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Short Note

Dichloro{4-(4-chlorophenoxy)phthalazin-1-yl} methylphosphonic dichloride

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Abstract: As part of our ongoing scaffold-hopping work on an antiplasmodial 2-trichloromethylquinazoline scaffold, we aimed to explore the 1-trichloromethylphthalazine scaffold as a potential new antimalarial series. Using previously chlorination conditions described by our lab to obtain a trichloromethyl group from a methyl group, we did not obtain the target compound; instead, we obtained a dichloro methylphosphonic dichloride side product **3**. The nature of this compound was then characterized by NMR, HRMS and X-ray crystallography. The same issue was previously reported by Kato et al., starting from the 2-methyl-3-nitropyridine. Finally, compound **3,** although not cytotoxic, was not active against *P. falciparum*, the parasite responsible for human malaria.

Keywords: phthalazine; chlorination; side product; phosphonic dichloride; *P. falciparum*; HepG2

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1. Introduction

Malaria is still the leading cause of mortality in comparison to other parasitic diseases. In 2020, malaria deaths dramatically increased by 12% from 2019 to an estimated 627,000, among which 77% were children under 5 years old: this was mainly due to service disruptions during the COVID-19 pandemic. [1] To overcome the resistance of the causal agent *Plasmodium* to most of the marketed therapies, including the most recent ones such as artemisinin-combination therapies (ACTs), a huge effort has been made to highlight new derivatives active against *Plasmodium* and display original mechanisms of action [2]. With the aim of developing new antiplasmodial compounds, our laboratory explored different aza-heterocyclic scaffolds bearing a trichloromethyl group, which was mandatory for providing the antiparasitic activity [3–5]. We previously obtained a hit molecule in the 2-trichloromethylquinazoline series bearing a 4′-chlorophenoxy substituent at position 4, showing micromolar activity against *P. falciparum* and a low cytotoxicity against the human HepG2 cell line [6] (Figure 1).

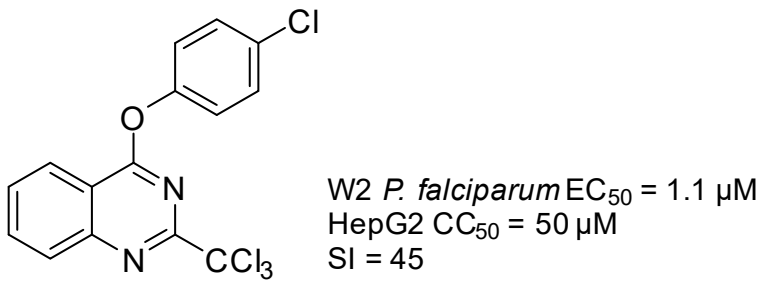


Figure 1. Previously described antiplasmodial Hit A.

In order to continue the pharmacomodulation work around this aza-heterocyclic scaffold, we performed a scaffold-hopping strategy using ring variation, among which we explored the phthalazine moiety. Indeed, phthalazines have recently gained some importance as privileged scaffolds in bioactive compounds, such as anticancer drugs, namely Olaparib [7] and Vatalanib [8], as well as the antihistaminic H_1 drug Azelastine [9] (Figure 2). Numerous other bioactive molecules are currently in development in various therapeutic areas [10].

