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One-Pot Synthesis and Characterization of 3,4-Dihydropyrimidin-2(1*H*)-one Derivatives using Sulphonated Reduced Graphene Oxide Nanoparticles *via* Biginelli Cyclocondensation and its Cytotoxic Studies

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A facile one-pot synthesis of 3,4-dihydropyrimidin-2(1*H*)-ones (**4a-h**) was performed in the presence of sulphonated reduced graphene oxide nanoparticles (SrGO NPs), using various substituted aromatic aldehydes (**1a-h**), β-keto ester (**2**) and urea (**3**). Methanol solvent mediated the reaction, under refluxing conditions. The synthesized SrGO NPs were characterized using XRD, SEM-EDS and Raman spectroscopic methods. The structural moieties of the organic compounds were confirmed by ^{1}H NMR, ^{13}C NMR, FTIR and Mass spectroscopic techniques. The *in vitro* anticancer activity of the synthesized derivatives (**4a-h**) was investigated using MTT (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric assay as per American Type Culture Collection protocol. The cytotoxic studies were conducted against MCF-7, SKNSH human cells, using doxorubicin as the standard. Majority of the derivatives have exhibited superior anticancer activity.

Keywords: One-pot synthesis, Dihydropyrimidones, Anticancer activity.

INTRODUCTION

Dihydropyrimidinones (DHPMs, Fig. 1) have been known to be versatile organic derivatives that can exhibit numerous biological profiles like, calcium channel blockers, antitumor, antiviral, antibacterial, anti-inflammatory activity [1], thrombin inhibition [2], tissue factor VIIa inhibition [3], antihypertensive [4], analgesic activity [5], human chymase inhibition [6], anti-tubercular activity [7] and human leukocyte elastase inhibition properties [8]. Antioxidants, α-1a-antagonists and neuro-peptide-Y (NPY) antagonists.

These derivatives were majorly reported obtained through Biginelli condensation reaction [9]. It involves a simple condensation of a mixture β-keto ester, benzaldehyde and urea, under acidic conditions. Using this as the basic template, many researchers have performed various synthetic protocols and the biological activity of the obtained derivatives was investigated. Zahed & Mohammad [10] reported the formation of 3,4-DHPMs and thione derivatives, formed through the catalysis of trichloro-acetic acid (TCAA, 20 mol%), under solvent free conditions.

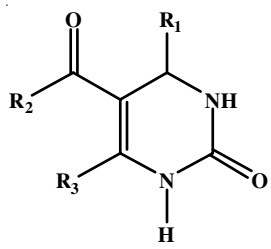

Fig. 1. Chemical structure of dihydropyrimidnones

In this method, 85% yield was obtained at 70 ºC, whereas only 20% yield was obtained in the absence of trichloro acetic acid. Bahekar *et al.* [11] have demonstrated the synthesis of DHPMs, through the catalysis of L-proline nitrate. A novel Brønsted acid based ionic liquid [Btto][*p*-TSA] (5 mol%) catalyzed synthesis of DHPMs was reported by Zhang *et al.* [12]. The major advantage of the method was observed to be, shorter reaction times, non-usage of toxic organic solvents and occur-rence of the reaction near the room temperature conditions. In another approach, nanosilica-supported tin(II)

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chloride catalyzed formation of DHPMs was conducted by Ziarati *et al.* [13] in the presence of ethanol solvent conditions. Excellent yields and easy recoverability of the catalyst were observed to be the important outcomes of this research work.

In all the above synthetic approaches, the development of a novel heterogeneous catalyst, to form the best yields of DHPMs, was identified to be major research point of interest. Based on this objective, several catalysts like Lewis acids [14-16], triflates [17-19], cerric ammonium nitrate [20], molecular I_2 [21], La(III)chloride-graphite [22], triphenylphosphine [23], Dowex [24], Baker's yeast [25], heteropolyacids [26], covalently anchored sulfonic acid on silica [27], Amberlyst-70 [28], DBSA [29], TCCA [30], ammonium carbonate [31], L-tyrosine [32], titania-carbon nanotutbes [33], silica-bonded *N*-propyl sulfamic acid [34] and *N*-sulfonic acid poly(4-vinylpyridinium) chloride [35], *etc*. were incorporated in their formation.

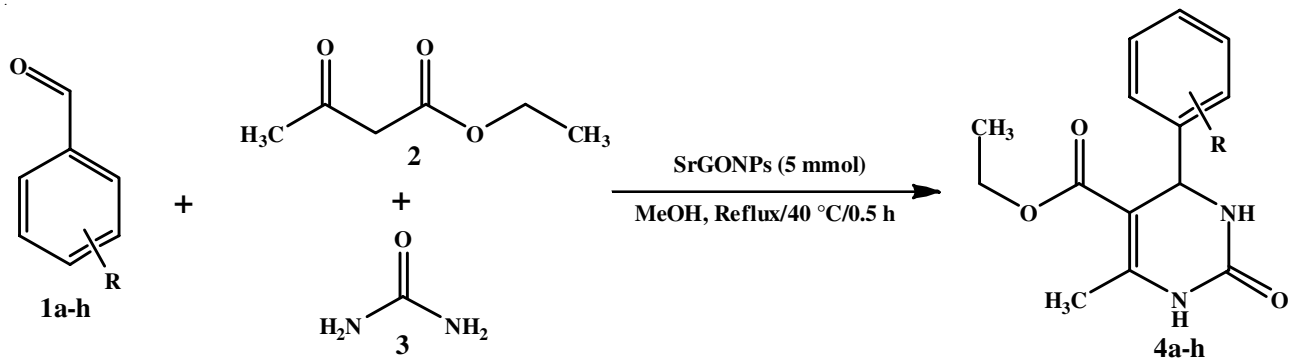
Among these catalysts, some of them are efficient and many of them are involved with lengthy methods of synthesizing the catalysts. Based on these inputs, it was planned to generate an efficient nanocatalyst in forming DHPMs, in good yields and which could display effective biological functions. In the process of developing newer heterogeneous catalysts, the molecular hybridization method has been prevailing as an alternative route in the formation of potential biological agents. In this view, the present work involves sulphonated reduced graphene oxide (SrGO) catalyzed synthesis of a novel series of 3,4-dihydropyrimidin-2(1*H*)-one derivatives and their anticancer activity was evaluated against MCF-7, SKNSH human cells.

