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# SYNTHESIS AND EVALUATION OF QUINOLIN-2(1*H*)-ONE FUSED OXAZOLE AS AN *IN VITRO* REACTIVATOR OF ORGANOPHOSPHORUS COMPOUND INHIBITED ACETYLCHOLINESTERASE

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## ABSTRACT

**Background:** Poisoning with organophosphorus (OP) compounds are frequent because OP are widely used as insecticide or pesticide. OP compounds exert inhibition on acetylcholinesterase (AChE) activity by irreversibly binding to the catalytic site of the enzyme.

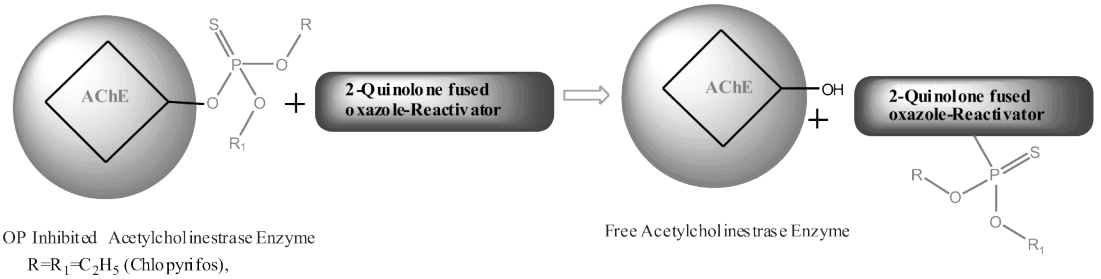
The inhibition of AChE leads to hyperstimulation of muscarnic and nicotinic receptors due to excess of acetylcholine (ACh).

**Methodology:**Various quinolin-2(1H)-one fused oxazole were synthesized by condensation and cyclization of chalcones with hydroxylamine hydrochloride in presence of piperidine. Synthesized compound were tested for *in vitro* reactivation of chlorpyrifos and methyl parathion inhibited AChE enzyme using pralidoxime (2-PAM) as standard reference.

**Result:**Among the synthesized compounds, the compound **3b**, **3f**, **3g**, and **5a** have showed promising activity as compared to standard against chlorpyrifos inhibited AChE. However, **3f** and **3g**showed good activity as compared to standard against methyl parathion inhibited AChE.

**Conclusion:** The derivatives having nitro and chloro substitution at 4$^{th}$ position gave potent activity against both OP compounds as compared to standard at concentration 0.001 M. Moreover, these quinolone fused oxazole seem to be very promising because of their sufficient reactivation potency at lower concentration ($10^{-3}$ M).

Reactivation of Organophosphorous Inhibited Acetylcholinestrase Enzyme by 2-Quinolone fused oxazole Reactivator




OP Inhibited Acetylcholinestrase Enzyme

$R=R_1=C_2H_5$ (Chlopyrifos),
$R=R_1=CH_3$ (Methyl Parathion)

Free Acetylcholinestrase Enzyme

**Key words:** *Acetylcholinesterase; Acetylcholine; 2-quinolone; reactivation; 2-PAM.*

## INTRODUCTION

Organophosphorus (OP) compounds are used as pesticides in agriculture, various purposes in industry[1]and DFP (diisopropylfluorophosphate) as therapeutic agent in the treatment of myasthenia gravis, and ecothiopate to treat glaucoma.[2,3] The inhibition of AChE by OP could result in severe intoxication and death of the exposed individual. These compounds block the active site by covalently binding with a serine hydroxyl group,[4]thus inhibiting its physiological action of hydrolyzing the acetylcholine (ACh) neurotransmitter at central and peripheral synapses.[5]ACh accumulation results in an over-stimulation of cholinergic receptors, depending on the type and dose of the incorporated OP, causes disturbance of numerous body functions and finally in respiratory arrest and death.[6] Poisoning is the common method of suicide, especially in developing world. OP pesticide self-poisoning is estimated to kill around 200,000 people each year and mortality rate varies from 10-20%.[7]

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Presently, a combination of an antimuscarinic agent, e.g. atropine and AChE reactivator such as one of the recommended pyridinium oximes (Pralidoxime, Trimedoxime, Methoxime, Obidoxime and HI-6) are used for the treatment of organophosphate poisoning in humans. However, these oximes have several disadvantages including toxicity, CNS penetrability, and limited reactivating efficacy towards the OP inhibited AChE.[8-9] In fact, none of the above oximes can be regarded as a broad spectrum antidote for all pesticide. So development and selection of new effective reactivators of AChE like antidotes of OP are very important due to the extended usage of pesticide in agriculture and therefore eventual intoxication of human.[10]

Thus, the present communication describes the synthesis of novel series of 2-quinolone fused oxazole derivatives and also their subsequent evaluation for reactivation efficacy against OP inhibited AChE. The compound **1a** and **1b** was synthesized according to our previously reported literature.[11] The acetyl group of compound **1a** and **1b** was treated with various substituted aromatic aldehydes to form respective chalcone **2a-2h**. The titled compounds were obtained by refluxing the **2a-2h** with hydroxylamine hydrochloride in presence of pyridine to yield **3a-3h**.

