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Design, Synthesis and In Vitro Anthelmintic Evaluation of Novel 3,5-Diaryl-4,5-dihydroisoxazole (Isoxazoline) Derivatives

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Abstract. Helminth infections remain a major public-health burden, and the emergence of resistance to benzimidazole anthelmintics demands new chemical scaffolds. In the present work, a series of six 3,5-diaryl-4,5-dihydroisoxazoles (4a–f) was designed and synthesised in two steps: Claisen–Schmidt condensation of 4′-chloroacetophenone with substituted benzaldehydes to give chalcones (3a–f), followed by cyclisation with hydroxylamine hydrochloride under basic conditions. Reaction progress and purity were monitored by thin-layer chromatography, and the structures were supported by melting point, solubility, UV, FT-IR and ^{1}H-NMR data; C, H and N values were within ±0.4% of theory. Anthelmintic activity was evaluated in vitro at 0.25% and 0.50% w/v using the earthworm model, with albendazole as the standard, by recording the time of paralysis and death. All compounds were active, with 4b (4-F) and 4d (4-OH) the most potent; at 0.50% w/v, 4d paralysed the worms in 27.33 min, comparable with albendazole (27.07 min). The 4,4′-dichloro (4a) and 2,4-dichloro (4e) analogues were least active. These isoxazolines are promising templates for further optimisation and in silico studies.

Keywords: *Isoxazoline; 4,5-dihydroisoxazole; chalcone; anthelmintic; albendazole; earthworm model*

I. Introduction

Helminthiases caused by nematodes, cestodes and trematodes are among the most widespread neglected tropical diseases. The World Health Organization estimates that more than 1.5 billion people are infected with soil-transmitted helminths, mainly in areas with poor sanitation and limited access to safe water [1,2]. Heavy infection leads to abdominal discomfort, diarrhoea, anaemia, malnutrition and impaired physical and cognitive development in children. Control relies on periodic mass administration of albendazole and mebendazole, which act mainly by binding to parasite β-tubulin and inhibiting microtubule polymerisation [3]. Because large-scale, repeated use of a few drug classes favours resistance, new anthelmintic chemotypes are urgently needed.

Five-membered heterocycles containing nitrogen and oxygen are privileged scaffolds in medicinal chemistry. Isoxazole and its partially saturated analogue, 4,5-dihydroisoxazole (isoxazoline), occur in compounds with antibacterial, antifungal, anticancer, anti-inflammatory and anticonvulsant properties [4,5]. Isoxazolines are conveniently accessed from α,β-unsaturated ketones (chalcones) and hydroxylamine, which allows the aryl substituents on both rings to be varied systematically. Despite this versatility, the anthelmintic potential of simple 3,5-diaryl-isoxazolines has been explored far less than their other activities.

The present study therefore aimed (i) to design and synthesise a series of 3,5-diaryl-4,5-dihydroisoxazoles bearing a constant 4-chlorophenyl ring at C-3 and different halogen or hydroxyl substituents on the C-5 aryl ring, (ii) to characterise them by physicochemical and spectral methods, and (iii) to evaluate their in vitro anthelmintic activity against albendazole.

II. Materials and Methods

2.1 Chemicals and instruments

4-Bromo-, 4-hydroxy-, 4-chloro-, 4-fluoro-, 2,4-dichloro- and 2,4-dibromobenzaldehyde, methanol, ethanol, acetone, pyridine, DMF, DMSO and aqueous ammonia were obtained from commercial suppliers (Otto Kemi, CDH, Loba Chemie, Qualigens and Paskem Fine Chem, India) and used as received. Melting points were determined by the open-capillary method. UV spectra were recorded on a Systronics double-beam spectrophotometer, FT-IR spectra on an FT-IR