EXPERIMENTAL

The reaction was performed under nitrogen atmosphere. Solvent evaporation was conducted with rotavapor, equipped with a water bath at below 40 ºC. All the required chemicals and solvents were procured from Sigma Aldrich (99% pure) and used without any further purification. The NMR spectra of the synthesized organic compounds were recorded with the help of Bruker ACF spectrophotometer (300 MHz). The residual solvent signals were taken as the reference (2.50 and 39.5 ppm for ^{1}H NMR and ^{13}C NMR spectra in DMSO-d_6, respectively, 7.26 and 77.0 ppm for ^{1}H NMR spectra and ^{13}C NMR spectra in $CDCl_3$). The chemical shift (δ) is referred in terms of ppm, coupling constants (*J*) in Hz. The IR spectra as wave number (ν_{max}, cm^{-1}) were recorded using KBr pellets in BRUKER spectrophotometer. HRMS spectra were recorded on a Xevo QT of mass spectrometer. Silica gel 60 F_{254} of Merck pre-coated plates were employed for their thin layer chromatography (TLC) analysis to check the progress of the reaction and also to analyze the purity of the compounds, the spot being located under UV light and iodine vapours.

Synthesis of sulfonated reduced graphene oxide (SrGO): Initially, graphite oxide (50 mg) was dispersed in 50 mL of deionized water and the contents were kept under reflux for 10-15 min. The graphene oxide (GO) obtained was cooled to room temper-ature and then transferred into a glass container for ultrasoni-cation (10 min). A solution of sodium borohydride (10 mL of 0.4 g/10 mL) was added to the GO solution, at near pH 10.0. These contents were heated to 100 ºC for about 1 h and a black coloured suspension was observed. After centrifuging the contents, the obtained partially reduced GO (rGO) particles were further kept under ultrasonication for about 30 min. On conducting diazotization, separately with calculated quantities of sulfanilic acid, HCl and KNO_2, the obtained diazotized salt was slowly added into the rGO solution near 0 ºC. The mixture was kept under magnetic stirring near the room temperature, for an overnight. A black coloured suspension was noticed and then centrifuged (2000 rpm) for about 15 min and washed several times with deionized water. The black residue was filtered off, dried near 75 ºC for about 2 h. The finally obtained sulphonated reduced graphene oxide (SrGO) particles were characterized.

Schematic procedure for the one-pot synthesis of 3,4-dihydropyrimidin-2(1*H*)-one derivatives (4a-h): A mixture of substituted aldehydes (**1a-h**, 10 mmol), β-keto ester (**2**, 10 mmol), urea (**3**, 15 mmol) and SrGO (5 mmol) dispersed in 20 mL of MeOH were refluxed at 40 ºC, in separate reaction vessels for 30 min. The progress of each set of reaction was verified through TLC and the catalyst, has been recovered by an external magnet. The solid products obtained in each reaction vessel were filtered, washed with cold water (2 × 50 mL) and a mixture of 1:1 of MeOH:H_2O (2 × 20 mL) followed by purification (**Scheme-I**). The products obtained (**4a-h**) were recrystallized with EtOH and were characterized by using ^{1}H, ^{13}C NMR, FTIR and mass spectral techniques and their melting points were determined.



1a, **4a**: R = 2-chlorophenyl, **1b**, **4b**: R = 3-chlorophenyl, **1c**, **4c**: R = 4-chlorophenyl, **1d**, **4d**: R = 4-nitrophenyl, **1e**, **4e**: R = 2-methoxyphenyl, **1f**, **4f**: R = 3-bromophenyl, **1g**, **4g**: R = 2-bromophenyl, **1h**, **4h**: R = 4-bromophenyl

Scheme-I: One-pot synthesis of 3,4-dihydropyrimidin-2(1*H*)-ones (**4a-h**)

Ethyl4-(2-chlorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4a): Yield: 95%; physical state/colour: solid/white powder; m.p.: 224-226 ºC; IR (KBr, ν_{max}, cm^{-1}): 3354, 3223, 3107, 2978, 1694, 1639, 1450, 1368, 1230, 1098, 744; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.25 (s, 1H, N-H), 7.68 (s, 1H, N-H), 7.40-7.24 (m, 4H, Ar-H), 5.61 (d, 1H, *J* = 2.7 Hz, CHAr), 3.87 (q, 2H, *J* = 7.1 Hz, CH_2CH_3), 2.28 (s, 3H, CH_3), 0.97 (t, 3H, *J* = 7.1 Hz, CH_2CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.47 (EtO C=O), 146.81 (C=O), 145.08 (Me(NH)C=C), Ar-Carbons: 136.27, 132.1, 128.27, 126.93,126.12, 125.48, 56.94 (CH_2-CH_3), 50.14 (CH-Ar), 20.55 (CH_3), 17.33 (CH_2-CH_3). ESI-MS: *m/z* 295 $[M+1]^+$; Elemental analysis: $C_{14}H_{15}N_2O_3Cl$ calcd. (found)%: C, 57.05 (57.03); H 5.13 (5.135), N 9.50 (9.476).

