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Cytotoxic effect of azole compounds bearing trifluorophenyl ring on MCF-7, MDA-MB-231, and HCT-116 cancer cell line

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Abstract

Cancer, a disease defined by rapid proliferation of cells, still remains one of the most feared diseases of the modern world. Many structurally different anti-carcinogenic drugs are used in several tumor types such as bladder, colon, ovary, breast, head and neck, testis, lung and prostate cancer. In this study, cytotoxic effects of the compounds with different linkers (ketone, oxime, alcohol, chlorine) between pyrazole and trifluoromethyl on MCF-7 (human breast adenocarcinoma), MDA-MB-231 (human breast adenocarcinoma), and HCT-116 (human colon cancer) cell lines were investigated. Compound a1 was observed to be the most potent compound with IC_{50} values of 5.84±0.76, 5.01±0.32, 5.57±0.02 $\mu g.ml^{-1}$ against these cell lines, respectively. It was found that all compounds were very effective against all the tested cancer cell lines.

Keywords: Cytotoxic effect, 4-trifluoromethylphenyl, pyrazole

Introduction

Cancer, which is characterized by uncontrolled proliferation of cells, is one of the leading health problems. Cancer ranks second after cardiovascular diseases among the top causes of death [1,2]. About 18.1 million people are diagnosed with cancer each year worldwide. However, the desired success cannot be achieved in cancer treatment [3]. There are several types of cancer and the most common cancer types vary different countires. While lung, breast, prostate and colorectal cancers are common in high-income countries; stomach, liver, oral cavity and cervical cancers are more common in the developing world. There are many factors that cause cancer, such as, genetic factors, lifestyle and external factors. Tobacco use, which is one of the most important causes of cancer, accounting for approximately one-third of all cancer types, while 10% of them are caused by chronic infections [4]. Studies on cancer in recent years have shown that cancer is a process influenced by differentiation at genotypic and phenotypic levels. It is thought that 10% of the causes of cancer is hereditary and 85% is caused by environmental and chemical factors [5].

Heterocyclic compounds are frequently used as the basic structure in medicinal chemistry, especially in the development of active biological compounds [6]. An important member of heterocyclic rings, pyrazole is a 5-membered heterocyclic aromatic ring. Compounds with pyrazole structure have many known effects such as anticonvulsant, antifungal, antioxidant, antiviral, antioxidant, antibacterial, apoptosis inducer, amylase inhibitor, as well as anticancer [7-13]. The importance of azole rings in cancer treatment is increasing. The chemical structures of the anticancer drugs criotinib, ruxolitinib, encorafenib and darolutamide, which have a pyrazole ring in their structure, are shown in Figure 1.

Apart from the pyrazole ring, compounds containing oxime, ketone or alcohol functional groups appear as active molecules. It has been reported in the literature that some oxime derivatives with arylalkyl azole backbone have anticonvulsant, antimicrobial and anticancer effects [9,14-21]. This demonstrates the importance

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of the compounds containing oxime, ketone, alcohol and chloro moieties in the development of potential anticancer compounds.

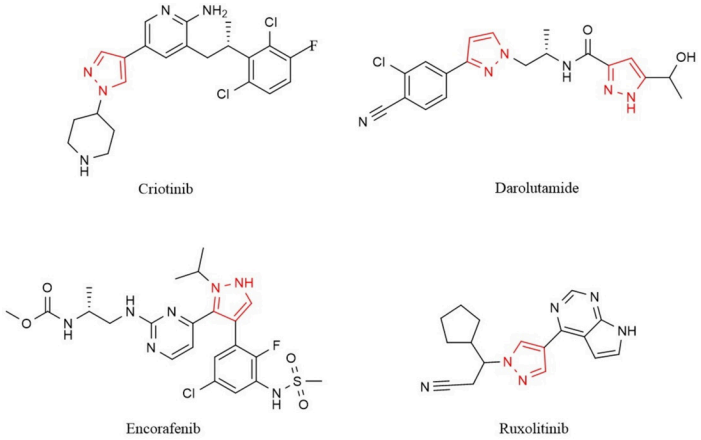


Figure 1. Molecular structures of anticancer drugs with pyrazole scaffols

It is known that some compounds carrying pyrazole ring and trifluoromethylphenyl also have important chemotherapeutic properties such as antiproliferative and anticancer (Figure 2) [22-24].

