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An Efficient One-pot Synthesis of some New Pyrazolyl Appended 1,3,4-Oxadiazole Derivatives as Antibacterial and Antioxidant agents

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ABSTRACT

Synthesis of 2(3,5-disubstituted)-1H-pyrazol-4-yl-thio-5-(pyridin-4-yl)1,3,4-oxadiazoles was achieved via one pot multi-component reaction of 1-(4-substitutedphenyl)-2-((5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl)thio)ethanone, substituted benzaldehydes and hydrazine hydrate by using Bleaching earth clay (pH 12.5, 10 wt%) and Polyethyleneglycol (PEG-400) as a green reaction media. The method is found an efficient, eco-friendly and catalyst recycled for five times with no significant loss in the yield of product. All the synthesized compounds were characterized by spectral data and screened in vitro for their antibacterial and antioxidant activities. The compounds 5s and 5l have shown considerable antioxidant and antimicrobial activity, however all other synthesized compounds demonstrated moderate activities.

Keywords: PEG-400, Bleaching Earth Clay (BEC), 1,3,4 Oxadiazole, Antibacterial, Antioxidant

INTRODUCTION

The chemistry of heterocyclic compounds has attracted researchers in recent times owing to its increasing significance in the field of pharmaceuticals and industrial applications [1,2]. In fact, the development of simple, elegant and facile methodologies for the synthesis of heterocycles is one of the most essential aspects in organic synthesis.

Oxadiazoles are very well inevitable class of heterocyclic compounds with assorted pharmaceutical applications. The 1,3,4-oxadiazoles have emerged as an important class of compound with resourceful applications in the meadow of pharmaceutical, pesticide and polymer sciences. The synthesis of 1,3,4-oxadiazole and its derivatives have established scrupulous attention for a long time because of its outstanding biological and pharmacological properties such as, antitubercular [3], antimicrobial, anti-HIV [4], antiviral [5], anti-inflammatory [6], antimalarial [7], antioxidant [8-10], antineoplastic [11] and hypoglycemic activity [12]. Thus the synthesis of oxadiazole offers a great impulsion to research on development of bioactive compounds of therapeutic importance.

On the other hand pyrazole and their derivatives have acknowledged a standing attention in heterocyclic chemistry and captivate substantial interest because of its versatile biological activities including antimalarial [13], antifungal [14,15], anti-inflammatory [16-21]. In recent times, some pyrazoles have been reported for their potential therapeutic bioactivities like antimicrobial, antiviral and anticancer [22-28].

In view of the above observations and considering the significant structural diversity in pharmaceutical chemistry, herein we design and develop some new compounds predicting significant biological activities based on molecular hybridization. It includes the hybridization of two important pharmacophores i.e., pyrazole and 1,3,4-oxadiazole into a single molecular skeleton leading to new prototype as a better drug candidate possibly having improved biological activities [29] and combat drug resistance [30].

Development of eco-friendly route for the synthesis of bioactive compounds is one of the prime goals of medicinal chemist. The classical method adapted for the synthesis of bioactive hybrid molecules suffers from many serious disadvantages like prolonged reaction time, use of toxic solvents and reagents, expensive ligands, poor yields and one or more side products. So as to overcome such issues and to make synthesis ideal, the principle of green chemistry attracted the attention of synthetic chemist. The use of green solvent is one of the important aspects of green chemistry. The PEG-400 is frequently used as a green solvent because of its one or more advantages over conventional solvent such as commercial availability, nonvolatile, thermal stability, miscibility with organic solvents and reusability. With these points of view we use PEG-400 as a green solvent to reduce the toxic effect of conventional solvents on the environment [31-35].

The utility of heterogeneous catalyst over the homogeneous catalyst offers many more advantages [36]. Recently we explored the utility of Bleaching earth clay pH (12.5) (BEC) as a heterogeneous green catalyst for synthesis of bioactive heterocyclic compounds [37-42]. BEC offers the advantages like reusability, eco-friendly, easily separable, economical, non-toxic in nature, shifts the reaction course towards the green approach and beat the conventional catalyst.

The above significances promoted us to design and synthesis of such scaffold which include the two bioactive moieties and may lead to a novel heterocycles with significant antibacterial and antioxidant activities. In the present study, herein we reported the "Eco-Friendly approach" for the synthesis of oxadiazole-pyrazole hybrid molecules by using a green catalytic media and study their antibacterial and antioxidant properties. The antibacterial evaluation was studied by using *Bacillus megaterium* and *Escherichia coli* species. The *in vitro* antioxidant appraisal was studied by using 2,2-diphenyl-1-picrylhydrazyl (DPPH), OH and SOR radical scavenging methods. Thus the title compound represent a new class of antibacterial and antioxidant agent and found to be worthy for further investigation.

EXPERIMENTAL

Chemistry

Melting points were determined by open capillary method and were uncorrected. The chemicals and solvents used were of laboratory grade and were purified prior to use. BEC was a gift from Supreme Silicones, Pune. Completion of the reaction was monitored by TLC on precoated sheets of silica gel-G (Merck, Germany) using iodine vapors for detection. IR spectra were recorded (in KBR pallets) on Shimadzu spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded (in DMSO) d_6 on Bruker Avance-400 MHz spectrometer using Tetramethylsilane (TMS) as an internal standard. The mass spectra were recorded on EI-Shimazdu-GC-MS spectrometer.

General procedure for the synthesis of 5-(pyridine-4-yl)-1,3,4-oxadiazole-2(3H)-thione(2)A

mixture of isoniazid (0.005 mol) and carbon disulfide (5 ml) in alcoholic KOH (20 ml, 10%) was refluxed 12 h. After completion of reaction (monitored by TLC) the solution was cooled at room temp and poured into ice cold water (50 ml) and acidified with dil. HCl. The solid product separated out was filtered and recrystallized from ethanol as white crystals.

