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Design, Synthesis and Biological evaluation of Pyrazole analogues of Natural Piperine

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Abstract

A series of pyrazole analogues of natural piperine were synthesized by removing the basic piperidine moiety from the piperine nucleus. Piperine upon hydrolysis and oxidation, converted to piperonal and allowed to condense with substituted acetophenone gave chalcone derivative and cyclized finally with thiosemicarbazide to form pyrazole derivatives of piperine. Docking studies were carried out against different targets like Cyclooxygenase, farnasyl transferase receptors. Majority of the synthesized chemical compounds showed good fit with the active site of all the docked targets.Compound 6a have shown significant anti inflammatory activity and 6d and 6c have shown significant anticancer activity when compared with standard drugs.

Key words: *Piperine, piperonal, pyrazole, anti-inflammatory, anticancer, Cyclooxygenase, farnasyl transferase.*

1. Introduction

Piperine has received enormous attention in the last two decades as a versatile bioactive molecule. The structure consists of three important components viz; methylene dioxy phenyl (MDP) ring, side chain with conjugated double bond and basic piperidine moiety attached through a carbonyl amide linkage. Piperine is also known to posses anti-inflammatory, analgesic, anti neoplastic, and anxiolytic activities. Pyrazole also posses anticancer and anti-inflammatory activities. Thus we become interested in the synthesis of piperine analogues that contains pyrazole moiety. Here we also take the advantage of microwave technique and evaluation of biological activities. Microwave assisted reaction have received great interest because of their simplicity in operation, enhanced reaction rates, product with high purity and better yield compared to those conducted by conventional method.

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2. Materials and methods

2.1. **Anti inflammatory activity:**

The anti inflammatory activity of the synthesized compounds was determined by Human Red Blood Cell (HRBC) membrane stabilization method at doses of 100 µgm, 500 µgm and 1000 µgm. Diclofenac sodium was used as standard drug[9, 10, 11, 12]. Control group was given Dimethyl sulphoxide (DMSO). The results obtained are reported in table no.8.

2.2. **Anticancer activity:**

The anticancer activity of the synthesized compounds were determined by MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-di phenyl tetrazolium bromide, a yellow tetrazole) assay Method[13]. We have investigated the cytotoxic activity of the compounds in various cancer cell lines such as HeLa (Cervical cancer cell line), A375 (Skin cancer cell line), HCT116 (Colon cancer cell line), MCF7 (Breast cancer cell line). This study was carried out in the department of Molecular Biology, Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, Kerala. The results are reported in table no.7.

3. Experimental

Melting points of the compounds were found out in an open capillary tube by electrically heated melting point apparatus were uncorrected. The synthesized compounds were purified by recrystallisation and Thin Layer Chromatography. IR spectra of the compounds were recorded using KBr pellets in the range of 4000- 500 cm^{-1} on a Jasco FTIR model 6200 in the College of Pharmaceutical Sciences, Medical College, Thiruvananthapuram. The ^{1}H NMR of the compounds were recorded in $CDCl_3$. Chemical shifts were reported in parts per million downfield with reference to internal standard Tetra Methyl Silane (TMS)on Bruker Ultra Sheild DPX 400 in the Institute of Intensive Research in Basic Sciences, Mahatma Gandhi University, Kottayam. SMILES and C Log P values, Physico Chemical Properties, Analysis of Lipinski rule of five, Drug Likeness Analysis, prediction of activity spectra (PASS) of the Novel proposed analogues were carried out by using Chemsketch and molinspiration software.

Insilco ADME properties were screened by using the application Qikprop in maestro molecular modeling software. Docking studies were carried out against different targets like Cycloxygenase and farnasyl transferase receptors using Schrodinger software in College of Pharmaceutical sciences, Medical college, Thiruvananthapuram. The reactions were carried out in catalyst synthetic microwave oven and by conventional method. The physical and spectral data of the synthesized compounds were reported in the table No. 4,5and 6.

3.1. **Synthesis of piperic acid (2) from piperine (1):**

Piperine 1 (11.4 g, 0.04 mol) was refluxed with methanolic KOH (20%, 500 ml) for 24 hrs. After completion of hydrolysis, methanol was distilled under reduced pressure. The resulting reaction mixture was suspended in hot water and acidified with HCl to pH less than 1.

Yellow precipitate obtained was collected by filtration, washed with cold water and recrystallized from methanol to yield crystals of piperic acid. [1, 2, 3.] Yield: 59%, mp 215-217^{0}c.

