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NEUROTROPIC AND ANTITUMOR ACTIVITY OF SOME NEW AMIDES OF SULPHOSALICYLIC ACID

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Abstract-The present manuscript covers the neurotropic and antitumor studies of some new amides of sulphosalicylic acid which were synthesized by reported method using reaction of suitable amines with 5-sulphosalicylic acid in 2:1 ratio. These compounds are found effective in nervous system. In the present paper we describe the neurotropic and antitumor activity of these amides against mice of both male and female sex and human breast adenocarcinoma cell line respectively.

Keywords: sulphosalicylic acid, neurotropic and antitumor activity.

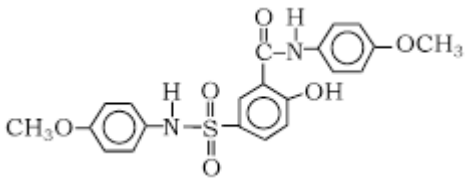
Introduction

In recent year due to emergence of mono and multidrugs resistant strains of mycobacterium tuberculosis and AIDS epidemic etc, there is a search for new drugs leading to new structural classes along with novel mechanism of action become the need of the present time. The literature survey revealed that nitrogen and sulfur containing compounds [1-3] are potentially active as anticancer, antiviral and antifungal agent [4, 5]. Amines in general have been known to be biologically active [6] and the effect of presence of various constituents in the amines increases their antimicrobial activity has been investigated [7, 8]. The compounds having amino acid have proven to be potentially active against various bacterial and fungal strains and many of them got wide acceptance in clinical trials [9, 10].

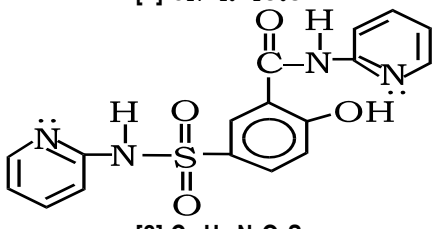
The differential inhibition of cytochrome P-450 between pathogenic bacterial and fungal strains and human beings is the basis for the clinically important amino acid as antimicrobial agents. It may be found that the inhibition can be determined by the differential complementarities between the structures of antimicrobial agent and the active sites of enzymes responsible for microbial activity. Further, the compounds containing amide moiety has also attracted attention due to their important role in various industrial and biological processes [11]. Moreover, the amide moieties having fundamental interest in order to understand the role of metalloproteins in the control of cell metabolism.

Experimental

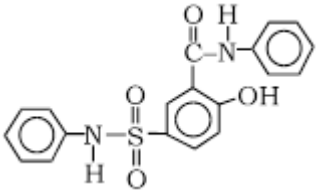
The synthesis of amides of 5-sulphosalicyclic acid was carried out by the reaction of respective amines with 5-sulphosalicylic acid as reported earlier by our group [12]. The amines which are taken here are: *p*-methoxyaniline, 2-aminopyridine, aniline, α-naphthylamine, *p*-chloroaniline and hydrazine. The amines were purified by crystallization before use. All the compounds were crystalline solid and quite stable at room temperature with good yield (70-75%). The compounds were soluble in polar solvents. They have sharp melting points. The molecular weights of the compounds were determined cryoscopically and their infra red spectra (FTIR) were recorded in a Perkin-Elmer spectrophotometer in 4000-200 cm^{-1} range. The structures of the synthesized compounds are given below.



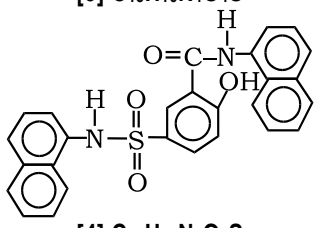
[1] $C_{21}H_{20}N_2O_6S$



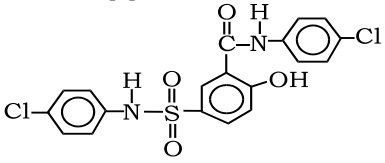
[2] $C_{17}H_{14}N_2O_4S$



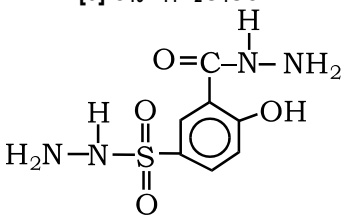
[3] $C_{18}H_{16}N_4O_4S$



[4] $C_{27}H_{20}N_2O_4S$



[5] $C_{19}H_{14}N_2O_4SCl$



[6] $C_7H_{10}N_4O_4S$

Neurotropic Activity

The neurotropic activity of the newly synthesized compounds was carried out on mice of both male and female sex weighing around 18-23 gm in winter season (in the month of December –January). The room temperature was maintained with in the limit that is 20-25°C. The solutions of compounds in the olive oil or the aqueous suspension of the remaining substance prepared with the addition of tween-80 as emulsifier. The solution of newly synthesized compounds, were injected intraperitoneally 30-45 min (In case of oily suspension 60 min) before the test. The same volume of sodium chloride (NaCl) isotonic solution was introduced into the control mice. The effect of the substance introduced in the doses of 50 mg/kg was compared in groups of animals consisting of 6-8 individuals. The experimental data were treated for their biostatic. The mean values of LD_{50} and ED_{50} for the observation were determined by reported method [13]. The arithmetical means and their standard deviations were calculated to assess the average duration of the anesthetic effect of the hexenal and phenamine stereotype, the protective properties in the corazol spasms and hypoxia, the degree of hypothermia. The significance of difference between mean values were assessed and considered as significant at a probability level $p<0.05$.

