

## Research Article

# Synthesis and Biological Evaluation of Novel Piperazine Containing Hydrazone Derivatives

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Some hydrazone derivatives were synthesized and their potential anticholinesterase activities were examined. A series of eleven new compounds of N'-(2,4-disubstitutedbenzylidene)-2-(4-(4-nitrophenyl)piperazin-1-yl)acetohydrazide derivatives were obtained via reaction of 2-[4-(4-nitrophenyl)piperazin-1-yl]acetohydrazide with aromatic aldehydes. The chemical structures of the compounds were enlightened by FT-IR, <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, and HRMS (ESI) spectral data. The inhibition potency of the compounds **3a-k** against AChE and BuChE was measured and evaluated using a modification of Ellman's spectrophotometric method. Among the tested compounds, compound **3c** was assigned to be the most active derivative. Galantamine was used as a standard drug.

## 1. Introduction

Alzheimer's disease (AD) is a chronic neurodegenerative and progressive disease responsible from 60% to 70% of cases of dementia [1] and characterized by progressive impairment of memory and cognition [2]. The primary lesions of AD are neurofibrillary tangles (NFT) and the senile plaques that is composed of central core of beta-amyloid [3, 4]. As the destruction of cholinergic neurons ends up with the decline in acetylcholine (ACh) level, a key aspect of the symptomatic therapy for AD is to increase acetylcholine concentration in presynaptic regions via blocking its metabolic enzyme acetylcholinesterase (AChE). In humans, cholinesterase is classified into two types: acetylcholinesterase (AChE) that hydrolyses acetylcholine selectively and more quickly and butyrylcholinesterase (BuChE) that metabolize butyrylcholine more quickly [5, 6]. The therapeutic approaches to date have tended to focus on AChE rather than BuChE that is considered to play a minor role in regulating ACh levels in brain. At the present time, reversible acetylcholinesterase inhibitors (AChEIs) like donepezil, rivastigmine, and galantamine are in clinical use in the therapy of AD as well as the N-methyl-D-aspartate-receptor (NMDA) antagonist memantine [7, 8].

Reversible AChE inhibitors perform a significant role in pharmacological manipulation of the enzyme activity [9]. Despite their efficacy, reversible AChE inhibitors suffer from several major drawbacks such as hepatotoxicity, short half life, and gastrointestinal tract excitement [10]. For medicinal chemists, hence, it has been an increasingly important area to discover novel and efficient AChE inhibitors with minimum side effects.

The past decades have seen increasingly rapid advances in the field of developing acetylcholinesterase inhibitors. Hydrazones, thus, have attracted great attention in the light of recent studies that reported compounds bearing hydrazone moiety exhibit various biological activities including acetylcholinesterase activity [11–18]. On the other hand, piperazine ring is known to act as bioisosteric replacement for piperidine ring that exist in donepezil and have also been revealed to have acetylcholinesterase activity [19–21]. Based on these observations, we designed a new series of piperazine containing hydrazone derivatives (Figure 1). This combination of studies provides support for designing novel and efficient agents for treatment of AD.

In view of the above-mentioned findings, herein, we reported the synthesis of and acetylcholinesterase

and butyrylcholinesterase inhibitory activity of *N'*-(2,4-disubstitutedbenzylidene)-2-(4-(4-nitrophenyl)piperazin-1-yl)acetohydrazide derivatives that may be of value in designing acetylcholinesterase inhibitors. The inhibitory potency of compounds was studied in comparison with the known AChE inhibitor drug galantamine.

## 2. Experimental

**2.1. Materials.** All chemicals were purchased from Sigma-Aldrich Chemical Co. (Sigma-Aldrich Corp., St. Louis, MO, USA) and Merck Chemicals (Merck KGaA, Darmstadt, Germany). All melting points (mp) were determined by Electrothermal 9100 digital melting point apparatus (Electrothermal, Essex, UK) and were uncorrected. All the reactions were monitored by thin-layer chromatography (TLC) using Silica Gel 60 F254 TLC plates (Merck KGaA, Darmstadt, Germany). Spectroscopic data were recorded with the following instruments: IR, Shimadzu 8400S spectrophotometer (Shimadzu, Tokyo, Japan); NMR, Bruker DPX 500 NMR spectrometer (Bruker Bioscience, Billerica, MA, USA), in DMSO-*d*<sub>6</sub>, using TMS as internal standard; M + 1 peaks were determined by LC-MS-IT-TOF system (Shimadzu, Tokyo, Japan).

**2.2. General Procedure for Ethyl 2-[4-(4-nitrophenyl)piperazin-1-yl]acetate (1).** 1-(4-Nitrophenyl)piperazine (0.015 mol) was dissolved in acetone (250 mL). Potassium carbonate (0.018 mol) was added. After addition of ethyl 2-chloroacetate (0.015 mol), the reaction mixture was stirred for 4 hours at room temperature. The solvent was evaporated under reduced pressure and then water was added to wash the resulting solid and the mixture was filtered to give compound **1**.

**2.3. General Procedure for 2-[4-(4-Nitrophenyl)piperazin-1-yl]acetohydrazide (2).** Ethyl 2-[4-(4-nitrophenyl)piperazin-1-yl]acetate (0.013 mol) was dissolved in ethanol (250 mL). Hydrazine hydrate (0.013 mol) dissolved in ethanol was added gradually and the mixture was stirred at room temperature. After completion of the reaction, the solvent was evaporated under reduced pressure; then water was added to wash the resulting solid and the mixture was filtered, dried, and recrystallized from ethanol to give compound **2**.