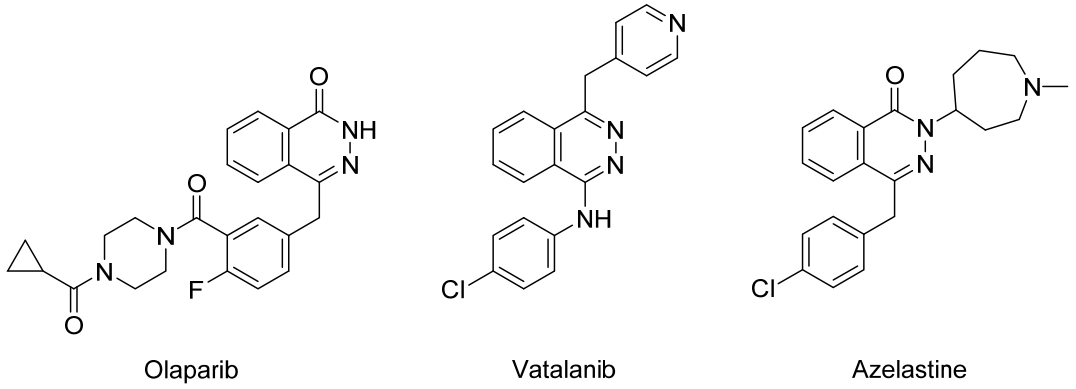
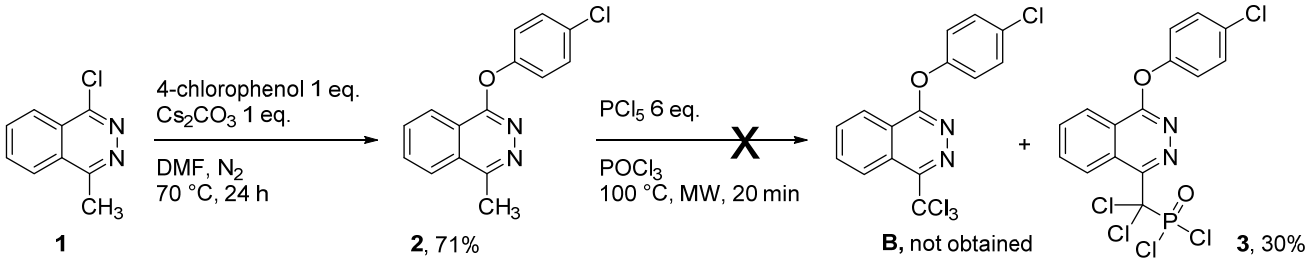


Figure 2. Some drugs based on phthalazine moiety.

2. Results

To obtain the target compound **B**, we followed our previously described reaction condition applied to the phthalazine scaffold (Scheme 1). Starting from the readily accessible 1-chloro-4-methylphthalazine **1** [11], we first introduced an S_NAr reaction to the 4-chlorophenoxy substituent at position 1, using the appropriate phenol and cesium carbonate as a base to yield **2** (71%). Then, we performed the chlorination reaction in order to obtain the 4-trichloromethyl group from the 4-methyl group, using a mixture of PCl_5 in $POCl_3$. This reaction is usually performed under microwave heating, which allows for the best yields in a short reaction time [12].



Scheme 1. Reaction conditions for the synthesis of compound **B** and structure of compound **3**.

A mixture of two compounds was obtained. Usually, the reaction leads to a mixture of the target compound -CCl_3 and the dichlorinated intermediate -$CHCl_2$. However, in our study, we noticed the formation of a new unexpected compound following the purification step. After complementary unambiguous analyses, we confirmed by NMR, HRMS and X-ray crystallography (Figure 3) [13] that this compound was dichloro{4-(4-chlorophenoxy)phthalazin-1-yl}methylphosphonic dichloride **3** (Scheme 1) (see Supplementary data).

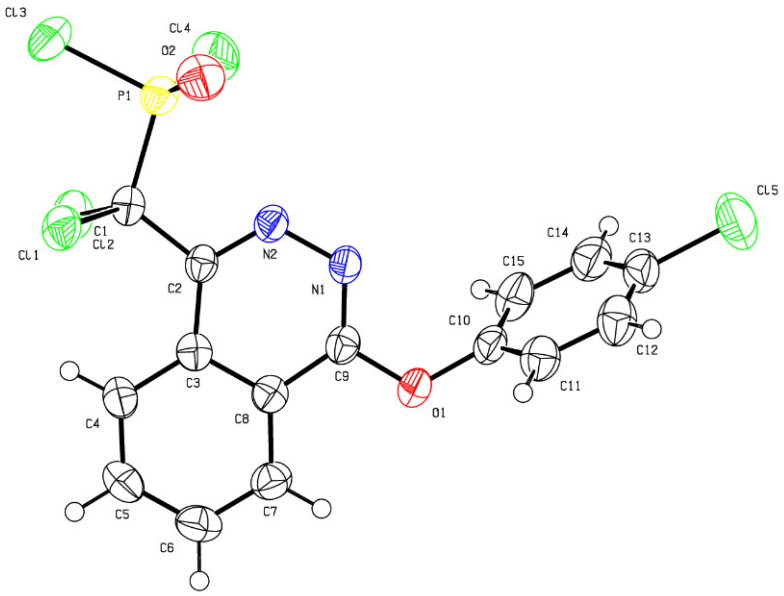
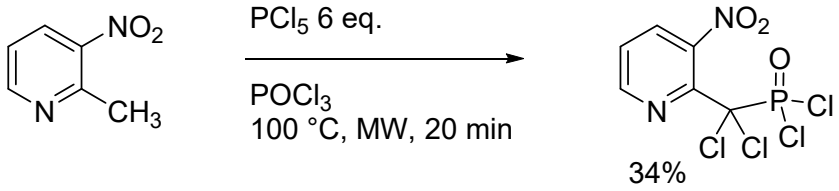


