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Microwave-assisted synthesis of some novel fluorinated pyrazolo[3,4-d]pyrimidine derivatives containing 1,3,4-thiadiazole as potential antitumor agents

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Abstract

A facile microwave-assisted procedure for synthesis of novel fluorinated pyrazolo[3,4-d]pyrimidine derivatives containing 1,3,4-thiadiazole is described. This protocol presented such advantages as short reaction time, high yields, simple purification and environmentally benign procedures. Their antitumor activities were evaluated against HL-60 by an MTT assay. The preliminary results indicated that some title compounds exhibit more potent antitumor inhibitory activity than doxorubicin (DOX).

Keywords: Pyrazolo[3,4-d]pyrimidine; 1,3,4-Thiadiazole; Microwave-assisted synthesis; Antitumor activity

Pyrazolo[3,4-d]pyrimidines have drawn considerable attention on the account of their structural similarity with purines and pharmacological importance. They are known to exhibit significant pharmacological activities, such as antimicrobial [1,2], antitumor [3–5], antiviral [6,7], and anti-inflammatory activities [8]. 1,3,4-Thiadiazole derivatives have attracted continuing interest over the last few decades due to their broad-spectrum biological activities [9–14], especially anticancer properties [15,16]. Moreover, in recent years, fluorinated compounds are one of the research hotspots in modern medicinal and agrochemistry chemistry [17]. In general, incorporation of a fluoro or trifluoromethyl group provides compounds with increased biological activity because of enhanced pharmacokinetic and physicochemical properties as compared to their non-fluorinated analogues [18,19].

It is well known that microwave (MW) irradiation technique possesses such advantages as short reaction time, environmentally benignity, easy work-up and high yield compared with the traditional heating methods [20]. MW irradiation is widely used in the field of medicinal chemistry and total syntheses of natural products.

Keeping these in mind, it was aimed in this paper to synthesize a series of novel fluorinated compounds containing both pyrazolo[3,4-d]pyrimidine and 1,3,4-thiadiazole nuclei under MW irradiation for evaluating their antitumor activity.

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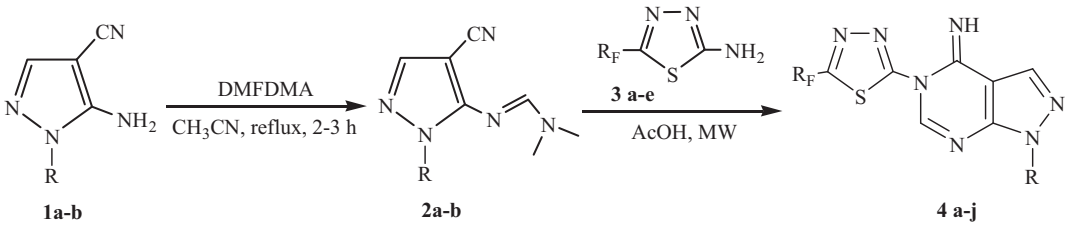

R = H (**1a**, **2a**, **4 a-e**); C_6H_5 (**1b**, **2b**, **4 f-j**)

Scheme 1. Synthetic route of the title compounds **4a**–**j**.

The title compounds **4a**–**j** were prepared using the synthetic strategy described in Scheme 1. The synthesis began by reacting commercially available 2-(ethoxymethylene)malononitrile with phenylhydrazine or hydrazine hydrate to deliver 1-phenyl or 1*H*-5-amino-4-cyanopyrazoles **1a** and **b** according to the literature methods [21,22]. The aminopyrazoles **1** were then refluxed with *N*,*N*-dimethylformamide dimethlyacetal (DMFDMA) in acetonitrile to afford the corresponding amidines **2a** and **b** which underwent a cyclo-condensation with the appropriate 2-amino-5-subsitituted-1,3,4-thiadiazoles **3a**–**e** [23] or their hydrochloride in acetic acid under MW irradiation to give the desired products **4a**–**j** with mp >300 °C, in good to excellent isolated yields (81–93%), as shown in Table 1. It only took 18–22 min to finish the reaction under microwave irradiation compared to the reaction time of about 6 h under the conventional heating.

The structures of the newly synthesized compounds **4a**–**j** [24] were confirmed by IR, ^{1}H NMR, mass spectroscopy and elemental analysis. The IR spectra contained the vibration absorption bands revealing the existence of the N–H and C=N groups, while no cyano group peak was observed. The NMR spectra of 4 showed all the expected signals for two =CH protons (δ 8.88–8.53), phenyl protons and NH singlets (δ 14.12–13.06). The EI mass spectra gave the anticipated molecular ion peaks and main fragmentation peaks, which were in accordance with the title structures.

The *in vitro* antitumor activities of the synthesized pyrazolo[3,4-d]pyrimidine derivatives containing 1,3,4-thiadiazole against HL-60 (human leukaemia cancer cell) were evaluated by the standard MTT assay [25]. As described in Table 1, compounds **4a**, **4f**, **4g**, **4i** and **4j** exhibit good anticancer activity against HL-60, especially **4f** and **4j** exhibit even higher activity than doxorubicin (DOX). As can be seen from the results, these compounds (**4f**–**j**) show better anticancer activity when H atom in position 1 of the pyrazole ring is substituted with a phenyl group. Further, incorporation of CF_3 group in the molecules enhanced biological activity significantly.

