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Synthesis and Investigation of Tricyclic Isoquinoline Derivatives as Antibacterial Agents

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Abstract: Isoquinoline derivatives exhibit a range of biological properties, including antibacterial activity, and are thus attractive as a scaffold for developing broad-spectrum antibacterial compounds. A series of six isoquinoline-based compounds were synthesized using the reaction of 6,7-dimethoxy-1-methyl-3,4-dihydroisoquinoline with dimethyl acetylenedicarboxylate (DMAD) to provide the tricyclic (2Z)-[2-oxo-5,6-dihydropyrrolo[2,1,a]isoquinolin-3-ylidene]-2-ethanoate. The [2 + 3] cycloaddition of DMAD with C-6 and C-7 substituted 1-methyl-3,4-dihydroisoquinolines proceeded using aryl ethers or unsubstituted compounds, but not with amine, amide or nitro moieties at the C-7 position. Compounds **8d** and **8f** were found to have antibacterial properties against some Gram-positive pathogens (*Staphylococcus aureus*—**8d** = 16 μg/mL, **8f** = 32 μg/mL; *Streptococcus pneumoniae*—**8f** = 32 μg/mL; and *Enterococcus faecium*—**8d** = 128 μg/mL, **8f** = 64 μg/mL). Evaluation of their cytotoxic properties against mammalian cell lines revealed some cytotoxic effects (**8b** and **8d**, 125 μM, 24 h, HEp-2 cells) and (**8a**, **8b**, **8d** = 125 μM, **8f** = 62.5 μM, 24 h, McCoy B cells), suggesting limitations in their antibacterial applications without further development.

Keywords: antibacterial; isoquinoline; 3,4-dihydroisoquinoline; cytotoxicity

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1. Introduction

Many bacterial pathogens are drug-resistant. A select few, known as the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumanni*, *Pseudomonas aeruginosa*, and *Enterobacter species*), present a particular threat due to their worldwide prevalence [1]. The World Health Organization (WHO) has listed *A. baumanni*, *P. aeruginosa*, and *Enterobacter* species as priority pathogens requiring new therapeutic treatments due to their resistance to carbapenem and extended spectrum β-lactamase resistance. *Enterococcus faecium* and *S. aureus* remain a high priority, while other common infections, such as *Streptococcus pneumoniae*, are of increasing concern [2].

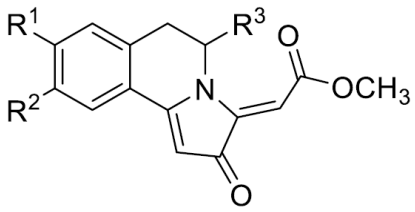
Very few antibacterial drugs have been approved in recent years. This has exacerbated the need for new drugs with novel structures. The WHO has published three main criteria that new antibiotic compounds should meet to reduce the risk of pathogens developing antibacterial resistance. New antibacterial agents should (i) have no cross-resistance (NCR), (ii) be a new chemical class, and (iii) target a new mode of action [3].

As of 2021, 27 antibacterial agents were in Phase I–III clinical trials that target priority pathogens, where 13 have noted activity against Gram-negative pathogens that are often

harder to treat. However, many of these 27 drugs are either derivatives of a parent molecular class, such as macrolides, tetracyclines, and β-lactamase inhibitors, or combinatorial therapeutics [4]. Molecular derivatization can be useful in the rapid development of new antibiotics, but because the same mechanism is targeted, this typically leads to resistance [5]. Combinatorial therapies frequently reduce the rate of resistance and, in some cases, effectively eliminate drug resistance. However, this increases the therapeutic complexity due to the potential of drug–drug interactions and drug antagonism [6,7].

Recent advancements have shown that antibacterial agents can incorporate isoquinolines into their structures with good efficacy [8] and, in some cases, without susceptibility to resistance in *S. aureus* after 30 days [9]. These include berberine, a natural product that may interfere with FtsZ polymerization [10], an antibacterial target, and key enzyme in bacterial replication. Isoquinoline-based *N*-ethyl ureas can inhibit Gram-positive and Gram-negative pathogens and interfere with DNA gyrase and topoisomerase IV activity [11].

No approved antibacterial agents to date have utilized isoquinoline compounds, although US Food and Drug Authority (FDA) approved drugs such as papaverine (an antispasmodic), which has been used since the 1800s. Other examples include praziquantel (an anthelmintic), solifenacin (an anticholinergic), and lifitegrast for dry-eye management. The successful use of these drugs as therapeutic agents indicates that druglike molecules can be constructed around an isoquinoline core structure. An attractive property of isoquinoline compounds is their straightforward synthesis, which has been investigated since the late 19th century. Reported reactions for the preparation of isoquinoline compounds date back to 1893 for the Bischler–Napieralski reaction [12] and 1911 for the Pictet–Spengler reaction [13]. Both reactions employ intramolecular electrophilic cyclization strategies to achieve their dihydro- and tetrahydroisoquinolines, respectively. A less common method to access dihydroisoquinoline structures uses the intracyclic Ritter reaction [14,15]. Of direct interest to the current work is the reported cyclization reaction that dihydroisoquinolines compounds undergo with dimethyl acetylenedicarboxylate, affording structures of the type shown in Figure 1 [16]. However, no biological activities have been investigated thus far.



Compound	R^1	R^2	R^3
8a	OCH_3	H	H
8b	H	OCH_3	H
8c	OCH_2O		H
8d	H	H	H
8e	OCH_3	OCH_3	H
8f	OCH_3	OCH_3	CH_3

Figure 1. Structures of the tricyclic isoquinoline compounds investigated in this work.

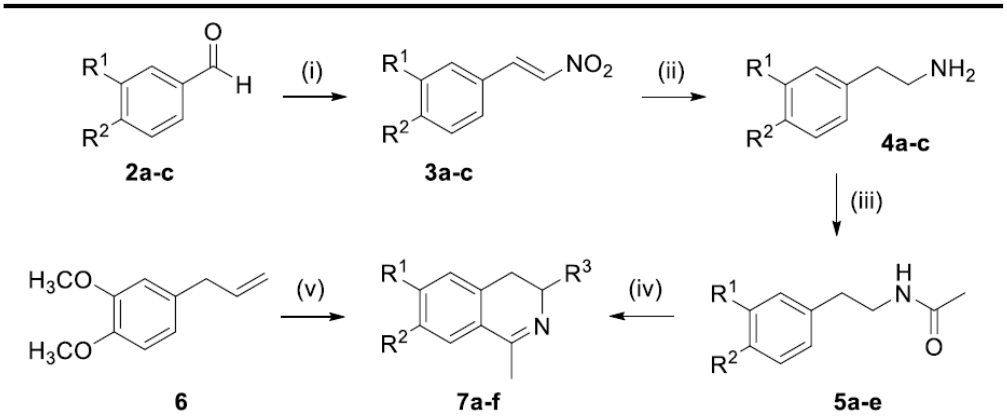
The unique tricyclic core structure of these compounds was of interest to investigate for potential antibacterial properties against a range of Gram-positive and Gram-negative bacteria. Therefore, we sought to synthesize and evaluate the antibacterial properties of the compounds outlined in Figure 1 and determine the scope of functional groups at R^2 in

dihydroisoquinoline precursors. Each of these compounds, except **8e** [16], has not been previously reported.

2. Materials and Methods

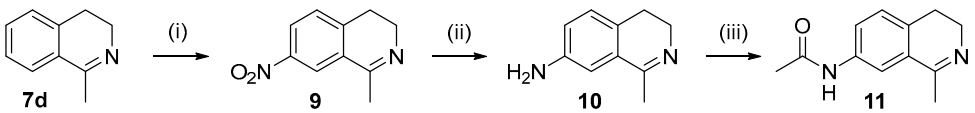
2.1. Chemistry

Chemical reagents were purchased from Merck (Darmstadt, Germany) and used without further purification, with the exception of dimethyl acetylenedicarboxylate, which was purchased from TCI chemicals. Compounds prepared using adapted literature procedures have the relevant citations included within the procedures below. Column chromatography used 60-mesh silica gel (Merck). NMR spectra were recorded on an Agilent 500 MHz or a Bruker 400 MHz spectrometer operating at 500 or 400 MHz for ^{1}H NMR, respectively, and 125 or 100 MHz for ^{13}C NMR, respectively. Spectra were referenced to the residual non-deuterated solvent signal using either $CDCl_3$ (^{1}H δ 7.26, ^{13}C δ 77.00) or DMSO-d_6 (^{1}H δ 2.50, ^{13}C δ 39.52). Multiplicity was assigned as singlet (s), doublet (d), triplet (t), quartet (q), quintet (quin), sextet (sxt), or multiplet (m). High-resolution mass spectra (HRMS) were recorded on an Agilent Technologies 6510 Q-TOF MS. Compound purity was confirmed to be >95% prior to biological assays using quantitative NMR spectroscopy (see Supplementary Materials). Schemes 1–3 (see Section 3.1) provide an outline of the synthetic routes described in detail below.

