DESIGN, SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF NOVEL 2-(1-BENZYL-2-OXOINDOLIN-3-YLIDENE)-N-PHENYLHYDRAZINECARBOXAMIDE DERIVATIVES.

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Abstract:
Novel 2-(1-benzyl-2-oxoindolin-6-substituted-3-ylidene)-n-phenyl hydrazine carboxamide derivatives are an important class of nitrogen containing heterocyclic's and were identified as the most active and potent classes of compounds with wide range of biological and pharmacological activities. A new series of 2-(1-benzyl-2-oxoindolin-3-ylidene)-n-phenyl hydrazine carboxamide derivatives were characterized by spectral data and evaluated for Anticancer activity, Anti-Alzheimer's activity and anti-microbial activity. The compounds **7d, 7i,** were found to be good inhibitors of HELa and MCF-7. Most of the compounds inhibited HELa and MCF-7with the IC_{50} values, ranging from 0.78µM 7.64 µM. Amongst them, compounds 7d, **7i** with fluoro substituted indole exhibited strongest inhibitory activity against HELa and MCF-7 with IC_{50}1.91±0.02 µM, 0.92±0.03 µM for 7d and 7i 1.31±0.02µM, 0.78±0.3 respectively, when compared to standard Cisplain with IC_{50} of 0.65±0.02 µM and 0.47±0.02. The compound 7i with fluoro substituted indole containing Schiff base showed potent anti-Alzheimer's activity with IC_{50} values 0.92±0.13, 1.01±0.12µM against AChE and BuChE respectively when compared to Standard drug donepazil showed IC_{50} 0.51±0.06 and 0.31±0.04µM against AChE and BuChE respectively. 7i ,7m & 7n showing potent activity for different strains of bacteria ranging from 10mm to 14mm when compared to ciprofloxacin 13- 16mm. Thus, these Novel 2-(1-benzyl-2-oxoindolin-3-ylidene)-n-phenyl hydrazine carboxamide derivatives have emerged as new cancer, AChE and BuChE inhibitors and showing anti microbial activity for further exploitation as anti-cancer and anti-Alzheimer's agents, anti bacterial agents. Docking studies of all the molecules disclosed close hydrogen bond interactions with in the binding site.

Keywords: Alzheimer's disease, anti-cancer, cholinesterase inhibitor, Anti-bacterial agents

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1.0. INTRODUCTION:

Indole is wide application in life sciences has stimulated the development of a number of methods for its derivative synthesis. It has a bicyclic structure, consisting of a six-membered benzene ring fused to a five-membered pyrrole ring.

In the last few decades, a series of molecular-targeted small-molecule cancer drugs have been introduced to the clinic. Various heterocyclic ring systems in the structure of these drugs have taken their place in the center of medicinal chemistry studies as very useful tools and building blocks for the synthesis of these small molecule cancer therapeutics. Indole is a very important heterocyclic system as the main structure of the essential amino acid tryptophan and is the building block of many compounds of natural origin. Therefore, it is included in the structure of many molecules such as naturally sourced proteins, receptors, hormones, enzymes, neurotransmitters, andalkaloids (7-9).

The wide variety and strong biologicalactivity of natural compounds containing the indolering has also attracted the attention of researchers over the years and has led to the isolation and/or synthesis of numerous compounds containing the indole ring. Animportant part of anti-cancer compounds are molecules that inhibit tubulin polymerization. After the isolation of vinca alkaloids vincristine and vinblastine and determination of their biological activities, one of the interesting and important biological activities of the compounds containing the indole ring is undoubtedly its anti-cancer effect.

Cediranib is an indole derivative withpotent inhibitor activity of vascular endothelial growth factor (VEGF) receptor tyrosine kinases (14). Osimertinib, for the treatment of NSCLC and advanced renal cellcarcinoma, and sunitinib, for the treatment of gastrointestinalstromal tumors, are also the indole containingdrugs (15). Additionally, anlotinib, a novel oral multitargettyrosine kinase inhibitor for advanced lung canceris also indole-derived small drug inhibitor (16).

2.0 NEED FOR THE PRESENT WORK

It is apparent from the foregoing survey that many new Schiff based isatin derivatives have been synthesized as potential anticancer agents. As the Schiff based indole nuclei are among the most extensively studied classes of heterocyclic compounds possessing anticancer, antiinfective, antibacterial and antifungal properties, also useful various other pathological conditions. It is thought worthwhile to synthesize Schiff based indole derivatives linked with hydrazinebridge as shown in **General StructureI** containing some electron-withdrawing/electron-releasing substituents on isatin.

3.0 MATERIALS AND METHODS:

3.1. Chemistry:

Melting points were determined by open capillary tubes using VEEGO VMP-D Digital melting point apparatus. FTIR spectra of the powdered compounds were recorded using KBr on JASCO FTIR 4100 series and are reported in cm^{-1}. ^{1}H NMR and ^{13}C NMR spectra were recorded on a BRUKER-II 400 (400 MHz NMR, ^{13}C NMR 100 MHz) spectrophotometer using TMS as an internal reference. Purity of the compounds was checked on pre-coated TLC plates using silica G as stationary phase, iodine vapors and ultra-violet rays as the visualizing agent. All chemicals including standard drugs and solvents were procured from Sigma-Aldrich, Hi Media, Bangalore, India and others. The estimation of biochemical parameters was carried out using kits (Sigma-Aldrich).

3.2. General synthetic procedure:

3.2.1. Synthesis of 1-benzylindoline-2,3-dione (3)

A mixture of Substituted indole(2.4 mmol), benzyl chloride (0.90gm, 5.0 mmol) in anhydrous DMSO (20ml) were refluxed for 20 h under reduced pressure. The reaction mixture was poured into water (50ml) followed by extracting with dichloromethane (10ml, 3x), the combined extracts were washed three times with distilled water. After removal of the solvent, the residue was purified with dichloromethane.

3.2.2. Synthesis of (Z)-1-benzyl-3-hydrazono-6-substitited-indolin-2-one (4)

In a cleaned dry round bottomed flask placed 80ml of absolute alcohol and equimolar quantities of 1-benzyl-6-substitited-indoline-2,3-dione and hydrazine hydrate and addition of glacial acetic acidby drop wise. Refluxed the mixture for about 2 h. During heating period itself, the crystals of compound (4) started separating out. Then the reaction mixture was cooled to room temperature and poured into crushed ice with stirring. After standing for 1 or 2h, the product separated was filtered and washed several times with small portions of cold water and then dried.

3.2.3. Synthesis of (Z)-phenyl 2-(1-benzyl-2-oxo-6-substituted-indolin-3-ylidene)hydrazinecarboxylate (5)

An appropriate (Z)-1-benzyl-3-hydrazono-6-substitited -indolin-2-one (4) (0.01mol) was heated

under reflux with phenyl chloroformate (0.01mol) in dry acetone in presence of potassium carbonate under anhydrous condition using calcium chloride guard tube for 2h. The product thus formed was filtered and washed with small portions of acetone to remove any unreacted ethylchloroformate. It was purified by recrystallization from ethanol.

3.2.4. Synthesis of 2-(1-benzyl-2-oxoindolin-6-substituted-3-ylidene)-N-phenylhydrazinecarboxamide (7a-o)

An appropriate (Z)-phenyl 2-(1-benzyl-2-oxo-6-substituted-indolin-3-ylidene) hydrazine carboxylate(5)(0.01mol) was heated under refluxwith various substituted amines (6, 0.01mol) in dry acetone under anhydrous conditions using calcium chloride guard tube for 2h. The progress wasmonitored by TLC using petroleum ether/ ethyl acetate (2:1). The solventwas evaporated in vacuum and the yellow product was collected,and on recrystallization with ethanol gave the desired product2-(1-benzyl-2-oxoindolin-6-substituted-3-ylidene)-N-phenylhydrazine carboxamide (7a-o).

