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**Preparation, Characterization, and In Vitro Cytotoxic Evaluation of
Pyrimidine-Loaded Mg-Ca-Fe Layered Double Hydroxides Against
Kasumi-1 Leukemia Cells**

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24 **Abstract**

25 Leukemia remains one of the most challenging hematological malignancies, necessitating the development
26 of efficient therapeutic systems capable of improving drug delivery and biological performance. In the
27 present study, a pyrimidine derivative (Pyr) was successfully incorporated into Mg-Ca-Fe layered double
28 hydroxides (LDHs) to produce a novel Pyr/Ca-Mg-Fe LDH nanocomposite with potential biomedical
29 applications. The synthesized materials were systematically characterized using Fourier-transform infrared
30 spectroscopy (FTIR), X-ray diffraction (XRD), scanning electron microscopy (SEM), transmission
31 electron microscopy (TEM), energy-dispersive X-ray spectroscopy (EDX), Brunauer-Emmett-Teller
32 (BET) surface area analysis, and thermogravimetric analysis (TGA). Structural characterization confirmed
33 the successful formation of the LDH framework and the incorporation of the pyrimidine derivative within
34 the nanocomposite matrix. Morphological investigations revealed a porous multilayered nanostructure with
35 enhanced surface characteristics, while BET analysis demonstrated an increase in surface area compared
36 with the individual components.

37 The biological activity of the synthesized materials was evaluated against human Kasumi-1 leukemia cells
38 using the MTT assay. The obtained results demonstrated concentration-dependent cytotoxic effects for Pyr,
39 Ca-Mg-Fe LDH, and Pyr/Ca-Mg-Fe LDH. The nanocomposite exhibited significant antiproliferative
40 activity toward leukemia cells, indicating effective interaction between the bioactive pyrimidine moiety
41 and the LDH carrier system. Although free Pyr displayed a lower IC₅₀ value than the corresponding
42 nanocomposite, the observed biological response suggests that the LDH matrix may provide a favorable
43 platform for intracellular delivery of pyrimidine derivatives. The improved physicochemical properties,
44 including nanoscale morphology, porous architecture, and increased surface area, are expected to facilitate
45 interactions with cellular membranes and contribute to the observed cytotoxic effects.

46 Overall, the developed Pyr/Ca-Mg-Fe LDH nanocomposite represents a promising pyrimidine-based
47 nanoplatform with potential applications in leukemia-directed drug delivery. Further investigations

involving drug-release studies, cellular uptake analysis, apoptosis evaluation, and mechanistic biological assays are warranted to fully elucidate its therapeutic potential.

Keywords:Pyrimidine, Layered double hydroxide, Drug delivery, Leukemia, Nanocomposite, Cytotoxicity.

1. Introduction

Cancer remains one of the leading causes of mortality worldwide after cardiovascular diseases and continues to represent a major global health challenge [1]. The development and progression of cancer are influenced by numerous factors, including aging, genetic alterations, environmental exposure, and family history [2–6]. A hallmark of malignant tumors is their ability to evade immune surveillance, invade surrounding tissues, and metastasize to distant organs, resulting in significant morbidity and mortality [4,7,8].

Among the various cancer types, leukemia is a particularly aggressive hematological malignancy affecting the bone marrow and blood-forming tissues. The disease disrupts normal hematopoiesis, leading to abnormal proliferation of immature leukocytes and impairment of immune function. Leukemia is broadly classified into acute and chronic forms, including acute lymphoblastic leukemia (ALL) [9], acute myeloid leukemia (AML) [10–12], chronic lymphocytic leukemia (CLL), and chronic myeloid leukemia (CML). Acute subtypes are generally characterized by rapid progression and require immediate therapeutic intervention because of their life-threatening nature [13].

Current treatment strategies for leukemia include chemotherapy [14,15], radiotherapy, targeted therapy, immunotherapy [7,16], and hematopoietic stem cell transplantation. Chemotherapy remains the most widely used therapeutic approach; however, its lack of selectivity often results in severe adverse effects, including myelosuppression, gastrointestinal toxicity, neuropathy, and secondary malignancies. Although targeted therapies and immunotherapeutic approaches have improved treatment outcomes by selectively targeting malignant cells or enhancing immune responses, their effectiveness may be limited by drug

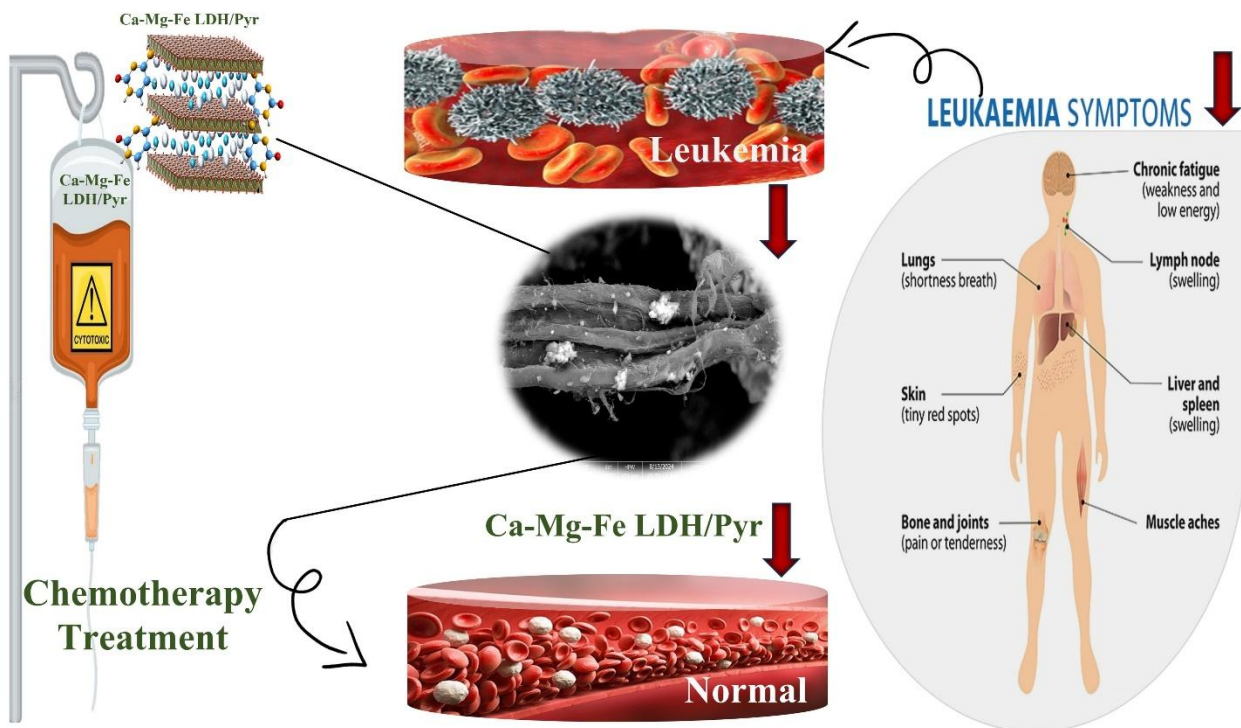
72 resistance, high treatment costs, and patient-specific variability. Therefore, the development of novel
73 therapeutic systems capable of enhancing treatment efficacy while minimizing systemic toxicity remains
74 an important research objective.

75 Pyrimidine derivatives have attracted considerable attention as promising anticancer agents owing to their
76 ability to interfere with nucleic acid biosynthesis and inhibit cellular proliferation [6,12,17]. Several
77 pyrimidine-based compounds exhibit potent cytotoxic activity against a wide range of cancer cells through
78 disruption of DNA and RNA synthesis pathways [9]. Despite their therapeutic potential, the clinical
79 performance of pyrimidine-containing compounds may be compromised by limited stability, rapid
80 degradation, poor bioavailability, and undesirable systemic side effects. Consequently, the development of
81 efficient delivery systems capable of improving the pharmacological performance of pyrimidine
82 derivatives has become an area of significant interest.

83 Layered double hydroxides (LDHs) have emerged as versatile nanomaterials for biomedical and drug-
84 delivery applications due to their layered structure, high loading capacity, biocompatibility, ion-exchange
85 properties, and controlled release behavior [18–21]. LDH nanoparticles have been extensively explored as
86 carriers for chemotherapeutic agents, genes, and photosensitizers because of their ability to protect
87 bioactive molecules and facilitate intracellular delivery [22–24]. Furthermore, the physicochemical
88 properties of LDHs, including particle size, morphology, and surface characteristics, can significantly
89 influence their accumulation within tumor tissues through passive targeting mechanisms such as the
90 enhanced permeability and retention (EPR) effect [25,26]. In addition, surface modification and elemental
91 doping can further improve their therapeutic versatility and applicability in cancer treatment [18,27].

92 A major advantage of LDH-based nanocarriers is their ability to provide controlled and pH-responsive drug
93 release under the acidic conditions commonly associated with tumor microenvironments [28–30]. Such
94 behavior may enhance drug stability, reduce premature drug leakage, and improve localized therapeutic
95 action while minimizing off-target toxicity [31,32]. These characteristics make LDHs attractive candidates
96 for the delivery of pyrimidine-based anticancer agents.

In the present study, a novel pyrimidine-loaded Mg-Ca-Fe layered double hydroxide nanocomposite was synthesized and investigated as a potential platform for leukemia therapy. The incorporation of magnesium, calcium, and iron within the LDH framework was expected to provide favorable physicochemical characteristics while maintaining the structural integrity of the carrier system [33,34]. The synthesized nanocomposite was comprehensively characterized using different physicochemical techniques to evaluate its structural, morphological, and surface properties. In addition, its antiproliferative activity against Kasumi-1 leukemia cells was assessed to explore its potential application as a pyrimidine delivery system. This work aims to contribute to the development of advanced LDH-based nanocarriers for leukemia-targeted therapeutic applications.



Graphical Abstract

2. Experimental

2.1. Materials and reagents

Chloride salts, ferric chloride (FeCl_3), calcium chloride (CaCl_2), and Magnesium sulphate (MgSO_4) were used to prepare all LDH catalysts. These salts were purchased from Alpha Chemika, India, and Oxford Laboratory Reagent, India. Hydrochloric acid (Carlo Erba reagents) and Sodium hydroxide (Piochem for Laboratory Chemicals in Egypt) were Laboratory Chemicals in Egypt. Chloroacetic acid (ClCH_2COOH), thionyl chloride (SOCl_2), thiourea (NH_2CSNH_2), ethyl cyanoacetate ($\text{C}_5\text{H}_7\text{NO}_2$), DMF, potassium carbonate K_2CO_3 , absolute alcohol 99%, all these chemicals were purchased from Sigma-Aldrich. All chemical materials are of a high analytical grade. Microanalysis was conducted at the microanalytical center, Cairo University, Egypt.

2.2. Preparation of pyrimidine derivative (Pyr)

6-(2,4-dichlorophenyl)-1,4,5,6-tetrahydro-4-hydroxy-2-mercapto-pyrimidine-5 carbonitrile 1 prepared according to the previous review [35]. 3,3'-(1,4-phenylenebis (azanediyl)) bis(7-(2,4-dichlorophenyl)-5-oxo-3,5,6,7-tetrahydro-2H-thiazolo[3,2-a]pyrimidine-6-carbonitrile) 3.

