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A Green Synthesis of 1,5-Benzodiazepines using Reusable-Heterogeneous Silica Sulfuric Acid Catalyst under Solvent-Free Conditions and their Antileukemic Activity

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1,5-Benzodiazepine derivatives are readily assembled from *o*-phenylene diamine and ketones containg α-hydrogen atoms by means of simple cyclocondensation *via* sp^3 C-H activation promoted by an efficient heterogeneous silica sulfuric acid catalyst. Eco-friendliness, good yields, easy workup, reusable catalyst, short reaction times, high atom economy and solvent-free conditions are the noteworthy features of this protocol. These benzodiazepines are chosen for the evaluation of antiproliferative activity against different leukemia cell lines. Among the investigated compounds, **3g** is the best antiproliferative agent against all the cell lines tested. Also, current preliminary analysis showed that compound **3g** phosphorylates ERK1/2 and induces G1 arrest in K562 cells.

Keywords: Benzodiazepines, Heterogeneous, Solvent-free, Anticancer, Leukemia.

INTRODUCTION

1,5-Benzodiazepines and their functionalized derivatives are potent antioxidants, lipid peroxidation inhibitors, peripheral cholecystokinin (CCK-1) receptor agonists, cholecystokinin-B receptor antagonists-B, anxiolytic, anti-neuroinflammatory, anticancer, analgesic and/or anti-inflammatory, antimicrobial and antianxiety agents. They are also used in the treatment of epilepsy with other applications in the field of agriculture as herbicides and pesticides [1-9]. In addition, they are also used as dyes for acrylic fibers [10] and are part of some drugs triaxol (anti-streptococcal), clobazam (antiepileptic), clazapine and Y-931 (antipsychotic) [11,12].

As a consequence of their wide range of applications, several synthetic protocols are available for their synthesis. Namely, condensation of *o*-phenylene diamines with ketones in the presence of polyfluorinated-Zn(II) phthalocyanine complex immobilized on silica [13], $Er(OTf)_3$ [14], CH_3COOH [15], $Yb(OTf)_3$ [16], $Ga(OTf)_3$ [17], $AgNO_3$ [18] and Fe_3O_4 [19] catalysts are known. Alternatively, they are synthesized from alkynes and *o*-phenylene diamines by using gold complexes [20-22], Fe-NPS/SiO_2 [23] and $Hg(OTf)_2$ [24]. Another popular approach is condensation of *o*-phenylene diamines with chalcones in the presence of catalysts [25]. Among the aforementioned catalysts, only $Er(OTf)_3$ and $Yb(OTf)_3$ catalyze the condensation of *o*-phenylene diamines with ketones and also with chalcones to give 1,5-benzodiazepines. Recently, Weers *et al.* [26] and Climent *et al.* [27] reported the one pot synthesis of 1,5-benzodiazepines directly from *N*-allyl-2-bromo anilines and nitro compounds instead of *o*-phenylene diamines. Most of the above reported methods suffer from one or the other disadvantages such as poor substrate scope, laborious and complex work-up, use of expensive and hazardous reagents or catalysts, undesirable side products and unsatisfactory yields.

Literature survey highlighted that silica sulfuric acid (SSA) is an efficient heterogeneous catalyst attracts researchers because of its special features like reusability, its use under solvent-free condition and solid phase synthesis. It is widely

used in the synthesis of various biologically active heterocycles [28-31]. For instance, it is used in the synthesis of benzodiazepines [32,33]. This inspired us to use this catalyst for the synthesis of biologically important benzodiazepine compounds. Unfortunately, we did not get yields of compounds in appreciable amounts according to earlier reported protocols [32,33] and we improved it by modification of reported protocol.

