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VIEWS

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Potential Energy Curves and Dissociation Energy of AlC Molecule

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ABSTRACT

The potential energy curves for the electronic ground state of AlC molecule have been constructed by using the RKRV method. The ground state dissociation energy for AlC molecule is determined by curve fitting techniques using the five parameters Hulbert-Hirschfelder (H-H) function. The estimated dissociation energy for AlC is 5.24± 0.117 eV. These values are good agreement with the literature values.

Key words: Potential energy, dissociation energies.

Introduction

The study of experimental potential energy curves for atomic interactions is fundamental importance in chemical physics for the understanding of various physical problems arising in Astrophysics, gas kinetics and aerodynamics. Also detail knowledge of experimental potential energy curve is needed for establishing the dissociation energy of molecule. In the present study the experimental potential energy curve for the $X^4\Sigma^-$, $A^4\Pi$ and $B^4\Sigma^-$ states of AlC molecule have been constructed by means of the RKRV method [1] .Then the dissociation energy (De) for the ground state of the molecule has been determined from the turning points of the experimental potential energy curve by fitting the five parameter Hulburt-Hirschfelder potential function [2].

Theory & Computational Procedure

Potential energy curves

Experimental observed vibrational levels are used to construct the potential energy curve. The molecular constants required for the present study have been taken from the literature [3-7] and are listed in Table I. Many authors employed RKRV method and constructed the potential energy curves [8-19]. The RKRV [20-24] method is an improved form of RKR method, which makes use of Wentzel-Kramers-Brillouin (WKB) approximation from which we obtain reliable potential energy curves with the observed vibrational and rotational constants. The potential energy curves are constructed using the method of RKRV [20-24] utilizing the molecular constants (listed in Table I) and the results are presented in Table II. The details of the said method were reported in literature, as such only the results of the present work are given Table I.

Dissociation energy

The RKRV turning points are inserted into the five parameter Hulburt-Hirschfelder's [25-27] function and the potential energies U(r) are calculated by varying the dissociation energy De values. An average percentage deviation is determined between the calculated U(r) and the experimental G (ν) values. An accurate estimation of the dissociation energy (Do) requires an empirical potential function, which provides the best reproduction of the experimental energy values. A critical evaluation of the

Table I
Spectroscopic constants of different electronic states of AlC molecule

Molecule	μ	State	T_e (cm^{-1})	ωe (cm^{-1})	Ωexe (cm^{-1})	Be (cm^{-1})	αe (cm^{-1})	re A^0
AIC	8.309	$X^4\Sigma^-$	0	654.89	4.347	0.531012	0.005094	1.954
		$A^4\Pi$	18012.1	843.66	10.914	0.63721	0.00802	1.784
		$B^4\Sigma^-$	22426.2	733.94	7.629	0.565807	0.006393	1.893

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Table II

Turning Points of the P-E curves of AlC molecule RKRV method

U	U (cm^{-1})	r max (A^0)	r min (A^0)
$X^4\Sigma^-$ State T_e=0			
0	326.35	2.038	1.880
1	972.55	2.106	1.831
2	1610	2.157	1.800
3	2238.86	2.200	1.776
4	2858.98	2.240	1.756
5	3470.39	2.277	1.738
6	4073.119	2.313	1.723
$A^4\Pi$ State T_e=18012.16 cm^{-1}			
0	419.10	1.859	1.719
1	1240.94	1.922	1.677
2	2040.96	1.969	1.651
3	2819.14	2.012	1.630
4	3575.50	2.051	1.613
5	4310.03	2.088	1.598
$B^4\Sigma^-$ State 22426.25 cm^{-1}			
0	365.06	1.973	1.824
1	1083.74	2.039	1.778
2	1787.17	2.089	1.749
3	2475.34	2.133	1.728
4	3148.25	2.173	1.708

Table III

Energy values obtained from Hulbert–Hirschfelder (H-H) function for the $X^4\Sigma^-$ electronic state of AlC molecule

r (A^0)	U (cm^{-1})	U (r) cm^{-1}		
		De=42421	De=42621	De=42821
2.038	326.31	324.48	326.01	327.54
2.106	972.41	967.47	972.03	976.59
2.157	1610.83	1602.93	1610.48	1618.04
2.200	2238.54	2231.31	2241.56	2252.35
2.240	2858.57	2852.98	2866.43	2879.89
2.27	3469.90	3468.54	3484.81	3501.16
2.313	4072.53	4078.02	4097.24	4116.47
1.880	326.31	324.44	325.97	327.50
1.831	927.41	966.58	971.14	975.70
1.800	1609.83	1599.66	1607.20	1618.04
1776	2238.54	2223.67	2234.15	2244.63
1756	2858.57	2838.71	2852.09	2865.47
1.738	3469.90	3444.74	3460.98	3477.22
1.723	4072.53	4041.96	4061.02	4080.08
Average percentage deviation		0.48	0.21	0.49

importance of these functions was given by Steele et al. [27] and they have shown that the potential function of Hulburt-Hirschfelder (H-H) [25-27] fits well with the RKRV curves of a large number of diatomic molecules. In the present investigation, it is observed that the H-H function [25, 26] functions fits best and reproduces the experimental energy values (Table III). Different De values are used in the H-H function and the De value, for which the best fit of the energy values U(r) is observed, which is taken as the dissociation energy (De) of the molecule.

Results and Discussion

The inherent error in the H-H function given by Steele et al [27] is 2%. The error involved in the evaluation of De is minimum average percentage deviation plus the inherent error in the potential function. The D0 values are found to be 5.24±0.117ev for AlC molecule. Here D0=De – G (0). The turning points obtained from RKRV [24-28] method are inserted in H-H [25-27, 28] function and evaluated U(r) values. The relevant U(r) values for the selected De values are given in Table III.

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Synthesis, Characterization and Biololgical Screening of (2-Dialkylamino-Ethoxy)-Acetic Acid (2-Oxo-1, 2-Dihydro-Indol-3-Ylidene)-Hydrazides

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ABSTRACT

A series of new (2-dialkylamino-ethoxy)-acetic acid (2-oxo-1,2-dihydro-indol-3-ylidene)-hydrazides VI(a-h) were synthesized in three phases. In the first phase isatins (indol-1H-2,3-diones) were converted to 3-hydrazono-1,3-dihydro-indol-2-ones using hydrazine hydrate. In the second phase 3-hydrazono-1, 3-dihydro-indol-2-one were converted to chloroacetic acid(2-oxo-1,2-dihydro-indol-3-ylidene)-hydrazides by treating with chloroacetyl chloride in dry benzene under anhydrous conditions. In the third phase chloroacetic acid (2-oxo-1,2-dihydro-indol-3-ylidene)-hydrazides were treated with 2-dialkylamino ethanols in dry acetone to get the desired compounds. The synthesized compounds were characterized for their structure by elemental analysis, FT-IR, mass and ^{1}H-NMR spectra. The compounds were screened for antibacterial and anticholinergic activities using standard protocols. The 2-(dialkylamino-ethoxy)-acetic acid (2-oxo-1, 2-dihydro-indol-3-ylidene)-hydrazides compounds possess remarkable antibacterial activity and no anticholinergic activity

Keywords: Anticholinergic, antibacterial activity, 3-hydrazono-1,3-dihydro-indol-2-ones, chloroacetic acid(2-oxo-1,2-dihydro-indol-3-ylidene)-hydrazide.

Introduction

Isatin, 1*H* -indole-2,3-dione, a synthetically versatile molecule that possess a diversity of biological activities including anticonvulsant [1,2] , anticancer [3,4], antioxidant [5-7], antiviral [8-10] and antibacterial [11-13] properties. Isatins are potential synthons for building synthetically a variety of chemical systems known for their broader biological and pharmacological applications [14]. Isatin one of the most studied chemical entities, is an endogenous compound found in blood, tissues, and various organs [15]. The Schiff bases several semicarbazone and hydrazone derivatives of isatin and 5-haloisatins were reported to possess antimicrobial properties [16-18]. It is also known from the literature compounds containing `dialkylaminoethyloxy' group as a pharmacophore exhibit antihistaminic and anticholinergic activities. In view of this, it has been felt worthwhile to prepare some novel (2-dialkylamino-ethoxy)-acetic acid(2-oxo-1, 2-dihydro-indol-3-ylidene)-hydrazides by making use of 3-keto group of isatins and screen them for anticholinergic and antibacterial activities. For this purpose eight new (2-dialkylamino-ethoxy)-acetic acid (2-oxo-1, 2-dihydro-indol-3-ylidene)-hydrazides VI (a-h) were synthesized as per the scheme given.

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Materials and Methods

All Chemicals and solvents utilized were of analytical grade and were purchased from authorized dealers of Merck, Sd-fine chemicals, India. Open capillaries tubes were used to determine melting points of all the synthesized compounds. Melting point determination was performed on Toshni wall melting point apparatus. The infrared (IR) spectra of the compounds were recorded on Perkin-Elmer FT-IR 240-C spectrometer using KBr optics.^{1}H NMR were recorded on an Gemini varian 200 MHz, Bruker AV 300 MHz instrument in DMSO-d_6 or $CDCl_3$using Tetra methyl silane (TMS) as an internal standard, EI and chemical ionization mass spectra were recorded on a VG 7070 H instrument at 70 ev. CHN analysis was recorded on a vario EL analyzer. Column chromatography was performed by using Qualigen's silica gel for column chromatography (60–120 mesh).

Experimental procedures

Synthesis of isatins (Indole- 2, 3-diones) ***(III)*****:** Different isonitrosoacetanilides were prepared from the respective

aromatic amines **(I)** viz. aniline, p-chloroaniline, p-toluidine and o-toluidine on reaction with chloral hydrate and hydroxylamine hydrochloride. Each of the isonitrosoacetanilide **(II)** was subjected to a dehydrative cyclization using sulphuric acid (d 1.84) to yield the corresponding isatin **(III)**. All these isatin thus prepared were identified by their physical constants reported in the literature [19].

*Synthesis of 3-hydrazono-1,3-dihydro-indol-2-one **(IV)***: An appropriate isatin (I, 0.01 mol) was dissolved in alcohol (20 ml) and added hydrazine hydrate (99%, 0.015 mol) while shaking, the reaction mixture was stirred well, warmed on a water bath for 10 min, and left in the refrigerator for 3 hours. The resultant yellow crystalline solid was filtered, washed repeatedly with small portions of cold alcohol. The product was dried and purified by recrystallization from chloroform.

The compounds thus obtained were characterized by comparison with their physical constants reported in the literature [19]**.**

*Synthesis of chloro-acetic acid (2-oxo-1, 2-dihydro-indol-3-ylidene)-hydrazide **(V):*** An appropriate isatin hydrazone (**IV**, 0.01 moles) was heated under reflux with chloroacetyl chloride (0.01mol) in dry benzene under anhydrous conditions using calcium chloride guard tube for 2 hrs. The product thus formed was filtered and washed with small portions of benzene to remove any unreacted chloroacetyl chloride. It was purified by recrystallization from suitable solvents. The compounds thus obtained were characterized by comparison with their physical constants reported in the literature [19].

*(2-Dialkylamino-ethoxy)-acetic acid (2-oxo-1,2-dihydro-indol-3-ylidene)-hydrazide **VI (a-h):*** A mixture of isatin 3-(N2-chloroacetyl) hydrazone (**V**, 0.01mol) and an appropriate 2-dialkylamino ethanol (0.012 mol) in dry acetone (20 ml) was heated under reflux for 2-3 hrs and the solvent was removed by evaporating. The residue was washed thoroughly with small portions of cold water to get the product. The purity of the compound was checked by TLC. The compounds were dried and purified by recrystallization from appropriate solvents.

The compounds thus obtained have been characterized as their respective (2-Dialkylamino-ethoxy)-acetic acid(2-oxo-1,2-dihydro-indol-3-ylidene)-hydrazides VI (a-h), by their physical, analytical and spectral data. All the synthesized compounds were screened for *in vitro* antibacterial activity by agar diffusion method using ampicillin as standard compound and *in vitro* anticholinergic activity.

Biological screening

Antibacterial activity

The antibacterial activity of the test compounds were studied systematically against four different strains of bacteria utilizing two gram positive (Bacillus subtilis, Staphylococcus aureas) and two gram negative (Escherichia coli and Klebsiella pneumonia) by agar diffusion method [20].

The test organisms were sub cultured using nutrient agar medium. The tubes containing sterilized medium was inoculated with respective bacterial strain. After incubation at 37 ± 10˚C for 24 hrs, they were stored in refrigerator. Bacterial inoculums were prepared by transferring a loopful of stock to nutrient broth (100 ml) in control flaks (250 ml). The flasks were incubated at 37 ± 10˚C for 18 hrs before the experimentation.

Solution of the test compounds were prepared by dissolved 10 mg each in 10 ml of dimethyl formamide. A reference standard for gram positive and gram negative bacteria were made by dissolving accurately weighed quantity of ampicillin in sterile distilled water, separately.

Anticholinergic activity

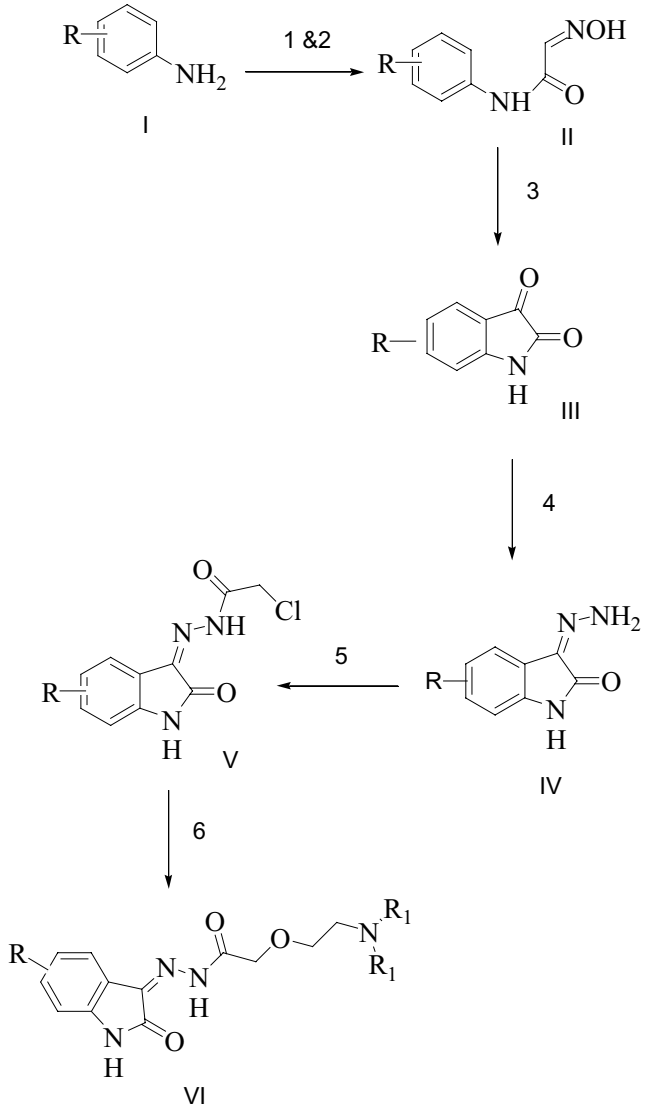
All the test compounds were also screened for their anticholinergic activity. The anticholinergic activity of the compounds was screened by using the Albino rat small intestine [21]. Acetylcholine chloride was used as an agonist for inducing the contractions. The test compounds and the agonist acetylcholine chloride were used in logarithmic doses.