3.2. **Synthesis of piperonal (3) from piperic acid:**

Piperic Acid 2.18g (0.01 mol) was suspended in 150ml boiling H_20, containing 4.2g (0.05 mol) sodium bicarbonate. To this hot solution, 3.16g (0.02 mol) $KMnO_4$ in 75ml warm H_20 added with a dropper over about 40 min, with constant stirring. Added 25ml of Isopropyl alcohol. (To remove any remaining oxidizer) The warm brown solution was filtered to leave a slightly yellow solution. This was chilled overnight and recrystallized[4, 5]. 75% yield,mp 36-38^{0}C.

3.3. **Procedure for the Synthesis of 3-(1, 3-Benzodioxol-5-yl)-1-phenylprop-2-en-1-one (chalcone):**

Into a 10 ml Erlenmeyer flask placed 0.24 g (2.0 mmoles) of acetophenone, 0.30 g (2.0 mmoles) of piperonal, 1 ml of 95% ethanol, and 1 ml of 10% sodium hydroxide solution. The mixture was stirred for 30 minutes during which time a solid forms. The reaction mixture was cooled and suction filtered using a Hirsch funnel, and recrystallized from a small amount (< 1mL) of 95% boiling hot ethanol and then allowed to cool slowly to room temperature[6].

3.4. **Synthesis of 5-(1, 3-Benzodioxol-5-yl)-3-(substituted) phenyl-4, 5-dihydro-1*H*-pyrazol-1-carbothioamide (6a-h):**

Conventional methods:

To a solution of NaOH (1 g, 0.025 mol) and chalcone derivatives (5a-h) (0.01 mol) in ethanol (25 ml), thiosemicarbazide (0.92 g, 0.01 mol) was added slowly under stirring. After addition was completed, the reaction mixture was refluxed for 1 h and the solution was added to crush ice. The resulting solid was washed with ether and water, then filtered[7]. The crystals were washed thoroughly with ice-cold water, dried and recrystallized from appropriate solvent to give 6a-h in 70% yield.

3.4.1. **Microwave method:**

A mixture of the chalcone 5a–h (2.2 mmol) and thiosemicarbazide (2 mmol) was dissolved in acetone (5 ml) and ethanol (5ml), then K_2CO_3 (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 3 min at 650 W. After completion of reaction as followed by T.L.C. examination, chilled water was added to the reaction mixture. The solid product (6a-h) was obtained, which was filtered, dried and crystallized from hot ethanol[8].

4. **Result and discussion**

Insilico molecular analysis of different pyrazole analogues of piperine were done, all these compounds obeyed "Lipinski rule of five". These analogues were taken for computing molecular descriptors and then for synthesis. The designed analogues were synthesized by conventional and microwave procedures. Microwave procedure was simple efficient and showed better yield. The purity of the synthesized molecules was ascertained routinely by TLC, and melting point determinations were noted with an open capillary tube method and are uncorrected.

Docking studies were carried out against different targets like *Cycloxygenase, farnasyl transferase* receptors. Majority of the synthesized chemical compounds showed good fit with the active site of all the docked targets. Compounds 6e, 6h, which showed a maximum G-score were taken out for wet laboratory validations for their anti-inflammatory activities. Compound 6d and 6c showed highest Glide score with anticancer drug target, *Farnasyl Transferase*.

Purity of the compound was done routinely by TLC and melting points. Acetone: Chloroform (3: 1) system was found to be ideal system for the development of compounds in TLC. The characterizations of the derivatives were carried out by various spectroscopic methods such as FTIR and NMR spectroscopy.

4.1. **Cytotoxicity Study:**

MTT assay was validated best method for screening cytotoxicity. The synthesized compound which shows good G-score was evaluated for the activity at different concentration 10-100µM. The drugs are administered as solution in DMSO and a DMSO control was also used. The drugs showed good cytotoxic behaviour and can be considered being potent cytotoxic agents.

4.2. **Anticancer activity of the compounds against various cancer cell lines:**

We have investigated the cytotoxic activity of the compounds in various cancer cell lines such as HeLa (Cervical cancer cell line), A375 (Skin cancer cell line), HCT116 (Colon cancer cell line), MCF7 (Breast cancer cell line). Compound 6d, 6c, 6b, 6f and 6a Sensitive to HCT116 (Colon cancer cell line), and compound 6g Sensitive to MCF7 (Breast cancer cell line).

4.3. **HRBC Membrane Stabilization Method:**

Anti inflammatory activities of the proposed analogue was carried out by using HRBC membrane stabilization method at doses of 100 µgm, 500 µgm and 1000 µgm. Diclofenac was used as the standard drug. Control group was given Dimethyl sulphoxide (DMSO). The analysis of the result shows that the compound 5-(1,3-benzodioxol-5-yl)-3-phenyl-1H-pyrazole-1-carbothioamide (6a) at doses of 100 µgm, 500 µgm, 1000 µgm shows significant activity. Study of the biological activity showed that the title compound is having significant anti inflammatory effect similar to that of Standard drug Diclofenac.

