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One-pot synthesis of pyrano chromene carbonitriles catalyzed by $NiFe_2O_4$ nanoparticles and their anticancer potential

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In the present paper, a rapid, clean, and highly efficient method was developed for the preparation of 2-amino-4-(2,4-alkylphenyl)-5-oxo-4,5-dihydropyrano [3,2-*c*]-3-carbonitrile and its derivatives by one-pot condensation of a three-component system consisting of malononitrile, 4-hydroxycoumarin and various aromatic aldehydes, using a novel $NiFe_2O_4$ nanocrystalline catalyst. The latter was synthesized by a simple sol-gel method. The obtained nanoparticles were characterized by spectroscopic methods such as SEM, XRD, TEM, a. o. The nanocatalyst is highly efficient and environmentally friendly. Some novel 2-amino-4-(2,4-alkylphenyl)-5-oxo-4,5-dihydropyrano [3,2-c] chromene carbonitrile derivatives were prepared by the described synthetic method and were analyzed by $_1$HNMR,$_{13}$CNMR, FTIR, mass spectroscopy, a. o. Anticancer activity of the synthesized compounds was checked against MCF-7 breast cancer cell line using the MTT assay.

Keywords: $NiFe_2O_4$ NPs, Three-component reactions, EtOH, Chromene carbonitriles, Anticancer activity

INTRODUCTION

The research is devoted to nanomaterials, including nanoparticles, nanofillers, nanowires, and various nanocomposites, owing to their distinctive chemical, optical, magnetic, and electrical properties [1]. Among these, spinel ferrites such as nickel ferrite ($NiFe_2O_4$) nanoparticles (NPs) have attracted considerable attention due to their intrinsic magnetic characteristics, which confer high electrical resistivity and excellent thermal stability. These properties render $NiFe_2O_4$ NPs highly suitable for a broad spectrum of technological applications, including magnetic sensors, power transformers, temperature-dependent magnetic devices, heterogeneous catalysts, gas sensing and microwave communication systems [2-5].

Nickel ferrite ($NiFe_2O_4$) is a well-known spinel-type magnetic material that has been widely investigated for its unique physicochemical properties. In the present study, $NiFe_2O_4$ NPs were synthesized using the sol–gel method. The precursors employed included nickel nitrate, ferric nitrate and citric acid. This synthesis route is advantageous due to its simplicity, cost-effectiveness and mild reaction conditions, which facilitate the formation of homogeneous nanocrystalline powders. The resulting $NiFe_2O_4$ NPs exhibit several benefits, including uniform particle distribution, high crystallinity and economic feasibility of production [6].

The primary objective of this work is to establish a reliable, efficient, and environmentally sustainable strategy for the preparation of highly crystalline $NiFe_2O_4$ NPs. Such materials are expected to display desirable physicochemical characteristics, making them promising candidates for advanced applications in catalysis, biomedical fields, and other emerging technologies.

Chromenes represent a significant class of oxygenated heterocyclic compounds with broad biological importance. Among these, pyrano [3,2-c] chromene carbonitrile derivatives have gained notable interest in recent years due to their broad pharmacological potential. These molecules are reported to possess multiple biological activities, including anti-anaphylactic, anticoagulant, anticancer, antispasmodic, and diuretic effects, highlighting their relevance in heterocyclic drug discovery **[7]**. The pyrano [3,2-c] chromene carbonitrile scaffold is particularly attractive for investigation because of its ease of synthesis, structural versatility, and promising therapeutic applications. The present protocol provides a simple, sustainable and environmentally safe synthesis of pyranochromene carbonitrile derivatives [8].

Numerous studies on the synthesis of these compounds have been stimulated by the special qualities and wide-range applications of pyrano coumarins and the nanoparticles were tested for their catalytic effectiveness [9]. In this way not only a novel route to highly efficient synthesis of this series of heterocyclic compounds was