In summary, we described the facile microwave-assisted synthesis of novel fluorinated pyrazolo[3,4-d]pyrimidine derivatives containing 1,3,4-thiadiazole. This protocol presented many advantages, such as good to excellent yields, shorter reaction time (18–22 min), readily available starting material, simple purification and environmentally benign

Table 1
Data of synthesis, characterization, *in vitro* antitumor activity against HL-60 for the title compounds **4a**–**j**.

Compd.	R_F	Formula	Mode of activation	Time	Temp. (°C)	Yield (%)	EI-MS (*m*/*z*, M^+)	IC50 (μmol/L)
4a	CF_3	$C_8H_4F_3N_7S$	MW	18 min	140	88	287	1.05
4b	2-FC_6H_4	$C_{13}H_8FN_7S$	MW	18 min	140	87	313	3.16
4c	3-FC_6H_4	$C_{13}H_8FN_7S$	MW	18 min	140	89	313	10.16
4d	4-FC_6H_4	$C_{13}H_8FN_7S$	MW	18 min	140	93	313	4.47
4e	4-$CF_3C_6H_4$	$C_{14}H_8F_3N_7S$	MW	18 min	140	91	363	2.56
4f	CF_3	$C_{14}H_8F_3N_7S$	MW	22 min	150	83	363	0.08
4g	2-FC_6H_4	$C_{19}H_{12}FN_7S$	MW	22 min	150	81	389	0.90
4h	3-FC_6H_4	$C_{19}H_{12}FN_7S$	MW	22 min	150	82	389	2.75
4i	4-FC_6H_4	$C_{19}H_{12}FN_7S$	MW	22 min	150	87	389	1.01
4j	4-$CF_3C_6H_4$	$C_{20}H_{12}F_3N_7S$	MW	22 min	150	86	439	0.21
4a	CF_3		Traditional heating	6 h	118	85		
DOX								0.55

procedures. The results of antitumor inhibitory activity test indicated that this class of pyrazolo[3,4-d]-pyrimidine derivatives can be developed as novel antitumor candidate drugs. Further pharmacological evaluation, optimization and structure–activity relationships of the title compounds are underway.

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[24] General procedure for the synthesis of 5-(5-substituted-1,3,4-thiadiazol-2-yl)-1*H*-pyrazolo[3,4-d]pyrimidin-4(5*H*)-imine (**4a**–**e**): the solution of 1*H*-4-cyano-5-[(*N,N*-dimethylaminomethylene)amino]pyrazole **2a** (0.33 g, 2 mmol) and the appropriate aminothiadiazoles **3** (2 mmol) in 6 mL glacial acetic acid was irradiated at 140 °C for 18 min by microwave. The reaction mixture was then cooled to room temperature, and stirred with ice-water. The resulting suspension was filtered, washed with ethanol/water (2:1), and dried in vacuum to give the desired products **4a**–**e** as pale yellow or white solids. While, the corresponding aminothiadiazole hydrochloride needed to be used for the preparation of compounds **4f**–**j** by employing this method. The spectral data of selected compounds: **4a**: ^{1}H NMR (400 MHz, DMSO-d_6): δ 14.12 (s, 1H, NH), 13.63 (s, 1H, NH), 8.71 (s, 1H, N═CH), 8.53 (s, 1H, N═CH); IR (KBr) (ν_{max}, cm^{-1}): 3436 (N–H), 1629, 1585 (C═N), 1327, 1162 (CF_3); Anal. Calcd. for $C_8H_4F_3N_7S$: C 33.45, H 1.40, N 34.14; found: C 33.62, H 1.18, N 34.37. **4e**: ^{1}H NMR (400 MHz, DMSO-d_6): δ 14.02 (s, 1H, NH), 13.18 (s, 1H, NH), 8.69 (s, 1H, N═CH), 8.55 (s, 1H, N═CH), 8.23–7.90 (m, 4H, Ar–H); IR (KBr, ν cm^{-1}): 3435 (N–H), 1625, 1595 (C═N), 1328, 1163 (CF_3); Anal. Calcd. for $C_{14}H_8F_3N_7S$: C 46.28, H 2.22, N 26.99; found: C 46.49, H 2.45, N 27.24. **4f**: ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.86 (s, 1H, NH), 8.88 (s, 1H, N═CH), 8.80 (s, 1H, N═CH), 8.20−7.41 (m, 5H, Ar–H); IR (KBr) (ν_{max}, cm^{-1}): 3438 (N–H), 1617, 1590 (C═N), 1323, 1148 (CF_3); Anal. Calcd. for $C_{14}H_8F_3N_7S$: C 46.28, H 2.22, N 26.99; found: C 46.03, H 2.06, N 26.75. **4j**: ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.42 (s, 1H, NH), 8.84 (s, 2H, N═CH), 8.80 (s, 1H, N═CH), 8.24−7.40 (m, 9H, Ar-H); IR (KBr) (ν_{max}, cm^{-1}): 3432 (N–H), 1622, 1596 (C═N), 1327, 1163 (CF_3); Anal. Calcd. for $C_{20}H_{12}F_3N_7S$: C 54.67, H 2.75, N 22.31; found: C 54.85, H 2.94, N 22.52.
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