Content available at: https://www.ipinnovative.com/open-access-journals



International Journal of Pharmaceutical Chemistry and Analysis

Journal homepage: https://www.ijpca.org/



# **Original Research Article**

# Synthesis, crystal structure studies, characterization and *in vitro* study of novel heterocyclic thiadiazoles as caspase 3 inhibitors for anticancer activity

# Bhavini K Gharia<sup>1</sup>, Bhanubhai N Suhagia<sup>2</sup>, Vineet Jain<sup>1</sup>

<sup>1</sup>Dept. of Pharmaceutical Chemistry, Bhagwan Mahavir College, Surat, Gujarat, India <sup>2</sup>Dept. of Pharmaceutical Chemistry, Dharmsinh Desai University, Nadiad, Gujarat, India



#### ARTICLE INFO

Article history: Received 14-10-2023 Accepted 28-11-2023 Available online 21-12-2023

*Keywords:* Anticancer drugs Caspase family steine proteases Colorimeteric Caspase 3 inhibitor kit

#### ABSTRACT

In the present study we have reported the synthesis of some novel heterocyclic derivatives comprising imidazole and 1,3,4-thiadiazole containing cyclopropyl moiety. Imidazothiadiazoles are of interest because of their diverse biological activities and clinical applications. Primarily docking studies are carried out with reference structure Pdb code:2DKO. We have reported the new series of 5,6 diaryl substituted with imidazo[2,1- b] <sup>1–3</sup>thiadiazoles analogs to target caspase family cysteine proteases. The reaction was monitored by Thin-layer chromatography using suitable mobile phase. The Rf values were compared and determined the melting point of synthesized compounds. Further these derivatives were characterized and confirmed by IR, 1H-NMR, 13C-NMR, and Mass spectral (MS) studies. For anticancer activity, all the selected compounds submitted to National Cancer Institute (NCI) for in vitro anticancer assay were evaluated for their anticancer activity. Primary *in vitro* one dose anticancer assay was performed in full NCI 60 cell panel in accordance with the protocol of the NCI, USA. The compounds *Va* and *Vc* showed good activity against all cancer cell lines. The synthesized drugs were monitored with Caspase 3 inhibitor kit.(CASP3C-1KT)using colorimeteric assay.

This is an Open Access (OA) journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprint@ipinnovative.com

# 1. Introduction

Many of the more recently approved cancer treatments target either the cell surface receptors at the head of these signaling pathways, or the intermediate phosphoproteins and kinases in the pathway signaling cascades. Understanding the phosphoprotein activation state of key signaling molecules in tumor cells can yield critical information on the type, stage and status of those cells, aiding in the diagnosis, prognosis and treatment of an individual's disease.<sup>1</sup>

Caspases are aspartic acid-specific cysteine proteases, which become activated in most forms of apoptosis. In cells, they localise in nucleus, cytoplasm, and mitochondrial intermembrane space, and can also be translocated to the plasma membrane receptors via adapter proteins.<sup>4</sup>

Caspases are expressed in most tissues in an inactive pro-form, which has an amino- terminal prodomain, a large subunit ( $\sim$ 20 kDa) and a small subunit ( $\sim$ 10 kDa) (Figure

1A). Upon activation, a procaspase is proteolytically cleaved to remove the prodomain and to release the large and the small subunit. Two small and two large subunits are assembled together to yield an active caspase enzyme with two active sites.<sup>2</sup>

DED = Death Effector Domain (dotted), QACQG= consensus amino acid sequence of the

active site of caspases (black ellipses) in large subunit,

arrows = sites of processing to release large subunit (light grey) and small subunit

(dark grey) from prodomain (white).

\* Corresponding author.

E-mail address: bkgharia@gmail.com (B. K. Gharia).

https://doi.org/10.18231/j.ijpca.2023.045 2394-2789/© 2023 Author(s), Published by Innovative Publication.



Figure 1: Schematic presentation of the general structure of caspases.<sup>2</sup>



Figure 2: Active caspase tetramer.<sup>5</sup>

# 1.1. Role of caspase in cancer

Dying cells in the tumor mass provide the initial signals to promote tumor repopulation. Specifically, dying cells release growth-promoting signals to stimulate the proliferation of surviving cells.Caspases play a crucial role for intratumoral dying cells in promoting the rapid repopulation of tumors from a small number of live tumor cells. In addition, caspase 3, a cysteine protease involved in the 'execution' phase of cellular apoptosis, is a key regulator of growth-promoting signals generated from the dying cells. Caspase-mediated tumor repopulation mechanism has key roles in cytotoxic cancer therapy.<sup>3</sup>

# 1.2. Caspase inhibitors:<sup>6,7</sup>



Figure 3: Role of caspase in cancer<sup>3</sup>



269

5-(2-aryloxypyrrolidin-1-ylsulfonyl)-1-arylindoline-2,3-dione

# 1.3. Chemistry of imidazo $[2, 1-b]^{1-3}$ thiadiazoles

The bicyclic molecule many be viewed as 2-vinylimino-3H-3- alkyl-1, 3, 4- thiadiazole.

Important canonical structure of imidazo[2, 1-b]<sup>1-3</sup> thiadiazole are given. They indicate greater delocalisation of  $\pi$  electrone in the imidazole ring, while the double bond of the thiadiazole ring is almost localized in structure is maximum contributing structure. It is pseudo-aromatic in behavior containing imidazole moiety as electron rich center. Chlorine or bromine dose not add to the double bond at 2, 3-position on the other hand, the electrophillic substitution reactions like bromination, nitration etc take place at 5-position. His bicyclic ring system with desired substituents at 2, 5, and 6-position can be built by starting with appropriate synthons, for introduction of substitutents at 5 and 6-position, the synthons R1COCHR2Br is employed for subtituents at 2-position, 5-substituted 2aminothiadiazole is required. The ring nitrogen in imino form of the starting thiadiazole attacks the carbon carrying bromine in the synthon.<sup>8,9</sup> Imidazo[2,1 -b]<sup>1-3</sup> thiadiazoles contain nitrogen as a bridge head atom at 4th position electron charge density measurement indicated that the imidazole ring is rich in electron density having at N-7 position and then at C-5 position. This accounts for the preferable electrophilic substitution reaction at C-5 position. Of the three nitrogen atoms, N-7 is the most basic center. The order of basicity is N-7> 3> N-4. Therefore, protonation occurs first at N-7 then at N-3 position.