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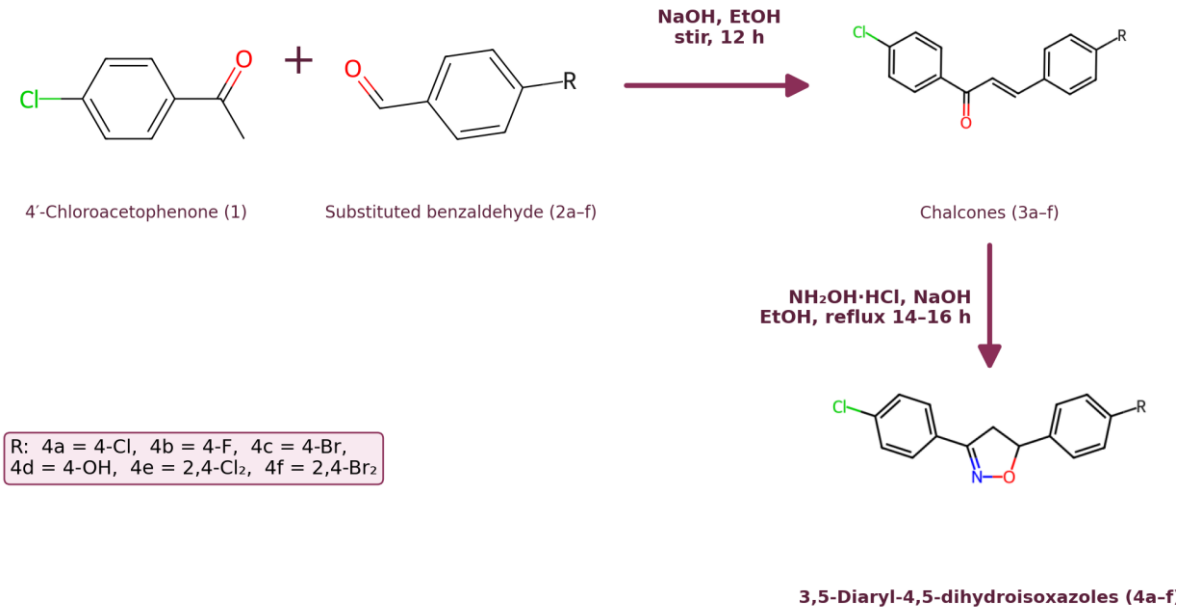
spectrometer using KBr, and ^{1}H-NMR spectra on a 300 MHz spectrometer with TMS as the internal standard and DMSO as solvent.

2.2 Synthesis of chalcones (3a–f)

4′-Chloroacetophenone (0.01 mol) was dissolved in absolute ethanol (25 mL) in a 100 mL conical flask, and ethanolic sodium hydroxide (0.01 mol) was added with stirring for 30 min. The appropriately substituted benzaldehyde (0.01 mol) was then added, and the mixture was stirred for about 12 h. The solid that separated was filtered, dried and recrystallised from ethanol (Scheme 1).

2.3 Synthesis of isoxazolines (4a–f)

A mixture of the purified chalcone (0.01 mol), hydroxylamine hydrochloride (0.03 mol) and sodium hydroxide (0.01 mol) in dry ethanol was refluxed for 14–16 h. After completion (TLC), most of the solvent was distilled off and the residue was poured into ice-cold water with vigorous stirring. The mixture was acidified with dilute hydrochloric acid and kept overnight in the cold. The solid obtained was filtered, washed with cold water, dried and recrystallised from ethanol.



Scheme 1. Synthetic route to 3,5-diaryl-4,5-dihydroisoxazoles (4a–f).

2.4 Physicochemical characterisation

Purity was checked by TLC on silica gel G plates (≈0.3 mm, activated at 105 °C for 30 min) with ethyl acetate–toluene (7:3 v/v) as the mobile phase and iodine vapour for visualisation. Solubility was assessed by adding 10 mg of each compound to 10 mL of water, methanol, chloroform, DMF or DMSO. Stock solutions (1000 µg/mL) for UV analysis were prepared in DMF or methanol and diluted to 100 µg/mL with methanol.