A mixture of thiopyrimidine 1 (0.02 mol, 5.58g), N,N'-(1,4-phenylene)bis(2-chloroacetamide) 2 (0.01 mol, 2.61 g) and K₂CO₃ (0.02 mol, 1.98g) in ethanol was refluxed for 24h and or in DMF for 12h. Under reduced pressure, the solvent was removed and HCl solution (0.01%) was used to acidify the alkaline residue, the formed precipitate filtered off, dried, and recrystallized from methanol to give dark yellow crystals of Pyr in 85% yield; m.p. > 360 °C; IR (KBr): ν /cm⁻¹ 3485 (NH), 1689 (C=O) and 2231 (CN); ¹H-NMR (DMSO-d₆): δ (ppm): 3.11- 3.49. (d, 4H, 2CH), 3.71-3.97. (d, 4H, 2CH₂-S), 3.98-4.11. (q, 2H, -2N-CH-NH), 6.96–7.80 (10H, m, Ar-H), 10.112-10.174 (d, 2H, 2NH); ESI MS m/z (rel. int.): 757.84 [M]⁺ (C₃₂H₂₂Cl₄N₈O₂S₂) Anal. calcd. for C₃₂H₂₂Cl₄N₈O₂S₂ (756.51): C, 50.80; H, 2.93; Cl, 18.75; N, 14.81; O, 4.32; S, 8.48. Found C, 50.69; H, 2.73; N, 14.54.

2.3. Preparation of Mg-Ca-Fe LDH

The coprecipitation technique was used to produce the Mg–Ca–Fe layered double hydroxide (LDH). In a molar ratio 1:1:2, a magnesium sulfate solution was combined with calcium chloride and ferric chloride solutions. A 2 mol/L sodium hydroxide solution was progressively added to the mixture under constant stirring until precipitation at a pH of 10. The precipitate was then kept at 70 ± 0.5 °C for 10 hours. It was then filtered and rinsed well with bi-distilled water to eliminate contaminants. For a full 24 hours, the purified precipitate was dried at 80 ± 0.5 °C [36].

2.4. Synthesis of pyrimidine derivatives Mg-Ca-Fe LDH composite.

A magnesium sulfate solution was mixed with calcium chloride, ferric chloride, and a pyrimidine derivative (Pyr) solution in a molar ratio of 1:1:2:0.75, respectively. Under continuous stirring, 2 mol/L sodium hydroxide solution was gradually added to the mixture until precipitation was achieved at a pH of 10. The resulting precipitate was maintained at 70 °C for 10 hours, then filtered and washed thoroughly with bi-distilled water to remove impurities. Finally, the purified precipitate was dried at 80 °C for 24 hours.

2.5. Characterization techniques

The prepared catalysts were characterized using a variety of tools. For instance, the crystallinity and structure of the synthesized materials were ascertained using a Panalytical (Empyrean) X-ray diffractometer with Cu-K radiation (wavelength 0.154 nm) operating at a current of 35 mA and voltage of 40 kV. When 0.50 mg of the obtained material was mixed with 300 mg of optically high-purity KBr in an agate mortar, the mixture was vacuumed for five minutes, and then the KBr pellet was pressed under ten tons of pressure for 15 min. This is produced in a 1.13 cm² area for the KBr pellet (Zeiss standard). Likewise, the produced samples' specific pore volume, surface area, and pore size distribution were all determined using BET (TriStar II 3020, Micrometric, USA). The microstructure was studied by a Jeol JEM-1230 transmission electron microscope (TEM) and a JSM-5410 scanning electron microscope (SEM). A light grey translucent pellet without discernible granules was obtained, and the IR spectra were captured using a (Bruker-Vertex 70, Germany) Spectrometer at the wave number range of 4000- 400 cm⁻¹.

2.6. Cell line

Human homo sapiens-Kasumi1; Leukemia cell line (ATCC: CRL-2724) established from peripheral blast cells. It is purchased from VACSERA holding Company for vaccine and serum production, Ministry of Health, Egypt. It is cultured in RPMI-1640 (Gibco®, CA, USA) supplemented with 10% Fetal bovine serum (Sigma-Aldrich, MO, USA) and 0.1% penicillin/streptomycin (Invitrogen, CA, USA) in humidified temperature containing 5% carbon dioxide (CO₂) at 37°C.

2.7. MTT assay for cell proliferation

The anticancer activity of pyrimidine and its composites on the Kasumi-1 leukemia cells was assessed using the MTT (3-(4, 5-dimethyl thiazol-2 yl)-2, 5-diphenyl tetrazolium bromide) assay to verify the cytotoxic activity by the protocol [37]. Cells for the MTT experiment were plated in 96-well plates at a density of 1x10⁵/100µl. The cells were treated with pyrimidine and pyrimidine composites at 31.25, 62.5, 125, 250, 500, and 1000µg/ml during a 24-hour incubation period. As controls, wells containing only serum-free media were designated. 20 µl of MTT reagent were added to the wells and incubated for four

hours in the dark following the 24-hour incubation period at 37°C. 200µl of DMSO was added to the wells to dissolve the formazan crystals. At 560 nm, the absorbance was measured. Each concentration was evaluated in triplicate during the three repetitions of the experiment. 5-fluorouracil was used as a positive control at 31.25–1000 µg/mL concentration. Cell viability for treated and non-treated Kasumi 1 cell lines is expressed as a percentage.

$$\% \text{ Cell viability} = (\text{Absorbance}_{\text{sample}} / \text{Absorbance}_{\text{control}}) \times 100$$

The concentration of the pyrimidine and its composites resulted in a 50% reduction of cell viability, and the half-maximal inhibitory concentration (IC₅₀ value) was calculated. Cell toxicity for pyrimidine and its composites was determined using the following formula:

$$\% \text{Cell toxicity} = ((\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}) / (\text{Absorbance}_{\text{control}})) \times 100$$

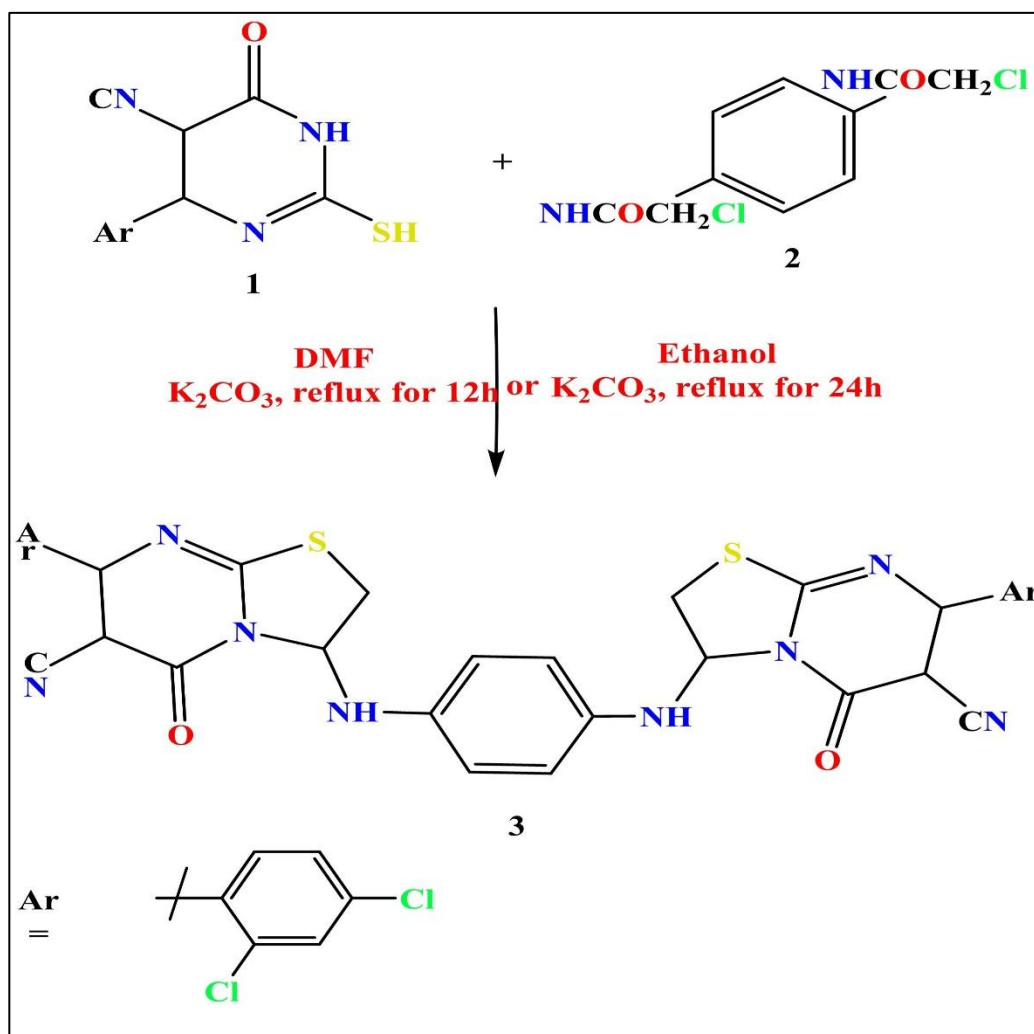
2.8. Statistical analysis

The difference in computed parameters between the concentrations was investigated using one-way ANOVA and Bonferroni's test (SPSS/10 Software Package; SPSS Inc., Chicago, IL, USA). Data expressed as mean ± SE for all treated and non-treated Kasumi 1 cell line concentrations. p<0.05 was rooted as the statistically significant value.

3. Results and discussion

3.1. Synthesis

Pyrimidine derivative (Pyr) was synthesized by [2+3] intramolecular cycloaddition of the pyrimidine **1** [35] to chloroacetyl chloride, gave the thiazolo[3,2-a]pyrimidine **3** (Scheme 1). The use of different analytical tools fully characterized it. Pyr was intercalated into Mg-Ca-Fe LDH using the simple coprecipitation method to form Mg-Ca-Fe LDH/Pyr.



Scheme 1. Synthesis of pyrimidine derivative (Pyr).

3.2. Catalyst characterization

3.2.1. FT-IR spectroscopy

The FTIR spectra of pyrimidine derivatives, Ca-Mg-Fe LDH, and the Ca-Mg-Fe LDH/Pyr composite were analyzed to confirm the structural features and interactions between the components. Fig. 1a shows the chemical structure of the pyrimidine derivatives, which is confirmed by the many distinctive peaks in their spectra. The N–H stretching vibrations [38], which are suggestive of amine groups, are responsible for the large bands at 3430 cm^{-1} and 3190 cm^{-1} [39]. A strong absorption band shows C–N stretching at 2215 cm^{-1} [40], implying nitrile functional groups exist. The peaks at 1510 cm^{-1} and 1397 cm^{-1} are linked to the

skeletal vibrations of the aromatic pyrimidine ring, whereas the band at 1675 cm^{-1} is typical of C=N stretching vibrations [40]. Additionally, absorptions caused by C–N stretching and C–H bending are between 1255 cm^{-1} and 505 cm^{-1} , supporting functional groups unique to pyrimidines [40].