Leukemia is one of the leading causes of cancer deaths worldwide and more diagnosed in children. Apart from the several advances in the surgical and irradiation treatment, chemotherapy still remains the backbone of treatment against cancer. Numerous pathways have been involved in the regulation of cancer. Among, ERK1/2 (extracellular signal-regulated kinase 1/2) cascades are one of the major pathways in mammals. ERK is one of the members of canonical MAPK family and centered on multiple signal transduction pathways to accomplish a variety of functions. Activation of ERK through different pathways leads to different cellular responses, including proliferation, differentiation, survival and memory consolidation [34-39]. The function of ERK1/2 in apoptosis is complex, depending on cell type, nature of death stimulus, duration of its activation and the activity of other signaling. Similarly, many cytotoxic drugs are known to induce a prolonged activation of ERK that is required for promoting apoptosis [40-43]. In addition ERK1/2 plays a major role in cell cycle progression. Furthermore, there are only few reports, which represents the anticancer activity of 1,5-benzodiazepines and no reports are available, which illustrates the anticancer activities of 1,5-benzodiazepines against leukemia. Consequently, we sought to explore the effect of these on cell proliferation in our ongoing research program [44-51]. In current study, the antiproliferative effect of 1,5-benzodiazepines against various leukemia cell lines is evaluated, which outlines an anticancer property of 1,5-benzodiazepines against leukemia.

EXPERIMENTAL

All chemicals were purchased from the Sigma-Aldrich. Silica sulfuric acid was prepared according to the reported procedure [52]. Purification of the reaction mass was carried out by normal column chromatography using SDFCL silica gel (60-120 mesh). Analytical thin layer chromatography was performed on merk silica gel 60 F_{254} plates. Visualization was accomplished with UV light and potassium permanganate. Melting points were recorded on a Thomas Hoover capillary melting point apparatus and are uncorrected. Infrared spectra were recorded on an ATI Mattson Genesis Series FT-Infrared spectrophotometer. Nuclear magnetic resonance spectra (^{1}H NMR, 400 MHz and ^{13}C NMR, 100 MHz) were recorded on Agilent NMR spectrometer and chemical shifts are given in ppm using DMSO-d_6 and $CDCl_3$ as solvent and tetramethylsilane as internal standard.

General procedure for the synthesis of 1,5-benzodiazepines (3): *o*-Phenylene diamine (**1a**, 1 mmol), ketone (**2**, 2 mmol) and silica sulfuric acid (0.5 g) were taken in a pestle and mortar and grinded well for a few minutes to obtain homogeneous reaction mixture in the case of solid starting material (if any one starting material was liquid, there is no need to grind). Homogeneous mixture was transferred to a round bottom flask equipped with condenser and stirred for 60 min at 120 ºC. The progress of the reaction was monitored by TLC. After the completion of reaction, the reaction mixture was diluted with dichloromethane and filtered through ordinary filter paper. The filtrate was washed with water and brine solution. The organic layer was concentrated under reduced pressure by using a rotary evaporator to afford the desired 1,5-benzodiazepine derivatives, which were purified by column chromatography using hexane: EtOAc (8:2) as eluent.

2-Methyl-2,4-di-*m*-tolyl-2,3-dihydro-1*H*-benzo[*b*][1,4]-diazepine (3a): Pale yellow crystals; yield 88%; (R_f = 0.38 in hexane/EtOAc:: 95:05 v/v); m.p.: 94-98 ºC; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 7.49 (d, *J* = 7.2 Hz, 1H), 7.37 (s, 2H), 7.31 (d, *J* = 7.6 Hz, 1H), 7.15-6.98 (m, 6H), 6.90 (d, *J* = 7.2 Hz, 1H), 6.83 (t, *J* = 7.6 Hz, 1H), 5.58 (s, 1H), 3.19 (d, *J* = 13.2 Hz, 1H), 2.82 (d, *J* = 13.6 Hz, 1H), 2.21 (s, 3H), 2.17 (s, 3H), 1.60 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ ppm: 166.42, 148.52, 139.96, 139.86, 138.89, 137.31, 137.09, 130.55, 129.07, 128.16, 128.09, 128.00, 127.31, 126.86, 126.43, 124.51, 123.10, 121.54, 120.09, 72.30, 43.06, 30.96, 21.63, 21.40; HRMS (ESI) $[M+H]^+$ calculated for $C_{24}H_{25}N_2$ 341.2017; found 341.2034.

