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Synthesis, Interpretation And Screening Of Some Heterocyclic Compounds For Nootropic Activity

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Abstract: ***Background:*** Coumarins are a class of heterocyclic compounds containing oxygen as a member of the heterocyclic ring. They are widely used for their antioxidant, antibacterial, anti-inflammatory, antifungal, antiviral, anti-HIV and anticancer activities. [1-10] Coumarins can exhibit antioxidant activity, which may contribute to neuroprotection and cognitive enhancement.[1] Nootropic drugs literally mean drugs that act on the mind.[22] Nootropic drugs increase glucose uptake into anaesthetised brain.[23] The purpose of this study is to develop new heterocyclic derivatives containing nitrogen, sulphur and oxygen moiety with increased pharmacological activities. The derivatives are synthesized by conventional method. The structures of the newly synthesized compounds are confirmed from IR and 1HNMR and Mass spectra. [1,2,20] The derivatives are screened for their anti-oxidant activity by Hydroxyl radical scavenging assay and Nootropic activity. The present studies focus on heterocycles in the treatment of various chronic diseases such as Alzheimer's disease, Dementia and Memory related functions. ***Objectives:*** The present study is designed to assess the antioxidant and reactive oxygen species (ROS) scavenging activities of Coumarins. The compounds are assessed for their memory learning. The compounds are also assessed for their anti-microbial activity. ***Materials and Methods:*** The evaluation of antioxidant properties is determined by Hydrogen Peroxide Scavenging Activity and 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. ***Results:*** Among the tested compounds, the maximum antioxidant activity is recorded in the coumarins having OH group. The compounds also showed significant Nootropic activity. ***Conclusion:*** This study suggests coumarins contain different potential antioxidant compounds capable of scavenge different types of free radicals. The study also reveals that the compounds show significant anti-bacterial activity.

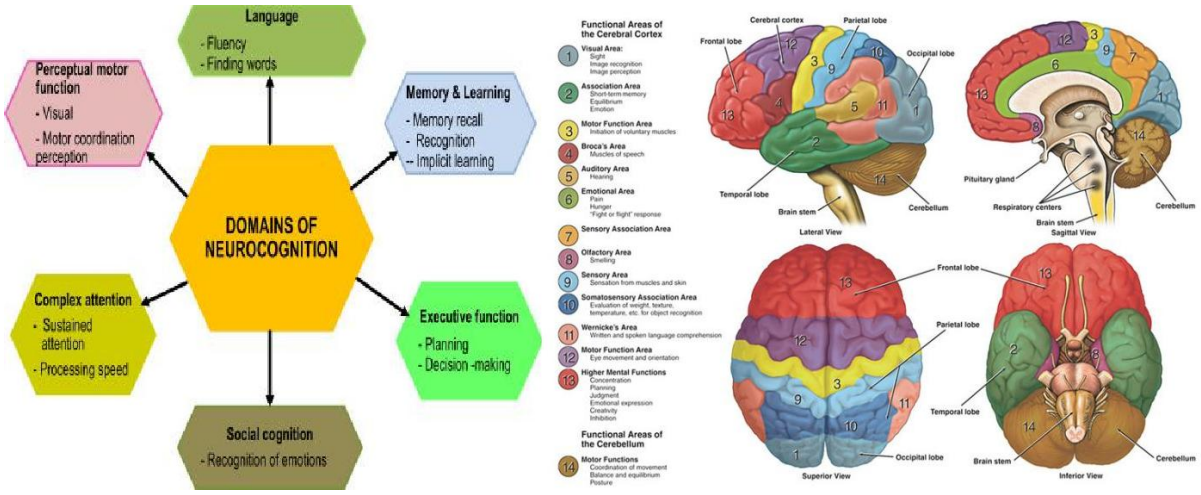
Keywords: Heterocyclic nucleus, Coumarin ring, Nootropic activity, Free radical scavenging, Antioxidant activity.