5. Summary and conclusion

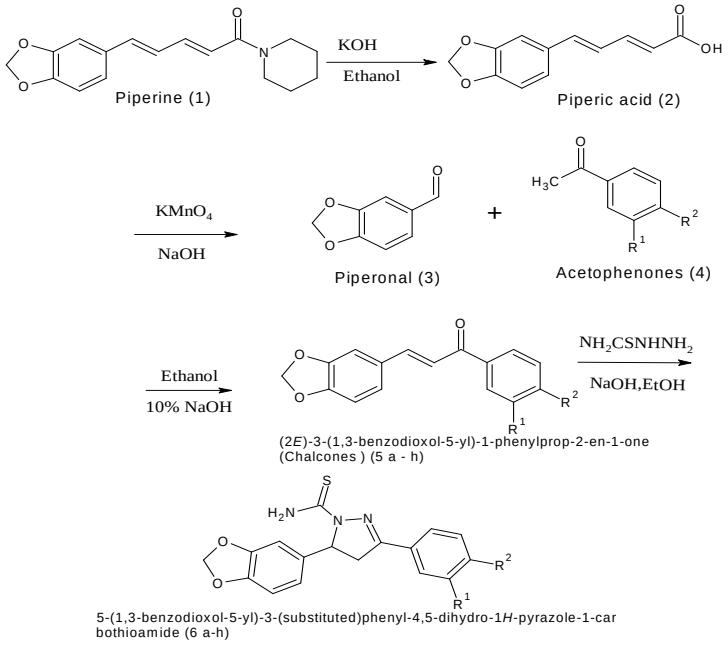
This research work was focused on design and development of pyrazole analogues of piperine as novel anticancer and anti inflammatory drugs. The present research work involved the preliminary insilico screening of various synthesized analogues for quantifying their drug likeness using molinspiration software. Eight analogues were synthesized in the wet lab by conventional and microwave procedures and comparative study for yield and reaction time was also carried out. Purity of the compounds were ascertained by consistency in melting point and *Rf* value and characterized by IR and ^{1}HNMR spectral studies.

The analogue also showed good binding affinity with Cycloxygenase and farnasyl transferase receptors, which was proved from the docking studies. The present study also highlights the importance of the structural features and C log P responsible for the activities.

Among the newly synthesized analogues 5-(1,3-benzodioxol-5-yl)-3-phenyl-1H-pyrazole-1-carbothioamide (6a) was screened for anti inflammatory and showed good activity when compared with standard diclofenac.

The compound having best Glide score -8.16, indicating good binding affinity with anticancer target, showed good activity in colon cancer cell lines (HCT116) which was proved from cytotoxic MTT assay.

Scheme 1



comp	R^1	R^2
a	H	H
b	H	O-CH_3
c	H	NH_2
d	NH_2	H
e	OCH_3	H
f	OCH_3	OCH_3
g	NO_2	H
h	H	NO_2

Table 1: Characterization data of synthesized derivatives

Compound Code	Molecular formula	Molecular Weight	mp (^{0}C)	R_f value
6a	$C_{17}H_{15}N_3O_2S$	323.377	226-229	0.56
6b	$C_{18}H_{17}N_3O_3S$	355.410	222-225	0.72
6c	$C_{17}H_{15}N_3O_2S$	338.392	227-230	0.41
6d	$C_{17}H_{15}N_3O_2S$	338.392	219-222	0.61
6e	$C_{18}H_{17}N_3O_3S$	355.410	223-226	0.73
6f	$C_{19}H_{19}N_3O_4S$	385.436	220-223	0.63
6g	$C_{17}H_{14}N_4O_4S$	370.382	225-228	0.77
6h	$C_{17}H_{14}N_4O_4S$	370.382	241-243	0.75

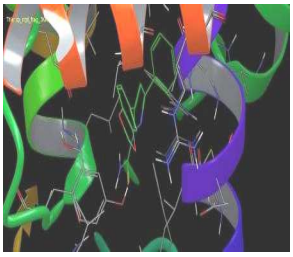
Table 2: Comparison of Different Synthetic Methods.

