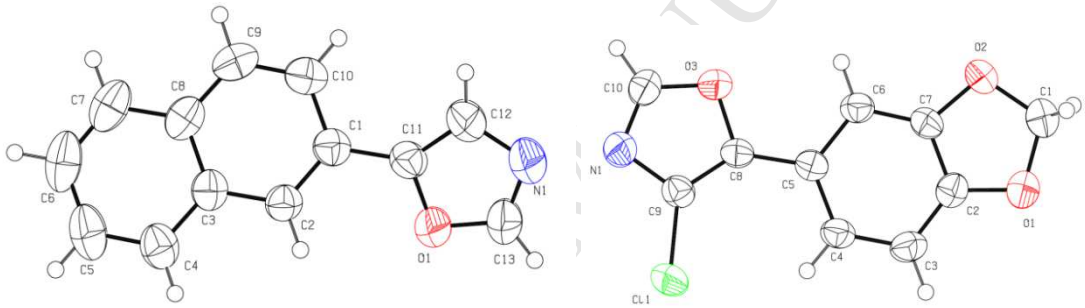


Figure 4. X-ray structures of compounds **6g** and **7e**

2.2. Biological Assays

The materials and methods used in the biological assays conducted at Syngenta were the same as those described in our earlier study[12]. Screening consists of seven different high throughput assays covering a selection of economically important plant pathogenic fungi and was performed using both leaf pieces and artificial media. The results of the biological testing are shown in **Table 1**.

Table 1. Antifungal activity of the target compounds

No.	Species	PHY[a]	ZYM[a]	URO[a]	PYT[a]	ALT[a]	BOT[a]	GIB[a]
	Rate (ppm)	200	100	100	20	20	20	20
	6a	0[b]	0	0	0	0	NC[c]	0
	6b	0	0	0	0	NC	0	0
	6c	0	0	0	0	0	0	0
	6d	0	0	0	0	**55**[d]	0	0

6e	0	27	0	27	0	0	0
6f	0	36	0	**55**	**99**	27	0
6g	0	36	0	**55**	27	0	0
6h	0	0	49	27	**55**	0	0
6i	0	18	0	0	0	0	0
6j	0	33	49	0	0	0	0
6k	0	0	0	0	0	0	0
6l	0	0	0	0	0	0	0
6m	0	0	0	0	0	0	0
6n	0	0	0	0	0	0	0
7c	0	0	0	0	NC	0	0
7d	0	0	0	0	0	0	0
7e	0	0	0	**55**	0	0	0
8c	0	0	0	0	0	0	0
8d	0	0	0	0	0	0	0
8e	0	36	49	**77**	0	27	0
8g	0	0	0	**55**	0	0	0
Pimprinine	0	51	27	0	0	0	0
Streptochlorin	49	36	**55**	**99**	**99**	**99**	**99**

[a]PHY, *Phytophthora infestans* (on tomato leaf pieces); ZYM, *Zymoseptoria tritici* (on wheat leaf pieces); URO, *Uromyces viciae-fabae* (on bean leaf pieces); PYT, *Pythium dissimile*; ALT, *Alternaria solani*; BOT, *Botryotinia fuckeliana*; GIB, *Gibberella zeae* (all in artificial media).

[b]The data are the mean of three replicates.

[c] Data not captured for this replicate (test failure).

[d] The bold means data equal to or above 55% control.

3. Results and discussion

3.1. Synthetic Chemistry

A series of indole-substituted streptochlorin analogues was efficiently synthesized based on the methods we had developed in our previous work. The synthetic routes to intermediates **6** and the streptochlorin analogues **7**-**8** are depicted in **Scheme 1**. The substituted heterocycle-carboxaldehydes **5** were either commercially available, or prepared by reported methods. In the key step of this sequence, the 5-linked oxazole was installed by Van Leusen's [3+2] cyclisation with TosMIC, under the catalytic influence of the ion exchange resin Ambersep® 900(OH), to give the heterocycle-5-oxazoles **6**. Some representative compounds of **6a-6n** (shown in **Scheme 1**) could be converted directly into the chlorinated or brominated streptochlorin analogues **7** or **8**, respectively by treatment with NCS or NBS.

