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Synthesis and Cytotoxic Evaluation of 6-Amino-4-Aryl-3-Methyl-2,4-Dihydropyrano[2,3-*C*]Pyrazole-Carbonitrile Derivatives Using Borax with Potential Anticancer Effects

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A green and efficient one-pot, four-component synthesis of 6-amino-4-aryl-3-methyl-2,4-dihydropyrano[2,3-*c*]pyrazole-carbonitrile derivatives catalyzed by borax in water has been examined and described. This method has several advantages such as environmental friendliness, shorter reaction time, excellent yields, and simple workup procedure. The *in vitro* cytotoxic activity of the synthesized compounds was investigated against cancer cell lines (SW48, A549, KB, HepG2) in comparison with doxorubicin, a well-known anticancer drug, using MTT colorimetric assay. The synthesized compounds showed good and reasonable cytotoxicity compared with doxorubicin in some studied cell lines. The compounds **5b**, **5c**, **5g** in KB cell line (IC_{50} = 8±2.217 μM, 7±2.77 μM, 7.5±1.49 μM respectively), **5f** in A549 cell line (IC_{50} = 31.5±2.02 μM), **5g** in HepG2 cell line (IC_{50} = 22.5±3.09 μM), **5e**, and **5i** in SW48 cell line (IC_{50} = 23±0.772 μM, 23±4.97 μM respectively) showed the best results in close to the control drug (IC_{50} = 6.8±0.78 μM, 6.3±0.65 μM, 5.4±0.5 μM, 4.3±0.12 μM in A549, HepG2, KB, and SW48 cell lines respectively).

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Introduction

Cancer is a disease of worldwide concern and its incidence is on the rise. According to information from the World Health Organization (WHO), more than eleven million people are diagnosed with cancer and, also, more than 13% of overall deaths, are directly caused by cancer every year worldwide[1]. Cancer cells are characterized by unlimited replicative potential, self-sufficiency in-growth signals, and insensitivity to antigrowth signals, sustained angiogenesis, metastasis, and evasion of apoptosis[2]. Apoptosis or programmed cell death is an important phenomenon for remission of damaged cells. Apoptosis can be activated by two major pathways: the extrinsic pathway, in which specific cell death receptors located on the cell surface membrane are activated by specific ligands; and the intrinsic pathway, where primarily mitochondria are involved[3]. Apoptosis plays a vital role in normal embryonic development as well as in adult life, such as elimination of dispensable or excess cells. It has been known that defects in the apoptosis pathways and the ability to evade cell death is one of the hallmarks of cancers, which results in uncontrollable tumor cell growth, as well as tumor resistance to chemotherapeutic agents[4]. Therefore, finding new therapeutic agents for neoplastic diseases with focus on the apoptosis pathways is one of the top subjects in this area of research. It has been well documented that many of the clinically useful cytotoxic agents induce apoptosis in cancer cells. The pro-apoptotic chemotherapeutic agents that target tubulin polymerization such as taxol and vinca alkaloids including vincristine, vinblastine, and vinorelbine are among the most potent and commonly prescribed antineoplastic agents. The development of chemo-resistance, as well as dose-limiting neurologic and bone marrow toxicity, however, has limited the use of tubulin targeting agents. This clearly highlights the urgent need for novel chemotherapeutic agents for more effective treatment of cancer[5]. Pyranopyrazoles are the fused heterocyclic compounds that exhibit bactericidal[6], molluscicidal[7,8], analgestic[9] and act as hypoglycemic, and anticancer agents[10,11]. They are also potential inhibitors of human Chk1 kinase[12]. Due to their biological significance, there has been considerable interest in developing synthetic methods for the preparation of pyranopyrazole derivatives[13-24].

Recently, the anti-tumor efficacy of 4-aryl-4*H*-chromenes (Fig. 1), as effective apoptosis inducers, has been characterized. They were found to induce tubulin destabilization as well as to arrest cells at the G2/M stage with high selectivity against proliferating versus resting cells. This series of compounds, also, were found as tumor vasculature targeting agents[25-27]. Green chemistry techniques continue to gain importance. Alternative processes help to conserve resources and can reduce costs. The replacement of conventional solvents with water, which is harmless to health and is available in large quantities, is an interesting basic approach along these lines[28-30]. One of the tools used to combine economic aspects with the environmental ones is the multi component reaction (MCR) strategy, the process consists of two or more synthetic steps which are carried out without isolation of any intermediate, thus reducing time, saving money, energy and raw materials[31]. As part of our program aimed at developing new selective and environmentally friendly methodologies for the preparation of fine chemicals[32-35], we performed the synthesis of 6-Amino-4-aryl-3-methyl-2,4-dihydropyrano[2,3-*c*]pyrazole-carbonitrile derivatives through a four-component reaction with borax as catalyst. Then, the *in vitro* cytotoxic activity of the synthesized test compounds was investigated against cancer cell lines (SW48, A549, KB, HepG2) in comparison with doxorubicin, a well-known anticancer drug, using MTT colorimetric assay.

