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Application of Chlorophyll Extracted from Spinach as a Green and affordable Catalyst for the Synthesis of Tetrahydrobenzo[*b*]pyran and Pyrano[*c*]chromene

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Chlorophyll is a natural pigment used for the synthesis of organic substances such as sugars. At mild conditions, chlorophyll was employed as a green catalyst for the synthesis of tetrahydrobenzo[b]pyran and 3,4-dihydropyrano[*c*]chromene from the condensation between aromatic aldehydes, malononitrile, and 4-hydroxy-2H-chromen-2-one which is an active enolic compound. This methodology has a considerable number of advantages such as mild conditions, natural, affordable catalyst, excellent yields, eco-friendly, and short reaction time. Another point worth mentioning is that purification of the products does not require sophisticated methods like column chromatography.

Keywords: Chlorophyll, Natural catalyst, Tetrahydrobenzo[*b*]pyran, 3,4-Dihydropyrano[*c*]chromene

INTRODUCTION

In this modern industrial world, the amount of CO_2 has been increased exponentially due to developing industries and destroying forests that conjure oxygen. Plants can overcome this superiority by generating as much oxygen as they can. Oxygen is made in photosynthesis reactions executed by chlorophyll. It is a green photosynthesis molecule found in most plants, algae, and cyanobacteria that absorbs sunlight and uses energy to "remove" carbon dioxide to produce carbohydrates and oxygen. This process is known as photosynthesis. Interestingly, it is the driving force of life in plants. Chlorophyll is a cyclic tetrapyrrole that is similar in structure to that of hemoglobin with the exception that the central metal is magnesium versus iron. There are two main types of chlorophyll found in most plants and most cyanobacteria, chlorophyll a and chlorophyll b in the ratio of 3:1, respectively. The discrepancy between them is that the methyl side chain in (a) is replaced by a formyl group in (b) (Scheme 1).

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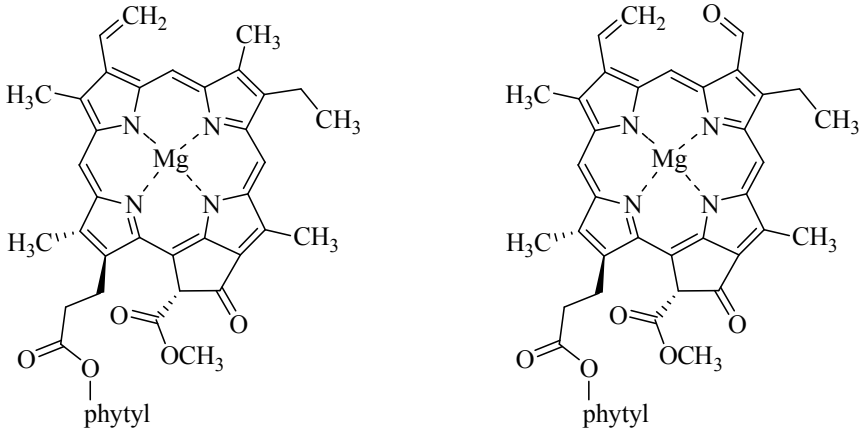
Multicomponent reactions (MCRs) have become a powerful tool in chemical synthesis and have attracted much attention because by this method drugs and complex compounds can be made from simple and cheap available raw materials [1-5]. They are more desirable than conventional multiples in several aspects including flexibility, time and energy saving, high yields, and no separation of intermediates as well [6-8]. Recently, tetrahydrobenzo[b]pyran and 3,4-dihydropyrano [c]chromene derivatives have been attracting interest because of their wide range of biological properties such as anti-cancer, antimicrobial, anticoagulant, anti-anaphylactic, *etc.* [9-14]. The importance of these compounds has led to a variety of methods for their synthesis such as ultrasonic irradiation [15], microwave [16] or using catalysts such as starch solution [17], hexadecyltrimethylammonium bromide (HTMAB) [18], acyclic acidic ionic liquids [19], cerium(III) chloride ($CeCl_3 \cdot 7H_2O$) [20] and iodine/DMSO [21] were found to be effective to promote them. Recently, some methods have been presented by other research groups. Mosaddegh, *et al.* reported some derivatives with $Zn(Phen)_2Cl_2$ as a catalyst in the water at room temperature [22]. Gurumurthi *et al.* applied tetrabutylammonium bromide for the former compound in different conditions



