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Synthesis and Biological Evaluation of 4-hydroxychromenyl arylmethyl-6-hydroxy pyrimidine-2, 4-dione Derivatives

Khalil Eskandari, Ph.D.[1], Bahador Karami, Ph.D.[2], Yaghoub Pourshojaei, Ph.D.[1], Zahra Lori-Gooini, Ph.D.[3], Elham Soltanipour-Joneghani, M.Sc.[4], Mohammad-Taghi Moradi, Ph.D.[3], Ali Asadipour, Ph.D.[5]

1- Assistant Professor, Department of Medicinal Chemistry, Faculty of Pharmacy and Pharmaceutics Research Center, Kerman University of Medical Sciences, Kerman, Iran (Corresponding author; khalileskandari@yahoo.com)

2- Professor, Department of Chemistry, Faculty of Basic Sciences, Yasouj University, Yasouj, Iran.

3- Assistant Professor, Medical Plants Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran.

4- Student's research committee, Shahrekord University of Medical Sciences, Shahrekord, Iran.

5- Professor, Department of Medicinal Chemistry, Faculty of Pharmacy and Pharmaceutics Research Center, Kerman University of Medical Sciences, Kerman, Iran

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Abstract

Background: An efficient, promoted tri-component catalytic reaction between barbituric acid (or N,N-dimethyl barbituric acid), 4-hydroxy coumarin, and a wide range of aryl aldehydes using zinc oxide nanowires (ZnO NWs) to obtain some new 4-hydroxychromenylarylmethyl-6-hydroxypyrimidine-2,4-diones is described.

Method: The reactants were successfully condensed via three C-C bond formation by zinc oxide nanowires (ZnO NWs) as an efficient, environmentally safe and recyclable nano catalyst to produce target molecules. In addition, the biological effects of synthesized products by the use of DPPH and acyclovir as positive controls and also Hep-2, vero cell, HSV-1, and adenovirus as four applied cell lines have been evaluated.

Results: The results showed that synthesized products have anti-oxidant, cytotoxic and anti-viral activities and can offer promising prospect as biologically active agents.

Conclusion: This achievement in an efficient and eco-friendly synthesis of novel analogous of hybrid molecules in aqueous media with special biological properties may engross chemists and pharmacologists as well as pharmacists in future.

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Introduction

Barbituric acid is one of the most important heterocyclic compounds which its discovery has attracted the attention of both chemists and pharmaceutics (1-4). So far, a wide range of biological and pharmaceutical activities related to barbituric acid and its derivatives have been disclosed. For example, the effect on the central nervous system (2,5), drugs side-effects (2,5), application against non-alcoholic fatty liver disease (4) and anti-cancer activity (5,6), are some of these properties which can be mentioned. Furthermore, this compound and its

derivatives have shown metal sequestering properties (7) and are considered as a new anchor unit for dye-sensitized solar cells (8). About fifty-five chemical compounds having barbituric acid moiety have been prescribed as medicines in all over the world. For instance, Barbital, Phenobarbital, Merbarone, and Bucolome are considered as important biological and pharmacological agents (Figure 1) and are used as medicine (3-5, 7).

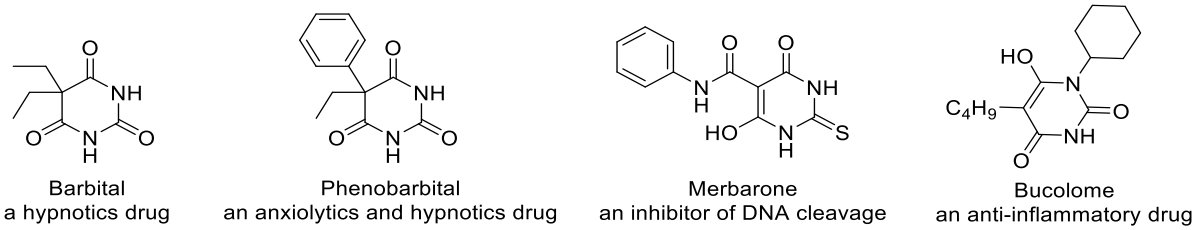


Figure 1. Some selected chemical compounds having pharmacological activities containing barbituric acid scaffold

Combination of barbituric acid moiety with other pharmacophoric groups provides the possibility to synthesize numerous derivatives with potential biological effects. Similarly, 4-hydroxycoumarin and its derivatives have exhibited antibacterial (9), anti-HIV (10-12), antiviral (13), anticoagulant (14), and antioxidant (15) properties. Also, it is interesting to mention that 4-hydroxycoumarin scaffold has been found in numerous natural products such as warfarin, phenprocoumon, coumatetralyl, carbochromen, bromadialone, etc. (9, 16). In other words, such biological and pharmaceutical properties provide effectively a high level of motivation to extend this domain of science (17, 18).

For example, 4-hydroxycoumarin scaffold has been found in Novobiocin (an antibiotic drug) (19, 20), Isopimpinellin (a natural product which has been considered as an anticarcinogen agent|) (21, 22), Pongavilleanine (a natural product isolated from root extracts of Pongamiopsis pervilleana and has been prescribed as antiproliferative drug), as well as Warfarin and Coumatetralyl which are well known as anticoagulant drugs (Figure 2) (23, 24).

