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Synthesis, Molecular Structure and Anti-Onchocercal Studies of 1-(Phthalazin-1(2H)-one) [(Pyridin-2-yl)ethylidene]hydrazone

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Abstract

The novel ligand, 1-(phthalazin-1(2H)-one)[(pyridin-2-yl)ethylidene]hydrazone,(APN), derived from the antihypertensive drug, hydralazine hydrochloride, was synthesized and characterized by spectroscopic methods (IR, ^{1}H NMR). The X-ray crystallographic data indicates that APN has an exocyclic C=N bond on the hydralazine moiety. APN revealed significant anti-onchocercal activity with IC_{50} values of 0.3125 µg/mL on microfilaria and 10 µg/mL on adult worms compared to the standard drug, ivermectin.

Keywords

Hydralazine Hydrochloride, Schiff Bases, Anti-Onchocercal Activity

1. Introduction

Hydralazine, a 1-substituted phthalazine, commonly called apresoline, is a powerful areterial vasodilator that has been effectively used for the treatment of hypertensive disorders particularly in pregnant patients and also in the treatment of patients with congestive heart failure. Schiff bases of hydralazine (hydrazones) have shown important medicinal properties including antifungal activities [1] [2], antimicrobial properties [3], antihypertensive agents [4], carbonyl scavenger and antiapoptotic activities [5], luminescence properties [6]. Hydrazones have been reported to be less toxic than hydrazines from which they are derived since the- NH_2 group is blocked [7] [8].

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Human onchocerciasis or subcutaneous filariasis, commonly known as river blindness, is one of the 17 neglected tropical diseases caused by the filarial worm *Onchocerca volvulus* and transmitted by the black fly, *Simulium damnosum*. By World Health Organization (WHO) estimates about 123 million people are at risk of becoming infected with river blindness and 25 million people worldwide are already infected with *Onchocera volvulus*, 300,000 of whom are blind and 800,000 are suffering from some kind of visual impairment [9].

The drug of choice for the treatment of onchocerciasis is ivermectin which is very effective against microfilariae with no discernible effect on macrofilariae. A yearly dose of prescribed ivermectin for 15 - 18 years may eradicate microfilariae and break the chain of transmission. This long period of treatment may lead to patients' noncompliance and drug resistance in the parasite. The emergence of ivermectin resistance in parasitic nematodes in veterinary medicine [10] may be an indication that this may extend to the human *O. volvulus* [11].

These challenges coupled with adverse effects of ivermectin to humans have prompted an urgent search for other sources of new and more potent drugs for the treatment of river blindness. Our objective in this study is to synthesize a new hydralazine-derived Schiff base and to test it against *Onchocerca ochengi*, a close relative of *Onchocerca volvulus*, the causative agent of human onchocerciasis.

2. Experimental

2.1. Materials

Hydralazine hydrochloride, 2-acetylpyridine and other solvents were used as purchased from commercial sources without further purification. Elemental analysis was performed on a VARIO EL (Heraeus) analyzer. IR spectra were obtained from a Perkin-Elmer System 2000 FT-IR spectrophotomer using KBr pellets. The mass spectra (ESI) were recorded with an FT-IR (APEX II) mass spectrometer from Bruker Daltonics and the ^{1}H NMR spectra were run in MeOD on a 400-MHz spectrometer

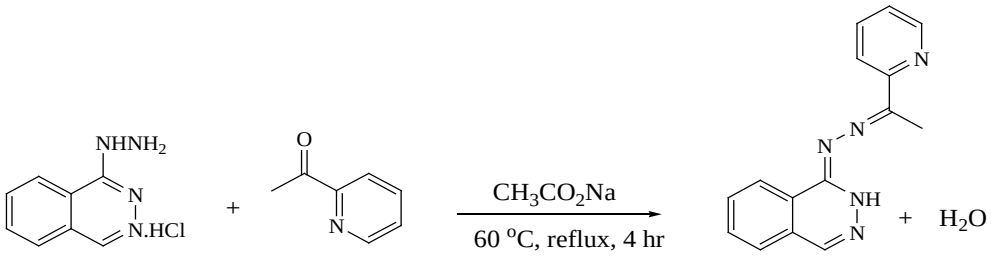
2.2. Synthesis of 1-(Phthalazin-1(2H)-One)[Pyridine-2-yl)Ethylidene]Hydrazine (APN)

A mixture of equimolar amounts of hydralazine hydrochloride (500 mg, 2.54 mmol) and 2-acetylpyrideine (307 mg, 2.54 mmol) and sodium acetate (buffering agent) (350 mg, 2.56 mmol) in 50 mL of ethanol was refluxed for 4 hours while stirring (**Scheme 1**). The mixture was left to cool overnight. The resultant solid was vacuum-filtered, washed several times with distilled water and ethanol to give a yellow precipitate on air-drying.

