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ORIGINAL PAPER

Zn/MCM-41-catalyzed unsymmetrical Hantzsch reaction and the evaluation of optical properties and anti-cancer activities of the polyhydroquinoline products

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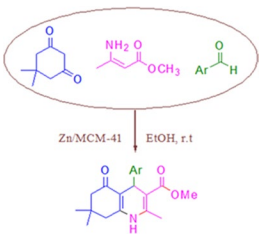
Abstract

The three-component Hantzsch condensation of various aryl aldehydes, dimedone, and methyl 3-aminocrotonate was investigated in the presence of MCM-41-supported $ZnNO_3$. The polyhydroquinoline products which were obtained under mild conditions and very easy workup were evaluated for anti-cancer activities against MCF-7, SK-BR-3, and HT-29 of breast and colon cancer cells. The fluorescence emission of some products was also studied and their optical parameters were reported.

Graphic abstract



Keywords Hexahydroquinolines · Hantzsch reaction · Heterocycles · Fluorescence spectroscopy · Multicomponent reactions · Anti-cancer activity

Introduction

Hexahydroquinoline-5-ones are the modified structures based on both quinolines and 1,4-dihydropyridines (DHPs), with many medicinal and biological properties [1, 2]. They have been shown to possess antimicrobial [3], anti-inflammatory [4], and neuroprotective [5] activities. Figure 1 shows the structure of some members of these compounds as potent anti-cancers (**1**–**3**) [6–8], antioxidant (**4**) [9], and calcium channel modulator agents (**5**) [10]. The catalytic-modified Hantzsch reaction between aromatic aldehydes, 1,3-cyclohexanediones, β-ketoesters, and ammonium acetate is the known one-pot method for the synthesis of these heterocycles [11]. Development of several synthetic methodologies to achieve various diversities

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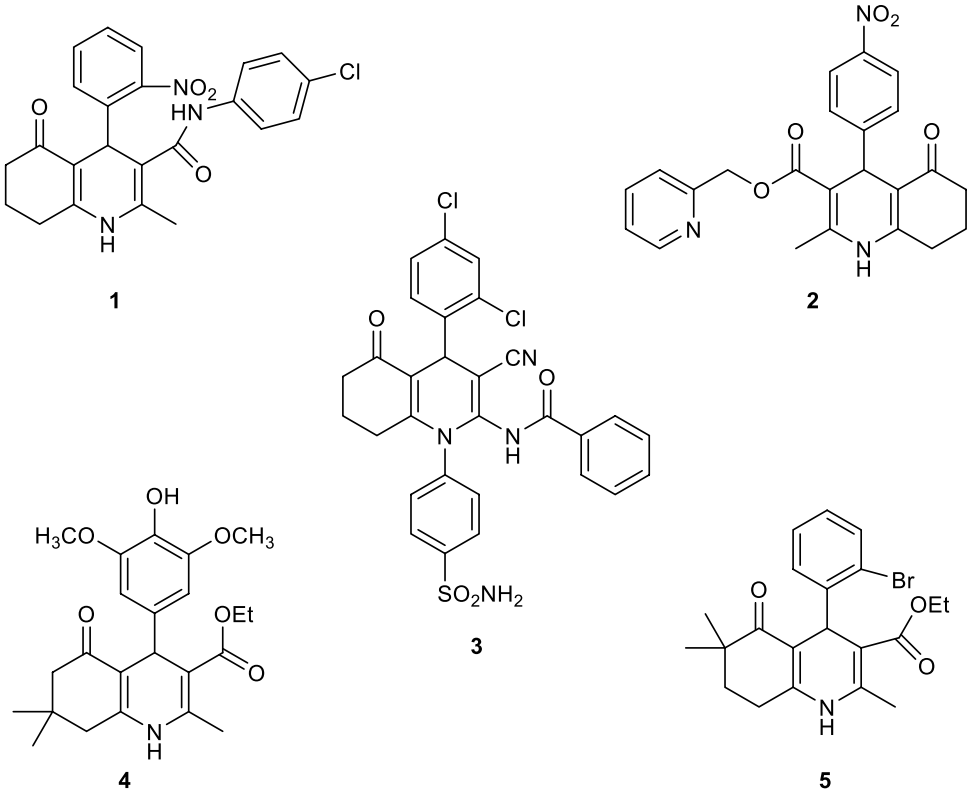