2,4-*bis*(4-Chlorophenyl)-2-methyl-2,3-dihydro-1*H*-benzo[*b*][1,4]diazepine (3b): Yellow solid; yield 90%; (R_f = 0.36 in hexane/EtOAc:: 95:05 v/v); m.p.: 144-146 ºC; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 7.68 (d, *J* = 8 Hz, 2H), 7.49 (d, *J* = 8 Hz, 2H) 7.30 (d, *J* = 8.4 Hz, 2H), 7.20 (d, *J* = 8.4 Hz, 2H), 7.10 (d, *J* = 7.6 Hz, 1H), 7.0-6.99 (m, 2H), 6.82-681 (m, 1H), 5.79 (s, 1H), 3.37 (d, *J* = 13.6 Hz, IH), 2.79 (d, *J* = 13.6 Hz, 1H), 1.61 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 164.44, 147.30, 139.80, 138.48, 137.83, 134.89, 131.44, 129.75, 128.37, 128.13, 128.03, 126.95, 121.13, 119.91, 71.15, 42.65, 31.0; HRMS (ESI) $[M+H]^+$ calculated for $C_{22}H_{19}N_2Cl_2$ 381.0925; found 381.0926.

4,4′-(2-Methyl-2,3-dihydro-1*H*-benzo[*b*][1,4]diazepine-2,4-diyl)dibenzonitrile (3c): Yellow solid; yield 94%; (R_f = 0.38 in hexane/EtOAc:: 80:20 v/v); m.p.: 178-182 ºC; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 7.80 (d, *J* = 8.4 Hz, 2H), 7.70 (d, *J* = 8.4 Hz, 2H), 7.60 (q, J_1 = 8.8 Hz, J_2 = 5.2 Hz, 4H), 7.13 (d, *J* = 8.4 Hz, 1H), 7.07-7.03 (m, 2H), 6.82-6.78 (m, 1H), 6.15 (br, s, 1H), 3.61 (d, *J* = 14 Hz, 1H), 2.82 (d, *J* = 14 Hz, 1H), 1.66 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 163.37, 153.53, 143.66, 139.83, 136.63, 132.32, 132.18, 130.65, 127.88, 127.68, 127.36, 120.84, 119.60, 119.16, 119.08, 112.09, 109.62, 70.61, 42.81, 30.97; HRMS (ESI) $[M+H]^+$ calculated $C_{24}H_{19}N_4$ 363.1609; found 363.1612.

2-Methyl-2,4-*bis*(4-(trifluoromethyl)phenyl)-2,3-dihydro-1*H*-benzo[*b*][1,4]diazepine (3d): Yellow solid; Yield 92%; (R_f = 0.34 in hexane/EtOAc:: 95:05 v/v); m.p.: 104-108 ºC; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 7.78 (d, *J* = 8.4 Hz, 2H), 7.65 (d, *J* = 8 Hz, 2H), 7.54 (d, *J* = 8.4 Hz, 2H), 7.49 (d, *J* = 8 Hz, 2H), 7.13 (d, *J* = 8 Hz, 1H), 7.05-7.04 (m, 2H), 6.84-6.82 (m, 1H), 5.96 (s, 1H), 3.53 (d, *J* = 13.6 Hz, 1H), 2.86 (d, *J* = 13.6, 1H), 1.68 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ ppm: 164.39, 152.62, 143.26, 139.76, 137.33, 130.08, 129.91, 129.59, 127.88, 127.68, 127.35, 127.13, 125.91, 125.04,

125.0, 124.96, 121.03, 119.82, 71.45, 43.05, 30.72; HRMS (ESI) $[M+H]^+$ calculated for $C_{24}H_{19}N_2F_6$ 449.1452; found 449.4459.

2-Methyl-2,4-*bis*(3-nitrophenyl)-2,3-dihydro-1*H*-benzo-[*b*][1,4]diazepine (3e): White crystals; yield 90%; (R_f = 0.36 in hexane/EtOAc:: 85:15 v/v); m.p.: 158-160 ºC; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 8.32 (t, *J* = 2.0 Hz, 1H), 8.25 (t, *J* = 2 Hz, 1H), 8.10-8.04 (m, 2H), 7.94 (d, *J* = 9.2 Hz, 1H), 7.87 (d, *J* = 9.6 Hz, 1H), 7.50 (t, *J* = 7.6 Hz, 1H), 7.41 (t, *J* = 7.6 Hz, 1H), 7.15 (d, *J* = 7.6 Hz, 1H), 7.10-7.06 (m, 2H), 6.88-6.84 (m, 1H), 6.10 (s, 1H), 3.70 (d, *J* = 13.6 Hz, 1H), 2.85 (d, *J* = 13.6 Hz, 1H), 1.73 (s, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 163.74, 150.37, 148.09, 147.77, 141.07, 139.53, 137.23, 133.39, 133.17, 130.04, 129.98, 129.88, 127.52, 124.35, 121.77, 121.44, 121.18, 120.08, 71.82, 42.78, 30.64; HRMS (ESI) $[M+H]^+$ calculated for $C_{22}H_{19}N_4O_4$ 403.1406; found 403.1408.