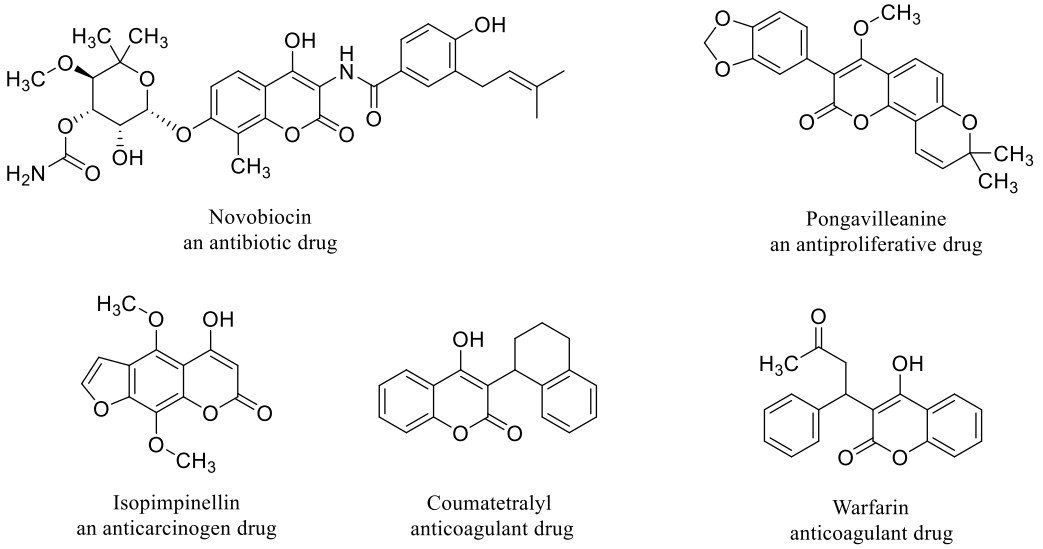


Figure 2. Some important natural products and drugs containing 4-hydroxycoumarin framework

Observing important biological activities among chemical structures belonging to barbituric acid and 4-hydroxycoumarin scaffolds encourage us to design and synthesize some noteworthy biological active compounds from hybridization of both barbituric acid and 4-hydroxycoumarin scaffolds.

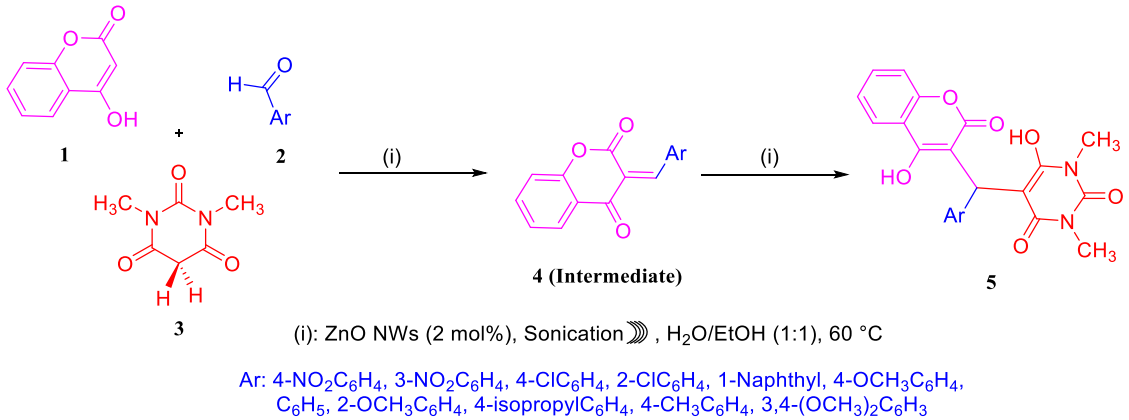
Results and discussion

Chemistry

Considering the high importance of zinc oxide nanowires (ZnO NWs) among nano-scale metal oxides as an efficient heterogeneous catalyst, it has attracted a great interest in organic syntheses because ZnO NWs as a heterogeneous Lewis catalyst can be easily separated from the reaction mixture, reused in further cycles without producing problematic side products. Furthermore, thermal stability, non-corrosive and non-toxic activity of ZnO NWs in response to the demand for more environmentally benign organic syntheses are other benefits of this green nanocatalyst (25).

In green chemistry approach, working up organic synthesis in aqueous medium has received considerable attention (26-29). Loading organic reactions in watery moiety has some advantages such as the use of a solvent which is non-toxic, easily available, economic and harmless and can also provide green and eco-friendly conditions for chemical syntheses and processes (26-29).

In the domain of our interest, the new highly efficient catalytic approach introducing the novel heterocyclic compounds and extension of their area (30-34), and also the synthesis of biologically important organic compounds (35-37) herein, we report a one pot catalytic condensation reaction of barbituric acid/*N*,*N*-dimethyl barbituric acid, as well as a variety of aryl aldehydes and 4-hydroxy coumarin in the presence of catalytic amounts of Zinc oxide nanowires (Scheme 1). However, there are some reported methods on the synthesis of these kinds of compounds with other catalysts and conditions (38,39), but in the current protocol we were successfully able to improve the reaction conditions and enhance the yields of products by an efficient strategy using ZnO NWs under ultrasound irradiation. Also, in this work we synthesized products **5c** and **4k** as novel compounds, developed the scope of these products, and evaluated their biological activities including anti-oxidant, cell toxicity and anti-viral effects on Hep-2, Vero Cell, HSV-1 and Adenovirus for the first time.



