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Synthesis and in vitro antifungal and cytotoxicity evaluation of substituted 4,5-dihydronaphtho[1,2-*d*][1,2,3]thia(or selena)diazoles

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Abstract

Unsubstituted 4,5-dihydronaphtho[1,2-*d*][1,2,3]thia (or selena)diazoles (**2a**, **2b**), prepared from the semicarbazone (**1a**), were nitrated using fuming nitric acid at 0 °C to yield various mono-nitrated dihydronaphthalenes (**3a**–**3e**). Related sulfamoyl derivatives (**4a**, **4b**) were prepared using chlorosulfonic acid, followed by the addition of ammonia solution. Synthesis of 6,9-dimethoxy-4,5-dihydronaphtho[1,2-*d*][1,2,3]thiadiazole derivative (**2c**) was performed using 5,8-dimethoxy-α-tetralone semicarbazone (**1b**) and thionylchloride at low temperature. At 10 ppm concentration, all compounds showed low toxicity (higher than 80% survival) on brine shrimps, while at 100 ppm concentration compounds **2d**, **3d**, and **4b** exhibited toxicity (less than 60% survival). Compounds **3a**, **3e**, and especially **4a** showed significant antifungal activity against *Cryptococcus neoformans*. Compound **4a**, while being the most active antifungal agent in this series, possessed low toxicity.

Keywords: Antifungal activity; Cytotoxicity; 1,2,3-Thiadiazole; 1,2,3-Selenadiazole

1. Introduction

The substantial increase in the incidence of fungal infections during the past two decades has been suggested as the major cause of morbidity and mortality among immunocompromized patients [1]. Aggressive immunosuppression, HIV-1 infection, cancer, myelotoxic therapeutic regimens, and organ transplantation have opened the door to pathogenic fungal organisms. Disseminated candidiasis, pulmonary aspergillosis, and infections caused by emerging opportunistic fungi are the most common of the serious mycoses [2]. Amphotericin B has been the gold standard in antifungal therapy for the past half a century, and newer chemically modified azoles, such as fluconazole and itraconazole, have provided the clinicians with favorable therapeutic options. Despite remarkable efficacy of both classes of compounds in the treatment of invasive mycoses, severe toxic reactions to amphotericin B and emergence of fungal resistance to azoles has made the pursuit of safe and effective therapies in the past decade [3,4]. In this respect, search for new antifungal agents, which are fungicidal, have a broad-spectrum of activity, and have fewer side effects has become critical. Small heterocyclic molecules with potential phamacophoric properties have attracted the attention of scientific communities as feasible alternatives to conventional drugs. Their synthetic diversity, easy to manipulate skeletons and economical feasibility has made these molecules the top runners in drug discovery endeavors. In this context, the syntheses and antifungal activity of a series of 1,2,3-selenadiazole, 1,2,3-thiadiazole, 1,2,4-triazolo[4,3-*a*]pyrimidine, and other relevant azoloazine derivatives has been reported [5]. Also, the syntheses and antibacterial/antifungal activity of a series of arylsulfonyl-1,2,3-selenadiazoles was previously reported [6]. We have recently reported the syntheses and antifungal activity of a series of thiazolo-4*H*-1,2,4-triazoles and 1,2,3-thiadiazolo-4*H*-1,2,4-triazole derivatives [7]. Based on the above reports and in continuation of our research on the syntheses of biologically-active small heterocyclic

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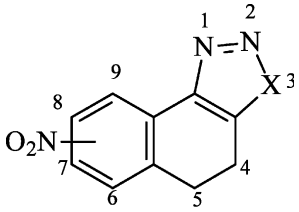
Table 1
Physical constants for compounds **3a** to **3e**