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introduced, but also the scope of these medicinally important products was broadened [10]. The utilization of magnetic nanoparticles is an exponentially expanding domain in the pursuit of sustainable and environmentally friendly methodologies [11]. Chromene is recognized as a privileged structure and a useful template for the design of novel compounds with potential pharmacological interest especially in the field of neurodegenerative, inflammatory and infectious diseases [12]. In this study, particular emphasis was placed on the synthesis of pyrano [3,2-c] chromene carbonitrile derivatives using $NiFe_2O_4$ NPs as a catalytic system. The target compounds were obtained *via* a three-component condensation reaction involving 4-hydroxycoumarin, aldehydes, and malononitrile. Although several catalysts such as DABCO [13], $Mg(ClO_4)_2$ [14], and MNPs@Cu [15] have previously been employed in this reaction, these methods often suffer from limitations including low product yields, reliance on toxic solvents, high operational costs, elevated reaction temperatures, and need for specialized equipment. To overcome these drawbacks, $NiFe_2O_4$ NPs were applied as an efficient and environmentally benign catalyst. This approach provided a simple, cost-effective, and eco-friendly route to pyrano [3,2-c] chromene carbonitriles, with the resulting compounds demonstrating notable biological, pharmacological, and therapeutic potential.

EXPERIMENTAL

Materials and methods

Chemicals of analytical grade were used. Aromatic aldehyde, 4-hydroxy coumarin, and malononitrile were purchased from Sigma Aldrich. Ethanol, n-hexane, and pet ether were acquired from a local provider and purified by a standard procedure. $NiFe_2O_4$ NPs (10 mol %) and 3 ml of EtOH were mixed; TLC was used for checking reaction progress. The solvent system used was hexane/ethyl acetate (8:2). After completion of the reaction the catalyst was separated by using an external Nd magnet, the residue was used as recovered catalyst. Open capillary tubes were used for melting point determination. All synthesized products were analyzed by SEM, TEM, XRD, IR, $_1$HNMR and $_{13}$CNMR spectral data.

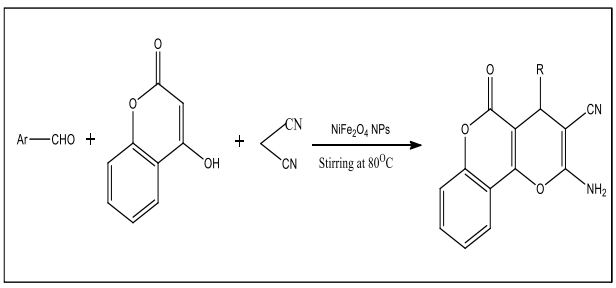
General procedure for obtaining $NiFe_2O_4$ NPs

$$4\ Fe(NO_3)_3.9H_2O + 2\ Ni(NO_3)_2.6H_2O + 2\ C_6H_8O_7 \longrightarrow 2\ NiFe_2O_4 + 12\ CO_2 + 56\ H_2O + 8\ N_2 + 11\ O_2$$

Nickel ferrite ($NiFe_2O_4$) NPs were prepared using a sol–gel synthesis route under ambient conditions. The starting materials consisted of iron nitrate nonahydrate [$(FeNO_3)_3.9H_2O$], nickel nitrate hexahydrate [$(Ni\ (NO_3)_3.6H_2O$] and citric acid [$C_6H_8O_7.H_2O$]. A molar ratio of 1:2 was maintained between the metal nitrates and citric acid. The precursors were dissolved in distilled water in stoichiometric proportions and stirred magnetically on a hot plate to obtain a uniform solution. The pH of the solution was adjusted to 7 by dropwise addition of ammonia under continuous stirring. The mixture was then heated at 80 °C for 3 h under constant stirring. The resulting solution was dried in a hot-air oven at 120 °C for 12 h, which led to gel formation. The dried gel was ground with a mortar and pestle and subsequently calcined at 600 °C for 4 h in a muffle furnace. After cooling to room temperature, $NiFe_2O_4$ NPs were obtained. The latter were utilized as catalysts in the synthesis of pyrano [3,2-c] chromene carbonitrile derivatives.

General procedure for obtaining pyranochromene carbonitrile compounds

As shown in Scheme 1, the reaction mixture was prepared by combining aromatic aldehyde (1 mmol), 4-hydroxycoumarin (1 mmol), malononitrile (1.2 mmol), and ethanol (3 mL) in a round-bottom flask. Nickel ferrite ($NiFe_2O_4$) NPs (10 mol%) were introduced as a catalyst, and the mixture was magnetically stirred at 80 °C. Under these conditions, the reaction proceeded to completion within 30 min. Progress was monitored by TLC using hexane/ethyl acetate (8:2) as the mobile phase. Upon completion of the reaction the catalyst was separated using an external Nd magnet. The reaction mixture was then cooled to room temperature, the crude product was collected and recrystallized from hot ethanol (10 ml) to afford the pure pyrano [3,2-c] chromene carbonitrile derivative. The purified product was filtered and dried in an oven at 60 °C.



