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단 신

항암활성이 있는 7-Trifluoromethyl-Substituted Pyrazolo[1,5-*a*]Pyrimidines의 효율적인 합성

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Novel and Efficient Synthesis of 7-Trifluoromethyl-Substituted Pyrazolo[1,5-*a*] Pyrimidines with Potent Antitumor Agents

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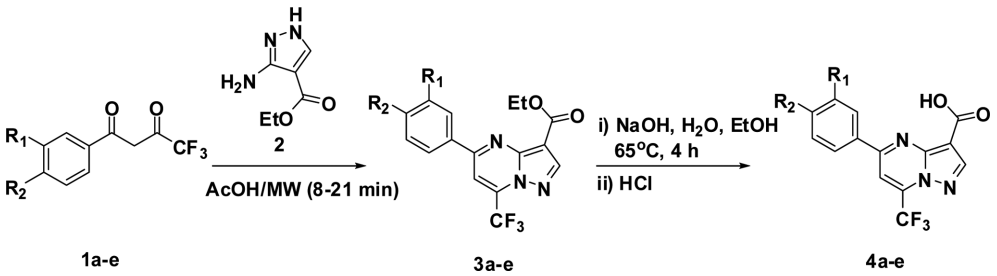
INTRODUCTION

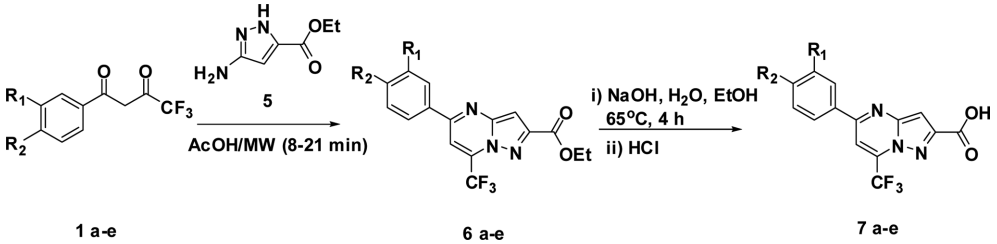
The pyrazolo[1,5-*a*]pyrimidine structural motif may be found in a large number of pharmaceutical agents with a diverse range of physiological activities, for example, antiepileptic agents,[1] anxiolytics,[2] antidepressants,[3] agents for treatment of sleep disorders[4] and oncolytics.[5] Recently, a series of antagonist of protease-activated PAR2 receptors were reported.[6] These compounds were expected to be useful for the treatment of arthritis, dermatitis, fever, asthma, bone resorption-related disorders, cardiovascular diseases, dysmenorrhea, nephritis, nephrosis, atherosclerosis, hypotension, shock, pain, neuroinflammation, cancer, Alzheimer's disease and other PAR2-mediated disorders. The trifluoromethyl group is one of the most attractive functional groups in organic chemistry and introduction of this group efficiently is a growing interest in organofluorine chemistry.[7] However, several methods are available for the synthesis of pyrazolo[1,5-*a*]pyrimidines.[8] There is a great need for new, simple and facile procedures that can incorporate a number of points of structural diversity and a variety of substitution patterns in the targeted pyrazolo pyrimidines. In this paper, we report a successful strategy for the synthesis of 7-trifluoromethyl-substituted pyrazolo[1,5-*a*]pyrimidines. The principal advantages, scope and limitations of the involved synthetic methods are discussed. The general synthetic approach is depicted in *Scheme* 1 and *Scheme* 2. The trifluoromethyl-β-diketones were treated under microwave condition with 5-aminopyrazolecarboxylic acid derivatives to provide the pyrazolo[1,5-*a*]pyrimidine carboxylates.

RESULTS AND DISCUSSION

The key amino pyrazole intermediates **2** and/or **5** can be obtained from commercial sources. A total of five different trifluoromethyl-β-diketones **1 a-e** were synthesized as reported [9] and used in this work. The choice of these particular-β-diketones was determined by their synthetic accessibility. The general synthetic approach was shown in *Scheme* 1 and 2. The trifluoromethyl-β-diketones **1a-e** were treated with 5-amino pyrazole carboxylates **2** and/or **5** under microwave condition, using acetic acid as a solvent to provide the pyrazolo[1,5-*a*]pyrimidine carboxy-