The Ca-Mg-Fe LDH's FTIR spectrum (Fig. 1b) displays characteristics typical of layered double hydroxides (LDHs). The O–H stretching vibrations [36] responsible for the wide absorption band that spans $3709\text{--}3148\text{ cm}^{-1}$ come from the hydroxyl groups in the LDH layers and interlayer water molecules [41]. A strong band at 1120 cm^{-1} corresponds to the S–O stretching vibrations of intercalated sulfate anions (SO_4^{2-}). Additionally, a peak at 618 cm^{-1} represents the bending mode of SO_4^{2-} [41]. The presence of interlayer water molecules is confirmed by the H–O–H bending vibrations linked to the band at 1639 cm^{-1} [42]. The LDH is confirmed by vibrational bands in the lower wavenumber area, such as 889 cm^{-1} and 634 cm^{-1} , which are assigned to metal-oxygen (M–O) [43] and metal-hydroxyl (M–OH) stretching [41]. However, the Ca-Mg-Fe LDH/Pyr composite's FTIR spectrum shows distinctive characteristics of the LDH and pyrimidine derivatives, indicating that the pyrimidine was successfully incorporated into the LDH structure. The wide band at 3406 cm^{-1} , which is associated with O–H stretching vibrations, shows that interlayer water and hydroxyl groups have been retained [44]. A small peak at 2520 cm^{-1} is likely due to weak interactions with interlayer anions. A prominent signal at 2115 cm^{-1} , corresponding to C≡N stretching vibrations [38], verifies that the pyrimidine's nitrile groups are present. The C=N stretching-related peak at 1665 cm^{-1} overlaps with the LDH's H–O–H bending vibrations. The strong band at 1120 cm^{-1} [44], associated with SO_4^{2-} stretching, persists in the hybrid material, indicating that sulfate anions remain intercalated. Peaks at 1110 , 1020 , 886 , 720 , and 577 cm^{-1} highlight interactions between pyrimidine derivatives and the LDH layers, with shifts or new bands indicating structural modifications due to composite formation, as illustrated in Fig. 1c.

3.2.2. X-ray diffraction analysis

Fig. 2. illustrates the X-ray diffraction (XRD) patterns of the produced materials, which include pyrimidine derivatives, Ca-Mg-Fe LDH, and the composite of Ca-Mg-Fe LDH/Pyr. For pyrimidine derivatives, the

amorphous character of the material is shown in the occurrence of discrete peaks at 2θ values of 8° , 23.6° , and 36.5° , which correspond to (100), (122), and (321) as shown in Fig. 2a. A lack of long-range crystalline order, which is typical of organic compounds like pyrimidine derivatives, is suggested by this wide reflection [45]. The crystalline domains are small, ranging from 49 to 51 nm, and have a monoclinic lattice structure with distinct characteristics. Fig. 2b displays the Ca-Mg-Fe LDH's crystalline character, which shows distinct, sharp peaks at 2θ values. LDHs are characterized by prominent peaks at (003), (006), (104), and (110), respectively. The basal spacing, which reflects the stacking structure of the brucite-like layers, is matched by the high reflection at (003). The existence of a well-ordered LDH structure is further supported by the other peaks, which include (012), (113), and (018). The crystallite size ranged from 19.5 to 24.5 nm with rhombo lattice structure [46]. The composite of Ca-Mg-Fe LDH/Pyr's XRD pattern (Fig. 2c) shows characteristics of both LDH and pyrimidine derivatives, suggesting that the two components successfully hybridized. The retention of the distinctive LDH reflections (003), (006), and (104) attests to the layered material's structural integrity. However, the addition of pyrimidine derivatives appears to have altered the crystalline structure, as seen by a slight widening and decrease in the intensity of these peaks. New reflections are also seen, including (202) and (024), which might be the result of interactions between the intercalated organic molecules and LDH layers [47-49].

X-ray diffraction (XRD) patterns verified that the crystalline structure of the Ca-Mg-Fe LDH was preserved during composite synthesis, as seen by the presence of distinctive, well-defined peaks [55]. Incorporating pyrimidine derivatives caused a little broadening and decreased peak intensity, which indicated effective intercalation and modest lattice distortion, implying structural change without violating the general order.

3.2.3. High field scanning electron microscopy

Pyr's SEM picture (Fig. 3a) shows a granular or plate-like morphology with irregularly shaped particles [50, 51]. Small granules and layered structures form the surface texture, which gives the surface a rough, inconsistent look. Ca-Mg-Fe LDH's SEM picture (Fig. 3b) shows a distinct layered structure with thin, hexagon-shaped platelets grouped well and orderly. Significant roughness and apparent holes and cavities

on the surface might increase the material's surface area, which could be advantageous for catalytic and adsorption applications. Pyr's successful integration into the LDH framework is shown by the SEM pictures of the Ca-Mg-Fe LDH/Pyr composite (Fig. 3c,d). Because of interactions between the Pyr molecules and the layered LDH structure, the composite material has a rougher surface than pure LDH. The observation of fibrous or network-like structures in Fig. 3c raises the possibility that Pyr was partly coated or incorporated in the LDH layers. Furthermore, agglomeration is seen in Fig. 3d, where adding Pyr causes LDH platelets to look crowded, suggesting robust intermolecular connections. This morphological change raises the possibility of changes to the composite's physicochemical characteristics, which might affect its catalytic or adsorption capabilities.

Images from Scanning Electron Microscopy (SEM) showed significant morphological changes in the composite, supporting these results even more. Unlike the clean LDH, the hybrid material showed a rougher surface shape with fibrous, network-like features, stressing significant connections between pyrimidine molecules and LDH layers [56]. Particularly in biomedical uses, this higher surface roughness is anticipated to improve the composite's surface area and responsiveness.

3.2.4. Brunauer-Emmett-Teller (BET) surface area measurements.

The whole surface area accessible for adsorption, comprising both internal (pore) and exterior surfaces, is measured by the Brunauer–Emmett–Teller (BET) surface area. Drug distribution, adsorption, and reactivity in biological systems are all impacted by a material's increased surface area, which generally improves its capacity to interact with its surroundings. The surface area, pore structure, and adsorption behavior of Ca-Mg-Fe LDH, pyrimidine derivatives, and their composite are shown in Table 1 and Fig. 4. Weak adsorption interactions and restricted porosity are indicated by the pyrimidine derivatives' Type III isotherm, moderate pore size (6.5 nm), and low BET surface area (4.7 m²/g). The material is primarily non-porous or macroporous, but the presence of micropores indicates a tiny interior surface area. The Ca-Mg-Fe LDH exhibits a Type IV isotherm with an H3 hysteresis loop, which is typical of mesoporous materials, along with a lower average pore size (5.0 nm) and a greater BET surface area (9.7 m²/g). Its layered structure

with holes that resemble slits improves its adsorption capacity. Synergistic properties of the Ca-Mg-Fe LDH and pyrimidine derivative composite include improved pore width (9 nm), more uniform pore size (5.3 nm), and higher surface area (19 m²/g). The Type III isotherm suggests a layered slit-like pore shape from Ca-Mg-Fe LDH and weak adsorption interactions from pyrimidine derivatives with an H3 hysteresis loop [52].

According to the BET surface area study, pyrimidine/Ca-Mg-Fe LDH had the largest surface area (19 m²/g) and pore volume (0.021 cm³/g), indicating increased porosity and the possibility of better drug delivery applications. With distinctive absorption bands that correspond to hydroxyl (-OH) groups, metal-oxygen linkages, and pyrimidine functional groups, FTIR spectrum analysis verified the effective retention of pyrimidine derivatives into the LDH framework [54]. Peaks in pyrimidine/Ca-Mg-Fe LDH at 3406 cm⁻¹ (O-H stretching), 2115 cm⁻¹ (C=N stretching), and 1655 cm⁻¹ (C=C aromatic stretching) show that the pyrimidine derivatives and the LDH matrix have significant interactions, which might improve stability and controlled release characteristics. Given that nitrogen-containing heterocycles (pyrimidine rings) are known to disrupt DNA synthesis and cellular metabolism, causing cancer cells to undergo apoptosis these functional groups may contribute to the observed antiproliferative activity through interference with cellular metabolic pathways and nucleic acid biosynthesis.

3.2.5. Transmission Electron Microscopy (TEM)

TEM images provide valuable insights into the morphology and structural characteristics of the studied materials. Fig. 5a presents the TEM image of Pyr, revealing a sheet-like morphology. This appearance suggests that Pyr exists as a thin-film or layered nanomaterial, where molecules may be arranged in a semi-ordered stacked configuration. The transparent, overlapping layers indicate the presence of ultrathin nanosheets, which may exhibit some degree of aggregation. Fig. 5b displays the TEM image of Ca-Mg-Fe LDH, highlighting the formation of nanoscale particles with distinct sizes (e.g., 19.55 nm, 15.19 nm, and 167.56 nm). Importantly, as shown in red, numerous particles have a unique hexagonal shape, an identifiable characteristic of LDH crystallites produced by brucite-like layers. The inherent crystal

symmetry of LDHs gives birth to this hexagonal form, which implies a well-defined ordered phase [53]. The presence of irregularly shaped particles and porous structures suggests a high surface area, which is beneficial for adsorption and catalytic applications. The darker regions in the image indicate areas where particles have aggregated or overlapped. Fig. 5c and 5d illustrate the morphology of the Ca-Mg-Fe LDH/Pyr composite, showing a multilayered, aggregated structure. The sheet-like formations suggest significant interaction between LDH and Pyr molecules. The observed fibrous and distorted regions imply that Pyr molecules may have intercalated into the LDH layers or adsorbed onto their surface, altering the material's structure and morphology. Denser patches and increased layer stacking indicate strong interactions between the two components, which may enhance the composite's stability and functional properties.

Provided nanoscale verification of these qualities, TEM pictures revealed a multilayered, aggregated structure with observable dense regions and higher stacking, indicating strong interfacial contacts among the components. The maintained hexagonal shape of LDH crystallites within the composite also implies structural stability [55]. These structural features, including retained crystallinity, enhanced surface roughness, and improved nanoscale integration, may contribute to improved drug stabilization, cellular interaction, and delivery performance of the composite. However, further biological investigations are required to establish their direct impact on therapeutic efficacy.

3.2.6. Energy Dispersive (EDX)

The EDX spectrum of Pyr (Fig. 6a) reveals strong carbon (C) and nitrogen (N) peaks, which are characteristic of its organic molecular structure. Additionally, the presence of oxygen (O), chlorine (Cl), and sulfur (S) suggests that these elements are associated with functional groups within the Pyr composition. In Fig. 6b, the EDX spectrum of Ca-Mg-Fe LDH confirms the presence of calcium (Ca), magnesium (Mg), and iron (Fe), which are key components of the layered double hydroxide (LDH) structure. The significant oxygen (O) peak corresponds to the hydroxide layers. In contrast, the detected small chlorine (Cl) and potassium (K) peaks may be attributed to residual precursors or contaminants from

the synthesis process. The EDX spectrum of the Ca-Mg-Fe LDH/Pyr composite (Fig. 6c) exhibits peaks corresponding to both the LDH framework and Pyr molecules. The C and N peaks are more pronounced compared to pure LDH, indicating the successful incorporation of Pyr into the composite structure. In addition, Fe and Ca signals remain prominent, confirming the stability of the LDH framework. The strong O peak is attributed to both the hydroxide layers of LDH and oxygen-containing functional groups from the Pyr molecules. Overall, the EDX analysis confirms the successful integration of Pyr into the Ca-Mg-Fe LDH structure, as evidenced by the increased intensity of C and N peaks in the composite compared to the pristine LDH. This composition suggests a strong interaction between the organic Pyr molecules and the inorganic LDH layers, which may influence the material's physicochemical properties [36].

3.3. Biological Activity

The anticancer activities of Pyr, Ca-Mg-Fe LDH, Pyr/Ca-Mg-Fe LDH, and the reference drug 5-FU were evaluated against the human Kasumi-1 leukemia cell line using the MTT assay. As shown in Figs. 8–11, all tested materials reduced cell viability in a concentration-dependent manner, indicating their ability to suppress leukemia cell proliferation. A progressive decrease in cell viability was observed with increasing concentrations from 31.25 to 1000 $\mu\text{g/mL}$. The calculated IC_{50} values confirmed the cytotoxic potential of the investigated samples against leukemia cells.