Ethyl-4-(3-chlorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4b): Yield: 99%; physical state/colour: Solid/white powder; m.p.: 210-212 ºC; IR (KBr, ν_{max}, cm^{-1}): 3250, 3113, 2940, 1711, 1647, 1475, 1429, 1223, 1090, 768; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.25 (s, 1H, N-H), 7.76 (s, 1H, N-H), 7.35-7.20 (m, 4H, Ar-H), 5.12 (s, 1H, Ar-C**H**), 3.98 (q, 2H, *J* = 6.8 Hz, -C**H**$_2$-CH_3), 2.23 (s, 3H, -CH_3), 1.07(t, 3H, *J* = 6.8 Hz, -CH_2C**H**$_3$). ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.21 (EtOC=O), 155.23 (C=O), 148.58 (Me(NH)-C=C), Ar-Carbons: 146.7, 134.35, 130.59, 129.65, 126.78, 125.58, 110.22 (C=C(CH)CO_2Et), 57.55 (CH_2-CH_3), 52.87 (CH-Ar), 20.51 (CH_3), 13.9 (CH_2-CH_3). ESI-MS: *m/z* 295 $[M+1]^+$; Elemental analysis: $C_{14}H_{15}N_2O_3Cl$ calcd. (found)%: C, 57.05 (57.046) H, 5.13 (5.135), N, 9.50 (9.476).

Ethyl-4-(4-chlorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4c): Yield: 95%; physical state/colour: Solid/white powder; m.p.: 220-222 ºC; IR (KBr, ν_{max}, cm^{-1}): 3237, 3117, 2978, 1701, 1647, 1460, 1288, 1221, 1088, 781; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.25 (s, 1H, N-H), 7.76 (s, 1H, N-H), 7.37 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.24 (d, 2H, *J* = 8.5 Hz, Ar-H), 5.12 (d, 1H, *J* = 3.2 Hz, CH-Ar), 3.97 (q, 2H, *J* = 7.1 Hz, CH_2-CH_3), 2.23 (s, 3H, CH_3), 1.07 (t, 3H, *J* = 7.1 Hz, CH_2-CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): δ 168.53 (EtOC=O), 149.92 (C=O), 148.12 (Me(NH)C=C), Ar-Carbons: 144.01, 132.72, 128.9, 127.31, 110.46 (C=C(CH)CO_2Et), 57.86 (CH_2-CH_3), 51.52 (CH-Ar), 20.83 (CH_3), 14.2 (CH_2-CH_3). ESI-MS: *m/z* 295 $[M+1]^+$; Elemental analysis: $C_{14}H_{15}N_2O_3Cl$ calcd. (found)%: C, 57.05 (57.02) H, 5.13 (5.12), N, 9.50 (9.46).

Ethyl-4-(4-nitrophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4d): Yield: 97%; physical state/colour: Solid/yellow powder; m.p.: 210-223 ºC; IR (KBr, ν_{max}, cm^{-1}): 3238, 3123, 2986, 1730, 1705, 1645, 1522, 1348, 1219, 1096, 854, 783; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.37 (s, 1H, N-H), 8.24(d, 2H, *J* = 8.7 Hz, Ar-H), 7.86 (s, 1H, N-H), 7.48 (d, 2H, *J* = 8.7Hz, Ar-H), 5.25 (d, 1H, *J* = 3.3 Hz, CH-Ar), 3.89 (q, 2H, *J* = 7.1 Hz, CH_2-CH_3), 2.25 (s, 3H, CH_3), 1.12 (t, 3H, *J* = 7.1 Hz, CH_2-CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 174.39 (EtOC=O), 157.5 (C=O), 143.65 (C-NO_2), 135.66 (Me(NH)C=C), Ar-Carbons: 129.26 (C-Ar), 128.88, 127.16, 126.18, 105.66 (C=C(CH)CO_2Et), 58.00 (CH_2-CH_3), 52.75 (CH-Ar), 18.32 (CH_3), 20.84 (CH_2-CH_3). ESI-MS: *m/z* 306. $[M+1]^+$; Elemental analysis: $C_{14}H_{16}N_3O_5$ calcd. (found)%: C, 55.08 (55.02) H, 4.95 (4.93), N, 13.76(13.71).

Ethyl-4-(2-methoxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4e): Yield: 96%; physical state/colour: Solid/white powder; m.p.: 201-203 °C; IR (KBr, ν_{max}, cm^{-1}): 3235, 3113, 2955, 1703, 1647, 1514, 1456, 1279, 1221, 1088, 837, 791; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.16 (s, 1H, N-H), 7.61 (s, 1H, N-H), 7.10 (d, 2H, *J* = 8.5 Hz, Ar-H), 6.90 (d, 2H, *J* = 8.5 Hz, Ar-H), 5.10 (d, 1H, *J* = 3.1 Hz, CH-Ar), 3.95 (q, 2H, *J* = 7.1 Hz, CH2-CH3), 3.75 (s, 3H, OCH_3), 2.25 (s, 3H, CH_3), 1.15 (t, 3H, *J* = 7.1 Hz, -CH_2-CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): δ 174.51 (EtO-C=O), 140.66 (Me(NH)C=C), Ar-Carbons: 153.87, 129.33, 127.17, 123.71, 78.76 (CH_2-CH_3), 59.6 (CH_2-CH_3), 57.93 (OCH_3), 52.76 (CH-Ar), 22.37 (CH_3), 18.40 (CH_2-CH_3). ESI-MS: *m/z* 291$[M+1]^+$; Elemental analysis: $C_{15}H_{18}N_2O_4$ Calcd. (Found)% C, 62.06 (62.04) H, 6.25 (6.23), N, 9.65(9.61).

