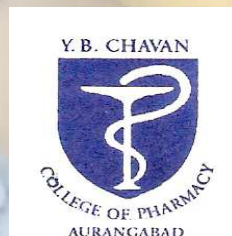


**“Microwave-assisted Facile Synthesis And Anticancer Evaluation Of N-((5-(substituted Methylene Amino)- 1,3,4-thiadiazol-2-yl)methyl) Benzamide Derivatives”**

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# ABSTRACT

Microwave induced synthesis has various advantages over conventional synthesis, such as highly accelerated reaction rate, reasonable better yields, simple open systems, no solvent or very less amount of solvents required, eco friendly method, clean heating system and control on reaction parameters. In the present work novel Schiff's bases containing thiadiazole scaffold and benzamide group, through appropriate pharmacophore were designed and synthesized, because of the important biological properties associated with these three moieties/groups. The coupling of these important moieties was achieved under microwave irradiation. A facile, solvent-free synthesis of a series of N-((5-(substituted methylene amino)-1,3,4-thiadiazol-2-yl)methyl)benzamide was carried out under microwave-irradiation. Solvent free synthesis of novel Schiff bases was achieved by cyclo addition of various aromatic aldehydes (0.01 mol) and N-((5-amino-1,3,4-thiadiazol-2-yl)methyl)benzamide(0.01 mol) in presence of catalytic amount of glacial acetic acid Under microwave irradiation. The same compounds were also synthesized using conventional approach. The conventional method required 15-18 hrs, while microwave irradiation method required only 15-20 minutes and gave better yields.

Total 12 final compounds were synthesized as per the scheme reported. Structures of synthesized compounds were confirmed by IR, NMR, and Mass spectral study. All the designed hybrids were evaluated for their *in vitro* anticancer activity against a panel of four human cancer cell lines viz SK-MEL-2(melanoma), HL-60 (leukemia), HeLa (cervical) and MCF-7 using MTT assays method. Most of the synthesized compounds exhibited promising anticancer activity with the some compounds having GI<sub>50</sub> values similar to that of the Adriamycin. The compounds **7k**, **7l**, **7b**, and **7a** were found to be the most promising in this study. A computational study of synthesized compounds **7(a-l)** was performed for prediction of ADMET. The absorption, distribution, metabolism, excretion and Toxicity (ADMET) properties of all compounds were predicted using Qikprop v3.5 (Schrödinger LLC).

**Keywords:** Micro-wave assisted synthesis, Schiff's bases, thiadiazoles, MTT assay, *in-vitro* anti-cancer activity

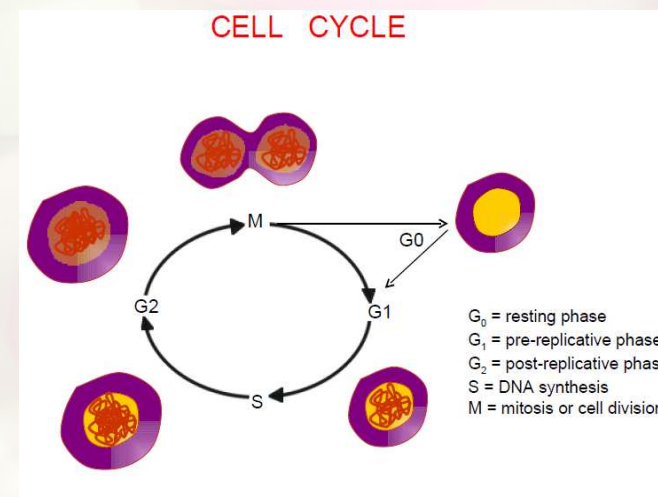
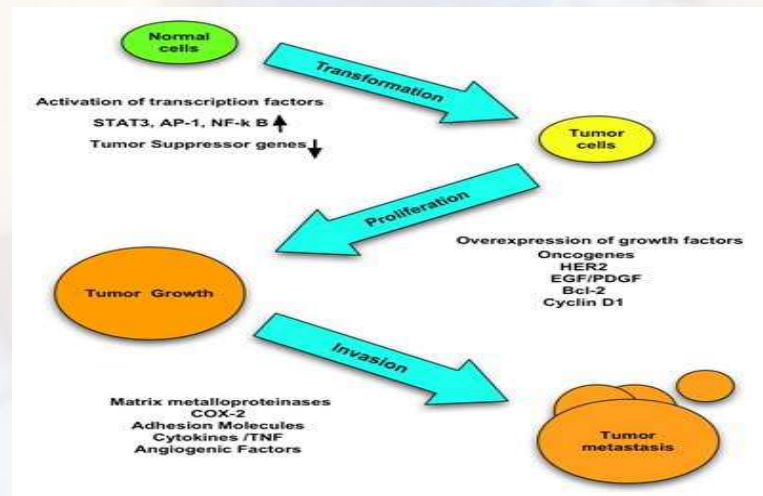
# INTRODUCTION

Cancer is a leading cause of death worldwide, accounting for 7.6 million deaths (around 13% of all deaths) in 2008<sup>h</sup>.

Cancer is a generic term for a large group of diseases that can affect any part of the body. Other terms used are malignant tumours and neoplasms.

One defining feature of cancer is the rapid creation of abnormal cells that grow beyond their usual boundaries, and which can then invade adjoining parts of the body and spread to other organs. This process is referred to as metastasis. Metastases are the major cause of death from cancer.