Two types of bicyclic imidazo[2,1b]<sup>1–3</sup>thiadiazole ring systems are possible.

#### 2. Materials and Methods

# 2.1. Docking studies

In our present study, we used computational approach to identify the potent and selective Caspase-3 inhibitors. Three-dimensional (3D) pharmacophore models were generated using the known set of Caspase-3 inhibitors, to reveal the chemical features required for its activity. Best pharmacophore is validated with docking and structure based pharmacophore studies. These models were used to rapidly screen compounds from database, for the







5-(2-benzylox ypyrrolidin-1-ylsulfonyl)-1-benzylindoline-2,3-dione

IMIDAZO[2,1-b][1,3,4]THIADIAZOLE



IMIDAZO[5,1-b][1,3,4]THIADIAZOLE

Figure 4: showing imidazolering system



Figure 5: Docking structure of caspase 3 inhibitor (PDB structure 2DKO)

identification of a series of novel and highly potent Caspase-3 inhibitors. Molecules were selected from virtual screening using pharmacophore as query and these molecules are selected for synthesis and in vitro screening studies based on the docking scores, predicted binding location and their drug like properties. 10,11

The identification and characterization of the compound were carried out by the following procedure to ascertain that all prepared compounds were of different chemical nature, than the respective parent compound.

- 1. Melting point
- 2. Solubility
- 3. Thin layer chromatography
- 4. Infrared spectroscopy
- 5. Proton nuclear magnetic resonance
- 6. Mass spectroscopy

The chemicals employed in the synthetic work i.e thiosemicarbazide and trifuoroaceticanhydride were purchased from Sigma-Aldrich while all other chemicals i.e. cyclopropanecarboxylic acid, Bromine, various acetophenones, DMF and POC13 etc. were purchased from Spectrochem. All the solvents were used after distillation. Most of the solvents and chemicals used were of LR grade. The purity of the compounds was confirmed by thin layer chromatography using precoated TLC plates and solvent systems of Benzene: Acetone (9:1), (7:3); T:E:F (5:4:1), and Chloroform: Methanol (9:1). The spots were visualized under ultraviolet lamp. Melting points were determined in one end open capillary tubes on a liquid paraffin bath and are uncorrected.

Infrared (IR) and 1H nuclear magnetic resonance (1H NMR) spectra were recorded for the compounds on Perkin Elmer IR 4000-400 (v max in cm-1) Spectrophotometer in KBr pellets and Bruker Model Advance II 400 (400 MHz, 1H NMR) instrument, respectively. Chemical shifts are reported as  $\delta$  parts per million (ppm) using tetramethylsilane (TMS) as an internal standard.

# 3. Reaction Scheme

# 3.1. Synthesis of

# 5-cyclopropyl-1,3,4-thiadiazol-2-amine (III)

Mixture of cyclopropanecarboxylic acid (I (0 05 mol, thiosemicarbazide (II (0.05 mol) and POCl3 (13 ml) was heated at 75 °C for 0.75 h. After cooling down to room temperature, water was added. The reaction mixture was refluxed for 4 h. After cooling, the mixture was neutralized to pH 7 by the drop wise addition of 50% NaOH solution under stirring. The precipitate was filtered and crystallized from ethanol.

# 3.2. Synthesis of

# 2-bromo-1,2-(substituted-aryl)ethanone (IVa-i)

To a mixture of phenyl acetic acid/p-substituted phenyl acetic acid (10a/b, 7.3 mmol), substituted aromatic hydrocarbon (one of *VIII a–i*, 8.8 mmol), and 88–93% orthophosphoric acid (8.8 mmol) was added trifluoroacetic anhydride (29.5 mmol) rapidly with vigorous stirring at 250 C. The mixture turned into a dark colored solution with vigorous exothermic reaction. The reaction mixture was stirred for 1 min at the same temperature and poured into ice-cold water (50 mL) with stirring. Then it was washed with cold hexane (2·10 mL) to obtain (IV a-i) as solid.

# *3.3.* Synthesis of 2-cyclopropyl-5,6-diarylsubstituted imidazo[2,1-b]-1,3,4- thiadiazole(Va-i)

A mixture of 2-amino-5-substituted-1,3,4-thiadiazole(III, 10 mmol) and an appropriate a-bromo-1-(4"substituted)phenyl-2-(4' substituted)phenyl-1-ethanone (one of IVa-i, 10 mmol) in dry ethanol (150 mL) was heated to reflux on a water bath for 6-8 h, phosphorus pentoxide (3 mmol) was added, and refluxing was continued for another 4-6 h. The reaction mixture was cooled overnight at room temperature. Excess of solvent was removed under reduced pressure and the solid hydrobromide separated was filtered, washed with cold ethanol, and dried. Neutralization of hydrobromide salts with cold aqueous solution of Na2CO3 yielded the corresponding free bases (Va-i), which were purified by recrystallization from dry ethanol. Further, the compounds were purified by column chromatography using 200-400 mesh silica gel and eluted either with ethyl acetate/ hexane (2:8) or chloroform/hexane (1:9) as mobile phase.