3.2. Antifungal activity and the structure-activity relationships (SAR)

The results of the biological testing against seven phytopathogenic fungi are given in **Table 1**. For the purposes of an analysis of structure-activity relationships, the antifungal activities of compounds **6**, **7** and **8** were compared to the lead compounds, the natural products streptochlorin and pimprinine.

In the structure-activity point of view, although the antifungal activity of most of the indole-replaced streptochlorin analogues has been proven to be rather poor, making it difficult to extract a clear structure-activity relationship analysis, some broad conclusions can still be drawn. Firstly, *Pythium dissimile* and *Alternaria solani* were noticeably more sensitive to the indole-replaced streptochlorin analogues than the other tested fungi, and compound **6f** was identified as the most promising candidate, as it possessed significant activity to *Alternaria solani* and also a broad antifungal spectrum. Secondly, most of the target compounds were inactive against the tested fungi, and this is highlighted by compounds **7c** and **7d**, compared these structures with streptochlorin, just replaced the N of the indole ring by O or S, but the corresponding compounds **7c** and **7d** were totally inactive to all the tested fungi, the other target compounds also dramatically eliminated the antifungal activity. Thirdly, the effect on the antifungal activity of halogen atom (Cl, Br) at the 4-position of the oxazole ring was uncertain due to the absence of biological activity, though the potency of streptochlorin analogues could be generally improved by introducing a halogen atom in our previous research.

4. Conclusions

In order to find new lead candidates from natural products for further structural optimization, we have designed and synthesized a series of streptochlorin analogues based on our reported methods. In this study, the indole ring of streptochlorin was replaced by other heterocycles, including benzofuran, benzothiophene, piperonal, naphthalene and quinoline. Biological testing showed that most of the designed compounds had dramatically reduced antifungal activity in comparison with the natural products, though compound **6f** had a broad spectrum of antifungal activity with significant activity against *Alternaria solani*. The SAR study demonstrates that indole moiety has an important effect on the antifungal activity of the streptochlorin analogues, promoting the idea of the indole ring as a framework that might be exploited in the future. Further step-by-step structural optimization of streptochlorin analogues is well under way, in order to

better define their levels of antifungal activity and carry out the following QSAR studies in the future.

Acknowledgments

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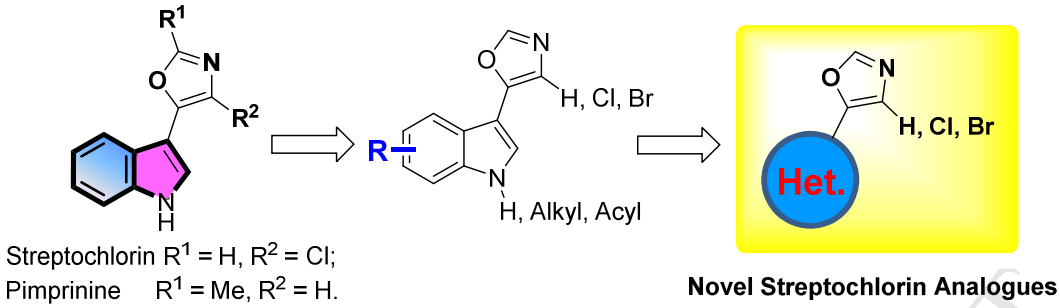

