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DNA-directed alkylating agents: synthesis, antitumor activity and DNA affinity of bis-*N,N′*-trisubstituted 1,2,4-triazolo-piperazines

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Abstract

A series of 1,4-bis-(1,5-dialkyl-1*H*-1,2,4-triazol-ylmethyl)piperazines and *N*-methyl-piperazine analogs were prepared spontaneously from the cycloaddition of various reactive cumulenes with the piperazino-1,4-(bis-ethanenitrile) and 1-cyanomethyl-4-methyl-piperazine, respectively. The new compounds were evaluated for their DNA affinity and antitumor activity.

1. Introduction

During the last decade, several piperazine derivatives have been synthesized as useful chemotherapeutic agents for various diseases. Examples of such compounds bearing the piperazine residues are: Crivixan® (Indinavir sulfate) **1** [1,2] and delaviridine (Rescriptor) **2** [3], the powerful inhibitors for the protease and reverse transcriptase HIV enzymes, respectively. Bis-(indolyl)piperazine (BHAP) **3** [4] and the trovirdine derivative **4** [5] are another examples with a highly specific inhibitory of HIV-1 replication. An extensive studies on the synthesis of various bis-1,4-dialkyl-piperazines have been reported due to their widely potential biological importance, particularly their activity as antibacterial [6–8], antiprotozoacidal [9], antihypertensive [10,11], coronary dilators [12], antiplatelet agents with vasodilatory activity [13], inhibitor of 17-hydroxylase/C17,20-lyase, e.g., ketoconazole [14], sedative properties and as tranquilizers [15], as well as antineoplastic agents [7,16,17], in addition to their antihelmintic and antifilarial properties [18]. Furthermore, recently, new pharmacologically interesting compounds within the series of piperazine, such as NAN-190 **5** [19], N^4-substituted 1-(2,3-dihydro-1,4-benzodioxin-5-yl)piperazine [20] and some phenyl-piperazinyl-coumarines [21] are reported as a well-recognized antagonist of postynaptic receptors 5-HT_{1A}. In addition, some azaindole derivatives such as piperazinylmethyl substituted pyrazolo[1,7-a]pyridines [22] as well as the antipsychotic agent, clozapine [23] were found to be highly selective dopamine D_4 receptor antagonists, while some 2-piperazinyl benzothiazole analogs proved to be effective as histamine H_3 antagonists [24]. On the basis of these pharmacological activity of the substituted piperazine derivatives, as well as the antifungal activity of some 1,2,4-triazole compounds such as Fluconazole **6** [25] prompted us to synthesize a series of new 1,4-disubstituted piperazines bearing substituted 1*H*-1,2,4-triazoles and/or methyl group as potential antiAIDS and/or antitumor, via the cycloaddition of the 1,4-bis-*N*-methylcyano- and 1-*N*-methylcyano-4-methyl-piperazines with different reactive cumulenes (scheme 1).

2. Results and discussion

Recently, the short-lived 1-(chloroalkyl)-1-aza-2-azoniaallenes **9** [26–28] played an important role in the synthesis of the 4,5-dihydro-3*H*-pyrazolium salts by the cycloaddition with various electron-rich alkenes in the presence of Lewis acids, such as $SbCl_5$. Our recent work dealt with synthesis of different 1,2,4-triazole C-nucleosides [29,30], acyclic C-nucleosides [31], pyrimidines [32], *N*-alkylphthalimides [33], D-*manno*-pentitol-1-yl-1,2,4-triazoles [34], 1*H*-indoles [35], quinolones [35], benzotriazoles [36] and 3′-triazolo-thymidines [37], from cycloaddition of the reactive intermediate **9** with the corresponding nitriles. In the present study, the intermediate **9**, piperazino-1,4-bis-