## Cancerogenesis



- Review of literature shows that 1,3,4-Thiadiazole has gained prominence by exhibiting a wide variety of biological activities. It has interesting pharmacophores that display a broad spectrum biological activity. The lower toxicity and in vivo stability of 1,3,4-thiadiazole nucleus are attributed to its aromaticity. Diverse chemical structures containing 1,3,4-Thiadiazole nucleus have been reported with potential anticancer activity. The 1,3,4-thiadiazole ring in anticancer agents performs its role in pharmacophores of apoptosis inducers and caspase activators, tyrosine kinase inhibitors, carbonic anhydrase inhibitors and etc. Hence, various mechanisms could be imagined for anticancer chemical structures that containing the 1,3,4-thiadiazole ring.
- In review of literature the Schiff base derivative have been found to be more potent molecules in inhibiting cancer cell lines. This is due to the presence of carbon–nitrogen double bond having potential receptor binding ability. Schiff bases are also one of the intensively investigated classes of aromatic and heteroaromatic compounds. This class of compounds showed a variety of applications ranging from anticancer, antibacterial, diuretic (Supran et al., 1996), antifungal and anti parasitic activity. They have also medicinal importance and are used in drug design due to their activity against a wide range of organisms. This importance of thiadiazole nucleus and Schiff's bases and continuing demand for new anticancer agents, prompted us to synthesize different Schiff base derivatives of 1, 3, 4-thiadiazole ring.

# Microwave Induced Green Synthesis

**In the present work microwave-assisted synthetic protocol is reported.**

**Microwave** is an important tool in **Green synthesis**. Microwave reactions involve selective absorption of electromagnetic waves by polar molecules, non-polar molecules being inert to microwave. Microwave induced organic reaction enhanced more. Green synthesis is a simplest method for conducting microwave assisted reactions which involves irradiation of reactants in an open vessel at fixed frequency of 2.45GHz. This method was developed by Bose *et al.*

## **Advantages of microwave induced synthesis:**

Microwave induced synthesis has various advantages over conventional synthesis. These are as follows:

- ❖ Highly accelerated reaction rate
- ❖ Reasonable good yields
- ❖ Simple open systems
- ❖ No solvent or very less amount of solvents required
- ❖ Eco friendly method
- ❖ Clean heating system
- ❖ Control on reaction parameters

# Mechanism Of Action Of Anticancer Drugs

- ❖ Block nucleic acid (DNA, RNA) biosynthesis
- ❖ Directly destroy DNA and inhibit DNA reproduction
- ❖ Interfere transcription and block RNA synthesis
- ❖ Interfere protein synthesis and function
- ❖ Influence hormone homeostasis

# Need For Study

Discovery of new anticancer agents is the need of the hour due to:

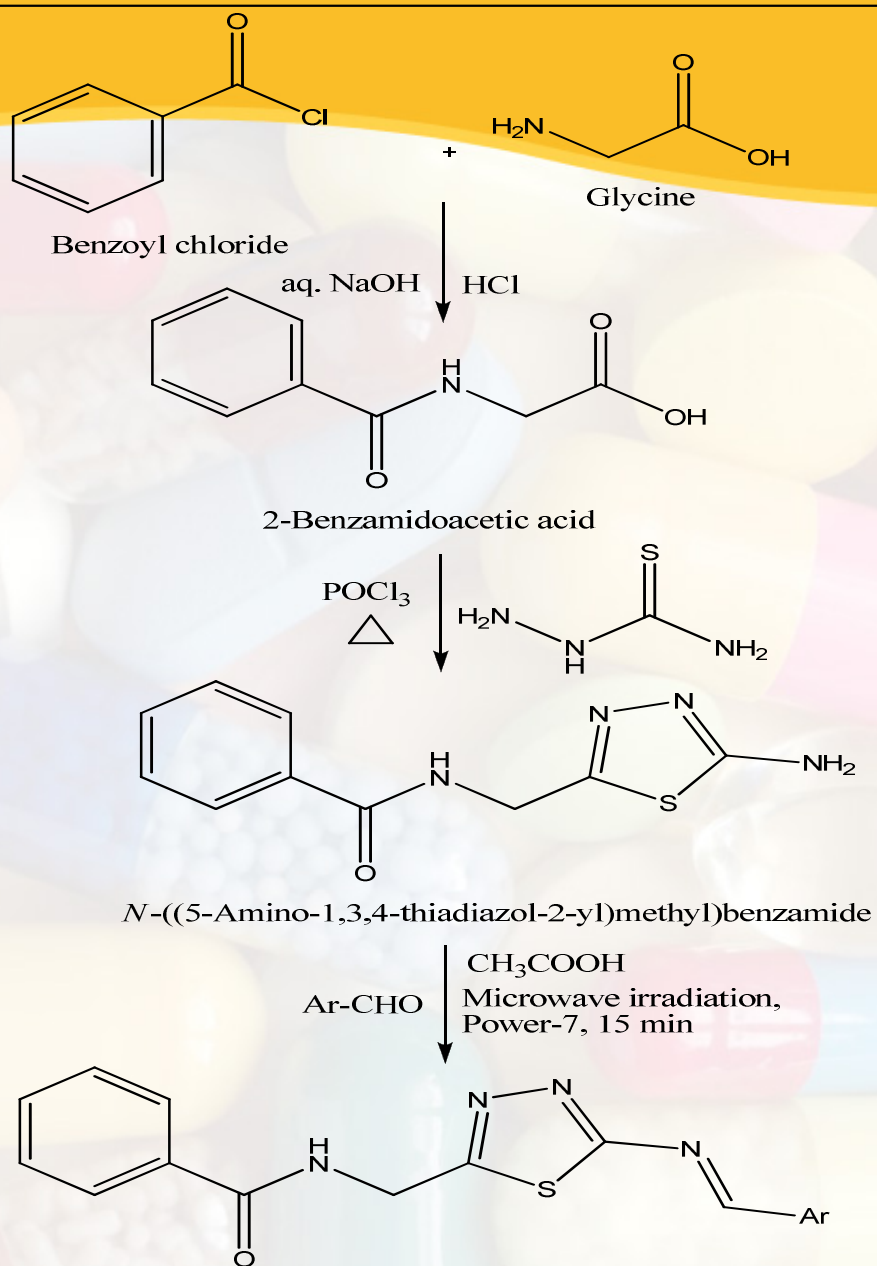
1. Development of resistance by cancer cells.
2. Abnormal nature of cancer cells.
3. High toxicity and side effects by anticancer agents.
4. High rate of mortality due to cancer.
5. Changes in lifestyle. With the recent advent in technology, it is possible to harness the presently available molecules for different pharmacological actions.
6. Anticancer drugs are one such class of drugs which still has a lot of scope for modulation and discovery.
7. Many heterocyclic compounds show multiple pharmacological activities including the anticancer activity.
8. Keeping the current medical needs in mind, the heterocyclic thiadiazole derivatives can be targeted for study.