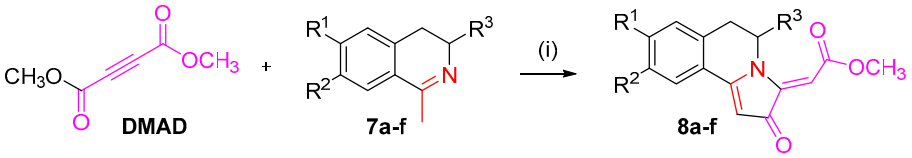


Compound	R¹	R²	R³
7a	OCH_3	H	H
7b	H	OCH_3	H
7c	OCH_2O		H
7d	H	H	H
7e	OCH_3	OCH_3	H
7f	OCH_3	OCH_3	CH_3

Scheme 1. Synthesis of compounds **7a**–**f**. Conditions: (**i**) CH_3COOH, NH_4CH_3COO (1.1 equiv.), CH_3NO_2 (3 equiv.), reflux, 4 h (**ii**) THF, $LiAlH_4$ (3 equiv.), reflux, 3 h (**iii**) CH_2Cl_2, CH_3COCl (1.5 equiv.), NEt_3 (2 equiv.), r.t., 2 h (**iv**) for **7a**–**e** only: P_2O_5 (1.5 *w*/*w*), $POCl_3$ (3 equiv.), toluene, 110 °C, 2–16 h. (**v**) for **7f** only: CH_3CN (25 equiv.), H_2SO_4/benzene (3:4), r.t., 3 h.



Scheme 2. Synthesis of compounds **9**–**11**. Conditions: (**i**) KNO_3 (1.1 equiv.), H_2SO_4, 60 °C, 4 h (**ii**) $SnCl_2$ (4 equiv.), HCl (32%), EtOH, 60 °C, 2 h (**iii**) CH_3COOH (1 equiv.), CDI (1.2 equiv.), THF, N_2, r.t., 2 h.



Scheme 3. Synthesis of compounds **8a**–**f**, and attempted cyclisation reactions with compounds **9** and **11**. Conditions: (**i**) DMAD (1.5 equiv.), methanol (see Section 2).

2.1.1. Synthesis of 3-Methoxybenzaldehyde (**2**)

3-Hydroxybenzaldehyde (**1**) (5.00 g, 40.9 mmol) was dissolved in anhydrous acetone (30 mL), to which potassium carbonate (11.82 g, 2 equiv.) was added, and stirred at room temperature for five minutes. Methyl iodide (8.72 g, 3.82 mL, 1.5 equiv.) was added, and the reaction was heated under reflux for three hours. Volatiles were removed with reduced pressure, and water (50 mL) was added. The aqueous phase was washed with chloroform (2 × 25 mL), and the combined organic extracts were washed with water (50 mL) and brine (50 mL) and then dried using anhydrous potassium carbonate. The solvent was removed with reduced pressure, affording a light orange liquid that was used without further purification. Spectral data were consistent with reported values [17]. ^{1}H NMR: (500 MHz, $CDCl_3$) δ 9.97 (s, 1H, CHO), 7.44 (m, 2H, H-4, 6), 7.39 (d, *J* = 2.0 Hz, 1H, H-2), 7.17 (dt, *J* = 6.5, 2.5 Hz, 1H, H-5). ^{13}C NMR: (125 MHz, $CDCl_3$) δ 192.1, 160.1, 137.8, 130.0, 123.5, 121.5, 112.0, 55.5.

2.1.2. General Procedure for the Synthesis of Nitrostyrene Compounds (**3a**–**3c**)

The general procedure for the synthesis of nitrostyrenes from arylaldehydes (**2a**–**c**) was adapted from a literature procedure [18]. The respective aldehyde was dissolved in glacial acetic acid (5 mL/g), to which nitromethane (3 equiv.) and ammonium acetate (1.1 equiv.) were added. The solution was heated under reflux for three hours, then poured into a beaker of crushed ice (50 g), and then filtered once all the ice had melted. The bright yellow cake was washed with water (2 × 20 mL) and recrystallized from ethanol to afford bright yellow needles.

- 3-Methoxy-β-nitrostyrene (**3a**) (6.80 g, 93%) from 3-hydroxybenzaldehyde over two steps. ^{1}H NMR: (500 MHz, $CDCl_3$) δ 7.97 (d, *J* = 14.0 Hz, 1H, H-2′), 7.57 (d, *J* = 13.5 Hz, 1H, H-1′), 7.36 (dd, *J* = 9.0, 7.5 Hz, 1H, H-5), 7.05–7.03 (m, 2H, 2H-Ar), 3.85 (s, 3H, OCH_3). ^{13}C NMR: (125 MHz, $CDCl_3$) δ 160.1, 139.0, 137.3, 131.3, 130.4, 121.7, 117.9, 114.0, 55.4. Spectral data were consistent with reported values [19].
- 4-Methoxy-β-nitrostyrene (**3b**) (5.21 g, 79%). ^{1}H NMR: (500 MHz, $CDCl_3$) δ 7.90 (d, *J* = 13.5 Hz, 1H, H-2′), 7.51 (d, *J* = 13.5 Hz, 1H, H-1′), 7.49 (d, *J* = 9.0 Hz, 2H, H-2, 6), 6.95 (d, *J* = 9.0 Hz, 2H, H-3, 5), 3.86 (s, 3H, OCH_3). ^{13}C NMR: (125 MHz, $CDCl_3$) δ 162.9, 139.0, 135.0 131.1, 122.5, 114.9, 55.5. Spectral data were consistent with reported values [20].
- 3,4-Dimethoxy-β-nitrostyrene (**3c**) (2.75 g, 71%) ^{1}H NMR: (500 MHz, $CDCl_3$) δ 7.93 (d, *J* = 13.5 Hz, 1H, H-2′), 7.47 (d, *J* = 13.0 Hz, 1H, H-1′), 7.07 (dd, *J* = 8.0, 1.5 Hz, 1H, H-6), 7.00 (d, *J* = 1.0 Hz, 1H, H-2), 6.87 (d, *J* = 8.0 Hz, 1H, H-5), 6.06 (s, 2H, CH_2). ^{13}C NMR: (125 MHz, $CDCl_3$) δ 151.4, 148.8, 139.1, 135.4, 126.6, 124.2, 109.1, 107.0, 102.0. Spectral data were consistent with reported values [21].

2.1.3. General Procedure for the Synthesis of Phenylethylamines (**4a**–**c**)

The general procedure for synthesizing phenylethylamines from nitrostyrenes (**3a**–**c**) was adapted from a literature procedure [22]. The respective nitrostyrene was dissolved in anhydrous tetrahydrofuran (c.a. 5 mL/g) and added dropwise at a rate of approximately 1 mL/minute to a stirring slurry of lithium aluminum hydride (3 equiv.) in tetrahydrofuran

(5–10 mL/g) at 0 °C under a nitrogen atmosphere. After the addition, the reaction was heated under reflux for three hours and then cooled to 0 °C. Aqueous hydrochloric acid (1 M) was added slowly with stirring under nitrogen until bubbling subsided and the pH was <1 (typically 20–30 mL). The aqueous phase was then washed with dichloromethane (3 × 30 mL). The aqueous phase was cooled to 0–5 °C with an ice bath and stirred vigorously. Sodium hydroxide pellets were slowly added until the pH was >11 and a white precipitate appeared. The solution was vacuum-filtered, and the filtrate was washed with dichloromethane (3 × 30 mL). The combined organic extracts were washed sequentially with saturated potassium sodium tartrate (50 mL), water (50 mL), and brine (50 mL) and then dried using anhydrous sodium carbonate. The solvent was removed with reduced pressure to afford the desired amine as a yellow oil and was used without further purification.