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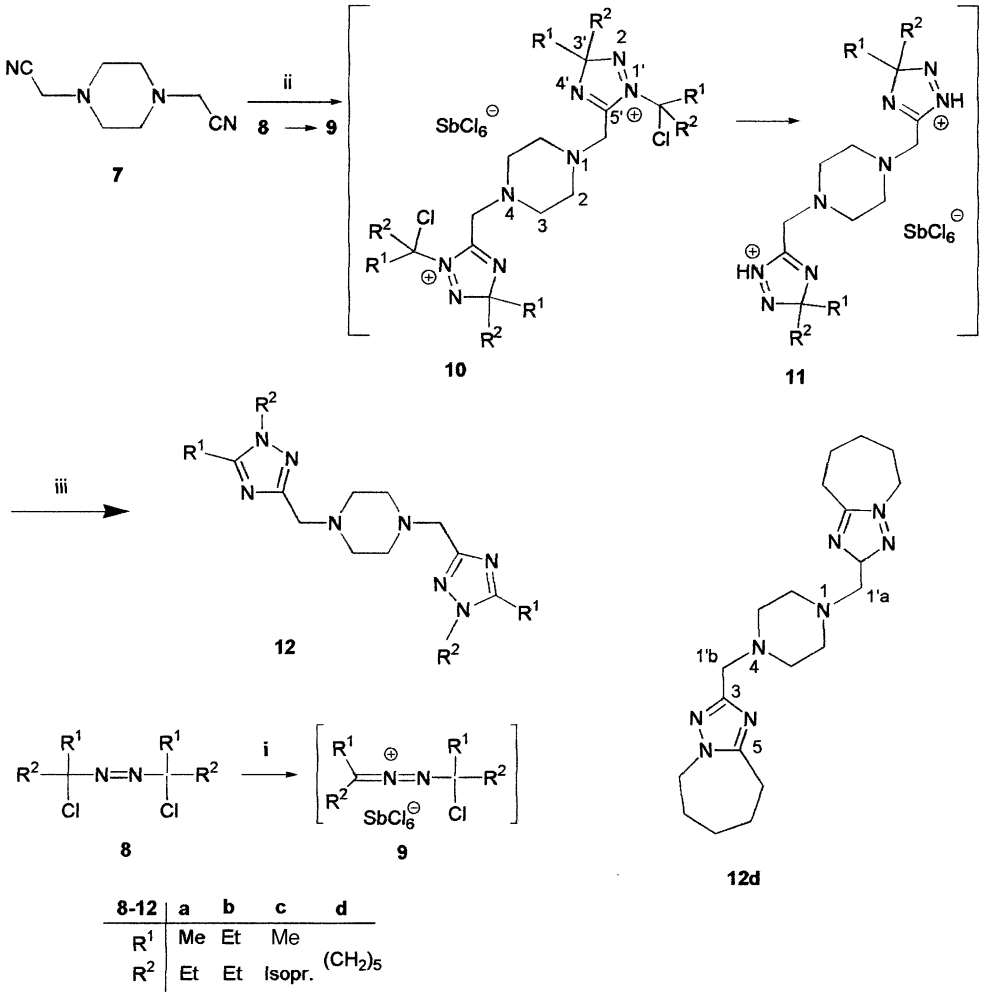
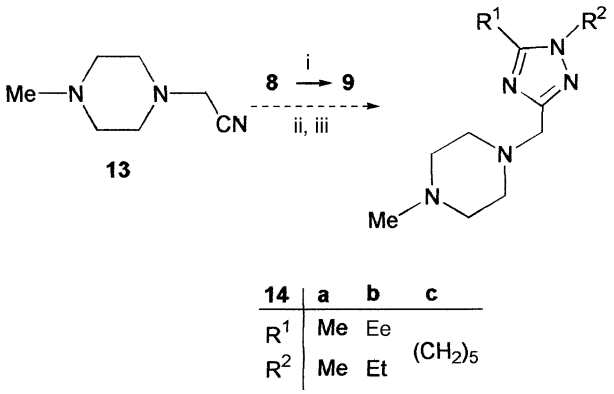