Chlorophyll a Chlorophyll b

Scheme 1. Chlorophylls a and b

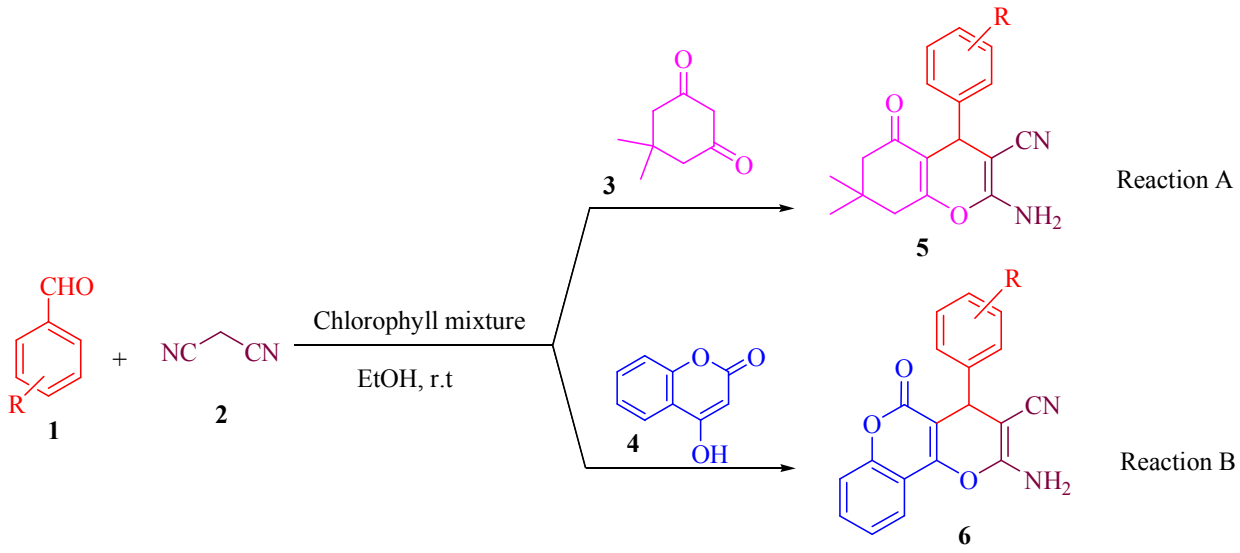
[23]. Mehrabi and Abusaidi used sodium dodecyl sulfate (SDS) in water to produce latter one [24]. Given an efficient benign method needed to synthesize these compounds, we synthesized them intelligently. Chlorophyll is the most numerous natural visible light photocatalyst on the earth. It is a main photo acceptor in the chloroplasts playing an imperative role in photosynthesis reaction. In that extraordinary reaction, sunlight is applied as the energy source by plants to transmute CO_2 and H_2O to organic substances. This condition can be used for the synthesis of other organic substances. Chlorophyll was exerted as a green photosensitizer in a visible light photoredox catalysis for the efficient polymerization by Shanmugam and co-workers [25]. To synthesize tetrahydrobenzo[b]pyran and 3,4-dihydroxy[c]chromene derivatives, a similar condition was provided, accordingly. In this work, spinach as a rich chlorophyll substance (in order to obtain the mixture of that as a catalyst) is used to synthesize the two aforementioned compounds. Despite the merits of these procedures, each of them suffers from at least one of the following constraints: unavailability of the catalyst, long reaction time, sewage pollution, harsh reaction conditions, and tedious work-up. To catch all benefits of chlorophyll and continue our research on multicomponent reactions [26-30], we introduce a green catalyst for the synthesis of tetrahydrobenzo[*b*] pyran and 3,4-dihydropyrano[*c*]chromene derivatives.

