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Design, synthesis, characterization and evaluation of anticancer activity of thiadiazole derivatives

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Abstract:
Cancer cells start to grow out of control and form new, abnormal cells. Cancer cells can also invade other tissues. Cancer is a world most threaten problem, therefore the development of potent anticancer agents is the urgent need of society. Compounds with heterocyclic nucleus showed potent anticancer activity, in this continuation of work we designed a series of newer derivatives containing thiadiazole heterocyclic scaffold as potent anticancer agent. All the derivatives were synthesized, characterized for their structure identification and evaluated for anticancer activity. The anticancer activity was evaluated against MCF-7 cancer cell lines by MTT assay. The comp A4 showed the highly potent anticancer activity against MCF-7 breast cancer cell line with IC_{50}= 38.59 ± 4.4. The structure activity relationship study revealed that the thidiazole ring is essential for anticancer activity and the substitution of electron withdrawing group at R_1 position exhibited more potent activity against breast cancer cell lines. Methyl group at R_2 and R_3 position showed more potent activity compared to unsubstituted ring. The results of *in silico* ADMET properties revealed that all the synthesized derivatives not violated any rules of Lipinski and all are drug like compounds. Overall activity result revealed that these derivatives will be used in future as lead molecules for development of anticancer agents.

Keywords: Thiadiazole, anticancer, MCF-7 cancer cell line, MTT assay, Pharmacokinetic.

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1. Introduction

Cancer is the most challenging public health harms worldwide. The global cancer prevalence is expected to raise about 47% cancer cases from 2020 to 2040, which will be expected about 28.4 million cases [1]. Now days, scientist and researcher are focusing on anticancer therapy significantly, there is an urgent need for the development of potent anticancer derivatives which effectively kill cancer cells, as well as improved anticancer therapeutic strategies.

Five member heterocyclic compounds containing sulfur and nitrogen have become an area of developing newer anticancer agents [2,3]. Among these, thiadiazole received greater interest. Various literature reviews reported the thiadiazole derivatives as anticancer therapeutic. Many derivatives have been reported with promising anticancer activity against many cancer cell lines. Thiadiazole are the bioisoster of pyrimidine, which is the backbone of the structures of three nucleobases. Hence, thiadiazoles have the ability to interfere with DNA replication processes [4,5,6]. Depending on the position of the nitrogen and sulfur atoms, the thiadiazole may occur in four different isomeric forms: 1,2,3-, 1,2,4-, 1,2,5 and 1,3,4-thiadiazole [2,6], as presented in **Figure 1**.

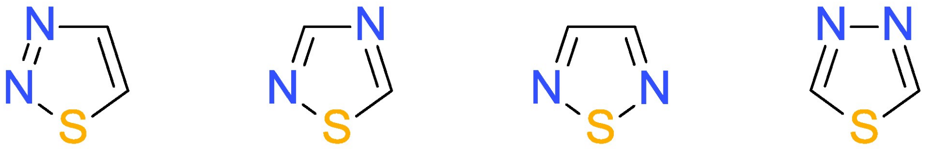


Figure 1. Isomeric form of thiadiazole 1,2,3-, 1,2,4-, 1,2,5 and 1,3,4-thiadiazole

In this research work we designed and synthesized a series of 5-benzyl-1,3,4-thiadiazol-2-amine urea derivatives (**Comp A1 –A13 and Comp B1-B5)**. The chemical structures of all the synthesized derivatives were confirmed by FT-IR, ^{1}HNMR and MASS spectroscopy. Further, the anti-cancer activity of all the synthesized derivatives was evaluated against MCF-7 breast cancer cell line. The pharmacokinetic activity of all the derivatives was also predicted by In-silico method.