**2.4. General Procedure for *N'*-(2,4-Disubstitutedbenzylidene)-2-(4-(4-nitrophenyl)piperazin-1-yl)acetohydrazide Derivatives (3a-k).** 2-[4-(4-Nitrophenyl)piperazin-1-yl]acetohydrazide (0.7 mmol) was dissolved in ethanol (100 mL). After addition of acetic acid (1 mL) and appropriate aldehydes (0.7 mmol), the reaction mixture was refluxed for 8 hours. After TLC screening, at the end of this period, the reaction mixture was cooled, filtered, and recrystallized from ethanol to give the target compounds **3a-k**.

**2.4.1. *N'*-(4-Fluorobenzylidene)-2-(4-(4-nitrophenyl)piperazin-1-yl)acetohydrazide (3a).** IR (KBr)  $\nu_{\max}$  (cm<sup>-1</sup>): 3259 (N-H), 3066 (Aromatic C-H), 2885 (Aliphatic C-H), 1695

(C=O), 1597 (C=N), 1581 and 1305 (NO<sub>2</sub>), 1473 (C=C), 1230 (C-N), 1143, 1076 and 827 (C-H out of plane deformation).

<sup>1</sup>H-NMR (400 Mhz, DMSO-*d*<sub>6</sub>, ppm)  $\delta$  2.61 (2H, t, *J* = 4.4 Hz, piperazine protons), 2.70 (2H, t, *J* = 4.4 Hz, piperazine protons), 3.15 and 3.61 (2H, two s, CO-CH<sub>2</sub>), 3.44–3.49 (4H, m, piperazine protons), 6.97 (2H, dd, *J*<sub>1</sub> = 9.2 Hz, *J*<sub>2</sub> = 4 Hz, phenyl protons), 7.20–7.27 (2H, m, phenyl photons), 7.66–7.73 (2H, m, phenyl protons), 7.93 and 8.31 (1H, two s, N=CH), 8.01 (2H, dd, *J*<sub>1</sub> = 9.2 Hz, *J*<sub>2</sub> = 3.6 Hz, phenyl protons), 11.23 and 11.32 (1H, two s, NH).

<sup>13</sup>C-NMR (100 Mhz, DMSO-*d*<sub>6</sub>, ppm)  $\delta$  46.17 and 46.37 (2CH<sub>2</sub>), 52.03 and 52.19 (2CH<sub>2</sub>), 56.85 and 60.07 (COCH<sub>2</sub>), 112.47 and 112.55 (2CH), 115.68 and 115.90 (2CH), 125.63 (2CH), 128.75 and 128.83 (CH), 129.07 and 129.16 (CH), 130.71, 130.73 and 130.80, 130.83 (C), 136.72 and 136.83 (C), 141.69 and 146.04 (C=N), 154.64 (C), 161.60, 161.79 and 164.07, 164.25 (C-F), 165.45 and 170.63 (C=O).

HRMS (*m/z*): [M + H]<sup>+</sup> calcd for C<sub>19</sub>H<sub>20</sub>FN<sub>5</sub>O<sub>3</sub>: 386.1623; found 386.1618.

**2.4.2. *N'*-(4-Methoxybenzylidene)-2-(4-(4-nitrophenyl)piperazin-1-yl)acetohydrazide (3b).** IR (KBr)  $\nu_{\max}$  (cm<sup>-1</sup>): 3271 (N-H), 3070 (Aromatic C-H), 2835 (Aliphatic C-H), 1681 (C=O), 1581 (C=N), 1504 and 1303 (NO<sub>2</sub>), 1471 (C=C), 1240 (C-N), 1207–1078 (C-O), 1078 and 825 (C-H out of plane deformation).

<sup>1</sup>H-NMR (400 Mhz, DMSO-*d*<sub>6</sub>, ppm)  $\delta$  2.64 (2H, t, *J* = 4.8 Hz, piperazine protons), 2.73 (2H, t, *J* = 4.8 Hz, piperazine protons), 3.17 and 3.64 (2H, 2s, CO-CH<sub>2</sub>), 3.48 (2H, t, *J* = 4.8 Hz, piperazine protons), 3.52 (2H, t, *J* = 4.8 Hz, piperazine protons), 3.80 (3H, d, *J* = 3.6 Hz, OCH<sub>3</sub>), 6.98–7.04 (4H, m, phenyl protons), 7.59–7.65 (2H, m, phenyl protons), 7.92 and 8.28 (1H, two s, N=CH), 8.05 (2H, dd, *J*<sub>1</sub> = 9.2 Hz, *J*<sub>2</sub> = 3.6 Hz, phenyl protons), 11.12 and 11.22 (1H, two s, NH).

<sup>13</sup>C-NMR (100 Mhz, DMSO-*d*<sub>6</sub>, ppm)  $\delta$  46.17 and 46.36 (2CH<sub>2</sub>), 52.03 and 52.18 (2CH<sub>2</sub>), 55.19 (OCH<sub>3</sub>), 56.86 and 60.08 (COCH<sub>2</sub>), 112.45 and 112.54 (2CH), 114.22 (2CH), 125.63 (2CH), 126.70 and 126.73 (C), 128.20 and 128.55 (2CH), 136.69 and 136.81 (C), 142.71 and 147.04 (C=N), 154.60 and 154.65 (C), 160.49 and 160.71 (C), 165.13 and 170.37 (C=O).

HRMS (*m/z*): [M + H]<sup>+</sup> calcd for C<sub>20</sub>H<sub>23</sub>N<sub>5</sub>O<sub>4</sub>: 398.1823; found 398.1838.

