

RESEARCH ARTICLE

Schiff Base Oxime Derivatives Reactivate Chlorpyrifos-induced Acetylcholinesterase Inhibition

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Abstract: Background: The biological effects of organophosphorus compounds are connected with the irreversible inhibition of acetylcholinesterase (AChE), an important neuromediator acetylcholine (ACh) splitting enzyme in the human body at the synaptic clefts. Due to this inhibition, AChE is unable to fulfill its physiological function resulting in the accumulation of ACh, which, in turn, over stimulates the parasympathetic nerve receptors and causes fatal cholinergic crisis.

Objective: The objective of the study was to synthesize a series of Schiff base oximes and to assess their *in vitro* reactivating potency against chlorpyrifos inhibited AChE.

Methods: The amino group of 4-amino acetophenone was exploited by treating with substituted benzaldehyde in the presence of glacial acetic acid to form Schiff base (**1a-1f**). The titled compounds (**2a-2f**) were prepared by treating Schiff base with hydroxylamine hydrochloride in the presence of alcohol. Through physical and spectral analysis, the structure of compounds was confirmed. The synthesized compounds were evaluated for their reactivation efficacy against chlorpyrifos-inhibited rat brain AChE by Ellman's method.

Results: The pralidoxime (2-PAM) showed potent reactivation against chlorpyrifos-inhibited AChE at the concentration tested (0.001 M). In this case, the compounds **2a** (40.4%, 60 min) and **2d** (37.9%, 60 min) showed promising reactivation as compared to 2-PAM (40.6%, 60 min) against chlorpyrifos-inhibited AChE.

Conclusion: Compounds having chloro (**2a**) and nitro (**2d**) substitution on the 4th position gave good activity against chlorpyrifos-inhibited AChE. Moreover, these Schiff base oximes appear to be very promising due to their sufficient reactivation strength at lower concentration (10⁻³ M).

Keywords: Schiff base, oxime, acetylcholine, acetylcholinesterase, reactivation.

1. INTRODUCTION

Most commonly used insecticides or pesticides in the agriculture field are organophosphorus (OP) compounds. Unfortunately, these compounds are poisons when ingested either accidentally or intentionally. The irreversible inhibition caused by OP compounds with catalytic site of the enzyme

acetylcholinesterase (AChE) leads to hyperstimulation of muscarinic and nicotinic receptors. The resultant effects depend on the type and dose of the OP, which causes impairment of numerous body functions and finally lead to respiratory arrest and death [1]. This warrants the need of invention of a broad range of new and efficacious deactivators of AChE [2].

Schiff base derivatives which have been reported to have an array of medicinal properties attract curiosity for both synthetic and biological viewpoints. These derivatives of Schiff base demonstrate antibacterial and antifungal properties [3], properties of drugs used for ulceration [4], as well as anti-HIV [5] and anticonvulsant properties [6]. In general, drug discovery involves modification of lead compounds to establish expected activity. The research on Schiff base has been attracting biochemists since the molecule exerts the properties similar to biological substances. Further, the ease of its synthesis allows tailoring of the molecule with desired structural properties [7-9]. The applications of Schiff's bases are not only limited to electrochemistry, bioinorganic, catalysis, metallic deactivators, separation processes, and environmental chemistry [10] but also impact the fields including pharmaceuticals, dyes, plastic industries, and liquid crystal technology [11].

The present work describes the synthesis of a series of Schiff base oxime derivatives and also their effectiveness to reactivate OP-inhibited AChE. The amino group of 4-aminoacetophenone was exploited by treating with substituted benzaldehyde in the presence of glacial acetic acid to form Schiff base (**1a-1f**) [12]. The titled compounds (**2a-2f**) were prepared by treating Schiff base with hydroxylamine hydrochloride in the presence of alcohol. The structure of compounds was established by their physical and spectral analysis.

2. MATERIALS AND METHODS

At regular intervals, the completions of reactions were monitored with the help of thin-layer chromatography (TLC) using aluminum sheets (E. Merck) precoated with GF₂₅₄ silica gel with 0.2 mm layer thickness. Veego melting point apparatus was used to check physical constants like melting point. Shimadzu infrared (IR) Affinity-1 was used to record IR spectra using KBr. Using Bruker Avance II 400 nuclear magnetic resonance (NMR) spectrometer (with TMS as internal references) at sophisticated analytical instrumentation facility, Panjab University (Chandigarh), the ¹H NMR spectra of the synthesized compounds were recorded. Mass spectra were recorded on Shimadzu liquid chromatography-mass spectrometry (LC/MS)-2010A at Quest Research and Training Institute (Pvt.) Ltd., Bengaluru.

