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Original Research Article

Synthesis of Hydroxy Aryl Ketones via a Novel Low Temperature Variant of the Fries Rearrangement

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A R T I C L E I N F O

A B S T R A C T

The aim of the current study was to examine the protection of phenol with various acyl chloride derivatives and anhydride derivatives at different temperatures in the presence of different simpler catalytic agents to produce corresponding ester in good yield after workup. All prepared esters were isolated and identified by their physical properties and spectrophotometrically methods such as FT IR, ^{1}H-NMR, and ^{13}C-NMR. Rearrangement of phenolic esters was done by Fries rearrangement with 5 equivalents of anhydrous Lewis acid, aluminum chloride in nitromethane, at low temperature. Aluminum chloride pre-dissolved in nitromethane occurred smoothly, enabling the desired regioselective *para*–isomer in moderate to good yield. All the products of the rearrangement were isolated and established by detecting their physicochemical properties and by analyzing their FT-IR, ^{1}H-NMR, and ^{13}C-NMR spectroscopy. *In vitro* four compounds, phenyl benzoate (3), phenyl 4-methoxy benzoate (4), 4-hydroxybenzophenone (4a), and (4-hydroxyphenyl)4-methoxyphenyl)methanone (4b) were evaluated for inhibition extracting DNA from HepG2 and MEF cells at IC_{50} for 24 hours. The results showed that 4-hydroxybenzophenoneand 4-hydroxyphenyl)(4-methoxyphenyl)methanone were induced apoptosis by mitochondrial Intrinsic pathway after 24 hours of exposure.

G R A P H I C A L A B S T R A C T
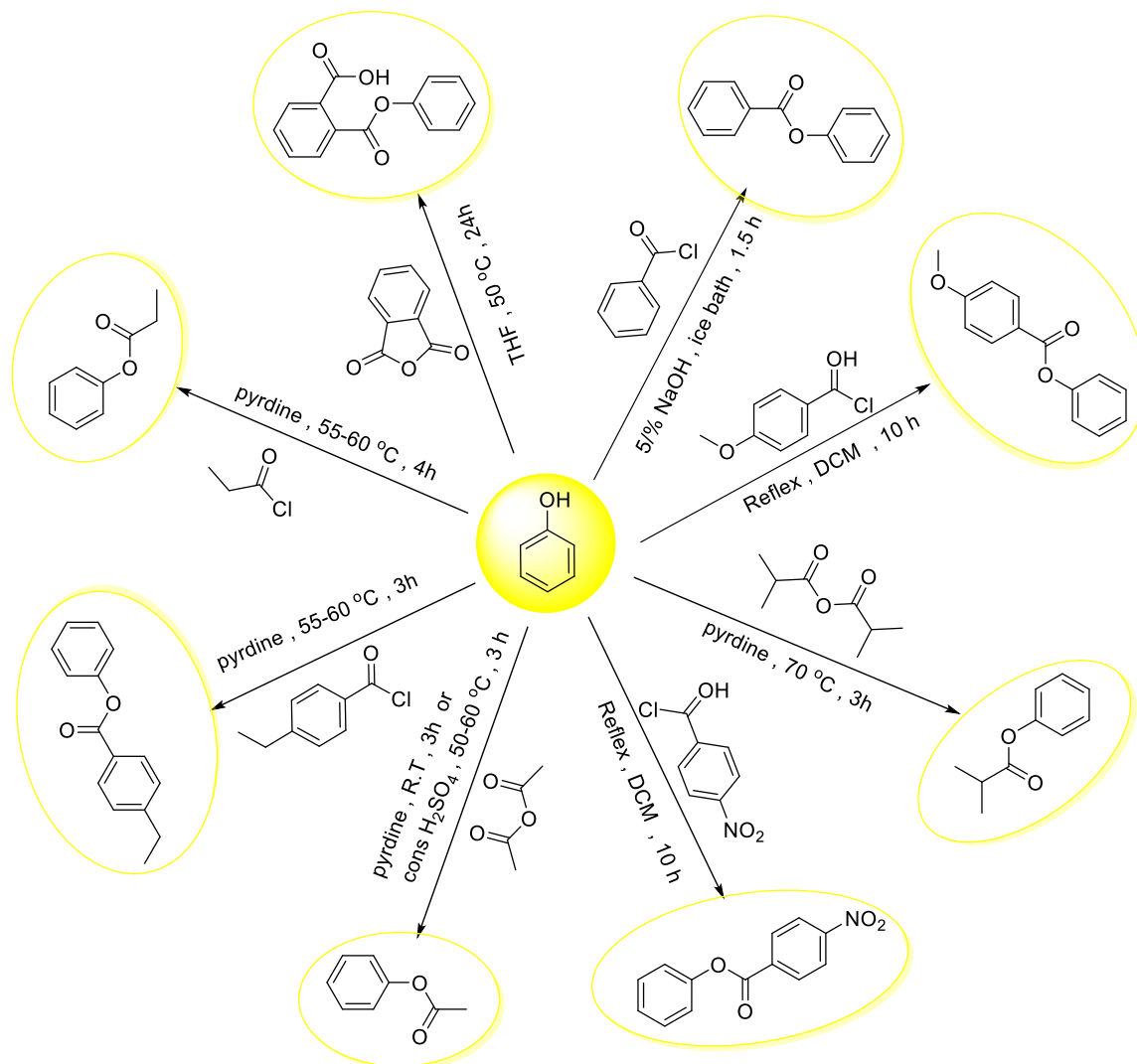
Introduction