2.5 In vitro anthelmintic assay

Adult earthworms (4–6 cm long) were collected from moist soil and washed with 0.9% normal saline; they were used as a model for intestinal roundworms (*Ascaris lumbricoides*). Test compounds and the standard, albendazole, were dissolved in a minimal amount of DMSO and made up to 10 mL with normal saline to give 0.25% and 0.50% w/v solutions; normal saline served as the control. Worms of near-identical size were placed in Petri dishes containing the test solutions at room temperature. The time to paralysis (no movement except when shaken vigorously) and the time to death (no movement on shaking or dipping in warm water) were recorded in minutes. Data are expressed as mean ± SEM and were analysed by one-way ANOVA.

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III. Results and Discussion

3.1 Chemistry

Six chalcones were prepared by base-catalysed Claisen–Schmidt condensation and were converted to the corresponding 3-(4-chlorophenyl)-5-aryl-4,5-dihydroisoxazoles (Fig. 1). The reaction is believed to proceed through conjugate addition of hydroxylamine to the enone, followed by intramolecular cyclisation with loss of water. Single spots on TLC indicated that the products were free from starting materials. The compounds were crystalline solids, freely soluble in DMSO, sparingly soluble in ethanol and acetone, and insoluble in water. Their melting points (135–191 °C) and Rf values are given in Table 1 and Fig. 2.

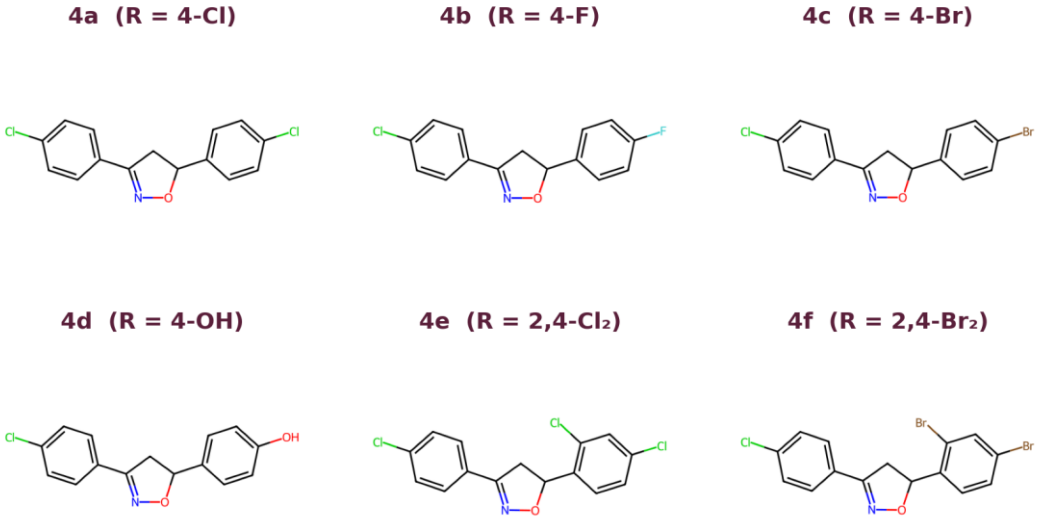

Fig. 1. Structures of the synthesised isoxazolines 4a–f.

Table 1. Physicochemical data of the synthesised compounds 4a–f.

Code	Name	Formula	M. wt.	M.p. (°C)	Rf
4a	3,5-Bis(4-chlorophenyl)-4,5-dihydroisoxazole	$C_{15}H_{11}Cl_2NO$	292	188–191	0.73
4b	3-(4-Chlorophenyl)-5-(4-fluorophenyl)-4,5-dihydroisoxazole	$C_{15}H_{11}ClFNO$	275	149–151	0.61
4c	5-(4-Bromophenyl)-3-(4-chlorophenyl)-4,5-dihydroisoxazole	$C_{15}H_{11}BrClNO$	336	156–158	0.63
4d	4-[3-(4-Chlorophenyl)-4,5-dihydroisoxazol-5-yl]phenol	$C_{15}H_{12}ClNO_2$	273	163–165	0.56
4e	3-(4-Chlorophenyl)-5-(2,4-dichlorophenyl)-4,5-dihydroisoxazole	$C_{15}H_{10}Cl_3NO$	326	148–150	0.50
4f	3-(4-Chlorophenyl)-5-(2,4-dibromophenyl)-4,5-dihydroisoxazole	$C_{15}H_{10}Br_2ClNO$	415	185–187	0.49

