Appl. Sci. **2014**, *4*, 380-389; doi:10.3390/app4030380

OPEN ACCESS

applied sciences

ISSN 2076-3417

www.mdpi.com/journal/applsci

Article

Comparisons of Halogenated β-Nitrostyrenes as Antimicrobial Agents

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Received: 7 July 2014; in revised form: 22 August 2014 / Accepted: 22 August 2014 / Published: 29 August 2014

Abstract: The influence of three types of halogen-substituted *E*-β-methyl-β-nitrostyrenes (such as Compounds **B**, **D**, **H**) to overcome bacterial activity that is currently a significant health threat was studied. The evaluations of their bio-potency was measured and related to their structure and activity relationships for the purposes of serving to inhibit and overcoming resistant microorganisms. In particular, fluorine-containing β-nitrostyrenes were found to be highly active antimicrobial agents. The addition of the β-bromo group enhanced the antibacterial activity significantly. Our work has illustrated that halogen substituents at both the 4-position in the aromatic ring and also at the β-position on the alkene side chain of nitropropenyl arenes enhanced the antimicrobial activity of these compounds.

Keywords: antimicrobial agents; medicinal chemistry; halogenated β-methyl-β-nitrostyrenes; Minimum Inhibitory Concentration

1. Introduction

An alarming analysis revealed that in 2007, more than 1500 people in Europe died from an invasive infection caused by a strain of *Escherichia coli* that was resistant to third-generation cephalosporins and originated in poultry [1]. Furthermore the mortality from carbapenem-resistant Enterobacteriaceae (CREs) is anticipated to be even higher should it spread in poultry on an equivalent scale, becoming a

major threat to human health in the world. Currently, no reliable treatment is available for humans infected with CREs. As these antibiotic-resistant bacterial pathogens are already entering the food chain [2,3] and can be transmitted through oral consumption [4], a call has been made for zero-tolerance on CREs in retail food to stop the situation from getting out of control. Unfortunately, the regulations on antimicrobial usage are largely ineffective, because they function only at a national level, whereas antimicrobial consumption and sales are global. An international/global ban on the sale of food items containing CREs could make imported and locally produced food meet the same standards. The health risks and consequences of the spread of these enterobacterial potential illnesses and diseases are enormous, and every effort must be made to prevent their increase to endemic proportions, especially for vulnerable individuals in communities and their threat to human life. Therefore, greater scientific efforts are urgently needed to focus on finding effective generic antibacterial agents.

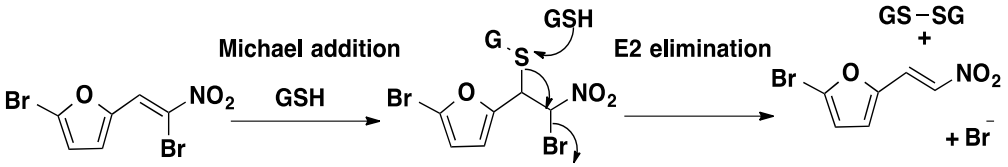
An analysis carried out in the United Kingdom predicts that if antibiotics become ineffective, everyday operations, such as hip replacements, could be fatal for around one in six [5].

Nitrostyrene derivatives have attracted considerable attention for the past several decades due to their diverse biological activities [6,7]. The resurgence of interest in the antibacterial activity of nitrovinyl containing compounds, including β-nitrostyrene derivatives, is attributed to their ready synthesis and significant antibacterial/antifungal activity with applications in veterinary and human medicine [8].

The introduction of the styryl functionality at position 2 of 5-nitroimidazoles potently enhanced the antimicrobial/antigiardial activity having low cytotoxicity against *Giardia lamblia* [9]. Indeed, an extensive SARs study of 5-nitroimidazoles related to metronidazole has uncovered compounds with improved drug activities against several clinically important microbes and unexpectedly overcame different forms of resistance. The positive performance of some of these research products in experimental disease testing has been promising. They could lead to better health outcomes [10].

The dibrominated nitrovinylfurans, 2-bromo-5-(2-bromo-2-nitrovinyl)furan [11], showed broad-spectrum antibacterial activity against Gram-positive (multi-resistant strains of *Staphylococcus aureus*) and Gram-negative bacteria with a minimum inhibitory concentration (MIC) of 4 μg/mL or less. Reactions of the dibromo-nitrovinylfuran with proteins and glutathione (GSH) indicated that the intermediate thiol adducts reacted further with GSH to give glutathione disulfide and the mono-bromo-nitrovinylfuran, as shown in Scheme 1.