Figure 1. Design strategies of target molecules

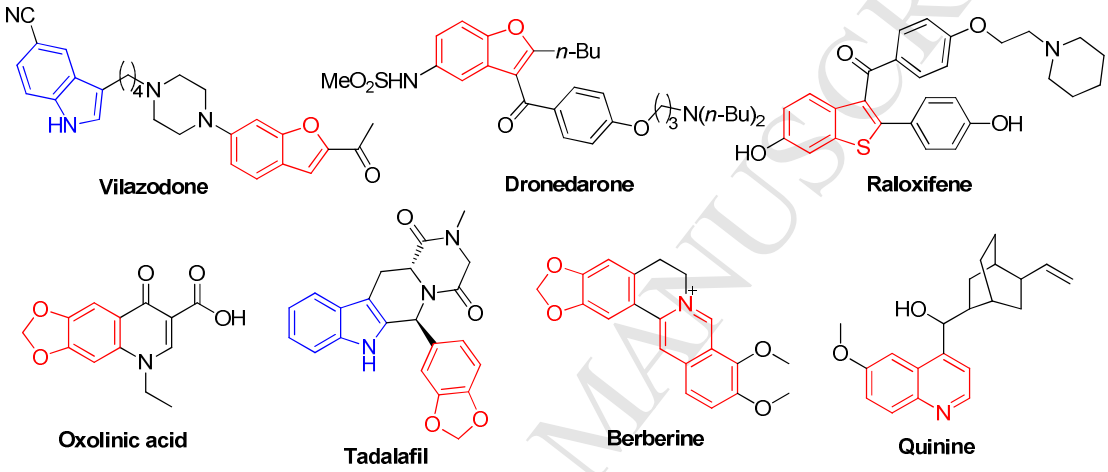

Figure 2.Structures of drugs and agrochemicals containing active heterocycles

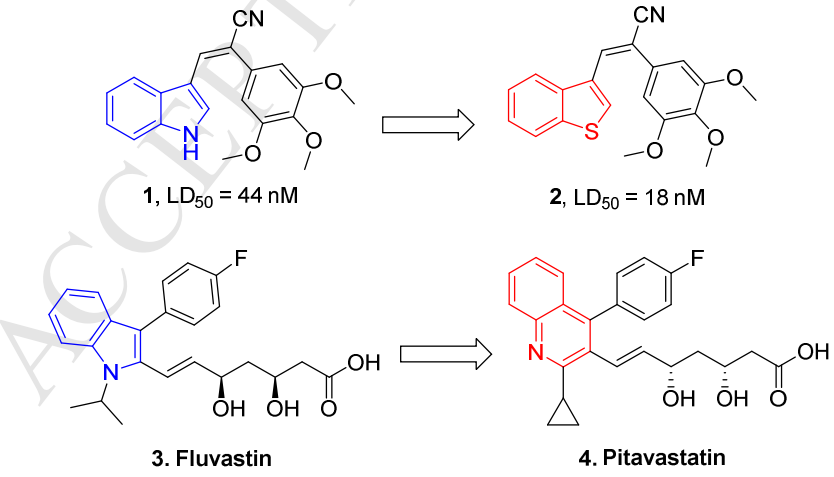

Figure 3. Structural optimization–replacement of indole by alternative heterocycles

