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(breast) using MTT assays method. Most of the synthesized compounds exhibited promising anticancer activity with the some compounds having GI_{50} 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

1. INTRODUCTION

Cancer is a disease in which cells grow and proliferate in an uncontrolled manner. Cancer disease evokes a high level of mortality regardless of recent advances in the development of clinically authorized anticancer agents [1]. Management of cancer still represents a major challenge in medicine despite of significant progress achieved in anticancer therapy. Therefore, many scientists are intensively engaged in the development of new anticancer active agents that reveal a selective cytotoxicity for cancer cells over normal cells which is undoubtedly needed to treat the severe cancer disease more efficiently and is also less toxic, since many of the marketed anticancer drugs are toxic in nature [2-4].

The class of organic compounds containing the azomethine (–HC=N–) group in their structure is called imine compounds or the molecule containing carbon nitrogen (HC=N) double bond is called as imine or alternatively a Schiff base. Schiff bases, derived mostly from variety of heterocyclic rings, were reported to possess a broad spectrum of pharmacological activities with a wide variety of biological properties, development of a new chemotherapeutic Schiff bases is now attracting the attention of medicinal chemist. They are known to exhibit a variety of potent activities.

Heterocyclic nucleus 1,3,4-thiadiazole constitutes an important class of compounds for new drug development because of its interesting biological properties such as anticancer [5], antibacterial [6] and antifungal activity [7]. Especially, the structure of ‘‘N–C–S’’ in 1,3,4-thiadiazole derivatives can work as the active center, chelate certain metal ions in vivo, and show good tissue permeability.. The synthesis of novel thiadiazole derivatives and investigation of their chemical and biological behavior have gained more importance in recent decades [8-11].

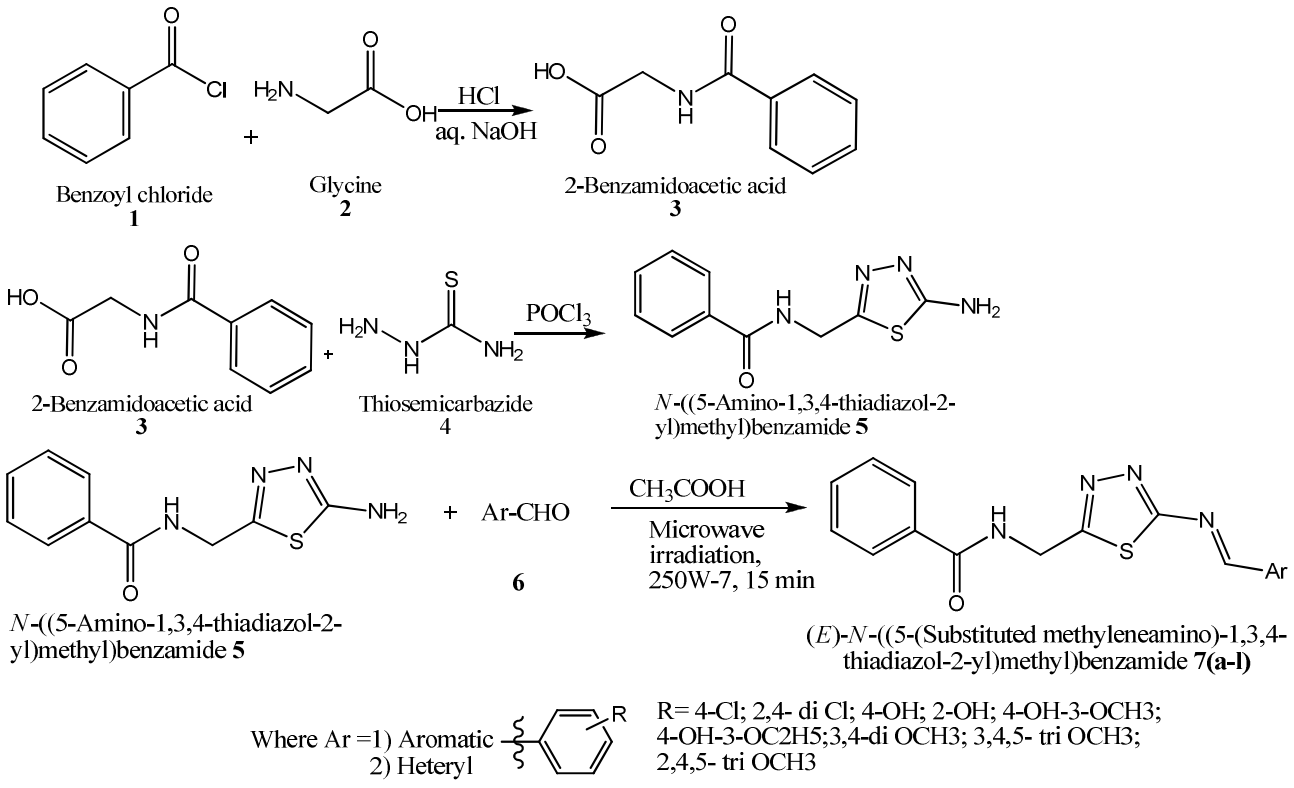
In the present work we planned to develop Schiff’s bases containing thiadiazole ring and benzamide group, because of the important biological properties associated with these groups. The coupling of these important moieties was achieved under microwave irradiation. Microwave induced synthesis has various advantages over conventional synthesis, such as 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 and control on reaction parameters [12].