Scheme 1. Michael addition of GSH to dibromo-nitrovinylfuran.



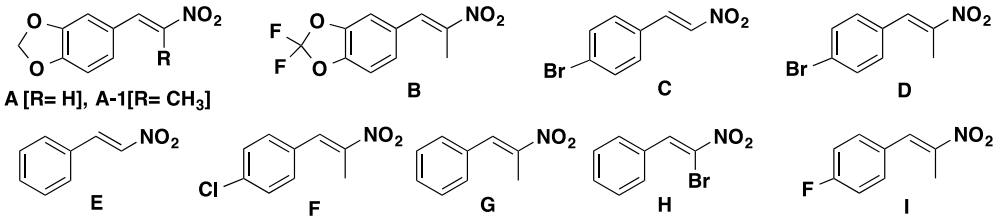
Our previous research on the structure activity relationships (SARs) of nitrostyrene derivatives for antibacterial activity revealed that the presence of a fluorine atom at the *para* position on the benzene ring showed the highest activity against Gram-negative bacteria [12,13]. The aim of the present research work is to study the effect of various halogen substitutions at different ring and side chain positions of β-nitrostyrene derivatives on antibacterial activity.

2. Experimental Section

2.1. Methods and Materials

Three assays, as previously described [12,13], were used to determine the antibacterial efficacy of the β-nitrostyrenes by their activity against a panel of 4 bacteria and a fungus (*Candida albicans*). The results appear as the minimum inhibitory concentration (MIC) for each compound and are presented in the Tables 1 and 2. The chemical structures of the test compounds are shown in Figure 1.

Figure 1. The structures of the *E*-β-nitrostyrene derivative test compounds.



The three assays were performed as follows:

Assay 1. The testing of the effect of the 2,2-difluoro-3,4-methylenedioxy substituent (**B**) on 3,4-methylenedioxy-β-nitrostyrene (**A**) and on 3,4-methylenedioxy-β-methyl-β-nitrostyrene (**A-1**). **A-1** was used for comparative purposes in the same assay.

Assay 2. The testing of 4-bromo β-nitrostyrene (**C**) and 4-bromo-β-methyl-β-nitrostyrene (**D**) derivatives compared against 4-chloro-β-methyl-β-nitrostyrene (**F**) and β-bromo-β-nitrostyrene (**H**). The β-nitrostyrene **E** and **A-1** were used for comparative purposes in the same assay.

Assay 3. The comparison of the antibacterial activities of the halogen substituents, F-, Br- and Cl-, at the *para* position of the benzene ring of nitrostyrenes.

All commercial chemicals and reagents used were of analytical reagent quality. The prepared purified compounds exhibited sharp melting points. Reactions were monitored by thin layer chromatography using Merck 60 F_{254} silica gel plates in (3:1) isopropanol-H_2O eluting solvent. All of the prepared compounds were found to be *E*-β-nitrostyrenes from NMR data and were also characterized by MS analysis, as previously described [12]. The compounds shown in Figure 1 were prepared according to published methods [12] and are briefly described here.

3,4-Methylenedioxy-β-methyl-β-nitrostyrene (**A-1**) was prepared by piperidine-catalysed condensation of piperonal with nitroethane, as previously described [13].

2.2. General β-Nitrostyrene Synthesis Method

A mixture of 1 equivalent of the aldehyde, nitroethane, (2 equivalents) 1-butylamine (0.03 g) and glacial acetic acid (0.03 g) was heated at 100 °C under reflux conditions for 2 h (reaction progress monitored by TLC). The cooled reaction mixture was concentrated under vacuum, extracted with ethyl acetate (10 mL) and the organic residue consecutively washed with dilute solutions of HCl (0.1 mol L^{-1}) and NaOH (0.1 mol L^{-1}). The organic phase was concentrated under vacuum, and the residue was recrystallized three times from 95% ethanol to furnish the product, melting point (MP) determined and characterized by spectrometric analysis.

