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Synthesis and Evaluation of Antimicrobial, Antioxidant Activities of Pyridopyrazolo Pyrimido Pyrimido Benzothiazole Derivatives

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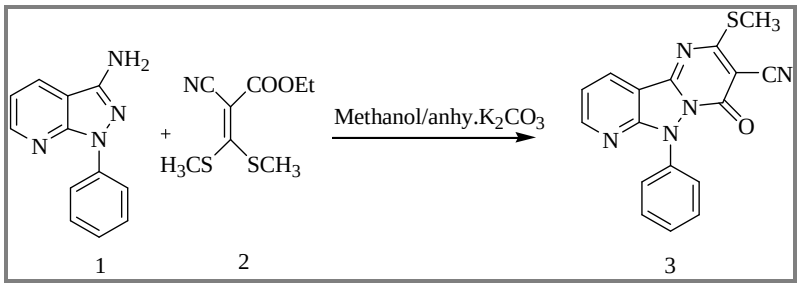
ABSTRACT

The objective of the present investigation was to synthesize 3-cyano-4-oxo-2-(methylthio)-6-N-phenyl pyrido[3,2-d] pyrazolo[3,2-b]pyrimidine (3) by condensation of 1-phenyl-1H-pyrazolo[3,4-b] pyridine-3-amine (1) with ethyl cyano bis(methylthio) acrylate (2). Which on further condensation with various 2-amino 1/2/3/4–substituted benzothiazoles(4a-f) gives 15-imino-14-oxo-12-N-phenyl pyrido [3,2-d] pyrazolo [3,2-b]-4H-pyrimido[5,6-e]-4H-pyrimido[2,3-b]benzothiazole and their 1/3 substituted derivatives (5a-f). These newly synthesized compounds were further screened for antimicrobial and antioxidant properties.

Graphical Abstract



Synthesis of 3cyano-4-oxo2-(methylthio)-6-phyenylpyrido[3,2-d]pyrimidine

Keywords: Pyrido pyrazolo pyrimidine, Ethyl cyano bis(methylthio)acrylate, Anhydrous K_2CO_3.

INTRODUCTION

Pyridopyrazolo pyrimidines are the tricyclic heterocycles shows divers biological activities like antitumor [**1**], antiproliferative [**2, 3**], proteinkinase inhibiter [**4**], antimicrobial [**5**] and due to these activities these pyridopyrazolopyrimidines tricyclic compounds have gained significant attention. These tricyclic pyridopyrazolo pyrimidine containing pyridine, pyrazole and pyrimidine when fused with other heterocycles to form polycyclic heterocycles and their impact on activity is of present

interest. In the literature survey there are lots of different derivatives of pyrido compounds pyrazolopyrimidine [**6-10**]. Thus literature survey reveals that pyridopyrazolopyrimidine derivatives are associated with broad range of biological activities which encourage us to synthesis new derivatives of these heterocycles.

MATERIALS AND METHODS

Open capillary tubes was used for recording melting points of newly synthesized compounds and were uncorrected. FTIR spectrometer was used for the IR spectra of compounds, were as on Bruckeradvance 300 MHz spectrometer was used for 1H-NMR spectra. FT-VG-7070 Hz mass spectrometer has been utilized for Mass spectra using ESI technique.

General Procedure

Synthesis of 3-cyano-4-oxo-2-(methylthio)-6-*N*-phenylpyrido[3,2-*d*]pyrazolo[3,2-*b*] pyrimidine (3): To a solution of 1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-3-amine (1) (2.1 g, 0.01 mol) in 20 mL of methanol, ethyl cyano bis(methylthio)acrylate (2) (2.17 g, 0.01 mol) and anhydrous K_2CO_3 (10 mg) was added and allowed to reflux for 4 hours. The process of reaction was monitored by TLC. After completion of reaction, the reaction mixture was cooled and poured into ice-cold water. The separated solid product was filtered washed with water and recrystallized using ethanol-DMF mixture to give (3) as grey solid.

