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In-silico Design, Synthesis, Anti-inflammatory and Anticancer Evaluation of Pyrazoline Analogues of Vanillin

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ABSTRACT
A series of novel pyrazoline derivatives of vanillin were synthesized. The hydroxyl group in vanillin was masked by converting into methyl vanillin. The methyl vanillin was allowed to condense with different acetophenone derivatives gave chalcone derivatives and finally cyclized with thiosemicarbazide to form the pyrazoline derivatives of vanillin. Docking studies were carried out against anti-inflammatory cyclooxygenase receptor and anticancer farnesyl transferase receptor. Majority of the synthesized compounds showed good fitting with the active site of all the docked targets. The synthesized compounds had shown significant anti inflammatory and anticancer activities.

Keywords: Vanillin, methylvanillin, pyrazoline, cyclooxygenase, farnesyl transferase, anti-inflammatory, anticancer.

INTRODUCTION

Pyrazolines are nitrogen containing 5-membered heterocyclic compounds. It is present in many biologically active compounds and shows a wide spectrum of biological activities. Pyrazoline derivatives are known to possess anti inflammatory [1], anticancer [2], antiviral, antifungal, MAO inhibitory [3], antimicrobial [4] activities. Vanillin is a natural compound used as flavouring agent and possess antioxidant, antimicrobial, antimutagenic activities. Now researchers are going on to explore the potential of vanillin in the treatment of sickle cell anaemia and as anti-mutagenic agent. Due to the biological importance of both moieties we become interested in the synthesis of novel vanillin analogues that contain pyrazoline moiety. The synthesis was also carried out by microwave technique, because of their enhanced reaction rates, high purity products and better yield compared to that of conventional method.

MATERIALS AND METHODS

SMILES and clogP values, physicochemical properties, Analysis of Lipinski rule of five, Drug likeness analysis of the novel analogues were carried out by using Chemsketch and Molinspiration software. The prediction of activity spectra of the compounds were carried out using the PASS Online software. The docking studies were carried out using Schrodinger software (9.3) on cyclooxygenase and farnesyl transferase receptor. The ADME of the compounds were studied using QikProp under Maestro.

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The synthesis of compounds were carried out by conventional and microwave method. All the chemicals and solvents used were of laboratory grade. Melting points were determined in theils tube and are uncorrected. The homogeneity of the products was checked by TLC using Silica Gel GF254 (E.Merck) and the eluent system was a mixture of n-hexane: acetone (3:1), chloroform: ethylacetate: formic acid (5:4:1) proportions. The IR spectra of the compounds were recorded on FTIR Spectrum model 400. The ^{1}H NMR of the compounds was recorded on Bruker Ultra Shield DPX 400 NMR spectrometer using TMS as an internal standard (chemical shift in δ ppm) in $CHCl_3$.

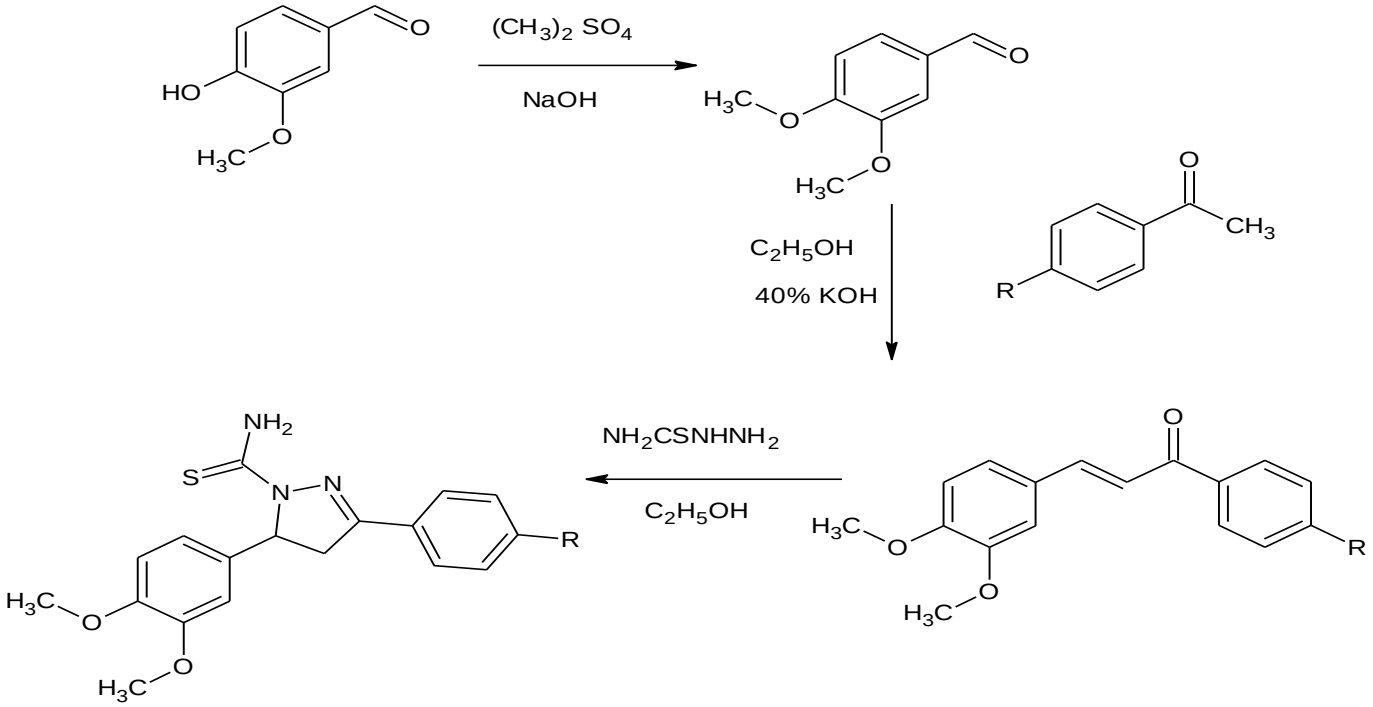
General procedure for the preparation of methyl vanillin (1) [5]