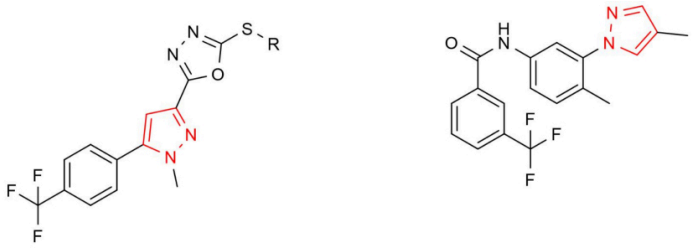


Figure 2. Some anticancer compounds with pyrazole and trifluoromethylphenyl rings

It has been reported in the literature that compounds containing 3,5-dimethyl pyrazole ring in their structure have strong antibacterial, antioxidant and anticancer effects [14,25]. Researchers synthesized 2-(3,5-dimethyl-^{1}H-pyrazol-1-yl)-1-aryletanone compounds and examined their anticancer activities (Figure 3). The antitumor activities of these compounds in HCT-116 (human colon), A549 (human lung adenocarcinoma), DU145 (human prostate), SKOV3 (ovarian carcinoma) and cancer cell lines were evaluated and they were determined to be active at various concentrations. In addition, the effects of various substituents (F, Cl, Br, CH_3, NO_2) attached to aryl groups on the activity were investigated [25].

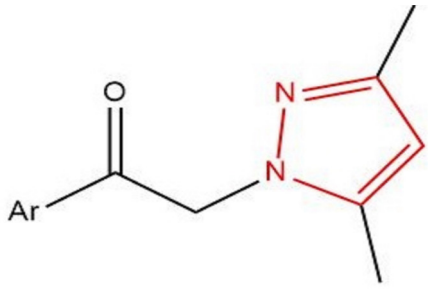


Figure 3. Structure of pyrazol-1-ylethanone derivatives

In this study, the effects of different functional groups (ketone, oxime, alcohol, alkyl halides) as the linker between the trifluoromethylphenyl ring and the pyrazole ring on various cancer cell lines were investigated. For this purpose, four compounds expected to have anticancer effects were designed, synthesized and their activities were tested in MDA-MB-231 (human breast adenocarcinoma), MCF-7 (human breast adenocarcinoma) and HCT-116 (human colon cancer) cell lines. The structures of the compounds are shown in figure 4.

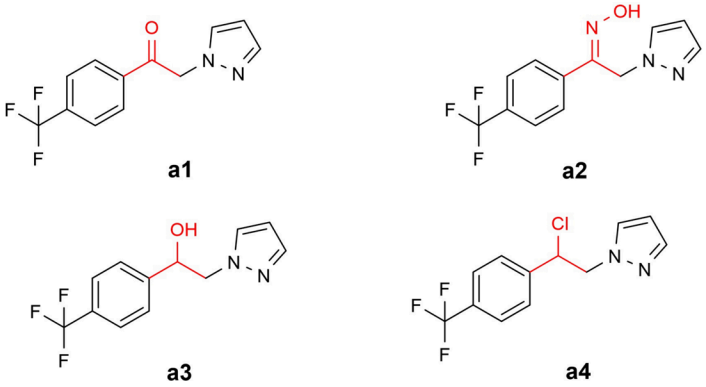


Figure 4. Structures of the synthesized compounds (a1-a4)

Materials and Methods

Chemistry

E. Merck, Fluka AG, and Aldrich provided all the compounds employed in this investigation. TLC on Merck Kieselgel 60 F254 aluminum plates were used to evaluate the purity of all the compounds. Kieselgel 60 (0.040–0.063mm) (230–400mesh ASTM) was used for column chromatography (Merck). A Bruker Avance 300MHz FT NMR spectrometer was used to record ^{1}H-NMR and ^{13}C-NMR spectra. Agilent 6400 Series Triple Quadrupole was used to determine Mass spectra (LC/MSMS) of the compounds.