# 3.4. Caspase colorimetric kit

The Caspase 3 Colorimetric Assay Kit is based on the hydrolysis of acetyl-Asp-Glu-Val-Asp p-nitroanilide (Ac-DEVD-pNA) by caspase 3, resulting in the release of the p-nitroaniline (pNA) moiety. p-Nitroaniline is detected at 405 nm ( $\varepsilon_{mM}$ =10.5). The concentration of the pNA released from the substrate is calculated from either the absorbance values at 405 nm or from a calibration curve prepared with pNA standards (pNA standard included with the kit).

The assay can be performed (a) in 1 mL volume and measured using a spectrophotometer, or (b) in 100  $\mu$ L volume in a 96-well plate using an ELISA reader.<sup>12,13</sup>

# 4. Result and Discussion

We have tried to synthesized a series of 9derivatives of imidazo  $[2,1,b]^{1-3}$  thiadiazole using cyclopropanecarboxylic acid as starting material. Synthesis was carried according to reaction shown in Reaction Scheme. The compounds *Va-Vi* containing substituted

aryl group at 5th and 6th position by reacting 2-amino 5-substituted 1, 3, 4-thiadiazole of general formula *III* with substituted a- haloaryl/heteroaryl ketones as depicited in scheme. The reaction was monitored by Thin-layer chromatography using suitable mobile phase such as Benzene: Acetone (7:3) n-hexane: Ethyl acetate: Formic acid (5:4:1); Chloroform: Methanol (9.5:0.5). The Rf values were compared and found that they were different from each others. The melting point of the derivatives was determined.

The supplementary material is attached of characterization of compounds.



2-cyclopropyl-5,6-dimethylimidazo[2,1-b][1,3,4]thiadiazole

**Table 1:** The docking scorewith various details

Sr. No.	Structure code	Docking Score	HBD	HBA
a.	Va	-79.2	2	6
b.	Vb	-21.9	3	7
c.	Vc	-82.1	3	7
d.	Vd	-49.6	2	6
e.	Ve	-13.6	2	6
f.	Vf	-69.4	3	7
g.	Vg	-46.3	2	7
h.	Vh	-19.4	3	6
i.	Vi	-64.3	2	7
	2DKO	-88.32	3	7

# 5. NCI-60 DTP Human Tumor Cell Line Screen

The screening is a two stage process, beginning with the evaluation of all compounds against the 60 cell lines at

Compound	Ar	Ar 1			
IV/Va	4-(OCH3)C6H4	C6H5			
IV/Vb	C6H5 C6H5	C6H5			
IV/Vc	4-(CH3)C6H4	C6H5			
IV/Vd	4-(SCH3)C6H4	C6H5			
IV/Ve	4-ClC6H4	C6H5			
IV/Vf	4-BrC6H4	4-OCH3C6H4			
IV/Vg	4-(OCH3)C6H4	4-OCH3C6H4			
IV/Vh	4-(SCH3)C6H4	4-OCH3C6H4			
IV/Vi	4-(Cl)C6H4				

 Table 2: List of proposed derivatives.

a single dose of  $10\mu$ M. The output from the single dose screen is reported as a mean graph and is available for analysis by the compare program. Compounds which exhibit significant growt inhibition are further evaluated against the 60 cell panel at five concentration level.<sup>8,14</sup>

a) Methodology of the in vitro cancer screen:<sup>5</sup> The human cancer cell lines of the cancer screening panel are grown in RPMI 1640 medium containing 5% fetal bovine serum at 2 mM L-glutamine. For a typical screening experiment, cells are inoculated into 96 well microtiter plates in  $100\mu$ L at plate densities ranging from 5,000 to 40,000 cells/well depending upon the doubling time of individed cell lines. After cell inoculation, the microtiter plates are incubated at 37 °C, 5% CO2, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs. After 24 h, two plates of each cell lines are fixed in situ with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition (Tz). Experimental drugs are solubilized in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate is thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50µg/ml gentamicin. Additional four, 10-fold or  $\frac{1}{2}$  log serial dilutions are made to provide a total of five drug concentrations plus control. Aliquots of  $100\mu$ l of these different drug dilutions are added to the appropriate microtiter wells already containing  $100\mu$ l of medium, resulting in the required final drug concentration.

Following drug addition, the plates are incubated for an additional 48 h at 37 °C, 5% CO2, 95% air and 100% relative humidity. For adherent cells, the assay is terminated by the addition of cold TCA. Cells are fixed in situ by the gentle addition of  $50\mu$ l of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 minutes at

4 °C. The supernatant is discarded and the plates are washed five times with tap water and air dried. Sulforhodamine (SRB) solution ( $100\mu$ l) at 0.4% (w/v) in 1% acetic acid is added to each well and plates are incubated for 10 minutes at room temperature. After staining, unbound dye is removed by washing five times with 1% acetic acid and the plates are air dried. Bound stain is subsiquently solubilized with 10mM trizma base, and the absorbance is read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology is the same except that the assay is terminated by fixing settled cells at the bottom by the wells by gentle adding  $50\mu$ l of

80% TCA (final concentration 16% TCA). Using the seven absorbance measurements

[time zero, (Tz), control growth, (C) and the test growth in the presence of drug at five concentration levels (Ti), the percentage growth is calculated at each of the drug concentrations levels. Percentage growth inhibition is calculated as:



**Figure 6:** Showing the result with caspase 3 inhibitor Xaxis Concentration( $\mu g/mL$ ) Yaxis Absorbance(Au)

[(Ti-Tz)/(C-Tz)] X 100 for concentrations for which Ti  $\geq$ Tz [(Ti-Tz)/Tz] X 100 for concentrations for which Ti<Tz

Three dose response parameters are calculated for each experimental agent. Growth inhibition of 50% (GI50) is calculated from [(Ti-Tz)/(C-Tz)] X 100 = 50 which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (TGI) is calculated from Ti=Tz. The LC50 (concentration of the drug resulting in 50% reduction in the measured protein at the end of the drug treatment as compared from.

