4-Halo-*N*-(5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl)benzamide and Benzothioamide Derivatives: Synthesis and *in vitro* Anticancer Assessment

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ABSTRACT: *Cancer is a lethal disorder that has caused a serious threat to human health and nowadays there is a crucial need for the development of novel anticancer agents. A new series of 1,3,4-thiadiazole-based compounds were synthesized and evaluated for anti-cancer properties in vitro. The synthesis of 5-(Trifluoromethyl)-1,3,4-thiadiazol-2-amine (3) was carried out via solvent-free conditions and consequently, benzamide (4a-4f) and benzothioamide (5a-5f) derivatives bearing halogen moieties (Cl, F) were synthesized. MTT assay was applied for in vitro cytotoxicity assessment against three cancerous cell lines consist of PC3 (Prostate cancer), HT-29 (Colon cancer), and SKNMC (Neuroblastoma). All tested derivatives exhibited equal or more (IC_{50} = 3-7 µM) cytotoxic activity than doxorubicin (IC_{50} = 7 µM) as a reference drug against PC3 cell line. Chlorine containing benzamide as well as benzothioamide derivatives (IC_{50} = 14-36 µM) were also exerted a higher cytotoxic activity against SKNMC cell line compared to doxorubicin (IC_{50} = 40 µM).*

KEYWORDS: *Synthesis; 1,3,4-thiadiazole; Anticancer; MTT assay.*

INTRODUCTION

Cancer is a lethal disorder that has caused serious threat to human health. In this disease cells can be aggressive (grow and divide without respect to normal limits), invasive (invade and destroy adjacent tissues) and/or

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metastatic (spread to other locations in body). These three malignant properties of cancer differentiate them from benign tumors, which are self-limited in their growth and do not invade or metastasize [1, 2]. Cancer may affect people at all ages, even fetuses, but risk for the more common varieties tends to increase with age. Cancer causes about 13% of all deaths. According to the American Cancer Society, 7.6 million people died from cancer in the world during 2007. In fact, cancer is the second leading cause of death in the western world. Despite advances in medical procedures for diagnosis and treatment of cancer, overall survival of patients still remains poor. Until recently surgery, chemotherapy, radiotherapy, and endocrine therapy have been the standard treatment options available for patients. This has improved survival in several types of solid tumours, but treatment-related side effects and emergence of drug resistance have been the major causes of morbidity and mortality [3, 4]. Therefore, the development of novel and efficacious anticancer drugs devoid of the mentioned limitations remains a major challenge.

1,3,4-thiadiazole nucleus is the isomer of thiadiazole series and have been widely used by the medicinal chemist in the past to explore its biological activities. The development of 1,3,4-Thiadiazole chemistry is linked to the discovery of phenylhydrazines and hydrazine in the late nineteenth century. The first 1,3,4-thiadiazole was described by *Fischer* in 1882 but the true nature of the ring system was demonstrated first in 1890 by *Freund* and *Kuh* [5]. During recent years a wide and intense investigation of different classes and derivatives of 1,3,4-thiadiazole have been carried out. Many of these derivatives possess interesting biological properties such as antimicrobial, antitubercular, antiviral, antiinflammatory, anticonvulsant, antihypertensive, antioxidant, antifungal and anticancer activity and now there are in the market as common used drugs (Fig. 1) [5-21].

A large numbers of chemical structures containing 1,3,4-thiadiazole pharmacophore have been reported with potential anticancer effects (Fig. 2). Anticancer 1,3,4-thiadiazole-based compounds exert their anticancer activity via several mechanisms like inhibition of protein tyrosine kinases (PTKs), activation of caspase enzymes and induction of apoptosis, inhibition of Cyclin Dependent Kinases (CDKs), inhibition of Carbonic Anhydrase (CA) and etc [22-28]. According to the facts mentioned above and in continuation of our efforts in synthesizing of novel 1,3,4-thiadiazole derivatives as anticancer agents, herein, we report the preparation and *in vitro* cytotoxicity evaluation of two classes of benzamide and benzothioamide derivatives containing 1,3,4-thiadiazole core. In the present research, we investigated the impact of substitution of halogen atoms like fluorine (F) and chlorine (Cl) as electron withdrawing moieties at different positions (*ortho*, *meta*, *para*) of the phenyl ring to exactly study the structure-activity relationships of these compounds. Cytotoxicity of prepared compounds was studied against PC3 (Prostate cancer), HT-29 (Colon cancer) and SKNMC (Neuroblastoma) as cancerous cell lines.

EXPERIMENTAL SECTION

Chemistry

All chemical materials such as starter materials, reagents and solvents were purchased from commercial vendors like Merck and Sigma-Aldrich companies. The purity of the prepared compounds was proved by Thin Layer Chromatography (TLC) using various solvents of different polarities. Merck silica gel 60 F_{254} plates were applied for analytical TLC. Column chromatography was applied on Merck silica gel (70-230 mesh) for purification of intermediate and final compounds. ^{1}H-NMR spectra were recorded using a Bruker 400 spectrometer in deutrated solvents, and chemical shifts are expressed as δ (ppm) with tetramethylsilane (TMS) as internal standard. The IR spectra were obtained on a Shimadzu 470 spectrophotometer using potassium bromide (KBr) disks. Melting points were determined using Electrothermal 9001 elemental analyzer apparatus and are uncorrected. The mass spectra were run on a Finigan TSQ-70 spectrometer (Finigan, USA) at 70 eV. All intermediate and final compounds were prepared according to the Scheme 1.