# Objective

- ❖ To design and synthesize Schiff base derivatives of heterocyclic 1,3,4 thiadiazole scaffold.
- ❖ To conduct physicochemical characterization of intermediates and synthesized compounds.
- ❖ To confirm the structures of synthesized compounds by chemical tests, and spectral techniques such as FT-IR, ES-MS and NMR.
- ❖ *In-vitro* anticancer screening of the synthesized compound on human cancer cell lines viz. HL-60(leukemia), MCF-7 (breast), HeLa (cervical) and SK-MEL-2 (melanoma)

# Scheme of Synthesis



(*E*)-*N*-((5-(Substituted methyleneamino)-1,3,4-thiadiazol-2-yl)methyl)benzamide

# EXPERIMENTAL

## **Step I: General process for synthesis of 2-Benzamidoacetic acid**

0.33mol of glycine (2) was dissolved in 250ml of 10% NaOH solution contained in a conical flask. 0.385mol of benzoyl chloride (1) was added in 5-portion to the solution and shaken vigorously until all the chloride has reacted. The solution was transferred to a beaker containing crushed ice and dil. HCl was added until the solution was acidic to congo red paper. The resulting crystalline solid was collected and boiled with 10 ml of CCl<sub>4</sub> for 10 min. The product was filtered and washed with CCl<sub>4</sub>. The solid product obtained was dried and recrystallized from ethanol. The melting point and yield were recorded.

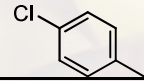
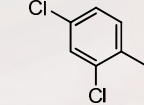
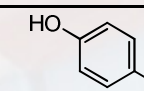
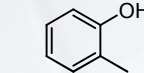
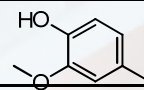
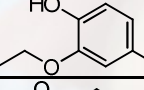
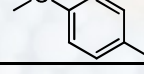
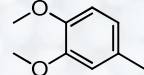
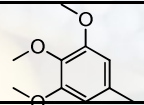
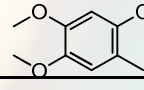
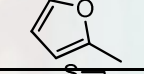
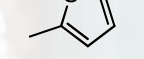
## **Step II: General procedure for synthesis of N-((5-amino-1,3,4-thiadiazol-2-yl)methyl) benzamide**

2- Benzamidoacetic acid (3) (0.05mol) was refluxed with thiosemicarbazide (4) (0.05mol) and phosphorus oxychloride (15 ml) for 1hr. The mixture was cooled and diluted with water (90 ml) and again refluxed for 4 hrs. Then the mixture was filtered and filtrate was basified with potassium hydroxide solution. The precipitate was filtered off and recrystallized from ethanol.

# Microwave-assisted method

Solvent free synthesis of Schiff bases was achieved by cycloaddition of various aromatic aldehydes (0.01 mol) and N-((5-amino-1,3,4-thiadiazol-2-yl)methyl)benzamide (0.01 mol) in presence of catalytic amount of glacial acetic acid under microwave irradiation. The synthesized products were recrystallized from ethanol. The same compounds were also synthesized using conventional approach. A comparative study in terms of yield and reaction period has been reported using conventional method. The reaction carried out using conventional method required about 15-18 hrs, while microwave irradiation method required only 15-20 min. The yield was about 95% using microwave method while conventional method yield was around 35%.

**Table 1** Physical characterization of the synthesized compounds **7 (a-l)**

Entry	Ar	Molecular formula	Molecular weight.	% Yield	Melting point(°C)	R <sub>f</sub> value
7a		C <sub>17</sub> H <sub>13</sub> ClN <sub>4</sub> O <sub>2</sub> S	356.83	95	124-128	0.44
7b		C <sub>17</sub> H <sub>12</sub> Cl <sub>2</sub> N <sub>4</sub> O <sub>2</sub> S	391.27	92	112-114	0.56
7c		C <sub>17</sub> H <sub>14</sub> N <sub>4</sub> O <sub>2</sub> S	338.38	94	112-114	0.59
7d		C <sub>17</sub> H <sub>14</sub> N <sub>4</sub> O <sub>2</sub> S	338.38	94	106-108	0.43
7e		C <sub>18</sub> H <sub>16</sub> N <sub>4</sub> O <sub>3</sub> S	368.41	92	122-126	0.66
7f		C <sub>19</sub> H <sub>18</sub> N <sub>4</sub> O <sub>3</sub> S	382.44	92	130-132	0.45
7g		C <sub>18</sub> H <sub>16</sub> N <sub>4</sub> O <sub>2</sub> S	352.41	95	112-118	0.57
7h		C <sub>19</sub> H <sub>18</sub> N <sub>4</sub> O <sub>3</sub> S	382.44	88	114-118	0.64
7i		C <sub>20</sub> H <sub>20</sub> N <sub>4</sub> O <sub>4</sub> S	412.26	86	138-140	0.48
7j		C <sub>20</sub> H <sub>20</sub> N <sub>4</sub> O <sub>4</sub> S	412.26	88	134-138	0.70
7k		C <sub>15</sub> H <sub>12</sub> N <sub>4</sub> O <sub>2</sub> S	312.35	85	124-126	0.55
7l		C <sub>15</sub> H <sub>12</sub> N <sub>4</sub> OS <sub>2</sub>	328.41	84	136-138	0.42

