Full Paper

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

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In the current research of medicinal chemistry, apoptosis induction is one of the novel strategies for the development and discovery of novel anticancer therapeutics. In the present study, a new series of 1,3,4-thiadiazole derivatives (**4a–4p**) were synthesized and their *in vitro* anticancer activities were evaluated against three cancer cell lines: PC3 (prostate cancer), MCF7 (breast cancer), and SKNMC (neuroblastoma). These cell lines were utilized in MTT assays and the obtained results were compared to doxorubicin. Apoptosis induction was also investigated through exploration of the activation of caspases 3, 8, and 9. According to the obtained results, compounds **4b** (3-Cl) and **4c** (4-Cl) demonstrated the best caspase activation. In fact, compounds **4b** and **4c** enhanced the activity of caspases 3 and 9 in the MCF7 cell line.

Keywords: Anticancer / Apoptosis / Caspase enzymes / Synthesis / 1,3,4-Thiadiazole

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Introduction

Cancer is a major global problem and is the second leading cause of death in developed countries. Since many of the current pharmacotherapeutic methods have problems with toxicity and drug resistance, there is a strong demand for the discovery and development of effective new anticancer drugs [1]. Chemotherapy involves the use of low-molecular-weight drugs (small molecules such as cyclophosphamide, capecitabine, etoposide, etc.) to selectively destroy tumor cells or at least prevent their proliferation or metastasis. The research for identification and discovery of novel chemical structures that can act as more effective and reliable anticancer agents is still a major challenge to medicinal chemists. Despite the important advances achieved over recent decades in the research and development of various anticancer drugs, current anticancer drugs still have major limitations such as drug resistance, lack of selectivity, and unwanted side effects (bone marrow depression, nausea, vomiting, infertility, neuropathy, etc.) [2–7].

Apoptosis or programmed cell death plays an important role in promoting tissue homeostasis by eliminating damaged or excessive cells. Defects in apoptosis signaling pathways could result in uncontrolled tumor cell growth as well as resistance to cancer treatment. Therefore, restoration of normal apoptosis or promotion of apoptosis could lead to cancer cell death as well as increase the response to chemotherapeutics. In addition, it is known that many chemotherapeutics kill cancer cells through the induction of apoptosis [8]. It is known that the anti-tumor efficacy of many chemotherapeutic agents is associated with their ability to induce apoptosis. Novel approaches in medicinal chemistry for design of effective anticancer agents are focused on apoptosis induction in cancer cells. In fact, targeting regulators of apoptosis could lead to the development of new anticancer treatments. In addition, overcoming tumor resistance is another advantage of these new agents over conventional anti-cancer drugs [9].

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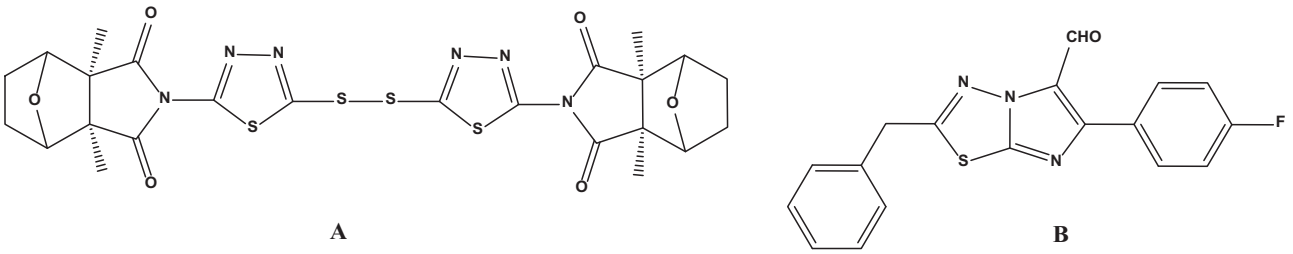


Figure 1. Structures of two 1,3,4-thiadiazole-based compounds as apoptotic inducers.

During recent years a deep and vast exploration of different pharmacophores and chemical structure containing 1,3,4-thiadiazole ring has been carried out. Many of these derivatives exhibited interesting and potential biological effects such as antimicrobial, antitubercular, antiviral, antiinflammatory, anticonvulsant, antihypertensive, antioxidant, antifungal, and anticancer activity and now they are on the market as commonly used drugs [10–19].

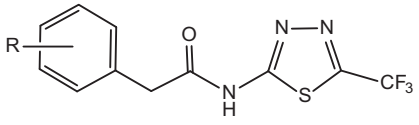
Diverse chemical structures containing the 1,3,4-thiadiazole nucleus have been reported with potential anticancer activity and as apoptotic inducers (Fig. 1). The 1,3,4-thiadiazole ring in anticancer agents performs its role in pharmacophores of apoptosis inducers and caspase activators, tyrosine kinase inhibitors, carbonic anhydrase inhibitors, etc. [20–28]. Hence, various mechanisms could be imagined for anticancer chemical structures containing the 1,3,4-thiadiazole ring.