## MATERIALS

All the chemicals and solvents were supplied by Merck, S.D Fine-Chem. Limited, Mumbai and used without further purification.DTNB [5,5'-dithiobis-(2-nitrobenzoic acid)] and acetylthiocholine iodide were purchased from Sigma-Aldrich, USA and used without further purification. Potassium dihydrogen phosphate and dipotassium-hydrogen phosphate were obtained from E. Merck (India) and used without further purification. Organophosphate Chlorpyrifos and Methyl parathion gift sample was obtained from Indian Institute of Horticulture, Bangalore, Karnataka, India. 2-PAM was prepared according to the reported method.[12]

## METHODS

The reactions were monitored with the help of thin layer chromatography using pre-coated aluminum sheets with $GF_{254}$ silica gel, 0.2mm layer thickness (E.Merck). The melting points were taken on the Veego (VMP-MP) melting point apparatus and are uncorrected. The IR spectra of the compounds were recorded using KBr on Shimadzu IRAFFINITY-1. The $^{1}H$ NMR and $^{13}C$ NMR spectra of the synthesized compounds were recorded on Bruker avance II 400 NMR spectrometer (with TMS as internal references) at Sophisticated Analytical and Instrumentation Facility (SAIF), Panjab University (Chandigarh). The Mass spectra were recorded on Waters, Q-TOF Micromass. Since the derivatives prepared were having similar structural features, hence only one final derivative was confirmed mass spectroscopy.

**General procedure for synthesis of 3-substituted cinnamoyl-4-hydroxy-1-phenyl (2a-2d)/methyl (2e-2h) quinolin-2(1*H*)-one derivative:**

A mixture of 1a/1b 0.01mol, substituted aldehydes 0.015 mol and piperidine1 mL in ethanol 5 mL was refluxed for 6-8 hours and progress of reaction was monitored by TLC (n-hexane: ethyl acetate 7:3). Solution obtained was cooled and allowed to stand overnight; solid separated was filtered and recrystallized using suitable solvent.

**3-Cinnamoyl-4-hydroxy-1-phenylquinolin-2(1*H*)-one (2a):** This was prepared and purified as per the above mentioned procedure: IR (KBr, $cm^{-1}$): 3107.32(O-H stretch), 3076.46, 3024.38 (aromatic C-H stretch); 3003.17, 2927.94 (aliphatic-C-H str.); 1654.92 (C=O stretch); 1608.63 (C=O amide stretch); $^{1}H$ NMR ($CDCl_3$, δ ppm): 7.2-8.6 (m, 14H, Ar-*H*); 7.3 (d, 1H, C*H*β); 6.5 (d, 1H, C*H*α).

**3-[3-(4-chlorophenyl)acryloyl]-4-hydroxy-1-phenylquinolin-2(1*H*)-one (2b):** This was prepared and purified as per the above mentioned procedure: IR (KBr, $cm^{-1}$): 3101.54-O-H stretch), 3064.89, 3051.39 (aromatic C-H stretch); 2902.87, 2798.71 (aliphaticC-H str.); 1653.00 (C=O stretch); 1610.56 (-C=O amide stretch); 603.72 (-C-Cl)**;** $^{1}H$ NMR (DMSO-$d_6$, δ ppm) : 18.1 (s, 1H, -O*H* enol); 7.2-8.5 (m, 13H, Ar-*H*); 7.6 (d, 1H, C*H*β); 6.5 (d, 1H, C*H*α).

**4-Hydroxy-1-phenyl-3-[3-(4-nitrophenyl)acryloyl]quinolin-2(1*H*)-one (2c):** This was prepared and purified as per the above mentioned procedure:IR (KBr, $cm^{-1}$): 3095.75(-OH enolic), 3080.32, 3041.74 (aromatic -C-H); 2935.66, 2848.86 (aliphatic-C-H str.); 1651.07 (-C=O acetyl); 1612.49 (-C=O amide)**;** $^{1}H$ NMR (DMSO-$d_6$, δ ppm): 8.6 (d, 1H, CHβ); 8.2-7.2 (m, 13H, Ar-H); 6.5 (d, 1H, CHα).

**3-[3-(4-Hydroxyphenyl)acryloyl]-4-hydroxy-1-phenylquinolin-2(1*H*)-one (2d):** This was prepared and purified as per the above mentioned procedure:IR (KBr, $cm^{-1}$): 3205.69 (-OH enolic); 3124.68, 3066.82, 3014.74 (aromatic -C-H); 2951.09, 2806.43 (aliphatic-C-H str.); 1631.78 (-C=O acetyl); 1598.99 (-C=O amide); $^{1}H$ NMR (DMSO-$d_6$, δ ppm): 18.6 (s, 1H, -OH enol); 9.9 (s, 1H, -OH aromatic); 6.8- 8.4 (m, 13H, Ar-H); 7.9 (d, 1H, CHβ); 6.5 (d, 1H, CHα).