All the designed hybrids were evaluated for their *in vitro* anticancer activity against a panel of three human cancer cell lines viz SK-MEL-2(melanoma), HL-60 (leukemia). Molecular docking study has also been performed to support the effective binding of compound at the active site of the enzyme. Most of the synthesized compounds exhibited promising anticancer activity with the some compounds having GI_{50} values similar to that of the Adriamycin. The compounds **7k**, **7l, 7b**, and **7a** were found to be the most promising in this study.

2. RESULTS AND DISCUSSION

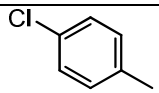
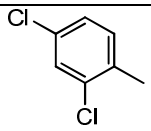
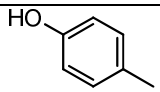
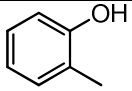
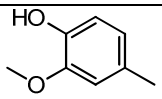
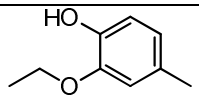
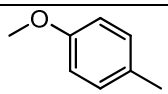
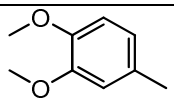
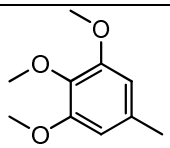
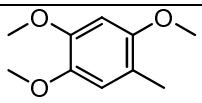
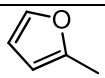
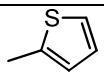
2.1. Chemistry

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 ^{1}H-NMR, ^{13}C-NMR, mass spectroscopy and IR.



Scheme 1 Synthesis of N-((5-(substituted Methylene Amino)-1,3,4-thiadiazol-2-yl)methyl) Benzamide Derivatives **7(a-l).**

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

Entry	Ar	Molecular formula	Molecular weight.	% Yield	Melting point(^{o}C)	R_f value
7a		$C_{17}H_{13}ClN_4OS$	356.83	95	124-128	0.44
7b		$C_{17}H_{12}Cl_2N_4OS$	391.27	92	112-114	0.56
7c		$C_{17}H_{14}N_4O_2S$	338.38	94	112-114	0.59
7d		$C_{17}H_{14}N_4O_2S$	338.38	94	106-108	0.43
7e		$C_{18}H_{16}N_4O_3S$	368.41	92	122-126	0.66
7f		$C_{19}H_{18}N_4O_3S$	382.44	92	130-132	0.45
7g		$C_{18}H_{16}N_4O_2S$	352.41	95	112-118	0.57
7h		$C_{19}H_{18}N_4O_3S$	382.44	88	114-118	0.64
7i		$C_{20}H_{20}N_4O_4S$	412.26	86	138-140	0.48
7j		$C_{20}H_{20}N_4O_4S$	412.26	88	134-138	0.70
7k		$C_{15}H_{12}N_4O_2S$	312.35	85	124-126	0.55
7l		$C_{15}H_{12}N_4OS_2$	328.41	84	136-138	0.42

2.2. *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 as shown in Table 2. The GI_{50} values (concentration required to Growth inhibition of 50%) for the synthesized compounds were determined using MTT assays method. The anticancer evaluation results and GI_{50} values were listed in Table 2 and the well-known anticancer drug Adriamycin was used as positive control.