Results and discussion

Structure–activity relationship (SAR)

A new series of 1,3,4-thiadiazole derivatives were synthesized and their *in vitro* anticancer activities were evaluated against three cancerous cell lines (Tables 1 and 2), namely, PC3 (prostate cancer), MCF7 (breast cancer), and SKNMC (neuroblastoma). These cell lines were utilized to test these compounds using MTT assay. Totally, none of the tested compounds **4a–4p** showed significant potency toward these cell lines. Various moieties with different electronic properties were applied to investigate the role of the electron-withdrawing and electron-donating activity of the corresponding substituents. Chlorine (Cl), fluorine (F), bromine (Br), trifluoromethyl (CF_3), and nitro (NO_2) groups were introduced on the phenyl ring as electron-withdrawing

Table 1. Cytotoxicity results (IC_{50}, $\mu M \pm SD$) of compounds **4a–4p**.



Compound	R	PC3	MCF7	SKNMC
4a	2-Cl	>300	>300	>300
4b	3-Cl	>300	49.5 ± 6.001	>300
4c	4-Cl	>300	56.2 ± 1.30	>300
4d	2-F	90 ± 8.13	>300	90 ± 4.33
4e	3-F	65 ± 0.97	240 ± 11.19	63 ± 1.08
4f	4-F	100 ± 1.45	>300	>300
4g	2-OCH_3	>300	119 ± 3.07	>300
4h	3-OCH_3	107 ± 9.50	80 ± 4.62	>300
4i	4-OCH_3	210 ± 6.69	>300	>300
4j	2-OH	>300	>300	140 ± 14.03
4k	3-OH	>300	>300	105 ± 6.20
4l	2-NO_2	152 ± 5.71	100 ± 9.35	70 ± 6.72
4m	4-NO_2	>300	125 ± 8.61	80 ± 1.91
4n	2-CH_3	>300	>300	61 ± 4.45
4o	4-Br	38 ± 5.07	>300	>300
4p	4-CF_3	>300	>300	75 ± 2.34
Doxorubicin	–	3.7 ± 0.5	7.2 ± 0.4	1 ± 0.03

Table 2. Properties of compounds **4a–4p**.

Compound	R	Molecular formula	Molecular weight (g/mol)	Yield (%)	Melting point (°C)
4a	2-Cl	$C_{11}H_7ClF_3N_3OS$	321.7	30	157
4b	3-Cl	$C_{11}H_7ClF_3N_3OS$	321.7	37	152
4c	4-Cl	$C_{11}H_7ClF_3N_3OS$	321.7	48	199
4d	2-F	$C_{11}H_7F_4N_3OS$	305.3	53	135
4e	3-F	$C_{11}H_7F_4N_3OS$	305.3	44	130
4f	4-F	$C_{11}H_7F_4N_3OS$	305.3	43	169
4g	2-CH_3O	$C_{12}H_{10}F_3N_3O_2S$	317.3	52	161
4h	3-CH_3O	$C_{12}H_{10}F_3N_3O_2S$	317.3	59	140
4i	4-CH_3O	$C_{12}H_{10}F_3N_3O_2S$	317.3	49	158–161
4j	2-OH	$C_{11}H_8F_3N_3O_2S$	303.3	53	142–145
4k	3-OH	$C_{11}H_8F_3N_3O_2S$	303.3	61	170–173
4l	2-NO_2	$C_{11}H_7F_3N_4O_3S$	322.3	43	195
4m	4-NO_2	$C_{11}H_7F_3N_4O_3S$	322.3	48	220
4n	2-CH_3	$C_{12}H_{10}F_3N_3OS$	301.3	87	140
4o	4-Br	$C_{11}H_7BrF_3N_3OS$	366.2	48	196
4p	4-CF_3	$C_{12}H_7F_6N_3OS$	355.3	62	198

moieties. On the other hand, electron-donating moieties such as hydroxyl (OH), methoxy (OCH_3), and methyl (CH_3) were also substituted to improve the cytotoxic effects. Chlorine moiety was applied at different positions of the phenyl ring. Chlorine moiety caused the compounds **4b** ($IC_{50} = 49.5\,\mu M$) and **4c** ($IC_{50} = 56.2\,\mu M$) to exert an acceptable activity against MCF7 cell line. *Ortho* positioning of the chlorine moiety was quite detrimental for cytotoxic potency. Replacement of the chlorine moiety with fluorine atom enhanced the anticancer activity especially toward SKNMC cell line. *Meta* positioning of the fluorine atom was so beneficial for *in vitro* anticancer activity as observed in compound **4e**. A moderate activity was rendered with this compound against SKNMC cell line ($IC_{50} = 63\,\mu M$). Probably, the increase in electron-withdrawing activity of the moiety is beneficial for activity. Utilization of methoxy group as an electron-donating moiety did not cause a favorable enhancement in potency. Only compound **4h** (*meta* methoxy) demonstrated a moderate cytotoxic activity toward MCF7 cell line ($IC_{50} = 80\,\mu M$). Increasing the electron-donating activity of the corresponding moiety was detrimental for anticancer activity. In other words, replacement of the methoxy group with hydroxyl moiety decreased the cytotoxic effects. Compounds **4j** (*ortho* hydroxyl, $IC_{50} = 140\,\mu M$) and **4k** (*meta* hydroxy, $IC_{50} = 105\,\mu M$) showed a weak activity against SKNMC cell line only. Nitro substituent at positions *ortho* (compound **4l**) and *para* (compound **4m**) of the phenyl ring also caused a weak potency against tested compounds. Introduction of the methyl group (compound **4n**) as a weak electron-donating and also lipophilic moiety increased the potency against SKNMC cell line ($IC_{50} = 61\,\mu M$). In fact, compound **4n** was the most active compound against SKNMC cell line. Compound **4o** with *para* bromine substituent was the most potent compound compared to other synthesized derivatives in this series against PC3 cell line ($IC_{50} = 38\,\mu M$). Compound **4p** with *para* trifluoro (p-CF_3) moiety was not so active against the examined cell lines.

