chemistry proceedings

MDPI

Proceeding Paper

Synthesis and Evaluation of Biological Activities of Schiff Base Derivatives of 4-Aminoantipyrine and Cinnamaldehydes †

Esteban Aguilar-Llanos [1], Saskya E. Carrera-Pacheco [2], Rebeca González-Pastor [2], Johana Zúñiga-Miranda [2], Cristina Rodríguez-Pólit [2], Juan Carlos Romero-Benavides [3] and Jorge Heredia-Moya [2,*]

[1] Facultad de Ciencias Químicas, Universidad Central del Ecuador, Francisco Viteri s/n y Gilberto Gato Sobral, Quito 170521, Ecuador
[2] Centro de Investigación Biomédica (CENBIO), Facultad de Ciencias de la Salud Eugenio Espejo, Universidad UTE, Quito 170527, Ecuador
[3] Departamento de Química, Facultad de Ciencias Exactas y Naturales, Universidad Técnica Particular de Loja, Loja 1101608, Ecuador
* Correspondence: jorgeh.heredia@ute.edu.ec; Tel.: +593-2-2990800 (ext. 2538)
† Presented at the 26th International Electronic Conference on Synthetic Organic Chemistry, 15–30 November 2022; Available online: https://sciforum.net/event/ecsoc-26.

Abstract: Schiff bases have been important compounds ever since their discovery and are both found in nature and synthesized in the laboratory. They participate in a variety of synthetic processes and possess desirable biological activity, including antibacterial, anti-inflammatory, antioxidant, and anticancer activity, among others. In this study, eight Schiff bases derived from the reaction of 4-aminoantipyrine with various cinnamaldehydes have been synthesized and characterized. All derivatives were tested in vitro on several human carcinoma cell lines to determine their antitumor activity and against different bacteria strains of clinical and food industry importance to evaluate their antibacterial activity. Several of the Schiff bases evaluated inhibited tumor cell growth in a dose-dependent manner. The compound that exhibited the most activity against all cell lines had IC_{50} values of less than 18 µM. On the other hand, during the evaluation of the antibacterial activity, only two Schiff base derivatives showed interesting antibacterial effects, with MIC values under 250 µM. These two Schiff base derivatives mainly exhibited a bacteriostatic effect against most of the studied bacterial strains. It is interesting to note that the same Schiff base presents the best activity in both biological evaluations.

Keywords: Schiff base; 4-aminoantypirine; cinnamaldehydes; antibacterial; anticancer

check for updates

Citation: Aguilar-Llanos, E.; Carrera-Pacheco, S.E.; González-Pastor, R.; Zúñiga-Miranda, J.; Rodríguez-Pólit, C.; Romero-Benavides, J.C.; Heredia-Moya, J. Synthesis and Evaluation of Biological Activities of Schiff Base Derivatives of 4-Aminoantipyrine and Cinnamaldehydes. *Chem. Proc.* **2022**, *12*, 43. https://doi.org/10.3390/ecsoc-26-13684