4,4′-(2-Methyl-2,3-dihydro-1*H*-benzo[*b*][1,4]diazepine-2,4-diyl)*bis*(*N*,*N*-dimethylaniline) (3f): White solid; yield 89%; (R_f = 0.42 in hexane/EtOAc:: 95:05 v/v); m.p.: 136-140 ºC; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 7.76 (d, *J* = 9.2 Hz, 1H), 7.67 (d, *J* = 9.2 Hz, 2H), 7.35 (d, *J* = 8.8 Hz, 2H), 6.92-6.89 (m, 2H), 6.68 (d, *J* = 9.2 Hz, 1H), 6.59 (d, *J* = 8.8 Hz, 4H), 5.24 (s, 1H), 2.91 (s, 6H), 2.88 (d, *J* = 6.8 Hz, 2H), 2.79 (s, 6H), 1.53 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ = 166.08, 151.6, 149.53, 140.42, 140.06, 137.22, 135.36, 130.59, 128.85, 128.21, 127.39, 126.29, 125.32, 121.74, 120.38, 117.71, 114.97, 112.45, 111.48, 111.07, 71.95, 42.11, 40.74, 31.98, 31.02; HRMS (ESI) $[M+H]^+$ calculated for $C_{26}H_{31}N_4$ 399.2548; found 399.2532.

2-Methyl-2,4-di(thiophen-2-yl)-2,3-dihydro-1*H*-benzo-[*b*][1,4]diazepine (3g): Pale yellow crystals; yield 92%; (R_f = 0.40 in hexane/EtOAc:: 95:05 v/v); m.p.: 90-92 ºC; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 7.66 (d, *J* = 4.8 Hz, 1H), 7.60 (d, *J* = 2.8 Hz, 1H), 7.13-7.11 (m, 2H), 6.84-6.80 (m. 2H), 6.69 (d, *J* = 8.4 Hz, 1H), 6.55-6.50 (m, 3H), 4.55 (s, 1H), 3.20 (d, *J* = 13.6 Hz, 1H), 2.29 (d, *J* = 13.6 Hz, 1H), 1.61 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 161.14, 146.90, 140.30, 139.71, 135.46, 130.86, 129.66, 128.30, 127.11, 125.26, 125.19, 122.95, 120.07, 118.53, 116.51, 114.95, 107.21, 79.76, 44.19, 17.61; HRMS (ESI) $[M+H]^+$ calculated for $C_{18}H_{17}N_2S_2$ 325.0833; found 325.0836.

2-Methyl-2,4-*bis*(5-methylfuran-2-yl)-2,3-dihydro-1*H*-benzo[*b*][1,4]diazepine (3h): White solid; yield 90%; (R_f = 0.38 in hexane/EtOAc:: 95:05 v/v); m.p.: 94-94 ºC; ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 7.04 (d, *J* = 8.8 Hz, 1H), 6.93-6.84 (m, 4H), 6.18 (d, *J* = 4 Hz, 1H), 6.03 (d, *J* = 3.2 Hz, 1H), 5.85 (d, *J* = 4 Hz, 1H), 5.28 (s, 1H), 2.89 (d, *J* = 13.6 Hz, 1H), 2.70 (d, *J* = 13.6 Hz, 1H), 2.30 (s, 3H), 2.15 (s, 3H), 1.52 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 158.45, 156.90, 155.30, 152.61, 150.43, 140.11, 139.09, 127.94, 125.83, 122.14, 121.21, 115.14, 109.12, 106.41, 105.64, 69.66, 28.68, 14.03, 13.76; HRMS (ESI) $[M+H]^+$ calculated for $C_{20}H_{21}N_2O_2$ 321.1603; found 321.1604.

