Synthesis, pharmacological screening and computational analysis of some 2-(1*H*-Indol-3-yl)-*N'*-[(un)substituted phenylmethylidene] acetohydrazides and 2-(1*H*-Indol-3-yl)-*N'*-[(un)substituted benzoyl/2-thienylcarbonyl]acetohydrazides

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Abstract: The undertaken research was initiated by transforming 2-(1*H*-Indol-3-yl)acetic acid (1) in catalytic amount of sulfuric acid and ethanol to ethyl 2-(1*H*-Indol-3-yl)acetate (2), which was then reacted with hydrazine monohydrate in methanol to form 2-(1*H*-Indol-3-yl)acetohydrazide (3). Further, The reaction scheme was designed into two pathways where, first pathway involved The reaction of 3 with substituted aromatic aldehydes (4a-o) in methanol with few drops of glacial acetic acid to generate 2-(1*H*-Indol-3-yl)-*N*□-[(un)substitutedphenylmethylidene]acetohydrazides (5a-o) and in second pathway 3 was reacted with acyl halides (6a-e) in basic aqueous medium (pH 9-10) to afford 2-(1*H*-Indol-3-yl)-*N*□-[(un)substitutedbenzoyl/2-thienylcarbonyl]acetohydrazides (7a-e). All The synthesized derivatives were characterized by IR, EI-MS and ^{1}H-NMR spectral techniques and evaluated for their anti-bacterial potentials against Gram positive and Gram negative bacterial strains and it was found that compounds 7a-d exhibited antibacterial activities very close to standard Ciprofloxacin. The synthesized derivatives demonstrated moderate to weak anti-enzymatic potential against α-Glucosidase and Butyrylcholinesterase (BChE) where, compounds 7c and 5c exhibited comparatively better inhibition against these enzymes respectively. Compounds 7a, 7d and 7e showed excellent anti-enzymatic potentials against Lipoxygenase (LOX) and their IC_{50} values were much lower than the reference standard Baicalein. Enzyme inhibitory activities were also supported by computational docking results. Compounds 5c, 7a, 7b and 7c also showed low values of % hemolytic activity as well, showing that these molecules were not toxic, indicating that these molecules can be utilized as potential therapeutic agents against inflammatory ailments.

Keywords: 2-(1*H*-Indol-3-yl)-*N'*-[(un)substituted phenylmethylidene]acetohydrazides, 2-(1*H*-Indol-3-yl)-*N'*-[(un)substituted benzoyl/2-thienylcarbonyl]acetohydrazides, Anti-enzymatic activity, Antibacterial activity, % age Hemolytic activity and Computational docking.

INTRODUCTION

Indole nucleus, an aromatic heterobicycle is present in most of the compounds which are involved in research aimed at evaluation of new compounds which have remarkable biological activities as anti-viral, anti-microbial, anti-inflammatory, anti-tubercular, anti-diabetic, anti-convulsant and anti-depressant activities. Indole and its derivatives are used primarily in research and industry (Dhani *et al*, 2011). It is a major constituent of fragrances and the precursor of many pharmaceutical compounds (Fischer and Jourdan, 1983). Indole derivatives represent many important classes of therapeutic agents in medicinal chemistry such as anti-cancer (Chen *et al*, 1996), anti-oxidant (Suzen and Buyukbingol, 2000), anti-rheumatoidal (Giagoudakis and Markantonis, 2005), and anti-HIV agents (Buyukbingol *et al*, 1994; Suzen and Buyukbingol, 1998). Studies showed that some of the 2-phenylindole sulfamates are inhibitors of steroid sulfatase with anti-proliferative activity in breast cancer cells (Walter, 2004; Leichtl and von Angerer, 1998). Some of the sulfur containing 2-phenylindole derivatives showed *in vivo* anti-neoplastic and anti-estrogenic activity (Biberger and von Angerer, 1996). Indomethacin (Anti-inflammatory drug), Pindolol (beta-blocker) and Dimethyltryptamine (naturally

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occurring hallucinogen) are some significant indole derivatives which are being used for curing different ailments.

Schiff's bases have also been reported to possess a wide range of biological activities, including anti-fungal, anti-malarial, anti-proliferative, anti-bacterial and anti-pyretic activity (Hussain *et al*, 2014; Przybylski *et al*, 2009). Imine or azomethine groups are present in various natural, naturally derived, and synthetic compounds. The imine group present in such compounds has been shown to possess a variety of biological activities (Bringmann *et al*, 2004; Salimon *et al*, 2010; Guo *et al*, 2007).

The presented work was aimed to synthesize Schiff's bases having indole moiety and substituted benzoylacetohydrazides. Their pharmacological screening along with docking simulation studies were carried out to bring forth some potent molecules which could be included in further pharmaceutical investigations saving lives against different diseases.

MATERIALS AND METHODS

General

The chemicals used in this study were obtained from Alfa Aesar (acyl halides), Merck (aldehydes) and DEA JUNG (2-(1*H*-Indol-3-yl)acetic acid 1). All solvents were distilled prior to use. Melting points of the synthesized compounds were recorded by open capillary tube on a Gallen kamp melting point apparatus and are presented here without further correction. Progress of the reactions and purity of compounds was monitored by thin layer chromatography (TLC) on pre-coated silica gel G-25-UV_{254} plates, using *n*-hexane and ethyl acetate in various proportions as mobile phase. HYPER IR spectrometer was used to record IR spectra of synthesized compounds by KBr pellet method (wave number in cm^{-1}). Mass spectra (EI-MS) were taken on a JEOL JMS 600-H spectrometer. ^{1}H-NMR spectra were taken in DMSO-d_6 on Bruker spectrometers operating at 300-600 MHz and chemical shifts (δ) are given in ppm and coupling constants (*J*) are given in Hertz (Hz).

Synthesis

Ethyl 2-(1H-indol-3-yl)acetate (2)

2-(1*H*-Indol-3-yl)acetic acid (1) (20.0g; 0.11mol;) solublized in absolute ethanol (70mL) and catalytic amount of concentrated sulfuric acid (10mL; 0.18 mol) was taken in a 500mL round bottomed flask and refluxed for 8h. At completion, the reaction mixture was neutralized with 10% sodium carbonate. The ester was obtained by solvent extraction using chloroform (50mL × 3) as brownish liquid at room temperature (20-40^{o}C).

2-(1H-Indol-3-yl)acetohydrazide (3)

Ethyl 2-(1*H*-Indol-3-yl) acetate (2) (17.0mL;) and hydrazine monohydrate (80%, 25mL) was taken in 60mL methanol in a round bottomed flask. Complete conversion was carried out after simple stirring for about 3 h at room temperature (Abbasi et al., 2013). Methanol was distilled off and the hydrazide was filtered, washed with cold *n*-hexane and dried to get pure 2-(1*H*-Indol-3-yl) acetohydrazide.

2-(1H-Indol-3-yl)-N'-[(un)substituted phenylmethylidene]acetohydrazides (5a-o)

2-(1*H*-Indol-3-yl)acetohydrazide (3) (0.2g; 1.0mmol;) was dissolved in 30mL methanol and 2-3 drops of glacial acetic acid. Aromatic aldehydes (1.0mmol; 4a-o) were subsequently added and stirred for about 3 h at room temperature. Precipitates were filtered and washed with cold distilled water to afford 2-(1*H*-Indol-3-yl)-*N*□-[(un)substitutedphenylmethylidene]acetohydrazides (5a-o) in good yields.

2-(1H-Indol-3-yl)-N'-[(un)substituted benzoyl/2-thienylcarbonyl]acetohydrazides (7a-e)

2-(1*H*-Indol-3-yl)acetohydrazide (0.2g; 1.0mmol; 3) was dissolved in 30 mL distilled water with few drops of 10 % NaOH solution. Different acyl halides (1.0mmol; 6a-e) were added and the contents were stirred for 25min at room temperature. The precipitates were filtered off, washed using cold distilled water and dried to get 2-(1*H*-Indol-3-yl)-*N'*-[(un)substitutedbenzoyl/2-thienylcarbonyl] acetohydrazides (7a-e).

Spectral Characterization

Ethyl 1H-indole-3-acetate (2)