Yield 79%. *Anal.Calcd* for $C_{15}H_{13}N_5$: C 58.40%; H 5.60%; N 22.70%. *Experimental*: C 58.40%; H 4.40%; N 33.43%. *IR* (KBr, cm^{-1}) 3438.6, 3317.1, 3045, 3006, 2922.1, 1605.6, 1591.5, 1569.1, 1532.6.1468.1, 1432.8, 1250.1, 1146.8, 1023, 780.5. *ESI* [m/z (%)]: 286.1 (100%) $[M + Na]^+$; 264.2 (35%) $[M]^+$. ^{1}H NMR (δ ppm): 2.64 (s, 3H at position 8'), 7.74 (m, H at positions 6,7), 8.05 (d, H at position 5), 8.12 (d,H at position 8), 8.56 (s, H at position 4), 7.79, 7.74 (m, Hs at positions 4' and 5'), 7.96 (d, H at position 3'), 8.71 (d, proton at position 6') (**Figure 1**).

2.3. Single Crystal X-Ray Diffraction Analysis and Structure Determination

The crystallographic data of **APN** were collected on a Gemini diffractometer (Agilent Technologies) using Mo-Kα radiation (λ = 71.073 pm), ω-scan rotation. CrysAlis Pro [12] was used for data including the program



Hydrazaline hydrochloride 2-Acetylpyridine

Scheme 1. Synthesis of **APN**.

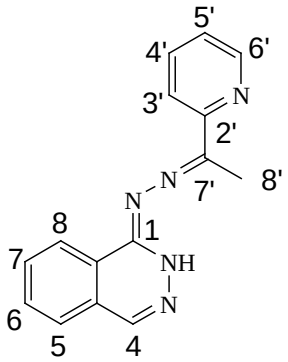


Figure 1. Structural features of **APN**.

SCALE3 ABSPACK [13] for empirical absorption correction. The refinement of all non-hydrogen atoms was performed with SHELXL-97 [14] and structure was solved by direct methods with SHELXS-97. All non-hydrogen atoms were refined with anisotropic thermal parameters and a difference-density Fourier map was used to locate all hydrogen atoms. CCDC 1007670 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44)1223-336-033; or deposit@ccdc.cam.uk). The molecular graphics were done with ORTEP-3 [15] and Mercury (version 3) [16].

2.4. Anti-Onchocercal Activity

Both O. ochengi microfilariae and macrofilariae were isolated and cultured by the known methods [17] [18]. The monkey kidney epithelial cells (LLCMK2) obtained from American Type Culture Collection (ATCC, Virginia, USA) and proliferated as instructed by the supplier served both for cytotoxicity studies and as feeder layer. The cells were cultured in a complete culture medium (CCM) and after becoming confluent, the parasites and drugs were added. The effect of the drugs on the integrity of the cells was determined by microscopy. The selectivity index (SI) of each compound was calculated as the ratio of CC_{50} of drug to these mammalian cells to the $IC_{50}$of the drug on the parasites.

All assays were conducted for 120 hours after adding compound. The FDA-approved compound, auranofin which has been reported to be active on adult worms at 10 µM, was used as positive control for the adult worm assays [19]. Ivermectin (Mectizan®) was used as the positive control drug for the microfilariae assays. Negative wells received only the diluents (2% dimethyl sulfoxide). Inhibition of microfilariae motility was assessed by microscopy every 24 hours post addition of drugs. Scores were assigned following observation under inverted microscope recorded as 100% (complete inhibition), 75% (only head or tail of worm moving or vibrating), 50% (worm sluggish) or 25% (little change in motility), 0% (no observable inhibition of motility). The motion of adult worms was determined by the MTT/formazan assay and a compound was considered active if there was 90% or greater mean inhibition of formazan formation, moderately active if there was 50% - 89% mean inhibition of formazan formation or inactive if there was less than 50% mean inhibition of formazan formation.