*Synthesis of 5-(Trifluoromethyl)-1,3,4-thiadiazol-2-amine (**3**)*

In a flat bottom flask 3g (0.03 mmol) of thiosemicarbazide was stirred under solvent free condition and 4.6 mL (0.03 mmol) of trifluoroacetic anhydride was added dropwise via a dropping funnel during 5 minutes. After a vigorous reaction, refluxing conditions was applied for 3 hours at 60-70^{0}C. Continuation of the reaction was done at room temperature

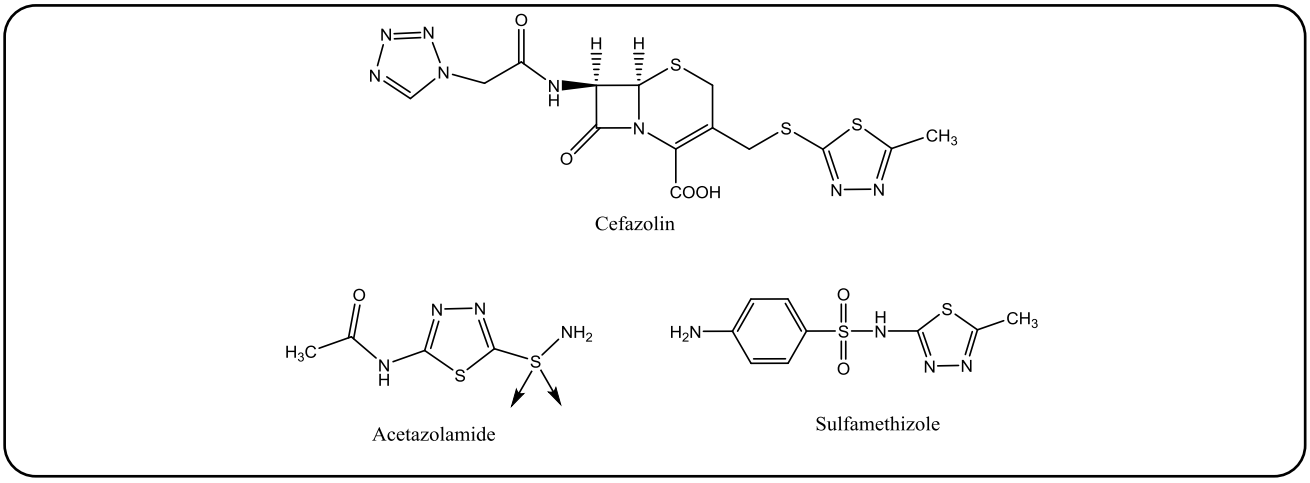


Fig. 1: Current drugs in the market containing 1,3,4-thiadiazole ring; Cefazolin (antibacterial agent, a first generation cephalosporin), acetazolamide (carbonic anhydrase inhibitor, antiglaucoma, anticonvulsant), sulfamethizole (a sulfonamide antibacterial agent).

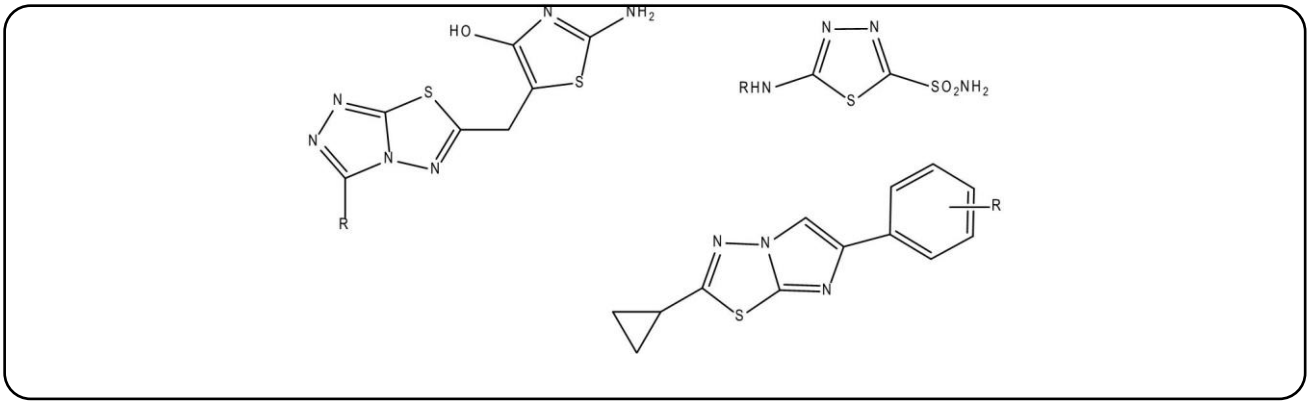
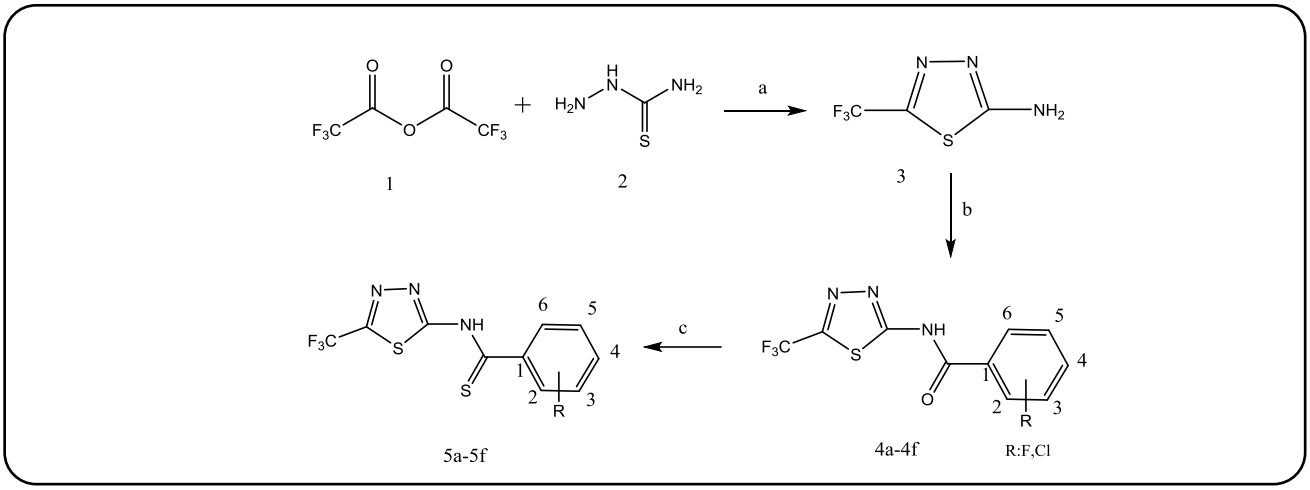