IR (KBr) υ cm^{-1}: 3415 (NH), 1602 (C=N), 1365 (C-O-C), 1227 (C=S); ^{1}H NMR (400MHz, DMSO-d_6, ppm): δ 7.82 (d, *J*=43 Hz , 2H, Ar-H), 8.82 (d, *J*=33 Hz, 2H, Ar-H), 14.66 (s, 1H, NH).

General procedure for the synthesis of 1-(substituted phenyl)-2((5-(pyridin-4-yl))-1,3,4-oxadiazole-2-yl) thio)ethanone(3a-e)

A mixture of **2** (0.015 mol), substituted phenacyl bromide (0.015 mol) and BEC (10 wt%) was stirred in PEG-400 for 1.5 h. After completion of reaction (monitored by TLC), the catalyst was isolated by simple filtration and the reaction mixture poured into ice cold water (50 ml) and neutralize with dil HCl. The separated solid was filtered and recrystallized by ethanol/chloroform as pink colored crystals.

1-(4-chlorophenyl)-2-((5-(pyridin-4-yl)-1,3,4-oxadiazole-2-yl)thio)ethanone(3a)

It was obtained as pink solid in 89 % yield; mp. 131-133°C; IR (KBr, cm^{-1}): 3088 (CH_2), 1678 (C=O), 1615 (C=N), 1227 (C=S), 1393 (C-O-C oxadiazole), 1185 (C-S-C); ^{1}H NMR: (400 Mz, DMSO- d_6, ppm): δ 5.21 (s, 2H, CH_2), 7.67 (d, *J*=8 Hz, 2H, Ar-H), 7.88 (d, *J*=4 Hz, 2H, Ar-H), 8.08 (d, *J*=8 Hz, 2H, Ar-H), 8.81 (d, *J*=4 Hz, 2H, Ar-H); MS (m/z): 331.90 (M+).

1-(4-nitrophenyl)-2-((5-(pyridin-4-yl)-1,3,4-oxadiazole-2-yl)thio)ethanone(3c)

It was obtained as yellow solid in 93% yield; mp. 136-138°C; IR (KBr, cm^{-1}): 3096 (CH_2), 1682 (C=O), 1620 (C=N), 1235 (C=S), 1378 (C-O-C oxadiazole), 1178 (C-S-C); ^{1}H NMR: (400 Mz, DMSO- d_6, ppm): δ 5.30 (s, 2H, CH_2), 7.78 (d, *J*=8 Hz, 2H, Ar-H), 7.91 (d, *J*=4 Hz, 2H, Ar-H), 8.12 (d, *J*=8 Hz, 2H, Ar-H), 8.92 (d, *J*=4 Hz, 2H, Ar-H); MS (m/z): 342.04 (M+).

1-(4-bromophenyl)-2-((5-(pyridin-4-yl)-1,3,4-oxadiazole-2-yl)thio)ethanone(3e)

It was obtained as orange solid in 87% yield; mp 158-160°C; IR (KBr, cm^{-1}): 3092 (CH_2), 1672 (C=O), 1624 (C=N), 1232 (C=S), 1380 (C-O-C oxadiazole), 1192 (C-S-C); ^{1}H NMR: (400 Mz, DMSO- d_6, ppm): δ 5.38 (s, 2H, CH_2), 7.89 (d, *J=8* Hz, 2H, Ar-H), 7.94 (d,*J* = 4 Hz, 2H, Ar-H), 8.16 (d, *J*=8 Hz, 2H, Ar-H), 8.97 (d, *J*=4 Hz, 2H, Ar-H); MS (m/z): 374.97(M+).

4.4 General procedure for the synthesis of 2-((3,5 disubstituted (4-substitutedphenyl)-1H-pyrazol-4-yl) thio)-5-(pyridin-4-yl)-1,3,4-oxadiazole (5a-s)

A mixture of **3** (1 mmol) and substituted benzaldehyde 4a-d (1 mmol) was stirred in PEG-400 in the presence of BEC (10 % wt) at 80°C for 1 h. After completions of reaction (indicated by TLC) hydrazine hydrate (99%) (1 mmol) were added to the reaction mixture and the reaction mixture was further stirred for 1 h at 80°C. After completion of reaction (monitored by TLC) the solid catalyst was isolated by simple filtration and the mother liquor was poured into ice cold water and then neutralize with dil. HCl. The solid separate out was filtered, wash with 10 ml ice cold water and recrystallized by chloroform to get pure product.

2-((3,5-bis(4-chlorophenyl)-1H-pyrazol-4-yl)thio)-5-(pyridin-4-yl)-1,3,4-oxadiazole (5a)

It was obtained as pale yellow solid in 87% yield; mp. 152-154°C; IR (KBr, cm^{-1}): 3345 (N-H), 1625 (C=N), 1393 (C-S-C), 1090 (C-O-C); ^{1}H NMR: (400 MHz, DMSO- d_6, ppm): δ 7.36 (d, *J*=8 Hz, 2H, Ar-H), 7.41 (d, *J*=12 Hz, 4H, Ar-H), 7.64 (d, *J*=12 Hz, 2H, Ar-H), 7.98 (d, *J*=8 Hz, 2H, Ar-H), 8.98 (s, 1H, NH); ^{13}C NMR: (100 MHz, DMSO- d_6, ppm): δ118.87, 120.18, 121.31, 122.53, 123.62, 126.00, 127.97, 128.05, 130.68, 130.71, 140.16, 140.66, 146.03, 148.53, 151.80, 153.71, 161.37, 163.84; MS (m/z): 465.40 (M+), 467.13 (M+2).Anal.Calcd for $C_{22}H_{13}Cl_2N_5OS$: C, 56.66; H, 2.81; N, 15.02. Found: C, 56.62; H, 2.85; N, 15.05.