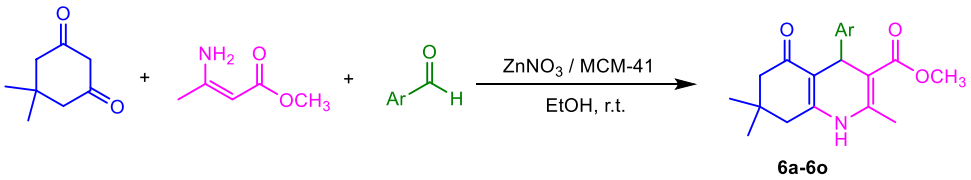
Fig. 1 Some hexahydroquinoline-5-one derivatives with potent biological activities

of hexahydroquinoline-5-one derivatives exhibits the growing interests on these compounds [12–17]. Using inexpensive and available reagents as well as structural variability have also increased the attractiveness of their synthesis [18, 19]. Among the reported catalytic conditions, application of heterogeneous and reusable catalysts has been extensively concerned for multicomponent reactions [20, 21]. The mesoporous materials such as MCM-41 (Mobil Composition of Matter) with high surface capacity and nano-sized pores have been widely utilized as proper support for many metal cations [22–25]. We have already reported the use of $ZnNO_3$-incorporated MCM-41 as an efficient catalyst for Biginelli reaction at solvent-free conditions [26]. Zn^{2+} is a potential Lewis acid to activate organic reactants to promote many transformations [27, 28]. In this work, we report the three-component reactions between various aromatic aldehydes, methyl 3-aminocrotonate and dimedone in the presence of $ZnNO_3$-impregnated MCM-41 at room temperature. We found that our dihydropyridine products show emission properties which have not been already reported. The optical parameters and fluorescence spectra of some synthetic products are herein reported. The anti-cancer activities of the products were then evaluated against MCF-7, SK-BR-3, and HT-29 of breast and colon cancer cells for which the results are presented.

Results and discussion

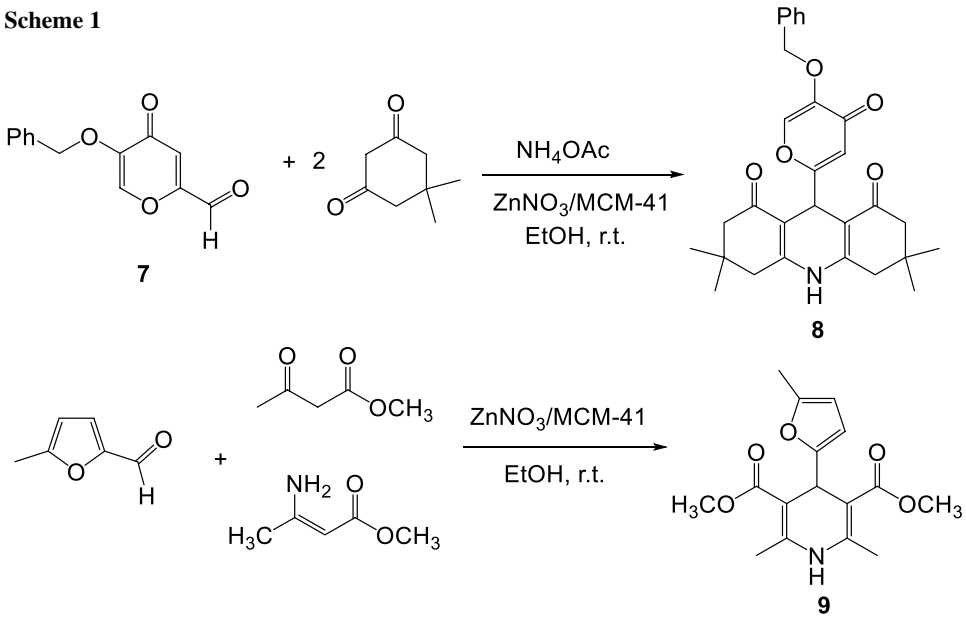
The modified three-component Hantzsch reaction between aromatic aldehydes, dimedone, and methyl 3-aminocrotonate in the presence of $Zn(NO_3)_2$/MCM-41 (prepared by impregnation method [26]) in ethanol at room temperature, afforded the dihydropyridine products **6a**–**6o** in good to excellent yields (Table 1). Using not-impregnated MCM-41, aqueous media or solvent-free conditions resulted in the complex mixtures or poor yields. Among the used aldehydes, 3- and 4-fluorobenzaldehyde, salicylaldehyde, 4-hydroxybenzaldehyde, and also 3-indolecarbaldehyde showed the least reactivity (entries 4–7 and 13). Although a variety of aromatic aldehydes with electron-withdrawing or -donating substituents and also heterocyclic aldehydes underwent this conversion efficiently, utilizing 3-benzyloxy-2-formyl-4*H*-pyran-4-one (**7**) [29, 30] gave only the Knoevenagel product resulted from the condensation of aldehyde and dimedone with no participation of aminocrotonate ester. By adding ammonium acetate to this mixture, acridine-based product **8** was obtained (Scheme 1). Furthermore, we examined the efficiency of the used catalyst for the condensation of methyl acetoacetate (instead of dimedone), methyl 3-aminocrotonate and 5-methylfurfural that gave the symmetrical product **9** in 97% yield within 2 h (Scheme 1).