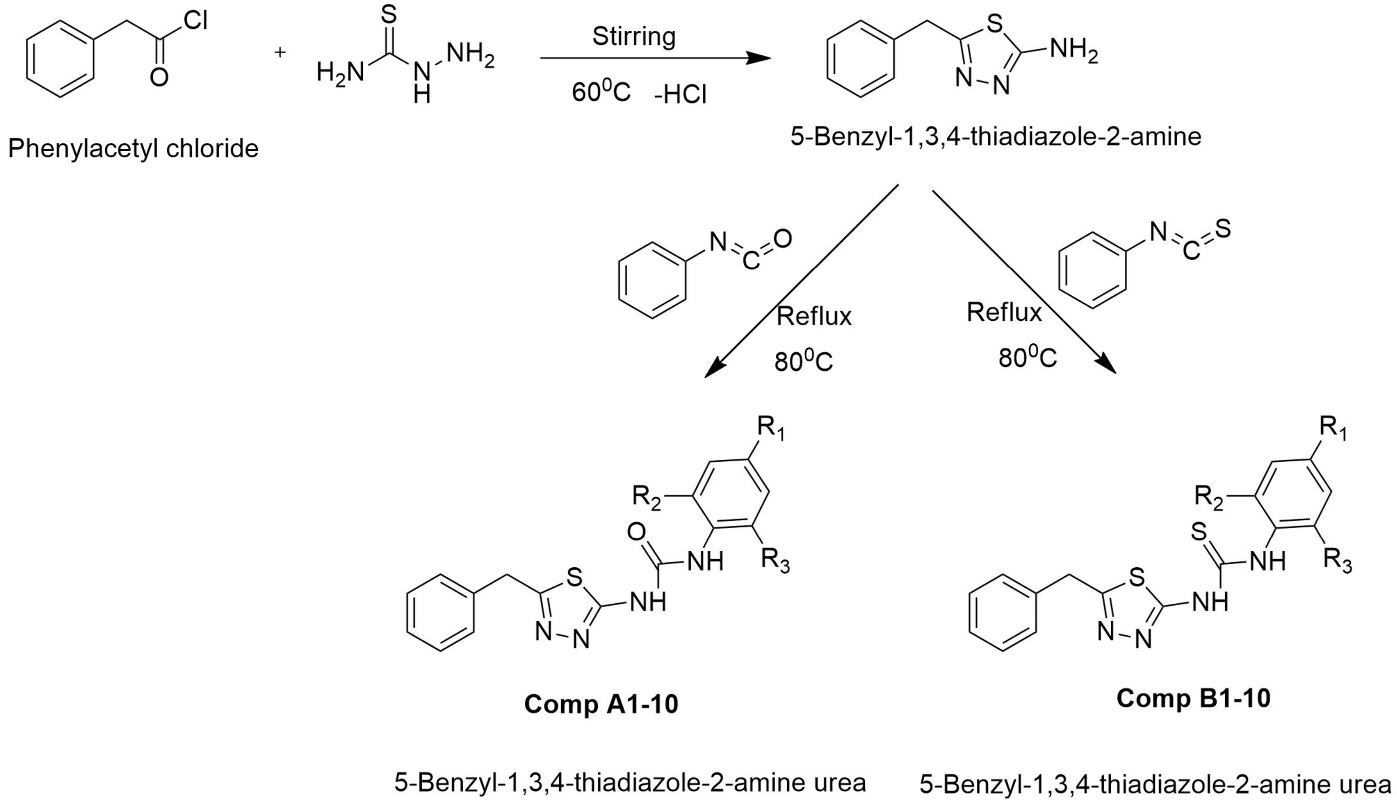
2. Materials and methods

2.1. Analytical procedure

The melting points (M.P.) of all the synthesized derivatives were determined using a melting point apparatus in open capillary tubes and are reported without any manipulations. The ^{1}H-NMR spectra were acquired on Bruker system of 500 MHz. Chemical shifts were recorded as δ values in ppm units, referenced to tetramethylsilane (TMS) used as the internal standard. DMSO-d6 was employed as solvent. The coupling constants (J) were reported in hertz (Hz), and the splitting patterns were abbreviated as follows: s, singlet; d, doublet; t, triplet; q, quartet; and b, broad. The infrared (IR) spectra were obtained on a FT/IR-4200 spectrometer. The molecular weight of synthesized derivatives was determined by mass spectroscopy (MASS) using methanol as solvent.

2.2. Chemistry and synthetic scheme

In this research work we synthesized eighteen 5-benzyl-1,3,4-thiadiazol-2-amine urea fifteen derivatives, the synthesis scheme are shown in **Scheme 1**. We design total eighteen thiadiazole derivatives by substituting different functional group at R_1, R_2 and R_3 position (**Table 1**).



Scheme 1: Synthesis of 5-benzyl-1,3,4-thiadiazol-2-amine urea derivatives

Table 1: List of 5-benzyl-1,3,4-thiadiazol-2-amine urea derivatives

S. No.	Comp. No.	R_1	R_2	R_3
1.	Comp A1	Chloro	Methyl	Methyl
2.	Comp A2	Chloro	Methyl	Hydro
3.	Comp A3	Methyl	Chloro	Hydro
4.	Comp A4	Bromo	Methyl	Methyl
5.	Comp A5	Bromo	Methyl	Hydro
6.	Comp A6	Bromo	Chloro	Hydro
7.	Comp A7	Methyl	Methyl	Hydro
8.	Comp A8	Hydro	Hydro	Methyl
9.	Comp A9	Isopropyl	Methyl	Hydro
10.	Comp A10	Nitro	Hydro	Methyl
11.	Comp A11	Hydro	Nitro	Methyl
12.	Comp A12	Methoxy	Hydro	Methyl
13.	Comp A13	Ethoxy	Hydro	Methyl
14.	Comp B1	Methyl	Hydro	Hydro
15.	Comp B2	Chloro	Methyl	Hydro
16.	Comp B3	Methoxy	Methyl	Hydro
17.	Comp B4	Ethoxy	Methyl	Hydro

18.	Comp B5	Isopropyl	Methyl	Hydro

1. Synthesis of 5-benzyl-1,3,4-thiadiazol-2-amine (3)

A mixture of 0.5 mole of phenylacetyl chloride **(1)** and 0.25 mole of thiosemicarbazide **(2)** was taken in RBF and heated with continuous stirring until the evolution of hydrogen chloride had ceased. Then the reaction mixture was cooled at room temperature and dissolved in 100 ml of distilled water and filtered. In cooled filtrate add 50% sodium hydroxide solution to make alkaline solution and to get precipitate. The solid material was filtered off, washed with water and dried [7, 8].