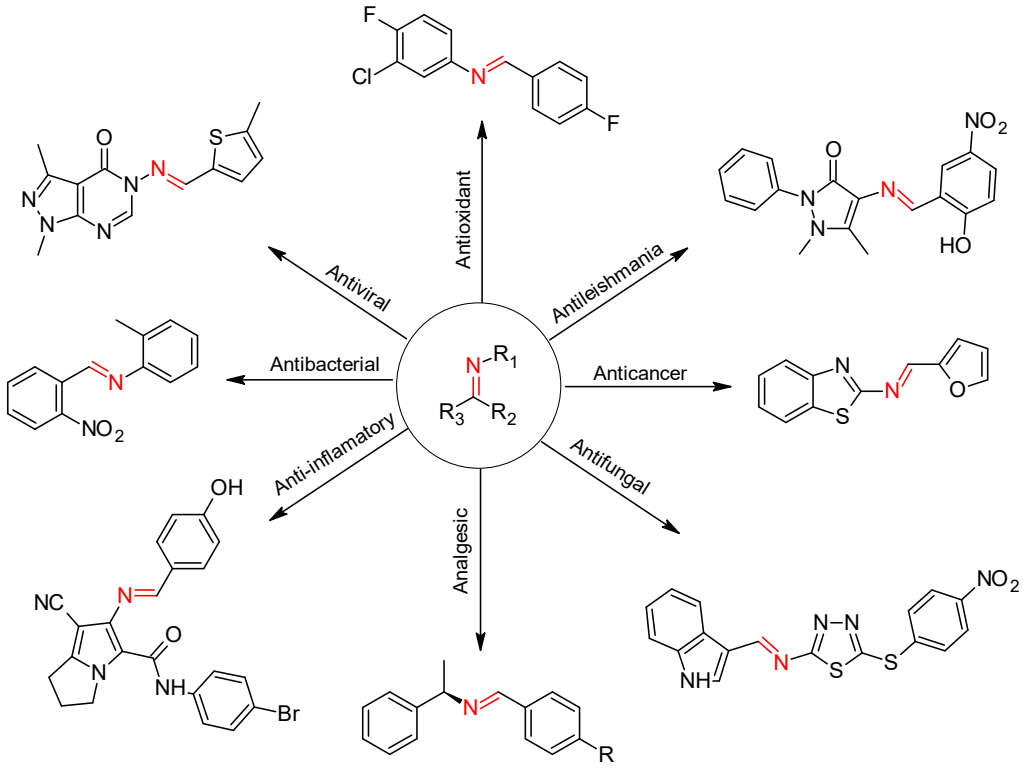
Academic Editor: Julio A. Seijas

Published: 17 November 2022

1. Introduction

Schiff bases, also known as imines or azomethines, have gained a lot of interest due to their wide range of applications, including pigments and dyes, catalysts, polymer stabilizers, luminescence chemosensors, corrosion inhibitors [1], organic synthesis intermediates, and new drug development [2,3]. The electron-donating nitrogen in the azomethine bond also makes these compounds L-type ligands that can interact with virtually any metal to create complexes [4,5]. One of the factors contributing to the popularity of Schiff bases in organic chemistry may be the simplicity of their synthesis. Condensation of primary amines with carbonyl compounds under reflux conditions can yield a large number of compounds in high yields; however, new methodologies have been developed that include the use of microwave, solvent-free synthesis or the use of Lewis or Bronsted–Lowry acids as catalysts, such as $ZnCl_2$, $TiCl_4$, alumina, P_2O_5/Al_2O_3, or $Er(OTf)_3$ [6].

Schiff bases have shown a wide range of biological activities [7] (Scheme 1), such as antileishmanial [8], analgesic [9], anti-inflammatory [10], antioxidant [11], antiviral [12], antifungal [13], and antibacterial activities [3], and for their biological activities, the imine or azomethine group (>C=N-) seems to be crucial.



Scheme 1. Different bioactive Schiff base derivatives.

Infections caused by the development of antimicrobial resistance (AMR) to existing antibiotics are a serious public health problem all over the world. According to recent data, an estimated 4.95 million people died from diseases associated with AMR in 2019 [14,15]. Moreover, the fast spread of multi-resistant bacteria worldwide is a serious topic that needs an immediate response [16]. Clinical strains and those associated with foodborne diseases become more dangerous due to the widespread and uncontrolled use of antibiotics for human health and livestock [17–19]. As a result, the necessity for effective treatments has been a driving factor in the study, design, and synthesis of novel biologically active compounds. Schiff bases have been reported to offer better anti-tumoral properties against a broad variety of tumor cells compared to standard chemotherapeutic drugs, such as cisplatin and doxorubicin [20]. They are capable of interacting with the nuclear DNA and trigger apoptosis, as well as modulating the intracellular redox equilibrium without significantly interfering with normal cell growth. Such mechanisms are particularly relevant in the context of cancer, where drug resistance and the high toxicity of conventional treatments has encouraged scientists to develop new and more effective anti-tumoral drugs [21].

Among Schiff bases, those derived from 4-aminoantipyrine have been shown to have interesting bioactivities, and the synthesis of new derivatives has caught the interest of many researchers, particularly in medicinal chemistry, due to their broad-spectrum biological activities [22,23]. Based on the facts presented above, this study was conducted in order to identify new antibacterial and anticancer drug candidate compounds. The synthesis of a variety of Schiff base derivatives from 4-aminoantipyrine with different cinnamaldehydes is described. To determine the biological significance of the synthesized compounds, we tested them against several bacteria strains of clinical and food industry interest, as well as against several human carcinoma cell lines.

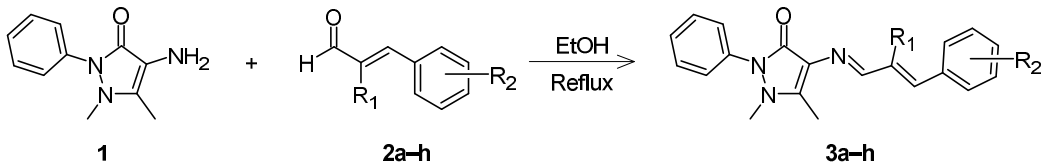
2. Methods

2.1. General

All solvents and reagents were acquired from Sigma-Aldrich (St. Louis, MO, USA) and were used without further purification. All melting points are uncorrected and were determined on a Fisher-Johns analog melting point apparatus. FTIR spectra were recorded by a Perkin Elmer FTIR Spectrum One using an ATR system (4000–650 cm^{-1}). The ^{1}H and ^{13}C NMR spectra were recorded at 298 K on a Bruker Advance 500 MHz spectrometer equipped with a z-gradient, triple-resonance (^{1}H, ^{13}C, ^{15}N) cryoprobe using DMSO-d6 or $CDCl_3$ as solvents. Chemical shifts are expressed in ppm with TMS as an internal reference (TMS, δ = 0 ppm) for protons. Reactions were monitored by TLC on silica gel using ethyl acetate/hexane mixtures as a solvent and compounds visualized by UV lamp. The reported yields are for the purified material and are not optimized.

