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Fe-Co-V/Zeolite nano composite catalyzed biginelli compounds synthesis and evaluation of their drug cytotoxic activity

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ABSTRACT

An effective one-pot synthesis of bis (dihydropyrimidinonoe) benzenes through Biginelli condensation reaction of terephthalic aldehyde, 1,3-dicarbonyl compounds and (thio) urea under solvent free conditions is described. Excellent yields of the products and simple work-up are attractive features of this green protocol. Then, the cytotoxic activities of these compounds were evaluated on 5 different human cancerous cell lines (Raji, HeLa, LS-180, SKOV-3 and MCF7). Their cytotoxic study indicated that they possessed a weak to moderate activity. Furthermore, the higher activity of compound **4b** bearing sulfur in C_2 position of pyrimidine ring showed the importance of this site for cytotoxic activity of these compounds.

Keywords: Biginelli reaction; nanozeolite; terephthalic aldehyde; multi-component; Cytotoxicity.

1. Introduction

Multi-component reactions (MCRs) can be distinguished from classical, sequential two component chemistry synthesis processes in that they use three or more chemical starting materials as the input for product formation. Up to seven starting components have been

used, and MCRs have often been shown to produce higher product yields than classical chemistry [1-3].

Multi-component reactions are finding increasing use in the discovery process of new drugs and agrochemicals [4-6] and offer significant advantages over conventional linear-type syntheses.

MCR condensations involve three or more compounds reacting in a single one pot reaction, but consecutively to form a new product, which contains the essential parts of all the starting materials. The search and discovery for new MCR's on one hand, [7] and the full exploitation of already known multi-component reactions on the other hand, is therefore of considerable current interest. One such MCR that belongs in the latter category is the venerable Biginelli dihydropyrimidine synthesis. A few years ago, Pietro Biginelli reported on the one pot cyclocondensation reaction of an aldehyde, a β-ketoester, and urea (or thiourea), a procedure known as the Biginelli reaction, [9] is receiving increased attention. More than a century ago, Biginelli intuitively anticipated the synthetic potential of multicomponent reactions by combining in a single flask the reactants of two different reactions having one component in common.[9] The result of the three-component reaction was a new product that was correctly characterized as a substituted 3,4-dihydropyrimidine-2(1*H*)-one (DHPM).

3,4-Dihydropyrimidinones (DHPMs) constitute a very important class of organic compounds due to their attractive pharmacological properties, including antiviral, antitumour, antibacterial activities [10].

They have emerged as integral backbones of several calcium channel blockers, antihypertensive agents, α-1a-antagonists, and neuropeptide Y (NPY) antagonists. [11-12] Moreover, several alkaloids containing the dihydropyrimidine core unit have been isolated from marine sources, which also exhibit interesting biological properties. Most notably, among these are the batzelladine alkaloids,

which were found to be potent HIV gp-120-CD4 inhibitors. [13-15]In the frame work of our program to develop the chemistry of heterocyclic compounds and in connection with our ongoing interests in MCRs, we would like to introduce a facile procedure for the synthesis of bis(dihydropyrimidinone)benzenes via one-pot condensation of terephthalic aldehyde or isophthalic aldehyde with (thio)urea, guanidine and 1,3- dicarbonyl compounds via catalytic nano zeoilte. Furthermore, the cytotoxic activity of synthesized compound was in 5 different cell lines.