2. Synthesis of 1-(5-benzyl-1,3,4-thiadiazol-2-yl)-3-(phenyl) urea derivatives (Comp A1-A13)

The 1-(5-benzyl-1,3,4-thiadiazol-2-yl)-3-(phenyl) **(6)** urea was prepared by the mixture of compound (**3**) 25 mmoles and different derivatives of phenylisocyanate 24 mmoles in 100 ml of acetonitrile was refluxed for 24 hrs and the hot reaction mixture was filtered, the residue obtained was washed with hot methanol, and dried.

3. Synthesis of 1-(5-benzyl-1,3,4-thiadiazol-2-yl)-3-(phenyl) urea derivatives (Comp B1-B5)

The 1-(5-benzyl-1,3,4-thiadiazol-2-yl)-3-(phenyl) **(6)** urea was prepared by the mixture of compound (**3**) 27 mmoles and different derivatives of phenylisothiocyanate 27 mmoles in 100 ml of acetonitrile was refluxed for 24 hrs and the hot reaction mixture was filtered, the residue obtained was washed with hot methanol, and dried.

2.3. Structural analysis of the synthesized compounds

2.3.1. 1-(5-benzyl-1,3,4-thiadiazol-2-yl)-3-(2,6-dimethyl,4-chlorophenyl) urea (Comp A1)

White solid, Yield 78%, M.P. 282-284°C, FTIR ($CHCl_3$, υ/cm^{-1}): 3364 (NH), 1635 (C=O), 1539 (C=N), 1487 (CH_2), 3025 (Ar-H), 697 (CCl), ^{1}HNMR (600 MHz, DMSO): δ ppm 10.9 (s, 1H, NH), 9.1 (s, 1H, NH), 7.4 (d, 2H, J = 7.84Hz, Ar-H), 7.36-7.32 (m, 6H, Ar-H), 7.2 (t, 1H, Ar-H), 4.2 (s, 2H, CH_2), LC/MS (m/z): 372.87 (M+H+), Anal. Calc. For $C_{18}H_{17}ClN_4OS$.

2.3.2. 1-(5-benzyl-1,3,4-thiadiazol-2-yl)-3-(6-methyl,4-chlorophenyl) urea (Comp A2)

White solid, Yield 74%, M.P. 275-277°C, FTIR ($CHCl_3$, υ/cm^{-1}): 3320 (NH), 1650 (C=O), 1550 (C=N), 1494 (CH_2), 3100 (Ar-H), 660 (CCl), ^{1}HNMR (600 MHz, DMSO): δ ppm 10.8 (s, 1H, NH), 9.2 (s, 1H, NH), 7.3 (d, 2H, J = 7.8 Hz, Ar-H), 7.36-7.32 (m, 6H, Ar-H), 7.2 (t, 1H, Ar-H), 4.2 (s, 2H, CH_2), LC/MS (m/z): 358.85 (M+H+), Anal. Calc. For $C_{17}H_{15}ClN_4OS$.

2.3.3. 1-(5-benzyl-1,3,4-thiadiazol-2-yl)-3-(4-methyl,6-chlorophenyl) urea (Comp A3)

White solid, Yield 76%, M.P. 270-272°C, FTIR ($CHCl_3$, υ/cm^{-1}): 3345 (NH), 1640 (C=O), 1535 (C=N), 1470 (CH_2), 3115 (Ar-H), 655 (CCl), ^{1}HNMR (600 MHz, DMSO): δ ppm 10.8 (s, 1H, NH), 9.2 (s, 1H, NH), 7.3 (d, 2H, J = 7.8 Hz, Ar-H), 7.36-7.32 (m, 6H, Ar-H), 7.2 (t, 1H, Ar-H), 4.2 (s, 2H, CH_2), LC/MS (m/z): 358.85 (M+H+), Anal. Calc. For $C_{17}H_{15}ClN_4OS$.

2.3.4. 1-(5-benzyl-1,3,4-thiadiazol-2-yl)-3-(2,6-dimethyl,4-bromophenyl) urea (Comp A4)

White solid, yield 80%, M.P. 290-292°C, FTIR ($CHCl_3$, υ/cm^{-1}): 3363 (NH), 1699 (C=O), 1532 (C=N), 1483 (CH_2), 3025 (Ar-H), 696 (CBr), ^{1}H NMR (600 MHz, DMSO-d6): δ ppm 10.9 (s, 1H, NH), 9.1 (s, 1H, NH), 7.47 – 7.45 (m, 4H, Ar-H), 7.365 – 7.323 (m, 4H, Ar-H), 7.2 (t, 1H, Ar-H), 4.2 (s, 2H, CH_2), LC/MS (m/z): 417.32 (M-H+), Anal. Calc. For $C_{18}H_{17}BrN_4OS$.