15-Imino-14-oxo-12-*N*-phenyl pyrido[3,2-*d*] pyrazolo[3,2-*b*]-4*H*-pyrimido[5,6-*e*]-4*H*-Pyrimido [2,3- *b*]benzothiazole and their 1/3 substituted derivatives (5a-f): A mixture of (3) (0.333 g, 0.001 mol) and independently with 2-aminobenzothiazole (4a), 2-amino-6-methyl benzothiazole (4b), 2-amino-4,6-dimethylbenzothiazole (4c), 2-amino-6-methoxy benzothiazole (4d), 2-amino-6-chloro benzothiazole (4e), 2-amino-6-nitro benzothiazole (4f), (0.001 mol) in 15 mL of DMF and anhydrous K_2CO_3 (10 mg) was refluxed for 4-5 h. The reaction mixture was cooled to room temperature and poured into ice cold water. The separated solid product was filtered, washed with water and recrystallized using ethanol-DMF mixture to give pure (5a-f) respectively.

3-cyano-4-oxo-2-(methylthio)-6-*N*-phenylpyrido[3,2-*d*]pyrazolo[3,2-*b*] pyrimidine (3): Gray solid, 75 % yield, M.P. 240°C, IR (KBr) cm-1 2194.84 (CN), 1697.24 (C=O), ^{1}H NMR (DMSO*d6*, δ ppm) 2.27 (s, 3H, SCH_3), 6.94-7.46 (m, 8H, -Ar-H), CI-MS (m/z) 334 (M+1).

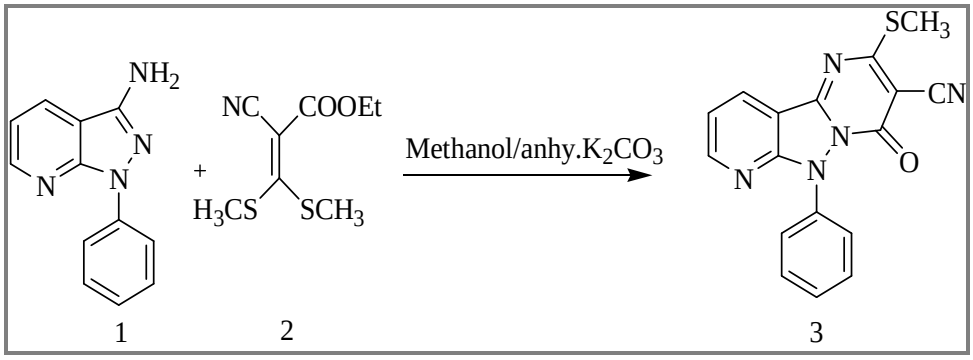
15-Imino-14-oxo-12-N-phenyl pyrido[3,2-*d*]pyrazolo[3,2-*b*]-4*H*- pyrimido[5,6-*e*]-4*H*-pyrimido [2,3-*b*]benzothiazole (5a): Gray solid, 77% yield, M.P. 231°C, IR (KBr) 3417, 3460 (=NH),1697.24 (C=O), ^{1}H NMR (DMSO*d6*, δ ppm) 6.96-7.47 (m, 12H, -Ar-H), 9.1(s, 1H, =NH), CI-MS (m/z) 436 (M+1).

15-Imino-3-methoxy-14-oxo-12-N-phenyl pyrido [3,2-*d*] pyrazolo [3,2-*b*] -4*H*-pyrimido [5,6-*e*]-4*H*- pyrimido[2,3-*b*] benzothiazole (5d): Brown solid, 74% yield, M.P. 240°C, IR (KBr) 3290, 3390, 3463.9(=NH), 1697.24 (C=O), ^{1}H NMR (DMSO*d6*, δ ppm3.73 (s, 3H, $-OCH_3$), 6.78-7.29 (m, 11H, -Ar-H),8.96 (s, 1H, =NH) CI-MS (m/z) 466 (M+1).

