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Facile green one-pot synthesis of pyrano [2, 3-c] pyrazole and 1, 8-dioxo-decahydroacridine derivatives using graphene oxide as a carbocatalyst and their biological evaluation as potent antibacterial agents

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ABSTRACT

In this study, graphene oxide was prepared as a carbocatalyst. The carbocatalyst is used for the synthesis of derivatives of pyrano [2, 3-c] pyrazole and 1, 8-dioxo-decahydroacridine in water as the green solvent under ultrasonic irradiation conditions. This method provides many advantages such as short reaction time with highly yields, mild reaction conditions and environmental friendliness. The characterization of the synthesized compounds was established by melting point, IR, ^{1}H NMR and ^{13}C NMR spectra. *In vitro* antibacterial activity of 1, 8-dioxo-decahydroacridine derivatives compared with cefazolin by minimum inhibitory concentration (MIC), all of these compounds were more active than cefazolin.

Keywords: *Pyranopyrazoles, Multicomponent Reactions, Heterogeneous carbocatalyst, Green Chemistry, Graphene oxide, 1, 8-dioxo-decahydroacridine, MIC.*

1. INTRODUCTION

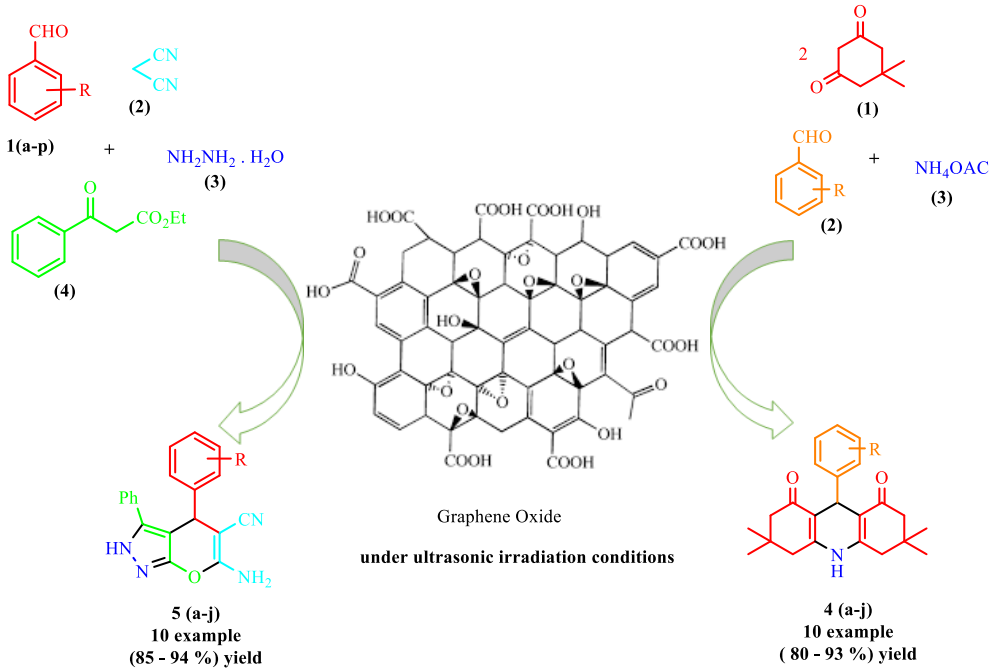
Recently, the use of nanomaterials has been considered by the researchers as a catalyst, for example, carbon nanotubes such as carbon nanotubes and graphene. Graphene oxide has been widely used in various fields due to its desirable attributes such as low cost material, high effective surface, reactivity and thermal stability.[1-3] Heterogeneous catalysts appear to be a potential solution and carbon based materials can be useful for this purpose. Graphene oxide (GO, graphite oxide sheet), is the product of chemical exfoliation of graphite powder using strong oxidants [4-6]. Multicomponent reactions (MCRs) consist of two or more starting materials react together to give a single product without isolating the intermediates which are one-step processes [7]. These reactions are efficient green tool for the synthesis molecular complexity and diversity coupled with shorter reaction time, atom economy, low cost and minimum wastage production [8-10].

In recent years, pyrano pyrazoles have interested synthetic organic chemists and biochemists of their biological [11] and pharmacological activities [12] such as antimicrobial [13], anti-cancer [14], analgesic and anti-inflammatory properties [15], anti-Alzheimer's disease [14], antioxidant agents [16], and also as potential inhibitors of human Chk1 kinase [17].