A healthy and adult rat of either sex was fasted overnight and sacrificed by a blow on its head. The abdomen was cut open and the caecum was lifted to trace the ileocaecal junction. A few centimeter long ileal portions was cut and placed immediately in a petriplate containing the Tyrode's solution. The mesentery was trimmed carefully and ileum cleaned with gentle care by passing the warm Tyrode's solution using a 10 ml bulb-pipette through the lumen of the ileum. A 2 cm piece of the ileum was taken from petriplate and tied with a thread to the top and the bottom ends without occluding the lumen and mounted in the organ bath containing the Tyrode's solution. Temperature of the bath was maintained at 37±1° C and was aerated continuously. A tension of 0.5 g was applied and the tissue was allowed to equilibrate for 30 minutes before starting the experiment.

The response of the tissue to increasing doses of acetylcholine was recorded on a smoked drum using a frontal writing lever with a magnification of 1:10. The sub maximal dose of the agonist was selected and the response of the tissue to this dose in the presence of increasing concentrations (logarithmic doses) of the test compounds was recorded. The agonist used in this experiment was standard acetylcholine solution (prepared in Tyrode's solution). The duration of action of the agonist on the tissue was one minute. The compound under test was added to the organ bath in the form of a suspension prepared in 0.8% sodium carboxy methyl cellulose solution. A blank solution was also used for recording the effect of the blank and the tissue. The test compounds were allowed to act on the tissue for 2 minutes before the agonist was added to the bath.

Contraction induced by the sub maximal dose of the agonist in the presence of the test compounds were recorded and the blockade, if any, produced by the compound was noted. Each time the tissue was allowed to rest for 3 minutes and the effect of the agonist was ensured before the next dose is attempted. It has been observed that none of the compound was inhibited acetylcholine induced contractions.

Scheme-I



1. Chloralhydrate
2. Hydroxylamine-HCl
3. Conc.H_2SO_4
4. Hydrazine hydrate (99%)
5. Chloroacetylchloride
6. Dialkylamino ethanol

R = H, 5-CH_3, 7-CH_3, 5-Cl ; R_1 = CH_3, C_2H_5

Results and Discussion

A series of novel and new (2-Dialkylamino-ethoxy)-acetic acid (2-oxo-1,2-dihydro-indol-3-ylidene)-hydrazides VI (a-h) were synthesized as per the scheme in three phases. In the first phase isatins (indol-1H-2,3-diones) were converted to 3-hydrazono-1,3-dihydro-indol-2-ones using hydrazine hydrate. In the second phase 3-hydrazono-1, 3-dihydro-indol-2-one were converted to chloroacetic acid (2-oxo-1,2-dihydro-indol-3-ylidene)-hydrazides by treating with chloroacetyl chloride in dry benzene under anhydrous conditions. The physical data of these compounds is given in table1. In the third phase chloroacetic acid (2-oxo-1,2-dihydro-indol-3-ylidene)-hydrazides were treated with 2-dialkylamino ethanols in dry acetone to get the desired compounds. The synthesized compounds were characterized for their structure by elemental analysis, FT-IR, mass and ^{1}H-NMR spectra. The data of the same compounds is as follows:

(2-Dimethylamino-ethoxy)-acetic acid (2-oxo-1,2-dihydro-indol-3-ylidene)-hydrazide ***(VIa)****:* IR (KBr) 3358.35 (-NH), 3082.29 (aromatic –CH streching), 1687.35 (-C=O, acid hydrazide),

1656.54 (-C=O, Isatin), 1590 (C=N), 1190 (C-O-C); ^{1}HNMR (DMSO-d6) ppm:1.84 (s, 6H, CH_3-N-CH_3), 2.17 (m, 4H, O-CH_2-CH_2-N), 2.28 (S, 2H, OCH_2), 6.8-7.3(m, 4H, Ar-H), 10.7 (s, 1H, NH-C=O), 11.2 (s, 1H, NH); MS m/z:210.32 (M+).

(2-Diethylamino-ethoxy)-acetic acid (2-oxo-1,2-dihydro-indol-3-ylidene)-hydrazide ***(VIb)****:* IR (KBr) 3365.35 (-NH), 3082.29 (aromatic –CH stretching), 1689.35 (-C=O, acid hydrazide), 1666.54 (-C=O, Isatin), 1598(C=N), 1195.50 (C-O-C); ^{1}HNMR (DMSO-d6) ppm:1.91 (s, 10H, C_2H_5-N-C_2H_5), 2.17 (m, 4H, O-CH_2-CH_2-N), 2.28 (S, 2H, OCH_2), 6.8-7.3(m, 4H, Ar-H), 10.7 (s, 1H, NH-C=O), 11.2 (s, 1H, NH); MS m/z : 319.37 (M+).

(2-Dimethylamino-ethoxy)-acetic acid (5-methyl-2-oxo-1,2-dihydro-indol-3-ylidene)-hydrazide ***(VIc)****:* IR (KBr) 3370.35 (-NH), 3082.29 (aromatic –CH streching), 1689.35 (-C=O, acid hydrazide), 1666.54 (-C=O, Isatin), 1598(C=N), 1195 (C-O-C); ^{1}HNMR (DMSO-d6) ppm:1.85 (s, 6H, CH_3-N-CH_3), 2.19 (m, 4H, O-CH_2-CH_2-N), 2.31 (S, 2H, OCH_2), 2.57 (s, 3H, 5-CH3), 6.90-7.35 (m, 3H, Ar-H), 10.72 (s, 1H, NH-C=O), 11.32 (s, 1H, NH); MS m/z : 332.40 (M+).

(2-Diethylamino-ethoxy)-acetic acid (5-methyl-2-oxo-1,2-dihydro-indol-3-ylidene)-hydrazide ***(VId)****:* IR (KBr) 3374.35 (-NH), 3087.29 (aromatic –CH streching), 1690.35 (-C=O, acid hydrazide), 1667.54 (-C=O, Isatin), 1598.23 (C=N), 1198.50 (C-O-C); ^{1}HNMR (DMSO-d6) ppm:1.91 (s, 10H, C_2H_5-N-C_2H_5), 2.17 (m, 4H, O-CH_2-CH_2-N), 2.28 (S, 2H, OCH_2), 2.53 (s, 3H, 5-CH3), 6.8-7.3(m, 3H, Ar-H), 10.7 (s, 1H, NH-C=O), 11.2 (s, 1H, NH); MS m/z:304.34 (M+).

*(2-Dimethylamino-ethoxy)-acetic acid (7-methyl-2-oxo-1,2-dihydro-indol-3-ylidene)-hydrazide **(VIe)**:* IR (KBr) 3370.35 (-NH), 3082.29 (aromatic –CH streching), 1689.35 (-C=O, acid hydrazide), 1666.54 (-C=O, Isatin), 1598(C=N), 1195 (C-O-C); ^{1}HNMR (DMSO-d6) ppm: 1.87 (s, 6H, CH_3-N-CH_3), 2.23 (m, 4H, O-CH_2-CH_2-N), 2.39 (S, 2H, OCH_2), 2.61 (s, 3H, 7-CH3), 6.95-7.38 (m, 3H, Ar-H), 10.76(s, 1H, NH-C=O), 11.36(s, 1H, NH); MS m/z : 332.40 (M+).

*(2-Diethylamino-ethoxy)-acetic acid (7-methyl-2-oxo-1,2-dihydro-indol-3-ylidene)-hydrazide **(VIf)**:* IR (KBr) 3384.67 (-NH), 3076.65 (aromatic –CH streching), 1687.45 (-C=O, acid hydrazide), 1669.43 (-C=O, Isatin), 1572.34 (C=N),

Table 1

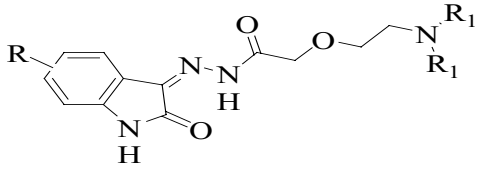
Physical data of chloroacetic acid (2-oxo-1,2-dihydro-indol-3-ylidene)-hydrazides V (a-d)

S.No.	Compound	R	M.P. (^{0}C)	Yield (%)	Mol.formula
1.	Va	H	204	75	$C_{10}H_8N_3O_2Cl$
2.	Vb	5-CH_3	212	82	$C_{11}H_{10}N_3O_2Cl$
3.	Vc	7-CH_3	226	80	$C_{11}H_{10}N_3O_2Cl$
4.	Vd	5-Cl	232	78	$C_{10}H_7N_3O_2Cl$

Note: R: substituent, M.p: Melting point
All the compounds were recrystallized from acetone.

Table: 2

Physical data of (2-dialkylamino-ethoxy)-acetic acid (2-oxo-1,2-dihydro-indol-3-ylidene)-hydrazides VI (a-h)



S.No	Compd code	R	R_1	Mole form	Molecular weight	Yield (%)	MP (^{0}C)	CHN analysis Found (Calc.)		
								%C	%H	%N
1	**VIa**	H	Me	$C_{14}H_{18}N_4O_3$	209.32	63	227	57.99 (57.92)	6.47 (6.25)	19.53 (19.30)
2	**VIb**	H	Et	$C_{16}H_{22}N_4O_3$	318.37	60	232	60.57 (60.36)	7.04 (6.97)	17.92 (17.60)
3	**VIc**	5-CH3	Me	$C_{15}H_{20}N_4O_3$	304.34	55	249	(59.38) 59.20	6.84 (6.62)	18.54 (18.41)
4	**VId**	5-CH3	Et	$C_{17}H_{24}N_4O_3$	332.40	57	255	61.65 (61.47)	7.47 (7.28)	16.93 (16.86)
5	**VIe**	7-CH3	Me	$C_{15}H_{20}N_4O_3$	304.34	59	233	59.42 (59.20)	6.85 (6.62)	18.62 (18.41)
6	**VIf**	7-CH3	Et	$C_{17}H_{24}N_4O_3$	332.40	51	239	61.68 (61.43)	7.50 (7.28)	17.06 (16.86)
7	**VIg**	5-Cl	Me	$C_{14}H_{17}N_4O_3Cl$	324.76	63	241	51.91 (51.78)	5.51 (5.28)	17.53 (17.25)
8	**VIh**	5-Cl	Et	$C_{16}H_{21}N_4O_3Cl$	352.13	65	247	54.62 (54.47)	6.27 (6.00)	10.26 (10.05)

Note: All the compounds were recrystallized from alcohol

1182.23 (C-O-C); ^{1}HNMR (DMSO-d6) ppm:1.96 (s, 10H, C_2H_5-N-C_2H_5), 2.19 (m, 4H, O-CH_2-CH_2-N), 2.41 (S, 2H, OCH_2), 2.64 (s, 3H, 7-CH3), 6.82-7.41(m, 3H, Ar-H), 10.56 (s, 1H, NH-C=O), 11.29 (s, 1H, NH); MS m/z : 332.40 (M+).

(2-Dimethylamino-ethoxy)-acetic acid (5-chloro-2-oxo-1,2-dihydro-indol-3-ylidene)-hydrazide ***(VIg)****:* IR (KBr) 3387.35 (-NH), 3065.29 (aromatic –CH streching), 1693.35 (-C=O, acid hydrazide), 1659.54 (-C=O, Isatin), 1557(C=N), 1198 (C-O-C); ^{1}HNMR (DMSO-d6) ppm: 1.92 (s, 6H, CH_3-N-CH_3), 2.45 (m, 4H, O-CH_2-CH_2-N), 2.48 (S, 2H, OCH_2), 6.87-7.56 (m, 3H, Ar-H), 10.83(s, 1H, NH-C=O), 11.61(s, 1H, NH); MS m/z : 324.76 (M+)

(2-Diethylamino-ethoxy)-acetic acid (5-chloro-2-oxo-1,2-dihydro-indol-3-ylidene)-hydrazide ***(VIh)****:* IR (KBr) 3363.35 (-NH), 3065.76 (aromatic –CH streching), 1682.35 (-C=O, acid hydrazide), 1663.54 (-C=O, Isatin), 1545.23(C=N), 1174.27 (C-O-C); ^{1}HNMR (DMSO-d6) ppm: 1.99 (s, 10H, C_2H_5-N-C_2H_5), 2.57 (m, 4H, O-CH_2-CH_2-N), 2.51 (S, 2H, OCH_2), 6.70-7.74 (m, 3H, Ar-H), 10.87(s, 1H, NH-C=O), 11.58 (s, 1H, NH); MS m/z : 352.13 (M+).

As many as eight new compounds were synthesized by adopting similar above procedure and then characterized by their physical, analytical and spectral data. The details of some of the representative compounds are given in the experimental section. Their physical and elemental analysis data are presented in Table 2.

Antibacterial activity

All the test compounds exhibited the antibacterial activity at a concentration of 100 µg / cup which was compared with reference compound ampicillin used at concentration 10 µg / cup.