Experimental

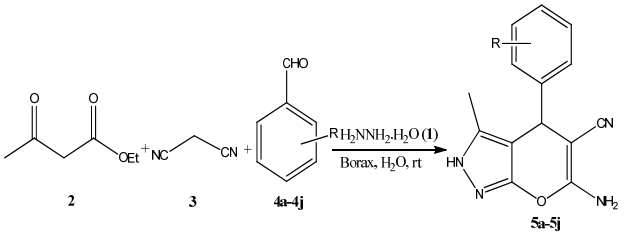
Chemistry

All starting materials, reagents and solvents were purchased from Merck and Aldrich Chemical Companies. All yields refer to isolated yield. The structure of compounds was characterized by IR, ^{1}H NMR spectra and MS. Merck silica gel 60 F254 plates were used for TLC. ^{1}H-NMR spectra were recorded using a Bruker 250 spectrometer and chemical shifts are expressed as (ppm) with tetramethylsilane (TMS) as internal standard using DMSO-d_6 as solvent. The IR spectra were obtained on a Shimadzu 470 spectrophotometer (potassium bromide disks). The melting point was taken on a Kofler hot stage apparatus and is uncorrected. k). The mass spectra was run on a Finigan TSQ-70

spectrometer at 70 eV. The purity of the compound was monitored by thin layer chromatography.

General procedure for the synthesis of pyranopyrazoles with borax as catalyst in water

Pyranopyrazole derivatives were synthesized by the one-pot four component condensation of an aromatic aldehyde (1.0 mmol, **4a-4j**), ethyl acetoacetate (1.0 mmol, **2**), hydrazine hydrate (1.0 mmol, **1**), malononitrile (1.0 mmol, **3**), and borax (10 mol%) as the catalyst, in water for a designated time less than 15 min listed in Table 1. After completion of the reaction followed by TLC analysis, the reaction mixture was cooled and the precipitated solid was filtered and washed several times with cooled water. The obtained products (**5a-5j**) were crystallized in appropriate solvent (EtOH) and the corresponding pyranopyrazoles were obtained in 70-98% yields (Scheme 1, Table 1). The spectral data of the compounds **5a-5j** are as followed:



2,5-dimethoxy	5a	3-chloro	5f
2,4-dimethoxy	5b	4-chloro	5g
3,4,5-trimethoxy	5c	2-nitro	5h
2,3,4-trimethoxy	5d	4-nitro	5i
2-chloro	5e	2-hydroxy	5j

Compound	R	Compound	R
5a	2,5-dimethoxy	**5f**	3-chloro
5b	2,4-dimethoxy	**5g**	4-chloro
5c	3,4,5-trimethoxy	**5h**	2-nitro
5d	2,3,4-trimethoxy	**5i**	4-nitro
5e	2-chloro	**5j**	2-hydroxy

Scheme 1. Reagents and conditions for the synthesis of pyranopyrazoles.

6-Amino-4-(2,5-dimethoxyphenyl)-3-methyl-2,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (5a)

Chemical Formula: $C_{16}H_{16}N_4O_3$; Molecular Weight: 312.32; m.p. = 210-212 ^oC; IR (KBr, cm^{-1}): $\bar{\upsilon}_{max}$ 3400, 3100, 2924, 2202, 1581, 1500, 1496, 1087, 1045, 871, 800; ^1H NMR (DMSO-d_6, 250 MHz): δ 1.79 (s, 3H, CH_3), 3.71 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 5.2 (s, 2H, CH), 6.90 (s, 2H, NH_2), 7.10-7.19 (m, 2H, Ar-H), 7.48-7.49 (m, 1H, Ar-H), 12.10 (s, 1H, NH) ppm; MS (*m/z,* %): 312 (M^+, 5), 297 (95), 270 (25), 215 (30), 199 (20), 150 (90), 77 (50), 51 (20).

6-Amino-4-(2,4-dimethoxyphenyl)-3-methyl-2,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (5b)

Chemical Formula: $C_{16}H_{16}N_4O_3$; Molecular Weight: 312.32; m.p. = 224-226 ^oC; IR (KBr, cm^{-1}): $\bar{\upsilon}_{max}$ 3400, 3100, 2924, 2168, 1570, 1504, 1423, 1087, 1041, 821, 802, 756; ^1H NMR (DMSO-d_6, 250 MHz): δ 1.78 (s, 3H, CH_3), 3.87 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 4.78 (s, 1H, CH), 6.65 (s, 2H, NH_2), 7.89-7.92 (m, 2H, Ar-H), 8.26 (s, 1H, Ar-H), 12.01 (s, 1H, NH) ppm; MS (*m/z*, %): 312 (M^+), 297 (30), 149 (100), 120 (50), 77 (25), 51 (10).

6-Amino-4-(3,4,5-trimethoxyphenyl)-3-methyl-2,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (5c)

Chemical Formula: $C_{17}H_{18}N_4O_4$; Molecular Weight: 342.35; m.p. = 210-212 ^oC; IR (KBr, cm^{-1}): $\bar{\upsilon}_{max}$ 3400, 3100, 2924, 2187, 1543, 1500, 1400, 1126, 1095; ^1H NMR (DMSO-d_6, 250 MHz): δ 1.87 (s, 3H, CH_3), 3.72 (s, 3H, OCH_3), 3.73 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 4.58 (s, 1H, CH), 6.47 (s, 1H, Ar-H), 6.85 (s, 2H, NH_2), 7.22 (s, 1H, Ar-H), 12.08 (s, 1H, NH) ppm; MS (*m/z*, %): 342 (M^+, 15), 311 (3), 276 (10), 241 (5), 181 (100), 77 (4), 53 (3).