In order to implement the preparation of tetrahydrobenzo[b]pyran and 3,4-dihydroxy[*c*] chromene derivatives in a more effectual method, we used chlorophyll mixture as a green, low-price catalyst. Both reactions A and B (Scheme 1) were investigated in three different states that all results are depicted in Table 1. As it can be seen, reactions in the shortage of catalyst are involved with low yields and long times. The application of the mixture of chlorophyll was caused to improve the time and yield. However, this rate was an insignificant proportion. Since, plants are exerted light and air in photosynthesis. As a result, we exploited them simultaneously. The UV lamp was manipulated as a light source. The significant yields demonstrated that light and chlorophyll are required for reaction.

Afterward, we optimized the reaction conditions concerning catalyst dosage and solvent. The catalyst dosage was evaluated. Increasing moderately, the amount of chlorophyll from 0.04 to 0.08, the yield of both products increased significantly. When the amount of chlorophyll was further escalated from 0.08 to 0.09, the yield was almost remained at the same level (Tables 2 and 3). Once this stage is completed, H_2O/ethanol was uncovered to be the most appropriate solvent among the others (Table 3). Hence, H_2O/ethanol and 0.08 mg of catalyst were selected as the suitable conditions for the model reaction. Other solvents were not tested under these conditions because we were supposed to provide a green and eco-friendly protocol

Table 1. The Optimized Reaction Conditions

		5a		6a	
Entry	Catalyst	Time	Yield	Time	Yield
1	-	2.5 h	56	3 h	51
2	Chlorophyll	1 h	71	1.5 h	69
3	Chlorophyll + UV	11 min	89	13 min	87



Scheme 2. Utilization of chlorophyll as a natural catalyst for the synthesis of tetrahydrobenzo[b]pyran and 3,4-dihydropyrano[c]chromene derivatives

Table 2. Effect of the Catalyst amount on the Synthesis Product of Tetrahydrobenzo[b]pyran under UV Irradiation

Entry	Catalyst (g)	Product	Time (min)	Isolated yield (%)
1	0.04	5a	65	51
2	0.05	5a	46	62
3	0.06	5a	28	74
4	0.07	5a	19	84
5	0.08	5a	9	90
6	0.09	5a	11	89

Table 3. The Effect of Solvent Type on the Synthesis Product of Tetrahydrobenzo[*b*]pyran and 3,4-Dihydropyrano[*c*]chromene in the Presence of Chlorophyll Mmixture at Rroom Temperature under UV Irradiation

		Product 5a		Product 6a	
Entry	Solvent	Catalyst (g)	Isolated yield (%)	Catalyst (g)	Isolated yield (%)
1	H_2O	0.08	70	0.09	72
2	H_2O/EtOH (1:1)	0.08	76	0.09	75
3	H_2O/EtOH (2:1)	0.08	79	0.09	78
4	EtOH	0.08	90	0.09	87
5	CH_3CN	0.08	73	0.09	80
6	CH_2Cl_2	0.08	49	0.09	48

Table 4. Comparison the Catalytic Effect of Chlorophyll with Different Magnesium Compounds

Entry	Catalyst	Amount of catalyst (g)	Time (min)	Yield (%)
1	Mg	0.08	21	85
2	$MgCl_2$	0.08	17	83
3	$MgSO_4$	0.08	16	87
4	Chlorophyll	0.08	9	90

Table 5. Comparison the Results of Chlorophyll with other Catalysts Reported

Entry	Products	Catalyst	Conditions	Time (min)	Yield (%)	Ref.
1	5	$RhB(OH)_2$	5 mol%, EtOH:H_2O, Reflux	30	88	[31]
2	5	Fructose	20 mol%, EtOH:H_2O, 40 °C	45	86	[32]
3	5	TBAF	10 mol%, H_2O, Reflux	30	97	[33]
4	5	Pectin	0.05 g EtOH:H_2O, r.t.	40	94	[34]
5	5	TFE	TFE, Reflux	5h	90	[35]
6	5	Chlorophyll	0.08 g, EtOH, r.t.	9	90	This work