**2.4.3. *N'*-(4-Hydroxybenzylidene)-2-(4-(4-nitrophenyl)piperazin-1-yl)acetohydrazide (3c).** IR (KBr)  $\nu_{\max}$  (cm<sup>-1</sup>): 3250 (N-H), 3115.04 (Aromatic C-H), 2823 (Aliphatic C-H), 1651 (C=O), 1595 and 1305 (NO<sub>2</sub>), 1504 (C=N), 1473 (C=C), 1238 (C-N), 1116 and 823 (C-H out of plane deformation).

<sup>1</sup>H-NMR (400 Mhz, DMSO-*d*<sub>6</sub>, ppm)  $\delta$  2.64 (2H, t, *J* = 4.4 Hz, piperazine protons), 2.71–2.76 (2H, m, piperazine protons), 3.15 and 3.62 (2H, two s, CO-CH<sub>2</sub>), 3.48–3.52 (4H, m, piperazine protons), 6.80–6.85 (2H, m, phenyl protons), 7.00–7.05 (2H, m, phenyl protons), 7.35–7.60 (2H, m, phenyl protons), 7.84 and 8.22 (1H, two s, N=CH), 8.05 (2H, dd, *J*<sub>1</sub> = 9.6 Hz, *J*<sub>2</sub> = 2.4 Hz, phenyl-H), 9.85 (1H, br s, OH), 11.04 and 11.14 (1H, two s, NH).

<sup>13</sup>C-NMR (100 Mhz, DMSO-*d*<sub>6</sub>, ppm)  $\delta$  46.16 and 46.35 (2CH<sub>2</sub>), 52.03 and 52.16 (2CH<sub>2</sub>), 56.85 and 60.07 (COCH<sub>2</sub>),

112.44 and 112.53 (2CH), 115.59 (2CH), 125.10 and 125.13 (C), 125.63 (2CH), 128.33 and 128.70 (2CH), 136.68 and 136.79 (C), 143.15 and 147.45 (C=N), 154.60 and 154.65 (C), 159.05 and 159.29 (C), 165.00 and 170.24 (C=O).

HRMS ( $m/z$ ):  $[M + H]^+$  calcd for  $C_{19}H_{21}N_5O_4$ : 384.1666; found 384.1650.

2.4.4.  $N'$ -(4-Ethoxybenzylidene)-2-(4-(4-nitrophenyl)piperazin-1-yl)acetohydrazide (**3d**). IR (KBr)  $\nu_{\max}$  ( $\text{cm}^{-1}$ ): 3253 (N-H), 3069 (Aromatic C-H), 2976 (Aliphatic C-H), 1693 (C=O), 1598.99 (C=N), 1585 and 1305 ( $\text{NO}_2$ ), 1477 (C=C), 1240 (C-N), 1207–1041 (C-O), 829 (C-H out of plane deformation).

$^1\text{H-NMR}$  (400 Mhz, DMSO- $d_6$ , ppm)  $\delta$  1.31 (3H, t,  $J$  = 7 Hz,  $\text{CH}_3$ ), 2.61 (2H, t,  $J$  = 4.4 Hz, piperazine protons), 2.70 (2H, t,  $J$  = 4.8 Hz, piperazine protons), 3.12 and 3.59 (2H, 2s, CO- $\text{CH}_2$ ), 3.27 (1H, s, piperazine proton), 3.42–3.60 (3H, m, piperazine protons), 4.00–4.06 (2H, m,  $\text{OCH}_2$ ), 6.92–7.04 (4H, m, phenyl protons), 7.54–7.62 (2H, m, phenyl protons), 7.88 and 8.24 (1H, two s, N=CH), 8.03 (2H, dd,  $J_1$  = 9.6 Hz,  $J_2$  = 3.2 Hz, phenyl protons), 11.03 and 11.13 (1H, two s, NH).

$^{13}\text{C-NMR}$  (100 Mhz, DMSO- $d_6$ , ppm)  $\delta$  14.47 ( $\text{CH}_3$ ), 46.21 and 46.40 (2 $\text{CH}_2$ ), 52.02 and 52.17 (2 $\text{CH}_2$ ), 56.84 and 60.07 (CO $\text{CH}_2$ ), 63.17 ( $\text{OCH}_2$ ), 112.49 and 112.57 (2CH), 114.65 (2CH), 125.60 (2CH), 126.61 (C), 128.18 and 128.52 (2CH), 136.74 and 136.85 (C), 142.73 and 147.08 (C=N), 154.62 (C), 159.79 and 160.01 (C), 165.08 and 170.33 (C=O).

HRMS ( $m/z$ ):  $[M + H]^+$  calcd for  $C_{21}H_{25}N_5O_4$ : 412.1979; found 412.1971.

2.4.5.  $N'$ -(2,4-Dichlorobenzylidene)-2-(4-(4-nitrophenyl)piperazin-1-yl)acetohydrazide (**3e**). IR (KBr)  $\nu_{\max}$  ( $\text{cm}^{-1}$ ): 3259 (N-H), 3093 (Aromatic C-H), 2862 (Aliphatic C-H), 1683 (C=O), 1597 and 1303 ( $\text{NO}_2$ ), 1508 (C=N), 1490 (C=C), 1232 (C-N), 1099 and 821 (C-H out of plane deformation).

$^1\text{H-NMR}$  (400 Mhz, DMSO- $d_6$ , ppm)  $\delta$  2.62 (2H, t,  $J$  = 4.4 Hz, piperazine protons), 2.64–2.67 (2H, m, piperazine protons), 3.17 and 3.63 (2H, two s, CO- $\text{CH}_2$ ), 3.27 (1H, s, piperazine proton), 3.42–3.52 (3H, m, piperazine protons), 6.99 (2H, dd,  $J_1$  = 9.6 Hz,  $J_2$  = 4 Hz, phenyl protons), 7.42–7.48 (1H, m, phenyl proton), 7.62–7.66 (1H, m, phenyl proton), 7.92 (1H, t,  $J$  = 8.4 Hz, phenyl proton), 8.00–8.05 (2H, m, phenyl protons), 8.27 and 8.65 (1H, 2s, N=CH), 11.50 and 11.53 (1H, two s, NH).