Fig. 2: Structure of some 1,3,4-thiadiazole-based compounds with anticancer activity.



Scheme 1: Synthetic pathway for compounds 4a-4f and Sa-Sf; Reagents and conditions: a) solvent free conditions; b) Benzoic acid derivatives, 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), Hydroxybenzotirazole (HOBt), CH_3CN, rt, 24 h; c) Lawesson's reagent, toluene, reflux, 48 h.

for 1 hour. Work-up procedure was performed by adding cool water to the reaction medium and following alkalinization by ammonia solution. Finally, the formed precipitate was filtered and washed by cool water and recrystallized from ethanol [29].

^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.1 (s, NH_2). IR (KBr, cm^{-1}) ῡ: 3302, 3128, 2958, 2924, 2854, 1639, 1519, 1327, 1192, 1149, 1037, 744, 686, 621. MS (*m/z*, %): 169 (M^+), 69, 60.

*General procedure for synthesis of compounds **4a-4f**:*

According to the scheme 1, equivalent quantities of EDC, HOBt and appropriate benzoic acid derivative were mixed in a flat bottom flask in acetonitrile solvent. The reaction mixture was stirred at room temperature for 30 minutes. Then, equivalent quantity of 5-(Trifluoromethyl)-1,3,4-thiadiazol-2-amine (**3**) was added to the reaction medium and stirring was continued at room temperature for 24 hours. The termination of reaction was approved by Thin Layer Chromatography (TLC). Acetonitrile was evaporated under reduced pressure using rotary evaporator and ethyl acetate/water mixture was added to the residue. The aqueous phase was discarded and the organic phase was washed two times by diluted sulfuric acid (2%), sodium bicarbonate (5%) and brine. The organic layer was dried over anhydrous sodium sulfate. Then, sodium sulfate was removed by filtration and ethyl acetate was evaporated using rotary evaporator apparatus. Column chromatography or crystallization was applied if needed. A white-creamy powder was obtained for all compounds **4a-4f** [30, 31].

2-Chloro-N-(5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl)benzamide (4a)

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.47 (t, 1H, H_4-2-Chlorophenyl), 7.54 (m, 2H, $H_{5,6}$-2-Chlorophenyl), 7.88 (d, *J* = 8 Hz, H_3-2-Chlorophenyl), 12.39 (brs, NH). IR (KBr, cm^{-1}) ῡ: 3111, 3080, 2924, 1723, 1683, 1535, 1489, 1421, 1330, 1301, 1190, 1138, 1037, 900, 742, 706. MS (*m/z*, %): 307 (M^+, Weak), 272 (40), 141 (70), 139 (100), 111 (90), 75 (60), 50 (20).

3-Chloro-N-(5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl)benzamide (4b)

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.53 (t, 1H, *J* = 8 Hz, 3-Chlorophenyl), 7.66 (d, *J* = 8 Hz, 3-Chlorophenyl), 8.18 (m, 3-Chlorophenyl), 12.77 (brs, NH). IR (KBr, cm^{-1}) ῡ: 3145, 3111, 2924, 1732, 1681, 1670, 1533, 1521, 1490, 1458, 1421, 1332, 1303, 1190, 1143, 1037, 744, 705. MS (*m/z*, %): 309 (M^++2, 15), 307 (M^+, 5), 139 (100), 111 (80), 75 (45), 50 (30).

4-Chloro-N-(5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl)benzamide (4c)

^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.68 (d, *J* = 8 Hz, 4-Chlorophenyl), 8.16 (d, *J* = 8 Hz, 4-Chlorophenyl), 13.20 (brs, NH). IR (KBr, cm^{-1}) ῡ: 3149, 3111, 2920, 1723, 1681, 1670, 1533, 1521, 1490, 1458, 1421, 1330, 1303, 1190, 1143, 1037, 740, 705. MS (*m/z*, %): 309 (M^++2, 10), 307 (M^+, 3), 139 (100), 111 (100), 75 (60), 50 (15).