3a-3e

Number [a]	Position	X	Yield (%)	M.p. [b] (°C)	R_f [c]	^{1}H NMR ($CDCl_3$)
3a	7	S	55	173–176	0.5	8.42 (d, 1H, H_9, $J_{8,9}=9.4$ Hz), 8.27 (dd, H_8, $J_{6,8}=3$ Hz, $J_{8,9}=9.4$ Hz), 8.21 (d, 1H, H_6, $J_{6,8}=3$ Hz), 3.36 (t, 2H, CH_2), 3.24 (t, 2H, CH_2)
3b	8	S	30	160–163	0.35	9.09 (d, 1H, H_9, $J_{7,9}=2.4$ Hz), 8.18 (dd, 1H, H_7, $J_{7,9}=2.4$ Hz, $J_{6,7}=6.7$ Hz), 7.48 (d, 1H, H_6, $J_{6,7}=6.7$), 3.35 (t, 2H, CH_2), 7.23 (t, 2H, CH_2)
3c	6	Se	21	142–144	0.70	8.58 (dd, 1H, H_9, $J_{8,9}=7.6$ Hz, $J_{7,9}=2.24$ Hz), 7.88 (dd, 1H, H_7, $J_{7,8}=8.2$ Hz, $J_{7,9}=2.24$), 7.52 (dd, 1H, H_8, $J_{8,9}=7.6$ Hz, J_7, 8 = 8.2 Hz), 3.44–3.29 (m, 4H, CH_2)
3d	7	Se	35	167–169	0.45	8.42 (d, 1H, H_9, $J_{8,9}=8.5$ Hz), 8.27 (dd, 1H, H_8, $J_{8,6}=2.1$ Hz, $J_{9,8}=8.5$ Hz), 8.20 (d, 1H, H_6, $J_{6,8}=2.1$ Hz), 3.38 (t, 2H, CH_2), 3.25 (t, 2H,CH_2)
3e	8	Se	41	155–157	0.2	9.10 (d, 1H, H_9, $J_{9,7}=2.1$Hz), 8.17 (dd, 1H, H_7, $J_{7,6}=7.1$ Hz, $J_{7,9}=2.1$ Hz), 7.48 (d, 1H, H_6, $J_{6,7}=7.1$ Hz), 3.39 (t, 2H, CH_2), 3.22 (t, 2H, CH_2)

[a] All compounds were crystallized from ethyl acetate:hexane (65:35) mixture.
[b] Crystals decomposed at their melting points.
[c] Mobile phase: ethyl acetate: hexane (40:60).

molecules, the syntheses and anitfungal evaluation of the title compounds are reported.

2. Chemistry

Reaction of thionyl chloride with the **1a** according to the procedure reported previously gave compound **2a** [8]. Compound **2b** was synthesized by the reaction of selenium dioxide with **1a** in acetic acid [9,10]. Compound **2c** was obtained through the reaction of semicarbazone **1b** with thionyl chloride at low temperature. Compound **2d** was synthesized according to the procedure reported previously [11]. Compounds **3a**–**3e** were synthesized through the nitration of compounds **2a** and **2b**. Reaction of compounds **2a** or **2b** with chlorosulfonic acid followed by ammonia gave compounds **4a** or **4b**, respectively.

3. Experimental

3.1. Chemistry

Melting points were determined on a Reichert–Jung hot stage microscope and are uncorrected. ^{1}H NMR spectra were obtained on a Brucker FT-80 (80 MHz) or a Varian Unity Plus (300 MHz) instrument, with tetramethylsilane as the internal standard. Infrared spectra were taken on a Perkin–Elmer 781 (KBr disks). Elemental microanalyses were within $\pm 0.4\%$ of theoretical values for C, H and N. Thin layer chromatography (TLC) was performed on silica gel polymer-backed (F 1500/LS 254, 20 × 20 cm, TLC Ready Foil, Schleicher and Schuell). Flash chromatography was performed using silica gel 60, class 70–230, 0.063–0.2 mm mesh, Rose Scientific.

3.1.1. 4,5-Dihydronaphtho[1,2-d][1,2,3]-thiadiazole (**2a**)

This compound was prepared in 46% yield from the relevant semicarbazone according to literature [8].

3.1.2. 6,9-Dimethoxy-4,5-dihydronaphtho[1,2-d][1,2,3]thiadiazole (**2c**)

To a recrystallized portion of 5,8-dimethoxy-α-tetralone semicarbazone [12] (**1b**) (2.63 g, 0.01 mol) in a cooled flask in an ice-salt bath at −5 °C, distilled thionyl chloride (10 ml) was added through a dropping funnel. After addition, the flask was kept at above temperature and the mixture was stirred vigorously for 24 h until a homogenous yellow suspension was formed. Chloroform (30 ml) was then added to the mixture. The final mixture was added dropwise to a beaker containing

Table 2
Minimum inhibitory concentration (MIC) (μg/ml) of different compounds on some fungal species

