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ORIGINAL ARTICLE

Synthesis and biological activity of some fluorinated arylhydrazotriazoles

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KEYWORDS

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Diazonium salts;
Antifungal activity

Abstract Potential biologically active derivatives of arylhydrazotriazole (**3a–l**) were prepared by the condensation reaction of diazonium salts using various aromatic amines (**1a–l**) and 1-(2,4-difluorophenyl)-2-(1*H*-1,2,4-triazol-1-yl) ethanones (2). The synthesized products were obtained in 75–85% yield. All the synthesized products were having good-excellent antifungal activity as compared with standard (Fluconazole and Ketoconazole) drugs.

1. Introduction

Diazonium salts could react readily with nucleophiles containing an amino group, which have been extensively researched and widely used for the preparation of molecules with significance for both academia and industry (Abdelrazek et al., 2001; Pellicciari et al., 1996). The most important and most studied arene diazoamino compounds prepared by the coupling of diazonium ions with primary and secondary aromatic amines for their special stability (Burgart et al., 1998). A common character of aromatic diazonium ions is the high electrophilicity of the α-nitrogen which effects easy azo-coupling with appropriate nucleophiles. Since molecular nitrogen is an extremely good leaving group, some arene diazonium salts lose it very easily. In general, the stability of arene diazonium salts is strongly influenced by the pH of the solution, the anions, trace amount of contaminants such as, transition metal ions and the presence or absence of water (Patai, 1978). Furthermore, the stability also depends on the electronic character of "R" to which nitrogen is attached. Enaminones, regarded as potential agrochemicals or intermediates in dye and pharmaceutical industries, are attracting more and more attention and have been successfully utilized to be good building blocks in the synthesis of a wide range of heterocycles (Holschbach et al., 2005; Tomita et al., 2002; Al-Awadi et al., 2001; Al-Mousawi et al., 2003; Al-Omran et al., 1997). Due to the potential versatility of these reactions for the construction of heterocycles, the demand for exploiting them has increased over the last

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20 years. Thus, several reports on nucleophilic coupling of enaminones with diazonium salts have appeared (Ghozlan et al., 2005; Al-Shiekh et al., 2004; Al-Mousawi et al., 2001). Moreover, it is well known that various diazonium salts could also couple with fluorine-containing 1,3-dicarbonyl compounds, including fluorinated 1,3-keto esters, 1,3-diketones and so on, yielding the corresponding hydrazones that could be used to synthesize multifarious significant compounds such as fluorinated heterocycles with antifungal and anti-inflammatory activity (Burgart et al., 1998; Khudina et al., 2005; Urmila et al., 2002, 2005; Shivanyuk et al., 1981).

Azoles antifungal agents are the largest class of antimycotics today. The characteristic chemical features of azoles are presence of a five-membered aromatic ring containing at least one aromatic ring (Williams and Lemke, 2002) examples are in Chart 1, such as Butoconazole (A), Flucanozole (B) and Chlormidazole (C). Recent studies Leyden, 1999; Gruz et al., 2002 showed that a combination of amphotericin B with Flucanozole (B) and other azoles may potentiate the antifungal activity due to the synergetic effect. Numerous 1,2,4-triazole analogs have been reported to exhibit antifungal activity (Itoh et al., 2001; Arnoldi et al., 2000; Srivastava et al., 1999; Etran et al., 1995; Invidiata et al., 1997) Chart 1.

In consideration with the activity of azole compounds, we have carried out the reaction via formation of diazonium salt by using various aryl amine (**1a-l**) and condensed it with 1-(2,4-difluorophenyl)-2-(1*H*-1,2,4-triazol-1-yl)ethanones (**2**) in the presence of sodium acetate and methanol. The Literature survey revealed that there is no single report on the condensation reaction of aromatic amines (diazonium salts) with 1-(2,4-difluorophenyl)-2-(1*H*-1,2,4-triazol-1-yl) ethanones.