for the synthesis of those products. Furthermore, room temperature was found to be the most proper temperature for these reactions. The reactions were performed using Mg and Mg-salt to compare their catalytic effects with the chlorophyll (Table 4). As it can be concluded, chlorophyll has the best result in this regard. In order to illustrate the versatility the reported catalyst in comparison with the other catalysts, some of the results are reported (Table 5). Based on the results, chlorophyll has the best efficacy in terms of time and yield. Under the optimized conditions, the substrate scope of the reaction was tried to get to the bottom of by using a wide range of aromatic aldehydes. As shown in Table 6, aromatic aldehydes containing distinctive substituents (electron donating or electron-withdrawing groups) have different influences on those reactions. Electron-withdrawing substituents like chloro, bromo, fluoro, and, in particular, nitro showed higher reactivates and gave better yields than those bearing electron-donating substituents such as methyl, methoxyl, and hydroxyl. It should be noted that all the reactions were conducted under UV irradiation.

A possible mechanism for the synthesis of products 5 is considered (Scheme 3). Preliminary, the Mannich reaction between a and 2 gives intermediate b. The chlorophyll is excited from its ground state to excited state with the absorption of a photon leading to the formation of singlet oxygen. A single-electron transfer from Dimedone to the singlet oxygen leads to the c formation. The singlet oxygen atom accepts an electron to form superoxide anion radicals, simultaneously. The radical addition of c to d gives e, and subsequently single electron transfer from e to Dimedone gives intermediate f. Finally, intermolecular cyclization leads to compound 5. In this process, the protonation of superoxide radical anion would generate HOO^- and then H_2O_2 [39].

EXPERIMENTAL

General

All reagents were purchased from Merck and Sigma-Aldrich and applied without any purification. Melting point (m.p.) and IR spectra of all compounds were measured on an Electro-thermal 9100 apparatus and an IR Shimadzu-460 plus spectrometer, respectively. The ^{1}H NMR spectra were recorded on a BRUKER DRX-400 Advance spectrometer using $CDCl_3$ and DMSO-d6 as a solvent.

Preparation of Chlorophyll Mixture

To prepare chlorophyll mixture, 1 Kg of spinach leaves were bought, washed and placed in boiling water for 30-45 s. The boiling water is able to destroy enzymes and break chloroplasts, making chlorophyll extraction easier. They were dried with a paper towel, and then petioles and central veins were eliminated (these parts of leaves do not contain a lot of pigments). The rest of them were cut into the small 1-2 mm pieces and then were expanded evenly on the foil and heated for 30 min at 60 °C. Dried leaf pieces were ground until they turn into greenish-yellow powder. Leaf grinds will release more pigment if cell walls are broken and expanded the area of the surface simultaneously by grinding. The resulting powder was placed inside a tube with a tight lid and 10 ml ethanol was added and closed the lid to shake it. After 10 min, an emerald-green slightly liquid on top of dark green powder was obtained; this is chlorophyll mixture. Then, the liquid was overflowed and placed under vacuum to evaporate the solvent to be used for the next steps.

General Procedure for the Preparation of Tetrahydrobenzo[*b*]pyrans, and 3,4-Dihidropyrano [*c*]chromene Derivatives