2-Fluoro-N-(5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl)benzamide (4d)

^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.42 (m, 2-Fluorophenyl), 7.55 (t, *J* = 4 Hz, 2-Fluorophenyl), 7.7 (q, 2-Fluorophenyl), 7.82 (t, *J* = 8 Hz, 2-Fluorophenyl), 13.83 (brs, NH). IR (KBr, cm^{-1}) ῡ: 3151, 3111, 2924,1681, 1533, 1489, 1458, 1330, 1303, 1186, 1138, 1035, 900, 740. MS (*m/z*, %): 291 (M^+, 10), 272 (7), 123(100), 95(70), 75 (40).

3-Fluoro-N-(5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl)benzamide (4e)

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.4 (t, H_5-3-Fluorophenyl), 7.59 (t, H_6-3-Fluorophenyl), 7.92 (d, *J* = 8 Hz, H_4-3-Fluorophenyl), 8.08 (d, *J* = 8 Hz, H_4-3-Fluorophenyl). IR (KBr, cm^{-1}) ῡ: 3151, 3111, 2920, 1681, 1521, 1490, 1456, 1328, 1303, 1186, 1138, 1035, 750. MS (*m/z*, %): 291 (M^+, 15), 272 (15), 123(100), 95(50), 75 (45).

4-Fluoro-N-(5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl)benzamide (4f)

^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.97 (t, 2H, *J* = 8 Hz, 4-Flurophenyl), 8.24 (t, 2H, *J* = 8 Hz, 4-Flurophenyl), 13.70 (brs, NH). IR (KBr, cm^{-1}) ῡ: 3155, 3111, 2920, 1680, 1674, 1521, 1490, 1458, 1330, 1303, 1186, 1143, 1037, 747. MS (*m/z*, %): 291 (M^+, 10), 272 (10), 123(100), 95(65), 75 (20).

*General procedure for synthesis of compounds **5a-5f**:*

According to scheme 1, In a flat bottom flask 1 mole of each obtained amide derivative (**4a-4f**) from the

previous step was treated with 1.5 mole of Lawesson's reagent and toluene was added as solvent. The reaction mixture was encountered with reflux conditions for 48 h. The completion of the reaction was proved by Thin Layer Chromatography (TLC). Then, toluene was evaporated by rotary evaporator under reduced pressure and ethyl acetate/water mixture was added to the residue and aqueous phase was removed. The organic layer was washed two times by brine. Ethyl acetate phase was separated and dried over anhydrous sodium sulfate. Filtration was applied for removing the sodium sulfate and ethyl acetate was evaporated under reduced pressure. A yellow-orange powder was obtained for all compounds **5a-5f**. All obtained compounds (**5a-5f**) were washed by diethyl ether and *n*-hexane for removing the impurities [32, 33].

2-Chloro-N-(5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl)benzothioamide (5a)

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.35 (t, 1H, H_4-2-Chlorophenyl), 7.44 (m, 2H, $H_{5,6}$-2-Chlorophenyl), 7.75 (d, *J* = 8 Hz, H_3-2-Chlorophenyl), 12.27 (brs, NH). IR (KBr, cm^{-1}) ῡ: 3429, 2927, 2846, 1597, 1504, 1300, 1261, 1184, 1122, 1026, 929, 810, 759, 717, 609, 524, 497, 447. MS (*m/z*, %): 325 (M^++2, 3), 323 (M^+, 10), 228 (10), 155 (75), 139 (70), 111 (100), 75 (50), 50 (35).

3-Chloro-N-(5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl)benzothioamide (5b)

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.43 (t, 1H, *J* = 8 Hz, H_5-3-Chlorophenyl), 7.66 (d, 1H, *J* = 8 Hz, H_4-3-Chlorophenyl), 7.89 (d, 1H, *J* = 8 Hz, H_6-3-Chlorophenyl), 7.93 (s, 1H, H_2-3-Chlorophenyl). IR (KBr, cm^{-1}) ῡ: 3406, 2920, 1631, 1516, 1423, 1377, 1080, 798, 547. MS (*m/z*, %): 325 (M^++2, 2), 323 (M^+, 7), 228 (20), 155 (65), 139 (90), 111 (100), 75 (70), 50 (25).

4-Chloro-N-(5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl)benzothioamide (5c)

^{1}H NMR ($CDCl_3$, 400 MHz) δ: ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.55 (d, *J* = 8 Hz, 4-Chlorophenyl), 8.04 (d, *J* = 8 Hz, 4-Chlorophenyl), 12.06 (brs, NH). IR (KBr, cm^{-1}) ῡ: 3429, 2924, 2854, 1670, 1597, 1523, 1492, 1404, 1307, 1153, 1099, 1037, 894, 829, 748, 675. MS (*m/z*, %): 325 (M^++2, 5), 323 (M^+, 15), 228 (20), 155 (40), 139 (80), 111 (100), 75 (70), 50 (35).

2-Fluoro-N-(5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl)benzothioamide (5d)

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.29 (m, 1H, 2-Fluorophenyl), 7.41 (t, 1H, 2-Fluorophenyl), 7.7 (m, 1H, 2-Fluorophenyl), 8.22 (m, 1H, 2-Fluorophenyl). IR (KBr, cm^{-1}) ῡ: 3157, 3111, 2920, 1681, 1521, 1490, 1458, 1328, 1305, 1186, 1145, 1037, 750. MS (*m/z*, %): 307 (M^+, 5), 301 (20), 282 (20), 245 (60), 226 (30), 135 (20), 123 (100), 113 (20), 107 (75), 95 (90), 91 (55)75 (40), 63 (40), 52 (30).