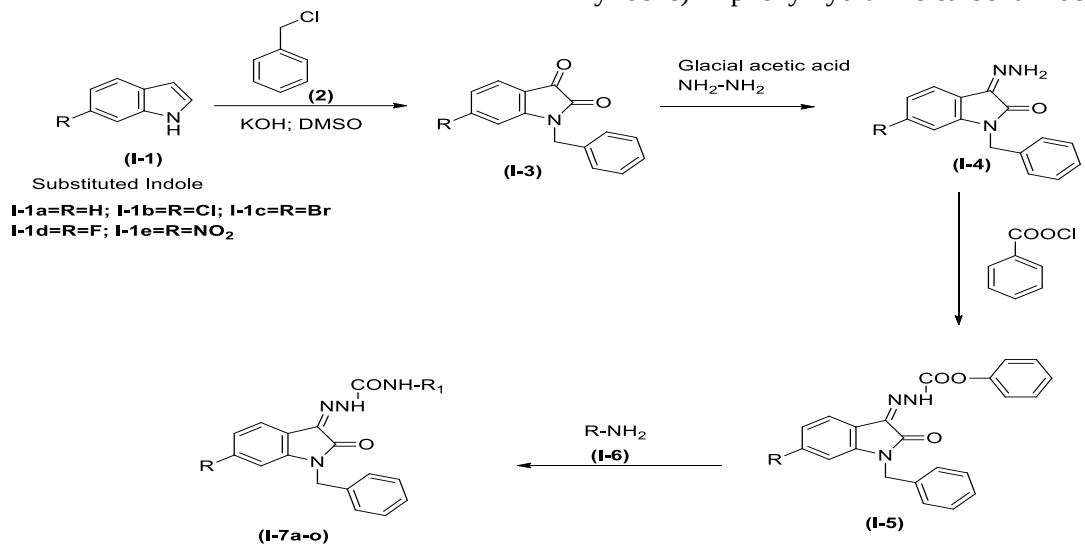
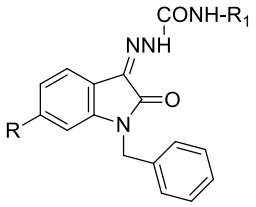


Table-1.Physical data of Synthesized compounds7a-7o.



General structure-I

Compound	R	R_1	M. Form	M.Wt	M.P	Rf*	% Yield
7a	H	3-Nitro phenyl	$C_{22}H_{17}N_5O_4$	415	235-237	0.7	57
7b	Cl	3-Nitro phenyl	$C_{22}H_{16}ClN_5O_4$	449	268-270	0.4	60
7c	Br	3-Nitro phenyl	$C_{22}H_{16}BrN_5O_4$	494	203-205	0.6	75
7d	F	3-Nitro phenyl	$C_{22}H_{16}FN_5O_4$	433	210-212	0.5	68
7e	NO_2	3-Nitro phenyl	$C_{22}H_{16}ClN_5O_4$	449	198-200	0.8	52
7f	H	3- chloro phenyl	$C_{22}H_{17}ClN_4O_2$	404	248-250	0.6	61
7g	Cl	3- chloro phenyl	$C_{22}H_{16}Cl_2N_4O_2$	439	263-265	0.4	38
7h	Br	3- chloro phenyl	$C_{22}H_{16}BrClN_4O_2$	483	270-272	0.5	78
7i	F	3- chloro phenyl	$C_{22}H_{16}ClFN_4O_2$	422	169-171	0.7	57
7j	NO_2	3- chloro phenyl	$C_{22}H_{16}ClN_5O_4$	449	188-190	0.5	72
7k	H	3,4dichloro phenyl	$C_{22}H_{16}Cl_2N_4O_2$	439	230-232	0.5	84
7l	Cl	3,4dichloro phenyl	$C_{22}H_{15}Cl_3N_4O_2$	478	195-197	0.8	65
7m	Br	3,4dichloro phenyl	$C_{22}H_{15}BrCl_2N_4O_2$	474	263-265	0.4	59
7n	F	3,4dichloro phenyl	$C_{22}H_{15}Cl_2FN_4O_2$	457	190-192	0.8	81
7o	NO_2	3,4dichloro phenyl	$C_{22}H_{15}Cl_2N_5O_4$	484	214-216	0.5	48

* Mobile phase: hexane: ethyl acetate

3.4.1 Characterization of compounds:

3.4.1.12-(1-benzyl-2-oxoindolin-3-ylidene)-N-(3-nitrophenyl)hydrazinecarboxamide (7a): Compound **7a** obtained as yellowish orange solid.

^{1}H NMR (400MHz DMSO, δ ppm):8.821-8843 (d, 2H,indole amide, Ar-H), 8.228-8.290 (m, 5H,Ar-H), 7.808-7.846 (t, 2H,*J*=4.0 Hz, Ar-H), 7.697-7.737(m, 3H,Ar-H),7.550-7.582(d, 3H, Ar-H), 7.430-7.455 (d,1H, Ar-H),4.082 (s, 2H Aliphatic CH).

^{13}C NMR (100MHz, DMSO):157.81, 152.64, 142.08, 132.54, 131.08, 129.35, 129.09, 128.56, 128.14, 125.88, 125.27, 124.48, 114.02,101.28, 57.20, MASS spectrum m/z: 371[M+1]$^+$,Calc. for $C_{22}H_{17}N_5O_4$; CHN: 60.15; H, 3.67N, 12.75; O, 7.28; Found: C, 60.10; H, 3.61; N, 12.71; O, 7.20. IR (KBr, cm^{-1}): 3080.32 (C-H, Aromatic), 2931.73 (C-H, Aliphatic), 1606.35(C=O**),** 1585.20 (C=C, Aromatic), 1158.40 (C-O).

3.4.1.2(E)-2-(1-benzyl-6-chloro-2-oxoindolin-3-ylidene)-N-(3-nitrophenyl)-hydrazinecarboxamide (7b).
Compound **7b** obtained as yellowish orange solid.

^{1}H NMR (400MHz DMSO, δ ppm):9.35 (s, 1H, indole amide), 7.85-7.91 (m, 5H,Ar-H) 7.63-7.65 (d, 1H,*J*=4.0 Hz, Ar-H), 7.53-7.55 (d, 3H,*J*=4.0 Hz, Ar-H),7.38-7.42 (m, 5H, Ar-H), 7.25 (s,1H, amide), 4.85 (s, 1H).

^{13}C NMR (100MHz, DMSO): 163.3, 155.10, 136.10, 134.05, 132.12, 130.20, 128.23 126.14, 127.18, 126.75, 126.25, 126.18, 124.52, 119.70, 117.12, 115.8, 48.58.**MASS spectrum m/z:**406.14[M+2]$^+$Calc. for $C_{22}H_{17}ClN_4O_2$; CHN: C, 65.27; H, 4.23; Cl, 8.76; N, 13.84; O, 7.90; Found: C, 65.20; H, 4.13; Cl, 8.16; N, 13.44; O, 7.95. IR (KBr, cm^{-1}): 3401.25 (NH, amide) 3060.21 (C-H, Aromatic), 2968.10 (C-H, Aliphatic), 1716.96 (C=O), 1516.01 (C=C, Aromatic), 1265.51 (C-O).

3.4.1.3(E)-2-(1-benzyl-6-bromo-2-oxoindolin-3-ylidene)-N-(3-nitrophenyl)-hydrazine-carboxamide (7c)
Compound **7c** obtained as yellowish white solid.

^{1}H NMR (400MHz DMSO, δ ppm):9.41 (s, 1H, indole amide), 7.80-7.85 (m, 4H,Ar-H) 7.72-7.75 (d, 2H,*J*=4.0 Hz, Ar-H), 7.55-7.58 (d, 4H,*J*=4.0 Hz, Ar-H),7.32-7.38 (m, 4H, Ar-H), 7.21 (s,1H, amide), 4.35 (s, 1H).

^{13}C NMR (100MHz, DMSO): 161.3, 150.10, 138.10, 135.01, 130.10, 129.42, 128.23 126.14, 127.18, 126.75, 126.25, 126.18, 124.52, 118.70, 115.10, 114.3, 45.18. **MASS spectrum m/z:**485.14[M+4]$^+$487.32Calc. for $C_{22}H_{16}BrClN_4O_2$; CHN: C, 54.62; H, 3.33; N, 11.58; O, 6.61Found: C, 54.60; H, 3.30; 7.33; N, 11.50; O, 6.55. IR (KBr, cm^{-1}): 3401.25 (NH, amide) 3060.21 (C-H, Aromatic), 2968.10 (C-H, Aliphatic), 1716.96 (C=O), 1516.01 (C=C, Aromatic), 1265.51 (C-O).

3.4.1.4(E)-2-(1-benzyl-6-fluoro-2-oxoindolin-3-ylidene)-N-(3-nitrophenyl)-hydrazinecarboxamide (7d):
^{1}H NMR (400MHz DMSO, δ ppm):9.42 (s, 1H, indole amide), 7.83-7.86 (m, 5H,Ar-H) 7.70-7.74 (d, 4H,*J*=4.0 Hz, Ar-H), 7.50-7.52 (d, 2H,*J*=4.0 Hz, Ar-H),7.32-7.38 (m, 3H, Ar-H), 7.23 (s,1H, amide), 4.34 (s, 1H).