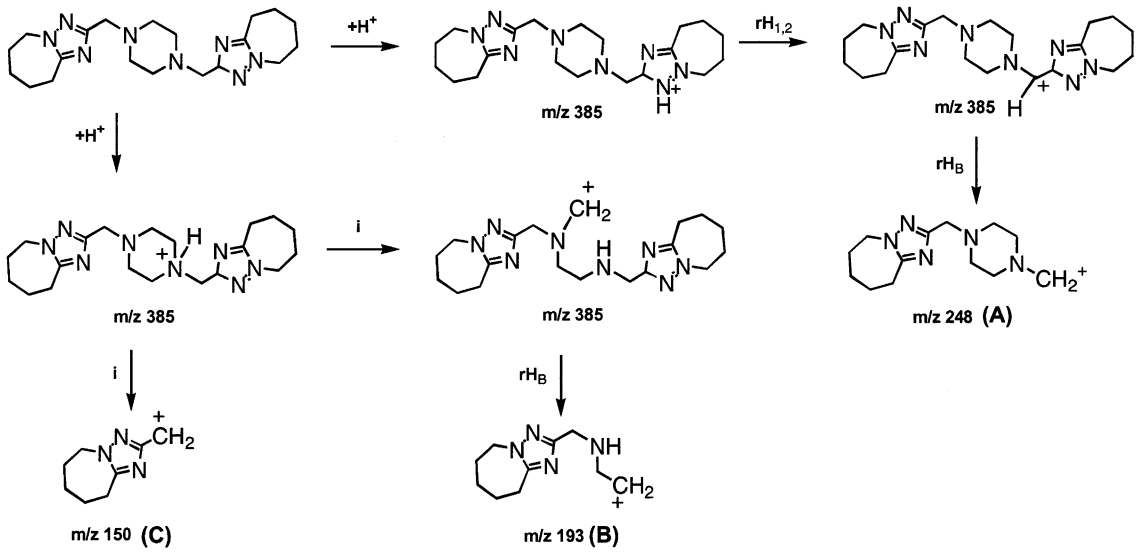
Scheme 1. Reagents and conditions: i: $SbCl_5$, CH_2Cl_2, –60 °C; ii: CH_2Cl_2, –60→23 °C; iii: $NaHCO_3$, NH_3, MeCN, 0 °C, 2 h.

(ethanenitrile) **6** [38] and 1-cyanomethyl-4-methyl-piperazine **13** [39] were selected for the synthesis of new 1,2,4-triazole precursors. The dichloride **8** was converted, at approximately –60 °C, to the salts **5** in the presence of $SbCl_5$. At –30 °C, the color changed indicating that **9** underwent cycloaddition with the bis-nitrile **7** to give the unseparable 1,4-bis-(3*H*-[1,2,4]triazolium-5-ylmethyl)piperazine hexachloroantimonates **10**. When the temperature was raised above –30 °C, **10** furnished the protonated 1,4-bis-triazolo-piperazines **11** by [1,2] migration [40,41] of the alkyl group (R^2) from C-3′ to N-2′, accompanied by elimination of the $CClR^1R^2$ group. In situ hydrolysis of **11** with aqueous $NaHCO_3$ and NH_3 solution gave the triazolo-piperazines **12a–d** in 83%, 81%, 78% and 86% yield, respectively. Similarly, the cycloaddition of **13** with **9** in the presence of $SbCl_5$ afforded, after a spontaneous rearrangement, the triazolo-piperazines **14a–c** in 75%, 70% and 75% yield, respectively (scheme 2).

The structures of these compounds were determined by homo- and heteronuclear NMR spectroscopic methods and by mass spectra. The ^1H NMR spectra of **12a–c** showed similar patterns. The CH_2-1′ appeared as singlets at the region δ 3.51–3.55. The alkyl groups at $N^{1''}$ and C-5″ of both triazole rings were assigned. The CH_2-piperazine groups of **12a,c** were assigned as broad singlets at δ 2.59 and 2.63, respectively, while the same groups of **12b,d** were appeared as multiplets between δ 2.62–2.55 and 2.34–2.25, respectively. Similarly, the CH_2-1′s at **14a–c** were appeared as singlets at δ 3.46, 3.52 and 3.48, respectively, while the singlets at δ 2.32, 2.34 and 2.41 were attributed, respectively, to the *N*-methyl-piperazine groups. The piperazine protons appeared as multiplets at the regions δ 2.57–2.52, 2.60–



Scheme 2. Reagents and conditions: i–iii as in scheme 1.



Scheme 3. Mass fragmentation ions of compound **12d**.