- The effect of the substance on the central nervous system was estimated-From their influence on the coordination of movements and the tone of muscle by the test “rotating rod”(8 rpm for 2-3 min on an Ugo basil apparatus), “tube”(30X2 cm of glass tube for 30 sec.) and by “tightening in cross beam”(2mm metal wire for 5-6 sec.).
- From the influence on the temperature of the body, by measuring the rectal temperature with an electric thermometer.
- From the analgesic effect which was determined by hot plate method.
- From the anti spasmodic activity which was estimated by the maximum electric shock test.
- From the corazol spasms caused by the intravenous titration with 1% corazol solution at a rate of 0.01 ml/ sec.
- From the influence on the duration of the hexenal anesthesia (70mg /kg i.v.) and the ethanol anesthesia (25% solution of ethanol i.a., the dose of 5gm/kg).
- From the influence of the life time of animals under hypoxic hypoxia, created by placing the mouse in a separate chamber with a volume of 220 cm^3 without absorption of CO_2.
- From the change in locomotors activity informed by phenamine (10mg/kg).
- The acute toxicity was determined by intra peritoneal injection of new synthesized compound and by establishing their lethal dose (LD_{50}).

Anti-tumor activity

This method was carried out to estimate the effect of test compound on the growth of tumor cells. The human breast cancer cells lines (MCF-7) were employed. The human breast cancer cell line (MCF-7)and mammary cancer cell line (EVSA-7), were co-incubated with the test compounds at 1 μg/ml doses for 96 hrs and the cell growth count was measured by MTT assay[14]. The basic principle involved in this assay depends upon the reduction of tetrazoleum salt. The yellow colored tetrazoleum MTT, [3-(4, 5-dimethylthiazol-2-yl)-2, 5,-diphenyl tetrazoleum bromide] is reduced by metabolically active cells in part by the action of dehydrogenase enzymes to generate reducing equivalents such as NADH and NADPH. The resulting intra cellular purple colour zones was solubilized and quantified by spectrophotometer method. The MTT was dissolved in PBS at a concentration of 5 mg/ml. Then 50 μl of the MTT solution was added to each well of the 96 well culture plate, containing the 100 μl culture along with test compound and incubated at 37°C for 4 hrs. The medium was then removed carefully without disturbing the purple colored formazon crystals. Then, 50 ml of dimethylsulfoxide (DMSO) was added to each well and mixed thoroughly to dissolve the crystals of the formazon. The plates were then read on ELISA plate reader at a wavelength of 570 mm. The readings were presented as optical density/ cell count.

Results and discussion

Neurotropic Activity

The neurotropic activity of the newly synthesized compounds was carried out on mice of both male and female sex weighing around 18-23 gm in winter season (in the month of December –January). The room temperature was maintained with in the limit that is 20-25°C. The results of these compounds show that these compounds are moderately active and show better efficacy in nervous system of mice. Depending on the nature of compound it was found that the compounds show predominantly psychotropic activity.

***In-vitro* Antitumor Activity**

The antitumor activity of these compounds was studied against the human breast adenocarcinoma (MCF-7) and mammary cancer (EVSA-7) cell lines. The compounds show moderate to higher activity against tumor cell lines. It was found that the slight variation in their activity is due to different amides. The compound generally interacts with the receptor site of multienzyme complex responsible for the cytostatic and cytotoxic conditions for a cell. The compounds can easily binds with the receptor site. It may be noted that the compound generally interact with purine bases in DNA molecule and form complex with DNA strands affecting replication and transcription of DNA molecule and stop the cell division along with protein synthesis.

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Table 1- Neurotropic Activity of amides of sulphosalicylic acid

S.N.	Compounds	Hypoxia	Hexenal anesthesia	Ethanol anesthesia	Corazole convulsions Clonic/tonic	Phenamine hyperactivity (as antagonist)	LD_{50}
1	$C_{21}H_{20}N_2O_6S$	176.4	130.0	112.0	86.5/148.6	105.6	30-55.2
2	$C_{17}H_{14}N_2O_4S$	126.0	112.0	29.3	171.6/239.6	128.7	76-136
3	$C_{18}H_{16}N_4O_4S$	152.1	136.2	35.0	154.6/162.3	76.3	560-1120
4	$C_{27}H_{20}N_2O_4S$	136.4	172.2	132.5	86.7/254.6	42.5	2050-5060
5	$C_{19}H_{14}N_2O_4SCl$	174.6	156.4	280.5	203.4/214.6	66.7	262
6	$C_7H_{10}N_4O_4S$	125.6	110.0	126.4	323.8/189.2	106.8	346-980

Table 2- In-vitro Anti-tumor activity of amides of sulphosalicylic acid

S. No.	Compounds	Cell No. x 10^4 (MCF-7)	Activity	Cell No. x 10^4 (EVSA-7)	Activity
1.	$C_{21}H_{20}N_2O_6S$	12.34 ± 1.05	-	11.74 ± 1.22	-
2.	$C_{17}H_{14}N_2O_4S$	11.69 ± 1.02	-	10.68 ± 1.08	-
3.	$C_{18}H_{16}N_4O_4S$	9.17 ± 0.87	+	9.69 ± 0.92	+
4.	$C_{27}H_{20}N_2O_4S$	9.34 ± 0.65	+	9.66 ± 0.90	+
5.	$C_{19}H_{14}N_2O_4SCl$	9.89 ± 0.85	+	8.28 ± 0.46	+
6.	$C_7H_{10}N_4O_4S$	9.25 ± 0.86	+	8.22 ± 0.42	+
7.	Negative Control	10.21 ± 1.01	-	10.23 ± 1.03	-
8.	Positive Control	40.26 ± 3.23	-	42.24 ± 4.22	-

*Negative Control- Culture Medium only, **Positive Control – 17 β estradiol