The vanillin was dissolved in 10% aqueous sodium hydroxide solution at room temperature. Then the residue relative to the theoretical amount of dimethylsulfate was added in one portion to the solution and the mixture was being stirred with a magnetic stirrer for 7 hours at 20°C. After that the mixture was left in the refrigerator at 4-5°C for 12 hours. The precipitate was filtered with the help of a glass filter and washed with 20 ml of ice water and was recrystallized with ethanol.

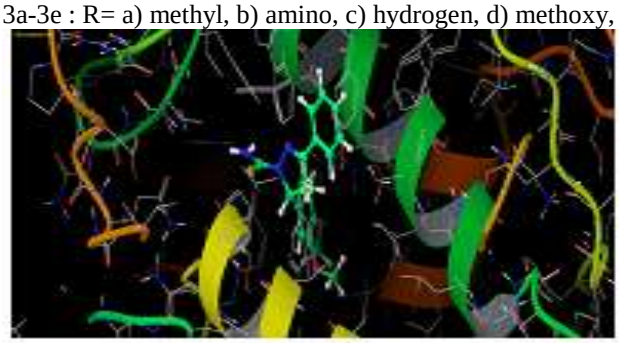
General procedure for the preparation of (2*E*)-3-(3,4-dimethoxyphenyl)-1-phenylprop-2-en-1-one (2a-2e) [6]

The equimolar portions of the 3,4-dimethoxy benzaldehyde (10 mmol, 1 equiv) and appropriate ketones (10 mmol, 1 equiv) were dissolved in approximately 15 ml of ethanol. The mixture was allowed to stir for several minutes at 5-10°C. A 10 ml aliquot of a 40% aqueous potassium hydroxide solution was then slowly added drop wise to the reaction flask *via* a self-equalizing addition funnel. The reaction

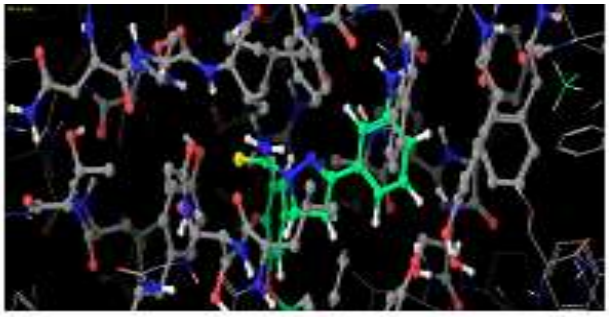
Scheme



3a-3e : R= a) methyl, b) amino, c) hydrogen, d) methoxy, e) nitro

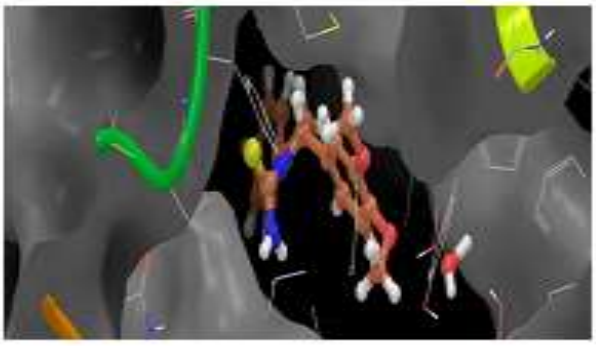


3b with 3KK6



3c with 3KK6

3c with 3E33



3b with 3E33

Fig. I: Compounds showing greater binding with the receptor

solution was allowed to stir at room temperature for approximately 4 hours. The precipitate formed was collected by suction filtration and the crude product was recrystallized from ethanol.