2. Experimental

2.1. Reagents and analysis

All the reagents and aromatic amines were obtained from commercial suppliers and were not purified. Melting points were determined in open capillaries and are uncorrected. The completion of reactions was monitored by TLC. IR spectra were recorded on a matrix of KBr with Perkin-Elmer 1430 spectrometer.^{1}H NMR spectra were recorded on Varian NMR spectrometer, Model Mercury Plus (200 MHz), CHNS analysis on CHNS analyzer, Mass spectra [ES-MS] were recorded on a Water-Micro mass Quattro-II spectrophotometer. DSC of compound **3g** taken on DSC Q2000 V23.12 Build 103. The in vitro activity of the antifungal agents was determined by the *E*-test (AB Biodisk), in accordance with the manufacturer's instructions.

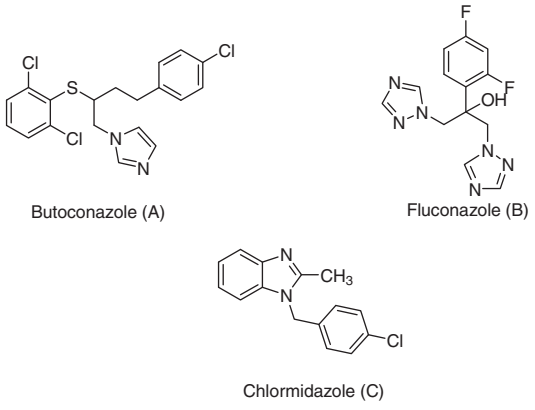


Chart 1 Some azoles containing antifungal agents.

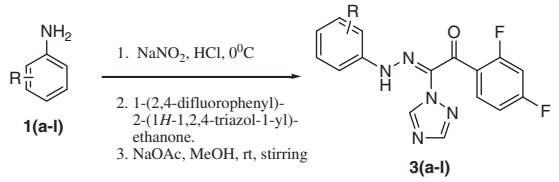
2.2. General procedure

A mixture of hydrochloric acid (2–3 mmol) and aromatic amine (1 mmol) was cooled at 0 C by using sodium chloride and ice mixture. The temperature was maintained at 0–5 °C and an aqueous solution of sodium nitrite (2 mmol) was added portion wise until, 3–4 min. The solution gives an immediate positive test for excess of nitrous acid with an external indicator -moist potassium iodide-starch paper. The precipitated amine hydrochloride was obtained, in which a solution of active methylene i.e. 1-(2,4-difluorophenyl)-2-(1*H*-1,2,4-triazol-1-yl) ethanones using sodium acetate as a base in methanol was added. The progress of the reaction was monitored by TLC, after the completion of reaction, the reaction mass was poured in 20 mL ice cold water and the product was separated by simple filtration. The obtained crude products were purified by recrystallization using ethanol. All the obtained products were screened for spectral as well as biological testing.