INTRODUCTION

Nowadays modernization in human lifestyle may affects various common habits of humans which may increase heavy burden on common people thinking abilities and may cause stress situation. Memory is critical to both human survival and personal identity. Various situations may affect common people daily routine and may cause heavy burden on families financially and such situations may cause mental disturbance to human being. Overstressed people may suffer from various ulcerative diseases.

Nootropics are also known as smart drugs or Cognition enhancers. A nootropic is a substance that has been proven to improve cognition and support healthy brain function. These compounds can be naturally occurring, like Brahmi and Ginkgo biloba, or synthetic, such as piracetam and modafinil. A variety of agents, such as cholinergic, serotonergic, dopaminergic, and antioxidant drugs, fall into this category. They probably act by altering the levels of neurotransmitters, hormones, and enzymes that are available to the brain, through improvement of brain's oxygen supply or stimulation of nerve. Piracetam, 2-pyrrolidinoneacetamide (Merck Index, X edition, page 1080, no. 7363) is a compound described in Belgian patent no. 667906 (Union Chimique Beige) as central stimulant. For its action at cerebral level on

glucose metabolism as well as, especially, in increasing acetylcholine release, Piracetam is considered the parent compound of nootropic drugs and it is used in therapy in the treatment of cerebral efficiency disorders, particularly in elderly patients.[7]



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In the discovery of new, effective, medicinally important heterocyclic compounds, the present study is basically focused on nitrogen-sulphur heterocycles of potential therapeutic interest, especially with thiazole, thiazine, pyrimidine, morpholine and piperazine heterosystems, benzothiazines, pyrazole-benzothiazines, morpholine-benzothiazines, piperazine-benzothiazines and pyrimidine-benzothiazoles, mainly due to their unique structural features, which enable them to exhibit a number of biological and pharmacological activities. Due to a novel mode of action, a broad spectrum of activity, lesser toxicity towards mammalian cells, and suitable profiles towards humans have triggered. The antioxidant capacities of novel heterocyclic compounds, including the compounds acting either by prevention of formation or catalysed decomposition of per-oxynitrite anion (ONOO-), namely the per-oxynitrite decomposition catalysts or as superoxide (O_2^{-})-scavengers which are the functional mimetics of superoxide dismutase (SOD) enzymes (SODm), as well as the derivatives of 6-nitro-3,4-methylenedioxyphenyl-N-acylhydrazone (LASSBio-881) or γ-butyrolactone (LPP1, BM113, BM113A, BM138 and BM138A) are also discussed as potent and promising future heterocyclic analgesics.[9]

MATERIALS AND METHODS

In this study the pharmacological evaluation of novel derivatives is carried out for nootropic activity. All reagents are used as received from commercial sources without purification. Resorcinol, Ethyl acetoacetate, conc. Sulphuric acid, Piperidine (catalytic grade), Benzaldehyde and its substituted derivatives are obtained from Dr. A.P.J. Abdul Kalam College of Pharmacy. All chemicals used are of L.R. grade. The experimental protocol is approved by Institutional Animal Ethics Committee (IAEC) with Ref. APJ/IAEC/2023/12 and care of animals according to CPCSEA guidelines with food and water and under hygienic conditions.

List of drugs used in the present study are Piracetam (UCB India Pvt. Ltd.) and Scopolamine (Cadila Healthcare Ltd., India) dissolved in distilled water. The newly synthesized compounds are dissolved in Corn oil and administered orally.

CHEMISTRY:

Melting points of the newly synthesized compounds are determined with a Veego electronic melting point apparatus (model VMP-D).^{1}HNMR spectra are recorded with the aid of BRUKER AVANCEV II 400 NMR spectrometer in DMSO as solvent and TMS as internal standard, Chandigarh. The IR spectra of compounds are recorded on a SHIMADZU Happ-Ganzel spectrometer at 4cm-1 frequency. The mass spectra are obtained on WATERS QTOF MICROMASS (LC-MS). Progress of reaction is monitored by Thin Layer Chromatography (TLC) using glass plates pre-coated with Silica Gel-G.