2.3.5. 1-(5-benzyl-1,3,4-thiadiazol-2-yl)-3-(6-methyl,4-bromophenyl) urea (Comp A5)

White solid, yield 82%, M.P. 295-297°C, FTIR ($CHCl_3$, υ/cm^{-1}): 3355 (NH), 1650 (C=O), 1565 (C=N), 1470 (CH_2), 3040 (Ar-H), 710 (CBr), ^{1}HNMR (600 MHz, DMSO-d6): δ ppm 10.7 (s, 1H, NH), 9.2 (s, 1H, NH), 7.38 – 7.36 (m, 4H, Ar-H), 7.343 – 7.291 (m, 4H, Ar-H), 7.1 (t, 1H, Ar-H), 4.2 (s, 2H, CH_2), LC/MS (m/z): 403.3 (M-H+), Anal. Calc. For $C_{17}H_{15}BrN_4OS$.

2.3.6. 1-(5-benzyl-1,3,4-thiadiazol-2-yl)-3-(6-chloro,4-bromophenyl) urea (Comp A6)

White solid, yield 82%, M.P. 295-297°C, FTIR ($CHCl_3$, υ/cm^{-1}): 3370 (NH), 1640 (C=O), 1575 (C=N), 1475 (CH_2), 3068 (Ar-H), 735 (CBr), 685 (CCl), ^{1}HNMR (600 MHz, DMSO-d6): δ ppm 10.7 (s, 1H, NH), 9.2 (s, 1H, NH), 7.38 – 7.36 (m, 4H, Ar-H), 7.343 – 7.291 (m, 4H, Ar-H), 7.1 (t, 1H, Ar-H), 4.2 (s, 2H, CH_2), LC/MS (m/z): 423.71 (M-H+), Anal. Calc. For $C_{16}H_{12}BrClN_4OS$.

2.3.7. 1-(5-benzyl-1,3,4-thiadiazol-2-yl)-3-(4,6-dimethylphenyl) urea (Comp A7)

White solid, yield 73%, M.P. 274-276ºC, FTIR ($CHCl_3$, υ/cm^{-1}): 3370 (NH), 1712 (C=O), 1538 (C=N), 1450 (CH2), 2866 (CH_3), 3029 (Ar- H), ^{1}HNMR (600 MHz, DMSO-d6): δ ppm 10.7 (s, 1H, NH), 8.8 (s, 1H, NH), 7.364– 7.259 (m, 7H, Ar-H), 7.0 (d, 2H, Ar-H), 4.2 (s, 2H, CH_2), 2.238 (s, 3H, CH_3), LC/MS (m/z): 338.43 (M+H+), Anal. Calc. For $C_{18}H_{18}N_4OS$.

2.3.8. 1-(5-benzyl-1,3,4-thiadiazol-2-yl)-3-(2-methylphenyl) urea (Comp A8)

White solid, yield 81%, M.P. 278-280ºC, FTIR ($CHCl_3$, υ/cm^{-1}): 3365 (NH), 1728 (C=O), 1526 (C=N), 1444 (CH2), 2872 (CH_3), 3039 (Ar- H), ^{1}HNMR (600 MHz, DMSO-d6): δ ppm 10.7 (s, 1H, NH), 8.8 (s, 1H, NH), 7.364– 7.259 (m, 7H, Ar-H), 7.0 (d, 2H, Ar-H), 4.2 (s, 2H, CH_2), 2.238 (s, 3H, CH_3), LC/MS (m/z): 324.4 (M+H+), Anal. Calc. For $C_{17}H_{16}N_4OS$.

2.3.9. 1-(5-benzyl-1,3,4-thiadiazol-2-yl)-3-(6-methyl,4-isopropylphenyl) urea(Comp A9)

White solid, yield 63%, M.P. 252-254ºC, FTIR ($CHCl_3$, υ/cm^{-1}): 3370 (NH), 1652 (C=O), 1517 (C=N), 3030 (Ar-H), 1473 (CH_2), 2862 (CH, aliphatic), 2945 (CH_3, aliphatic), ^{1}H NMR (600 MHz, DMSO-d6): δ ppm 10.7 (s, 1H, NH), 8.8 (s, 1H, NH), 7.364 – 7.322 (m, 6H, Ar-H), 7.2 (t, 1H, Ar-H), 7.1 (d, 2H, J = 8.4 Hz, Ar-H), 4.2 (s, 2H, CH_2), 2.8 (t, 1H, CH), 1.169 (s, 6H, CH3), LC/MS (m/z): 366.48 (M-H+), Anal. Calc. For $C_{20}H_{22}N_4OS$.

2.3.10. 1-(5-benzyl-1,3,4-thiadiazol-2-yl)-3-(2-methyl,4-nitrophenyl) urea (Comp A10)

White solid, yield 70%, M.P. 299-301ºC, FTIR ($CHCl_3$, υ/cm^{-1}): 3363 (NH), 1715 (C=O), 1556 (C=N), 3010 (Ar-H), 1493 (CH_2), 1507 (NO_2), ^{1}HNMR (600 MHz, DMSO-d6): δ ppm 11.1 (s, 1H, NH), 9.8 (s, 1H, NH), 8.1 (d, 2H, Ar-H), 7.7 (d, 2H, Ar-H), 7.373 – 7.332 (m, 4H, Ar-H), 7.2 (t, 1H, Ar- H), 4.2 (s, 2H, CH_2), LC/MS (m/z): 369.4 (M-H+), Anal. Calc. For $C_{17}H_{15}N_5O_3S$.