Scheme 1. One-pot selective synthesis of 4-hydroxychromenyl arylmethyl-6-hydroxypyrimidine-2,4-diones catalysed by ZnO NWs

It was found that ZnO NWs not only can efficiently catalyze the synthesis of 4-hydroxychromenyl arylmethyl-6-hydroxypyrimidine-2,4-diones via a convenient work-up, but also has some advantages such as safety, recyclability, high stability, and easy handling. Accordingly, in this work, preparation, high activation, and regeneration of ZnO NWs as eco-friendly and effective catalysts in the synthesis of newly prepared organic compounds have been explained. Furthermore, the efficiency of this catalyst with those of other catalysts was compared.

Zinc oxide nanowires were synthesized by a slight modification of the solvothermal reported method (40). Figure 3 shows the X-ray diffraction pattern (XRD) of the ZnO NWs. In the XRD pattern, the distinguished diffraction peak centered at $2\theta \sim 32°$, 34.5° and 36.5° were related respectively to the (100), (002) and (101) plane of the ZnO with a wurtzite structure which is in agreement with the standard data for the ZnO structure (41).

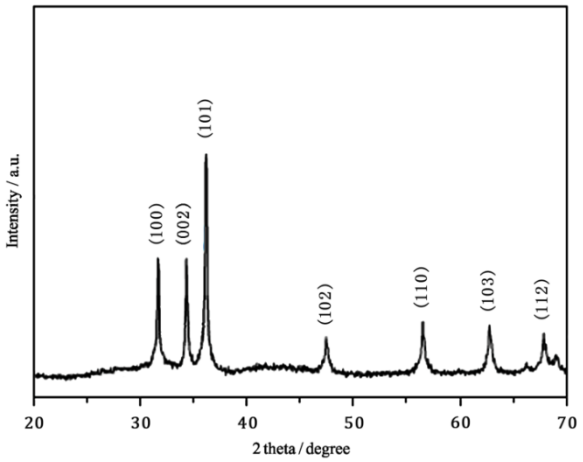


Figure 3. XRD pattern of ZnO NWs

Scanning electron microscopy (SEM) was used to observe the morphology of ZnO NWs (Figure 4). SEM images of the as-prepared ZnO NWs show that the ZnO nanowires have a diameter of about 20 nm without any amorphous or other kinds of crystallized phase particles. For more investigation of ZnO NWs morphology, the TEM image of ZnO NWs was studied (Figure 5). TEM image of ZnO NWs clearly reveals that the ZnO nanowire has a homogeneous diameter size about 20 nm that does not vary significantly along the wire length.

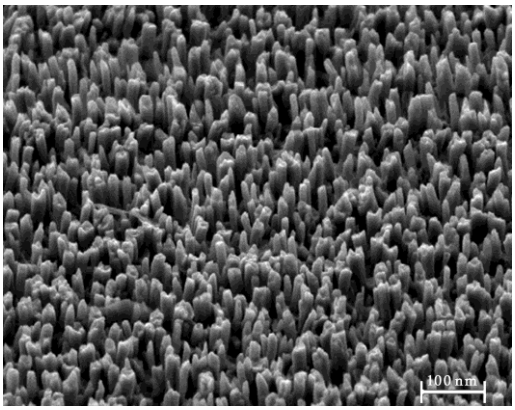


Figure 4. SEM image of ZnO NWs

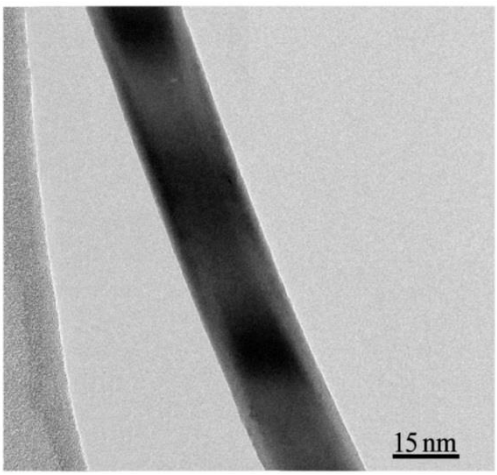


Figure 5. Transmission electron microscopy of ZnO NWs

After preparation and characterization of catalyst, ZnO NWs, as Lewis acid nano catalyst, was applied to selective synthesis of 4-hydroxychromenyl arylmethyl-6-hydroxypyrimidine-2,4-diones via three component one-pot reaction (Scheme 1). Prior to expanding the general scope,

compound 5g was opted as a model to find the best conditions for reaction accomplishment (Table 1). Loading reaction in various solvents reveals that it could proceed successfully in water and ethanol (v/v = 1:1). After finding out suitable solvent, from investigations on reaction temperature, it was found that reaction progress in 60°C under ultrasound irradiation was faster and led to obtain the product with a higher yield. Among different catalysts which were used to model reaction with identical amounts (2 mol%), ZnO NWs showed the best efficiency in the synthesis of the product (Table 1). Besides, in absolute water and solvent-less conditions no remarkable yield was observed even in the presence of different catalysts such as *p*-TSA, SSA, $Y(NO_3)_3.6H_2O$, $In(OTf)_3$, Na_2HPO_4, and Na_2CO_3.