2,2-Difluoro-5-[(1*E*)-2-nitroprop-1-en-1-yl] Benzodioxole (B): A mixture of 2,2-difluoro-5-formylbenzodioxole (1.0 g, 5.3 mmol) and nitroethane (2.5 g) was reacted according to the general synthesis protocol to furnish a yellow powder, MP 65–66 °C, in a 65% yield.

^{1}H NMR (300 MHz, $CDCl_3$): δ_H (ppm): 8.05 (s, 1H, *H*–C=C), 7.18–7.19 (multiplet, 3Ar–H), 2.46 (s, 3H, C–*H*, *E*), 1.56 (s, 3H, C–*H*, *Z*). ^{13}C NMR (300 MHz, $CDCl_3$) δ_C (ppm): 148.0 (Ar), 144.6 (Ar), 144.2 (Ar), 132.2 (CH–Ar), 131.6 (t, J = 257.2 Hz, *C*–F_2), 128.5 (C=*C*, β carbon), 126.5 (Ar), 131.6 (*C*=C, α carbon), 128.5 (Ar), 128.2 (Ar), 126.5 (CH–Ar), 110.5 (CH–Ar), 110.0 (CH–Ar), 14. 0 (–*C*H_3). **B** Hires mass spectrum GC/MS *m*/*z* (M^+) $C_{10}H_7F_2NO_5$: calculated 243.0338, found 243.0371.

1-Bromo-4β-[(*E*)-2-nitroethenyl] Benzene (C): A mixture of 4-bromobenzaldehyde (0.74 g, 4 mmol) and nitromethane (0.5 g, 8 mmol) was reacted according to the general synthesis protocol to furnish yellow crystals, MP 145–146 °C in a 33% yield. GC/MS *m*/*z* (M^+) 228.

1-Bromo-4β-[(1*E*)-2-nitroprop-1-en-1-yl] Benzene (D): A mixture of 4-bromobenzaldehyde (0.93 g, 5 mmol) and nitroethane (0.6 g, 8 mmol) was reacted according to the general synthesis protocol to furnish yellow crystals, MP 148–149 °C, in a 24% yield. GC/MS *m*/*z* (M^+) 242.

[(*E*)-2-nitroethenyl] Benzene (E): A mixture of benzaldehyde (0.42 g, 4 mmol) and nitromethane (0.49 g, 8 mmol) was reacted according to the general synthesis protocol to furnish yellow crystals, MP 51–52 °C, in a 24% yield. GC/MS *m*/*z* (M^+) 149.

1-Chloro-4β-[(1*E*)-2-nitroprop-1-en-1-yl] Benzene (F): A mixture of 4-chlorobenzaldehyde (0.70 g, 5 mmol) and nitroethane (0.6 g, 8 mmol) was reacted according to the general synthesis protocol to furnish yellow crystals, MP 89–91 °C, in a 25% yield.

^{1}H NMR (300 MHz, $CDCl_3$): δ_H (ppm): 8.05 (s, 1H, *H*–C=C), 7.28–8.05 (multiplet, 4Ar–H), 2.46 (s, 3H, C–*H*, *E*), 1.55 (s, 3H, C–*H*, *Z*). ^{13}C NMR (300 MHz, $CDCl_3$) δ_C (ppm): 148.1 (Ar), 136.1 (Ar), 132.2 (Ar), 131.2 (CH–Ar), 130.9 (CH–Ar), 129.3 (CH–Ar), 14. 0 (–*C*H_3). GC/MS *m*/*z* (M^+) 197.

[(*E*)-2-Nitropropenyl] Benzene (G): A mixture of benzaldehyde (0.53 g, 5 mmol) and nitroethane (0.6 g, 8 mmol) was reacted according to the general synthesis protocol to furnish yellow crystals, MP 60–62 °C, in a 26% yield. GC/MS *m*/*z* (M^+) 163.

[2-Bromo-(*E*)-2-nitroethenyl] Benzene (H): A mixture of benzaldehyde (1.06 g, 10 mmol), bromonitromethane (1.0 g, 7 mmol), glacial acetic acid (0.05 g) and NH_4OAc (0.19 g) was heated at 100 °C under reflux conditions for 2 h (reaction progress monitored by TLC). The cooled reaction mixture was concentrated under vacuum, extracted with ethyl acetate (10 mL) and the organic residue consecutively washed with dilute solutions of HCl (0.1 mol L^{-1}) and NaOH (0.1 mol L^{-1}). The organic phase was concentrated under vacuum, and the residue was recrystallized three times from 95% ethanol to furnish orange flakes, MP 61–62 °C, in a 20% yield.