3-Fluoro-N-(5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl)benzothioamide (5e)

^{1}H NMR (DMSO-d_6, 400 MHz) δ: 6.99 (q, 1H, 3-Fluorophenyl), 7.6 (m, 2H, 3-Fluorophenyl), 7.97 (t, 1H, 3-Fluorophenyl). IR (KBr, cm^{-1}) ῡ: 3151, 3111, 2924, 1680, 1521, 1490, 1458, 1328, 1303, 1186, 1143, 1037, 745. MS (*m/z*, %): 307 (M^+, 15), 282 (25), 245 (35), 226 (40), 123 (100), 107 (55), 95 (80), 91 (35), 75 (40), 63 (50), 52 (20).

4-Fluoro-N-(5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl)benzothioamide (5f)

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.24 (t, 2H, 4-Fluorophenyl), 8.31 (t, 2H, 4-Fluorophenyl). IR (KBr, cm^{-1}) ῡ: 3429, 3213, 3174, 2924, 2854, 1674, 1600, 1504, 1330, 1303, 1249, 1161, 1103, 1037, 933, 898, 856, 829, 802, 752, 536, 470. MS (*m/z*, %): 307 (M^+, 10), 301 (30), 245 (50), 226 (20), 135 (30), 123 (100), 113 (15), 107 (55), 95 (70), 91 (35), 75 (30), 63 (40), 52 (35).

Cytotoxicity assessment

All synthesized derivatives (compounds **4a-4f** and **5a-5f**) were tested for cytotoxic activity at 0.1-250 mcg/ml concentration in three human cancer cell lines of PC3 (Prostate cancer), HT-29 (Colon cancer) and SKNMC (Neuroblastoma). Cells from different cell lines were seeded in 96-well plates at the density of 8000-10,000 viable cells per well and incubated for 48 hours to allow cell attachment. The cells were then incubated for another 48-96 hours (depends to cell cycle of each cell line) with various concentrations of compounds **4a-4f** and **5a-5f**.

Cells were then washed in PBS, and 20 µL of MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide solution (5 mg/mL) were added to each well. An additional 4 hours of incubation at 37°C were done, and then the medium was discarded. Dimethyl sulfoxide (60 µL) was added to each well, and the solution was vigorously mixed to dissolve the purple tetrazolium crystals. The absorbance of each well was measured by plate reader (Anthous 2020; Austria) at a test wavelength of 550 nm against a standard reference solution at 690 nm. The amount of produced purple formazan is proportional to the number of viable cells [30].

RESULTS AND DISCUSSION

Chemistry

According to the Table 1, all intermediate and final compounds were prepared with 22-76% yield. Compound 3 was prepared with accordance to the literature and to some extent a modified procedure as reported herein [29] Solvent free condition was an advantage for performing this reaction and modification of the conditions improve the obtained yield. Easy work-up with ice water and following alkalinization with ammonia was an extra advantage of this reaction. Consequent amidation of compound 3 with various benzoic acid derivatives bearing chlorine and fluorine substituents at various positions on the phenyl ring were done through a clean reaction. EDC as a coupling reagents and HOBt as an additive substance helped us to perform a direct coupling of various benzoic acid derivatives with compound 3. The reaction was feasible at room temperature and in acetonitrile solvent. Obtained amide derivatives (**4a-4f**) was tested against cancerous cell lines and also reacted with Lawesson's reagent for preparation of compounds **5a-5f**. Thionation of compounds **4a-4f** was needed a long reflux conditions (24 hours) in toluene solvent.

Amidic and thioamidic derivatives were afforded a white and yellow powder respectively. Compound **3** was also recorded as a creamy-white powder. Melting point related to each compound was measured by electrothermal 9001 melting point analyser using open capillary tubes. All compounds rendered a sharp melting point except for compound 5c (mp. 228-230˚C). n-Hexane and diethyl ether (Et_2O) were applied for trituration of synthesized compounds. All compounds were characterized by ^{1}HNMR, IR and MS spectroscopic methods. Proton signal related to the hydrogen atom of NH group was not observed in some cases. Electron withdrawing effects of the neighboring carbonyl group as well as the trifluoromethyl-1,3,4-thiadiazole group enhanced the acidic property of the hydrogen atom of the NH moiety and caused an upfield signaling with low or no integration. According to the presence of chlorine atom in some derivatives, a M^++2 peak was recorded in MS spectra interpretation.

