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Efficient synthesis of novel Pyrazolo thiazole derivatives and its antifungal activity studies

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ABSTRACT

Novel pyrazolo thiazole derivatives were synthesized by thioamide treated with various α-haloketones in ethanol. The structures of the newly synthesized compounds are characterized by ^{1}HNMR, ^{13}C NMR, LC-MS and screened for their antifungal activity against Aspergillus flavus (NCIM No.524), Fusarium oxysporum (NCIM No.1072) and Candida albicans (NCIM No.3102).

Keywords: *Tert-butyl 6, 7-dihydro-1-methyl-3-thiocarbamoyl-1-H-pyrazolo [4, 3-c] pyridine-5-(4H)carboxylate, Synthesis, Antifungal activity.*

INTRODUCTION

Nitrogen containing heterocyclic compounds- especially pyrazoles and its derivatives are broad spectrum of biologically active such as antimicrobial agents[1], anti-inflammatory[2], antifungal[3], herbicidal[4], antiviral[5], analgesic, antitumour, cytotoxic, antipyretic and obesity[6].Much attention was paid to pyrazole as a potential antimicrobial agent after the discovery of the natural pyrazol-c-glycoside, pyrazofurin which demonstrated a broad spectrum of antimicrobial activity[7].With growing application on their synthesis and bioactivity, Chemists and biologists recent years have directed considerable attention on the research oh pyrazole derivatives. We report in the present work the synthesis and biological activity of novel pyrazole derivatives containing thiazole skeleton.

Chemistry:

The required pyrazolothiazole derivatives prepared from N-Boc-4-piperidone by five steps. The β-diketo ester(2) was synthesized from N-Boc-4 piperidone by using LiHMDS and diethyl oxalate[8], then it was condensed with methyl hydrazine in ethanol to get pyrazole carboxylic acid ethyl ester(3)[9] which upon treated by liquor ammonia in tetrahydrafuran to afford amide(4)[10]. The amide was converted into Boc protected thioamide(5)[11] by using Lawesson's reagent in toluene, which under the refluxing condition with various α-bromo ketones in ethanol to afford the expected Boc cleaved pyrazolo-thiazole derivatives(6)[12][Scheme 1]. The expected yields of final compounds are mentioned in Table 1.

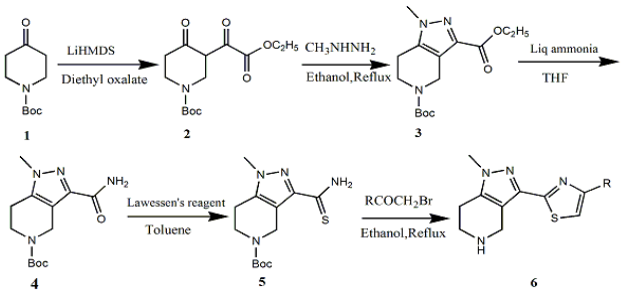
EXPERIMENTAL

All reagents were purchased from Aldrich and used as received. Dry THF, Ethanol, Toluene were supplied by Spectrochem. All chemistry was performed under a nitrogen atmosphere using standard techniques. All the NMR spectra were measured using either Bruker AMX 400 instrument with 5mm PABBO BB-1H tubes. 1H and 13C NMR spectra were measured for approximately 0.03M solutions in d6-DMSO at 400MHZ with TMS as internal reference. The IR spectra were measured as potassium bromide pellets using a Perkin-Elmer 1600 series FTIR spectrometer. LCMS were obtained using Agilent 1200 series LC and Micro mass zQ spectrometer. Column chromatography was performed using a silica gel (230-400 mesh).

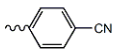
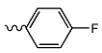
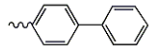
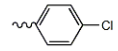
Tert-butyl 3-(ethyl formylformyl)-4-oxopiperidine-1-carboxylate (2):

LiHMDS (65.3 mL, 1.0 M in THF, 65.3 mmol) was added to the solution of N-Boc-4-piperidinone (10g, 50mmol) in tetrahydrofuran (100 mL) at –40 °C with agitation and the anion formed was allowed to stand approximately 20 min., and then Diethyl oxalate (10.99 g, 75.3 mmol) was added slowly with stirring. The reaction mixture (RM) was removed from acetone-dry ice bath and stirred for 1h. The reaction was monitored by TLC, then 2 mL of Acetic acid was added with stirring. RM was dissolved in Ethyl Acetate; the organic layer was washed with saturated brine solution, dried over Na_2SO_4, evaporated under reduced pressure. Crude product was purified by Column chromatography using pet ether: Ethyl Acetate (1:99). Yield of the product was 8.5g (56%) as yellow liquid. Mol.Wt.: 299.32; LCMS : M^++1(300.2). ^{1}H NMR (DMSO-d^6, 400MHz) δ 4.2 (q, J=5.6Hz, 2H), 3.9(s, 2H), 3.49(t, J=5.85Hz, 1H), 2.48(m, 2H), 1.41(s,9H), 1.25(t, J=7.3Hz, 3H). ^{13}C NMR (DMSO-d^6, 100MHz) 207.91, 173.83, 166.14, 154.32, 105.46, 79.69, 66.83, 40.83, 39.17, 30.28, 14.17.