**4-Hydroxy-1-methyl-3-€-3-phenylacryloyl)quinolin-2(1H)-one (2e):** This was prepared and purified as per the above mentioned procedure:IR (KBr, $cm^{-1}$): 3109.25(-OH enolic), 3080.32, 3059.10 (aromatic -C-H); 3005.10, 2951.09 (aliphatic-C-H str.); 1654.00 (-C=O acetyl); 1624.06 (-C=O amide).*

**3-[3-(4-Chlorophenyl)acryloyl]-4-hydroxy-1-methylquinolin-2(1*H*)-one (2f)**: This was prepared and purified as per the above mentioned procedure:IR (KBr, $cm^{-1}$): 3055.24, 3032.10 (aromatic -C-H stretch); 2976.16, 2943.37 (aliphatic-C-H str.); 1647.21 (-C=O acetyl stretch); 1618.28 (-C=O amide stretch); $^{1}H$ NMR ($CDCl_3$, δ ppm); 17.8 (s, 1H, -O*H* enol); 8.6 (d, 1H, C*H*β); 7.2-8.2 (m, 13H, Ar-*H*); 7.8 (d, 1H, C*H*α); 3.6 (s, 3H, -N-C$H_3$).

**4-Hydroxy-1-methyl-3-[3-(4-nitrophenyl) acryloyl]quinolin-2(1*H*)-one (2g):** This was prepared and purified as per the above mentioned procedure:IR(KBr, $cm^{-1}$):3097.68(O-H stretch), 3072.60,

3055.24 (aromatic -C-H stretch); 2989.66, 2927.94, 2841.15 (aliphatic-C-H str.); 1649.14 (-C=O acetyl stretch); 1620.21 (-C=O amide stretch)**;** $^1$H NMR ($CDCl_3$, δ ppm): 17.46 (s, 1H, O-*H* enol); 7.2-8.2 (m, 13H, Ar-*H*); 7.8 (d, 1H, C*H*β); 7.2 (d, 1H, C*H*α); 3.6 (s, 3H,-N-C$H_3$)**.**

**3-[3-(4-hydroxyphenyl)acryloyl]-4-hydroxy-1-methylquinolin-2(1*H*)-one (2h):** This was prepared and purified as per the above mentioned procedure:IR (KBr, $cm^{-1}$): 3219.19(O-H stretch); 3113.11, 3014.74, 3078.39 (aromatic -C-H stretch); 2925.02, 2812.21 (aliphatic-C-H str.); 1639.49 (-C=O acetyl stretch); 1624.06 (-C=O amide stretch); $^1$H NMR (DMSO-$d_6$, δ ppm): 18.2 (s, 1H, -O*H* enol); 10.0 (s, 1H, –O*H* aromatic stretch); 8.5-6.8 (m, 13H, Ar-*H*); 7.8 (d, 1H, CHβ); 7.4 (d, 1H, CHα); 3.6 (s, 3H,-N-C$H_3$).

***Table 1:*** *Physicochemical data of 4-Hydroxy-1-phenyl (**2a-2d**)/methyl (**2e-2h**)-3-((E)-3-substituted phenylacryloyl) quinolin-2(1H)-one.*

| Compound | X | R | Molecular Formula | M.W. | M.P. °C | % Yield | *$R_f$ Value |
|---|---|---|---|---|---|---|---|
| 2a | -$C_6H_5$ | H | $C_{24}H_{17}NO_3$ | 367.39 | 292-94 | 54.59 | 0.69 |
| 2b | -$C_6H_5$ | 4-Cl | $C_{24}H_{16}ClNO_3$ | 401.84 | 264-66 | 50.00 | 0.78 |
| 2c | -$C_6H_5$ | 4-$NO_2$ | $C_{24}H_{16}N_2O_5$ | 412.39 | 254-56 | 56.25 | 0.69 |
| 2d | -$C_6H_5$ | OH | $C_{24}H_{17}NO_4$ | 383.39 | 280-82 | 46.15 | 0.32 |
| 2e | -$Ch_3$ | H | $C_{19}H_{15}NO_3$ | 305.32 | 170-72 | 50.80 | 0.60 |
| 2f | -$Ch_3$ | 4-Cl | $C_{19}H_{14}ClNO_3$ | 339.77 | 204-06 | 57.57 | 0.66 |
| 2g | -$Ch_3$ | 4-$NO_2$ | $C_{19}H_{14}N_2O_5$ | 350.32 | 246-50 | 67.73 | 0.50 |
| 2h | -$Ch_3$ | OH | $C_{19}H_{15}NO_4$ | 321.32 | 268-70 | 37.38 | 0.20 |