Ethyl-4-(3-bromophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4f): Yield: 98%; physical state/colour: Solid/pale green powder; m.p.: 210-212 ºC; IR (KBr, ν_{max}, cm^{-1}): 3238, 3098, 2930, 1709, 1653, 1474, 1285, 1224, 1092, 768; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.25 (s, 1H, N-H), 7.76 (s, 1H, N-H), 7.45-7.21 (m, 4H, Ar-H), 5.14 (d, 1H, *J* = 3.2 Hz, CH-Ar), 3.9 (q, 2H, *J* = 7.1 Hz, CH_2-CH_3), 2.24 (s, 3H, CH_3), 1.09 (t, 3H, *J* = 7.1 Hz, CH_2-CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.3 (EtOC=O), 152.31 (C=O), 149.1 (Me(NH)C=C), Ar-Carbons: 147.70, 132.10, 130.5, 129.6, 125.5, 121.9, 99.3 (C=C(CH)CO_2Et), 59.7 (CH_2-CH_3), 54.3 (CH-Ar), 18.4 (CH_3), 14.4(CH_2-CH_3). ESI-MS: *m/z* 339 $[M+1]^+$; Elemental analysis: $C_{14}H_{15}N_2O_3Br$ calcd. (found)%: C, 49.57 (49.52) H, 4.46 (4.43), N, 8.26(8.22).

Ethyl-4-(2-bromophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4g): Yield: 94%; physical state/colour: Solid/pale green powder; m.p.: 210-212 ºC; IR (KBr, ν_{max}, cm^{-1}): 3221, 3099, 2978, 1709, 1653, 1474, 1285, 1224, 1092, 768; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.27 (s, 1H, N-H), 7.66 (s, 1H, N-H), 7.55-7.21 (m, 4H, Ar-H), 5.60 (d, 1H, *J* = 3.2 Hz, CH-Ar), 3.92 (q, 2H, *J* = 7.1 Hz, CH_2-CH_3), 2.28 (s, 3H, CH_3), 1.01 (t, 3H, *J* = 7.1 Hz, CH_2-CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.41 (EtOC=O), 151.71 (C=O), 149.73 (Me(NH)C=C), Ar-Carbons: 143.85, 133.05, 129.83, 129.23, 128.91, 122.73, 98.71 (C=C(CH)CO_2Et), 59.5 (CH_2-CH_3), 54.47 (CH-Ar), 18.11 (Me), 14.44 (CH_2-CH_3). ESI-MS: *m/z* 339 $[M+1]^+$; Elemental analysis: $C_{14}H_{15}N_2O_3Br$ calcd. (found) %: C, 49.57 (49.53) H, 4.46 (4.44), N, 8.26 (8.23).

Ethyl-4-(4-bromophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4h): Yield: 96%; physical state/colour: solid/pale green powder; m.p.: 215-217 ºC; IR (KBr, ν_{max}, cm^{-1}): 3246, 3111, 2949, 1701, 1649, 1458, 1288, 1221, 1088, 781; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.21 (s, 1H, N-H), 7.73 (s, 1H, N-H), 7.53 (d, 2H, *J* = 8.3 Hz, Ar-H), 7.15 (d, 2H, *J* = 8.3 Hz, Ar-H), 5.11 (d, 1H, *J* = 3.1 Hz, -CH-Ar), 4.0 (q, 2H, *J* = 7.09 Hz, CH_2-CH_3), 2.23 (s, 3H, CH_3), 1.1 (t, 3H, *J* = 7.1 Hz, CH_2-CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.77 (EtOC=O), 152.5 (C=O), 149.3 (Me(NH)C=C), Ar-Carbons: 144.1, 132.0, 129.2, 121, 99.3 (C=C(CH)CO_2Et), 59.8 (CH_2-CH_3), 54.06 (CH-Ar), 18.4 (Me), 14.6 (CH_2-CH_3). ESI-MS: *m/z* 339.0336 $[M+1]^+$; Elemental analysis: $C_{14}H_{15}N_2O_3Br$ calcd. (found) %: C, 49.57 (49.54) H, 4.46 (4.42), N, 8.26 (8.24).

***in vitro* anticancer activity:** The *in vitro* anticancer activity of the synthesized compounds (**4a-h**) was investigated using MTT (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric assay by following the American Type Culture Collection (ATCC) protocol [36-39]. The cell lines that were used in these studies are, MCF-7 (human breast tumor cell line) derived from ATCC No. CCL-185 and SKNSH (Human Neuroblastoma cell line) derived from Human lung adenocarcinoma epithelial cell line (ATCC No. HTB-11). The process was conducted at 37 ºC, in the presence of 5% humidified carbon dioxide incubator.