Scheme-1



Table

S.No	R	S.No	R
6a		6f	
6b		6g	
6c		6h	
6d		6i	
6e		6j	

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5-tert-butyl-6, 7-dihydro-1-methyl-1-H-pyrazolo [4, 3-c] pyridine-3, 5 -(4H) dicarboxylate (3):

To a solution of Tert-butyl 3-(ethyl formyl formyl)-4-oxopiperidine-1-carboxylate (6gm,20.mmol) in 60 mL ethanol was added methyl hydrazine (0.92g, 20 mmol) and contents were refluxed for 3hrs. After the completion of reaction (monitored by TLC), Reaction media was concentrated to residue stage under reduced pressure and re-dissolved to Ethyl Acetate. The organic layer was washed with brine solution, dried over sodium sulphate and evaporated under reduced pressure. Crude product was purified by Column chromatography using Pet ether: Ethyl Acetate. Yield 5g (82%) as yellow gammy mass. Mol.Wt. 309.36; LCMS: 310.2 (M+1). ^{1}HNMR (DMSO-d^6, 400 MHz) δ 4.2(q, J=5.6Hz, 2H), 3.9 (s, 2H), 3.75(s, 3H), 2.67(m, 2H), 1.41(s, 9H), 1.25(t, J=7.28Hz,3H).

Tert-butyl-6,7-dihydro-1-methyl-3-carbamoyl-1-H-pyrazolo[4,3-c]pyridine-5-(4H)carboxylate (4):

To the solution of 5-tert-butyl -6,7-dihydro-1-methyl -1-H-pyrazolo[4,3-c]pyridine-3,5-(4H)dicarboxylate(5g, 16.2mmol) in tetrahydrofuran (30 mL) was added 40ml liquor ammonia. It was stirred for overnight at ambient temperature and the RM was concentrated under reduced pressure. The crude sample was saturated with diethyl ether. Yield 3.5g (77.7%) as off white powder. M.pt:: 240-241ºC. Mol.Wt. 280.32; LCMS: 281.2 (M+1). ^{1}H NMR (DMSO-d^6, 400 MHz) δ 4.64 (s, 2H), 3.76 (s, 3H), 3.71 (t, J=5.5Hz, 2H), 2.67 (t, J=5.4Hz, 2H), 1.47(s, 9H). ^{13}C NMR (DMSO-d^6, 100MHz) 64.44, 138.93, 116.46, 80.15, 77.33, 77.22, 77.02, 76.70, 41.39, 36.11, 29.67, 28.43, 21.88.

Tert-butyl6,7-dihydro-1-methyl-3-thiocarbamoyl-1-H-pyrazolo[4,3-c]pyridine-5-(4H)carboxylate (5):

To a solution of Tert-butyl -6,7-dihydro-1-methyl -3-carbamoyl- 1- H- pyrazolo[4, 3-c] pyridine -5-(4H) carboxylate (3.2gm, 11.4mmol) in 40 mL of toluene was added Lawsessn's reagent (0.92g, 12.8 mmol) was added and refluxed for 4hrs. The reaction was monitored by TLC, then RM was dissolved in Ethyl Acetate, then organic layer washed with brine solution, dried over Na_2SO_4 and evaporated under reduced pressure. Crude product was purified by Column chromatography using Pet ether: Ethyl Acetate. Yield 2.4g (71%) as white solid. M.pt:252-253ºC. Mol.Wt. 296.39; LCMS: 297 (M+1). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.78 (s, 2H), 3.76 (s, 3H), 3.71(t, J=5.5Hz, 2H), 2.68 (t, J=5.4Hz, 2H), 1.48 (s, 9H). ^{13}C NMR ($CDCl_3$, 100MHz) 119.77, 118.48, 80.08, 77.33, 77.02, 76.70, 43.00, 36.21, 28.4, 21.95.

General procedure to synthesize pyrazolo-thiazole derivative (6):

To a solution of Tert-butyl 6,7-dihydro-1-methyl-3-thiocarbamoyl-1-H-pyrazolo[4,3-c]pyridine-5-(4H) carboxylate (0.2gm, 0.674mmol) in 10 mL of ethanol was added corresponding halo ketone (0.674 mmol) was added and refluxed for 3hrs. After completion of reaction (monitored by TLC) RM was concentrated to dryness under reduced pressure and re-dissolved in Ethyl Acetate, then organic layer washed with brine solution, dried over sodium Sulphate and evaporated under reduced pressure. Crude product was purified by Column chromatography using Pet ether: Ethyl Acetate.