3. Results and Discussion

The condensation reaction between hydralazine hydrochloride and 2-acetylpyridine gave **APN** in good yield (**Scheme 1**).

3.1. Elemental Analyses

Elemental analysis of the ligand was in good agreement with literatures values [1]. The molecular ion peak appeared at m/z 286.1 (M + Na) and the (M + H) ion at m/z 264.2.

3.2. Infrared Spectra

The IR spectra of the **APN** (**Figure 2**) was taken in 4000 - 400 cm^{-1} usual region. Strong bands are seen at 3439

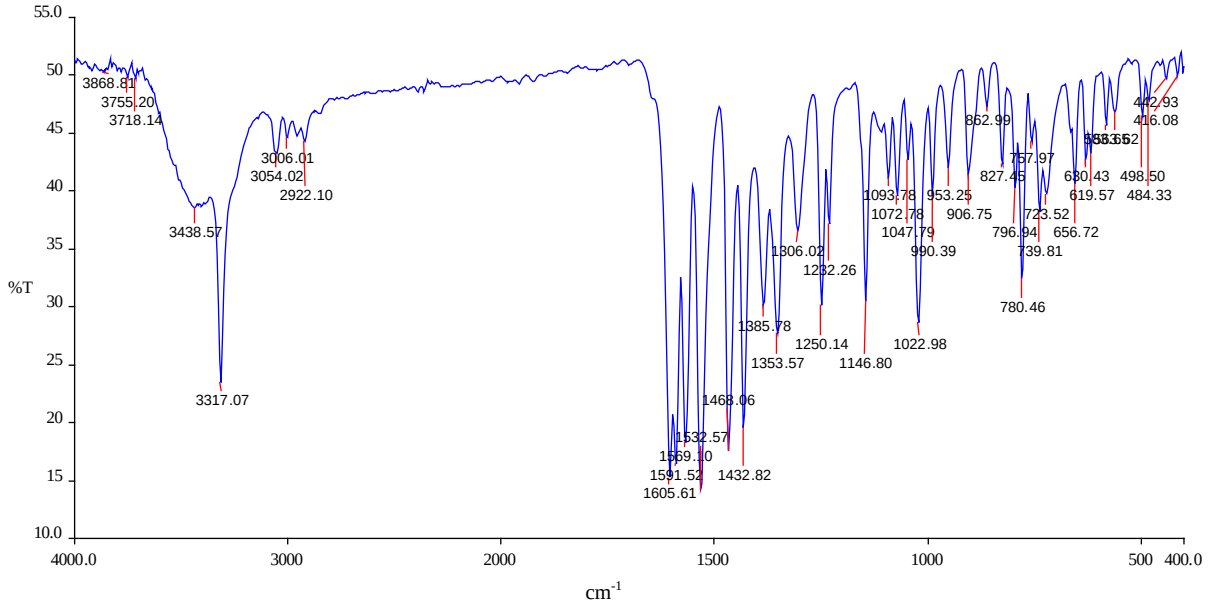


Figure 2. IR Spectrum of **APN**.

and 3317 cm^{-1} corresponding to the stretching vibrations of the O-H bond of residual water molecules and N-H stretching vibrations respectively. The bands that cluster around 3000 cm^{-1} are the stretching vibrations of =CH and the sharp bands at 2992 cm^{-1} can be assigned to the methyl group bonds. The strong bands in the range 1532 - 1606 cm^{-1} are due to the C=C vibrations respectively [20]. The lower vibrations may be attributed to the pyridine breathing modes.

3.3. ^{1}H NMR Spectra

The proton NMR spectra of **APN** (**Figure 3**) were recorded using MeOD as the solvent. The single peak at 2.64 ppm is attributed to the methyl group at position 8' (**Figure 1**). The multiplet in the region 7.74 - 7.79 ppm is observed for position 6 and 7 for the hydralazine moiety and 4' and 5' for the pyridine moiety. The proton at position 4 gives a singlet (δ 8.56) and the proton at position 6' gives a doublet (δ 8.71). These protons are highly deshielded due to the presence of the adjacent nitrogen atom. The doublet at δ 8.05 and δ 8.12 are assigned to protons at 5 and 8 respectively which are influenced by long range coupling. Due to the tautomeric nature of the ligand, the N-H proton of the hydralazine moiety may be highly deshielded. The insert in **Figure 3** shows the expanded ^{1}H NMR spectrum of the ligand.