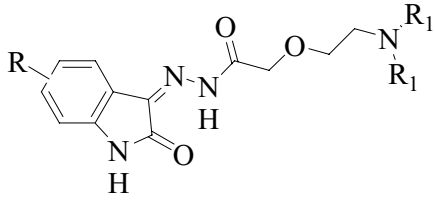
The results are summarized in Table 3, the tested compounds exhibited mild to moderate antibacterial activity against all four strains of bacteria. All the test compounds showed activity against gram positive bacteria than gram negative bacteria. Among the test compounds, compound VIg and VIh containing chlorine group in indole molecule exhibited relatively more antibacterial activity against *B. subtilis* and *S. aureus* and rest of the compounds showed moderate activity against gram-positive organisms and mild activity against gram-negative organisms. Compounds at a concentration 50 µg / cup exhibited mild activity against the test organisms employed.

Anticholinergic activity

It has been observed that no compound has produced any anticholinergic activity.

Table 3

Antibacterial Activity of (2-dialkylamino-ethoxy)-acetic acid (2-oxo-1,2-dihydro-indol-3-ylidene)-hydrazides VI (a-h)



Sl.No.	Compd.No.	Substituents		Zone of inhibition (in mm)			
		R	R1	B.subtilis	S. aureus	E. coli	K.P
1.	Via	H	Me	9.0	11.5	8.2	8.0
2.	VIb	H	Et	8.2	9.5	9.2	8.5
3.	Vic	5-CH_3	Me	10.0	9.8	12.8	11.0
4.	VId	5-CH_3	Et	9.2	11.2	8.5	9.5
5.	Vie	7-CH_3	Me	7.8	9.3	9.2	6.0
6.	VIf	7-CH_3	Et	11.4	7.5	7.5	8.2
7.	VIg	5-Cl	Me	14.3	13.3	9.9	6.4
8.	VIh	5-Cl	Et	13.9	14.2	7.2	9.5
9.	Ampililllin (10 ug/cup)			24.0	26.0	21.0	28.0

Note: Concentration of the test compounds 100 (ug/cup)

Conclusions

Synthetic work was positive as per the planning and as such in all the reactions carried out the expected compound alone was obtained. All the test compounds at a concentration of 100 µg / cup showed inhibitory activity against both gram-positive and gram-negative bacteria. All the test compounds exhibited moderate activity against gram-positive bacteria and mild activity against gram-negative bacteria. The compounds IVg and IVh (R=Cl; R_1=Me, Et) showed relatively more activity against gram-positive bacteria. All the compounds showed mild activity against gram-negative bacteria. All the test compounds exhibited mild antibacterial activity at a concentration 50 µg / cup. None of the compounds showed anticholinergic activity.

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Evaluation of Acute Toxicity And Hepatoprotective Effect of *Brassica Oleracea* Var*. Botrytis* in Experimental Animals

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ABSTRACT

Paracetamol is a known chemical agent with a high incidence of hepatotoxicity. The objective of this work was to investigate the acute toxicity and hepatoprotective activity of aqueous extract of leaves of *Brassica oleracea* var. *botrytis* against paracetamol induced hepatotoxicity. No mortality was observed in acute toxicity studies conducted as per OECD guidelines 423 and was found to be safe up to 2000 mg/kg b. wt. Animals were pretreated with the aqueous extract (200 and 400 mg/kg b. wt) for the period of 6 days and challenged with paracetamol (2 g/kg body weight) on 7th day. Serum marker enzymes (SGPT, SGOT, ALP, total Protein, direct bilirubin and total bilirubin) were estimated in all the study groups. The results of biochemical parameters revealed the elevation of serum marker enzymes (SGPT, SGOT, ALP, total protein, direct bilirubin and total bilirubin) and decreased total protein in toxicant group indicating the paracetamol induced damage to the liver. Pretreatment with the aqueous extract of leaves of *Brassica oleracea* var. *botrytis* (200 and 400 mg/kg *p.o*) significantly reduced the elevated levels of SGPT, SGOT, ALP, direct bilirubin, total bilirubin and significantly increased total protein in a dose dependent manner in treatment groups. There was no significant difference in the biochemical parameters of the extract when compared to the standard LIV 52. Histopathological studies are also further supported the hepatoprotective effect of plant extract.

Key words: *Brassica oleracea* var. *botrytis*, LIV 52, Paracetamol, Serum marker enzymes.

Introduction

Liver is the largest organ in human body, plays an important role in maintenance of human homeostasis mechanism through metabolic pathway [1]. Liver disease is a worldwide problem. No reliable drugs are available in allopathic medicine. Liver diseases are mainly caused by environmental pollutants, toxic chemicals, many allopathic preparations, excess consumption of alcohol, infections and autoimmune disorders. In absence of a reliable liver protective drug in the modern system of medicine, a number of medicinal preparations in ayurveda, the Indian system of medicine are recommended for the treatment of liver disorders. Natural remedies from medicinal plants are considered to be effective and safe alternative treatments for hepatotoxicity [2].

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Hippocrates, father of medicine quoted "Let food be thy medicine and medicine be thy food" and 'Ayurveda' told "Let food be your medicine". Recently, a number of investigations on the health benefits associated with fruits, vegetables demonstrated that they possess potent antioxidant [3], anti-inflammatory [4], antidiabetic [5], hepatoprotective [6], anticarcinogenic [7], immunomodulatory [8], antigout [9] and nephroptrotective [10] effects. Cauliflower is one of the most commonly used edible plant material in world wide.

Cauliflower traces its ancestry back to the wild cabbage, a plant coming from Syria by a Spanish Arab in the 12th century. It spread from Crete and Malta to the Italian mainland in the late 14th century. It gained popularity in France in the mid-16th century and was subsequently cultivated in Europe and England. The United States, France, Italy, India and China are countries that produce significant quantities of cauliflower. Earlier reports were suggested that, it is proved as anticancer agent, antiobesity, antioxidant and anti-inflammatory agent [11].

Scientific studies of leaf extract of *Brassica oleracea* var. *botrytis* as hepatoprotective were lacking; therefore in this investigation the leaves extract was tested against paracetamol induced liver injuries to validate its use against hepatoceullar damage.

Materials and Methods

Preparation of Extrac

The fresh leaves of *Brassica oleracea* var. *botrytis* was collected from local market, Kadapa, Kadapa District, Andhra Pradesh, India during the month of November 2012 and was shade dried. The leaves were cleaned and milled into coarse powder by a mechanical grinder. The coarse powder plant material was extracted with water by using soxhlet apparatus. The solvent was removed under reduced pressure to get solid residue (Yield 40 % w/w). The extract stored in a airtight container and used for further studies. The extract was subjected to qualitative phytochemical screening for the identification of phytoconstituents [3].

Animals

Female wistar rats weighing between 160–180 g were used for this study. The animals were obtained from Sri Raghavendra Enterprises Pvt Ltd, Bangalore, Karnataka, India. The animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2^{0}C and relative humidity of 70 ± 5%. A 12:12 light: dark cycle was followed. They were fed with standard commercial pellet rat chaw and water *ad libitum* during the experiment. All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (IAEC) and were in accordance with the guidelines of the IAEC.

Acute Toxicity Studies

Acute toxicity study was performed for aqueous extract according to the acute toxic class method described by OECD 423.

Hepatoprotective Activity

The animals were divided into 5 groups of 6 animals each.

Group 1- Normal control: The animals received 2% Carboxyl Methyl Cellulose (CMC) for 6 days.

Group 2- Induction of hepatotoxicity by using Paracetamol: The animals received 2% CMC for 6 days and given Paracetamol single dose, 2000 mg/kg b.w orally on day 7.

Group 3- Pretreatment with aqueous extract of *Brassica oleracea* var. *botrytis* at an oral dose of 200 mg/kg b.w/day for 6 days (p.o) followed by a single dose of PCM on day 7.

Group 4- Pretreatment with aqueous extract of *Brassica oleracea* var. *botrytis* at an oral dose of 400 mg/kg b.w/day for 6 days (p.o) followed by a single dose of PCM on day 7.

Group 5- Pretreatment with polyherbal formulation (LIV-52) at 5 ml/kg b.w/day for 6 days (p.o) followed by a single dose of Paracetamol on day 7.

After 48 h of Paracetamol administration, blood was collected by puncturing the retro orbital plexus. Blood samples were allowed to clot for approximately 1 h at room temperature and then centrifuge for 2500 rpm for 15 min to obtain the serum and various biochemical parameters like Serum Glutamate oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), Serum Alkaline Phosphatase (ALP), direct bilirubin, total bilirubin and total protein level were estimated by standard procedures.

Histopathology: The liver samples were excised from the experimental animals of each group and washed with normal saline. Initially the materials were fixed in 10 % buffered formalin, dehydrated in ethanol (50-100 %), cleared in xylene, and embedded in paraffin. Sections (4-5 μm) were prepared and stained with haematoxylin and eosin (H-E) dye. The sections were examined microscopically for the evaluation of histopathological changes.

Statistical Analysis

The values were expressed as Mean ± SEM, n=6 in each group. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnett's test. $P< 0.001$ was considered significant.

Results and Discussion

Phytochemical Screening

The preliminary Phytoconstituents were identified by chemical tests, which showed the presence of following Phytoconstituents carbohydrate, proteins, alkaloids, glycosides, flavonoids, Saponins.

Acute Toxicity Studies

An attempt was made to determine LD_{50} of aqueous extract of *Brassica oleracea* var. *botrytis*. Since no mortality was observed at 2000 mg/kg, it was thought that 2000 mg/kg was the cut off dose. Therefore 1/10th (200 mg/kg) and 1/5th (400 mg/kg) of cut off dose were selected for screening hepatoprotective property.

Biochemical parameters

Table - 1

Effect of aqueous extract of *Brassica oleracea* var. *botrytis* on Paracetamol induced hepatotoxicity in rats

Treatment Groups	SGPT (U/ml)	SGOT (U/ml)	ALP (KA units)	Total Protein (g/dl)	Direct Bilirubin (mg/dl)	Total Bilirubin (mg/dl)
Control	22.57± 1.857	32.88± 0.3439	17.92± 1.248	9.982± 0.3085	0.1517± 0.02167	0.2383±0.02167
Paracetamol (2 g/kg)	67.37± 2.395	52.12± 3.140	35.42± 3.604	7.450± 0.1147	0.3683± 0.02167	0.4550±0.02907
B. oleracea (200mg/kg)	22.33± 2.329***	50.82± 4.668	27.88± 2.747	8.533± 0.1116**	0.2383± 0.02167**	0.3667±0.01476*
B. oleracea (400mg/kg)	20.07± 0.5018***	41.42± 1.784*	21.19± 1.078***	9.333± 0.1706***	0.1517± 0.02167***	0.3033±0.02741***
LIV-52 (5 ml/kg)	17.22± 0.9311***	31.47± 0.7965***	17.75± 1.642***	9.417± 0.1851***	0.1733± 0.02741***	0.2817±0.02167***

Values are expressed as Mean ± SEM (n = 6).

***P < 0.001, ** P < 0.001, *P< 0.001 vs. CCl_4 treated group.

Overindulge of paracetamol causes a potentially fatal, hepatic centrilobular necrosis. Therefore, paracetamol has been used as a tool to induce hepatotoxicity in experimental animals. It was attributed to the formation of a toxic metabolite, *N-acetyl-p-benzoquinoneimine* (NAPQI) by the action of cytochrome P4502E1. NAPQI, in turn, is detoxified by conjugating with glutathione (GSH). Thus, GSH constitute the first line of defense against paracetamol induced generation of free radicals. In paracetamol toxicity, total hepatic GSH was found to be depleted due to the damage caused to hepatic cells. As a result, formation of NAPQI-glutathione conjugate is diminished.

In the present study, paracetamol administration resulted in elevated levels of SGOT, SGPT and ALP in serum (Group II) against their respective normal control values (Group I). Similarly, serum bilirubin level was also found to be increased significantly as a result of paracetamol toxicity (Group II). On the other hand, total serum protein level was lowered in response to paracetamol administration when compared with normal control. Abnormally higher activities of serum SGPT, SGOT and ALP after paracetamol administration as observed in the present study is an indication of the development of hepatic injury, which is responsible for leakage of cellular enzymes into the blood. When liver plasma membrane gets damaged, a variety of enzymes normally located in the cytosol are released into the circulation. Reduction in level of SGPT, SGOT enzymes towards the respective normal values is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by paracetamol. Suppression of increased ALP activity with concurrent depletion of raised Bilirubin level suggests that stability of the biliary dysfunction in rat liver. Treatment of animals with the known hepatoprotective agent LIV 52 as a reference standard resulted in significant decrease in elevated levels of SGPT, SGOT, ALP, Bilirubin, and increased concentration of Total Protein ($p<0.001$). Oral administration of 200 and 400 mg/kg doses of aqueous extract of *Brassica oleracea* var. *botrytis* was administered prior to paracetamol administrated resulted in gradual normalization of the activities of SGPT, SGOT, ALP, Direct and Total Bilirubin, and Total Protein in dose dependent manner. This evidently suggests the protective effect of the extract in improving the functional integrity of liver cells (**Table-1**).

Histopathological studies

Histopathological examination of liver sections of the normal control group (Group I) showed normal cellular architecture with distinct hepatic cells (Fig. 1). However, distinct hepatic necrosis was noted after paracetamol administration (Group II) with destruction of hepatic cells (Fig. 2). *Brassica oleracea* var. *botrytis* extract treatment (Group III, IV) showed recovery of the hepatocytes from necrosis. This also suggests that the plant extract has a magnificent potential to reverse the changes induced by paracetamol toxicity back to normal condition. (**Fig 1-5**).