2.1. General Procedure for Synthesis of (1E)-1-(4-{{(1E)-2-Substituted phenylethylidene} amino}phenyl)ethanone Oxime

The synthesized compounds **1a-1f** (0.5 g) were refluxed for 6–8 h with 0.5 g of hydrochloride salt of hydroxylamine. The TLC method with mobile phase (chloroform:methanol, 6:4) adopted to ensure the progress of the reaction. After the completion of reaction, the obtained solution was cooled and transferred into the beaker containing ice cubes with stirring. The solution was left for overnight for crystallization. Then, the obtained precipitate was filtered and recrystallized using a suitable solvent. The physico-chemical properties are shown in **Table 1**.

2.1.1. (1Z)-1-(4-{{(1E)-(4-Chlorophenyl)Methylene} Amino}Phenyl)Ethanone Oxime **2a**

IR (KBr, cm⁻¹): 3288.11 (-OH oxime), 2978.72, 2846.68 (aliphatic-CH str.), 1605.83, 1512.79 (-C=N of Schiff base and oxime); ¹H NMR (DMSO-*d*₆, δ ppm): 8.60 (s, 1H, oxime -OH), 7.82 (d, 1H, -CH), 7.25-7.95 (m, 8 H, Ar-H), 2.32 (s, 3H, CH₃). LCMS: *m/z* 272.73; calculated 271.21.

2.1.2. (1Z)-1-(4-{{(1E)-(2-chlorophenyl)Methylene} Amino}Phenyl)Ethanone Oxime **2b**

IR (KBr, cm⁻¹): 33651.53 (-OH of oxime), 3223.51, 3064.77 (aromatic -CH str.), 2917.17, 2848.71 (aliphatic -CH str.), 1588.43, 1515.33 (-C=N of Schiff base and oxime); ¹H NMR (DMSO-*d*₆, δ ppm): 8.42 (s, 1H, oxime -OH), 7.75 (d, 1H, -CH), 7.25-8.05 (m, 8H, Ar-H), 2.32 (s, 3H, CH₃).

2.1.3. (1Z)-1-(4-{{(1E)-(2,4-dichlorophenyl)Methylene} Amino}Phenyl)Ethanone Oxime **2c**

IR (KBr, cm⁻¹): 3225.93 (-OH of oxime), 3062.65, 2980.49 (aromatic -CH str.), 2919.85, 2850.32 (aliphatic -CH str.), 1604.83, 1580.14 (-C=N of Schiff base and oxime); ¹H NMR (DMSO-*d*₆, δ ppm): 8.48 (s, 1H, oxime -OH), 7.78 (d, 1H, -C-H), 7.19-7.77 (m, 7H, Ar-H), 2.38 (s, 3H, CH₃); LCMS: *m/z* 307.17; calculated 306.03.

2.1.4. (1Z)-1-(4-{{(1E)-(4-nitrophenyl)Methylene} Amino}Phenyl)Ethanone Oxime **2d**

IR (KBr, cm⁻¹): 3295.48 (-OH of oxime), 1659.08, 1590.08 (-C=N of Schiff base and oxime); ¹H NMR (DMSO-*d*₆, δ ppm): 8.27 (s, 1H, -OH), 8.25 (d, 1H, -CH) 7.26-8.23 (m, 8H, Ar-H), 2.24 (s, 3H, CH₃); LCMS: *m/z* 282.46; calculated 283.28.

2.1.5. (1Z)-1-(4-{{(1E)-(3-hydroxyphenyl)Methylene} Amino}Phenyl)Ethanone Oxime **2e**

IR (KBr, cm⁻¹): 3247.52 (-OH of oxime), 2980.29 (aromatic -C-H str), 2849.15, 2359.94 (aliphatic -C-H str), 1581.44, 1512.90 (C=N Schiff base and oxime); ¹H NMR (DMSO-*d*₆, δ ppm): 9.43 (s, 1H, -OH), 8.93 (s, 1H, -OH), 8.65 (d, 1H, -C-H), 7.28-8.45 (m, 8H, Ar-H), 2.32 (s, 3H, CH₃), LCMS: *m/z* 254.11; calculated 254.28.

2.1.6. (1Z)-1-(4-{{(1E)-(4-fluorophenyl)Methylene} Amino}Phenyl)Ethanone Oxime **2f**

IR (KBr, cm⁻¹): 3294.46 (-OH of oxime), 2980.50 (aromatic -C-H str), 1664.09, 1592.90 (-C=N Schiff base and oxime); ¹H NMR (DMSO-*d*₆, δ ppm): 8.45 (s, 1H, -O-H), 8.35 (d, 1H, -CH) 7.62-8.23 (m, 8H, Ar-H) 2.28 (s, 3H, CH₃); LCMS: *m/z* 256.16; calculated 256.27.