Caspase activity

Compounds **4b** and **4c** were evaluated by caspase activation assay in MCF7 cell line. In fact, the ability of these compounds was explored toward activation of caspases 3, 8, and 9. According to Fig. 2, a remarkable activation of caspase enzymes was recorded for both compounds **4b** and **4c** especially caspases 3 and 9. Compound **4b** (3-Cl) could cause a better activation of caspase 3 as well as caspase 9. Compounds **4b** and **4c** did not render a significant activation of caspase 8. According to the obtained results, it is obvious that compounds **4b** and **4c** could be an apoptosis inducer via intrinsic pathway. The obtained results of investigation about the activation of caspase 8 implied that compounds **4b** and **4c** do not have enough ability to activate caspase 8. In other words, these compounds could not induce the apoptosis through the extrinsic pathway.

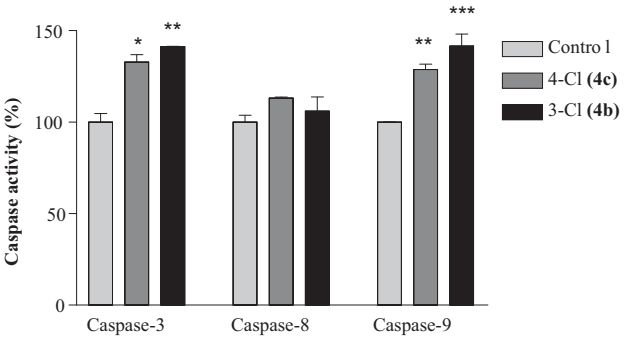
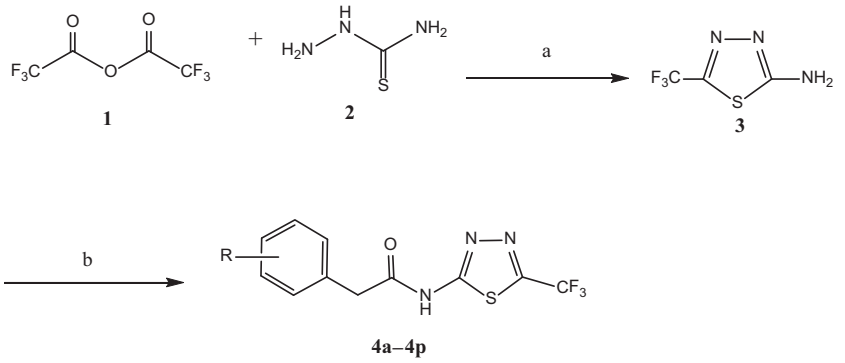


Figure 2. Caspase activation of compounds **4b** (3-Cl) and **4c** (4-Cl).



Scheme 1. Synthetic pathway of compounds **4a–4p**. Reagents and conditions: (a) solvent free condition, rt, 5 h; (b) phenylacetic acid derivatives, EDC, HOBt, CH_3CN, rt, 24 h.

Experimental

Chemistry

All chemical substances such as starter materials, reagents, and solvents were purchased from commercial suppliers 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 performed on Merck silica gel (70–230 mesh) for purification of intermediate and final compounds. ^{1}H NMR spectra were recorded using a Varian 400 spectrometer, and chemical shifts are expressed as δ (ppm) with tetramethylsilane (TMS) as internal standard. The IR spectra were obtained on a Shimadzu 470 spectrophotometer (potassium bromide disks). Melting points were determined using an elemental analyzer apparatus and are uncorrected. The mass spectra were run on a Finigan TSQ-70 spectrometer (Finigan, USA) at 70 eV. All cell lines were purchased from Pasteur Institute of Iran. All intermediate and final compounds were prepared according to Scheme 1.