Table 1 Zn/MCM-41-catalyzed synthesis of methyl 4-aryl-5-oxo-hexahydroquinoline-3-carboxylates **6a–o**



Entry	Ar	Product	Yield/%	Reaction time/h
1	4-ClC_6H_4	**6a**	86	2
2	3-BrC_6H_4	**6b**	89	2
3	2-BrC_6H_4	**6c**	93	2
4	4-FC_6H_4	**6d**	74	5
5	3-FC_6H_4	**6e**	75	5
6	4-HOC_6H_4	**6f**	77	10
7	2-HOC_6H_4	**6g**	77	10
8	3-$O_2NC_6H_4$	**6h**	87	2
9	2-$O_2NC_6H_4$	**6i**	92	2
10	3,4-$(OMe)_2C_6H_3$	**6j**	82	7
11	4-$MeOC_6H_4$	**6k**	85	5
12	5-Methylfuran-2-yl	**6l**	85	5
13	Indole-3-yl	**6m**	76	10
14	4-$Me_2NC_6H_4$	**6n**	87	6
15	4-MeC_6H_4	**6o**	96	1

Scheme 1



The proposed mechanism can involve the activation of aldehyde and diketone components for Knoevenagel condensation by coordination of their carbonyl groups with zinc cation (Fig. 2). The Michael attack of enamine to the resulted intermediate **I** and then tautomerization of the adduct gives species **II**, which under catalytic intramolecular cyclization and then elimination of H_2O is converted to the desired products **6a–6o**.

We also noticed the emission properties of our synthesized dihydropyridines. We recorded the UV–Vis and