Number [a]	*Candidia albicans*	*Saccharomyces cerevisiae*	*Cryptococcus neoformans*	*Aspergilus fumigatus*
2b	12.5	25	12.5	25
2c	12.5	25	12.5	25
2d	12.5	25	12.5	25
3a	12.5	0.53	3.12	25
3b	12.5	12.5	12.5	25
3c	12.5	3.12	12.5	25
3d	12.5	12.5	25	25
3e	12.5	3.12	3.12	25
4a	12.5	3.12	0.53	25
4b	12.5	3.12	12.5	25
Amphotricin B	0.14	0.06	0.13	0.14
Fluconazole	0.26	25	0.53	3.25
DMSO	12.5	3.12	12.5	25

[a] All compounds were soluble in media and sterility of media was checked carefully.

saturated sodium bicarbonate solution (30 ml). After neutralization, the mixture was decanted and the organic layer was washed with water (3 × 10 ml). The organic layer was then dried over sodium sulfate and filtered. The filtrate was evaporated at reduced pressure at 40 °C and the residue was immediately purified by flash column chromatography with a gradient of ethyl acetate: hexane mixture (from 10:90 to 90:10) to give 1.5 g (55%) of **2a**; m.p.: 43–45 °C. ^{1}H NMR ($CDCl_3$) δ 7.03 (ABq, 2H, aromatic, $J_{7,8} = 7.8$ Hz), 4.17 (s, 3H, OCH_3), 4.05 (s, 3H, OCH_3), 3.19 (t, 2H, CH_2, $J_{4,5} = 7.8$ Hz), 3.04 (t, 2H, CH_2, $J_{4,5} = 7.8$ Hz); UV(CH_3OH) λ_{max} 254 nm (log $\varepsilon = 3.5$).

3.1.3. 4,5-Dihydronaphtho[1,2-d][1,2,3]selenadiazole (**2b**)

This compound was prepared according to the literature [10].

3.1.4. Nitration of 4,5-dihydronaphtho[1,2-d][1,2,3]thia(or selena)diazoles

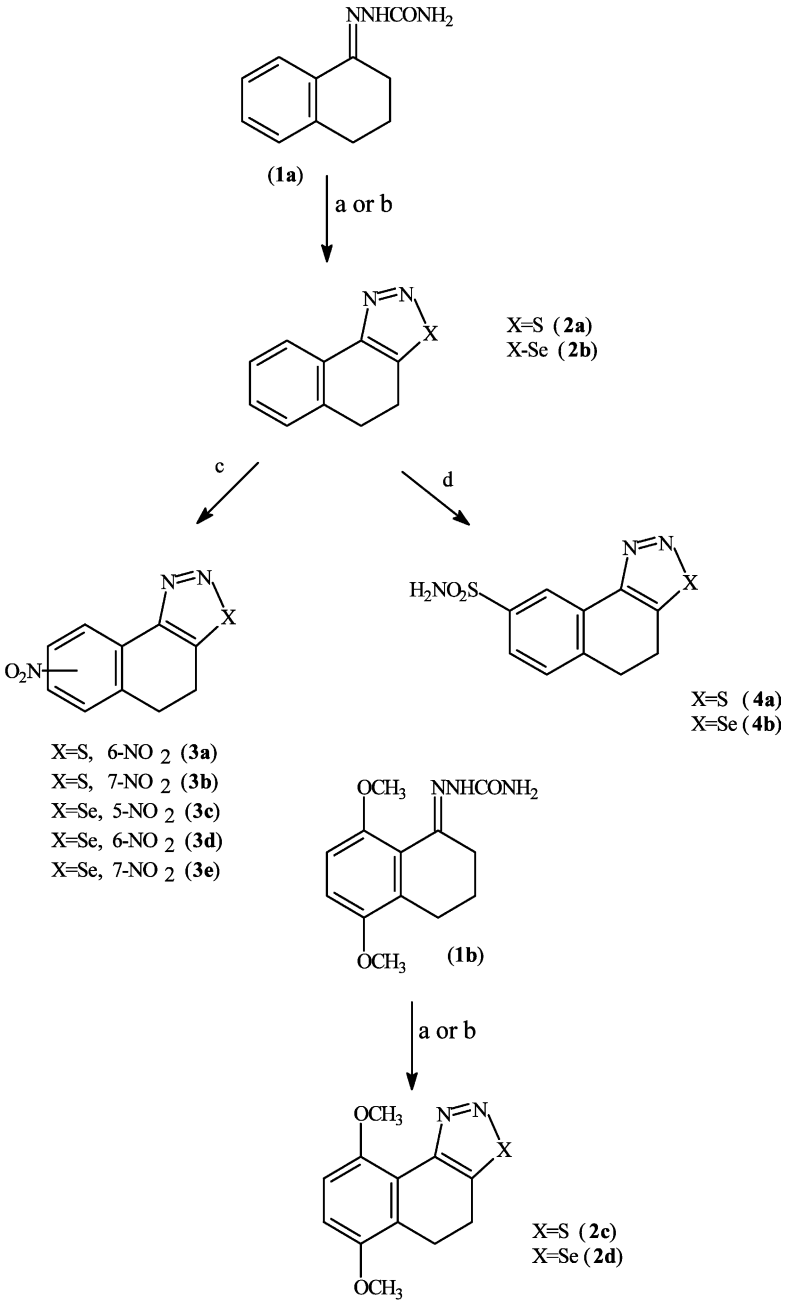
Fuming nitric acid (5 ml) was added dropwise to **2a** or **2b** (1 mmol) with stirring in an ice bath during 10 min. After stirring for 1 h, the mixture was warmed to room temperature (r.t.) and the reaction mixture was added to ice-water (50 ml). The resulting mixture was then successively extracted with chloroform (3 × 10 ml) and the organic layer was dried over sodium sulfate. After filteration the solvent was evaporated at reduced pressure to give a yellow solid which was purified by flash chromatography using ethyl acetate:hexane (from 25:75 to 100%) as eluent to yield different mono-nitrated compounds (Table 1).