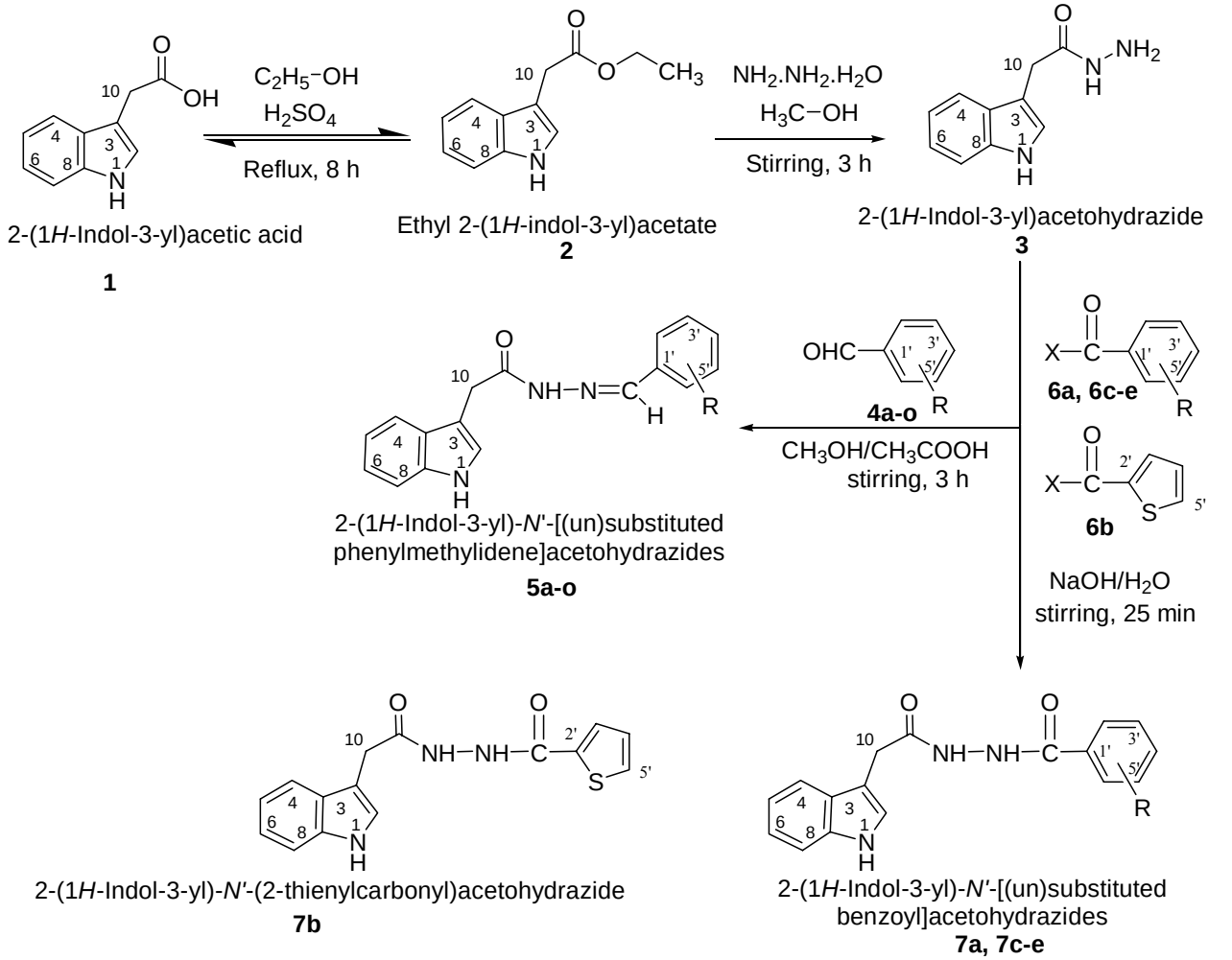
Brownish liquid; Yield: 80-85%; Molecular formula: $C_{12}H_{13}N_2O$; Molecular Weight: 203 g/mol; IR (KBr)ν_{max}: 3417 (N-H), 3036 (C-H Ar),1630 (C=O), 1539 (Ar C=C); ^{1}H-NMR (400 MHz): δ 10.8(s, 1H, NH-1), 7.47 (br.d, *J*= 8.0 Hz, 1H, H-4), 7.35 (br.d, *J*=8.2 Hz, 1H, H-7), 7.21 (br.s, 1H, H-2), 7.04 (t, *J*=7.6 Hz, 1H, H-5), 6.99 (t, *J*= 7.2 Hz, 1H, H-6), 4.18 (q, *J*=7.2, 2H, -OCH_2CH_3), 3.76 (s, 2H, CH_2-10), 1.19 (t, *J*=7.2 Hz, 3H, -OCH_2CH_3); EIMS: *m/z* 203 $(C_{12}H_{13}N_2O)^{\cdot+}$ $[M]^+$, 158 $(C_{10}H_8NO)^+$, 130 $(C_9H_8N)^+$, 59 $(C_3H_5O_2)^+$.

2-(1H-Indol-3-yl)acetohydrazide (3)

Brownish crystals; Yield: 89%; m.p: 113°C; Molecular formula: $C_{10}H_{11}N_3O$; Molecular Weight: 189g/mol; IR (KBr)ν_{max}: 3433 (N-H), 3037 (C-H Ar),1632 (C=O), 1526 (Ar C=C); ^{1}H-NMR (400 MHz): δ 10.6 (s, 1H, NH-1), 9.09 (s, 1H, $NHNH_2$), 7.54 (br.d, *J*=7.6 Hz, 1H, H-4), 7.34 (br.d, *J*=8.0 Hz, 1H, H-7), 7.18 (br.s, 1H, H-2), 7.05 (t, *J*=7.2 Hz, 1H, H-5), 6.98 (t, *J*=7.6 Hz, 1H, H-6), 4.16 (br.s,1H, $NHNH_2$) 3.44 (s, 2H, CH_2-10); EIMS: *m/z* 189 $(C_{10}H_{11}N_3O)^{\cdot+}$ $[M]^+$, 158 $(C_{10}H_8NO)^+$, 130 $(C_9H_8N)^+$, 59 $(C_3H_5O_2)^+$.

2-(1H-Indol-3-yl)-N'-[(2-methylphenyl)methylidene]acetohydrazide (5a)

Cream colored amorphous powder; Yield: 79%; m.p: 157^{o}C; Mol. Formula: $C_{18}H_{17}N_3O$; Mol. Weight: 291



Scheme 1: Outline for the synthesis of 2-(1*H*-Indol-3-yl)-*N*□-[(un)substituted phenylmethylidene]acetohydrazides (5a-p), 2-(1*H*-Indol-3-yl)-*N*□-[(un)substituted benzoyl]acetohydrazides (7a, 7c-e) and 2-(1*H*-Indol-3-yl)-*N*□-(2-thienylcarbonyl)acetohydrazide (7b).

g/mol; IR (KBr)ν_{max}: 3432 (N-H), 3034 (C-H Ar), 1673 (C=O), 1528 (C=C Ar); ^{1}H-NMR (400 MHz): δ 11.76 (s, 1H, CONH), 11.11 (s, 1H, NH-1), 8.68 (s, 1H, H-7'), 7.74 (dd, *J*=8.8, 1.6 Hz, 1H, H-6'), 7.54 (br.d, *J*=7.6 Hz, 1H, H-4), 7.38-7.34 (m, 2H, H-4' & H-5'), 7.32 (br.d, *J*=8.0 Hz, 1H, H-7), 7.25 (d, *J*=7.6 Hz, 1H, H-3'), 7.21 (br.s, 1H, H-2), 7.06 (t, *J*=7.2 Hz, 1H, H-5), 6.95 (t, *J*=7.6 Hz, 1H, H-6), 3.41 (s, 2H, CH_2-10), 2.45 (s, 3H, CH_3-2'); EIMS (*m/z*): 291 $[C_{18}H_{17}N_3O]^{\cdot+}$ $[M]^+$, 173 $[C_{10}H_9N_2O]^+$, 161$[C_9H_9N_2O]^+$, 158 $[C_{10}H_8NO]^+$, 133 $[C_8H_9NO]^+$, 130 $[C_9H_8N]^+$, 91 $[C_7H_7]^+$, 76 $[C_6H_4]^{\cdot+}$.

2-(1H-Indol-3-yl)-N -[(3-methylphenyl)methylidene]acetohydrazide (5b)

Cream colored amorphous powder; Yield: 80%; m.p: 160 ^{o}C; Mol. Formula: $C_{18}H_{17}N_3O$; Mol. Weight: 291 g/mol; IR (KBr)ν_{max}: 3432 (N-H), 3035 (C-H Ar), 1670 (C=O), 1528 (C=C Ar); ^{1}H-NMR (400 MHz): δ 11.78 (s, 1H, CONH),11.12 (s, 1H, NH-1), 8.66 (s, 1H, H-7'), 7.53 (br.d, *J* = 7.6 Hz, 1H, H-4), 7.43 (d, *J*=7.6 Hz, 1H, H-6'), 7.32 (br.d, *J*=8.0 Hz, 1H, H-7), 7.30 (t, *J*=7.6 Hz, 1H, H-5'), 7.26 (br. s, 1H, H-2'), 7.23 (d, *J*=7.6 Hz, 1H, H-4'), 7.20 (br. s, 1H, H-2), 7.06 (t, *J*=7.2 Hz, 1H, H-5), 6.95 (t, *J*=7.6 Hz, 1H, H-6), 3.41 (s, 2H, CH_2-10), 2.35(s, 3H, CH_3-3'); EIMS (*m/z*): 291 $[C_{18}H_{17}N_3O]^{\cdot+}$ $[M]^+$, 173 $[C_{10}H_9N_2O]^+$, 161$[C_9H_9N_2O]^+$, 158 $[C_{10}H_8NO]^+$, 133 $[C_8H_9NO]^+$, 130 $[C_9H_8N]^+$, 91 $[C_7H_7]^+$, 76 $[C_6H_4]^{\cdot+}$.

2-(1H-Indol-3-yl)-N -[(3-hydroxyphenyl)methylidene]acetohydrazide (5c)

Cream colored amorphous powder; Yield: 79%; m.p: 114^{o}C; Mol. Formula: $C_{17}H_{15}N_3O_2$; Mol. Weight: 293 g/mol; IR (KBr)ν_{max}: 3436 (N-H), 3134 (O-H), 3035 (C-H Ar), 1675 (C=O), 1527 (C=C Ar); ^{1}H-NMR (400 MHz): δ 11.73 (s, 1H, CONH),11.10 (s, 1H, NH-1), 9.84 (s, 1H, OH-3'), 7.85 (s, 1H, H-7'), 7.53 (br. d, *J*=7.6 Hz, 1H, H-4), 7.35 (br. d, *J*=8.0 Hz, 1H, H-7), 7.23 (br. t, *J*= 7.6 Hz, 1H, H-5'), 7.21 (br. s, 1H, H-2), 7.19 (br. s, 1H, H-2'), 7.08 (d, *J*=7.6 Hz, 1H, H-6'), 7.00 (t, *J*=7.2 Hz, 1H, H-5), 6.96 (t, *J*=7.6 Hz, 1H, H-6), 6.87 (dd, *J*=7.6, 2.0 Hz, 1H, H-4'), 3.41 (s, 2H, CH_2-10); EIMS (*m/z*): 293 $[C_{17}H_{15}N_3O_2]^{\cdot+}$ $[M]^+$, 173 $[C_{10}H_9N_2O]^+$, 163$[C_8H_7N_2O_2]^+$,

158 $[C_{10}H_8NO]^+$, 135 $[C_7H_7N_2O]^+$, 130 $[C_9H_8N]^+$, 93 $[C_6H_5O]^+$.