***Scheme* 1.** Synthesis of 7-trifluoromethyl-substituted pyrazolo[1,5-*a*]pyrimidines **4a-e**.



***Scheme* 2.** Synthesis of 7-trifluoromethyl-substituted pyrazolo[1,5-*a*]pyrimidines **7a-e**.

***Table* 1.** Synthesis of 7-trifluoromethyl-substituted pyrazolo[1,5-*a*]pyrimidines **4a-e**

Starting Material No	R_1	R_2	Reaction Time (min)	Product No	Yield (%)
1a	H	H	5	4a	74
1b	H	Me	15	4b	71
1c	H	Cl	17	4c	64
1d	H	F	11	4d	68
1e	H	Br	21	4e	66

***Table* 2.** Synthesis of 7-trifluoromethyl-substituted pyrazolo[1,5-*a*]pyrimidines **7a-e**

Starting Material No	R_1	R_2	Reaction Time (min)	Product No	Yield (%)
1a	H	H	8	7a	65
1b	H	Me	18	7b	60
1c	H	Cl	15	7c	67
1d	H	F	11	7d	65
1e	H	Br	21	7e	61

lates **3a-e** and/or **6a-e** respectively. The obtained pyrazolo [1,5-*a*]pyrimidine carboxylates **3a-e** and/or **6a-e** were treated with sodium hydroxide solution in ethanol at 65 °C for 4 h to furnish desired pyrazolo[1,5-*a*]pyrimidine carboxylic acids **4a-e** and/or **7a-e** in 60-74% yield.

Antitumor screening

Human chronic myeloid leukemia cells, K562, human colon carcinoma cells, Colo-205 and human embryonic kidney cells, HEK293, were procured from National Center for Cell Sciences, Pune, India. All cells were grown in RPMI-1640 supplemented with 10% heat inactivated fetal bovine serum (FBS), 100 IU/mL penicillin, 100 mg/mL streptomycin and 2 mM-glutamine. Cultures were maintained in a humidified atmosphere with 5% CO_2 at 37 °C. The cells were subcultured twice each week, seeding at a density of about 2×10^3 cells/mL.

MTT Assay

Cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells (5×10^3 cells/well) were seeded to 96-well culture plate and cultured with or without compounds at 1 μM and 10 μM concentration for 24 h in a final volume of 200 μL. After treatment, the medium was removed and 20 μL of MTT (5 mg/mL in PBS) was added to the fresh medium. After 2 h incubation at 37 °C, 100 μL of DMSO was added to each well and plates were agitated for 1 min.

***Table* 3.** *In vitro* cytotoxic activity of the synthesized compounds **4a-e** and **7a-e** against leukemiacells, K562, humancolon carcinoma cells, Colo-205

Compound	Leukemiacells	Humancolon carcinoma cells
4a	22	10
4b	31	13
4c	24	8
4d	19	10
4e	35	15
7a	20	11
7b	10	9
7c	32	14
7d	28	12
7e	25	10
Chlorambucil	14	-
Doxotrobicin		1.2

Absorbance was read at 570 nm on a multi-well plate reader (Victor3, Perkin Emler). Percent inhibition of proliferation was calculated as a fraction of control (without compound).

The cytotoxic effects of the newly synthesized pyrazolo[5.1-*a*]pyrimidines **4a-e** and **7a-e** against leukemiacells, K562 and humancolon carcinoma cells, Colo-205 were evaluated at Department of Bio-Chemistry, Sri Krishnadevaraya University, Anantapur. Chlorambucil was used as a reference to evaluate the potency of the tested compounds against leukemiacells, K562. Doxorubicin was used as a reference to evaluate the potency of the tested compounds against humancolon carcinoma cells, Colo-205. Two different concentrations of each compound (1 mM, 10 mM) and the reference were used in such screening tests and determination of IC_{50} values. The results are given in *Table* 3. As shown in this table, compounds have lower antitumor activity against humancolon carcinoma cells, Colo-205 as their IC_{50} values are much higher (8-15) than that of the reference doxotrubicin (IC_{50} = 1.2). However, the data shows that the antitumor activity of the studied pyrazolo[5.1-*a*]pyrimidines having electron withdrawing groups is little bit more than that of others.