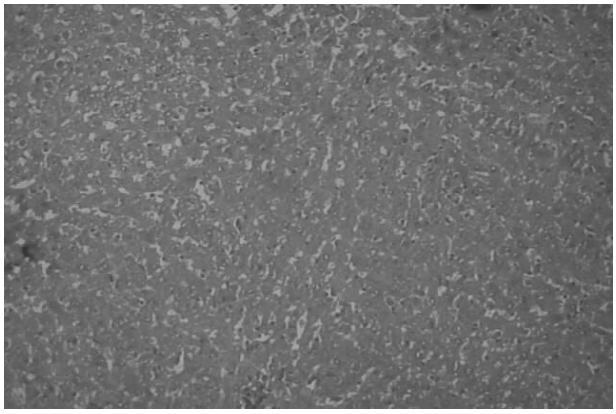

Fig. 1: Section of liver in normal control rat (1% CMC) showing normal histological architecture, H&E x10

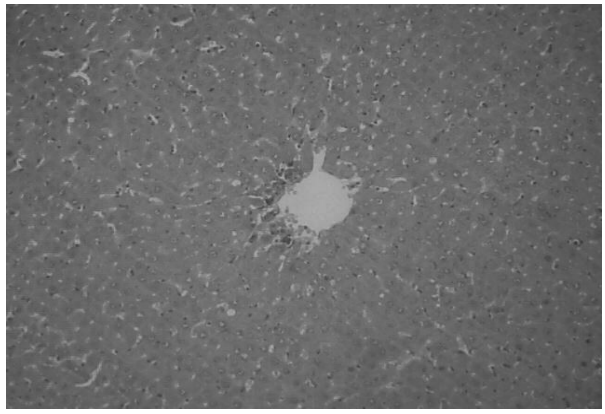

Fig. 2: Section of liver in in toxicant rat (Paracetamol) showing areas of necrosis, H&E x10

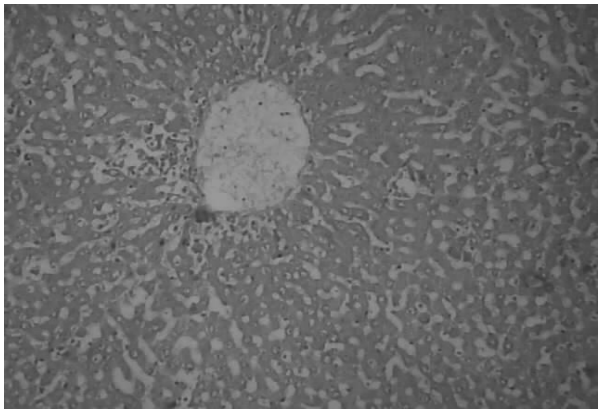

Fig. 3: Section of liver in extract treated rat (*Brassica oleracea* var. *botrytis* 200 mg/kg) showing recovery of necrosis, H&E x10

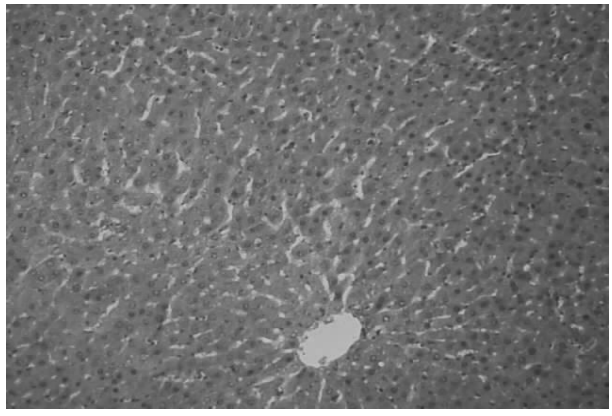

Fig. 4: Section of liver in extract treated rat (*Brassica oleracea* var. *botrytis* 400 mg/kg) showing complete protection of hepatocytes, H&E x10

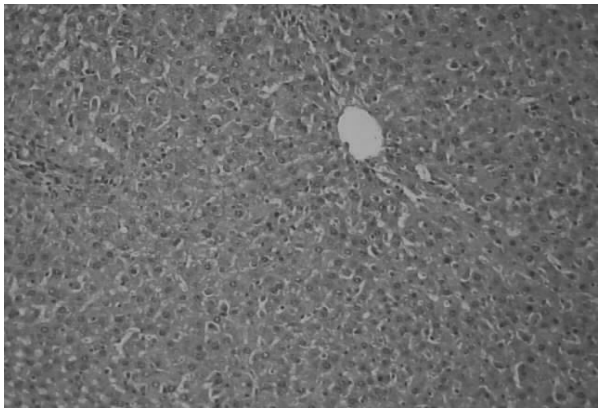

Fig. 5: Section of liver in Standard treated rat (LIV 52) showing normalization of liver architecture, H&E x10

Conclusion

In this study, paracetamol induced hepatotoxicity method to elucidate the protective effect of *Brassica oleracea* var. *botrytis* extract has been attempted. The data obtained clearly indicated that plant extract has hepatoprotective activity by the highly significant responses. Further work in progress to isolate and purify the active principle involved in hepatoprotective activity.

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Dual HDAC/RR Inhibitor: Design, Synthesis and Evaluation as Anticancer Agents

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ABSTRACT

The primary objective of the presented investigation is to design and synthesize compounds with anticancer activity by interfering with HDAC and RR. Hydroxamates are a class of HDAC inhibitors and thisemicarbazoneswere reported for their RR inhibitory activity. We designed molecules that incorporates pharmacophoric features of both HDAC and RR inhibitors. The *N*-hydroxythiazolecarboxamide (**4a-4i**) derivatives were synthesized and characterized by physic-chemical and spectral data. They were evaluated for their *in-vitro* anticancer activity against NCIH460, HCT116 and U251 cell lines by MTT method. All the compounds exhibited moderate to potent anticancer activity with mean GI_{50} between 20.80±1.92 and 63.54±14.76 μM. Among all thiazole derivatives of *N*-hydroxythiazole carboxamides, **4a, 4e** and **4f** were potent with mean GI_{50} value less than or equal to 24 μM and the remaining compounds were moderate potency.

Key Words:*N*-hydroxythiazole carboxamides, Anticancer, Dual inhibitors, HDAC and RR.

Introduction

Cancer is a leading cause of death worldwide, according to the WHO survey of 2008 and approximately 7.6 million people were reported die due to cancer.Several rational pharmacological strategies have emerged for the treatment of cancer, including vaccination, gene therapy, immunotherapy, and new target identification and validation. In spite of these progresses, the primary choice of treatment for most of the cancer cases is chemotherapy. But, nearly all chemotherapeutic agents suffer from undesirable side-effects and other severe toxicities. Drugs hitting a single target may be insufficient for the treatment of diseases because multiple pathogenic factors may be involved in diseases like cancer. To address these problems, the cancer medicine of the future will incorporate, a single chemical entity can have ability to hit multiple cancer-fighting targets simultaneously, which can reduce the risk of side effects and can increase the possibility of patient compliance[1].This recognition has continued to seed huge efforts in the literature. Studies aimed at identifying multivalent ligands as promising pharmacological tools, that may be more efficacious for various human diseases than highly selective single-target drugs, are ongoing in several academic and commercial labs. A subset of these studies has revealed that balanced modulation of a small number of targets may have superior efficacy and fewer side effects than single-target treatments [2].

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Epigenetic modification and control has become extensively recognized as a mechanism for cell regulation such as DNA methylation, post-translational modification of histone proteins, and chromatin remolding regulate gene expression through change of chromatin structure without change of gene sequence [3, 4]. Histone acetylation is believed to be associated with activation of gene transcription which dependent on the activities of histone aceytltransferases (HTAs) and histone deacetylases (HDACs) [5, 6]. HATs acetylate cationic histone lysine substrates in nucleosome associated histones neutralizes charge and cause chromatin to relax, which allows access of various transcription factors and leads to transcriptional expression. HDACs deacetylate histone lysine substrates results in formation of condensed chromatin, which prevents access of transcription factors and leads to transcriptional repression. This dynamic system is controlled by the balance of the activity between HATs and HDACs[7]. Deactivation of HATs an over-expression of HDACs may cause various forms of malignancy [8]. Mammalian HDACs consist of four classes, which are classified by their catalytic activity, sub-cellular distribution and sequence homology. Class I (HDAC1, -2, -3, -8), class II (HDAC4, -5, -6, -7, -9, -10), and class IV (HDAC11) enzymes are zinc-dependent HDACs, whereas class III (Sirtuins 1-7) enzymes require NAD^+ for activity. Class II enzymes are subdivided into class IIa (HDAC4, -5, -7, -9) and class IIb (HDAC6, -10) [9].

HDAC activity is invariably increased in cancer cells and inhibitors of HDAC were proven to be novel class of antiproliferative agents [10]. HDAC inhibition has recently been clinically validated as a new therapeutic strategy for cancer treatment with the FDA approval of SuberoylAnilide Hydroxamic Acid (SAHA) and Romidepsin (FK-228) for the treatment of cutaneous T cell lymphoma [11]. Several potentially useful class of HDAC inhibitors were reported which includes [10]: (a) Hydroxamates-based derivatives: SAHA, LBH-589, SB-939, etc., (b) Short-chain fatty acids: butyrate and valproate, etc., (c) Cyclic tetrapeptides: FK-228, trapoxin (TPX), and apicidin, etc., (d) 2-aminobenzamides: MS-275 and MGCD0103, etc.

Ribonucleotide reductase (RR) catalyzes the reduction of ribonucleotides to their corresponding deoxyribo nucleotides, which are the building blocks for DNA in almost all the living cells [12].Since the reduction of ribonucleotides is the rate-limiting step of DNA synthesis, inactivation of RR stops DNA synthesis, which inhibits cell proliferation and human RR belongs to Class Ia. This along with the fact that RR has low activity in resting cells, high activity in rapidly growing normal cells, and very high activity in cancer cells has made it an important target for cancer therapy [13].Also studies have indicated the critical role of RR in tumor promotion. Therefore, RR is considered as a relevant molecular target for the design and development of antitumor agents [13]. Several potentially useful class of RR inhibitors were reported which includes: (a) Free-radical scavengers: Hydroxy urea, Trimidox, Didox, etc., (b) Iron chelators: Triapine, PIH, 311, etc., and (c) Substrate analogs: Gemcitabin, Azido CDP, etc [13]. A class of α-(N)-Heterocyclic carboxaldehyde thiosemicarbazones (HCT) were reported to be carcinostaticpossibly due to their iron chelation property[14].HCTs were reported to inhibit RR and are suggested to interact with the iron in the R2 subunit of RR [15].

The primary objective of the presented investigation is to design and synthesize compounds with anticancer activity by interfering with HDAC and RR. Hydroxamates are a class of molecules that are widely reported for their HDAC inhibitory activity (SAHA, a clinically approved drug [11, 16]). On the other side thisemicarbazones (Triapine, a clinically approved drug [17])were reported for their RR inhibitory activity. Moreover the RR inhibitors in clinical use, hydroxyurea [18] and didox [19] are having hydroxamate pharmacophoric features similar to the one present in HDACinhibitors. Based on the above factors we designed molecules that incorporates pharmacophoric features of both HDAC and RR inhibitors (**Figure 1**).

Materials and Methods

Chemistry

Melting points were determined using Thermonik Melting Point Apparatus (Campbell electronics, India) by capillary method and are uncorrected. FT-IR spectra were taken on an IR-Prestige21 (Shimatzu Corporation, Japan) from 4000-400 cm^{-1} using KBr discs. ^{1}H-NMR spectra were recorded at 400 MHz in DMSO-d_6 using a BrukerAvance 400 instrument (Bruker Instruments Inc., USA). Chemical shifts were measured in δ (ppm) unit relative to tetramethylsilane (TMS). FAB-MS spectra were recorded on a Jeol SX 102/ DA-6000 Mass Spectrometer (Jeol Ltd. Akishima, Tokyo, Japan) using argon/xenon (6 kV, 10 mA) as FAB gas, m-nitrobenzyl alcohol as matrix, and 10 kV as accelerating voltage at room temperature. Elemental analyxsis was performed on Vario EL III Elemental Analyser (Elementar, Germany) using Sulfanilamide as standard. All chemicals were purchased from Aldrich, E Merck, Spectrochem or CDH, India. Solvents were of reagent grade and were purified and dried by standard procedure. Reactions were monitored using Thin-layer chromatography on silica gel plates in either iodine or UV chambers. Final compounds

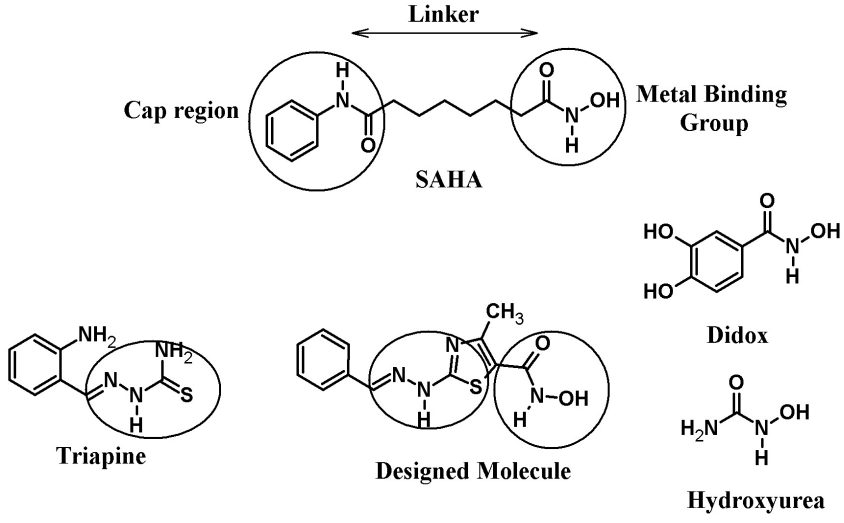


Figure 1. Designing strategy for dual inhibitors

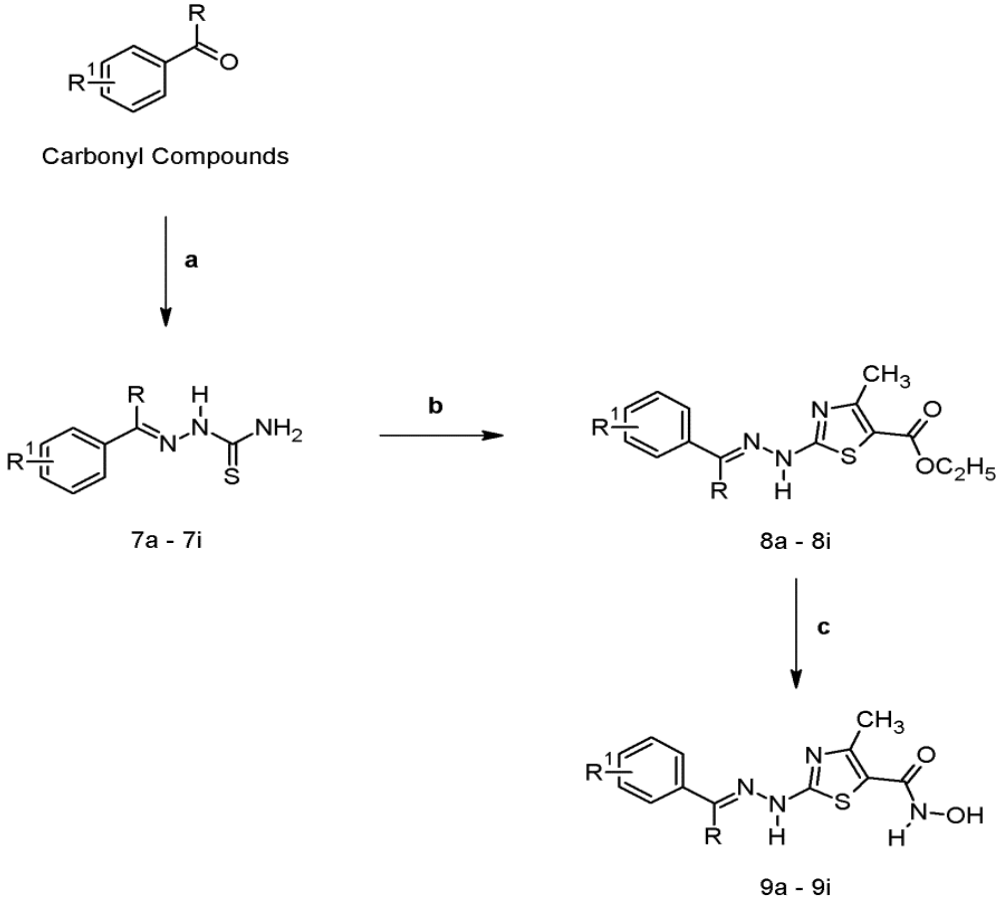
were characterized by ^{1}H-NMR, FAB-MS and elemental analysis. In the elemental analysis, the observed values were within ±0.4% variation of the calculated values.