Scheme 1. Synthesis of pyranochromene derivatives catalyzed by $NiFe_2O_4$ NPs.

RESULTS AND DISCUSSION

The synthesized pyranochromene carbonitrile derivatives were obtained in very good yields Obtained spectral data confirmed the successful formation of the products. All the details are shown in Tables 1 and 2.

SEM analysis

The microstructure of the nickel ferrite sample and its chemical composition were analyzed using SEM. The SEM analysis confirmed the formation of $NiFe_2O_4$ NPs. The particles were uniformly distributed but showed significant agglomeration due to magnetic interactions and high surface energy. The average particle size was estimated to be in the range of 40-100 nm as shown in Fig. 1. The rough, porous surface texture suggests the presence of fine crystallites forming clustered aggregates. Overall, the microstructure is characteristic of spinel ferrite nanomaterials and supports the successful synthesis of nanosized $NiFe_2O_4$.

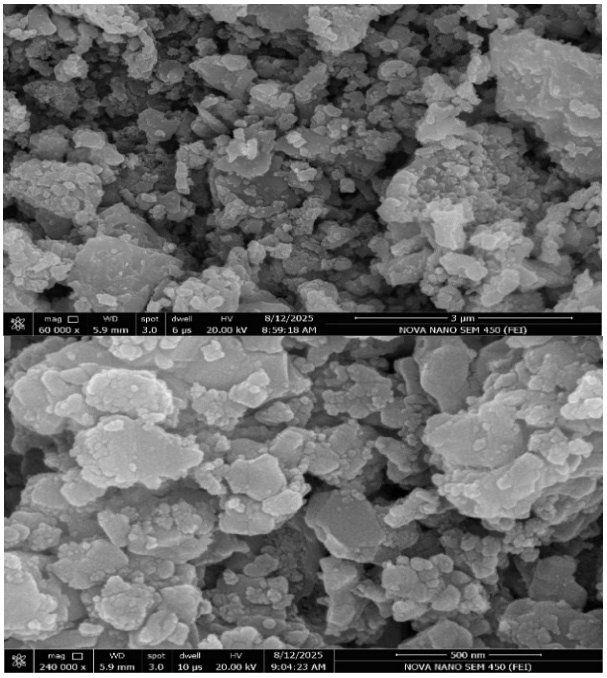


Figure 1. SEM images of the synthesized $NiFe_2O_4$ NPs

XRD analysis

The synthesized $NiFe_2O_4$ nanoparticles exhibit excellent diffraction peaks at 2θ values coordinating with (111), (220), (311), (222), (400), (511) and (440) crystallographic planes, as shown in Fig. 2. The standard JCPDS data for cubic spinel ferrite $NiFe_2O_4$ confirmed the formation of single-phase nickel ferrite because reflections were perfectly matched. The most intense peak. referred to a (311) plane. indicates high crystallinity of the prepared nanoparticles. The absence of extra peaks suggests phase purity without any detectable secondary phases. The peak broadening confirms the nanocrystalline nature of the material.

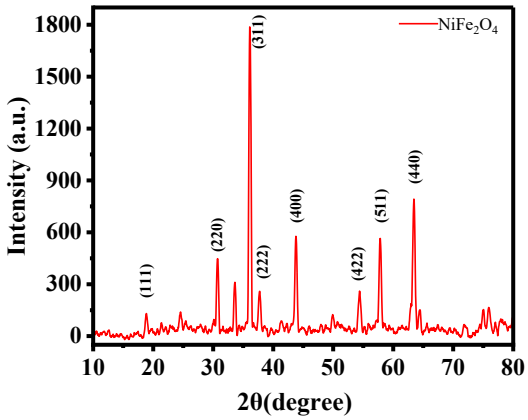


Figure 2. XRD spectrum of the $NiFe_2O_4$ NPs

TEM analysis

The TEM micrograph of the $NiFe_2O_4$ nanoparticles reveals the formation of nearly spherical to irregularly shaped particles with noticeable agglomeration as shown in Fig. 3, The particle size varies from 28 nm to 45 nm with a regular size around 35-40 nm, The observed aggregation is attributed to strong magnetic dipole interactions and high surface energy of ferrite nanoparticles. The uniform contrast and well-defined particle boundaries confirm the formation of crystalline $NiFe_2O_4$ nanoparticles.