 $100 = -50.^{15}$ 

Values are calculated for each of these parameters if the level of activity is reached; however, if the effect is not reached or is exceeded, the value for that parameters is expressed as greater or less than the maximum or minimum concentration tested.

Further structures of the synthesized compounds were established on the basis of spectral data. (IR, 1H-NMR, 13C-NMR, and Mass) Compounds III showed peaks at Cm-1 for NH2, compound Va-Vi showed absorption bands ranging from1600-Cm-1 for C=N stretch, in their respective

One Dose Mean Graph         Experiment ID:         12:04084         Report Date::May 00, 20:2           Panel/Cell Line         Growth Percent         Mean Growth Percent         Mean Growth Percent         Mean Growth Percent           Level         0:0400         0:040         0:040         0:040         0:040           Machine         0:040         0:040         0:040         0:040         0:040           Machine         0:040         0:040         0:040         0:040         0:040           Non-Small Cell Ling         0:040         0:040         0:040         0:040         0:040           Non-Small Cell Ling         0:040         0:040         0:040         0:040         0:040           Non-Small Cell Ling         0:040         0:050         0:050         0:040         0:040           Non-Small Cell Ling         0:040         0:050         0:040         0:040         0:040           Non-Statistic         0:040         0:050         0:050         0:040         0:040         0:040           Non-Statistic         0:040         0:040         0:040         0:040         0:040         0:040           Non-Statistic         0:040         0:040         0:040         0:040         0:040	Developmental Thera	apeutics Program	NSC: D-754956 / 1	Conc: 1.00E-5 Molar	Test Date: Apr 10, 2012	
Parelic Cell Line Growth Percent Mean Growth Percent - Gr	One Dose Mean Graph		Experiment ID: 120	Experiment ID: 1204OS46		
Law Corresting         12,43           Mod 14         100,14           Mod 14	Panel/Cell Line	Growth Percent	Mean Growth	Percent - Growth Perc	ent	
CERCENT       6243         Nordicitat       6244         Nordicitat       6243         Nordicitat       6243         Nordicitat       6243         Nordicitat       6243         Nordicitat       6244         Nor	Leukemia					
MOL 104         Concer         100 cm           MOL 105         Concer         100 cm <td< th=""><th>CCRF-CEM</th><th>62.43</th><th></th><th></th><th></th></td<>	CCRF-CEM	62.43				
Non-Additional Cancer         45.9           Non-Additional Cancer         45.9           Holp-R2         45.9           State-R3         55.9           State-R3         55.9           State-R3         55.9           Holp-R4         55.9           Holp-R4         55.9           Holp-R4         55.9           Holp-R4         55.9           Holp-R4         55.9           Holp-R4	K-562	70.34				
No.         Association         Association           Association         4.50         4.50           PVV         5.50         4.32           PVV         5.50         4.32           SV4.50         5.51         5.51           SV4.50         5.52         5.52           SV4.50         5.52         5.52           SV4.50         5.52           SV4.50         5.52           SV4.51         5.52           SV4.51         5.52           SV4.51         5.52	MOLT-4	60.14				
ABM/TCC       44.89         PHO-R2       41.89         NCH-R22       13.81         NCH-R23       61.92         NCH-R23       14.61         NCH-R23       14.61         NCH-R23       14.61         NCH-R23       14.62         NCH-R23       15.64         NCH-R23       15.76         NCH-R23       15.76         NCH-R23       15.76         NCH-R23       15.76	Non-Small Cell Lung Cancer	43.85				
HOP Ac2 HOP Ac2	A549/ATCC	44.89				
NG1-62       -13.81         NG1+622       -23.81         NG1+623       -15.51         NG1+623       -15.52         NG1+623       -15.52         NG1-623       -15.52         NG2-767       -15.52         NG2-767       -15.52         NG2-767       -15.52         NG2-767       -15.52         NG2-767       -15.54 <th>HOP-82</th> <th>43.39</th> <th></th> <th></th> <th></th>	HOP-82	43.39				
NCH202m       0104         NCH202m       0104         NCH400       35.51         NCH202       0104         HCC3066       10.35         HCC3066       10.35         HCC3066       10.35         HCC3066       10.35         HCC3066       10.35         HCC307       10.47         HCC307       10.47         MMERONA       10.47         MARONA       10.47         MARONA       10.47         MARONA       10.47         MARONA <th>HOP-92</th> <th>-13.81</th> <th></th> <th></th> <th>• • • •</th>	HOP-92	-13.81			• • • •	
NC1+822M       100         NC1+822       20.91         NC1+822       30.91         NC1+822       30.91         NC1+822       30.91         NC1+823       91.91         SNR-19       56.52         SNR-10       57.62         OVCAR4       57.62         OVCAR5       57.62         SNR-19       56.52         SNR-19       56.52         SNR-10       57.62         OVCAR4       57.62         SNR-10       57.62         SNR-10       57.62         SNR-10 <t< th=""><th>NCI-H226 NCI-H23</th><th>61.04</th><th></th><th></th><th></th></t<>	NCI-H226 NCI-H23	61.04				
NC1+H800       35.51         Color C0	NCI-H322M	65.80				
Concentration of the state of t	NCI-H460	35.51				