2-((3-(4-chlorophenyl)-5-(4-fluorophenyl)-1H-pyrazol-4-yl)thio)-5-(pyridin-4-yl)-1,3,4-oxadiazole (5b)

It was obtained as yellow solid in 82% yield; mp. 142-145°C; IR (KBr, cm^{-1}): 3409 (N-H), 1625 (C=N), 1294 (C-S-C), 1089 (C-O-C); ^{1}H NMR: (400 MHz, DMSO- d_6, ppm): δ 7.31(d, *J*=8 Hz, 2H, Ar-H), 7.42 (d, *J*=12 Hz, 4H, Ar-H), 7.68 (d, *J*=12 Hz, 2H, Ar-H), 7.91 (d, *J*=8 Hz, 2H, Ar-H), 8.79 (s, 1H, NH); ^{13}C NMR: (100 MHz, DMSO- d_6, ppm): δ 118.76, 120.94, 127.89, 128.03, 128.54, 129.38, 129.84,130.19, 130.72, 131.83, 134.68, 136.24, 138.76, 144.37, 149.48, 151.69, 154.78, 161.15, 163.28; MS (m/z): 449.05(M+). Anal.Calcd for $C_{22}H_{13}ClFN_5OS$: C, 58.73; H, 2.91; N, 15.57. Found: C, 58.78; H, 2.94; N, 15.54.

Was obtained as pale yellow solid in 92% yield; mp 156-158°C; IR (KBr, cm^{-1}) 3303 (N-H), 1595 (C=N), 1344 (C-S-C), 1078 (C-O-C);^{1}H NMR: (400 MHz, DMSO- d_6, ppm): δ 7.20 (d, *J*=8 Hz, 2H, Ar-H), 7.37 (d, *J*=12 Hz, 4H, Ar-H), 7.59 (d, *J*=12 Hz, 2H, Ar-H), 7.72 (d, *J*=8 Hz, 2H, Ar-H), 9.28 (s, 1H, NH); ^{13}C NMR: (100 MHz, DMSO- d_6, ppm): δ 121.47, 125.56, 126.81, 127.34, 128.16, 128.91, 129.31, 129.92, 130.23, 130.86, 131.78 132.56, 138.41, 139.35, 144.36, 148.62, 153.64, 156.25, 163.12; MS (m/z): 476.05 (M+). Anal.Calcd for $C_{22}H_{13}ClN_6O_3S$: C, 55.41; H, 2.75; N, 17.62. Found: C, 55.44; H, 2.73; N, 17.65.

2-((3-(4-chlorophenyl)-5-(3-nitrophenyl)-1H-pyrazol-4-yl)thio)-5-(pyridin-4-yl)-1,3,4-oxadiazole (5d)

It was obtained as pale yellow solid in 90% yield; mp 162-164°C; IR (KBr, cm^{-1}): 3386 (N-H), 1615 (C=N), 1345 (C-S-C), 1120 (C-O-C); ^{1}H NMR: (400 MHz, DMSO- d_6, ppm): δ 7.38 (d, *J* = 8 Hz, 2H, Ar-H), 7.48 (d, *J*= 12 Hz, 4H, Ar-H), 7.66 (d, *J*=12 Hz, 2H, Ar-H), 7.95 (d, *J* 8 Hz, 2H, Ar-H), 9.35 (s, 1H, NH); ^{13}C NMR: (100 MHz, DMSO- d_6, ppm): δ 118.86, 119.96, 120.38, 121.56, 123.48, 128.24, 128.79, 129.11, 129.83, 130.07, 130.74, 134.91, 149.28, 150.94, 154.36, 156.78, 158.21, 159.26, 160.52, 163.62; MS (m/z): 476.05 (M+). Anal.Calcd for $C_{22}H_{13}ClN_6O_3S$: C, 55.41; H, 2.75; N, 17.62. Found: C, 55.39; H, 2.72; N, 17.64.

2-((5-(4-chlorophenyl)-3-(4-fluorophenyl)-1H-pyrazol-4-yl)thio)-5-(pyridin-4-yl)-1,3,4-oxadiazole (5e)

It was obtained as pale yellow solid in 80% yield; mp. 148-151°C; IR (KBr, cm^{-1}): 3390 (N-H), 1620 (C=N), 1415 (C-S-C), 1080 (C-O-C); ^{1}H NMR: (400 MHz, DMSO- d_6, ppm): δ 7.40 (d, *J*=8 Hz, 2H, Ar-H), 7.52 (d, *J*=12 Hz, 4H, Ar-H), 7.70 (d, *J*=12 Hz, 2H, Ar-H), 7.96 (d, *J*=8 Hz, 2H, Ar-H), 8.90 (s, 1H, NH); ^{13}C NMR: (100 MHz, DMSO- d_6, ppm): δ 118.33, 121.74, 122.48, 124.67, 126.27, 127.58,128.17, 128.83, 129.23, 129.94, 130.46, 131.85, 132.54, 143.76, 149.91, 150.97,156.42, 160.48, 161.89; MS (m/z): 449.05 (M+). Anal.Calcd for $C_{22}H_{13}ClFN_5OS$:C, 58.73; H, 2.91;N, 15.57. Found:C, 58.76; H, 2.88; N, 15.60.