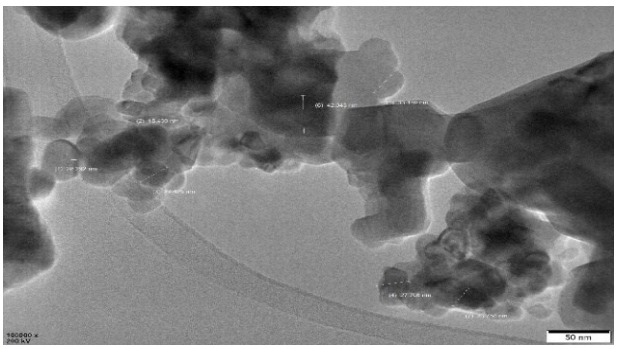


Figure 3. TEM image of the synthesized $NiFe_2O_4$ NPs

Spectral data of pyrano chromene carbonitrile derivatives. (supplementary information ^{1}HNMR,^{13}C NMR & mass spectrometric graphs)

(1) 2-Amino-4-(2,6-dichorophenyl)-5-oxo-4,5-dihydropyrano [3,2-c] chromene-3-carbonitrile

Yield 0.90 g (88 %), color white, m. p. 235–238°C; IR spectrum ν, cm^{-1}: 756 (C-Cl), 1246 (C-O-C), 1602, 1514 (C=C), 1684 (C=O), 2223 (C≡N), 2924 (C-H), 3062 (Ar-CH), 3421, 3328 (NH_2); ^{1}H NMR spectrum δ, ppm: 5.52 (s,1H, CH) 6.90 (s, 1H,

pyran-H), 7.45-7.35 (d, 2H, Ar-H), 7.70-7.30 (m, 4H, Ar-H), 7.90-7.75 (d, 2H, Ar-H); ^{13}CNMR spectrum δC, ppm 38.7 (CH_3), 78.5, 104.3, 133.8, 131.6, 129.4, 128.7, 126.9, 124.8, 123.5, 121.9. 137.9, 136.4, 158.6, 154.2, 162.8(C=O); Mass spectrum *m*/*z* 384.

(2) 2-Amino-4-(2,4-dimethoxyphenyl)-5-oxo-4,5-dihydropyrano [3,2-c] chromene-3-carbonitrile

Yield 0.90 g (89 %), color yellow, m. p. 234-239 °C. IR spectrum, ν, cm^{-1}: 1134 (C–C), 1435 (C–N), 1542 (C–O), 1632 (C–O), 1747 (C=O), 2242 (C≡N), 3189 (=C–H), 3284 and 3381 (NH_2); ^{1}H NMR spectrum δ, ppm 3.24 s (6H, CH_3O), 5.02 s (1H, 4-H), 7.44 d (2H, *J* = 8.0, 7.2 Hz,), 7.85 d (2H, *J* = 8.0, 7.2 Hz), 9.79 s (2H, NH_2); ^{13}C NMR spectrum, δC, ppm: 35.6 (CH3), 103.6, 110.5, 112.8, 112.9, 116.2, 118.3, 120.2, 122.6, 124.6, 132.2, 136.2, 140.2, 142.8, 152.3,157.2, 162.6, 172.2 (C=O); Mass spectrum *m*/*z* 378.23.

(3) 2-Amino-4-(4-methoxyphenyl)-5-oxo-4,5-dihydropyrano [3,2-c] chromene carbonitrile

Yield 0.98 g (91%), color whitish yellow, m. p. 242-246°C, IR spectrum ν, cm^{-1}; 832, 1032 (C-O-C), 1178(C-O), 1251 (C-O-C), 1360 (C-O), 1718 (C=O), 2218 (C≡N), 3452 (N-H); ^{1}H NMR δ, ppm (DMSO-d6): d 3.73 (s, 3H, OCH_3), 4.41 (s, 1H, CH), 6.72-8.33 (m, 10H, Ar, NH_2); ^{13}CNMR spectrum δ C, ppm 55.10 (OCH_3), 116.43, 135.27, 113.69, 158.28, 157.78, 152.95, 151.9, 159.40 (C=O); MS (ESI) m/z 346 (MC).