2.2. Synthesis

All Schiff bases **3** were synthesized according to the reported procedures by our research group (Scheme 2) [8]. The synthesis of the Schiff base derivatives **3a–h** starts with a mixed equimolar reaction of 4-amino-1,5-dimethyl-2-phenylpyrazol-3-one (**1**) (1.722 mmol) and (1.722 mmol) of substituted cinnamaldehydes **2a–h**, dissolved in 5.00 mL of EtOH, and the mixture was refluxed for 1h to 24h. The exception was the reaction of **3f**, where reflux was not used. The progress of the reaction was monitored by TLC. The precipitates formed were collected by filtration and purified by recrystallization with ethanol, then the products were dried under vacuum to obtain the pure compounds.



1 **2a–h** **3a–h**

	R_1	R_2
3a	H	H
3b	H	2-NO_2
3c	H	2-OMe
3d	H	4-NMe_2
3e	H	3-OMe-4-OAc
3f	Br	H
3g	Me	H
3h	H	4-NO_2

Scheme 2. General reaction for the synthesis of Schiff bases **3a–h**.

4-[(3-Phenyl)allylideneamino]-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one (**3a**); yield 90% as yellow crystals; m.p. 162–163 °C (Lit [8] 165.5–165.9 °C); ^{1}H-NMR (300 MHz, DMSO-d_6) δ 9.40 (d, *J* = 8.2 Hz, 1H), 7.64 (dd, *J* = 8.3, 1.4 Hz, 2H), 7.57–7.49 (m, 2H), 7.43–7.29 (m, 6H), 7.11 (d, *J* = 16.1 Hz, 1H), 7.01 (dd, *J* = 16.1, 8.3 Hz, 1H), 3.17 (s, 3H), 2.39 (s, 3H).

4-[3-(2-Nitrophenyl)allylideneamino]-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one (**3b**); yield 84.8% as red crystals; m.p. 164–165 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.56 (d, *J* = 9.0 Hz, 1H), 7.92 (dd, *J* = 8.2, 1.0 Hz, 1H), 7.72 (d, *J* = 7.9 Hz, 1H), 7.57 (t, *J* = 7.4 Hz, 1H), 7.50 (d, *J* = 15.8 Hz, 1H), 7.45 (t, *J* = 7.9 Hz, 2H), 7.40 (dt, *J* = 7.7, 1.1 Hz, 1H), 7.37 (dd, *J* = 8.0, 1.0 Hz, 2H), 7.30 (t, *J* = 7.4 Hz, 1H), 6.94 (dd, *J* = 15.8, 9.0 Hz, 1H), 3.14 (s, 3H), 2.41 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 160.4, 158.3, 151.8, 148.0, 135.3, 134.7, 134.5, 133.1, 132.0, 129.3, 128.9, 128.3, 127.2, 124.9, 124.7, 118.9, 35.7, 10.1; FTIR (cm^{-1}) 3052, 1642, 1556, 1339, 977.

4-[3-(2-Methoxyphenyl)allylideneamino]-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one (**3c**); yield 85.7% as yellow crystals; m.p. 175–176 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.57 (d, *J* = 9.2 Hz, 1H), 7.55 (dd, *J* = 7.7, 1.6 Hz, 1H), 7.45 (dd, *J* = 8.3, 7.4 Hz, 2H), 7.43 (d,

J = 16.1 Hz, 1H), 7.39 (dd, *J* = 8.6, 1.3 Hz, 2H), 7.29 (tt, *J* = 7.1, 1.3 Hz, 1H), 7.25 (ddd, *J* = 8.4, 7.5, 1.7 Hz, 1H), 7.03 (dd, *J* = 16.1, 9.2 Hz, 1H), 6.94 (td, *J* = 7.6, 0.9 Hz, 1H), 6.87 (dd, *J* = 8.3, 0.8 Hz, 1H), 3.84 (s, 3H), 3.09 (s, 3H), 2.40 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 160.9, 160.8, 157.5, 151.3, 136.6, 135.0, 130.6, 130.0, 129.2, 127.4, 126.8, 125.5, 124.3, 120.8, 119.6, 111.2, 55.6, 35.9, 10.2; FTIR (cm^{-1}) 3035, 1642, 1237, 1049, 990, 764.