Derivatives of 1, 8-dioxo-decahydroacridine are interesting heterocyclic compounds that have many applications in the field of organic and medicinal chemistry because of their remarkable biological properties. These compounds are used as anti-malaria [18], antifungal [19], anti-tumor [20], calcium β-blockers [21].

A number of methods have been reported one-pot cyclocondensations for the synthesis of the pyrano [2,3-c] pyrazole and 1,8-dioxo-decahydroacridin derivatives employing different catalysts such as γ-alumina [22], DABCO [23, 24], bmim[OH] [25], TrCl (trityl chloride) [26], sodium bisulfite under ultrasound irradiations in solvent free [27], magnetic Fe_3O_4 nanoparticles [28], PS-PSTA [29], catalyst free [30], Nano TiO_2 [31], DES [32], Amberlyst-15[33], ammonium chloride or L-proline, $Zn(OAc)_2{\cdot}2H_2O$ [34], nano CeO_2 [35], nano-Fe_3O_4@SiO_2–SO_3H [36], Brønsted acidic imidazolium salts containing perfluoroalkyl tails[37], benzyltriethyl ammonium chloride (TEBAC) [38] and CAN [39].

This study has examined synthesis of the pyrano[2, 3-c] pyrazole derivatives using from aryl aldehydes (1), malonitrile (2), hydrazine hydrate (3), and ethyl benzoyl acetate (4) under ultrasonic irradiation conditions in the presence of graphene oxide (GO) as a heterogeneous catalyst in short reaction time with high yields. Also, the present study has investigated synthesis of derivatives of 1, 8-dioxo-decahydroacridine using reactions of aromatic aldehydes, dimedone and ammonium acetate under ultrasonic irradiation conditions in the presence of graphene oxide (GO) as a heterogeneous catalyst in short reaction time with high yields (Scheme 1). In this work the best results according to the reaction conditions in the presence of catalyst graphene oxide is reported. The synthesized compounds were characterized using spectroscopic analyses and investigated for their antibacterial activities.



Scheme 1. Synthesis of compounds.

2. EXPERIMENTAL SECTION

All melting points were uncorrected and measured using capillary tubes on an Electrothermal digital apparatus. IR spectra were recorded on a Shimadzo (FT)-IR 300 spectrophotometer in KBr. NMR spectra were recorded on a Brucker 500 and 400 MHz spectrometer in DMSO-d_6 as solvent with TMS as an internal standard. Chemical shifts (δ) for ^{1}H and ^{13}C NMR was reported in parts per million (ppm) and was referenced to the solvent peak; DMSO-d_6 (2.50 ppm for ^{1}H and 39.70 ppm for ^{13}C). Multiplicities are represented by s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). The progress of the reaction was monitored by thin-layer chromatography TLC (Thin-Layer Chromatography) using n-hexane/EtOAc (1:1) as an eluent.

Synthesis of graphene oxide: According to the reference [40], we have synthesized graphene oxide (GO) as heterogeneous catalyst. GO was synthesized by the oxidation of graphite powder using the modified Hummers method, followed by exfoliation in an aqueous solution. In order to carry out the quantitative characterization of GO, such as the amounts of –COOH and –OH groups, a solid based titrimetry method was used [41]. Based on the titration curves the value of –COOH and –OH were measured to be 0.17 ± 0.01% and 2.65 ± 0.02%, respectively. The pH value of a dispersion containing GO was 4.6, at about 0.1 mg ml^{-1}, which is consistent with that reported in the literature [42, 43].

General procedure for synthesis of 1, 8-dioxo-decahydroacridin derivatives 4 (a-j): To a mixture of dimedone (2 mmol), aromatic benzaldehyde (1 mmol) and ammonium acetate (1.2 mmol) was added graphene oxide (GO) as a heterogeneous catalyst (10%mol) in 5 mL H_2O as the green solvent and under ultrasonic irradiation until completion of the reaction. The progress of the reaction was followed by thin layer chromatography (TLC). After the completion of the reaction, mixture was added hot ethanol. Then, the reaction mixture was filtered and the catalyst was easily separated. Finally, the resulting solid was washed with H_2O without further purification and dried in an oven at 60 °C. The pure 1, 8-dioxo-decahydroacridin derivatives were obtained in excellent yields.