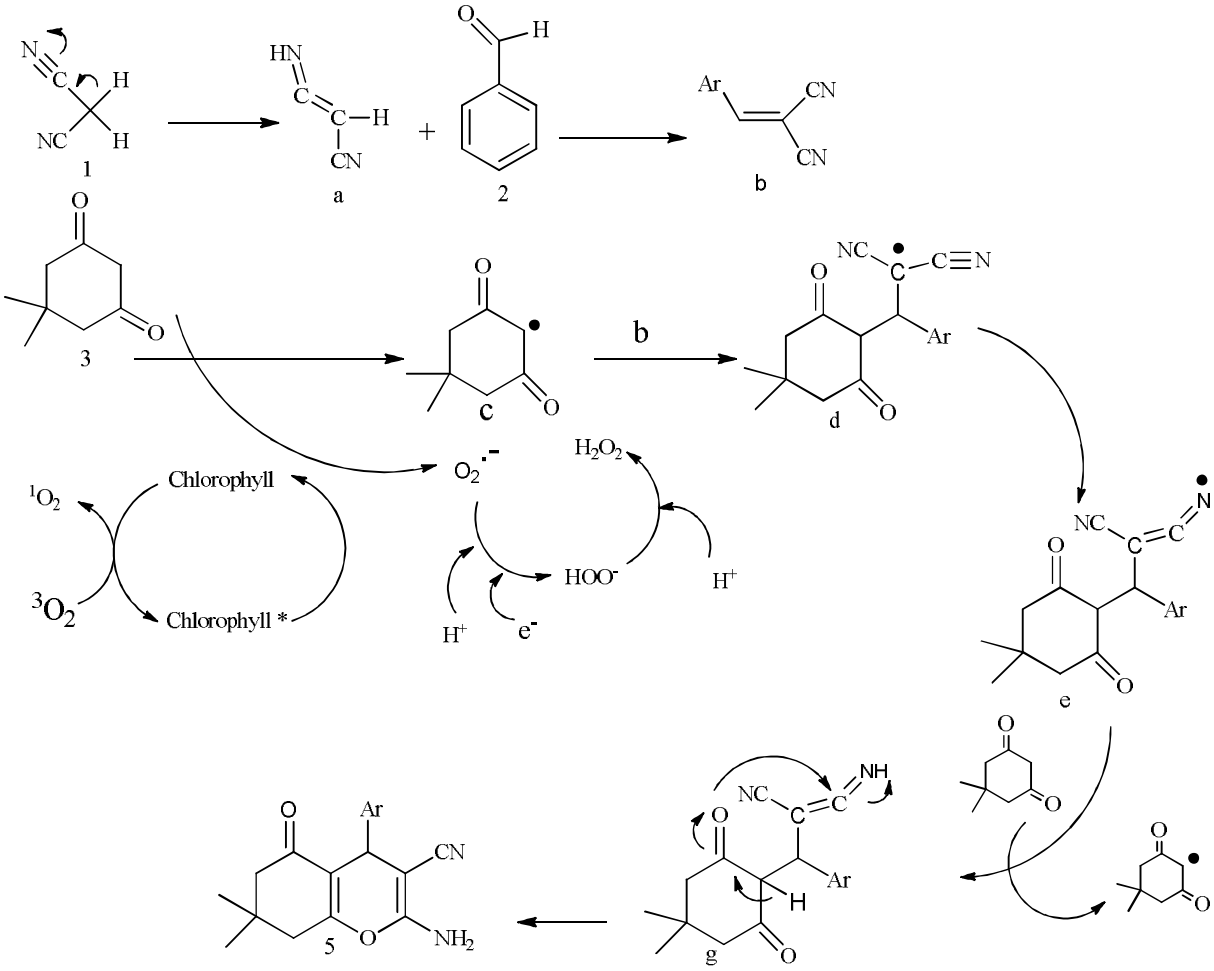
To a magnetical mixture of aromatic aldehyde (1.0 mmol), malononitrile (1.0 mmol) and 1,3-diketone (1.0 mmol) in EtOH, 0.08 g of chlorophyll mixture was added under UV irradiation and held for an appropriate time. Thin-layer chromatography (TLC) is a good tool for monitoring the reactions processes. After completion the reactions, in order to remove catalyst, unreacted starting materials and affording the pure products, the products were filtered and washed with H_2O and EtOH (99.7%), and recrystallized by ethanol.

2-Amino-5,6,7,8-tetrahydro-7,7-dimethyl-5-oxo-4-phenyl-4H-chromene-3-carbonitrile (5a). IR (KBr) v/cm^{-1} 3391, 3246, 2959, 2189, 1675, 1210; ^{1}H NMR (400 MHZ, $CDCl_3$) 1.07 (s, 3H, CH_3), 1.14 (s, 3H, CH_3), 2.23 (dd, J = 8.2, 1.2 Hz 2H, CH_2), 2.48 (s, 2H, CH_2), 4.44 (s, 1H, CH), 4.56 (s, 2H, NH_2), 7.2-7.4 (m, 5H, Ar).