4-[3-(4-Dimethylaminophenyl)allylideneamino]-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one (**3d**); yield 97.5% as orange crystals; m.p. 179–180 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.52 (d, *J* = 9.1 Hz, 1H), 7.46 (t, *J* = 7.8 Hz, 2H), 7.40 (dd, *J* = 8.5, 1.4 Hz, 2H), 7.39 (d, *J* = 8.8 Hz, 2H), 7.29 (tt, *J* = 7.0, 1.3 Hz, 1H), 6.98 (d, *J* = 15.8 Hz, 1H), 6.81 (dd, *J* = 15.7, 9.1 Hz, 1H), 6.67 (d, *J* = 8.9 Hz, 2H), 3.09 (s, 3H), 2.99 (s, 6H), 2.41 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.09, 161.07, 151.02, 150.96, 142.4, 135.1, 129.2, 128.8, 126.8, 125.8, 124.7, 124.3, 120.0, 112.2, 40.4, 36.2, 10.2; FTIR (cm^{-1}) 3019, 1649, 1600, 1367, 1147, 980, 808.

4-[3-(4-Acetoxy-3-methoxyphenyl)allylideneamino]-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one (**3e**); yield 81.7% as yellow crystals; m.p. 240–241 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.55 (d, *J* = 8.6 Hz, 1H), 7.47 (dd, *J* = 8.3, 7.4 Hz, 2H), 7.39 (dd, *J* = 8.5, 1.2 Hz, 2H), 7.32 (tt, *J* = 7.0, 1.2 Hz, 1H), 7.11 (d, *J* = 1.8 Hz, 1H), 7.07 (dd, *J* = 8.2, 1.8 Hz, 1H), 7.01 (d, *J* = 8.1 Hz, 1H), 7.00 (d, *J* = 15.9 Hz, 1H), 6.93 (dd, *J* = 15.9, 8.6 Hz, 1H), 3.86 (s, 3H), 3.14 (s, 3H), 2.43 (s, 3H), 2.31 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.0, 160.8, 159.5, 151.5, 151.4, 140.4, 140.4, 135.6, 134.9, 130.7, 129.3, 127.1, 124.7, 123.2, 120.5, 119.4, 110.6, 56.0, 35.9, 20.8, 10.2. ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.0, 160.8, 159.5, 151.5, 151.4, 140.4, 140.4, 135.6, 134.9, 130.7, 129.3, 127.1, 124.7, 123.2, 120.5, 119.4, 110.6, 56.0, 35.9, 20.8, 10.2; FTIR (cm^{-1}) 3011, 1755, 1640, 1417, 1289, 1199, 1032, 991.

4-[2-bromo-(3-phenyl)allylideneamino]-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one (**3f**); yield 86.9% as yellow crystals; m.p. 149–150 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.48 (s, 1H), 7.86 (d, *J* = 7.5 Hz, 2H), 7.50–7.43 (m, 3H), 7.41–7.36 (m, 4H), 7.36–7.33 (m, 2H), 7.34–7.28 (m, 2H), 3.12 (s, 3H), 2.46 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 160.3, 155.0, 152.3, 139.0, 135.1, 134.6, 130.0, 129.2, 129.1, 128.3, 127.1, 125.7, 124.6, 117.9, 35.5, 10.1; FTIR (cm^{-1}), 3066, 1644, 1591, 1492, 1310, 1136, 756, 693.

4-[2-Methyl-(3-phenyl)allylideneamino]-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one (**3g**); yield 94.59% as yellow crystals; m.p. 169–170 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.51 (s, 1H), 7.47 (dd, *J* = 8.3, 7.3 Hz, 2H), 7.45 (d, *J* = 7.2 Hz, 2H), 7.41 (dd, *J* = 8.5, 1.3 Hz, 2H), 7.38 (t, *J* = 7.7 Hz, 2H), 7.30 (tt, *J* = 7.4, 1.3 Hz, 1H), 7.27 (tt, *J* = 7.3, 1.3 Hz, 1H), 6.94 (s, 1H), 3.10 (s, 3H), 2.43 (s, 3H), 2.24 (d, *J* = 1.2 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.6, 161.0, 151.9, 139.2, 138.5, 137.3, 135.1, 129.6, 129.2, 128.4, 127.5, 126.8, 124.3, 119.3, 36.1, 12.3, 10.1; FTIR (cm^{-1}) 3066, 1640, 1587, 1480, 1455, 1302, 754, 693.

4-[3-(4-Nitrophenyl)allylideneamino]-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one (**3h**); yield 98.2% as red crystals; m.p. 217–218 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.55 (d, *J* = 8.5 Hz, 1H), 8.18 (d, *J* = 8.8 Hz, 2H), 7.60 (d, *J* = 8.8 Hz, 2H), 7.47 (dd, *J* = 8.3, 7.4 Hz, 2H), 7.37 (dd, *J* = 8.5, 1.1 Hz, 2H), 7.33 (tt, *J* = 7.0, 1.1 Hz, 1H), 7.08 (dd, *J* = 16.0, 8.5 Hz, 1H), 7.01 (d, *J* = 16.0 Hz, 1H), 3.17 (s, 3H), 2.43 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 160.3, 157.7, 151.7, 147.3, 142.9, 137.3, 134.7, 134.5, 129.3, 127.6, 127.3, 124.8, 124.1, 118.8, 35.5, 10.0; FTIR (cm^{-1}) 3071, 1645, 1511, 1335, 972, 825.