**TLC solvent system; n-Hexane: Ethyl acetate (7:3).*

**Synthesis of 3-[3-(furan-2-yl) prop-2-enoyl]-4-hydroxy-1-phenyl (4a)/methyl (4b) quinolin-2(1*H*)-one derivative:**

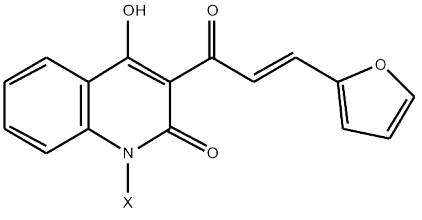
A mixture of1a/1b 0.01mol, furaldehyde 0.015mol and piperidine1mL in ethanol 5mL was refluxed for 8-10 hours and progress of reaction was monitored by TLC (n-hexane: ethyl acetate 7:3). Solution obtained was cooled and allowed to stand overnight and solid separated was filtered and recrystallized using suitable solvent.

**3-[3-(furan-2-yl) prop-2-enoyl]-4-hydroxy-1-phenylquinolin-2(1*H*)-one (4a):** This was prepared and purified as per the above mentioned procedure:IR (KBr, $cm^{-1}$):3107.32(-OH enolic), 3010.88 (aromatic -C-H); 2927.94, 2912.51 (aliphatic-C-H str.); 1654.92 (-C=O acetyl); 1608.63 (-C=O amide); 1068.56 (-C-O-C); $^1$H NMR (DMSO-$d_6$, δ ppm): 18.33 (s, 1H, -OH enol); 8.3-6.6 (m, 12H, Ar-H); 6.9 (d, 1H, CHβ); 6.4 (d, 1H, CHα).

Note: The excessive downfield shift in the enolic proton may be due to presence of keto group, and it may shift to 16 to 18 for all the respective derivatives.[11]

**3-[3-(furan-2-yl) prop-2-enoyl]-4-hydroxy-1-methylquinolin-2(1*H*)-one (4b):** This was prepared and purified as per the above mentioned procedure:IR (KBr, $cm^{-1}$): 3130.47(-OH enolic), 3109.25, 3039.81(aromatic -C-H); 2945.30, 2889.37 (aliphatic-C-H str.); 1633.71 (-C=O acetyl); 1610.56 (-C=O amide); 1068.56 (-C-O-C).

***Table 2:*** *Physicochemical data of 3-[3-(furan-2-yl) prop-2-enoyl]-4-hydroxy-1-phenyl (**4a**)/methyl (**4b**) quinolin-2(1H)-one derivative.*




X=Phenyl(**4a**))/Methyl(**4b**)

| Compound | R | Molecular formula | M.W. | M.P. °C | % Yield | $R_f$Value |
|---|---|---|---|---|---|---|
| 4a | -$C_6H_5$ | $C_{22}H_{15}NO_4$ | 357.35 | 282-86 | 52.63 | 0.695 |
| 4b | -$Ch_3$ | $C_{17}H_{13}NO_4$ | 295.28 | 166-68 | 44.3 | 0.541 |

**TLC solvent system; n-Hexane: Ethyl acetate (7:3).*

**General procedure for synthesis of 5-phenyl(3a-3d)/methyl(3e-3h)-2-[2-phenyl ethenyl][1,3]oxazolo[4,5-*c*]quinolin-4(5*H*)-one:**

Mixture of 0.5g of 2a-2h, 0.5g of hydroxylamine hydrochloride, 5mL of dimethylformamide and 5mL of pyridine was refluxed for 8-16 hours, progress of the reaction was monitored by TLC (ethyl acetate: n-hexane 1:1). Solution obtained was cooled and poured on to the crushed ice while stirring and allowed to stand overnight. Precipitate thus obtained was filtered and recrystallized using a suitable solvent.

**5-phenyl-2-[2-phenylethenyl][1,3]oxazolo[4,5-*c*]quinolin-4(5*H*)-one (3a):** This was prepared and purified as per the above mentioned procedure**:** IR (KBr, $cm^{-1}$): 3059.10, 3030.17 (aromatic -C-H); 2926.01, 2854.65 (aliphatic-C-H str.); 1683.36 (-C=N); 1637.56 (-C=O amide); $^1$H NMR (DMSO-$d_6$, δ ppm): 7.2-7.5 (m, 14H, Ar-H); 7.3 (d, 1H, CHβ); 6.6 (d, 1H, CHα).

**2-[2-(4-chlorophenyl)ethenyl]-5-phenyl[1,3]oxazolo[4,5-*c*]quinolin-4(5*H*)-one(3b):** This was prepared and purified as per the above mentioned procedure**:** IR (KBr, $cm^{-1}$): 3082.25, 3022.45 (aromatic -C-H); 2916.37, 2848.86 (aliphatic-C-H str.); 1681.93 (-C=N); 1633.71(-C=O amide); 748.38 (-C-Cl); $^1$H NMR ($CDCl_3$, δ ppm): 7.2-8.0 (m, 13H, Ar-H); 7.0 (d, 1H, CHβ); 6.7 (d, 1H, CHα). EI-MS (m/e, M+1): 399.