To A mixture of 1 gm. Of compound and 3.5ml of 33% NaOH solution a test tube, 2.5 ml of chloro acetic acid solution is added and heated gently on boiling water bath for an hour. After cooling the mixture is cooled and diluted with 10 ml of water and acidified to Congo red with dil. HCl. It is extracted with 10 ml of ether. The ethereal extract is ished with 2.5 ml of sodium carbonate solution and acidified with dil. HCl to Congo red. The aryl oxy acetic acid derivatives which separate out are collected and are recrystallized from aq. Ethanol. Melting points of

newly synthesized compounds are determined and are recorded uncorrected. [7]

IR spectrum interpretation data:

2-[2-oxo-4(4-methoxystyryl)-2H-chromen-7-yl] oxy acetic acid, 3317cm-1(CH=CH), 2993cm-1(C-H), 1672 cm-1 (C=O), 1511cm-1(C=C), 3025cm-1(OCH3).

1H NMR Spectrum interpretation:

1H NMR Spectrum interpretationof2- [2-oxo-4(4-methoxy styryl)-2H-chromen-7-yl] oxy acetic acid (**3c**). 6.5-7.5δ ppm [m, 1H, C-H (Ar-H)], 3.7δ ppm [s,3H, C-H (methyl)],6.8δ ppm [d,2H, C-H(ethylene)], 8.7δ ppm [s,1H, OH (carboxylic)], 4.7δ ppm [d, 2H, C-H(methylene)].

Mass Spectra:

Mass Spectra of 2-[2-oxo-4-styryl-2H-chromen-7-yl] oxy acetic acid. 328(100%) M+1Peak, Molecular weight is found to be 328 which is almost equal to calculated molecular weight 322.

BIOLOGICAL SCREENING:

ANTI-OXIDANT ACTIVITY:

a) Hydrogen Peroxide Scavenging Activity:

A solution of hydrogen peroxide (40 mM) is prepared in phosphate buffer (pH 7.4). Different concentrations ((250, 500, 750 and 1000 µg/mL)) of synthesized compounds are added to a hydrogen peroxide solution (0.6 mL, 40 mM). Ascorbic acid is used as a reference standard. Absorbance of hydrogen peroxide at 230 nm is determined after 10 min. against a blank solution containing phosphate buffer without hydrogen peroxide. Hydrogen peroxide percentage scavenging activity is then calculated using Equation:

$$\text{Scavenging effect (\%)} = \frac{A_0 - A_t}{A_t} * 100$$

Where A_o is the absorbance of control and A_t is the absorbance of test compounds or standard.

b). DPPH radical scavenging:

The effect of the different synthetic compounds on DPPH radical scavenging is compared to ascorbic acid using as positive control and appreciated by the determination of the IC50 values. DPPH test is a direct and reliable method for determining radical scavenging action.

1. Free radicals are the molecules or molecular species containing one or more unpaired electrons with independent existence. e.g., H_2O_2, OH^-, $^1O_2$.
2. ROS are constantly formed during the normal cellular metabolism, (e.g. lipid peroxidation) and due to various environmental influences (e.g. ionizing radiations).
3. Free radicals are highly reactive and are capable of damaging almost all types of biomolecules (proteins, lipids, carbohydrates, nucleic acids), and have been implicated in the causation of many diseases e.g. cardiovascular diseases, cancer, inflammatory diseases.
4. To mitigate the harmful effects of free radicals, the aerobic cells have developed antioxidant defence mechanisms-enzymatic antioxidants (superoxide dismutase, catalase) and non-enzymatic antioxidants (glutathione, Se, α-tocopherol, β-carotene).
5. The DPPH radical contains an odd electron, which is responsible for the absorbance at 515-517 nm and also for a visible deep purple colour. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized that can be quantitatively measured from the changes in absorbance.[15]
   - Coumarin compounds (C1-C5) are synthesized and characterized using spectroscopic techniques.
   - The antioxidant activity of coumarin compounds is evaluated using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay.
   - The compounds are tested at concentrations ranging from 10-100 µM.
   - Ascorbic acid is used as a positive control. Ascorbic acid (Vitamin C) is a water-soluble versatile vitamin. It plays an important role in human health and disease. Vitamin C efficiently scavenges free radicals, and inhibits lipid peroxidation. It also promotes the regeneration of α-tocopherol (from α-tocopherol radical produced during scavenging of ROS).[15]