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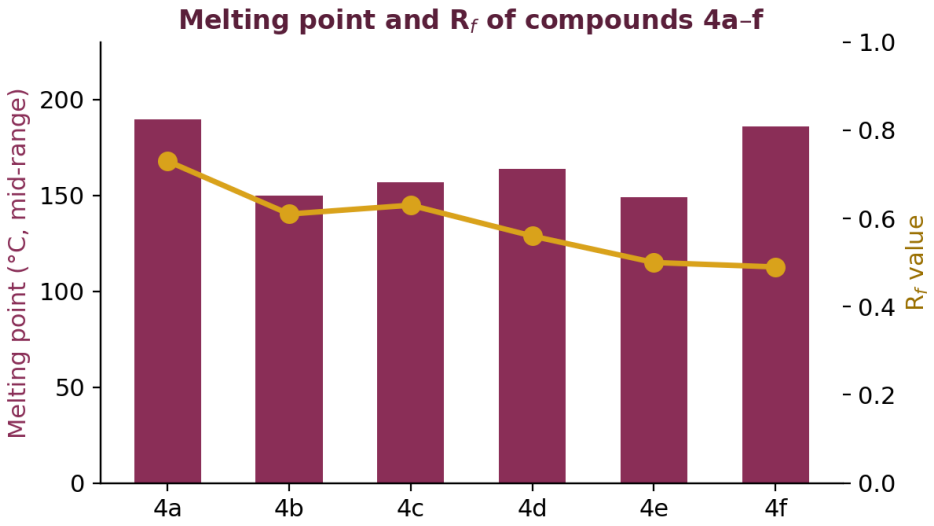

Fig. 2. Melting point (bars) and Rf value (line) of compounds 4a–f.

3.2 Spectral characterisation

The FT-IR spectra of all compounds showed aromatic C=C stretching at 1590–1664 cm^{-1}, aromatic C–H stretching near 3064 cm^{-1} (2920 cm^{-1} in 4d), C–N stretching at about 1320 cm^{-1} and C–Cl absorption between 525 and 651 cm^{-1} (Table 2; representative spectra in Fig. 3). In the ^{1}H-NMR spectra, the isoxazoline ring protons (CH_2–CH, 3H) and the aromatic protons (7–8H) were observed in the expected regions, and compound 4d additionally showed the phenolic OH singlet at δ 5.30. The proton counts of all compounds are consistent with the proposed structures.

Table 2. Key FT-IR (KBr, cm^{-1}) and ^{1}H-NMR (δ, ppm) data of compounds 4a–f.

Code	C=C (Ar)	C–H (Ar)	C–N	C–Cl	Ring protons (3H)	Aromatic H (δ)
4a	1591.07	3063.94	1320.03	642.07	5.30	6.80–7.62 (8H)
4b	1589.80	3063.92	1319.93	524.62	6.04	7.10–8.31 (8H)
4c	1591.96	3064.92	1320.27	525.60	4.95–5.01	5.78–6.20 (8H)
4d	1664.01	2919.52	1322.69	651.22	3.79 (OH: 5.30, 1H)	7.55–7.99 (8H)
4e	1658.69	3063.60	1385.97	641.61	4.59	7.55–7.98 (7H)
4f	1663.73	3064.01	1320.82	641.92	4.69	7.55–7.99 (7H)