2-((3,5-bis(4-fluorophenyl)-1H-pyrazol-4-yl)thio)-5-(pyridin-4-yl)-1,3,4-oxadiazole (5f)

It was obtained as faint yellow solid in 82% yield; mp. 138-140°C; IR (KBr, cm^{-1}): 3355(N-H), 1635 (C=N), 1380 (C-S-C), 1095 (C-O-C); ^{1}H NMR: (400 MHz, DMSO- d_6, ppm): δ 7.10 (d, *J*=8 Hz, 2H, Ar-H), 7.34 (d, *J*=12 Hz, 4H, Ar-H), 7.44 (d, *J*=12 Hz, 2H, Ar-H) 7.69 (t, *J*=8 Hz, 2H, Ar-H), 8.75 (s, 1H, NH); ^{13}C NMR: (100 MHz, DMSO- d_6, ppm): δ 119.51, 121.22, 124.73, 127.64, 128.97, 129.42, 130.20, 130.94, 131.28, 131.73, 134.34, 136.62, 138.43, 141.28, 145.67, 149.87, 151.79, 154.21, 161.13, 164.58; MS (m/z): 433.08 (M+).Anal.Calcd for $C_{22}H_{13}F_2N_5OS$:C, 60.96; H, 3.02;N, 16.16. Found:C, 60.99; H, 3.05;N, 16.13.

2-((3-(4-fluorophenyl)-5-(4-nitrophenyl)-1H-pyrazol-4-yl)thio)-5-(pyridin-4-yl)-1,3,4-oxadiazole (5g)

It was obtained as yellow solid in 91% yield; mp. 156-159°C; IR (KBr, cm^{-1}): 3395 (N-H), 1595 (C=N), 1448 (C-S-C), 1115 (C-O-C);^{1}H NMR: (400 MHz, DMSO- d_6, ppm): δ 7.32 (d, *J*=8 Hz, 2H, Ar-H), 7.44 (d, *J*=12 Hz, 4H, Ar-H), 7.58 (d, *J*=12 Hz, 2H, Ar-H), 7.89 (d, *J*=8 Hz, 2H, Ar-H), 8.86 (s, 1H, NH); ^{13}C NMR: (100 MHz, DMSO- d_6, ppm): δ 118.37, 120.74, 121.42, 126.36, 127.58, 128.86, 131.76, 132.87, 136.28, 138.39, 139.86, 141.36, 148.48, 150.30, 151.42, 153.72, 156.35, 159.46, 160.40, 161.36; MS (m/z): 460.08 (M+). Anal.Calcd for $C_{22}H_{13}FN_6O_3S$:C, 57.39; H, 2.85;N, 18.25. Found: C, 57.36; H, 2.82;N, 18.21.

2-((3-(4-fluorophenyl)-5-(3-nitrophenyl)-1H-pyrazol-4-yl)thio)-5-(pyridin-4-yl)-1,3,4-oxadiazole (5h)

It was obtained as yellow solid in 88% yield; mp. 162-164°C; IR (KBr, cm^{-1}): 3415 (N-H), 1606 (C=N), 1434 (C-S-C), 1130 (C-O-C); ^{1}H NMR: (400 MHz, DMSO- d_6, ppm): δ 7.18 (d, *J*=8 Hz, 2H, Ar-H), 7.29 (d, *J*=12 Hz, 4H, Ar-H), 7.54 (d, *J*=12 Hz, 2H, Ar-H), 7.78 (d, *J*=8 Hz, 2H, Ar-H), 8.79 (s, 1H, NH); ^{13}C NMR: (100 MHz, DMSO- d_6, ppm): δ 116.56, 120.35, 121.42, 126.74, 127.89, 128.87, 129.10, 129.87, 130.25, 130.91, 134.46, 140.84, 145.68, 149.98, 150.34, 154.21, 156.39, 158.67, 160.92, 134.12; MS (m/z): 460.08 (M+). Anal.Calcd for $C_{22}H_{13}FN_6O_3S$:C, 57.39; H, 2.85;N, 18.25. Found:C, 57.35; H, 2.87;N, 18.27.

2-((5-(4-chlorophenyl)-3-(4-nitrophenyl)-1H-pyrazol-4-yl)thio)-5-(pyridin-4-yl)-1,3,4-oxadiazole (5i)

It was obtained as faint yellow solid in 93% yield; mp. 166-168°C; IR (KBr, cm^{-1}): 3405 (N-H), 1610 (C=N), 1380 (C-S-C), 1095 (C-O-C); ^{1}H NMR: (400 MHz, DMSO- d_6, ppm): δ 7.25 (d, *J*=8 Hz, 2H, Ar-H), 7.38 (d, *J*=12 Hz, 4H, Ar-H), 7.54 (d, *J*=12 Hz, 2H, Ar-H), 7.76 (d, *J*=8 Hz, 2H, Ar-H), 8.72 (s, 1H, NH); ^{13}C NM:R (100 MHz, DMSO- d_6, ppm): δ 116.97, 120.27, 121.24, 124.75, 126.78, 127.98, 129.24, 129.86, 130.35, 130.73,131.67, 139.86, 143.68, 149.74, 150.24, 153.74, 155.82, 157.45, 160.32, 162.78; MS (m/z): 476.05 (M+).Anal.Calcd for $C_{22}H_{13}ClN_6O_3S$:C, 55.41; H, 2.75; N, 17.62. Found:C, 55.43; H, 2.71; N, 17.65.