The observed cytotoxic activity of Pyr and Pyr/Ca-Mg-Fe LDH can be attributed primarily to the biological properties of pyrimidine derivatives, which represent an important class of heterocyclic compounds with well-documented anticancer activity. Pyrimidine-based compounds are known to interfere with nucleotide biosynthesis and nucleic acid metabolism, leading to inhibition of DNA replication and suppression of cellular proliferation in rapidly dividing malignant cells. Furthermore, several pyrimidine analogs have been reported to induce cell-cycle arrest and apoptosis through disruption of essential metabolic pathways and inhibition of DNA synthesis [9,12,17,50,51]. Therefore, the reduction in cell viability observed following treatment with Pyr-containing samples is consistent with the established biological activities of pyrimidine derivatives.

In addition to the pharmacological role of Pyr, layered double hydroxides (LDHs) have attracted considerable attention as promising nanocarrier systems for biomedical and drug-delivery applications due to their biocompatibility, high surface area, anion-exchange capacity, and ability to transport therapeutic molecules into living cells [18,22,23]. Previous investigations have demonstrated that LDH nanoparticles can efficiently interact with cellular membranes and undergo endocytosis-mediated internalization, thereby facilitating intracellular delivery of loaded bioactive compounds [22,23,26]. Following cellular uptake, the acidic microenvironment of endosomes and lysosomes may promote gradual dissolution of the LDH layers, allowing controlled release of incorporated molecules and increasing their intracellular availability [18,30,32].

The physicochemical characteristics of the synthesized Pyr/Ca-Mg-Fe LDH nanocomposite may also contribute significantly to its biological performance. BET analysis demonstrated that the nanocomposite possessed a larger surface area compared with the individual components, which may enhance contact between the material and cellular membranes. Increased surface area and porosity often favor adsorption processes, facilitate cellular interaction, and improve transport efficiency of biologically active molecules [22,28]. Furthermore, SEM and TEM analyses (Figs 4–6) revealed porous multilayered nanoscale structures that provide a large interfacial area capable of promoting nanoparticle-cell interactions and enhancing cellular uptake.

Interestingly, Pyr/Ca-Mg-Fe LDH exhibited a slightly higher IC₅₀ value than free Pyr under the investigated experimental conditions. Although free Pyr produced a stronger immediate cytotoxic response, this observation does not necessarily indicate lower therapeutic potential of the nanocomposite. Nanocarrier systems are frequently designed to improve drug stability, protect bioactive compounds from premature degradation, and provide sustained or controlled release rather than maximize acute in vitro cytotoxicity [18,22,31,32]. Therefore, the observed reduction in immediate cytotoxicity may be attributed to the gradual release of Pyr molecules from the LDH matrix, resulting in prolonged rather than instantaneous exposure of leukemia cells to the active compound.

Morphological examination further supported the cytotoxicity findings. Kasumi-1 cells treated with increasing concentrations of Pyr, Ca-Mg-Fe LDH, and Pyr/Ca-Mg-Fe LDH exhibited progressive alterations in cellular morphology, including reduced cell density, loss of normal cellular architecture, cellular shrinkage, and membrane damage, as illustrated in Figs 8–11. Such morphological changes are frequently associated with cellular stress and activation of programmed cell death pathways, including apoptosis [29]. Although apoptosis-specific assays were not performed in the present study, the substantial reduction in cell viability together with the observed cellular deformation suggests the involvement of cell-death-related mechanisms.

The contribution of Mg, Ca, and Fe ions present within the LDH structure should also be considered. Besides providing structural stability, these metal ions may influence several intracellular biochemical processes. In particular, iron-containing nanomaterials have been reported to participate in redox-related reactions that can generate reactive oxygen species and oxidative stress, thereby increasing the susceptibility of cancer cells to therapeutic intervention [22,23]. Consequently, the biological activity of the developed Pyr/Ca-Mg-Fe LDH nanocomposite is likely governed by a combination of pyrimidine-mediated antiproliferative effects, LDH-assisted intracellular delivery, and physicochemical interactions between the nanocomposite and leukemia cells.

Collectively, the obtained findings demonstrate that Pyr/Ca-Mg-Fe LDH possesses significant antiproliferative activity against Kasumi-1 leukemia cells. The enhanced surface characteristics, porous structure, and carrier properties of the LDH matrix may support efficient intracellular delivery of pyrimidine derivatives [18,22,26,32]. Nevertheless, further investigations involving cellular uptake studies, apoptosis analysis, reactive oxygen species determination, and drug-release experiments are required to elucidate the precise mechanisms responsible for the observed biological effects and to further validate the potential of this nanocomposite as a leukemia-directed drug-delivery platform.

3.4. Study Limitations

426 The present study has several limitations that should be acknowledged. Although significant
427 antiproliferative activity against Kasumi-1 leukemia cells was demonstrated, the precise molecular
428 mechanisms responsible for the observed cytotoxic effects were not investigated. In addition, drug-release
429 behavior, cellular uptake pathways, apoptosis induction, and oxidative stress-related responses were not
430 evaluated. Furthermore, the cytotoxicity of the developed nanocomposite was not assessed against normal
431 cell lines; therefore, its selectivity toward cancer cells cannot be fully established. Consequently, further in
432 vitro and in vivo investigations are required to achieve a comprehensive understanding of the biological
433 performance, safety profile, and therapeutic potential of the developed Pyr/Ca-Mg-Fe LDH
434 nanocomposite.

435 **Conclusion**

436 In this study, a novel pyrimidine-loaded Mg-Ca-Fe layered double hydroxide (Pyr/Ca-Mg-Fe LDH)
437 nanocomposite was successfully synthesized and characterized using FTIR, XRD, SEM, TEM, EDX, and
438 BET analyses. The obtained results confirmed the successful formation of the LDH structure and the
439 incorporation of the pyrimidine derivative within the nanocomposite matrix. The developed material
440 exhibited favorable physicochemical characteristics, including porous morphology and enhanced surface
441 properties, which may support its potential application as a nanostructured delivery platform.

442 Biological evaluation against Kasumi-1 leukemia cells demonstrated that both free pyrimidine and the
443 Pyr/Ca-Mg-Fe LDH nanocomposite possess antiproliferative activity. Although the nanocomposite
444 exhibited a slightly higher IC₅₀ value than the free pyrimidine derivative, the developed LDH system may
445 offer potential advantages related to physicochemical stability and cellular interaction. The observed
446 biological activity is likely associated with the combined contribution of the pyrimidine derivative and the
447 LDH carrier matrix.

Overall, the synthesized Pyr/Ca-Mg-Fe LDH nanocomposite represents a promising nanomaterial for leukemia-related applications. Nevertheless, further investigations involving cellular uptake analysis, apoptosis studies, selectivity assessment using normal cell lines, and in vivo evaluation are required to achieve a more comprehensive understanding of its biological behavior, safety profile, and therapeutic potential.

Data availability

Data details can be provided by the corresponding author on request.

Declaration of Interests

Authors have nothing to declare.

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References

1. Mattiuzzi, C. and G. Lippi, *Current cancer epidemiology*. Journal of epidemiology and global health, 2019. **9**(4): p. 217-222. <https://doi.org/10.2991/jegh.k.191008.001>
2. Siegel, R.L., et al., *Cancer statistics, 2022*. CA: a cancer journal for clinicians, 2022. **72**(1): p. 7-33. <https://doi.org/10.3322/caac.21708>
3. Chae, H.-S. and S.-T. Hong, *Overview of cancer metabolism and signaling transduction*. International journal of molecular sciences, 2022. **24**(1): p. 12. <https://doi.org/10.3390/ijms24010012>
4. Siegel, R.L., et al., *Cancer statistics, 2023*. CA: a cancer journal for clinicians, 2023. **73**(1): p. 17-48. <https://doi.org/10.3322/caac.21763>
5. van der Burg, S.H., et al., *Vaccines for established cancer: overcoming the challenges posed by immune evasion*. Nature Reviews Cancer, 2016. **16**(4): p. 219-233. <https://doi.org/10.1038/nrc.2016.16>

6. Bedirian, K., et al., *Overview of cancer control in Armenia and policy implications*. *Frontiers in Oncology*, 2022. **11**: p. 782581. <https://doi.org/10.3389/fonc.2021.782581>
7. Shah, J.R., D.U. Kapoor, and B.G. Prajapati, *Emerging Innovations in Nanomedicine for Cancer Immunotherapy*. *Journal of Drug Delivery Science and Technology*, 2024: p. 106584. <https://doi.org/10.1016/j.jddst.2024.106584>
8. Zeng, Q., T. Jiang, and J. Wang, *Role of LMO7 in cancer*. *Oncology reports*, 2024. **52**(3): p. 1-12. <https://doi.org/10.3892/or.2024.8776>
9. Stoll, G.R., et al., *TP53 Aberrant B-Cell Precursor Acute Lymphoblastic Leukemia (BCP-ALL) Are Uniquely Sensitive to a Combination of Pyrimidine De Novo Synthesis Blockade and Replication Checkpoint Inhibition*. *Blood*, 2023. **142**: p. 4189. <https://doi.org/10.1182/blood-2023-179159>
10. Khwaja, A., et al., *Acute myeloid leukaemia*. *Nature reviews Disease primers*, 2016. **2**(1): p. 1-22. <https://doi.org/10.1038/nrdp.2016.10>
11. Stirewalt, D.L. and J.P. Radich, *The role of FLT3 in haematopoietic malignancies*. *Nature Reviews Cancer*, 2003. **3**(9): p. 650-665. <https://doi.org/10.1038/nrc1169>
12. Liu, W., et al., *Discovery of 3-amide-pyrimidine-based derivatives as potential fms-like tyrosine receptor kinase 3 (FLT3) inhibitors for treating acute myelogenous leukemia*. *Bioorganic & Medicinal Chemistry Letters*, 2025. **117**: p. 130082. <https://doi.org/10.1016/j.bmcl.2024.130082>
13. Delamarre, L., I. Mellman, and M. Yadav, *Neo approaches to cancer vaccines*. *Science*, 2015. **348**(6236): p. 760-761. <https://doi.org/10.1126/science.aab3465>
14. Zeng, Q., J. Cheng, and W. Yue, *Estimating hourly all-sky surface longwave upward radiation using the new generation of Chinese geostationary weather satellites Fengyun-4A/AGRI*. *IEEE Transactions on Geoscience and Remote Sensing*, 2024. <https://doi.org/10.1109/TGRS.2024.3399781>