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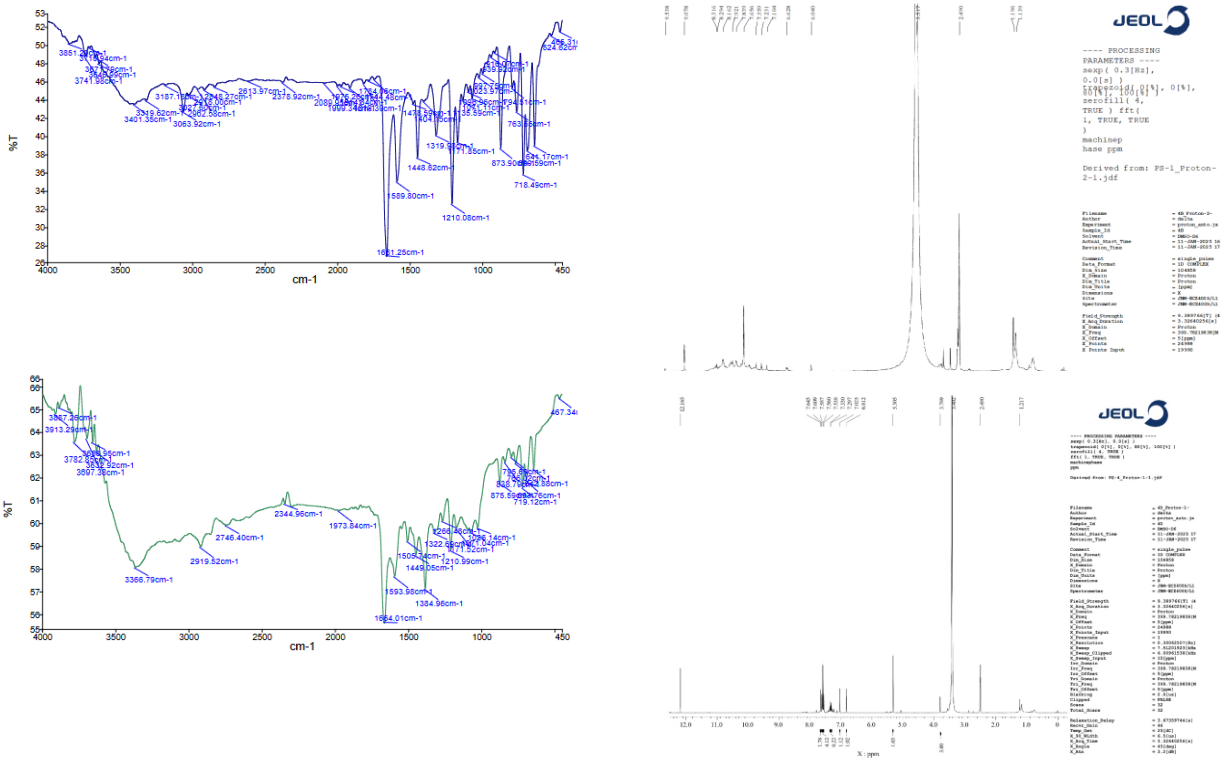

Fig. 3. FT-IR (left) and ^{1}H-NMR (right) spectra of the most active compounds 4b (top) and 4d (bottom).

3.3 Anthelmintic activity

All compounds paralysed and killed the worms at both concentrations, whereas the saline control showed no effect during the observation period. Activity increased with concentration for most compounds (Table 3, Fig. 4). Compounds 4b and 4d were the most potent: at 0.25% w/v their paralysis times (34.42 and 34.59 min) were close to that of albendazole (32.44 min), and at 0.50% w/v 4d (27.33 min) was practically equal to the standard (27.07 min), while 4b (29.31 min) was slightly slower. Compound 4f produced paralysis at 0.25% w/v (32.19 min) as fast as albendazole, although its death time was longer. Compounds 4a and 4e were the least active, requiring 41–55 min.

Table 3. Anthelmintic activity of compounds 4a–f (mean ± SEM, min).