2.3.11. 1-(5-benzyl-1,3,4-thiadiazol-2-yl)-3-(2-methyl,6-nitrophenyl) urea (Comp A11)

White solid, yield 70%, M.P. 299-301ºC, FTIR ($CHCl_3$, υ/cm^{-1}): 3343 (NH), 1768 (C=O), 1522(C=N), 3030 (Ar-H), 1460 (CH_2), 1520 (NO_2), ^{1}HNMR (600 MHz, DMSO-d6): δ ppm 11.2 (s, 1H, NH), 9.7 (s, 1H, NH), 8.2 (d, 2H, Ar-H), 7.6 (d, 2H, Ar-H), 7.274 – 7.233 (m, 4H, Ar-H), 7.2 (t, 1H, Ar- H), 4.3 (s, 2H, CH_2), LC/MS (m/z): 369.4 (M-H+), Anal. Calc. For $C_{17}H_{15}N_5O_3S$.

2.3.12. 1-(5-benzyl-1,3,4-thiadiazol-2-yl)-3-(2-methyl,4-methoxyphenyl) urea (Comp A12)

White solid, yield 68%, M.P. 253-255ºC, FTIR ($CHCl_3$, υ/cm^{-1}): 3371 (NH), 1598 (NH), 1691 (C=O), 1548 (C=N), 1490 (CH2), 2992 (Ar-H), 1043 (CO- C), ^{1}HNMR (600 MHz, DMSO-d6): δ ppm 10.7 (s, 1H, NH), 8.8 (s, 1H, NH), 7.363 – 7.319 (m, 6H, Ar-H), 7.2 (t, 1H, Ar-H), 6.875 (d, 2H, J = 9Hz, Ar-H), 4.2 (s, 2H, CH2), 3.7 (s, 3H, CH_3), LC/MS (m/z): 354.43 ((M-H+), Anal. Calc. For $C_{18}H_{18}N_4O_2S$.

2.3.13. 1-(5-benzyl-1,3,4-thiadiazol-2-yl)-3-(2-methyl,4-ethoxyphenyl) urea (Comp A13)

White solid, yield 65%, M.P. 249-251ºC, FTIR ($CHCl_3$, υ/cm^{-1}): 3381 (NH), 1592 (NH), 1721 (C=O), 1546 (C=N), 2972 (Ar-H), 1495 (CH2), 1044 (CO- C), ^{1}HNMR (600 MHz, DMSO-d6): δ ppm 10.7 (s, 1H, NH), 8.7 (s, 1H, NH), 7.363 – 7.320 (m, 6H, Ar-H), 7.2 (t, 1H, Ar-H), 6.8 (d, 2H, J = 9Hz, Ar-H), 4.2 (s, 2H, CH_2), 3.9 (q, 2H, CH_2), 1.2 (t, 3H, CH_3), LC/MS (m/z): 368.45 (M-H+)1, Anal. Calc. For $C_{19}H_{20}N_4O_2S$.

2.3.14. 1-(5-benzyl-1,3,4-thiadiazol-2-yl)-3-(4-methylphenyl) thiourea (Comp B1)

White solid, yield 75%, M.P. 260-262ºC, FTIR ($CHCl_3$, υ/cm^{-1}): 3627 (NH), 1336 (C=N), 1453 (CH_2), 1035 (C=S), 2882.17 (CH3), ^{1}HNMR (600 MHz, DMSO-d6): δ ppm 14.2 (s, 1H, NH), 10.4 (s, 1H, NH), 7.6 (d, 2H, J = 9Hz, Ar-H), 7.376 – 7.258 (m, 8H, Ar-H), 4.1 (s, 2H, CH_2), 2.037 (s, 6H, CH3), LC/MS (m/z): 340.47 (M-H+), Anal. Calc. For $C_{17}H_{16}N_4S_2$.

2.3.15. 1-(5-benzyl-1,3,4-thiadiazol-2-yl)-3-(6-methyl, 4-chlorophenyl) thiourea (Comp B2)

White solid, yield 66%, M.P. 260-262ºC, FTIR ($CHCl_3$, υ/cm^{-1}): 3627 (NH), 2890 (CH3), 1430 (C=N), 1489 (CH_2), 1048 (C=S), 822 (C-Cl), ^{1}HNMR (600 MHz, DMSOd6): δ ppm 14.3 (s, 1H, NH), 10.4 (s, 1H, NH), 7.7 (d, 2H, J = 8.4Hz, Ar-H), 7.376 – 7.329 (m, 6H, Ar-H), 7.2 (t, 1H, Ar-H), 4.2 (s, 2H, CH_2), LC/MS (m/z): 374.91 (M+H+), Anal. Calc. For $C_{17}H_{15}ClN_4S_2$.