Table 3
Survival percentage of shrimps in presence of different concentrations (100 and 10 ppm) of test compounds

Number	10 ppm	100 ppm
2c	97	80
2d	81	23
3a	97	77
3b	97	60
3c	81	77
3d	81	50
3e	100	80
4a	100	70
4b	93	57
5b	93	63
Sea water	100	100
DMSO	100	100
Taxol	0	0

3.1.5. 8-Sulfamoyl-4,5-dihydronaphtho[1,2-d][1,2,3]thiadiazole (**4a**)

Chlorosulfonic acid (25 ml) was added to **2a** (188 mg, 1 mmol) with stirring in an ice bath. After stirring for 10 min, the mixture was warmed at 60–80 °C for 2.5–3 h. Ethyl acetate (25 ml) was added to the reaction mixture and the mixture was added to ice-water (50 ml). The organic layer was decanted. It was washed with water (3 × 10 ml) and dried over sodium sulfate. The mixture was filtered and the solvent evaporated under reduced pressure to give a yellow–brown oil. To the latter residue, a mixture of water (25 ml), methanol (25 ml) and ammonia solution (25 ml, 25% v/v) was added. The reaction mixture was stirred at r.t. for 0.5 h, then warmed at 60–80 °C for 0.5 h. The mixture was evaporated at reduced pressure to remove methanol, then cooled to r.t. and acidified (pH 5) by the addition of concentrated hydrochloric acid. The resulting mixture was extracted with ethyl acetate. The organic layer was



Scheme 1. Reagents and condition a, $SOCl_2$; b, SeO_2, AcOH; c, HNO_3 (fuming) 5–0 °C; d, HSO_3Cl, NH_3.

dried (sodium sulfate), filtered and evaporated at reduced pressure to give a yellow residue which was crystallized from ethyl acetate:hexane (3:1) to give 144 mg (54%) of **4a**; m.p. 225–227 °C, ^{1}H NMR (DMSO-d_6) δ 8.55 (d, 1H, H_9, $J_{7,9} = 1.86$ Hz), 7.77–7.84 (dd, H_7, $J_{6,7} = 7.9$ Hz, $J_{7,9} = 1.86$ Hz), 7.59 (d, 1H, H_6, $J_{7,6} = 7.9$ Hz), 7.46 (bs, 2H, NH_2), 3.43 (t, 2H, CH_2), 3.16 (t, 2H, CH_2).