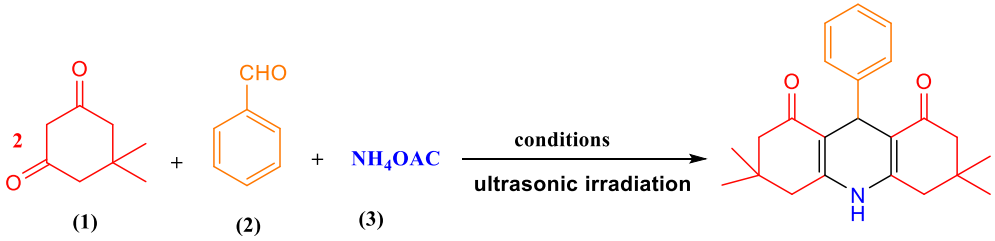
General procedure for the synthesis of pyrano [2, 3-c]pyrazole derivatives 5 (a–j): To a mixture of aromatic benzaldehyde (1 mmol), malonitrile (1mmol), hydrazine hydrate (1.5 mmol) and ethyl benzoyl acetate (1mmol) was added graphene oxide (GO) as a heterogeneous catalyst (10%mol) in 5 mL H_2O as the green solvent and under ultrasonic irradiation until completion of the reaction. The progress of the reaction was followed by thin layer chromatography (TLC). After the completion of the reaction, mixture was added hot ethanol. Then, the reaction mixture was filtered and the catalyst was easily separated. Finally, the resulting solid was washed with H_2O without further purification and dried in an oven at 60 °C. The pure pyrano [2, 3-c] pyrazole derivatives were obtained in excellent yields.

3. RESULTS SECTION

In order to optimize the method, the reaction of dimedone (2 mmol), aromatic benzaldehyde (1 mmol) and ammonium acetate (1.2 mmol) was examined. This model reaction for various solvents and catalyst amounts was studied to find the best conditions. As shown in Table 1, the nature of the solvent was observed to have a profound effect on both the activity of the catalyst and the yield of product. The best performance was achieved using H_2O as the solvent. In the reactions of chemistry time and yield are important, so without catalyst time is long and yield is low. Thus, the reaction does not proceed completely.

Participation of the catalyst in the reaction was confirmed by conducting a blank experiment without the catalyst. This blank experiment gave a 10 % yield after 2 h (Table 1, entry 1). The effect of the catalyst amount of the reaction was also investigated. Use of a higher amount of catalyst had no significant effect on the reaction rate or the isolated yield of product (Table 1, entry 7, 8), while a decrease in the amount of catalyst decreased the product yield. The best result was obtained using 10% mol of catalyst. Overall, the ideal reaction conditions for the formation of 1, 8-dioxo-decahydroacridine derivatives were H_2O as the solvent and 10% mol of the catalyst under ultrasonic irradiation. With respect to yield of products and reaction time, the best result is achieved at the room temperature using 10% mol of geraphene oxide as a catalyst in 5 mL H_2O as the solvent with respect to green nature, polarity and clean workup procedure for this synthesis under ultrasonic irradiation (Table 1, entry 6).

Table 1. Optimization of reaction conditions for preparation of 1, 8 -dioxo-decahydroacridine derivatives.

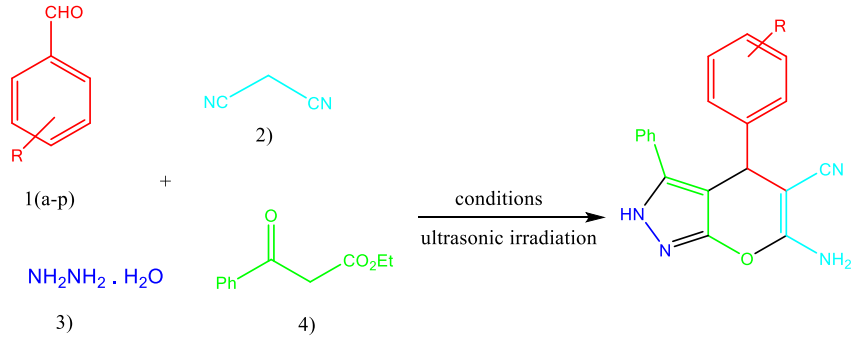


Entry	Catalyst (mol %)	Solvent	Time (min)	Yield (%)[a] 4a
1	-----	H_2O	120	<10
2	GO (5)	EtOH	120	<10
3	GO (5)	CH_3CN	120	60
4	GO (5)	CH_2Cl_2	120	62
5	GO (5)	H_2O	120	80
6	GO (10)	H_2O	10	93
7	GO (15)	H_2O	10	88
8	GO (20)	H_2O	10	80
9	Graphite (10)	H_2O	60	45

[a]Isolated yield

Also, we began our study with the optimization of the four-component reaction in H_2O as the green solvent between amount of arylaldehyde, malononitrile, hydrazine hydrate and ethylbenzylacetate. This reaction was initially carried out in the presence of geraphene oxide (10% mol) as a heterogeneous catalyst under ultrasonic irradiation. It also was studied under several conditions. This model reaction for various solvents and catalyst amounts was studied to find the best conditions (Table 2, entry 7).