Solvent system chosen for R<sub>f</sub> value determination was Chloroform: methanol (8:2).

# Spectral characterization

- ❖ Synthesized compounds were confirmed by FTIR,  $^1\text{H}$ NMR,  $^{13}\text{C}$  NMR and Mass spectroscopic studies.
- ❖ All the spectral data were in accordance with assumed structures IR spectra were scanned on JASCO made FTIR-PS 4000, within 4000-400  $\text{cm}^{-1}$  wavelength range. KBr powder technique was used for the sampling purpose.
- ❖ The  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra of synthesized compounds were recorded on BrukerAvance II 400 NMR Spectrometer at 400 MHz Frequency in deuterated DMSO and  $\text{CDCl}_3$  and using TMS as internal standard (chemical shift  $\delta$  in ppm. Mass spectra of some compounds were scanned on Water's Micromass Q-ToF system. The spectral data are in accordance with assumed structures.

# Spectral study

## **(E)-N-((5-(4-chlorobenzylideneamino)-1,3,4-thiadiazol-2-yl)methyl)benzamide (7a)**

Yield: 95%; M.P: 126-128<sup>0</sup>C ; IR (KBr<sub>v</sub><sub>max in</sub> cm<sup>-1</sup>): 3350.41 (NH), 2970.76 (C=H), 1810.26 (C=O of amide); <sup>1</sup>H NMR (DMSO) δ ppm: 3.42 (s, 2H, CH<sub>2</sub>), 6.69-5.2 (m, 9H), 8.21 (s, 1H, NH), 10.33 (s, 1H, N=CH); <sup>13</sup>C NMR (DMSO) δ ppm: 168.00, 167.89, 160.00, 136.67, 134.51, 134.34, 133.23, 131.22, 130.63, 130.00, 128.98, 128.78, 127.51, 127.34, 127.36, 39.11; m/z: 356.05 (100.0%), 358.05 (37.1%), 357.05 (20.7%), 359.05 (7.1%), 358.06 (1.6%), 360.04 (1.5%); Molecular Formula: C<sub>17</sub>H<sub>13</sub>ClN<sub>4</sub>OS Elemental Analysis: Calculated: (C, H, Cl, N, O, S) 57.22, 3.67, 9.94, 15.70, 4.48, 8.99, Found: 57.20, 3.65, 9.97, 15.73, 4.45, 8.98.

## **(E)-N-((5-(2,4-dichlorobenzylideneamino)-1,3,4-thiadiazol-2-yl)methyl)benzamide (7b)**

Yield: 92%; M.P: 112-114<sup>0</sup>C ; IR (KBr<sub>v</sub><sub>max in</sub> cm<sup>-1</sup>): 3352.41 (NH), 2975.76 (C=H), 1818.26 (C=O of amide); <sup>1</sup>H NMR (DMSO) δ ppm: 3.45 (s, 2H, CH<sub>2</sub>), 6.69-5.2 (m, 8H), 8.29 (s, 1H, NH), 10.33 (s, 1H, N=CH); <sup>13</sup>C NMR (DMSO) δ ppm: 169.00, 168.89, 161.00, 136.77, 134.41, 134.39, 133.13, 131.22, 130.53, 130.00, 128.98, 128.68, 127.51, 127.34, 127.36, 40.11; m/z: 390.01 (100.0%), 392.01 (68.9%), 391.01 (20.7%), 393.01 (13.2%), 394.00 (13.1%), 395.01 (2.5%), 392.02 (1.8%), 394.01 (1.5%), 393.00 (1.0%); Molecular Formula: C<sub>17</sub>H<sub>12</sub>Cl<sub>2</sub>N<sub>4</sub>OS; Elemental Analysis: Calculated: (C, H, Cl, N, O, S) 52.18, 3.09, 18.12, 14.32, 4.09, 8.20, Found: 52.17, 3.07, 18.14, 14.30, 4.08, 8.22.

**(E)-N-((5-(4-hydroxybenzylideneamino)-1,3,4-thiadiazol-2-yl)methyl)benzamide (7c)**

Yield: 94%; M.P: 112-114<sup>o</sup>C ; IR (KBr<sub>v</sub><sub>max in</sub> cm<sup>-1</sup>): 3350.41 (NH), 3179.92 (OH), 2970.76 (C=H), 1810.26 (C=O of amide); <sup>1</sup>H NMR (DMSO) δ ppm: 3.44 (s, 2H, CH<sub>2</sub>), 5.43 (s, 1H, OH), 6.69-5.2 (m, 9H), 8.21 (s, 1H, NH), 10.34 (s, 1H, N=CH); <sup>13</sup>C NMR (DMSO) δ ppm: 190.00, 168.54, 165.05, 163.36, 156.36, 134.60, 131.90, 129.41, 128.96, 125.20, 121.93, 116.19, 115.80, 40.12; m/z: 338.08 (100.0%), 339.09 (18.6%), 340.08 (4.8%), 339.08 (2.3%), 340.09 (2.2%); Molecular Formula: C<sub>17</sub>H<sub>14</sub>N<sub>4</sub>O<sub>2</sub>S Elemental Analysis: Calculated: (C, H, N, O, S) 60.34, 4.17, 16.56, 9.46, 9.48, Found: 60.30, 4.18, 16.59, 9.43, 9.45.