2-(1H-Indol-3-yl)-N -[(4-hydroxyphenyl)methylidene]acetohydrazide (5d)

Cream colored amorphous powder; Yield: 78%; m.p: 247 ^oC; Mol. Formula: $C_{17}H_{15}N_3O_2$; Mol. Weight: 293g/mol; IR (KBr)ν_{max}: 3439 (N-H), 3137 (O-H), 3034 (C-H Ar), 1671 (C=O), 1528 (C=C Ar); ^{1}H-NMR (600 MHz): δ 11.36 (s, 1H, CONH),10.92 (s, 1H, NH-1),9.89 (s, 1H, OH-4'), 7.92 (s, 1H, H-7'), 7.60 (br. d, *J*=8.3 Hz, 1H, H-4), 7.56 (d, *J*=8.5 Hz, 2H, H-2' & H-6'), 7.35 (br. d, *J*= 8.1 Hz, 1H, H-7), 7.23 (br. s, 1H, H-2), 7.06 (t, *J*=7.5 Hz, 1H, H-5), 6.97 (t, *J*=7.3 Hz, 1H, H-6), 6.85 (d, *J*=8.5Hz, 2H, H-3' & H-5'), 4.05 (s, 2H, CH_2-10); EIMS (*m/z*): 293 $[C_{17}H_{15}N_3O_2]^{\cdot+}$ $[M]^+$, 173 $[C_{10}H_9N_2O]^+$, 163$[C_8H_7N_2O_2]^+$, 158 $[C_{10}H_8NO]^+$, 135 $[C_7H_7N_2O]^+$, 130 $[C_9H_8N]^+$, 93 $[C_6H_5O]^+$.

2-(1H-Indol-3-yl)-N -[(2-nitrophenyl)methylidene]acetohydrazide (5e)

Light yellow colored amorphous powder; Yield: 85%; m.p: 161^{o}C; Mol. Formula: $C_{17}H_{14}N_4O_3$; Mol. Weight: 322 g/mol; IR (KBr)ν_{max}: 3437 (N-H), 3034 (C-H Ar), 1670 (C=O), 1525 (C=C Ar); ^{1}H-NMR (400 MHz): δ 11.74 (s, 1H, CONH),11.46 (s, 1H, NH-1), 8.72 (s, 1H, H-7'), 8.26 (d, *J*=8.8 Hz, 1H, H-3'), 7.97 (dd, *J*=8.8, 2.0 Hz, 1H, H-6'), 7.94-7.90 (m, 2H, H-4' & H-5'), 7.73 (br.d, *J*=7.6 Hz, 1H, H-4), 7.33 (br.d, *J*=8.0 Hz, 1H, H-7), 7.20 (br. s, 1H, H-2), 7.05 (t, *J*=7.2 Hz, 1H, H-5), 6.96 (t, *J*= 7.6 Hz, 1H, H-6'), 4.05 (s, 2H, CH_2-10□); EIMS (*m/z*): 322 $[C_{17}H_{14}N_4O_3]^{\cdot+}$ $[M]^+$, 173 $[C_{10}H_9N_2O]^+$, 164 $[C_7H_6N_3O_2]^+$, 158 $[C_{10}H_8NO]^+$, 149 $[C_7H_5N_2O_2]^+$, 135 $[C_7H_5NO_2]^+$, 130 $[C_9H_8N]^+$, 122 $[C_6H_4NO_2]^+$, 118 [C7H6N2]$^{\cdot}$+, 103 $[C_7H_5N]^{\cdot+}$, 89 $[C_7H_5]^{\cdot+}$, 76$[C_6H_4N]^{\cdot+}$

2-(1H-Indol-3-yl)-N -[(3-nitrophenyl)methylidene]acetohydrazide (5f)

Yellow colored amorphous powder; Yield: 83%; m.p: 211^{o}C; Mol. Formula: $C_{17}H_{14}N_4O_3$; Mol. Weight: 322 g/mol; IR (KBr)ν_{max}: 3400 (N-H), 3057 (C-H Ar), 1667 (C=O), 1487 (C=C Ar); ^{1}H-NMR (500 MHz): δ 11.75 (s, 1H, CONH), 11.49 (s, 1H, NH-1), 8.70 (s, 1H, H-7'), 8.49 (br. s, 1H, H-2'), 8.36 (dd, *J*=6.9, 1.2 Hz, 1H, H-6'), 8.21 (br. d, *J*=8.5 Hz, 1H, H-4'), 7.82 (t, *J*=7.9 Hz, 1H, H-5'), 7.75 (d, *J*=7.5 Hz, 1H, H-4), 7.33 (br.d, *J*=8.7 Hz, 1H, H-7), 7.21 (br.s, 1H, H-2), 7.04 (t, *J*=7.3 Hz, 1H, H-5), 6.97 (t, *J*=7.5 Hz, 1H, H-6), 4.07 (s, 2H, CH_2-10); EIMS (*m/z*): 322 $[C_{17}H_{14}N_4O_3]^{\cdot+}$ $[M]^+$, 173 $[C_{10}H_9N_2O]^+$, 164 $[C_7H_6N_3O_2]^+$, 158 $[C_{10}H_8NO]^+$, 149 $[C_7H_5N_2O_2]^+$, 135 $[C_7H_5NO_2]^+$, 130 $[C_9H_8N]^+$, 122 $[C_6H_4NO_2]^+$, 118 [C7H6N2]$^{\cdot}$+, 103 $[C_7H_5N]^{\cdot+}$, 89 $[C_7H_5]^{\cdot+}$, 76 $[C_6H_4N]^{\cdot+}$.

2-(1H-Indol-3-yl)-N -[(4-nitrophenyl)methylidene]acetohydrazide (5g)

Yellow colored amorphous powder; Yield: 81%; m.p: 198^{o}C; Mol. Formula: $C_{17}H_{14}N_4O_3$; Mol. Weight: 322 g/mol; IR (KBr)$\nu_{max:}$ 3400 (N-H), 3057 (C-H Ar), 1667 (C=O), 1487 (C=C Ar); ^{1}H-NMR (600 MHz): δ 11.87 (s, 1H, CONH), 10.91 (s, 1H, NH-1), 8.33 (s, 1H, H-7'), 8.28 (br. d, *J*=8.8 Hz, 2H, H-3' & H-5'), 7.98 (br. d, *J*=8.2 Hz, 2H, H-2' & H-6'), 7.59 (br. d, *J*=8.0 Hz, 1H, H-4), 7.35 (br. d, *J*=8.1 Hz, 1H, H-7), 7.26 (br.s, 1H, H-2), 7.07 (t, *J* =7.2 Hz, 1H, H-5), 6.98 (t, *J*=7.5 Hz, 1H, H-6), 4.11 (s, 2H, CH_2-10); EIMS (*m/z*): 322 $[C_{17}H_{14}N_4O_3]^{\cdot+}$ $[M]^+$, 173 $[C_{10}H_9N_2O]^+$, 164 $[C_7H_6N_3O_2]^+$, 158 $[C_{10}H_8NO]^+$, 149 $[C_7H_5N_2O_2]^+$, 135 $[C_7H_5NO_2]^+$, 130 $[C_9H_8N]^+$, 122 $[C_6H_4NO_2]^+$, 118 [C7H6N2]$^{\cdot}$+, 103 $[C_7H_5N]^{\cdot+}$, 89 $[C_7H_5]^{\cdot+}$, 76 $[C_6H_4N]^{\cdot+}$.

2-(1H-Indol-3-yl)-N -[(4-dimethylaminophenyl)methylidene]acetohydrazide (5h)

Bright yellow colored amorphous powder; Yield: 80%; m.p: 195^{o}C; Mol. Formula: $C_{19}H_{20}N_4O$; Mol. Weight: 320 g/mol; IR (KBr)ν_{max}: 3431 (N-H), 3036 (C-H Ar), 1683 (C=O), 1528 (C=C Ar); ^{1}H-NMR (500 MHz): δ 10.95 (s, 1H, CONH),10.87 (s, 1H, NH-1), 8.48 (s, 1H, H-7'), 7.56 (br. d, *J*=7.0 Hz, 1H, H-4), 7.51 (d, *J*=8.7 Hz, 2H, H-2' & H-6'), 7.32 (br. d, *J*=7.2 Hz, 1H, H-7), 7.23 (br. s, 1H, H-2), 7.04 (t, *J*=8.5 Hz, 1H, H-5), 6.94 (t, *J*=8.0 Hz, 1H, H-6), 6.73 (d, *J*=8.7 Hz, 2H, H-3' & H-5'), 4.00 (s, 2H, CH_2-10), 2.97 (s, 6H, $(CH_3)_2$N-4'); EIMS (*m/z*): 320 $[C_{19}H_{20}N_4O]^{\cdot+}$ $[M]^+$, 173 $[C_{10}H_9N_2O]^+$, 190 $[C_{10}H_{12}N_3O]^+$, 162 $[C_9H_{12}N_3]^+$, 158 $[C_{10}H_8NO]^+$, 130 $[C_9H_8N]^+$, 120 $[C_8H_{10}N]^+$, 79 $[C_7H_7N]^+$.

2-(1H-Indol-3-yl)-N -[(4-diethylaminophenyl)methylidene]acetohydrazide (5i)

Orange yellow colored amorphous powder; Yield: 78%; m.p: 171^{o}C; Mol. Formula: $C_{21}H_{24}N_4O$; Mol. Weight: 348 g/mol; IR (KBr)ν_{max}: 3433 (N-H), 3036 (C-H Ar), 1686 (C=O), 1526 (C=C Ar); ^{1}H-NMR (500 MHz): δ 11.51 (s, 1H, CONH),11.38 (s, 1H, NH-1), 8.44 (s, 1H, H-7'), 7.64 (d, *J*=8.9 Hz, 1H, H-4), 7.47 (d, *J*=8.8 Hz, 2H, H-2' & H-6'), 7.33 (br. d, *J*=8.3 Hz, 1H, H-7), 7.21 (br. s, 1H, H-2), 7.05 (t, *J*=8.8 Hz, 1H, H-5), 6.98 (t, *J*=7.9 Hz, 1H, H-6), 6.69 (d, *J*=8.9 Hz, 2H, H-3' & H-5'), 3.99 (s, 2H, CH_2-10), 3.39-3.35 (m, merged in signal of DMSO, 4H, $(CH_3CH_2)_2$N-4'), 1.10 (br. t, *J*=7.3 Hz, 6H, $(CH_3CH_2)_2$N-4'); EIMS (*m/z*): 348 $[C_{21}H_{24}N_4O]^{\cdot+}$ $[M]^+$, 218 $[C_{12}H_{16}N_3O]^+$, 190 $[C_{11}H_{16}N_3]^+$, 173 $[C_{10}H_9N_2O]^+$, 158 $[C_{10}H_8NO]^+$, 134 $[C_9H_{12}N]^+$, 130 $[C_9H_8N]^+$, 105 $[C_8H_9N]^+$.