Table 1. The effect of different conditions in the synthesis of 5g

Entry	Conditions[a]	Yield[b] (%)
1	Catalyst-free, EtOH/H_2O (1:1), 70 °C	---
2	ZnO NWs, EtOH/H_2O (1:1), 60 °C	70
3	ZnO NWs, EtOH/H_2O (1:1), Sonication, 60 °C	88
4	ZnO NWs, Solvent-free, 70 °C	45
5	ZnO NWs, H_2O, 80 °C	40
6	ZnO NWs, CH_2Cl_2, Reflux	30
7	ZnO NWs, CH_3CN, 70 °C	35
8	ZnO NWs, EtOH, Reflux	40
9	ZnO NWs, DMF, 70 °C	30
10	*p*-TSA, Solvent-free, 70 °C	25
11	SSA, H_2O, 70 °C	35
12	$In(OTf)_3$, H_2O, 80 °C	38
13	Na_2CO_3, H_2O, 80 °C	Trace
14	Na_2HPO_4, H_2O, 80 °C	Trace
15	$Y(NO_3)_3.6H_2O$, H_2O, 80 °C	30
16	SSA, EtOH/H_2O (1:1), 70 °C	45
17	Na_2HPO_4, EtOH/H_2O (1:1), 70 °C	40

[a] Reaction time: 150 min. [b] Isolated Yield

In another assessment, the effect of catalyst amounts on model reaction was investigated and the results are summarized in table 2. As it can be seen in table 2, no remarkable target product in the absence of catalyst was obtained (31%), while using just 1 mol% of ZnO NWs improved the yield significantly (65%). Increasing the catalyst amount to 2 mol% led to produce target product with the highest yield (88%). Therefore, based on the obtained results, we chose 2 mol% of ZnO NWs as optimum catalyst amount for the reaction.

Table 2. Optimization of catalyst amounts on model reaction

Entry	ZnO NWs/mol%	Time/min	Yield/%[a]
1	---	180	31
2	1	180	65
3	1.5	150	80
4	2[b]	150	88
5	2.5	150	88
6	3	150	87
7	3.5	180	87
8	4	180	86

[a] Isolated yields
[b] The catalyst amount that leads to the best results

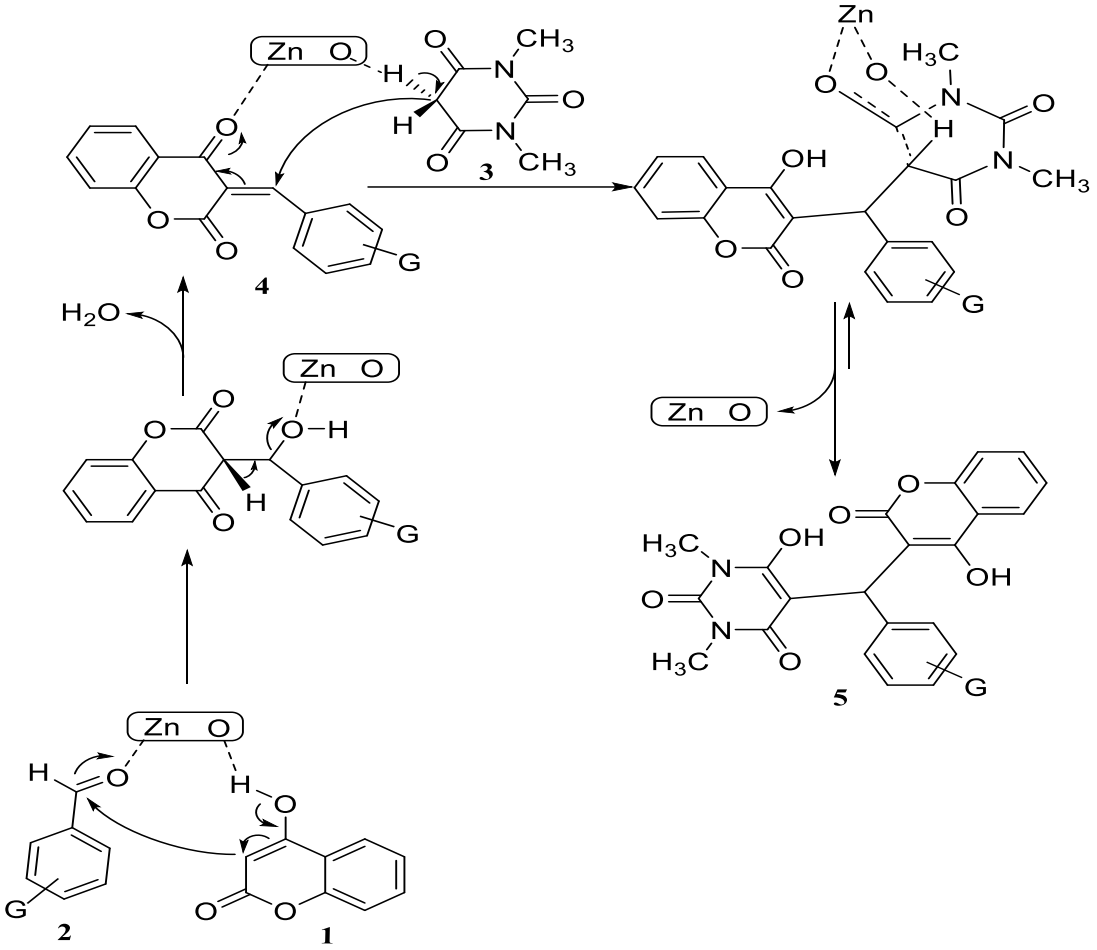
As mentioned above, the reaction in the presence of ZnO NWs (2 mol%) at 60 °C in H_2O/EtOH (1:1) under ultrasound irradiation was carried out. In order to expand the reaction scope, a wide range of aryl aldehydes, *N*,*N*-dimethyl barbituric acid and/or barbituric acid with 4-hydroxycoumarin were reacted together in the obtained optimum conditions, which led to the synthesis of 11 newly prepared products (5a-j and 4k) with good yields (Table 3). It was comprehensively understood that the process tolerates both electron-withdrawing and electron-donating groups on aromatic rings of aldehydes, and products 5 can be formed with good yields. In addition, when 3,4-dimethoxy-benzaldehyde was used as aryl aldehyde, compound 4k was obtained as the only product which this observation also strongly confirmed the proposed mechanism of this reaction (Scheme 2) (42).