Experimental

General Information: Melting points (°C) were measured with Koeffler melting point apparatus and are uncorrected. Thin-layer chromatography (TLC) was performed on aluminum sheets precoated with silica gel (Merck, Kieselgel 60 F-254). NMR spectra were recorded at Mercury Plus (Varian-400 MHz) for ^{1}H NMR and 200 MHz for ^{13}C NMR, in DMSO-d_6 using TMS as an internal standard (chemical shifts in parts per million). LC/MS spectra were recorded with a PE SCIEX API 150EX liquid chromatograph equipped with a UV detector (max 215 and 254 nm). Elution started with water and ended with acetonitrile/water (95:5, v/v) and used a linear gradient at a flow rate of 0.15 mL/min and an analysis cycle time of 25 min. According to LC/MS data, all the synthesized compounds have purity >95%. All solvents and reagents were obtained from commercial sources and were used without purification. 4-ethoxycarbonyl-5-aminopyrazole **2** and 5-ethoxycarbonyl-5-aminopyrazole **5** were purchased from Aldrich.

General procedure for synthesis of 3-carboxy-7-trifluoromethylpyrazolo[5.1-a]pyrimidines 4a-e and/or 7a-e: A solution of 3-amino-1*H*-pyrazole-4-carboxylic acid ethyl ester **2** and/or **5** (0.01 mol) and diketone **1a-e** (0.01 mol) in acetic acid (5 mL) was exposed to MWI in the CATA-4R—Scientic Microwave oven (Catalyst Systems) at 490 W in ambient pressure (monitored by TLC). After cooling to room temperature, the reaction mixture was poured onto ice. The formed precipitate was filtered off, washed with water, and dried. The resulting ethyl carboxylates **3a-e and/or 6a-e** were added to a mixture of NaOH in EtOH/water (1:3), and the reaction mixture was stirred at 60-70 °C for 4 h (monitored by TLC). The mixture was cooled to room temperature and acidified with concentrated HCl until pH 1 was reached. The formed precipitate was filtered off, washed with water, and recrystallized from acetonitrile to give pure **4a-e and/or 7a-e** in 60-74% yields.

5-Phenyl-7-(trifluoromethyl)pyrazolo[1,5-*a*]-pyrimidine-3-carboxylic acid 4a: (Yield 74%) mp 250-252 °C; ^{1}H NMR (400 MHz): δ 2.32-2.50 (bs, 1H), 7.22-7.55 (m, 5H), 8.1 (s, 1H), 8.41 (s, 1H); ^{13}C NMR (100 MHz): δ 166.40, 162.10, 155.20, 148.31, 145.66, 133.73, 132.10,130.27 (2C), 120.16, 116.12 (2C), 106.33,104.21; LC/MS *m*/*z* 308 (M+1).

5-(4-Methylphenyl)-7-(trifluoromethyl)pyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid 4b: (Yield 71%) mp 255-257 °C; ^{1}H NMR(400 MHz): δ 2.22-2.38 (bs, 1H), 2.49 (s, 3H), 7.22 (s, 1H), 7.33 (d, J = 7.8 Hz, 2H), 8.08 (s, 1H), 8.33 (d, J = 7.8 Hz, 2H); ^{13}C NMR (100 MHz): d 163.20, 157.10, 149.60, 149.40, 143.10, 133.80, 133.40 (2C), 131.20, 127.60, 121.40, 107.10 (2C),100.10. 21.45; LC/MS m/z 322 (M+1).

5-(4-Chlorophenyl)-7-(trifluoromethyl)pyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid 4c: (Yield 64%) mp 223-225 °C; ^{1}H NMR (400 MHz): δ 2.4-2.8 (bs,1H), 7.22 (s,1H), 7.54 (d, J = 8.4 Hz, 2H), 8.24 (s,1H), 8.35 (d, J =

8.4 Hz, 2H); ^{13}C NMR (100 MHz): δ 164.80, 156.40, 149.50, 149.20, 138.10, 135.40, 133.20 (2C), 129.90, 129.16, 119.90, 108.10 (2C), 101.2; LC/MS m/z 342 (M+1).

5-(4-Fluorophenyl)-7-(trifluoromethyl)pyrazolo[1,5-*a*]-pyrimidine-3-carboxylic acid 4d: (Yield 68%) mp 252-253 °C; ^{1}H NMR (400 MHz): δ 2.35-2.55 (s, 1H), 7.25 (d, *J* = 8.2 Hz, 2H, ArH), 8.2 (s, 1H, ArH), 8.5 (m, 2H, ArH), 8.52 (s, 1H, ArH); ^{13}C NMR (100 MHz): d 166.23, 163.41, 155.14, 148.31, 145.66, 133.73, 132.10, 130.27 (2C), 120.16, 116.12 (2C), 104.21, 106.33; LC/MS m/z 326 (M+1).