Synthesis

2-bromo-1-(4-(trifluoromethyl)phenyl)ethan-1-one: A solution of 0.05 mol of 1-(4-(trifluoromethyl)phenyl)ethan-1-one in acetic acid was prepared in ice bath. A few drops of hydrobromic acid were mixed to the solution. 0.05mol of bromine diluted with acetic acid was added dropwise with a dropping funnel by continuous mixing. After the bromine was added, it was agitated for roughly 3 hours at room temperature. In ice water, the reaction media was poured. Washing with sodium bicarbonate solution neutralized the precipitate, which was then dried in a dark atmosphere. It was crystallized from a methanol/water mixture [19].

2-(1H-pyrazol-1-yl)-1-(4-(trifluoromethyl)phenyl)ethan-1-one (a1): A solution of 0.03 mol of pyrazole in 2.5ml of dimethylformamide was prepared and cooled to 0oC in an ice bath. A solution of 0.01 mol of 1-(4-trifluoromethylphenyl)-2-bromoethanone in 2.5ml of dimethylformamide (DMF) was slowly added to it and mixed. Stirring was continued in an ice bath for 2h and at rt for 1 day. Drop by drop, the medium was poured into freezing water. The resulting precipitate was filtered, washed, dried, and purified [19].

2-(1H-pyrazol-1-yl)-1-(4-(trifluoromethyl)phenyl)ethan-1-one oxime (a2): 0.015 mol of 1-(4-(trifluoromethyl)phenyl)-2-(1H-pyrazol-1-yl)ethanone was dissolved in 75 ml of ethanol. It was

heated under reflux and 0.03 mol of hydroxylamine hydrochloride was added. It was alkalized up to pH:10 with 15 N sodium hydroxide solution. It was mixed under refluxing for 1-2 hours. The ethanol was evaporated to dryness in the rotary evaporator using the resultant solution. The resultant solid was filtered after 4 hours of stirring in freezing water. The substance was obtained in pure form [19].

2-(1H-pyrazol-1-yl)-1-(4-(trifluoromethyl)phenyl)ethan-1-ol (a3): While 1.8 mmol 1-(4-(trifluoromethyl)phenyl)-2-(1H-pyrazol-1-yl)ethanone solution in 18 ml ethanol was mixed at 0-5 ^{0}C, 5.4 mmol sodium borohydride ($NaBH^4$) was added in a closed system and mixed in an ice bath for 1 hour. The ethanol was evaporated until it was completely dry, then 100 mL of water was added. The final product was filtered before being rinsed with water [18].

1-(2-Chloro-2-(4-(trifluoromethyl)phenyl)ethyl)-1H-pyrazole (a4): 1.5 mmol 2-(1H-pyrazol-1-yl)-1-(4-(trifluoromethyl)phenyl) ethanol and 1 ml thionyl chloride were agitated for 30 minutes at room temperature before being evaporated to dryness. Ethyl acetate was used to treat the residue. The white crystalline precipitate was filtered off, was recrystallized with the acetone/methanol mixture [18].

2-(1H-pyrazol-1-yl)-1-(4-(trifluoromethyl)phenyl)ethan-1-one (a1) [26]: White crystals, yield 90%, ^{1}H-NMR (DMSO-d6,400MHz) δ ppm:5.92 (^{2}H;s;-CH^2), 6.34 (H;t;pyrazole-H^4;J=2.05), 7.46-7.75 (^{2}H;m;pyrazole), 7,92-8.27 (4H;m;phenyl). ^{13}C-NMR (DMSO-d6,100MHz) δ ppm:193.83,139.50,138.26,133 .71,132.00,129.36,126.30,122.36,106.13,58.34. HRMS (ESI): m/z calculated for $C_{12}H_9F_3N_2O$=254.21 found:254.90.