Table 3. Investigations of the scope of substrates to the synthesis of 4-hydroxychromenyl arylmethyl-6-hydroxy pyrimidine-2,4-diones[a]

Product[b]	Aldehyde	Ar	Time (min)	Yield[c] (%)
5a	2a	4-$NO_2C_6H_4$	130	88
5b	2b	3-$NO_2C_6H_4$	130	85
5c	2c	4-ClC_6H_4	130	84
5d	2d	2-ClC_6H_4	130	84
5e	2e	1-Naphthyl	140	85
5f	2f	4-$OCH_3C_6H_4$	150	81
5g	2g	C_6H_5	150	88
5h	2h	2-$OCH_3C_6H_4$	145	83
5i	2i	4-isopropylC_6H_4	150	82
5j	2j	4-$CH_3C_6H_4$	150	80
4k	2k	3,4-$(OMe)_2$-C_6H_3	150	80

[a] Reaction conditions: 4-hydroxycoumarin **1** (1 mmol), aryl aldehyde **2** (1.2 mmol), pyrimidine-2,4,6-trione 3 (1 mmol), ZnO NWs (2 mol%), EtOH/H_2O (10 mL, v/v = 1:1), under ultrasound irradiation at 60 °C. [b] The products have been ordered by their antioxidant activities. [c] Isolated Yield.

A commonly agreed mechanism for the syntheses of 4-hydroxychromenyl arylmethyl-6-hydroxypyrimidine-2,4-diones is described in Scheme 2. In this proposed cascade pathway, in the first step, 4-hydroxycoumarin in the presence of ZnO NWs reacted with aryl aldehyde which was also activated by ZnO NWs and lose water in a Knoevenagel condensation reaction to obtain intermediate 4. In the next step, activated barbituric acid (or *N*,*N*-dimethyl barbituric) was attacked to Int. 4 by the key effect of ZnO NWs and followed by keto-enol tautomerism to afford product 5.



Scheme 2. A proposed mechanism for the ZnO NWs catalytic synthesis of product 5

The structures of compounds 5 were confirmed by the basis of comprehensive spectroscopic analyses such as IR, ^{1}H and ^{13}C NMR spectroscopy, and elemental analysis. The ^{1}H NMR spectrum of 5c for example exhibited two identification singlets with different highly deshielded in chemical shift signals (δ = 12.53 and 11.97 ppm) corresponding to OH groups (2×OH). This observation provides a strong evidence for the formation of structure 5 and clearly reveals that ring closure has not taken place. Other signals which appeared at δ = 8.03-7.29 ppm are correlated to aromatic CH groups. The signal of the methine group (CH) appeared at δ = 6.32 ppm as a sharp singlet. Also, the signals of two CH_3 groups appeared at δ = 3.23 and 3.18 ppm as two sharp singlets. The proton-decoupled ^{13}C NMR spectrum of 5c showed also 22 distinct resonances in agreement with the proposed structure. Furthermore, IR spectroscopies and elemental analyses (C,H,N) data also were in agreement and confirmed structure of 5 (See experimental).

One of the noteworthy aspects of this procedure is the capability of catalyst to recycle which makes it economically significant. From this viewpoint, the recyclability of ZnO NWs was also investigated on model reaction (Figure 6). Focusing on catalyst reusability in reaction cycle for the synthesis of 5g, we came to know that catalyst remained

active and effectively catalyzed reaction up to six times without significant loss of activity.