**(E)-N-((5-(2-hydroxybenzylideneamino)-1,3,4-thiadiazol-2-yl)methyl)benzamide (7d)**

Yield: 94%; M.P: 106-108<sup>o</sup>C ; IR (KBr<sub>v</sub><sub>max in</sub> cm<sup>-1</sup>): 3350.58 (NH), 3179.90 (OH), 2970.66 (C=H), 1811.16 (C=O of amide); <sup>1</sup>H NMR (DMSO) δ ppm: 4.12 (s, 2H, CH<sub>2</sub>), 5.38 (s, 1H, OH), 7.02-7.71 (m, 4H), 7.81-8.10 (m, 5H), 8.16 (s, 1H, NH), 9.91(s, 1H, N=CH); <sup>13</sup>C NMR (DMSO) δ ppm: 170.11, 169.09, 160.99, 160.55, 135.51, 132.41, 131.00, 129.80, 128.17, 127.91, 127.45, 126.89, 121.46, 120.52, 118.82, 40.03; m/z: 338.08 (100.0%), 339.09 (18.6%), 340.08 (4.8%), 339.08 (2.3%), 340.09 (2.2%); Molecular Formula: C<sub>17</sub>H<sub>14</sub>N<sub>4</sub>O<sub>2</sub>S; Elemental Analysis: Calculated: (C, H, N, O, S) 60.34, 4.17, 16.56, 9.46, 9.48, Found: 60.30, 4.19, 16.58, 9.49, 9.50.



**(E)-N-((5-(4-hydroxy-3-methoxybenzylideneamino)-1,3,4-thiadiazol-2-yl)methyl)benzamide (7e)**

Yield: 92%; M.P: 122-126<sup>0</sup>C ; IR (KBr $\nu_{\max}$  in  $\text{cm}^{-1}$ ): 3350.31 (NH), 2971.76 (C=H), 1810.16 (C=O of amide); <sup>1</sup>H NMR (DMSO)  $\delta$  ppm: 3.83 (s, 3H, OCH<sub>3</sub>), 4.10 (s, 2H, CH<sub>2</sub>), 5.35 (s, 1H, OH), 6.93 (d, 1H), 7.34 (d, 1H), 7.52 (s, 1H), 7.70-8.08 (m, 5H), 8.19 (s, 1H, NH), 10.00 (s, 1H, N=CH); <sup>13</sup>C NMR (DMSO)  $\delta$  ppm: 169.18, 167.71, 159.99, 152.09, 149.71, 135.09, 130.16, 129.98, 128.28, 127.76, 127.01, 126.81, 122.15, 118.16, 113.17, 56.17, 39.87; m/z: 368.09 (100.0%), 369.10 (19.8%), 370.09 (4.8%), 370.10 (2.6%), 369.09 (2.3%); Molecular Formula: C<sub>18</sub>H<sub>16</sub>N<sub>4</sub>O<sub>3</sub>S; Elemental Analysis: Calculated: (C, H, N, O, S) 58.68, 4.38, 15.21, 13.03, 8.70, Found: 58.70, 4.39, 15.25, 13.00, 8.72.

**(E)-N-((5-(3-ethoxy-4-hydroxybenzylideneamino)-1,3,4-thiadiazol-2-yl)methyl)benzamide (7f)**

Yield: 92%; M.P: 130-132<sup>0</sup>C; IR (KBr $\nu_{\max}$  in  $\text{cm}^{-1}$ ): 3350.31 (NH), 2971.76 (C=H), 1810.16 (C=O of amide); <sup>1</sup>H NMR (DMSO)  $\delta$  ppm: 1.32 (t, 3H, CH<sub>3</sub>), 4.09 (q, 2H, CH<sub>2</sub>), 4.46 (s, 2H, CH<sub>2</sub>), 5.35 (s, 1H, OH), 6.91-7.52 (m, 3H, CH), 7.63-8.09 (m, 5H, CH), 8.13 (s, 1H, NH), 10.00 (s, 1H, N=CH); <sup>13</sup>C NMR (DMSO)  $\delta$  ppm: 168.78, 161.66, 151.88, 148.83, 134.47, 132.55, 130.70, 128.88, 128.19, 127.00, 122.34, 116.59, 112.38, 64.57, 39.99, 14.87; m/z: 382.11 (100.0%), 383.11 (22.9%), 384.11 (5.6%), 384.12 (2.1%); Molecular Formula: C<sub>19</sub>H<sub>18</sub>N<sub>4</sub>O<sub>3</sub>S; Elemental Analysis: Calculated: (C, H, N, O, S) 59.67, 4.74, 14.65, 12.55, 8.38, Found: 59.64, 4.72, 14.61, 12.57, 8.39.