^{13}C NMR (100MHz, DMSO): 161.3, 150.10, 138.10, 135.01, 130.10, 129.42, 128.23 126.14, 127.18, 126.75, 126.25, 126.18, 124.52, 118.70, 115.10, 114.3, 45.18. **MASS spectrum m/z:**485.14[M+4]$^+$487.32Calc. for $C_{22}H_{16}BrClN_4O_2$; CHN: C, 62.49; H, 3.81; N, 13.25; O, 7.57. Found: C, 62.40; H, 3.85; N, 13.20; O, 7.53**.** IR (KBr, cm^{-1}): 3051.89 (C-H, Aromatic), 2922.61 (C-H, Aliphatic), 1722.32 (C=O), 1588.12 (C=C, Aromatic), 1253.72(C-O).

3.4.1.5(E)-2-(1-benzyl-6-nitro-2-oxoindolin-3-ylidene)-N-(3-nitrophenyl)-hydrazinecarboxamide (7e)
Compound **7e** obtained as yellowish white solid.**^{1}H NMR (400MHz DMSO, δ ppm):**9.45 (s, 1H, indole amide), 7.90-7.94 (m, 2H,Ar-H) 7.72-7.75 (d, 2H,*J*=4.0 Hz, Ar-H), 7.55-7.60 (d, 6H,*J*=4.0 Hz, Ar-H),7.30-7.34 (m, 4H, Ar-H), 7.21 (s,1H, amide), 4.32 (s, 1H).**^{13}C NMR (100MHz, DMSO):** 161.3, 150.10, 138.10, 135.01, 130.10, 129.42, 128.23 126.14, 127.18, 126.75, 126.25, 126.18, 124.52, 118.70, 115.10, 114.3, 45.18. **MASS spectrum m/z:**449.14[M+2]$^+$451.32Calc. for $C_{22}H_{16}ClN_5O_4$; CHN: C, 58.74; H, 3.59; N, 15.57; O, 14.23Found: C, 58.56; H, 3.55; N, 15.51; O, 14.28 IR (KBr, cm^{-1}): 3058.56 (C-H, Aromatic), 2976.58 (C-H, Aliphatic), 1724.84 (C=O**),** 1591.59 (C=C, Aromatic), 1080.34 (C-O).

3.4.1.6(E)-2-(1-benzyl-2-oxoindolin-3-ylidene)-N-(3-chlorophenyl)hydrazine-carboxamide (7f)
Compound **7f** obtained as white solid.

^{1}H NMR (400MHz DMSO, δ ppm):9.41 (s, 1H, indole amide), 7.85-7.90 (m, 5H,Ar-H) 7.74-7.76 (d, 2H,*J*=4.0 Hz, Ar-H), 7.55-7.58 (d, 4H,*J*=4.0 Hz, Ar-H),7.32-7.38 (m, 4H, Ar-H), 7.21 (s,1H, amide), 4.35 (s, 1H). **^{13}C NMR (100MHz, DMSO):** 160.3, 152.10, 140.10, 138.11, 135.12, 129.42, 128.23 126.14, 127.18, 126.75, 126.25, 126.18, 124.52, 118.70, 115.10, 114.3, 48.18. **MASS spectrum m/z:**404.14[M+4]$^+$406.32Calc. for $C_{22}H_{16}BrClN_4O_2$; CHN: C, 54.62; H, 3.33; N, 11.58; O, 6.61Found: C, 54.60; H, 3.30; 7.33; N, 11.50; O, 6.55. IR (KBr, cm^{-1}): 3401.25 (NH, amide) 3060.21 (C-H, Aromatic), 2968.10 (C-H, Aliphatic), 1716.96 (C=O), 1516.01 (C=C, Aromatic), 1265.51 (C-O).

3.4.1.7(E)-2-(1-benzyl-5-chloro-2-oxoindolin-3-ylidene)-N-(3-chlorophenyl)hydrazine-carboxamide(7g): Compound **7g** obtained as cream colour solid.**^{1}H NMR (400MHz DMSO, δ ppm):**9.35 (s, 1H, indole amide), 7.75-7.80 (m, 4H,Ar-H) 7.64-7.66 (d, 2H,*J*=4.0 Hz, Ar-H), 7.55-7.58(d, 5H,*J*=4.0 Hz, Ar-H),7.35-7.39 (m, 3H, Ar-H), 7.05 (s,1H, amide), 4.90 (s, 1H). **^{13}C NMR (100MHz, DMSO):** 165.5, 150.23, 138.16, 136.15, 132.10, 130.38, 129.8 128.2, 127.08, 126.15, 126.25, 126.18, 124.52, 119.70, 118.12, 114.5, 47.18, **MASS spectrum m/z:**441.14[M+2]$^+$,443.25[M+4]$^+$,Calc. for $C_{22}H_{17}ClN_4O_2$; CHN: C, 60.15; H, 3.67; N, 12.75; O, 7.28 Found: C, 60.10; H, 3.61; N, 12.71; O, 7.20. IR (KBr, cm^{-1}): 3103.33 (C-H, Aromatic), 2922.15 (C-H, Aliphatic), 1718.28 (C=O**),** 1590.56 (C=C, Aromatic), 1184.54 (C-O).

3.4.1.8.(E)-2-(1-benzyl-5-bromo-2-oxoindolin-3-ylidene)-N-(3-chlorophenyl)hydrazine-carboxamide (7h)

Compound **7h** obtained as yellowish white solid.**^{1}H NMR (400MHz DMSO, δ ppm):**9.36 (s, 1H, indole amide), 7.82-7.85 (m, 5H,Ar-H) 7.63-7.65 (d, 1H,*J*=4.0 Hz, Ar-H), 7.55-7.58(d, 3H,*J*=4.0 Hz, Ar-H),7.40-7.45(m, 5H, Ar-H), 7.28(s,1H, amide), 4.80 (s, 1H).**^{13}C NMR (100MHz, DMSO):** 161.5, 156.15, 138.15, 136.15, 132.12, 130.20, 128.23 126.14, 127.18, 126.75, 126.25, 126.18, 124.52, 119.70, 117.12, 115.8, 48.58. **MASS spectrum m/z:**485.14[M+2]$^+$, 487.15 [M+4]$^+$.Calc. for $C_{22}H_{16}BrClN_4O_2$; CHN: C, 54.62; H, 3.33; Br, 16.52; N, 11.58;Found: C, 54.62; H, 3.33; Br, 16.52; N, 11.58;IR (KBr, cm^{-1}):3086.15 (C-H, Aromatic), 2922.83 (C-H, Aliphatic), 1722.81(C=O), 1549.75 (C=C, Aromatic), 1269.79 (C-O).

3.4.1.9(E)-2-(1-benzyl-5-fluoro-2-oxoindolin-3-ylidene)-N-(3-chlorophenyl)-hydrazine-carboxamide (7i)

Compound **7i** obtained as yellowish white solid.**^{1}H NMR (400MHz DMSO, δ ppm):**9.41 (s, 1H, indole amide), 8.328-8.374 (m, 2H,Ar-H), 8.132-8.232 (m, 2H, Ar-H), 7.816-7.895(m, 3H, Ar-H),7.675-7.716(t, 2H, Ar-H), 7.536-7.622(m, 3H, Ar-H),7.293-7.314(d, 1H, Ar-H), 7.197-7.218(d, 1H, Ar-H),3.360 (s, 1H). **^{13}C NMR (100MHz, DMSO):**196.34, 168.13, 164.53, 153.78, 152.34, 143.63, 142.44, 139.59 138.74, 136.03, 129.96, 129.40, 127.96, 126.73, 125.01, 119.69, 48.15, **MASS spectrum m/z:** 424.14[M+2]$^+$,426.14[M+4]$^+$Calc. for $C_{22}H_{16}ClFN_4O_2$; CHN: C, 62.49; H, 3.81; N, 13.25; Found: C, 62.44; H, 3.85; N, 13.25;IR spectrum (KBr, cm^{-1}): 3071.66(C-H, Aromatic), 2981.22(C-H, Aliphatic), 1730.51 (C=O), 1591.45(C=C, Aromatic, 1294.70(C-O).