CC0.0206       77.31         HCC7.110       20.94         HCC3.0206       77.31         HT7.0       60.16         KM12       61.12         SW-620       72.21         SF-539       -16.82         SN-73       -10.47         U231       19.50         MAK       64.17         MALE-35       67.72         SK-MEL-30       52.72         SK-MEL-30       52.72         SK-MEL-30       52.72         SK-MEL-30       52.72         SK-MEL-30       52.72         UACC-287       60.22         UACC-287       60.22         UACC-287       60.22         UACC-287       60.22         UACC-287       60.22         UACC-287       60.21         SK-000       77.85         OVCARA-3       63.76         OVCARA-4       67.92         SK-000       71.85         Brast Cancer       30.35         MC7       31.41         OVCARA-5       63.73         SK-60       71.85         Brast Cancer       30.35         MC7       31.41	Colon Cancer	20.01				
HCC-5080       1/3.34         HCC-5080       1/3.34         HCC-5080       63.16         KM12       51.12         SW-620       72.51         SF-305       43.41         SF-305       43.41         SF-305       40.47         U251       10.47         MCDNM       50.47         MLMC-500       52.5         SNB-19       65.52         SNB-19       65.52         SNB-19       65.52         SNB-19       65.52         SNB-19       56.52         SNB-19       52.53         SNB-10       52.61         UACC-20       77.95         OVCAR-5       68.93         OVCAR-6       56.42         ACN-1       52.44         CAS1       40.65         MD2-48-2314       52.44         OVCAR-6 <td< th=""><th>COLO 205</th><th>86.35</th><th></th><th></th><th></th></td<>	COLO 205	86.35				
HT27:16 HT22 CH 67:06 SF-208 S	HCC-2998 HCT-118	20.94				
HV20       63.10         HV20       72.51         GK8 Cancer       72.51         SF-205       43.41         Mainona       61.52         Mainona       61.52         SF-205       43.41         MALME-SM       20.47         MALME-SM       61.52         SK-MEL-3       35.75         SK-MEL-3       35.75         SK-MEL-3       35.76         OVCAR-3       83.56         OVCAR-3       65.75         SK-MEL-3       35.76         OVCAR-3       65.92         UACC-42       57.92         OVCAR-3       65.93         OVCAR-4       67.92         OVCAR-5       90.65         DV-401       57.76         SK-601       90.65         DV-401       57.76         SK-775       90.65         DV-401       90.65         DV-402 <th>HCT-15</th> <th>67.05</th> <th></th> <th></th> <th></th>	HCT-15	67.05				
CNS Cancer SF-288 SF	KM12	63.10				
CMS Cancer SF-288 SF-289 SF	SW-620	72.51				
SF-235       -13.41         SF-235       -10.52         SNB-19       -56.52         SNB-75       -10.47         MELDOOTS       -11.7         OVCAR-3       -11.7         OVCAR-4       -11.7         OVCAR-5       -11.7         OVCAR-6       -11.7         OVCAR-7       -11.7         MEROOT       -11.7         MEROOT       -11.7         MEROOT       -11.7	CINS Canoer SE-268	38.32				
SN-539 SNB-75 SNB-75 SNB-75 SNB-75 SNB-75 MC-12 MM-S-3M MC-12 SN-MEL-23 SN-MEL	SF-295	43.41				
358:75       -0047         Weisnems       19251         Meisnems       2047         MALME-SM       2047         M4       61.93         MCA.MB-435       47.79         SK.MEL-2       59.22         SK.MEL-3       52.20         UACC-257       96.22         UACC-257       96.22         UACC-257       96.22         UACC-257       96.22         UACC-257       96.22         UACC-257       96.22         OVCAR-4       57.89         OVCAR-5       66.93         OVCAR-6       59.24         OVCAR-7       59.26         OVCAR-8       30.65         OVCAR-9       59.42         OVCAR-9       59.42         OVCAR-9       30.35         Protate Cancer       30.05         DU-343       71.85         Bread Cancer       30.05         DU-345       71.85         Bread Cancer       30.33         MCP7       204.0         MCP7       204.0         MCP7       30.05         Du-343       63.93         Bread Cancer       30.33	SF-539	-16.82			•	
U251       10.56         Melanoma       64.17         MALME-3M       20.47         M4       61.03         M5.ME-3M       20.47         M4.ME-3A5       61.72         SK.MEL-3B       52.75         SK.MEL-3B       52.62         UACC-257       56.22         UACC-267       56.22         UACC-267       56.22         UACC-267       56.22         OVCAR-3       83.56         OVCAR-3       83.56         OVCAR-3       83.66         OVCAR-3       63.02         OVCAR-3       63.02         OVCAR-3       63.02         OVCAR-4       57.92         OVCAR-5       64.02         SK-0V-3       81.42         OVCAR-5       93.05         OVCAR-5       30.05         OVCAR-5       30.05         OV-146       57.75         Bread Canoer       -10.44         HS 578T       -57.5         Bread Canoer       -150         M0A-MB-231/ATOC       -10.44         HS 578T       57.35         Bread Canoer       -150         M0A-MB-231/ATOC       -10.	SNB-75	-10.47				
Image: Construction of the second	U251	19.56				
MALME-SM 20.47 M4 01.93 MDA.MB-435 47.79 SK.MEL-2 83.22 SK.MEL-2 83.22 SK.MEL-3 22.02 UACC-257 52.02 UACC-257 59.22 UACC-227 59.22 UACC-210 41.51 Breast Cancer PC-3 90.05 UC-31 59.55 Mean 44.52 Deta 41.54 Dot 50 0 -50 -100 -150	LOX IMVI	54.17		_		
With ME-135       0779         SK-MEL-23       56375         SK-MEL-23       56375         SK-MEL-23       56375         SK-MEL-23       56375         UAOC-257       59.22         UAOC-257       50.22         Overain Cancer       53.76         OVCAR-3       63.350         OVCAR-5       68.33         OVCAR-6       68.33         OVCAR-7       68.35         OVCAR-8       38.42         NCIADR-RES       47.40         SK-00-3       222         AAGE       -0.56         ACAN       2241         SNT2C       50.44         CAN-1       40.21         SNT2C       50.44         CAN       22.40         MOA-ME-231MTCC       -10.44         HS 5731       37.33         T-701       103.17         MDA-ME-488       65.95         MEan       41.34	MALME-3M	20.47				
SK-MEL-28 SK-MEL-28	MDA-MB-435	47.79				