*3.1.6. 8-Sulfamoyl-4,5-dihydronaphtho[1,2-d][1,2,3]selenadiazole (**4b**)*

This compound was prepared similar to **4a** in 39% yield; m.p. 201–203 °C.^{1}H NMR (DMSO-d_6) δ 8.54 (d, 1H, H_9, $J_{7,9} = 1.86$ Hz), 7.74–7.82 (dd, H_7, $J_{6,7} = 7.9$ Hz, $J_{7,9} = 1.86$ Hz), 7.56 (d, 1H, H_6, $J_{7,6} = 7.9$ Hz), 7.40 (bs, 2H, NH_2), 3.40 (t, 2H, CH_2), 3.12 (t, 2H, CH_2).

3.2. Biological in vitro tests

3.2.1. Antifungal tests

3.2.1.1. Agar dilution method. The method used for this test was adopted from Muanza et al. (1994) and Mitscher et al. (1972) with little modifications [13,14]. The test was done in duplicates and, the positive

antifungal results were read based on no growth compared with solvent control.

3.2.1.2. Microbroth dilution method. Recommendations of NCCLS (1992) were mostly used to measure the minimum inhibitory concentration (MIC) values. The fungal organisms, taken from SDA plates, were suspended in normal saline to obtain $T = 75–77\%$ at 530 nm, which was equal to 106 CFU/ml. The fungal suspension was diluted 1000-times in the medium and 100 μl aliquots were added to each well. Compounds were dissolved in DMSO to make a concentration of 250 μg/ml, leaving 100 μl in each well. The 96-well plates were incubated at 35 °C for 24–96 h (Table 2).

3.2.1.3. Brine shrimp test. Brine shrimp toxicity test was determined on different compounds by a modification of the previously reported methods [15,16]. Two different concentrations (10 and 100 ppm) of test compounds were prepared by dissolving in DMSO as the solvent. Seawater was prepared by dissolving commercially available sea salt (3.8 g) into tap water (1 l). Brine shrimps hatched in seawater media at r.t. for 48 h as an air flow was bubbling through the media. Ten shrimps, seawater (5 ml) and different amounts of each test compound, were put in a test tube. Two blank samples were prepared containing, DMSO (50 μl in 5 ml sea water) and of sea water (5 ml). Taxol (10 ppm in DMSO) was used as the positive standard. All test tubes were left at r.t. for 24 h and the survived brine shrimps were counted and reported as survival percentage (Table 3).

4. Results and discussion

We were not previously successful in preparing methoxylated dihydronaphtho[1,2-*d*][1,2,3]thiadiazole (**2c**), instead only demethylated compound, 5,8-dihydroxydihydronaphtho[1,2-*d*][1,2,3]thiadiazole, was separated [11]. Preparation of compound (**2c**) was successfully performed using thionyl chloride at low temperature and rapid work up. Nitrated, sulfamoylated and methoxylated 4,5-dihydronaphtho[1,2-*d*][1,2,3]thia-(or selena)diazoles were synthesized in order to assess their toxicity and antifungal effects.

Dihydronaphthalene derivative (**2c**), bearing an electron-donating group, showed no antifungal effect, whereas those having nitro or sulfamoyl groups exhibited moderate effect. Compounds **3a**, **3e**, and especially **4a** showed significant antifungal activity against *Cryptococcus neoformans* and in the nitrated series **3a** showed significant effect on *Saccharomyces cerevisiae* (Table 2). In addition, compounds **3a**, **3c**, **3e** **4a** and **4b** exhibited slight toxicity (Table 3).

On brine shrimp toxicity test only **2d** showed a strong toxicity at 100 ppm, while the rest of the compounds exhibited slight toxicity. The minority of the compounds demonstrated considerable toxicity against brine shrimps. The detailed data are tabulated in Table 3.

5. Conclusion

In order to obtain new chemotherapeutic heterocyclic cores, we have synthesized substituted-naphtho[1,2-*d*][1,2,3]thia(or selena)diazoles that showed antifungal activities against some pathogenic fungal organisms, in vitro. The toxicity test on brine shrimps demonstrated that most of the compounds have low toxicity. Compound **4a** exhibited significant antifungal activity against *Cryptococcus neoformans* (**4a**, MIC: 0.53 mg/ml, compared with 0.53 mg/ml for fluconazole and 0.13 mg/ml for amphotericin B), while other compounds, such as **3a** and **3e** (MIC = 3.12 mg/ml) showed moderate effects (Scheme 1).

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