2-(1H-Indol-3-yl)-N -[(2,3-dimethoxyphenyl)methylidene]acetohydrazide (5j)

Cream colored amorphous powder; Yield: 76%; m.p: 191^{o}C; Mol. Formula: $C_{19}H_{19}N_3O_3$; Mol. Weight: 337 g/mol; IR (KBr)ν_{max}: 3431 (N-H), 3034 (C-H Ar), 1679 (C=O), 1529 (C=C Ar); ^{1}H-NMR (400 MHz): δ 11.34 (s, 1H, CONH),11.16 (s, 1H, NH-1), 8.63 (s, 1H, H-7'), 7.54 (d, *J*=8.8 Hz, 1H, H-4), 7.33 (br.d, *J*=8.3 Hz, 1H, H-7), 7.27 (d, *J*=8.4 Hz, 1H, H-6'), 7.25 (dd, *J*=7.6, 1.2 Hz, 1H, H-4'), 7.21 (br. s, 1H, H-2), 7.13 (t, *J*=7.2 Hz, 1H, H-5'), 7.03 (t, *J*=8.8 Hz, 1H, H-5), 6.95 (t, *J*=7.6 Hz, 1H, H-6), 3.98 (s, 2H, CH_2-10), 3.84 (s, 3H, CH_3O-3'), 3.78 (s, 3H,

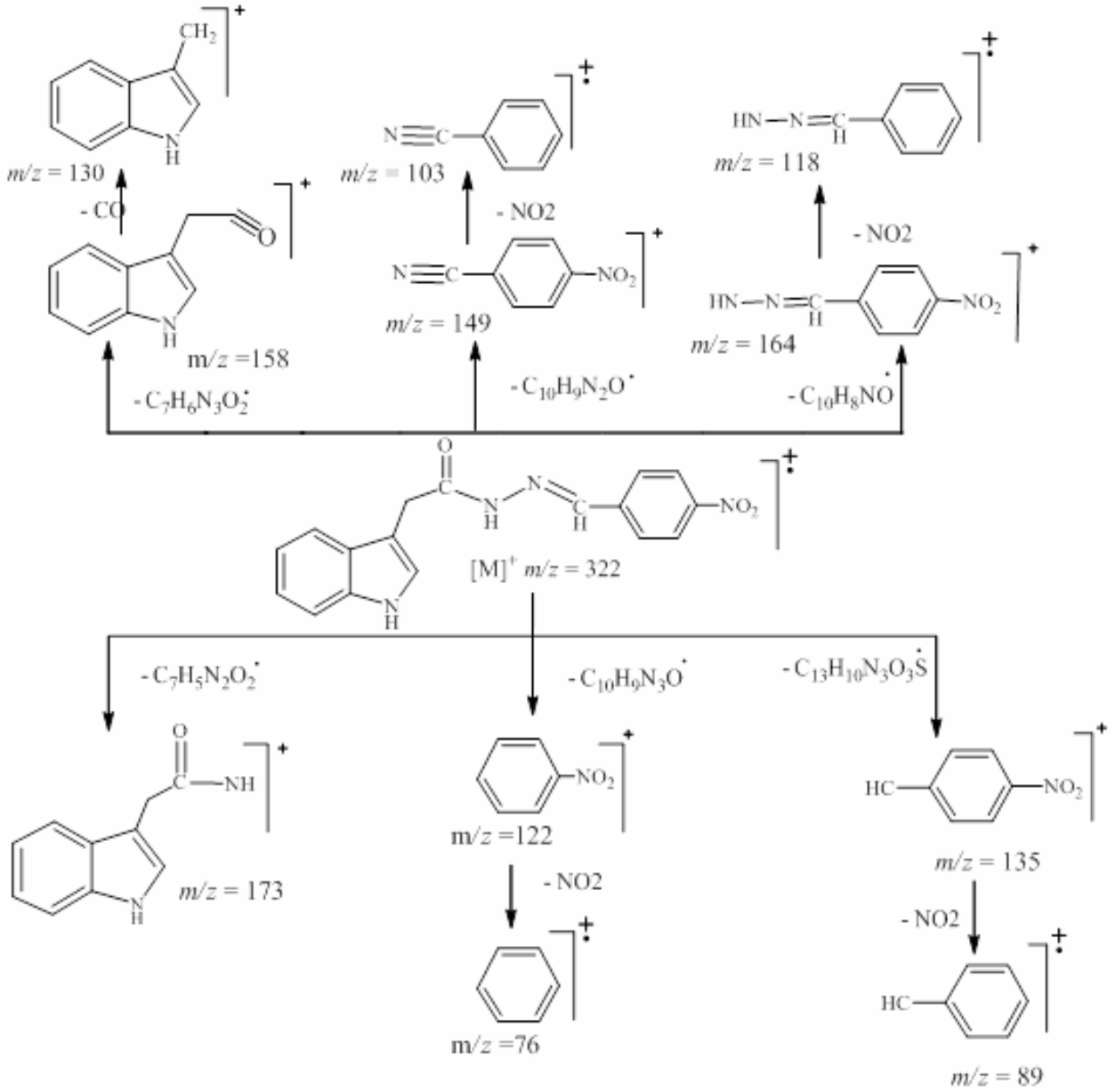


Fig. 1: Suggested mass fragmentation pattern of [2-(1*H*-indol-3-yl)-*N*□-[(4-nitrophenyl)methylidene]acetohydrazide (**5g**).

CH_3O-2'); EIMS (*m/z*): 337 $[C_{19}H_{19}N_3O_3]^{\cdot+}$ $[M]^+$, 207 $[C_{10}H_{11}N_2O_3]^+$, 179 $[C_9H_{11}N_2O_2]^+$, 173 $[C_{10}H_9N_2O]^+$, 158 $[C_{10}H_8NO]^+$, 137 $[C_8H_9O_2]^+$, 130 $[C_9H_8N]^+$, 106 $[C_7H_6O]^{\cdot+}$, 75 $[C_6H_3]^+$.

2-(1H-Indol-3-yl)-N -[(2,4-dimethoxyphenyl)methylidene]acetohydrazide (5k)

Cream colored amorphous powder; Yield: 74%; m.p: 190°C; Mol. Formula: $C_{19}H_{19}N_3O_3$; Mol. Weight: 337 g/mol; IR (KBr)ν_{max}: 3435 (N-H), 3034 (C-H Ar), 1678 (C=O), 1526 (C=C Ar); ^{1}H-NMR (400 MHz): δ 11.75 (s, 1H, CONH),11.53 (s, 1H, NH-1), 8.68 (s, 1H, H-7'), 7.54 (d, *J*=8.8 Hz, 1H, H-4), 7.33 (br. d, *J*=8.3 Hz, 1H, H-7), 7.25 (d, *J*=8.0 Hz, 1H, H-6'), 7.21 (br. s, 1H, H-2), 7.03 (t, *J*=8.8 Hz, 1H, H-5), 6.95 (t, *J*=7.6 Hz, 1H, H-6), 6.71 (dd, *J*=8.8, 1.6 Hz, 1H, H-5'), 6.44 (d, *J*=2.0 Hz, 1H, H-3'), 3.98 (s, 2H, CH_2-10), 3.85 (s, 3H, CH_3O-2'), 3.80 (s, 3H, CH_3O-4'); EIMS (*m/z*): 337 $[C_{19}H_{19}N_3O_3]^{\cdot+}$ $[M]^+$, 207 $[C_{10}H_{11}N_2O_3]^+$, 179 $[C_9H_{11}N_2O_2]^+$, 173 $[C_{10}H_9N_2O]^+$, 158 $[C_{10}H_8NO]^+$, 137 $[C_8H_9O_2]^+$, 130 $[C_9H_8N]^+$, 106 $[C_7H_6O]^{\cdot+}$, 75 $[C_6H_3]^+$.

2-(1H-Indol-3-yl)-N -[(2,5-dimethoxyphenyl)methylidene]acetohydrazide (5l)

Cream colored amorphous powder; Yield: 79%; m.p: 192°C; Mol. Formula: $C_{19}H_{19}N_3O_3$; Mol. Weight: 337 g/mol; IR (KBr) ν_{max}: 3432 (N-H), 3036 (C-H Ar), 1671 (C=O), 1529 (C=C Ar); ^{1}H-NMR (600 MHz): δ 11.55 (s,

1H, CONH),10.87 (s, 1H, NH-1), 8.29 (s, 1H, H-7'), 7.58 (br. d, *J*=8.8 Hz, 1H, H-4), 7. 40 (d, *J*=3.0 Hz, 1H, H-6'), 7.33 (br. d, *J*=7.3 Hz, 1H, H-7), 7.20 (br.s, 1H, H-2), 7.05 (d, *J*=7.6 Hz, 1H, H-3'), 7.03 (t, *J*=8.8 Hz, 1H, H-5), 7.02 (d, *J*=8.0 Hz, 1H, H-4'), 6.96 (t, *J*=7.6 Hz, 1H, H-6), 4.05 (s, 2H, CH_2-10), 3.79 (s, 3H, -OCH_3-2'), 3.74 (s, 3H, -OCH_3-5'); EIMS (*m/z*): 337 $[C_{19}H_{19}N_3O_3]^{\cdot+}$ $[M]^+$, 207 $[C_{10}H_{11}N_2O_3]^+$, 179 $[C_9H_{11}N_2O_2]^+$, 173 $[C_{10}H_9N_2O]^+$, 158 $[C_{10}H_8NO]^+$, 137 $[C_8H_9O_2]^+$, 130 $[C_9H_8N]^+$, 106 $[C_7H_6O]^{\cdot+}$, 75 $[C_6H_3]^+$.