- 2-(3-Methoxyphenyl)ethylamine (**4a**) Yellow oil (2.00 g, 35%). ^{1}H NMR: (500 MHz, $CDCl_3$): δ 7.22 (t, *J* = 7.5 Hz, 1H H-3), 6.79 (d *J* = 7.5 Hz, H-2), 6.77–6.76 (m, 1H, H-4), 6.75 (s, 1H, H-6), 3.80 (s, 3H, OCH_3), 2.97 (t, *J* = 7.0 Hz, 2H, H-1″), 2.73 (t, *J* = 7.0 Hz, 2H, H-1′). ^{13}C NMR: (125 MHz, $CDCl_3$) δ 159.7, 141.4, 129.4, 121.2, 114.6, 111.4, 55.1, 43.4, 40.0. Spectral data were consistent with reported values [23].
- 2-(4-Methoxyphenyl)ethylamine (**4b**) Yellow oil (2.13 g, 63%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.11 (d, *J* = 9.0 Hz, 2H, H-2, 6), 6.84 (d, *J* = 8.5 Hz, 2H, H-3, 5), 3.78 (s, 3H, OCH_3), 2.92 (t, *J* = 7.0 Hz, 2H, H-2′), 2.68 (t, *J* = 7.0 Hz, 2H, H-1′). ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.0, 131.8, 129.7, 133.8, 55.2, 43.7, 39.1. Spectral data were consistent with reported values [24].
- 2-(3,4-Methylenedioxyphenyl)ethylamine (**4c**) Pale yellow oil (0.731 g, 71%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.73 (d, *J* = 8.0 Hz, 1H, H-5), 6.68 (d, *J* = 2.0 Hz, 1H, H-2), 6.63 (dd, *J* = 8.0, 1.5 Hz, 1H, H-6), 5.91 (s, 2H, OCH_2O), 2.90 (t, *J* = 7.0 Hz, 2H, H-2′), 2.65 (t, *J* = 7.0 Hz, 2H, H-1′) (NH_2 Not observed). ^{13}C NMR (125 MHz, $CDCl_3$) δ 147.6, 145.8, 133.5 121.6, 109.1, 108.1, 100.7, 43.6, 39.7. Spectral data were consistent with reported values [21].

2.1.4. General Procedure for the Synthesis of *N*-Phenylethylacetamides (**5a**–**e**)

The respective phenylethylamine* was dissolved in anhydrous chloroform (25 mL/g) and cooled to 0 °C. Acetyl chloride (1.5 equiv.) was added dropwise over one minute with stirring and maintaining temperature. Triethylamine (2 equiv.) was added dropwise with vigorous stirring over five minutes and then warmed slowly to room temperature and stirred for two to three hours. Water (50 mL) was added and stirred vigorously for five minutes. Then, the aqueous layer was removed. The organic phase was washed sequentially with water (2 × 50 mL) and brine (50 mL) and dried using anhydrous sodium carbonate. The solvent was removed with reduced pressure and purified using silica gel column chromatography where specified. (For **5d**, commercially available hydrochloride salt was used. Three equivalents of triethylamine were used to generate the free base in situ).

- *N*-[2-(3-Methoxyphenyl)ethyl]acetamide (**5a**) The dark oil was purified by gradient silica gel column chromatography (50–100% ethyl acetate in hexane) to afford a yellow oil (1.86 g, 74%). ^{1}H NMR: (500 MHz, $CDCl_3$) δ 7.23 (t, *J* = 7.5 Hz, 1H, H-3), 6.77 (d, *J* = 8.0 Hz, 2H, H-2,4) 6.74 (s, 1H, H-6), 5.50 (br s, 1H, NH), 3.80 (s, 3H, OCH_3), 3.51 (q, *J* = 6.5 Hz, 2H, H-1″), 2.79 (t, *J* = 7.0 Hz, 2H, H-1′), 1.94 (s, 3H, $NHCOCH_3$). ^{13}C NMR: (125 MHz, $CDCl_3$) δ 170.0, 159.8, 140.4, 129.6, 121.0, 114.4, 111.8, 55.2, 40.5, 35.6, 23.3. Spectral data were consistent with reported values [23].
- *N*-[2-(4-Methoxyphenyl)ethyl]acetamide (**5b**) The dark oil was purified by gradient silica gel column chromatography (40–100% ethyl acetate in hexane) to afford a light yellow solid (2.08 g, 77%). ^{1}H NMR: (500 MHz, $CDCl_3$) δ 7.11 (dt, *J* = 6.5, 1.5 Hz, 2H,

H-2, 6), 6.85 (dt, *J* = 7.0 Hz, 1.5 Hz, 2H, H-3, 5), 5.52 (br s, 1H, NH), 3.79 (s, 3H, OCH_3), 3.46 (q, *J* = 7.0 Hz, 2H, H-2′), 2.75 (t, *J* = 7.0 Hz, 2H, H-1′), 1.93 (s, 3H, $NHCOCH_3$). ^{13}C NMR: (125 MHz, $CDCl_3$) δ 170.0, 158.2, 130.8, 129.6, 114.0, 55.2, 40.8, 34.7, 23.3. Spectral data were consistent with reported values [25].

- *N*-[2-(3,4-Methylenedioxyphenyl)ethyl]acetamide (**5c**) Pale yellow solid (0.543 g, 96%) was used, requiring no further purification. 1H NMR (500 MHz, $CDCl_3$) δ 6.74 (d, *J* = 8.0 Hz, 1H, H-5), 6.67 (d, *J* = 2.0 Hz, 1H, H-2), 6.62 (dd, *J* = 8.0, 2.0 Hz, 1H, H-6), 5.93 (s, 2H, OCH_2O), 5.50 (br s. 1H, NH), 3.45 (q, *J* = 7.0 Hz, 2H, H-2′), 2.72 (t, *J* = 7.0 Hz, 2H, H-1′), 1.95 (s, 3H, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.0, 147.8, 146.2, 132.6, 121.6, 109.0, 108.4, 100.9, 40.8, 35.3, 23.3 Spectral data were consistent with reported values [26].
- *N*-(2-Phenyl)ethylacetamide (**5d**) Brown solid requiring no further purification (2.84 g, >99%). ^1H NMR: (500 MHz, $CDCl_3$) δ 7.28 (t, *J* = 7.5 Hz, 2H, 2Ar), 7.20 (d, *J* = 7.5 Hz, 1H, H-4), 7.17 (t, *J* = 7.5 Hz, 2H, 2Ar), 6.09 (br s, 1H, NH), 3.46 (q, *J* = 7.5 Hz, 2H, H-2′), 2.79 (t, *J* = 7.5 Hz, 2H, H-1′), 1.90 (s, 3H, CH_3). ^{13}C NMR: (125 MHz, $CDCl_3$) δ 170.0, 138.8, 128.7, 128.6, 126.5, 40.6, 35.6, 23.3. Spectral data were consistent with reported values [27].
- *N*-[2-(3,4-Dimethyoxyphenyl)ethyl]acetamide (**5e**) Yellow solid (3.70 g, >99%). ^1H NMR: (500 MHz, $CDCl_3$) δ 6.80–6.79 (m, 1H, H-Ar), 6.72 (m, 2H, 2Ar), 5.56 (br s, 1H, NH), 3.86 (s, 6H, 2 × OCH_3), 3.48 (q, *J* = 7.0 Hz, 2H, H-1′), 2.75 (t, *J* = 7.0 Hz, 2H, H-2′), 1.93 (s, 3H, $COCH_3$). ^{13}C NMR: (125 MHz, $CDCl_3$) δ 170.0, 149.0, 147.7, 131.3, 129.6, 111.8, 111.4, 111.3, 55.9, 55.8, 40.7, 35.1, 23.3. Spectral data were consistent with reported values [28].