Initially, they were subjected to trypsinization, in order to remove the adhered cells and then centrifuged to obtain the cell pellet. To this cell pellet, a fresh media (100 µL) was slowly dispersed through haemocytometer, with cells in the range of 5,000 to 6,000 per well in a 96-well plate. The entire set up was incubated in the carbon dioxide incubator for an overnight, so as to achieve the adherence and regaining the shape of the cells. After the incubation period, the cells were treated with the DHPMs (**4a-h**) at the concentration of 25 µM, to investigate the percentage inhibition on the cancer and normal human cells. These cells under study were kept under incubation for about 48 h, in order to examine the influence of the synthesized DHPMs (**4a-h**) on the selected cell lines. With the untreated cell lines, the zero hour reading was noted and also with DMSO solvent (1%), the control was subtracted from the reading obtained after 48 h. After the incubation period (48 h), the cell lines were made to interact with MTT, dissolved in phosphate buffered saline (PBS, 5 mg/mL) and further incubated for about 3-4 h near 37 ºC. Formazan crystal formation was observed in 100 µL of DMSO and the viability was measured on a multimode reader (spectramax) near 540 nm.

RESULTS AND DISCUSSION

Characterization of SrGO: The morphology of GO and SrGO were verified through SEM analysis. SEM images (Fig. 2a-b) exhibit the wrinkled sheet-type morphology and these images also advise that the microstructure of GO nanolayers was well-preserved even after the sulfonation procedure [36] (Fig. 2b). The EDS elemental analysis of both GO (Fig. 2c) and SrGO (Fig. 2d) was accomplished. On the other hand the Raman spectra (Fig. 3) gives the I_D/I_G ratio for GO and SrGO particles was found to be 1.51 and 2.04, respectively, which implies that some of the oxygen functionalities were disconnected from the surface with the help of $NaBH_4$ to generate the sulfonated nanomaterial.

XRD studies: XRD patterns revealed that the characteristic peak of GO was observed at 2θ = 12.6º (d = 6.90 Å) and 2θ = 26.5º (d = 3.36 Å) for the SrGO NPs (Fig. 4), suggesting that the GO nano layers were associated through the π-π interaction upon sulfonation [40]. The catalytic potentiality of the produced SrGO particles was assessed towards the synthesis of DHPM derivatives.

IR studies: The respective absorption peaks were observed for all the related functional groups. The absorption peaks for the –N-H stretching and aromatic C-H stretching were observed around 3250-3230 cm^{-1} and 3100 cm^{-1}, respectively. These vibrations were obtained in common for all the compounds.

NMR spectra: The aromatic C-H, protons were observed at δ 6.68-8.13 ppm, 3.8 ppm for the aldehyde proton (-CHO)

