Journal of Fluorine Chemistry 131 (2010) 584–586

Contents lists available at ScienceDirect

Journal of Fluorine Chemistry

journal homepage: www.elsevier.com/locate/fluor

Synthesis and anti-microbial activity of some new fluorinated 1*H*-pyrazoles

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ARTICLE INFO

Article history:
Received 24 October 2009
Received in revised form 10 January 2010
Accepted 13 January 2010
Available online 22 January 2010

Keywords:
Trifluoromethyl-1*H*-pyrazole
Synthesis
1,3-Diketones
Anti-microbial activity

ABSTRACT

Several new trifluoromethyl-1*H*-pyrazoles were prepared by reaction of hydrazine monohydrate with 1,3-diketones. Their structures were confirmed by elemental analysis, IR, ^{1}H NMR and EI-MS spectroscopy. The anti-microbial activities of the newly synthesized compounds were examined by disc diffusion method against *Escherichia coli*, *Staphylococcus aureus*, *Pyricularia oryzae* and *Rhizoctnia solani*. All the trifluoromethyl-1*H*-pyrazoles exhibited a certain degree of anti-bacterial and anti-fungal activities.

1. Introduction

Strategically positioned fluorine substituents, especially the trifluoromethyl group, in heterocyclic compounds play important roles in medicine and agrochemicals [1–4]. In particular, the fluorinated pyrazoles have been shown to possess high biological activities [5–7] as herbicides, fungicides, insecticides, analgesics, antipyretics and anti-inflammatory agents. Accordingly, much recent attention has been paid to the synthesis of trifluoromethyl substituted pyrazoles [8–12]. Among the reported routes, the reaction of hydrazine monohydrate with 1,3-diketones is still the most frequently used and efficient method for the synthesis of important pyrazole heterocycles [13,14]. Recently, we studied the solid state structures of some new substituted pyrazoles [15]. As a continuation of our work, herein we report the preparation of several new fluorinated 1*H*-pyrazoles and their anti-microbial activities against *Escherichia coli*, *Staphylococcus aureus*, *Pyricularia oryzae*, *Rhizoctnia solani*.

2. Results and discussion

2.1. Synthesis of trifluoromethyl-1H-pyrazoles

Synthesis of the title compounds is outlined in Scheme 1. Compounds **3a**–**h** were synthesized from the corresponding trifluoromethyl-1,3-diketones obtained according to the our published procedures [16,17]. Nucleophilic attack of hydrazine monohydrate with the 1,3-diketone precursors in hot ethanol led to formation of the expected trifluoromethyl-1*H*-pyrazoles. As shown in Table 1, the starting materials 1,3-diketones were converted completely and the substituted 1*H*-pyrazoles were obtained as major products accompanying minor unidentified compounds. After recrystallization from dilute ethanol solution, pure 1*H*-pyrazoles were isolated in moderate to good yields (54–72%). And all products were characterized by element analysis, FTIR, ^{1}H NMR, and mass spectroscopy.

2.2. Spectral characterizations

The IR spectra of the title compounds showed broad bands in the region of 3350–3150 cm^{-1}, due to N–H stretching vibrations. Strong absorption bands in the ranges of 1616–1578 cm^{-1} and 1519–1482 cm^{-1} were assigned to the C=N stretching vibrations and N–H bending vibrations, respectively. Several medium bands corresponding to C=C stretching vibrations of the aromatic ring were always found in the region of 1600–1450 cm^{-1}. The absorption of C–F stretching vibrations appeared as a very strong band around 1246–1286 cm^{-1}. The absorption of N–N stretching vibrations also exhibited a strong band in the range of 1058–1071 cm^{-1} [18,19].