- 494 15. Zhang, H., et al., *Inflammatory factors predict infection risk during chemotherapy in leukemia*
495 *patients*. Microbial Pathogenesis, 2025. **199**: p. 107210.
496 <https://doi.org/10.1016/j.micpath.2024.107210>
- 497 16. Kawai, H., et al., *Acute myeloid leukemia with a ZMYND11:: MBTD1 fusion gene following*
498 *chemotherapy and radiotherapy for breast cancer: A case report*. Leukemia Research Reports,
499 2024. **22**: p. 100478. <https://doi.org/10.1016/j.lrr.2024.100478>
- 500 17. Sanglikar, G. and A. KumarTengli, *A critical review on purine and pyrimidine heterocyclic*
501 *derivatives and their designed molecules in cancer*. Results in Chemistry, 2025: p. 102210.
502 <https://doi.org/10.1016/j.rechem.2025.102210>
- 503 18. Cao, Z., et al., *2D layered double hydroxide nanoparticles: recent progress toward*
504 *preclinical/clinical nanomedicine*. Small Methods, 2020. **4**(2): p. 1900343.
505 <https://doi.org/10.1002/smt.201900343>
- 506 19. Zhao, P., et al., *Electrochemical deposition to construct a nature inspired multilayer*
507 *chitosan/layered double hydroxides hybrid gel for stimuli responsive release of protein*. Journal of
508 Materials Chemistry B, 2015. **3**(38): p. 7577-7584. <https://doi.org/10.1039/C5TB01056J>
- 509 20. Xu, M. and M. Wei, *Layered double hydroxide-based catalysts: recent advances in preparation,*
510 *structure, and applications*. Advanced Functional Materials, 2018. **28**(47): p. 1802943.
511 <https://doi.org/10.1002/adfm.201802943>
- 512 21. Wang, Q. and D. O'Hare, *Recent advances in the synthesis and application of layered double*
513 *hydroxide (LDH) nanosheets*. Chemical reviews, 2012. **112**(7): p. 4124-4155.
514 <https://doi.org/10.1021/cr200434v>
- 515 22. Yan, L., et al., *Layered double hydroxide nanostructures and nanocomposites for biomedical*
516 *applications*. Journal of Materials Chemistry B, 2019. **7**(37): p. 5583-5601.
517 <https://doi.org/10.1039/C9TB01312A>

23. Jing, G., et al., *Interference of layered double hydroxide nanoparticles with pathways for biomedical applications*. Advanced Drug Delivery Reviews, 2022. **188**: p. 114451. <https://doi.org/10.1016/j.addr.2022.114451>
24. Tian, D.-Y., et al., *synthesis of methotrexatum intercalated layered double hydroxides by different methods: Biodegradation process and bioassay explore*. Applied Clay Science, 2015. **118**: p. 87-98. <https://doi.org/10.1016/j.clay.2015.09.007>
25. Deng, X., et al., *Cancer immunotherapy boosted by layered double hydroxide nanoparticles*. RSC Pharmaceutics, 2024. <https://doi.org/10.1039/D4PM00179F>
26. Forano, C., et al., *Interactions between biological cells and layered double hydroxides: Towards functional materials*. The Chemical Record, 2018. **18**(7-8): p. 1150-1166. <https://doi.org/10.1002/tcr.201700102>
27. Hu, T., et al., *2D MOFs nanosheets derived from 2D LDHs as a multifunctional sensitizer for sonodynamic therapy-mediated tumor treatment*. Chemical Engineering Journal, 2025: p. 159771. <https://doi.org/10.1016/j.cej.2025.159771>
28. Kiani, M., et al., *Multifunctional green synthesized Cu–Al layered double hydroxide (LDH) nanoparticles: anticancer and antibacterial activities*. Scientific Reports, 2022. **12**(1): p. 9461. <https://doi.org/10.1038/s41598-022-13431-7>
29. Lewinski, N., V. Colvin, and R. Drezek, *Cytotoxicity of nanoparticles*. small, 2008. **4**(1): p. 26-49. <https://doi.org/10.1002/smll.200700595>
30. Zhang, L.-X., et al., *Efficient delivery of clay-based nanovaccines to the mouse spleen promotes potent anti-tumor immunity for both prevention and treatment of lymphoma*. Nano Research, 2021. **14**: p. 1326-1334. <https://doi.org/10.1007/s12274-020-3175-0>
31. Wang, Z., et al., *Layered double hydroxide eliminate embryotoxicity of chemotherapeutic drug through BMP-SMAD signaling pathway*. Biomaterials, 2020. **230**: p. 119602. <https://doi.org/10.1016/j.biomaterials.2019.119602>

32. Yan, L., et al., *A novel type of aqueous dispersible ultrathin-layered double hydroxide nanosheets for in vivo bioimaging and drug delivery*. ACS Applied Materials & Interfaces, 2017. **9**(39): p. 34185-34193. <https://doi.org/10.1021/acsami.7b05294>
33. Chen, K., et al., *In vitro and in vivo degradation behavior of Mg–2Sr–Ca and Mg–2Sr–Zn alloys*. Bioactive Materials, 2020. **5**(2): p. 275-285. <https://doi.org/10.1016/j.bioactmat.2020.02.014>
34. Ding, P., et al., *In vitro and in vivo biocompatibility of Mg–Zn–Ca alloy operative clip*. Bioactive Materials, 2019. **4**: p. 236-244. <https://doi.org/10.1016/j.bioactmat.2019.07.002>
35. Ragab, I., Y.A. Selim, and M.H. Abd El-Azim, *Synthesis of some New Dihydropyrimidines Derivatives by Cyclization of Polarized Unsaturated Systems*. Journal of Heterocyclic Chemistry, 2018. **55**(12): p. 2727-2731. <https://doi.org/10.1002/jhet.3332>
36. Moaty, S.A., A. Farghali, and R. Khaled, *Preparation, characterization and antimicrobial applications of Zn-Fe LDH against MRSA*. Materials Science and Engineering: C, 2016. **68**: p. 184-193. <https://doi.org/10.1016/j.msec.2016.05.110>
37. Ghasemi, M., et al., *The MTT assay: utility, limitations, pitfalls, and interpretation in bulk and single-cell analysis*. International journal of molecular sciences, 2021. **22**(23): p. 12827. <https://doi.org/10.3390/ijms222312827>
38. Awate, R., A. Mishra, and A. Mansuri. *Synthesis and Characterization of Transition Metal complexes with pyrimidine based ligand derivative*. in *Journal of Physics: Conference Series*. 2016. IOP Publishing. <https://doi.org/10.1088/1742-6596/755/1/012019>
39. Zaman, H., et al., *Novel pyrimidine linked acyl thiourea derivatives as potent α -amylase and proteinase K inhibitors: design, synthesis, molecular docking and ADME studies*. RSC advances, 2024. **14**(45): p. 33235-33246. <https://doi.org/10.1039/D4RA05799F>
40. Bryndal, I., et al., *Pyrimidine Schiff bases: Synthesis, structural characterization and recent studies on biological activities*. International Journal of Molecular Sciences, 2024. **25**(4): p. 2076. <https://doi.org/10.3390/ijms25042076>

41. Wei, X., et al., *Fabrication of a novel nanofiltration membrane using an Mg-Fe layered double hydroxide for dye/salt separation*. RSC advances, 2024. **14**(33): p. 24055-24065. <https://doi.org/10.1039/D4RA03366C>
42. Altalhi, A.A., E.A. Mohamed, and N.A. Negm, *Recent advances in layered double hydroxides (LDH)-based materials: Fabrications, modification strategies, characterization, promising environmental catalytic applications, and prospective aspects*. Energy Advances, 2024. <https://doi.org/10.1039/D4YA00272E>
43. Liang, M., et al., *The phosphorus adsorption and recovery of Mg/Fe-LDHs mulberry rod biochar composite*. Separations, 2024. **11**(3): p. 86. <https://doi.org/10.3390/separations11030086>
44. Peng, C., et al., *Calcined Mg-Fe layered double hydroxide as an absorber for the removal of methyl orange*. Aip Advances, 2015. **5**(5). <https://doi.org/10.1063/1.4921455>
45. Powder, R.F., *INTERNATIONAL REPORTS*. Powder Diffr, 1999. **14**(4). <https://doi.org/10.1017/S0885715600021229>
46. Shannon, R., *Acta Crystallogr., Sect. A: Cryst. Phys., Diffr., Theor. Gen. Cryst.* 1976.
47. Reddy, A.J., et al., *Structural, EPR, photo and thermoluminescence properties of ZnO: Fe nanoparticles*. Materials chemistry and Physics, 2012. **133**(2-3): p. 876-883. <https://doi.org/10.1016/j.matchemphys.2012.01.111>
48. Nuss, J., et al., *The structural anomaly of zinc: evolution of lattice constants and parameters of thermal motion in the temperature range of 40 to 500 K*. 2010, Wiley Online Library. <https://doi.org/10.1002/zaac.200900460>
49. Tutton, A., *Crystallography and mineralogy*. Annual Reports on the Progress of Chemistry, 1922. **19**: p. 234-266. <https://doi.org/10.1039/ar9221900234>
50. Natarajan, R., et al., *Structure-activity relationships of pyrimidine derivatives and their biological activity-A review*. Medicinal Chemistry, 2023. **19**(1): p. 10-30. <https://doi.org/10.2174/1573406418666220509100356>

51. Tolba, M.S., et al., *synthesis, reactions, and applications of pyrimidine derivatives*. Current Chemistry Letters, 2022. **11**(1): p. 121-138. <https://doi.org/10.5267/j.ccl.2021.8.002>
52. Thommes, M., et al., *Physisorption of gases, with special reference to the evaluation of surface area and pore size distribution (IUPAC Technical Report)*. Pure and applied chemistry, 2015. **87**(9-10): p. 1051-1069. <https://doi.org/10.1515/pac-2014-1117>
53. Phan, V.G., et al., *Nanoengineered injectable hydrogels derived from layered double hydroxides and alginate for sustained release of protein therapeutics in tissue engineering applications*. Journal of Nanobiotechnology, 2023. **21**(1): p. 405. <https://doi.org/10.1186/s12951-023-02160-2>
54. Hubetska, T., V. Demchenko, and N. Kobylinska, *Surface engineering: binary Mg, Fe-LDH· x Fe 3 O 4 nanocomposites for improved magnetic solid-phase extraction of pharmaceuticals from aqueous solution*. Materials Advances, 2024. **5**(20): p. 8145-8163. <https://doi.org/10.1039/D4MA00609G>
55. Zhang, Y., H. Xu, and S. Lu, *Preparation and application of layered double hydroxide nanosheets*. RSC advances, 2021. **11**(39): p. 24254-24281. <https://doi.org/10.1039/D1RA03289E>
56. Alagha, O., et al., *Magnetic Mg-Fe/LDH intercalated activated carbon composites for nitrate and phosphate removal from wastewater: Insight into behavior and mechanisms*. Nanomaterials, 2020. **10**(7): p. 1361. <https://doi.org/10.3390/nano10071361>

Table 1. Surface area parameters of prepared samples.

	pyrimidine derivatives	Ca-Mg-Fe LDH	pyrimidine derivatives/ Ca-Mg-Fe LDH
BET surface area [m ² g ⁻¹]	4.7	9.7	19
Total pore volume [cm ³ g ⁻¹]	0.01118	0.001551	0.021
Average pore diameter [nm]	6.5	5.0	5.3
Average pore width [nm]	4.3	8.2	9

613 **Table 2.** % Cell viability, % Toxicity, and IC50 of in vitro synthesized drugs at different concentrations by
614 MTT assay.