2-(1H-pyrazol-1-yl)-1-(4-(trifluoromethyl)phenyl)ethan-1-one oxime (a2): White crystals, yield 80%,1H-NMR (DMSO-d6, 400 MHz) δ ppm:5.26 ($_2$H;s;-CH_2;%19),5.49 (2H;s;-CH_2;%81), 6.15 (H;t;pyrazole-H4;%19,J=2.6), 6.20 (H;d; pyrazole-H4;%81, J=2.9), 7.33-7.41 (H;m;pyrazole), 7.54-7.93 (5H; m; pyrazole and phenyl), 11.51 (H,s,N-OH, %19), 12.21 (H,s,N-OH,%81). $_{13}$C-NMR (DMSO-d6, 100MHz) δ ppm: 151.72, 139.33,139.15, 131.32,129.59,129.54,127.44,125.66,125.61,105.98,105.88,54. 76,44.93. HRMS (ESI): m/z calculated for $C_{12}H_{10}F_3N_3O$=238.90 found: 238.90.

2-(1H-pyrazol-1-yl)-1-(4-(trifluoromethyl)phenyl)ethan-1-ol (a3): White powder, yield 64%, 1H-NMR (DMSO-d6, 400MHz) δ ppm: 4.28 (2H,d,-CH_2,J=6.52), 5.05 (H;s;-OH), 5.88 (H;s;CH-), 6.19 (H; t; pyrazole-H4;J=2.05), 7.40-7.73 (6H;m;pyrazole and phenyl). ^{13}C-NMR (DMSO-d6,100MHz) δ ppm:147.96,139.04,131.17,12 8.57,127.31,125.43,125.38,105.30,71.70,58.67. HRMS(ESI):m/z calculated for $C_{12}H_{11}F_3N_{20}$=274.67 found: 274.70.

1-(2-Chloro-2-(4-(trifluoromethyl)phenyl)ethyl)-1H-pyrazole (a4): White crystals, yield 58%, 1H-NMR (DMSO-d6,400MHz) δ ppm:4.68-4.85 (2H,m,-CH_2), 5.68 (H;t;CH-,J=6.4), 6.21 (H;t; pyrazole-H4;J=2.04), 7.46-7.84 (6H;m;pyrazole and phenyl). $_{13}$C-NMR(DMSO-d6, 100MHz) δ ppm:143.27,139.82,143.27, 139.82,131.35,129.45,129.02,126.03,122.65,105.66,60.57,57.2 5. HRMS (ESI):m/z calculated for $C_{12}H_{10}ClF_3N_2$=270.08 found: 270.00.

Activity studies

Cell lines studies

In this study, MCF-7 (human breast adenocarcinoma), MDA-MB-231 (human breast adenocarcinoma), HCT-116 (human colon cancer) and L929 (mouse fibroblast) cell lines were evaluated. Cell lines were seeded in 25 cm² bottles. DMEM medium containing 10% FBS, 1% Penicillin-Streptomycin, L-Glutamine was used for cell culture. Cells were incubated and grown at 37°C, 5%CO_2 and 95% humidity. Cells were passaged for migration to different media when grown. DMEM was taken first and washed with DPBS to remove FBS in the pass. Then, Trypsin-EDTA solution was added to the balloon to remove the cells from the balloon and the balloon was kept in a CO_2 oven for about 1 minute. Media containing FBS was placed in the flask to stop the activity of trypsin-EDTA. Cells were collected in a hawk tube and centrifuged at rt for 5min at 800 rpm. The supernatant was discarded and the precipitated pellet was suspended in medium and inoculated into cell culture plates according to the procedure [27].

The "Cell Proliferation Kit" was used to assess the IC_{50} dose of the compounds using an XTT test. XTT and activation agent are both included in the XTT kit. The 50/1 XTT agent (labelling reagent)/activation agent mixture was used to make the XTT solution (electron coupling reagent). It is broken down by the dehydrogenase enzyme in the mitochondria of metabolically active cells and turns into water-soluble formazan. The amount of orange color produced by formazan is proportional to the number of viable cells. Depending on the degree of orange color formation at the end of the incubation period, cell viability was determined by reading in a microplate reader at a wavelength of 450nm and a reference range of 630nm [27].

Fluorescence scanning

DAPI (4', 6-diamidino-2-phenylindole) dye was used to detect cell death by synthesized compounds. 1x104 cells were seeded into plates, and 100µl ethanol was added to the cells, which were then incubated for 30minutes. The fixed cells were stained with DAPI (2ug/ul) and fluorescence images were obtained [28].