Biology assays

Antibacterial screening

The antibacterial activities of the synthesized compounds were screened by using the agar diffusion method against two different bacterial species such as *B. megaterium* and *E. coli*. Penicillin was used as a standard drug for antibacterial activities. DMSO was used as a solvent. Nutrient agar plates are used for the evaluation of antibacterial activities which was seeded with respective bacterial culture strain (0.1 ml) of the suspension prepared in sterile saline. All the plates were incubated at 37 ± 0.5°C for 24 h. The minimum inhibitory Concentrations (MIC) are noted and the results are summarized in Table 1.

Antioxidant screening

In the present study we investigate *in vitro* DPPH, OH and SOR (superoxide anion) radical scavenging assay to evaluate the antioxidant potential of substituted pyrazolyl thio pyridinyl 1,3,4-oxadiazolederivatives (5a-s) with respect to standard AA using spectrophotometer and the results are summarized in Table 2.

DPPH radical scavenging assay

The synthesized compound was added to 10^{-4} M ethanol solution of DPPH for the preparation of solution having equimolar concentration (0.5-1 mM). After incubation (30 min) at room temperature the sample absorbance was measured on spectrophotometrically at 517 nm. AA was used as standard.

OH radical scavenging assay

For the generation of OH radicals, the ferric ion (Fe^{+3})/AA system was used. The OH radical detection being carried out by measuring the amount of formaldehyde generated from the oxidation of Dimethyl Sulfoxide (DMSO).

The reaction mixture contain 0.1 mM Ethylenediaminetetraacetic acid (EDTA), 167 mM Fe^{+3}, 33 mM DMSO in phosphate buffer (50 mM pH 7.4), 0.05-0.1 ml individual 2(3,5-disubstituted)-1H-pyrazol-4-yl-thio-5-(pyridin-4-yl)1,3,4-oxadiazole derivatives (0.5-1 mM) solution. The reaction was initiated by the addition of AA (150 ml, 10 mM in phosphate buffer). The reaction was terminated by using Trichloroacetic acid (17% w/v). The generated formaldehyde was detected spectrophotometrically at 412 nm. For comparative study AA (1 mM) was used as reference compound.

Table 1: Antibacterial activity of the synthesized compounds (5a-s)

Entry	Product	Minimum Inhibition Concentration (MIC) in µg/ml	
		Bacillus megaterium	***Escherichia coli***
1	5a	250	250
2	5b	500	500
3	5c	125	62.78
4	5d	78.23	250
5	5e	500	500
6	5f	500	500
7	5g	250	250
8	5h	250	250
9	5i	89.11	125
10	5j	500	500
11	5k	125	30.12
12	5l	21.40	250
13	5m	250	82.69
14	5n	125	125
15	5o	250	44.20
16	5p	50.12	125
17	5q	125	125
18	5r	500	500
19	5s	11.15	10.21
STD	Penicillin	3.9 µg	3.9 µg

Table 2: Antioxidant activity of the synthesized compounds (5a-s)

Entry	Product	DPPH (%)	OH (%)	SOR (%)
1	5a	52.88	40.39	35.20
2	5b	51.55	42.76	41.34
3	5c	49.02	50.66	51.20
4	5d	51.33	49.22	48.24
5	5e	35.39	34.29	31.88
6	5f	39.77	35.22	34.29
7	5g	41.88	42.38	30.97
8	5h	44.27	46.56	35.17
9	5i	52.76	49.76	48.40
10	5j	54.21	32.86	36.60
11	5k	58.20	54.65	50.70
12	5l	57.82	52.25	58.40
13	5m	49.92	49.26	42.25
14	5n	53.95	45.56	40.89
15	5o	51.45	61.25	47.69
16	5p	51.76	55.95	54.68
17	5q	49.68	50.65	49.51
18	5r	50.32	42.14	41.88
19	5s	68.80	58.89	52.02
STD	Ascorbic acid	76.45	69.37	72.58

Superoxide Anion Radical (SOR) scavenging assay

The superoxide anion radical was generated by PMS/NADH system. Afterward the superoxide anion was made to diminish NBT, which yields a chrogenic products having λ_{max} at 560 nm. The radical scavenging activities which are concentration dependent were performed separately. The reaction cocktail contain NBT (300 mM), NADH (936 mM), PMS (120 mM), and individual concentration of substituted pyrazolyl thio pyridinyl 1,3,4-oxadiazolederivatives (0.5-1 mM) in Tris HCl (buffer 100 mM, pH 7.4). The reaction was initiated by the addition of PMS to the reaction mixture. After incubation period (5 min) at room temperature the reaction mixture was read at 560 nm by using Thermo Make Automatic Ex-Microplate Reader (M51118170).

The percentage activity of DPPH, OH and SOR radical scavenging activity was calculated by using the following equation:

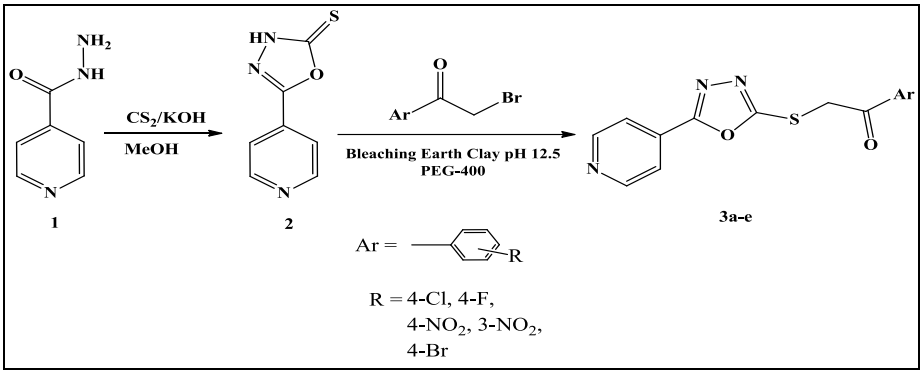
$$Activity(\%) = 1 - \frac{T}{C} \times 100$$

Where, T=Absorbance of the test sample and C=Absorbance of the standard sample.