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

In a flat bottom flask, 3 g (0.03 mmol) of thiosemicarbazide was stirred under solvent-free conditions and 4.6 mL (0.03 mmol) of trifluoroacetic anhydride was added dropwise via a dropping funnel during 5 min. After a vigorous reaction, refluxing condition was applied for 3 h in an oil bath and the temperature was adjusted at 60–70°C. Continuation of the reaction was done at room temperature for 1 h. 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 with cool water and recrystallized from ethanol [29].

M.p. 224°C, yield: 73%, IR (KBr, cm^{-1}) $\bar{\upsilon}$ 3302 (N–H, stretch, asymmetric), 3128 (N–H, stretch, symmetric), 2958 (C–H, asymmetric, aliphatic), 2924 (C–H, asymmetric, aliphatic), 2854 (C–H, symmetric, aliphatic), 1639, 1519 (N–H, bend), 1327, 1192, 1149, 1037, 744, 686, 621. MS (*m*/*z*, %): 169 (M^+, 100), 166 (8), 168 (45), 127 (5), 113 (10), 104 (12), 91 (10), 74 (30), 69 (65), 68 (35), 60 (50), 59 (35).

*General procedure for the synthesis of compounds **4a–4p***

All compounds **4a–4p** (Table 2) were synthesized according to the Scheme 1. In a flat bottom flask, equimolar quantities of *N*-ethyl-*N*-dimethylaminopropyl carbodiimide (EDC), hydroxybenzotriazole (HOBt), and appropriate phenylacetic acid derivative were stirred in acetonitrile (CHCN) for 30 min. Then, equivalent amount of compound **3** derivative was added and stirring was continued for 24 h. The progress of the reaction was checked using TLC. After completion, the solvent was evaporated under reduced pressure and equal portions of water and ethyl acetate were added to the residue. The aqueous phase was removed and the organic phase was treated two times with sodium bicarbonate 5% solution, sulfuric acid 5%, and brine. Anhydrous sodium sulfate was used for drying and then ethyl acetate was evaporated using a rotary evaporator apparatus. The obtained product was triturated by diethyl ether and *n*-hexane for eliminating the impurities [30].

*2-(2-Chlorophenyl)-N-(5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl)acetamide (**4a**)*

^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 4.05 (s, 2H, $-CH_2-$), 7.33 (m, 2H, $H_{4,6}$-2-chlorophenyl), 7.47 (m, 2H, $H_{3,5}$-2-chlorophenyl). IR (KBr, cm^{-1}) $\bar{\upsilon}$: 3425 (N–H, stretch), 3143 (C–H, aromatic), 2924 (C–H, asymmetric, aliphatic), 2854 (C–H, symmetric, aliphatic), 1697 (C=O, stretch), 1539 (N–H, bend), 1192 (C–N, stretch), 1153 (stretch, C–F). MS (*m*/*z*, %): 323 (M^++2, 1), 321 (M^+, 3), 154 (10), 152 (30), 127 (30), 125 (100), 113 (5), 99 (5), 89 (25), 69 (5), 63 (12).

*2-(3-Chlorophenyl)-N-(5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl)acetamide (**4b**)*

^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 3.95 (s, 2H, $-CH_2-$), 7.25 (d, 1H, $J = 8$ Hz, H_5-3-chlorophenyl), 7.37 (m, 2H, $H_{4,6}$-3-chlorophenyl), 7.44 (s, 1H, H_2-3-chlorophenyl), 13.33 (br s, NH). IR (KBr, cm^{-1}) $\bar{\upsilon}$: 3425 (N–H, stretch), 3147 (C–H, aromatic), 2927 (C–H, asymmetric, aliphatic), 2858 (C–H, symmetric, aliphatic), 1701 (C=O, stretch), 1543 (N–H, bend), 1485 (C=C, aromatic), 1195 (C–N, stretch), 1153 (stretch, C–F).

*2-(4-Chlorophenyl)-N-(5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl)acetamide (**4c**)*

^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 3.85 (s, 2H, $-CH_2-$), 7.35 (d, 2H, $J = 8$ Hz, $H_{2,6}$-4-chlorophenyl), 7.41 (d, 2H, $J = 8$ Hz, $H_{3,5}$-4-chlorophenyl). IR (KBr, cm^{-1}) $\bar{\upsilon}$: 3410 (N–H, stretch), 3151 (C–H, aromatic), 2924 (C–H, asymmetric, aliphatic), 2858 (C–H, symmetric, aliphatic), 1701 (C=O, stretch), 1545 (N–H, bend), 1492 (C=C, aromatic), 1199 (C–N, stretch), 1149 (stretch, C–F).

*2-(2-Fluorophenyl)-N-(5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl)acetamide (**4d**)*

^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 3.95 (s, 2H, $-CH_2-$), 7.16–7.43 (m, 4H, 2-fluorophenyl). IR (KBr, cm^{-1}) $\bar{\upsilon}$: 3410 (N–H, stretch), 3155 (C–H, aromatic), 2920 (C–H, asymmetric, aliphatic), 2858 (C–H, symmetric, aliphatic), 1708 (C=O, stretch), 1550 (N–H, bend), 1492 (C=C, aromatic), 1199 (C–N, stretch), 1157 (stretch, C–F). MS (*m*/*z*, %): 305 (M^+, 2), 303 (15), 277 (25), 252 (8), 196 (5), 169 (12), 136 (45), 122 (10), 109 (100), 99 (5), 83 (10), 63 (12), 52 (5).