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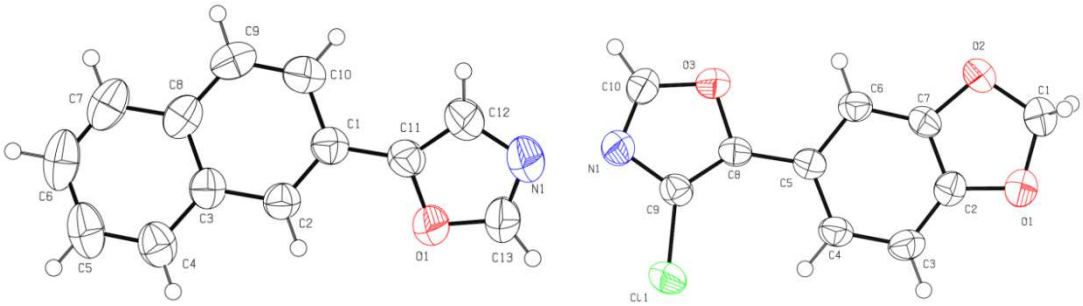
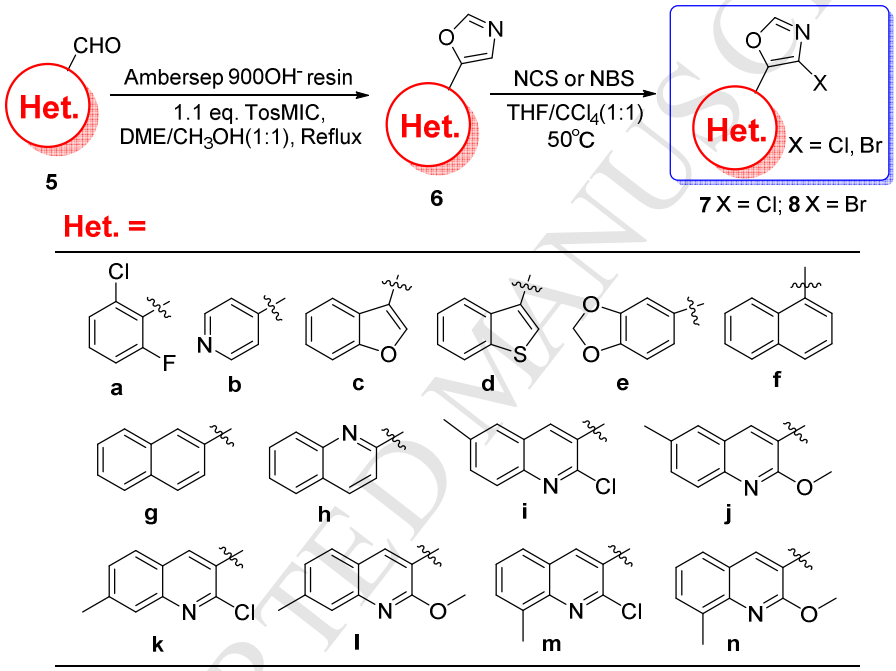


Figure 4. X-ray structures of compounds **6g** and **7e**



Scheme 1: Synthetic routes for the target compounds

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Table 1. Antifungal activity of the target compounds

No.	Species	PHY[a]	ZYM[a]	URO[a]	PYT[a]	ALT[a]	BOT[a]	GIB[a]
	Rate (ppm)	200	100	100	20	20	20	20
	6a	0[b]	0	0	0	0	NC[c]	0
	6b	0	0	0	0	NC	0	0
	6c	0	0	0	0	0	0	0
	6d	0	0	0	0	**55**[d]	0	0
	6e	0	27	0	27	0	0	0
	6f	0	36	0	**55**	**99**	27	0
	6g	0	36	0	**55**	27	0	0
	6h	0	0	49	27	**55**	0	0
	6i	0	18	0	0	0	0	0
	6j	0	33	49	0	0	0	0
	6k	0	0	0	0	0	0	0
	6l	0	0	0	0	0	0	0
	6m	0	0	0	0	0	0	0
	6n	0	0	0	0	0	0	0
	7c	0	0	0	0	NC	0	0
	7d	0	0	0	0	0	0	0
	7e	0	0	0	**55**	0	0	0
	8c	0	0	0	0	0	0	0
	8d	0	0	0	0	0	0	0
	8e	0	36	49	**77**	0	27	0
	8g	0	0	0	**55**	0	0	0
	Pimprinine	0	51	27	0	0	0	0
	Streptochlorin	49	36	**55**	**99**	**99**	**99**	**99**

[a]PHY, *Phytophthora infestans* (on tomato leaf pieces); ZYM, *Zymoseptoria tritici* (on wheat leaf pieces); URO, *Uromyces viciae-fabae* (on bean leaf pieces); PYT, *Pythium dissimile*; ALT, *Alternaria solani*; BOT, *Botryotinia fuckeliana*; GIB, *Gibberella zeae* (all in artificial media).

[b]The data are the mean of three replicates.

[c] Data not captured for this replicate (test failure).

[d] The bold means data equal to or above 55% control.

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Highlights

A series of streptochlorin analogues in which indole has been replaced by other heterocycles has been designed and synthesized.

Biological testing showed most of the designed streptochlorin analogues were inactive.

SAR study demonstrated that indole ring is essential for the antifungal activity of streptochlorin analogues, promoting the idea of indole as the framework that might be exploited in the future.

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