Phenols are important starting materials, intermediates, and functional elements of a very broad range of chemicals and materials [1] in different industries such as dyes, pharmaceuticals [2], and pesticides industries [3]. Usually phenols are functionalized through electrophilic aromatic substitution reactions, such as Friedel-Crafts alkylations and Acylations. The Friedel-Crafts alkylations and acylations of phenols gives ortho- and para- substuted phenols, the regioselectivity being dependent on the catalyst used. Numerous catalysts have been

explored for efficient preparation of the alkylphenols, both bronsted and lewis acids can be used as the catalysts. Friedel-Crafts acylations are moreregioselective than the alkyations [4]. Phenols are extremely valuable building blocks in the areas of pharmaceuticals, and natural products to carry out modifications on phenols, the phenolic oxygen is routinely protectedto prevent unwanted side reactions. At present, a wide range of compounds are available that can be used as protection groups for hydroxyl phenol [5]. Phenol can be protectedas ether or ester. Aromatic ethers and esters are more readily cleaved than the corresponding aliphatic

compounds [6]. Phenolic esters [7] can be rearranged to hydroxyl aryl ketone [8] by synthetically useful reaction known as the Fries rearrangement. The reaction is *ortho,para*-selective and the ratio depends on temperature, solvent, and other reaction conditions. Generally at low temperature *P*-products and at high temperature *O*-products are predominately formed [9]. The present study is a fundamental investigation into a potentially game-changing modification- but under-investigated - variant of the Fries rearrangement, the intramolecular analogue of the more commonly encountered Friedel-Crafts reaction. Not only does the mildness of the conditions (aluminum chloride in nitromethane solvent at room temperature as opposed to molten aluminum chloride as reaction medium) mean that a far wider range of substrates can become synthetically viable, but also the regiochemical outcome of the room temperature reaction differs from that of the high temperature (>300 °C) classical Fries reaction.

This study will investigate the basis of both substrate scope and regioselectivity with simple model substrates, but the results of this study can rapidly extrapolated to more complex systems leading to targets of agrochemical and pharmaceutical interest, resulting in more efficient and cost-effective commercial production of key complex organic materials. It has been proposed, but not conclusively demonstrated, that the greater activity of this catalytic system is a result of a co-operative interaction between the aluminum chloride catalyst and the nitromethane solvent. Thus, these synthetic and mechanistic studies should lead to interaction with chemists from other disciplines, most notably in the spectroscopic and physical analytical areas.

## Materials and Instruments

Chemicals used in this work are supplied from Merck, Sigma-Aldrich, BDH and Fluka companies and are used without further purification. FT-IR spectra were recorded on Perkin Elmer spectrum -65 using KBr discs in the (500-4000) cm^{-1} spectral range. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker 400 MHz instrument using DMSO-d6 as a solvent and TMS as internal reference. Thin layer chromatography (TLC) analyses were performed using plastic backed plates with 0.25 mm Merck 60 G silica gel with fluorescent indicator, and the plates were developed either by the quenching of UV fluorescence at 254 nm or by treatment with KMnO$_4$ solution and heating.