^{1}H NMR (300 MHz, $CDCl_3$): δ_H (ppm): 8.67 (s, 1H, *H*–C=C), 7.89–7.93 (multiplet, 2H, 2Ar–H), 7.48–7.56 (multiplet, 3H, 3Ar–H), ^{13}C NMR (300 MHz, $CDCl_3$) δ_C (ppm): 136.5 (1*CH*=C), 131.9 (1CH–Ar), 130.9 (2CH–Ar), 130.2 (Ar), 128.1 (Ar). GC/MS *m*/*z* (M^+) 228.

1-Fluoro-4β-[(1*E*)-2-nitroprop-1-en-1-yl] benzene (**I**) was prepared as previously described [13].

Compound **I** was prepared in a 30% yield, yellow crystals, MP 65–66 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H (ppm): 8.06 (s, 1H, *H*–C=C), 7.46 (dd, *J* = 3.30, 5.37 Hz, 2H, Ar–*H*), 7.17 (t, *J* = 8.66 Hz, 2H, Ar–*H*), 2.46 (s, 3H, C–*H*, *E*), 1.59 (s, 3H, C–*H*, *Z*). ^{13}C NMR (300 MHz, $CDCl_3$) δ_C (ppm): 165.2 (d, *J* = 252.21 Hz, 1C, *C*–F), 147.5 (C=*C*, β carbon), 132.5 (*C*=C, α carbon), 132.2 (Ar), 128.5 (Ar), 128.5 (Ar), 116.3 (Ar), 116.0 (Ar), 14 .0 (*C*H_3). GC/MS *m*/*z* (M^+) 181.

3. Results and Discussion

3.1. Analysis of Assays

Assay 1. The effect of the two fluorine atoms in 2,2-difluoro-3,4-methylenedioxy-β-methyl-β-nitrostyrene (**B**) reduced the antimicrobial activity compared to **A-1**. All of the test compounds showed very weak antimicrobial activity against *E. coli*. However, the activity of **B** against *S. aureus* and *Bacillus subtilis* was high and comparable to **A-1** (refer to Table 1). The activity of 2,2-difluoro-3,4-methylenedioxy-β-nitrostyrene was very weak compared with that of the same compound, including the β-methyl group (**B**), and therefore, the data were not included in Table 1.

Table 1. Assays 1 and 2: minimum inhibitory concentrations (MICs) of β-nitrostyrene analogues.

Strain	A-1	B	C	D	E	F	G	H
Staphylococcus aureus ATCC 29213	16	16	32	16	128	32	32	32
Bacillus subtilis ATCC 6633	16	16	32	8	128	32	64	64
Enterococcus faecalis ATCC 29212	32	512	64	64	128	256	128	128
Escherichia coli ATCC 25922	362	512	197	181	362	128	128	128
Candida albicans ATCC 10231	23	44	23	23	362	23	44	23

Assay 2. The highest activity was observed with 4-bromo-β-methyl-β-nitrostyrene (**D**), which performed better than **A-1** against *E. coli* and was comparable to **A-1** against the other species. Compounds **G** and **H** also showed good all-round activity. Improvement in activity is also observed by substitution of a β-methyl group (**G**) or a β-bromine group (**H**) to Compound **E**.

Compound **C** (4-bromo–β-nitrostyrene) also showed fairly high activity, except against *E. coli*. It is, however, more active that **A-1**, **B** and **E** against this species. Between Compounds **D**, **F**, **G** and **H**, **D** is clearly superior against Gram-positive species, but there is little difference in activity against *C. albicans*. The fact that **D** is better than **F** suggests that 4-bromo substitution leads to higher activity than 4-chloro substitution. Only the 4-bromo derivative (**D**) was more active than the parent compound (**G**) against Gram-positive bacteria. Only **F** (4-chloro-), **G** (β-methyl) and **H** (β-bromo) derivatives gave satisfactory activity against the Gram-negative *E. coli*. Compounds **C** (4-bromo) and **D** (4-bromo-β-methyl), derivatives of nitrostyrene, are still significantly better than the methylenedioxy derivative (**A-1**) (refer to Table 1). The same comments apply to Compound **I**, which is evaluated in Assay 3. This compound has been evaluated in different assays, and the results in Table 3 are from an assay in which **I** was evaluated against 4-fluoro-β-nitrostyrene (without the β-methyl group), which

showed that **I** was much better than 4-fluoro-β-nitrostyrene and also **A-1**. In all of our work, we have found this to be the case (see [12,13]).