$^{13}\text{C-NMR}$  (100 Mhz, DMSO- $d_6$ , ppm)  $\delta$  46.18 and 46.41 (2 $\text{CH}_2$ ), 51.97 and 52.17 (2 $\text{CH}_2$ ), 56.79 and 60.12 (CO $\text{CH}_2$ ), 112.50 and 112.57 (2CH), 125.60 (2CH), 127.86 (C), 128.00 (C), 129.25 (C), 130.48 and 130.67 (C), 133.44 and 133.71 (C), 134.66 and 134.93 (C), 136.77 and 136.87 (C), 137.87 and 141.90 (CH), 154.61 (C), 165.79 and 170.79 (C).

HRMS ( $m/z$ ):  $[M + H]^+$  calcd for  $C_{19}H_{19}Cl_2N_5O_3$ : 436.0938; found 436.0928.

2.4.6.  $N'$ -(4-Cyanobenzylidene)-2-(4-(4-nitrophenyl)piperazin-1-yl)acetohydrazide (**3f**). IR (KBr)  $\nu_{\max}$  ( $\text{cm}^{-1}$ ): 3261 (N-H), 3065 (Aromatic C-H), 2833 (Aliphatic C-H), 2223 (C $\equiv$ N), 1683 (C=O), 1597 and 1305 ( $\text{NO}_2$ ), 1508 (C=N), 1471 (C=C), 1240 (C-N), 1087 and 825 (C-H out of plane deformation).

$^1\text{H-NMR}$  (400 Mhz, DMSO- $d_6$ , ppm)  $\delta$  2.66 (2H, t,  $J$  = 4.8 Hz, piperazine protons), 2.74 (2H, t,  $J$  = 4.8 Hz, piperazine protons), 3.22 and 3.68 (2H, two s, CO- $\text{CH}_2$ ), 3.37 (1H, s, piperazine proton), 3.49–3.58 (3H, m, piperazine protons), 7.03 (2H, dd,  $J_1$  = 9.2 Hz,  $J_2$  = 4 Hz, phenyl protons), 7.82–7.92 (4H, m, phenyl protons), 8.01 and 8.40 (1H, two s, N=CH), 8.05 (2H, dd,  $J_1$  = 9.2 Hz,  $J_2$  = 3.2 Hz, phenyl protons), 11.51 and 11.59 (1H, two s, NH).

$^{13}\text{C-NMR}$  (100 Mhz, DMSO- $d_6$ , ppm)  $\delta$  46.15 and 46.35 (2 $\text{CH}_2$ ), 51.98 and 52.15 (2 $\text{CH}_2$ ), 56.80 and 60.02 (CO $\text{CH}_2$ ), 111.50 and 111.78 (CN), 112.47 and 112.54 (2CH), 118.56 and 118.61 (C), 125.62 (2CH), 127.21 and 127.48 (2CH), 132.62 (2CH), 136.71 and 136.82 (C), 138.54 and 138.70 (C), 140.89 and 145.14 (C=N), 154.58 and 154.64 (C), 165.84 and 170.95 (C=O). HRMS ( $m/z$ ):  $[M + H]^+$  calcd for  $C_{20}H_{20}N_6O_3$ : 393.1670; found 393.1654.

2.4.7. 2-(4-(4-Nitrophenyl)piperazin-1-yl)- $N'$ -(4-(trifluoromethyl)benzylidene)acetohydrazide (**3g**). IR (KBr)  $\nu_{\max}$  ( $\text{cm}^{-1}$ ): 3267 (N-H), 3035 (Aromatic C-H), 2835 (Aliphatic C-H), 1683 (C=O), 1597 and 1303 ( $\text{NO}_2$ ), 1506 (C=N), 1473 (C=C), 1242 (C-N), 1116, 1083 and 825 (C-H out of plane deformation).

$^1\text{H-NMR}$  (400 Mhz, DMSO- $d_6$ , ppm)  $\delta$  2.67 (2H, t,  $J$  = 4.8 Hz, piperazine protons), 2.75 (2H, t,  $J$  = 4.4 Hz, piperazine protons), 3.22 and 3.69 (2H, two s, CO- $\text{CH}_2$ ), 3.38 (1H, s, piperazine proton), 3.46–3.56 (3H, m, piperazine protons), 7.01–7.06 (2H, m, phenyl protons), 7.79 (2H, t,  $J$  = 8.4 Hz, phenyl protons), 7.86–8.09 (2H, m, phenyl protons), 8.06 (2H, dd,  $J_1$  = 9.2 Hz,  $J_2$  = 3.6 Hz, phenyl protons), 8.43 (1H, s, C=N), 11.47 and 11.56 (1H, two s, NH).

$^{13}\text{C-NMR}$  (100 Mhz, DMSO- $d_6$ , ppm)  $\delta$  46.19 and 46.40 (2 $\text{CH}_2$ ), 52.02 and 52.21 (2 $\text{CH}_2$ ), 56.86 and 60.08 (CO $\text{CH}_2$ ), 112.51 and 112.59 (2CH), 122.69 ( $\text{CF}_3$ ), 125.40 and 125.60 (2CH), 125.65 (2CH), 127.24 and 127.55 (2CH), 129.22, 129.45, 129.53, 129.77 (C), 136.76 and 136.87 (C), 138.09 and 138.25 (C), 141.20 and 145.41 (C=N), 154.64 and 154.69 (C), 165.81 and 170.91 (C=O).