Figure 3. X-ray crystallography structure of compound **3**.

3. Discussion

After conducting bibliographical research, we noticed that Kato et al. previously reported the formation of a similar unwanted compound upon the chlorination of 2-methyl-3-nitropyridine using a mixture of PCl_5 in $POCl_3$, leading to dichloro-(3-nitro-2-pyridyl)methylphosphonic dichloride [14] (Scheme 2).

Finally, we wanted to explore the biological potential of **3**: this compound was not active against *P. falciparum* (EC_{50} = 28.9 µM), nor was it cytotoxic on the HepG2 cell line (CC_{50} = 62.5 µM).



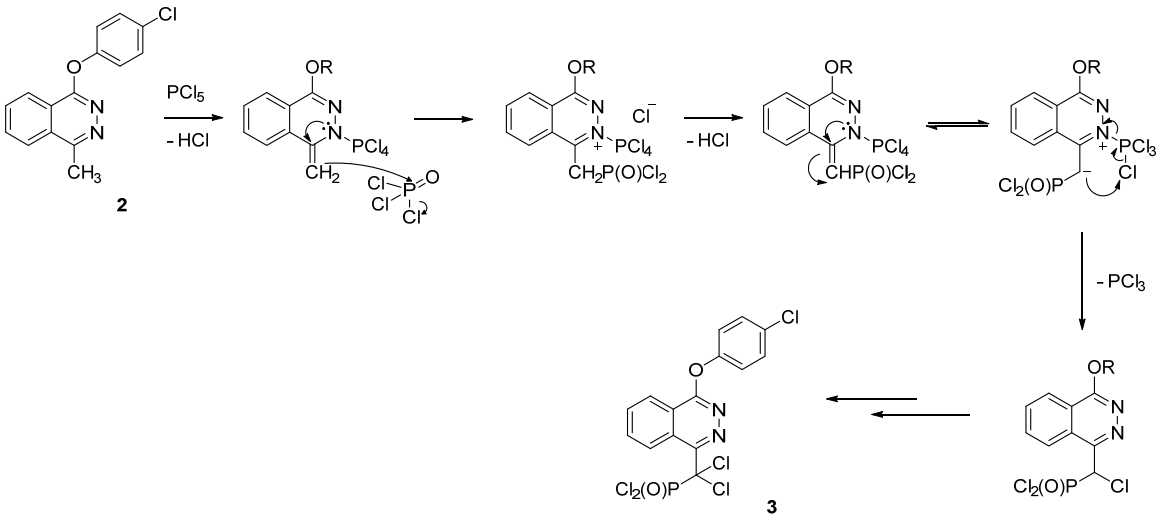
Scheme 2. Similar issue observed by Kato et al. [14] in pyridine series.

Several factors could explain the direct phosphonylation of methylphthalazine **2**, although the mechanism of the reaction is not clearly established:

(1) The substituent effect of the 4-chlorophenoxy group to the nucleophilic behavior of the 1-methyl group;

(2) The ability of the phthalazine to form a complex with the strongest available electrophile, i.e., PCl_5, if sterically possible.

The chlorination by PCl_5 is usually favored, thanks to a lower enthalpy of activation; however, it is sterically more constrained than phosphonylation by $POCl_3$, because oxygen atoms are much smaller than chlorine atoms [14]. Thus, PCl_5 cannot react due to its size, but $POCl_3$ can come close enough to react with the nucleophilic carbon, giving the intermediate phosphonic dichloride. The next step could involve intramolecular chlorination to give the monochlorophosphonic dichloride. Repeating the chlorination step finally gave the product **3** (Scheme 3).



Scheme 3. Hypothesized reaction mechanism.

