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In-Silico Molecular Docking Study of Substituted Imidazo 1,3,4 Thiadiazole Derivatives: Synthesis, Characterization, and Investigation of their Anti-Cancer Activity

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Insilico, anticancer, hormones, cell signaling, antimicrobial

Abstract
Alternate derivatives, N-{[5-(substituted)-1, 3, 4-thiadiazole-2-yl] carbamothioyl} (3a-t), were developed and synthesized. These derivatives incorporate the 1, 3, 4-thiadiazole, and were specifically designed as a means to facilitate green chemistry practices. he synthesized compounds were subjected to molecular docking studies with the receptors of lipoxygenase-3 soybean complex in order to explore their potential as inhibitors for silencing hormone signaling in breast cancer (PDB Code-3k59) and antimicrobial doings (PDB Code-3ave). Compounds 3r Displayed heavy action against receptors of lipoxygenas-3 soybean complex for anti-inflammatory activity (PDB Code- 4tuk), Compounds 3p Displayed heavy action against inhibitors of NUDTs silence hormone signaling in Brest cancer (PDB Code-3k59) and Compounds 3n Displayed heavy action against microbial (PDB Code-3ave) for antimicrobial activity. These synthesized 3a-t compounds were inveterate through spectral characterization. The result showed significance for these compounds antimicrobial activity, anti-cancer activity and Comparison anti-inflammatory activity to standard drugs was used. PLS advanced method of analysis was opted for a set of 10 derivatives to figure out the co-relationship between hydrophobic, steric and electrostatic descriptors.

1. Introduction

1,3,4-Thiadiazole

1,3,4-Thiadiazole has emerged as a significant building block within the realm of heterocyclic compounds, finding widespread application in the development of novel pharmaceuticals[1-3]. The inclusion of the 1,3,4-thiadiazole scaffold in compounds has imparted a diverse range of biological activities[4-10]. The unique chemical reactivity exhibited by 1,3,4-thiadiazole heterocyclic compounds has made them highly valuable for molecular design, owing to their inherent structural advantages and vast biological potential[11-14]. Acetazolamide and methazolamide are two notable examples of compounds containing the 1,3,4-thiadiazole unit, used as carbonic anhydrase inhibitors .[15-17]

The discovery of 1,3,4-thiadiazole dates back to 1882 when Fischer first reported its existence, followed by subsequent advancements by Busch and his research team. Thiadiazole is exists in four isomeric forms: 1,2,3-thiadiazole (1), 1,2,4-thiadiazole (2), 1,2,5-thiadiazole (3), and 1,3,4-thiadiazole[18, 19]..

1,3,4- thiadiazole have been most promised isomer than the other. The inductive effect was developed by the sulfur atom of 1,3,4-thiadiazole ring- very low base property and possessing relatively high

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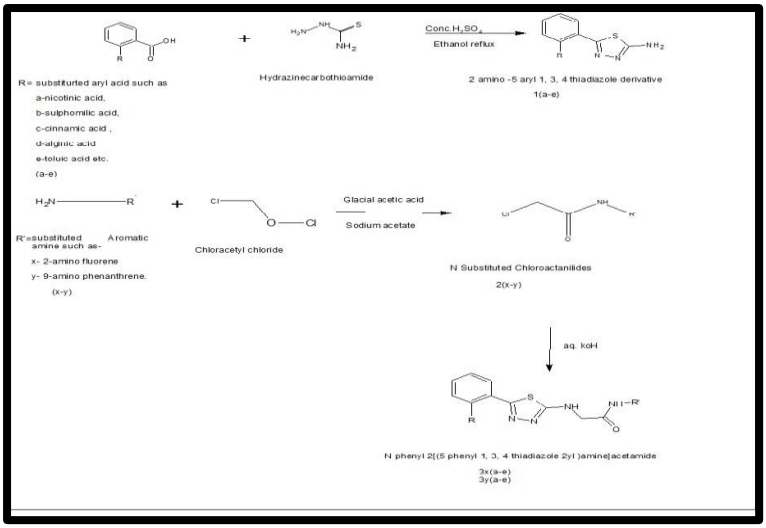
aromaticity. However, the 1,3,4-thiadiazole ring nitrogen atoms are also shown low provided electron due to the impact of electron withdrawal and comparatively even towards electrophilic substitution, but susceptible to nucleophile . So, making the substitution in this ring's 2′ or 5' position and certain substitutions involves strongly activating reaction.[20-45]

2. Materials and Methods:

The chemicals and solvents were obtained from commercial suppliers.