Table 2. Optimization of reaction conditions for preparation of pyrano [2, 3- c] pyrazole derivatives.



Entry	Catalyst (mol %)	Solvent	Time (min)	Yield (%)[a]5a
1	-----	H_2O	120	<8
2	GO (5)	EtOH	120	<8
3	GO (5)	CH_3CN	120	55
4	GO (5)	CH_2Cl_2	120	59
5	GO (5)	H_2O	120	82
6	GO (10)	EtOH	30	85
7	GO (10)	H_2O	5	94
8	GO (15)	H_2O	5	85
9	GO (20)	H_2O	5	80
10	Graphite (10)	H_2O	60	52

[a]Isolated yield

The conditions optimized for the production of the 1, 8-dioxo-decahydroacridine derivatives were evaluated using the geraphene

oxide as catalyst. The reaction of aromatic aldehydes carrying either electron-donating or electron-withdrawing substituents with dimedone and ammonium acetate is done (Table 3).

Table 3. Multicomponent one-pot synthesis of 1,8-dioxo-decahydroacridine derivatives using geraphene oxide as catalyst [a].

Entry	Aromatic aldehyde (R)	Product	Time (min)	Yield (%)[b]	Melting point (°C)	
					Found	Reported
1	H	**4a**	20	93	288-290	290-291 [44]
2	4-Me	**4b**	18	82	279-281	278-280 [44]
3	4-$CH(Me)_2$	**4c**	25	80	235-237	---
4	3-NO_2	**4d**	20	80	306-308	307-308 [44]
5	4-$N(Me)_2$	**4e**	20	87	280-282	280-282 [45]
6	3-OH	**4f**	17	82	308-310	---
7	2-NO_2	**4g**	15	93	297-299	297-299 [46]
8	4-Cl	**4h**	20	92	298-301	298-300 [47]
9	2-Me	**4i**	25	85	299-302	299-302 [46]
10	3,4-$(OMe)_2$	**4j**	25	91	288-290	287-290 [48]

[a]Aromatic aldehyde (1 mmol), dimedone (2 mmol), ammonium acetate (1.2 mmol) and 10% mol geraphene oxide, under ultrasonic irradiation.
[b]Isolated yield.

The conditions optimized for the production of the pyrano [2, 3-c] pyrazole derivatives were evaluated using the geraphene oxide as catalyst. The reaction of aromatic aldehydes carrying either electron-donating or electron-withdrawing substituents with malononitrile, hydrazine hydrate and ethylbenzylacetate is done (Table 4).

Table 4. The preparation of pyrano [2, 3-c] pyrazole (5a-j) derivatives using grapheme oxide as catalyst.

Entry	Aromatic aldehyde (R)	Product	Time (min)	Yield (%)[b]	Melting point (°C)	
					Found	Reported
1	H	**5a**	5	94	242-243	242-244 [32]
2	4-Me	**5b**	3	85	237-238	237-238 [32]
3	3-NO_2	**5c**	2	89	254-255	255-256 [49]
4	3-OH	**5d**	3	84	258-260	258-260 [32]
5	2-Me	**5e**	5	87	238-240	238-240 [32]
6	2-NO_2	**5f**	2	92	256-258	255-257 [32]
7	4-OMe	**5g**	2	85	241-243	241-243 [32]
8	4-Br	**5h**	6	93	255-256	255-256 [32]
9	4-$CH(Me)_2$	**5i**	5	86	250-252	250-251 [32]
10	2-Cl	**5j**	6	90	271-273	271-273 [32]

aromatic benzaldehyde (1 mmol), malonitrile (1mmol), hydrazine hydrate (1.5 mmol), ethyl benzoyl acetate (1mmol) and graphene oxide (GO) as a heterogeneous catalyst (10%mol) in 5 mL H_2O
[b]Isolated yield.

The recovery and reusability of the catalyst are very important for commercial and industrial applications as well as green process considerations. Thus, after completion of the reaction, the geraphene oxide catalyst was recycled by simple extraction of the product with hot ethanol from the reaction mixture. The geraphene oxide catalyst that remains in the reaction test tube is thoroughly washed with water and reused in subsequent reactions without further purifications. Moreover, the catalyst is reusable for the next catalytic cycles after activating the geraphene oxide catalyst at 60 °C under vacuum in each cycle (Table 5).