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_{50} 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_{50} 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. Compound **7a** containing 4-Chlorophenyl substituent (MCF-7 GI_{50} 22.9µg/ml, HeLa GI_{50} 32.8µg/ml, SKMEL-2 GI_{50} 21.9µg/ml and HL-60 GI_{50} 21.7µg/ml) and compound **7b** 2,4 dichlorophenyl substituent (MCF-7 GI_{50} 28.7µg/ml, HeLa GI_{50} 39.0µg/ml, SKMEL-2 GI_{50} 22.9µg/ml and HL-60 GI_{50} 28.2µg/ml) were found to have good anticancer activity.

Structural activity relationship (SAR) studies for these compounds demonstrated that electron withdrawing groups such as chloro (**7a, 7b**) exhibited good activity compared to electron donating, polar groups. Compound **7c** with 4-hydroxyphenyl substituent (MCF-7 GI_{50} 32.4 µg/ml, HeLa GI_{50} 41.1µg/ml, SKMEL-2 GI_{50} 27.5µg/ml and HL-60 GI_{50} 33.3µg/ml) was found to be more active than compound **7e** with 4-hydroxy-3-methoxyphenyl substituent (MCF-7 GI_{50} 35.2µg/ml, HeLa GI_{50} 46.8 µg/ml, SKMEL-2 GI_{50} 28.1µg/ml and HL-60 GI_{50} 39.6µg/ml) and compound **7f** with 4-hydroxy-3-ethoxyphenyl substituent (MCF-7 GI_{50} 38.4µg/ml, HeLa GI_{50} 49.2µg/ml, SKMEL-2 GI_{50} 30.0µg/ml and HL-60 GI_{50} 37.5µg/ml). 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. From SAR it can be considered that 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**. It is also clear that the replacement of the phenyl ring with the furan ring (**7k**) and thiophene ring (**7l**) significantly increased the anticancer activity.

Compound **7k** and **7l** have shown significantly good anticancer activity in comparison of standard anticancer drug Adriamycin against MCF-7, HeLa, SKMEL-2 and HL-60 cancer cell lines and can be developed as anticancer agents in the future.

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

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

2.4. 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. ADMET studies have always played a critical role in helping to optimize the pharmacokinetic properties of new drugs, thereby increasing their success rate. The analysis of Lipinski's rule of five was performed to indicate whether a chemical compound could be an orally active drug in humans. It was observed that the compounds exhibited a good % absorption (% ABS) ranging from 75% to 100% Table 3. A parameter used to evaluate aqueous solubility is Log S (S in mol/L). All compounds present solubility values within the range -6.1 to -4.2. The efficiency and distribution of a drug may be affected by the degree to which it binds to the proteins within blood plasma. Log Khsa is used for prediction of binding to human serum albumin. The compounds showed Log Khsa 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. Human ether-a-go-go related gene (HERG) encodes a potassium ion (K^+) channel that is implicated in the fatal arrhythmia known as *torsade de pointes* or the long QT syndrome [13]. The HERG K^+ channel, which is

best known for its contribution to the electrical activity of the heart that coordinates the heart's beating, appears to be the molecular target responsible for the cardiac toxicity of a wide range of therapeutic drugs [14]. HERG has also been associated with modulating the functions of some cells of the nervous system and with establishing and maintaining cancer-like features in leukemic cells [15]. Thus, HERG K^+ channel blockers are potentially toxic and the predicted IC_{50} values often provide reasonable predictions for cardiac toxicity of drugs in the early stages of drug discovery [16]. None of the synthesized compounds **7 (a-l)** are toxic as shown in Table 3. All synthesized compounds **7 (a–l)** had good absorption and were found to be nontoxic.