Reaction Scheme:



3. Procedure:

Synthesis: By Conventional method:

Step 1st: - Creating 5-(substituted)-1,3,4-thiadiazole-2-amine through synthesis:

A total of 9.11 grams of tiosemicarbazide and 1 gram of aryl acid dissolved in 50 ml of ethanol. Mixture was then cooled to temperature range of 2-5ºC, and a gradual dropwise addition of 5 ml of concentrated H2SO4 was performed under cold conditions. The entire reaction mixture was subsequently refluxed for one hour at a temperature of 70ºC. The resulting product was recrystallized using ethanol.

Step 2nd: - Synthesis of substituted benzoyl isothiocyanate:

An aromatic amine solution (0.05 mol) was prepared by dissolving it in 25 ml of glacial acetic acid and 25 ml of a saturated sodium acetate solution. The resulting solution was then cooled to 5ºC. To this cooled mixture, 6.2 ml of chloroacetyl chloride was added drop wise while maintaining a temperature range of 0-5ºC, with continuous stirring and kept at room temperature for 5-6 hours.

Step3rd: -Creating N-{[5-(substituted)-1,3,4-thiadaizole-2-yl]carbamothioyl}:

A quantity of 2-amino-5-aryl 1,3,4-thiadiazole (1.79 gm) dissolved in an aqueous solution of KOH (0.619 gm/10 ml water) with stirring to get yellow result. Small portions of various aromatic N-substituted α-chloro-acetanilides were gradually added to the solution with constant shaking at a temperature of 50-60ºC for a period of 4-5 hours. After completion, mixture was left overnight, and precipitate was collected.

B. Microwave-assisted method for synthesis of 1,3,4-thiadiazole derivatives.

Step 1st: - Synthesis of 5-(substituted)-1,3,4-thiadiazole - 2-amine:

4.8 grams of tiosemicarbazide and 0.5 grams of aryl acid dissolved in 25 ml of ethanol. Mixture was cooled to 2-5ºC, and a gradual dropwise addition of 2.5 ml of concentrated H2SO4. The reaction mixture

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was refluxed for 15 to 20 minutes at 340 watts and confirmed by TLC.

The solid precipitate was dried and subsequently recrystallized.

Step 2nd: - Synthesis of substituted benzoyl isothiocyanate:

An aromatic amine (0.05 mol) dissolved in 25 ml

of glacial acetic acid and 25 ml of saturated sodium acetate. It was cooled to a temperature of 5ºC and 6.2 ml of C2H2Cl2O was added dropwise while maintaining a temperature range of 0-5ºC, with continuous stirring. The reaction mixture kept at room temperature for 5-6 hours and TLC was performed.

Step 3rd: - Synthesis of N-{[5-(substituted)-1,3,4-thiadiazole-2-yl] carbamothioyl} derivatives:

2-amino-5-aryl 1,3,4-thiadiazole (1.79 gm) dissolved in an aqueous solution of KOH (0.619 gm in 10 ml water) under continuous stirring until yellow solution was obtained and was filtered. Small portions of various aromatic N-substituted α-chloro-acetanilides were added to the solution while shaking at a temperature range of 50-60ºC for duration of 4-5 hours.

The resulting precipitate filtered and washed with cold water to remove any excess KOH and TLC testing was carried out.

Table No. 1. Physicochemical data: 2-amino-5 aryl 1, 3, 4 thiadiazole by Conventional and microwave method (1a-e).

S. no	-Compounds	Molecular Formula	Molecular Weight	MP 0_C	Percentage Yield%		Rf value	Mobile Phase
					- Conventional	icro- wave		
1	1a	C8H8O3	152.14	276-278	85%	90%	0.73	A: T (7:3)
2	1b	C7H4N2O6	212.12	202-204	82%	92%	0.75	A: T (7:3)

Table No. 2 Physicochemical data: N-phenyl 2[(5-phenyl 1, 3, 4-thiadiazole 2yl) amine] acetamide by conventional and microwave method 3x (a-j), 3y (a-j).