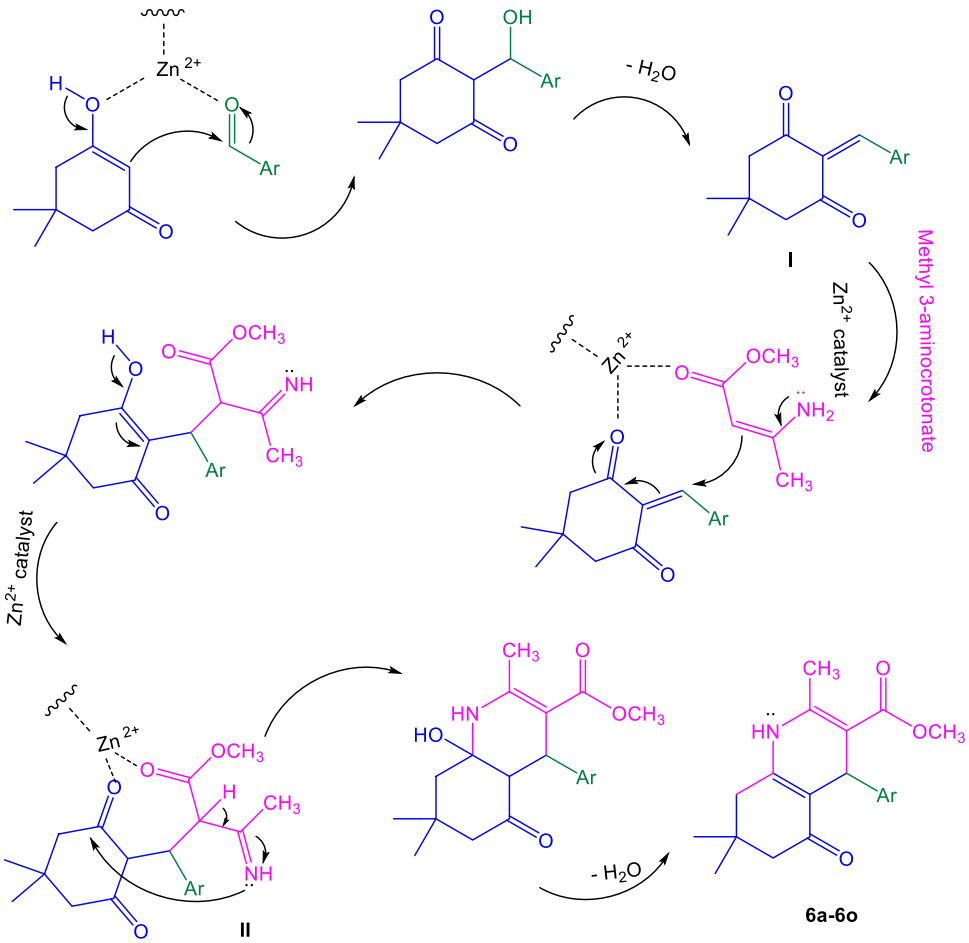


Fig. 2 The proposed mechanism for the generation of products **6a–6o**

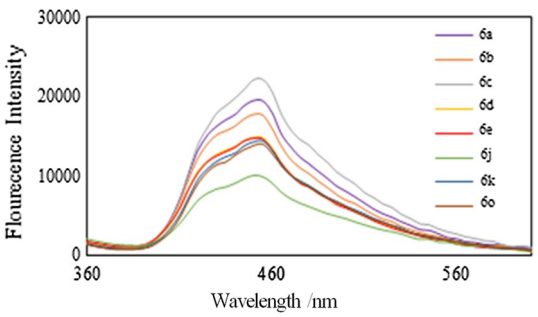


Fig. 3 The fluorescence spectra of some chosen products in dilute (1.0×10^{-5} M) ethanol solutions

fluorescence spectra of some chosen products as dilute (1.0×10^{-5} M) solutions in ethanol, to access their optical parameters. Figure 3 shows the emission spectra of the selected derivatives, which reveals that the emission wavelengths of the compounds, λ_{em}, are close to each other and in the visible area. As seen, product **6c** containing 2-bromophenyl group at the 4th position of the molecule showed higher emission quantum yield.

Table 2 IC_{50} values for cell lines/μM

Product	MCF 7	SK BR 3	HT 29	HEK 293
6a	200	40	130	1350
6b	100	35	130	990
6c	30	35	95	795
6d	45	145	135	1236
6e	100	100	100	980
6f	100	100	130	710
6g	70	120	45	1450
6h	100	80	125	998
6i	17	65	30	700
6j	50	100	100	1100
6k	100	100	100	854
6l	40	50	100	1019
6m	45	45	130	1211
6n	60	120	120	878
6o	40	125	100	1334
8	125	150	140	1452
9	100	115	120	1322
Doxorubicin	2.4 ± 0.12	1.29 ± 0.16	0.49	1.30

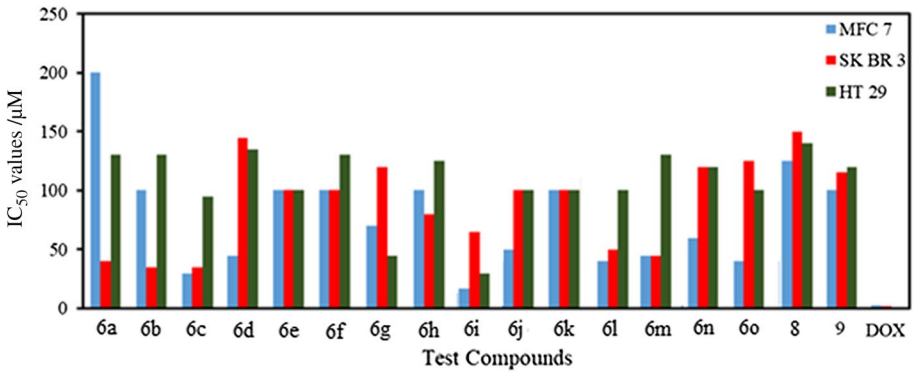


Fig. 4 Graphical presentation of the cytotoxic activity of the 1,4-dihydropyridines