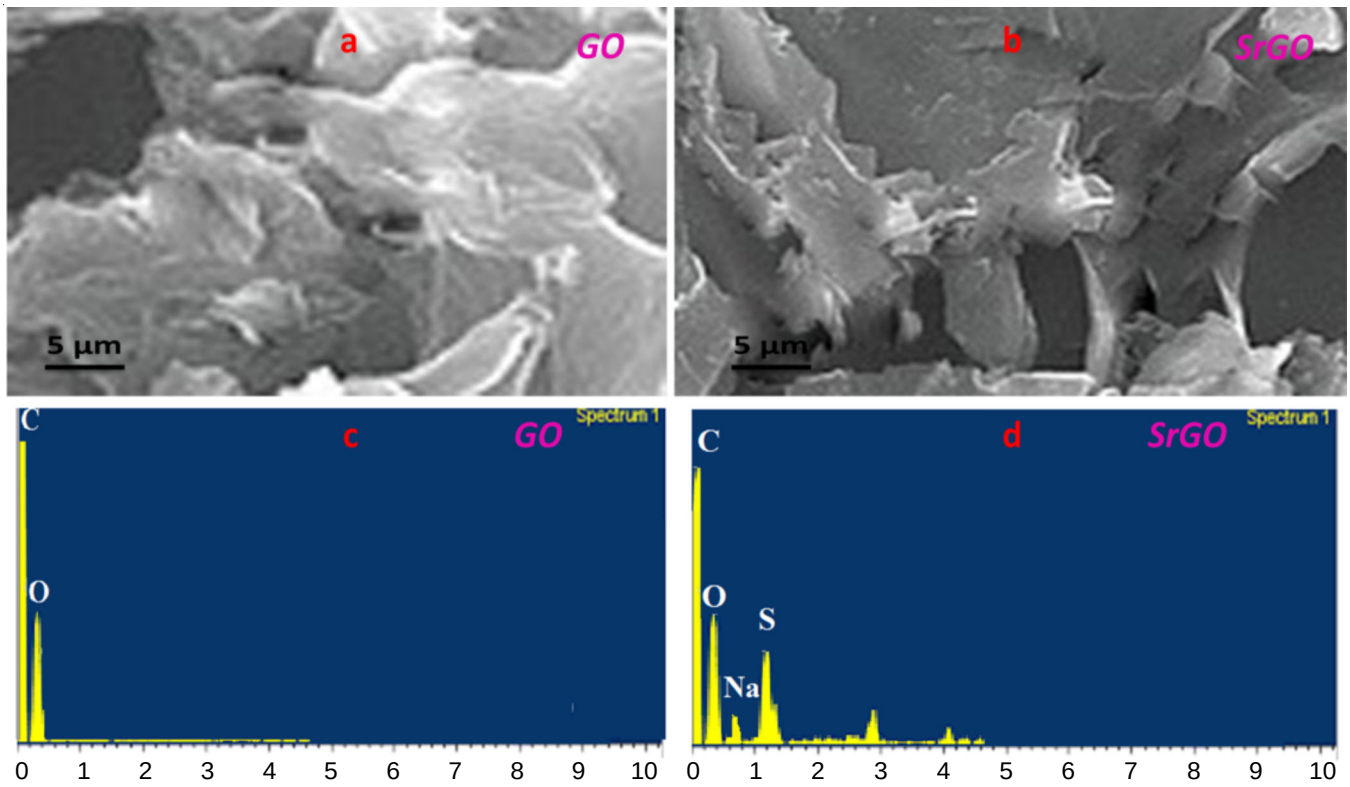


Fig. 2. SEM images of (a) GO and (b) SrGO

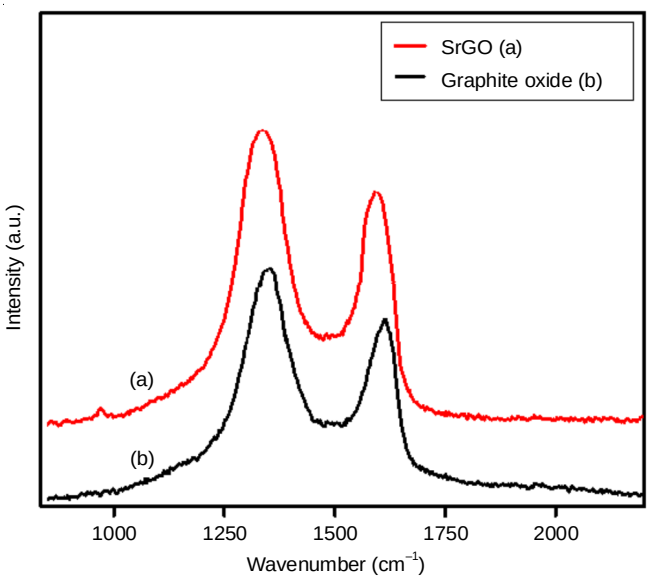


Fig. 3. Raman spectra for GO and SrGO

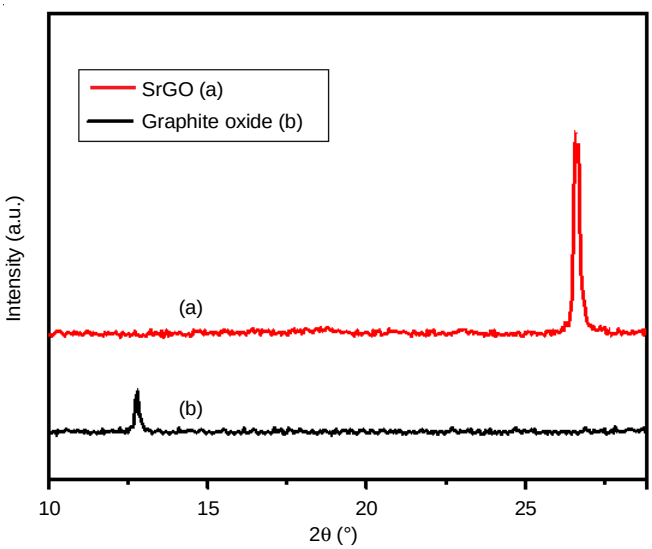


Fig. 4. XRD patterns for the GO and SrGO

was observed for all the synthesized compounds. Similarly, 165 ppm for ester carbonyl carbon and 55 ppm for CH_3-**C**H_2-O were observed in ^{13}C NMR for all the synthesized compounds.