6-Amino-4-(2,3,4-trimethoxyphenyl)-3-methyl-2,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (5d)

Chemical Formula: $C_{17}H_{18}N_4O_4$; Molecular Weight: 342.35; m.p. = 188-190 ^oC; IR (KBr, cm^{-1}): $\bar{\upsilon}_{max}$ 3400, 3100, 2924, 2168, 1543, 1500, 1400, 1091, 806; ^1H NMR (DMSO-d_6, 250 MHz): δ 1.87 (s, 3H, CH_3), 3.79 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 4.77 (s, 1H, CH), 6.85 (s, 2H, NH_2), 6.93 (s, J = 8.1 Hz, 1H, Ar-H), 7.73 (s, J = 8.1 Hz, 1H, Ar-H), 11.98 (s, 1H, NH) ppm; MS (*m/z*, %): 342 (M^+), 311 (3), 241 (1), 179 (100), 150 (40), 107 (15), 77 (25), 51 (12).

6-Amino-4-(2-chlorophenyl)-3-methyl-2,4-dihydropyrano[2,3-*c*]pyrazole-5-carbonitrile (5e)

Chemical Formula: $C_{14}H_{11}ClN_4O$; Molecular Weight: 286.72; m.p. = 145-147 ^oC; IR (KBr, cm^{-1}): $\bar{U}_{max}$ 3500, 3300, 2924, 2191, 1400-1500, 1527, 1087, 750, 790; ^1H NMR (DMSO-d_6, 250 MHz): δ 1.82 (s, 3H, CH_3), 5.06 (s, 1H, CH), 6.75 (s, 2H, NH_2), 7.05-7.15 (m, 2H, Ar-H), 8.50 (s, 1H, Ar-H), 8.52-8.60 (m, 1H, Ar-H) ppm; MS (*m/z*, %): 286 (M^+, 25), 260 (5), 206 (20), 175 (100), 125 (50), 66 (90), 51 (60).

6-Amino-4-(3-chlorophenyl)-3-methyl-2,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (5f)

Chemical Formula: $C_{14}H_{11}ClN_4O$; Molecular Weight: 286.72; m.p. = 159-161 ^oC; IR (KBr, cm^{-1}): $\bar{U}_{max}$ 3100-3400, 2927, 2195, 1400-1500, 1519, 1087, 678 & 790; ^1H NMR (DMSO-d_6, 250 MHz): δ 2.01 (s, 3H, CH_3), 4.45 (s, 1H, CH), 6.55 (s, 2H, NH_2), 7.09-7.83 (m, 4H, Ar-H) ppm; MS (*m/z*, %): 286 (M^+), 250 (6), 220 (30), 205 (4), 175 (43), 165 (75), 125 (100), 75 (60), 51 (23).

6-Amino-4-(4-chlorophenyl)-3-methyl-2,4-dihydropyrano[2,3-*c*]pyrazole-5-carbonitrile (5g)

Chemical Formula: $C_{14}H_{11}ClN_4O$; Molecular Weight: 286.72; m.p. = 173-175 ^oC; IR (KBr, cm^{-1}): $\bar{U}_{max}$ 3300-3500, 2924, 2205, 1400-1500, 1087, 821; ^1H-NMR (DMSO-d_6, 250 MHz): δ 1.79 (s, 3H, CH_3), 4.63 (s, 1H, CH), 6.93 (s, 2H, NH_2), 7.18 (d, J = 8.0 Hz, 2H, Ar-H), 7.36 (d, J = 8.0 Hz, 2H, Ar-H) ppm; MS (*m/z*, %): 286 (M^+, 12), 250 (4), 220 (23), 175 (99), 153 (45), 125 (50), 66 (46), 51 (22).

6-Amino-4-(2-nitrophenyl)-3-methyl-2,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (5h)

Chemical Formula: $C_{14}H_{11}N_5O_3$; Molecular Weight: 297.27; m.p. = 185-187 ^oC; IR (KBr, cm^{-1}): $\bar{U}_{max}$ 3100-3400, 2924, 2187, 1350, 1527, 1500, 1400, 1080, 1040, 790, 740; ^1H- NMR (DMSO-d_6, 250 MHz): δ 1.86 (s, 3H, CH_3), 4.26 (s, 1H, CH), 6.40 (s, 2H, NH_2), 7.34-7.81 (m, 4H, Ar-H,), 12.15 (s, 1H, NH) ppm; MS (*m/z*, %): 297 (M^+, 4), 280 (18), 251 (80), 231 (11), 185 (97), 77 (46), 66 (35), 51 (30).