Synthetic methods

Synthesis of phenyl benzoate (**3a**)

A Mixture of phenol (0.5 g) (5.3 mmol) and Benzoyl chloride (2.4 mL) in solution of 5% NaOH (10 mL) was added to round bottom flask and left stirred in ice bath for 1.5 h. The reaction mixture was left to precipitate for 3 days, washed thoroughly with cold distal water (3×3 mL) to furnish the title compound as a white precipitate (0.95 g); yield: (90%); m.p. 75-77 °C.

FT-IR (KBr) $ν_{max}/cm^{-1}$: 3071, 3017, 2876, 2088, 1989, 1729, 1598, 1453, 1199, 705. ^{1}H-NMR (400 MHz, DMSO-d$_6$, ppm): $δ$ = 8.3-7.3 (m, 10H, Ar-H); **^{13}C-NMR (400 MHz, DMSO-d$_6$, ppm):$δ$ =** 165.06, 151.14, 134.45, 130.03, 129.75, 129.51, 129.09, 128.79, 127.44 126.68,122.38 .
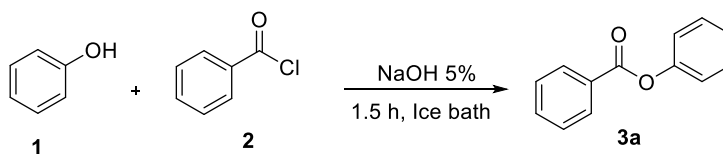
Scheme 1. Synthesis of phenyl benzoate.

Synthesis of(4-Hydroxybenzophenone) (4a)

Phenyl benzoate (0.5 g) (2.52 mmol) was dissolved in nitromethane (8 mL) and cooled to (-10 ˚C) with stirring. A solution of anhydrous aluminum chloride (1.68 g) (12.6 mmol) in nitromethane (8 mL) was added drop wise over 15 min and the mixture allowed warm up to room temperature. Stirring was continued at room temperature for 3 h. When no starting material was observed by TLC, the mixture was then quenched with ice-cold 5M hydrochloric acid (15 mL) and extracted with diethyl ether (30 mL), and the organic extract washed with saturated sodium bicarbonate (2×30), washed with brine water until neutral and dried over anhydrous sodium sulfate. Complete removal of the solvent below 40 ˚C afforded the title compound as light brown precipitate (0.46 g); yield: (92%); m.p. 136-138 °C.

FT-IR (KBr) ν_{max}/cm^{-1}: 3332, 3042, 2008, 1961,1910 , 1644, 1601 , 1486 , 1198 .**^{1}H-NMR (400 MHz, DMSO-d$_6$, ppm): δ =** 10.44 (1H) , 8.35-6.75 (m , 9H Ar-H); **^{13}C-NMR (400 MHz, DMSO-d$_6$, ppm):δ =** 194.77, 165.06, 151.12, 138.59, 143.49, 132.95 , 130.24 ,129.57, 128.82, 126.47, 122.38, 116.73, and 115.73.
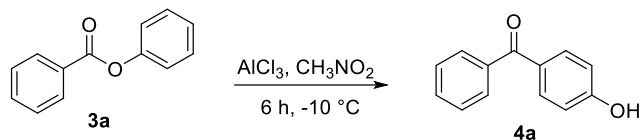
Results and Discussion

