APPLICATION OF SBA-15 FUNCTIONALIZED SULFONIC ACID (SBA-Pr-SO_3H) AS AN EFFICIENT NANOREACTOR IN THE ONE-POT SYNTHESIS OF PYRIDO[2,3-*d*]PYRIMIDINE

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ABSTRACT

Sulfonic acid functionalized nanoporous silica (SBA-Pr-SO_3H) with a pore size of 6 nm was synthesized using surface modification of Santa Barbara Amorphous (SBA-15). It is a solid acid hexagonally nano-reactor with high surface area and is reusable, high selective, non-corrosiveness which can easily isolate from the products. An efficient and green one-pot three component reaction was developed for the synthesis of pyrido[2,3-*d*]pyrimidine derivatives *via* a simple, mild and effective solvent free reaction of 4(6)-aminouracil, barbituric acid and aromatic aldehydes in the presence using SBA-Pr-SO_3H. In this reaction, SBA-Pr-SO_3H was an effective nano-catalyst which gave the products in good to high yields in a shorter reaction time compared to the other published reports. Some of the products were strong antibiotics against *Bacillus subtilis* and *Staphylococcus aureus*.

Keywords: SBA-Pr-SO_3H; Pyrido[2,3-*d*]pyrimidine compound; Green synthesis; Antibiotic.

INTRODUCTION

Synthesis of MCM-41 by Mobil Corporation scientists in 1992[1, 2] and then SBA-15 by Zhao et al. in 1994[3] were the first approaches to achieve mesoporous molecular sieves. SBA-15 is a hexagonal nanoporous silica with good accessibility due to its large pore size, high surface area, excellent chemically and thermally stability.[3] Surface modification of SBA-15 makes it an effective inorganic-organic hybrid which may be acting as a catalyst; up to day, different modified SBA-15 has been synthesized, including Ionic liquid supported SBA-15 (SBA-IL)[4], propyl amine functionalized SBA-15 (SBA-Pr-NH_2)[5], propyl thiol functionalized and propyl sulfonic acid functionalized SBA-15 (SBA-Pr-SH and SBA-Pr-SO_3H, respectively)[6] and so on. SBA-Pr-SO_3H is prepared by the reaction of silanol groups on the SBA-15 surface with 3-mercaptopropyltrimethoxysilane following oxidation of thiol groups.[6] It maintains as a hexagonal structure after modification and can act as a solid acid nanoreactor; therefore, it is a heterogeneous, non-corrosiveness and reusable solid acid catalyst, which can isolate from the products easily.[7] SBA-Pr-SO_3H has been applied as an effective nanoreactor in the multi-component reactions.[6-12]

The increase in bacterial strains that are resistant to antibacterial therapies has prompted the development of drugs to treat bacterial diseases. One of the usual strategies to address bacterial drug resistance is the synthesis of new compounds which modify to common antibacterial. Heterocyclic compounds with pyrimidine skeleton are important class of chemical products since they have wide variety of potentially biological activities, especially antimicrobial properties.[13-15] In addition, the pyridopyrimidine structure and their derivatives are famous pharma core in the drug design.[16] In connection with such studies, the present paper reports the synthesis of pyrimidine-fused heterocycles, with antibacterial properties in the presence of SBA-Pr-SO_3H as an efficient nanoreactor.

EXPERIMENTAL

The chemicals employed in this work were obtained from Merck Company and were used with no purifications. IR spectra were recorded from KBr disk using a FT-IR Bruker Tensor 27 instrument. Melting points were measured by using the capillary tube method with an electro thermal 9200 apparatus. The ^{1}H NMR (250 MHz) was run on a Bruker DPX. Mass analysis was performed on a model: 5973 network Agilent. Weight change curve in nitrogen was measured on a TA instrument of TGA Q50 V6.3 with maximum heating rate of 20 °C/min. Nitrogen adsorption and desorption isotherms were measured at -196 °C using a Japan Belsorb II system after the samples were vacuum dried at 150 °C overnight. SEM analysis was performed on a PhilipsXL-30 field-emission scanning electron microscope operated at 16 kV while TEM was carried out on a Tecnai G^2 F30 at 300 kV.