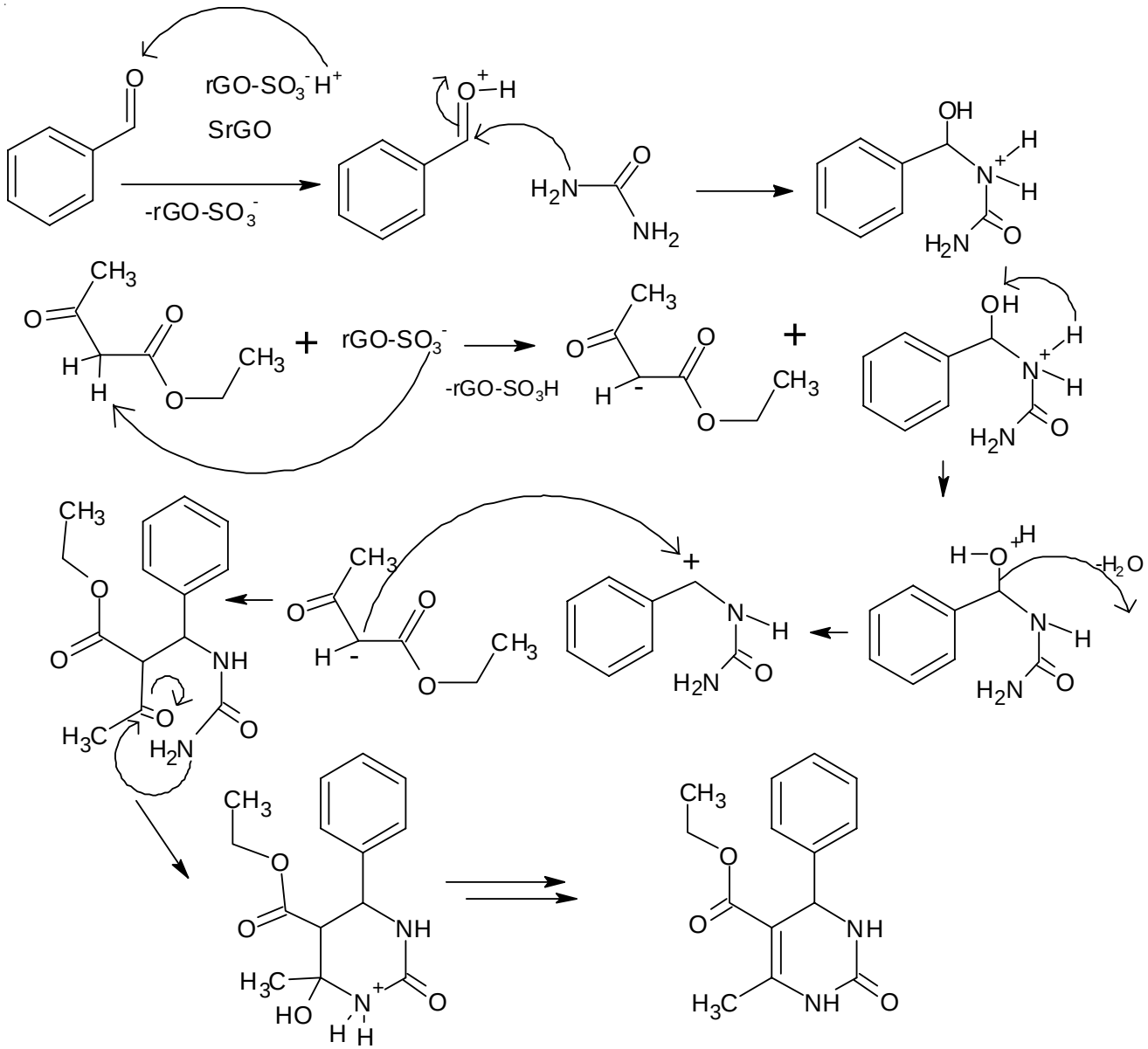
Effect of recyclability of SrGO NPs: The reaction scheme was performed with the recycled SrGO NPs, in 5 consecutive steps. The nanoparticles were separated, using a simple bar magnet and then the separated nanoparticles were washed with ethanol:water mixture, thoroughly. In each step, the% yields of the obtained products was determined and the results of the derivative **4a** are 95%, 92%, 90%, 88% and 84%, respectively in five consecutive steps. It shows that there was no much large difference in the yields, indicating the effectiveness of the nanoparticles as a novel heterogeneous nanocatalyst, in forming the best yields, even with its recycled forms.

In general, Biginelli reaction is performed in homogeneous catalytic medium where the major drawback is the recovery of the catalyst. The main objective of the present research work is to conduct the reaction through three component one-pot synthetic approach to produce DHPMs under heterogeneous catalytic medium with the advantage of recovering the used catalyst. The catalyst employed in this synthetic protocol is SrGO (5 mmol), which can exhibit magnetic property and could be easily recovered after formation of the desired products. The cyclocondensation was implemented through the heterogeneous catalysis and forms excellent yields of DHPMs.

A plausible mechanism of the formation of the derivatives, **4a-h** is presented in **Scheme-II**. Initial protonation of aromatic aldehydes, occurs with SrGO NPs, followed by electron pair donation by the urea-N to the electrophilic carbonyl carbon. This step might have occurred due to the high acidic character of the SrGO NPs, because of the resonance stability of the rGO-SO_3^- ion, which could occur between the π-system in the GO and the SO_3^- ion. Thereafter, a proton exchange could occur from amine nitrogen to the alcoholic oxygen and an immediate loss of water molecule occurs to form the carbocation. The anionic form of the SrGO can abstract the acidic methylene proton from the β-ketoester, to form a carbanion. These two forms of carbon intermediates, couples through C-C bond formation, followed by cyclization through condensation and form the DHPM derivatives.

The recyclability of the catalyst was examined in further reaction cycles and reasonable yields were obtained. On reusing the catalyst in these cycles, the efficiency of the yields has not reduced. Different aromatic aldehydes (**1a-h**) have been used to synthesize DHPM derivatives (**4a-h**). Aromatic aldehydes containing either electron withdrawing or electron donating groups in the *ortho*-, *meta*-, *para*-positions affords high yields of products. Another vital feature of this procedure is the survival of variety of functional groups such as nitro, methoxy, halides, *etc*. The main advantage of this reaction is its moderate reaction condition, efficiency of SrGO NPs, formation of the best yields, low cost and environmentally benign.

On thorough analysis of few scientific reports (Table-1), it was observed that the SrGO NPs were effective in bringing out the desired organic derivatives in shorter time, with more yields. The nanoparticles were prominent in forming the DHPMs derivatives (**4f** and **4h**) in best yields, when compared with those obtained in the entries 1 to 3 [41-43]. In these studies, though the catalyst incorporated are effective, the major drawback is the long reaction times and lesser yields. Furthermore, the Fe_3O_4 NPs and $LaCl_3$-graphite composites were effective in obtaining the DHPMs in less time [22,44]. However, the SrGO NPs were able to form nearly 99% yield of compound **4b** in 30 min time, though the reaction time is slightly higher than that of the entries 4 and 5. Hence, the SrGO NPs were identified to be the novel nanocatalysts, towards the formation of better yields of DHPM derivatives. On further comparing its effici-ency with few reported catalysts that formed the DHPM deriva-tives (Table-2), it was observed that Ziarati *et al.* [13] conducted trichloroacetic acid catalysis and reported the maximum yield of 4-chlorophenyl derivative (92%) [10]. With [Btto][*p*-TSA] catalysis, Zhang *et al*. [12] presented the formation of almost 99% and 97% of 3-bromo and 3-chlorophenyl derivatives. The $SnCl_2$/nano SiO_2 catalysis has formed almost 92% yields of the DHPM derivatives, as demon-