2.1.5. General Procedure for the Synthesis of 1-Methyl-3,4-dihydroisoquinolines (**6a**–**e**)

The respective *N*-(1-phenylethyl)acetamide (**5a**–**d**) was dissolved in anhydrous toluene (10 mL/g) (except **5e**, which was dissolved in anhydrous acetonitrile), to which phosphorus pentoxide (1.5 *w*/*w*) and phosphorus oxychloride (3 equiv.) were added, and heated under reflux for the duration specified below. The solution was cooled to 0 °C, poured onto crushed ice (c.a. 20 g), and stirred vigorously for five minutes. The mixture was transferred to a separatory funnel, and the aqueous layer was removed. Then, the organic phase was washed with an additional portion of water (30 mL). The combined aqueous extracts were washed with dichloromethane (2 × 20 mL), and then the aqueous phase was cooled to 0 °C and stirred vigorously. Sodium hydroxide pellets were slowly added to the solution until the pH was >10, and then the mixture was extracted with dichloromethane (3 × 30 mL). The combined extracts were washed sequentially with water (50 mL) and brine (50 mL) and dried using anhydrous sodium carbonate. Products that required purification were purified by silica gel column chromatography with 0–25% methanol in ethyl acetate (Table 1).

Table 1. Reaction times used in the synthesis of compounds **7a**–**e** from **5a**–**e**.

Compound	Reaction Time (h)
7a	4
7b	16
7c	24
7d	3
7e	3

- 6-Methoxy-1-methyl-3,4-dihydroisoquinoline (**7a**) Yellow oil (1.06 g, 65%). ^1H NMR: (500 MHz, $CDCl_3$) δ 7.41 (d, *J* = 8.5 Hz, 1H, H-8), 6.78 (dd, *J* = 8.5, 2.0 Hz, 1H, H-7), 6.70 (br s, 1H, H-5), 3.83 (s, 3H, OCH_3), 3.62, (t, *J* = 7.5 Hz, 2H, H-3), 2.68 (t, *J* = 7.5 Hz,

2H, H-4), 2.34 (br s, 3H, H-1′). ^{13}C NMR: (125 MHz, $CDCl_3$) δ 164.0, 131.1, 139.6, 128.2, 123.1, 112.7, 111.8, 55.3, 46.7, 26.6, 23.2. Spectral data were consistent with reported values [29].

- 7-Methoxy-1-methyl-3,4-dihydroisoquinoline (**7b**) Yellow oil (0.430 g, 24%). ^{1}H NMR: (500 MHz, $CDCl_3$) 7.09 (d, *J* = 8.5 Hz, 1H, H-5), 7.02 (d, *J* = 2.5 Hz, 1H, H-8), 6.90 (dd, *J* = 8.5, 2.5 Hz, 1H, H-7), 3.82 (s, 3H, OCH_3), 3.64 (tq, *J* = 7.5, 1.5 Hz, 2H, H-3), 2.63 (t, *J* = 7.5 Hz, 2H, H-4), 2.37 (t, *J* = 1.5 Hz, 3H, H-1′). ^{13}C NMR: (125 MHz, $CDCl_3$) δ 164.2, 158.4, 130.2, 129.4, 128.1, 115.6, 111.4, 55.4, 47.2, 25.1, 23.2. Spectral data were consistent with reported values [30].
- 6,7-Methylenedioxy-1-methyl-3,4-dihydroisoquinoline (**7c**) Brown solid that was used without further purification (0.450 g, 62%). ^{1}H NMR: (500 MHz, $CDCl_3$) δ 6.97 (s, 1H, H-8), 6.66 (s, 1H, H-5), 5.97 (s, 2H, OCH_2O), 3.59 (td, *J* = 7.5, 1.5 Hz, 2H, H-3), 2.60 (t, *J* = 7.5 Hz, 2H, H-4), 2.32 (t, *J* = 1.5 Hz, 3H, H-1′). ^{13}C NMR: (125 MHz, $CDCl_3$) δ 163.5, 146.3, 132.8, 123.7, 107.8, 106.0, 101.2, 46.9, 26.3, 23.6. Spectral data were consistent with reported values [31].
- Methyl-3,4-dihydroisoquinoline (**7d**) Brown oil required no further purification. (0.635 g, 74%). ^{1}H NMR: (500 MHz, $CDCl_3$) δ 7.47 (dd, *J* = 7.5, 1.5 Hz, 1H, H-Ar) 7.34 (td, *J* = 7.5, 1.5 Hz, 1H, H-Ar), 7.30 (td, *J* = 7.5, 1.5 Hz, 1H, H-Ar), 7.17, (dd, *J* = 7.5, 1.5, 1H, H-Ar), 3.66 (tq, *J* = 7.5, 1.5 Hz, 2H, H-3), 2.70 (t, *J* = 7.5 Hz, 2H, H-4), 2.38 (s, 3H, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.3, 137.4, 130.5, 129.5, 127.4, 126.8, 125.3, 46.9, 26.0, 23.3. Spectral data were consistent with reported values [32].
- 6,7-Dimethoxy-1-methyl-3,4-dihydroisoquinoline (**7e**) Brown oil was used in the next reaction step without further purification (2.40 g, 72%). ^{1}H NMR: (500 MHz, $CDCl_3$) δ 6.98 (s, 1H, H-8), 6.68 (s, 1H, H-5), 3.90 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 3.62 (td, *J* = 7.5, 1.5 Hz, 2H, H-3), 2.63 (t, *J* = 7.5 Hz, 2H, H-4) 2.36 (s, 3H, CH_3). ^{13}C NMR: (125 MHz, $CDCl_3$) δ 163.9, 151.0, 147.5, 131.2, 122.3, 110.2, 109.1, 56.2, 56.0, 46.8, 25.7, 23.3. Spectral data were consistent with reported values [33].

2.1.6. Synthesis of (±)-6,7-Dimethoxy-1,3-dimethyl-3,4-dihydroisoquinoline (**7f**)

Anhydrous acetonitrile (35.6 mL, 25 equiv.) was cooled in a flask to −10 °C using an ice bath with sodium chloride. Sulfuric acid (98%, 15.0 mL) was added dropwise and stirred for two minutes. Then, methyl eugenol (**6**) (4.90 g, 27.5 mmol) was added dropwise as a mixture in benzene (20 mL), and the flask was fitted with a drying tube. The reaction mixture was warmed to room temperature over one hour and stirred for another two hours. Water (20 mL) was then added, and the mixture was washed with diethyl ether (20 mL) twice, allowing ten minutes of vigorous stirring for each wash. The aqueous layer was cooled in an ice bath and basified with aqueous sodium hydroxide (4 M). The mixture was then washed with chloroform (3 × 20 mL), and the combined extracts were washed with brine (50 mL) and dried using anhydrous sodium sulfate. The solvent was then removed in vacuo, and the product was used without further purification. Yellow oil (3.58 g, 16.3 mmol, 59%). ^{1}H NMR: (500 MHz, $CDCl_3$) δ 6.93 (s, 1H, H-5), 6.61 (s, 1H, H-8), 3.86 (s, 3H, -OCH_3), 3.84 (s, 3H, -OCH_3), 3.47–3.42 (m, 1H, H-3), 2.58 (dd, *J* = 5.5, 15.5 Hz, 1H, H-4a), 2.35 (dd, *J* = 13.0, 15.5 Hz, 1H, H-4b), 2.30 (d, *J* = 2.0 Hz, 3H, H-1′), 1.36 (d, *J* = 6.5 Hz, 3H, H-3′). ^{13}C NMR: (100 MHz, $CDCl_3$) δ 162.8, 150.8, 147.4, 130.9, 122.1, 110.3, 108.9, 56.2, 55.9, 51.9, 33.1, 23.3, 21.9. HRMS (ESI/Q-TOF) *m*/*z*: $[M + H]^+$ Calcd for $C_{13}H_{19}NO_2$ 220.1332; found 220.1339.

2.1.7. Synthesis of 7-Nitro-1-methyl-3,4-dihydroisoquinoline (**9**)

This compound was prepared from **5d** using a literature method [34] to afford a pink solid that was used without further purification (0.508 g, 79%). ^{1}H NMR: (500 MHz, $CDCl_3$)

δ 8.32 (d, *J* = 2.0 Hz, 1H, H-8), 8.22 (dd *J* = 8.0, 2.5 Hz, 1H, H-6), 7.37 (t, *J* = 8.5 Hz, 1H, H-5), 3.74 (tq, *J* = 7.5, 1.5 Hz, 2H, H-4), 2.82 (t, 3H, *J* = 8.0 Hz, H-3), 2.47 (t, *J* = 7.5 Hz, 3H, CH_3).^{13}C NMR: (125 MHz, $CDCl_3$) δ 162.5, 144.7, 130.0. 128.5, 125.2, 120.1, 46.3, 26.1, 23.2. Spectral data were consistent with reported values.