2-(1H-Indol-3-yl)-N -[(3,4-dimethoxyphenyl)methylidene]acetohydrazide (5m)

Cream colored amorphous powder; Yield: 78%; m.p: 159°C; Mol. Formula: $C_{19}H_{19}N_3O_3$; Mol. Weight: 337 g/mol; IR (KBr)ν_{max}: 3433 (N-H), 3031 (C-H Ar), 1674 (C=O), 1530 (C=C Ar); ^{1}H-NMR (400 MHz): δ 11.36 (s, 1H, CONH),11.15 (s, 1H, NH-1), 8.13 (s, 1H, H-7'), 7.58 (d, *J*=8.0 Hz, 1H, H-4), 7.47 (d, *J*=1.2 Hz, 1H, H-2'), 7.34 (br. d, *J*=8.4 Hz, 1H, H-7), 7.31 (br. s, 1H, H-2), 7.24 (dd, *J*=8.0, 1.2 Hz, 1H, H-6'), 7.05 (t, *J*=8.0 Hz, 1H, H-5), 6.99 (d, *J*=8.4 Hz, 1H, H-5'), 6.93 (t, *J*=7.6 Hz, 1H, H-6), 4.03 (s, 2H, CH_2-10), 3.82 (s, 3H, CH_3O-3'), 3.81 (s, 3H, CH_3O-4'); EIMS (*m/z*): 337 $[C_{19}H_{19}N_3O_3]^{\cdot+}$ $[M]^+$, 207 $[C_{10}H_{11}N_2O_3]^+$, 179 $[C_9H_{11}N_2O_2]^+$, 173 $[C_{10}H_9N_2O]^+$, 158 $[C_{10}H_8NO]^+$, 137 $[C_8H_9O_2]^+$, 130 $[C_9H_8N]^+$, 106 $[C_7H_6O]^{\cdot+}$, 75 $[C_6H_3]^+$.

2-(1H-Indol-3-yl)-N -[(2,4-dichlorophenyl)methylidene]acetohydrazide (5n)

Cream colored amorphous powder; Yield: 77%; m.p: 194°C; Mol. Formula: $C_{17}H_{13}N_3OCl_2$; Mol. Weight: 346 g/mol; IR (KBr)ν_{max}: 3436 (N-H), 3034 (C-H Ar), 1676 (C=O), 1527 (C=C Ar); ^{1}H-NMR (300 MHz): δ 11.81 (s, 1H, CONH),11.53 (s, 1H, NH-1), 8.55 (s, 1H, H-7'), 8.03 (br. t, *J*=8.4 Hz, 1H, H-6'), 7.91 (br. d, *J*=8.4 Hz, 1H, H-5'), 7.71 (br. s, 1H, H-3'), 7.54 (d, *J*=7.2 Hz, 1H, H-4), 7.34 (br.d, *J*=6.9 Hz, 1H, H-7), 7.24 (br.s, 1H, H-2), 7.04 (t, *J*=6.3 Hz, 1H, H-5), 6.95 (t, *J*=7.8 Hz, 1H, H-6), 4.05 (s, 2H, CH_2-10; EIMS (*m/z*): 350 $[C_{17}H_{13}N_3OCl_2+4]^{\cdot+}$ $[M+4]^+$, 348 $[C_{17}H_{13}N_3OCl_2+2]^{\cdot+}$ $[M+2]^+$, 346 $[C_{17}H_{13}N_3OCl_2]^{\cdot+}$ $[M]^+$, 220 $[C_8H_5N_2OCl_2+4]^+$, 218 $[C_8H_5N_2OCl_2+2]^+$, 216 $[C_8H_5N_2OCl_2]^+$, 192 $[C_7H_5N_2Cl_2+4]^+$, 190 $[C_7H_5N_2Cl_2+2]^+$, 188 $[C_7H_5N_2Cl_2]^+$, 173 $[C_{10}H_9N_2O]^+$, 158 $[C_{10}H_8NO]^+$, 149 $[C_6H_3Cl_2+4]^+$, 147 $[C_6H_3Cl_2+2]^+$, 145 $[C_6H_3Cl_2]^+$, 130 $[C_9H_8N]^+$.

2-(1H-Indol-3-yl)-N -[(2,6-dichlorophenyl)methylidene]acetohydrazide (5o)

Cream colored amorphous powder; Yield: 79%; m.p: 207°C; Mol. Formula: $C_{17}H_{13}N_3OCl_2$; Mol. Weight: 346 g/mol; IR (KBr)ν_{max}: 3435 (N-H), 3033 (C-H Ar), 1681 (C=O), 1528 (C=C Ar); ^{1}H-NMR (300 MHz): δ 11.80 (s, 1H, CONH),11.51 (s, 1H, NH-1), 8.53 (s, 1H, H-7'), 7.91-7.72 (m, 3H, H-3' to H-5'), 7.53 (d, *J*=7.2 Hz, 1H, H-4), 7.33 (br.d, *J*=7.2 Hz, 1H, H-7), 7.20 (br.s, 1H, H-2), 7.04 (t, *J*=8.4 Hz, 1H, H-5), 6.96 (t, *J*=7.2 Hz, 1H, H-6), 4.04 (s, 2H, CH_2-10; EIMS (*m/z*): 350 $[C_{17}H_{13}N_3OCl_2+4]^{\cdot+}$ $[M+4]^+$, 348 $[C_{17}H_{13}N_3OCl_2+2]^{\cdot+}$ $[M+2]^+$, 346 $[C_{17}H_{13}N_3OCl_2]^{\cdot+}$ $[M]^+$, 220 $[C_8H_5N_2OCl_2+4]^+$, 218 $[C_8H_5N_2OCl_2+2]^+$, 216 $[C_8H_5N_2OCl_2]^+$, 192 $[C_7H_5N_2Cl_2+4]^+$, 190 $[C_7H_5N_2Cl_2+2]^+$, 188 $[C_7H_5N_2Cl_2]^+$, 173 $[C_{10}H_9N_2O]^+$, 158 $[C_{10}H_8NO]^+$, 149 $[C_6H_3Cl_2+4]^+$, 147 $[C_6H_3Cl_2+2]^+$, 145 $[C_6H_3Cl_2]^+$, 130 $[C_9H_8N]^+$.

2-(1H-Indol-3-yl)-N -(benzoyl)acetohydrazide (7a)

Cream colored amorphous powder; Yield: 73%; m.p: 174°C; Mol. Formula: $C_{17}H_{15}N_3O_2$; Mol. Weight: 293 g/mol; IR (KBr):ν_{max}: 3437 (N-H), 3031 (C-H Ar), 1679 (C=O), 1528 (C=C Ar); ^{1}H-NMR (500 MHz): δ 11.12 (s, 1H, NH-1), 10.26 (s, 1H, -CH_2CONH),10.21 (s, 1H, -CONH),7.88 (br. d, *J*=7.1 Hz, 2H, H-2' & H-6'), 7.65 (br.d, *J*=8.0 Hz, 1H, H-4), 7.57 (br. t, *J*=8.0 Hz, 1H, H-4'), 7.49 (br. t, *J*=7.7 Hz, 2H, H-3' & H-5'), 7.34 (br.d, *J* =8.1 Hz, 1H, H-7), 7.29 (br.s, 1H, H-2), 7.08 (t, *J*=7.0 Hz, 1H, H-5), 6.98 (t, *J*=7.0 Hz, 1H, H-6), 3.64 (s, 2H, CH_2-10); EIMS (*m/z*): 293 $[C_{17}H_{15}N_3O_2]^{\cdot+}$ $[M]^+$, 158 $[C_{10}H_8NO]^+$, 157 $[C_{10}H_7NO]^+$, 130 $[C_9H_8N]^+$, 105 $[C_7H_5O]^+$, 77 $[C_6H_5]^+$.

2-(1H-Indol-3-yl)-N -(2-thienylcarbonyl)acetohydrazide (7b)

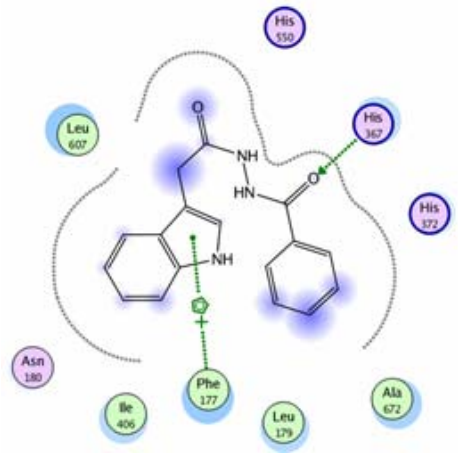
Cream colored amorphous powder; Yield: 70%; m.p: 157°C; Mol. Formula: $C_{15}H_{15}N_3O_2S$; Mol. Weight: 301 g/mol; IR (KBr)ν_{max}: 3434 (N-H), 3039 (C-H Ar), 1678 (C=O), 1525 (C=C Ar); ^{1}H-NMR (600 MHz): δ 10.90 (s, 1H, NH-1), 10.38 (s, 1H, -CH_2CONH),10.12 (s, 1H, -CONH)), 7.83 (d, *J*=3.3 Hz, 1H, H-5'), 7.82 (br. d, *J*=4.8 Hz, 1H, H-3'), 7.64 (br.d, *J*=7.8 Hz, 1H, H-4), 7.35 (br.d, *J*=8.0 Hz, 1H, H-7), 7.29 (br.s, 1H, H-2), 7.16 (t, *J*=4.2 Hz, 1H, H-4'), 7.06 (t, *J*=7.5 Hz, 1H, H-5), 6.99 (t, *J*=7.4 Hz, 1H, H-6), 3.63 (s, 2H, CH_2-10); EIMS (*m/z*): 301 $[C_{15}H_{13}N_3O_2S+2]^{\cdot+}$ $[M+2]^+$, 299 $[C_{15}H_{13}N_3O_2S]^{\cdot+}$ $[M]^+$, 173 $[C_{10}H_9N_2O]^+$, 158 $[C_{10}H_8NO]^+$, 130 $[C_9H_8N]^+$, 113 $[C_5H_3OS+2]^+$, 111$[C_5H_3OS]^+$, 85$[C_4H_3S+2]^+$, 83 $[C_4H_3S]^+$.