3.4. X-Ray Data

A single x-ray analysis performed on APN (**Figure 4**) confirmed the proposed structure of the hydralazine Schiff base showing clearly the exocyclic C=N double bond (bond length 1.308 A˚) of the hydralazine moiety [1] [21] and the N-H bond of bond length 0.918 A˚ [19]

3.5. Activity of Compounds on Microfilariae/Macrofilariae and Cytotoxicity

APN was screened on the juvenile form of the *O. ochengi* beginning at the highest compound concentration of 20 µg/mL. It inhibited microfilariae motility completely at 1.25 µg/mL (**Table 1**) but it was only moderately active at the highest concentration of 20 µg/mL against adult worms.

APN compound was also seen to be most cytotoxic on LLCMK2 cells. **APN** killed the cells completely at 1.25 µg/mL and had a selectivity index of 2 (**Table 1**). **APN** demonstrated a dose-dependent progressive motility inhibition characteristic. This progressive killing of the parasites is a good aspect of the compound as severe immune response to dead mfs might be avoided. Ivermectin, currently used in the control of onchocerciasis is only microfilaricidal and sub-optimal responses have been reported of this drug [22]. The need for alternative

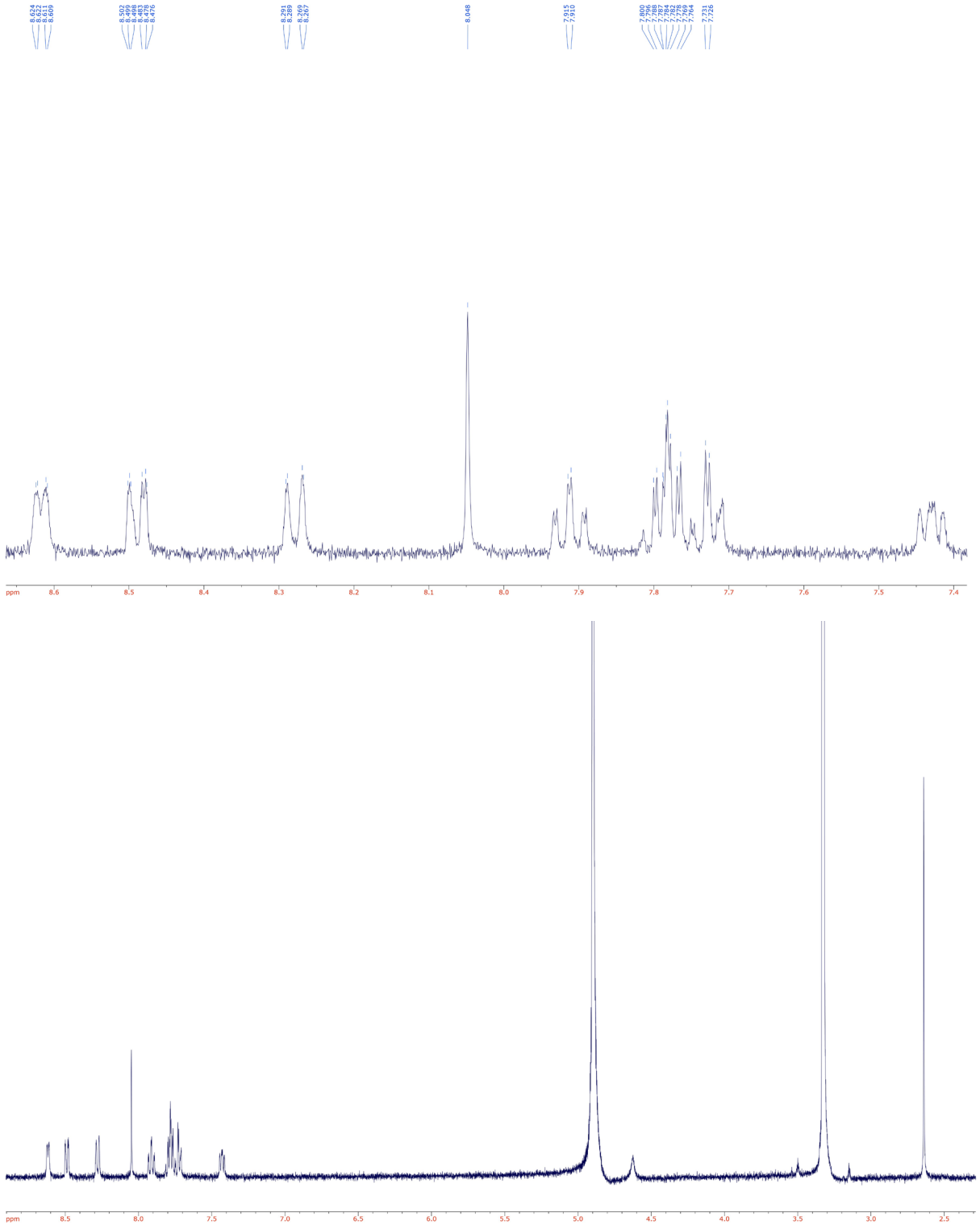


Figure 3. 1H NMR Spectrum of **APN**.

drugs which are macro-/microfilaricidal is still highly solicited.