Table 5. Catalyst reusability study.

Cycle	1st	2nd	3rd	4th	5th
Yield (%)	93	92	90	88	85

Antibacterial studies. Antibacterial activity of the pyrano [2, 3-c] pyrazoles derivatives was investigated [32]. In continuation of our work, all the synthesized 1, 8-dioxo-decahydroacridine derivatives assessed for their antibacterial activity against two Gram-positive bacteria (*Staphylococcus saprophyticus* and *S. aureus*) and two Gram-negative bacteria (*Esherichia coli* and *Pseudomonas aeruginosa*). Normal saline was used for preparation of inoculants having 0.5 McFarland standards. The 1, 8-dioxo-decahydroacridine derivatives were dissolved in dimethylsolfuxide (DMSO) for bioassay. The microplates were incubated at 37^0 C for 24 h. Values of minimum inhibitor concentration (MIC) were recorded as the lowest concentration of substance, which gives no growth of inoculated bacteria. All the compounds showed antibacterial activity against both Gram-positive and Gram-negative standard strains and their MICs ranged between 20 and 45 μg/ml. The MICs of these compounds and cefazolin were

determined by using the standard protocol of the NCCLS Broth Microdilution MIC method [50] and the results are presented in Tables 6.

Tables 6. MIC values of the 1, 8-dioxo-decahydroacridine derivatives.[a]

Comp No	MIC (μg.ml^{-1})			
	E.Coli	*P.aeruginosa*	*S.saprophyticus*	*S.aureus*
5a	20	30	25	20
5b	25	35	45	25
5c	30	45	45	40
5d	30	30	30	30
5e	25	40	35	35
5f	35	45	35	20
5g	35	30	25	35
5h	40	25	45	45
5i	45	40	35	40
5j	35	30	45	35

[a] Cefazolin is taken as a standard drug and its MIC is >35 μg/mL against all the four strains.

4. CONCLUSIONS

The structures of newly synthesized compounds were assigned on the basis on their spectral data and those reported compounds by comparing with earlier literature. This one-pot green chemistry protocol is advantageous requiring shorter reaction time with highly yields, easily available and cheap catalyst, simple and practical which proceeds without any special handing technique and requiring routine reagents. All the compounds showed antibacterial activity against both Gram-positive and Gram-negative standard strains.

Spectroscopic data for selected products:

3,3,6,6-tetramethyl-9-phenyl-3,4,6,7,9,10-hexahydroacridine-1,8(2H,5H)-dione (4a). White solid; m.p: 288–290 ^{0}C; Yield 93%; IR (KBr, cm^{-1}) ν_{max}; 1591 (C=O), 2879-2961 (CH_3 str.), 3068 (NH); ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 1.11 (6H, s, $2CH_3$), 1.24 (6H, s, $2CH_3$), 2.30-2.49 (8H, m, $4CH_2$), 7.10-7.29 (5H, m, Ar-H), 11.91 (1H, s, NH); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ ppm: 27.4, 29.6, 31.4, 32.7, 46.4, 47.0, 115.6, 125.8, 126.7, 128.2, 138.0, 189.4, 190.5.

3,3,6,6-tetramethyl-9-(p-tolyl)-3,4,6,7,9,10-hexahydroacridine-1,8(2H,5H)-dione (4b). White solid; m.p: 279–281 ^{0}C; Yield 85%; IR (KBr, cm^{-1}) ν_{max}; 1596 (C=O), 2878-2961 (CH_3 str.), 3021 (NH); ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 1.11 (6H, s, $2CH_3$), 1.24 (6H, s, $2CH_3$), 2.31 (3H, s, CH_3), 2.32-2.49 (8H, m, $4CH_2$), 5.52 (1H, s, CH), 6.99-7.09 (4H, dd, Ar-H, J= 7.7 Hz), 11.93 (1H, s, NH); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ ppm: 20.7, 20.9, 27.2, 27.3, 29.1, 29.4, 31.2, 31.3, 32.0, 32.3, 40.7, 46.3, 46.9, 50.6, 115.5, 115.6, 126.5, 128.1, 128.6, 128.8, 134.8, 135.1, 135.5, 141.1, 161.9, 189.2, 190.2, 196.2.