HRMS ( $m/z$ ):  $[M + H]^+$  calcd for  $C_{20}H_{20}F_3N_5O_3$ : 436.1591; found 436.1577.

2.4.8.  $N'$ -(4-(Dimethylamino)benzylidene)-2-(4-(4-nitrophenyl)piperazin-1-yl)acetohydrazide (**3h**). IR (KBr)  $\nu_{\max}$  ( $\text{cm}^{-1}$ ): 3250 (N-H), 3078 (Aromatic C-H), 2962 (Aliphatic C-H), 1681 (C=O), 1583 and 1315 ( $\text{NO}_2$ ), 1595 (C=N), 1477 (C=C), 1232 (C-N), 1114, 1093 and 827 (C-H out of plane deformation).

$^1\text{H-NMR}$  (400 Mhz, DMSO- $d_6$ , ppm)  $\delta$  1.21 (6H, dd,  $J_1$  = 7.2 Hz,  $J_2$  = 2.8 Hz,  $\text{N}(\text{CH}_3)_2$ ), 2.65 (2H, t,  $J$  = 4.8 Hz, piperazine protons), 2.74 (2H, t,  $J$  = 4.8 Hz, piperazine protons), 3.18 and 3.65 (2H, two s, CO- $\text{CH}_2$ ), 3.39 (1H, s, piperazine proton), 3.44–3.56 (3H, m, piperazine protons), 7.00–7.05 (2H, m, phenyl protons), 7.30 (2H, t,  $J$  = 8.2 Hz, phenyl protons), 7.58 (1H, d,  $J$  = 8 Hz, phenyl protons), 7.62 (1H, d,  $J$  = 8 Hz, phenyl proton), 7.96 and 8.32 (1H, two s, N=CH), 8.05 (2H, dd,  $J_1$  = 9.2 Hz,  $J_2$  = 3.6 Hz, phenyl protons), 11.21 and 11.31 (1H, two s, NH).

$^{13}\text{C}$ -NMR (100 Mhz, DMSO- $d_6$ , ppm)  $\delta$  23.56 and 33.29 (2CH<sub>3</sub>), 46.18 and 46.38 (2CH<sub>2</sub>), 52.03 and 52.20 (2CH<sub>2</sub>), 56.87 and 60.09 (COCH<sub>2</sub>), 112.47 and 112.55 (2CH), 125.63 (2CH), 126.69 and 126.73 (2CH), 127.07 (C), 131.81 and 131.89 (2CH), 136.71 and 136.83 (C), 142.91 and 147.20 (C=N), 150.26 and 150.54 (C), 154.61 and 154.66 (C), 165.33 and 170.51 (C).

HRMS ( $m/z$ ): [M + H]<sup>+</sup> calcd for C<sub>22</sub>H<sub>28</sub>N<sub>6</sub>O<sub>3</sub>: 424.4961; found 424.4967.

2.4.9. *N'*-(4-(Diethylamino)benzylidene)-2-(4-(4-nitrophenyl)piperazin-1-yl)acetohydrazide (**3i**). IR (KBr)  $\nu_{\text{max}}$  (cm<sup>-1</sup>): 3203 (N-H), 3049 (Aromatic C-H), 2985 (Aliphatic C-H), 1660 (C=O), 1587 and 1315 (NO<sub>2</sub>), 1548 (C=N), 1494 (C=C), 1240 (C-N), 1195, 1097, 815 (C-H out of plane deformation).

$^1\text{H}$ -NMR (400 Mhz, DMSO- $d_6$ , ppm)  $\delta$  2.60 (2H, t,  $J$  = 4.8 Hz, piperazine protons), 2.70 (2H, t,  $J$  = 4.8 Hz, piperazine protons), 3.09 and 3.56 (2H, two s, CO-CH<sub>2</sub>), 3.28–3.38 (10H, m, N(C<sub>2</sub>H<sub>5</sub>)<sub>2</sub>), 3.43–3.55 (4H, m, piperazine protons), 6.65 (2H, t,  $J$  = 9.2 Hz, phenyl protons), 7.01 (2H, dd,  $J_1$  = 9.2 Hz,  $J_2$  = 3.2 Hz, phenyl protons), 7.38–7.45 (2H, m, phenyl protons), 7.78 and 8.10 (1H, two s, N=CH), 8.03 (2H, dd,  $J_1$  = 9.6 Hz,  $J_2$  = 3.2 Hz, phenyl protons), 10.86 and 10.98 (1H, two s, NH).

$^{13}\text{C}$ -NMR (100 Mhz, DMSO- $d_6$ , ppm)  $\delta$  12.31 (2CH<sub>3</sub>), 43.58 (2CH<sub>2</sub>), 46.20 and 46.39 (2CH<sub>2</sub>), 52.00 and 52.14 (2CH<sub>2</sub>), 56.83 and 60.08 (COCH<sub>2</sub>), 111.00 and 111.06 (2CH), 112.45 and 112.53 (2CH), 120.54 and 120.61 (C), 125.57 (2CH), 128.17 and 128.57 (2CH), 136.71 and 136.83 (C), 143.69 and 148.04 (C=N), 148.54 and 148.76 (C), 154.60 and 154.65 (C), 164.58 and 169.91 (C=O).

HRMS ( $m/z$ ): [M + H]<sup>+</sup> calcd for C<sub>23</sub>H<sub>30</sub>N<sub>6</sub>O<sub>3</sub>: 439.2452; found 439.2436.