2.1.8. Synthesis of 7-Amino-1-methyl-3,4-dihydroisoquinoline (**10**)

This method was adapted from a literature procedure [35]. Compound **9** (0.500 g, 2.65 mmol) was dissolved in ethanol (50 mL), to which tin(II) chloride dihydrate (2.72 g, 4 equiv.) and hydrochloric acid (32%, 5 mL) were added, and stirred at 60 °C for two hours. The reaction mixture was poured onto crushed ice (30 g) and washed with chloroform (2 × 20 mL). The aqueous phase was then basified with chilled aqueous sodium hydroxide (4 M) and extracted with chloroform (2 × 20 mL). The combined extracts were washed with water (50 mL) and brine (50 mL), dried using anhydrous sodium carbonate, and concentrated with reduced pressure to afford a brown solid that was used without further purification. ^{1}H NMR: (500 MHz, $CDCl_3$) δ 6.97 (d, *J* = 7.5 Hz, 1H, H-5), 6.82 (d *J* = 2.5 Hz, 1H, H-8), 6.70 (dd, *J* = 8.0, 2.5 Hz, 1H, H-6), 3.66 (br s, 2H, NH_2), 3.62 (tq, 2H, *J* = 7.5, 1.5 Hz, H-3), 2.58 (t, 8.0 Hz, 2H, H-4) 2.47 (t, *J* = 8.0 Hz, 3H, CH_3). ^{13}C NMR: (125 MHz, $CDCl_3$) δ 164.3, 145.1, 130.2, 128.1, 127.4, 117.2, 112.1, 47.5, 25.1, 23.3. Spectral data were consistent with reported values.

2.1.9. Synthesis of 7-Acetamido-3,4-dihydro-1-methylisoquinoline Synthesis (**11**)

Acetic acid (188 mg, 179 µL, 1 equiv.) was dissolved in dry tetrahydrofuran (10 mL), to which carbonyl diimidazole (607 mg, 1.2 equiv.) was added, and stirred in a nitrogen atmosphere for two hours. The solution was transferred to a mixture of compound **10** (0.500 g, 3.12 mmol, 1 equiv.) in dry tetrahydrofuran (10 mL) and left to stir for 22 h at room temperature under a nitrogen atmosphere. The solvent was removed with reduced pressure, diluted with aqueous hydrochloric acid (20 mL, 0.25 M), and then washed with chloroform (20 mL). The aqueous phase was basified with aqueous sodium hydroxide (1 M) and extracted with chloroform (3 × 20 mL). The extracts were combined and washed sequentially with water (30 mL) and brine (50 mL), dried using anhydrous potassium carbonate, and then concentrated with reduced pressure to afford a dark yellow wax that was used without further purification. ^{1}H NMR: (400 MHz, $CDCl_3$) δ 8.61 (br s, 1H, NH), 7.73 (d, *J* = 1.6 Hz, H-8), 7.45 (dd, *J* = 1.6, 8.0 Hz, 1H, H-6), 7.08 (d, *J* = 8.4 Hz, 1H, H 5), 3.61 (t, *J* = 7.2 Hz, 2H, H-3), 2.63 (t, *J* = 7.6 Hz, 2H, H-4), 2.30 (s, 3H, H-1′), 2.15 (s, 3H, $NHCOCH_3$). ^{13}C NMR: (100 MHz, $CDCl_3$) δ 168.9, 164.4, 137.1, 133.0, 129.6, 127.7, 122.3, 117.4, 46.7, 25.4, 24.2, 23.0. HRMS (ESI/Q-TOF) *m*/*z*: $[M + H]^+$ Calcd for $C_{12}H_{16}N_2O_4$ 203.1177; found 203.1179.

2.1.10. Synthesis of Methyl (2Z)-[2-oxo-5,6-dihydropyrrolo[2,1,a]isoquinolin-3-ylidene]-2-ethanoates (**8a**–**f**)

Compounds **8a**-**f** were synthesized using an adapted literature procedure [16]. The respective dihydroisoquinoline **7a**-**f** was dissolved in methanol (10 mL), to which DMAD (1.5 equiv.) was added dropwise, and stirred at room temperature for the duration specified below. Reactions that formed bright red precipitates were filtered, and those that did not were concentrated with reduced pressure. Crude products were purified by silica gel column chromatography, with mobile phases specified for each compound except for **8e**, which was triturated with cold diethyl ether (2 × 5 mL) (Table 2).

Table 2. Reaction times used in the synthesis of compounds **8a–f** from **7a–f**.

Compound	Reaction Time (h)
8a	1
8b	16
8c	12
8d	16
8e	2
8f	16

- Methyl (2Z)-8-methoxy-[2-oxo-5,6-dihydropyrrolo[2,1,a]isoquinolin-3-ylidene]-2-ethanoate (**8a**) (20–100% ethyl acetate in hexane) to afford a bright red/orange solid (0.817 g, 52%). ^{1}H NMR: (500 MHz, $CDCl_3$) δ 7.66 (d, *J* = 8.5 Hz, 1H, H-8), 6.90 (d, *J* = 9.0 Hz, 1H, H-7), 6.80 (br s, 1H, H-5), 6.05 (s, 1H, C=CH), 5.73 (s, 1H, C=CH), 4.31 (t, *J* = 6.5 Hz, 2H, H-3), 3.89 (s, 3H, OCH3), 3.78 (s, 3H, OCH3), 3.10 (t, *J* = 6.5 Hz, 2H, H-4). ^{13}C NMR: (125 MHz, $CDCl_3$) δ 187.0, 166.5, 166.3, 163.2, 143.4, 138.9, 130.4, 117.6, 114.1, 113.3, 98.6, 94.3, 55.6, 51.9, 42.2, 29.0. HRMS (ESI/Q-TOF) *m*/*z*: $[M + H]^+$ Calcd for $C_{16}H_{16}NO_4$ 286.1073; found 286.1110. m.p. 150 °C (decomp.)
- Methyl (2Z)-9-methoxy-[2-oxo-5,6-dihydropyrrolo[2,1,a]isoquinolin-3-ylidene]-2-ethanoate (**8b**) (20–40% ethyl acetate in hexane) to afford the desired product as a bright red solid (0.070 g, 11%). ^{1}H NMR: (500 MHz, $CDCl_3$) δ 7.21 (d, *J* = 8.0 Hz, 1H, H-5), 7.16 (d, *J* = 2.5 Hz, 1H, H-8), 7.05 (dd, *J* = 8.0, 2.5 Hz), 6.05 (s, 1H, C=CH), 5.78 (s, 1H, C=CH), 4.27 (t, *J* = 6.0 Hz, 2H, H-3), 3.85 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 3.04 (t, *J* = 6.0 Hz, 2H, H-4). ^{13}C NMR: (125 MHz, $CDCl_3$) δ 187.5, 166.3, 166.1, 158.7, 142.8, 129.7, 128.8, 125.7, 119.4, 111.9, 99.3, 95.7, 55.5, 51.9, 42.5, 27.9. HRMS (ESI/Q-TOF) *m*/*z*: $[M + H]^+$ Calcd for $C_{16}H_{16}NO_4$ 286.1073; found 286.1099. m.p. 151 °C (decomp.)
- Methyl (2Z)-8,9-methylenedioxy-[2-oxo-5,6-dihydropyrrolo[2,1,a]isoquinolin-3-ylidene]-2-ethanoate (**8c**) (9:1 dichloromethane/ethyl acetate) affording a bright red solid (0.162 g, 24%). ^{1}H NMR: (500 MHz, $CDCl_3$) δ 7.09 (s, 1H, H-8), 6.75 (s, 1H, H-5), 6.06 (s, 2H, OCH_2O), 6.03 (s, 1H, C=CH), 5.67 (s, 1H, C=CH), 4.28 (t, *J* = 6.5 Hz, 2H, H-3), 3.78 (s, 3H, OCH_3), 3.04 (t, *J* = 6.5 Hz, 3H, H-4). 13C NMR: (125 MHz, $CDCl_3$) δ 187.1, 166.5, 166.0, 151.8, 147.4, 147.4, 143.3, 133.0, 118.5, 108.6, 107.1, 102.0, 98.7, 94.9, 51.9, 42.2, 29.0. HRMS (ESI/Q-TOF) *m*/*z*: $[M + H]^+$ Calcd for $C_{16}H_{14}NO_5$ 300.0866; found 300.0850. m.p. 199 °C (decomp.)
- Methyl (2Z)-[2-oxo-5,6-dihydropyrrolo[2,1,a]isoquinolin-3-ylidene]-2-ethanoate (**8d**) (20–40% ethyl acetate in hexane) to afford a bright red solid (0.080 g, 18%). ^{1}H NMR: (500 MHz, $CDCl_3$) δ 7.71 (d, *J* = 7.5 Hz, 1H, H-8), 7.48 (t, *J* = 7.5 Hz, 1H, H-7), 7.37 (t, *J* = 7.0 Hz, 1H, H-6), 7.31 (d, *J* = 7.5 Hz, 1H, H-5), 6.06 (s, 1H, C=CH), 5.82 (s, 1H, C=CH), 4.31 (t, *J* = 6.0 Hz, 2H, H-3), 3.78 (s, 3H, OCH_3), 3.12 (t, *J* = 6.0 Hz, 2H, H-4). ^{13}C NMR: (125 MHz, $CDCl_3$) δ 187.5, 166.4, 166.2, 142.8, 136.4, 132.6, 128.6, 128.1, 127.4, 124.9, 99.3, 95.6, 51.9, 42.3, 28.7. HRMS (ESI/Q-TOF) *m*/*z*: $[M + H]^+$ Calcd $C_{15}H_{14}NO_3$ 256.0968; found 256.1009. m.p. 138 °C (decomp.)
- Methyl (2Z)-8,9-dimethoxy-[2-oxo-5,6-dihydropyrrolo[2,1,a]isoquinolin-3-ylidene]-2-ethanoate (**8e**) Triturated with diethyl ether to afford a bright red solid (0.416 g, 83%). ^{1}H NMR: (500 MHz, $CDCl_3$) δ 7.09 (s, 1H, H-8), 6.76 (s, 1H, H-8), 6.03 (s, 1H, C=CH), 5.70 (s, 1H, C=CH), 4.31 (t, *J* = 6.5 Hz, 2H, H-3), 3.96 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 3.06 (t, *J* = 6.5 Hz, 2H, H-4). ^{13}C NMR: (125 MHz, $CDCl_3$) δ 187.0, 166.5, 166.1, 153.2, 148.5, 143.5, 131.2, 117.1, 110.8, 109.8, 98.5, 94.5, 56.2, 56.2, 51.9, 42.3, 28.4. HRMS (ESI/Q-TOF) *m*/*z*: $[M + H]^+$ Calcd $C_{17}H_{18}NO_5$ 316.1179; found 316.1183. Spectral data were consistent with reported values [16].