The synthesized ester compounds (**3a-h**) are shown in (Scheme 3).
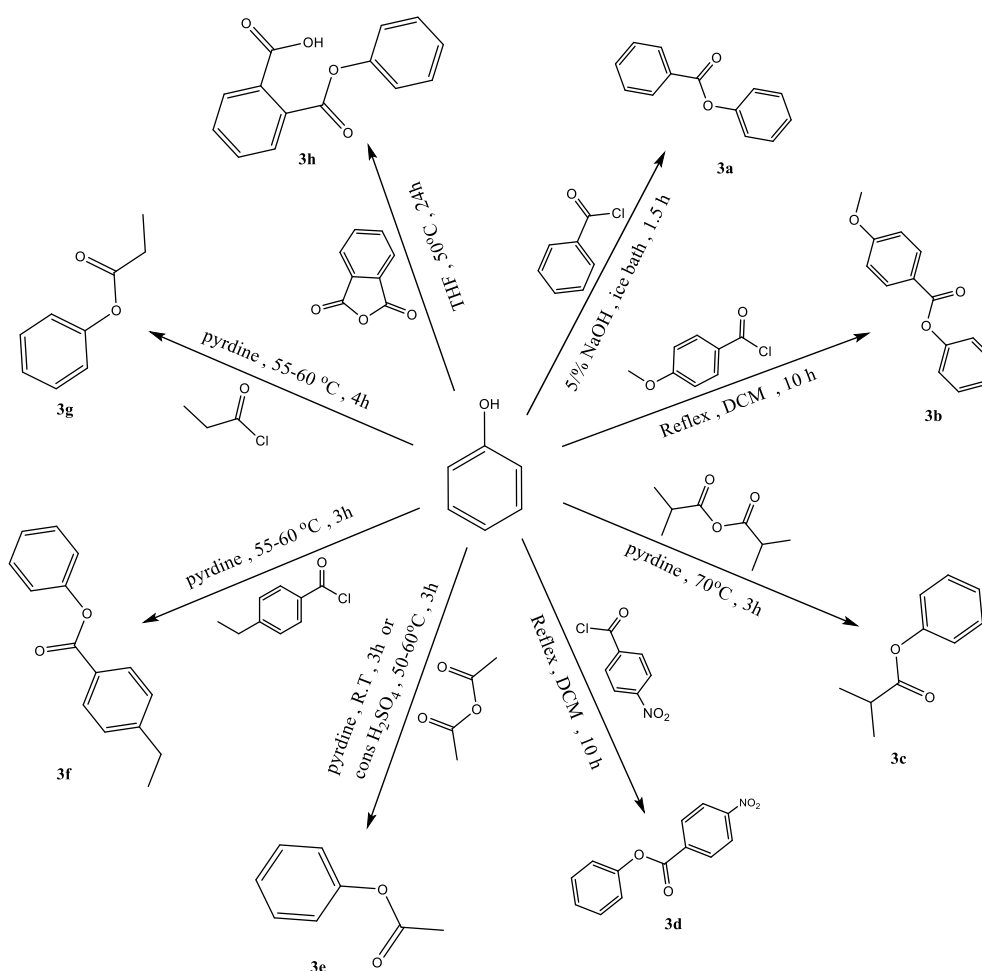
Scheme 3. Proposal synthesis of compounds **3a-h.**

The synthesized compounds were subject to TLC; spectral studies like ^{1}H-NMR, ^{13}C-NMR, and FTIR and results of some synthesized compound are discussed below. The IR spectrum of compound (Phenyl benzoate) (**3a**) showed absorption corresponding to the carbonyl group of ester at 1729 cm^{-1}. Analysis of the ^{1}H-NMR spectrum of crude materials compound (**3**) (Figure 1) was done after work showed signals characteristic at δ8.3-7.3 ppm corresponding to protons of aromatic protons. Furthermore, appearance signals at δ 165.06 ppm in ^{13}C-NMR spectrum corresponding to the resonances of the carbon of C=O of ester (Figure 2).

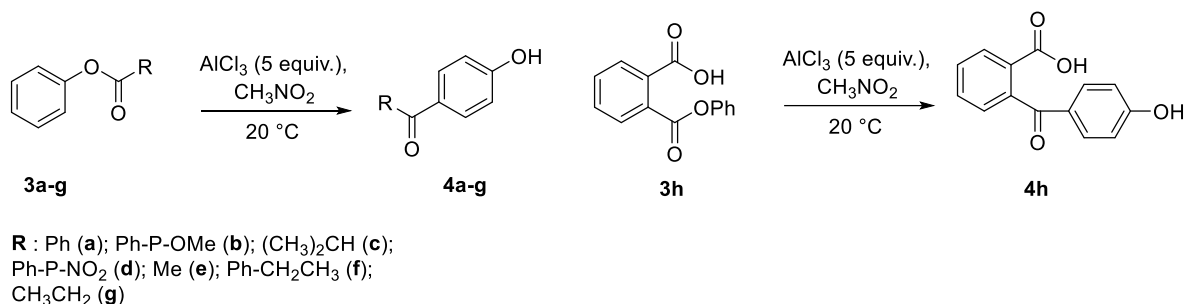**Scheme 2.** Synthesis of (4-Hydroxybenzophenone).

Figure 1. ^{1}H-NMR spectrum of compound (**3a**).