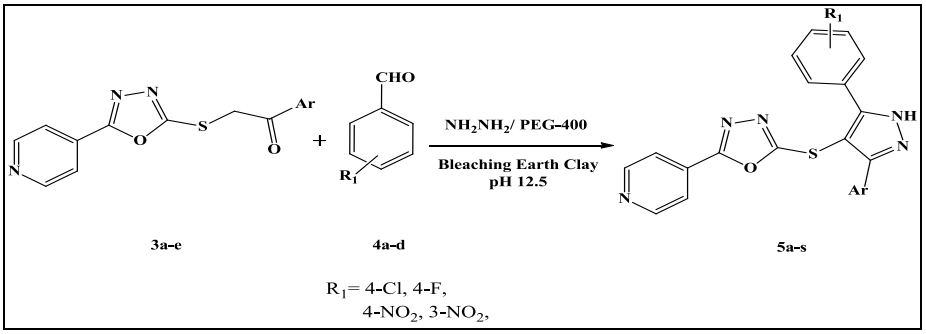
RESULTS AND DISCUSSION

Chemistry

The synthetic route of the title compounds were depicted in the Schemes 1 and 2. The precursor 2 was prepared by the method reported elsewhere in the literature [43-46]. Furthermore, the compounds 3a-e were prepared by reported method [47,48] with some modification wise reaction of 2 with the substituted phenacyl bromide in the presence of BEC and PEG-400 as green reaction solvent.

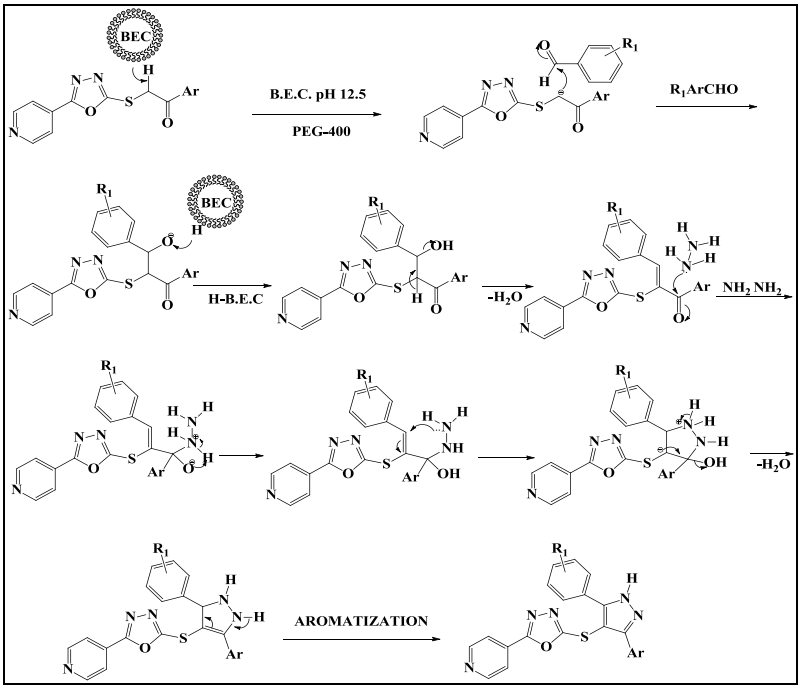


Scheme 1: Synthesis of compounds (3a-e)



Scheme 2: Synthesis of compounds (5a-s)

The structures of compounds 3a-e were confirmed by the IR, ^{1}H NMR and Mass spectral analysis. The IR spectrum of compound 3a shown a characteristic absorption bands at 1678 cm^{-1} clearly indicates the presence of C═O (carbonyl) group. The ^{1}H NMR spectrum of compound 3a revealed a singlet at δ 5.21 ppm was attributed to aliphatic -CH_2 protons. Whereas all aromatic protons are resonate in the range of δ 6.80-7.90 ppm. The molecular ion peak in the mass spectrum corresponds to the molecular mass of the compound.



Scheme 3: Possible mechanism of compounds (5a-s)

Furthermore, one pot condensation of 3a-e with substituted benzaldehyde (4a-d) and hydrazine hydrate in the presence of BEC and PEG-400 offer title compound in quantitative yield. Initially, *in situ* generation of α, β-unsaturated compound by the BEC catalyzed Knoevenagel condensation of 3a-e with substituted benzaldehyde (4a-d) followed by the Michael addition of hydrazine hydrate to give the title compounds 5a-s. The structures of compounds 5a-s were established by the satisfactory spectral analysis.

In an IR spectrum, the compounds 5a shown a strong stretching vibration band at 3345 cm^{-1} indicate the presence of -NH group. A strong stretching vibration observes at 1625 cm^{-1} due to the presence of C=N and a strong stretching vibration at 1090 cm^{-1} confirms the presence of C–O–C linkage. The ^{1}HNMR spectrum of compound 5a revealed a singlet at δ 8.65 ppm was attributed to -NH of pyrazole, whereas the remaining protons are present in the aromatic region at δ 7.20-8.43 ppm. The compound 5a in the mass spectrum gave a peak at 467 due to [M+1]. The plausible mechanism was outlined in Scheme 3.

In order to synthesize title compounds (5a-s) in quantitative yield, the model reaction was studied for the optimization of the newly designed cyclization route. Keeping in mind "Principles of Green Chemistry" initially the reaction was carried out under solvent-free and catalyst free condition. We observed that in the absence of catalyst, there was no reaction at room temperature or even at higher temperature. Moreover, the model reaction was studied under different basic catalyst such as K_2CO_3, Triethylamine (TEA), Piperidine, Morpholine and BEC. Among the five catalysts studied for the optimization of reaction, BEC was found to be effective and offer the best yield (92% entry 9, Table 3).