The ^{1}H NMR spectra of all trifluoromethyl-1*H*-pyrazoles in $CDCl_3$ showed a typical proton chemical shift of 3,5-disubstituted pyrazole (4-H) at δ = 6.47–6.97 ppm. The phenyl ring protons of compounds **3a**–**3c**, **3e**, **3f** appeared as an AA′BB′ system with J_{ortho} = 7.2–8.8 Hz at δ = 6.98–8.36 ppm, consistent with the characteristic coupling pattern of *p*-disubstituted benzenes [20]. The naphthenyl ring protons of compounds **3d** and **3h** exhibited multiple peaks at δ = 7.17–8.07 ppm and the pyridyl ring protons

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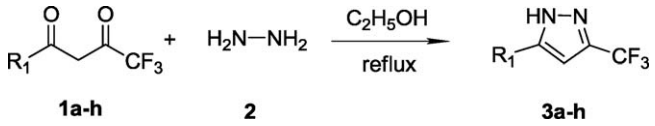
doi:10.1016/j.jfluchem.2010.01.005



1a-h 2 3a-h

Scheme 1. Reaction of trifluoromethyl-1,3-diketones with hydrazine.

Table 1
Preparation of trifluoromethyl-1*H*-pyrazoles[a].

1*H*-Pyrazole	R_1	mp (°C)	Yield[b] (%)
3a	4-$CH_3OC_6H_4$–	139–141	65
3b	4-FC_6H_4–	117–118	57
3c	4-$NO_2C_6H_4$–	155–156	70
3d	6-CH_3O-2-naphthyl	190–192	72
3e	4-$C_6H_4CH_2OC_6H_4$–	163–165	67
3f	4-$C_2H_5OC_6H_4$–	136–137	54
3g	4-Pyridyl	151–152	62
3h	2-Naphthyl	162–163	58

[a] Standard rection conditions: 1,3-diketones (2.0 mmol), hydrazine monohydrate (2.0 mmol), ethanol (10 mL), reflux, 4 h.
[b] Recrystallized isolated yields. All starting materials reacted completely, and there are some unidentified products in the mixture.

Table 2
Anti-microbial data of trifluoromethyl-1*H*-pyrazoles.

1*H*-Pyrazole	Anti-bacterial activity zone of inhibition in mm		Anti-fungal activity zone of inhibition in mm	
	E. coli	*S. aureus*	*P. oryzae*	*R. solani*
3a	15	13	12	13
3b	18	20	19	16
3c	10	9	11	10
3d	11	10	11	12
3e	12	13	11	12
3f	14	12	11	13
3g	16	15	17	19
3h	12	11	13	12
Norfloxacin	32	29	–	–
Triadimefon	–	–	27	31

of compound **3g** displayed a multiplet at δ = 7.43–8.57 ppm. However, the N–H resonance signals for the pyrazolyl ring of all compounds were not observed in $CDCl_3$ at room temperature, in agreement with results in the literature [21].

The EI mass spectra of compounds **3a**–**h** were measured and compared to confirm elemental compositions. The molecular ion peaks (M^+) for these compounds were observed in accordance with the Nitrogen Rule.

2.3. Anti-microbial activity

Compounds **3a**–**h** are screened in vitro for their anti-microbial activity against *E. coli*, *S. aureus*, *P. oryzae* and *R. solani*. The zones of inhibition formed for the compounds against bacteria and fungi were presented in Table 2. All the title compounds showed certain degree of anti-bacterial and anti-fungal activities. Compound **3b** and **3g** exhibited slightly higher activities against *E. coli*, *S. aureus*, *P. oryzae* and *R. solani*, compared with compounds **3a**, **3c**–**f** and **3h** [22]. All test data in Table 2 were the average values from triplicate runs. Although these compounds showed less anti-microbial activities compared to their respective standards, additional compounds in this series will be prepared to try to improve activity.

3. Conclusions

In this paper, several new 5-substituted-3-(trifluoromethyl)-1*H*-pyrazoles were synthesized by the condensation of trifluoromethyl-1,3-diketones with hydrazine in ethanol. Their structures were confirmed by elemental analysis, IR, ^{1}H NMR and EI-MS spectroscopy. Their preliminary anti-microbial activities against *E. coli*, *S. aureus*, *P. oryzae* and *R. solani* were tested in a concentration of 50 mg/L by disc diffusion method. The results showed that compounds **3b** and **3g**, in particular, exhibited significant anti-bacterial and anti-fungal activities.