3.4.1.10(E)-2-(1-benzyl-5-nitro-2-oxoindolin-3-ylidene)-N-(3-chlorophenyl)hydrazine-carboxamide (7j):

^{1}H NMR (400MHz DMSO, δ ppm):9.45 (s, 1H, indole amide), 7.80-7.85 (m, 4H,Ar-H) 7.70-7.72 (d, 4H,*J*=4.0 Hz, Ar-H), 7.54-7.56(d, 2H,*J*=4.0 Hz, Ar-H),7.32-7.38 (m, 4H, Ar-H), 7.23 (s,1H, amide), 4.35 (s, 1H).**^{13}C NMR (100MHz, DMSO):** 161.3, 150.10, 138.10, 135.01, 130.10, 129.42, 128.23 126.14, 127.18, 126.75, 126.25, 126.18, 124.52, 118.70, 115.10, 114.3, 45.18. **MASS spectrum m/z:** [M+2]$^+$Peak observed at 451.14.Calc. for $C_{22}H_{16}BrClN_4O_2$; CHN: C, 58.74; H, 3.59; N, 15.57;Found: C, 58.70; H, 3.55; N, 15.57;IR (KBr, cm^{-1}): 3051.89 (C-H, Aromatic), 2922.61 (C-H, Aliphatic), 1722.32 (C=O), 1588.12 (C=C, Aromatic), 1253.72(C-O).

3.4.1.11(E)-2-(1-benzyl-2-oxoindolin-3-ylidene)-N-(3,4-dichlorophenyl)hydrazinecarboxamide (7k); Compound **7k**obtained as yellowish white solid.**^{1}H NMR (400MHz DMSO, δ ppm):**9.44(s, 1H, indole amide), 7.89-7.91 (m, 4H,Ar-H) 7.70-7.73 (d, 2H,*J*=4.0 Hz, Ar-H), 7.58-7.61(d, 4H,*J*=4.0 Hz, Ar-H),7.30-7.34 (m, 3H, Ar-H), 7.20(s,1H, amide), 4.35 (s, 1H).**^{13}C NMR (100MHz, DMSO):** 160.1, 155.12, 137.10, 136.08, 132.10, 129.42, 128.23 126.14, 127.18, 126.75, 126.25, 126.18, 124.52, 118.70, 115.10, 112.3, 43.18. **MASS spectrum m/z:**[M+2]$^+$Peak observed at 441.25 and [M+4]$^+$443.25; Calc. for $C_{22}H_{16}Cl_2N_4O_2$; CHN: C, 60.15; H, 3.67; N, 12.75;Found: C, 60.15; H, 3.67; N, 12.75;IR (KBr, cm^{-1}): 3068.52(C-H, Aromatic), 2980.20 (C-H,

Aliphatic), 1716.34(C=O**),** 1535.12(C=C, Aromatic), 1135.18(C-O).

3.4.1.12(E)-2-(1-benzyl-5-chloro-2-oxoindolin-3-ylidene)-N-(3,4-dichlorophenyl)-hydrazine-carboxamide (7l):

Compound **7l** obtained as cream colour solid.**^{1}H NMR (400MHz DMSO, δ ppm):**9.36 (s, 1H, indole amide), 7.75-7.80 (m, 4H,Ar-H) 7.64-7.66 (d, 2H,*J*=4.0 Hz, Ar-H), 7.55-7.58 (d, 4H,*J*=4.0 Hz, Ar-H),7.35-7.39 (m, 3H, Ar-H), 7.05 (s,1H, amide), 4.90 (s, 1H). **^{13}C NMR (100MHz, DMSO):** 165.5, 150.23, 138.16, 136.15, 132.10, 130.38, 129.8 128.2, 127.08, 126.15, 126.25, 126.18, 124.52, 119.70, 118.12, 114.5, 47.18, **MASS spectrum m/z:**473.12 $[M+]^+$,475.14$[M+2]^+$,477.25$[M+4]^+$,Calc. for $C_{22}H_{15}Cl_3N_4O_2$; CHN: C, 55.78; H, 3.19; N, 11.83; Found: C, 55.78; H, 3.19; N, 11.83; IR (KBr, cm^{-1}): 3103.33 (C-H, Aromatic), 2922.15 (C-H, Aliphatic), 1718.28 (C=O**),** 1590.56 (C=C, Aromatic), 1184.54 (C-O).

3.4.1.13(E)-2-(1-benzyl-5-bromo-2-oxoindolin-3-ylidene)-N-(3,4-dichlorophenyl)-hydrazine-carboxamide (7m):

Compound **7m** obtained as white solid.**^{1}H NMR (400MHz DMSO, δ ppm):**9.32 (s, 1H, indole amide), 7.80-7.83 (m, 4H,Ar-H) 7.60-7.63 (d, 2H,*J*=4.0 Hz, Ar-H), 7.58-7.60 (d, 3H,*J*=4.0 Hz, Ar-H),7.40-7.45 (m, 4H, Ar-H), 7.28 (s,1H, amide), 4.80 (s, 1H). **^{13}C NMR (100MHz, DMSO):** 160.5, 158.15, 138.15, 136.15, 132.12, 130.20, 128.23 126.14, 127.18, 126.75, 126.25, 126.18, 124.52, 119.70, 117.12, 115.8, 48.58. **MASS spectrum m/z:**518.12 $[M+]^+$, 520.25 $[M+2]^+$, 522.31$[M+4]^+$, 524.45 $[M+6]^+$.Calc. for $C_{22}H_{16}BrClN_4O_2$; CHN: C, 54.62; H, 3.33; Br, 16.52; N, 11.58;Found: C, 54.62; H, 3.33; Br, 16.52; N, 11.58;IR (KBr, cm^{-1}): 3082.10 (C-H, Aromatic), 2975.15 (C-H, Aliphatic), 1720.12(C=O**)**, 1541.20 (C=C, Aromatic), 1150.68(C-O).

3.4.1.14(E)-2-(1-benzyl-5-fluoro-2-oxoindolin-3-ylidene)-N-(3,4-dichlorophenyl)hydrazine-carboxamide (7n): Compound **7n** obtained as yellowish solid.**^{1}H NMR (400MHz DMSO, δ ppm):**9.39 (s, 1H, indole amide), 7.79-7.84 (m, 4H,Ar-H) 7.73-7.75 (d, 2H,*J*=4.0 Hz, Ar-H), 7.56-7.60 (d, 4H,*J*=4.0 Hz, Ar-H),7.35-7.38 (m, 3H, Ar-H), 7.20 (s, 1H, amide), 4.38 (s, 1H). **^{13}C NMR (100MHz, DMSO):** 164.5, 154.10, 140.10, 138.01, 135.10, 130.42, 129.23 128.14, 127.18, 126.75, 126.25, 126.18, 124.52, 118.70, 115.10, 114.3, 43.18. **MASS spectrum m/z:**457.14$[M+]^+$,459.28 $[M+2]^+$, 461.32 $[M+4]^+$; Calc. for $C_{22}H_{15}Cl_2FN_4O_2$; CHN: C, 57.78; H, 3.31; N, 12.25; Found: C, 57.70; H, 3.35; N, 12.20; IR spectrum (KBr, cm^{-1}): 3023.18(C-H, Aromatic), 2974.62(C-H, Aliphatic), 1732.35 (C=O), 1585.25 (C=C, Aromatic, 1280.24 (C-O).

3.4.1.15(E)-2-(1-benzyl-5-nitro-2-oxoindolin-3-ylidene)-N-(3,4-dichlorophenyl)-hydrazine-carboxamide (7o):

^{1}H NMR (400MHz DMSO, δ ppm):9.42 (s, 1H, indole amide), 7.79-7.84 (m, 4H,Ar-H), 7.72-7.74 (m, 5H,*J*=4.0 Hz, Ar-H), 7.54-7.56 (d, 2H,*J*=4.0 Hz, Ar-H),7.30-7.35 (m, 2H, Ar-H), 7.23 (s,1H, amide), 4.35 (s, 1H). **^{13}C NMR (100MHz, DMSO):** 160.3, 151.10, 140.10, 138.21, 134.10, 130.42, 128.23 126.14, 127.18, 126.75, 126.25, 126.18, 124.52, 118.70, 115.10, 114.3, 45.18. **MASS spectrum m/z:**484.25 $[M+]^+$, 486.13 $[M+2]^+$, 488.32 $[M+4]^+$.Calc. for $C_{22}H_{15}Cl_2N_5O_4$; CHN: C, 54.56; H, 3.12; N, 14.46;Found: C, 54.50; H, 3.18; N, 14.40;IR (KBr, cm^{-1}): 3050.19 (C-H, Aromatic), 2928.65 (C-H, Aliphatic), 1720.30 (C=O), 1589.18 (C=C, Aromatic), 1263.72(C-O).