**2-[2-(4-nitrophenyl)ethenyl]-5-phenyl[1,3]oxazolo[4,5-*c*]quinolin-4(5*H*)-one (3c):** This was prepared and purified as per the above mentioned procedure**:** IR (KBr, $cm^{-1}$): 3064.89, 3099.61, 3041.74 (aromatic -C-H); 2933.73, 2846.93 (aliphatic-C-H str.); 1681.93 (-C=N); 1639.49 (-C=O amide).

**2-[2-(4-Hydroxyphenyl)ethenyl]-5-phenyl[1,3]oxazolo[4,5-*c*]quinolin-4(5*H*)-one(3d):**This was prepared and purified as per the above mentioned procedure**:** IR (KBr, $cm^{-1}$): 3219.19 (aromatic -OH); 3064.89, 3016.67(aromatic -C-H); 2953.02, 2814.14 (aliphatic-C-H str.); 1666.50 (-C=N); 1633.71(-C=O amide); $^1$H NMR (DMSO-$d_6$, δ ppm): 9.8 (s, 1H –OH aromatic); 6.6-8.3 (m, 13H, Ar-H); 7.7 (d, 1H, CHβ);7.0 (d, 1H, CHα); $^{13}$C NMR (DMSO-$d_6$, δ ppm): 162.61 (1C, C=O amide); 159.28 (1C, C=N); 116.57 -

156.81 (19C of aromatic Carbon); 115.76 (1C, C-3 of Quinolin-2-one); 110.51 (1C, CHβ); 108.75 (1C, CHα).

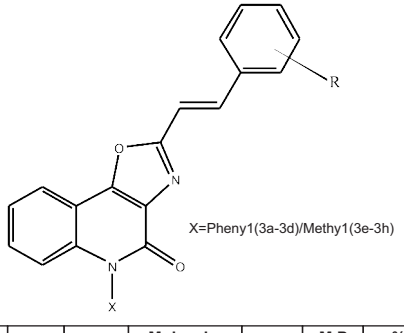
**5-methyl -2-[2-phenylethenyl][1,3]oxazolo[4,5-*c*]quinolin-4(5*H*)-one(3e):** This was prepared and purified as per the above mentioned procedure**:** IR (KBr, $cm^{-1}$): 3078.39, 3055.24, 3024.38 (aromatic -C-H); 2941.44, 2889.37 (aliphatic-C-H str.); 1672.28 (-C=N); 1635.64 (-C=O amide).

**2-[2-(4-chlorophenyl)ethenyl]-5-methyl [1,3]oxazolo[4,5-*c*]quinolin-4(5*H*)-one (3f):** This was prepared and purified as per the above mentioned procedure**:** IR (KBr, $cm^{-1}$): 3064.89, 3080.32, 3030.17 (aromatic -C-H); 2943.37, 2887.44 (aliphatic-C-H str.); 1674.21 (-C=N); 1635.64 (-C=O amide); 752.24 (-C-Cl).$^{13}$C NMR ($CDCl_3$, δ ppm): 161.92 (1C, C=N); 157.39 (1C, C=O amide); 121.64 -151.76 (13C of aromatic Carbon); 115.33 (1C, C-3 of Quinolin-2-one); 113.72 (1C, CHβ); 111.09 (1C, CHα); 29.71 (1C, -N-$CH_3$).

**2-[2-(4-nitrophenyl)ethenyl]-5-methyl [1,3]oxazolo[4,5-*c*]quinolin-4(5*H*)-one (3g):** This was prepared and purified as per the above mentioned procedure**:** IR (KBr, $cm^{-1}$): 3105.39, 3076.46, 3039.81 (aromatic -C-H); 2939.52, 2856.58 (aliphatic-C-H str.); 1635.64 (-C=O amide); 1678.07 (-C=N).

**2-[2-(4-hydroxyphenyl)ethenyl]-5-methyl [1,3]oxazolo[4,5-*c*]quinolin-4(5*H*)-one (3h):** This was prepared and purified as per the above mentioned procedure**:** IR (KBr, $cm^{-1}$): 3182.25 (aromatic -OH), 3018.60, 3066.82 (aromatic -C-H); 2947.23, 2893.22, 2816.07 (aliphatic -C-H str.); 1633.71(-C=O amide); 1662.64 (-C=N);$^{1}$H NMR (DMSO-$d_6$, δ ppm): 9.8 (s, 1H, -OH aromatic), 6.6-8.1 (m, 8H, Ar-H); 7.9 (d, 1H, CHβ); 7.4 (d,1H,CHα);3.7(s,3H,-N-$CH_3$).