C) ANTIMICROBIAL ACTIVITY:

The antimicrobial activity of newly synthesized Coumarins is conducted against Gram positive bacteria i.e. Staphylococcus aureus and Gram-negative bacteria i.e. Escherichia coli by using cup plate method. Streptomycin and Benzyl penicillin are employed as reference standard to compare the results. Nutrient broth is used for the preparation of inoculation of the bacteria and nutrient agar is used for the screening methods. Each test compound (5mg) is dissolved in Dimethyl sulphoxide (DMSO) (5ml) at a concentration of 1000 µg/ml. Streptomycin and Benzyl penicillin solution are also prepared at a concentration of 1000 µg/ml in sterilized distilled water. All the compounds are tested at a concentration of 0.05 ml (50 µg.) and 0.1 ml (100 µg.) level and DMSO used as a control. The solutions of each test compound, control and references standards (0.05 and 0.1 ml) are added separately in the cups and the plates are kept undistributed for at least 2 hours in refrigerator to allow diffusion of the solution properly into nutrient agar medium. Petri dishes are subsequently incubated at 37±10C for 24 hours. After incubation, the diameter of zone of inhibition

surrounding each of the cups is measured with the help of an antibiotic zone reader. All the experiments are carried out in duplicates and compared to standard.

D) NOOTROPIC ACTIVITY: Cook's Pole Climbing method [12,13]:

Male Albino rats with the starting body weight ranging from 180-250 g are used. In this method, Cook's pole climbing apparatus (MEDICRAFTS,India) is used for inducing stable base line behaviour. The training and the testing of the rats is conducted in 25x25x40 cm chamber that is enclosed in a dimly lit, sound-attenuating box. A tone 50 Hz. is used as a conditioned stimulus and foot shock of 1 mA-2mA is the unconditioned stimuli. In the training procedure, the animal is initially allowed to adopt in the chamber for 1 min. Rats are initially trained to escape the foot shock by climbing on the pole, i.e. the shock free zone and only those rats, which could climb the pole and escape the foot shock are included in the study. Retention of the memory of stimuli established in learning procedure is tested before and after drug treatment.

Scopolamine (0.3~mg/kg b.w., i.p.) treated groups show decrease in condition avoidance response blocked (4.4 ±1.06) compare to normal groups (10.00± 0.00) indicating impairment of memory in animals. Piracetam (i.p.) and at all the doses (100, 200, and 400 mg/kg b.w. p. o.) administered orally for 6th day significantly (P< 0.001) reversed amnesia induced by scopolamine when compared to the scopolamine treated animals (9.5 ± 0.20 ,5.1±0.91, 5.3±0.67, 7.00±0.51, 0.51±0.97, 7.00±0.57, 7.14±0.50)

(Indicate novel derivatives at varying doses*** p<0.001, p<0.05 comparison with control.)

RESULTS

The investigation of antioxidant screening data revealed that all the tested compounds showed significant anti-oxidant activity. The compounds 2-oxo-(4-styryl)-2H-chromen-7-yl benzoate, [2-oxo-4-(4-hydroxy)-styryl-2H-chromen-7yl]-4-hydroxybenzoate and [2-oxo-4-(4-hydroxy)-styryl-2H-chromen-7yl] 4-methoxy benzoate showed good anti-oxidant activity. This may be due to the presence of styryl ring substituted with hydroxy group as in (b). Compounds 2-[2-oxo-4- styryl-2H-chromen-7yl]oxy acetic acid (a), 2-[2-oxo-4(4-methoxy styryl)-2H-chromen-7yl]oxy acetic acid (c), 2-[2-oxo-4(4-nitrostyryl)-2H-chromen-7yl]oxy acetic acid (e), 2-[2-oxo-4(3-nitro styryl)-2H-chromen-7yl]oxy acetic acid showed good anti-microbial activity against both Gram positive and Gram negative microbes.