2. Experimental

Materials and methods

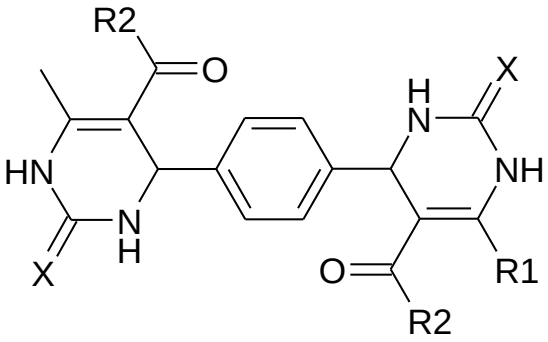
The reactions were carried out with a microwave oven (Microsynthesis, Milstone, 2.45GHz, 1500w). Melting points were measured on the Electrothemal 9100 apparantus and are uncorrected. IR spectra were measured on a Bomem FT-IR-MB 100 spectrometer. ^{1}H and ^{13}C NMR spectra were measured with a Bruker DRX-300 Avance spectrometer in DMSO-d_6 at 300 MHz and 75 MHz using TMS as internal standard. Chemical shifts are reported (δ) relative to TMS and coupling constant (J) is reported in hertz (Hz). Mass spectra were recoeded on a MS model 5973 Network apparatus at ionization potential of 70 eV. All other reagents were purchased from commercial sources and were freshly used after being purified by standard procedures.

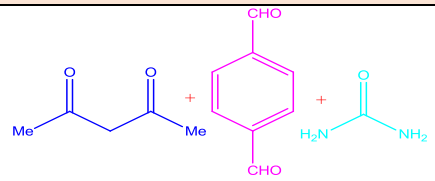
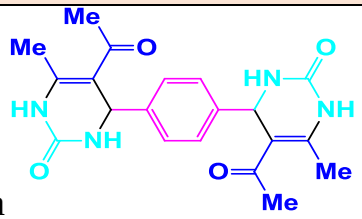
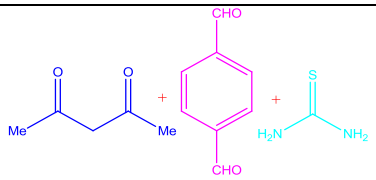
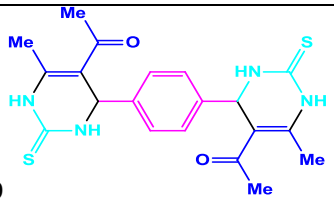
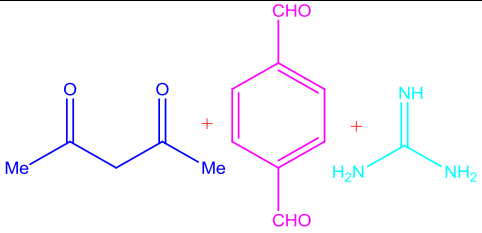
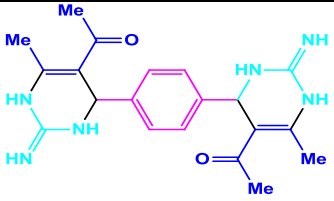
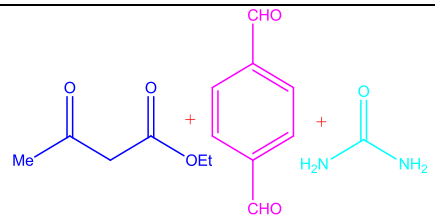
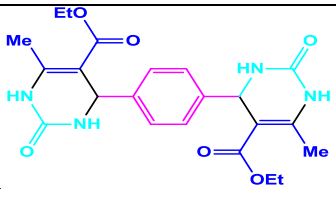
Typical procedure for the preparation of (4a-4g)