6-Amino-4-(4-nitrophenyl)-3-methyl-2,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (5i)

Chemical Formula: $C_{14}H_{11}N_5O_3$; Molecular Weight: 297.27; m.p. = 190-192 ^oC; IR (KBr, cm^{-1}): $\bar{U}_{max}$ 3100-3400, 2924, 2189, 1597, 1519, 1350, 1500, 1400, 1087, 806; ^1H- NMR (DMSO-d_6, 250 MHz): δ 1.80 (s, 3H, CH_3), 4.83 (s, 1H, CH), 6.20 (s, 2H, NH_2), 7.05 (d, J = 8.2 Hz, 2H, Ar-H), 8.12 (d, J = 8.2 Hz, 2H, Ar-H), 11.90 (s, 1H, NH_2), ppm; MS (*m/z*, %): 297 (M, 35), 271 (6), 251 (16), 214 (13), 175 (100), 109 (45),76 (38).

6-Amino-4-(2-hydroxyphenyl)-3-methyl-2,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (5j)

Chemical Formula: $C_{14}H_{12}N_4O_2$; Molecular Weight: 268.27; m.p. = 208-210 ^oC; IR (KBr, cm^{-1}): $\bar{U}_{max}$ 3500, 3100, 2924, 2187, 1400-1500, 1543, 1257, 1091, 750 & 790; ^1H- NMR (DMSO-d_6, 250 MHz): δ 1.96 (s, 3H, CH_3), 4.63 (s, 1H, CH), 5.60 (s, 1H, OH), 6.56 (s, 2H, NH_2), 6.66-7.18 (m, 2H, Ar-H), 7.21-7.71 (m, 2H, Ar-H) ppm; MS (*m/z*, %): 268 (M^+, 5), 240 (98), 223 (47), 170 (10), 121 (65), 93 (64), 65 (83), 51 (42).

Cell culture conditions

HepG2, NCBI-C158 (Human Liver Carcinoma), KB, NCBI-C152 (Human Mouth Carcinoma), SW48, NCBI-C480 (Human Colon Adenocarcinoma) and A549, NCBI-C137 (Human Lung Carcinoma) cell lines, were purchased from Pasture Institute (Iran). Cell lines were grown and maintained in a humidified incubator at 37 ^oC with 5% CO_2 atmosphere. Cells were cultured in DMEM-F12 (Dulbecco's Modified Eagle's Medium) supplemented with 10% (V/V) heat inactivated fetal bovine serum (FBS), and antibiotics (100 IU/ml penicillin and 100 µl/ml streptomycin). Doxorubicin, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT), penicillin and streptomycin were purchased from Sigma-Aldrich. The test compounds were dissolved in dimethylsulfoxide (DMSO), and stored as the stock solutions with a concentration of 1.0 mg/mL at –20

^{o}C (The concentration of DMSO was less than 1%)[33-34]. [36-38].

MTT cytotoxicity assay

Cellular toxicities of pyranopyrazole derivatives were analyzed against four cell lines (A549, SW48, KB and HepG2) and compared to DMSO and doxorubicin as negative and positive controls respectively. To evaluate the number of live and dead cells, the cells were stained with trypan blue and counted using hemocytometer. To determine the growth inhibitory activity of the test compounds, 1×10^4 cells were plated into each well of 96-well plates in 180 µl of growth medium. After 24 h of seeding, the cells in each set of three wells were treated with certain concentrations of pyranopyrazoles derivatives. After 24 hours of incubation, the growth rate was determined using the MTT assay as described in literature[36-38]. Cells were then washed in PBS (Phosphate Buffer Saline) and 100 µL of fresh media 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. Additional 4 h of incubation at 37 °C were done and then the medium was discarded. Dimethylsulfoxide (60 µL) was added to each well and the solution was vigorously mixed to dissolve the purple formazan crystals. The absorbance of each well was measured by plate reader (Synergy 1; BioTek) at a wavelength of 540 nm. The amount of produced purple formazan is proportional to the number of viable cells. IC_{50} (µM) were calculated by Prism analysis, expressed in mean±SEM (Figures 1-8)[36-38].

Results and discussion

Although, the reported methods for the synthesis of pyranopyrazole derivatives are effective, they are confronted with certain drawbacks of environment compatibility by the use of toxic and expensive catalysts, which also lack recyclability. While catalyst used in this project (borax) is eco-friendly and inexpensive catalyst. In this approach we used water instead of large amounts of organic solvents, work-up is simplified, and the reaction time is considerably decreased. The experimental procedure is very simple and convenient, and can withstand a variety of functional groups under the reaction conditions used. Simply by adding catalyst to a mixture of reactants, rapid and convenient condensation is achieved at 25 ^{o}C in water. After the completion of the reaction (monitored by TLC), the residue was filtered and washed with cooled water then was purified by recrystallization from ethanol to produce the desired solid and confirmed by physical and spectral data.

The *in vitro* cytotoxic activity of pyranopyrazole derivatives **5a-5j** was evaluated by MTT assay on A549, SW48, KB and HepG2 cell lines (Table 1). The dose effect curves were generated and the sensitivity to pyranopyrazole derivatives expressed as a drug concentration that caused 50% growth inhibition (Figures 1-8). Pyranopyrazole derivatives efficiently inhibited all cell growth tested in a dose-dependent manner (Table 1 and Figures 1-8).