2.3.16. 1-(5-benzyl-1,3,4-thiadiazol-2-yl)-3-(6-methyl, 4-methoxyphenyl) thiourea (Comp B3)

White solid, yield 62%, M.P. 277-279ºC, FTIR ($CHCl_3$, υ/cm^{-1}): 3636 (NH), 2866 (CH3), 1534 (NH), 1450 (C=N), 1333 (CH_2), 1281 (C-O-C), 1055 (C=S), ^{1}HNMR (600 MHz, DMSO-d6): δ ppm 14.1 (s, 1H, NH), 10.2 (s, 1H, NH), 7.4 (d, 2H, J = 7.8, Ar-H), 7.371 – 7.199 (m, 7H, Ar-H), 4.1 (s, 2H, CH_2), 3.6 (s, 3H, CH3), LC/MS (m/z): 370.49 (M+H+), Anal. Calc. For $C_{18}H_{18}N_4OS_2$.

2.3.17. 1-(5-benzyl-1,3,4-thiadiazol-2-yl)-3-(6-methyl, 4-ethoxyphenyl) thiourea (Comp B4)

White solid, yield 62%, M.P. 285-287ºC, FTIR ($CHCl_3$, υ/cm^{-1}): 3660 (NH), 2845 (CH3), 1555 (NH), 1438 (C=N), 1340 (CH_2), 1267 (C-O-C), 1022 (C=S), ^{1}HNMR (600 MHz, DMSO-d6): δ ppm 14.2 (s, 1H, NH), 10.1 (s, 1H, NH), 7.4 (d, 2H, J = 7.8, Ar-H), 7.271 – 7.099 (m, 7H, Ar-H), 4.2 (s, 2H, CH_2), 3.967 (q, 2H, CH2), 3.7 (s, 3H, CH_3), LC/MS (m/z): 383.53 (M+H+), Anal. Calc. For $C_{20}H_{21}N_3OS_2$.

2.3.18. 1-(5-benzyl-1,3,4-thiadiazol-2-yl)-3-(6-methyl, 4-isopropylphenyl) thiourea (Comp B4)

White solid, yield 63%, M.P. 252-254ºC, FTIR ($CHCl_3$, υ/cm^{-1}): 3320 (NH), 1546 (C=N), 3040 (Ar-H), 1450 (CH_2), 2887 (CH, aliphatic), 2924 (CH_3, aliphatic), 1055 (C=S), ^{1}HNMR (600 MHz, DMSO-d6): δ ppm 10.7 (s, 1H, NH), 8.8 (s, 1H, NH), 7.364 – 7.322 (m, 6H, Ar-H), 7.2 (t, 1H, Ar-H), 7.1 (d, 2H, J = 8.4 Hz, Ar-H), 4.2 (s, 2H, CH_2), 2.8 (t, 1H, CH), 1.169 (s, 6H, CH3), LC/MS (m/z): 382.55 (M-H+), Anal. Calc. For $C_{20}H_{22}N_4S_2$.

2.4. In vitro cytotoxicity assay

All the eighteen derivatives of 1-(5-benzyl-1,3,4-thiadiazol-2-yl)-3-(phenyl) were evaluated for anti-cancer activity. The sulforhodamine B (SRB) assay was used to evaluate anticancer activity against MCF-7 human breast cancer cell line. Doxorubicin (DOX) was used as a reference drug. National Cancer Institute protocol was used for cytotoxicity assay. The anticancer activity results were represented as IC_{50} values and shown in **Table 2**. The RPMI 1640 medium with 10% fetal bovine serum and 2mM L-glutamine were used for cancer cell growth. Then cells will inoculated into 96 well plates followed by incubation at 37°C, 5% CO_2, 95% air and 100% relative humidity for 24 h. After the incubation all the synthesized derivatives will add (10 µL) in each well containing medium (90 µL) with different concentration (10^{-2}). These 96 well plated were incubated for 48 h followed by addition of cold TCA to terminate assay. Monolayers of cells were fixed with cold TCA and incubated for 1 hour at 4°C. After that supernatant will disposed off and washed the plates with tap water and then dried. Sulforhodamine B (SRB) solution (50 µL) at 0.4% (w/v) in 1% acetic acid was prepared and added to each of the wells and then plates were incubated for 20 min at room temperature. After staining, unbound dye was recovered and the residual dye was removed by washing repeatedly with 1% (v/v) acetic acid then plates were air dried. The protein bound dye subsequently eluted by dissolving in 10 mM trizma base, and the absorbance was read on an Elisa plate reader at a wavelength of 540 nm with 690 nm reference wavelength [9, 10].

The percentage of growth was calculated on a plate-by-plate basis for test wells relative to control wells. Percentage of growth was expressed as the ratio of average absorbance of the test well to the average absorbance of the control wells multiplied by 100. The percentage of growth inhibition of (GI_{50}) was calculated:

$[(Ti-Tz)/(CTz)] \times 100=50$

Where,

Tz = time zero

C = Control growth

Ti = Test growth in the presence of drug at the four concentration levels.

2.5. ADMET prediction by In-silico method

The pharmacokinetic properties absorption, distribution, metabolism, and excretion (ADME) of all the synthesized derivatives of 11-(5-benzyl-1,3,4-thiadiazol-2-yl)-3-(phenyl) were predicted using freely available online software tool SWISS-ADME [11, 12]. For toxicity prediction we used ProTox free online server (https://tox.charite.de/protox3/) [13]. A structure of all the compounds was drawn in chemdraw for the prediction of physicochemical properties.