2.3. Biological Evaluation

2.3.1. Evaluation of Antitumoral Activity

HeLa (human cervical carcinoma), HCT116 and HT29 (human colorectal carcinoma), SK-MEL103 (human melanoma), MDA-MB-231 (human breast carcinoma), and NIH3T3 (mouse NIH/Swiss embryo fibroblasts) were obtained from ATCC and cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) (Eurobio, Les Ulis, France) and 1% penicillin/streptomycin (Thermo Fisher Scientific, Gibco, Miami, FL, USA). All cell lines were maintained at 37 °C in a humidified atmosphere at 5% CO_2.

To assay the effect of the compounds on cell proliferation, cells were seeded at a density of 1×10^4 cells/well in 96-well plates and incubated for 72 h with 100 µL of the eight Schiff

bases at 4–250 μM final concentrations. Derivatives were dissolved in DMSO at a stock concentration of 20 mM. The final working concentration of DMSO (<1%, *v*/*v*) did not affect cell growth. After the incubation period, the MTT (thiazolyl blue tetrazolium bromide) dye assay (Sigma, St. Louis, MO, USA) was used following the standard protocol provided by the supplier. Briefly, 10 μL of MTT solution (5 mg/mL) was added to each well. After 1–2 h incubation in a humidified atmosphere, media was removed and 50 μL of DMSO were added to each well to solubilize the formazan crystals. Agitation was performed for 5 min before measuring the absorbance with a Cytation5 multi-mode detection system (BioTek, Winooski, VT, USA) at 570 nm. Each data point was generated from triplicate samples, and experiments were repeated four times. To determine the concentration of compound inhibiting 50% of cell proliferation (IC_{50}), dose-response curves were generated in GraphPad Prism (GraphPad Software, San Diego, CA, USA) using untreated cells as 100% cell proliferation control.

2.3.2. Evaluation of Antibacterial Activity

The antibacterial activity of all synthesized Schiff bases was tested against the Gram-positive bacteria *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Bacillus cereus*, *Listeria monocytogenes* ATCC 13932 and the Gram-negative bacteria *Escherichia coli* ATCC 25922, using the microdilution method [24].

The bacterial inoculum was prepared in brain–heart infusion broth (BHI) to a final cell density of 5×10^5 cfu/mL. Stock solutions of the tested compounds were prepared by dissolving them in DMSO at 10 mM. Tested volumes were adjusted so the final concentration of the DMSO in each well was always 2.5% *v*/*v*. This concentration was shown to not affect bacterial growth previously [8]. As control, bacterial cells were grown with 2.5% DMSO to rule out any potential growth inhibitory effect. Additionally, several antibiotics were used as controls for growth inhibition at the recommended working concentrations for the tested strains (Table 1). Both BHI alone and supplemented with the compounds at different concentrations were used as blanks.

Table 1. List of antibiotics and concentrations used as controls during the evaluation of antibacterial activity.

Bacteria Strain	Antibiotic
E. coli ATCC 25922 *S. aureus* ATCC 25923 *L. monocytogenes* ATCC 13932	Carbenicillin (100 μg/mL)
B. cereus	Chloramphenicol (20 μg/mL)
E. faecalis ATCC 29212	Tetracycline (10 μg/mL)

The range of concentrations (0.5 μM–250 μM) used for the Schiff bases was selected based on previous findings for Schiff base derivatives [8]. Drug sensitivities were assessed via the microdilution method [25] and according to the Clinical and Laboratory Standards Institute (CSLI) guidelines [24], with the following modifications: first, the compounds were serially diluted in DMSO, then 5 μL of each dilution was added to 195 μL of bacterial suspension (5×10^5 cfu/mL) to a total volume of 200 μL. The plates were then incubated at 37 °C for 20 h with constant shaking at 300 cpm (double orbital setting), and the OD_{600} was monitored every 30 min in a Cytation5 multi-mode detection system (BioTek). The minimal inhibitory concentration (MIC) was determined after tracking the bacterial growth over 20 h in samples exposed to the tested compound at different concentrations. The MIC was defined as the lowest concentration of the antibacterial agent, which completely inhibited the growth of the microorganism as determined by the optical density at 600 nm. These assays were performed at least in triplicate.

To determine if the inhibitory effect was either bactericidal or bacteriostatic, the bacteria were grown with the respective compound at the established MIC, and in a compound-free medium for 20 h in a microplate with the same shaking and temperature

conditions as above. Finally, the bacterial suspension was pelleted, washed in 500 µL of BHI, and plated as a drop on plain BHI agar overnight at 37 °C. Bacterial survival was registered as bacteriostatic effect, whereas bacterial absence was registered as bactericidal effect.