RESULTS AND DISCUSSION

Title compounds 15-imino-14-oxo-12-*N*-phenyl pyrido[3,2-*d*]pyrazolo[3,2-*b*]-4*H*-pyrimido[5,6-*e*]-4*H*- pyrimido[2,3-*b*]benzothiazole and their 1/3 substituted derivatives (5a-f) in two steps. The reaction starts with 1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-3-amine (1). The compound (1) was reacted with ethyl cyano bis(methylthio) acrylate (2) in methanol and anhydrous K_2CO_3. Compound (1) having nucleophilic centre at 1,3 position undergoes cycloaddition with (2), which is a functionalized α-oxo-ketene dithioacetal possessing 1-3 bielectrophilic character which is

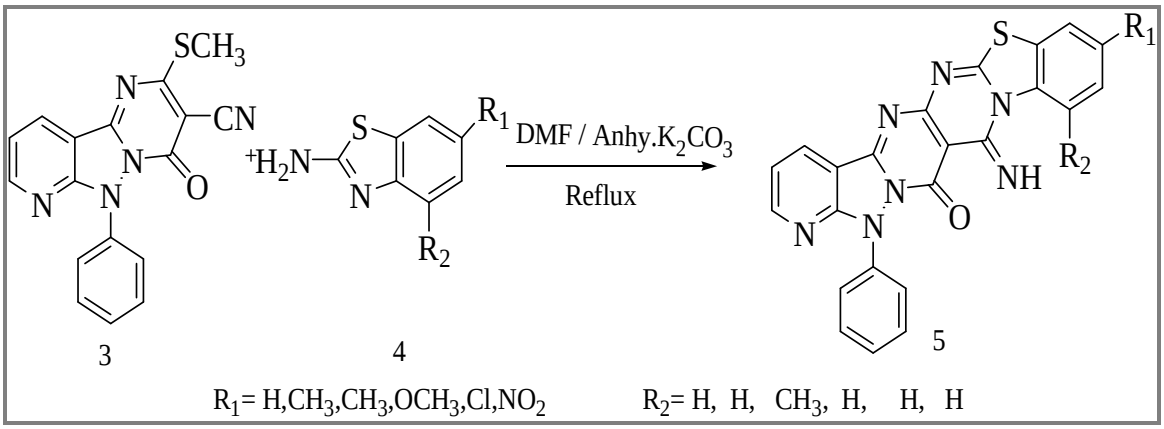
conveniently cyclized with1,3-dinitrogen compounds to afford 3-cyano-4-oxo-2-(methylthio)-6-*N*-phenylpyrido [3,2-*d*]pyrazolo[3,2-*b*] pyrimidine (3) (Scheme 1).



Scheme 1. Synthesis of 3cyano-4-oxo2-(methylthio)-6-phyenylpyrido[3,2-d]pyrimidine.

The structure of the compound (3) was assigned on the basis of analytic and spectral data. IR spectrum shows absorption bonds at 2194.84 cm^{-1} and 1697.24 cm^{-1}, which can be assigned to –CN and C=O stretching respectively. The ^1H NMR spectrum of the compound was recorded in DMSO d_6 and shows singlet at δ 2.27 ppm assignable to $–SCH_3$ group, multiplet at δ 6.94-7.46 ppm due to eight aromatic protons respectively. Mass spectrum exhibits molecular ion peak at m/z 334 (M+1) which corresponds to its molecular weight.

Synthesized parent compound (3) acts as bis-electrophilic reacted with nucleophilic reagents like substituted 2-amino benzothiazoles (4a-f) for 4-5 hour in DMF and anhydrous K_2CO_3 resulting in 15-imino-14-oxo pyrido[2,3-*d*]pyrazolo[2,3-*b*]-4*H*-pyrimido[5,6-*d*]-4*H*-pyrimido[2,3-*b*]benzothiazole and their 1/3 substituted derivative (5a-f) (Scheme 2).



Scheme 2. Synthesis of 15-imino-14 oxo-12-N-phenyl pyrido[3,2-d]pyrazolo[3,2-b]-4h-pyrimido[5,6-e]-4H-pyrimido [2,3-b]benzothiazole and their 1/3 substituted derivatives.