The long duration of treatment of onchocerciasis with ivermectin is because the drug is only microfilaricidal. **APN** has shown both micro- and macrofilaricidal properties raising hope for an effective anti-onchocercal drug.

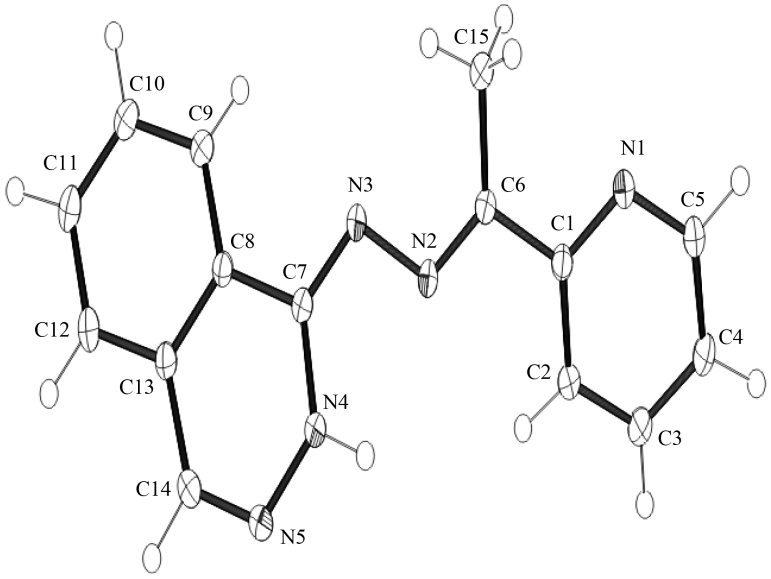


Figure 4. Perspective view (ORTEP) of APN.

Table 1. Dose dependent inhibition of microfilariae (mfs) mobility and cytotoxicity of the active compounds by the 120th hour.

Compound code	Concentration of compound (µg/mL)	Percent inhibition of mfs motility	Cytotoxicity on LLCMK2 cells	IC_{50} of parasite (µg/mL)	CC_{50} of LLCMK2 cell (µg/mL)	Selectivity Index
APN	20	100	100	0.3125	0.625	2
	10	100	100			
	5	100	100			
	2.5	100	100			
	1.25	100	100			
	0.625	50	50			
	0.3125	50	0			
	0	0	0			
Ivermectin	10	100				

4. Conclusion

APN derived from hydralazine hydrochloride was synthesized and characterized by spectroscopic methods. The X-ray crystallographic data show that **APN** has an exocyclic carbon-nitrogen double bond on the hydralazine moiety indicating the stable form of the compound in the solid state. **APN** showed significant anti-onchocercal activity with IC_{50} value of 0.3125 µg/mL on microfilariae and 10 µg/mL on adult worms. The anti-onchocercal activities of this compound are reported for the first time and the results indicate that this novel ligand can be exploited as an anti-onchocercal agent.

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