Biological studies

The prepared 1,4-dihydropyridine (DHPs) **6a–6o** were tested for in vitro cytotoxicity analyses against three different cancer cell lines: human breast adenocarcinoma (MCF 7), human breast cancer (SK BR-3), and human colon adenocarcinoma (HT 29) using the MTT assay [31, 32]. Doxorubicin, a well-known anti-cancer agent, was used as the positive control in our study. The antiproliferative efficacy records are presented as IC_{50} values which describe the concentration of the product that reduces cell proliferation at 50% (Table 2). The IC_{50} values for cell lines were determined after at least three individual experiments. Cell survival was ascertained by MTT assay as explained in "**Cell cytotoxicity assay by MTT**". All of the synthetic compounds (**6a–6o**, **8**, **9**) were investigated for cytotoxicity effects and some of them showed interesting results. The results proved the considerable activity of derivatives **6c** and **6i** against all of the three cell lines. The compounds **6a** and **6b** exhibited promising activities against SK BR-3 cell line, while compound **6g** against HT 29 cell line. It has been also observed that the products containing 4-chlorophenyl (**6a**), 3- or 2-bromophenyl (**6b** and **6c**), and 2-nitrophenyl (**6i**) substituents at the 4th position of the 1,4-dihydropyridines showed relatively good activity, but those with 3-fluorophenyl (**6e**), 4-hydroxyphenyl (**6f**), and 4-methoxyphenyl (**6k**) groups, as well as the symmetric compounds **8** and **9**, were completely inactive. In addition, the attendance of a heterocyclic moiety (such as **6l** and **6m**) enhanced the cytotoxic properties against MCF-7 and SK BR-3 cell lines. The cytotoxic activities of all the 1,4-dihydropyridines have been graphically exhibited in Fig. 4. The effects of the synthetic compounds on normal cells (HEK 293) were also investigated. As could be seen in Table 2, the desired compounds have low activity.

Conclusion

As a result, we have reported the synthesis of some hexahydroquinoline-5-one derivatives by three-component reactions of aryl aldehydes, dimedone, and methyl 3-aminocrotonate in the presence of $ZnNO_3$-incorporated MCM-41 mesoporous bed. We then studied the UV–Vis and fluorescence spectra of some chosen products in dilute (1.0×10^{-5} M) solutions in ethanol and calculated their optical parameters. By considering the inherent biological properties of dihydropyridines, we have also evaluated the in vitro cytotoxicity of our products against three different cancer cell lines: human breast adenocarcinoma (MCF 7), human breast cancer (SK BR-3), and human colon adenocarcinoma (HT 29) using the MTT assay. Some of the synthesized hexahydroquinolines show considerable activities.

Experimental

All the reagents were purchased from commercial suppliers and used without further purification. The progress of the reactions was monitored using thin-layer chromatography (TLC) performed on Merck Silica Gel 60 F_{254} plates. Melting points were measured on an Electrothermal MEL-TEMP apparatus (model 1202D). FT-IR spectra were obtained with a Bruker Tensor 27 spectrometer. ^1H and ^{13}C NMR spectra were recorded with a Bruker Spectrospin Avance 400 spectrometer operating at 400 and 100 MHz, respectively; chemical shifts are given in parts per million (ppm, δ) relative to solvent peaks as internal standards ($CDCl_3$: 7.26 ppm (^1H), 76 ppm (^{13}C); DMSO-d_6: 2.50 ppm (^1H), 39.5 ppm (^{13}C)). Elemental analyses were measured by Vario EL III apparatus (Elementar Co.), their results agreed favorably with the calculated values. UV–Vis spectra were recorded on an Analytic Jena Specorel 250 spectrometer. Fluorescence emission spectra were acquired on a Jasco FP-750 spectrofluorimeter. For biological studies, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and phosphate buffer solution (PBS) were purchased from Sigma-Aldrich Co. Fetal bovine serum (FBS), penicillin–streptomycin, trypsin–EDTA (25%) and Roswell Park Memorial Institute 1640 growth medium (RPMI) were purchased from Gibco BRL Life Technologies (USA). All cancer cells including breast (MCF 7 and SK BR 3) and colon (HT 29) were

provided from the standard cell banks of the National Cell Bank of Iran (NCBI) (Tehran, Iran). All cancer cells were cultured in a sterilized flask with RPMI media supported by 10% FBS and 1% penicillin–streptomycin.