9-(3-hydroxyphenyl)-3,3,6,6-tetramethyl-3,4,6,7,9,10-hexahydroacridine-1,8(2H,5H)-dione (4f). Yellow solid; m.p: 308–310 ^{0}C; Yield 89%; IR (KBr, cm^{-1}) ν_{max}; 1617 (C=O), 2878-2928 (CH_3 str.), 3282 (NH), 3447 (OH); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ ppm: 0.87 (6H, s, $2CH_3$), 0.99 (6H, s, $2CH_3$), 1.97-2.00 (2H, d, CH_2, J= 16.1 Hz), 2.14-2.17 (2H, d, CH_2, J= 16.1 Hz), 2.28-2.31 (2H, d, CH_2, J= 17.0 Hz), 2.41-2.44 (2H, d, CH_2, J= 17.0 Hz), 4.73 (1H, s, CH), 6.39-6.42 (1H, d, Ar-H, J= 11.0 Hz), 6.55-6.57 (1H,d, Ar-H, J= 7.65 Hz), 6.61 (1H, s, Ar-H), 6.89-6.92 (1H, t, Ar-H, J= 7.5 Hz), 9.03 (1H, s, NH), 9.23 (1H, s, OH); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ ppm: 26.5, 26.6, 27.9, 28.8, 31.9, 32.2, 32.6, 40.0, 46.6, 50.1, 50.3, 111.5, 112.5, 114.5, 114.7, 118.4, 128.7, 148.5, 149.3, 156.7, 157.1, 162.9, 194.5, 196.1.

3,3,6,6-tetramethyl-9-(2-nitrophenyl)-3,4,6,7,9,10-hexahydroacridine-1,8(2H,5H)-dione (4g). Yellow solid; m.p: 297–299 ^{0}C; Yield 92%; IR (KBr, cm^{-1}) ν_{max}; 1343 and 1520 (NO_2), 1723 (C=O), 2900-2955 (CH_3 str.), 3381 (NH); ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 1.02 (6H, s, $2CH_3$), 1.15 (6H, s, $2CH_3$), 2.20-2.51 (8H, m, $4CH_2$), 6.04 (1H, s, CH), 7.24-7.55 (4H, m, Ar-H), 11.6 (1H, s, NH); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ ppm: 28.1, 28.5, 30.0, 31.9, 46.2, 46.8, 114.6, 124.3, 127.2, 129.6, 131.3, 132.1, 149.7, 189.4, 190.4.

9-(4-chlorophenyl)-3,3,6,6-tetramethyl-3,4,6,7,9,10-hexahydroacridine-1,8(2H,5H)-dione (4h). White solid; m.p: 298–301 ^{0}C; Yield 91%; IR (KBr, cm^{-1}) ν_{max}; 673-885 (Cl), 1590 (C=O), 2881-3005 (CH_3 str.), 3049 (NH); ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 1.09 (6H, s, $2CH_3$), 1.21 (6H, s, $2CH_3$), 2.29-2.47 (8H, m, $4CH_2$), 5.47 (1H, s, CH), 7.00-7.02 (2H, d, Ar-H, J= 8.0 Hz), 7.21-7.23 (2H, d, Ar-H, J= 8.0 Hz), 11.87 (1H, s, NH); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ ppm: 27.2, 27.4, 29.2, 29.5, 31.3, 31.4, 32.1, 32.4, 40.8, 46.4, 47.0, 50.6, 115.2, 115.3, 128.1, 128.3, 129.7, 131.5, 131.9, 136.7, 142.6, 162.4, 189.3, 190.5, 196.2.

6-amino-2, 4-dihydro-3-phenyl-4-p-tolylpyrano [2, 3-c] pyrazole-5-carbonitrile (5b). Yellow solid; m.p: 237-238 ^{0}C; Yield 98%; IR (KBr, cm^{-1}) ν_{max}: 3480(NH), 3212 and 3123 (NH_2), 2196 (C≡N). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ ppm: 2.18 (s, 3H, CH_3); 4.94 (s, 1H, CH); 6.90 (brs, 2H, NH_2, D_2O-exchangable); 6.98-7.024 (dd, 4H, C_6H_4): 6.98-7.00 (d_1, 2H, $^3J_{H2,3}$=8.0 Hz, $H_{2,3}$); 7.02-7.00 (d_2, 2H, $^3J_{H4,5}$=8.0 Hz, $H_{4,5}$, C_6H_4); 7.24-7.48 (m, 5H, C_6H_5); 12.88 (s, 1H, NH, D_2O-exchangable). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ ppm: 160.4, 156.5, 142.2, 138.3, 136.1, 129.4, 129.2, 129.1, 128.7, 127.6, 126.6, 121.1, 97.9, 58.9, 36.9, 21.3.