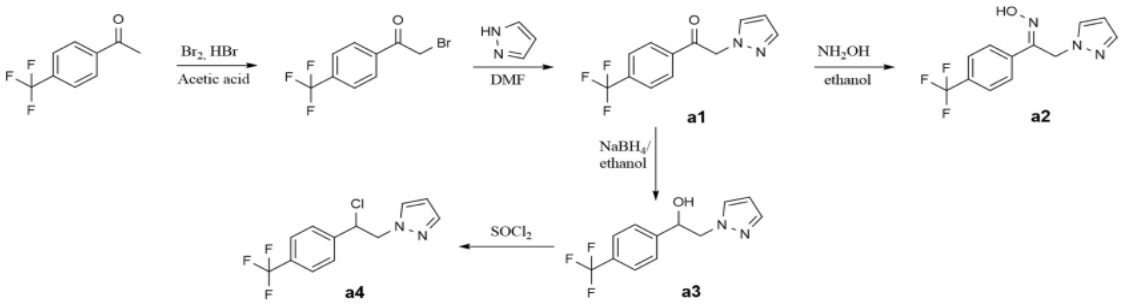
Results

Chemistry

Compounds were synthesized according to the literature methods as outlined in Scheme 1 [18,19]. The structures, crystallization solvents, yields, melting points of the synthesized compounds are given in Table 1. Structures of the compounds were confirmed by 1H-NMR, 13C-NMR and Mass spectral data.

Table 1. Structures, yields and melting points of compounds

Compounds	Yield (%)	M.W.	Molecular formula
a1	90	254.21	$C_{12}H_9F_3N_2O$
a2	80	238.90	$C_{12}H_{10}F_3N_3O$
a3	64	274.67	$C_{12}H_{11}F_3N_2O$
a4	58	270.08	$C_{12}H_{10}F_3N_2$



Scheme 1. Synthesis of the compounds

The compound **a1** was previously synthesized by Dhiman et al. in 32% yield, but its anticancer activity was not investigated [26]. Compound a1 was obtained in 90% yield as a result of N-alkylation reaction of pyrazole with 2-bromo-1-(4-(trifluoromethyl)phenyl) ethan-1-one. Excess pyrazole was used to allow the reaction to take place in basic medium. As a result of the reaction of the ketone group in the structure of the compound a1 with hydroxylamine hydrochloride, compound **a2** containing the oxime fragment was synthesized. This reaction is thought to occur by the SN2 mechanism. Hydroxylamine hydrochloride, which became a nucleophile in the basic medium, attacked the partially positively charged carbon atom in the carbonyl group and the addition reaction took place. As a result of the removal of water from the molecule, a double bond was formed between carbon and nitrogen, and an elimination reaction occurred with 80% efficiency. As a result of the reaction, 81% and 19% Z/E isomers were formed. Compound a3 was obtained by reduction of compound a1 with NaBH4. Three times more sodium borohydride was used in the reduction and the reaction was carried out in ice bath as it was exothermic reaction. Halogen derivative compound **a4** was synthesized in 58% yield from the reaction of compound **a3** with thionyl chloride.

Activity studies

The cytotoxic activity of the compounds **(a1-a4)** was tested on three cancer cell lines MCF-7 (human breast adenocarcinoma), MDA-MB-231 (human breast adenocarcinoma), and HCT-116 (human colon cancer) as well as one healthy (L929, mouse fibroblast) cell line in this study (Table 2). For 24 hours, the cell lines were treated with five different doses of the chemicals (5,10,25,50, and 100µg.ml^{-1}), as well as doxorubicin and cisplatin serving as positive controls. All compounds were found to be effective against three cancer cell lines. In the MCF-7 cell line, the IC_{50} values of compounds **a1–a4** ranged from 5.84-15.34 µg.ml^{-1}, 5.01–9.92 µg.ml^{-1} in the MDAMB-231 cell line, and 5.57–14.18 µg.ml^{-1} in the HCT-116 cell line. While the IC_{50} values of doxorubicin were determined 17.73,21.84 and 13.85µg.ml^{-1} in the MCF-7, MDA-MB-231 and HCT-116 cell lines respectively

Table 2. IC_{50} values (µg.ml-1) of compounds a1-a4 and standart compounds against MCF-7, MDA-MB-231, and HCT-116 cancer and L929 healty cell lines after 24 h