5-(4-Bromophenyl)-7-(trifluoromethyl)pyrazolo[1,5-*a*]-pyrimidine-3-carboxylic acid 4e: (Yield 66%) mp 260-262 °C; ^{1}H NMR (400 MHz): δ 2.30-2.56 (s, 1H), 7.29 (d, *J* = 8.4 Hz, 2H, ArH), 8.22 (s, 1H, ArH), 8.53 (d, *J* = 8.5 Hz, 2H, ArH), 8.56 (s, 1H, ArH); ^{13}C NMR (100 MHz): δ 167.30 , 158.20, 148.10, 146.90, 139.70, 133.90, 133.40, 131.70 (2C), 127.20, 122.40, 107.80 (2C), 104.40; LC/MS m/z 387 (M+1).

5-Phenyl-7-(trifluoromethyl)pyrazolo[1,5-*a*]-pyrimidine-2-carboxylic acid 7a: (Yield 65%) mp 240-242 °C; ^{1}H NMR (400 MHz): δ 2.9-3.6 (s,1H), 7.22 (s, 1H), 7.64 (d, *J* = 7.4 Hz, 3H), 8.15 (s, 1H), 8.29 (d, *J* = 5 Hz, 2H); ^{13}C NMR (100 MHz): δ 162.8, 156.4, 149.1, 148.6, 136.3, 133.5, 131.6, 129.1 (2C), 127.6 (2C), 120.7, 108.7, 101.8; LC/MS m/z 308(M+1).

5-(4-Methylphenyl)-7-(trifluoromethyl)pyrazolo[1,5-*a*]-pyrimidine-2-carboxylic acid 7b: (Yield 60%) mp 296-297 °C; ^{1}H NMR (400 MHz): δ 2.48 (bs, 3H), 7.20 (s, 1H, ArH), 7.31 (d, *J* = 7.8 Hz, 2H, ArH), 8.06 (s, 1H, ArH), 8.23 (d, *J* = 7.8 Hz, 2H, ArH); ^{13}C NMR (100 MHz): δ 162.98, 156.14, 149.56, 149.23, 142.07, 133.72, 133.40, 130.09 (2C), 127.52 (2C), 120.42, 107.13, 100.12, 21.45; LC/MS m/z 322 (M+1).

5-(4-Chlorophenyl)-7-(trifluoromethyl)pyrazolo-[1,5-*a*]-pyrimidine-2-carboxylic acid 7c: (Yield 67%) mp 223-225 °C; ^{1}H NMR (400 MHz): δ 2.4 (bs, 1H), 7.21 (s, 1H, ArH), 7.51 (d, *J* = 8.4 Hz, 2H, ArH), 8.22 (s, 1H, ArH), 8.33 (d, *J* = 8.4 Hz, 2H, ArH); ^{13}C NMR (100 MHz): δ 164.53, 155.41, 149.25, 149.20, 142.07, 136.71, 134.54, 133.72, 129.29 (2C), 129.16 (2C), 119.69, 107.15, 100.92; LC/MS m/z 342 (M+1).

5-(4-Fluorophenyl)-7-(trifluoromethyl)pyrazolo-[1,5-*a*]-pyrimidine-2-carboxylic acid 7d: (Yield 71%) mp 218-220 °C; ^{1}H NMR (400 MHz): δ 2.75-3.38 (bs, 1H), 7.21 (s, 1H, ArH), 7.25 (d, *J* = 8.2 Hz, 2H, ArH), 8.19 (s, 1H, ArH), 8.39 (m, 2H, ArH); ^{13}C NMR (100 MHz): δ 164.93, 162.43, 155.54, 149.25, 149.06, 133.11, 132.11, 129.99 (2C), 119.76, 116.06 (2C), 107.10, 100.52; LC/MS m/z 326 (M+1).

5-(4-Bromophenyl)-7-(trifluoromethyl)pyrazolo-[1,5-a]-pyrimidine-2-carboxylic acid 7e: (Yield 65%) mp 220-222 °C; ^{1}H NMR (400 MHz): δ 2.30 (bs, 1H), 7.20 (d, *J* = 8.4 Hz, 2H, ArH), 8.20 (s, 1H, ArH), 8.50 (d, *J* = 8.5 Hz, 2H, ArH), 8.54 (s, 1H, ArH); ^{13}C NMR (100 MHz): δ 167.32 , 158.21, 148.17, 146.82, 139.16, 133.78, 133.42, 131.75 (2C), 127.23, 122.44, 107.82 (2C), 104.41; LC/MS m/z 387 (M+1).

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