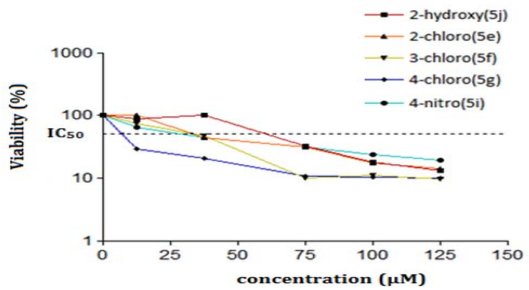


Fig.1. The percentage of cytotoxicity versus concentration by MTT exclusion on cancer cell line KB (Human Mouth Carcinoma). IC_{50} value was obtained by plotting the $\log_{10}$ of the percentage of proliferation values versus drug concentrations. Data are expressed as the mean±SEM of three separate experiments (n = 3).

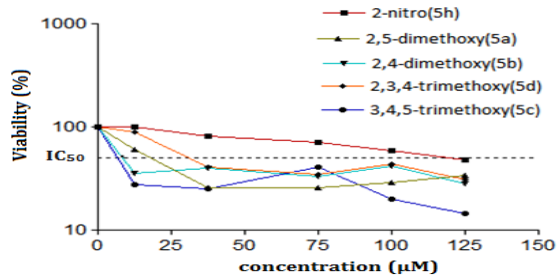


Fig. 2. The percentage of cytotoxicity versus concentration by MTT exclusion on cancer cell line KB (Human Mouth Carcinoma). IC_{50} value was obtained by plotting the $\log_{10}$ of the percentage of proliferation values versus drug concentrations. Data are expressed as the mean±SEM of three separate experiments (n = 3).

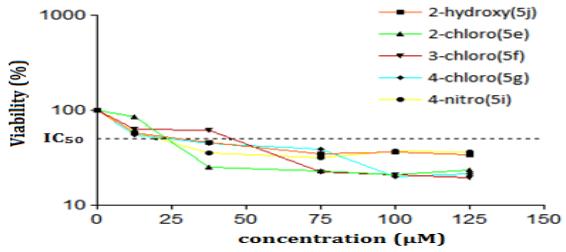


Fig. 3. The percentage of cytotoxicity versus concentration by MTT exclusion on cancer cell line SW48 (Human Colon Adenocarcinoma). IC_{50} value was obtained by plotting the $\log_{10}$ of the percentage of proliferation values versus drug concentrations. Data are expressed as the mean±SEM of three separate experiments (n = 3).

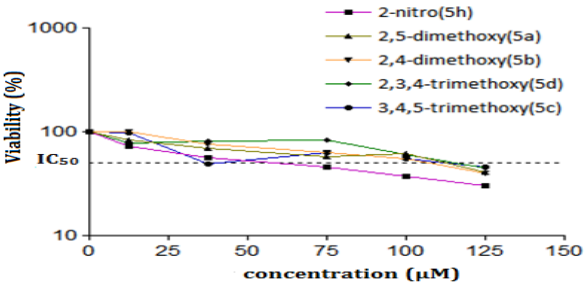


Fig.4. The percentage of cytotoxicity versus concentration by MTT exclusion on cancer cell line SW48 (Human Colon Adenocarcinoma). IC_{50} value was obtained by plotting the $\log_{10}$ of the percentage of proliferation values versus drug concentrations. Data are expressed as the mean±SEM of three separate experiments (n = 3).

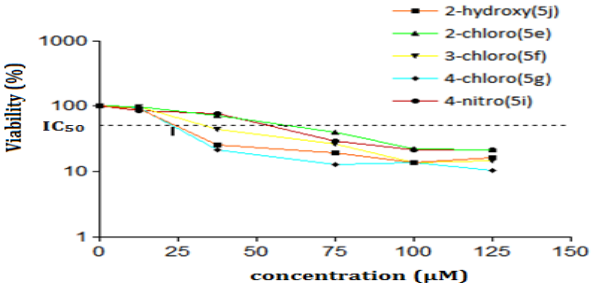


Fig. 5. The percentage of cytotoxicity versus concentration by MTT exclusion on cancer cell line HepG2 (Human Liver Carcinoma). IC_{50} value was obtained by plotting the $\log_{10}$ of the percentage of proliferation values versus drug concentrations. Data are expressed as the mean±SEM of three separate experiments (n = 3).

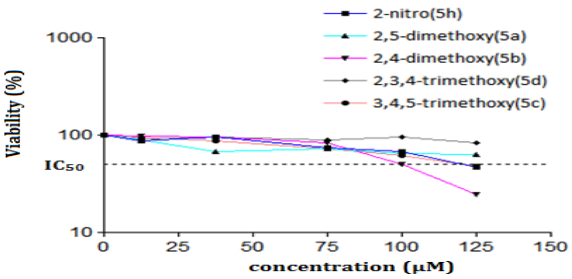


Fig. 6. The percentage of cytotoxicity versus concentration by MTT exclusion on cancer cell line HepG2 (Human Liver Carcinoma). IC_{50} value was obtained by plotting the $\log_{10}$ of the percentage of proliferation values versus drug concentrations. Data are expressed as the mean±SEM of three separate experiments (n = 3).