*2-(3-Fluorophenyl)-N-(5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl)acetamide (**4e**)*

^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 3.97 (s, 2H, $-CH_2-$), 7.09 (t, 1H, $J = 8$ Hz, H_5-3-fluorophenyl), 7.20 (t, 1H, $J = 8$ Hz, H_6-3-fluorophenyl), 7.42 (m, 2H, $H_{2,4}$-3-fluorophenyl), 13.56 (br s, NH). IR (KBr, cm^{-1}) $\bar{\upsilon}$: 3414 (N–H, stretch), 3147 (C–H, aromatic), 2927 (C–H, asymmetric, aliphatic), 2858 (C–H, symmetric, aliphatic), 1705 (C=O, stretch), 1546 (N–H, bend), 1489 (C=C, aromatic), 1188 (C–N, stretch), 1145 (stretch, C–F).

*2-(4-Fluorophenyl)-N-(5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl)acetamide (**4f**)*

^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 3.90 (s, 2H, −CH2−), 7.18 (t, 2H, $J = 8$ Hz, $H_{2,6}$-4-fluorophenyl), 7.38 (t, 2H, $J = 8$ Hz, $H_{3,5}$-4-fluorophenyl). IR (KBr, cm^{-1}) $\bar{\upsilon}$: 3406 (N–H, stretch), 3151 (C–H, aromatic), 2920 (C–H, asymmetric, aliphatic), 2858 (C–H, symmetric, aliphatic), 1705 (C=O, stretch), 1550 (N–H, bend), 1512 (C=C, aromatic), 1199 (C–N, stretch), 1153 (stretch, C–F).

*2-(2-Methoxyphenyl)-N-(5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl)acetamide (**4g**)*

^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 3.74 (s, 3H, OCH_3), 3.85 (s, 2H, $-CH_2-$), 6.87–7.00 (m, 2H, 2-methoxyphenyl), 7.23–7.28 (m, 2H, 2-methoxyphenyl). IR (KBr, cm^{-1}) $\bar{\upsilon}$: 3410 (N–H, stretch), 3159 (C–H, aromatic), 2939 (C–H, asymmetric, aliphatic), 2855 (C–H, symmetric, aliphatic), 1712 (C=O, stretch), 1546 (N–H, bend), 1496 (C=C, aromatic), 1195 (C–N, stretch), 1161 (stretch, C–F).

*2-(3-Methoxyphenyl)-N-(5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl)acetamide (**4h**)*

^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 3.75 (s, 3H, OCH_3), 3.91 (s, 2H, $-CH_2-$), 6.85 (d, 1H, $J = 8$ Hz, H_4-2-methoxyphenyl), 6.92 (d, 1H, $J = 8$ Hz, H_6-2-methoxyphenyl), 6.95 (s, 1H, H_2-2-methoxyphenyl), 7.26 (t, 1H, $J = 8$Hz, H_5-2-methoxyphenyl), 13.59 (br s, NH). IR (KBr, cm^{-1}) $\bar{\upsilon}$: 3398 (N–H, stretch), 3155 (C–H, aromatic), 2927 (C–H, asymmetric, aliphatic), 2889 (C–H, symmetric, aliphatic), 1701 (C=O, stretch), 1597 (C=C, aromatic), 1550 (N–H, bend), 1492 (C=C, aromatic), 1489 (C=C, aromatic), 1184 (C–N, stretch), 1157 (stretch, C–F).

*2-(4-Methoxyphenyl)-N-(5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl)acetamide (**4i**)*

^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 3.74 (s, 3H, OCH_3), 3.84 (s, 2H, $-CH_2$), 6.91 (d, 2H, $J = 8$ Hz, $H_{3,5}$-4-methoxyphenyl), 7.26 (d, 2H, $J = 8$ Hz, $H_{3,5}$-4-methoxyphenyl), 13.41 (br s, NH). IR (KBr, cm^{-1}) $\bar{\upsilon}$: 3371 (N–H, stretch), 3147 (C–H, aromatic), 2927 (C–H, asymmetric, aliphatic), 2854 (C–H, symmetric, aliphatic), 1701 (C=O, stretch), 1543 (C=C, aromatic), 1516 (N–H, bend), 1489 (C=C, aromatic), 1192 (C–N, stretch), 1153 (stretch, C–F). MS (*m*/*z*, %): 317 (M^+, 5), 149 (10), 148 (100), 121 (60), 120 (20), 91 (10), 78 (12), 77 (12), 69 (5), 51 (5).