Preparation of SBA-15 and SBA-Pr-SO_3H

The synthesis of SBA-15 and its modification to SBA-Pr-SO_3H was carried out in accordance with our earlier reports.[17-19]

General procedure for the preparation of the products

The SBA-Pr-SO_3H (0.02 gr) was activated in vacuum at 100 °C and then after cooling of catalyst to room temperature, barbituric acid (0.128 g, 1 mmol), uracil (0.127 g, 1mmol) and aryl aldehydes (1 mmol) were added to it. The mixture was heated in an oil bath (140 °C) in appropriate time as shown in Table 2. After completion of the reaction, which was monitored by thin layer chromatography (TLC), the crude product was dissolved in hot EtOH and H_2O and then the mixture was filtered for removing the solid catalyst. The cooled filtrate gave the pure product. The solid acid catalyst subsequently was washed with a diluted acid solution, distilled water and then acetone, dried under vacuum that it can be used for several times without loss of significant activity.

Selected Spectral Data

5-(4-OH)C_6H_4-3,5,7,9,10-pentahydro-(1H)-pyrimido[5′,4′:5,6]pyrido[2,3-d]pyrimidine-2,4,6,8-tetrone (1d)

IR (KBr): υ_{max} = 3270, 3191, 2923, 2816, 1718, 1667, 1612, 1530, 1448, 1407 and 1347 cm^{-1}. ^{1}H NMR (250 MHz, DMSO): d_H = 5.21 (s, 1H, CH), 5.81 (s, 1H, OH), 6.6 (m, 2H, ArH), 6.72 (bs, 2H, NH), 6.9 (m, 2H, ArH), 11.2 (d, 3H, NH) ppm. MS (m/e): 341 (M^+, 1 %), 231 (40 %), 128 (90 %), 107 (90 %), 94 (100 %).

5-(2-OMe)C_6H_4-3,5,7,9,10-pentahydro-(1H)-pyrimido[5′,4′:5,6]pyrido[2,3-d]pyrimidine-2,4,6,8-tetrone (1e)

IR (KBr): υ_{max} = 3389, 3193, 2969, 1711, 1664, 1606, 1456 and 1389 cm^{-1}. ^{1}H NMR (250 MHz, DMSO): d_H = 3.63 (s, 3H, OCH_3), 5.25 (s, 1H, CH), 6.5 (bs, 2H, NH), 6.82 (d, 1H, ArH), 7.1 (t, 3H, ArH), 10.39 (d, 3H, NH) ppm. MS (m/e): 355 (M^+, 4 %), 353 (10 %), 322 (30 %), 213 (100 %), 170 (95 %), 143 (100 %), 127 (98 %).

5-(3-OMe)C_6H_4-3,5,7,9,10-pentahydro-(1H)-pyrimido[5′,4′:5,6]pyrido[2,3-d]pyrimidine-2,4,6,8-tetrone (1f)

IR (KBr): υ_{max} = 3310, 3190, 2950, 2850, 1711, 1610, 1466 and 1385 cm^{-1}. ^{1}H NMR (250 MHz, DMSO): d_H = 3.6 (s, 3H, OCH_3), 5.25 (s, 1H, CH), 6.5 (bs, 2H, NH), 6.8 (d, 2H, ArH), 7.1 (t, 2H, ArH), 10.4 (d, 3H, NH) ppm. MS (m/e): 355 (M^+, 1 %), 353 (5 %), 213 (100 %), 170 (50 %), 143 (95 %), 127 (50 %).