Table 1 shows the MICs in μg mL^{-1} of β-nitrostyrene analogues tested against four strains of bacteria and a fungus. The values are the geometric means of two determinations. In our MIC data, the geometric mean represents a mean or average, indicative of the central tendency or typical value of duplicate-measured MIC values by using the product of their values. It is defined as the ½ root of the product of two determinations [14].

Assay 3. In the third assay, 4-fluoro-β-methyl-β-nitrostyrene showed comparable activity to **A-1**, with the advantage against *E. coli*, but this was offset by lower activity against *E. faecalis*. No real advantage of fluorine over chlorine or bromine substitution could be claimed (refer to Table 2). All compounds showed equivalent activity to **A-1** against the bacteria and the fungus.

Table 2. Assay 3: MICs of halogenated derivatives **I**, **D** and **F** compared against **A-1**.

Strain	A-1	I	D	F
Staphylococcus aureus ATCC 29213	32	32	32	32
Bacillus subtilise ATCC 6633	16	16	16	32
Enterococcus faecalis ATCC 29212	64	128	128	256
Escherichia coli ATCC 25922	512	256	256	128
Candida albicans ATCC 10231	32	32	32	32

Table 2. Assay 3: MICs in μg mL^{-1} of the halogenated derivatives of β-methyl-β-nitrostyrene compared against **A-1** using four strains of bacteria and a fungus. The values are the geometric means of two determinations. Note: Assays 1, 2 and 3 were all within the ciprofloxacin acceptable range for *S. aureus*, *E. faecalis* and *E. coli*.

3.2. Mechanism of Antibacterial Activity

Nicoletti *et al*. [13,15] investigated the MIC to bacteria and fungi of 20 compounds based on the β-nitrostyrene scaffold to bacteria and fungi and found that they were broadly antimicrobial, with greater activity overall against Gram-positive bacteria and fungi. Whilst it was established from SAR studies that their lipophilic and the nucleophilic addition of thiol groups to the nitroolefin are the key physicochemical features, their antimicrobial mechanisms are not well understood.

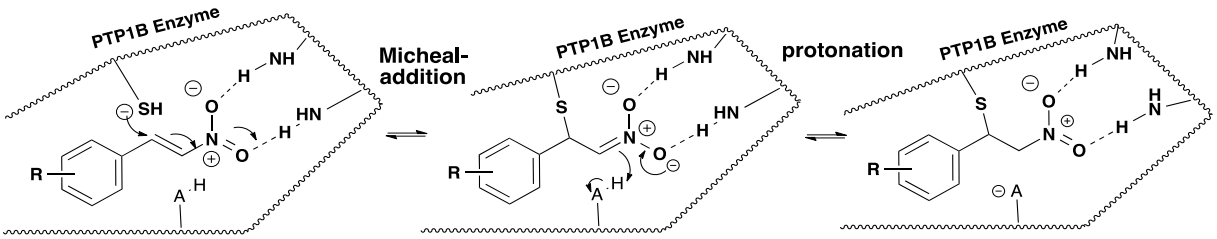
Various β-nitrostyrene derivatives are used as tyrosine kinase inhibitors with applications in potent antiplatelet activity [16,17]. Furthermore, pronounced substituent effects were observed. Specific mono- and di-substitutions on the aromatic ring of β-nitrostyrene tended to increase the anti-platelet activity and decrease the cytotoxic activity [17]. 3,4-Methylenedioxy-β-nitrostyrene (MNS) blocks the assembly of NLRP3 inflammasome by the inhibition of NLRP3 ATPase activity [18,19]. The nitrovinyl group is essential for the inhibitory activity of MNS.