2-Amino-5,6,7,8-tetrahydro-4-(4-nitrophenyl)-7,7-di-

Table 6. Preparation of Tetrahydrobenzo[*b*]pyran and 3,4-Dihydroxy[*c*]chromene Derivatives in the Presence of Chlorophyll Mixture at Room Temperature

Entry	1,3-Dicarbonyl	Product	Ar	Isolated yield (%)	Time (min)	M.P Observed (°C)	M.P Reported (°C)
1	3	5a	C_6H_5	89	11	229-231	228-230[27]
2	3	5b	2-NO_2-C_6H_4	88	11	228-229	224-226[28]
3	3	5c	3-NO_2-C_6H_4	92	10	209-211	208-210[34]
4	3	5d	4-NO_2-C_6H_4	93	8	183-184	183-185[34]
5	3	5f	4-Me-C_6H_4	84	10	209-211	212-215[35]
6	3	5g	2,4-$(Cl)_2C_6H_3$	91	21	175-177	180-182[24]
7	3	5h	4-OH-3-$OMeC_6H_3$	82	33	227-229	226-230[35]
8	3	5i	2,3-$OMeC_6H_3$	84	29	215-217	217-219[28]
9	3	5j	Thiophene-2-carbaldehyde	87	27	211-213	210-212[35]
10	3	5k	4-OHC_6H_4	79	56	205-207	204-206[34]
11	3	5l	2-Furaldehyde	91	28	219-221	218-220[25]
12	4	6a	C_6H_5	87	13	257-259	258-260[26]
13	4	6b	4-$NO_2C_6H_4$	90	20	256-258	255-256[26]
14	4	6c	3,4-$(OMe)_2C_6H_3$	80	39	223-235	230-232[38]
15	4	6d	2-ClC_6H_4	81	27	274-276	273-274[34]
16	4	6f	4-ClC_6H_4	88	19	260-262	258-260[26]
17	4	6g	4-OHC_6H_4	90	18	261-263	265-266[36]
18	4	6h	2-FC_6H_4	89	20	219-221	220-223[34]
19	4	6i	Thiophene-2-carbaldehyde	86	24	230-232	226-230[34]
20	4	6j	Thiophene-3-carbaldehyde	81	29	254-257	255-260[34]



Scheme 3. The suggested mechanism for the synthesis of tetrahydrobenzo[*b*]pyran in the presence of chlorophyll mixture, as a catalyst, and UV

methyl-5-oxo-4-phenyl-4H-chromene-3-carbonitrile (5d). IR (KBr) ν/cm^{-1} 3286, 3160, 2959, 2185, 1675, 1209; ^{1}H NMR (400 MHZ, $CDCl_3$) 0.97 (s, 3H, CH_3), 1.028 (s, 3H, CH_3), 2.12 (d, J = 1.5 Hz, 2H, CH_2) 2.27 (s, 2H, CH_2), 4.36 (s, 1H, CH), 7.19 (s, 2H, NH_2), 7.44 (d, J = 5.3 Hz, 2H, Ar), 8.16 (d, 2H, Ar).

2-Amino-5,6,7,8-tetrahydro-4-(4-methyl)-7,7-dimethyl-5-oxo-4-phenyl-4H-chromene-3-carbonitrile (5f). IR (KBr) ν/cm^{-1} 3465, 3320, 2956, 2189, 1675, 1276; ^{1}H NMR (400 MHZ, $CDCl_3$) 1.06 (s, 3H, CH_3), 1.12 (s, 3H, CH_3), 2.26 (dd, , J = 7.8, 1.6 Hz, 2H, CH_2), 2.29 (s, 3H), 2.46 (s, 2H, CH_2), 4.56 (s, 2H, NH_2), 7.11 (d, J = 7.9 Hz, 2H, Ar), 7.15 (d, 2H, Ar).

2-Amino-5,6,7,8-tetrahydro-4-(4-hydroxyphenyl)-7,7-dimethyl-5-oxo-4-phenyl-4H-chromene-3-carbonitrile (5k). IR (KBr) ν/cm^{-1} 3286, 3160, 2960, 2186, 1676, 1210; ^{1}H NMR (400 MHZ, $CDCl_3$) 1.04 (s, 3H, CH_3), 1.13 (s, 3H, CH_3), 2.25 (dd, J = 7.8, 4.7 Hz, 2H, CH_2), 2.25 (dd, J = 4.6, 1.6 Hz, 2H, CH_2), 2.45 (dd, J = 6.4 Hz, 2.2 Hz, 2H, CH_2), 4.35 (s, 1H, CH), 4.55 (s, 2H, NH_2), 5.27 (s, 1H, OH), 6.72-7.26 (m, 4H, Ar).