Sick MEL 5 UKCC-12 UKCC-12 Ovarian GROV1 GROV1 GROV1 GROV1 GROV1 GROV1 GROV1 GROV1 GROV1 GROV1 GROV1 GROV1 GROV1 GROV1 GROV2	SK-MEL-2	83.22				
UACC-257 59.22 UACC-42 57.91 IGROV1 53.76 OVCAR-3 83.56 OVCAR-4 57.92 OVCAR-5 88.93 OVCAR-5 88.93 OVCAR-5 88.93 OVCAR-5 84.93 NCUADR-RES 47.40 NCUADR-RES 47.40 NCUADR-RES 47.40 Remal Cancer 222 A498 - 6.56 ACHN 40.21 SNCOV-3 41.51 Remal Cancer 70 Prostate Cancer 90.05 DU-145 71.85 Breat Cancer 90.05 BT-37 28.40 MCF7 48.50 MCF7 4	SK-MEL-5	22.62				
Ovarian Cancer IGROVI 63.76 OVCAR-3 63.76 OVCAR-3 68.03 OVCAR-5 68.03 OVCAR-5 68.03 OVCAR-5 68.03 OVCAR-5 68.03 OVCAR-5 68.03 OVCAR-6 69.02 SK-0V-3 41.51 Renal Cancer PC-3 69.44 GAK-11 40211 SN12C 50.44 GAK-11 40211 SN12C 50.44 TK-10 77.45 SN12C 50.44 HS 5781 5.73 Breast Cancer PC-3 5.73 DU-145 71.85 Breast Cancer MCA-MB-231IATCC -10.44 HS 5781 6.75 Breast Cancer MDA-MB-231IATCC -10.44 HS 5781 6.75 Breast Cancer MDA-MB-408 63.95 Mean 44.52 Deta 61.34 Range 103.17	UACC-257	59.22				
IGROV1         03.76           OVCAR43         63.76           OVCAR4         57.92           OVCAR5         68.33           OVCAR5         68.33           OVCAR5         30.42           NCIADR-RES         41.51           Reval Cancer         705-0           705-0         2.22           A08         -656           ACHN         52.44           CAK1         49.21           SN12C         50.44           TK-10         77.45           DU-31         30.35           PC-3         39.05           DU-145         71.85           Breast Cancer	Ovarian Cancer	07.01				
OVCAR-4 OVCAR-8 OVCAR-8 OVCAR-8 NCIADR-RES NCIADR	IGROV1 OVICAR-3	53.76				
OVCAR-8 OVCAR-8 NCIIADR-RES SK-OV-3	OVCAR-4	57.92				
OCUMPR-RES       43740         SK-OV-3       41.51         Renal Cancer       786-0         A498       -6.56         ACHN       52.44         CAKL1       40.21         SN12C       50.44         TK-10       77.45         DU-145       71.85         Breast Cancer       90.05         PC-3       30.05         DU-145       71.85         Breast Cancer       70.60         MCF7       28.40         MDA-MB-231/ATCC       -10.44         HS 5781       61.34         BT-540       37.33         T-470       17.66         MDA-MB-468       05.95         Mean       44.52         Deta       61.34         Range       103.17         150       100       50       0       -50       -100       -150	OVCAR-5	68.93				
SK-CV-3 41.51 Renal Cancer 786-0 2.22 A498 - 0.56 ACHN 52.44 CAKI-1 40.21 SN12C 50.44 TK-10 77.45 UO-31 30.35 Prostate Cancer PC-3 30.05 DU-145 71.85 Breast Cancer MCF7 28.40 MDA-MB-231/ATCC -10.44 HS 578T -5.75 BT -549 37.33 T-47D 17.66 MDA-MB-468 65.95 Mean 44.52 Deta 61.34 Range 103.17 150 100 50 0 -50 -100 -150	NCI/ADR-RES	47.40				
10786-0       222         A498       -6.56         ACHN       52.44         CAN-1       40.21         SN12C       50.44         TK-10       77.45         UO-31       30.35         Prostate Cancer       PC-3         PC-3       30.05         DU-145       71.85         Breast Cancer	SK-OV-3 Renal Cancer	41.51				
A4980.50 ACHN 52.44 CAK0-1 40.21 SN12C 50.44 TK-10 77.45 UO-31 30.35 Prostate Cancer PC-3 30.05 DU-145 71.85 Breast Cancer MCF7 28.40 MDA-MB-231/ATCC -10.44 HS 578T 37.33 T-47D 17.66 MDA-MB-468 65.95 Mean 44.52 Deta 61.34 Range 103.17 150 100 50 0 -50 -100 -150	786-0	2.22				
CARG-1 4021 SN12C 50.44 TK-10 77.45 UO-31 30.35 Prostate Cancer PC-3 30.05 DU-145 71.85 Breast Cancer MCF7 28.40 MDA-MB-231/ATCC -10.44 HS 778T 6.75 BT-549 37.33 T-47D 17.66 MDA-MB-468 65.95 Mean 44.52 Delta 61.34 Range 103.17 150 100 50 0 -50 -100 -150	A498 ACHN	-6.56				
SN12C 50.44 TK-10 77.45 UO-31 30.35 Prostate Cancer PC-3 39.05 DU-145 71.85 Breast Cancer MC7 28.40 MDA-MB-231/ATCC -10.44 HS 578T -5.75 BT-649 77.33 T-47D 17.66 MDA-MB-468 65.95 Mean 44.52 Deta 61.34 Range 103.17 150 100 50 0 -50 -100 -150	CAKI-1	49.21				
UO-31 30.35 Prostate Cancer PC-3 39.05 DU-145 71.85 Breast Cancer MC7 29.40 MDA-MB-231/ATCC -10.44 HS 578T -5.75 BT-549 37.33 T-470 17.86 MDA-MB-468 65.95 Mean 44.52 Delta 61.34 Range 103.17 150 100 50 0 -50 -100 -150	SN12C TK-10	50.44				
Prostate Cancer PC-3 39.05 DU-145 71.85 Breast Cancer MCF7 28.40 MDA-MB-231/ATCC -10.44 HS 578T -5.75 BT-549 37.33 T-47D 17.66 MDA-MB-468 65.95 Mean 44.52 Delta 61.34 Range 103.17 150 100 50 0 -50 -100 -150	UO-31	30.35				
DU-145       71.85         Breast Cancer       28.40         MCF7       28.40         MDA-MB-231/ATCC       -10.44         HS 578T       -5.75         BT-549       37.33         T-47D       17.66         MDA-MB-468       65.95         Mean       44.52         Delta       61.34         Range       103.17         150       100       50       0       -50       -100       -150	Prostate Canoer PC-3	30.05				
Breast Cancer MCF7 28.40 MDA-MB-231/ATCC -10.44 HS 578T -5.75 BT-540 37.33 T-47D 17.66 MDA-MB-468 65.95 Mean 44.52 Delta 61.34 Range 103.17 150 100 50 0 -50 -100 -150	DU-145	71.85				
MDA-MB-231/ATCC -10.44 HS 578T -5.75 BT-549 37.33 T-47D 17.66 MDA-MB-468 65.95 MDA-MB-468 65.95 Mean 44.52 Delta 61.34 Range 103.17 150 100 50 0 -50 -100 -150	Breast Cancer MCE7	28.40				
HS 578T -6.75 BT-549 37.33 T-47D 17.66 MDA-MB-468 65.95 Mean 44.52 Deta 61.34 Range 103.17 150 100 50 0 -50 -100 -150	MDA-MB-231/ATCC	-10.44				
T-470 MDA-MB-468     17.68 05.95 Mean     17.68 05.95 1.34 Range     103.17       Mean     44.52 0.134 Range     103.17       150     100     50     0       -50     -100     -150	HS 578T	-5.75				
MDA-M8-468 65.95 Mean 44.52 Deta 61.34 Range 103.17 150 100 50 0 -50 -100 -150	T-47D	17.66				
Mean 44.52 Deta 61.34 Range 103.17 150 100 50 0 -50 -100 -150	MDA-MB-468	65.95				
Range 103.17 150 100 50 0 -50 -100 -150	Mean	44.52			_	
150 100 50 0 -50 -100 -150	Range	103.17	•		•	
150 100 50 0 -50 -100 -150						
		150	100 50	0 -50	-100 -150	