- (±)-Methyl (2Z)-8,9-dimethoxy-[2-oxo-5-methyl-5,6-dihydropyrrolo[2,1,a] isoquinolin-3-ylidene]-2-ethanoate (**8f**) Compound **7f** (0.200 g, 1.26 mmol) was dissolved in anhydrous methanol (10 mL) and stirred in a flask at room temperature. DMAD (194 µL, 168 mg, 1.1 equiv.) was added and stirred for 16 h at room temperature. The product precipitated out of the solution as the reaction progressed and was cooled in an ice bath and then filtered. The solid was then washed with cold diethyl ether and dried to give a bright red powder (0.084 g, 28%). ^{1}H NMR: (400 MHz, $CDCl_3$) δ 7.09 (s, 1H, H-6), 6.74 (s, 1H, H-9), 6.05 (s, 1H, H-4″), 5.71 (s, 1H, H-1″), 5.57–5.54 (m, 1H, H-3), 3.96 (s, 3H, -OCH_3), 3.93 (s, 3H, -OCH_3), 3.78 (s, 3H, -OCH_3), 3.42 (dd, *J* = 6.4, 16.4 Hz, 1H, H-4a), 2.72 (dd, *J* = 1.6, 16.4 Hz, 1H, H-4b), 1.09 (d, *J* = 6.8 Hz, 3H, H-3′). ^{13}C NMR: (100 MHz, $CDCl_3$) δ 187.1, 166.4, 164.8, 153.5, 148.3, 142.3, 129.2, 116.6, 111.6, 109.7, 98.6, 94.8, 56.2, 56.1, 51.9, 47.4, 34.4, 18.0 HRMS (ESI/Q-TOF) *m*/*z*: $[M + H]^+$ Calcd $C_{18}H_{21}NO_5$ 330.1336; found 330.1343. m.p. 189 °C (decomp.)

2.2. Biological Studies

2.2.1. MIC Evaluation for Other Bacterial Species (Microdilution Assay)

The MIC was determined by broth microdilution according to Clinical Laboratory Standards Institute guidelines and performed in biological triplicate [36]. The respective pathogen was streaked onto an agar plate of cation-adjusted Mueller–Hinton broth (CAMHB) and allowed to incubate at 37 °C for 16 h. A single colony was suspended in 10 mL of CAMHB media (with the exception of *Enterococcus faecium*, where Brain-heart infusion media were used) and incubated at 37 °C for 16 h.

The respective drug concentration (or DMSO solvent control) was added to each well of a 96-well plate in media (95 µL), to which 5 µL of the pathogen suspended in media was added (5×10^5 CFU/mL). The plate was covered with a sealing film and incubated at 37 °C for 24 h. The absorbance of each well was analyzed on a TECAN Spark 10M plate reader at a wavelength of 600 nm (OD_{600}). Growth inhibition was calculated using Microsoft Excel™ relative to uninhibited controls, using the following equation. Compounds that reduced the OD_{600} by ≥90% relative to vehicle controls were considered active for the tested concentration.

$$\text{Bacterial inhibition}(\%) = \frac{\text{Absorbance}_{\text{ Drug}} - \text{Absorbance}_{\text{ Media control}}}{\text{Absorbance}_{\text{ Vehicle control}} - \text{Absorbance}_{\text{ Media control}}}$$

2.2.2. Cytotoxicity Assay (MTS)

The MTS assay was performed according to a reported protocol [37]. HEp-2 (human epithelial type 2, ATCC® CCL-23) or McCoy B (mouse fibroblast, ATCC® CRL-1696) cells were grown to 80–100% confluence, and cell density was determined with a hemocytometer. Cells were then diluted to 1×10^5 cells/mL, and 100 µL was added to each well in a 96-well plate, with three wells left as controls. Cells were grown for 24 h, and the media were removed and then replaced with fresh media containing 0.1% DMSO and the drug for testing in triplicate. Drugs and cells were incubated for 24 h, and then 20 µL of MTS and PMS solution was added and incubated for a further four hours. Absorbance was measured at 490 nm on a TECAN M200 Infinite plate reader, and data were processed using Microsoft Excel™ with the following equation to determine cell viability relative to DMSO controls. Then, statistical analysis was performed using GraphPad PRISM version 8.0.1. Statistical significance was determined using Tukey's one-way analysis of variance (ANOVA), followed by Tukey's post hoc test, with $p < 0.05$ considered significant.

$$\text{Cell viability}(\%) = \frac{\text{Absorbance}_{\text{ Drug}} - \text{Absorbance}_{\text{Dye}}}{\text{Absorbance}_{\text{DMSO}} - \text{Absorbance}_{\text{Dye}}}$$

3. Results and Discussion

3.1. Design and Synthesis of Tricyclic Isoquinolines

We designed and synthesized a small library of new tricyclic isoquinoline structures using 3,4-dihydro-1-methylisoquinoline compounds as substrates for [2 + 3] cyclization reactions with dimethyl acetylenedicarboxylate (DMAD) [16]. The Bischler–Napieralski reaction was used to access the intermediate dihydroisoquinolines **7a**–**e**, and an intracyclic Ritter reaction was used to access compound **7f**.