2.4.10. *N'*-(4-Methylbenzylidene)-2-(4-(4-nitrophenyl)piperazin-1-yl)acetohydrazide (**3j**). IR (KBr)  $\nu_{\text{max}}$  (cm<sup>-1</sup>): 3255 (N-H), 3082 (Aromatic C-H), 2829 (Aliphatic C-H), 1683 (C=O), 1589 and 1319 (NO<sub>2</sub>), 1508 (C=N), 1489 (C=C), 1240 (C-N), 1089, 823 (C-H out of plane deformation).

$^1\text{H}$ -NMR (400 Mhz, DMSO- $d_6$ , ppm)  $\delta$  2.31 (3H, s, CH<sub>3</sub>), 2.62 (2H, t,  $J$  = 4.8 Hz, piperazine protons), 2.71 (2H, t,  $J$  = 4.8 Hz, piperazine protons), 3.14 and 3.61 (2H, two s, CO-CH<sub>2</sub>), 3.45 (2H, t,  $J$  = 4.8 Hz, piperazine protons), 3.49 (2H, t,  $J$  = 4.8 Hz, piperazine protons), 6.99 (2H, dd,  $J_1$  = 9.6 Hz,  $J_2$  = 4 Hz, phenyl protons), 7.21 (2H, t,  $J$  = 7.2 Hz, phenyl protons), 7.50–7.57 (2H, m, phenyl protons), 7.91 and 8.27 (1H, two s, N=CH), 8.02 (2H, dd,  $J_1$  = 9.2 Hz,  $J_2$  = 3.6 Hz, phenyl protons), 11.11 and 11.19 (1H, two s, NH).

$^{13}\text{C}$ -NMR (100 Mhz, DMSO- $d_6$ , ppm)  $\delta$  20.87 (CH<sub>3</sub>), 46.18 and 46.38 (2CH<sub>2</sub>), 51.96 and 52.13 (2CH<sub>2</sub>), 56.79 and 60.04 (COCH<sub>2</sub>), 112.45 and 112.53 (2CH), 125.56 (2CH), 126.57 and 126.88 (2CH), 129.27 (2CH), 131.38 and 131.48 (C), 136.73 and 136.85 (C), 139.37 and 139.68 (C), 142.90 and 147.19 (C=N), 154.58 and 154.63 (C), 165.23 and 170.44 (C=O).

HRMS ( $m/z$ ): [M + H]<sup>+</sup> calcd for C<sub>20</sub>H<sub>23</sub>N<sub>5</sub>O<sub>3</sub>: 382.1874; found 382.1885.

2.4.11. *N'*-(4-(Methylthio)benzylidene)-2-(4-(4-nitrophenyl)piperazin-1-yl)acetohydrazide (**3k**). IR (KBr)  $\nu_{\text{max}}$  (cm<sup>-1</sup>): 3244 (N-H), 3055 (Aromatic C-H), 2833 (Aliphatic C-H), 1683

(C=O), 1593 and 1307 (NO<sub>2</sub>), 1508 (C=N), 1471 (C=C), 1242 (C-N), 1116, 1087, 819 (C-H out of plane deformation).

$^1\text{H}$ -NMR (400 Mhz, DMSO- $d_6$ , ppm)  $\delta$  2.51 (3H, s, SCH<sub>3</sub>), 2.63–2.68 (2H, m, piperazine protons), 2.72–2.76 (2H, m, piperazine protons), 3.18 and 3.65 (2H, 2s, CO-CH<sub>2</sub>), 3.33 (1H, br s, piperazine proton), 3.44–3.56 (3H, m, piperazine protons), 7.01 (2H, dd,  $J_1$  = 9.6 Hz,  $J_2$  = 4 Hz, phenyl protons), 7.30 (2H, t,  $J$  = 7.6 Hz, phenyl protons), 7.57–7.64 (2H, m, phenyl protons), 7.94 and 8.30 (1H, two s, N=CH), 8.05 (2H, dd,  $J_1$  = 9.6 Hz,  $J_2$  = 3.6 Hz, phenyl protons), 11.17 and 11.26 (1H, two s, NH).

$^{13}\text{C}$ -NMR (100 Mhz, DMSO- $d_6$ , ppm)  $\delta$  14.40 (CH<sub>3</sub>), 46.18 and 46.37 (2CH<sub>2</sub>), 51.97 and 52.13 (2CH<sub>2</sub>), 56.80 and 60.04 (COCH<sub>2</sub>), 112.42 and 112.50 (2CH), 125.55 and 125.58 (C), 125.63 (2CH), 127.00 and 127.31 (2CH), 130.60 and 130.65 (2CH), 136.74 and 136.85 (C), 140.40 and 140.77 (C), 142.43 and 146.76 (C=N), 154.56 and 154.60 (C), 165.24 and 170.44 (C=O). HRMS ( $m/z$ ): [M + H]<sup>+</sup> calcd for C<sub>20</sub>H<sub>23</sub>N<sub>5</sub>O<sub>3</sub>S: 414.1594; found 414.1589.