Cytotoxicity assay

According to the Table 2, all synthesized compounds **4a-4f** and **5a-5f** were tested against three cancerous cell lines consist PC3 (Prostate cancer), HT-29 (Colon cancer) and SKNMC (Neuroblastoma). The obtained results were expressed as IC50 (µM) compared to doxorubicin as a standard anticancer drug. Totally, PC3 cell line showed more sensitivity to all compounds. All tested derivatives exhibited equal or more cytotoxic activity than doxorubicin as reference drug against PC3 cell line. Compound **5b** rendered the highest anticancer activity towards PC3 cell line with IC50 = 3 µM. In fact, in this compound meta substitution of chlorine on the phenyl ring in thioamide derivatives enhanced the cytotoxic potency significantly against this cell line. Comparison between amide derivatives with chlorine moiety (compounds **4a-4c**) and amide derivatives with fluorine moiety (compounds **4d-4f**) demonstrated that chlorine could better enhance the anticancer effect than fluorine in amidic series. It is notable to state that a similar trend was also observed for thioamidic derivatives (**5a-5f**). In this series the derivatives **5a-5c** with chlorine substituent exerted better activity than derivatives 5d-5f with fluorine substituent. Although, a significant difference was observed in cytotoxic effect among the types of moiety (F or Cl) on the phenyl ring, did not seen any impact about the variation of the position of the related moiety. Approximately, all positions of the phenyl ring (ortho, meta, para) showed a similar potency for a distinct moiety. It means that movement of a distinct substituent from one position of the phenyl ring to the other positions could not affect the cytotoxic effect significantly.

Comparison of current halogenated compounds with previously reported benzamide congeners demonstrated that these derivatives have more cytotoxic effects [34] The halogenated derivatives that presented here caused

Table 1: Physicochemical properties of synthesized compounds 4a-4f and 5a-5f.

Compound	R	X	Yield(%)	MW(g/mol)	Closed Formula	Melting Point(°C)
3	-	-	73	169	C3H2F3N3S	224
4a	2-Cl	O	22	307	C10H5ClF3N3OS	162
4b	3-Cl	O	58	307	C10H5ClF3N3OS	170
4c	4-Cl	O	76	307	C10H5ClF3N3OS	249
4d	2-F	O	31	291	C10H5F4N3OS	155
4e	3-F	O	43	291	C10H5F4N3OS	188
4f	4-F	O	46	291	C10H5F4N3OS	188
5a	2-Cl	S	73	323	C10H5ClF3N3S2	110
5b	3-Cl	S	33	323	C10H5ClF3N3S2	125
5c	4-Cl	S	76	323	C10H5ClF3N3S2	229
5d	2-F	S	48	307	C10H5F4N3S2	164
5e	3-F	S	61	307	C10H5F4N3S2	215
5f	4-F	S	44	307	C10H5F4N3S2	189

Table 2: Cytotoxicity results (IC50, µM) of compounds 4a-4f and 5a-5f against cancerous cell lines. Compounds with higher activity than doxorubicin (reference drug) were highlighted.

Compound	R	X	PC3	HT-29	SKNMC
4a	2-Cl	O	5.12	8.12	23.21
4b	3-Cl	O	5.20	10.23	24.17
4c	4-Cl	O	4.86	19.09	36.84
4d	2-F	O	6.23	8.54	52.76
4e	3-F	O	7.07	9.32	27.32
4f	4-F	O	7.31	164.21	57.01
5a	2-Cl	S	4.43	9.43	29.25
5b	3-Cl	S	3.13	63.86	15.16
5c	4-Cl	S	4.87	13.21	14.12
5d	2-F	S	5.94	25.90	9.73
5e	3-F	S	6.25	208.37	45.18
5f	4-F	S	7.65	10.16	41.53
Doxorubicin	-	-	7.84	8.74	40.24

superior cytotoxicity than former one especially towards PC3 cell line. In the other words, replacement of the nitro and methoxy moieties with chlorine as well as fluorine enhanced the anticancer potency *in vitro*. Furthermore, a beneficial impact was also observed against the rest of cell lines namely HT-29 and SKNMC.

Utilization of the benzamide residue on position 2 of the 1,3,4-thiadiazole ring instead of phenylacetamide was also a positive change that concluded in this research. Replacement of the phenylacetamide residue that applied in the previous project with benzamide residue led to a significant and outstanding increment in the cytotoxic activity [7].

CONCLUSIONS

A new series of 1,3,4-thiadiazole-based benzamide and benzothioamide compounds were synthesized and their anticancer effects were evaluated in vitro. The first synthetic step of compound **3** as solvent free reaction fascilitated the achievement to the final derivatives. All target derivatives were prepared with an acceptable yield. Amidic derivatives (**4a-4f**) afforded higher yield in synthesis compared to thioamidic congeners (5a-5f). Thionation reaction caused a remarkable decline in the total yield. Three cancerous cell lines (PC3, HT-29, SKNMC) were applied and related results were presented as IC50 (μM). All tested derivatives exhibited equal or more (IC50 = 3-7 μM) cytotoxic activity than doxorubicin (IC50 = 7 μM) as reference drug against PC3 cell line. Compound 5b with meta chlorine substituent demonstrated the best anticancer activity with IC50 = 3 μM against PC3 cell line. Chlorine containing benzamide as well as benzothioamide derivatives (IC50 = 14-36 μM) were also exerted a higher cytotoxic activity against SKNMC cell line compared to doxorubicin (IC50 = 40 μM). The study of structure activity relationships revealed that chlorine moiety can be better improved the cytotoxic activity in both amidic series (4a-4f) and thioamidic series (5a-5f) in comparison with fluorine moiety. Further investigation was also showed that there is not any significant difference in the view of potency between different positions of the phenyl ring. Unfortunately, none of the synthesized compounds showed higher IC50 than doxorubicin in HT-29 cell line. Only compounds **4a** (o-Chlorine) and 4d (o-Fluorine) showed equal potency with doxorubicin in this cell line. Totally, synthesized compounds 4a-4f as well as **5a-5f** in the current survey can be proposed as potential anticancer lead compounds especially for prostate cancer and neuroblastoma. But further explorations such as in vivo tests are needed to prove this statement.