5-(2,3-OMe_2)C_6H_4-3,5,7,9,10-pentahydro-(1H)-pyrimido[5′,4′:5,6]pyrido[2,3-d]pyrimidine-2,4,6,8-tetrone (1h)

IR (KBr): υ_{max} = 3305, 3196, 2990, 2850, 1710, 1618, 1472, 1384 and 1053 cm^{-1}. ^{1}H NMR (250 MHz, DMSO): d_H = 3.6 (6H, OCH_3), 5.25 (s, 1H, CH), 6.5 (bs, 2H, NH), 6.8 (d, 2H, ArH), 7.1 (t, 1H, ArH), 10.4 (d, 3H, NH) ppm. MS (m/e): 385 (M^+, 1 %), 243 (100 %), 214 (95 %), 142 (70 %), 128 (80 %).

RESULT AND DISCUSSION

In this manuscript, 5-aryl-3,5,7,9,10-pentahydro-(1*H*)-pyrimido[5′,4′:5,6]

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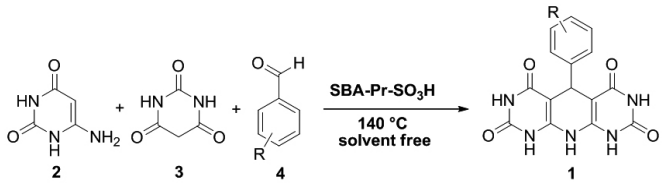
pyrido[2,3-*d*]pyrimidine-2,4,6,8-tetrones **1** were synthesized *via* a simple, mild and effective solvent free reaction of 4(6)-aminouracil **2**, barbituric acid **3** and aromatic aldehydes **4** in the presence of solid acid nanoreactor (SBA-Pr-SO_3H) (Scheme 1 and Fig. 1). In this reaction, solvent free system was the best condition with high yield of the product in a shorter reaction time (Table 1). For generality investigation of this method, benzaldehyde derivatives with electron withdrawing and donating groups were subjected to this reaction and the products were obtained in appropriate times and yields as shown in Table 2. After completion of the reaction (monitored by TLC), the crude product was poured in hot EtOH and H_2O until no solid were observed, then, the heterogeneous solid acid catalyst was removed by a simple filtration and after cooling the filtered, the pure crystal of the product was obtained.



Scheme 1. Synthesis of pyrido[2,3-*d*]pyrimidine

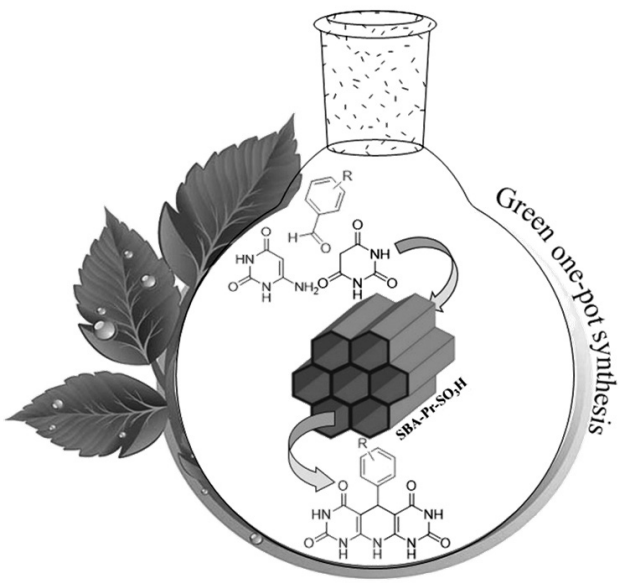


Fig. 1. SBA-Pr-SO_3H acts as nanoreactor.