4. Materials and Methods

Melting points were determined on a Köfler melting point apparatus (Wagner & Munz GmbH, München, Germany) and were uncorrected. Elemental analyses were carried out at the Spectropole, Faculté des Sciences de Saint-Jêrome (Marseille) with a Thermo Finnigan EA1112 analyzer (Thermo Finnigan, San Jose, CA, USA). NMR spectra were recorded on a Bruker Avance NEO 400MHz NanoBay spectrometer at the Faculté de Pharmacie of Marseille (^{1}H NMR: reference $CDCl_3$ δ = 7.26 ppm and ^{13}C NMR: reference $CHCl_3$ δ = 76.9 ppm). The following adsorbent was used for column chromatography: silica gel 60 (Merck KGaA, Darmstadt, Germany, particle size 0.063–0.200 mm, 70–230 mesh ASTM). TLC was performed on 5 cm × 10 cm aluminum plates coated with silica gel 60F-254 (Merck) in an appropriate eluent. Visualization was performed with ultraviolet light (234 nm). The purity of synthesized compounds was checked by LC/MS analyses, which were performed at the Faculté de Pharmacie of Marseille with a Thermo Scientific Accela High Speed LC System® (Waltham, MA, USA), coupled to a single quadrupole mass spectrometer Thermo MSQ Plus®. The RP-HPLC column was a Thermo Hypersil Gold® 50 × 2.1 mm (C18 bounded), with particles of a diameter of 1.9 mm. The volume of sample injected on the column was 1 µL. The chromatographic analysis with a total duration of 8 min, was performed on the following solvents' gradients: t = 0 min, methanol/water 50:50; 0 < t < 4 min, linear increase in the proportion of methanol to a methanol/water ratio of 95:5; 4 < t < 6 min, methanol/water 95:5; 6 < t < 7 min, linear decrease in the proportion of methanol to return to a methanol/water ratio of 50:50; 6 < t< 7 min, methanol/water 50:50. The water used was buffered with ammonium acetate 5 mM. The flow rate of the mobile phase was 0.3 mL/min. The retention times (t_R) of the molecules analyzed were indicated in min. The microwave reactions were performed using multimode reactor ETHOS Synth Lab station (Ethos start, MLS GmbH, Leutkirch, Germany) in an open vessel with a power output of 0 to 800 W. Reagents were purchased and used without further purifications from Sigma-Aldrich or Fluorochem.

*1-(4-Chlorophenoxy)-4-methylphthalazine (***2***).* To a solution of 1-chloro-4-methylphthalazine (**1**) [11], (500 mg, 2.8 mmol) and 4-chlorophenol (360 mg, 2.8 mmol, 1.0 equiv) in anhydrous DMF (5 mL), Cs_2CO_3 (912 mg, 2.8 mmol, 1.0 equiv) was added under an inert atmosphere. The mixture was stirred at 70 °C for 24 h. After completion of the reaction, water was added, leading to a precipitate which was separated by filtration. The resulting yellow precipitate was then thoroughly washed with water. The precipitate was dissolved in CH_2Cl_2 and dried with Na_2SO_4. After filtration and evaporation, the resulting solid was purified by silica-gel column chromatography (Petroleum ether/CH_2Cl_2, 1:1 *v*/*v*) to afford the desired compound **2**.

Yield 71% (540 mg). Yellow solid. Mp 132–133 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46–8.34 (m, 1H), 8.08–8.02 (m, 1H), 8.00–7.93 (m, 2H), 7.39 (d, *J* = 8.9 Hz, 2H), 7.27 (d, *J* = 8.9 Hz, 2H), 2.93 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) *δ* 160.7, 155.1, 152.1, 132.9, 132.5, 130.6, 129.7, 129.1, 124.9, 123.6, 123.2, 119.9, 19.5. LC-MS (ESI+) t_R 5.42 min; *m*/*z* $[M+H]^+$ 271.11/273.12. HRMS (ESI): *m*/*z* calcd. for $C_{15}H_{12}ClN_2O$ $[M + H]^+$ 271.0633. Found: 271.0632.