2.5. *Enzymatic Assay.* AChE and BuChE inhibitory activity was determined by Ellman's method with minor modifications [22]. Compounds **3a–k** were dissolved in 2% DMSO and were tested at final concentrations of 5, 10, 20, 40, and 80 mg/mL. 20 mL (1 unit/mL) of AChE (from *Electrophorus electricus*, electric eel) or BuChE (from equine serum) and 10 mL sample were added to 2.4 mL buffer; the mixture was incubated at 37°C for 15 min. After the 15 min incubation, 50 mL of 0.01 M DTNB and 20 mL of 75 mM ATCI or 10 mM BTCI were added, and the final mixture was incubated at room temperature for 30 min. A control mixture (blank) was prepared using 10 mL of 2% DMSO instead of the test sample, with all other procedures similar to those used in the case of the sample mixture. Absorbance was measured at 412 nm and 37°C using polystyrol cuvettes with spectrophotometer (Shimadzu, UV-1700). Experiment was done in triplicate. Data are expressed as mean standard deviation (SD). The inhibition (percent) of AChE and BuChE was calculated using the following equation. The statistical analysis was evaluated using Microsoft Office Excel 2013 and the data were expressed as mean  $\pm$  SD:

$$I (\%) = 100 - \left( \frac{\text{OD}_{\text{sample}}}{\text{OD}_{\text{control}}} \right) \times 100. \quad (1)$$

### 3. Results and Discussion

3.1. *Chemistry.* The synthesis of the compounds (**3a–k**) was carried out in three steps as shown in Figure 1. In the first step, ethyl 2-[4-(4-nitrophenyl)piperazin-1-yl]acetate (**1**) was synthesized via the reaction of 1-(4-nitrophenyl)piperazine with ethyl chloroacetate in the existence of potassium carbonate. To obtain corresponding hydrazide (**2**) compound **1** reacted with excess of hydrazine hydrate in ethanol. Finally, the reaction of 2-[4-(4-nitrophenyl)piperazin-1-yl]acetohydrazide (**2**) with aromatic aldehydes gave the target compounds (**3a–k**). Some characteristic properties of the compounds were given in Table 1.

TABLE I: Some properties of the compounds (3a–k).

Compound	R	R'	Yield (%)	Mp (°C)	Molecular formula	Molecular weight
3a	H	F	74	181	C <sub>19</sub> H <sub>20</sub> FN <sub>5</sub> O <sub>3</sub>	385
3b	H	OCH <sub>3</sub>	80	207	C <sub>20</sub> H <sub>23</sub> N <sub>5</sub> O <sub>4</sub>	397
3c	H	OH	70	262	C <sub>19</sub> H <sub>21</sub> N <sub>5</sub> O <sub>4</sub>	383
3d	H	OC <sub>2</sub> H <sub>5</sub>	76	204	C <sub>21</sub> H <sub>25</sub> N <sub>5</sub> O <sub>4</sub>	411
3e	Cl	Cl	72	165	C <sub>19</sub> H <sub>19</sub> Cl <sub>2</sub> N <sub>5</sub> O <sub>3</sub>	436
3f	H	CN	78	226	C <sub>20</sub> H <sub>20</sub> N <sub>6</sub> O <sub>3</sub>	392
3g	H	CF <sub>3</sub>	82	212	C <sub>20</sub> H <sub>20</sub> F <sub>3</sub> N <sub>5</sub> O <sub>3</sub>	435
3h	H	N(CH <sub>3</sub> ) <sub>2</sub>	75	95	C <sub>21</sub> H <sub>26</sub> N <sub>6</sub> O <sub>3</sub>	410
3i	H	N(C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub>	78	221	C <sub>23</sub> H <sub>30</sub> N <sub>6</sub> O <sub>3</sub>	438.5
3j	H	CH <sub>3</sub>	73	227	C <sub>20</sub> H <sub>23</sub> N <sub>5</sub> O <sub>3</sub>	381
3k	H	SCH <sub>3</sub>	81	203	C <sub>20</sub> H <sub>23</sub> N <sub>5</sub> O <sub>3</sub> S	413.5

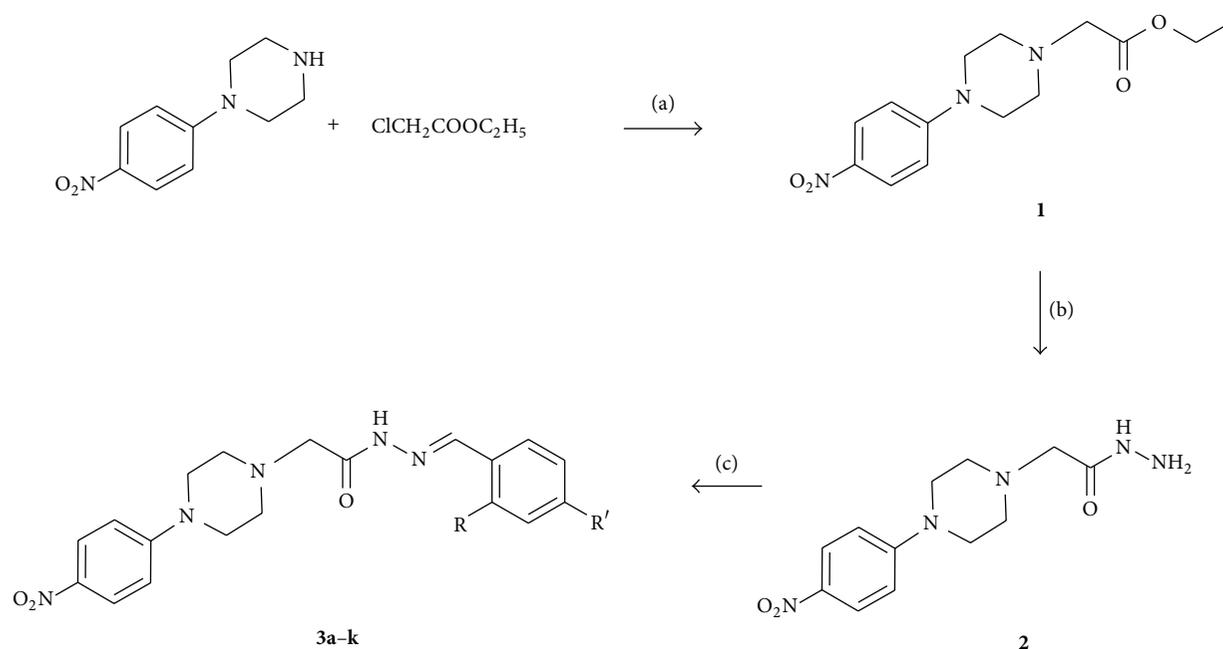


FIGURE 1: Synthesis of the compounds (3a–k). Reactants, reagents, and conditions; (a) K<sub>2</sub>CO<sub>3</sub>, acetone rt; (b) NH<sub>2</sub>NH<sub>2</sub>·H<sub>2</sub>O, ethanol, rt; (c) aromatic aldehyde, ethanol, reflux.