4.0 ANTICANCER ACTIVITY:

4.1 MTT ASSAY:

Procurement of cell line: The HELa and MCF-7 cell line with passage number 45 was procured from NCCS, Pune.

Media Preparation: Pour 20-30 ml MEM media in centrifuge. To this add 10ml of bovine serum, 0.5 ml antibiotic solution, 1.25ml HEPES and make up volume up to 50ml by appropriate media. Mix it and store at 208ºC (for up to 4 weeks).

Sub culturing cells: Take above solution and remove the media and wash with PBS. Remove PBS and add 1ml trypsin-EDTA solution. Incubate the flask at 37ºC in CO2 incubator(17).

Table -2 Protocol for MTT assay:

Day 1 (Cells Suspended)	Day 2 (Drug Treatment)	Day 3 (MTT Assay)
Washing with PBS	Removed the plate form incubator to remove the media from each well	25 µl of freshly prepared MTT added in each well
Trypsinization	Drug treatment (10, 25, 50,100,200 and 300µM)	Keep in nncubator at 37°C for 2-3h
Cell Counting	Untreated / DMSO	Media was removed
Filled 96 well Plate (10000 cells/well)	Incubation (24h)	DMSO (100 µl) was added

Incubation (24h)		Kept the plate in Incubator at 37°C for overnight and recorded plate at 570nm in ELISA plate reader

Traditionally, the determination of cell growth is done by counting viable cells after staining with a vital dye. Several approaches have been used in the past. Trypan blue staining is a simple way to evaluate cell membrane integrity (and thus assume cell proliferation or death) but the method is not sensitive and cannot be adapted for high throughput screening. Measuring the uptake of radioactive substances, usually tritium-labelled thymidine, is accurate but it is also time-consuming and involves handling of radioactive substances. Yellow MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) is reduced to purple formazan in the mitochondria of living cells. The absorbance of this coloured solution can be quantified by measuring at a certain wavelength (usually between 500 and 600 nm) by a spectrophotometer.

The absorption max is dependent on the solvent employed. This reduction takes place only when mitochondrial reductase enzymes are active, and therefore conversion can be directly related to the number of viable (living) cells. When the amount of purple formazan produced by cells treated with an agent is compared with the amount of formazan produced by untreated control cells, the effectiveness of the agent in causing death of cells can be deduced, through the production of a dose-response curve. Solutions of MTT solubilized in tissue culture media or balanced salt solutions, without phenol red, are yellowish in colour.

Mitochondrial dehydrogenases of viable cells cleave the tetrazolium ring, yielding purple MTT formazan crystals which are insoluble in aqueous solutions. The crystals can be dissolved in acidified isopropanol. The resulting purple solution is spectrophotometrically measured. An increase in cell number results in an increase in the amount of MTT formazan formed and an increase in absorbance. The use of the MTT method does have limitations influenced by:

(1) the physiological state of cells.
(2) Variance in mitochondrial dehydrogenase activity in different cell types.

Nevertheless, the MTT method of cell determination is useful in the measurement of cell growth in response to mitogens, antigenic stimuli, growth factors and other cell growth promoting reagents, cytotoxicity studies, and in the derivation of cell growth curves.

Table 2

Compound	**R**	**R_1**	**M. Form**	**MCF-7 IC_{50}(μM)**	**HELa IC_{50}(μM)**
7a	H	3-Nitro phenyl	$C_{22}H_{17}N_5O_4$	1.10±0.02	6.17±0.02
7b	Cl	3-Nitro phenyl	$C_{22}H_{16}ClN_5O_4$	1.52±0.04	5.02±0.04
7c	Br	3-Nitro phenyl	$C_{22}H_{16}BrN_5O_4$	1.12±0.02	2.78±0.04
7d	F	3-Nitro phenyl	$C_{22}H_{16}FN_5O_4$	**0.92±0.03**	**1.91±0.02**
7e	NO_2	3-Nitro phenyl	$C_{22}H_{16}ClN_5O_4$	2.80±0.02	5.35±0.03
7f	H	3- chloro phenyl	$C_{22}H_{17}ClN_4O_2$	1.36±0.03	4.26±0.03
7g	Cl	3- chloro phenyl	$C_{22}H_{16}Cl_2N_4O_2$	1.35±0.02	3.45±0.03
7h	Br	3- chloro phenyl	$C_{22}H_{16}BrClN_4O_2$	1.08±0.03	2.38±0.03
7i	F	3- chloro phenyl	$C_{22}H_{16}ClFN_4O_2$	**0.78±0.03**	**1.31±0.02**
7j	NO_2	3- chloro phenyl	$C_{22}H_{16}ClN_5O_4$	2.90±0.02	7.64±0.02
7k	H	3,4dichloro phenyl	$C_{22}H_{16}Cl_2N_4O_2$	2.02±0.04	5.59±0.02
7l	Cl	3,4dichloro phenyl	$C_{22}H_{15}Cl_3N_4O_2$	1.65±0.03	3.24±0.03
7m	Br	3,4dichloro phenyl	$C_{22}H_{15}BrCl_2N_4O_2$	1.91±0.02	5.86±0.03
7n	F	3,4dichloro phenyl	$C_{22}H_{15}Cl_2FN_4O_2$	1.58±0.03	4.15±0.04
7o	NO_2	3,4dichloro phenyl	$C_{22}H_{15}Cl_2N_5O_4$	2.74±0.03	4.25±0.04
Cisplatin	-	-	-	0.47±0.02	0.65±0.02

4.2. ANTI-ALZHEIMER'S ACTIVITY:

The tests were carried out in accordance with CPCSEA standards and were approved by the institutional animal ethics committee (IAEC/40/SURA/HYD/2018). 5-6 Weeks For behavioural experiments, Swiss albino male mice weighing 20-25 g were used. The animals were kept in standard environments, which include a temperature of 20-25 °C, a 12-hour light/dark cycle, a standard pellet diet, and water available at all times. Before the test, the animals were given a week to get acclimated to the lab surroundings. They were then randomly divided into five groups of six animals each, as shown below:

Group 1: (vehicle control): Animals received Phosphate Buffer Solution, per oral

Group 2: (negative control): Mice administered with β-amyloid peptide by cerebroventricular injection.
Group 3: Mice injected with β-amyloid peptide and rivastigmine (per oral) 5 mg/kg.
Group 4:Mice injected with β-amyloid peptide and treated with 200 mg/kg and 400 mg/kg of test compounds (per oral)

Weidentified the bregma site in the skull using stereotaxic equipment and delivered 10 µL containing 10 µg Aβ (25-35) peptideto all but one of the groups to induce neurotoxicity by intra-cerebroventicular injection (INCO, India). Test compounds and standards were given on the fourteenth day after the β-amyloid peptide was started. Behavior studies were examined on days 7 and 21 following β-amyloid peptide administration (18).

***4.2.1. In vitro AChE and BuChE inhibitory studies*(19):**

The investigation of AChE and BuChE was conducted using ready-to-use kits and standard specified techniques, all of which had been provided by Sigma Aldrich. The test compounds were dissolved in DMSO and 1% bovine serum before being diluted with phosphate buffer. Ellman's reagent (5, 5'-dithiobis- 2-nitrobenzoic acid, DTNB) solution (5, 5'-dithiobis- 2-nitrobenzoic acid, DTNB) was prepared by dissolving 2 mg of DTNB in 200 mL of buffer.The modified Ellman method employs the production of thiocholine by the action of AChE on the substrate, acetylthiocholine iodide, to determine the IC50 of test chemicals. When tested spectroscopically at 412 nm, thiocholine quickly interacts with DTNB to create yellow colour, and the intensity of the colour is proportional to the enzyme activity in the presence of test substances. The assay mixture of 10 Ml AChE and 30 L DTNB solution was combined in a test tube with occasional shaking. After adding concentrations (5 M, 10 M, and 25 M) of test compounds and standards, the solution was incubated for 20 minutes before adding 30 L of DTNB reagent and 30 L of substrate acetyl thiocholine iodide. The intensity of the yellow hue generated was measured in triplicate at 412 nm one minute gap at 37 °C. The intensity of the yellow hue produced in the stoichiometric reaction indicates the amount of acetyl thiocholine hydrolyzed in the enzymatic reaction. The aforementioned protocol was used to test BuChE activity utilising butyrylthiocholine iodide as a substrate.Linear regression plot drawn between % inhibition vs log concentration of test compounds on MS Excel determines the IC_{50} of compounds. The results are presented as mean ± standard deviation of the triplicates.