Compound Code	Conventional		Microwave	
	Time(hr)	Yield %	Time(min)	Yield%
6a	6	70	3	91
6b	6	73	3	86
6c	6	76	3	89
6d	6	75	3	90
6e	6	80	3	92
6f	6	77	3	88
6g	6	75	3	87
6h	6	78	3	85

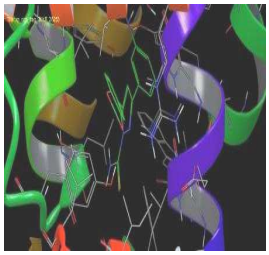
Table 3: Glide score of synthesized compounds based on G-score

Targets	PDB ID	Compounds	G-Score
Cycloxygenase	3KK6	6a	-12.41
		6d	-10.51
		6b	-10.47
Farnasyl Transferase	3E33	6d	-8.16
		6c	-7.68
		6b	-7.68

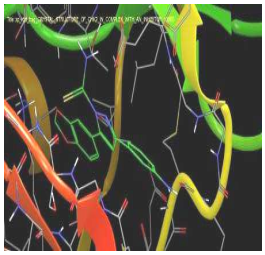
Fig. 1: Compounds Showing Stronger Binding with the Receptors



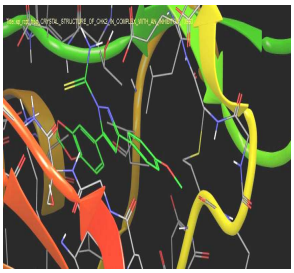
Compound (6a) with 3KK6



Compound (6d) with 3KK6



Compound (6d) with 3E33



Compound (6c) with3E33

Table 4: Characteristic IR peaks of the Synthesized Compounds

Compound	IR (KBr v cm^{-1})
Piperonal(3)	2985(Ar-CH), 1601.59(Ar C=C), 1037.52(sym C-O-C), 1261.22(asym C-O-C),1686.44(C=O), 2820,2720(CH)
5a	2956(Ar-CH) 1659.45(C=O), 1590.32(Ar-C=C), 1018.23(Sym C-O-C Str), 1253.5, 1105.98(asym C-O-C).
5b	2956(Ar-CH)1652.7(C=O),1583.27(Ar-C=C),1018.23(SymC-O-CStr), 1253.5, 1105.98(asym C-O-C),1098.26(Ar-OCH)
5c	1642(C=O), 1597.73(C=C), 3457.74, 3346.85(ArNH), 1034.02(sym C-O-C), 1243(asy m C-O-C).
5d	3424.96(Ar-CH),1655.59(C=O),1580.38(C=C),1036.55(sym C-O-C),1267.97(asym C-O-C)
5f	2925.1(Ar-CH)1652.7(C=O),1583.27(Ar-C=C), 1018.23(Sym C-O-C Str), 1253.5,1105.98(asym C-O-C),1495.38(Ar-OCH)
5g	2956(Ar-CH)1659.45(C=O),1590.32(Ar-C=C), 1018.23(Sym C-O-C Str), 1253.5,1105.98(asym C-O-C).1521.41(Ar-NO_2)
6a	2925(CH Str)3343.96(NH),2360.44, 1100 (C=S)1468.53-1579.41(C=N,N-N combined vib)1247.72(asym C-O-C),1040.41(symC-O-C),
6b	3337.21(N-H), 1173.47,2361.41(C=S)1356.68(C-N),1579.41(C=N)1469.49,1579(C=N,N-N combined vib)1248.68(asymC-O-C), 1038.48(sym C-O-C),1000-1248.68(s, m Aryl O-CH3)
6c	2362.37,1050.11(C=S),1483.96,1595.81(C=N,N-N combined vib)1234.22symC-O-C),1250.10(asymC-O-C)
6d	3330.46(NH), 2360.44(C=S)1484.92,1572.66(C=N,N-N combined vib)
6f	3337.21(N-H),1173.47,2361.41(C=S)1356.68(C-N),1579.41(C=N)1469.49,1579(C=N,N-Ncombinedvib) 1248.68 (asymC-O-C),1038.48(symC-O-C),1000-1475.49(ArylO-CH3)
6g	3237.14(CH Str)3343.96(NH),2360.44,1459.57(C=S)1468.53-1579.41(C=N,N-N combined vib)1247.72(asym C-O-C),1040.41(symC-O-C),