6-amino-2, 4-dihydro-4-(3-nitrophenyl)-3-phenylpyrano [2, 3-c] pyrazole-5-carbonitrile (5c). Yellow solid; m.p: 254-255^{0}C; Yield 93%; IR (KBr, cm^{-1}) ν_{max}: 3472(NH), 3247 and 3096 (NH_2), 2190 (C≡N), 1530 and 1349 (NO_2). ^{1}H-NMR (DMSO-d_6 , 400 MHz) δ ppm: 5.33 (s, 1H, CH); 7.11 (brs, 2H, NH_2, D_2O-exchangable); 7.23-7.48 (m, 5H, C_6H_5); 7.492-7.514 (dd, 1H, $^3J_{H6,5}$=1.2 Hz, H_6, C_6H_4); 7.576-7.602 (dt, H_5): 7.576-7.579 (dt_1,

1H, $^3J_{H5,4}$=1.2 Hz, H_5, C_6H_4); 7.599-7.602 (dt_2, 1H, $^3J_{H5,6}$=1.2 Hz, H_5, C_6H_4); 7.96-7.969 (dd, 1H, $^4J_{H2,4}$=1.6 Hz, H_2, C_6H_4); 7.978-7.986 (dd): 7.978-7.980 (d_1, 1H, $^3J_{H4,5}$= 0.8 Hz, H_4, C_6H_4); 7.984-7.986 (d_2, 1H, $^4J_{H4,2}$= 0.8 Hz, H_4, C_6H_4); 12.96 (s, 1H, NH, D_2O-exchangable). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ ppm: 160.9, 156.1, 147.9, 147.1, 138.9, 134.7, 130.4, 129.0, 128.9, 128.7, 126.9, 122.3, 122.2, 120.8, 96.9, 57.5, 36.5.

6-amino-2, 4-dihydro-4-(3-hydroxyphenyl)-3-phenylpyrano [2, 3-c] pyrazole-5-carbonitrile (5d). White solid; m.p: 258-260 ^{0}C; Yield 94%; IR (KBr, cm^{-1}) ν_{max}: 3833 (OH), 3472(NH), 3331 and 3205 (NH_2), 2193 (C≡N). ^{1}H-NMR (DMSO-d_6 ,400 MHz) δ ppm: 4.85 (s, 1H, CH); 6.91 (brs, 2H, NH_2, D_2O-exchangable); 6.473-6.482 (dd, 1H, $^4J_{H2,4}$= 2Hz, H_2, C_6H_4); 6.5-6.528 (dd): 6.5-6.502 (d_1, 1H, $^3J_{H4,5}$= 0.8 Hz, H_4); 6.526-6.528 (d_2, 1H, $^4J_{H4,6}$= 0.8 Hz, H_4); 6.571-6.59 (dd, 1H, $^3J_{H6,5}$= 7.6 Hz, H_6); 6.98-7.02 (t, 1H, $^3J_{H5,6}$= 7.6 Hz, H_5); 7.25-7.48 (m, 5H, C_6H_5); 9.26 (1H, s, OH, D_2O-exchangable); 12.89 (s, 1H, NH, D_2O-exchangable). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ ppm: 160.5, 157.8, 156.5, 146.7, 138.3, 129.7, 129.2, 129.1, 128.8, 126.6, 121.1, 118.5, 114.4, 114.3, 97.9, 58.9, 37.2.

6-amino-2, 4-dihydro-4-(4-methoxyphenyl)-3-phenylpyrano [2, 3-c] pyrazole-5-carbonitrile (5g). White solid; m.p: 241-243 ^{0}C; Yield 98%; ^{1}H-NMR (DMSO-d_6 ,400 MHz) δ ppm: 3.34 (s, 3H, OCH_3); 5.05 (s, 1H, CH); 6.98 (brs, 2H, NH_2, D_2O-exchangable); 7.116-7.267 (dd, 4H, C_6H_4): 7.116-7.137 (d_1, 2H, $^3J_{H2,3}$=8.4 Hz, $H_{2,3}$): 7.240-7.261 (d_2, 2H, $^3J_{H5,6}$= 8.4 Hz, $H_{5,6}$); 7.274-7.466 (m. 5H, C_6H_5); 12.90 (s, 1H, NH, D_2O-exchangable).^{13}C-NMR (DMSO-d_6 , 100 MHz) δ ppm: 161.06, 160.6, 156.3, 144.04, 138.5, 136.5, 133.07, 131.5, 130.5, 129.6, 129.5, 129.06, 128.9, 128.8, 128.7, 126.7, 120.9, 97.4, 58.2, 36.5.