4-(2-4, 5, 6, 7 -tetrahydro-1-methyl-1H-pyrazolo [4, 3-c] pyridine-3-yl)thiazol -4-yl) benznitrile (6a):

Yield: 85% as white powder. M.pt:322-323ºC. Mol. Wt. : 321.4 for $C_{17}H_{15}N_5S$, LCMS : 322.2(M+1); ^{1}H NMR (DMSO-d^6, 400 MHz) δ 8.42 (s, H), 8.22(m, 2H), 7.98(m, 2H), 4.53(s, 2H), 3.85(s, 3H), 3.51(t, J=5.76Hz, 2H), 3.05(t, J=5.68Hz, 2H).^{13}C NMR (DMSO-d^6, 100MHz) 161.7 ,153.22, 140.68, 138.34, 137.68, 133.38,127.09, 119.32, 117.41, 110.9, 108.41 ,40.81,40.42, 39.8, 36.79,18.95.

3-(4-(2-fluprophenyl) thiazol-2-yl)-4, 5, 6, 7-tetrahydro-1-methyl- 1H-pyrazolo [4,3-c]pyridine (6b):

Yield: 89% as yellowish powder. M.pt:317-318ºC. Mol. Wt: 314.38 for $C_{16}H_{15}FN_4S$, LCMS: 315.2 (M+1). ^{1}H NMR (DMSO-d^6, 400 MHz) δ 8.25 (s, H), 7.82(m, 2H), 7.56(d, J=7.84Hz, 1H), 7.2 (d, J=6.28Hz, 1H), 4.53(s, 2H), 3.84(s, 3H), 3.51 (t, J=5.76Hz, 2H), 3.05 (t, J=5.68Hz, 2H). ^{13}C NMR (DMSO-d^6, 100MHz) 164.31, 161.89, 161.28, 123.72, 140.81, 137.61, 136.66, 131.41, 122.51, 115.5, 113.22, 108.37, 40.81, 39.8, 36.76, 18.95.

4, 5, 6, 7- tetrahydro-1-methyl- 3 - (4-phenylthiazol -2 -yl) 1H - pyrazolo[4,4-c] pyridine (6c):

Yield:81%. M.pt:305-306ºC. Mol.Wt:296.39 for $C_{16}H_{16}N_5S$. LCMS: 270.2 (M+1). ^{1}H NMR (DMSO-d^6, 400 MHz) δ 8.01 (m, 2H), 7.8(s, 1H), 7.46(m, 2H), 7.37 (t, J=6.16Hz, 1H), 4.67(s, 2H), 3.89(s, 3H), 3.65(t, J=6.24Hz,2H), 3.14(t, J=6.08Hz, 2H).

4, 5, 6, 7-tetrahydro-1-methyl-3-(4-phenylthiazol-2-yl) 1H-pyrazolo [4, 4-c] pyridine (6d):

Yield: 80%, M.pt: 330-331ºC.Mol.Wt :372.49 for $C_{22}H_{20}N_4S$.LCMS: 373.2(M+1); ^{1}H NMR (DMSO-d^6, 300 MHz) δ 8.08 (m, 2H),7.82 (s, 1H), 7.71(m,4H), 7.47 (m,2H), 7.37(t, J=7.26Hz, 1H), 4.68(s, 2H), 3.87(s, 3H), 3.64(t, J=6.21Hz, 2H), 3.14 (t, J=6.21Hz, 2H).

3-(4-(4-chlorophenyl) thiazol-2-yl)-4, 5, 6, 7-tetrahydro-1-methyl-1H-pyrazolo [4,3,-c]pyridine(6e):

Yield: 88% as white solid. M.pt:325-326ºC.Mol. Wt: 330.07 for $C_{16}H_{15}ClN_4S$, LCMS: 331.2(M+1); ^{1}H NMR (CD3OD, 400MHz) δ 8.02(m, 2H), 7.85(s, 1H), 7.45(m, 2H), 4.67(s, 2H), 3.90(s, 3H), 3.64(t, J=5.97Hz, 2H), 3.14 (t, J=5.9Hz, 2H). ^{13}C NMR (CD3OD, 100MHz) 160.29, 153.32, 140.33, 137.2, 132.7, 132.66, 128.84, 127.66, 114.12, 107.81, 40.21, 39.49, 36.21, 18.41.