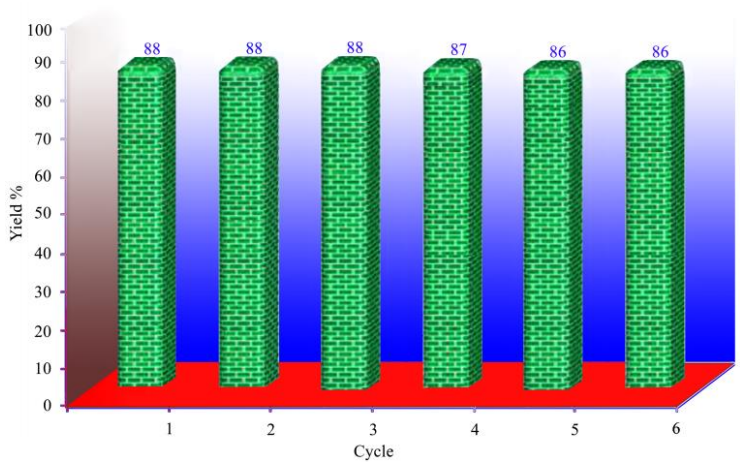


Figure 6. Reusability of ZnO NWs over six runs

Antioxidant and antiviral studies

Based on antioxidant activity test, all synthetic derivatives have high radical scavenging effect (Table 4). Among them, compounds 5a and 5b were the strongest compounds at low concentrations.

It is generally accepted that the NO_2 group presented in compounds 5a and 5b inhibits the oxidation of macromolecules by donation of H atoms to scavenge free radicals. Its reason may be correlated to the fact that electron-withdrawing nature of NO_2 group via both induced and conjugation effects leads to easily separation of H radical from molecule and subsequently makes generated free radical more stable by electron-withdrawing effects than other products. All of these facts are definitely compatible with our obtained results from antioxidant assay of target compounds in the presence of DPPH as positive control (Table 4).

Table 4. Antioxidant evaluation of synthesized compounds using DPPH

Entry	Sample[a]	MW ($g.mol^{-1}$)	IC_{50} (mM)
1	5a	451	$0.15\times10^{-3}\pm0.01$
2	5b	451	$0.16\times10^{-3}\pm0.01$
3	5c	440	0.08±0.01
4	5d	440	0.12±0.04
5	5e	456	0.13±0.01
6	5f	436	0.17±0.22
7	5g	406	0.29±0.03
8	5h	436	0.31±0.01
9	5i	448	1.09±0.07
10	5j	420	4.75±0.85
11	4k	310	5.09±0.90
12	DPPH[b]	394.32	7.92±0.06

[a] All samples have been ordered according to their activities. [b] Positive control

As it can be seen from table 4, the best anti-oxidant activities are related to ones that have electron withdrawing such as NO_2 and Cl groups. On the other hand, when electron-withdrawing groups are located in para position of phenyl ring, their effects on the stability of radicals are stronger than those located in ortho or metha positions. These observations could be more understood with considering the fact that electron-withdrawing by conjugative effect acts more effectively from para position than from ortho and metha ones. In contrast, products bearing electron donating such as OCH_3 group especially those belonging to non-polar including CH_3 or *i*-propyl groups showed the lowest anti-oxidant activities than other molecules. The reason of this observation may be directly dependent on the stability of produced radicals in the presence of electron-withdrawing groups.

Assessment of cytotoxic and antiviral activities

Based on MTT analysis results, cytotoxicity effects of compound 5a which was considered and selected as the most effective anti-oxidant agent in the above antioxidant assessments were evaluated on two cell lines (hep-2 and vero cell) . As it is seen in table 5, the CC_{50} values of the compound 5a on hep-2 and verro cells were 139.7 $\mu g.mL^{-1}$ and 262.66 $\mu g.mL^{-1}$ respectively. The result on vero cell showed that the cytotoxicity effect of compound 5a was better than acyclovir which was applied as positive control. Even CC_{50} of compound 5a in the presence of hep-2 was lower than its value in the presence of vero cell. Overall, it is concluded that cytotoxicity of compound 5a is as good as the positive control. However, it could be considered as a more effective agent than acyclovir as positive control. In addition, anti-viral studies of compound 5 against HSV-1 and adenovirus were performed and the results are summarized in table 6.

Table 5. Cytotoxicity evaluation of the most effective synthesized compound 5 on Cell Lines Hep-2 and Vero Cells

Entry	Sample	Cell line	CC_{50} ($\mu g.mL^{-1}$)
1	5a	Hep-2	139.7
2	Acyclovir[a]	Hep-2	n.d.[b]
3	5a	Vero Cell	262.66
4	Acyclovir[a]	Vero Cell	841.3

[a] Positive control; [b] no detection

Table 6. Anti-viral assessment of the most effective synthesized compound 5 against HSV-1 and adenovirus

Entry	Sample	Test virus	IC_{50} (μM)	SI[a]
1	5a	HSV-1	262.66	>1
2	Acyclovir[b]	HSV-1	15400	94
3	5a	Adenovirus	139.7	>1
4	Acyclovir[b]	Adenovirus	>100	n.d.[c]

[a] Selectivity index; [b] positive control; [c] not done; NOTE. IC_{50} is the in vitro concentration of inhibitor required to inhibit viral plaque formation by 50%; Selectivity index (SI) = CC_{50}/IC_{50}.