2-Amino-5,6,7,8-tetrahydro-(4-metyl)-7,7-dimethyl-5-oxo-4H-chromene-3-carbonitrile (5i). IR (KBr) ν/cm^{-1} 3328, 3257, 3215, 2194, 1679, 1214. ^{1}H NMR (400MHz, DMSO-d_6): δ 1.06 (s, 3H, CH_3), 1.3 (s, 3H, CH_3), 2.28 (dd, J = 4.5 Hz, 1.8 Hz, 2H, CH_2), 2.41 (s, 3H), 2.51 (2H, CH_2), 4.39 (s, 1H, CH), 4.52 (s, 2H, NH_2), 7.09 (d, J = 1.5 Hz, 2H, Ar), 7.28 (d, J = 5.3 Hz, 2H, Ar).

2-Amino-4,5-fihydro-4-(thiophen-2-yl)-5-oxo-pyrano [3,2-c]-chromene-3-carbonitrile (6i). IR (KBr) ν/cm^{-1} 3401, 3323, 2202, 1702, 1677, 1611, 1531, 1379 ^{1}H NMR (400 MHz, *δ)* 4.84 (s, 1H), 7.04-7.87 (m, 9H, Ar, NH_2), ^{13}C NMR (100 MHz, DMSO, d_6), 32.3, 57.4, 104.7, 114.0, 117.5 (CN), 119.9, 122.5, 125.0, 125.6, 127.5, 133.5, 147.4, 152.6, 158.6, 160.1 (CO).

2-Amino-4,5-dihydro-4-(-2-fluorophenyl)-5-oxo-pyrano[3,2-c] chromenr-3-carbonitrile (6h). IR ν/cm^{-1} 3401, 3319, 2102, 1749, 1665, 1610, 1529, 1376. ^{1}H NMR (400 MHZ, DMSO-d_6) 4.75 (s, 1H, CH), 7.11-8.38 (m, 9H, Ar, NH_2). ^{13}C NMR (100MHZ, DMSO-d_6): 31.7 (CH), 56.8, 103.1, 113.4, 117.2 (CN), 119.5, 122.9, 125.1, 125.2, 125.2, 129.6, 130.2, 130.4, 133.5, 152.7, 154.4, 158.7, 159.9, 162.3 (CO).

2-Amino-4,5-dihydro-4-(thiophen-3-yl)-5-oxopyrano [3,2-c] chromene-3-carbonitrile (6j). IR ν/cm^{-1} 3402, 3324, 2202, 1703, 1677, 1611, 1531, 1380. ^{1}H NMR (400 MHZ, DMSO-d_6) 4.64 (s, 1H, CH), 7.03-7.9 (m, 9H, Ar, NH_2). ^{13}C NMR (100MHZ, DMSO-d_6): 32.4 (CH), 57.9, 104.4, 113.5, 117.1 (CN), 119.8, 122.7, 122.9, 125.1, 127.0, 127.5, 131.3, 144.4, 152.6, 153.8, 158.7, 160.1 (CO).

CONCLUSIONS

To sum up, we are presented a green and cheap catalyst in order to develop an efficient approach to the synthesis of tetrahydrobenzo [b]pyran and 3,4-dihydropyrano[*c*] chromene derivatives via one-pot three-component reaction of aromatic aldehydes, 4-hydroxy-2*H*-chromen-2-one as an active enolic compound, and malononitrile. The catalytic activity is remarkable, and usage of environmental protocol, commercially available chlorophyll as a catalyst in this work in good yield is also significant. There are many advantages in this regard, such as avoidance of discharging harmful organic solvents, the simplicity of the methodology, easy work-up, short times and high yield, and interestingly usage of an inexpensive catalyst.

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