ID	µg/ml	O.D ± SE	Viability %	Toxicity %	IC50 ± SD
Kasumi-1	-----	0.51 ± 0.002	100	0	ug
Pyrimidine (Sample 1, A)	1000	0.01± 0.000	3.45	96.54	90.51 ± 1.38
	500	0.01 ± 0.000	3.65	96.34	
	250	0.01 ± 0.001	3.783	96.21	
	125	0.11 ± 0.004	23.02	76.97	
	62.5	0.35 ± 0.008	70.25	29.74	
	31.25	0.50 ± 0.001	99.41	0.58	
Ca- Mg- Fe LDH (Sample 2, B)	1000	0.01 ± 0.000	3.65	96.34	95.5 ± 1
	500	0.01 ± 0.001	3.84	96.15	
	250	0.10 ± 0.003	20.61	79.38	
	125	0.15 ± 0.007	30.98	69.01	
	62.5	0.35 ± 0.008	70.18	29.81	
	31.25	0.48 ± 0.009	94.06	5.93	
Pyrimidine/Ca- Mg- Fe LDH (Sample 3, C)	1000	0.01 ± 0.000	3.84	96.15	109.16 ± 1.62
	500	0.01 ± 0.001	3.78	96.21	
	250	0.10 ± 0.003	21.06	78.93	
	125	0.19 ± 0.006	38.48	61.51	
	62.5	0.45 ± 0.005	89.88	10.11	
	31.25	0.48 ± 0.006	94.97	5.02	
5-FU, (Reference drug, D)	1000	0.01 ± 0.001	3.78	96.21	82.19 ± 3.3
	500	0.01 ± 0.000	3.58	96.41	
	250	0.07 ± 0.030	14.41	85.58	
	125	0.09 ± 0.001	17.67	82.32	
	62.5	0.34 ± 0.011	66.53	33.46	
	31.25	0.46 ± 0.003	91.06	8.93	

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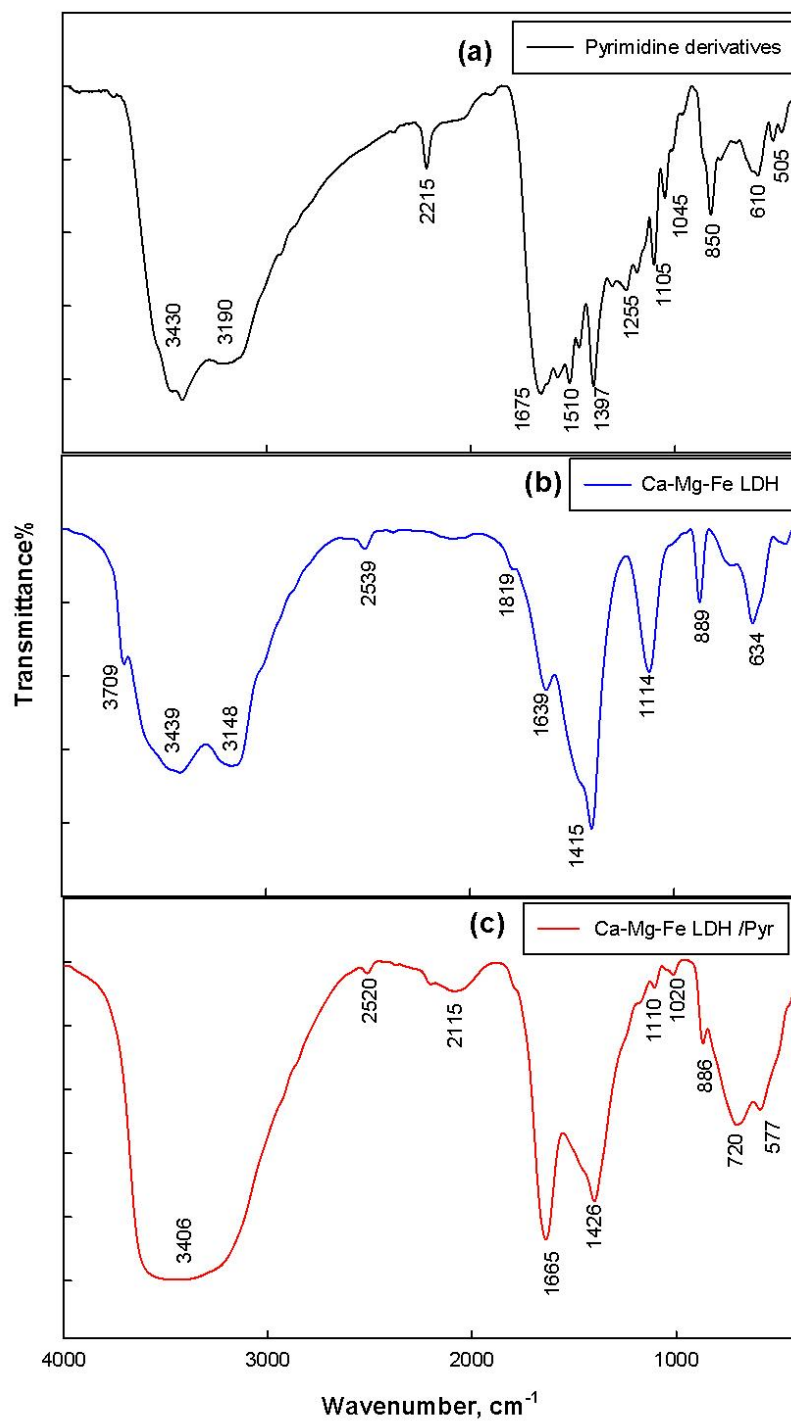


Figure 1. FTIR spectra of >(a) pyrimidine derivatives, (b) Ca-Mg-Fe LDH, and (c) Ca-Mg-Fe LDH/Pyr.

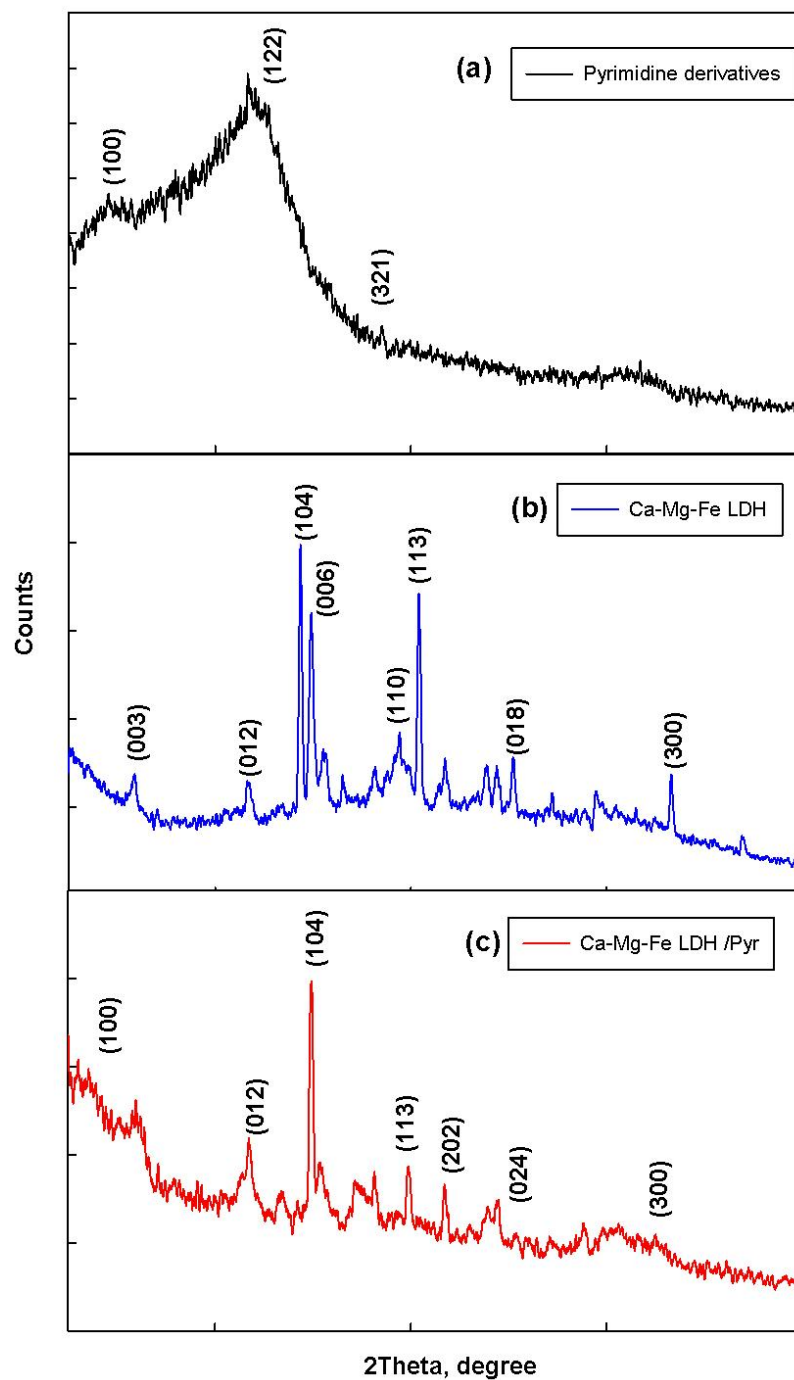


Figure 2. XRD analysis of (a) pyrimidine derivatives, (b) Ca-Mg-Fe LDH, and (c) Ca-Mg-Fe LDH/Pyr