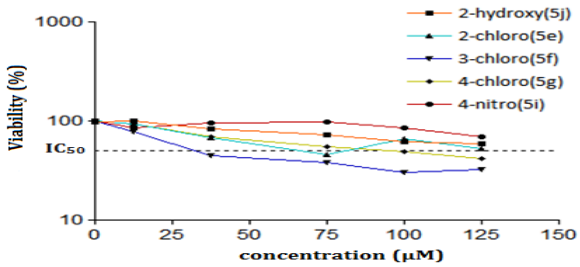


Fig.7. The percentage of cytotoxicity versus concentration by MTT exclusion on cancer cell line A549 (Human Lung Carcinoma). IC_{50} value was obtained by plotting the $\log_{10}$ of the percentage of proliferation values versus drug concentrations. Data are expressed as the mean±SEM of three separate experiments (n=3).

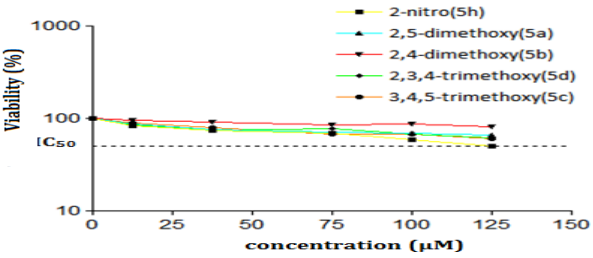


Fig. 8. The percentage of cytotoxicity versus concentration by MTT exclusion on cancer cell line A549 (Human Lung Carcinoma). IC_{50} value was obtained by plotting the $\log_{10}$ of the percentage of proliferation values versus drug concentrations. Data are expressed as the mean±SEM of three separate experiments (n = 3).

The data for doxorubicin as a positive control was included for comparison. The most potent compounds were **5b**, **5c**, **5g** against KB cell line (IC_{50} = 8±2.217 μM, 7±2.77 μM, 7.5± 1.49 μM respectively), **5f** against A549 cell line (IC_{50} = 31.5±2.02 μM), **5g** against HepG2 cell line (IC_{50} = 22.5±3.09 μM), **5e** and **5i** against SW48 cell line (IC_{50} = 23± 0.772 μM, 23± 4.97 μM respectively) which seem to be the best ones compared with the control drug. It should be mentioned that both electron-donating substituent (methoxy) and electron-withdrawing one (chlorine) are effective on the potency of the test compounds so that the best results were obtained for these groups. According to Table 1, compound **5b** with 2,4-dimethoxy substituent was more potent than compound **5a** against all cell lines. Substitution of dimethoxy group with trimethoxy one at 3,4,5 positions f the phenyl ring increased the cytotoxic effect on KB and SW48 cell lines with IC_{50} equal to 7±2.77 μM and 35±6.38 μM, respectively. Substitution of chlorine at different positions of the phenyl ring caused a moderate activity so that compound **5g** (*p*-Chloro) showed the best results against KB cell line (IC_{50} = 7.5±1.49 μM) compared to other positions. Replacement of chlorine atom with nitro group led to lower cytotoxic activity (compounds **5h**, **5i**, Table 1). Compound **5j** with hydroxyl group at *ortho* position could not significantly improve the cytotoxicity in comparison with other derivatives, but showed good cytotoxicity profile against HepG2 and SW48 cell lines. The overall activity profiles of derivatives demonstrated that the KB cell line has more sensitivity with respect to other cell lines.

Conclusion

In conclusion, we have been able to introduce an efficient and environmentally friendly approach for the synthesis of pyranopyrazoles via a four component condensation, using borax as a solid base catalyst in heterogeneous conditions. The reactions are characterized by non-corrosiveness, safety, low cost and waste, ease of separation, and high yields and short reaction times. Replacement of liquid acids with a solid base catalyst is a desirable feature of the reactions that may be important in their industrial manufacturing process. The reaction in water has special advantages over existing solvent and is a green chemistry approach to the preparation of biological compounds. Furthermore, *in vitro* cytotoxic activity of the test compounds was evaluated against a panel of cancer cell lines comparable to doxorubicin. Some of the pyranopyrazoles showed the high tumor-specific cytotoxicity, indicating a new drug candidate for cancer chemotherapy. Also, in our laboratory, further work is in process to design more potent anticancer agents containing pyranopyrazole moiety.

Conflict of interest

Authors certify that no actual or potential conflict of interest in relation to this article exists.

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References

[1] Anand P, Kunnumakkara AB, Sundaram C, Harikumar KB, Tharakan ST, Lai OS, Sung B, Aggarwal BB. Cancer is a preventable disease that requires major lifestyle changes. Pharm. Res. 2008;25:2097–116.

[2] Hanahan D, Weinberg RA. The Hallmarks of Cancer. Cell. 2000;100:57–70.

[3] Zhang HZ, Kasibhatla S, Wang Y, Herich J, et al. Discovery, characterization and SAR of gambogic acid as a potent apoptosis inducer by a HTS assay. Bioorg. Med. Chem. 2004;12:309–317.

