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Synthesis and Estimation of Enzyme Inhibitory Activity of Isatin Derivatives

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Abstract
In the present study several derivatives of Isatin (A-L) were synthesized. All the synthesized derivatives (A-L) were Schiff bases and show vital pharmacological properties, suppose beneficial in Alzheimer's disease (AD). The structures were confirmed spectroscopically by infrared (IR) and nuclear magnetic resonance (NMR). The purpose of the current study was to synthesized derivatives which possess acetylcholinesterase and butryl cholinesterase inhibitory potential. Several compounds possess anti-acetylcholinesterase and anti-butryl cholinesterase activity, particularly the compound (J) with malononitrile substituent on Isatin ring was most active in suppressing acetyl cholinesterase enzyme activity and the compound (F) with M-Toluidine substituent on isatin ring was potent inhibitory activity against butryl cholinesterase enzyme.
Keywords: Isatin, amines, Schiff bases, Alzheimer's diseases, BChE inhibitor, AChE inhibitor.

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Introduction

Isatin (1-*H* indole 2-3 Dione) is synthetically multipurpose compound that is use for the production of different heterocyclic compounds. The isatin derivatives indicated variety of biological activities, e.g. anticonvulsants [1], antitubercular [2], antibacterial [3], antiviral [4] activities. Because of various biological activities of isatin derivatives our attention has focused to synthesis series of derivatives of isatin which showed remarkable BChE inhibitory and AChE inhibitory activities and used for the cure of Alzheimer's disease (AD).

Acetylcholinesterase performs a noticeable role as it participates in finishing of acetylcholine based signal transmission through neurosynaptic cleft or neuromuscular junction [5], [6].

A shortage of AChE levels in CNS leads to circumstances such as Alzheimer's disease (AD).Most common form of dementia is Alzheimer's disease, an advanced neurologic disease of the brain that leads to loss of mental capability unadorned to affect with ordinary activities of daily living and weakening in mental functions such as memorizing, reasoning and planning. It disturbs brain parts that control thought, remembrance and language. It is categorized by nerve-cell loss, irregular tangles and plaques within nerve cells and lacks of several neurochemicals such as acetylcholine (ACh) and butryl choline (BuCh), that's are vital for the spread of nerve messages.

The most favorable approaches for handling the Alzheimer's disease (AD) is to increase the acetylcholine level in brain using acetylcholinesterase inhibitors [7].Many AChE inhibitors are studied for the treatment of Alzheimer's disease. There are also tacrine, donezepil, rivastigmine and galanthamine inhibitors that have been used for cure of Alzheimer's disease (AD) [8].

It was assumed that blocking the enzyme cholinesterase (ChE) persuaded hydrolysis of ACh and successive increase in Ach concentration in central synapses and improvement of cholinergic functions provides the symptomatic improvement to AD patient [9], [10].

Materials and Methods

General procedure

Thin layer chromatography (TLC) was performed by using thin layer aluminum plate pre-coated silica gel of 0.2 mm in thickness. The spots were analyzed under ultraviolet light. The melting points of the compounds were verified with Leica Galen melting apparatus.

Instrumentation

Several instruments were used to characterize the compounds synthesized in the experiments. Infrared (IR) spectra were confirmed on shimadzu 8000. KBr pellet were used for solid sample. Nuclear magnetic resonance (NMR) spectra of all compounds were documented by using Bruker Advance spectrometer.

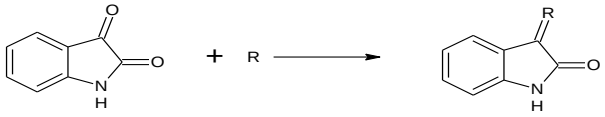
Solvent and Chemicals

The chemical used in the synthesis of derivatives were isatin, 2-amino acetophenone, 1,5 diamino naphthalene, 2-amino benzoic acid, 2-aminoethanethiol hydrochloride, p-toluidine, m-toluidine, hydroxyl amine, phenyl hydrazine, aniline, thiosemicarbazide, O-Anisidine and malononitrile. The organic solvents used were ethanol, methanol, n-Hexane, ethyl acetate and acetone.

General Synthesis of Isatin Derivatives

2 mmol of isatin was dissolve in 10 ml of ethyl alcohol at room temperature in a 50 ml of round bottom flask. Then 2.4 mmol of amine was added in it. The reaction mixture was heated at 210^0C. The reaction was monitor with TLC. After stirring precipitate was appeared. The precipitates were filtered and dried. The melting point of the compound was confirmed in an open capillary tube. The compound was recrystallized from ethanol.

General reaction:



Isatin *Amine* *Isatin Derivative*

In vitro AChE and BChE Assay

The Acetylcholinesterase (AChE) inhibition activity was performed according to the method of Ellman, 1961 with slight modifications [11]. The total volume of the reaction mixture was 100 µL and confined 60 µL Na2H PO4 buffer with concentration of 50 mM and pH 7.7. Ten µL test compound (0.5 mM well-1) was added, followed by the addition of 10 µL (0.005 unit well-1) enzyme. The contents were mixed and pre-read at 405 nm. Then contents were pre-incubated for 10 min at 37ºC. The reaction was started by the addition of 10 µL of 0.5 mM well-1 substrate (acetylthiocholine iodide), followed by the addition of 10 µL DTNB (0.5 mM well-1). After 30 min of incubation at 37ºC, absorbance was measured at 405 nm. Eserine (0.5 mM well-1) was used as a positive control. In vitro BChE assay was similar with the method used for AChE but in BChE substrate (butyrylthiocholine bromide) was used instead of substrate (acetylthiocholine iodide).