4. Experimental

4.1. Chemicals and methods

Elemental analysis (C, H, N) was performed using a Perkin-Elmer 2400 elemental analyzer. ^{1}H NMR spectra were measured on a Varian Mercury-Plus 400 NMR nuclear magnetic resonance instrument in $CDCl_3$ solution with TMS as internal standard. Infrared spectra (4000–400 cm^{-1}) were recorded on a Nicolet FTIR 5700 spectrophotometer with KBr pellets. Mass spectra were measured on a Finnigan Trace EI-MS 2000 spectrometer. Melting points were determined by an X-4 digital melting point apparatus, which are uncorrected. Reagents were of analytical grade and were used without further purification.

4.2. General procedure for trifluoromethyl-1H-pyrazoles

The corresponding trifluoromethyl-1,3-diketone (2.0 mmol) was dissolved in hot ethanol (10 mL). To this solution hydrazine monohydrate (2.0 mmol) in ethanol was added and the mixture was refluxed under stirring for 4 h. After completion of the reaction (detected by thin layer chromatography, TLC), the solvent was removed by evaporation. The residual solid was recrystallized from dilute ethanol solution to give the corresponding trifluoromethyl-1*H*-pyrazole.

4.2.1. 5-(4-Methoxyphenyl)-3-(trifluoromethyl)-1H-pyrazole (3a)

White crystals, yield 65%, mp 139–141 °C; R_f = 0.40 (TLC, ethyl acetate/hexane = 1:3), IR (KBr): ν 3243 (b, s), 3046 (w), 2974 (m), 2836 (w), 1616 (s), 1518 (s), 1493 (vs), 1464 (s), 1282 (vs), 1248 (vs), 1163 (vs), 1130 (vs), 1058 (s), 983 (vs), 838 (s), 800 (s), 744 (s) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.87 (s, 3H, OCH_3), 6.72 (s, 1H, pyrazloyl C–H), 7.00 (d, 2H, Ar–H, J = 8.4 Hz), 7.52 (d, 2H, Ar–H, J = 8.4 Hz) ppm, N–H not found; EI-MS (70 eV): m/z (%) 242.1 (M^+, 100), 227.1 (54), 199.0 (73), 151.1 (67), 74.6 (50); Anal. Calcd. for $C_{11}H_9N_2OF_3$: C, 54.55; H, 3.74; N, 11.56; Found C, 54.82; H, 3.68; N, 11.69.

4.2.2. 5-(4-Fluorophenyl)-3-(trifluoromethyl)-1H-pyrazole (3b)

White crystals, yield 57%, mp 117–118 °C; R_f = 0.53 (TLC, ethyl acetate/hexane = 1:3), IR (KBr): ν 3242 (b,s), 3050 (m), 2937 (w), 1614 (s), 1515 (s), 1493 (vs), 1432 (s), 1279 (vs), 1250 (vs), 1163 (vs), 1125 (vs), 1058 (s), 984 (vs), 840 (vs), 810 (vs), 745 (s) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.76 (s, 1H, pyrazloyl C–H), 7.18 (d, 2H, Ar–H, J = 8.4 Hz), 7.57 (d, 2H, Ar–H, J = 8.8 Hz) ppm, N–H not found; EI-MS (70 eV): m/z (%) 230.1 (M^+, 63), 181.9 (52), 161.2 (60), 133.1 (100), 94.9 (64), 74.7 (58), 68.9 (42); Anal. Calcd. for $C_{10}H_6N_2F_4$: C, 52.19; H, 2.63; N, 12.17; Found C, 52.46; H, 2.59; N, 12.33.

4.2.3. 5-(4-Nitrophenyl)-3-(trifluoromethyl)-1H-pyrazole (3c) [7]

Colorless needles, yield 70%, mp 155–156 °C; R_f = 0.46 (TLC, ethyl acetate/hexane = 1:3), IR (KBr): ν 3148 (m), 3060 (w), 3029 (w), 1610 (m), 1531 (s), 1519 (m), 1496 (m), 1344 (vs), 1286 (s), 1257 (vs), 1171 (s), 1133 (vs), 1071 (m), 989 (s), 854 (s), 833 (s), 755 (m), 693 (m) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.97 (s, 1H, pyrazloyl C–H), 7.81 (d, 2H, Ar–H, J = 8.4 Hz), 8.36 (d, 2H, Ar–H, J = 8.8 Hz) ppm, N–H not found; EI-MS (70 eV): m/z (%) 257.1 (M^+, 100), 227.1 (41), 199.0 (40), 191.1 (44), 141.8 (43), 114.4 (41); Anal.