General procedure for the synthesis of methyl 4-aryl-1,4,5,6,7,8-hexahydro-2,7,7-trimethyl-5-oxoquinoline-3-carboxylates 6a–6o The mixture of the dimedone (1 mmol), aromatic aldehydes (1 mmol), methyl 3-aminocrotonate (1 mmol), and 0.02 g catalyst ($Zn(NO_3)_2$/MCM-41/calcinated/wet impregnated [26]) in 4 cm^3 ethanol was stirred at room temperature for the specific time (Table 1). At the end of the reaction (controlled by TLC), the obtained solid was filtered, washed with ethanol for several times and then poured into a stirred chloroform–methanol solution (2:1 v/v, 6 cm^3). Filtration of the mixture allowed the separation of unsolved catalyst. Concentration of the filtrate with rotary evaporator resulted in the solid product which was washed twice with diethyl ether (2×5 cm^3) to give the pure products **6a**–**6o** (**6g**, **6l**, **6m**, and **8** are new compounds).

Methyl 4-(2-hydroxyphenyl)-1,4,5,6,7,8-hexahydro-2,7,7-trimethyl-5-oxoquinoline-3-carboxylate (6g, $C_{20}H_{23}NO_4$) White solid; yield 262 mg (77%); m.p.: 232–234 °C; FT-IR (KBr): $\bar{\nu}=3436$, 3018, 2959, 1737, 1644, 1524, 1216 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta=0.78$ (s, 3H, dimedone-CH_3), 1.00 (s, 3H, dimedone-CH_3), 2.07 (d, $J=16.4$ Hz, 1H, CH_2), 2.27 (d, $J=16.4$ Hz, 1H, CH_2), 2.29 (d, $J=17.1$ Hz, 1H, CH_2), 2.34 (s, 3H, Py-CH_3), 2.42 (d, $J=17.0$ Hz, 1H, CH_2), 3.45 (s, 3H, OCH_3), 4.94 (s, 1H, CH), 6.67–6.72 (m, 2H, Ar–H), 6.89 (dd, $J=8.3$, 1.5 Hz 1H, Ar–H), 6.92–6.97 (m, 1H, Ar–H), 9.38 (s, 1H, N–H), 9.42 (s, 1H, O–H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta=18.1$, 18.2, 26.3, 28.7, 30.0, 32.1, 39.4, 49.6, 50.8, 103.6, 109.3, 116.9, 117.0, 119.7, 127.1, 128.4, 133.8, 145.2, 152.2, 153.2, 167.3, 196.9 ppm.

Methyl 4-(5-methylfuran-2-yl)-1,4,5,6,7,8-hexahydro-2,7,7-trimethyl-5-oxoquinoline-3-carboxylate (6l, $C_{19}H_{23}NO_4$) White solid; yield 279 mg (85%); m.p.: 184–186 °C; FT-IR (KBr): $\bar{\nu}=3441$, 3087, 2942, 1690, 1619, 1547, 1218 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=1.04$ (s, 3H, dimedone-CH_3), 1.09 (s, 3H, dimedone-CH_3), 2.15 (s, 3H, Fur-CH_3), 2.21–2.26 (m, 3H, CH_2), 2.34 (s, 3H, Py-CH_3), 2.37 (d, $J=16.8$ Hz, 1H, CH_2), 3.68 (s, 3H, OCH_3), 5.17 (s, 1H, CH), 5.76 (d, $J=1.9$ Hz, 1H, Fur-H), 5.84 (d, $J=2.9$ Hz, 1H, Fur-H), 6.40 (s, 1H, N–H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=12.7$, 18.3, 25.7, 28.6, 29.0, 31.7, 40.1, 49.7, 50.0, 101.9, 104.2, 105.0, 107.8, 143.6, 148.4, 149.1, 155.1, 166.8, 194.5 ppm.