(4) 2-Amino-4-(3,5-dibromo-4-hydroxy-phenyl)-5-oxo-4,5-dihydropyrano[3,2-c] chromene-3-carbonitrile

Yield 0.89 (87%), color white, m. p. 222-226°C; IR spectrum ν, cm^{-1} 592, 528 (C-Br), 1268 (C-O), 1604,1512 (C=C), 1708 (C=O), 2224 (C≡N), 3062 (C-H), 3356 (NH_2), 3428 (O-H); ^{1}H NMR δ, ppm (400 Hz, DMSO-d6): 3.32(br s, 2H, NH_2), 4.45 (s, 1H, H-4), 7.1-7.3 (m, 2H, ArH), 9.88 (s, 1H, NH);

^{13}C NMR spectrum δ C, ppm: 55.69 (-CH), 104.53, 103.34, 119.31 (-CN), 132.54, 129.69, 124.51 (C-Br), 159.16, 158.01, 155.92, 153.61 (Ar-CH), 159.61 (C=O); MS (ESI): m/z: 490.

(5) 2-Amino-4-(3-iodophenyl)-5-oxo-4,5-dihydropyrano [3,2-c] chromene-3-carbonitrile

Yield 0.91 g (89%), color whitish, creamy, m. p. 260-265°C; IR spectrum ν, cm^{-1}: 856 (C-H), 1068 (C-O-C), 1159 (C-I), 1358 (C-O), 1527 (CN), 1632 (C=C), 1725 (C=O), 2221 (C≡N), 3348 (N-H); ^{1}H NMR, δ, ppm (400 Hz, DMSO-d6): 4.62 (s,1H), 7.28 (m,2H),7.45 (m,2H),7.68 (d, J=8.0 Hz, 2H), 7.92 (d,J= 8.0 Hz, 2H); ^{13}CNMR spectrum δ C ppm: 38.6 (CH2), 112.4, 115.7, 119.3, 121.9, 124.2, 126.4, 128.6, 129.8, 139.1, 147.6 (Ar-CH), 155.8 (C-O), 159.4 (C=O); MS (ESI): m/z 346.

(6) 2-Amino-4-(4-chlorophenyl)-5-oxo-4,5-dihydropyrano [3,2-c] chromene-3-carbonitrile

Yield 0.98 g (91%), color dark yellow, solid, m. p. 266–269°C; IR spectrum ν; cm^{-1}: 775 (C–Cl), 1044 (C–O), 1234 (C–O), 1600 (C=C), 1652 (C=C), 1735 (C=O), 2242 (C≡N), 3120 (=C–H), 3216 and 3312 (NH_2); ^{1}H NMR spectrum δ, ppm: 4.96 s (1H, 4-H), 7.46 d (2H, *J* = 8.4, 7.2 Hz),7.8 d (2H,*J* = 8.2, 7.4 Hz), 9.78 s (2H, NH_2); ^{13}C NMR spectrum, δC, ppm: 105.5, 114.6, 115.8, 115.9, 116.8, 118.6, 121.3, 124.3, 122.8, 133.3,134.4, 138.9,143.9, 152.9, 156.4, 161.8, 173.3 (C=O); Mass spectrum: *m*/*z* 351.28.

(7) 2-Amino-4-(4-nitrophenyl)-5-oxo-4,5-dihydropyrano [3,2-c] chromene-3-carbonitrile

Yield 0.90 g (90%), color dark yellow, solid, m. p. 255–257°C; IR spectrum ν, cm^{-1}:1032 (C–O), 1246 (C–O), 1340 (NO_2), 1655 (C=C), 1736 (C=O), 2258 (C≡N), 3219 (=C–H), 3328 (NH_2), 3415 (NH_2); ^{1}H NMR spectrum δ, ppm: 5.10 s (1H, 4-H), 7.46 d (2H, *J* = 8.0, 7.2 Hz), 7.87 d (2H, *J* = 8.0, 7.6 Hz, Harom), 8.49 s (2H, NH_2); ^{13}C NMR spectrum, δC, ppm: 101.3, 112.6, 114.4, 114.6, 117.2, 120.8, 122.4,124.6,127.6, 130.4,132.5, 137.6, 148.7, 158.9, 160.8, 163.2, 176.8 (C=O); Mass spectrum: *m*/*z* 363.43.