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)**
7a	356.8	4.82	5.5	1	76.2	0.31	-5.8	98	2	-6.8	0
7b	391.2	5.34	5.5	1	74.4	0.37	-6.1	99	2	-6.6	0
7c	338.3	5.18	6.2	2	98.4	0.02	-4.7	89	3	-6.7	0
7d	338.3	5.17	6	2	98.2	0.03	-4.3	88	3	-6.5	0
7e	368.4	5.27	7.2	1	133.3	-0.04	-4.7	75	3	-6.6	0
7f	382.4	5.09	7	2	105.7	0.16	-5.5	91	4	-6.9	0
7g	352.4	4.91	6.2	1	83.9	0.16	-5.1	100	3	-6.7	0
7h	382.4	5.20	7	1	89.0	0.19	-5.4	100	4	-6.7	0
7i	412.2	4.05	7.7	1	95.4	0.17	-5.4	100	5	-6.5	0
7j	412.2	4.08	7.7	1	97.6	0.19	-5.6	100	5	-6.6	0
7k	312.3	3.91	6	1	84.7	-0.11	-4.0	94	3	-6.3	0
7l	328.4	4.11	5	1	85.6	-0.21	-4.2	95	3	-6.2	0

3. MATERIALS AND METHODS

3.1. General Information

All the chemicals used for synthesis were of Merck, Sigma, Research lab, Qualigens make and Himedia. The reactions were carried out by conventional method and in synthetic microwave oven CATA-R microwave. Melting points were determined in open capillaries using melting point apparatus and are uncorrected. All the reactions were performed in oven-dried glassware's. Phosphorus oxychloride was used by distilling under reduced pressure. The synthetic protocol employed for the synthesis of N-((5-(substituted Methylene Amino)-1,3,4-thiadiazol-2-yl)methyl) Benzamide derivatives **7 (a-l)** is presented in Scheme 1. The purity of the synthesized compounds was checked by TLC and melting points were determined in open capillary tubes and are uncorrected. The physical characterization data of the synthesized compounds are presented in Table 1. The NMR spectra of final titled compounds were recorded on Brucker Advance II (400 MHz) and Infrared (IR) spectra were recorded for the compounds on JASCO FTIR (PS 4000) using KBr pallet. The mass spectra were recorded on a waters Micro Mass ZQ 2000 spectrometer.

Step I: General process for synthesis of 2-Benzamidoacetic acid (17)

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 congored paper. The resulting crystalline solid was collected and boiled with 10 ml of CCl_4 for 10 min. The product was filtered and washed with CCl_4. 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 [18]

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.

Step III: General procedure for synthesis of(E)-N-((5-(substituted methyleneamino)-1,3,4-thiadiazol-2-yl)methyl) benzamide (7a-7l)

A. Conventional method [19]:

Equimolar quantities of N-((5-amino-1,3,4-thiadiazol-2-yl)methyl) benzamide (0.01 mol) **(5)** and different suitable aldehydes **(6)** (0.01mol) were refluxed in the presence of glacial acetic acid (0.02mol) in absolute ethanol (25 ml) for 6-8 h. The completion of reaction was monitored by TLC. The reaction mixture was concentrated and cooled. The obtained solid was filtered and dried. The product was recrystallized from ethanol. The melting point and yield were recorded.

B. Microwave-assisted method [20, 21]:

Solvent free synthesis of Schiff bases was achieved by cycloaddtion of various suitable aldehydes **(6)** (0.01 mol) and N-((5-amino-1,3,4-thiadiazol-2-yl)methyl) benzamide **(5)** (0.01 mol) in presence of catalytic amount of glacial acetic acid under microwave irradiation at 250 W for 15-20 min as shown in scheme 1. 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%.

2-benzamidoacetic acid **(3)**

IR (KBr$\nu_{max\ in}$ cm^{-1}): 3350.41 (NH), 3179.92 (OH), 2970.76 (C=H), 1810.26 (C=O of amide), 1725.01 (C=O of carboxylic acid); ^{1}H NMR (DMSO) δ ppm: 3.84 (s, 2H, CH_2), 7.77-8.00 (m, 5H), 8.05 (s, 1H, NH), 11.35 (s, 1H, OH); ^{13}C NMR (DMSO) δ ppm: 172.11, 167.87, 134.23, 132.89, 128.61, 128.00, 127.87, 127.31, 41.34; m/z: 179.06 (100.0%), 180.06 (10.3%), 181.06 (1.1%); Molecular Formula: $C_9H_9NO_3$; Elemental Analysis: Calculated: (C, H, N, O) 60.33, 5.06, 7.82, 26.79; Found: 60.35, 5.08, 7.80, 26.76.