Scheme 1 outlines the synthesis of key compounds in this investigation. Compound **2a** was prepared from methylation of 3-hydroxybenzaldehyde (**1**). Then, aryl-aldehydes (**2a**–**c**) were reacted with nitromethane to afford the corresponding nitrostyrene compounds (**3a**–**c**), which were subsequently reduced with lithium aluminum hydride to the corresponding phenylethylamine compounds (**4a**–**c**). Acylation with acetyl chloride afforded the corresponding amides (**5a**–**e**), which were cyclized to the dihydroisoquinolines compounds (**7a**–**7e**) using phosphoryl chloride and phosphorus pentoxide. Compound **7f** was synthesized from the intracyclic Ritter reaction using methyl eugenol (**6**) and acetonitrile under strongly acidic conditions.

Compound **7d** was further derivatized using literature procedures (shown in Scheme 2) to afford the corresponding 7-nitro-3,4-dihydro-1-methylisoquinoline (**9**), and then the nitro group was reduced to 7-amino-3,4-dihydro-1-methylisoquinoline (**10**) and subsequently acylated using an amide coupling reaction to afford compound **11** (*N*-(1-methyl-3,4-dihydroisoquinolin-7-yl)acetamide). These compounds were used to investigate the scope of reactions with DMAD, as the current literature reports only the reaction occurring when aryl-ethers are substituted with $R^1 = R^2 = OCH_3$.

The synthesis of compounds **8a**–**8f** was performed using a modified literature procedure [16] with dihydroisoquinolines (**7a**–**7f**), as shown in Scheme 3. The reaction was found to proceed rapidly and exothermically when R^1 acted as electron-donating substituents, affording the desired product in good yield. However, compounds bearing no donating substituent at R^1 produced lower yields. Compound **7d** ($R^1 = R^2 = H$) reacted slowly and afforded compound **8d** in an 18% yield. The reaction of DMAD with compounds **9**-**11** did not form the desired tricyclic product.

An investigation using compound **8e** as a substrate was undertaken to ascertain potential methods of derivatization. However, attempts to hydrolyze the terminal ester using acidic hydrolysis at room temperature or at reflux in aqueous 1M hydrochloric acid did not afford any conversation from the starting material. Alkaline hydrolysis (1M NaOH/THF (1:3)) led to the formation of multiple indiscernible and inseparable products, likely due to the formation of multiple diastereoisomers from the nucleophilic addition of hydroxide at several α–β unsaturated carbonyl positions. Attempts to transesterify the terminal ester were unsuccessful (conditions: EtOH, p-TsOH (5 mol%), reflux, 4 h).

Alkylation of the nitrogen was attempted using methyl iodide (conditions: CH_3I (5 equiv.), acetone, reflux, 16 h), and a literature method was used to functionalize the nitrogen to the corresponding *N*-oxide [38] (conditions: 30% H_2O_2, MeOH, r.t., 16 h). Both reactions were unsuccessful. However, the reported crystal structure of **8e** highlights that the nitrogen is in a trigonal planar geometry, suggesting that the lone pair is highly delocalized throughout the structure and may not behave as a typical tertiary nitrogen, preventing its use in derivatization reactions [39].

3.2. Cytotoxicity Evaluation of Synthesized Compounds Against Mammalian Cell Lines

The capacity of compounds **8a**–**f** to reduce the viability of two mammalian cell lines was investigated to determine if the antibacterial activity was selective. Compounds **8a**–**f** were screened against HEp-2 and McCoy cells, and their effects against cell viability were

assessed using the MTS assay. **8a**, **b**, **d**, and **8f** showed mild cytotoxic effects against HEp-2 cells, as shown in Figure 2. Bridging the methoxy group to a methylenedioxy group (**8c** and **8e**) was found to have a small effect on the cytotoxicity at 125 µM (**8c** = 65% vs. **8e** = 76%). All other compounds were found to have larger cytotoxic effects at 125 µM, suggesting that removal of aryl ethers from R^1 or R^2 may enhance these effects (**8e** vs. **8a** = 36% and **8b** = 17%).

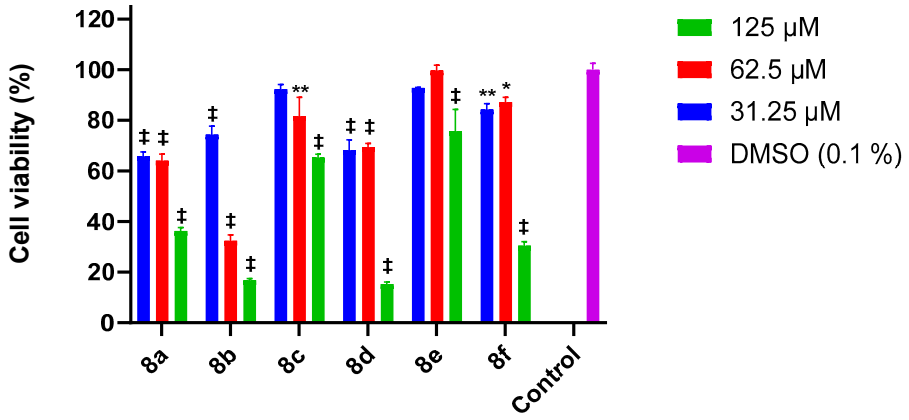


Figure 2. Cytotoxicity of compounds **8a**–**8f** against HEp-2 cells (24 h; 31.25 µM, 62.5 µM, and 125 µM). Values represent the average ± SEM from three independent experiments. Significance shown is relative to solvent (0.1% DMSO) vehicle controls: (*) $p \leq 0.05$, (**) $p \leq 0.01$, (‡) $p \leq 0.001$ vs. vehicle control ($n = 3$, one-way ANOVA, Tukey's post hoc).

The cytotoxic properties against McCoy cells were found to correlate strongly with those observed in HEp-2 cells (Figure 3); however, compound **8f** was significantly more cytotoxic to McCoy B cells at all tested concentrations (31.25 µM = 39%). Compounds **8c** and **8e** were not significantly cytotoxic at all assay concentrations, suggesting that the electron-rich nature of the aromatic ring may moderate its biological effects. Further study into various substituents on the ring may provide insight into this. Compounds **8a** (125 µM, 17%), **8b** (125 µM, 10%), and **8d** (125 µM, 12%) were found to have similar cytotoxic properties, suggesting that lipophilicity may be a driving factor for the observed cytotoxicity in McCoy cells. Removing the C-5 methyl group significantly decreased the cytotoxicity (**8e** vs. **8f**). Compounds **8a**–**f** possess an electron-poor Michael acceptor, which might allow non-specific covalent binding to cellular proteins, leading to non-selective cytotoxicity [40].

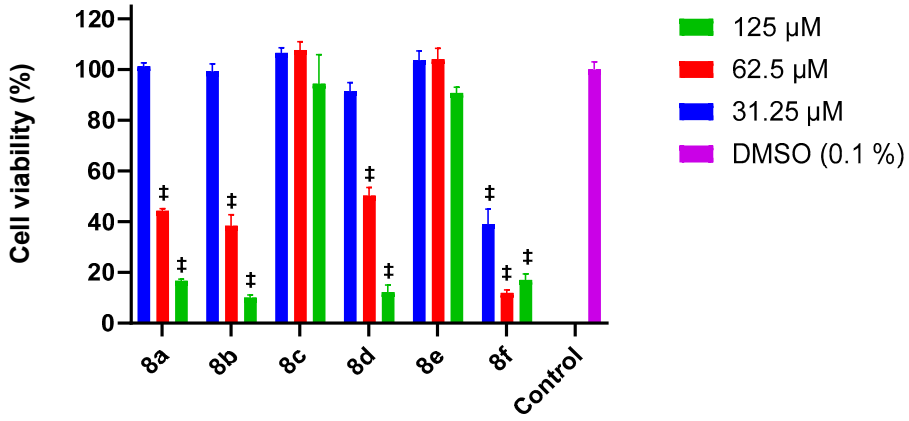


Figure 3. Cytotoxicity of compounds **8a**–**8f** against McCoy B cells (24 h; 31.25 µM, 62.5 µM, and 125 µM). Values represent the average ± SEM from three independent experiments. Significance shown is relative to solvent (0.1% DMSO) vehicle controls: (‡) $p \leq 0.001$ vs. vehicle control ($n = 3$, one-way ANOVA, Tukey's post hoc).

3.3. Evaluation of Antibacterial Properties

Compounds **8a–f** were screened against a panel of bacteria, and the results are shown in Table 3.

Table 3. Minimum inhibitory concentration (MIC) of compounds **8a-f** against Gram-positive and Gram-negative pathogens.