Treatment	Paralysis 0.25%	Paralysis 0.50%	Death 0.25%	Death 0.50%
Control (saline)	–	–	–	–
Albendazole	32.44 ± 0.44	27.07 ± 0.10	33.21 ± 0.61	30.24 ± 0.22
4a	51.01 ± 0.71	45.18 ± 0.36	55.14 ± 0.65	51.45 ± 0.56
4b	**34.42 ± 0.17**	**29.31 ± 0.01**	**35.06 ± 0.47**	**36.44 ± 0.13**
4c	34.22 ± 0.65	33.34 ± 0.42	38.54 ± 0.54	34.18 ± 0.35
4d	**34.59 ± 0.24**	**27.33 ± 0.51**	**36.46 ± 0.25**	**33.24 ± 0.64**
4e	41.13 ± 0.54	44.32 ± 0.51	46.37 ± 0.35	52.24 ± 0.44
4f	32.19 ± 0.87	31.37 ± 0.45	36.25 ± 0.31	33.25 ± 0.55

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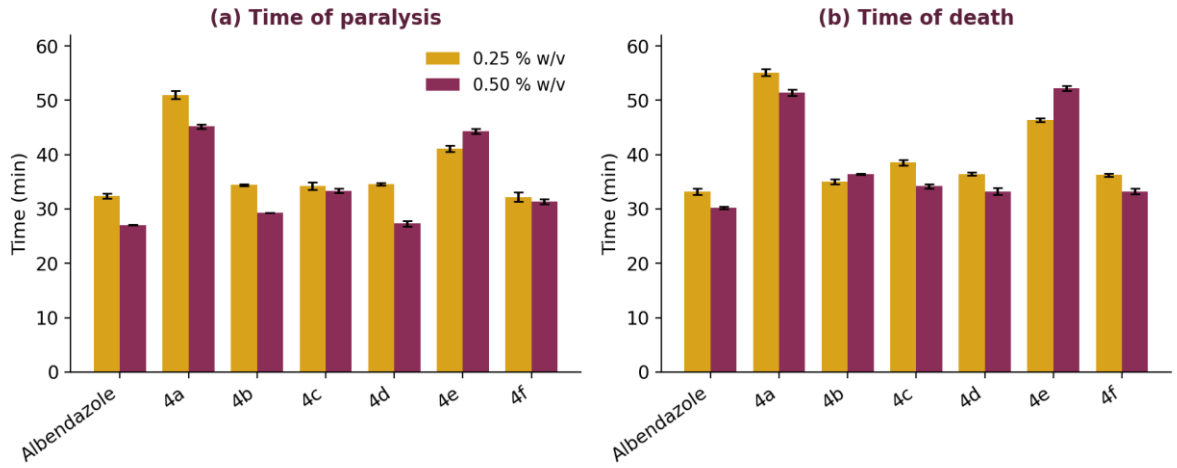

Fig. 4. Time of paralysis (a) and time of death (b) of earthworms treated with albendazole and compounds 4a–f at 0.25% and 0.50% w/v (mean ± SEM).

3.4 Structure–activity relationship

Because the 4-chlorophenyl ring at C-3 is constant, the differences in activity arise from the C-5 aryl substituent. Small, polar or strongly electronegative para substituents (4-F in 4b, 4-OH in 4d) gave the best activity, suggesting that a smaller hydrophobic footprint and, for 4d, the hydrogen-bonding ability of the phenolic group may favour interaction with the parasite target. The 4-bromo analogue (4c) was intermediate. In contrast, the 4-chloro (4a) and 2,4-dichloro (4e) derivatives were the weakest, and these were also the more lipophilic, higher-melting or more sterically crowded analogues. These observations are preliminary and should be confirmed by docking and by testing additional analogues.

IV. Conclusion

Six new 3,5-diaryl-4,5-dihydroisoxazoles (4a–f) were synthesised in two steps and characterised by physicochemical and spectral methods. All showed in vitro anthelmintic activity in the earthworm model; compounds 4b (4-F) and 4d (4-OH) were the most active, with 4d approaching the potency of albendazole at 0.50% w/v. These compounds can serve as templates for in silico design and the synthesis of more potent anthelmintic isoxazolines. Confirmation on parasitic nematodes and toxicity studies are the next steps.

Acknowledgements

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