3. Results and Discussion

3.1. Synthesis of Schiff Base of 4-Aminoantipyrine

Schiff bases **3** were synthesized as previously reported [8]. The condensation of 4-amino-1,5-dimethyl-2-phenylpyrazol-3-one (**1**) with various cinnamaldehydes **2a–h**, using ethanol as solvent, affords the corresponding Schiff base **3a–h** in good to excellent yield as pure products after recrystallization in EtOH (Scheme 2) (Table 2). All compounds were characterized, and all the data obtained agreed with the proposed structures. The ^{1}H-NMR spectra for **3a**–**h** shows a doublet between 9.40 and 9.57 ppm, corresponding to the azomethine –CH=N proton, except for **3f** and **3g**, which appear as a singlet at 9.48 and 9.51 ppm, respectively. Despite the type of the substituent, the signal shifts downfield when the substituent is in position 2 compared to when it is in another position.

Table 2. General reaction for the synthesis of Schiff bases **3a–h**.

Compound	R_1	R_2	Appearance/Color	m.p. (°C)	Yield [1] (%)
3a	H	H	yellow crystals	162–163	90.0
3b	H	2-NO_2	red crystals	164–165	84.8
3c	H	2-OMe	yellow crystals	175–176	85.7
3d	H	4-NMe_2	orange crystals	179–180	97.5
3e	H	3-OMe-4-OAc	yellow crystals	240–241	81.7
3f	Br	H	yellow crystals	149–150	86.9
3g	Me	H	yellow crystals	169–170	94.5
3h	H	4-NO_2	red crystals	217–218	98.2

[1] Isolated yield.

3.2. Antitumor Activity Evaluation

Cells were exposed to different concentrations of each derivative for 72 h and proliferation was monitored through the MTT assay. Based on the IC_{50} values (Table 3) obtained in tumor cells compared to the IC_{50} values obtained in non-tumor cells, the most efficient derivatives were **3h** > **3c**. The compound with the highest toxicity profile was **3f**; in contrast, derivatives **3e** and **3g** did not show any effect against tumor or non-tumor cells.

Table 3. Inhibitory concentration values (IC_{50}) [a] of Schiff bases **3a–h** against tumor and non-tumor cell lines at 72 h.

Compounds	MDA-MB-231	SK-MEL-103	HCT116	HT29	HeLa	NIH3T3
3a	68.5	49.2	53.9	137.7	62.9	168
3b	30.2	24.5	44.2	72.7	30.7	116
3c	41.3	25.6	46.6	137	43.4	164
3d	114	101	71	123	113	131
3e	204	139	320	NA	204	NA
3f	18.1	5.9	4.8	NA	6.5	20.1
3g	NA	NA	NA	NA	NA	NA
3h	47.6	44.5	24.8	125	90.9	NA
DMSO [b]	2.4	2.2	1.3	2.3	1.6	2.0

[a] µM; [b] %*v*/*v*; NA: not active.

3.3. Antibacterial Activity Evaluation

Antibacterial activities were determined by testing the eight Schiff bases against the Gram-positive bacteria *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus cereus*, and *Listeria monocytogenes* and the Gram-negative bacteria *Escherichia coli*. However, only the Schiff bases **3f** and **3h** showed inhibition of the bacterial growth, and their range of minimum inhibitory concentration (MIC) are detailed in Table 4. MIC values above 250 μM were not considered as effective and were labeled as "non-effective" (NE).

Table 4. Minimal inhibitory concentration (MIC) [a] and type of inhibition determined by growth kinetics over 20 h (OD_{600}) after serial microdilution in 96-well plates for Schiff bases.

Bacteria strain	3a	3b	3c	3d	3e	3f	3g	3h
E. faecalis ATCC 29212	NE	NE	NE	NE	NE	<100 [b]	NE	NE
E. coli ATCC 25922	NE	NE	NE	NE	NE	15.6 [b]	NE	NE
S. aureus ATCC 25923	NE	NE	NE	NE	NE	<100 [b]	NE	NE
L. monocytogenes ATCC 13932	NE	NE	NE	NE	NE	<100 [c]	NE	250 [b]
B. cereus	NE	NE	NE	NE	NE	<100 [b]	NE	250 [b]

[a] μM; [b] Bacteriostatic; [c] Bactericidal; NE: non-effective.

Compounds **3a**, **3b**, **3c**, **3d**, **3e**, and **3g** did not show any activity against the tested bacteria, whereas **3f** showed antibacterial activity for all the tested strains (<100 μM). The lowest MIC value identified so far (15.6 μM) corresponds to **3f** against *E. coli.* In general, **3f** showed increased antibacterial activity compared to **3h** within the same strain, with MIC values at least 2.5 times lower. This result could be attributed to the presence of a bromine atom in the structure, which is known to have antibacterial properties due to its oxidant potential [26]. On the other hand, when the nitro group is present (**3h**), the activity is limited to *L. monocytogenes* and *B. cereus*, which suggests a different mode of action compared to **3f**.