*Dichloro(4-(4-chlorophenoxy)phthalazin-1-yl)methyl)phosphonic dichloride (***3***).* To a solution of 1-(4-Chlorophenoxy)-4-methylphthalazine (**2**) (500 mg, 1.85 mmol) in $POCl_3$ (10 mL) PCl_5 was added (2.31 g, 11.1 mmol). The reaction mixture was heated by a microwave reactor at a reflux of POCl3 for 20 min at 800 W. After cooling down, the reaction mixture was poured into ice, and then the pH was adjusted to neutrality with Na_2CO_3. The resulting solution was extracted three times with CH_2Cl_2. The organic phase was then washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in a vacuum to afford the crude product, which was purified by silica-gel flash chromatography (using dichloromethane/petroleum ether from 5/5 to 7/3 *v*/*v*) to afford compound **3**.

Yield 30% (250 mg). Yellow solid. Mp 250 °C (degradation). ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 8.75 (d, *J* = 7.9 Hz, 1H), 8.55 (d, *J* = 7.9 Hz, 1H), 8.10–8.06 (m, 2H), 7.42 (d, *J* = 8.5 Hz, 2H), 7.27 (d, *J* = 8.5 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) *δ* 162.3, 151.6, 151.2, 133.4, 133.3, 131.6, 130.0, 126.1, 126.0, 125.2, 124.6, 123.3, 121.5. ^{31}P NMR (162 MHz, $CDCl_3$) *δ* 35.73. HRMS (ESI): *m*/*z* calcd. for $C_{15}H_9Cl_5N_2O_2P$ $[M + H]^+$ 456.8810. Found: 456.8806.

Crystal Data for $C_{15}H_8Cl_5N_2O_2P$ (*M* = 456.45 g/mol): monoclinic, space group $P2_1/n$ (no. 14), *a* = 7.9208(3) Å, *b* = 23.1270(9) Å, *c* = 9.9621(5) Å, *β* = 96.368(4)°, *V* = 1813.64(13) Å^3, *Z* = 4, *T* = 295 K, μ(MoKα) = 0.900 mm^{-1}, *Dcalc* = 1.672 g/cm^3, 15,418 reflections measured ($6.244° \leq 2\Theta \leq 56.808°$), 3992 unique ($R_{int}$ = 0.0343, R_{sigma} = 0.0295), which were used in all calculations. The final R_1 was 0.0400 ($I > 2\sigma(I)$) and wR_2 was 0.1021 (all data).

Supplementary Materials: The following are available online: Figure S1, ^{1}H NMR spectra of 1-(4-Chlorophenoxy)-4-methylphthalazine; Figure S2, ^{13}C NMR spectra of 1-(4-Chlorophenoxy)-4-methylphthalazine; Figure S3, HRMS (ESI) spectra of 1-(4-Chlorophenoxy)-4-methylphthalazine; Figure S4, ^{1}H NMR spectra of Dichloro(4-(4-chlorophenoxy)phthalazin-1-yl)methyl)phosphonic dichloride; Figure S5, ^{13}C NMR spectra of Dichloro(4-(4-chlorophenoxy)phthalazin-1-yl)methyl)phosphonic dichloride; Figure S6, ^{31}P NMR spectra of Dichloro(4-(4-chlorophenoxy)phthalazin-1-yl)methyl)phosphonic dichloride; Figure S7, HRMS (ESI) spectra of Dichloro(4-(4-chlorophenoxy)phthalazin-1-yl)methyl)phosphonic dichloride.

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Sample Availability: Samples of the compounds are available from the authors.