**(E)-N-((5-(4-methoxybenzylideneamino)-1,3,4-thiadiazol-2-yl)methyl)benzamide (7g)**

Yield: 95%; M.P: 114-118<sup>0</sup>C ; IR (KBr<sub>v<sub>max</sub> in</sub> cm<sup>-1</sup>): 3352.18 (NH), 2971.66 (C=H), 1810.17 (C=O of amide); <sup>1</sup>H NMR (DMSO) δ ppm: 3.83 (s, 6H, OCH<sub>3</sub>), 4.19 (s, 2H, CH<sub>2</sub>), 7.06 (d, 1H), 7.16 (d, 1H), 7.60-8.06 (m, 7H), 8.17 (s, 1H, NH), 10.00 (s, 1H, N=CH); <sup>13</sup>C NMR (DMSO) δ ppm: 170.13, 169.99, 163.09, 161.00, 135.01, 132.96, 131.91, 130.71, 128.89, 128.01, 127.97, 127.52, 127.03, 114.45, 114.01, 55.89, 40.00; m/z: 352.10 (100.0%), 353.10 (21.8%), 354.10 (5.4%), 354.11 (1.8%); Molecular Formula: C<sub>18</sub>H<sub>16</sub>N<sub>4</sub>O<sub>2</sub>S; Elemental Analysis: Calculated: (C, H, N, O, S) 61.35, 4.58, 15.90, 9.08, 9.10, Found: 61.33, 4.56, 15.93, 9.04, 9.13.

**(E)-N-((5-(3,4-dimethoxybenzylideneamino)-1,3,4-thiadiazol-2-yl)methyl)benzamide (7h)**

Yield: 88%; M.P: 114-116<sup>0</sup>C ; IR (KBr<sub>v<sub>max</sub> in</sub> cm<sup>-1</sup>): 3352.18 (NH), 2971.66 (C=H), 1810.17 (C=O of amide); <sup>1</sup>H NMR (DMSO) δ ppm: 3.83 (s, 6H, OCH<sub>3</sub>), 4.19 (s, 2H, CH<sub>2</sub>), 6.98-7.61 (m, 3H), 7.69-8.05 (m, 5H), 8.12 (s, 1H, NH), 9.98 (s, 1H, N=CH); <sup>13</sup>C NMR (DMSO) δ ppm: 170.09, 169.96, 159.91, 152.10, 149.92, 134.26, 132.10, 130.66, 128.81, 127.99, 127.58, 126.96, 124.69, 111.77, 108.92, 56.11, 39.96; m/z: 382.11 (100.0%), 383.11 (22.9%), 384.11 (5.6%), 384.12 (2.1%); Molecular Formula: C<sub>19</sub>H<sub>18</sub>N<sub>4</sub>O<sub>3</sub>S; Elemental Analysis: Calculated: (C, H, N, O, S) 59.67, 4.74, 14.65, 12.55, 8.38, Found: 59.65, 4.72, 14.69, 12.58, 8.36.

**(E)-N-((5-(3,4,5-trimethoxybenzylideneamino)-1,3,4-thiadiazol-2-yl)methyl)benzamide (7i)**

Yield: 86%; M.P: 138--140°C ; IR (KBr $\nu_{\max}$  in  $\text{cm}^{-1}$ ): 3350.18 (NH), 2972.66 (C=H), 1810.17 (C=O of amide);  $^1\text{H}$  NMR (DMSO)  $\delta$  ppm: 3.85 (s, 9H,  $\text{OCH}_3$ ), 4.19 (s, 2H,  $\text{CH}_2$ ), 6.98-7.61 (m, 3H), 7.69-8.05 (m, 5H), 8.12 (s, 1H, NH), 9.98 (s, 1H, N=CH);  $^{13}\text{C}$  NMR (DMSO)  $\delta$  ppm: 170.09, 169.96, 159.91, 152.10, 149.92, 134.26, 132.10, 130.66, 128.81, 127.99, 127.58, 126.96, 124.69, 111.77, 108.92, 56.11, 39.96; m/z: 412.12 (100.0%), 413.12 (24.1%), 414.12 (5.9%), 414.13 (2.3%), 415.12 (1.1%); Molecular Formula:  $\text{C}_{20}\text{H}_{20}\text{N}_4\text{O}_4\text{S}$ ; Elemental Analysis: Calculated: (C, H, N, O, S) 58.24, 4.89, 13.58, 15.52, 7.77, Found: 58.22, 4.85, 13.54, 15.53, 7.79.

**(E)-N-((5-(2,4,5-trimethoxybenzylideneamino)-1,3,4-thiadiazol-2-yl)methyl)benzamide (7j)**

Yield: 88%; M.P: 134-138°C ; IR (KBr $\nu_{\max}$  in  $\text{cm}^{-1}$ ): 3350.18 (NH), 2972.66 (C=H), 1810.17 (C=O of amide);  $^1\text{H}$  NMR (DMSO)  $\delta$  ppm: 3.86 (s, 9H,  $\text{OCH}_3$ ), 4.20 (s, 2H,  $\text{CH}_2$ ), 6.99-7.65 (m, 3H), 7.67-8.07 (m, 5H), 8.17 (s, 1H, NH), 9.99 (s, 1H, N=CH);  $^{13}\text{C}$  NMR (DMSO)  $\delta$  ppm: 171.09, 168.96, 158.91, 153.10, 148.92, 135.26, 133.10, 131.66, 129.81, 128.99, 127.58, 126.96, 125.19, 110.97, 109.12, 55.35, 37.96; m/z: 412.12 (100.0%), 413.12 (24.1%), 414.12 (5.9%), 414.13 (2.3%), 415.12 (1.1%); Molecular Formula:  $\text{C}_{20}\text{H}_{20}\text{N}_4\text{O}_4\text{S}$ ; Elemental Analysis: Calculated: (C, H, N, O, S) 58.24, 4.89, 13.58, 15.52, 7.77, Found: 58.23, 4.85, 13.55, 15.54, 7.78.