Figure 2. ^{13}C-NMR spectrum of compound (**3a**).
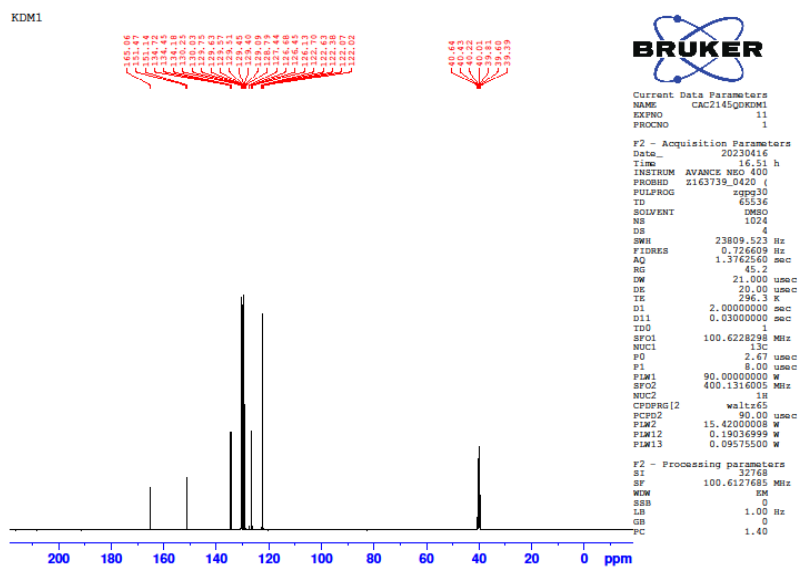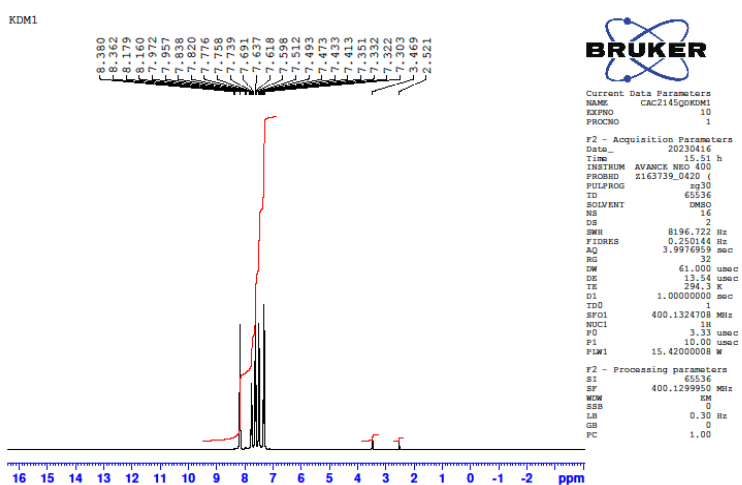
As seen earlier, we successfully synthesized phenolic esters therefore, we decided to investigate Fries rearrangements of related substrates in order to ascertain if such rearrangement could be achieved to synthesis hydroxyl aryl ketone in a regioselective manner. Subsequent rearrangement of phenolic esters using 5 equivalents of aluminum chloride predissolved in nitromethane occurred smoothly, enabling the desired product (**4a-h**) to be isolated with no isomeric product being detected in the crude reaction mixture (Scheme 4).

The compounds (**4a-g**) were treated with 5 equ. of $AlCl_3$ in CH_3NO_2 at room temperature for 6-8 h. Fries rearrangement was observed and the yield of desired products was 80-92%.

Programmed cell death (Apoptosis)

Is a highly regulated mechanism that generally occurs during development and aging to keep cells in tissue? It can also act as a protective mechanism when the cells are damaged by toxic agents or by diseases. Drugs and irradiation cause DNA damage, which results in triggering apoptosis, and some hormones (e.g., Corticosteroids) can cause apoptotic death in several cells. Apoptosis occurs in damaged cells as a result of exposure to carcinogenic factors or when infected with viruses and it is genetic systems that facilitate the destruction of abnormal cells, and then it is one of the mechanisms of inhibition of cancer cells [20-23].

R : Ph (**a**); Ph-P-OMe (**b**); $(CH_3)_2CH$ (**c**); Ph-P-NO$_2$ (**d**); Me (**e**); Ph-CH$_2$CH$_3$ (**f**); CH$_3$CH$_2$ (**g**)

Scheme 4. Rearrangement of the compound (**4a-g, 4h**).