Additionally, after 20 h exposure of the bacteria to the compounds, they were plated on drug-free agar to determine the type of inhibitory effect. All the strains subjected to **3f** and **3h** showed a bacteriostatic effect, except from *L. monocytogenes* exposed to compound **3f**, which showed a bactericidal effect. All effects are reported in Table 4.

4. Conclusions

The Schiff base derivatives **3a–h** can be readily synthesized with high yields by the condensation reaction between 4-aminoantipyrine (**1**) and various cinnamaldehydes. Schiff base derivatives **3h** and **3c** inhibited tumor cell proliferation while having no effect on non-tumoral cells utilized as controls. As a result, these compounds show potential as antitumor agents and may benefit from additional research. Furthermore, Schiff bases **3f** and, to a lesser extent, **3h** have promising activity against different Gram-positive and Gram-negative bacteria and should be investigated further. The antibacterial potential of **3f** could be attributed to the oxidative properties of the bromine atom, which benefits pathogen growth inhibition.

Author Contributions: Conceptualization, J.H.-M.; formal analysis, E.A.-L., S.E.C.-P., R.G.-P., J.C.R.-B. and J.H.-M.; investigation, E.A.-L., S.E.C.-P., R.G.-P., J.Z.-M., C.R.-P., J.C.R.-B. and J.H.-M.; writing—original draft preparation, E.A.-L., R.G.-P. and J.Z.-M.; writing—review and editing, S.E.C.-P., R.G.-P. and J.H.-M.; supervision, J.H.-M.; project administration, J.H.-M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data generated or analyzed during this study are included in this published article.