3. Result and discussion

3.1. Sulforhodamine B (SRB) assay

Anti-cancer activities of all the eighteen derivatives of thiadiazole were determined against MCF-7 human breast cancer cell line by SRB method. SRB assay results of derivatives are represented in terms of 50% inhibition concentration (IC_{50} µM) values and which are shown in **Table 2**.

Comp A4 reported as most potent derivative against breast cancer line with IC_{50} value 38.59 ± 4.4 µM. Substitution of bromo group at R_1 position showed more potent anti-cancer activity compared to chloro, methyl and unsubstituted ring. Compounds with isopropyl group at para position of phenyl ring (R_1) reported as less active derivatives against MCF-7 breast cancer cell line.

Table 2: The *in vitro* anti-proliferative activity (GI_{50}) of synthesized derivatives of 1,2,4 oxadiazole against MDA-MB-231 human breast cancer cell line

S. No.	Compound No.	IC_{50} (µM)$^{+}$ (± standard deviation)
1.	Comp A1	8.39 ± 1.59
2.	Comp A2	7.92 ± 0.10
3.	Comp A3	6.24 ± 0.72
4.	Comp A4	38.59 ± 4.4
5.	Comp A5	37.62 ± 0.79
6.	Comp A6	32.21 ± 0.42
7.	Comp A7	28.32 ± 1.65
8.	Comp A8	27.36 ± 1.78
9.	Comp A9	22.63 ± 2.2
10.	Comp A10	21.83 ± 2.16
11.	Comp A11	20.93 ± 2.22
12.	Comp A12	18.73 ± 1.29
13.	Comp A13	17.82 ± 0.79
14.	Comp B1	17.40 ± 0.72
15.	Comp B2	15.64 ± 0.62
16.	Comp B3	14.47 ± 4.12
17.	Comp B4	13.68 ± 4.22
18.	Comp B5	10.55 ± 1.77
19.	$^{++}$DOX	1.28 ± 1.19

$^{+}IC_{50}$ = Value are the mean of three separate experiment.

$^{++}$DOX = Doxorubicin, use as positive control.

3.2. Structure activity relationship

The substitution of different group on thiadiazole ring showed different anticancer potency gainst breast cancer cell lines (MCF-7). **Figure 2** showed the effect of different substituent's on anticancer potency of synthesized derivatives of thiadiazole.

1. Urea derivatives of thiadiazole showed potent anticancer activity compared to thiourea derivatives.

2. Substitution of electron withdrawing group at R_1 position showed potent anticancer activity.
3. Methyl group at both R_2 and R_3 position showed potent anticancer activity compared to H and other group.
4. Thiadiazole ring are essential for anticancer activity.