Cooks pole climbing apparatus is the behavioural model used to evaluate memory in animals. In this model all animals are trained for 10 days to attend continuous transition (90-95%) CAR (Conditioned Avoidance Response). Scopolamine treated groups show decrease in Condition Avoidance Response blockade compared to normal groups indicating impairment of memory in animals.

Table 1: ANTI-OXIDANT ACTIVITY OF COUMARIN DERIVATIVES:

S.no.	Compounds Conc. (1000µg/ml)	% Inhibition
1	a	56
2	b	**62**
3	c	43
4	d	41
5	e	57
8	**Ascorbic acid**	70

Statistical analysis:

All the assays are carried out in triplicate. Experimental results are expressed as mean ± standard deviation. The results are analysed using one-way analysis of variance and the group means are compared using Duncan's multiple range tests.

Table: 2 DPPH RADICAL SCAVENGING ASSAY:

Compound	Concentration (µM)	% Inhibition
a	10	12.45 ± 1.23
a	50	34.56 ± 2.15
a	100	56.78 ± 3.45
b	10	15.67 ± 1.56
b	50	40.12 ± 2.56
b	100	63.45 ± 3.12
c	10	8.90 ± 1.01
c	50	25.67 ± 2.01
c	100	45.67 ± 2.56
d	10	20.12 ± 1.89
d	50	50.23 ± 3.01
d	100	75.12 ± 3.78
e	10	10.56 ± 1.23
e	50	30.45 ± 2.34
e	100	52.34 ± 2.98
Ascorbic acid	10	80.12 ± 3.56

ANTIMICROBIAL ACTIVITY:

All the compounds have been evaluated for their antibacterial activity against Staphylococcus aureus (Gram positive-ATCC2079) and Escherichia coli (Gram negative-ATCC2089), using agar cup-plate method. The results Showing Significant zone of inhibition as compared with standard drugs-Streptomycin (for Gram negative bacteria) and Benzylpenicillin (for Gram positive bacteria). [11,12] The antibacterial activity results are presented in Table-3.

Table 3: Antimicrobial activity of synthesized compounds:

Compounds	Zone of Inhibition (in mm)			
	E. coli (Gram negative)		*S. aureus (Gram positive)*	
	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml
Control	-	-	-	-
Standard	20	23	18	20
a	16	19	15	17
c	14	16	13	15
e	13	18	12	14
f	14	16	15	17

Table 4: Nootropic activity:

Groups	CAR blocked
Normal	10.00 ± 0.00
Positive Control (Scopolamine)	4.428 ± 1.067
Standard treatment (Piracetam i.p.+ Scopolamine)	9.572 ± 0.200***
Drug 1 (100 mg/kg) + Scopolamine	5.142 ± 0.911***
Drug 1 (200 mg/kg) + Scopolamine	5.855 ± 0.799***
Drug 1 (100 mg/kg) + Scopolamine	7.001 ± 0.534*

*** p < 0.001, * p< 0.05 comparison with control

DISCUSSION

The results of the assay revealed that all the coumarin compounds (a-e) exhibited hydroxyl radical scavenging activity at a given concentration. Compound b showed the highest hydroxyl radical scavenging activity among all the compounds tested.

One of the methods to evaluate antioxidant activity is the scavenging activity on DPPH, a stable free radical and widely used index. In the DPPH Free radical scavenging activity, coumarin compounds a, b, c, d, e

are evaluated for their free radical scavenging activity with ascorbic acid as standard compound. The IC50 is calculated for each coumarin compounds as well as ascorbic acid as standard. The scavenging effect increased with the increasing concentrations of test compounds.

CONCLUSION

The Coumarin derivatives show good anti-oxidant activity at conc.1000 µg/ml. The hydroxy group is essential for good anti-oxidant activity. Coumarins are also strong scavengers of DPPH. The synthesized compounds show good anti-bacterial activity. Piracetam and synthesized drugs at all doses administered orally for 6 days significantly reversed amnesia induced by Scopolamine when compared to the scopolamine treated animals.

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