Materials and methods

Table 1. IC_{50} concentration values in the cancer cell lines HePG2, MEF cell line

Cell Line	IC_{50} con. µg/mL
HepG2	100
MEF	200

Electrophoresis of genetic material (DNA)

Cellsseeding and harvesting of the cancer cell lines

DNA Extraction

DNA extraction was carried out using Magnesia Genomic DNA Large Volume Kit from (Anatolia, Turkey) according to method. This diagnostic kit contains all the reaction components needed to purify and extract DNA using the Magnetic-particle technique.

Detection of morphological changes using acridine orange\propidium iodide (AO/PI) stain

This stain is used to dye DNA and detect changes that occur in cells due to programmed cell death. (AO) stain is considered a positive ion with the ability to stain the nuclei of living and dead cells, as the nuclei of living cells appear in a bright green, spherical shape, and a healthy structure when examined under a fluorescent microscope by using a wavelength filter (blue), while PI stain enters the nuclei of dead cells in which a breakdown occurs in their membranes and which appear bright red color when examined under a fluorescence microscope using

a wavelength filter (green). This method was performed to assess morphological changes (imaging nuclear changes and the formation of apoptotic bodies) and to identify living cells and dead cells in which programmed cell death has occurred.

The solution used

(1) Acridine orange stain solution at a concentration of 5 mg/mL.
(2) Propidium Iodide stock stain solution at a concentration of 3 mg/mL.
(3) PBS (pH 7.2).

Preparation of Working Solution

100 μL of (AO) and 100 μL of (PI) were added to 1000 μL of (PBS), mix well, and keep at room temperature for two weeks.

Procedure

1- Grown the cancer cells in a 24-hole tissue culture plate, 2 mL of cell suspension is added to each hole and incubated for 24 hours at 37 °C.

2- When the cells adhere to the plate, the cells are treated with alkaloid extract at IC50 concentration and incubated for 24 hours.

3- After the incubation period is over, the culture medium is discarded and 500 μL of AO/PI stain mixture is added to each hole and then incubated at 37 °C for 20-30 minutes in the dark.

4- After that, the cells are washed with PBS solution, and the washing process is repeated more than once until the excess stain is removed.

5- Finally, 500 μL of PBS is added to each hole, and then the cells are visualized with an inverted fluorescence light microscope by using blue and green filters.

Detection of biological activity of synthesized compound (3a, 4a)

In this study, the DNA fragmentation was detected by extracting DNA from HepG2 and MEF cells treated with (**3a**) and (**4a**) at IC_{50} for 24 hours, then the DNA fragmentation was examined by using agarose gel electrophoresis, shows a significant indicator of programmed cell death (DNA fragmentation) in the cancer lines treated with the (**3a**) and (**4a**). The results showed that damages occurred, represented by a break in the DNA in the agarose gel of the treated cells, while the control group showed minimal damage. According to acridine orange/ propidium iodide mixed fluorescent staining assay, the used (**3a**) and (**4a**) were able to induce highest apoptosis percentage in the treated cells after 24 hrs of exposure. This was confirmed by DNA fragmentation assay as well. The all results showed that (**4a**) better than compound (**3a**) was induced apoptosis by mitochondrial Intrinsic pathway after 24 hours of exposure.

Detection of biological activity of synthesized compound (3b, 4b)