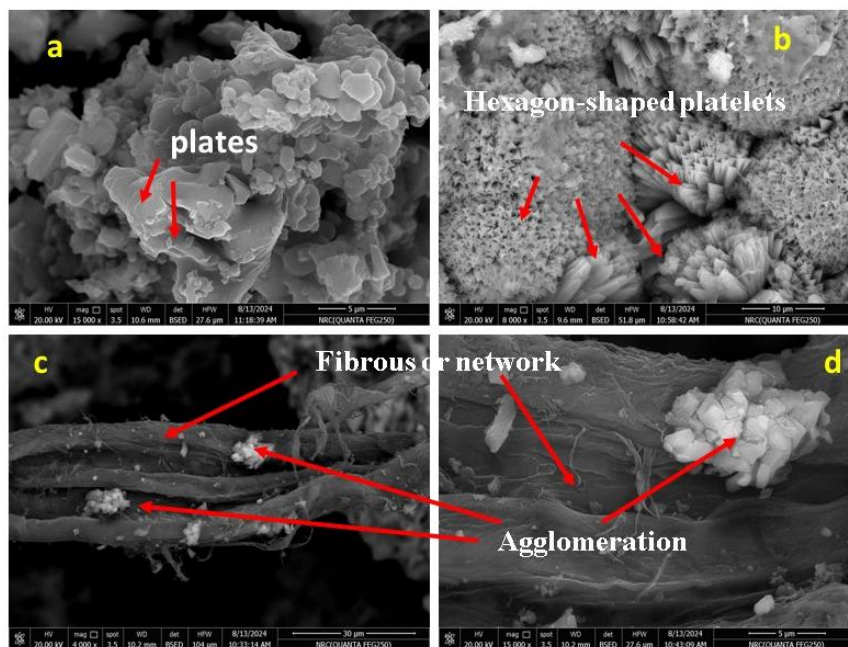


Figure 3. SEM images of (a) Pyr, (b) Ca-Mg-Fe LDH, and (c, d) Ca-Mg-Fe LDH/Pyr

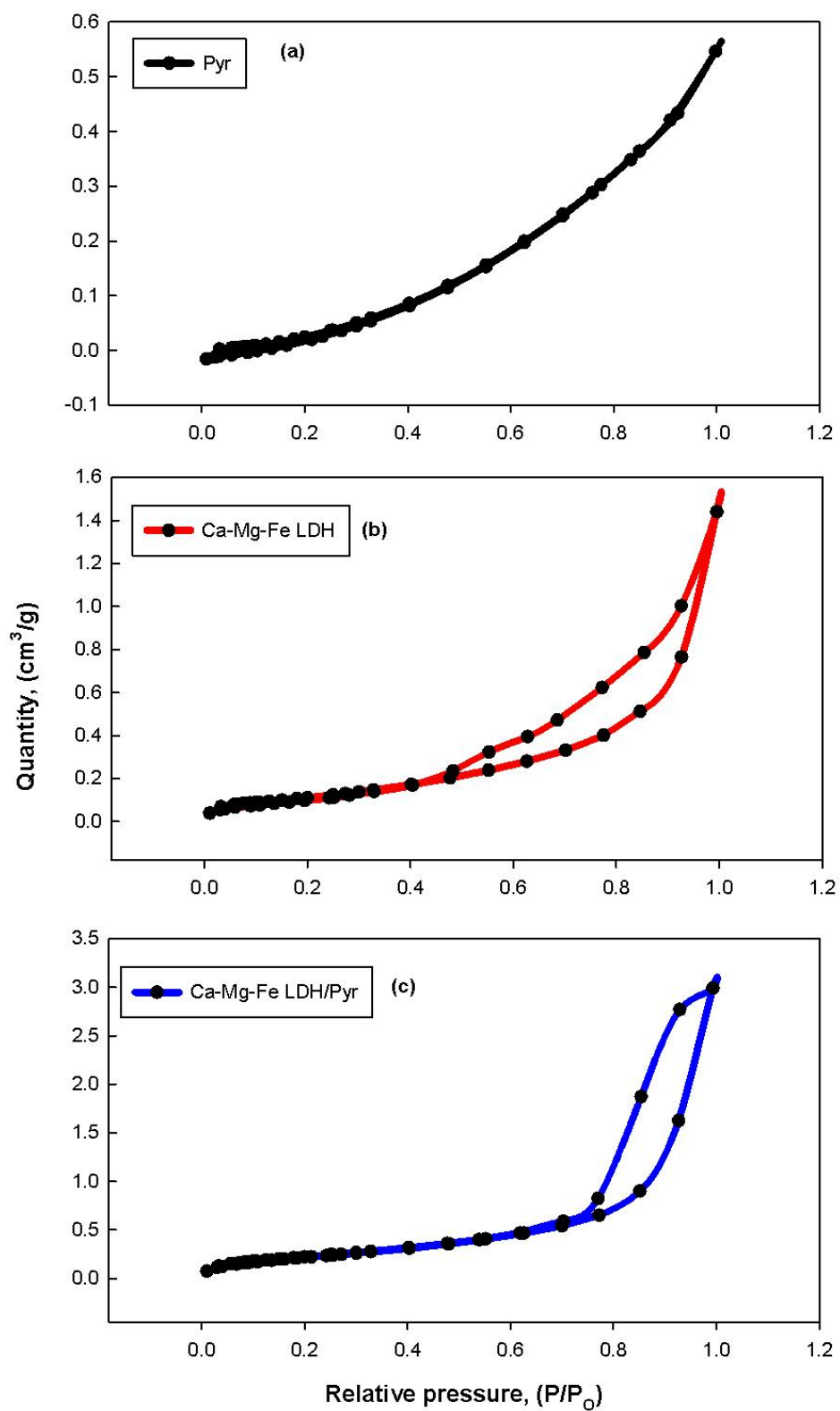


Figure 4. BET adsorption-desorption isotherms of (a) pyrimidine derivatives, (b) Ca-Mg-Fe LDH, and (c) Ca-Mg-Fe LDH/Pyr.

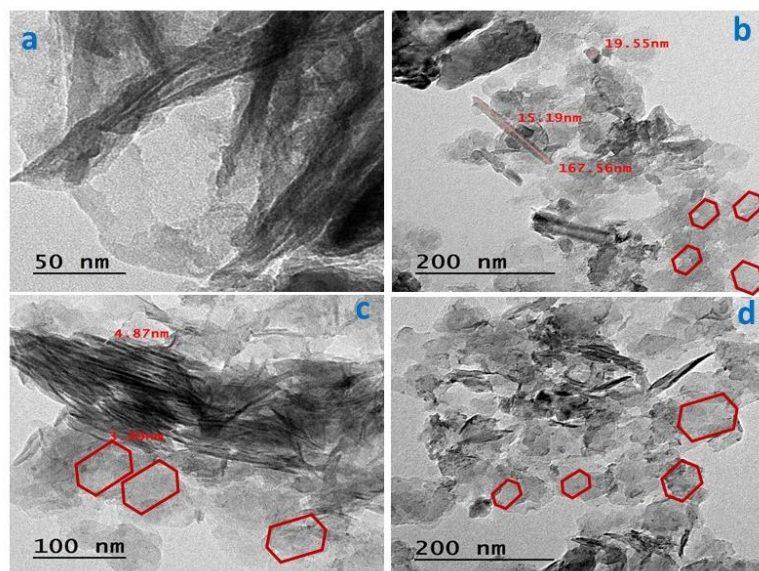


Figure 5. TEM images of (a) Pyr, (b) Ca-Mg-Fe LDH, and (c, d) Ca-Mg-Fe LDH/Pyr

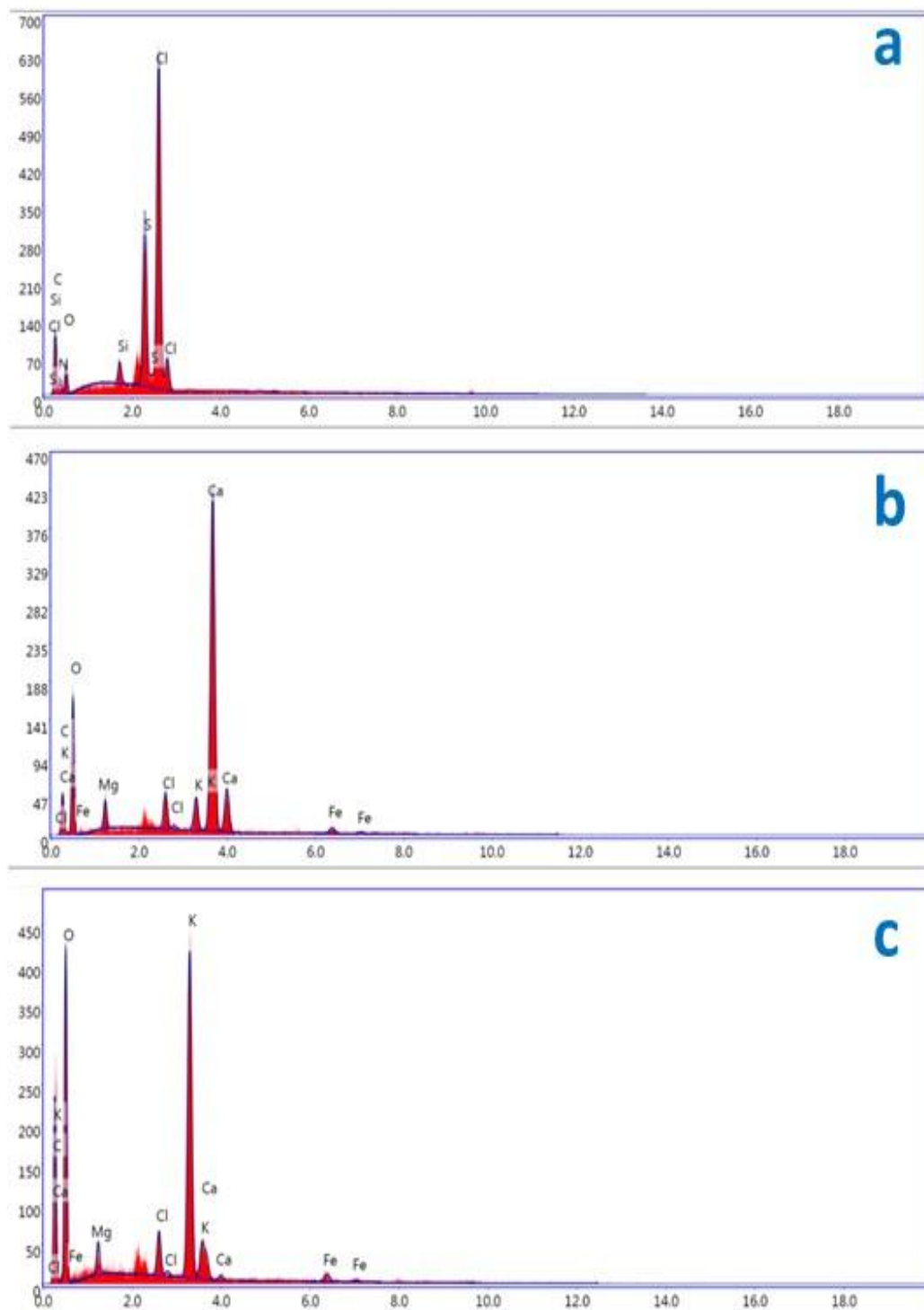
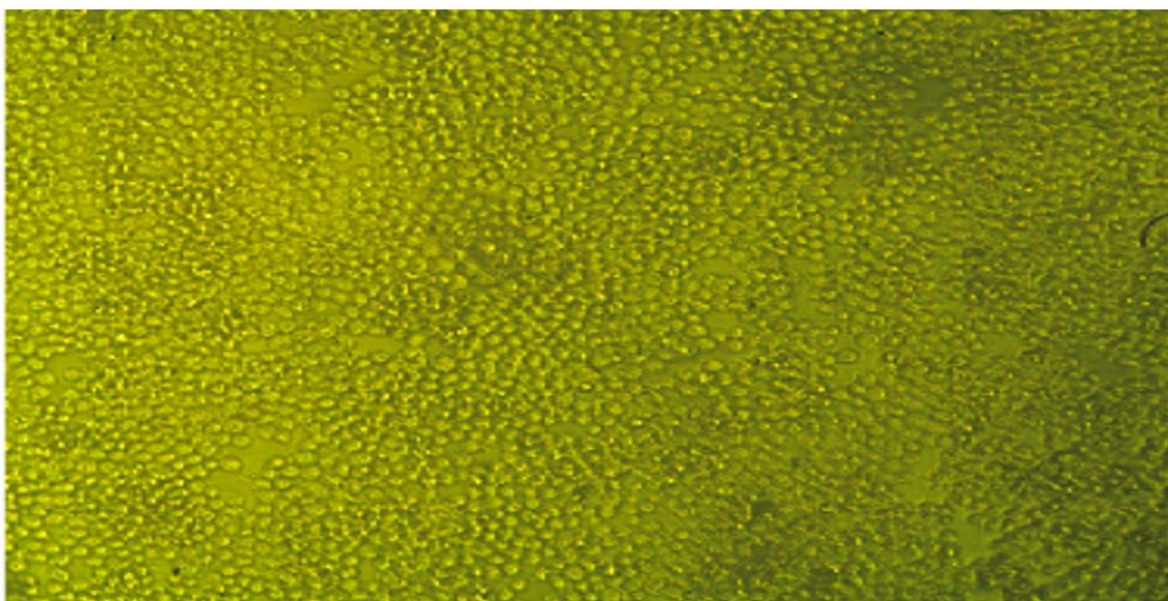


Figure 6. EDX analysis of (a) Pyr, (b) Ca-Mg-Fe LDH, and (c) Ca-Mg-Fe LDH/Pyr



Kasumi 1

Organism :	<i>Homo sapiens</i> , human
Tissue :	Peripheral blood
Cell Type :	myeloblast
Culture Properties :	suspended
Disease :	Acute myeloblastic leukemia
ATCC :	CRL-2724

Figure 7. Photomicrographic image of untreated human homo sapiens-Kasumi1 leukemia cell line (control) as obtained from MTT assay.