3. Spectral data

- (**3a**) IR (KBr, cm^{-1}): 3175, 1665; ^{1}H NMR (DMSO-d_6, 200 MHz, ppm): 6.20–7.32 (m, 8H, Ar–H), 8.25 (s, 1H, Heteroaryl-H), 8.65 (s, 1H, Heteroaryl-H), 11.25 (s, 1H, —NH); CHN data: Calc. C 58.72, H 3.39, N 21.40, Found C 58.62, H 3.49, N 21.00; ES-MS (m/z): 328 (M + 1).
- (**3b**) IR (KBr, cm^{-1}): 3160, 1670; ^{1}H NMR (DMSO-d_6, 200 MHz, ppm): 2.25 (s, 3H, —CH_3), 6.40–7.15 (m, 7H, Ar–H), 8.35 (s, 1H, Heteroaryl-H), 8.80 (s, 1H, Heteroaryl-H), 11.10 (s, 1H, —NH); CHN data: Calc. C 63.35, H 4.07, N 21.73, Found C 63.21, H 4.05, N 21.55; ES-MS (m/z): 342 (M + 1).
- (**3c**) IR (KBr, cm^{-1}): 3160, 1670; ^{1}H NMR (DMSO-d_6, 200 MHz, ppm): 2.15 (s, 3H, —CH_3), 6.38–7.10 (m, 7H, Ar–H), 8.55 (s, 1H, Heteroaryl-H), 8.90 (s, 1H, Heteroaryl-H), 11.15 (s, 1H, —NH); CHN data: Calc. C 63.35, H 4.07, N 21.73, Found C 62.92, H 4.15, N 22.05; ES-MS (m/z): 342 (M + 1).
- (**3d**) IR (KBr, cm^{-1}): 3175, 1679, 765; ^{1}H NMR (DMSO-d_6, 200 MHz, ppm): 6.80–7.35 (m, 7H, Ar–H), 8.60 (s, 1H, Heteroaryl-H), 9.05 (s, 1H, Heteroaryl-H), 11.30 (s, 1H, —NH); CHN data: Calc. C 53.13, H 2.79, N 19.36, Found C 52.93, H 2.99, N 19.00; ES-MS (m/z): 362 (M + 1), 364 (M + 3).
- (**3e**) IR (KBr, cm^{-1}): 3160, 1670; ^{1}H NMR (DMSO-d_6, 200 MHz, ppm): 3.65 (s, 3H, —OCH_3), 6.35–7.30 (m, 7H, Ar–H), 8.40 (s, 1H, Heteroaryl-H), 8.85 (s, 1H, Heteroaryl-H), 11.20 (s, 1H, —NH); CHN data: Calc. C 57.13, H 3.67, N 19.63, Found C 57.02, H 3.78, N 19.15; ES-MS (m/z): 358 (M + 1).
- (**3f**) IR (KBr, cm^{-1}): 3167, 1672; ^{1}H NMR (DMSO-d_6, 200 MHz, ppm): 2.18 (s, 3H, —CH_3), 6.28–7.23 (m, 7H, Ar–H), 8.63 (s, 1H, Heteroaryl-H), 9.10 (s, 1H, Heteroaryl-H), 11.30 (s, 1H, —NH); CHN data: Calc. C 63.35, H 4.07, N 21.73, Found C 63.02, H 4.45, N 21.35; ES-MS (m/z): 342 (M + 1).

- (**3g**) IR (KBr, cm^{-1}) 3185, 1670; ^1H NMR (DMSO-d_6, 200 MHz, ppm): 1.37(t, 3H), 4.20 (q, 2H), 6.87–7.86 (m, 7H, Ar–H), 8.53 (s, 1H, Heteroaryl-H), 9.24 (S, 1H, Heteroaryl-H), 11.86 (s,1H, —NH); Mass: ES-MS E/Z 372 (M^+).



Scheme 1 Synthesis of various arylhydrazotriazole (**3a**–**l**).

Table 1 Characterization data of synthesis of various arylhydrazotriazole (**3a**–**l**).

Entry	Compound	*R*	Time (h)	Yield (%)[a]	M.P.(°C)
1	**3a**	H	4	79	135–137
2	**3b**	4-Me	2	83	125–127
3	**3c**	3-Me	1.40	81	120–122
4	**3d**	4-Cl	1	85	174–176
5	**3e**	4-MeO	3	80	115–117
6	**3f**	2-Me	2	82	124–126
7	**3g**	4-EtO	1	84	126–128
8	**3h**	2-EtO	1	82	108–110
9	**3i**	3-Cl, 4-F	3	75	138–140
10	**3j**	3-F	2.30	86	136–138
11	**3k**	2-Br	2	79	146–148
12	**3l**	4-NO_2	4	77	226–228

[a] Products yield are isolated yield.