General procedure for the preparation of 5-(3,4-dimethoxyphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (3a-3e) [6]

Conventional method

A mixture of chalcone (0.01 mol), thiosemicarbazide (0.01 mol), and sodium hydroxide (0.025 mol) was refluxed in ethanol (25 ml) for 8 hours. The solution was poured into ice-water. The precipitate was filtered and recrystallized from methanol.

Microwave method

A mixture of the chalcone (2.2 mmol) and thiosemicarbazide (2 mmol) was dissolved in acetone (5 ml) and ethanol (5ml), and then potassium carbonate (4.0 g) was added and stirred vigorously. After 5 min, the solvent was removed under vacuum and the dry powder was irradiated in a microwave oven for the appropriate time at 650 W. After completion of

reaction as followed by TLC examination, chilled water was added to the reaction mixture. The solid product was obtained, which was filtered, dried and crystallized. [7]

Anti inflammatory activity

The anti inflammatory activity of the synthesized compounds was determined by proteinase inhibitory assay and cyclooxygenase assay.

Proteinase inhibitory assay

The reaction mixture (2 ml) was containing 0.06 mg trypsin, 1 ml of 20 mM Tris HCl buffer (pH 7.4) and 1 ml test sample of different concentrations. The reaction mixture was incubated at 37°C for 5 min and then 1ml of 0.8% (W/V) casein was added. The mixture was inhibited for an additional 20 min, 2 ml of 70% perchloric acid was added to terminate the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210 nm against buffer as blank. The experiment was performed in triplicate. The percentage of inhibition of proteinase inhibitory activity was calculated. [8]

Cyclooxygenase assay

The monocytes were incubated with Tris-HCl buffer, 5mM glutathione, and 5mM haemoglobin for 1 minute at 25°C. The reaction was started by the addition of 200µM arachidonic acid and terminated after 20 min incubation at 37ºC by addition of 0.2ml of 10% trichloroacetic acid in 1N hydrochloric acid, mixed and 0.2ml of thiobarbituric acid was added and contents heated in a boiling water bath for 20 minutes, cooled and centrifuged at 1000 rpm for 3 minutes. The supernatant was measured at 530 nm for COX activity. [9]

Anticancer activity

The anticancer activity of the synthesized compounds were determined by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, a yellow tetrazole) assay method. We have investigated the cytotoxic activity of the compounds in cervical cancer cell line (HeLa cells). [10] The results are reported in Table 7.

RESULTS AND DISCUSSION

This research work was focused on design and development of pyrazoline analogues of vanillin as novel anti inflammatory and anticancer agents. *In-silico* analysis of pyrazoline analogues of vanillin were done, all these compounds obeyed Lipinski rule of five. The PASS online software predicted that the compounds were having anti inflammatory and anticancer activities. The docking studies were carried out against different targets like cyclooxygenase and farnesyl transferase receptors. Majority of the synthesized compounds showed good fitting with the active site of the receptors. The ADME of the compounds were studied using QikProp software. The compounds which showed maximum Glide score were taken for wet lab synthesis. The microwave synthesis showed high yield in lesser time. The compounds 3a-3c which showed a maximum G score were taken for cyclooxygenase assay and 3a-3e for MTT assay in cervical cancer cell line.

The purity of the compounds was done routinely by TLC and melting points. The characterization of the derivatives was carried out by spectroscopic methods.

Anti-inflammatory activity

Proteinase inhibitory assay

The anti-inflammatory activity of the proposed analogues was carried out by proteinase inhibitory assay at the dose of 100-500µg/ml. The analysis of the result showed that the compounds show good activity. Diclofenac was used as the standard drug.

Cyclooxygenase assay

The anti inflammatory activity of the proposed analogues was carried out by cyclooxygenase assay at the dose of 100, 200 and 500µg/ml. The analysis of the result showed that the compound 3b shows maximum activity. Study of the biological activity showed that the compound is having significant anti inflammatory effect similar to that of the standard drug diclofenac.

Cytotoxic study

MTT assay was the best method for screening cytotoxicty. The synthesized compound which showed good Glide score was evaluated for the activity at different concentration of 10-100µg/ml. The drugs showed good cytotoxic behaviour and can be considered as potent cytotoxic agents. The compound 3b having best Glide score -6.9560 kcal/mol, indicating good binding affinity with anticancer target, showed good activity in cervical cancer cell lines (HeLa cells).