In this study, the DNA fragmentation was detected by extracting DNA from HepG2 and MEF cells treated with (**3b**) and (**4b**) at IC_{50} for 24 hours, then the DNA fragmentation was examined by using agarose gel electrophoresis. shows a significant indicator of programmed cell death (DNA fragmentation) in the cancer lines treated with the (**3b**) and (**4b**). The results showed that damages occurred, represented by a break in the DNA in the agarose gel of the treated cells, while the control group showed minimal damage. According to acridine orange/ propidium iodide mixed fluorescent staining assay, the used (**3b**) and (**4b**) were able to induce highest apoptosis percentage in the treated cells after 24 hours of exposure. This was confirmed by DNA fragmentation assay as well. The all results showed that (**4b**) better than compound (**3b**) was induced apoptosis by mitochondrial Intrinsic pathway after 24 hours of exposure.
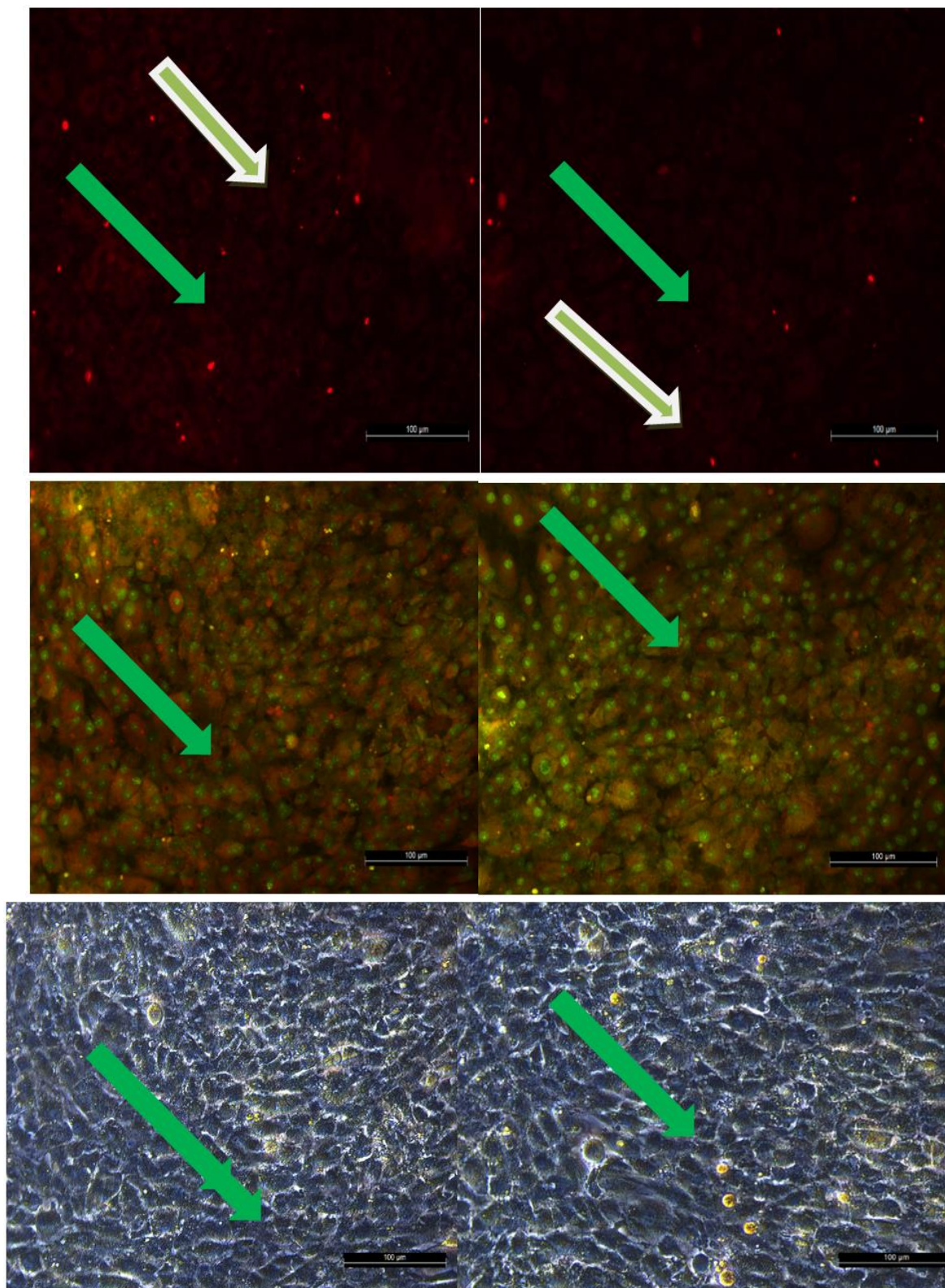
Figure 3. Detection of morphological changes of programmed cell death using (AO\PI) stain in HepG2 and MEF normal cell line that treated with (**3a**).