N-((5-Amino-1,3,4-thiadiazol-2-yl)methyl)benzamide **(5)**

IR (KBr$\nu_{max\ in}$ cm^{-1}): 3360.45 (NH_2), 2970.76 (C=H), 1810.26 (C=O of amide), ^{1}H NMR (DMSO) δ ppm: 3.45 (s, 2H, CH_2), 6.89 (s, 2H, NH_2), 7.70-8.00 (m, 5H), 8.05 (s, 1H, NH); ^{13}C NMR (DMSO) δ ppm: 168.00, 167.89, 161.66, 134.21, 132.11, 128.76, 128.42, 127.69, 127.26, 39.11; m/z: 234.06 (100.0%), 235.06 (11.8%), 236.05 (4.5%), 235.05 (1.5%); Molecular Formula: $C_{10}H_{10}N_4OS$; Elemental Analysis: Calculated: (C, H, N, O, S) 51.27, 4.30, 23.91, 6.83, 13.69; Found: 51.29, 4.34, 23.89, 6.80, 13.71.

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

Yield: 95%; M.P: 126-128^{0}C ; IR (KBr$\nu_{max\ in}$ cm^{-1}): 3350.41 (NH), 2970.76 (C=H), 1810.26 (C=O of amide); ^{1}H NMR (DMSO) δ ppm: 3.42 (s, 2H, CH_2), 6.69-5.2 (m, 9H), 8.21 (s, 1H, NH), 10.33 (s, 1H, N=CH); ^{13}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: $C_{17}H_{13}ClN_4OS$ 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^{0}C ; IR (KBr$\nu_{max\ in}$ cm^{-1}): 3352.41 (NH), 2975.76 (C=H), 1818.26 (C=O of amide); ^{1}H NMR (DMSO) δ ppm: 3.45 (s, 2H, CH_2), 6.69-5.2 (m, 8H), 8.29 (s, 1H, NH), 10.33 (s, 1H, N=CH); ^{13}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: $C_{17}H_{12}Cl_2N_4OS$; 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^{0}C ; IR (KBr$\nu_{max\ in}$ cm^{-1}): 3350.41 (NH), 3179.92 (OH), 2970.76 (C=H), 1810.26 (C=O of amide); ^{1}H NMR (DMSO) δ ppm: 3.44 (s, 2H, CH_2), 5.43 (s,1H, OH), 6.69-5.2 (m, 9H), 8.21 (s, 1H, NH), 10.34 (s, 1H, N=CH); ^{13}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: $C_{17}H_{14}N_4O_2S$ 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^{0}C ; IR (KBr$\nu_{max\ in}$ cm^{-1}): 3350.58 (NH), 3179.90 (OH), 2970.66 (C=H), 1811.16 (C=O of amide); ^{1}H NMR (DMSO) δ ppm: 4.12 (s, 2H, CH_2), 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); ^{13}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: $C_{17}H_{14}N_4O_2S$; 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^{0}C ; IR (KBr$\nu_{max\ in}$ cm^{-1}): 3350.31 (NH), 2971.76 (C=H), 1810.16 (C=O of amide); ^{1}H NMR (DMSO) δ ppm: 3.83 (s, 3H, OCH_3), 4.10 (s, 2H, CH_2), 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); ^{13}C NMR (DMSO) δ 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: $C_{18}H_{16}N_4O_3S$; 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^{0}C; IR (KBr$\nu_{max\ in}$ cm^{-1}): 3350.31 (NH), 2971.76 (C=H), 1810.16 (C=O of amide); ^{1}H NMR (DMSO) δ ppm: 1.32 (t, 3H, CH_3), 4.09 (q, 2H, CH_2), 4.46 (s, 2H, CH_2), 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); ^{13}C NMR (DMSO) δ 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: $C_{19}H_{18}N_4O_3S$; 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^{0}C ; IR (KBr$\nu_{max\ in}$ cm^{-1}): 3352.18 (NH), 2971.66 (C=H), 1810.17 (C=O of amide); ^{1}H NMR (DMSO) δ ppm: 3.83 (s, 6H, OCH_3), 4.19 (s, 2H, CH_2), 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); ^{13}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: $C_{18}H_{16}N_4O_2S$; 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^{0}C ; IR (KBr$\nu_{max\ in}$ cm^{-1}): 3352.18 (NH), 2971.66 (C=H), 1810.17 (C=O of amide); ^{1}H NMR (DMSO) δ ppm: 3.83 (s, 6H, OCH_3), 4.19 (s, 2H, 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}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: $C_{19}H_{18}N_4O_3S$; 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^{0}C ; IR (KBr$\nu_{max\ in}$ cm^{-1}): 3350.18 (NH), 2972.66 (C=H), 1810.17 (C=O of amide); ^{1}H NMR (DMSO) δ ppm: 3.85 (s, 9H, OCH_3), 4.19 (s, 2H, 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}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: 412.12 (100.0%), 413.12 (24.1%), 414.12 (5.9%), 414.13 (2.3%), 415.12 (1.1%); Molecular Formula: $C_{20}H_{20}N_4O_4S$; 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^{0}C ; IR (KBr$\nu_{max\ in}$ cm^{-1}): 3350.18 (NH), 2972.66 (C=H), 1810.17 (C=O of amide); ^{1}H NMR (DMSO) δ ppm: 3.86 (s, 9H, OCH_3), 4.20 (s, 2H, 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}C NMR (DMSO) δ 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: $C_{20}H_{20}N_4O_4S$; 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^{0}C ; IR (KBr$\nu_{max\ in}$ cm^{-1}): 3350.41 (NH), 2970.76 (C=H), 1810.26 (C=O of amide); ^{1}H NMR (DMSO) δ ppm: 3.48 (s, 2H, CH_2), 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); ^{13}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: $C_{15}H_{12}N_4O_2S$; 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^{0}C; IR (KBr$\nu_{max\ in}$ cm^{-1}): 3350.18 (NH), 2975.66 (C=H), 1815.17 (C=O of amide); ^{1}H NMR (DMSO) δ ppm: 4.46 (s, 2H, CH_2), 7.17 (t, 1H, CH), 7.63-8.09 (m, 6H), 8.29 (s, 1H, NH); ^{13}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: $C_{15}H_{12}N_4O_2S_2$; 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.