[4] Zhang HZ, Drewe J, Tseng B, Kasibhatla S, Cai SX. Discovery and SAR of indole-2-carboxylic acid benzylidene-hydrazides as a new series of potent apoptosis inducers using a cell-based HTS assay. Bioorg. Med. Chem. 2004;12:3649–3655.

[5] Broxterman HJ, Georgopapadakou NH. Anticancer therapeutics: "Addictive" targets, multi-targeted drugs, new drug combinations. Drug Resist. Updat. 2005;8:183–197.

[6] Nasr MN, Gineinah MM. Pyrido[2,3-*d*]pyrimidines and pyrimido[5′,4′:5,6]pyrido[2,3-*d*]pyrimidines as new antiviral agents: Synthesis and biological activity. Arch. Pharm. Med. Chem. 2002;335:289–295.

[7] Abdelrazek FM, Metz P, Metwally NH, El-Mahrouky SF. Synthesis and molluscicidal activity of new cinnoline and pyrano[2,3-*c*]pyrazole derivatives. Arch. Pharm. Med. Chem. 2006;339:456.

[8] Abdelrazek FM, Metz P, Kataeva O, Jaeger A, El-Mahrouky SF. Synthesis and molluscicidal activity of new chromene and pyrano[2,3-*c*]pyrazole derivatives. Arch. Pharm. Med. Chem. 2007;340:543.
[9] Kuo SC, Huang LJ, Nakamura HJ. Studies on heterocyclic compounds. 6. Synthesis and analgesic and anti-inflammatory activities of 3,4-dimethylpyrano[2,3-*c*]pyrazole-6-one derivatives. Med. Chem. 1984;27:539.
[10] Nadia MR, Nahed YK, Fahmyb A, El-Sayeda AAF. Facile synthesis of fused nitrogen containing heterocycles as anticancer agents. Pharma. Chem. 2010;2:400-406.
[11] Wang JL, Liu D, Zheng ZJ, Shan S, Han X, Srinivasula SM, Croce CM, Alnemri ES, Huang Z. Structure-based discovery of an organic compound that binds Bcl-2 protein and induces apoptosis of tumor cells. Proc. Natl. Acad. Sci. U.S.A. 2000;97:7124.
[12] Foloppe N, Fisher LM, Howes R, Potter A, Robertson AGS, Surgenor AE. Identification of chemically diverse Chk1 inhibitors by receptor-based virtual screening. Bioorg. Med. Chem. 2006;14:4792.
[13] Mecadon H, Rohman MDR, Rajbangshi M, Myrboh B. γ-Alumina as a recyclable catalyst for the four-component synthesis of 6-amino-4-alkyl/aryl-3-methyl-2,4-dihydropyrano[2,3-*c*]pyrazole-5-carbonitriles in aqueous medium. Tetrahedron Lett. 2011;52:2523.
[14] Kanagaraj K, Pitchumani K. Solvent-free multicomponent synthesis of pyranopyrazoles: per-6-amino-β-cyclodextrin as a remarkable catalyst and host. Tetrahedron Lett. 2010;51:3312.
[15] Shaabani A, Sarvary A, Rezayan AH, Keshipour S. Synthesis of fully substituted pyrano[2,3-*c*]pyrazole derivatives via a multicomponent reaction of isocyanides. Tetrahedron 2009;65:3492.
[16] Litvinov YM, Shestopalov AA, Rodinovskaya LA, Shestopalov, AM. J. Comb. Chem. New convenient four-component synthesis of 6-amino-2,4-dihydropyrano[2,3-*c*] pyrazole-5-carbonitriles and one-pot synthesis of 6'-aminospiro[(3*H*)-indol-3, 4'pyrano[2,3-*c*]pyrazole]-(1*H*)-2-on-5'-carbonitriles.2009.
[17] Mecadon H, Rohman MDR, Kharbangar I, Laloo BM, Kharkongor I, Rajbangshi M, Myrboh B. L-Proline as an efficicent catalyst for the multi-component synthesis of 6-amino-4-alkyl/aryl-3-methyl-2,4-dihydropyrano[2,3-*c*]pyrazole-5-carbonitriles in water. Tetrahedron Lett. 2011;52:3228.
[18] Dyachenko VD, Rusanov EB. Novel approaches to synthesis of 4-alkyl-6-amino-5-cyano-3-methyl(propyl,phenyl)-*2H*,*4H*-pyrano[2,3-*c*]pyrazoles. Chem. Heterocycl. Compd. 2004;40:231.
[19] Al-Matar HM, Khalil KD, Adam AY, Elnagdi MH. Green one pot solvent-free synthesis of pyrano[2,3-c]pyrazoles and pyrazolo[1,5-a]pyrimidines. Molecules 2010;15:6619.
[20] Litvinov YM, Rodinovskaya LA, Shestopalov AM. A new convenient four-component synthesis of 6-amino-2**H**,4**H**-pyrano[2,3-**c**]pyrazole-5-carbonitriles and one-pot synthesis of 6′-amino-5-cyano-1,2-dihydrospiro-[(3**H**)-indole-3,4′-(4′**H**)-pyrano[2,3-**c**]pyrazole]-2-ones. Russ. Chem. Bull., Int. Ed. 2009;58:2362.
[21] Vasuki G, Kumaravel K. Rapid four-component reactions in water: synthesis of pyranopyrazoles. Tetrahedron Lett. 2008;49:5636.
[22] Shestopalov AM, Emeliyanova YM, Shestopalov AA, Rodinovskaya LA, Niazimbetova ZI, Evans DH. Cross-condensation of derivatives of cyanoacetic acid and carbonyl compounds. Part 1: Single-stage synthesis of 1′-substituted 6-amino-spiro-4-(piperidine-4′)-2*H*,4*H*-pyrano[2,3-*c*]pyrazole-5-carbonitriles. Tetrahedron 2003;59:7491.
[23] Gogoi S, Zhao CG. Organocatalyzed enantioselective synthesis of 6-amino-5-cyanodihydropyrano[2,3-*c*]pyrazoles. Tetrahedron Lett. 2009;50:2252.
[24] Siddekha A, Nizam A, Pasha MA. An efficient and simple approach for the synthesis of pyranopyrazoles using imidazole(catalytic) in aqueous medium, and the vibrational spectroscopic studies on 6-amino-4-(4′-methoxyphenyl)-5-cyano-3-methyl-1-phenyl-1,4-dihydropyrano[2,3-*c*]pyrazole using density functional theory. Spectrochem. Acta: A. 2011;81:431.
[25] Kemnitzer W, Drewe J, Jiang S, Zhang H, Wang Y, Zhao J, et al. Discovery of 4-aryl-4H-chromenes as a new series of apoptosis inducers using a cell- and caspase-based high-throughput screening assay. Structure-activity relationship of the 4-aryl group. J. Med. Chem. 2004;47:6299-6310.
[26] Kemnitzer W, Kasibhatla S, Jiang S, Zhang H, Wang Y, Zhao J, et al. 4-Aryl-4H-chromenes as a new series of apoptosis inducers using a cell- and caspase-based high throughput screening assay, structure–activity relationships of the 7,8-positions. Bioorg. Med. Chem. Lett. 2005;15:4745–4751.
[27] Kemnitzer W, Drewe J, Jiang S, Zhang H, Wang Y, Zhao J, et al. Discovery of 4-aryl-4H-chromenes as a new series of apoptosis inducers using a cell- and caspase-based high throughput screening assay. 3. Structure–activity relationships of fused rings at the 7,8-positions. J. Med. Chem. 2007;50:2858–2864.
[28] Li CJ, Chan TH. Comprehensive Organic Reactions in Aqueous Media. Eds. John Wiley & Sons, 2007.
[29] Chanda A, Fokin VV. Organic synthesis "on water"**.** Chem. Rev. 2009;109:725.
[30] Breslow R. Hydrophobic effects on simple organic reactions in water**.** Acc. Chem. Res. 1991;24:159.
[31] Domling A, Herdtweck E, Ugi I. Multi-component reactions V: The seven-component reaction**.** Acta. Chem. Scand. 1998;52:107.
[32] Gholivand K, Jafari H, Adibi H. Simple and new method for the synthesis of β-acetamido ketones on a solid surface. Synth. Commun. 2011;41:1786-1793.