*2-(2-Hydroxyphenyl)-N-(5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl)acetamide (**4j**)*

^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 3.72 (s, 2H, $-CH_2-$), 3.79 (s, 1H, 7.40 (t, 1H, $J = 8$ Hz, H_3-2-hydroxyphenyl), 7.40 (d, 1H, $J = 8$ Hz, H_3-2-hydroxyphenyl). IR (KBr, cm^{-1}) $\bar{\upsilon}$: 3302 (O–H, stretch), 3128 (C–H, aromatic), 2958 (C–H, asymmetric, aliphatic), 1639 (C=O, stretch), 1519 (N–H, bend), 1492 (C=C, aromatic), 1192 (C–N, stretch), 1165 (C–F, stretch).

*2-(3-Hydroxyphenyl)-N-(5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl)acetamide (**4k**)*

^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 3.81 (s, 2H, $-CH_2-$), 6.64–6.76 (m, 4H, 3-hydroxyphenyl), 9.43 (br s, OH), 13.51 (br s, NH). IR (KBr, cm^{-1}) $\bar{\upsilon}$: 3460 (O–H, stretch), 3128 (C–H, aromatic), 2920 (C–H, asymmetric, aliphatic), 1701 (C=O, stretch), 1600 (C=C, aromatic), 1546 (N–H, bend), 1489 (C=C, aromatic), 1157 (C–F, stretch).

*2-(2-Nitrophenyl)-N-(5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl)acetamide (**4l**)*

^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 4.39 (s, 2H, $-CH_2-$), 7.41 (t, 1H, $J = 8$ Hz, H_4-2-nitrophenyl), 7.54 (t, 1H, $J = 8$ Hz, H_5-2-nitrophenyl), 7.64 (d, 1H, $J = 8$ Hz, H_6-2-nitrophenyl), 7.72 (d, 1H, $J = 8$ Hz, H_3-2-nitrophenyl), 13.74 (br s, NH). IR (KBr, cm^{-1}) $\bar{\upsilon}$: 3414 (N–H, stretch), 3159 (C–H, aromatic), 2920 (C–H, asymmetric, aliphatic), 2893 (C–H, symmetric, aliphatic), 2854 (C–H, symmetric, aliphatic), 1701 (C=O, stretch), 1550 (N–H, bend), 1523 (stretch, asymmetric, NO_2), 1492 (C=C, aromatic), 1342 (stretch, symmetric, NO_2), 1195 (C–N, stretch), 1165 (C–F, stretch).

*2-(4-Nitrophenyl)-N-(5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl)acetamide (**4m**)*

^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 4.11 (s, 2H, $-CH_2-$), 7.64 (d, 2H, $J = 8$ Hz, $H_{2,6}$-4-nitrophenyl), 8.21 (d, 2H, $J = 8$ Hz, $H_{2,6}$-4-nitrophenyl). IR (KBr, cm^{-1}) $\bar{\upsilon}$: 3429 (N–H, stretch), 3155 (C–H, aromatic), 2924 (C–H, asymmetric, aliphatic), 2858 (C–H, symmetric, aliphatic), 1697 (C=O, stretch), 1554 (N–H, bend), 1519 (stretch, asymmetric, NO_2), 1492 (C=C, aromatic), 1350 (stretch, symmetric, NO_2), 1199 (C–N, stretch), 1149 (C–F, stretch). MS (*m*/*z*, %): 332 (M^+, 15), 196 (10), 170 (10), 169 (10), 164 (15), 163 (100), 150 (8), 137 (12), 136 (80), 113 (10), 106 (30), 90 (25), 89 (40), 78 (40), 69 (12), 63 (20), 51 (5).

*2-o-Tolyl-N-(5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl)-acetamide (**4n**)*

^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 2.27 (s, 3H, $-CH_3$), 3.95 (s, 2H, $-CH_2-$), 7.10–7.24 (m, 4H, 2-methylphenyl), 13.52 (br s, NH). IR (KBr, cm^{-1}) $\bar{\upsilon}$: 3429 (N–H, stretch), 3140 (C–H, aromatic), 2924 (C–H, asymmetric, aliphatic), 2858 (C–H, symmetric, aliphatic), 1697 (C=O, stretch), 1543 (N–H, bend), 1492 (C=C, aromatic), 1192 (C–N, stretch), 1149 (C–F, stretch). MS (*m*/*z*, %): 301 (M^+, 5), 196 (15), 169 (3), 150 (5), 132 (5), 105 (100), 90 (10), 77 (30), 69 (5), 63 (12), 52 (12).

*2-(4-Bromophenyl)-N-(5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl)acetamide (**4o**)*

^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 3.92 (s, 2H, $-CH_2-$), 7.31 (d, 2H, $J = 8$ Hz, $H_{2,6}$-4-bromophenyl), 7.56 (d, 2H, $J = 8$ Hz, $H_{3,5}$-4-bromophenyl), 13.54 (br s, NH). IR (KBr, cm^{-1}) $\bar{\upsilon}$: 3410 (N–H, stretch), 3151 (C–H, aromatic), 2920 (C–H, asymmetric, aliphatic), 2862 (C–H, symmetric, aliphatic), 1701 (C=O, stretch), 1546 (N–H, bend), 1489 (C=C, aromatic), 1203 (C–N, stretch), 1145 (C–F, stretch). MS (*m*/*z*, %): 367 (M^+2, 5), 365 (M^+, 5), 198 (95), 196 (100), 171 (85), 169 (90), 150 (5), 136 (5), 113 (10), 90 (55), 77 (5), 69 (10), 63 (30), 52 (5).