S. no	Compound	Molecular Formula	olecular Weight	MP 0_C	Percentage Yield%		Rf value
					Conventional	Microwave	
1	3a	$C_{16}H_{12}BrNO_3S$	434.26	176-180	61%	75%	0.57
2	3b	$C_{18}H_{17}N_5O_3S$	383.42	173-178	50%	88%	0.66
3	3c	$C_{15}H_{12}N_6O_3S$	356.35	165-170	66%	89%	0.78
4	3d	$C_{16}H_{14}N_6O_3S_2$	402.45	163-168	58%	86%	0.46

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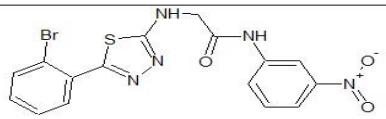
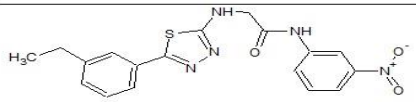
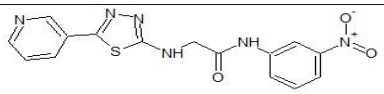
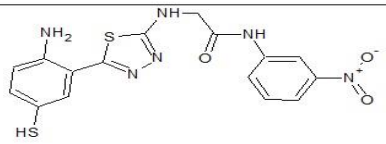
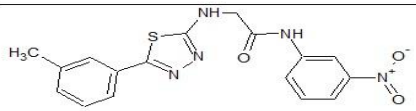
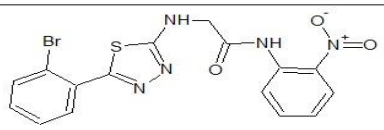
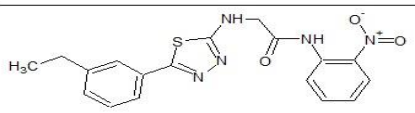
5	3e	$C_{17}H_{15}N_5O_3S$	369.39	182-188	65%	72%	0.37
6	3f	$C_{16}H_{12}BrN_5O_3S$	434.26	173-176	78%	77%	0.48
7	3g	$C_{18}H_{17}N_5O_3S$	383.42	170-175	74%	90%	0.44
8	3h	$C_{15}H_{12}N_6O_3S$	356.35	174-179	59%	85%	0.47
9	3i	$C_{16}H_{14}N_6O_3S_2$	402.45	163-168	80%	82%	0.63
10	3j	$C_{17}H_{15}N_5O_3S$	369.39	162-167	79%	77%	0.55

Anticancer activity docking results:

Table No.3: Anticancer activity : Molecular docking using GRIP Batch docking.

Sr.no	Compound	Structure	Final Energy	Final GRMS	Dock score
1	3a		69.1451	0.0099	-24.04
2	3b		67.8078	0.0093	-31.44
3	3c		67.6498	0.0074	-16.95
4	3d		76.9614	0.0095	-24.94
5	3e		68.2124	0.0099	-30.03
6	3f		75.3026	0.0082	-18.69
7	3g		73.9093	0.0081	-24

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The synthesized derivatives 3a-3j assessed for antitumor potential against MCF-7. The docking scores of the composite compounds given with compound 3b exhibiting the lowest score of -31.44, indicating strong binding affinity compared to other compounds. Upon comparing these results with existing literature, it is evident that the designed compounds possess significant binding affinity for the NUDTs receptor, serving as potent inhibitors in silencing hormone signaling in breast cancer (PDB Code-1dls). The best docking pose, as shown in figure 5.2.4.1, highlights the key interactions between the receptor and ligand. All designed compounds exhibit a similar conformation within the binding pocket, forming hydrogen bond interactions with GLU257A, aromatic interactions with PHE265A, and van der Waals interactions with TRP255A, ARG256A, ARG365A, GLU257A, PHE265A, and DT802T, as depicted in the 2D representation diagram (figure 5.2.4.2). The superimposed image of compound 3b with the receptor is illustrated in figure 5.2.4.3. The docking score of the standard drug, 5-fluorouracil, was found to be -53.65.