Scheme-II: Plausible mechanism for the formation of DHPMs

TABLE-1
COMPARISON OF THE CATALYTIC EFFICIENCY OF SrGO WITH FEW SCIENTIFIC REPORTS

Entry	Nanocatalyst	Reaction time	Yield of DHPMs (%)	Ref.
1	$[Al(H_2O)_6](BF_4)_3$ (10 mol%)	20 h	85	[41]
2	*p*-Sulfonic acid calixarenes (0.5 mol%)	8 h	69	[42]
3	Fe_3O_4@mesoporous SBA-15 (50 mg)	6 h	85	[43]
4	Fe_3O_4 NPs (20 mol%)	16 min	90	[44]
5	$LaCl_3$–graphite (35 mol%)	10 min	85	[22]
6	SrGO (5 mmol)	30 min	99[a]	Present work

[a]Maximum% yield of compound **4b** synthesized in present work.

strated by Ziarati *et al*. [13]. Competing with these catalysts, the SrGO NPs were also found the be effective in bringing the maximum % yields of the designed DHPM derivatives as shown in Table-1.

Anticancer activity: The results of the *in vitro* cytotoxic studies are presented in Table-3. Doxorubicin was used as the standard for these studies. From the results, it was observed that compounds **4c** (R = 4-chlorophenyl), **4d** (R = 4-nitrophenyl) have displayed effective cytotoxic activity against the MCF-7 cell line. Whereas, compound **4g** (R = 2-bromophenyl) have shown superior activity against the SKNSH cell line.

Among the tested cell lines, MCF-7 cells have shown a positive response, against all the derivatives (**4a-h**) under investigation than the SKNSH cells. Compound **4c** displayed

TABLE-2
COMPARISON OF THE % YIELDS OF DHPMs WITH SrGO NPs AND FEW REPORTED CATALYSTS

Compound	R	CCl_3COOH [10]	[Btto][*p*-TSA] [12]	$SnCl_2$/nano SiO_2 [13]	SrGO NPs (Present work)
4a	2-Cl-C_6H_4	85	–	92	95
4b	3-Cl-C_6H_4	–	97	–	99
4c	4-Cl-C_6H_4	92	93	–	95
4d	4-NO_2-C_6H_4	85	–	95	97
4e	2-OCH_3-C_6H_4	94	–	92	96
4f	3-Br-C_6H_4	–	99	–	98
4g	2-Br-C_6H_4	–	–	–	94
4h	4-Br-C_6H_4	90	–	92	96

TABLE-3
In vitro CYTOTOXIC ACTIVITY OF THE COMPOUNDS **4a-h**

Entry	MCF-7 (IC_{50} values)	SKNSH (IC_{50} values)
4a	2.37	44.29
4b	20.56	32.27
4c	31.28	34.13
4d	22.31	22.17
4e	5.54	30.68
4f	18.29	45.71
4g	18.10	65.04
4h	4.18	14.83
4i	11.64	17.86
Doxorubicin	2.1 µM	3.3 µM

promising activity and compounds **4d, 4e** (R = 2-methoxyphenyl), **4b** (R = 3-chlorophenyl), demonstrated moderate to good activity against MCF-7 cells.

Structure-activity relationship (SAR) study of **4c** to **4d** in turn to **4g** (R = 2-bromo phenyl) decreased the activity profile for MCF-7 cell and similar model was not observed for SKNSH cancer cell line, demonstrating the selectivity of these derivatives to a particular cell line. Therefore, it was strongly affirmed the synthesized DHPM derivatives acts as effective anticancer agents by the established experimental conditions.

Conclusion

A series of 3,4-dihydropyrimidinones (**4a-h**) have been synthesized using novel SrGO nanoparticles, through Biginelli cyclocondensation. The reaction was performed in the presence of methanol solvent and reflux conditions (40 ºC) for about 30 min. The structural moieties of all the compounds were characterized through spectroscopic techniques like ^1H & ^{13}C NMR, FTIR and HRMS methods. The SrGO NPs were easily separable from the reaction mixture and the synthesis of compound **4a** was conducted with the recycled SrGO NPs in 5 cycles. It was observed that the% yield of the product has decreased with a small difference at each trial. Further, the cytotoxic studies were conducted against the MCF-7 and SKNSH cell lines with doxorubicin as the standard. In these studies, it was observed that the compounds **4c** (R = 4-chlorophenyl), **4d** (R = 4-nitrophenyl) have displayed effective cytotoxic activity against the MCF-7 cell line. Whereas, compound **4g** (R = 2-bromophenyl) have shown superior activity against the SKNSH cell line. The major outcomes of the present research work are: (i) facile synthesis of SrGO nanoparticles and confirming its formation through, XRD, SEM-EDS and Raman spectral analysis; (ii) efficiency of SrGO nanoparticles as heterogeneous nanocatalysts in the formation of highest% yields of various 3,4-DHPM derivatives, through cyclocondensation reaction; (iii) the nanoparticles are easily separable and also effective in catalyzing itself in its recycled forms; (iv) synthesized DHPMs were effective towards exhibiting good anticancer activity.

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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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