Methyl 4-(1*H*-indole-3-yl)-1,4,5,6,7,8-hexahydro-2,7,7-trimethyl-5-oxoquinoline-3-carboxylate (6m, $C_{22}H_{24}N_2O_3$) Yellow solid; yield 276 mg (76%); m.p.: 182–184 °C; FT-IR (KBr): $\bar{\nu}=3358$, 3077, 2941, 1689, 1618, 1494, 1223 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta=0.77$ (s, 3H, dimedone-CH_3), 0.99 (s, 3H, dimedone-CH_3), 1.94 (d, $J=16.1$ Hz, 1H, CH_2), 2.14 (d, $J=16.1$ Hz, 1H, CH_2), 2.26 (s, 3H, Py-CH_3), 2.29 (d, $J=17.6$ Hz, 1H, CH_2), 2.41 (d, $J=17.0$ Hz, 1H, CH_2), 3.50 (s, 3H, OCH_3), 5.13 (s, 1H, CH), 6.87–6.90 (m, 2H, Ar–H), 6.95–6.99 (m, 1H, Ar–H), 7.24 (d, $J=8.0$, 1H, Ar–H), 7.52 (d, $J=7.9$, 1H, Ar–H), 9.16 (s, 1H, N–H), 10.67 (s, 1H, N–H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta=18.1$, 18.2, 26.6, 27.2, 29.2, 32.1, 39.4, 50.4, 50.6, 103.6, 109.8, 111.2, 118.0, 119.6, 120.3, 121.2, 122.5, 122.6, 125.7, 136.2, 144.0, 149.0, 167.7, 194.4 ppm.

9-(5-Benzyloxy-4-oxo-4*H*-pyran-2-yl)-3,4,6,7-tetrahydro-3,3,6,6-tetramethylacridine-1,8-dione (8, $C_{29}H_{31}NO_5$) According to the general procedure, using dimedone (2 mmol), aldehyde **7** (1 mmol), ammonium acetate (1 mmol), and 0.02 g $Zn(NO_3)_2$/MCM-41/calcinated (WI) in 4 cm^3 ethanol and stirring the mixture at room temperature for 4 h, the compound **8** was obtained as a white solid. Yield 425 mg (90%); m.p.: 194–196 °C; FT-IR (KBr): $\bar{\nu}=3440$, 3083, 2957, 1739, 1642, 1547, 1262 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=1.08$ (s, 6H, dimedone-CH_3), 1.14 (s, 6H, dimedone-CH_3), 2.26–2.42 (m, 8H, CH_2), 5.02 (s, 2H, CH_2OPh), 5.30 (d, $J=1.1$ Hz, 1H, CH), 6.23 (d, $J=1.4$ Hz, 1H, pyrone-H3), 7.31–7.39 (m, 6H, Ph-H, pyrone-H6), 11.93 (s, 1H, NH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=25.8$, 28.4, 30.4, 31.7, 45.1, 45.6, 70.7, 111.5, 112.2, 126.8, 127.3, 127.6, 134.6, 139.7, 145.7, 163.3, 173.4, 188.9, 189.3 ppm.

Cell cytotoxicity assay by MTT

The MCF 7, SK BR-3, and HT 29 cancer cells were cultured in Roswell Park Memorial Institute 1640 (RPMI) media supplemented with 10% FBS and 1% penicillin and streptomycin at 37 °C under 5% CO_2. The cells were washed with phosphate buffer solution (PBS) to remove the excess RPMI and then the cells were treated with trypsin–EDTA solution and incubated for 5 min at 37 °C under 5% CO_2 to separate the cell from the flask bottom surface. Then the separated cancer cells were transferred to a tube and centrifuged to wash out excess trypsin–EDTA. Finally, the separated cells were resuspended to the fresh RPMI medium. Cell viability of MCF 7, SK BR-3, and HT 29 cancer cells were tested by adding 1.0×10^4 cells per each well of a 96-well texture plate. Afterwards, the cancer cells were incubated for about 24 h to grow and cover at least 40% of each well. Then the wells were treated with different concentrations of the 1,4-dihydropyridines and incubated for 48 h. Next, the wells were washed with PBS buffer solution, and subsequently 20 mm^3 of MTT (2.5 mg cm^{-3}) reagent and 180 mm^3 RPMI refresh media were added to each well and incubated for 4 h at 37 °C under 5% CO_2. Afterwards, the RPMI medium

was replaced with 200 mm^3 of DMSO and incubated for 0.5 h. Finally, the absorbance of the wells was measured at 570 nm.

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