The enzyme 4-oxalocrotonate tautomerase (4-OT), which contains an amino-terminal proline (Pro1), promiscuously catalyses the asymmetric Michael-type additions of aldehydes and ketone donors to β-nitrostyrene to yield γ-nitroaldehydes, with 89% enantiomeric excess [20]. Significantly, this Michael addition reaction has been designed and performed *in vivo* in resting *E. coli* BL21 whole

cells expressing 4-OT. This implies that the β-nitrostyrene used in the context as a reactant/substrate had no antibacterial effect on the host *E. coli* BL21 cells [21].

Park and Pei [22] proposed that their nitroalkenyl aromatic compounds act as tyrosine mimetics, thereby inhibiting protein tyrosine phosphatases and interfering with downstream cell signaling in microorganisms. They proposed that β-nitrostyrene acts as a reversible inhibitor of this enzyme by interaction and formation of a covalent complex via nucleophilic attack of PTP1B cysteine on the β-nitro group at the catalytic site. However, sulphur ylides, like thiol nucleophiles, are found to undergo Michael addition to a wide range of β-nitrostyrenes [23–26]. The known reactivity of β-nitrostyrenes with sulfhydryl groups is consistent with these observations. A more attractive mechanistic proposal is that the conjugated nitroalkene is a good Michael acceptor of nucleophiles owing to the non-covalent binding capability of the nitro group with PTP1B, as shown in Scheme 2 below.

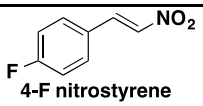
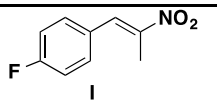
Scheme 2. Modified Michael addition mechanism: *E*-β-nitrostyrene-PTP1B binding complex and inhibition mechanism.



Furthermore, in contrast to what was reported by Park and Pei, research published by Milhazes *et al.* [27] and supported from our work [12,13] found that the antibacterial activity was substantially improved by the addition of a β-methyl group, as shown by the MIC comparisons in Table 3. The β-methyl group would be expected to enhance the above mechanism [12].

Table 3 shows the enhanced antibacterial activity (MIC values) of Compound **I** by the introduction of the β-methyl group to 4-F-nitrostyrene [12]. The values are the geometric means of two determinations.

Table 3. Comparative MIC (μg/mL) values of 4-F-nitrostyrene and Compound **I**.

Strain	 4-F nitrostyrene	 I
Staphylococcus aureus	128	2
Bacillus subtilis	256	2
Enterococcus faecalis	64	5.5
Escherichia coli	256	27
Candida albicans	32	2

3.3. Summary of SARs of the Antimicrobial Activity of Nitrostyrenes

a. The fluorination of the methylenedioxy substituent of **A-1** and Compound **A** was counterproductive in improving the bioactivity of **A-1** against all organisms.

b. The highest antimicrobial activity was found with Compound **D** (4-bromo-β-methyl-β-nitrostyrene), which gave good protection against most strains, except *E. coli*; however, it performed better than **A-1**, which was poor against *E. coli*. Compound **D** gave good protection against *S. aureus*, *B. subtilis* and *C. albicans*, but only marginal protection against *E. faecalis*.

c. Furthermore, Compound **D** gave identical results to **I** (4-fluoro-β-methyl-β-nitrostyrene), supporting previous data that **I** is superior to **A-1**, although still not being very effective against *E. coli*.

d. The relative effects of halogen substitution at the 4-position (Compounds **D**, **F** and **I**) indicate small differences from substitution by Br-, Cl- and F- for protection against the microorganisms tested.

e. The substitution of Br- for CH_3 at the β-carbon side chain (Compounds **G** and **H**) gave almost identical results for all strains, except for *C. albicans*. Good protection was provided by Compound **H** against this fungus.

f. The parent compound, β-nitrostyrene (**E**), was found to offer little antimicrobial protection against all strains, justifying the testing of β-nitrostyrene derivatives, and it was the least effective antibacterial of all compounds tested.

4. Conclusions

The predominant reaction of β-nitrostyrenes to undergo Michael addition reactions with nucleophiles, such as cysteine sulfhydryls, thiols, aldehydes and biocatalysts, may be a feature of their general biological activity, including their antimicrobial mode of activity, particularly their inhibition of cysteine-rich enzyme systems. Additional β-substitution by methyl or bromine significantly enhances the antimicrobial activity of β-nitrostyrenes consistent with that reported by others for nitrovinylfurans, and this supports the Michael addition reaction mechanism. From our previous SAR studies and from this work, we have found that the nature of the substituents on the aromatic ring plays a major role in the efficacy of the microbial toxicity of β-methyl-β-nitrostyrenes.