***Table 3:*** *Physicochemical data of 5-phenyl (**3a-3d**)/methyl (**3e-3h**)-2-[2-phenyl ethenyl][1,3]oxazolo[4,5-c]quinolin-4(5H)-one derivatives.*

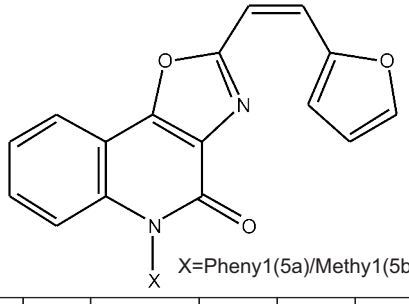



| Compound | X | R | Molecular formula | M.W. | M.P. °C | % Yield | $R_f$ Value |
|---|---|---|---|---|---|---|---|
| 3a | $-C_6H_5$ | H | $C_{24}H_{16}N_2O_2$ | 364.39 | 154-56 | 54.05 | 0.692 |
| 3b | $-C_6H_5$ | 4-Cl | $C_{24}H_{15}ClN_2O_2$ | 398.84 | 250-52 | 57.97 | 0.743 |
| 3c | $-C_6H_5$ | 4-$NO_3$ | $C_{24}H_{15}N_3O_4$ | 409.39 | 284-86 | 42.02 | 0.692 |
| 3d | $-C_6H_5$ | OH | $C_{24}H_{16}N_2O_3$ | 380.39 | 180-82 | 65.21 | 0.375 |
| 3e | $-CH_3$ | H | $C_{19}H_{14}N_2O_2$ | 302.32 | 118-20 | 56.56 | 0.414 |
| 3f | $-CH_3$ | 4-Cl | $C_{19}H_{13}ClN_2O_2$ | 336.77 | 150-54 | 69.56 | 0.50 |
| 3g | $-CH_3$ | 4-$NO_3$ | $C_{19}H_{13}N_3O_4$ | 347.32 | 280-82 | 75.36 | 0.341 |
| 3h | $-CH_3$ | OH | $C_{19}H_{14}N_2O_3$ | 318.32 | 190-94 | 73.91 | 0.56 |

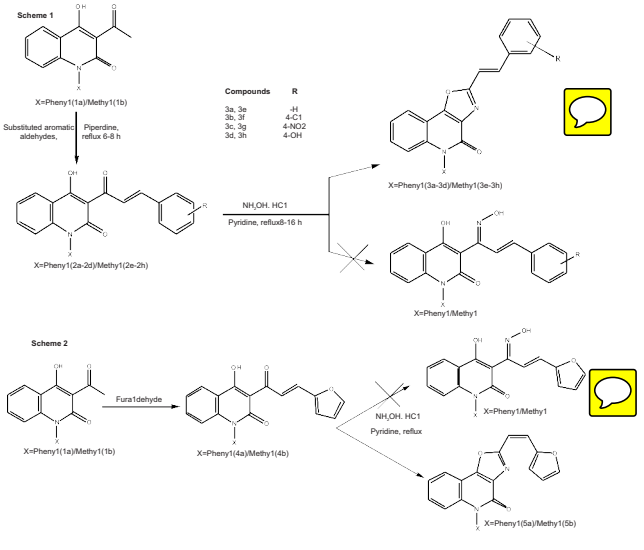
**General procedure for synthesis of 2-[2-(furan-2-yl)ethenyl]-5-phenyl (5a)/methyl (5b)-[1,3] oxazolo[4,5-*c*]quinolin-4(5*H*)-one:**
Mixture of 0.5g of 4a-4b, 0.5g of hydroxylamine hydrochloride, 5mL of dimethylformamide and 5mL of pyridine was refluxed for 8-16 hrs, progress of the reaction was monitored by TLC (ethyl acetate: n-hexane 1:1). Solution obtained was cooled and poured on to the crushed ice while stirring and allowed to stand overnight. Precipitate thus obtained was filtered and recrystallized using a suitable solvent.

**2-[2-(Furan-2-yl)ethenyl]-5-phenyl [1,3]oxazolo[4,5-*c*]quinolin-4(5*H*)-one (5a):**
IR (KBr, $cm^{-1}$):3064.89, 3014.74, (aromatic -C-H); 2929.87, 2860.43 (aliphatic-C-H str.); 1631.78 (C=N); 1608.63 (-C=O amide); 1070.49 (-C-O-C).