6-amino-4-(4-bromophenyl)-2, 4-dihydro-3-phenylpyrano [2, 3-c] pyrazole-5-carbonitrile (5h). White solid; m.p: 255-256 ^{0}C; Yield 89%; IR (KBr, cm^{-1}) ν_{max}: 3431(NH), 3287 and 3134 (NH_2), 2185 (C≡N), 1064 (Br). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ ppm: 5.05 (s, 1H, CH); 6.99 (brs, 2H, NH_2, D_2O-exchangable); 7.052-7.085 (dd, 4H, C_6H_4): 7.052-7.063 (d_1, 1H, $^3J_{H3,2}$=2.4 Hz, H_3); 7.075-7.085 (d_2, 1H, $^3J_{H2,3}$=2.4 Hz, H_2); 7.25-7.40 (m, 5H, C_6H_5); 7.449-7.472 (d_1, 1H, $^3J_{H5,6}$=1.6 Hz, H_5); 7.075-7.085 (d_2, 1H, $^3J_{H6,5}$=1.6 Hz, H_6); 12.93 (s, 1H, NH, D_2O-exchangable). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ ppm: 160.6, 156.3, 144.5, 138.5, 131.7, 130.1, 129.0, 128.9, 126.7, 121.0, 120.1, 97.3, 58.2, 36.6 .

6-amino-2, 4-dihydro-4-(4-isopropylphenyl)-3-phenylpyrano [2, 3-c] pyrazole-5-carbonitrile (5i). White solid; m.p: 250-251 ^{0}C; Yield 97%; IR (KBr, cm^{-1}) ν_{max}: 3481(NH), 3222 and 3108 (NH_2), 2196 (C ≡ N). ^{1}H-NMR (DMSO-d_6, 500 MHz) δ ppm:1.094-1.09 (d, 3H, $^3J_{H(CH3,\ CH)\ Isopropyl}$= 2.4 Hz, CH_3); 1.1084-1.1132 (d, 3H, $^3J_{H(CH3,\ CH)\ Isopropyl}$ = 2.4 Hz, CH_3); 2.48-3.34 (m, 1H, $CH_{Isopropyl}$); 4.92 (s, 1H, CH); 6.89 (brs, 2H, NH_2, D_2O-exchangable); 7.0102-7.0822 (dd, 4H, C_6H_4): 7.0102-7.0265 (d_1, 2H, $^3J_{H2,3}$=8.15 Hz, $H_{2,3}$); 7.0659-7.0822 (d_2, 2H, $^3J_{H5,6}$= 8.15 Hz, $H_{5,6}$); 7.228-7.455 (m, 5H, C_6H_5); 12.87 (s, 1H, NH, D_2O-exchangable). ^{13}C-NMR (DMSO-d_6, 125 MHz) δ ppm: 160.1, 156.1, 146.5, 142.2, 137.6, 128.7, 128.6, 128.5, 128.2, 127.1, 126.9, 126.3, 126.2, 126.1, 126.1, 120.7, 97.6, 58.5, 36.4, 32.8, 23.8, 23.6.

6-amino-4-(2-chlorophenyl)-2, 4-dihydro-3-phenylpyrano [2, 3-c] pyrazole-5-carbonitrile (5j). White solid; m.p: 271-273 ^{0}C; Yield 91%; IR (KBr, cm^{-1}) ν_{max}: 3442 (NH), 3268 and 3120 (NH_2), 2190 (C≡N), 1058 (Cl). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ ppm: 5.43 (s, 1H, CH); 6.99 (brs, 2H, NH_2, D_2O-exchangable); 7.086-7.274 (m, 5H, C_6H_5); 7.294-7.298 (dd, 1H, $^3J_{H6,5}$= 1.6 Hz, H_6, C_6H_4); 7.305-7.314 (dt, 1H, J_{H4}=3.6 Hz, $H_{4(5)}$, C_6H_4); 7.361-7.385(dd, 1H, $^3J_{H3,4}$= 1.6Hz, H_3, C_6H_4); 12.86 (s, 1H, NH, D_2O-exchangable). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ ppm: 160.9, 156.6, 141.5, 138.5, 132.5, 131.1, 129.8, 128.9, 128.8, 128.0, 126.5, 120.5, 97.1, 56.9, 34.5.

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6. ACKNOWLEDGEMENTS

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