Acknowledgments

We are indebted to Frank Antolasic and Paul Morrison for their skill and technical support for the HRMS, GC/MS data and operation of GC-MS instrumentation.

Author Contributions

Hugh Cornell performed the chemical syntheses; Thu Nguyen carried out the assays/testing of all the compound antimicrobial efficacies; Gina Nicolletti and Neale Jackson contributed to the practical aspects of the research work; Hugh Cornell and Helmut Hügel analyzed the data and wrote the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Klutmans, J. Enterobacteria: Ban resistant strains from food chain. *Nature* **2013**, *501*, 316.

2. McKenna, M. Antibiotic resistance: The last resort. *Nature* **2013**, *499*, 394–396.
3. Fischer, J.; Rodrïguez, I.; Schmoger, S.; Friese, A.; Roesler, U.; Helmuth, R.; Guerra, B. *Salmonella enterica* subsp. *Enterica* producing VIM-1 carbapenemase isolated from livestock farms. *J. Antimicrob. Chemother*. **2013**, *68*, 478–480.
4. Magnes, A.R.; Johnson, J.R. Food-borne origins of *Escherichia coli* causing extra intestinal infections. *Clin. Infect. Dis*. **2012**, *55*, 712–719.
5. Smith, R.; Coast, J. The true cost of antimicrobial resistance. *Br. Med. J*. **2013**, *346*, f1493.
6. Pettit, R.K.; Pettit, G.R.; Hamel, E.; Hogan, F.; Moser, B.R.; Wolf, S.; Pon, S.; Chapuis, J.C.; Schmid, J.M. E-Combretastatin and E-resveratrol structural modifications: Antimicrobial and cancer cell growth inhibitory β-*E*-nitrostyrenes. *Bioorg. Med. Chem*. **2009**, *17*, 6606–6612.
7. Galano, J.J.; Alias, M.; Pérez, R.; Velåzquez-Campoy, A.; Hoffman, P.S.; Sanch, J. Improved flavodoxin inhibitors with potential therapeutic effects against *Heliobacter pylori* infection. *J. Med. Chem*. **2013**, *56*, 6248–6258.
8. Nicoletti, A.; White, K.S. β-Nitrostyrene Derivative Protein Tyrosine Phosphatase Modulators, and Their Therapeutic Use. Patent WO/2008/061308, 29 May 2008.
9. Valdez, C.A.; Tripp, J.C.; Miyamoto, Y.; Kalisiak, J.; Hruz, P.; Anderson, Y.S.; Brown, S.E.; Kangas, K.; Arzu, L.V.; Davids, B.J.; *et al*. Synthesis and electrochemistry of 2-Ethenyl and 2-Ethanyl derivatives of 5-Nitroimidazole and antimicrobial activity against *Giardia lamblia*. *J. Med. Chem.* **2009**, *52*, 4038–4053.
10. Miyamoto, Y.; Kalisiak, J.; Korthals, K.; Lauwaet, T.; Cheung, D.Y.; Lozano, R.; Cobo, E.R.; Upcroft, P.; Upcroft, J.A.; Berg, D.E.; *et al*. Expanded therapeutic potential in activity space of next-generation 5-nitroimidazole antimicrobials with broad structural diversity. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 17564–17569.
11. Scholz, T.; Heyl, C.L.; Bernardi, D.; Zimmermann, S.; Kattner, L.; Klein, C.D. Chemical, biochemical and microbiological properties of a brominated nitrovinylfuran with broad-spectrum antibacterial activity. *Bioorg. Med. Chem*. **2013**, *21*, 795–804.
12. Lo, K.; Cornell, H.; Nicoletti, G.; Jackson, N.; Hügel, H. A study of fluorinated β-nitrostyrenes as antimicrobial agents. *Appl. Sci*. **2012**, *2*, 114–128.
13. Nicoletti, G.; Cornell, H.; Hügel, H.M.; White, K.S.; Nguyen, T.; Zalizniak, L.; Nugegoda, D. Synthesis and antimicrobial activity of nitroalkenyl arenes. *Anti-Infective Agents* **2013**, *11*, 179–191.
14. Fleming, P.J.; Wallace, J.J. How not to lie with statistics: The correct way to summarize benchmark results. *Commun. ACM* **1986**, *29*, 218–221.
15. White, K.S. The Antimicrobial Mechanism of Action of 3,4-Methylenedioxy-β-Nitropropene. Ph.D. Thesis, RMIT University, Melbourne, Australia, March 2008.
16. Wang, W.Y.; Hsieh, P.W.; Wu, Y.C.; Wu, C.C. Synthesis and pharmacological evaluation of novel β-nitrostyrene derivatives as tyrosine kinase inhibitors with potent antiplatelet activity. *Biochem. Pharmacol*. **2007**, *74*, 601–611.
17. Hsieh, P.W.; Chang, Y.T.; Chuang, W.Y.; Shih, H.C.; Chiang, S.Z.; Wu, C.C. The synthesis and biologic evaluation of anti-platelet and cytotoxic β-nitrostyrenes. *Bioorg. Med. Chem*. **2010**, *18*, 7621–7627.