**(E)-N-((5-(furan-2-ylmethyleneamino)-1,3,4-thiadiazol-2-yl)methyl)benzamide (7k)**

Yield: 85%; M.P: 124-126<sup>0</sup>C ; IR (KBr<sub>v</sub><sub>max</sub> in cm<sup>-1</sup>): 3350.41 (NH), 2970.76 (C=H), 1810.26 (C=O of amide); <sup>1</sup>H NMR (DMSO) δ ppm: 3.48 (s, 2H, CH<sub>2</sub>), 6.52 (t, 1H), 6.93 (d, 1H), 7.65 (d, 1H), 7.80 (s, 1H, N=CH), 7.75-8.05 (m, 5H), 8.19 (s, 1H, NH); <sup>13</sup>C NMR (DMSO) δ ppm: 168.09, 167.34, 150.44, 146.99, 144.48, 134.29, 131.11, 128.57, 128.04, 127.54, 126.99, 118.90, 112.26, 40.12; m/z: 312.07 (100.0%), 313.07 (18.7%), 314.06 (4.5%), 314.07 (2.0%); Molecular Formula: C<sub>15</sub>H<sub>12</sub>N<sub>4</sub>O<sub>2</sub>S; Elemental Analysis: Calculated: (C, H, N, O, S) 57.68, 3.87, 17.94, 10.24, 10.27, Found: 57.64, 3.89, 17.92, 10.28, 10.25.

**(E)-N-((5-(thiophen-2-ylmethyleneamino)-1,3,4-thiadiazol-2-yl)methyl)benzamide (7l)**

Yield: 84%; M.P: 136-138<sup>0</sup>C; IR (KBr<sub>v</sub><sub>max</sub> in cm<sup>-1</sup>): 3350.18 (NH), 2975.66 (C=H), 1815.17 (C=O of amide); <sup>1</sup>H NMR (DMSO) δ ppm: 4.46 (s, 2H, CH<sub>2</sub>), 7.17 (t, 1H, CH), 7.63-8.09 (m, 6H), 8.29 (s, 1H, NH); <sup>13</sup>C NMR (DMSO) δ ppm: 39.91, 127.00, 127.44, 127.99, 128.34, 128.89, 130.73, 132.77, 134.55, 142.98, 152.69, 167.98; m/z: 328.05 (100.0%), 329.05 (16.4%), 330.04 (9.1%), 329.04 (3.1%), 330.05 (2.0%), 331.04 (1.7%); Molecular Formula: C<sub>15</sub>H<sub>12</sub>N<sub>4</sub>O<sub>2</sub>S<sub>2</sub>; Elemental Analysis: Calculated: (C, H, N, O, S) 54.86, 3.68, 17.06, 4.87, 19.53, Found: 54.84, 3.64, 17.03, 4.88, 19.55.

# Biological Evaluation:

## *In-Vitro* Anticancer Evaluation

- ❖ The anticancer evaluation of synthesized compounds on selected cell lines i. e. HL-60, HeLa, MCF-7 and SK-MEL-2 was conducted at ACTREC (Advanced Centre for Treatment Research and Education in Cancer), Mumbai.
- ❖ MTT assay of the compounds **7(a-l)** was performed using Adriamycin as the standard drug. The experiments were performed at the concentration of 10, 20, 40 and 80  $\mu\text{g/ml}$ .
- ❖ The inhibition of cell growth was calculated in terms of:
- ❖  **$\text{GI}_{50}$  = Concentration of the drug that produces 50% inhibition of the cells;**

**Table 2** *In-vitro* anticancer activity of synthesized compounds **7 (a-l)**.

Compound	GI <sub>50</sub> µg/ml			
	MCF-7	HeLa	SK-MEL-2	HL-60
<b>7a</b>	22.9	32.8	21.9	21.7
<b>7b</b>	28.7	39.0	22.9	28.2
<b>7c</b>	32.4	41.1	27.5	33.3
<b>7d</b>	36.7	52.4	34.0	40.2
<b>7e</b>	35.2	46.8	28.1	39.6
<b>7f</b>	38.4	49.2	30.0	37.5
<b>7g</b>	41.0	66.1	46.4	42.4
<b>7h</b>	46.2	71.7	49.1	48.2
<b>7i</b>	49.0	78.0	52.6	45.8
<b>7j</b>	51.4	78.8	55.7	49.9
<b>7k</b>	11.7	23.8	19.6	35.5
<b>7l</b>	19.0	28.8	22.0	29.9
ADR	<10	<10	<10	<10

## *In Silico* ADMET Prediction

A computational study of synthesized compounds **7(a–l)** was performed for prediction of ADMET. The absorption, distribution, metabolism, excretion and Toxicity (ADMET) properties of all compounds were predicted using Qikprop v3.5 (Schrödinger LLC). In the present study, we have calculated the molecular weight (MW), Predicted octanol-water partition coefficient ( $\log P_{o/w}$ ), number of hydrogen bond acceptors (n-ON), number of hydrogen bonds donors (n-OHNH), Percentage human oral absorption (% ABS), Polar surface area (PSA), Aqueous solubility (Log S), Prediction of binding to human serum albumin (Log K<sub>hsa</sub>) and *in silico* cardiac toxicity study (Log HERG). The above described properties help us in understanding the ADMET properties of any drug/synthesized molecule. A molecule likely to be developed as an orally active drug candidate should show no more than one violation of Lipinski rule of 5