Procedure for synthesis of Ethyl-2-bromo acetoacetate: A mixture of ethyl acetoacetate(1.5 mM), potassium bromide (835 mg, 7.5 mM), Hydrochloric acid (7.5 mL, 7.5 mM) and 30% hydrogen peroxide (3.4 mL, 30 mM) in toluene (7.5 mL) was placed in a 50 mL flask and stirred at room temperature. The completion of reaction was monitored by TLC. The saturated solutions of sodium thiosulphate (10 mL) and sodium bicarbonate (10 mL) were added to the reaction mixture. The organic layer was separated and the aqueous phase was extracted with ethyl acetate (2×20 mL). The combined organic phase was washed with brine, dried over anhydrous magnesium sulphate, and evaporated [20].

General procedur for synthesis of thiosemicarbazones (2a-2i): An equimolar quantity of (un)substituted aryl aldehydes or ketones (0.012 M) and thiosemicarbazide (0.01 M) are dissolved in methanol. To this mixture catalytic amount of concentrated sulphuric acid was added and heated under reflux for 16-24 hours. Completion of reaction was monitored by TLC. After completion of reaction, the reaction mixture was drop wise added to crushed ice. The solid obtained was filtered, dried and recrystallized form suitable solvent [21].

General Procedure for synthesis of ethyl-2-(substituted 2-benzy-lidenehydrazinyl)-4-methylthiazole-5-carboxylate (3a-3i): To the solution of Schiff's base of Thiosemicarbazide (0.01 M) in absolute ethanol (25 mL), sodium acetate (0.03 M) was added. Catalytic amount of acetic acid and ethyl 2-bromoacetoacetate (0.01 M) were added to the above solution then heated and refluxed for about 80-96 hours. Completion of reaction was confirmed by TLC. After the completion the reaction, the reaction mixture allowed to reach room temperature. The solid obtained was collected, washed with ice-cold water then cold ethanol, and dried and recrystallized with suitable solvent [21].

Synthesis of 2-(substituted 2-benzylidene-hydrazinyl)-N-hydroxy-4-methylthiazole-5-carboxamide derivatives (4a-4i): Derivatives of 2-(substituted 2-benzy-lidenehydrazinyl)-4-methylthiazole-5-carboxylate (0.01mol) were dissolved in methanol. To that equimolar quantity (0.01) of Hydroxylamine hydrochloride and potassium



Scheme 1. Reagents and conditions: (a) R^1-C_6H_4-CO-R/MeOH, H_2SO_4 [cat], reflux, 16-24h; (b) Ethyl 2-Bromoacetoacetate, Sodium Acetate, EtOH, reflux, 80-96h; (d) NH_2OH. HCl, KOH, Methanol, stirring, rt.

hydroxide were added and stirred for overnight. Reaction was monitored by TLC. After completion of reaction the reaction mixture was evaporated to get the product and recrystallized from ethanol [22].

2-(2-benzylidenehydrazinyl)-N-hydroxy-4-methylthiazole-5-carboxamide (4a):IR (in KBr, cm^{-1}) 3186.64 (CONH), 2921 .13 (C-H in CH_3), 1694.59 (C=O), 1661.74 (C=N), 1419.61 (Aromatic C=C), 1083.46 (C-S); ^{1}H NMR (DMSO-d_6, δ ppm) 1.4 (s, 3H, C<u>H</u>$_3$), 2.4 (s, 1H, -NH-O<u>H</u>), 4.2 (s, 1H, =N-N<u>H</u>), 6.8 (s, 1H, -N<u>H</u>-OH), 7.2 -7.4 (m, 5H, Ar<u>H</u>), 8.2 (s, 1H, =C<u>H</u>); EI-MS (m/z) 277 $[M+1]^+$; Elemental Analysis (Calcd/Found) C 52.16/52.28, H 4.38/4.36, N 20.28/20.34, S 11.60/11.64.

N-hydroxy-2-(2-(2-hydroxybenzylidene) hydrazinyl)-4-methyl thiazole-5-carboxamide (4b): IR (in KBr, cm^{-1}) 3176.53 (CONH), 2932.45 (C-H in CH_3), 1624.73 (C=O), 1651.23 (C=N), 1399.47 (Aromatic C=C), 1038.46 (C-S); ^{1}H NMR (DMSO-d_6, δ ppm) 1.4 (s, 3H, -C<u>H</u>$_3$), 2.0 (s, 1H, -NH-O<u>H</u>), 2.2 (s, 1H, ArO<u>H</u>), 4.0 (s, 1H, =N-N<u>H</u>), 6.0 (s, 1H, -N<u>H</u>-OH), 7.2 -7.4 (m, 4H, Ar<u>H</u>), 8.2 (s, 1H, =C<u>H</u>); EI-MS (m/z) 293 $[M+1]^+$; Elemental Analysis(Calcd/Found) C 49.31/49.50, H 4.14/4.15, N 19.17/19.22, S 10.97/11.00.

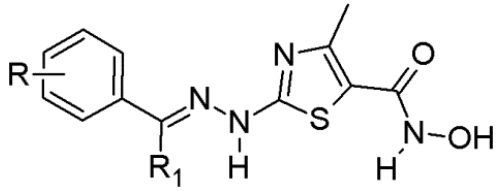
N-hydroxy-2-(2-(3-methoxybenzylidenehydrazinyl) -4- methyl thiazole-5-carboxamide (4c): IR (in KBr, cm^{-1}) 3068.64 (CONH), 2931.43 (C-H in CH_3), 1620.18 (C=O), 1656.23 (C=N), 1513.69 (Aromatic C=C), 1055.68 (C-S); ^{1}H NMR (DMSO-d_6, δ ppm) 1.8 (s, 3H, C<u>H</u>$_3$), 2.6 (s, 1H, NH-O<u>H</u>), 4.0 (s, 1H, =N-N<u>H</u>), 4.8 (s, 3H, OCH_3), 6.7 (s, 1H, N<u>H</u>-OH), 7.0-7.8 (m, 4H, Ar<u>H</u>), 8.1 (s, 1H, =C<u>H</u>); EI-MS (m/z) 307 $[M+1]^+$; Elemental Analysis (Calcd/Found) C 50.97/51.10, H 4.61/4.60, N 18.29/18.34, S 10.47/10.50.

N-hydroxy-2-(2-(4-methoxybenzylidene) hydrazinyl)-4-methyl thiazole-5-carboxamide (4d):IR (in KBr, cm^{-1}) 3127.14 (CONH), 2941.53 (C-H in CH_3), 1598.17 (C=O), 1601.03 (C=N), 1409.56 (Aromatic C=C), 1036.24 (C-S); ^{1}H NMR (DMSO-d_6, δ ppm) 1.4 (s, 3H, C<u>H</u>$_3$), 2.2 (s, 1H, NH-O<u>H</u>), 3.4 (s, 3H, OC<u>H</u>$_3$) 4.0 (s, 1H, =N-N<u>H</u>), 5.8 (s, 1H, N<u>H</u>-OH) 7.2 -7.6 (2d, 4H, Ar<u>H</u>), 8.0 (s, 1H, =C<u>H</u>); EI-MS (m/z) 307 $[M+1]^+$; Elemental Analysis (Calcd/Found)C 50.97/50.88, H 4.61/4.62, N 18.29/18.28, S 10.47/10.48.

N-hydroxy-2-(2-(4-(dimethyamino)benzylidene) hydrazinyl)-4-methylthiazole-5-carboxamide (4e):IR (in KBr, cm^{-1}) 3397.49 (CONH), 2919.75 (C-H in CH_3), 1603.70 (C=O), 1580 (C=N), 1450 (Aromatic C=C), 1040 (C-S); ^{1}H NMR (DMSO-d_6,δ ppm) 1.8 (s, 3H, C<u>H</u>$_3$), 2.4 (s, 1H, NH-O<u>H</u>), 3.0 (s, 6H, N(C<u>H</u>$_3$)$_2$) 4.4 (s, 1H, =N-N<u>H</u>), 6.2 (s, 1H, NH-O<u>H</u>), 6.6-6.8 (m, 2H, Ar<u>H</u>), 7.4-7.8 (m, 2H, Ar-H), 8.1 (s, 1H, =C<u>H</u>); EI-MS (m/z) 320 $[M+1]^+$; Elemental Analysis (Calcd/Found) C 52.65/52.78, H 5.37/5.39, N 21.93/22.02, S 10.04/9.99.

N-hydroxy-2-(2-(4-chlorobenzylidene)hydrazinyl)-4-methyl thiazole-5-carboxamide (4f):IR (in KBr, cm^{-1}) 3198.02 (CONH), 2923.48 (C-H in CH_3), 1660.62 (C=O), 1594.28 (C=N) 1553.29 (Aromatic C=C), 1088.11 (C-S);

Table 1:
Physical data of 2-(substituted 2-benzylidene-hydrazinyl)-N-hydroxy-4-methylthiazole-5-carboxamide derivatives (4a-4i):



Code	R^1	R	MF	MW	% Y	*R_f	M.P (°C)
4a	H	H	$C_{12}H_{12}N_4O_2S$	276	47	0.61	142-144
4b	2-OH	H	$C_{12}H_{12}N_4O_3S$	292	54	0.46	136-138
4c	3-OCH_3	H	$C_{13}H_{14}N_4O_3S$	306	55	0.69	128-130
4d	4-OCH_3	H	$C_{13}H_{14}N_4O_3S$	306	52	0.69	138-140
4e	4-$N(CH_3)_2$	H	$C_{14}H_{17}N_5O_2S$	319	47	0.56	156-158
4f	4-Cl	H	$C_{12}H_{11}ClN_4O_2S$	310	32	0.66	176-178
4g	4-NO_2	H	$C_{12}H_{11}N_5O_4S$	321	49	0.57	122-124
4h	H	CH_3	$C_{13}H_{14}N_4O_2S$	290	39	0.56	152-154
4i	4-Cl	CH_3	$C_{13}H_{13}ClN_4O_2S$	324	52	0.56	166-168

^{1}H NMR (DMSO-d_6, δ ppm) 1.4 (s, 3H, CH_3), 2.2 (s, 1H, NH-OH), 4.2 (s, 1H, =N-NH), 6.0 (s, 1H, NH-OH), 7.2-7.6 (m, 4H, ArH), 8.0 (s, 1H, =CH); EI-MS(m/z) 311 $[M+1]^+$; Elemental Analysis (Calcd/Found) C 46.38/46.42, H 3.57/3.56, N 18.03/18.08, S 10.32/10.30.

N-hydroxy-2-(2-(4-nitrobenzylidene)hydrazinyl)-4-methyl thiazole-5-carboxamide (4g): IR (in KBr, cm^{-1}) 3146.44 (CONH), 2931.65 (C-H in CH_3), 1626.68 (C=O), 1656.34 (C=N), 1416.65 (Aromatic C=C), 1008.13 (C-S); ^{1}H NMR (DMSO-d_6, δ ppm) 1.8 (s, 3H, CH_3), 2.3 (s, 1H, NH-OH), 4.1 (s, 1H, NH), 5.8 (s, 1H, NH-OH), 7.0-7.5 (m, 4H, ArH), 8.0 (s, 1H, =CH); EI-MS (m/z) 322 $[M+1]^+$; Elemental Analysis (Calcd/Found) C 44.86/44.78, H 3.45/3.46, N 21.80/21.76, S 9.98/10.02.

N-hydroxy-4-methyl-2-(2-(1-phenylethylidene) hydrazinyl) thiazole-carboxamide (4h):IR (in KBr, cm^{-1}) 3226.14 (CONH), 2964.26 (C-H in CH_3), 1642.46 (C=O), 1664.89 (C=N), 1409.51 (Aromatic C=C), 1036.66 (C-S); ^{1}H NMR (DMSO-d_6, δ ppm) 1.4 (s, 3H, CH_3), 1.8 (s, 3H, CH_3), 2.0 (s, 1H, NH-OH), 4.0 (s, 1H, =N-NH), 5.8 (s, 1H, NH-OH), 7.4-7.6 (m, 5H, ArH); EI-MS(m/z) 291 $[M+1]^+$;Elemental Analysis (Calcd/Found) C 53.78/53.84, H 4.86/4.88, N 19.30/19.36, S 11.04/11.08.

2-(2-(1-(4-chlorophenyl)ethylidene)hydrazinyl)-N-hydroxy-4-methyl thiazole-5-carboxamide (4i):IR (in KBr, cm^{-1}) 3246.62 (CONH), 2962.22 (C-H in CH_3), 1640.34 (C=O), 1578.42 (C=N), 1409.12 (Aromatic C=C), 1088.28 (C-S); ^{1}H NMR (DMSO-d_6, δ ppm) 1.5 (s, 3H, CH_3), 1.7 (s, 3H, CH_3), 2.1 (s, 1H, NH-OH), 4.2 (s, 1H, =N-NH), 5.7 (s, 1H, NH-OH), 7.4-7.6 (2d, 4H, ArH); EI-MS (m/z) 325 $[M+1]^+$; Elemental Analysis (Calcd/Found) C 48.07/47.92, H 4.03/4.02, N 17.25/17.30, S 9.87/9.90.