Table 5: Characteristic ^{1}H NMR peaks of the synthesized analogues

compound	^{1}H NMR($CDCl_3$) δ ppm
6a	6.5-7(8H, Ar-H), 5.9(2H,CH_2), 3.9(1H,CH), 1.5-2.2(4H,CH_2,NH_2)
6b	6.5-7(7H, Ar-H), 5.9)2H, CH_2)3.9-4(4H, CH, Ar-OCH_3), 1.8-2(4H(CH_2,NH_2).
6c	6.5-7(7H, Ar-H), 5.9(2H, CH_2), 3.9-4.2(3H, CH,Ar-NH_2), 1.8-2(4H,CH_2,NH_2).
6d	6.5-7(7H, Ar-H), 5.9(2H, CH2), 3.9-4(3H,CH,Ar-NH_2), 1.8-2(4H,CH_2,NH_2).
6f	6.5-7(7H, Ar-H), 5.9)2H,CH_2)3.5-4.1(6H,CH, Ar-OCH_3), 1.8-2(4H(CH_2,NH_2).
6g	6.5-7(7H, Ar-H), 5.9(2H,CH_2), 3.9(1H,CH), 1.5-2.2(4H,CH_2,NH_2)

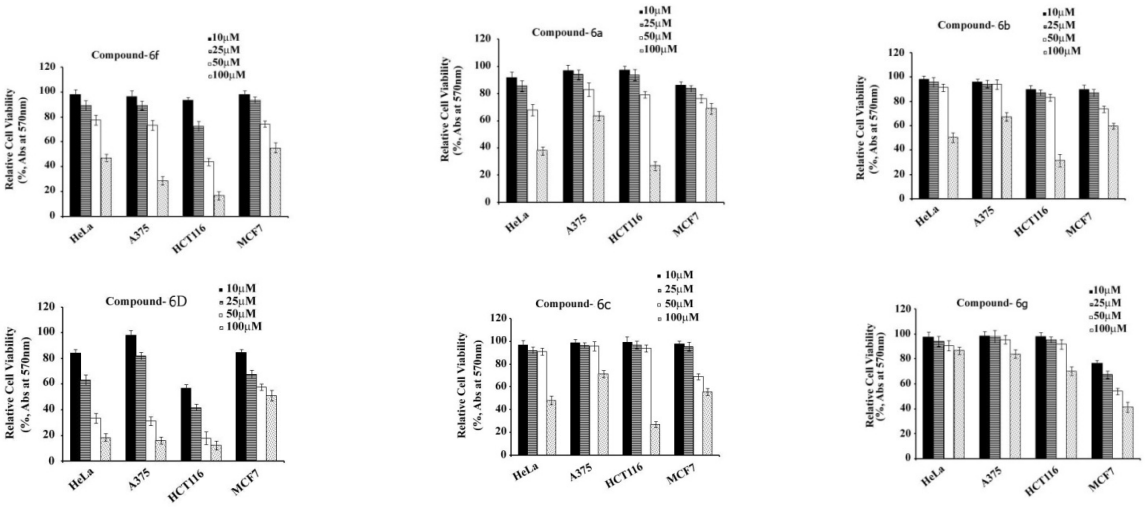


Fig: 2 .Relative cell viability in various cancer cell lines.

Table 7: Cells and their IC50 based on sensitivity.

Compound	IC50 (µM)			
	HeLa	A375	HCT116*	MCF7*
6d	37.57	36.32	11.59**	102.58
6c	96.46	175.37	68.42*	112.94
6b	100.76	154.08	73.20*	124.40
6g	372.57	309.40	166.44	54.32*
6f	94.80	70.10	44.42*	111.45
6a	78.36	137.40	68.45*	161.29

* Sensitive cells and their IC50.

Table 8: Anti-inflammatory study of 5-(1, 3-benzodioxol-5-yl)-3-phenyl-1H-pyrazole-1-carbothioamide (6a) compared with Diclofenac.

Treatment	Concentration in µg/ml	Absorbance	% inhibition of Membrane lysis
Compound 6a	100	0.888 ± 0.012	50*
	500	0.592 ± 0.025	66.66*
	1000	0.273 ± 0.017	84.62**
Diclofenac	100	0.202 ± 0.02	88.62

* P <0.01 compared to Diclofenac.

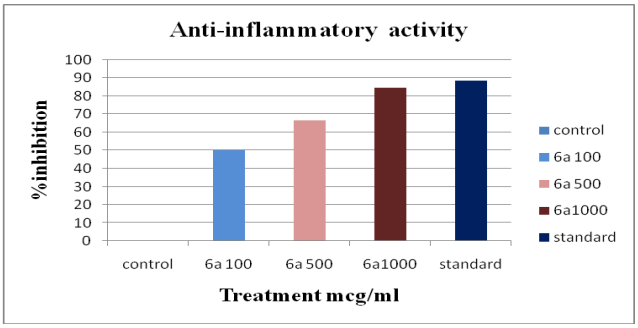


Fig: 3: Anti-inflammatory study of 5-(1, 3-benzodioxol-5-yl)-3-phenyl-1H-pyrazole-1-carbothioamide (6a) compared with Diclofenac.

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