Graph 1: Mean graph of the one dose screen for the compound (Va)

Developmental Therapeutics Program			NSC: D-754955/1 Conc: 1.00E-5 Molar		E-5 Molar	Test Date: Apr 10, 2012			
One Dose Mean Graph			Experiment ID: 1204OS46			Report Date: May 09, 2012			
Panel/Cell Line	Panel/Cell Line Growth Percent		Mean Growth Percent - Growth Perc			ent			
Leukemia CCRF-CEM	70.86				_				
HL-60(TB) K-582	98.10 75.91								
MOLT-4	67.74								
RPMI-8226	70.09								
Non-Small Cell Lung Cancer	01.00								
EKVX HOD 62	46.66						_		
NCI-H226	73.71								
NCI-H23	49.14								
NCI-H322M NCI-H480	70.91				_				
NCI-H522	23.53				-	_			
Colon Cancer COLO 205	137.04								
HCC-2998	65.05				_	_			
HCT-116 HCT-15	34.56								
HT29	82.50								
KM12 SW-820	92.03								
CNS Cancer						_			
SF-268 SF-295	30.54								
SF-530	62.93				_				
5NB-19 SNB-75	30.79								
Melanoma									
MALME-3M	51,84				_				
M14	73.04								
SK-MEL-2	-14.72						-		
SK-MEL-28	83.01					_			
UACC-62	33.19								
Ovarian Cancer	50.50								
OVCAR-3	69.33				_				
OVCAR-4	78.60								
NCI/ADR-RES	62.46				_	.			
Renal Cancer	39.56								
786-0	32.88					_			
ACHN	0.43								
CAKI-1	48.89								
SN12C	57.18				_				
TK-10	12.53								
Prostate Cancer	14.40								
PC-3 DU-145	58.24 58.73				-				
Breast Cancer	00.70				1				
MCF7 MDA-MB-231/ATCC	44.04								
HS 578T	56.00				- 1_	.			
T-47D	50.56					- I			
Mean	51.57								
Delta	86.57					-			
Range	172.04								
	150		10	0 50		-50	.4	20	-150
	150				v	-30			100

Graph 2: Mean graph of the one dose screen for the compound (VC)

spectra.

In particular, it must be seemed that in 1H NMR the disappearance of a singlet between  $\delta$ - 6.0-6.5ppm indicates the formation of bridgehead nitrogen heterocycle (Va-i) by cyclodehydration process via III. Compounds showed singlet peaks at around 3.8ppm indicating presence of methoxy group of the compound Va, Vg-i and the characteristics peaks at  $\delta$ 1.16-2.39, 3.73, 2.47 ppm indicated the presence of cyclopropane group, methoxy and methyl groups in their respective structure. The compounds Va-i showed prominent signals of aaromatic protons around  $\delta$  7.09- 7.8ppm In<sup>8</sup> C-NMR spectrum it was observed most characteristic signals appear around  $\delta$  123.961, 159.137, 162.905ppm for the C-N of imidazo, and C-S of the thiadiazole carbon in their respective structure. The carbon signals of phenyl group at  $\delta$  around 128.0-132 ppm were seemed. Also (M+1) peak of compound Va appeared at 348.0.