2.2. *In vitro* experiments

The test compounds (**2a-2f**) were subjected to *in vitro* reactivation of chlorpyrifos-inhibited AChE in phosphate buffer solution (0.1 M, pH 8.0 at 37°C) by Ellman method [13]. Values shown in Table 2 are averages of three readings that appear to

be significant as the maximum relative standard deviation does not exceed $\pm 2\%$. Chlorpyrifos (1.4×10^{-3} M) solution prepared in isopropanol was preserved in refrigerator until further use, whereas dimethylformamide was the solvent to prepare the test stock solutions. The stock solutions of DTNB (0.01 M) and acetylthiocholine iodide (0.075 M) were prepared using phosphate buffer (pH 8.0, 0.1 M) and distilled water, respectively. About 50 μ l of chlorpyrifos (1.4×10^{-3} M) and 50 μ L of enzyme were added to 350 μ L of phosphate buffer solution (0.1 M, pH 8.0) to obtain the incubation mixture, which was incubated for 15 min at room temperature to yield $96 \pm 1\%$ inhibition of enzyme activity. Storage at this condition for even more than 1 h does not change enzyme inhibition activity. Test solution (50 μ L, 0.001 M) was then added to the incubation mixture up to the final volume of 500 μ L to initiate reactivation. Finally, the activity was analyzed by Ellman's method soon after 10 min.

From the above solution, 20 ml was mixed with 50 μ L of DTNB in phosphate buffer (pH 8.0, 0.1 M) in a cuvette, for which 50 μ L of substrate was added to check reactivation efficacy. Simultaneously, blank without substrate was used. In both cases, the final volume was made up to 3 mL using phosphate buffer (pH 8.0). The enzyme reactivation activity was observed up to 1 h with an interval of 10 min. The calculation of percentage reactivation was carried out using the equation in the following:

$$\% \text{ Reactivation} = \frac{E_r - E_i}{E_o - E_i} \times 100$$

where, E_o = Control enzyme activity at 0 min

E_i = Inhibited enzyme activity

E_r = Activity of reactivated enzyme after incubation with the test compounds.

3. RESULTS AND DISCUSSION

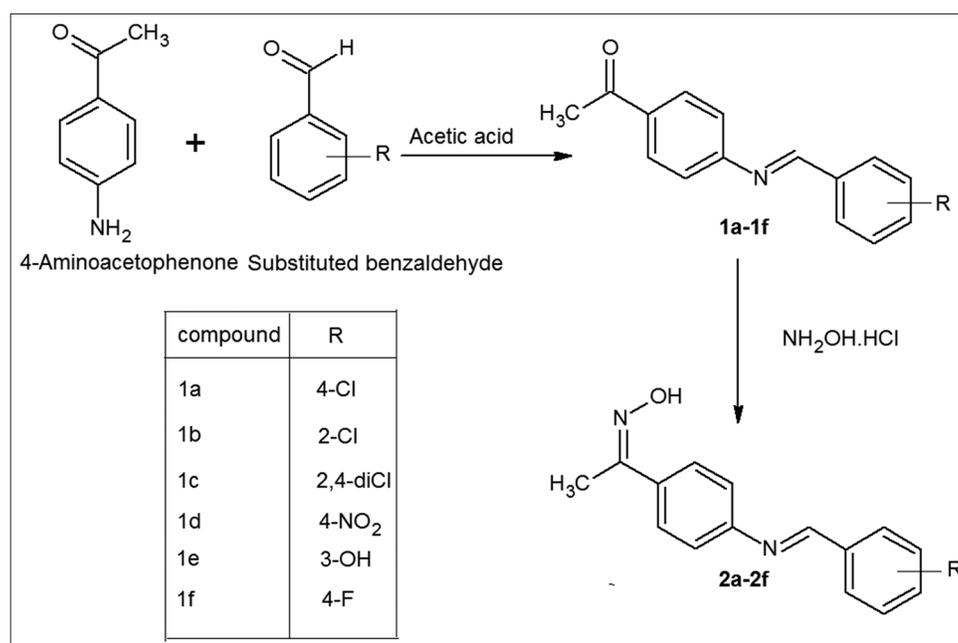
The titled compounds were successfully synthesized as shown in **Scheme 1** and characterized on the basis of physicochemical and spectral analysis. The 4-amino reactive

center (NH_2) in compound 4-aminoacetophenone has been utilized for the condensation with various substituted benzaldehyde in the presence of glacial acetic acid to give the Schiff base (**1a-1f**) in a good yield. Further, the ketone group of Schiff base (**1a-1f**) was treated with hydroxylamine hydrochloride to yield six derivatives of Schiff base oxime (**2a-2f**).