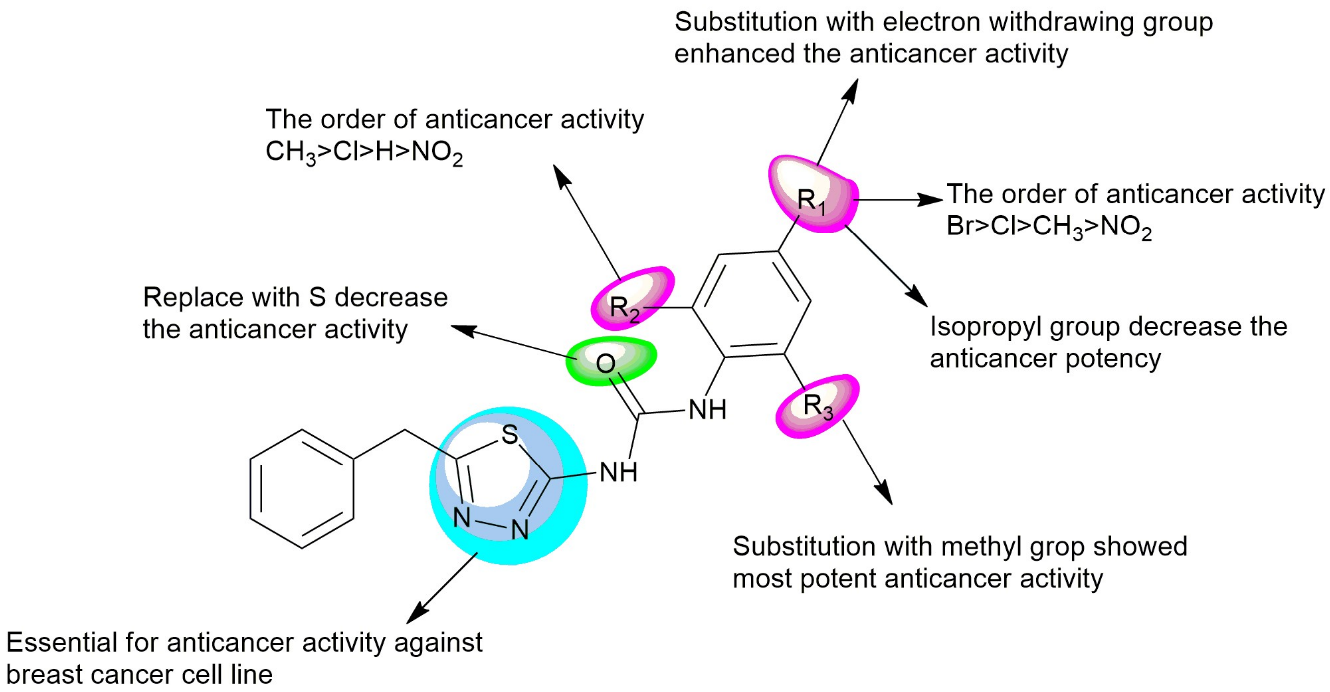


Figure 2: SARs of synthesized thiadiazole derivatives

3.3. ADMET prediction

The prediction ADMET properties of all the synthesized derivatives are shown in **Table 3**. All the thiadiazole derivatives displayed a molecular weight between 340 to 423 g/mol, which indicates that all the synthesized derivatives are within the range and they can be easily distributed within the body. All the compounds showed hydrogen bond acceptor <5 and hydrogen bond donor 2 respectively. All the thiadiazole derivatives showed consensus logP values in the range of 2.74-4.81. All the compounds showed good GIT absorption and no blood–brain barrier permeation. LD_{50} values of all the derivatives are within the range. Overall results of pharmacokinetic property revealed that all the compounds not violated lipinski rules and and can be considered as ‘drug-like’ for further development of a hit or lead molecules.

Table 3: Data obtained from predicted absorption, distribution, metabolism, excretion and toxicity parameters

Comp. No.	MW	No. of HBA	No. of HBD	TPSA	Consensus LogP	GIT Absorption	ESOL class	BBB permeation	BA score	Lipinski violations	LD_{50} (mg/kg)
Comp A1	372.87	3	2	95.15	4.17	High	Moderately soluble	No	0.55	0	2000
Comp A2	358.85	3	2	95.15	3.85	High	Moderately soluble	No	0.55	0	2000
Comp A3	358.85	3	2	95.15	3.85	High	Moderately soluble	No	0.55	0	2580

Comp A4	417.32	3	2	95.15	4.26	High	Moderately soluble	No	0.55	0	2000
Comp A5	403.3	3	2	95.15	3.97	High	Moderately soluble	No	0.55	0	2000
Comp A6	423.71	3	2	95.15	4.14	High	Moderately soluble	No	0.55	0	2580
Comp A7	338.43	3	2	95.15	3.69	High	Moderately soluble	No	0.55	0	2000
Comp A8	324.4	3	2	95.15	3.33	High	Moderately soluble	No	0.55	0	2000
Comp A9	366.48	3	2	95.15	4.32	High	Moderately soluble	No	0.55	0	2000
Comp A10	369.4	5	2	140.97	2.74	Low	Moderately soluble	No	0.55	0	2000
Comp A11	369.4	5	2	140.97	2.78	Low	Moderately soluble	No	0.55	0	2000
Comp A12	354.43	4	2	104.38	3.37	High	Moderately soluble	No	0.55	0	2000
Comp A13	368.45	4	2	104.38	3.51	High	Moderately soluble	No	0.55	0	2000
Comp B1	340.47	2	2	110.17	3.83	High	Moderately soluble	No	0.55	0	2000
Comp B2	374.91	2	2	110.17	4.37	High	Moderately soluble	No	0.55	0	2000
Comp B3	370.49	3	2	119.4	3.77	High	Moderately soluble	No	0.55	0	2000
Comp B4	383.53	2	2	106.51	4.45	High	Moderately soluble	No	0.55	0	3200
Comp B5	382.55	2	2	110.17	4.81	High	Moderately soluble	No	0.55	0	2000

BA: Bioavailability; BBB: Blood–brain barrier; ESOL class: Solubility class in LogS scale; ESOL LogS: ESOL model logarithm of molar solubility in water; HBA: Hydrogen bond acceptor; HBD: Hydrogen bond donor; LogP: Logarithm of the partition coefficient MS: Moderately soluble; MW: Molecular weight; PS: Poorly soluble; TPSA: Topological polar surface area.

4. Conclusion

All the designed derivatives of thiadiazole **(Comp A1-A13 and Comp B1- B5)** were synthesized by suitable scheme and structures of synthesized derivatives were confirmed by FT-IR, ^{1}HNMR, and MASS spectral analysis. The anticancer activity of all the synthesized derivatives was evaluated by *in-vitro* SRB assay against MCF-7 breast cancer cell line and compared the anticancer activity with doxorubicin standard drug. All the derivatives exhibited good to moderate activity against breast cancer cell lines. All the derivatives showed high GIT absorption and no BBB permeation. No derivatives violated the linpinski rule of 5, which indicates that all the derivatives have drug like property and will be used in future as lead molecule. The comp A4 showed highest anticancer activity compared to other derivatives with IC_{50}= 38.59 ± 4.4. The SARs study revealed that the thidiazole ring is essential for anticancer activity and the substitution of electron withdrawing group at R_1 position showed more potent activity against breast cancer cell lines. .

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