Cytotoxicity assay: Cell survival was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay as described [53]. This is based on the ability of viable cells to metabolize a yellow tetrazolium salt to violet formazan. Briefly, exponentially growing K562 cells (1×10^4 cells/well) were plated in triplicates and incubated with 2, 4 and 8 μM of compound **3g**. Cells were harvested after 48 h of treatment and incubated with MTT (5 mg/mL) at 37 ºC. The blue MTT formazan precipitate was then solubilized in detergent (50% final concentration of *N,N*-dimethylformamide and 10% of sodium dodecyl sulphate). The absorbance was measured at 570 nm using ELISA plate reader. The mean absorbance of culture medium was used as the blank and was subtracted. IC_{50} values (concentration of compound causing 50% inhibition of cell growth) were estimated after 48 h of compound treatment.

Western blot analysis: To examine the effect of compound **3g** on the expression of ERK1/2, cell lysates were prepared as described earlier with slight modifications [53]. Cells with or without treatment were harvested and lyzed using RIPA buffer as per the manufacturer's protocol with protease inhibitor cocktail and cleared by centrifugation at 10,000 rpm for 10 min at 4 ºC. Protein concentrations were determined by Bio-Rad Protein Assay and equal amount of protein (50 μg) was electrophoresed on SDS-polyacrylamide gels and transferred onto PVDF membrane. Membranes were blocked with 5% milk in Tris-buffered saline containing Tween-20 before probing with primary antibody according to the instructions of the manufacturer. Subsequently, the membranes were incubated with the corresponding horseradish peroxidase conjugated secondary antibody for 1 h. Protein bands were detected by enhanced ECL reagent (Thermo Scientific) and visualized by BioRad Imaging system. For reprobing, blots were stripped with Restore Western Blot stripping buffer (Thermo-Scientific).

Cell cycle analysis: Cellular DNA content was measured by flow cytometry as described earlier [53]. Approximately 0.75×10^5 cells/mL were cultured and treated with 2, 4 and 8 μM concentrations of compound **3g**. The cells were harvested after 24 h of treatment, washed, fixed in 70% ethanol and incubated with RNase A (Sigma-Aldrich, USA). Propidium iodide (50 μg/mL, Sigma-Aldrich, USA) was added 0.5 h before acquiring the flow cytometric reading (FACScan, BD Biosciences, USA). A minimum of 10,000 cells were acquired per sample and histograms were analyzed by using WinMDI 2.8 software.

RESULTS AND DISCUSSION

Initially, we considered the reaction of *o*-phenylene diamine (**1a**) with acetophenone (**2a**) on 0.1 g SSA per mmol of *o*-phenylene diamine, which afforded a product in trace amount (entry 1, Table-1). After the increase of reaction temperature and time, the yield of same product was improved to 20% (entries 2-4) which was characterized as 2-methyl-2,4-di-*m*-tolyl-2,3-dihydro-1*H*-benzo[*b*][1,4]diazepine (**3a**). On increasing the quantity of silica sulfuric acid (SAA) (entries 5-11), the highest yield (90%) was obtained by 0.5 g of SAA/mmol of *o*-phenylene diamine. Further increase in reaction tempearature reduced the yields of compound **3a** (entries 12-14). Finally, use of solvents lowered the yields of compound **3a** (entries 15-19).

With this optimized reaction condition, we next investigated the substrate scope by using various methyl ketones **2** bearing electron donating and withdrawing groups, which afforded respective benzodiazepines **3b-h** in 89-94% yields

TABLE-1
OPTIMIZATION OF REACTION CONDITIONS
FOR THE SYNTHESIS OF COMPOUND **3a**

Entry	SSA (g)	Temp. (°C)	Time (min)	Yield[b] (%)
1	0.1	100	30	trace
2	0.1	110	40	10
3	0.1	120	50	20
4	0.1	120	60	20
5	0.2	120	60	25
6	0.3	120	60	50
7	0.4	120	60	75
8	0.5	120	60	90
9	0.6	120	60	88
10	0.7	120	60	80
11	0.8	120	60	74
12	0.5	130	70	89
13	0.5	140	80	86
14	0.5	150	90	78
15	H_2O	Reflux	60	35
16	EtOH	Reflux	60	40
17	EtOAc	Reflux	60	25
18	CH_3CN	Reflux	60	30
19	DMSO	Reflux	60	35

[a]Reactions were performed with *o*-PD **1a** (144 mg, 1 mmol), ketone **2a** (240 mg, 2 mmol) were used to afford the **3a**; [b]Isolated yield after purification by column chromatography.