Materials and Methods

Chemicals

The Chemicals were bought from research lab, Mumbai, India.

Cell lines:

Observations:

- Cell lines were acquired from hospital.
- The cells formed 37 a.c..in a 5 percent CO2 incubator humidified atmosphere.

Method

- Preparing hemocytometer :

1. Before use clean hemocytometer& cover slip and wipe with 70% alcohol.

2. Slip the cover with water and add to the hemocytometer to moisten.

Cell suspension: Prepared by swirling the flask for even distribution of cells, extracting 0.5 mL of the suspension using a sterile pipette, and transferring it to an Eppendorf tube. Then, mix 100 µL of cells with 400 µL of 0.4% Trypan Blue solution (0.32% final concentration) in a separate Eppendorf tube.

Counting:

100 µL counted by hemocytometer.

Viability:

1. Determine the average cell count per set of 16-corner squares.
2. Multiply the average count by 10^4 and then by 5 to account for the 1:5 Trypan Blue dilution.

To calculate viability: To estimate viability, add the count of live cells to the count of dead cells in each set of 16-corner squares to obtain the total cell count.

Table No. 4. Anticancer activity of synthesized compound (3a-i) against MCF-7 cell line.

Sr. no	compound	10mcg	50mcg	100mcg
1	3a	87.69	78.62	82.31
2	**3b**	**89.76**	**80.60**	**80.00**
3	3c	82.00	79.44	83.51
4	3d	80.56	82.90	79.83
5	3e	85.71	77.81	79.50

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6	3f	83.21	77.42	84.44
7	3g	82.01	77.90	82.01
8	3h	83.21	82.90	80.56
9	3i	85.44	86.36	79.00
10	**Standard (5-F-uracil)**	**90.72**	**88.21**	**85.14**

"The statistical significance of difference across the groups is determined using ANOVA followed comparisons test."

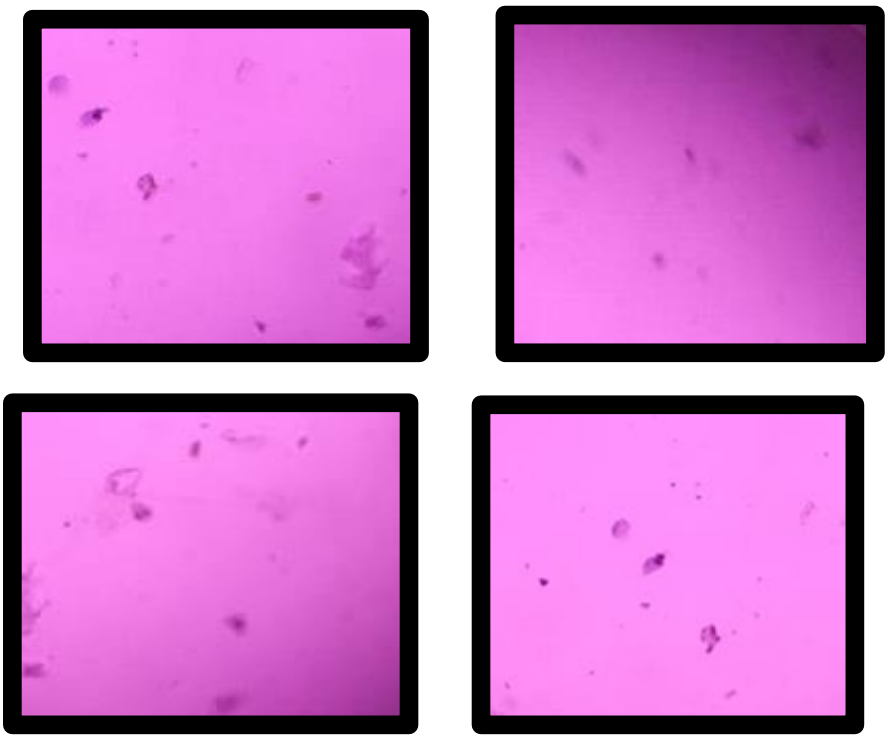

Figure No. 1: Anticancer Activity of Synthesized Compound

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