The structures of these newly synthesized cyclized compounds (5a-f) were confirmed on the basis of elemental analysis, IR, ^1H NMR and Mass spectral data. In the compounds (5a-f) disappearance of IR signal for CN stretching absorption band in the region 2194.84 cm^{-1} indicates that cyclisation takes place. All compounds (5a-f) showed the presence of absorption bands in the region 3058-3464 cm^{-1} and 1697.24 cm^{-1} which can be assigned to (=NH) and (C=O) respectively. The ^1H NMR spectra exhibited a singlet at δ 8.96-9.21, exchangeable with D_2O, which can be assigned to (=NH) proton. Mass spectra of compounds exhibited the molecular ion peaks which correspond to their molecular weights.

Antioxidant Activity

DPPH assay: DPPH (2, 2, diphenyl-1-picrylhydrazyl) radical scavenging assay was carried out as per reported methods with slight modification [**11**]. Briefly, 1 mL of test solution (Test compound) added to equal quantity of 0.1 mmol solution of DPPH in ethanol. After 20 min incubation at room temperature, the DPPH reductions were measured by reading the absorbance at 517 nm. Ascorbic acid used as reference compound.

Hydroxyl radical scavenging assay: Hydroxyl radical scavenging activities were determined by the earlier reported method [**12**]. The reaction cocktail contained 60 µL of 1 mmol, $Fecl_3$, 90 µL of 1 mmol 1,10-phenanthroline, 2.4 mL of 0.2 M phosphate buffer (pH 7.8), 150 µL of 0.17 M H_2O_2 and 1.5 mL of various concentration of individual compound. Reaction mixture kept at room temperature for 5 min incubation and absorbance was measured at 560 nm using spectrophotometer. α-Tocopherol was used a reference compound.

Table 1. Antioxidant potential of pyrido[2,3-*d*]pyrazolo[2,3-*b*]-4*H*-pyrimido[5,6-*d*]-4*H*-pyrimido[2,3-*b*]benzothiazoles and their substituted derivatives

S. No.	Compound	DPPH radical scavenging activity (%)	OH radical scavenging activity (%)
1	3	46 ± 0.09	60 ± 0.65
2	5a	32 ± 0.29	38 ± 0.08
3	5d	24 ± 0.12	29 ± 0.28
4	Ascorbic Acid	86 ± 0.88	NA
5	α- Tocopherol	NA	81 ± 0.19

NA: Not applicable

APPLICATION

Antimicrobial Activity

Disc diffusion method: Kirby-Bauer method was followed for disc diffusion assay. *In vitro* antimicrobial activity was screened by using Mueller Hinton Agar (MHA) obtained from Himedia (Mumbai). The MHA plates were prepared by pouring 15 mL of molten media into sterile petriplates. The plates were allowed to solidify for 5 min and 0.1 % inoculum suspension was swabbed uniformly and the inoculum was allowed to dry for 5 min. The concentration of compounds were set at (10 µg $disc^{-1}$) were loaded on 5 mm sterile individual discs. The loaded discs were placed on the surface of medium and the compound was allowed to diffuse for 5 min and the plates were kept for incubation at 37°C for 24 h. Penicillin (10 µg $disc^{-1}$) was used as positive control. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter.

Table 2. Antimicrobial potential of pyrido[2,3-*d*]pyrazolo[2,3-*b*]-4*H*-pyrimido[5,6-*d*]-4*H*-pyrimido[2,3-*b*]benzothiazoles and their substituted derivatives

S.No.	Name of compound	Zone of inhibition in mm	
		E. coli	*B. subtilis*
1	3	12	NR
2	5a	11	10
3	5d	14	09
4	*penicillin*	26	28

NR: Not reported

CONCLUSION

Simple and efficient synthesis 14,15-diimino-12-*N*-phenyl pyrido[3,2-d]pyrazolo[3,2-*b*]-4*H*-pyrimido [5,6-e]-4*H*- pyrimido[2,3-*b*]benzothiazole and their 1/3 substituted derivatives (5a-f) has been

presented. Among these synthesized compounds (5d) showed remarkable antioxidant activity and compound (5d), exhibit promising antimicrobial activity against *E.coli*. The result of the present work demonstrated that pyrido pyrazolo pyrimidines are potent antioxidant and antimicrobial agents and it will attract researchers to design new potent pharmacological pyrido pyrazolo pyrimidines.

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