Table 1: Docking score of the synthesized compounds

Targets	PDB ID	Compounds	Glide score (kcal/mol)
Cyclooxygenase	3KK6	3a	-9.7345
		3b	-10.0505
		3c	-9.4357
Farnesyl transferase	3E33	3a	-6.2150
		3b	-6.9560
		3c	-5.8297

Table 2: Characterization data of synthesized derivatives

Compound code	Molecular formula	Molecular weight	R_f value	m.p
3a	$C_{19}H_{21}N_3O_2S$	355.45	0.74	171-172
3b	$C_{18}H_{20}N_4O_2S$	356.44	0.56	174-175
3c	$C_{18}H_{19}N_3O_2S$	341.43	0.71	146-148
3d	$C_{19}H_{21}N_3O_3S$	371.45	0.73	136-137
3e	$C_{18}H_{18}N_4O_4S$	386.42	0.53	110-112

Table 3: Comparison of different synthetic methods

Compound code	Conventional		Microwave	
	Time (hrs)	Yield (%)	Time (min)	Yield (%)
3a	8	43.45	6.30	79.62
3b	6	48.56	4.30	81.89
3c	8	57.78	6	84.21
3d	8	41.45	6.30	75.56
3e	6	39.34	4.30	69.82

Table 4: Characteristic ir peak of the synthesized compounds

Compound	IR data
3a	3451.36, 3330.37 (NH str.), 2966.57(CH str asym aliphatic), 1131.20(C-O-C str asym), 1596.03 (C-N str.).
3b	3447.62, 3304.84 (NH str.), 1017.93 (C-O-C str sym), 1619.09 (C-N str.)
3c	3451.36, 3330.37(NH str.),1596.03 (C-N str), 1131.20(C-O-C str asym), 1019.97(C-O-C str sym)
3d	3337.21, 3329.23 (NH str), 1251.40 (C-O-C str asym), 1022.55 (C-O-C str sym)
3e	3346.68, 3342.34 (NH str), 1259.33 (C-O-C str asym), 1018.39 (C-O-C str sym)

Table 5: Characteristic NMR Peak of 3C

Compound	Signal Position ∂ (ppm)
PV3	∂ = 3.936,3.909(s,6H, 2 -OCH_3)
	∂ = 4.898 (q, 1H, CH of pyrazole)
	∂ = 3.44 (q, 1H, Ha of CH_2 of pyrazole)
	∂ = 3.021(q, 1H, Hb of CH_2 of pyrazole)
	∂ = 6.838 -7.435 (m, 8H, ArH of both aldehyde and acetophenone)

Table 6: Determination of percentage inhibition by Proteinase inhibitory method

S. No	Sample	Concentration (µg/ml)	Absorbance (MEAN ± S.D)	Percentage inhibition
1.	Control	-	0.284±0.002	
		100	0.147±0.001	48.36
		200	0.119±0.002	58.07
2.	Standard	300	0.093±0.001	67.56
		400	0.057±0.002	79.97
		500	0.040±0.001	85.94
		100	0.144±0.001	49.41
		200	0.132±0.002	53.39
3.	PV1	300	0.103±0.002	63.58
		400	0.065±0.001	76.93
		500	0.044±0.003	84.30
		100	0.144±0.003	49.18
		200	0.125±0.001	55.97
4.	PV2	300	0.102±0.001	64.05
		400	0.064±0.003	77.27
		500	0.043±0.002	84.89
		100	0.158±0.003	44.49
		200	0.131±0.003	53.74
5.	PV3	300	0.112±0.002	60.65
		400	0.072±0.002	74.47
		500	0.039±0.002	86.29
		100	0.164±0.003	42.38
		200	0.135±0.002	52.57
6.	PV4	300	0.119±0.002	57.84
		400	0.094±0.002	66.97
		500	0.063±0.003	77.86
		100	0.164±0.004	42.38
		200	0.144±0.004	49.29
7.	PV5	300	0.122±0.003	57.13
		400	0.102±0.002	63.93
		500	0.065±0.002	76.92

Table 7: Anti-inflammatory activity by COX assay

Sample	Concentration (µg/ml)	Optical Density	Percentage inhibition (Mean±S.D)
Standard	100	0.017±0.002	93.403±0.761
	100	0.140±0.002	45.663±0.929
3a	200	0.087±0.002	66.104±0.651
	500	0.022±0.001	91.588±0.497
	100	0.131±0.002	49.288±0.402
3b	200	0.075±0.003	70.893±0.975
	500	0.024±0.003	90.810±1.028
	100	0.164±0.002	36.348±0.964
3c	200	0.122±0.002	52.123±0.311
	500	0.053±0.002	79.298±0.716

Table 8: Compounds and their IC_{50} values by MTT Assay

Compound	IC_{50} values (µg/ml) *HeLa cells*
3a	22.131
3b	17.579
3c	23.988
3d	43.551
3e	57.809

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