Compounds	MCF-7	MDA-MB-231	HCT-116	L929
a1	5.84±0.76	5.01±0.32	5.57±0.02	16.19±0,26
a2	7.82±0.15	6.38±0.07	14.18±0.21	20.22±0.18
a3	15.34±0.65	9.92±0.24	11.61±0.23	19.37±0.32
a4	9.02±0.34	7.04±0.07	11.89±0.19	8.72±0.09
Doxorubicin	17.73	21.84	13.85	18.95
Cisplatin	>300	>200	14.68	20.96

Discussion

Chemistry

Compound **a1** was synthesized with a yield of 32% in the literature, whereas it was synthesized with a yield of 90% in our study. While the -CH_2 peaks adjacent to the carbonyl group in the structure of the compound were observed at 5.64ppm in the literature, it was observed at 5.92ppm in our study. While aromatic protons gave signals between 6.41 and 8.11ppm in the literature, they gave signals between 6.34ppm and 8.27ppm in our study in ^{1}H-NMR. Although the peak of carbonyl was observed at 191.7ppm in ^{13}C-NMR in the literature, it gave a signal at 193.83 ppm in our study.

Activity studies

Especially, compound **a1** bearing ketone functional group was observed as the most effective compound in all cancer cell lines. The activity of compound a3, obtained by reduction of the ketone group, was significantly decreased in all cell lines. The addition of chlorine and oxime groups did not cause a significant change in the activity. Also, compound a1 was determined the best cytotoxic compound with 5.01µg.ml^{-1} on the MDA-MB-231 cell line. It was also found that all compounds were very effective against MDA-MB-231 cancer cells. However, a disadvantage was that the compounds were also toxic to healthy cells. The fluorescens scan images of the most active compound (**a1**) and the reference drugs are given in Figure 5. Compound **a1** was shown to cause more cell death than cisplatin, doxorubicin and control group.

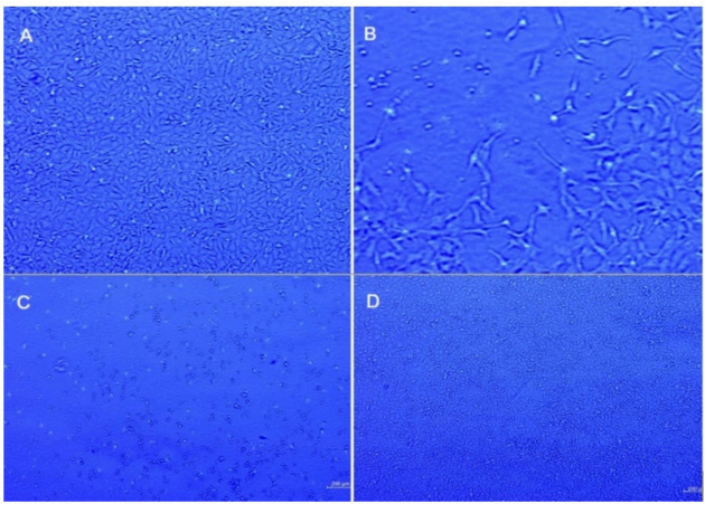


Figure 5. Fluorescence scanning images of compounds. A: control (live), B: compound a1, C: cisplatin, D: doxorubicin

Conclusion

In this study, four compounds containing ketone, oxime, alcohol and chlorine groups were synthesized and their structures were elucidated by spectrophotometric analysis. Their anticancer activities against MCF-7 (human breast adenocarcinoma), MDA-MB-231 (human breast adenocarcinoma), and HCT-116 (human colon cancer) cell lines were evaluated. All the synthesized compounds **(a1-a4)** were found to be effective against all the studied cancer cell lines. In addition, it has been determined that all the compounds were more effective than the reference drugs, cisplatin and doxorubicin. This suggests that the synthesized compounds **(a1-a4)** may have potential in vivo anticancer activity. In future studies, structural changes are planned to increase the activity potential of the compounds and reduce their toxicity to the healthy cells.

Conflict of interests
The authors declare that there is no conflict of interest in the study.

Financial Disclosure
The authors declare that they have received no financial support for the study.

Ethical approval
No consent of ethical is needed for this research

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