Calcd. for $C_{10}H_6N_3O_2F_3$: C, 46.70; H, 2.35; N, 16.34; Found C, 46.55; H, 2.30; N, 16.51.

4.2.4. 5-(6-Methoxynaphthalen-2-yl)-3-(trifluoromethyl)-1H-pyrazole (3d)

White crystals, yield 72%, mp 190–192 °C; R_f = 0.39 (TLC, ethyl acetate/hexane = 1:3), IR (KBr): ν 3312 (b,s), 3066 (m), 2962 (m), 1627 (s), 1606 (s), 1595 (m), 1485 (s), 1452 (s), 1337 (s), 1261 (s), 1206 (s), 1066 (s), 1034 (s), 915 (s), 887 (s), 806 (s), 713 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 3.95 (s, 3H, OCH_3), 6.88 (s, 1H, pyrazloyl C–H), 7.17 (s, 1H, Ar–H), 7.22 (d, 1H, Ar–H, *J* = 9.2 Hz), 7.63 (d, 1H, Ar–H, *J* = 8.8 Hz), 7.80 (d, 1H, Ar–H, *J* = 8.8 Hz), 7.84 (d, 1H, Ar–H, *J* = 8.8 Hz), 7.99 (s, 1H, Ar–H) ppm, N–H not found; EI-MS (70 eV): *m*/*z* (%) 292.2 (M^+, 100), 249.1 (81), 201.0 (28), 152.3 (27); Anal. Calcd. for $C_{15}H_{11}N_2OF_3$: C, 61.65; H, 3.79; N, 9.58; Found C, 61.47; H, 3.72; N, 9.74.

4.2.5. 5-(4-(Benzyloxy)phenyl)-3-(trifluoromethyl)-1H-pyrazole (3e)

Colorless needles, yield 67%, mp 163–165 °C; R_f = 0.26 (TLC, ethyl acetate/hexane = 1:3), IR (KBr): ν 3329 (s), 3136 (b,s), 2958 (w), 1609 (s), 1518 (s), 1485 (m), 1421 (m), 1383 (m), 1340 (s), 1260 (s), 1188 (s), 1071 (s), 1032 (s), 905 (m), 852 (m), 740 (s), 698 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 5.12 (s, 2H, OCH_2), 6.70 (s, 1H, pyrazloyl C–H), 7.07 (d, 2H, Ar–H, *J* = 7.2 Hz), 7.40–7.52 (m, 7H, Ar–H) ppm, N–H not found; EI-MS (70 eV): *m*/*z* (%) 318.2 (M^+, 6), 227.8 (5), 90.7 (100), 65.1 (23); Anal. Calcd. for $C_{17}H_{13}N_2OF_3$: C, 64.15; H, 4.12; N, 8.80; Found C, 64.38; H, 4.06; N, 8.93.

4.2.6. 5-(4-Ethoxyphenyl)-3-(trifluoromethyl)-1H-pyrazole (3f)

Colorless crystals, yield 54%, mp 136–137 °C; R_f = 0.44 (TLC, ethyl acetate/hexane = 1:3), IR (KBr): ν 3262 (b, s), 3068 (w), 2985 (m), 2888 (w), 1615 (s), 1516 (s), 1493 (s), 1439 (m), 1389 (m), 1281 (s), 1246 (s), 1153 (s), 1116 (s), 1058 (s), 982 (s), 838 (s), 809 (s), 746 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.85 (t, 3H, CH_3, *J* = 7.2 Hz), 3.84 (q, 2H, OCH_2, *J* = 7.2 Hz), 6.70 (s, 1H, pyrazloyl C–H), 6.98 (d, 2H, Ar–H, *J* = 8.4 Hz), 7.49 (d, 2H, Ar–H, *J* = 8.4 Hz) ppm, N–H not found; EI-MS (70 eV): *m*/*z* (%) 256.1 (M^+, 40), 228.0 (100), 151.1 (30), 131.0 (23); Anal. Calcd. for $C_{12}H_{11}N_2OF_3$: C, 56.25; H, 4.33; N, 10.93; Found C, 55.98; H, 4.26; N, 11.15.