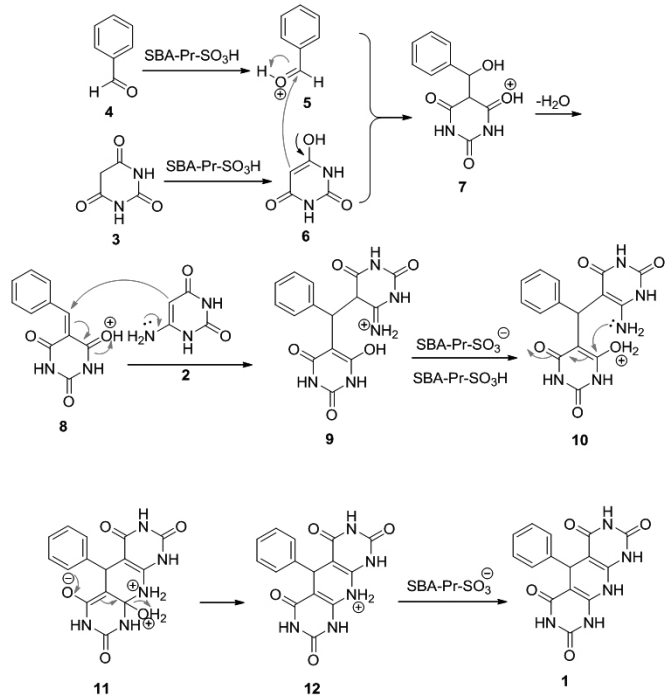
Table 1. The optimization of reaction conditions in the synthesis of **1a**.

No.	Solvent	Cat.	Time (h)	(C°) Temp.	(%) Yield
1	EtOH	SBA-Pr-SO_3H	1.5	80	50
2	H_2O	SBA-Pr-SO_3H	1.5	100	78
3	-	SBA-Pr-SO_3H	1.5	140	90
4	-	-	1.5	140	30

The most probable mechanism for this reaction is shown in Scheme 2. Initially, the solid acid catalyst protonates the carbonyl group of the aldehyde **4**. Then, barbituric acid **6** condenses with **5** to give intermediate **8** which is attacked by 6-aminouracil to produce **10**. Furthermore, an intra-Michael addition of the amino group of uracil to the barbituric moiety affords cyclization product **11** which after dehydration results in the formation of desired product **1**.

Table 2. SBA-Pr-SO_3H catalyzed the synthesis of **1a-k** under solvent free condition.

No.	Product	R	Time (h)	Yield (%)	mp (ºC)	Ref.
1	1a	H	1.5	90	160-162	[16]
2	1b	4-Cl	2	85	253-256	[21]
3	1c	2-OH	2.5	80	<300	[16]
4	1d	4-OH	2	82	242-244	-
5	1e	2-OMe	3	85	286-289	-
6	1f	3-OMe	4.5	65	276-279	-
7	1g	4-OMe	1	80	277-278	[21]
8	1h	2,3-$(OMe)_2$	2.5	45	264-266	-
9	1i	3-NO_2	2	84	239-241	[21]
10	1j	4-NO_4	3.5	65	216-218	[22]

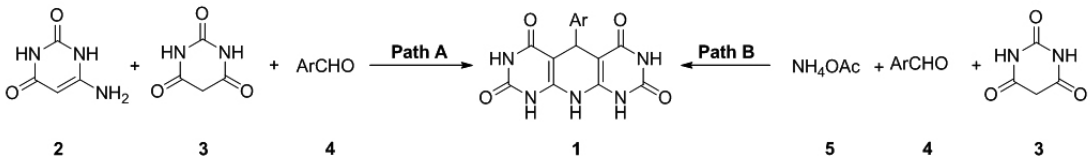


Scheme 2. Proposed mechanism

There are only two paths for the synthesis of pyridopyrimidine **1** in the literature (Scheme 3). As it illustrated, the present procedure (Path A) contains the reaction of 4(6)-aminouracil, barbituric acid and aromatic aldehydes, whereas in the second path in the literature (Path B), ammonium acetate was used instead of the 4(6)-aminouracil. Mosslemin *et al.* applied the path A (Table 3, Entry 2); the products were obtained in the presence of piperidine under ultrasonic condition (US) and heating.[21] The use of *p*-toluene sulfonic acid (*p*-TSA) as a catalyst (Entry 3, Table 3) did not diminish the reaction time.[23] In comparison with these published work,[21,22] the present methodology was performed using a non-toxic and efficient catalyst with high yield product. The other existing methods were conducted by Path B. Based on the Entry 4 in Table 3, the product **1a** was obtained without the use of catalyst in a longer reaction time.[21] However, in another publication (Entry 5, Table 3),[16] the reaction time is shorter due to the assistance of microwave (MW) irradiation. Nevertheless, the present methodology has several advantages such as a shorter reaction time without applying of the US or MW, in addition, SBA-Pr-SO_3H is a non-toxic and reusable catalyst. Thus, this approach is a greener reaction with a simple synthesis, easy work-up, and good yields of the high purity products.