- (**3i**) IR (KBr, cm^{-1}): 3175, 1679, 770; ^1H NMR (DMSO-d_6, 200 MHz, ppm): 6.30–7.05 (m, 6H, Ar–H), 8.50 (s, 1H, Heteroaryl-H), 9.15 (s, 1H, Heteroaryl-H), 11.39 (s, 1H, —NH); CHN data: Calc. C 50.61, H 2.39, N 18.44, Found C 51.00, H 2.05, N 18.00; ES-MS (m/z): 380 (M + 1), 382 (M + 3).
- (**3j**) IR (KBr, cm^{-1}) 3180, 1665; ^1H NMR (DMSO-d_6, 200 MHz, ppm): 6.82–7.81 (m, 7H, Ar–H), 8.40 (s, 1H, Heteroaryl-H), 8.93 (S, 1H, Heteroaryl-H), 11.26 (s,1H, —NH); Mass: ES-MS E/Z 346 (M^+).
- (**3k**) IR (KBr, cm^{-1}): 3175, 1679; ^1H NMR (DMSO-d_6, 200 MHz, ppm): 6.40–7.25 (m, 7H, Ar–H), 8.54 (s, 1H, Heteroaryl-H), 9.05 (s, 1H, Heteroaryl-H), 11.29 (s, 1H, —NH); CHN data: Calc. C 47.31, H 2.48, N 17.24, Found C 46.95, H 2.90, N 17.48; ES-MS (m/z): 406 (M + 1).
- (**3l**) IR (KBr, cm^{-1}): 3175, 1679; ^1H NMR (DMSO-d_6, 200 MHz, ppm): 6.50–7.45 (m, 7H, Ar–H), 8.46 (s, 1H, Heteroaryl-H), 8.69 (s, 1H, Heteroaryl-H), 10.96 (s, 1H, —NH); CHN data: Calc. C 51.62, H 2.71, N 22.57, Found C 51.50, H 2.54, N 22.12; ES-MS (m/z): 373 (M + 1).

4. Results and discussion

In concern with the role of various 1,2,4-triazole in biological activity, we have reported here arylhydrazotriazoles (**3a-l**) synthesis. Arylhydrazotriazoles (**3a-l**) were prepared via coupling aryl amines (**1a-l**) with appropriate active methylene 1-(2,4-difluorophenyl)-2-(1*H*-1,2,4-triazol-1-yl)ethanones (**2**).

Substituted aromatic amines (**1a-l**) in conc. hydrochloric acid were cooled at 0 °C and into which aq. solution of sodium nitrite was added slowly without increasing the temperature above 5 °C,which resulted in the corresponding diazonium salts. To these diazonium salts commercially available 1-(2,4-difluorophenyl)-2-(1*H*-1,2,4-triazol-1-yl)ethanones (**2**) and

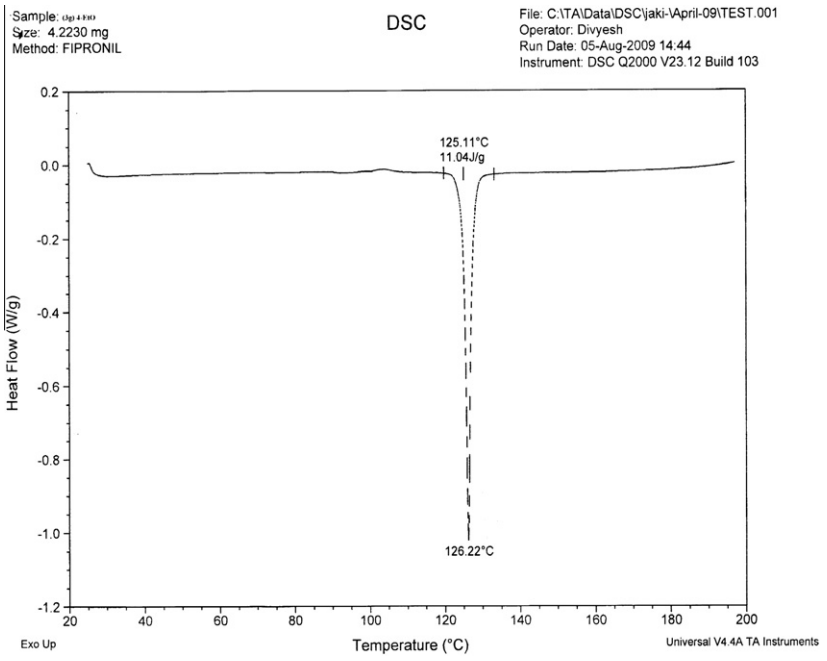


Figure 1 DSC of compound 3g.

sodium acetate as a base in methanol were added. The reaction mixture was stirred to obtain the corresponding fluorinated aryl hydrazoles (**3a-l**) (Scheme 1).