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REFERENCES

[1] Bhuva H.A., Kini S.G., Synthesis, Anticancer Activity and Docking of some Substituted Benzothiazoles as Tyrosine Kinase Inhibitors, J. *Mol. Graph. Model.*, **29(1)**: 32-37 (2010).

[2] Kumar D., Patel G., Johnson E.O., Shah K., Synthesis and Anticancer Activities of Novel 3, 5-Disubstituted-1, 2, 4-Oxadiazoles, *Bioorg. Med. Chem. Lett.*, **19(10)**: 2739-2741 (2009).

[3] Porter A.C., Vaillancourt R.R., Tyrosine Kinase Receptor-Activated Signal Transduction Pathways Which Lead to Oncogenesis, *Oncogene.*, **17(11)**: 1343 (1998).

[4] Madhusudan S., Ganesan T.S., Tyrosine Kinase Inhibitors in Cancer Therapy, *Clin Biochem*, **37(7)**: 618-635 (2004).

[5] Gupta J.K., Yadav R.K., Dudhe R., Sharma P.K., Recent Advancements in the Synthesis and Pharmacological Evaluation of Substituted 1, 3, 4-Thiadiazole Derivatives. *Int. J. Pharm. Tech. Res.*, **2(2)**: 1493-1507 (2010).

[6] Kumar D., Vaddula B.R., Chang K-H., Shah K., One-Pot Synthesis and Anticancer Studies of 2-Arylamino-5-Aryl-1, 3, 4-Thiadiazoles, *Bioorg. Med. Chem. Lett.*, **21(8)**: 2320-2323 (2010).

[7] Hosseinzadeh L, Khorand A, Aliabadi A. Discovery of 2-Phenyl-N-(5-(Trifluoromethyl)-1, 3, 4-Thiadiazol-2-Yl) Acetamide Derivatives as Apoptosis Inducers *via* the Caspase Pathway with Potential Anticancer Activity, *Arch. Pharm.*, **346**: 812-818 (2013).

[8] Rajak H., Deshmukh R., Aggarwal N., Kashaw S., Kharya M.D., Mishra P. Synthesis of Novel 2, 5-Disubstituted 1, 3, 4-Thiadiazoles for Their Potential Anticonvulsant Activity: Pharmacophoric Model Studies, *Arch. Pharm.*, **342(8)**: 453-461 (2009).

[9] Abdel-Aziz M., Aly O.M., Khan S.S., Mukherjee K., Bane S., Synthesis, Cytotoxic Properties And Tubulin Polymerization Inhibitory Activity of Novel 2-Pyrazoline Derivatives, *Arch. Pharm.*, **345(7)**: 535-548 (2012).

[10] Deng X.Q., Dong Z.Q., Song M.X., Shu B., Wang S.B., Quan Z.S., Synthesis and Anticonvulsant Activities of some Triazolothiadiazole Derivatives, *Arch. Pharm.*, **345(7)**: 565-573 (2012).

[11] Rostom S.A., Badr M.H., Abd El Razik H.A., Ashour H., Abdel Wahab A.E., Synthesis of Some Pyrazolines and Pyrimidines Derived from Polymethoxy Chalcones As Anticancer and Antimicrobial Agents, *Arch. Pharm.*, **344(9)**: 572-587 (2011).

[12] Mishra G., Singh A.K., Jyoti K., Review Article on 1, 3, 4-Thiadiazole Derivatives And its Pharmacological Activities, *Int. J. Chem. Tech. Res.*, **3**: 1380-1393 (2011).

[13] Singh A.K., Mishra G., Jyoti K., Review on Biological Activities of 1, 3, 4-Thiadiazole Derivatives, *J. App. Pharm. Sci.*, **5**: 44-49 (2011).

[14] Kharb R., Kaur P., Sharma P.C., Yar M.S., Significance of Thiadiazole Derivatives as Antimicrobial Agents, *Int. J. Res. Pharm. Biomed. Sci.*, **2(4)**: 1520-1540 (2011).

[15] Kalidhar U., Kaur A., 1, 3, 4-Thiadiazole Derivatives and Their Biological Activities: A Review, *Res. J. Pharm. Biol. Chem.*, **4**: 1091-1106 (2011).

[16] Siddiqui N., Ahuja P., Ahsan W., Pandeya S., Alam M.S., Thiadiazoles: Progress Report on Biological Activities, *J. Chem. Pharm. Res.*, **1(1)**: 19-30 (2009).

[17] Farshori N.N., Banday M.R., Ahmad A., Khan A.U., Rauf A., Synthesis, Characterization, and *in Vitro* Antimicrobial Activities of 5-Alkenyl/Hydroxyalkenyl-2-Phenylamine-1, 3, 4-Oxadiazoles and Thiadiazoles, *Bioorg. Med. Chem. Lett.*, **20(10)**: 1933-1938 (2010).

[18] Pattan S., Kekare P., Dighe N., Nirmal S., Musmade D., Parjane S., Daithankar A., Synthesis and Biological Evaluation of Some 1, 3, 4-Thiadiazoles, *J. Chem. Pharm. Res.*, **1(1)**: 191-198 (2009).