Table 3. Comparison of different conditions in the synthesis of **1a**

Entry	Catalyst	Solvent	Condition	Time	Yield (%)	Year
1	SBA-Pr-SO_3H	-	140 ºC	1.5 h	90	This Work
2	Piperidine	H_2O	US)))/60 ºC	1 h	87	2009[20]
3	*p*-TSA	H_2O	reflux	4 h	84	2009[22]
4	-	H_2O:EtOH	reflux	10 h	85	2012[21]
5	-	H_2O	MW	2 min	82	2007[16]



Scheme 3. The two paths synthesis of pyridopyrimidine **1**

Investigation of the catalyst reusability

As mentioned before, SBA-Pr-SO_3H is a heterogeneous catalyst which can be separated from the reaction mixture by a simple filtration. The reusability of the catalyst was investigated to prove its applicability. The collected catalyst, obtained from all derivatization reactions, was washed with hot EtOH to remove impurities. Afterward, it was reactivated by diluted HCl, and then, washed with water and acetone, respectively. The dried catalyst was reused in the model reaction and the yield of the product showed no significant change.

Antimicrobial activity

All synthesized compounds were screened for antimicrobial activity using the disc diffusion methods. The microorganisms that used in this study were *Pseudomonas aeruginosa* ATCC 85327 and *Escherichia coli* ATCC 25922 (Gram-negative bacteria), *Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 465 (Gram positive bacteria), and *Saccharomyces cerevisiae* ATCC 9763 and *Candida albicans* ATCC 10231 (fungi). All products were dissolved in DMSO (100 µg/ml), and 25 µl was loaded onto 6-mm paper discs. One hundred microliters of 10^9 cell/ml suspension of the microorganisms was spread on sterile Mueller–Hinton agar plates, and the discs were placed on the surface of culture plates. The minimum inhibitory concentration (MIC) of the selected compounds which showed antibiotic activity in disc diffusion tests was also determined by microdilution method and compared with three commercial antibiotics: Chloramphenicol, Gentamicin, and Nystatin. Table 4 shows the inhibition zones (IZ) of the products around the discs. The highest antibacterial activity was observed from compound **1e** against *Staphylococcus aureus* and *B. subtilis* with MIC equal to 2 micrograms per milliliter.

Table 4 Antimicrobial activities of **1a-1k** against some Gram-positive and Gram-negative bacteria and fungi, as determined by disc diffusion (IZ = 250 µg/disc) and MIC methods.

	1a	**1b**	**1c**	**1d**	**1e**	**1f**	**1g**
	IZ/MIC	IZ/MIC	IZ/MIC	IZ/MIC	IZ/MIC	IZ/MIC	IZ/MIC
Bacillus subtilis (ATCC 465)	15/64	0/-	15/64	0/-	27/2	0/-	0/-
Staphylococcus aureus (ATCC 25923)	22/8	0/-	24/4	0/-	32/2	0/-	0/-
Escherichia coli (ATCC 25922)	11/256	0/-	0/-	0/-	0/-	0/-	0/-
Pseudomonas aeruginosa (ATCC 85327)	10/512	0/-	0/-	0/-	0/-	0/-	0/-
Candida albicans (ATCC 10231)	8/1024	0/-	0/-	0/-	0/-	0/-	0/-
Saccharomyses cerevisiae (ATCC 9763)	0/-	0/-	0/-	0/-	0/-	0/-	0/-
	1h	**1i**	**1j**	**Chloramphenicol**		**Gentamicin**	**Nystatin**
	IZ/MIC	IZ/MIC	IZ/MIC	IZ/MIC		IZ/MIC	IZ/MIC
Bacillus subtilis (ATCC 465)	0/-	8/>512	0/-	26/4		28/0.125	0/-
Staphylococcus aureus (ATCC 25923)	0/-	11/256	0/-	22/8		20/0.5	0/-
Escherichia coli (ATCC 25922)	0/-	0/-	0/-	24/4		20/0.5	0/-
Pseudomonas aeruginosa (ATCC 85327)	0/-	0/-	0/-	8/256		18/1	0/-
Candida albicans (ATCC 10231)	0/-	0/-	0/-	0/-		0/-	0/-
Saccharomyses cerevisiae (ATCC 9763)	0/-	0/-	0/-	0/-		0/-	23/4