4.2.2. *In vitro* estimation of Brain Cholinesterases:

The Ellman approach was then used to estimate AChE and BuChE levels in the brains of experimental animals. To determine the cholinesterase inhibitory activity of test compounds, rats were sacrificed and their brains were homogenised in 0.1M phosphate buffer (pH 8.0). A test tube containing 2.6 mL of phosphate buffer was filled with 0.4 mL of brain homogenate, which was thoroughly mixed. 100 L of DTNB chromophore reagent was added to the aforementioned solution and stirred to ensure appropriate mixing and air bubbling. The absorbance of the produced colour was measured at 412 nm, and when the absorbance reached a steady value, it was recorded as the basal reading. Acetyl thiocholine substrate 20 µL was then transferred into test tube and alternation in absorbance was noted for a period of 10 minutes at intervals of 2 minutes and later change in the absorbance per minute was determined.

***4.2.3. In vivo* Behavior Pharmacological Activities:**

4.2.3.1. Jumping Avoidance Box (19):

A Plexiglas partition in a box was used to create two equal chambers, and a gate was installed to allow the animals access to the adjacent compartment through a 14*17 cm area. The test animals are exposed for 30 seconds to light which is followed by 10 seconds sound stimulus and a single low intensity foot shock (0.5 mA) for three seconds in each session. Each mouse in each group received 15 such trials each day, with a 15-second break between successive trials, which was repeated for five days.

4.2.3.2. Rectangular Maze Test(20):

Rectangular Maze test apparatus consists of three interconnected chambers A, B, and C, with chamber B containing the maze used to assess test animals' memory capacity. All hungry mice were allowed to move from chamber A to chamber C via chamber B. Chamber C held reward food for the animal as it moved from chamber A to chamber C, as indicated by the pilot light. Every test animal was trained daily to collect the reward food, and the time duration was recorded.The animals were termed trained when the time required to complete each maze remained consistent for three consecutive days, and the time to traverse for maze

completion was then recorded for each animal before and after treatment with test compounds and standard medication.

4.2.3.4. Y-Maze Test(21):

The special recognition memory of mice can be tested using a two-trial recognition Y-maze test that does not require a learning rule. The spontaneous Alternation behaviour of rodents is observed using a Y maze composed of black painted wood. Y maze with three arms, each 40 cm long, 12 cm high, 3 cm wide at the bottom, and 10 cm wide at the top, converged in an equilateral triangular central region. In the eight-minute experiment, a mouse is placed in one arm of a Y maze and permitted to walk freely around the maze, and arm entries (one entry is stated to be completed when the mouse hind paw completely enters the artm) are visually recorded. In Y maze, Alternation term is defined as successive entries into all the three arms and % Alternation is calculated by following expression.

%alternation = {(No. of alternations) / (Total arm entries - 2)} x 100 % Alternation indicates the depth of working memory, and the higher the%, the greater the rodents' memory level. Except for group 1, all mice in each group were injected with -amyloid protein (10g) on day one, and all animals in each group were trained through a maze. On the seventh day, maze counts were taken, and on the fourteenth day, conventional medications tacrine and rivastigmine, as well as test compounds, were delivered. On the 21st day, the mice were allowed to freely roam the maze once more, and entry counts were recorded.

4.3. Evaluation for Antimicrobial Activity:

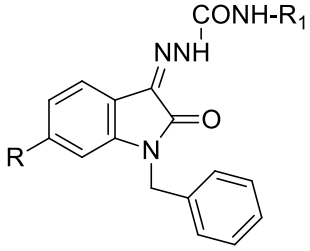
The antibacterial activity of azaindole derivatives (10a-o) and their derivatives had been assayed against four different strains of bacteria by cup-plate agar diffusion method by measuring the zone of inhibition.

Composition of the nutrient agar medium:

Peptone 5gm
Sodium chloride 5gm
Beefextact 1.5gm
Yeast extract 1.5gm
Agar 1.5gm
Distilled water up to 1000mL
pH 7.4±0.2

Procedure: The test organisms were sub cultured using nutrient broth mediun. The tubes containing sterilized medium were inoculated with respective bacterial strains. After incubation at 37±1ºc for 24 hours they were stored in refrigerator. The stock cultures were maintained. Bacterial inoculum was prepared by transferring a loopful of culture to nutrient broth in conical flask. The flasks were incubated at 37±lºc for 48 hours before the experimentation. Solution of test compound was prepared by dissolving the sample in DMSO. A reference standard for both Gram positive and Gram negative bacteria was made by dissolving accurately weighed quantity of Moxifloxacin in sterile distilled water. The nutrient agar medium was sterilized by autoclaving at 121ºc for 15min. The petriplates, tubes and flasks plugged with cotton were sterilized in hot air oven at 160ºc for an two hours. Into each sterilized petri plate, about 25mL of molten nutrient agar medium inoculated with the respective strains ofbacteria was transferred aseptically. The plates were left at room temperature to allow solidification. In each plate, cups of 5 mm diameter were made with sterile borer. Then 100µl of the test solution was added to the respective cups aseptically and labeled accordingly. The plates were kept undisturbed for at least 2 hours in refrigerator to allow diffusion of the solution properly into the nutrient agar medium. After the incubation of the plates at 37±1ºc for 24 hours, the diameter of the zone of inhibition surrounding each of the cups was measured with the help of the scale and tabulated.

Table:3: *In vitro* inhibitory activity of AChE/ BuChE of compounds **(7a-o)**

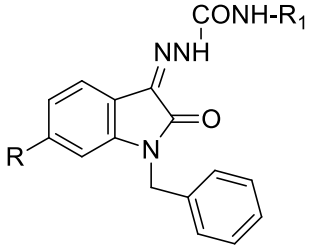


General structure-I

Compound	**R**	**R_1**	**M. Form**	**IC_{50} AChE**	**IC_{50} BChE**
7a	H	3-Nitro phenyl	$C_{22}H_{17}N_5O_4$	5.18±0.04	7.15±0.14
7b	Cl	3-Nitro phenyl	$C_{22}H_{16}ClN_5O_4$	2.52±0.03	6.22±0.24
7c	Br	3-Nitro phenyl	$C_{22}H_{16}BrN_5O_4$	3.12±0.01	2.70±0.08
7d	**F**	**3-Nitro phenyl**	**$C_{22}H_{16}FN_5O_4$**	**1.02±0.05**	**1.41±0.04**
7e	NO_2	3-Nitro phenyl	$C_{22}H_{16}ClN_5O_4$	3.25±0.12	4.15±0.13
7f	H	3- chloro phenyl	$C_{22}H_{17}ClN_4O_2$	4.16±0.13	4.16±0.03
7g	Cl	3- chloro phenyl	$C_{22}H_{16}Cl_2N_4O_2$	3.35±0.02	4.45±0.03
7h	Br	3- chloro phenyl	$C_{22}H_{16}BrClN_4O_2$	2.18±0.03	3.38±0.03
7i	**F**	**3- chloro phenyl**	**$C_{22}H_{16}ClFN_4O_2$**	**0.92±0.13**	**1.01±0.12**
7j	NO_2	3- chloro phenyl	$C_{22}H_{16}ClN_5O_4$	3.10±0.21	7.01±0.12
7k	H	3,4dichloro phenyl	$C_{22}H_{16}Cl_2N_4O_2$	3.02±0.04	5.59±0.02
7l	Cl	3,4dichloro phenyl	$C_{22}H_{15}Cl_3N_4O_2$	2.65±0.03	3.24±0.03
7m	Br	3,4dichloro phenyl	$C_{22}H_{15}BrCl_2N_4O_2$	1.61±0.02	2.86±0.03
7n	F	3,4dichloro phenyl	$C_{22}H_{15}Cl_2FN_4O_2$	1.58±0.03	1.15±0.04
7o	NO_2	3,4dichloro phenyl	$C_{22}H_{15}Cl_2N_5O_4$	2.65±0.06	4.32±0.0ZZ6
Donepezil	-	-	-	0.51±0.06	0.31±0.04

Data are expressed as (mean±SD in µM, n=3)

Table-4: *In vitro* inhibitory activity of brain AChE/ BuChE of compounds**7d, 7h, 7l, 7m, 7n** and**7o**

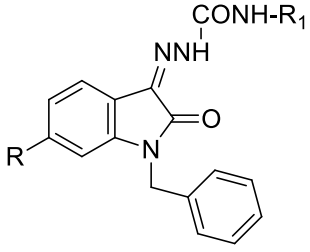


General structure-I

S.No	**Group**	**IC_{50} AChE[a]**	**IC_{50} BuChE[a]**
1	**7d**	**28.32**±0.18	**30.21**±0.12
2	7h	46.10±0.10	45.12±0.10
3	**7i**	**25.35±0.15**	**31.14±0.10**
4	7l	32.16±0.41	38.35±0.17
5	7m	34.14±0.12	38.20±0.11
6	7n	38.21±0.21	38.35±0.30
6	7o	36.10±0.18	34.13±0.41
8	Donepezil	20.20±0.14	26.25±0.25
9	Control	50.25±0.52	55.20±0.15

[a]Data are expressed as (mean±SD in µM.