(8) 2-Amino-4-(3-nitrophenyl)-5-oxo-4,5-dihydropyrano [3,2-c] chromene-3-carbonitrile

Yield 0.91 g (89%), color yellow, solid, m. p. 258–260°C; IR spectrum, ν, cm^{-1}: 986 (C–C),1045 (C–C), 1230 (C–O), 1346 (NO_2),1608 (C–O), 1636(C–O), 1732 (C=O), 2249 (C≡N), 3127 (=C–H), 3325 (NH_2), 3492 (NH_2); ^{1}H NMR spectrum δ, ppm: 4.80 s (1H, 4-H), 7.44 d (2H, *J* = 8.2, 7.5 Hz,), 7.56 d (2H, *J* = 8.2, 7.2 Hz,), 9.96 s (2H, NH_2); ^{13}C NMR spectrum δC, ppm: 105.5, 110.6, 112.8, 116.8, 118.8, 121.8,122.7, 124.6, 127.9, 134.3, 138.6, 140.2, 142.6, 154.5, 156.6, 164.2, 174.7 (C=O); Mass spectrum: *m*/*z* 363.28.

(9) 2-Amino-4-(4-methylphenyl)-5-oxo-4,5-dihydropyrano [3,2-c] chromene-3carbonitrile

Yield 0.84 g (80%), color white, solid, m. p. 266–269°C; IR spectrum ν, cm^{-1}: 1159 (C–O), 1236 (C–C), 1608(C–O), 1636 (C–O), 1746 (C=O), 2236 (C≡N), 3298 (=C–H), 3314 (NH_2); ^{1}H NMR spectrum δ, ppm:1.6 s (3H, CH_3), 4.96 s (1H, 4-H), 7.36 d (2H, *J* = 8.2, 7.4 Hz), 7.62 d (2H*J* = 8.2, 7.4 Hz), 6.74 s (2H, NH_2); ^{13}C NMR spectrum δC, ppm: 21.4 (CH_3), 106.6, 113.9, 116.7, 116.8, 118.6, 121.6, 122.8, 126.8, 128.2, 132.7, 136.4, 140.9, 142.4, 152.8, 158.8, 164.2, 176.6 (C=O); Mass spectrum: *m*/*z* 332.23.

(10) 2-Amino-4-(4-hydroxyphenyl)-5-oxo-4,5-dihydropyrano [3,2-c] chromene-3-carbonitrile

Yield 0.88 g (86%), color white, solid, m. p. 260–265°C; IR spectrum ν, cm^{-1}: 854 (C–H), 1125 (C–C), 1222 (C–C), 1444 (C–O),1636 (C–O), 1738 (C=O), 2236 (C≡N), 3182 (=C–H), 3292 and 3384 (NH_2); ^1H NMR spectrum δ, ppm: 5.20 s (1H, 4-H), 7.46 d (2H, J = 8.1, 7.3 Hz), 7.74 d (2H, J = 8.1, 7.3 Hz), 6.84 s (2H, NH_2); ^{13}C NMR spectrum δC, ppm: 104.6, 109.1, 110.4, 111.8, 112.4, 116.1, 118.6, 120.4, 122.2, 122.8, 126.1, 128.8, 139.4, 146.3, 152.2, 156.2, 176.5 (C=O); Mass spectrum *m*/*z* 334.62.

Anticancer activity

The MTT assay of the synthesized pyrano chromene carbonitrile derivatives against MCF-7 (human breast cell line) showed that all tested samples: 2-amino-4-(4-dichlorophenyl)-5-oxo-4,5-dihydropyrano [3,2-c] chromene-3-carbonitrile, 2-amino-4-(2,4-dimethoxyphenyl)-5-oxo-4,5-dihydropyrano [3,2-*c*] chromene-3-carbonitrile, and 2-amino-4-(4-3,5-dibromo-4-hydroxyphenyl)-5-oxo-4,5-dihydropyrano [3,2-c] chromene-3-carbonitrile exhibited dose-dependent cytotoxic activity. [2-Amino-4-(2,4-dimethoxyphenyl)-5-oxo-4,5-dihydropyrano [3,2-*c*] chromene-3-carbonitrile showed the strongest anticancer activity with lowest IC_{50} value (58.80 µg/ml) and significant reduction in cell viability. Compound (4) showed moderate activity, while (1) exhibited the least cytotoxic effect overall. Compound (2) gave the best result, as shown in Figs. 4 and 5.