MTT Assay

The assay was based on the method of Mosmann [23] and Monks *et al.*[24],stock solution of **4a-4i** were prepared in DMSO and serial dilution of the test and standared were prepared with growth medium. 100 µL of respective cell suspension in growth medium was added to each well of 96 well tissue culture plates and incubated at 37 °C with 5% CO_2 in the CO_2 incubatorfor 24 hours. 100 µL different dilutions prepared with **4a-4i** were then added to the respective wells in triplicate (control receives only growth medium) and incubated for 48 hours at 37 °C in CO_2 incubator. After incubation remove the plates from the incubator and add 20 µl of MTT (5 mg/ml stock) to each well of the plate and kept the plates in the CO_2 incubator and incubated for 3-4 hours. After incubation, the supernatant layer was removed carefully taking care that the fromazan crystals formed are not removed and added 150 µl of DMSO to each well. The plate was then shaken on a plate shaker to ensure complete dissolution of formazan crystals and the absorbance was recorded at 570 nm.

The three OD values are required for calculating the percentage growth of cells with the samples.

- Control (untreated cells incubated for 48 hrs): **C**
- Test (treated cells with compounds incubated for 48 hrs): **T**
- Time zero (untreated cells assayed before compound addition) from ZERO plate: $\mathbf{T_0}$
- If $\mathbf{T > T_0}$then percentage growth (**PG**) = $\mathbf{(T-T_0)/(C-T_0)*100}$
- If $\mathbf{T < T_0}$then percentage growth (**PG**) = $\mathbf{(T-T_0)/(T_0)*100}$

Graphs are plotted with concentrations on x-axis and percentage growth on y-axis using Microsoft excel package and the scale of the y-axis is fixed ranging from -100 to +100 with the intervals at 50. From this growth inhibition curves GI_{50}, TGI and LC_{50} were calculated. The GI_{50} value of a compound is obtained from the graph as the concentration, which decreases % growth to 50 (y = 50), the TGI value of a compound is obtained from the graph as the concentration, which decreases % growth to zero (y = 0) and The LC_{50} value of a compound is obtained from the graph as the concentration, which decreases the % growth to –50 (y = -50).

Results and Discussion

The designed dual inhibitors of nine N-hydroxythiazolecarboxamide (**4a-4i**) derivatives were synthesized as per the reaction outlined in the **Scheme 1**. The scheme starts with the synthesis of ethyl 2-bromoacetoacetate by chemoselectivebromination of ethyl acetoacetate with mixture of KBr, HCl and 30% H_2O_2 in toluene with high yields while producing only nontoxic KCl and water as waste [20]. The possible reaction mechanism of bromination of ethyl acetoacetate as follows, bromide ion (Br^-) formed from KBr was oxidized into the bromo ion (Br^+) equivalent by H_2O_2 [25, 26], and the resulting Br^+ reacted with the enol form of the active methylene group of ethyl acetoacetate. The HCl acts as a catalyst to form enol, and neutralizes KOH formed from KBr [20]. The hetero/aromatic aldehydes/ ketones thiosemicarbazones (**2a-2i**) were prepared in good yields by the reactions of hetero/aromatic aldehydes/ ketones with thiosemicarbazide. Hydrazone derivatives of thiazole carboxylate (**3a-3i**) were synthesized by the reaction between corresponding thiosemicarbazones (**2a-2i**) and ethyl 2-bromo-acetoacetate to yield the ethyl-2-(substituted 2-benzylidene-hydrazinyl)-4-methylthiazole-5-carboxylate(**3a-3i**). The thiosemi-carbazones (**2a-2i**) first reacted with ethyl 2-bromoacetoacetate to give the initial thiazoline derivatives and then undergoes rearrangement to produce the most stable thiazole derivatives (**3a-3i**). Finally the hydroxamates (**4a-4i**) were prepared by the reaction between esters (**3a-3i**) and hydroxylamine.

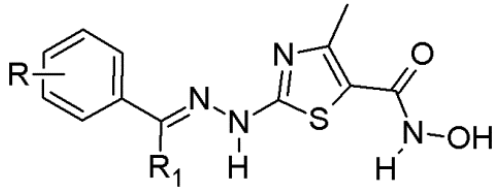
The final hydroxamate derivatives **4a-4i** were characterized by their physical data (given in **Table 1**), IR, ^{1}H-NMR and ES-MS spectral and elemental analysis data. CHNS microanalysis revealed that variation in experimental values compared with calculated values is

within ±0.4%. Analysis of IR spectra showed that all the hydroxamate derivatives **(4a-4i)** displayed characteristic CONH amide stretch (between 3068.64-3397.49), C-H stretch in CH_3 (between 2919.75-2964.26), C=O stretch in amides (between 1598.17-1694.59), C=N stretch (between 1578.42-1664.89), C=C aromatic stretch (between 1399.47-1553.29) and C-S stretch (between 1008.13-1088.28). All the hydroxamate derivatives **(4a-4i)** showed a characteristic peak for the methyl protons between δ 1.4-1.8 ppm as a singlet, hydroxamate -OH proton between δ 2.0–2.6 ppm as a singlet, =N-NH proton between δ 4.0-4.4 ppm as a singlet, NH-OH proton between δ 5.7-6.8 ppm as a singlet, aromatic protons between δ 6.6-7.8 ppm as doublet or multiplets, aldehydic CH proton between δ 8.0–8.2 ppm as a singlet and ketonic $–CH_3$ proton between δ 1.7–1.8 ppm as a singlet. Phenolic hydroxyl proton of **4b** appeared as a singlet at δ 2.2 ppm, methoxy protons of **4c** and **4d** appeared as a singlet atδ 3.4 ppm and δ 4.8 ppm as a singlet respectively and $N(CH_3)_2$ of six protons of **4e** appeared as a singlet at δ ٣,٠ ppm.

MTT Assay

Recent X-ray crystallographic analysis of histone deacetylase-like protein (HDLP) [27] and HDAC8 [28],complexed with SAHA and/or TSA, has revealed that the HDAC catalytic domain consists of a narrow tube-like pocket covering a length equivalent to 4-6 carbons straight chain, and the zinc ion is submerged near the bottom of the active site. Therefore, the structural requirements for an ideal HDAC inhibitor to display inhibitory activity involve three vital pharmacophores, i.e., *a zinc binding group*, which interacts with zinc which is in active site, *a linker*, which occupies the channel, and *a surface recognition group/area*, which interacts with residues on the border of the active site. Based on these concepts, many HDAC inhibitors have been reported [10]. The thiosemicarbazones may be complexes iron and inhibits the activity of ribonucleosidediphosphate reductase and some of these agents were found to possess potent tumor inhihitory potential [29]. In fact, ability of metal chelation properties thiosemicarbazones plays a

Table 2
In-vitro cell line assay of compounds 4a-4i



Code	R	R_1	NCIH460 (Lung)			HCT116 (Colon)			U251 (Glioma)			Mean GI_{50}
			GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	
4a	H	H	21.04	83.4	>100	22.58	>100	>100	28.13	>100	>100	23.92± 3.73
4b	2-OH	H	30.18	>100	>100	42.60	>100	>100	37.20	>100	>100	36.66± 6.23
4c	3-OCH_3	H	63.80	>100	>100	78.16	>100	>100	48.65	>100	>100	63.54± 14.76
4d	4-OCH_3	H	38.54	>100	>100	27.64	>100	>100	32.22	>100	>100	32.80± 5.47
4e	4-$N(CH_3)_2$	H	18.74	60.5	>100	22.55	78.6	>100	21.10	94.7	>100	20.80± 1.92
4f	4-Cl	H	21.05	76.8	>100	19.45	55.7	>100	24.37	98.2	>100	21.62± 2.51
4g	4-NO_2	H	55.12	>100	>100	38.48	>100	>100	28.61	>100	>100	40.74± 13.40
4h	H	CH_3	75.23	>100	>100	44.87	>100	>100	34.50	>100	>100	51.53± 21.17
4i	4-Cl	CH_3	69.15	>100	>100	43.54	>100	>100	38.76	>100	>100	50.48± 16.34

critical role in their biological activity [30].This is probably coordination of metal ions tridentate binding with NNS. Since the compounds that exhibited good activity are good iron chelators [31] and possibly due to the observation that structural alterations that hinder a thiosemicarbazone's ability to function as a chelating agent tend to destroy or reduce its medicinal activity [32]. Considering this, it is important to note that the all the derivatives (**4a-4i**) exhibited their anticancer activity may be due to the inhibition of HDAC and RR.

All the synthesized thiazole derivatives of *N*-hydroxypiperazine carboxamides derivatives (**4a-4i**) were evaluated for their anticancer activity in *in-vitro* cancer cell-line assay against NCIH460, HCT116 and U251 cell lines based on cell viability using the dye, 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). The MicrocultureTetrazolium Assay is based on metabolic reduction of 3-(4-, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to water insoluble blue formazan crystal by Mictochondrial dehydrogenase enzyme. The formation of formazan complex is directly proportional to number of viable cells and the results are presented in **Table 2.**

All the compounds exhibited moderate to potent anticancer activity with mean GI_{50} between 20.80±1.92 and 63.54±14.76 μM. Among all thiazole derivatives of *N*-hydroxypiperazine carboxamides, three derivatives (**4a, 4e**&**4f**) were potent withmean GI_{50} value less than orequalto 24 μM and the remaining compounds were moderate andhaving mean GI_{50} value in the range.

between32.80±5.47 and 63.54±14.76 μM. The **4b**, **4d** and **4g** were the moderate potent molecules in this series with meanGI_{50} value of36.66±6.23, 32.80±5.47 and 40.74±13.40 μM, respectively. The para substitution on phenyl ring with electron donating group (**4d**, **4e** and **4f**) shown more potent than electron withdrawing group (**4g**). The benzaldehyde derivatives (**4a** and **4f**, mean GI_{50} value of 23.92±3.73 and 21.62±2.51 respectively) are more potent than their corresponding acetophenone derivatives (**4h** and **4i**, mean GI_{50} value of 51.53±21.17 and 50.48±16.34).

Conclusion

Cancer is a major cause of death in the world and posing the real challenge to scientists for new drug discoveries due to mutation of cancer causing genes. HDAC is a protein inhibiting the tumor suppressor gene, a major cause of cancer and RR promotes the formation of DNA during replication and repair in all living cells. Dual inhibition of both enzymes was considered as the main approach of drug design in the current investigation. The approach is very challenging and was found difficult to get a molecule with balanced dual inhibitory activity. Even though, the present molecules provided novel leads against HDAC & RR and also insights into structural features required to be considered while designing the dual inhibitors.

Acknowledgement

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Formulation and Evaluation of Thermosensitive Intranasal *in situ* Gel of Sumatriptan Succinate by using A Blend of Polymers

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ABSTRACT

The prolonged residence of drug formulation in the nasal cavity is of utmost importance for intranasal drug delivery. To improve the nasal retention time of Sumatriptan Succinate, it has been formulated as in situ mucoadhesive gel by using blend of sodium CMC, Poloxamer 188 and carbopol 934P. The objective of this work was to improve the nasal bioavailability of antimigraine drug, Sumatriptan Succinate by increasing its nasal retention time as well as by means of nasal permeation. The in vitro tests performed for mucoadhesive strength and drug diffusion showed that nasal in situ gelling formulations prepared were having good mucoadhesive strength with nearly 100% drug diffusion. The formulations were evaluated for physiochemical parameter, gelation temperature, viscosity, gel strength, content uniformity, FTIR and DSC. So, this study points to the potential of mucoadhesive in situ nasal gel in terms of ease of administration, accuracy of dosing, prolonged nasal residence and improved nasal bioavailability.

Key words: Nasal drug delivery, Poloxamer 188, Carbopol 934P, Sumatriptan succinate.

Introduction

Sumatriptan is one of the first used antimigraine drug which prevents migraine and has been used by oral and injectable administration. It is having high first pass metabolism leading to low bioavailability. So there is a need to improve the bioavailability by improving nasal penetration because of long duration of retention. It is a drug of 5-HT agonist and mainly used in migraine so as it is acting in CNS and is not able to cross the Blood Brain Barrier [1]. Therefore, for better action or activity it is required to cross the Blood Brain Barrier. Intranasal administration allows transport of drugs to the brain circumventing (bypassing) BBB as well as first pass metabolism.

Nasal delivery has been paid attention as an alternative dosage form. The advantages of nasal route have been suggested as follows: rapid absorption, higher bioavailability allowing lower doses, avoidance of liver or gastrointestinal metabolism, avoidance of gastric irritation, non-invasive administration, ease of self-medication, improved patient compliance, and reduced risk of infectious disease transmission. The idea of mucoadhesive system came from the need to localize drug at a certain site in the body, often as the extent of drug absorption is limited due to the residence time of drug at the absorption site. The objective of present work was to formulate and develop thermo sensitive *in situ* gelling system for nasal administration of an antimigraine drug Sumatriptan Succinate, by different approaches. [2-6] Poloxamer is a block copolymer that consists of polyethylene oxide (PEO) and polypropylene oxide (PPO) units, is known for exhibiting the phenomenon of reverse thermal gelation under a certain concentration and temperature [7,8]. Sol-to-gel systems of Sumatriptan Succinate were prepared utilizing the phase transition properties of Poloxamer 188, sodium carboxy methyl cellulose as a viscosity enhancing agent and carbopol 934 P as mucoadhesive agent[9,10].

Experimental

Materials

Sumatriptan Succinate (Sun Pharmaceuticals Ltd, Baroda, India), Poloxamer 188 (BASF India Ltd., Mumbai), Sodium Carboxy Methyl Cellulose (Lobachemie, Mumbai),

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EDTA (Finar chemicals Ltd, Ahmadabad), Sodium Lauryl Sulphate (S.D.Fine-chemicals Ltd., Mumbai), Benzalkonium chloride (Lobachemie, Mumbai), Sodium metabisulphites (Lobachemie, Mumbai).