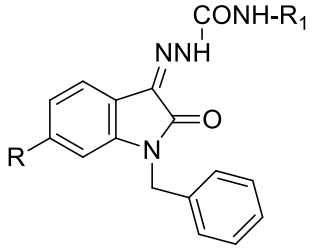
Table 5. Behavioral effects in Y-maze, rectangular maze and jumping box tests



General structure-I

Comp.	Y-maze test (% Alternations)		Rectangular maze (sec)		Jumping box (sec)	
	Before treatment	**After treatment**	**Before treatment**	**After treatment**	**Before treatment**	**After treatment**
7a	24.11±1.01	34.13±1.10	144.13±1.10	131.45±1.35	32.1±2.41	20.01±1.40
7b	25.11±1.05	35.84±1.15	146.20±1.03	140.01±2.40	30.23±2.50	22.43±2.10
7c	26.11±1.12	36.12±1.13	148.16±1.06	124.32±1.48	31.15±2.10	24.56±2.10
7d	**24.12±1.15**	**46.20±2.14**	**149.10±1.04**	**98.12±1.24**	**30.6±3.56**	**12.15±2.56**
7e	23.42±1.14	30.32±1.80	149.28±1.35	116.20±2.35	28.15±2.45	18.08±1.45
7f	22.10±1.30	33.24±3.14	142.28±1.10	130.18±2.40	29.18±2.54	16.12±1.54
7g	24.12±1.10	34.13±1.87	150.12±3.40	104.15±1.40	30.23±2.54	14.12±2.54
7h	22.10±2.10	35.14±2.65	152.72±2.37	108.75±2.37	32.05±1.23	14.17±1.23
7i	**21.12±1.02**	**50.61±1.87**	**150.15±1.85**	**94.15±1.82**	**32.6±1.62**	**10.24±1.62**
7j	24.22±1.20	34.20±2.10	149.21±1.98	132.07±2.35	31.20±2.15	20.43±2.15
7k	21.40±1.10	32.15±1.50	150.14±2.48	129.07±1.32	30.10±3.18	18.42±3.18
7l	24.47±1.12	30.65±1.46	149.76±1.37	126.75±1.37	29.50±0.22	16.17±0.22
7m	25.15±1.10	44.61±3.15	148.12±2.40	116.15±1.40	30.23±1.54	17.12±1.20
7n	22.12±1.29	48.12±1.46	146.28±1.87	111.18±1.25	30.18±2.54	15.16±1.54
Donepezil	20.18±2.10	54.32±2.58	151.42±1.21	92.61±2.21	30.32±0.71	9.30±0.71
-ve	23.19±2.18		142.87±2.53		34.12±1.09	
Vehicle control	47.61±3.15		47.83±2.53		12.5±2.18	

Table 6 Antibacterial activity of Azaindole derivatives. Series-I (I-7a-o)



General structure-I

S.No	Compound	Minimum Inhibitory Concentration (mm)			
		Gram +ve		Gram –ve	
		B.substilis	***S. aureus***	***K. pneumonia***	***E. coli***
1	7a	5	6	6	5
2	7b	4	4	5	5
3	7c	5	5	4	6
4	**7d**	**10**	**9**	**11**	**10**
5	7e	5	5	5	4
6	7f	4	5	5	5
7	7g	3	6	4	5
8	7h	8	5	9	8
9	**7i**	**12**	**10**	**12**	**14**
10	7j	4	5	4	4
11	7k	6	4	5	5
12	7l	9	8	9	8
13	7m	8	9	10	9
14	7n	9	12	9	11
15	7o	8	4	6	5
16	Ciprofloxacin	14	13	15	16

5.Results and discussions:

5.1 ANTI CANCER ACTIVITY:

Unsubstituted compound **7a** showed both HELa and MCF-7 with IC_{50} value of 2.10 and 6.17 μM. Most active compound among the series was found to be **7d** with IC_{50} of **0.92**and **1.91** μM and compared to 0.47, 0.65 μM of cisplatin against Hela and MCF-7 respectively. In general, increasing the electronegative group increasing the anti-cancer activity was found to increase significantly Effect of the nature of aryl group and substituents on HEL a and MCF-7 inhibitory actvity was studied to understand the structure activity relationship. The simple phenyl group in **7d** (R=F) when replaced with Cl resulted in marginal decrease in both HELa and MCF-7 inhibitory actvity. compound **7i** with F

substituted moiety showed potent activity with IC_{50} value of 0.78and 1.31 μM against HELa and MCF-7 cell lines respectively.

Insertion of electron withdrawing group (3-nitro) as in **7e, 7j, 7o**resulted in decreased HELa and MCF-7 cell lines inhibitory activity when compared with their corresponding unsustituted compound, **7a** which indicated that electron withdrawing groups hinder theHELa and MCF-7 cell lines inhibitory activity. To explore further the SAR, compounds with two Cl groups have been synthesized and to our expected observations. Three compounds with 3,4 di chlorophenyl groups as in **7k-o** have also shown reduced activity over unsubstitutedcompounds, in both HELa and MCF-7 cell lines**.**

5.2ANTI ALZEIMERS ACTIVITY:

5.2.1 Biochemical evaluation

5.2.1.1*In vitro* AChE and BuChE inhibitory activity:

All the fifteen compounds have been screened against human AChE and BuChE by following reported methods and compared the potency with standards Donepazil. AChE and BuChE inhibitory activity can be tangibly correlated with structure of all compounds depending on the attached to the Indole ring and substitution in the phenyl ring. AChE and BuChE inhibitory activities of all the compounds have been listed in **Table 3**.

Corbamide fusedwith chloro phenyl ring showed less AChE and BuChE inhibitory activities. Most active compound among the series was found to be **7d and 7i.**

Two compounds with 3,4 di chloro phenyl groups as in**7m-n** have also shown enhanced activity over 3-nitrophenyl substituted compounds, **7a-d,** in both *in vitro* cholinesterase inhibitory activity. Fluro substitution showed enhanced activity as in 7d 1.02μM& 1.41μM and 7i 0.92μM& 1.01μM for AChE and BuChE inhibitory activities respectively.

5.2.1.2.*In vitro* brain AChE and BuChE inhibitory activity

Six potent compounds, **7d, 7h, 7i, 7l, 7m, 7n**and **7o**were further tested for *in vitro* brain AChE and BuChE inhibitory activity along with donepazil as standards by following the reported methodology. The *in vitro* brain Acetyl cholinesterase and Butyrylcholinesterase inhibitory activities of tested compounds have been listed in **Table 3.**

5.2.2Behavioral Studies:

The statistical analysis was performed using the Graphpad Prism software version 5.0, and one-way ANOVA and Tukey's Multiple Comparison Test were used to compare the results. Statistical significance was defined as a P value <0.001.

Y maze model demonstrates a sensitive measure of spatial recognition memory in rodents. The result of the Y maze experiment is reported as % Alternations for both before and after the administration of test compounds . All the test compounds and standards Tacrine and donepazil have been administered with 400 mg/kg dose (p.o). The negative control group showed significant decrease in the Alternation when compared with the Normal control According to the learning scores (Transfer latency) obtained by each group in the rectangular maze, mice appeared to be taking less time on the last day of the experiment. When compared to the vehicle group, the negative control group had a higher transfer delay score because of the memory loss deficit induced by β-amyloid peptide. When compared to the negative control group, the transfer latency scores for the conventional test compounds **7d** and **7i** treated animals show a ($P \leq 0.001$) memory-improving potential. Similar to the negative control group, those treated with compounds 10m and 10n had a significant decrease in transfer latency ($P \leq 0.001$).

With the aim to assess avoidance behaviours, mice were subjected to conditioning tests in jumping box as this is reliable test for measuring the integrity of the learning and memory processes. Active avoidance behavioral responses, calculated as a cumulative number of shock avoidance during 15 trials in a day. In avoidance test, groups of animals administered with test compounds **7d** and **7i** showed significantly increased number of conditioned stimulus responses (avoidances) on the last day of study ($P \leq 0.001$) as compared with the negative control group animals. Animals treated with Donepazil showed significantly increased the number of conditioned stimulus responses (avoidances) on the last day of study ($P \leq 0.001$) as compared with the negative control group animals.While non-significant difference was observed between test group **7d** and **7i** as compared with these standard drugs signifies increase in the memory.