Compound	R^1	R^2	R^3	MIC (μg/mL)							
				S. aureus	*E. coli*	*E. coli ΔtolC*	*S. pneumoniae*	*E. faecium*	*P. aeruginosa*	*A. baumanii*	*A. baylyi*
8a	OCH_3	H	H	>32	>32	>32	>32	>32	>32	>32	>32
8b	H	OCH_3	H	>32	>32	>32	>32	>32	>32	>32	>32
8c	OCH_2O		H	>32	>32	>32	>32	>32	>32	>32	>32
8d	H	H	H	16	>128	>128	>32	128	>128	>32	>32
8e	OCH_3	OCH_3	H	>32	>32	>32	>32	>32	>32	>32	>32
8f	OCH_3	OCH_3	CH_3	32	>128	>128	32	64	>128	>128	>128
CIP [a] [11]	-	-	-	0.25	≤0.03	≤0.03	1	-	-	-	0.06 [41]
MER [b] [42]	-	-	-	0.12	≤0.06	-	-	-	0.5	0.5	-
VAN [c] [43]	-	-	-	-	-	-	-	1	-	-	-

[a] Ciprofloxacin, [b] meropenem, and [c] vancomycin.

For Gram-positive pathogens, compound **8d** was the most active, as it inhibited the growth of *S. aureus* with an MIC_{90} concentration half that of **8f**, with no observed change in potency relative to other screened pathogens. Since **8d** lacks substitution on the aromatic ring, the result suggests that the dimethoxy substituents in **8f** are not required for activity or lower potency. This finding is supported by the poor activity of the mono-methoxylated isoquinolines **8a** and **8b**, indicating that substitution of the aromatic ring at the R^1 position is not well tolerated but is acceptable at R^2, albeit with a reduction in potency.

Methoxylation of the aryl ring was found to abolish antibacterial activity (**8d** vs. **8a–c** and **8e**). It is proposed that with the presence of several Michael acceptors within the compounds, electron donating groups may weaken their electrophilicity due to their highly conjugated nature. Development of compounds with electron withdrawing substituents, as attempted in the current work, would be of benefit to evaluate the influence of this property on antibacterial activity.

For compounds **8a–b**, poor aqueous solubility prevented testing at higher concentrations. Bridging the dimethoxy analogue to a methylene linker (**8c**) was found not to influence potency, and although slightly less lipophilic than the dimethoxy analogue, solubility was not noticeably improved. The antibacterial properties were found to correlate with the cytotoxic properties of compounds **8d** and **8f**, potentially reducing their efficacy as antibacterial agents without further development to enhance their selectivity toward bacteria over mammalian cell lines.

The mechanism of action of these compounds has not been determined, although there are structural similarities with compounds such as berberine, which is known to inhibit bacterial replication by the inhibition of FtsZ [10]. As cytotoxicity was observed in mammalian cell lines, the mechanism of action may encompass a broader, more conserved mechanism of action, such as interfering with DNA replication. Highly conjugated and planar isoquinoline-based compounds such as HSN584 have been shown to interfere with cell-wall synthesis [8], and they could be explored as a potential mechanism of action of **8d** and **8f**. Future studies may warrant determination of the minimum bactericidal concentration (MBC) to ascertain if active compounds are bacteriostatic or bactericidal and could provide insight into the mechanism of action. Furthermore, it would be of interest to examine if tested compounds exhibit antibacterial activity on a broader range

of pathogens, including drug-resistant bacteria pertinent to clinical settings, including methicillin-resistant *S. aureus* and vancomycin-resistant enterococci.

There was no observed inhibitory activity against Gram-negative pathogens (*E. coli*, *E. faecium*, *P. aeruginosa*, *A. baumanii*, and *A. baylyi*). In the case of *E. coli*, screening against the Δ*tolC* mutant suggests the lack of activity is not due to drug efflux. Although the compounds may be benign upon uptake, for Gram-negative pathogens, uptake is one of the most challenging issues facing drug efficacy. As the scope of compatible chemical reactions with compounds **8a–f** was restricted, the use of drug-delivery systems such as nanoformulations including polyphenols [44], silica [45], and metallic nanoparticles [46] may simultaneously address drug uptake issues, as well as improve the limited solubility of these compounds in aqueous systems, opening the potential for assessing their antibacterial properties in vivo.

Moreover, drug uptake in Gram-negative bacteria could be improved by the exploitation of active transport systems. As the cyclisation step of compound **7** with DMAD to compound **8** employs mild reaction conditions, it can be envisioned that alkynes with an array of synthetic handles, or pre-functionalized moieties such as glycosides, peptides, or an ionizable functional group could be installed [47–49]. This may facilitate better transport across Gram-negative cell membranes, and could be utilized to improve the efficacy, and potentially, the selectivity of compounds toward various pathogens tested this work.

4. Conclusions

Access to novel scaffolds that afford unique compounds with antibacterial properties is critical in the development of antibacterial agents. Isoquinoline compounds and their fused-ring derivatives possess a wide range of pharmacological properties, including antibacterial applications. In this work, six methyl (2Z)-[2-oxo-5,6-dihydropyrrolo[2,1,a]-isoquinoline-3-ylidene]-2-ethanoate compounds were synthesized. Five of these were previously unreported. The scope of compatible chemistry for the synthesis of new aryl substituents was investigated and is thus far restricted to an unsubstituted aryl system and aryl ethers. Attempts to derivatize **8e** were unsuccessful, but the attempted reactions provide valuable insight into specific properties of these compounds, including the non-nucleophilic nature of the nitrogen and the limited scope of compatible aryl substituents on 1-methyl-3,4-dihydroisoquinolines that undergo cyclisation with DMAD. It was shown that compounds **9**, **10**, and **11** do not readily react with DMAD to afford tricyclic isoquinolines compounds.

The inhibition of bacterial growth was observed against the Gram-positive bacteria *S. aureus* and *E. faecium* for **8d** and **8f**. Additionally, **8f** weakly exhibited activity against *S. pneumoniae*. Gram-negative pathogens were found to be uninhibited by **8a–f**. As cytotoxicity was observed against mammalian cell lines, the utility of these compounds as antibacterial agents may be limited without further development to enhance the selectivity toward bacterial pathogens. This may be accomplished by functionalizing electron deficient alkynes with glycosides, peptides, or ionizable functional groups prior to cyclization with 1-methyl-3,4-dihydroisoquinolines to facilitate increased drug uptake, or by incorporating nanoencapsulation of active compounds to improve their uptake into pathogens of interest, and mitigate the observed cytotoxicity by improving selectivity.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/biochem5010001/s1, NMR spectra of new compounds and compounds for which data were not previously reported: Table S1: Purity of **8a–f** determined by qNMR. Figure S1: The 500 MHz 1H NMR spectrum of **7f** in CDCl3; Figure S2: The 100 MHz 13C NMR spectrum of **7f** in $CDCl_3$; Figure S3: The 400 MHz ^{1}H NMR spectrum of **8a** in $CDCl_3$; Figure S4: The 100 MHz ^{13}C NMR spectrum of **8a** in $CDCl_3$; Figure S5: The 400 MHz ^{1}H NMR spectrum of **8b**

in $CDCl_3$.; Figure S6: The 100 MHz ^{13}C NMR spectrum of **8b** in $CDCl_3$; Figure S7: The 400 MHz ^{1}H NMR spectrum of **8c** in $CDCl_3$; Figure S8: The 100 MHz ^{13}C NMR spectrum of **8c** in $CDCl_3$; Figure S9: The 400 MHz ^{1}H NMR spectrum of **8d** in $CDCl_3$; Figure S10: The 100 MHz ^{13}C NMR spectrum of **8d** in $CDCl_3$; Figure S11: The 400 MHz ^{1}H NMR spectrum of **8f** in $CDCl_3$; Figure S12: The 100 MHz ^{13}C NMR spectrum of **8f** in $CDCl_3$; Figure S13: The 400 MHz ^{1}H NMR spectrum of **11** in $CDCl_3$; Figure S14: The 100 MHz ^{13}C NMR spectrum of **11** in $CDCl_3$; Figure S15: The 400 MHz ^{1}H NMR spectrum of **12** in $CDCl_3$; Figure S16: The 100 MHz ^{13}C NMR spectrum of **12** in $CDCl_3$ [50,51].

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