3-(4-(4-fluorophenyl) thiazol-2-yl)-4, 5, 6, 7-tetrahydro-1-methyl-1H-pyrazolo [4,3,-c]pyridine(6f):

Yield: 78% as white solid.. M.pt:316-317ºC. Mol. Wt: 314.38 for $C_{16}H_{15}FN_4S$, LCMS: 315.2 (M+1). ^{1}H NMR (CD3OD,400MHz) δ,8.03(m, 2H), 7.75(m, 1H), 7.19(m, 2H), 4.64(s, 2H), 3.87(s, 3H), 3.59(t, J=576Hz, 2H), 3.13 (t, J=5.7Hz, 2H).

4, 5, 6, 7 -tetrahydro-1-methyl-3-(4-(thiazol-2-yl) thiazol-2-yl)-1H-pyrazolo [4,3-c]pyridine(6g):

Yield: 72% as white solid. M.pt:328-329ºC. Mol. Wt: 303.4 for $C_{13}H_{13}N_5S_2$; LCMS: 304.2(M+1). ^{1}H NMR (DMSO-d^6, 400 MHz) δ 8.24 (s, H), 7.96(d, J=3.2Hz,1H), 7.84(d, J=3.16Hz, 1H), 4.47(s, 2H), 3.85(s, 3H), 3.52(t, J=5.64Hz, 2H), 3.05(t, J=5.68Hz, 2H). ^{13}C NMR (DMSO-d^6, 100MHz) 162.09, 149.28, 144.5, 140.32, 137.8, 121.43, 116.51, 108.45, 40.71, 39.59, 36.83, 18.59.

2-(4,5,6,7-tetrahydro-1-methyl-1H-pyrazolo[4,3-c]pyridine-3-yl)thiazole-4-carboxylic acid(6h):

Yield: 83% as white powder M.pt:290-291ºC. Mol. Wt: 264.3 for $C_{11}H_{12}N_4O_2S$. LCMS: 265.2(M+1). ^{1}H NMR (DMSO-d^6, 300 MHz) δ 8.42 (s, H), 4.38(s,2H), 3.82(s,3H), 3.58(t,J=6.18Hz,2H), 3.01(t,J=5.79Hz,2H)

3-(4-ethylthiazol-2-yl)-4,5,6,7-tetrahydro-1-methyl-1H-pyrazolo [4,3-c]pyridine(6i):

Yield: 86% as off white solid M.pt:283-284ºC. Mol.For $C_{12}H_{16}N_4S$ Mol. Wt: 248.35, LCMS : 249.2(M+1);^{1}H NMR (CDCL3, 400 MHz) δ 7.10 (s,1H), 4.52(s, 2H), 3.85(s, 3H), 3.61(t, J=6.27Hz, 2H), 3.11(t, J=6.18Hz, 2H), 2.84 (q, J=7.56Hz, 2H), 1.34 (t, J=7.56Hz, 3H).

3-(4-(2-chlorophenyl) thiazol-2-yl)-4, 5, 6, 7-tetrahydro-1-methyl-1H-pyrazolo [4,3,-c]pyridine(6j):

Yield: 83% as white solid. M.pt:326-327ºC. Mol. Wt : 330.07 for $C_{16}H_{15}ClN_4S$. LCMS : 331.2(M+1).^{1}H NMR (CD3OD, 400MHz) δ8.20(m, 2H), 8.06(s, 1H), 1H), 7.82(m, 2H), 4.66(s, 2H), 3.89(s, 3H), 3.65(t, J=5.97Hz,2 H), 3.15 (t, J=5.9Hz, 2H).

Antifungal activity:

The synthesized compounds **6(a-j)** were evaluated for their antimicrobial activity against *Aspergillus flavus* 4), *Fusarium oxysporus* and *Candida albicans* arerepresenting fungal organisms by diffusion disc method[13]. The results of antifungal effect of all tested compounds were reported as zone of inhibition in mm and are shown in Table 2. Amphoterecin-B and Ketoconazole were used as the reference antifungal agent. The result evealed that most of newly synthesized pyrazolo thiazole copounds exhibited good antifungal activities against Fusarium oxysporusand Candida albicans.

Table No. 2: Antifungal activities of the pyrazolothiazole compounds 6a-j

Comp. No	*Aspergillus flavus*	*Fusarium oxysporuum*	*Candida albicans*
6a	3	10	12
6b	NZ	12	11
6c	NZ	9	13
6d	3	13	10
6e	6	13	15
6f	5	12	12
6g	NZ	7	13
6h	NZ	8	3
6i	NZ	5	7
6j	5	12	13
Amphoterecin-B	10	15	Not used
Ketoconazole	Not Used	Not Used	17

*Values are mean of three determinations, the ranges of which are less than 5% of the mean in all cases. * Compounds 5µg compound in 500 µL DMSO, used for experiments, NZ= No Zone of activity.

CONCLUSION

We synthesized a series of Novel pyrazolo-thiazole derivatives in high yields. The advantages are the usage of low cost starting chemicals and simple experimental procedure. These derivatives are having good antifungal activity.

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