References

1. *World Malaria Report 2021*; World Health Organization: Geneva, Switzerland, 2021.
2. Burrows, J.N.; Duparc, S.; Gutteridge, W.E.; Hooft van Huijsduijnen, R.; Kaszubska, W.; Macintyre, F.; Mazzuri, S.; Möhrle, J.J.; Wells, T.N.C. New developments in anti-malarial target candidate and product profiles. *Malar. J.* **2017**, *16*, 26. [CrossRef] [PubMed]
3. Amrane, D.; Gellis, A.; Hutter, S.; Prieri, M.; Verhaeghe, P.; Azas, N.; Vanelle, P.; Primas, N. Synthesis and Antiplasmodial Evaluation of 4-Carboxamido- and 4-Alkoxy-2-Trichloromethyl Quinazolines. *Molecules* **2020**, *25*, 3929. [CrossRef] [PubMed]
4. Amrane, D.; Arnold, C.-S.; Hutter, S.; Sanz-Serrano, J.; Collia, M.; Azqueta, A.; Paloque, L.; Cohen, A.; Amanzougaghene, N.; Tajeri, S.; et al. 2-Phenoxy-3-Trichloromethylquinoxalines Are Antiplasmodial Derivatives with Activity against the Apicoplast of Plasmodium Falciparum. *Pharmaceuticals* **2021**, *14*, 724. [CrossRef] [PubMed]
5. Amrane, D.; Primas, N.; Arnold, C.-S.; Hutter, S.; Louis, B.; Sanz-Serrano, J.; Azqueta, A.; Amanzougaghene, N.; Tajeri, S.; Mazier, D.; et al. Antiplasmodial 2-Thiophenoxy-3-Trichloromethyl Quinoxalines Target the Apicoplast of Plasmodium Falciparum. *Eur. J. Med. Chem.* **2021**, *224*, 113722. [CrossRef] [PubMed]
6. Castera-Ducros, C.; Azas, N.; Verhaeghe, P.; Hutter, S.; Garrigue, P.; Dumètre, A.; Mbatchi, L.; Laget, M.; Remusat, V.; Sifredi, F.; et al. Targeting the Human Malaria Parasite Plasmodium Falciparum: In Vitro Identification of a New Antiplasmodial Hit in 4-Phenoxy-2-Trichloromethylquinazoline Series. *Eur. J. Med. Chem.* **2011**, *46*, 4184–4191. [CrossRef] [PubMed]
7. Robson, M.; Im, S.-A.; Senkus, E.; Xu, B.; Domchek, S.M.; Masuda, N.; Delaloge, S.; Li, W.; Tung, N.; Armstrong, A.; et al. Olaparib for Metastatic Breast Cancer in Patients with a Germline BRCA Mutation. *N. Engl. J. Med.* **2017**, *377*, 523–533. [CrossRef] [PubMed]
8. Scott, E.; Meinhardt, G.; Jacques, C.; Laurent, D.; Thomas, A.L. Vatalanib: The Clinical Development of a Tyrosine Kinase Inhibitor of Angiogenesis in Solid Tumours. *Expert Opin. Investig. Drugs* **2007**, *16*, 367–379. [CrossRef] [PubMed]
9. Ratner, P.H.; Findlay, S.R.; Hampel, F.; van Bavel, J.; Widlitz, M.D.; Freitag, J.J. A Double-Blind, Controlled Trial to Assess the Safety and Efficacy of Azelastine Nasal Spray in Seasonal Allergic Rhinitis. *J. Allergy Clin. Immunol.* **1994**, *94*, 818–825. [CrossRef]
10. Zaib, S.; Khan, I. Synthetic and Medicinal Chemistry of Phthalazines: Recent Developments, Opportunities and Challenges. *Bioorg. Chem.* **2020**, *105*, 104425. [CrossRef]
11. Elmeligie, S.; Aboul-Magd, A.M.; Lasheen, D.S.; Ibrahim, T.M.; Abdelghany, T.M.; Khojah, S.M.; Abouzid, K.A.M. Design and Synthesis of Phthalazine-Based Compounds as Potent Anticancer Agents with Potential Antiangiogenic Activity via VEGFR-2 Inhibition. *J. Enzyme Inhib. Med. Chem.* **2019**, *34*, 1347–1367. [CrossRef] [PubMed]
12. Verhaeghe, P.; Rathelot, P.; Gellis, A.; Rault, S.; Vanelle, P. Highly Efficient Microwave Assisted α-Trichlorination Reaction of α-Methylated Nitrogen Containing Heterocycles. *Tetrahedron* **2006**, *62*, 8173–8176. [CrossRef]
13. Primas, N.; Vanelle, P.; Amrane, D. CCDC 2074292: Experimental Crystal Structure Determination. 2021. Available online: https://www.ccdc.cam.ac.uk/structures/search?id=doi:10.5517/ccdc.csd.cc27mgn0&sid=DataCite (accessed on 11 August 2022).
14. Kato, T.; Katagiri, N.; Wagai, A. Synthesis of Methylpyridine Derivatives—XXXIII Phosphonylation and Chlorination of Methylpyridine and 3-Nitro-Methylpyridine Derivatives. *Tetrahedron* **1978**, *34*, 3445–3449. [CrossRef]