2-(1H-Indol-3-yl)-N -(4-nitrobenzoyl)acetohydrazide (7c)

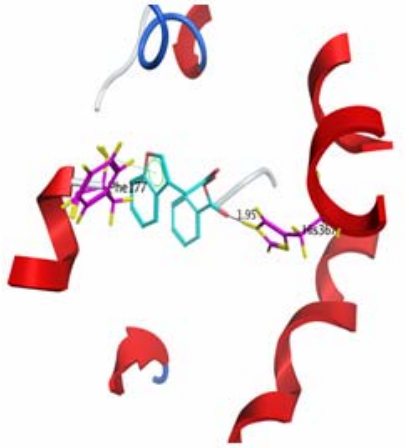
Brown colored amorphous powder; Yield: 81%; m.p: 204°C; Mol. Formula: $C_{17}H_{14}N_4O_4$; Mol. Weight: 338 g/mol; IR (KBr)ν_{max}: 3437 (N-H), 3035 (C-H Ar), 1681 (C=O), 1529 (C=C Ar);^{1}H-NMR (600 MHz): δ 10.91 (s, 1H, NH-1), 10.25 (s, 2H, -CH_2CONH, -CONH)),8.34 (d, *J*=8.2 Hz, 2H, H-3' & H-5'), 8.11 (d, *J*=8.2 Hz, 2H, H-2' & H-6'), 7.65 (br. d, *J*=7.6 Hz, 1H, H-4), 7.36 (br. d, *J*=7 .9 Hz, 1H, H-7), 7.30 (br. s, 1H, H-2), 7.08 (t, *J*=7.0 Hz, 1H, H-5), 7.00 (t, *J*=7.2 Hz, 1H, H-6), 3.66 (s, 2H, CH_2-10); EIMS (*m/z*): 338 $[C_{17}H_{14}N_4O_4]^{\cdot+}$ $[M]^+$, 216 $[C_{11}H_{10}N_3O_2]^+$, 188 $[C_{10}H_{10}N_3O]^+$, 208 $[C_8H_6N_3O_4]^+$, 180 $[C_7H_6N_3O_3]^+$, 173 $[C_{10}H_9N_2O]^+$, 165 $[C_7H_5N_2O_3]^+$, 150 $[C_7H_4NO_3]^+$, 158 $[C_{10}H_8NO]^+$, 130 $[C_9H_8N]^+$, 122 $[C6H4NO_2]^+$, 76 $[C_6H_4]^+$.

Table 1a: Different R groups in (4a-o) which are used in synthesis of 2-(1*H*-indol-3-yl)-*N*□-[(un)substituted phenylmethylidene]acetohydrazides (5a-o)

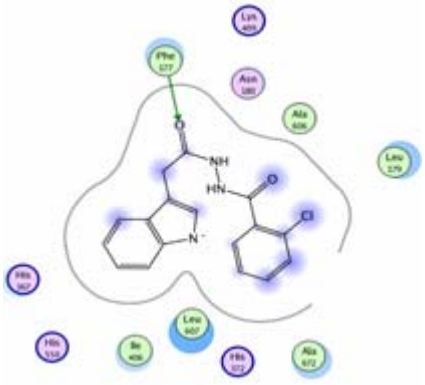
Code	R	Code	R	Code	R	Code	R
4a, 5a	2-CH_3	4e, 5e	2-NO_2	4i, 5i	4-*N*(C_2H_5)$_2$	4m, 5m	3-OCH_3, 4-OCH_3
4b, 5b	3-CH_3	4f, 5f	3-NO_2	4j, 5j	2-OCH_3, 3-OCH_3	4n, 5n	2-Cl, 4-Cl
4c, 5c	3-OH	4g, 5g	4-NO_2	4k, 5k	2-OCH_3, 4-OCH_3	4o, 5o	2-Cl, 5-Cl
4d, 5d	4-OH	4h, 5h	4-*N*(CH_3)$_2$	4l, 5l	2-OCH_3, 5-OCH_3		



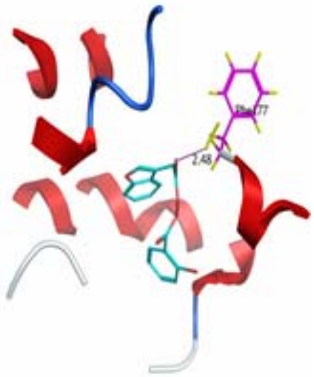
7a (a-1): 2D interacted image of LOX with the docked ligand after docking



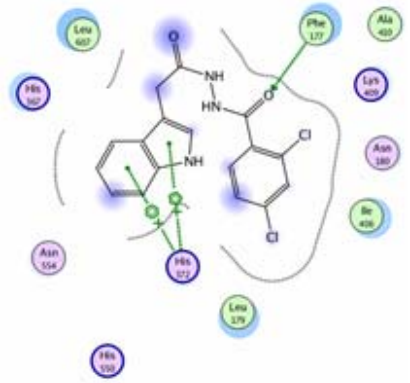
7a (a-2): 3D interacted image of LOX with the docked ligand after docking



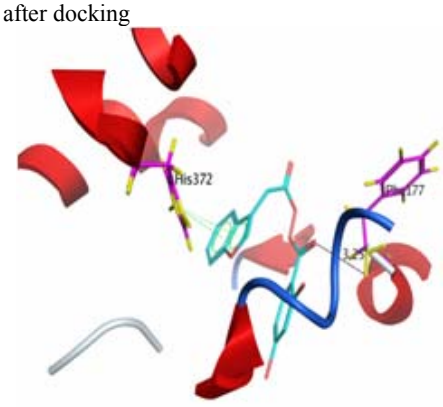
7d (b-1): 2D interacted image of LOX with the docked ligand after docking



7d (b-2): 3D interacted image of LOX with the docked ligand after docking



7e (c-1): 2D interacted image of LOX with the docked ligand after docking



7e (c-2): 3D interacted image of LOX with the docked ligand after docking

Fig 2 (a; 1-2): Binding modes of compound 7a against LOX (b; 1-2); Binding modes of compound 7d against LOX (c; 1-2); Binding modes of compound 7e against LOX.

2-(1H-Indol-3-yl)-N -(2-chlorobenzoyl)acetohydrazide (7d)

Cream colored amorphous powder; Yield: 79%; m.p: 176°C; Mol. Formula: $C_{17}H_{14}N_3O_2Cl$; Mol. Weight: 327 g/mol; IR (KBr)ν_{max}: 3438 (N-H), 3032 (C-H Ar), 1683 (C=O), 1529 (C=C Ar); ^{1}H-NMR (600 MHz): δ 10.90 (s, 1H, NH-1), 10.28(s, 1H, -CH_2CONH, -CONH),7.64 (d, *J* =7.8 Hz, 1H, H-6'), 7.51 (br. d, *J*=7.5 Hz, 1H, H-3'), 7.48-7.46 (m, 2H, H-4'& H-5'), 7.42 (br. d, *J*=7.7 Hz, 1H, H-4), 7.35 (br. d, *J*=8.1 Hz, 1H, H-7), 7.27 (br. s, 1H, H-2), 7.07 (t, *J*=7.3 Hz, 1H, H-5), 6.98 (t, *J*=7.2 Hz, 1H, H-6), 3.62 (s, 2H, CH_2-10); EIMS (*m/z*): 329 $[C_{17}H_{14}N_3O_2Cl+2]^{\cdot+}$ $[M+2]^+$, 327 $[C_{17}H_{14}N_3O_2Cl]^{\cdot+}$ $[M]^+$, 158 $[C_{10}H_8NO]^+$, 141 $[C_7H_4OCl+2]^+$, 139 $[C_7H_4OCl]^+$, 130 $[C_9H_8N]^+$, 113 $[C_6H_4Cl+2]^+$, 111 $[C_6H_4Cl]^+$, 76 $[C_6H_4]^+$.

2-(1H-Indol-3-yl)-N -(2,4-dichlorobenzoyl)acetohydrazide (7e)

Cream colored amorphous powder; Yield: 81%; m.p: 196°C; Mol. Formula: $C_{17}H_{13}N_3O_2Cl_2$; Mol. Weight: 362 g/mol; IR (KBr)ν_{max}: 3438 (N-H), 3036 (C-H Ar), 1680 (C=O), 1529 (C=C Ar); ^{1}H-NMR (600 MHz): δ 10.89 (s, 1H, NH-1), 10.67 (s, 1H, -CH_2CONH),10.28 (s, 1H, -CONH), 7.83 (d, *J*=8.4 Hz, 1H, H-6'),7.43 (d, *J*=1.3 *Hz*, 1H, H-3'), 7.74 (dd, *J*=1.9, 7.3 Hz, 1H, H-5'), 7.71 (d, *J*=7.5 Hz, 1H, H-4), 7.36 (br. d, *J*=7.9 Hz, 1H, H-7), 7.28 (br. s, 1H, H-2), 7.06 (t, *J*=7.4 Hz, 1H, H-5), 6.98 (t, *J*=7.2 Hz, 1H, H-6), 3.64 (s, 2H, CH_2-10); EIMS (*m/z*): 366 $[C_{17}H_{13}N_3O_2Cl_2+4]^{\cdot+}$ $[M+4]^+$, 364 $[C_{17}H_{13}N_3O_2Cl_2+2]^{\cdot+}$ $[M+2]^+$, 362 $[C_{17}H_{13}N_3O_2Cl_2]^{\cdot+}$ $[M]^+$, 236 $[C_8H_5N_2O_2Cl_2+4]^+$, 234 $[C_8H_5N_2O_2Cl_2+2]^+$, 232 $[C_8H_5N_2O_2Cl_2]^+$, 208 $[C_7H_5N_2OCl_2+4]^+$, 206 $[C_7H_5N_2OCl_2+2]^+$, 204 $[C_7H_5N_2OCl_2]^+$, 193 $[C_7H_4NOCl_2+4]^+$, 191 $[C_7H_4NOCl_2+2]^+$, 189 $[C_7H_4NOCl_2]^+$, 188 $[C_{10}H_{10}N_3O]^+$, 178 $[C_7H_3OCl_2+4]^+$, 176 $[C_7H_3OCl_2+2]^+$, 174 $[C_7H_3OCl_2]^+$, 173 $[C_{10}H_9N_2O]^+$, 158 $[C_{10}H_8NO]^+$, 149 $[C_6H_3Cl_2+4]^+$, 147 $[C_6H_3Cl_2+2]^+$, 145 $[C_6H_3Cl_2]^+$, 130 $[C_9H_8N]^+$.