1.1. *In-vitro* anticancer screening

The stock solutions of test compounds were prepared in DMSO. After 24 h incubation, different concentrations (2, 4, 6, 8 µM) of compounds, made by serial dilution in culture medium, were added in 48 h incubation. The final concentration of DMSO was 0.01% in each well. A separate well containing 0.01% DMSO only was run as DMSO control, which was found inactive under applied conditions. The cell growth was determined using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (Sigma) reduction assay, which is based on ability of viable cells to reduce a soluble yellow tetrazolium salt to blue farmazan crystal [22, 23]. Briefly, after 48 h of treatments, the10µl of MTT dye, prepared in phosphate buffered saline (PBS) were added to all wells. The plates were then incubated for 4h at 37 ^{o}C. Supernatant from each well was carefully removed, formazon crystals were dissolved in 100µL of DMSO and absorbance at 540nm wavelength was recorded (Sharma et al., 2010) and each concentration was tested in threefold. The GI_{50} values were determined as concentration of compounds that inhibited cancer cell growth by 50%.

1.2. *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 Po/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 Khsa) 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 [24].

CONCLUSION

Total 12 final compounds were synthesized under microwave irradiation in 7-15 minutes time, in better yields, as per the scheme reported. Structures of synthesized compounds were confirmed by spectral study such as IR, ^{1}HNMR, ^{13}C NMR and Mass. The synthesized compounds were evaluated for anticancer activity on SK-MEL-2 and HL-60 cell lines by MTT assay. The compounds **7k**, **7l, 7b**, and **7a** were found to be the most promising in this study.

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