All the performed reactions were completed within a 1–4 h. The progress of the reaction was monitored by thin layer chromatography (TLC). After the completion of the reaction, the desired reaction mass was poured in ice water. The yellow colored products were obtained in the good yields (75–85%) (Table 1).

All the synthesized compounds were purified by crystallization using ethanol as a solvent and characterized by spectral analysis (IR, ^{1}HNMR, Mass and CHN analysis). One of the obtained compounds **3g**, have been screened for DSC and it showed only one peak at 126.22 °C (Fig. 1).

It suggests the purity of the compound and the presence of only one form.

All the prepared compounds were screened for antifungal activity.

5. Biological activity

All the synthesized compounds were screened for biological activity. We have tested antifungal susceptibility by the *E*-test. The in vitro activity of the antifungal agents was determined by the *E*-test (AB Biodisk), in accordance with the manufacturer's instructions. The *E*-test was performed by inoculating a 150 mm Petri dish containing 60 mL RPMI agar supplemented with 2% glucose and buffered to pH 7.0 with MOPS. The inoculum was applied with cotton swabs using a growth suspension prepared in 0.85% NaCl with turbidity adjusted to 0.5 McFarland standard. The lates were incubated for 24 h at 35 °C and read after 24 h. Reference strains *C. albicansa* (TCC 14503) and *A. fumigatus* (ATCC 16424) using Fluconazole and Ketoconazole were used for quality control. Interpretive susceptibility criteria for Fluconazole were those recommended by the Clinical and Laboratory Standards Institute (CLSI), formerly the National Committee for Clinical Laboratory Standards (National Committee for Clinical Laboratory Standards, 2002). Antifungal activity against *Candida albicansa*, and *A. fumigatus* at two conc. (100 and 200 μg/mL). The compounds tested are compared against the standard (Fluconazole and Ketoconazole) by measuring the diameter in the zone of inhibition (mm). All the compounds tested exhibited good activity at 100 μg/mL and very few compounds at 200 μg/mL conc. were found to be active against the fungi. All the products were screened for antifungal activity and the results were summarized in Table 2.

By observing the results in Table 2, we conclude that, the synthesized compounds are active against *C. albicansa*, and *A. fumigatus* at two conc. (100 and 200 μg/mL). Some of the arylhydrazotriazole derivatives having good activities as compared to standard. Compounds 3c, 3h, 3k gave good activity against *C. albicansa*, as compared to fluconazole. Similarly, compounds 3a, 3d, 3h and 3j gives the same activity as like standard fluconazole activity for *A. fumigatus*. Compound 3b is active against *A. fumigatus* and it defeats the standard activity of fluconazole.

6. Conclusion

We conclude that, all synthesized new fluorinated arylhydrazotriazole compounds are biologically active and prepared at mild reaction condition.

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Table 2 Antifungal activity (10–20 mg/ml) of α-aminophosphonates (**3a–l**).

Sr. No.	Compounds	Zone of inhibition (mm)			
		Antifungal[a]			
		C. albicansa (TCC 14503)		*A. fumigatus (ATCC 16424)*	
		100 μg/mL	200 μg/mL	100 μg/mL	200 μg/mL
1	**3a**	14	18	18	21
2	**3b**	14	17	22	34
3	**3c**	18	26	16	24
4	**3d**	11	16	20	26
5	**3e**	16	26	15	21
6	**3f**	12	20	16	20
7	**3g**	15	28	14	24
8	**3h**	20	30	19	22
9	**3i**	14	20	16	22
10	**3j**	12	17	20	27
11	**3k**	18	21	16	26
12	**3l**	12	18	17	21
13	Fluconazole[a]	22	–	20	–
14	Ketoconazole[b]	28	–	24	–

[a] Fluconazole (100 μg/mL) was used as a standard and it shows 22 and 20 mm zone of inhibition for *C. albicansa* and *A. fumigatus*, respectively.

[b] Ketoconazole (100 μg/mL) was used as a standard and it shows 28 and 24 mm zone of inhibition for *C. albicansa* and *A. fumigatus*, respectively.

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