(**Scheme-I**). In general, acetophenones substituted with electron-withdrawing groups gave higher yields than those containing electron-donating substituents. Also, heteroarylmethyl ketones furnished corresponding products **3g** and **3h** in 92 and 90% yield respectively. The probable mechanism for the formation of benzodiazepines **3** is similar to the reported method [18]. The structure of one of the benzodiazepines, **3a** was confirmed by single crystal X-ray diffraction studies and its ORTEP diagram is given in Fig. 1.

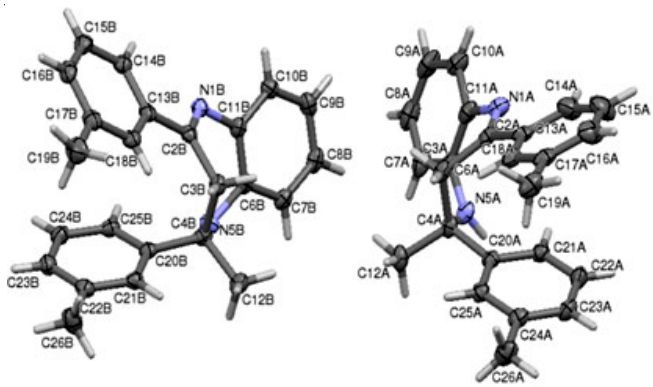
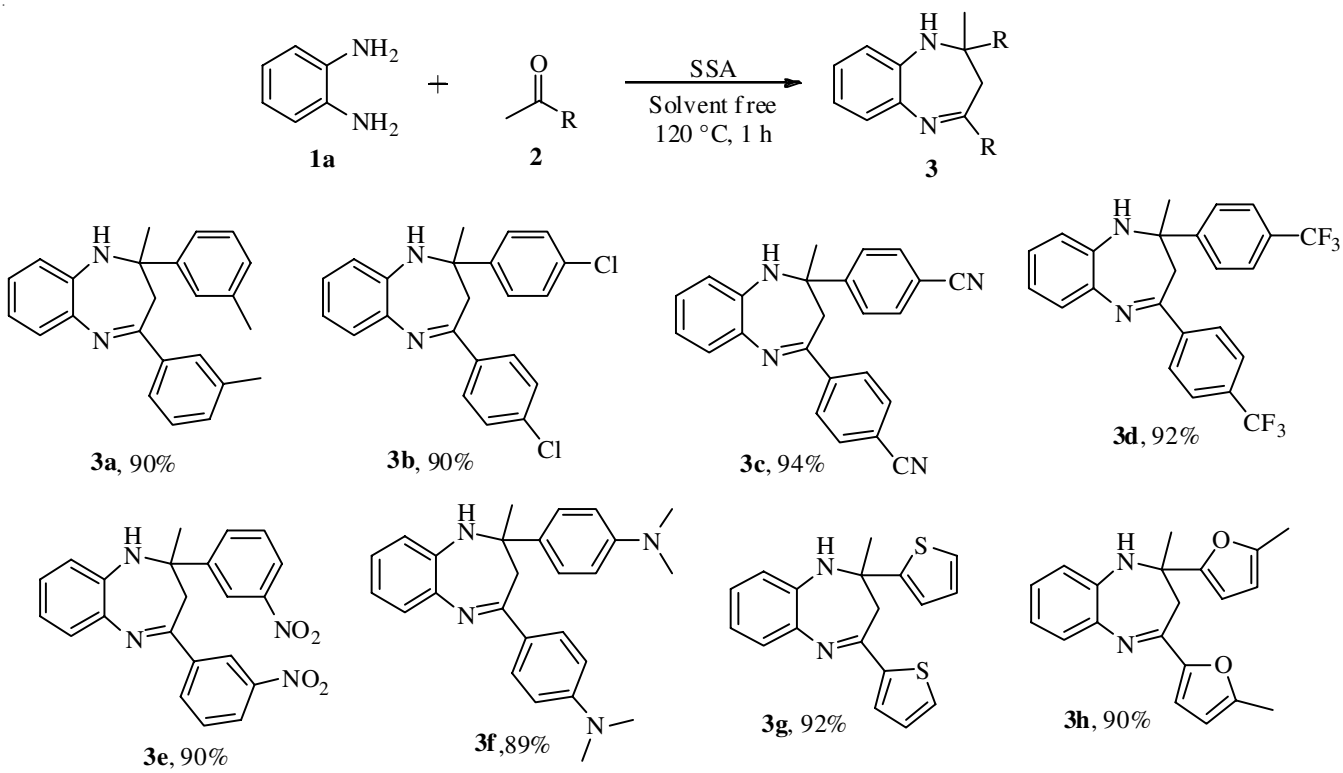