Table 3: Influence of the catalyst on the synthesis of (5c)

Entry	Catalyst (mole/wt%)	Temp. (°C)	Time (h)[a]	Yield(%)[b]
1	No Catalyst	RT	7	0
2	No Catalyst	80	7	0
3	K_2CO_3 (mole %)	70	6	60
4	TEA (mole %)	70	6	64
5	Piperidine (mole %)	70	5	65
6	Morpholine (mole %)	70	6	55
7	Bleaching Earth Clay (1 wt %)	80	3	73
8	Bleaching Earth Clay (5 wt %)	80	2.5	85
9	Bleaching Earth Clay (10 wt %)	80	2	92

[a]Reaction progress monitored by thin-layer chromatography (TLC); [b]Yields refer to isolated yield

The synthesis is said to be ideal if it is carried out under solvent free condition or by adopting use of green solvent. Initially the model reaction was studied under solvent free condition in the presence of BEC as a catalyst and it was observed that the yield of reaction at lower side. In order to enhance the yield of the reaction, the model reaction was carried out in water, considering water as green reaction media. It revealed that reaction was not clean and efficient as far the yield and purity of the product as concern (Tables 4 and 5, entry 1). In recent years PEG-400 attracted much attention of synthetic chemist for its use as green reaction media. Hence, we studied a model reaction in PEG-400 in the presence of BEC as catalyst and to our delight the combination of PEG-400 and BEC was found efficient for the conversion of reactant to product above 90% yield. With this optimized condition utilizing PEG-400 and BEC as a green catalytic media, we have efficiently synthesized all remaining derivative in good yield and purity.

Table 4: Influence of the solvent on the synthesis of (5c)

Entry	Solvent	Time(h)[a]	5c(%)[b]
1	Water	6	0
2	EtOH	5	71
3	CH_3COOH	6	62
4	DMF	6	67
5	PEG-400	2	92

[a]Reaction progress monitored by thin-layer chromatography (TLC); [b]Yields refer to isolated yield

Table 5: Synthesis of compounds (5a-s)

Entry	Product	Ar	R_1	Molecular formula	Yield (%)	M.P. (°C)
1	5a	4-Cl $C_6H_4^-$	4-Cl	$C_{22}H_{13}Cl_2N_5OS$	87	152-154
2	5b	4-Cl $C_6H_4^-$	4-F^-	$C_{22}H_{13}ClFN_5OS$	82	142-145
3	5c	4-Cl $C_6H_4^-$	4-NO_2^-	$C_{22}H_{13}ClN_6O_3S$	92	156-158
4	5d	4-Cl $C_6H_4^-$	3-NO_2^-	$C_{22}H_{13}ClN_6O_3S$	90	162-164
5	5e	4-F $C_6H_4^-$	4-Cl^-	$C_{22}H_{13}ClFN_5OS$	80	148-151
6	5f	4-F $C_6H_4^-$	4-F^-	$C_{22}H_{13}F_2N_5OS$	82	138-140
7	5g	4-F $C_6H_4^-$	4-NO_2^-	$C_{22}H_{13}FN_6O_3S$	91	156-159
8	5h	4-F $C_6H_4^-$	3-NO_2^-	$C_{22}H_{13}FN_6O_3S$	88	162-164
9	5i	4-NO_2 $C_6H_4^-$	4-Cl^-	$C_{22}H_{13}ClN_6O_3S$	93	166-168
10	5j	4-NO_2 $C_6H_4^-$	4-F^-	$C_{22}H_{13}FN_6O_3S$	89	162-164
11	5k	4-NO_2 $C_6H_4^-$	4-NO_2^-	$C_{22}H_{13}N_7O_5S$	92	168-170
12	5l	4-NO_2 $C_6H_4^-$	3-NO_2^-	$C_{22}H_{13}N_7O_5S$	92	167-169
13	5m	3-NO_2 $C_6H_4^-$	4-Cl^-	$C_{22}H_{13}ClN_6O_3S$	80	159-161
14	5n	3-NO_2 $C_6H_4^-$	4-F^-	$C_{22}H_{13}FN_6O_3S$	83	153-155
15	5o	3-NO_2 $C_6H_4^-$	4-NO_2^-	$C_{22}H_{13}N_7O_5S$	82	167-169
16	5p	3-NO_2 $C_6H_4^-$	3-NO_2^-	$C_{22}H_{13}N_7O_5S$	80	164-167
17	5q	4-Br $C_6H_4^-$	4-Cl^-	$C_{22}H_{13}BrClN_5OS$	82	176-178
18	5r	4-Br $C_6H_4^-$	4-F^-	$C_{22}H_{13}BrFN_5OS$	81	172-175
19	5s	4-Br $C_6H_4^-$	4-NO_2^-	$C_{22}H_{13}BrN_6O_3S$	84	181-183