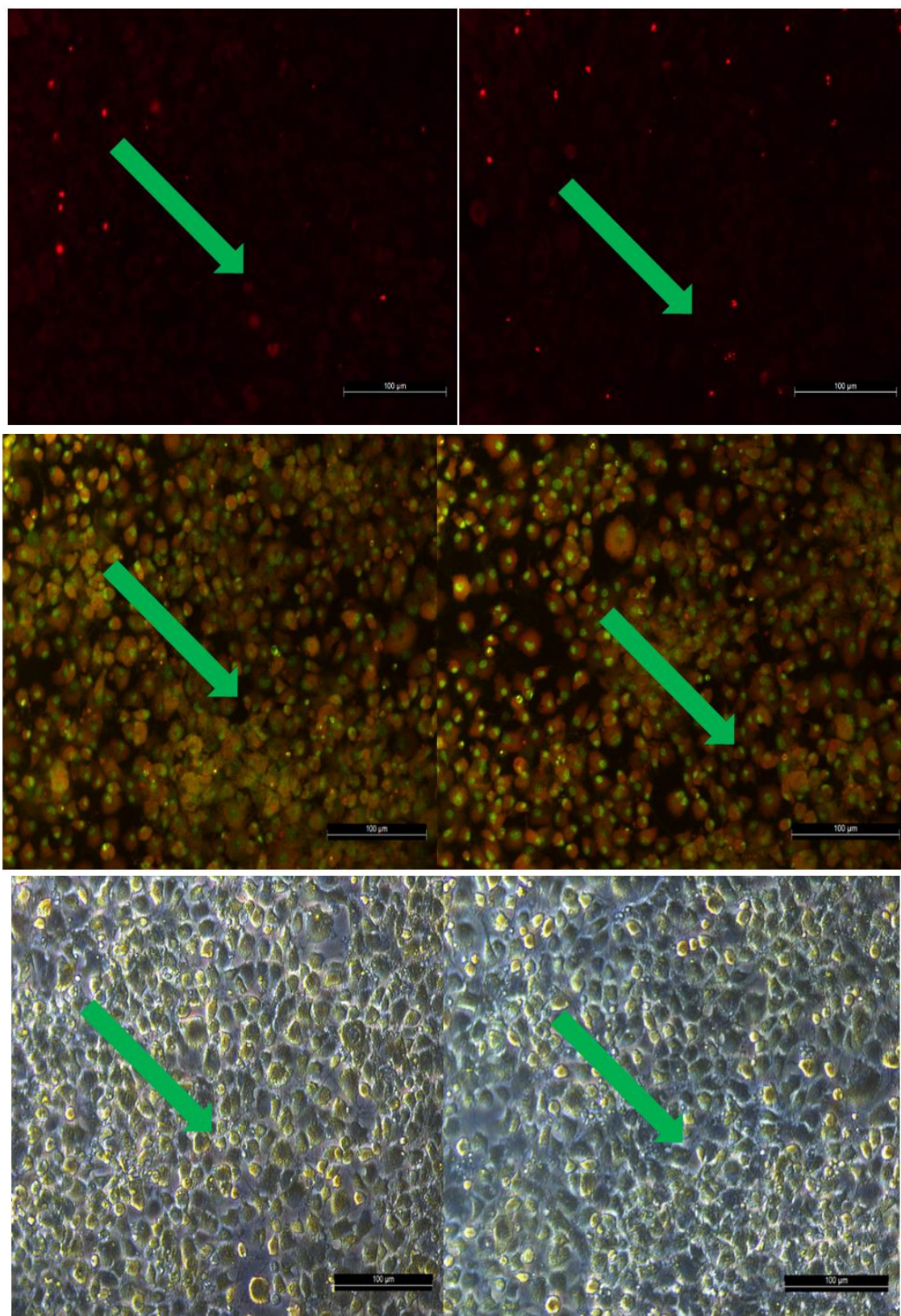
Figure 4. Detection of morphological changes of programmed cell death using (AO\PI) Stain in HepG2 and MEF normal cell line that treated with (**4a**).

Conclusion

To sum up, various series of ester that proved to be a useful intermediate to investigate the Fries Rearrangements were synthesized, characterized and their pharmacological and chemical activities have been evaluated. The Fries Rearrangements obtained under stander conditions like aluminum chloride and nitromethane as solvent under lower temperature that led to the appearance of an orange solution and it therefore seems that the solvent plays an important role in generating the active catalyst, which also gave the desired rearrangement. The synthesized compounds phenyl benzoate, phenyl 4-methoxy benzoate, 4-hydroxybenzophenone and (4-hydroxyphenyl)4-methoxyphenyl)methanone showed good activity in a human liver cancer cell line HepG2 inhibition at 37 °C within 24 h.

Acknowledgements

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