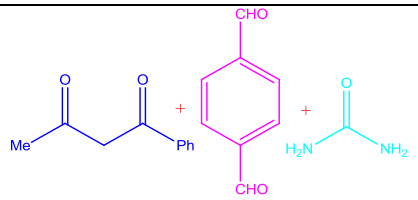
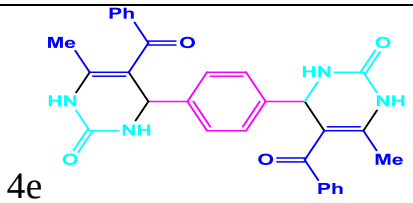
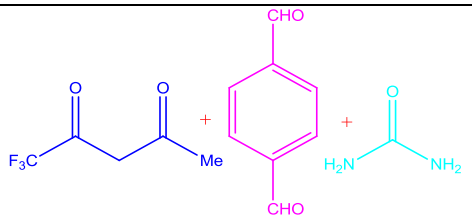
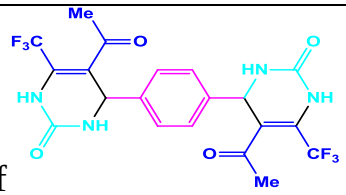
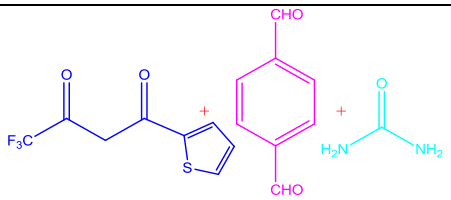
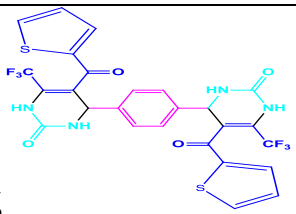
A mixture of dialdehyde (1 mmol), 1,3-dicarbonyl compounds (2 mmol), (thio)urea or guannidine (3 mmol) and nano zeolite (3 mol %) were mixed and sealed with a cap containing a septum. The loaded vial was then reacted in solvent free condition. After completion of the reaction (monitored by TLC, the ethyl acetate/n- hexane), reaction mixture was poured into cold water (25 ml) and stirred for 5-10 min. The precipitates were filtered and washed with cold water (2 ×15 ml) and then with 90% ethanol (2×10 ml) to give pure products. Products were characterized by analyzing their ^{1}H and ^{13}C NMR, and mass spectra

and their purity was confirmed by elemental analysis.

***Table* 1.** Nano zeolite catalyzed one- pot synthesis of compounds (4a-g) under solvent free conditions



Primary material	**product**	**Time (min)**	**Yield (%)**
	 4a	30	90
	 4b	25	85
	 4c	35	82
	 4d	48	90

	 4e	50	91
	 4f	55	83
	 4g	40	87

4.3.1. 4,4'-(1,4-phenylene)bis(5-acetyl-6-methyl-3,4-dihydropyrimidin-2(1H)-one) ***4a:*** Yield 91%, m.p. 315-317 °C (dec). IR (KBr): λ_{max} = 3279, 3103, 2923, 1706, 1601, 1326 cm^{-1}. ^{1}H NMR (DMSO-d_6) □: 9.17 (sbr, 2H, NH), 7.78 (sbr, 2H, NH), 7.18 (s, 4H, Ar), 5.21 (d, J=3 Hz, 2H, CH), 2.27 (s, 6H, COMe), 2.10 (s, 6H, Me). ^{13}C NMR (DMSO-d_6) □: 195.0, 152.9, 148.9, 144.3, 127.4, 110.5, 54.3, 31.3, 19.7. MS: (m/z) (%) 382 (M^+,7), 354(10), 259(17), 183(100), 155(45), 43(95).

4.3.2. 1,1'-(4,4'-(1,4-phenylene)bis(6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5,4-diyl)) diethanone **4b:** Yield 87%, m.p. 310-312 °C (dec). IR (KBr): λ_{max} = 3384, 3176, 2981, 1615, 1447 cm^{-1}. ^{1}H NMR (DMSO-d_6) □: 10.23 (s, 2H, NH), 9.66 (s, 2H, NH), 7.17 (s, 4H, Ar), 5.24 (s, 2H, CH), 2.29 (s, 6H, COM), 2.16 (s, 6H, Me). ^{13}C NMR (DMSO-d_6) □: 193.5, 174.6, 148.9, 144.2, 127.4, 110.6, 54.5, 31.2, 19.80. MS: (m/z) (%) 414 (M^+,6), 325(11), 314(17), 274(19), 140(94), 59(100).