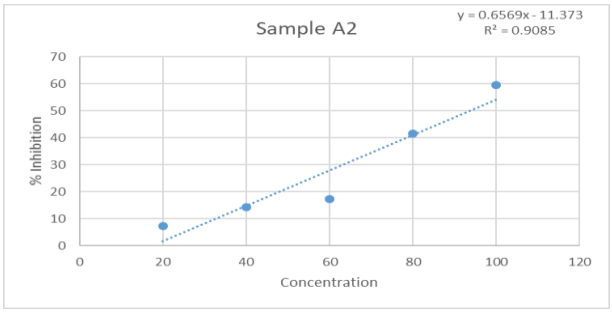


Figure 4. Concentration-dependent anticancer activity of sample (2) against MCF-7

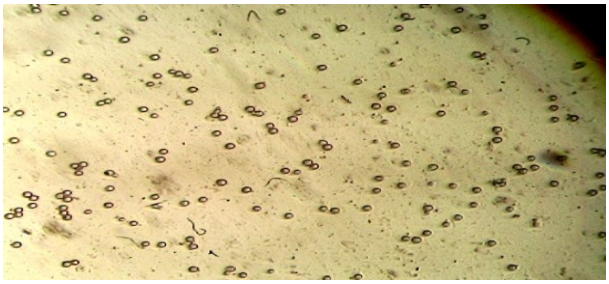


Figure 5. Cell morphology images against MCF-7

Table 1. Preparation of substituted pyrano [3,2-*c*] chromene carbonitrile derivatives (1-10) (ethanol, reflux) using $NiFe_2O_4$ catalyst

Compound	Time (min)	Yield (%)	M. p. (°C)
(1) 2,6-(Cl)$_2$C$_6$H$_4$ CHO	25	88	235-238
(2) 2,4-(OMe)$_2$ C$_6$H$_4$ CHO	27	89	234-239
(3) 4-OMe C$_6$H$_4$ CHO	20	92	242-246
(4) 3,5(Br)$_2$, 4-OH C$_6$H$_4$ CHO	30	87	222-226
(5) 3- I C$_6$H$_4$ CHO	20	89	260-265
(6) 4-Cl C$_6$H$_4$ CHO	20	91	266-269
(7) 4-NO$_2$ C$_6$H$_4$ CHO	20	90	255-257
(8) 3-NO$_2$ C$_6$H$_4$ CHO	30	89	258-260
(9) 4-Me C$_6$H$_4$ CHO	28	80	266-269
(10) 4-OH C$_6$H$_4$ CHO	35	86	260-265

Table 2. Preparation of pyrano [3,2-*c*] chromene carbonitriles compared with other catalysts

S. No.	Catalyst	Time (min)	Yield (%)	Ref.
1	DABCO	30	87	8
2	$Mg(ClO_4)_2$	35	85	9
3	MNPs@Cu	40	80	10
4	$NiFe_2O_4$	20	92	Present work

CONCLUSION

Nickel ferrite ($NiFe_2O_4$) NPs were successfully synthesized *via* the sol–gel method. The catalyst was comprehensively characterized using XRD, SEM, and TEM. The analytical data confirmed the successful formation of well-defined $NiFe_2O_4$ NPs. These nanoparticles were subsequently employed in the synthesis of 2-amino-4-arylphenyl-5-oxo-4,5-dihydro-pyrano [3,2-c] chromene-3-carbonitrile and its derivatives. The structures of the obtained compounds were verified through IR spectroscopy, ^1H NMR, ^{13}C NMR, and mass spectrometry, all of which provided consistent and reliable results. Cytotoxicity assays demonstrated that the pyrano [3,2-c] chromene carbonitrile derivatives exhibited promising anticancer potential, underscoring their relevance in medicinal chemistry. The methodology offers several advantages, including short reaction times, high product yields, and excellent purity (90–93%). The use of the nanocatalyst provides a highly

efficient and sustainable approach for the synthesis of bioactive heterocyclic compounds.

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Conflict of interest: *The authors declare that they have no conflict of interest.*

Author contributions: *All authors contributed to editing, reviewing, and sanctioning the finished version for publication.*

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