Data presented in table 6 show that the IC_{50} value on HSV-1 was >262.66 μM and that of adenovirus was >139.7 μM. The results showed that compound 5a acts more effectively than acyclovir as positive control on anti-herpes virus. Also, it can be stated that the activity of compound 5a on adenovirus was as good as acyclovir. Furthermore, the SI value of compound 5a on adenovirus and on HSV-1 was >1. In summary, according to the obtained results in anti-viral test assessments on compound 5a, it can be clearly realized that compound 5a possesses a slight anti-herpes virus and anti-adenovirus activities.

Experimental

Materials and methods

Chemicals were purchased from Fluka and Merck chemical companies. Scanning electron microscopy (SEM) studies of the nanostructures were carried out with a JEOL JEM 3010 instrument operating at an accelerating voltage of 300 kV. X-ray diffraction (XRD, D8, Advance, Bruker, AXS) patterns were obtained for characterization of the heterogeneous nano catalyst. The elecrtothermal KSB1N apparatus was handled for melting point measurements. JASCO FT-IR-680 plus spectrometer with KBr as matrix was used for recording IR spectra. FT-NMR Bruker Avance Ultra Shield Spectrometer (at 400.13 for ^{1}H NMR and at 100.62 MHz for ^{13}C NMR in DMSO as solvent) was used for recording ^{1}H and ^{13}C NMR. Elemental analyses (C,H,N,S) were performed on a Heraeus Rapid analyzer and the results were found in good agreement (±0.3%) with the calculated values. The ultrasound apparatus with cleaning bath Wiseclear 770W, operating frequency of 40 kHz and the output power of 200 W (Seoul, Korea) was used. TLC-Grade silica gel-G/UV (254 nm) plates were handled for monitoring of reaction progress.

Typical procedure to Prepare ZnO NWs

To prepare ZnO nanowires, firstly, 0.315 g (1.43 mmol) Zinc acetate dihydrate [$Zn(OAc)_2.2H_2O$] was dissolved in 66 mL ethanol in a 100 mL round bottom flask. Next, 1.67 g (41 mmol) NaOH was added to the flask and stirred for 1.5 h to make it being dissolved at room temperature. Then, the resulting cloudy solution was sealed in a 70 mL teflon-lined stainless-steel autoclave, heated at 120°C for 24 h, and then allowed to cool down at room temperature. The white obtained precipitate was collected by centrifugation, washed with water/ethanol (v/v = 1:1) several times until the washing solution showed negative results for NaOH. At the end, the prepared nanocatalyst was dried at 100 °C for 6 h, characterized by SEM, TEM and XRD spectroscopies and was successfully applied as an efficient green catalyst in the above three component reactions.

General procedure for the synthesis of 5

To a stirred mixture of 4-hydroxycoumarin 1 (0.162g, 1 mmol), proper arylaldehyde 2 (1 mmol) and ZnO NWs (0.0016 g, 2 mol%), as well as *N*,*N*-dimethylbarbituric acid 3 (0.156 g, 1 mmol) were added. The mixture was heated at 60 °C under ultrasound irradiation for appropriate time. The reaction progress was controlled by TLC (*n*-hexane/ethyl acetate; v/v = 1:1 as eluents). After completion of the reaction, the mixture was filtrated to separate catalyst, and then catalyst was washed with boiling THF (4 mL), dried at 100 °C for 2 h and reused in another cycle. The filtrate was concentrated by rotary evaporator, and it was transmitted in column

chromatography (*n*-hexane/ethyl acetate = 1:1 as eluents) to obtain pure 5.

Typical procedure for the synthesis of 4k

0.162 g 4-hydroxycoumarin 1 (1 mmol) was dissolved in 6 mL H_2O/EtOH (1:1); and ZnO NWs (0.0016 g, 2 mol%) along with 0.166 g 3,4-dimethoxybenzaldehyde 2k (1 mmol) were dissolved in 4 cm^3 H_2O/EtOH (1:1). The mixture was heated at 60 °C under ultrasound irradiation for the time indicated in table 2. After completion of the reaction which was confirmed by monitoring on TLC, the mixture was filtrated to separate catalyst. The filtrate was concentrated by rotary evaporator to afford crude product. The crystalline pure product 4k was obtained by recrystallization of crude product from boiling ethanol.

Representative spectral data

6-hydroxy-5-((4-hydroxy-2-oxo-2*H*-chromen-3-yl)(4-chlorophenyl)methyl)-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione (5c): Yield: 84%; ^{13}C NMR: (DMSO-d_6, 100 MHz): δ (ppm) = 27.9, 28.5, 35.6, 90.9, 103.7, 115.8, 118.1, 123.6, 123.9, 127.5, 127.8, 128.0, 128.6, 131.8, 134.3, 152.0, 152.2, 153.8, 160.2, 161.9, 164.6, 165.6; ^1H NMR (DMSO-d_6, 400 MHz; J:Hz): δ (ppm) = 3.18 (3H, s, Me), 3.23 (1H, s, Me), 6.32 (1H, s, CH), 7.29-7.37 (4H, m, CH_{Ar}), 7.57-7.61 (2H, m, CH_{Ar}), 7.90 (1H, dd, ^3J, ^4J = 7.8, 1.6, CH_{Ar}), 8.03 (1H, d, J = 8.8, CH_{Ar}), 11.97 (1H, s, OH), 12.53 (1H, s, OH); Found, %: C, 60.02; H, 4.60; N, 5.97. $C_{22}H_{17}ClN_2O_6$. Calculated, %: C, 59.94; H, 4.51; N, 5.95; FT-IR (KBr, cm^{-1}) $\bar{\upsilon}$: 728, 830, 1430, 1490, 1538, 1600, 2901, 3050 3415.