4.2.7. 4-(3-(Trifluoromethyl)-1H-pyrazol-5-yl) pyridine (3g) [23]

Colorless crystals, yield 62%, mp 151–152 °C; R_f = 0.14 (TLC, ethyl acetate/hexane = 1:3), IR (KBr): ν 3308 (s), 3051 (m), 2862 (m), 2708 (m), 1606 (s), 1578 (s), 1496 (m), 1425 (s), 1364 (s), 1255 (s), 1179 (vs), 1150 (s), 1062 (s), 1016 (s), 969 (s), 903 (m), 818 (s), 683 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 6.47 (s, 1H, pyrazloyl C–H), 7.43 (d, 2H, Ar–H, *J* = 4.8 Hz), 8.57 (d, 2H, Ar–H, *J* = 5.2 Hz) ppm, N–H not found; EI-MS (70 eV): *m*/*z* (%) 213.0 (M^+, 74), 162.0 (100), 78.1 (81), 51.6 (59); Anal. Calcd. for $C_9H_6N_3F_3$: C, 50.71; H, 2.84; N, 19.71; Found C, 51.02; H, 2.83; N, 19.91.

4.2.8. 5-(Naphthalen-2-yl)-3-(trifluoromethyl)-1H-pyrazole (3h) [24]

White microcrystalline power, yield 58%, mp 162–163 °C; R_f = 0.36 (TLC, ethyl acetate/hexane = 1:3), IR (KBr): ν 3312 (s), 3058 (m), 2904 (m), 1600 (m), 1482 (m), 1447 (m), 1337 (s), 1255 (s), 1170 (s), 1118 (s), 1065 (s), 1032 (s), 904 (m), 864 (s), 833 (s), 791 (s), 754 (s), 704 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 6.93 (s, 1H, pyrazloyl C–H), 7.55–7.57 (m, 2H, Ar–H), 7.67 (d, 1H, Ar–H, *J* = 8.4 Hz), 7.87–7.91 (m, 2H, Ar–H), 7.95 (d, 1H, Ar–H, *J* = 8.4 Hz), 8.07 (s, 1H, Ar–H) ppm, N–H not found; EI-MS (70 eV): *m*/*z* (%) 262.2 (M^+, 49), 211.1 (47), 164.9 (48), 151.4 (39), 126.9 (100), 105.6 (29), 62.7 (31); Anal. Calcd. for $C_{14}H_9N_2F_3$: C, 64.12; H, 3.46; N, 10.68; Found C, 64.37; H, 3.40; N, 10.76.

4.3. Procedure for anti-microbial activity

The preliminary anti-microbial activities of new trifluoromethyl-1*H*-pyrazoles were measured in a concentration of 50 mg/L by disc diffusion method [25,26]. Two bacterial microorganisms *E. coli* and *S. aureus*, and the two fungal microorganisms *P. oryzae* and *R. solani* were used. DMSO was used as a solvent control and the standard drugs used were Norfloxacin and Triadimefon. The disc diffusion method was performed using Muller-Hinton agar (Hi-Media) medium. The inhibition zones were measured in/mm at the end of an incubation period of 24 h at 37 °C for bacteria and 72 h at 24 °C for fungi.

Acknowledgement

This work was supported by the importantFoundation of the Educational Commission of Hubei Province, PR China (Q200822002).

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The procedure for the synthesis of the trifluoromethyl-1,3-diketones **1** is as follows: the mixture of aryl methyl ketones (0.04 mol), ethyl trifluoracetate (0.08 mol), CH_3ONa (0.08 mol) and benzene (150 ml) was stirred at 50 °C for 8 h. The reaction mixture was cooled to room temperature, acidified with dilute hydrochloric acid and then stirred until all solids dissolved. The benzene layer was separated, washed with a saturated $NaHCO_3$ solution, dried over anhydrous $MgSO_4$ and the solvent was removed by evaporation. The residual oil solidified on standing and the solid was recrystallized from ethanol to obtain the trifluoromethyl-1,3-diketones.
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