Pharmacological evaluation

Antibacterial assay

The antibacterial activity was performed according to the reported method, where the samples and organisms are mixed in sterile 96-well micro plates in aseptic environment after some dilution and the variation in turbidity is noted. The activity is mentioned as % inhibition and minimum inhibitory conc. (MIC) (Kaspady *et a.,* 2009; Yang *et a.,* 2006). The method is based on the principle that absorbance of broth medium increases with the increased number of microbial cells.

Enzyme Inhibition Assays

α-Glucosidase assay

The α-glucosidase inhibition activity was performed in accordance with the reported method (Pierre *et al.*, 1978). Total volume of the reaction mixture of 100μL contained 70μl 50mM phosphate buffer saline, pH 6.8, 10μl (0.5 mM) test compound, subsequently the addition of 10μl (0.057 units) enzyme. The contents were mixed, preincubated for 10 min at 37ºC and pre-read at 400 nm. The reaction was initiated by the addition of 10μl of 0.5 mM substrate (p-nitrophenylglucopyranoside). Acarbose was used as positive control. After 30min of incubation at 37ºC, absorbance was measured at 400nm using Synergy HT micro plate reader. All experiments were carried out in duplicates. The percent inhibition was calculated by the following equation:

$$\text{Inhibition}\,(\%) = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

IC_{50} values (concentration at which there is 50% in enzyme catalyzed reaction) compounds were calculated using EZ-Fit Enzyme Kinetics Software (Perrella Scientific Inc. Amherst, USA).

Butyrylcholinesterase (BChE) assay

The AChE and BChE inhibition activities were performed according to the reported method (Ellman *et al*, 1961). Total volume of the reaction mixture was 100μL. It contained 60μL Na_2HPO_4 buffer with concentration of (50mM and pH 7.7). Ten μL test compound (0.5mM well^{-1}) was added, followed by the addition of 10μL (0.005 unit well^{-1}) enzyme. The contents were mixed and pre read at 405 nm. Then contents were pre incubated for 10 mins at 37ºC. The reaction was initiated by the addition of 10μL of (0.5mM) well^{-1} substrate (acetylthiocholine iodide/ butyrylthiocholine iodide), after that the addition of 10μL DTNB (0.5mM well^{-1}). After 15mins of incubation at 37ºC absorbance was measured at 405nm. All experiments were carried out with their individual controls in triplicate. Eserine (0.5mM well^{-1}) was used as a positive control. The inhibition (%) and IC_{50} were calculated by the same method as described in α-glucosidase assay.

Lipoxygenase (LOX) assay

Lipoxygenase inhibition assay was done according to the cited methods (Baylac and Racine *et al*., 2003). Sodium phosphate buffer (100mM; pH 8.0, 150µL), test compound (10µL) and purified LOX enzyme (15µL) was taken to make total volume of 200µL. The mixture was thoroughly mixed, pre-incubated for 10 min at 25°C and absorbance was taken at 234 nm. Substrate solution (25 µL) was then added to start the reaction. The change in absorbance was noted at 234 nm after 6 minutes. All the experiments were done in triplicates. The negative ans positive controls were used in the assay. Baicalein (0.5 mM well^{-1}) was employed as a positive control. The % age inhibition and IC_{50} values were calculated by the same method as mentioned in α-glucosidase assay.

Hemolytic activity

Hemolytic activity was done by the reported method (Shahid *et al.*, 2013). Bovine blood was obtained from the Department of Clinical Medicine and Surgery, University

of Agriculture, Faisalabad, Pakistan. After centrifugation, separation and washing, the % RBCs lysis was computed by noting the absorbance.

Molecular docking methodology

To predict the bioactive conformations, various compounds (ligands) were docked into the binding pockets of the selected proteins (enzymes) by using the default parameters of MOE-Dock program. Prior to docking, the protein molecules of *α*-glucosidase, lipoxygenase and butyrylcholinesterase were retrieved from Protein Data Bank having PDB ID codes of 3NO4 (Resolution: 2.02Å), 1IK3 (Resolution: 2.0Å) and 1POP (Resolution: 2.0Å) respectively. All the water molecules were removed from receptor proteins and 3D protonation was carried out by using the Protonate 3D Option. The energies of protein molecules were minimized by using the default parameters of MOE 2009-10 energy minimization algorithm (gradient: 0.05, Force Field: MMFF94X). Then all the ligands were docked into the binding pockets (selective residues/amino acids as shown in the docking images) of the above mentioned proteins using Triangular Matching docking method. Re-docking procedure was also applied to validate the docking protocol. Each complex was analyzed for the type of interactions; bond distances and their 3D images were taken.

STATISTICAL ANALYSIS

Statistical analysis was performed by Microsoft Excel 2010. Results are given as mean ± SEM as all assays were carried out in triplicate.

RESULTS

The synthetic methodology was preceded as mentioned in (Scheme 1 & table 1a,1b) where ethyl 2-(1*H*-Indol-3-yl)acetate (**2**) was prepared by 2-(1*H*-Indol-3-yl)acetic acid (**1**) in absolute ethanol with catalytic amount of concentrated sulfuric acid and refluxed for 8 h. Ester 2 was stirred for 3 h at room temperature with hydrazine monohydrate in methanol to form 2-(1*H*-Indol-3-yl)acetohydrazide (3).The scheme was spliced in two pathways, where the acid hydrazide 3 was reacted with different aromatic aldehydes (4a-o) in methanol and 2-3 drops of glacial acetic acid (Scheme 1a) and with substituted acyl halides (6a-e) in distilled water and a few drops of NaOH (Scheme 1b). At completion cold distilled water was added and kept still for some time to complete precipitation, which were then filtered, washed and dried to get desired products. All compounds were characterized by IR, EI-MS and ^{1}H-NMR spectral data and screened for their enzyme inhibitory potentials against *α*-glucosidase, BChE and LOX enzymes. The enzyme inhibition data was also supported by computational docking which was done for all derivatives of parent molecule **3**. The antibacterial and % hemolytic activities were also performed for all the synthesized derivatives.

Pharmacological screening

Antibacterial activity

Two gram positive strains *Bacillus subtilis* and *Staphylococcus aureus* and three gram negative strains *Salmonella typhi, Pseudomonas aeruginosa* and *Euscheria coli* were used in this study to ascertain antibacterial potentials of synthesized compounds and their MIC values are given in (table 2). Ciprofloxacine was used as reference standard.

In vitro enzyme inhibition

All the derivatives were screened for inhibitory potentials against BChE, *α*-glucosidase and LOX enzymes and their % age inhibition and IC_{50} values are given in (table 3).

Hemolytic activity

Cytotoxicity was evaluated in terms of % age hemolytic activity (table 4) as compared to Triton-X and PBS which exhibit 100% and 0.0% hemolytic values respectively.

Computational docking

Synthesized 2-(1*H*-Indol-3-yl)-*N*□-[(un)substituted phenyl methylidene]acetohydrazides (5a-p) and 2-(1*H*-Indol-3-yl)-*N*□-[(un)substituted benzoyl]acetohydrazides (7a-e) were computationally docked and results were found in good agreement with their anti-enzymatic data.

DISCUSSION

Among 2-(1*H*-Indol-3-yl)-*N*□-[(substituted)phenylmethyl idene]acetohydrazides (5a-o) series, one of the compounds 5g is discussed in detail hereby for the expediency of the readers. It was obtained as yellow colored amorphous powder in 81% yield and the melting point was found to be 198^{o}C. The molecular formula $C_{17}H_{14}N_4O_3$ was established by molecular ion peak at *m/z* 322 in EI-MS spectrum and by the assigned protons in the ^{1}H-NMR spectrum. Different functionalities were confirmed by IR spectrum showing absorption bands at 3400 cm^{-1} for N-H stretching, at 3057 cm^{-1} for aromatic C-H stretching. The stretching bands observed at 1667 and 1487 cm^{-1} corresponded to C=O and C=C (aromatic) respectively. In the EI-MS spectrum, major fragment peaks were seen at *m/z* 173, 158 and 130 of cationic fragments indicating indole-3yl-methyl moiety and peaks at *m/z* 164 149, 135, 122, 118, 103, 89 and 76 belonged to cationic fragments of 4-nitrobenzyl group (fig. 1). ^{1}H-NMR spectrum showed two singlets in downfield region at *δ* 11.87 and 10.91 for two N-H protons pertaining to hydrazide and indole moiety respectively. A singlet at *δ* 8.33 with integration of one proton was assigned to H-7' (methylidene proton). An A_2B_2 spin system, characterized by two *ortho*-coupled doublets at *δ* 8.28 and *δ* 7.98, was

specific for 4-nitrophenyl group. Signals of indole-3yl-methyl appeared as two broad doublets at δ 7.59 and 7.35 having coupling constants 8.0 and 8.1 Hz for H-4 and H-7, respectively. A broad singlet at δ 7.26 was assigned to H-2 of this moiety. Two triplets appeared in relatively up field region at δ 7.07 and 6.98 with coupling constants 7.2 and 7.5 Hz, respectively, were assigned to H-5 and H-6 of indole moiety. In the aliphatic region, a singlet at δ 4.11 was assignable to methylene protons of C-10 in this molecule. On the basis of these cumulative evidences, 5g was confirmed as 2-(1*H*-Indol-3-yl)-*N*-[(4-nitrophenyl) methylidene]acetohydrazide.