5.3Anti-bacterial activity:

All indole derivatives tested (7a-o) exhibited significant antibacterial activity with zone of inhibition of 9-12mm when compared to 12-16mm

shown by the standard at 25µg/ml concentration. The compoundscontaining fluro substitution 7i showing zone of inhibition of 12mm,10mm, 12mm, 14mm for B.subtilis,s. aureus, k pneumonia, E coli respectively. 7d showing zone of inhibition 10mm, 9mm, 11mm, 10mm for 7i 12mm, 7d 11mm,B.subtilis, s.aureus, k.pneumonia, E coli respectively when compared to ciprofloxacin showing zone of inhibition of 14mm, 13mm, 15mm, 16mm for B.subtilis, s.aureus, k.peumonia, Ecoli respectively.

6. Docking and molecular dynamics studies:

Docking studies were carried out using Schrödinger software (Version 2019-1, Schrödinger) (Glide module). The ligands used as inputs for docking were sketched by using ChemDraw software. Ligands were prepared using OPLS3e force field in Ligprep(Dizdaroglu et al., 2020) (Version 2019-1, Schrödinger). This minimization helps to assign bond orders and addition of the hydrogens to the ligands. The generated output file (Best conformation of the ligands) was used for docking studies. Protein was prepared by using the protein preparation in Maestro wizard (Dizdaroglu et al., 2020)(Version 2019-1, Schrödinger). Hydrogen atom was added to the proteins and charges were assigned andalso generated Het states using epik at pH 7.2. Water molecules and other heteroatoms were excluded from the crystal structure as they were not significant for the function of the protein in docking studies. Finally, the protein was optimized by using optimized potential liquid simulations (OPLS3) force field. A receptor grid was generated around the cocrystal ligand (X-ray pose of the ligand in the protein). The centroid of grid box and Vander Waal radius of receptor atoms was scaled to 1.00 Å with a partial atomic charge of 0.25. Glide docking score was used to determine the bestdocked structure from the output. Poses of the generated ligands after docking were analyzed by the help of XP Visualizer (Version 2019-1, Schrödinger).

Table 4: Docking energies of 7a-t in6ENVand BuChE

Compound	**R**	**R_1**	Docking score 6ENV	Docking score EML4
7a	H	3-Nitro phenyl	-8.633	-10.015
7b	Cl	3-Nitro phenyl	-11.85	-8.803
7c	Br	3-Nitro phenyl	-10.666	-8.213
7d	F	3-Nitro phenyl	-6.579	-6.185
7e	NO_2	3-Nitro phenyl	-10.529	-12.78
7f	H	3- chloro phenyl	-9.607	-10.408
7g	Cl	3- chloro phenyl	-9.5	-9.388
7h	Br	3- chloro phenyl	-8.88	-9.311
7i	F	3- chloro phenyl	-6.84	-7.046
7j	NO_2	3- chloro phenyl	-10.381	-12.981
7k	H	3,4dichloro phenyl	-8.36	-8.914
7l	Cl	3,4dichloro phenyl	-8.278	-8.894
7m	Br	3,4dichloro phenyl	-8.049	-6.799
7n	F	3,4dichloro phenyl	-7.759	-5.68
7o	NO_2	3,4dichloro phenyl	-8.262	-10.517
7p	H	3,5 dibromo phenyl	-10.195	-8.483
7q	Cl	3,5 dibromo phenyl	-8.194	-7.098
7r	Br	3,5 dibromo phenyl	-9.046	-8.775
7s	F	3,5 dibromo phenyl	-6.595	-6.417
7t	NO_2	3,5 dibromo phenyl	-10.433	-10.015

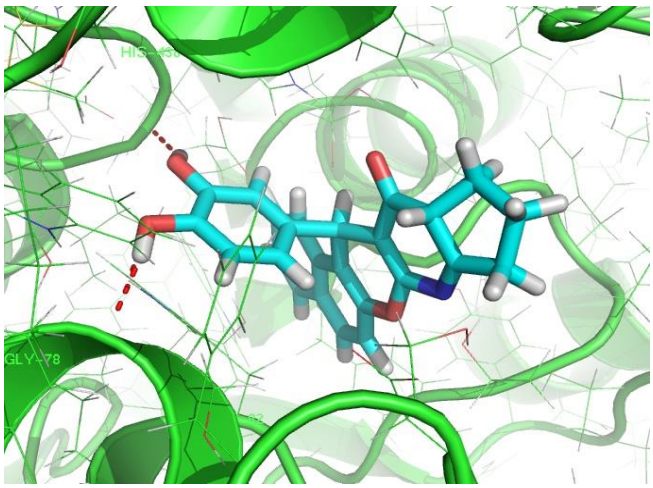


Figure-1: Docking pose of **7i** (cyan) with 6ENV (green) where hydrogen bonds are shown in red dotted line.

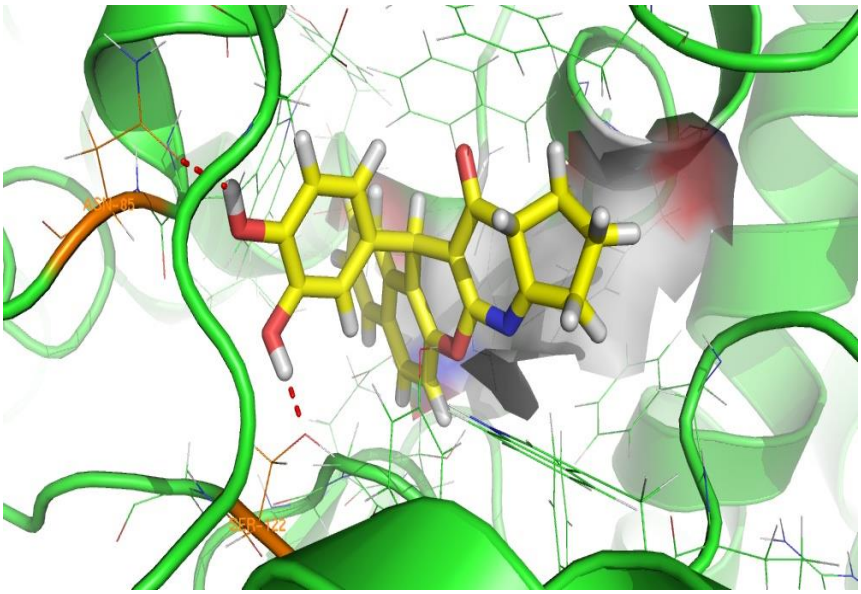


Figure-2: Docking pose of **7i** (yellow) with EML4 (green) where hydrogen bonds are shown in red dotted line.

Docking studies:

In vitro studies of synthesized compounds showed the potential anti-cancer activity and among all, the compound **7i** showed promising anti-cancer activity. These results encouraged us to perform docking studies to get the insight in to the binding mode of synthesized compounds within binding pocket of Hela and MCF-7. All structures of ligands were built using maestro and further prepared using LigPrep form Schrodinger package. Protein structures were obtained from the Protein Data Bank (PDB ID: EML4 and 6ENV) and necessary correction to the protein structure were done using Protein Preparation Wizard in Schrodinger package. Docking studies were performed using Glide docking software and docking protocol was validated by docking the cocrystal ligand which resulted with RMSD of docked conformation and cocrystal ligand pose was found to be 0.6. The binding interactions of compounds with HELa and MCF-7 have been listed in **Table 2**

Binding poses of synthesized compounds with HELahave shown that these molecules bind well within binding pocket of enzyme.Among theall synthesized molecules,**7i**with potent anti-cancer activity**,** has shown the highest binding score. In superimposed pose of **7i**with cocrystal ligand, aromatic ring was coinciding with skeleton of cocrystal ligand as depicted in **figure-2** However, Nitro substituted ring has occupied the empty additional space available in binding pocket. In binding pocket,**7i**was involved in π-π stacking interactions with Phe331 and His440. 3-Chloro group formed hydrogen bond with side chain of

Ser122 while 4-methoxy group was involved in hydrogen bonding with side chain carbonyl carbon of Asn80 (**figure-2**). Moreover, docking studies on the MCF-7showed the similar results . Compound **7i** has the highest binding score and fluoro substituted was involved in hydrogen bonding interactions with enzyme; 3-methoxy group with His148, whereas 4-methoxy was forming with Gly78 and Trp82.

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