***Table 4:*** *Physicochemical data of 2-[2-(furan-2-yl)ethenyl]-5-phenyl (**5a**)/methyl (**5b**)-[1,3] oxazolo[4,5-c]quinolin-4(5H)-one derivative.*




| Compound | R | Molecular formula | M.W. | M.P. °C | % Yield | $R_f$ Value |
|---|---|---|---|---|---|---|
| 5a | $-C_6H_5$ | $C_{22}H_{14}N_2O_3$ | 354.35 | 244-46 | 59.18 | 0.675 |
| 5b | $-CH_3$ | $C_{17}H_{12}N_2O_3$ | 292.28 | 180-82 | 44.11 | 0.525 |




## *In Vitro* Experiments

The *in vitro* reactivation of OP-inhibited AChE using test compounds **3a-3h** & **5a-5b** was carried out in triplicate in phosphate buffer solution (0.1 M, pH 8.0 at 37ºC) using the method of Ellman.[13] Values depicted in figures are

average of triplicate runs with a standard deviation. A freshly prepared stock solution of Chlorpyrifos (1.4 X $10^{-3}$ M) and Methyl parathion (1.4 X $10^{-3}$ M) in isopropanol was stored under refrigeration. All test stock solutions were prepared in dimethylformamide. DTNB stock solution (0.01 M) was prepared in phosphate buffer solution (pH 8.0, 0.1 M). The substrate stock (acetylthiocholine iodide, 0.075 M) was prepared in distilled water. The incubation mixture was prepared by the addition of 50 µL of OP compounds (1.4 X $10^{-3}$M) to a mixture of 50 µL enzyme in 350 µL phosphate buffer solution (0.1 M, pH 8.0). The mixture was allowed to stand for 15 min at ambient temperature to give 96 ± 1% inhibition of enzyme activity. No further increase in the inhibition of enzyme activity was observed even after 1 h of the incubation with Chlorpyrifos and Methyl parathion at this concentration. It was then followed by addition of 50 µL of test solution (0.001 M) to start reactivation. The final volume of the reactivation cocktail was 500 µL. After 10 min of reactivation, the enzyme activity was assayed by Ellman's method.

Twenty microliters of reactivation cocktail was transferred to a cuvette containing 50 µL DTNB in phosphate buffer solution (pH 8.0, 0.1 M). The enzyme activity was then assayed by addition of 50 µL of substrate to the cuvette against a blank containing reactivation cocktail without substrate. The final volume of the assay mixture was adjusted to 3 mL. The reactivation of inhibited enzyme was then studied at an interval of 10 min and followed up to 1 h.

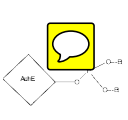
Percentage reactivation was calculated using the following equation:

$$\% \text{ Reaction} = \frac{Er - Ei}{Eo - Ei} \times 100$$

where, Eo is the control enzyme activity at 0 min (without inhibitor and reactivator), Ei is the inhibited enzyme activity (without reactivator) determined in the similar manner as described above, and Er is the activity of reactivated enzyme after incubation with the test compounds.

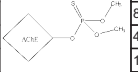
### Observation

***Table 5:*** *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 to 60 min; pH-8.0;25º C).*

| Compounds | Resulting Enzyme-inhibitor complex | Reactivation potency [%]±SD (0.001 M) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 10 min | 20 min | 30 min | 40 min | 50 min | 60 min |
| 3a |  | 2.3±0.4 | 8.1±0.8 | 9.3±0.2 | 8.6±0.7 | 7.4±0.5 | 8.6±0.9 |
| 3b | | 18.6±0.8 | 23.1±0.8 | 28.6±0.9 | 34.3±0.6 | 42.8±1.1 | 34.1±1.2 |
| 3c | | 14.4±1.2 | 19.3±1.5 | 28.6±1.1 | 31.0±1.2 | 33.6±1.8 | 34.2±1.5 |
| 3d | | 9.6±0.7 | 12.2±1.3 | 17.3±1.9 | 21.0±1.3 | 18.2±1.2 | 16.3±1.4 |
| 3e | | 13.9±0.6 | 21.0±0.5 | 28.4±1.2 | 26.1±1.5 | 22.4±1.4 | 21.3±0.9 |
| 3f | | 27.7±1.3 | 33.7±1.6 | 41.3±1.6 | 18.7±1.2 | 53.4±1.5 | 41.8±0.6 |
| 3g | | 12.9±0.5 | 24.1±1.6 | 29.5±1.0 | 37.7±1.5 | 41.6±1.3 | 44.0±0.9 |
| 3h | | 6.4±1.1 | 10.5±1.3 | 12.6±1.1 | 19.6±1.4 | 17.0±1.5 | 14.6±1.5 |
| 5a | | 10.9±0.5 | 15.3±0.9 | 21.1±1.6 | 27.3±1.4 | 36.5±1.4 | 43.2±1.8 |
| 5b | | 10.4±0.5 | 19.2±1.1 | 24.6±1.2 | 29.2±1.4 | 37.5±1.2 | 32.1±1.2 |
| 2-PAM | | 24.6±1.7 | 35.9±1.7 | 43.2±1.4 | 47.9±1.5 | 56.3±1.0 | 68.2±1.2 |