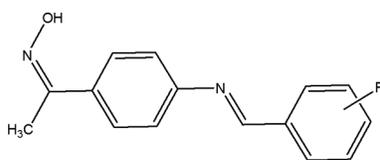
The characterized IR absorption peak for compound **2c** at 3225.93 cm^{-1} was due to hydroxyl group of oxime. The aromatic $-\text{C}-\text{H}$ stretch which appeared between the peak at 3062.5 and 2980.49 cm^{-1} , the aliphatic $-\text{C}-\text{H}$ stretch which appeared between the peak at 2919.85 and 2850.32 cm^{-1} , and the Schiff base of $-\text{C}=\text{N}$ bond which appeared between the peak at 1604.83 and 1580.14 cm^{-1} indicated the completion of the reaction. Further, structure assignment of **2c** is supported by NMR spectral data in which signal at 8.48 singlet of hydroxyl group of oxime, 7.78 signal for doublet of $-\text{C}-\text{H}$ proton and the appearance of aromatic proton in between 7.19 and 7.77 and further, it is supported by mass spectral studies, in which compound shows single molecule ion peak (m^+) at 307.16 which exactly matches with the molecular weight, and it is another evidence for the completion of the reaction.

The compounds **2a** to **2f** were assayed for their *in vitro* reactivation efficacy against chlorpyrifos-inhibited AChE using Ellman's method. All results obtained are summarized in **Table 2**.

As mentioned, the pralidoxime (2-PAM) was the most potent reactivator in the treatment of chlorpyrifos-inhibited AChE at concentration of 0.001 M. In this case, the compounds **2a** (40.4%, 60 min) and **2d** (37.9%, 60 min) achieved promising reactivation efficacy as compared to 2-PAM (40.6%, 60 min) against chlorpyrifos-inhibited AChE. It is worth to note that the Schiff base oxime showed better reactivation at 40 min, especially compounds such as **2a** (38.4%), **2d** (36.0%), and **2e** (28.5%). In addition, at a lower concentration (10^{-3} M),



Scheme 1: Synthesis of 2-substituted phenylethylidene] amino} phenyl) ethanone oxime derivatives.

Table 1. Physicochemical data of (1E)-1-(4-[(1E)-2-Substituted phenylethylidene] amino} phenyl) ethanone oxime compounds (2a-2f).

Com.	R	Mol. Form.	Mol. Wt.	Recrystallization solvent	M.P°C	% yield	RF value
2a	4-Cl	C ₁₅ H ₁₃ N ₂ OCl	272.71	Chloroform	100-102	32.5	0.79
2b	2-Cl	C ₁₅ H ₁₃ N ₂ OCl	272.71	Chloroform	78-80	26.96	0.92
2c	2,4-diCl	C ₁₅ H ₁₂ N ₂ OC ₁₂	307.16	Chloroform	118-120	62.38	0.66
2d	4-NO ₂	C ₁₅ H ₁₃ N ₂ O ₃	283.25	Alcohol	98-100	30.23	0.77
2e	3-OH	C ₁₅ H ₁₄ N ₂ O ₂	242.17	Methanol	88-90	42.28	0.74
2f	4-F	C ₁₅ H ₁₃ N ₂ OF	256.25	Chloroform	138-140	28.15	0.91

*TLC profile: Chloroform: methanol (6:4), TLC: Thin-layer chromatography

Table 2. The percentage reactivation of chlorpyrifos-inhibited AChE by test compounds (source of the enzyme: Rat brain AChE; time of inhibition by OP-15 min; time of reactivation; 10-60 min; pH – 8.0; 25°C).

Compounds (0.001 M)	Resulting enzyme –inhibitor complex	% reactivation*					
		10 min	20 min	30 min	40 min	50 min	60 min
2a		15.5	19.7	28.4	38.4	40.1	40.4
2b		2.1	4.1	6.2	7.3	6.9	5.6
2c		8.2	13.1	18.7	22.6	21.2	19.4
2d		16.5	20.7	26.8	36.0	37.5	37.9
2e		10.2	16.4	22.1	28.5	21.7	20.6
2f		10.4	14.9	17.1	21.6	18.8	18.3
2-PAM		18.0	25.0	36.2	41.8	42.5	40.6

*The values are the average of three runs with a maximum SD of $\pm 2\%$

the reactivation potential of the **2c** and **2e** Schiff base oximes showed nearly 21% of the reactivation potential in comparison to 42.5% regeneration by 2-PAM.

4. CONCLUSION

In the present study, we have synthesized six novel Schiff base oxime (**2a** to **2f**) derivatives which were biologically evaluated for the reactivation efficacy against chlorpyrifos-inhibited rat brain AChE by Ellman's method. The method of synthesis is efficient and provides the desired compounds in satisfactory yields. Compounds having chloro (**2a**) and nitro (**2d**) substitution at the 4th position gave good activity against chlorpyrifos-inhibited AChE. Moreover, these Schiff base oximes seem to be very promising due to their sufficient reactivation potency at lower concentration (10^{-3} M).

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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