3-(3,4-dimethoxybenzylidene)chromane-2,4-dione (4k): Yellowish crystals; Yield: 80%; ^{13}C NMR: (DMSO-d_6, 100 MHz): δ (ppm) = 55.4, 55.5, 104.4, 111.3, 111.5, 115.9, 117.7, 118.8, 123.7, 123.8, 131.8, 132.6, 147.1, 148.5, 152.1, 164.7, 164.9, 191.3; ^1H NMR (DMSO-d_6, 400 MHz; J:Hz): δ (ppm) = 3.57 (3H, s, OMe), 3.71 (3H, s, OMe), 6.30 (1H, s, CH), 6.69 (1H, d, J = 8.4, CH_{Ar}), 6.74 (1H, s, CH_{Ar}), 6.82 (1H, d, J = 8.4, CH_{Ar}), 7.32-7.39 (2H, m, CH_{Ar}), 7.58-7.62 (1H, m, CH_{Ar}), 7.92 (1H, dd, ^3J, ^4J = 7.8, 1.6, CH_{Ar}); MS (EI+): calcd. ($C_{18}H_{14}O_5$) [M^+]: 310.0; found: 310.0; Found, %: C, 69.65; H, 4.51. $C_{18}H_{14}O_5$. Calculated, %: C, 69.67; H, 4.55; FT-IR (KBr, cm^{-1}) $\bar{\upsilon}$: 765, 1375, 1450 1699, 1720, 2950, 3065.

Assay of antioxidant activity

The antioxidant activity of all synthetic compounds and the standard antioxidants were assessed on the basis of radical scavenging effect of the stable DPPH free radical. In a modified assay, 200 µL of a100 mM solution of DPPH radical in methanol was mixed with a soluation of synthetic derivatives (20 µL) or standard antioxidants (20 µL). After mixing, it was left for 30 min at room temperature. The DPPH radical inhibition was measured at 490 nm by using a microplate reader. The IC_{50} of each sample (concentration in mM required to inhibit DPPH radical formation by 50%) was calculated. Test was carried out in triplicate. The antioxidant activity (AOA) was given by:

$$100 - [\frac{(A)sample - (A)blank}{(A)control} \times 100]$$

where, A is the absorbance of the color formed in microplate wells. DPPH was used as control (without synthetic derivatives), and blank contained methanol (43).

Cell toxicity assay

The evaluation is based on the reduction of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide)

(Sigma-Aldrich, Saint Louis, Missouri, USA). The MTT colorimetric assay was performed in 96-well plates. Hep2 (cervix adenocarcinoma) and Vero (African green monkey kidney) cells were seeded in 96-well plates at a concentration of 10^5 cells per well and incubated for 24 h at 37 °C in a 5% CO_2 enriched atmosphere. After treatment with various concentrations of each extract, cells were incubated for an additional 48 h at 37 °C. After that, medium was removed and the cells in each well were incubated with 200 mL of MTT solution (5 mg/mL) for 2 h at 37°C. MTT solution was then discarded and 200 mL insoluble formazan crystal was added. Optical density (OD) was measured at 570 nm. Data were obtained from triplicate wells. The 50% cytotoxic concentration (CC_{50}) was defined as the cytotoxic concentration of the compound by regression analysis (44, 45).

Test viruses

Adenovirus (type 5) and HSV-1 (herpes simplex type-1) were grown on cells in DMEM medium until complete cytopathic effect (CPE). The titer viral was used at a final concentration of 100 $TCID_{50}$/mL.

Antiviral activity assay

A CPE reduction assay for screening the antiviral activities of the compound was employed. In brief, to confluent cell monolayers in a 96-well plate, 100 $TCID_{50}$ (50% tissue culture-infective dose) virus suspension and serial two-fold dilutions of the compound were added simultaneously. The dilution medium without samples and virus suspension were added, respectively, to the cell cultures to serve as cell control and virus control. The plates were incubated at 37°C in a humidified CO_2 atmosphere for 3 and 4 days (3 days for HSV1 and 4 days for adenovirus). The concentration that reduced 50% of CPE with respect to virus control was estimated from the plots of data and was defined as the 50% inhibitory concentration (IC_{50}). The selective index (SI) was calculated from the ratio CC_{50}/IC_{50} (45).

Conclusions

The current synthetic procedure described a green adapted catalytic method for the synthesis of new prepared organic products by the use of ZnO NWs as an efficient nano catalyst. By this achievement, the scope of pharmacologically important organic compounds was developed because by this procedure some important anti-oxidant, cytotoxic and anti-viral compounds were obtained. The anti-oxidant evaluations of all synthesized compounds comparing to radical scavenging effect of the stable DPPH free radical showed that all of them can be candidates as powerful anti-oxidant agents. The cytotoxicity and anti-viral assessments revealed that these types of compounds show cytotoxic and anti-viral activities and can be considered as newly introduced cytotoxic and anti-viral agents. We assume that this achievement may draw the attention of chemists, biologists and pharmacologists in the future.

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