The synthesized compounds were evaluated for their *in-vitro* anticancer activity at NCI, USA. The result of anticancer activity is presented in Graphs 1 and 2. We studied the effects of various substituents at 4' and 4" position of aromatic ring. Among them compound *Va* and *Vc* showed encouraging anticancer activity.

# 6. Conclusion

The present work, which has undertaken is bonafied, for the synthesis of Imidazo[2,1- b]-1,3,4-thiadiazole derivatives. A novel series of derivatives were synthesized comprising Imidazo[2,1-b]-1,3,4- thiadiazole containing cyclopropyl moiety by refluxing substituted 2-amino-1,3,4- thiadiazole with various substituted 2-bromo-1,2-diarylethanone in dry ethanol. The yield of the synthesized compounds was found to be in range from 70-80 %. The Phenyl derivatives were obtained in good yield as compared to substituted phenyl derivatives. All the newly synthesized compounds were characterized on the basis of their physical, spectral and analytical data.

The IR spectra, NMR spectra and Mass spectra of the representative compounds were analyzed.

#### 7. Source of Funding

None.

# 8. Conflict of Interest

None.

#### References

- Frantzi M, Latosinska A. Harald Mischak Proteomics in Drug Development: The Dawn of a New Era? *Proteomics Clin Appl.* 2019;13(2):e1800087.
- Aly AA, Samia M, Abdelzaher MY, Abdelhafez SESMN, Abdelhafez WN, et al. New quinoline-2-one/pyrazole derivatives; design,

synthesis, molecular docking, anti-apoptotic evaluation, and caspase-3 inhibition assay. *Bioorg Chem.* 2020;94:103348.

- Zhao M, Wang Y, Zhao Y, He S, Zhao R, Song Y, et al. Caspase-3 knockout attenuates radiation-induced tumor repopulation via impairing the ATM/p53/Cox-2/PGE2 pathway in non-small cell lung cancer. 2020;12(21):21758–76.
- Araya LE, Ishankumar V, Soni JA. Deorphanizing Caspase-3 and Caspase-9 Substrates In and Out of Apoptosis with Deep Substrate Profiling. ACS Chem Biol. 2021;16(11):2280–96.
- Kang J, Thompson RF, Aneja S, Lehman C, Obcemea C, Obcemea C, et al. Issam El Naqa, National Cancer Institute Workshop on Artificial Intelligence in Radiation Oncology. *Training the Next Gen Pract Radiati Oncol.* 2021;11(1):74–83.
- Firoozpour L, Gao L, Moghimi S, Pasalar P, Davoodi J, Wang MW. Massoud Amanlou & Alireza Foroumadi (2020) Efficient synthesis, biological evaluation, and docking study of isatin based derivatives as caspase inhibitors. *J Enzyme Inhib Med Chem.* 2020;35(1):1674–84.
- Zhang J, Liang Z. Metal-Free Synthesis of Functionalized Tetrasubstituted Alkenes by Three-Component Reaction of Alkynes, Iodine, and Sodium Sulfinates. ACS Omega. 2018;3(12):18002–801.
- Maher M, Kassab AE, Ashraf F. Zeinab Mahmoud Novel pyrazolo[3,4-d]pyrimidines: design, synthesis, anticancer activity, dual EGFR/ErbB2 receptor tyrosine kinases inhibitory activity, effects on cell cycle profile and caspase-3-mediated apoptosis. *J Enzyme Inhibition Med Chem.* 2019;34(1):2019–20.
- Ahmed BM, Mehany A, Shaaban G, Mohamed SF. Apoptotic and anti-angiogenic effects of propolis against human bladder cancer: molecular docking and in vitro screening. *Biomarkers*. 2022;27(2):138–50.
- Asadi M, Taghizadeh S, Kaviani E, Vakili O, Taheri-Anganeh M, Tahamtan M. Amir Savardashtaki Caspase-3: Structure, function, and biotechnological aspect. *Biotechnol Appl Biochem*. 2022;69(4):1633– 45.
- Shoaib S, Tufail S, Sherwani MA, Yusuf N. Phenethyl Isothiocyanate Induces Apoptosis Through ROS Generation and Caspase-3 Activation in Cervical Cancer Cells. *Front Pharmacol.* 2021;12:673103. doi:10.3389/fphar.2021.673103.
- Unnisa A, Greig NH, Kamal MA. Inhibition of Caspase 3 and Caspase 9 Mediated Apoptosis: A Multimodal Therapeutic Target in Traumatic Brain Injury. *Curr Neuropharmacol.* 2023;21(4):1001–12.
- Jiang M, Qi L, Li L, Li Y. The caspase-3/GSDME signal pathway as a switch between apoptosis and pyroptosis in cancer Mingxia Jiang1 , Ling Qi1 , Lisha Li1 and Yanjing Li1. *Cell Death Discovery*. 2020;6:112. doi:10.1038/s41420-020-00349-0.
- Cho SO, Lim JW. Oxidative stress induces apoptosis via calpain- and caspase-3-mediated cleavage of ATM in pancreatic acinar cells. *Free Radic Res.* 2019;11(12):799–809.
- Karlsson T, Gustafsson Å, Ekstrand B, Hammarström L, Elfsmark S. Chlorine exposure induces Caspase-3 independent cell death in human lung epithelial cells. *Toxicol Vitro*. 2022;80:105317.

# Author biography

Bhavini K Gharia, Associate Professor 💿 https://orcid.org/0000-0002-9382-6156

Bhanubhai N Suhagia, Dean

Vineet Jain, Principal

**Cite this article:** Gharia BK, Suhagia BN, Jain V. Synthesis, crystal structure studies, characterization and *in vitro* study of novel heterocyclic thiadiazoles as caspase 3 inhibitors for anticancer activity. *Int J Pharm Chem Anal* 2023;10(4):268-275.