Preparation of the catalyst

New nanoporous silica SBA-15 can be obtained by using commercially available triblock copolymer Pluronic P126 as a structure directing agent.[3, 24] As shown in Fig. 2, the SBA-15 silica was functionalized, using the grafting method, with (3-mercaptopropyl)trimethoxysilane (MPTS). Then, oxidation of the thiol groups to sulfonic acids was performed by hydrogen peroxide. TGA, BET and CHN methods were used for analyzing of the catalyst surface which demonstrated that the propyl sulfonic acids were immobilized into the pores. Using the BET method, pore volume of SBA-Pr-SO_3H was determined to 440 m^2g^{-1}, 6.0 nm and 0.660 cm^3g^{-1}, respectively, which are smaller than those of SBA-15 due to the functional sulfonosilane groups into the pores.[17] SEM image of SBA-Pr-SO_3H (Fig. 3) shows uniform particles about 1μm as same as SBA-15. It can be concluded that the morphology of solid was saved without change during the surface modifications. Furthermore, the TEM image reveals the parallel channels, which resemble the pores configuration of SBA-15. This indicates that the pore of SBA-Pr-SO_3H was not collapsed during two step reactions.

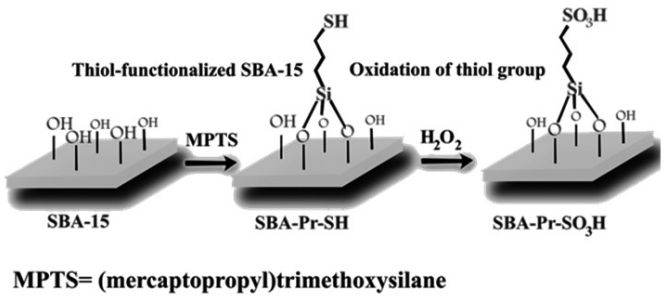


Fig. 2. Schematic illustration for the preparation of SBA-Pr-SO_3H

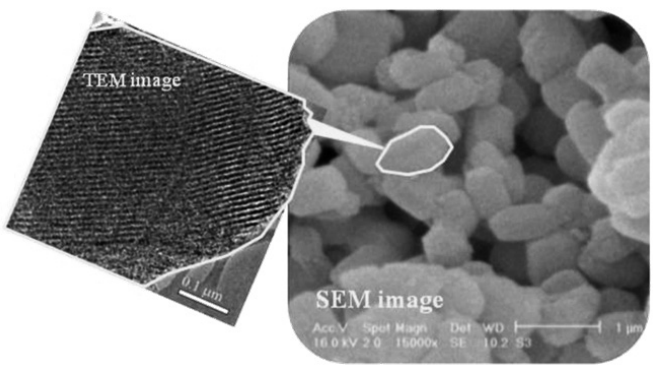


Fig. 3. SEM and TEM images of SBA-Pr-SO_3H

CONCLUSIONS

An efficient method for the synthesis of pyrido[2,3-*d*]pyrimidines has been developed in the solvent free reaction of barbituric acid, 6-aminouracil and aromatic aldehydes using recyclable and environmentally benign SBA-Pr-SO_3H as a solid acid nanoreactor. Some of the products were strong antibiotics against *Bacillus subtilis* and *Staphylococcus aureus*.

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