Fig. 1. Single crystal XRD of compound **3a** (CCDC 1821865)

Pristine silica gel showed a broad signal in the 2θ range of 20-28º (black curve, Fig. 2) which suggest that it is polycrystalline in nature. On the other hand, SSA showed almost same pattern (red curve, Fig. 2) indicating that no change even after the addition of surface sulfonic acid (SO_3H) groups. In addition, silica sulfuric acid (SSA) (after reuse three times) also showed similar pattern (blue curve, Fig. 2), pointing out that the catalyst remain unaltered even after the reaction, which is of major concern in catalysis reactions. Later, we conducted FT IR analysis of pristine silica gel (black curve, Fig. 3), SSA before and after use (red and green curve, respectively, Fig. 3), which suggested that the catalyst surface remain unaltered even after the reaction.

Finally, we investigated the reusability of the catalyst for the synthesis of compound **3a** (Table-2), which is of great



Scheme-I: Synthesis of 1,5-benzodiazepines from ketones

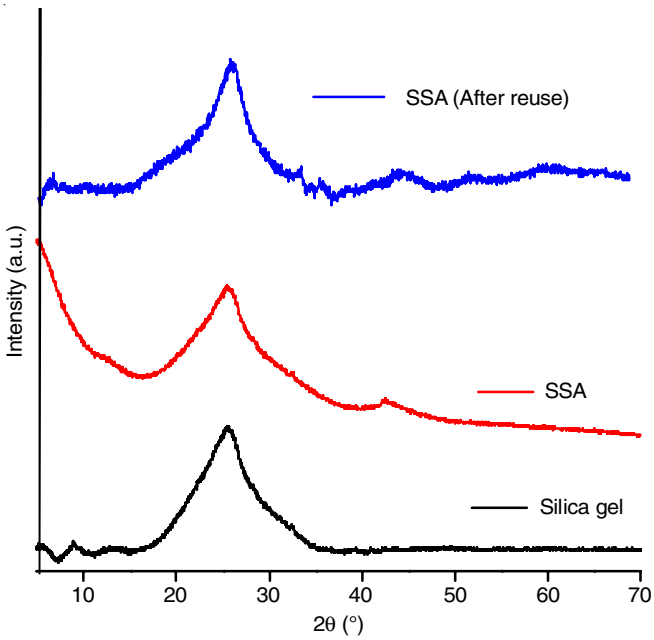


Fig. 2. PXRD patterns of silica gel, silica sulfuric acid before and after reuse

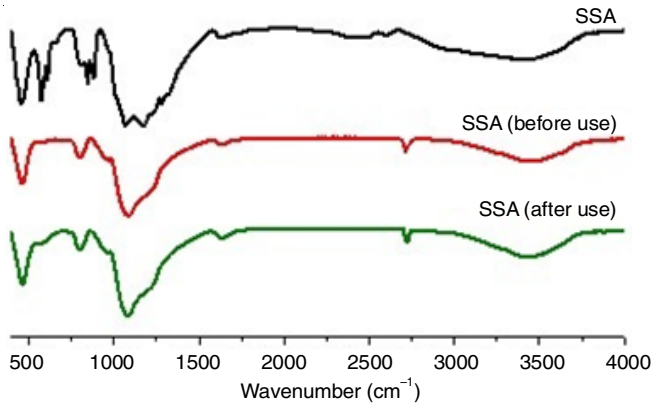


Fig. 3. FTIR spectra of silica gel, silica sulfuric acid before and after use

TABLE-2
REUSABILITY OF CATALYST

Number of runs	1	2	3	4
Product (%)	90	90	88	86

importance for any heterogeneous catalysis reactions. The catalyst was recovered after each run by washing with acetone and drying at 100 ºC for 30 min prior to each run. It was observed that there was no loss in the yield of compound **3a** for the first two cycles, thereafter the yield reduced very slightly. Thus, the catalyst has retained its catalytic activity up to four runs and it was confirmed by XRD.