Another compound 7d, among 2-(1*H*-Indol-3-yl)-*N*□-[(un)substituted benzoyll2-thienylcarbonyl] acetohydrazides (7a-e) series is also discussed thoroughly hereby. It was synthesized as a cream colored amorphous solid in 79% yield and its melting point was recorded as 176°C. The molecular formula $C_{17}H_{14}N_3O_2Cl$ was established by molecular ion peak at *m/z* 327 in EI-MS spectrum and by counting the number of protons in the ^{1}H-NMR spectrum. IR spectrum depicted the major absorption bands at 3438, 3032 and 1683 cm^{-1} due to N-H stretching, C-H aromatic stretching and stretching of unsaturated C=O, respectively, while stretching of aromatic C=C was observed at 1529 cm^{-1} in the molecule. The mass spectrum exhibited peaks at *m/z* 158, 157 and 130 related to indole containing fragments and peaks at *m/z* 139, 111 and 76 belonged to cationic fragments of *N*-substituted 2-chlorobenzoyl group in the molecule. In ^{1}H-NMR spectrum, two singlets were observed in the most down field region which belonged to one indole and two hydrazide protons. Two doublets at δ 7.64 and 7.51 were assigned to H-6' and H-4 respectively, while a broad triplet at δ 7.51 was assigned to H-3' of 2-chlorobenzoyl group. A multiplet ranging between δ 7.48-7.46 was assigned to H-4' and H-5'of 2-chlorobenzoyl group. Remaining signals of indole moiety appeared in relatively upfield aromatic region where, a broad doublet at δ 7.35 with coupling constant 8.1 Hz belonged to H-7 and a singlet of H-2 appeared at δ 7.27. Two more broad triplets at δ 7.07 and 6.98 with coupling constants 7.3 and 7.2 Hz were assigned to H-5 and H-6 of indole moiety. A singlet appearing in aliphatic region at δ 3.62 was assigned to methylene protons of C-10 of indole moiety. On the basis of These cumulative evidences, The structure of this compound was identified as *N*-(2-chlorobenzoyl)-2-(1*H*-Indol-3-yl)acetohydrazide. All other (2-(1*H*-Indol-3-yl)-*N*□-[(un)substituted phenylmethylidene] acetohydrazides (5a-o, table 1a) and 2-(1*H*-Indol-3-yl)-*N*□-[(un)substituted benzoyl/2-thienylcarbonyl] acetohydrazides (7a-e; table 1b) were characterized in the similar manner.

Antibacterial activity

Synthesized compounds 2-(1*H*-Indol-3-yl)-*N*□-[(un) substitutedphenylmethyli dene]acetohydrazides (5a-o) and 2-(1*H*-Indol-3-yl)-*N*□-[(un)substituted benzoyl/2-thienylcarbonyl]acetohydrazides (7a-e) were screened against two gram positive strains (*Bacillus subtilis* and *Staphylococcus aureus)* and three gram negative strains (*Salmonella typhi, Pseudomonas aeruginosa*, and *Escheria coli*) to ascertain Their antibacterial potentials. The % inhibition and MIC values are given in (table 2). Ciprofloxacin was used as reference standard. Compound 5h showed good antibacterial potential with MIC 09.79± 2.16μM against *P. aeruginosa* while all other compounds of this series (5a-o) exhibited moderate or no activity against all the strains used in this study. The compound 7a exhibited very good antibacterial activity having MICs 9.00±0.87, 9.68±0.69, 0.63±0.76 and 9.14±0.68 μM against *S. typhi, P. aeruginosa, B. subtilis* and *S. aureus,* respectively. This activity might be due to *N*-substituted benzoyl group in this molecule. The compound 7b also showed very good antibacterial activity having MIC 9.45 ±1.00 and 9.11±0.85μM against *P. aeruginosa* and *S. aureus,* respectively. This activity might be attributed to the presence of *N*-substituted 4-nitrobenzoyl group in the molecule. Likewise, compound 7c had shown very good antibacterial activity against *S. typhi, P.aeruginosa, B. subtilis* and *S. aureus* with MIC values 9.60±0.91, 9.98± 0.79, 9.45±1.05 and 9.22±0.89μM, respectively. The compound 7d showed very good antibacterial potentials against *P. aeruginosa* and *S. aureus* with MIC values 9.75 ±0.49 and 9.08±1.02μM, respectively. These good activities might be due to presence of 2-chlorobenzoyl group in the molecule. The compound 7e is also worth mentioning, that showed very good antibacterial activities against *S. typhi, P. aeruginosa* and *S. aureus* with MIC very close to the standard ciprofloxacin. This compound have 2,4-dichlorobenzoyl group that might be responsible for this activity.

Enzyme inhibition

The *in vitro* screening of 2-(1*H*-Indol-3-yl)-*N*□-[(un)substitutedphenylmethyli-dene]acetohydrazides (5a-o) and 2-(1*H*-Indol-3-yl)-*N*□[(un)substituted benzoyl/2-thienylcarbonyl)acetohydrazides (7a-e) against α-glucosidase, BChE and LOX revealed that these molecules exhibited variable inhibitory potentials as shown by their IC_{50} values (table 3). The molecule 7c, having *N*-substituted thienylcarbonyl group, showed moderate inhibitory potential with IC_{50} value of 127.71± 0.14 μM against α-glucosidase, relative to the reference standard Acarbose (IC_{50} 38.25±0.12μM), while the remaining compounds showed weaker or no inhibitory potential against this enzyme. The compound 5c was moderately active against BChE with IC_{50} value of 09.15±0.04μM as compared to standard Eserine (IC_{50} 0.85 ±0.0001μM) while other compound of this series (5a-o) showed weak or no activity against BChE enzyme. All 2-(1*H*-Indol-3-yl)-*N*□[(un)substituted-phenylmethylidene] acetohydrazides (5a-o) exhibited weak or no inhibitory

potential against LOX except 5l which showed moderate inhibition having IC_{50} value of 78.03±0.98μM. However, the compound 7b exhibited inhibitory potential (IC_{50}= 28.19±0.56 μM) very close to that of reference standard Baicalein (22.4±1.3μM), while compounds 7a, 7d and 7e showed excellent inhibitory potentials against LOX with IC_{50} values 3.59± 0.21, 4.43±0.31 and 13.51±0.36μM, respectively, which were even less than the standard Baicalein (22.4±1.3μM). These outstanding inhibitory potentials might be attributed to the presence of *N*-substituted benzoyl, 2-chlorobenzoyl and 2,4-dichlorobenzoyl groups respectively, in these molecules.

Hemolytic activity

All the synthesized compounds were screened for their cytotoxicity in terms of % hemolytic activities (table 5). It was inferred that 2-(1*H*-Indol-3-yl)-*N*□-[(un)substituted benzoyl/2-thienylcarbonyl]acetohydrazides (7a-c,7b & 7e), which exhibited very good antibacterial potentials against almost all the studied bacterial strains and also excellent inhibitory potentials against LOX, are very modest in cytotoxicity and hence can by utilized as potential therapeutic entrants for inflammatory ailments. Compound 7d revealed the highest % hemolytic activity (46.67%) among both the series of compounds. The compound 5l (0.33%), 5b (1.53%) and 5h (1.16%) also showed the lowest values of % hemolytic activities, which were very close to negative control which was phosphate buffer saline (PBS) having 0.00% hemolytic activity. The positive control used in this study was Triton-X which has 100% hemolytic activity.

Computational docking

In order to know about the validity of accuracy, the co-crystallized ligands of following enzymes were extracted and then re-docked into the binding pockets of the receptors. In all these cases, RMSD values between docked and co-crystallized ligands were less than 2A° which indicated about the reliability of docking method and thus our protocol can be used for further studies. Almost all the synthesized derivatives were computationally docked against α-glucosidase, BChE and LOX to explore the binding modes of all the ligands (fig. 2). The results were very much in favor of experimental work, for example, Compound 7a showed interactions with two active site amino acid residues of the target protein. Amino acid His367 showed a strong hydrogen bond donor (polar) interaction with acyl carbonyl oxygen residue having a bond distance of 1.95 angstrom, while Phe177 showed arene cation interaction with indole moiety. Other residues present in the nearby vicinity of the ligand were Leu607, His372, Ile406 and Leu179 (fig. 2a; 1, 2). One Amino acid residue interacted with compound 7d as Phe177 which interacted strongly with oxygen atom of acetohydrazide residue showing bond length of 2.48 angstroms. Other residues found in close contact with the ligand were Ala606, Asn180 and Leu607 etc (fig. 2b; 1, 2). Two amino acid residues interacted with compound 7e as His372 showed arene cation interaction with indole moiety, while Phe177 showed hydrogen bond donor interaction with acyl carbonyl oxygen having bond length of 3.25 angstroms. Other residues that existed in the near most region of the ligand were Leu179, Ile406, Lys409 and Asn180 (fig. 2c; 1, 2).

CONCLUSION

The presented work generally concluded that almost all compounds of 2-(1*H*-Indol-3-yl)-*N*□-[(un)substituted benzoyl/2-thienylcarbonyl]acetohydrazides (7a-e) series showed excellent antibacterial activity with MIC values very close to Ciprofloxacin. Compound 5h showed good antibacterial potential against *P. aeruginosa.* Enzyme inhibition data revealed that compounds 7a, 7d and 7e showed excellent inhibitory potentials against LOX, which were higher than the standard Baicalein. Their inhibitory potentials were also supported by computational docking results. These compounds showed low % hemolytic activity values except the compound 7d which showed comparatively higher value of % hemolytic activity.

On the basis of aforesaid results, it can be concluded that 2-(1*H*-Indol-3-yl)-*N*□-[(un)substitutedbenzoyl) acetohydrazides (7a, 7c-e) which are excellent LOX inhibitors may prove as potent drugs candidates for the treatment of various inflammatory diseases.

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