4.3.3. 1,1'-(4,4'-(1,4-phenylene)bis(2-imino-6-methyl-1,2,3,4-tetrahydropyrimidine-5,4-diyl)) diethanone ***4c:*** *Yield 85%, m.p. 298-300°C* (dec). IR (KBr): λ_{max} = 3353, 3220, 2973,

1694, 1606, 1374 cm-^{1}. ^{1}H NMR (DMSO-d_6) □: 9.99 (sbr, 2H, NH), 7.95 (s 2H, NH), 7.21 (s, 4H, Ar), 6.28 (sbr, 2H, NH), 5.23 (s, 2H, CH), 2.23 (s, 6H, COMe), 2.06 (s, 6H, Me). ^{13}C NMR (DMSO-d_6): 193.4, 178.2, 154.3, 144.6, 127.6, 109.5, 53.2, 31.1, 20.1. MS: (m/z) (%) 380 (M^+,9), 351(11), 307(19), 267(25), 183(78), 59(81), 43(100). Anal. Calcd for $C_{20}H_{24}N_6O_2$: C, 63.14; H, 6.36; N, 22.09. Found: C, 63.41, H, 6.71; N, 21.97.

3. Results and discussion

The original Biginelli protocol for the preparation of the DHMPs consisted of heating a mixture of the three components (aldehyde, β-keto-ester, and urea) in ethanol containing a catalytic amount of HCl.[10] The major drawback associated with this protocol is the low yields, particularly for substituted aromatic and aliphatic aldehydes. This has led to the development of multi-step synthetic strategies, involving combinations of Lewis acids and transition metal salts, e.g. $BF_3.OEt_2$, polyphosphate esters, and reagents like CuI, $InCl_3$, $Mn(OAc)_3$, trimethylsilyltriflate, $LaCl_3.7H_2O$, $CeCl_3.7H_2O$, $LiClO_4$, $Yb(OTf)_3$, clays, etc. However, many of these methods involve expensive reagents, long reaction times, and stoichiometric amount of catalysts, and difficult to handle especially on a large scale. Therefore, the discovery of a new and an inexpensive catalyst for the preparation of dihydropyrimidin-2-(1*H*)-ones under mild and efficient conditions is of prime importance. For the increasing environmental and economic concerns in recent years, it is now essential for chemists to search environmentally benign catalytic reactions as many as possible. Here we wish to report the use of a catalytic agent, chlorotrimethylsilane in the Biginelli's reaction under microwave assisted solvent-free conditions. Table **1** summarizes the results for reactions of terephthalic aldehyde with various derivatives of **1** and **2.** We initially examined the reaction of acetylacetone (**1a)** with urea (**2a**) and terephthalic aldehyde (**3)** in the presence of Nano zeolite under microwave irradiation conditions at 100 ^{0}C . Experiments

showed that 15mol% quantities of catalyst are enough for the reaction to complete in less than 6 minutes (as indicated by TLC). Alternatively, in the absence of catalyst did not yield any product. The structure of product **4a** in 91% yield was elucidated by spectroscopy methods and its purity was confirmed by elemental analysis. The optimized conditions utilize a 1: 2: 3: 0.3 ratio of dialdehyde, 1,3-dicarbonyl compounds, thiourea and TMSCl (Table 2).