Finally, we investigated the cytotoxic ability of selected benzodiazepine derivatives **3a-h** against different leukemia cell lines *viz.* HL60, THP1, OCI/A ML3, K562 and KU812 (Table-3). Amongst, compound **3g** was found to be the most potent cytotoxic agent with IC_{50} value of 1.8 ± 0.4 to 4.2 ± 1.0 µM against all cell lines. Further, it is found that compound **3g** activates ERK1/2 (Fig. 4) and induces cell cycle arrest at G1 phase in CML K562 cells in a dose-dependent manner (Fig. 5).

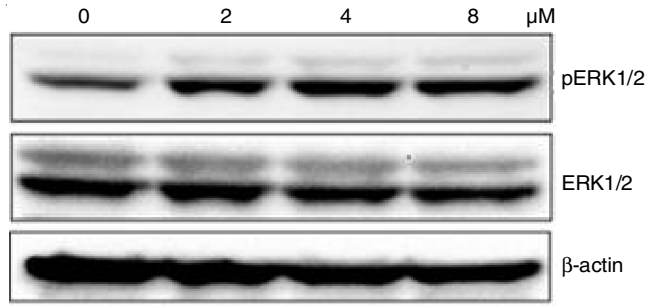


Fig. 4. Compound **3g** activates ERK1/2

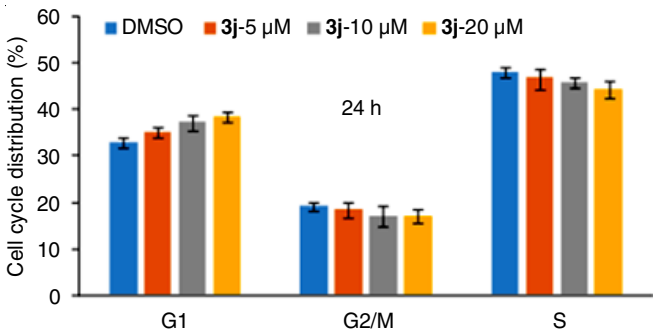


Fig. 5. Compound **3g** induces G1 arrest by declining G2/M and S phases in a dose-dependent manner

Conclusion

An efficient and environmentally benign protocol for the synthesis of 1,4-benzodiazepines from readily available precursors like *o*-phenylene diamine and aromatic/aliphatic/alicyclic ketones using heterogeneous reusable catalyst heterogeneous silica sulfuric acid (SSA) *via* simple cyclocondensation under

TABLE-3
IC_{50} VALUES OF SELECTED 1,5-BENZODIAZEPINE DERIVATIVES DETERMINED BY MTT ASSAY[a]

Entry	3	IC_{50} (µM)				
		HL60	THP1	OCI/AML3	K562	KU812
1	**3a**	58.18 ± 1.6	>250	88.64 ± 1.8	>250	39.01 ± 1.2
2	**3b**	36.11 ± 1.9	66.39 ± 1.3	41.78 ± 1.2	69.73 ± 1.1	41.31 ± 1.2
3	**3c**	26.32 ± 1.1	393.2 ± 1.5	>250	>250	15.31 ± 1.0
4	**3d**	34.87 ± 1.7	52.68 ± 0.4	55.76 ± 3.0	127.0 ± 2.3	31.59 ± 0.7
5	**3e**	68.34 ± 1.5	155.6 ± 0.9	>250	>250	1.076 ± 0.5
6	**3f**	51.85 ± 0.9	148.8 ± 2.4	126.7 ± 2.1	>250	79.15 ± 1.2
7	**3g**	4.20 ± 1.0	2.0 ± 0.8	1.9 ± 0.4	1.8 ± 0.2	1.8 ± 0.4
8	**3h**	71.56 ± 2.1	153.4 ± 2.5	130.4 ± 1.4	189 ± 1.7	51.19 ± 1.5

[a]The experiments were run in triplicate. Each value represents mean ± SD.

solvent-free condition is reported. This methodology demonstrated an application of SSA for the synthesis of a library of bioactive heterocycles. Preliminary investigations on cytotoxic effect of 1,5- benzodiazepine derivatives revealed the importance of thiophene ring in association with benzodiazepine moiety. Interference of compound **3g** in ERK1/2 pathway with G1 phase cell cycle arrest provides a potential insight to carry out further investigations to elucidate its anticancer property.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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