Acknowledgments: This study was funded by Universidad UTE and Universidad Técnica Particular de Loja (UTPL).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Okey, N.C.; Obasi, N.L.; Ejikeme, P.M.; Ndinteh, D.T.; Ramasami, P.; Sherif, E.-S.M.; Akpan, E.D.; Ebenso, E.E. Evaluation of some amino benzoic acid and 4-aminoantipyrine derived Schiff bases as corrosion inhibitors for mild steel in acidic medium: Synthesis, experimental and computational studies. *J. Mol. Liq.* **2020**, *315*, 113773. [CrossRef]
2. Raczuk, E.; Dmochowska, B.; Samaszko-Fiertek, J.; Madaj, J. Different Schiff Bases—Structure, Importance and Classification. *Molecules* **2022**, *27*, 787. [CrossRef] [PubMed]
3. Ceramella, J.; Iacopetta, D.; Catalano, A.; Cirillo, F.; Lappano, R.; Sinicropi, M.S. A Review on the Antimicrobial Activity of Schiff Bases: Data Collection and Recent Studies. *Antibiotics* **2022**, *11*, 191. [CrossRef] [PubMed]
4. Catalano, A.; Sinicropi, M.S.; Iacopetta, D.; Ceramella, J.; Mariconda, A.; Rosano, C.; Scali, E.; Saturnino, C.; Longo, P. A Review on the Advancements in the Field of Metal Complexes with Schiff Bases as Antiproliferative Agents. *Appl. Sci.* **2021**, *11*, 6027. [CrossRef]
5. Matela, G. Schiff Bases and Complexes: A Review on Anti-Cancer Activity. *Anticancer Agents Med. Chem.* **2020**, *20*, 1908–1917. [CrossRef]
6. Murtaza, G.; Mumtaz, A.; Khan, F.A.; Ahmad, S.; Azhar, S.; Najam-Ul-Haq, M.; Atif, M.; Khan, S.A.; Maalik, A.; Alam, F.; et al. Recent pharmacological advancements in Schiff bases: A Review. *Acta Pol. Pharm.-Drug Res.* **2014**, *71*, 531–535.
7. Kajal, A.; Bala, S.; Kamboj, S.; Sharma, N.; Saini, V. Schiff Bases: A Versatile Pharmacophore. *J. Catal.* **2013**, *2013*, 893512. [CrossRef]
8. Teran, R.; Guevara, R.; Mora, J.; Dobronski, L.; Barreiro-Costa, O.; Beske, T.; Pérez-Barrera, J.; Araya-Maturana, R.; Rojas-Silva, P.; Poveda, A.; et al. Characterization of Antimicrobial, Antioxidant, and Leishmanicidal Activities of Schiff Base Derivatives of 4-Aminoantipyrine. *Molecules* **2019**, *24*, 2696. [CrossRef]
9. Afridi, H.H.; Shoaib, M.; Al-Joufi, F.A.; Shah, S.W.A.; Hussain, H.; Ullah, A.; Zahoor, M.; Mughal, E.U. Synthesis and Investigation of the Analgesic Potential of Enantiomerically Pure Schiff Bases: A Mechanistic Approach. *Molecules* **2022**, *27*, 5206. [CrossRef]
10. Shawky, A.M.; Abourehab, M.A.S.; Abdalla, A.N.; Gouda, A.M. Optimization of pyrrolizine-based Schiff bases with 4-thiazolidinone motif: Design, synthesis and investigation of cytotoxicity and anti-inflammatory potency. *Eur. J. Med. Chem.* **2020**, *185*, 111780. [CrossRef]
11. Mermer, A.; Demirbas, N.; Uslu, H.; Demirbas, A.; Ceylan, S.; Sirin, Y. Synthesis of novel Schiff bases using green chemistry techniques; antimicrobial, antioxidant, antiurease activity screening and molecular docking studies. *J. Mol. Struct.* **2019**, *1181*, 412–422. [CrossRef]
12. Wang, Y.-Y.; Xu, F.-Z.; Zhu, Y.-Y.; Song, B.; Luo, D.; Yu, G.; Chen, S.; Xue, W.; Wu, J. Pyrazolo [3,4-d]pyrimidine derivatives containing a Schiff base moiety as potential antiviral agents. *Bioorg. Med. Chem. Lett.* **2018**, *28*, 2979–2984. [CrossRef] [PubMed]
13. Wang, C.; Fan, L.; Pan, Z.; Fan, S.; Shi, L.; Li, X.; Zhao, J.; Wu, L.; Yang, G.; Xu, C. Synthesis of Novel Indole Schiff Base Compounds and Their Antifungal Activities. *Molecules* **2022**, *27*, 6858. [CrossRef] [PubMed]
14. Laxminarayan, R. The overlooked pandemic of antimicrobial resistance. *Lancet* **2022**, *399*, 606–607. [CrossRef] [PubMed]
15. Nadimpalli, M.L.; Chan, C.W.; Doron, S. Antibiotic resistance: A call to action to prevent the next epidemic of inequality. *Nat. Med.* **2021**, *27*, 187–188. [CrossRef]
16. World Health Organization. *Global Antimicrobial Resistance and Use Surveillance System (GLASS) Report: Early Implementation 2020*; World Health Organization: Geneva, Switzerland, 2020; p. 180.
17. Cohen, T.L. The Next Pandemic: A pragmatic and ethical discussion about the looming threat of antibiotic resistance. *Voices Bioeth.* **2022**, *8*. [CrossRef]
18. Vaughn, V.M.; Gandhi, T.N.; Petty, L.A.; Patel, P.K.; Prescott, H.C.; Malani, A.N.; Ratz, D.; McLaughlin, E.; Chopra, V.; Flanders, S.A. Empiric Antibacterial Therapy and Community-onset Bacterial Coinfection in Patients Hospitalized with Coronavirus Disease 2019 (COVID-19): A Multi-hospital Cohort Study. *Clin. Infect. Dis.* **2021**, *72*, e533–e541. [CrossRef]
19. Ghimpețeanu, O.M.; Pogurschi, E.N.; Popa, D.C.; Dragomir, N.; Drăgotoiu, T.; Mihai, O.D.; Petcu, C.D. Antibiotic Use in Livestock and Residues in Food—A Public Health Threat: A Review. *Foods* **2022**, *11*, 1430. [CrossRef]
20. Iacopetta, D.; Ceramella, J.; Catalano, A.; Saturnino, C.; Bonomo, M.G.; Franchini, C.; Sinicropi, M.S. Schiff Bases: Interesting Scaffolds with Promising Antitumoral Properties. *Appl. Sci.* **2021**, *11*, 1877. [CrossRef]
21. Zhang, Z.; Zhou, L.; Xie, N.; Nice, E.C.; Zhang, T.; Cui, Y.; Huang, C. Overcoming cancer therapeutic bottleneck by drug repurposing. *Signal Transduct. Target. Ther.* **2020**, *5*, 113. [CrossRef]
22. Reşit, Ç.; Başaran, E.; Boğa, M.; Erdoğan, Ö.; Çınar, E.; Çevik, Ö. Schiff Base Derivatives of 4-Aminoantipyrine as Promising Molecules: Synthesis, Structural Characterization, and Biological Activities. *Russ. J. Bioorg. Chem.* **2022**, *48*, 334–344. [CrossRef]

23. Rashmi, A.; Rishi, S.; Abhishek, T.; Ajmer Singh, G.; Balraj, S.; Sandeep, A.; Rajwinder, K. Design and synthesis of novel 4-aminophenazone Schiff bases by grinding technique as prospective anti-inflammatory agents. *J. Appl. Pharm. Sci.* **2021**, *11*, 48–53. [CrossRef]
24. Clinical and Laboratory Standards Institute (CLSI). *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*, 11th ed.; Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2018; ISBN 1562388363.
25. Balouiri, M.; Sadiki, M.; Ibnsouda, S.K. Methods for in vitro evaluating antimicrobial activity: A review. *J. Pharm. Anal.* **2016**, *6*, 71–79. [CrossRef] [PubMed]
26. Gottardi, W.; Klotz, S.; Nagl, M. Superior bactericidal activity of *N*-bromine compounds compared to their *N*-chlorine analogues can be reversed under protein load. *J. Appl. Microbiol.* **2014**, *116*, 1427–1437. [CrossRef] [PubMed]