Preparation and optimization of intranasal *in situ* gel

The dose of Sumatriptan Succinate for preparation of In-*situ* gel was 200mg. This dose was fixed for preparation of the in situ gel formulation and in this dose of the drug there is no interference found throughout the study. The Gels were Prepared on a weight basis using the cold method. The Poloxamer 188 was slowly added to cold water (5^0c) maintaining at constant stirring. All other excipients were added with continuous stirring. The dispersions were then stored in a refrigerator until clear solution was obtained. The Sodium CMC with different concentrations (2%, 2.5% and 3%) were dissolved in distilled water and stirred for 1 hr. From the each prepared Sodium CMC solution, 200mg of the Sumatriptan Succinate was added. Then the Poloxamer 188 solution was slowly added to that Sodium CMC solution containing drug stirred for 1 hr. The composition of developed gel formulations was summarized in Table 1.

Evaluation of *in-situ* gel

The Sumatriptan Succinate *in-situ* gels were examined for not only for appearance in terms of clarity, texture and consistency. But also for viscosity, pH, gelation time, gel strength, gelation temperature and drug content and in-vitro drug release study.

Appearance

Appearance is an important characteristic in gel formulations as it increases the patient acceptability. The appearance of *in-situ* gels in terms of clear or turbid was evaluated visually.

Texture evaluation

Texture of the *in-situ* gel in terms of stickiness and grittiness was evaluated by mildly rubbing the gel between two fingers.

Rheological measurement

Viscosity of all the batches of *in-situ* gels was measured using Brookfield DV-E viscometer. The gel was taken in a 100ml beaker and the viscosity was measured using spindle no 64 at the rotation speed 0.6 RPM at room temperature.

pH of the gels

The pH of the *in-situ* gel was measured using Labindia SAB 5000 digital pH meter at room temperature.

Gel strength

Gel strength of the *in-situ* gel was measured by using ball (10 gm) placed in a 100 ml beaker containing *in-situ* gel and measured the time taken by the ball to penetrate 5 cm and it was determined in second. The gel strength was done in triplicate.

Gelation Temperature

The gelation temperature was measured by using water bath maintained at 37 ± 5^0c temperature. The temperature at which it forms the gel is recorded as the gelation temperature. It was measured when temperature induced polymer was used for *in-situ* gel formulation.

Drug content

Five gram of *in-situ* gel was accurately weighed on an electronic balance and transferred to 100 ml volumetric flask. Then 100 ml of Phosphate buffer of pH 6.4 was added to dissolve the gel. From that, 1 ml of the sample was withdrawn and diluted up to 100 ml with Phosphate buffer of pH 6.4 Samples were analyzed spectrophotometrically at 281.50 nm after filtering the sample in the whatmann filter paper.

***In-vitro* diffusion studies**

The rate of diffusion may be directly related to the efficacy of the *in-situ* gel formulation, as well as bioavailability differences between formulations. *In vitro* release studies of formulations were performed using the Franz diffusion cell with dialysis membrane. Phosphate buffer of pH 6.4 was used as diffusion media.

Table1

Composition of developed thermo reversible *in situ* gel of Sumatriptan Succinate

Ingredients	SP14	SP15	SP16	SP17
Sumatriptan Succinate (%)	0.2	0.2	0.2	0.2
Poloxamer 188 (%w/w)	18	18	18	18
Sodium CMC(%w/v)	2	2.5	3	-
Carbopol 934 (%w/v)	-	-	-	0.5
SLS(%w/v)	1	1	1	1
Propylene Glycol(% v/v)	1	1	1	1
Benzalkonium chloride (%w/v)	0.01	0.01	0.01	0.01
Sodium metabisulphites (%w/v)	0.1	0.1	0.1	0.1
Distilled Water % upto(ml)	100	100	100	100

Results and Discussion

Scanning of Sumatriptan Succinate solution in pH 6.4 Phosphate buffer by UV Spectrophotometer showed the λ_{max} 281.5 nm. Linearity was observed in the range of 5 to 50 μg/ml with the R2 value of 0.998. The appreance of prepared gels were examined by visually. From the study it was observed that all formulations having clear appearance. The pH of the developed formulations was in between the range of 5.8 to 6.3. Texture of the *in-situ* gel in terms of stickiness and grittiness was evaluated by mildly rubbing the gel between two fingers. All the formulations were sticky and Non-greasy. The gelation temperature of all the formulations was determined and shown in Table 2.

From viscosity study on formulations showed increase in viscosity at 37°C. This indicated the temperature induced gel structure formulation of Poloxamer. The results were reported in Table 2, and showed that viscosity resulted at physiological temperature at 0.6 RPM by using spindle no 64 of rotating type Brookfield viscometer. The drug content of all formulations was ranging from 98.13% to 100.04%. The results were shown in Table-2.

In vitro diffusion studies of formulations were performed using the Franz diffusion cell with dialysis membrane. Phosphate buffer of pH 6.4 was used as diffusion media. The study reveals that the formulation SP17 (contains Carbopol 0.5% w/v) shows a good release upto 93.63% at about 8 hour. The decrease in drug release from formulation SP16 may be due to increase in viscosity (contains Sodium CMC 3% w/v). The results were shown in Table-3.

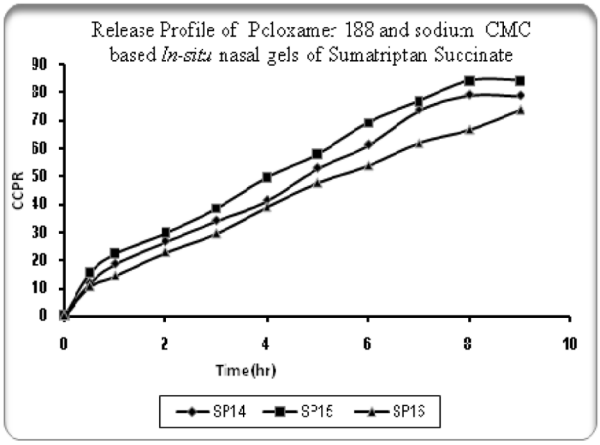
From the figures 1 and 2, it was conformed that the initial rates of drug release were very rapid due to incomplete gel formation, but as the time progresses the release rate decreases due to complete gel formation. By observing comparative in-vitro release study and all other evaluation parameters, the formulation SP17 considered as the optimized formulation. FTIR and DSC studies were carried out on pure drug as well as its combination with selected polymers and exhibited no interaction.

Table 2
Evaluation parameters of formulations

TEST PARAMETERS	RESULTS			
	SP14	SP15	SP16	SP17
Appearance	Clear	Clear	Clear	Clear
Texture	Sticky, Nongreasy	Sticky, Nongreasy	Sticky, Nongreasy	Sticky, Nongreasy
pH	6.1	5.4	6.0	6.3
Gelation Temperature (^{0}c)	33	37	47	36
Drug Content (%w/w)	98.1 3± 0.32	99.26± 1.52	100.04 ± 0.38	99.25 ± 0.87
Gel Strength (sec)	57 ± 0.12	66 ± 0.59	92 ± 1.22	68 ± 1.26
Viscosity (CPS)	646415	722351	764873	751956

Table-3
***In-vitro* diffusion studies of formulations from SP 14 – SP 1**

Sl No.	Time(hrs)	Cumulative Percentage Drug Released			
		SP14	SP15	SP16	SP17
1	0	0	0	0	0
2	0.5	11.73	15.33	10.55	12.67
3	1	18.58	22.27	14.31	18.91
4	2	26.63	29.69	22.73	29.30
5	3	34.01	38.42	29.42	42.41
6	4	41.22	49.56	38.89	51.72
7	5	52.81	57.82	47.51	66.17
8	6	61.09	69.15	53.66	73.74
9	7	73.52	76.77	61.73	82.63
10	8	78.93	84.08	66.42	93.63
11	9	78.65	84.26	73.56	92.61



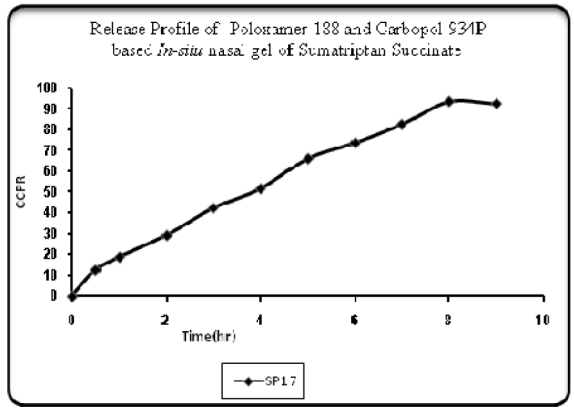


FIG.1 ***FIG.2***

Comparative In vitro drug release study of in situ nasal gels from formulations SP14 to SP17

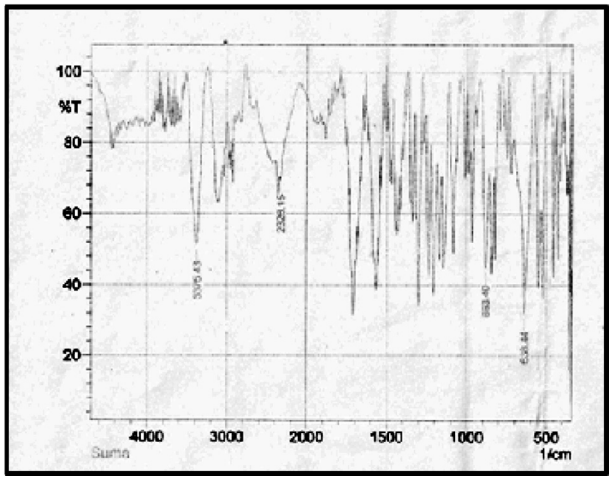


Fig.3: FTIR of Sumatriptan Succinate

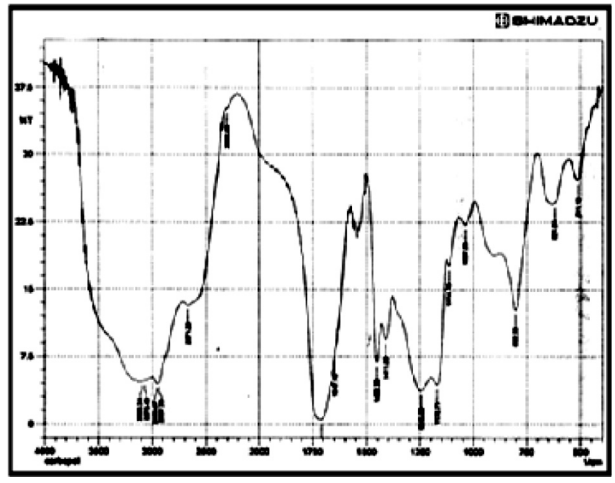


Fig. 4: FTIR of Carbopol

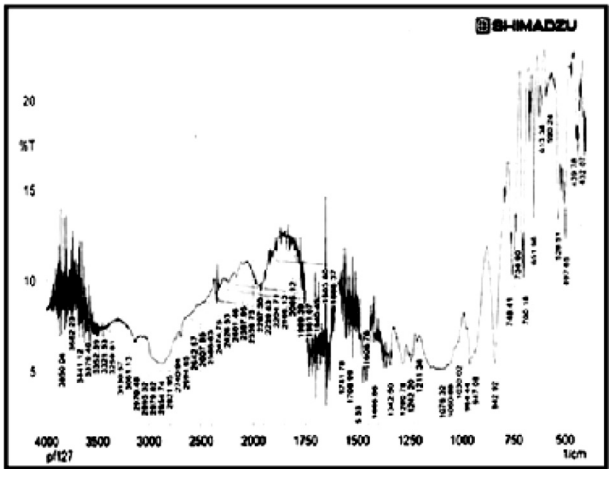


Fig. 5 : FTIR of Poloxamer 188

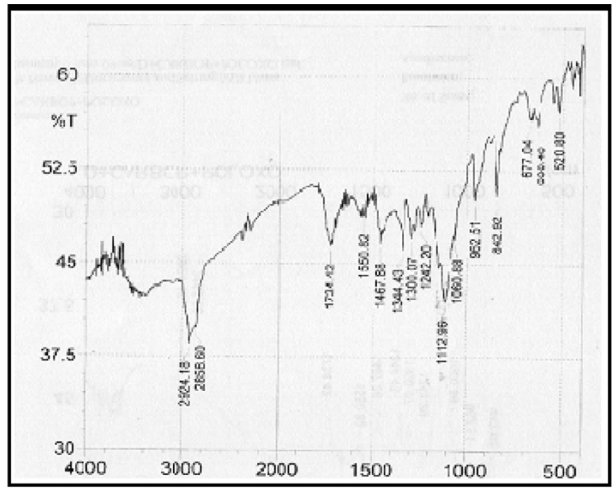


Fig. 6 : FTIR of Optimized Formulation SP17

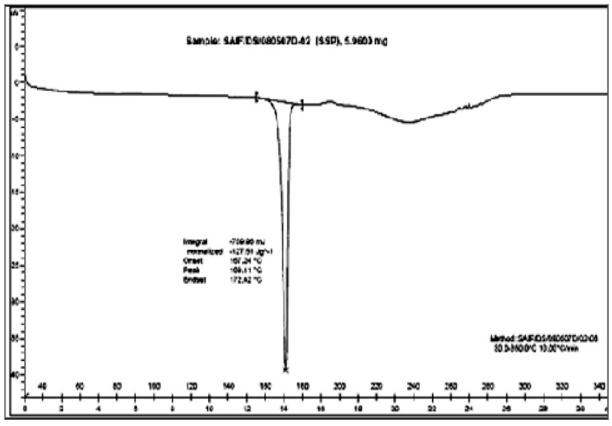


Fig. 7 : DSC thermogram of Sumatriptan Succinate

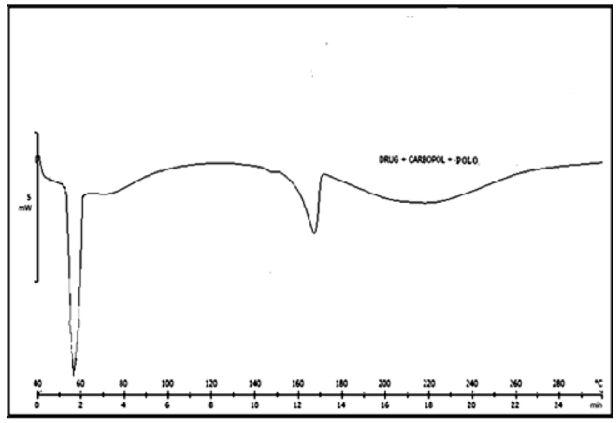


Fig. 8 : DSC thermogram of optimized Formulation SP17

Conclusion

Sumatriptan succinate is a 5-HT agonist and mainly used in treatment of migraine, was successfully formulated in thermo-responsive in situ nasal gel by using Poloxamer 188, mucoadhesive polymer Carbopol 934P and Sodium CMC in different ratios. From the study conducted, it was found that the batch SP17 was substantially stable and was able to release the drug in sustained manner. The methodology adopted for preparation of *in-situ* gel solution was very simple and cost effective. It is a newer approach to easy instillation by nasal route, improve the residence time, bioavailability and prolong drug release.