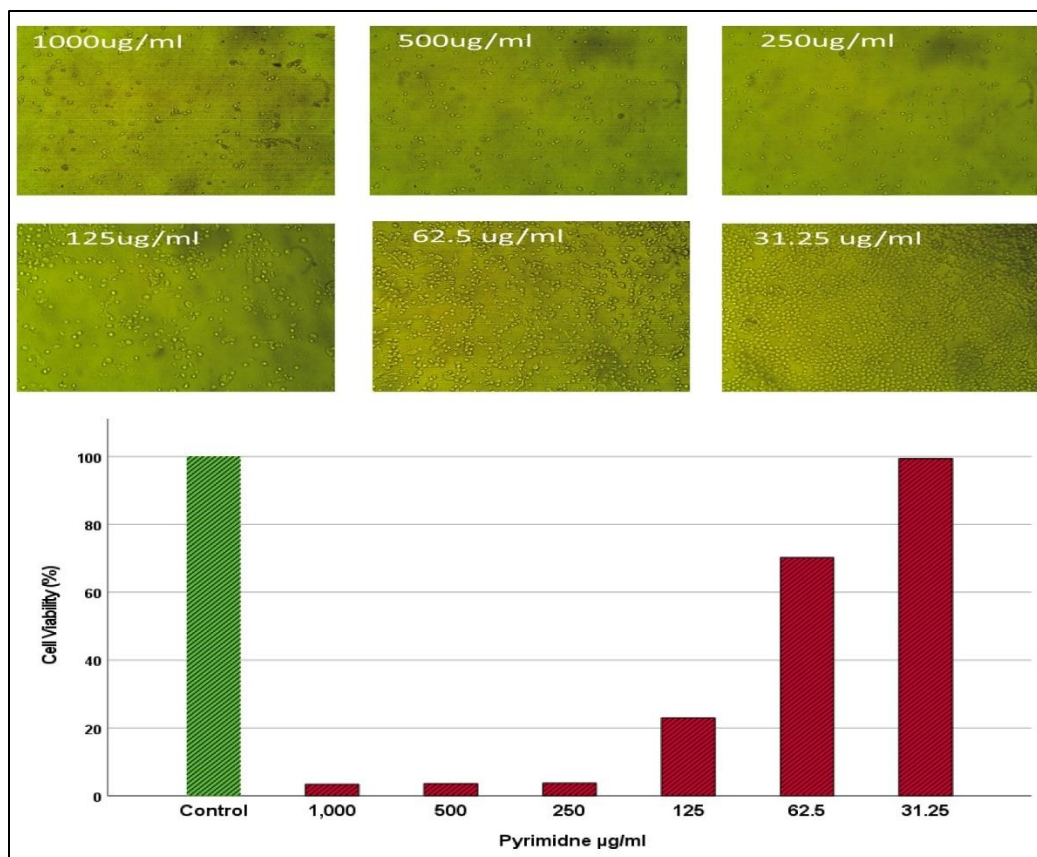
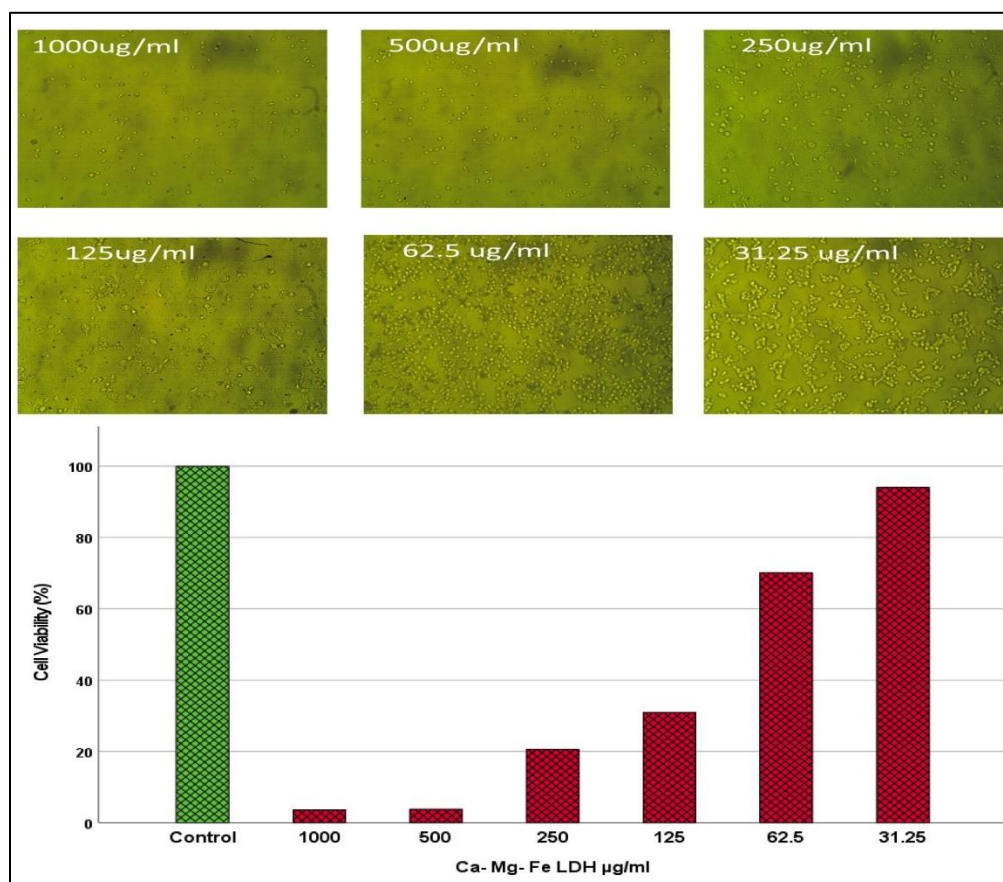


Figure 8. Photomicrographic images of anticancer activity of the human homo sapiens-Kasumi1 leukemia cell line were screened by different concentrations of the pyrimidine(Sample 1, A). The cell was treated for 24 h along with the control group, as obtained from the MTT assay. Data expressed as mean $\pm$ SE of three experiments done in triplicate.

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749 **Figure 9.** Photomicrographic images of anticancer activity of the human homo sapiens-Kasumi1 leukemia cell
 750 line were screened by different concentrations of the Ca- Mg- Fe LDH (Sample 2, B). The cell was treated for 24
 751 h along with the control group, as obtained from the MTT assay. Data expressed as mean $\pm$ SE of three experiments
 752 done in triplicate.

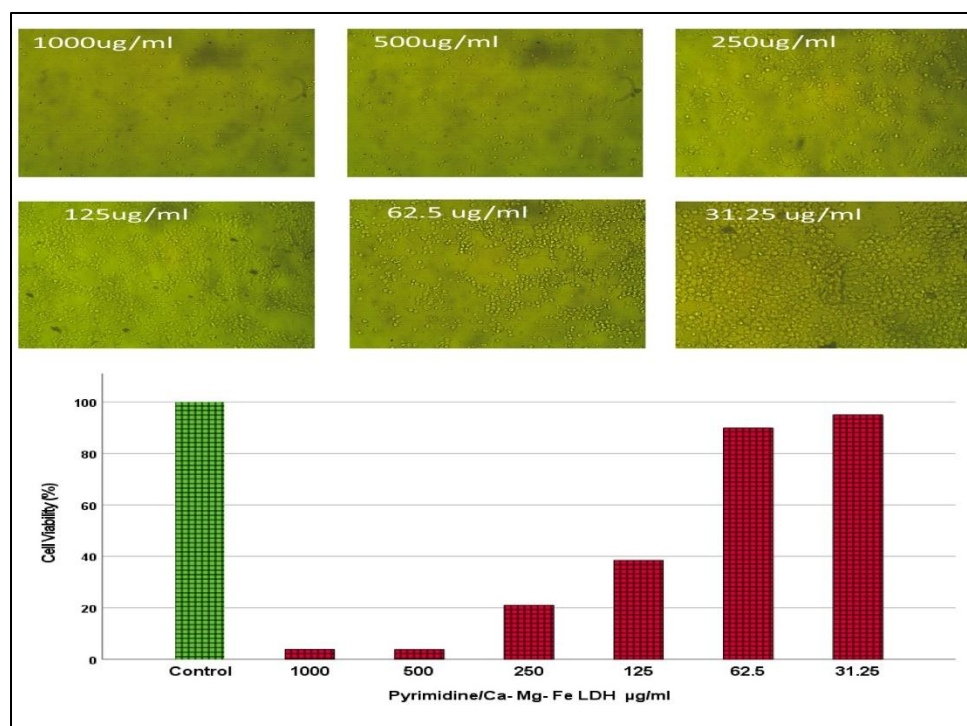


Figure 10. Photomicrographic images of anticancer activity of the human homo sapiens-Kasumi1 leukemia cell line were screened by different concentrations of the pyrimidine/Ca- Mg- Fe LDH (Sample 3, C). The cell was treated for 24 h along with the control group, as obtained from the MTT assay. Data expressed as mean $\pm$ SE of three experiments done in triplicate.

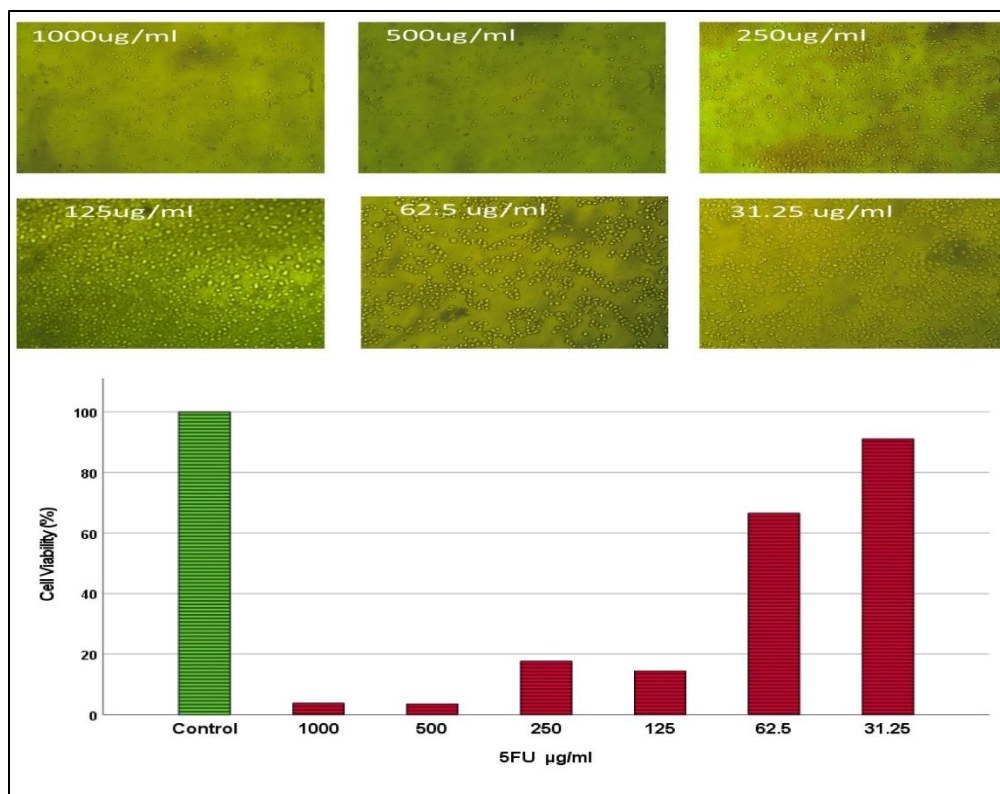


Figure 11. Photomicrographic images of anticancer activity of the human homo sapiens-Kasumi1 leukemia cell line were screened by different concentrations of the 5-FU (Reference drug, D). The cell was treated for 24 h along with the control group, as obtained from the MTT assay. Data expressed as mean $\pm$ SE of three experiments done in triplicate.