[19] Talath S., Gadad A., Synthesis, Antibacterial and Antitubercular Activities of Some 7-[4-(5-Amino-[1, 3, 4] Thiadiazole-2-Sulfonyl)-Piperazin-1-Yl] Fluoroquinolonic Derivatives, *Eur. J. Med. Chem.*, **41(8)**: 918-924 (2006).

[20] Jatav V., Mishra P., Kashaw S., Stables J., CNS Depressant and Anticonvulsant Activities of Some Novel 3-[5-Substituted 1, 3, 4-Thiadiazole-2-Yl]-2-Styryl Quinazoline-4 (3H)-Ones, *Eur. J. Med. Chem.*, **43(9)**: 1945-1954 (2008).

[21] Almajan G.L, Barbuceanu S-F., Bancescu G., Saramet I., Saramet G., Draghici C., Synthesis and Antimicrobial Evaluation of some Fused Heterocyclic [1, 2, 4] Triazolo [3, 4-B][1, 3, 4] Thiadiazole Derivatives, *Eur. J. Med. Chem.*, **45(12)**: 6139-6146 (2010).

[22] Noolvi M.N., Patel H.M., Singh N., Gadad A.K., Cameotra S.S., Badiger A., Synthesisand Anticancer Evaluation of Novel 2-Cyclopropylimidazo [2, 1-B] [1, 3, 4]-Thiadiazole Derivatives, *Eur. J. Med. Chem.*, **46(9)**: 4411-4418 (2011).

[23] Supuran C.T., Scozzafava A., Carbonic Anhydrase Inhibitors-Part 94. 1, 3, 4-Thiadiazole-2-Sulfonamide Derivatives as Antitumor Agents?, *Eur. J. Med. Chem.*, **35(9)**:867-874 (2009).

[24] Ibrahim D., Synthesis And Biological Evaluation of 3, 6-Disubstituted [1, 2, 4] Triazolo [3, 4-B][1, 3, 4] Thiadiazole Derivatives as a Novel Class of Potential Anti-Tumor Agents, *Eur. J. Med. Chem.*, **44(7)**: 2776-2781(2009).

[25] Radi M., Crespan E., Botta G., Falchi F., Maga G., Manetti F., Corradi V., Mancini M., Santucci M.A., Schenone S., Botta M., Discovery and SAR of 1, 3, 4-Thiadiazole Derivatives as Potent Abl Tyrosine Kinase Inhibitors and Cytodifferentiating Agents, *Bioorg. Med. Chem. Lett.*, **18(3)**: 1207-1211 (2008).

[26]Yang X-H., Wen Q., Zhao T-T., Sun J., Li X., Xing M., Lu X., Zhu H.-L., Synthesis, Biological Evaluation, And Molecular Docking Studies of Cinnamic Acyl 1, 3, 4-Thiadiazole Amide Derivatives as Novel Antitubulin Agents, *Bioorg. Med. Chem.*, **20(3)**: 1181-1187 (2012).

[27] Sun J., Yang Y-S., Li W., Zhang Y-B., Wang X-L., Tang J-F., Zhu H.L., Synthesis, Biological Evaluation and Molecular Docking Studies of 1, 3, 4-Thiadiazole Derivatives Containing 1, 4-Benzodioxan as Potential Antitumor Agents, *Bioorg. Med. Chem. Lett.*, **21**(20): 6116-6121 (2011).

[28] Matysiak J., Opolski A., Synthesis and Antiproliferative Activity of N-Substituted 2-Amino-5-(2, 4-Dihydroxyphenyl)-1, 3, 4-Thiadiazoles, *Bioorg. Med. Chem.*, **14**(13): 4483-4489 (2006).

[29] Lalezari I., Sharghi N., Synthesis Of 1, 3, 4-Thiadiazoles Containing the Trifluoromethyl Group, *J. Heterocycl. Chem.*, **3**(3): 336-337 (1966).

[30] Aliabadi A., Shamsa F., Ostad S.N., Emami S., Shafiee A., Davoodi J., Et Al., Synthesis and Biological Evaluation of 2-Phenylthiazole-4-Carboxamide Derivatives as Anticancer Agents, *Eur. J. Med. Chem.*, **45**(11): 5384-5389 (2011).

[31] Aliabadi A., Andisheh S., Tayarani-Najaran Z., Tayarani-Najaran M., 2-(4-Fluorophenyl)-N-Phenylacetamide Derivatives As Anticancer Agents: Synthesis and in-Vitro Cytotoxicity Evaluation, *Iran. J. Pharm. Res.*, **12(3)**: 267-271 (2013).

[32] Nishio T., Sekiguchi H., Thionation of Ω-Hydroxy Amides with Lawesson's Reagent: Synthesis of Thioenamides and Sulfur-Containing Heterocycles, Tetrahedron., **55(16)**: 5017-5026 (1999).

[33] Burke M.J., Trantow B.M., An Efficient Route to 3-Aminoindazoles and 3-Amino-7-Azaindazoles, *Tetrahedron. Lett.*, **49(31)**: 4579-4581 (2008).

[34] Aliabadi A., Afnanzade N., Hosseinzadeh L., Mohammadi-Farani A., Shafiee M.H., Nazari H., Ahmadi F., Foroumadi A., N-(5-(Trifluoromethyl)-1,3,4-Thiadiazol-2-Yl)Benzamide and Benzothioamide Derivatives Induce Apoptosis *via* Caspase-Dependent Pathway, *Pharm. Chem. J.*, **53**(6): 488-493 (2019).