**Table 3** *In silico* physicochemical pharmacokinetic parameters important for good oral bioavailability of synthesized compounds **7 (a–l)**

Entry	M.W	Log P o/w (-2.0-6.5)	n-ON (<10)	n-OHNH (<5)	PSA (7-200)	log Khsa (-1.5 - 1.2)	Log S (-6-0.5)	% ABS	# meta (1-8)	Log HERG below -5	Lipinski rule of 5 (≤1)
<b>7a</b>	356.8	4.82	5.5	1	76.2	0.31	-5.8	98	2	-6.8	0
<b>7b</b>	391.2	5.34	5.5	1	74.4	0.37	-6.1	99	2	-6.6	0
<b>7c</b>	338.3	5.18	6.2	2	98.4	0.02	-4.7	89	3	-6.7	0
<b>7d</b>	338.3	5.17	6	2	98.2	0.03	-4.3	88	3	-6.5	0
<b>7e</b>	368.4	5.27	7.2	1	133.3	-0.04	-4.7	75	3	-6.6	0
<b>7f</b>	382.4	5.09	7	2	105.7	0.16	-5.5	91	4	-6.9	0
<b>7g</b>	352.4	4.91	6.2	1	83.9	0.16	-5.1	100	3	-6.7	0
<b>7h</b>	382.4	5.20	7	1	89.0	0.19	-5.4	100	4	-6.7	0
<b>7i</b>	412.2	4.05	7.7	1	95.4	0.17	-5.4	100	5	-6.5	0
<b>7j</b>	412.2	4.08	7.7	1	97.6	0.19	-5.6	100	5	-6.6	0
<b>7k</b>	312.3	3.91	6	1	84.7	-0.11	-4.0	94	3	-6.3	0
<b>7l</b>	328.4	4.11	5	1	85.6	-0.21	-4.2	95	3	-6.2	0



# RESULTS AND DISCUSSION

## Chemistry

Herein we report the synthesis of novel N-((5-(substituted Methylene Amino)- 1,3,4-thiadiazol-2-yl)methyl) Benzamide Derivatives using microwave as shown in scheme1. The physical characterization data of the synthesized compounds **7 (a-l)** are as shown in Table 1. All the synthesized compounds were characterized by <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, mass spectroscopy and IR.

## *In Vitro* Anticancer Activity

The synthesized compounds (**7a-l**) were evaluated for their anticancer activity against MCF-7 (Human breast cancer cell line), HeLa (Human cervical cancer cell line), SKMEL-2 (Human Melanoma cancer cell line) and HL-60 (Human Leukemia cancer cell line) cancer cell lines.

The results indicated that the compounds, **7k**, **7l**, **7a** and **7b** exhibited significant cancer cell growth inhibition compared to reference standard Adriamycin against MCF-7, HeLa, SKMEL-2 and HL-60 cancer cell lines. From the anticancer activity results, it was observed that compound **7k**, which has furan ring was found to have the highest GI<sub>50</sub> values of 11.7µg/ml, 23.8µg/ml, 19.6µg/ml and 35.5µg/ml for MCF-7, HeLa, SKMEL-2 and HL-60 cancer cell lines respectively. Compound **7l** which has thiophene ring was found to have the good GI<sub>50</sub> values of 19.0µg/ml, 28.8µg/ml, 22.0µg/ml and 29.9µg/ml for MCF-7, HeLa, SKMEL-2 and HL-60 cancer cell lines respectively.

## Structural Activity Relationship

- ❖ Electron withdrawing groups such as chloro (**7a**, **7b**) exhibited good activity compared to electron donating, polar groups.
- ❖ Replacement of the phenyl group in the parent compound by furan ring in **7k** and thiophene ring in **7l** has shown significant increase in anticancer activity in comparison to the standard drug Adriamycin.
- ❖ Compounds containing electron donating, polar groups such as **7c**, **7d**, **7e**, **7f**, **7g**, **7h**, **7i** and **7j** are less active in comparison to electron withdrawing groups such as **7a** and **7b**.

# *In Silico* ADMET Prediction

- ❖ The prediction of the ADMET parameters prior to the experimental studies is one of the most important aspects of drug discovery and development of the drug molecule.
- ❖ The synthesized compounds exhibited a good % absorption (% ABS) ranging from 75% to 100%
- ❖ All the synthesized compounds have shown aqueous solubility values within the range -6.1 to -4.2.
- ❖ The compounds showed Log K<sub>hsa</sub> value ranges between -0.2 to 0.19 this is an indication that a significant proportion of the compounds are likely to circulate freely in the blood stream and hence reach the drug target sites.
- ❖ The HERG K<sup>+</sup> channel blockers are potentially toxic and the predicted IC<sub>50</sub> values often provide reasonable predictions for cardiac toxicity of drugs in the early stages of drug discovery . None of the synthesized compounds **7 (a-l)** are toxic in nature as shown in Table 3

# CONCLUSION

- ❖ Total 12 final compounds were synthesized under microwave irradiation as per the scheme reported.
- ❖ Structures of synthesized compounds were confirmed by spectral study such as IR,  $^1\text{H}$ NMR,  $^{13}\text{C}$  NMR and Mass.
- ❖ The synthesized compounds were evaluated for anticancer activity on SK-MEL-2, MCF-7, HeLa and HL-60 human cancer cell lines by MTT assay.
- ❖ It appears that, the hybrid molecule **(E)-N-((5-(substituted methyleneamino)-1,3,4-thiadiazol-2-yl)methyl)benzamide derivatives** possess very good potential for development as novel anticancer agents and can prove a benchmark for the development of potential anticancer agents.

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*Thank you*