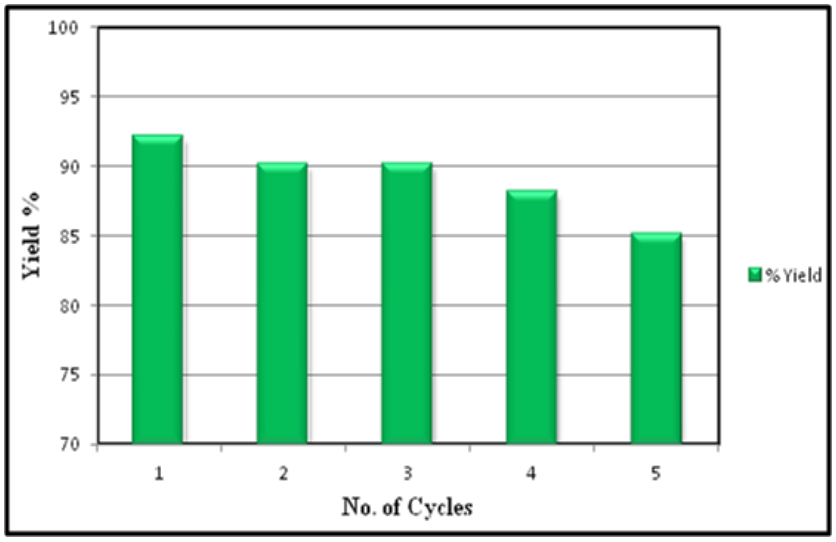


Figure 1: Relationship between yield of the reaction and reusability of catalyst

Biological evaluation

Finally we focus on the recycling of the catalyst and it was found that the catalyst BEC shows good catalytic power up to five run with minimum loss of activity (Figure 1). This shows the reusability of the catalyst. Thus, at the end of the model reaction, the catalyst was filter by simple filtration, washed thoroughly with ethanol, dried at room temperature and reuse for the next reaction.

Antibacterial screening

All the newly synthesized compounds (5a-s) were evaluated for their antibacterial activity against *B. megaterium* (Gram positive) and *E. coli* (Gram negative) bacterial species by using agar diffusion method. This study revealed that the compounds 5s and 5l demonstrated moderate antimicrobial activities, while all other synthesized compounds showed low antibacterial activity in comparison to penicillin (a standard drug) as shown in Table 1.

Among the synthesized compounds (5a-s) compound 5s showed excellent activity against *B. megaterium* (MIC=11.15 µg/ml) and *E. coli* (MIC=10.21 µg/ml). Compound 5k had excellent activity (MIC=30.12 µg/ml) against *E. coli*. These data revealed that compound 5l was highly active (MIC=21.40 µg/ml) against *B. megaterium* and compound 5o displayed strong activity (MIC=44.20 µg/ml) against *E. coli*. It is noteworthy that compound 5p exhibited good activity (MIC=50.12 µg/ml) against *B. megaterium*. Compound 5c (MIC=62.78 µg/ml) and 5m (MIC=82.69 µg/ml) showed moderate activity against *E. coli* while compound 5d (MIC=78.23 µg/ml) and 5i (MIC=89.11 µg/ml) displayed good activity against *B. megaterium*. Moreover the remaining compounds possessed feeble antibacterial activities. The above observation reveals that the compounds 5s having electron withdrawing groups especially bromo at para position shows significant antibacterial activity against *B. megaterium* and *E. coli.* The compounds 5c, 5d, 5i, 5k, 5l, 5m, 5o and 5p bearing Nitro group at para and meta position displayed considerable activity against both the species. Thus the chemical structure and antibacterial activity relationship showed that the compounds with the substituents like Bromo and Nitro group exhibited significant antibacterial activity as compared to the rest of synthesized compounds.

Antioxidant screening

From the result summarized in Table 2, the compounds 5s and 5l demonstrated considerable antioxidant activities, while all other synthesized compounds showed moderate antioxidant activity as compared to Ascorbic Acid (AA) as a standard antioxidant agent. The DPPH assay has been widely used to study the ability of the compound as free radical scavengers or hydrogen donors. Compounds 5s (68.80%), 5k (58.20%) and 5l (57.82%) showed significant DPPH radical scavenging activity as compared to standard AA (76.45%), while the rest compounds showed moderate DPPH radical scavenging activity.

The overall range of DPPH scavenging activity of all the tested compounds was in a range of 43.77-68.80%. OH radicals are highly hyperactive among the reactive oxygen species and play a key role in the physiological regulation and control of cell function [49-51]. It was observed that compound 5o (61.25%), 5s (58.89) and 5p (55.95) exhibited good OH radical scavenging activity as compared to standard AA (69.37%), whereas the remaining tested compound demonstrated moderate OH radical scavenging activities. The overall range of OH scavenging activity of all the tested compounds was in a range of 32.86-61.25%. The profile of SOR radical scavenging activities indicate that the compounds 5l (58.40%), 5p (54.68%) and 5s (52.02) exhibit considerable SOR radical scavenging activity as compared to standard AA (72.58%) whereas the rest compounds showed moderate SOR radical scavenging activities. The overall range of SOR scavenging activity of all the tested compounds was in a range of 30.97-58.40%. While discussing the SAR of the synthesized compounds and the antioxidant screening, all the above results confess that the compounds 5k, 5l, 5o, 5p and 5s having bromo and nitro substituent displayed significant antioxidant activity as compared to the other synthesized compounds.

CONCLUSION

In conclusions, we have found an efficient, clean and eco-friendly method for the synthesis of a new series of substituted pyrazolyl thio pyridinyl 1,3,4-oxadiazoleby using BEC and PEG-400. The method offer several advantages including short reaction time, simple isolation, reuse of the catalyst and easy work-up. Furthermore the biological screening result revealed that the newly synthesized compounds exhibited good to moderate biological activity. Compounds (5k, 5l, 5o, 5p, 5s) displayed comparable activity to penicillin used as an active comparator, whereas compounds (5s, 5o, 5l) showed considerable antioxidant activities as compared to standard AA.

Thus the present rout of green synthesis might contribute for the development of green strategy for the synthesis of a novel class of antibacterial and antioxidant agents.

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