*N-(5-(Trifluoromethyl)-1,3,4-thiadiazol-2-yl)-2-(4-(trifluoromethyl)phenyl)acetamide (**4p**)*

^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 4.08 (s, 2H, $-CH_2-$), 7.58 (d, 2H, $J = 8$ Hz, $H_{2,6}$-4-trifluorophenyl), 7.72 (d, 2H, $J = 8$ Hz, $H_{3,5}$-4-trifluorophenyl). IR (KBr, cm^{-1}) $\bar{\upsilon}$: 3429 (N–H, stretch), 3155 (C–H, aromatic), 2924 (C–H, asymmetric, aliphatic), 2862 (C–H, symmetric, aliphatic), 1705 (C=O, stretch), 1545 (N–H, bend), 1492 (C=C, aromatic), 1327 (C–N, stretch), 1161 (C–F, stretch). MS (*m*/*z*, %): 355 (M^+, 10), 196 (3), 186 (45), 167 (5), 159 (100), 150 (3), 119 (5), 109 (15).

Cell culture conditions

PC3 (prostate carcinoma), MCF7 (breast cancer), and SKMNC (neuroblastoma) cells were obtained from Pasteur Institute (Tehran, Iran) and maintained at 37°C in a humidified atmosphere (90%) containing 5% CO_2. PC3, MCF7, and SKNMC cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) with 5% v/v fetal bovine serum, 100 U/mL penicillin, and 100 mg/mL streptomycin. Cells were seeded overnight and then incubated with various concentrations of different derivatives **4a–4p** in a 95% CO_2 humidified incubator. The medium was changed every 2–3 days and subcultured when the cell population density reached 70–80% confluence. Cells were seeded at an appropriate density according to each experimental design [31].

Viability assay

The cytotoxic effects of compounds **4a–4p** were determined against cell lines by a colorimetric assay using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and compared with the untreated control. Cells were plated onto 96-well plates at a density of 2.0×10^4 cells/well and in a volume of 200 μL. Stock solutions of synthesized compounds were prepared in dimethyl sulfoxide (DMSO). The final concentration of the vehicle in the medium was always 0.5%. One day after seeding, 2 μL of the DMSO containing intended derivative at different concentrations was added to each well. At appropriate time intervals, the medium was removed and replaced by 100 μL of 0.5 mg/mL MTT in growth medium and then the plates were transferred to a 37°C incubator for 3–4 h. Supernatants were removed and the reduced MTT dye was solubilized with DMSO (100 μL/well). Absorbance was determined on an ELISA plate reader (Biotek, H1M) with a test wavelength of 570 nm and a reference wavelength of 630 nm to obtain sample signal (OD570-OD630). Percentage of proliferation was calculated using the following formula: percent of control proliferation = (OD test/OD control) × 100. IC_{50} values were calculated by plotting the log 10 of the percentage of proliferation versus drug concentration [32].

Detection of caspases 3, 8, and 9 activation

The activity of caspase 3 was determined by the Sigma colorimetric caspase 3 kit according to the manufacturer's instrument. This assay is based on spectrophotometric detection of the chromophore *p*-nitroanilide (pNA) after cleavage from the labeled substrate DEVD-pNA in equal amount of cells protein lysate. Briefly, 1×10^6 cells were collected and lysed with 50 μL of chilled lysis buffer and incubated on ice for 10 min. Cell lysates were centrifuged at maximum speed for 5 min at 4°C, after which 50 μL of 2× reaction buffer/DTT mix and 5 μL of 1 mM caspase 3 substrate (DEVD-pNA) were added to each reaction and incubated at 37°C for 1 h. The pNA light emission was quantified using a microplate reader at 400 or 405 nm. Comparison of the absorbance of pNA from an apoptotic sample with an uninduced control allowed determination of the fold increase in caspase 3 activity. The protein content was determined by the Bio-Rad protein assay kit using the bovine serum albumin as a standard. The above stages were also performed for determining the activation of caspases 8 and 9 [33].

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References

[1] A. Lawen, *Bioessays* **2003**, *25*, 888–896.

[2] G. Kaushik, T. Kaushik, S. Khanduja, C. M. Pathak, K. Khanduja, *Cancer Lett.* **2008**, *270*, 120–131.

[3] T. Albreht, M. Mckee, D. M. Alexe, M. P. Coleman, J. M. Martin-Moreno, *Eur. J. Cancer* **2008**, *44*, 1451–1456.

[4] J. Ferlay, P. Autier, M. Boniol, M. Heanue, M. Colombet, P. Boyle, *Ann. Oncol.* **2007**, *18*, 581–592.