Table2. Cytotoxic activity of compounds assessed by the MTT reduction assay

Compound	HeLa cells		SKOV-3 cells		LS-180 cells		MCF7 cells		Raji cells	
	IC_{16} (μM)	IC_{50} (μM)	IC_{16} (μM)	IC_{50} (μM)	IC_{16} (μM)	IC_{50} (μM)	IC_{16} (μM)	IC_{50} (μM)	IC_{16} (μM)	IC_{50} (μM)
4a	55.0 ± 43.3	> 100	37.5 ± 10.7	> 100	> 100	> 100	24.1 ± 12.9	> 100	> 100	> 100
4b	5.0 ± 5.3	26.4 ± 9.2	7.2 ± 1.9	23.9 ± 1.8	4.0 ± 1.4	29.2 ± 4.5	17.2 ± 6.8	45.3 ± 12.6	15.1 ± 0.8	32.9 ± 1.6
4c	15.0 ± 5.2	68.9 ± 0.9	> 100	> 100	> 100	> 100	28.3 ± 19.0	> 100	28.6 ± 9.6	> 100
4d	12.5 ± 1.6	40.1 ± 1.2	> 100	> 100	> 100	> 100	35.0 ± 11.4	> 100	32.8 ± 6.5	> 100
4e	> 25	> 25	> 25	> 25	> 25	> 25	> 25	> 25	19.9 ± 2.4	>25
4f	21.7 ± 3.6	61.5 ± 3.9	14.9 ± 5.1	66.0 ± 5.9	7.9 ± 2.6	92.6 ± 17.9	19.8 ± 3.7	> 100	> 100	> 100
4g	42.4 ± 16.5	> 100	41.6 ± 10.8	> 100	> 100	> 100	> 100	> 100	> 100	> 100
Doxorubicin	0.023 ± 0.001	0.055 ± 0.004	0.062 ± 0.016	0.233 ± 0.118	0.024 ± 0.007	0.135 ± 0.038	0.009 ± 0.006	0.063 ± 0.020	0.016 ± 0.004	0.050 ± 0.008

Values represent the mean ± S.D. of at least 3 different experiments. The maximum concentration of the compound tested for cytotoxicity was 100 μM, except for G, which due to lower solubility, were tested at the maximum concentrations of 25 μM.

The procedure is shown to be equally efficient when thiourea is replaced urea or guanidine. In addition, it can be concluded from both ^{1}H NMR and ^{13}C NMR spectra of the product that the reaction is stereospecific loading to exclusive formation of one the meso or dl diastereoproducts from which the meso product is shown here for the simplicity.

Cytotoxicity activity

The data of cytotoxicity is present in Table 2. IC_{16} and IC_{50} were calculated for each compound. The most cytotoxic effect was seen on HeLa cell line; in this manner, all

compounds except compound G had IC_{16} lower than 100 µM (due to low solubility, the maximum concentration tested for compound G was 25 µM). the lowest activity was observed in Raji, since only one compound had IC_{50} lower than 100 µM .

As seen in table 2, compound **4b** showed the best activity among the synthesized compounds against the different cell lines. For instance, this compound was the only compound having IC_{50} of lower than 100 µM in Raji and MCF7 cell lines. Furthermore, in all cell lines, its IC_{16} and IC_{50} were the lowest compared to other ones. A structural analysis indicated that the key point was presence of sulfur instead of oxygen or nitrogen in C_2 position of pyrimidine ring. This implies that this position might play a role in cytotoxic effect of these compounds which can be a helpful hint for future designs.

4. Conclusions

In conclusion, we have presented the first synthesis of novel derivatives of bis(dihydropyrimidinone)benzenes using the efficient, easily available, and low quantities of an inexpensive catalyst under solvent free conditions. This method not only preserved the simplicity of Biginelli's one-pot condensation but also remarkably improved the yields (>85%) of dihydropyrimidinones in shorter reaction times as against the longer reaction times required for other catalysts after the addition of a low catalyst concentration. The procedure gives the products in good yields and avoids problems associated with solvent use (cost, handling, safety and pollution), and easy experimental work–up procedure, hence, it is a useful addition to the existing methods. Development of the method to one-pot synthesis of products containing two different dihydropyrimidinone units and determination of the stereochemistry of the products by X–ray crystallography are currently under investigation in our laboratories.

On the other hand, the cytotoxic assays of these derivatives in particular compound **4b** revealed that these compounds can be assumed as good scaffold for future design as cytotoxic

agents since the results showed that the presence of special group in specific position can cause a great improvement in cytotoxic effects.

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