Structures of the obtained compounds were elucidated by <sup>1</sup>H NMR, <sup>13</sup>C NMR, and HRMS spectral data.

In the IR spectra of compounds (3a–k), significant stretching bands belonging to C=O were observed in between 1651–1695 cm<sup>-1</sup>, bands belonging to C=N and C=C were observed in between 1599–1471 cm<sup>-1</sup>, and bands belonging to NO<sub>2</sub> were observed at about 1597.06 and 1303.88 cm<sup>-1</sup>.

In the <sup>1</sup>H NMR spectra of the final compounds (3a–k), the hydrazone proton was observed in the area of 10.5–12 ppm. The protons belonging to CO-CH<sub>2</sub> (3.09–3.56 ppm), N=CH (7.78–8.10 ppm), and CO-NH (11.03–11.13 ppm) were detected as paired peaks with respect to the presence of the *E* and *Z* forms of the compounds [18, 19]. The other aromatic and aliphatic protons were observed at the expected regions.

In <sup>13</sup>C NMR spectra of the compounds, the carbons belonging to -N=CH- (140–147 ppm), carbonyl (165–170 ppm), and -CH<sub>2</sub>- attached to carbonyl (55–60 ppm) were observed as paired peaks due to *E* and *Z* isomer forms. Due to the presence of fluoro in compound 3a and 3g, splitting relating to neighbouring atoms was detected in the spectrum.

In the HRMS spectra of final compounds (3a–k), the M + 1 peak was observed in accordance with their molecular formula. The M + 1 peaks were determined in accuracy of 5–16 ppm.

**3.2. Enzyme Inhibition.** Therapeutic efficacy of novel hydrazone derivatives for the treatment of AD depends on their anticholinesterase activities. Inhibition of AChE and BuChE

TABLE 2: Cholinesterase inhibitory activity and IC<sub>50</sub> values ( $\mu\text{g/mL}$ ) of compounds (3a–k).

Compound	AChE		BuChE	
	80 mg/mL (% inhibition)	IC <sub>50</sub> mg/mL	80 mg/mL (% inhibition)	IC <sub>50</sub> mg/mL
3a	42.30 $\pm$ 4.70	>80	40.11 $\pm$ 3.72	>80
3b	16.30 $\pm$ 2.43	>80	20.16 $\pm$ 2.86	>80
3c	57.30 $\pm$ 0.66	29.5 $\pm$ 2.12	46.56 $\pm$ 3.20	>80
3d	—	—	15.53 $\pm$ 3.84	>80
3e	33.37 $\pm$ 4.45	>80	33.07 $\pm$ 3.41	>80
3f	37.22 $\pm$ 3.46	>80	27.63 $\pm$ 0.86	>80
3g	24.74 $\pm$ 1.81	>80	31.60 $\pm$ 2.05	>80
3h	12.11 $\pm$ 2.80	>80	—	—
3i	35.14 $\pm$ 4.43	>80	—	—
3j	11.39 $\pm$ 0.16	>80	—	—
3k	23.85 $\pm$ 1.85	>80	—	—
Galantamine	nt	0.46 $\pm$ 0.08	nt	6.28 $\pm$ 0.32

—: not active.  
nt: nontested.

by synthesized derivatives (3a–k) is compared with galantamine as reference drug and IC<sub>50</sub> values of individual compounds are listed in Table 2. The anticholinesterase activities of the compounds were detected via a modification of Ellman's spectrophotometric method. All compounds showed less anticholinesterase potency than reference drugs. Only compound 3c with substituent hydroxyl at *para* position exhibited available inhibitory effect against AChE having IC<sub>50</sub> value of 29.5  $\pm$  2.12. In addition, compound 3a, with substituent fluoro at *para* position also showed an inhibitory effect against AChE and BuChE. This conclusion supports that the hydroxyl and fluoro substituents on phenyl ring at *para* positions may have a considerable influence on anticholinesterase activity. It could be considered that hydrogen bonds contribute to the activity of compounds 3a and 3c. Substitution at *orto* and *para* positions with chloro did not increase the activity. Compound 3d was found to be nonactive against either AChE or BChE. When compared with reference drugs and other derivatives, compound 3j exhibited the lowest anticholinesterase activity.

#### 4. Conclusion

In the present paper, we synthesized some hydrazone derivatives and evaluated their AChE and BuChE enzyme inhibitory activities. The consequences verify that compound 3c, with an IC<sub>50</sub> value of 29.5  $\pm$  2.12, is the most promising inhibitory agent of AChE and BuChE among these compounds. As a result, it may be pointed out that hydroxyl and fluoro substituents at *para* position on the phenyl ring have a vital influence on anticholinesterase activity. Further investigations can be executed on the evaluation of new potent AChE and BuChE inhibitory agents bearing hydrazone moiety by modification of compound 3c.

#### Competing Interests

The authors declare that there are no competing interests regarding the publication of this paper.

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