*The values are average of three runs with Standard Deviation (SD).*

***Table 6:*** *Reactivation of Methyl parathion-inhibited AchE by test compounds (Source of the enzyme: rat brain AChE; time of inhibition by OP-15 min; time of reactivation; 10 to 60 min; pH-8.0; 25º C).*

| Compounds | Resulting Enzyme-inhibitor complex | Reactivation potency [%]±SD (0.001 M) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 10 min | 20 min | 30 min | 40 min | 50 min | 60 min |
| 3a |  | ---- | 4.8±0.3 | 4.3±0.5 | 5.8±0.4 | 4.3±0.3 | 3.2±0.3 |
| 3b | | 7.6±0.4 | 17.6±0.3 | 25.6±0.3 | 29.5±0.3 | 34.1±0.2 | 37.0±0.5 |
| 3c | | 2.8±0.3 | 3.74±0.6 | 7.6±0.1 | 11.2±0.3 | 37.3±0.4 | 36.5±1.2 |
| 3d | | 8.5±0.3 | 14.1±0.5 | 17.8±0.4 | 16.0±0.3 | 12.0±0.2 | 9.6±0.3 |
| 3e | | 4.3±0.6 | 8.3±0.5 | 12.3±0.3 | 16.0±0.3 | 14.4±0.1 | 12.9±0.5 |
| 3f | | 18.2±1.1 | 26.3±0.6 | 35.7±0.8 | 41.7±0.5 | 46.7±0.3 | 49.2±0.3 |
| 3g | | 21.7±0.6 | 32.4±0.5 | 27.8±0.4 | 49.8±0.8 | 54.4±0.4 | 58.4±0.4 |
| 3h | | 4.1±0.2 | 9.7±0.6 | 15.2±0.3 | 18.3±0.2 | 17.2±0.4 | 15.2±0.6 |
| 5a | | 8.3±0.2 | 6.7±0.5 | 20.7±0.6 | 23.2±0.6 | 28.0±0.6 | 34.2±0.7 |
| 5b | | 9.2±0.2 | 13.3±1.4 | 22.1±0.4 | 25.6±0.3 | 22.6±0.4 | 23.0±0.5 |
| 2-PAM | | 23.7±0.6 | 37.6±0.3 | 45.7±0.6 | 59.2±0.5 | 67.4±0.3 | 73.6±0.6 |

## Results and Discussions

4-Hydroxy-6-methyl/phenyl-2H-pyrano[3,2-c]quinoline-2,5(6*H*)-dione, were synthesized according to literature procedure[11] and subjected to hydrolysis to yield **1a** and **1b.** The acetyl group of compound **1a-1b** was treated with various substituted aromatic aldehydes to form respective chalcone **2a-2h, 4a** and **4b**.Physicochemical data of the respective chalcone is summarized in **Table 1** and **Table 2**. The titled compounds were obtained by refluxing the **2a-2h** with hydroxylamine hydrochloride in presence of pyridine to yield **3a-3h, 5a** and **5b**.The synthesis of compounds **3a-3h, 5a** and **5b** is represented in Scheme 1 and Scheme 2, respectively.Physicochemical data of the final derivatives are abridged in **Table 3** and **Table 4,** respectively.

The compounds **3a-3h, 5a** and **5b** were assayed for their *in vitro* reactivation efficacy against OP inhibited AChE. All results obtained are summarized in **Table 5** (Chlorpyrifos) and **Table 6** (Methyl parathion), respectively.As resulted, 2-PAM was the most potent reactivator in the treatment of OP-inhibited AChE at concentration tested (0.001 M). In this case, the compounds **3b** (42.8±1.1%, 50 min), **3c** (34.2±1.2%, 60 min), **3f** (53.4±1.5%, 50 min), **3g** (44.0±0.9%, 60 min), **5a** (43.2±1.8%, 60 min), and **5b** (37.5±1.2%, 50 min) achieved promising reactivation as compared to 2-PAM (56.3±1.0%, 50 min and 68.2±1.2%, 60min) against chlorpyrifos inhibited AChE.

The 2-quinolone fused oxazole derivatives **3b** (37.0±0.5%, 60 min), **3c** (37.3±0.4%, 50 min), **3f** (49.2±0.3%, 60 min), **3g** (58.4±0.4%, 60 min), and **5a** (34.2±0.7%, 60 min)showed promising reactivation formethyl parathion inhibited AChEas compared to standard (67.4±0.3%, 50 min and 73.6±0.6%, 60 min).

## CONCLUSION

Reactivation of OP inhibited acetylcholinesterase was performed by Ellman et al method. All the synthesized compounds were found to be good reactivator of inhibited enzyme. Compounds having nitro and chloro substitution at 4$^{th}$ position gave good activity against both OP inhibited AChE. Among the synthesized compound, compound **3b**, **3f**, **3g**, and **5a** have shown promising activity as compared to standard against

chlorpyrifos inhibited AChE. However, **3f** and **3g** showed good activity as compared to standard against methyl parathion inhibited AChE. Moreover, these quinolone fused oxazole seem to be very promising because of their sufficient reactivation potency at lower concentration ($10^{-3}$ M).

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