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Isolation, Charecterization of Secondary Metabolite from the Plant *Commelina Clavata*

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ABSTRCT

Nature's window has contributed many medicinal plants which can alleviate the wide spectrum of disorders. Till date 25% of medicinal plants gave a new molecular leads due its structural diversity of compounds. Now a day, standardized herbal preparations are gaining importance in the western countries due to their safety and efficacy. *Commelina clavata clarke* (Commelineaceae) is a perennial herb with thick, non tuberous roots. Shoots are rising to climbing, rooting on contact with ground. They grow up to 1 meter long. Leaves are narrowly lanceolate shaped to elliptic and 2.5 to10 cm long and 0.4 to1.5 cm wide with pointed or long pointed tip. Flowers are borne in 2-flowered clusters. Flowers are sky-blue, about 1.5 cm across, with sepals 3 mm long. Paired petals are 7 mm wide, sky-blue. The third petal is 5 mm wide. The third petal is 5 mm wide. The family is important for its ornaments as day flower (commelina). In South Africa the young shoots and leaves of *Commelina clavata* are edible. *Commelina* is a genus of approximately 170 species, commonly called dayflowers due to the short lives of their flowers. They are less often known as widow's tears. It is the largest genus of its family Commelinaceae. The preliminary Phytochemical Screening of *Commelina clavata Clarke* shows Alkaloids, Carbohydrates, Protines, Flavonoids and Terpenoids. The present study was sought to isolate Triterpenoid (Ursolic acid) from *Commelina clavata clarke*, its structure confirmation by spectral analysis

Key words: *Commelina clavata clarke*, Tri Terpenoids, Silica gel, Column chromatography, Spectroscopy.

Introduction

Nature is and will still serve as the man's primary source for the cure of his ailments. However, the potential of higher plants as sources for new drugs is still largely unexplored. Over 7500 plant species have been reported to be used in the Indian traditional systems including ethno medicines[1]. Nature has an enormous diversity of chemical structure, which is not waste products, but specialized secondary metabolites involved in the relationship of the organism with the environment[2,3]. *Commelina clavata clarke*[4] (Commelineaceae) is a perennial herb with thick, non tuberous roots. Shoots are rising to climbing, rooting on contact with ground. They grow up to 1 meter long. Leaves are narrowly lanceolate shaped to elliptic and 2.5 to10 cm long and 0.4 to1.5 cm wide with pointed or long pointed tip. Flowers are borne in 2-flowered clusters. Flowers are sky-blue, about 1.5 cm across, with sepals 3 mm long. Paired petals are 7 mm wide, sky-blue. The third petal is 5 mm wide. The third petal is 5 mm wide. The family is important for its ornaments as day flower (commelina). In South Africa the young shoots and leaves of *Commelina clavata* are edible. *Commelina* is a genus of approximately 170 species, commonly called dayflowers due to the short lives of their flowers. They are less often known as widow's tears. It is the largest genus of its family Commelinaceae. The preliminary Phytochemical Screening of *Commelina clavata Clarke* shows Alkaloids, Carbohydrates, Protines, Flavonoids, Tannins and Terpenoids[5]. The present study was designed for isolation of ursolic acid and spectral analysis.

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Materials And Methods

Plant Material

The whole plant of *Commelina clavata* (C) was collected from local supplier and was identified and authenticated by Dr. B. Reddy Raju Venkatapathi Raju, Botanist SK University, Anatapur, Andhra Pradesh, India. A

voucher specimen has been preserved in out laboratory for future reference. The aerial parts were dried under shade, powdered by a mechanical grinder and were passed through 40-mesh sieve and stored in airtight container for further use.

Preparation Of Extract

About 1kg of the powdered plant material was exhaustively extracted using Methanol (90%) in a Soxhlet extractor. The Methanolic extracts were concentrated and the traces of the solvent were completely removed under reduced pressure and were stored in vacuum desiccator for further use. The yield of Methanolic extract was found to be (12.3%) w/w with respect to dried powder.

Phytochemical Analysis

The dried extract was subjected to phytochemical analysis for constituent identification using standard protocol[5,6].

Experimentation

To the solution of methanolic extract of *Commelina clavata* 300 gm of silica gel of 60-120 mesh was added and mixed by stirring with the help of a glass rod. The mixture was dried by the help of rotavapor. The dried silica gel extract was carefully layered on the column gel bed. The column was first eluted with n-hexane and then n-hexane with 5% increments of ethyl acetate. 300 ml fractions were collected in glass beakers. Collected fractions were concentrated using rotavapor. Chemical tests and TLC were used to analyze the fractions and those with similar properties were combined. These fractions were dried in and their masses were determined. By analysing the results of Infra red, NMR and Mass spectrums of the isolated compounds from CCM extract (CC-1) were found as; may be Triterpenoids such as Ursolic acid (from CC-1) respectively.

Identification Test

The ursolic acid was subjected on to the precoated silica gel TLC plates. The mobile phase is Hexane: Ethyl acetate in 8:2 ratios. After the TLC run the yellow spot of Ursolic acid (from CC-1) were identified visually as well as in UV-visible light. Rf value was calculated[7].

Results and Discussion

Ursolic acid (3β-hydroxyurs-12-en-28-oic acid) is a pentacyclic triterpenoid. In the present study it was isolated as yellow needles, with melting point 283-284^{0}C (literature 283-285^{0}C). After isolation it is identified by TLC. The standard Rf- value of ursolic acid from the literature was 0.53. The Rf- value of purified ursolic acid from TLC was found to be 0.51. So it was confirmed that the product obtained from the *Commelina clavata* may be ursolic acid. The structure was further confirmed on the basis of the spectral data (IR, ^{1}H-NMR and MS) which are in agreement with the published data [8, 9].

IR spectral analysis

IR spectrum (**Fig- 1**) was run by using conventional KBr pellet method from 4,000-600cm^{-1}. It's characteristic peaks are at 3527.96cm^{-1} (free –OH stretching); symmetric stretching of -C=O ketone at 1741.79 cm^{-1}; stretching of –C-O at 1040 cm^{-1}.

Mass spectra

ESI-MS *m/z* in positive-ion mode for the ursolic acid peak gave a molecular ion at *m/z* 456.0 [M +] **(Fig- 2**).

^{1}H-NMR analysis

^{1}H-NMR spectrum **(Fig-3)** of ursolic acid exhibited a signal at δ1.14-0.77 (7H, s) resembles H on C23,24,25,26,27,29,30 and exhibited a signal at δ2.18 (1H, d) resembles H on C18 and exhibited a signal at δ3.34 (1H, d) resembles H on C3 and exhibited a signal at δ5.24 (1H, d) resembles H on C12 and exhibited a signal at δ11.94 (1H, d) resembles H on C28 . Peaks obtained were listed below in **Table-1.**

Experimental

Melting points were determined in open capillaries and

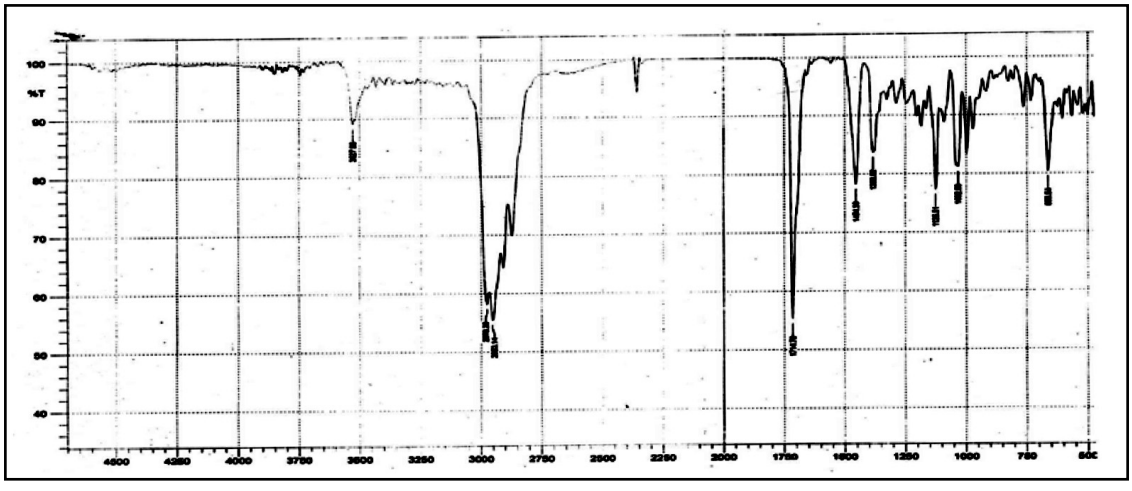

Fig- 1 : IR spectrum of Ursolic acid

are uncorrected. The purity of the compounds was routinely checked by TLC on silica gel coated plates. IR spectra were recorded in KBr pellets. ^{1}H-NMR spectra on a Varian 400 MHz instrument with DMSO-d_6 as internal standard, chemical shifts are expressed in δ ppm and Mass spectra on a Hewlett Packard Mass spectrometer operating at 70eV.

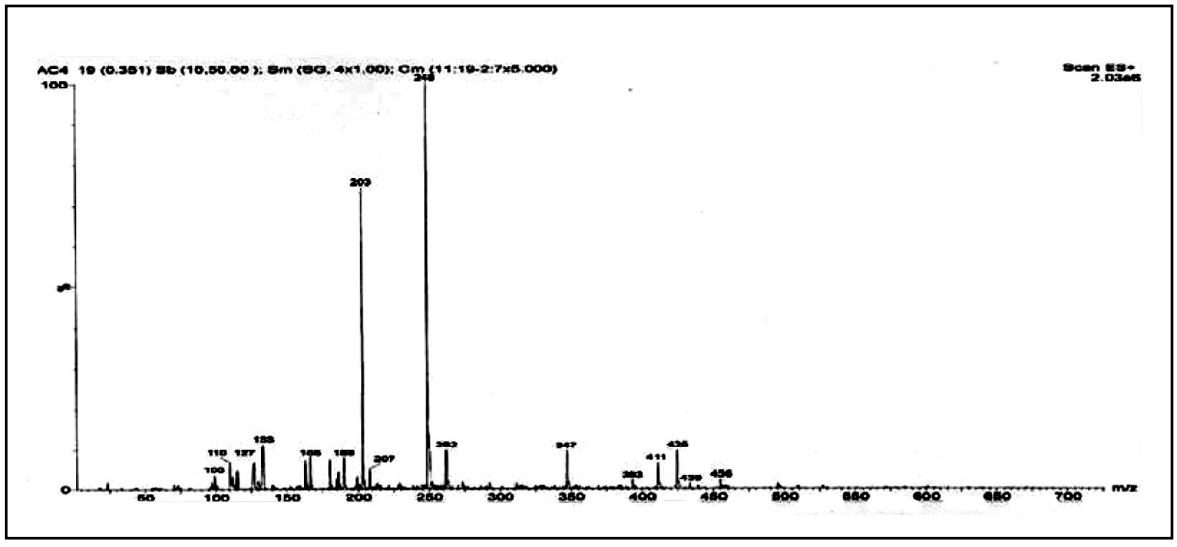

Fig- 2 MS spectrum of Ursolic acid

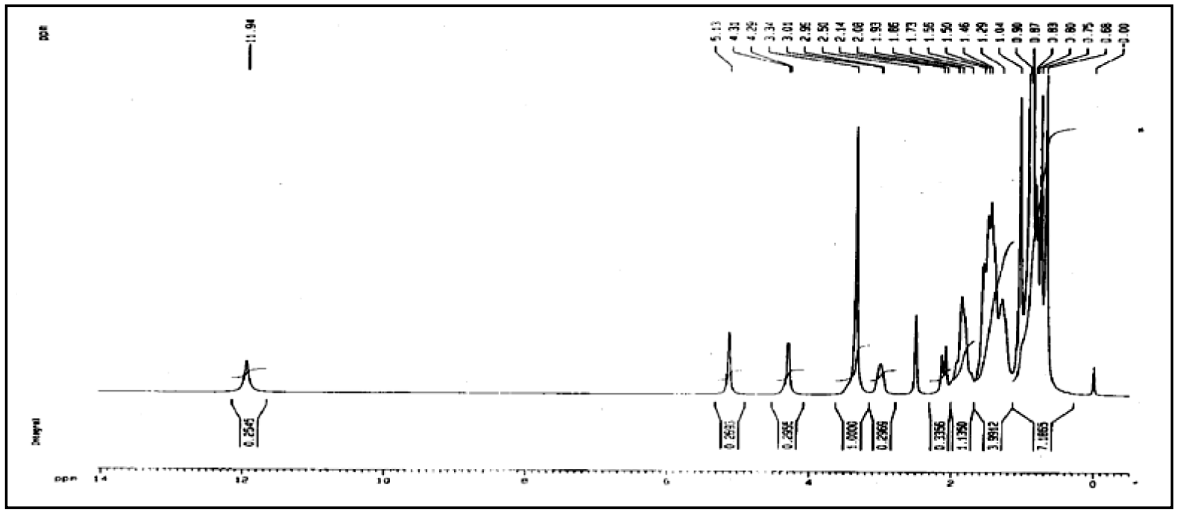

Fig- 3 ^{1}H-NMR spectra of Ursolic acid

Table - 1

^{1}H-NMR values of Ursolic acid

δ ppm	multiplicity	Number of H	Assignment
1.14-0.77		7	H23,24,25,26,27,29,30
2.18	D	1	18-H
3.34	D	1	3-H
5.24	D	1	12-H
11.94	D	1	28-H

Conclusion

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