18. He, Y.; Varadarajan, S.; Muñoz-Planillo, R.; Burberry, A.; Nakamura, Y.; Nüñez, G. 3,4-Methylenedioxy-β-nitrostyrene inhibits NLRP3 inflammasome activation by blocking assembly of the inflammasome. *J. Biol. Chem*. **2014**, *289*, 1142–1150.
19. Strowig, T.; Henao-Mejia, J.; Elinav, E.; Flavell, R. Inflammasomes in health and disease. *Nature* **2012**, *481*, 278–286.
20. Miao, Y.; Geertsema, E.M.; Tepper, P.G.; Zandvoort, E.; Poelarend, G.J. Promiscuous catalysis of asymmetric michael-type additions of linear aldehydes to β-nitrostyrene by the proline-based Enzyme 4-Oxalocrotonate tautomerase. *ChemBioChem* **2013**, *14*, 191–194.
21. Naranic, T.; Radiojevic, J.; Jovanovic, P.; Francuski, D.; Bigovic, M.; Maslak, V.; Savic, V.; Vasiljevic, B.; O'Connor, K.E.; Nikodinovic-Runic, J. Highly eficient Michael -type addition of acetaldehyde to β-nitrostyrenes by whole resting cells of *Escherichia coli* expressing 4-oxalocrotonate tautomerase. *Bioresour. Technol*. **2013**, *142*, 462–468.
22. Park, J.; Pei, D. Trans-β-nitrostyrene derivatives as slow-binding inhibitors of protein tyrosine phosphatases. *Biochemistry* **2004**, *43*, 15014–15021.
23. Lu, L.Q.; Cao, Y.J.; Liu, X.P.; An, J.; Yao, C.J.; Ming, Z.H.; Xiao, W.J. A new entry to cascade organocatalysis: Reactions of stable sulfur ylides and nitroolefins sequentially catalyzed by thiourea and dmap. *J. Am. Chem. Soc*. **2008**, *130*, 6946–6948.
24. Baker, L.M.S.; Baker, P.R.S.; Golin-Bisello, F.; Schopfer, F.J.; Fink, M.; Woodcock, S.R.; Branchaud, B.P.; Radi, R.; Freeman, B.A. Nitro-fatty acid reaction with glutathione and cysteine: Kinetic analysis of thiol alkylation by a Michael addition reaction. *J. Biol. Chem.* **2007**, *282*, 31085–31093.
25. Bernasconi, C.F.; Schuck, D.F. Kinetics of reversible thiolate ion addition to substituted beta-nitrostyrenes in water. Radicaloid transition state or principle of nonperfect synchronization? *J. Org. Chem*. **1992**, *57*, 2365–2373.
26. Berner, O.M.; Tedeschi, L.; Enders, D. Asymmetric michael additions to nitroalkenes. *Eur. J. Org. Chem*. **2002**, *2002*, 1877–1894.
27. Milhazes, N.; Calheiros, R.; Marques, M.P.M.; Garrido, J.; Cordeiro, M.N.D.S.; Rodrigues, C.; Quinteira, S.; Novais, C.; Peixe, L.; Borges, F. β-Nitrostyrene derivatives as potential antibacterial agents: A structure-property-activity relationship study. *Bioorg. Med. Chem*. **2006**, *14*, 4078–4088.