Results

Physical properties of all the twelve derivatives are represented in **Table 2** which includes molecular formula, molecular weight, color and appearance based on IR spectra and physical evaluation.

All the compounds (A-L) showed significant biological activity against AChE (acetylcholinesterase) as compare to Eserine except compound (G & K) where insignificant activity was observed as shown in **Table 3.** The control values of enzyme inhibitory activity for derivatives are given in **Table 4**. It was observed that all the compounds (A-L) showed significant biological activity against BChE (butrylcholinesterase) as compared to the Eserine.

Different types of functional groups and bonds present in the synthesized derivatives (absorb infrared radiation of different wavelengths) are show in the **Table 1** [12].

Table 1: IR Spectra of the Derivatives

Serial No.	Compounds	C=N	N-H
1	A	1617	3195
2	B	1613	3416
3	C	1673	3474
4	D	1616	5308
5	E	1615	3253
6	F	1656	3458
7	G	1659	3412
8	H	1617	3434
9	I	1620	3500
10	J	1621	3260
11	K	1555	3497
12	L	1609	3234

Discussion

The anticholinesterase activity of the derivatives (A-L) was assayed according to slight modification in Ellmann's method. BChE activity was also evaluated by the same method by using Eserine as a standard. The physical properties of all the derivatives were mentioned in **Table 2** Inhibition AChE and BChE activities of the synthesized derivatives is shown in **Table 3**. The IR spectra of all the derivatives presented in **Table 1**. The data in table No. 2 shows that most of the derivatives exhibited good to moderate AChE and BChE activities. Among the entire synthesized compound the derivative (J) with

malononitrile substituent on isatin ring was most active and suppressing 84.72% acetylcholinesterase enzyme inhibitory activity and the compound (F) with M-Toluidine substituent on isatin ring was showing 94.79% potent inhibitory activity against butrylcholinesteraseenzyme. Recent study indicated that the synthesized isatin derivatives with primary amines showed remarkable BChE inhibitory and AChE inhibitory activities and can be used for the treatment of Alzheimer's disease (AD). Among these derivatives, it was inferred that derivatives showing AChE and BChE enzyme inhibitory activity can be further revised to show superior potency than the standard drugs.

Table 2: Physical Properties of the Derivatives

Serial #	Synthesized Compounds	Molecular Formula	Molecular Weight	Color	Appearance	%age Yield	Melting point
1	A	$C_{16}N_2O_2H_{12}$	264	Reddish Orange	Crystalline	60%	200-205 ºC
2	B	$C_{18}N_3OH_{13}$	287	Brown	Powder	63%	180-183 ºC
3	C	$C_{15}O_3N_2H_{10}$	266	Orange	Crystalline	73%	130-135 ºC
4	D	$C_{10}N_2OS_2H_{10}$	206	Reddish Orange	Crystalline	61%	200-204 ºC
5	E	$C_{15}N_2OH_{12}$	236	Yellow	Powder	72%	226-230 ºC
6	F	$C_{15}N_2OH_{12}$	236	Mustard	Crystalline	57%	240-245 ºC
7	G	$C_8N_2O_2H_6$	162	Light Yellow	Crystalline	63%	170-175 ºC
8	H	$C15N_2O_2H_{12}$	252	Orange	Crystalline	61%	200-204 ºC
9	I	$C_9H_8N_4OS$	220	Pale Yellow	Powder	75%	235-240 ºC
10	J	$C_{10}H_7N_3O$	185	Reddish Brown	Powder	61%	232-236 ºC
11	K	$C_{14}ON_3H_{11}$	237	Yellow	Powder	72%	220-225 ºC
12	L	$C_{14}H_{10}ON_2$	222	Light Brown	Crystalline	53%	225-228 ºC

Table 3: Enzyme inhibitory activity for derivatives

Serial #	Compounds	AChE (%) at 0.5mM	AchE (IC50) umoles	BChE (%) at 0.5 mM	BChE (IC50) umoles
1	A	63.44±0.28	280.11±0.06	67.98±0.21	140.11±0.18
2	B	56.29±0.64	<400	83.55±0.34	63.11±0.25
3	C	53.97±0.82	<400	51.21±0.11	<400
4	D	64.02±0.31	244.21±0.41	73.13±0.44	127.31±0.11
5	E	59.38±0.15	<400	55.91±0.21	<400
6	F	63.83±0.21	251.81±034	94.79±0.34	35.11±0.32
7	G	24.76±0.34	NIL	59.59±0.11	306.51±0.45
8	H	60.15±0.11	281.31±0.44	70.65±0.44	157.31±0.19
9	I	74.66±0.41	151.91±0.22	88.50±0.41	48.11±0.19
10	J	84.72±0.39	119.91±0.30	87.99±0.55	61.91±0.36
11	K	36.36±0.55	NIL	73.06±0.61	150.41±0.22
12	L	55.90±0.29	<300	93.39±0.94	26.21±0.09

Table 4: Control values of enzyme inhibitory activity for derivatives

Control	Conc.	AchE (%)	AchE(IC_{50}) µmoles	BChE (%)	BChE(IC_{50}) µmoles
Eserine	0.25mM	91.29±1.17	0.04±0.0001	82.82±1.09	0.85±0.0001

Conclusion

It was inferred that most of the isatin derivatives tested showed enzyme inhibitory activities against Acetylcholinesterase, and Butrylcholinesterase and most of the derivatives could be supposed for advance studies in the treatment of AD. It was concluded that derivative (J) showed highest 84.72% activity against AchE. While, derivative (F) was found to be most active derivative showing 94% activity against BChE as compare to the Eserine. Further research is needed to explore the chemical

and biological properties of the derivatives in promising treatment for Alzheimer's diseases AD.

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