[5] A. Jemal, R. Siegel, E. Ward, T. Murray, J. Xu, C. Smigal, M. J. Thun, *Cancer J. Clin.* **2006**, *56*, 106–130.

[6] H. A. Bhuva, S. J. Kini, *J. Mol. Graph. Model.* **2010**, *29*, 32–37.

[7] S. Madhusudan, S. Trivadi, T. S. Ganesan, *Clin. Biochem.* **2004**, *37*, 618–635.

[8] I. M. Ghobrial, T. E. Witzig, A. A. Adjei, *CA Cancer J. Clin.* **2005**, *55*, 178–194.

[9] D. Sunil, A. M. Isloor, P. Shetty, K. Satyamoorthy, A. S. Bharath Prasad, *Arab. J. Chem.* **2010**, *3*, 211–217.

[10] H. Rajak, R. Deshmukh, N. Aggarwal, S. Kashaw, M. D. Kharya, P. Mishra, *Arch. Pharm. Chem. Life Sci.* **2009**, *342*, 453–461.

[11] M. Abdel-Aziz, O. M. Aly, S. S. Khan, K. Mukherjee, S. Bane, *Arch. Pharm. Chem. Life Sci.* **2012**, *345*, 535–548.

[12] X. Q. Deng, Z. Q. Dong, M. X. Song, B. Shu, S. B. Wang, Z. S. Qua, *Arch. Pharm. Chem. Life Sci.* **2012**, *345*, 565–573.

[13] S. A. F. Rostom, M. H. Badr, H. A. Abd El Razik, H. M. A. Ashour, A. E. Abdel Wahab, *Arch. Pharm. Chem. Life Sci.* **2011**, *344*, 572–587.

[14] G. Mishra, A. K. Singh, K. Jyoti, *Int. J. ChemTech Res.* **2011**, *3*, 1380–1393.

[15] A. K. Singh, G. Mishra, K. Jyoti, *J. Appl. Pharm. Sci.* **2011**, *5*, 44–49.

[16] R. Kharb, P. Kaur, P. C. Sharma, M. S. Yar, *Int. J. Res. Pharm. Biomed. Sci.* **2011**, *4*, 1520–1540.

[17] U. Kalidhar, A. Kaur, *Res. J. Pharm. Biol. Chem.* **2011**, *4*, 1091–1106.

[18] N. Siddiqui, P. Ahuja, W. Ahsan, S. N. Pandeya, M. S. Alam, *J. Chem. Pharm. Res.* **2009**, *1*, 19–30.

[19] S. K. Ardestani, F. Poorrajab, S. Razmi, A. Foroumadi, S. Ajdary, B. Gharegozlou, M. Behrouzi-Fardmoghadam, A. Shafiee, *Exp. Parasitol.* **2012**, *32*, 116–122.

[20] D. Kumar, B. Reddy, K. H. Vaddula, K. H. Chang, K. Shah, *Bioorg. Med. Chem. Lett.* **2011**, *21*, 2320–2323.

[21] N. N. Farshori, R. M. Banday, A. Ahmad, A. U. Khan, A. Rauf, *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1933–1938.

[22] J. Matysiak, A. Opolski, *Bioorg. Med. Chem.* **2006**, *14*, 4483–4489.

[23] M. Yusuf, P. Jain, *Arab. J. Chem.* **2011**, in press.

[24] W. Rzeski, J. Matysiakb, M. Kandefer-Szerszen, *Bioorg. Med. Chem.* **2007**, *15*, 3201–3207.

[25] J. Y. Chou, S. Y. Lai, S. L. Pan, G. M. Jow, J. M. Chern, J. H. Guh, *Biochem. Pharmacol.* **2003**, *66*, 115–124.

[26] D. A. Ibrahim, *Eur. J. Med. Chem.* **2009**, *44*, 2776–2781.

[27] C. T. Supuran, A. Scozzafava, *Eur. J. Med. Chem.* **2000**, *35*, 867–874.

[28] X. H. Yang, Q. Wen, T. T. Zhao, J. Sun, X. Li, M. Xing, X. Lu, H. L. Zhu, *Bioorg. Med. Chem.* **2012**, *20*, 1181–1187.

[29] I. Lalezari, N. Sharghi, *J. Heterocyclic Chem.* **1966**, *3*, 336–338.

[30] A. Aliabadi, F. Shamsa, S. N. Ostad, S. Emami, A. Shafiee, J. Davoodi, A. Foroumadi, *Eur. J. Med. Chem.* **2010**, *11*, 5384–5389.

[31] L. Hosseinzadeh, J. Behravan, F. Mosaffa, G. Bahrami, A. Bahrami, G. Karimi, *Food. Chem. Toxicol.* **2011**, *49*, 1102–1109.

[32] D. Gerlier, N. Thomasset, *J. Immunol. Methods* **1986**, *94*, 57–63.

[33] S. J. Riedl, Y. Shi, *Nat. Rev. Mol. Cell. Biol.* **2004**, *5*, 897–907.