[33] Massah AR, Adibi H, Khodarahmi R, Abiri R, Majnooni MB, Shahidi Sh, Asadi B, Mehrabi M, Zolfigol MA. Synthesis, in vitro antibacterial and carbonic anhydrase II inhibitory activities of N-acylsulfonamides using silica sulfuric acid as an efficient catalyst under both solvent-free and heterogeneous conditions. Bioorg. Med. Chem. 2008;16:5465-5472.
[34] Gholivand K, Zare K, Jafari H, Adibi H. A New and Efficient Procedure for Synthesis of 1,5-Benzodiazepine Derivatives in Solution and under Solvent-Free Conditions. Chin. J. Chem. 2011;29:2421-2427.
[35] Adibi H, Massah AR, Majnooni MB, Shahidi Sh, Afshar M, Abiri R, Javaherian HJ. Synthesis, Characterization, and Antimicrobial Evaluation of Sulfonamides Containing *N*-Acyl Moieties Catalyzed by Bismuth(III) Salts Under Both Solvent and Solvent-Free Conditions. Synth. Commun. 2010;40:2753–2766.
[36] Rajabalian S, Foroumadi A, Shafiee A, Emami S. Functionalized *N*-(2-oxyiminoethyl)piperazinyl quinolones as new cytotoxic agents. J. Pharm. Pharm. Sci. 2007;10:152-157**.**
[37] Hosseinzadeh L, Behravan J, Mosaffa F, Bahrami G, Bahrami A, Karimi G. Curcumin potentiates doxorubicin-induced apoptosis in H9c2 cardiac muscle cells through generation of reactive oxygen species. Food Chem. Toxicol. 2011;49(5):1102–1109.
[38] Adibi H, Hosseinzadeh L, Mahdian M, Foroumadi A, Zolfigol MA, Mallakpour S. Synthesis of 7-Substituted Fluoroquinolone Derivatives Containing Triazolidine Dione Moiety and In Vitro Evaluation of Their Cytotoxic Effects. J. Rep. Pharm. Sci. 2013;2: 59-66.