2.51 and 2.59–2.54, respectively. The ^{13}C NMR spectra of **12a–d** contained the resonance signals of the triazole ring (C-3″ and C-5″) at higher field between δ 159.2–157.6 and 157.5–150.9, respectively, meanwhile the same carbons of **14a–c** were appeared in the regions δ 158.6–157.7 and 157.4–151.4, respectively. C-1′ of **12a–d** and **14a–c** appeared between δ 55.9 and 54.5 while the carbons of piperazine ring of **12a–d** were resonated at the region δ 52.5, 50.9, 51.6 and 51.0, respectively. Due to the different groups attached at N-1 and N-4, the carbons of piperazine ring of **14a–c** were appeared as two singlets in the region δ 52.5–50.9. The alkyl groups were identified from their HMBC spectra [42]. Compound **12d** was selected for further mass fragmentation study, which showed correct molecular ion, M^+, as suggested by its molecular formula. The main fragmentation pathways are displayed in scheme 3. Under electron impact, the molecular ion protonated at triazole ring to give the fragment 385, followed by elimination of one of the triazolo-azepine precursor giving rise to ion (A) at $m/z = 248$. Alternatively, the protonated M^+ at piperazine ring leads to the carbonium ion ($m/z = 385$), followed by loss of triazolo-azepine ring giving the ion (B) at $m/z = 193$. The fragment at $m/z = 150$ is indicated to the ion (C), after loosing the piperazino-triazolo-azepine moiety from the protonated M^+, as shown in Scheme 3.

2.1. Antitumor activity

The compounds **12b–d** and **14a,c** selected by the National Cancer Institute (NCI), were tested using a one dose (10^{-4} M) primary anticancer in vitro assay [43] against tumor in the three-cell line panel consisting of MCF7 (breast), NCI-H460 (lung) and SF-268 (CNS) (Table 1). Compound **12a** was assayed in vitro against a panel consisting of 60 human tumor cell lines, derived from nine cancers types (leukemia, lung, colon, CNS, melanoma, ovarian, renal, prostate and breast cancers), using at five, 10-fold dilutions from a maximum concentration of 10^{-4} M (Table 1). Based on the requirement for cell line screening set by NCI is that the percent growth of tumor cells (PG%) is 30% or less, in at least one of the cell lines, it may be concluded that all the compounds are not active since did not approach this value except **12a** which showed a PG% of 39% in the CCRF-CEM (leukemia) cells at a concentration of 10^{-4}. Structure activity correlation of the obtained results revealed that the substitution of piperazine moiety with bis-1,4-(disubstituted-1,2,4-triazole) residues can play an important role in increasing antitumor activity. Accordingly, our current attempts is continued to modify the substituted piperazine with groups other than triazole like pyridine precursors.

Table 1
Growth inhibition

Compound	Growth (%)		
	(Lung) H460	(Breast) MCF7	(CNS) SF-268
12a *	107	127	86
12b	103	107	109
12c	106	124	105
12d	95	107	93
14a	98	98	97
14c	104	99	106

* PG% of **12a** against leukemia (CCRF-CEM) = 39%; against melanoma (UACC-62) = 59%.

Table 2
DNA affinity assay

Compound	DNA affinity (a_{24}/a_0) *
12a	2.2
12b	1.7
12c	1.9
12d	1.3
14a	1.1
14b	1.4
14c	1.1
m-AMSA	0.54
Bis-benzimide	0.57

* a_{24}: Final UV absorbance after 24 h; a_0: the UV absorbance at the same concentration at 0 time, then value $a_{24}/a_0 > 1$ indicates a total lack of affinity; $a_{24}/a_0 = 0$ indicates a total binding to DNA.

2.2. DNA affinity

The degree of affinity of the new synthesized compounds were studied, by following a procedure depending on the interference of their UV spectra after addition of calf thymus DNA [44]. The results were obtained by repeating the assays with well-known intercalating agents such as *m*-AMSA and bis-benzimide, the last closely binding to DNA along with the minor groove [45]. It was concluded from Table 2 that these compounds showed no or very low degree of affinity to DNA, in comparison to the above-mentioned standard compounds.

3. Experimental

3.1. General procedure [29–35]

3.1.1. Preparation of 1,4-bis-(1,5-dialkyl-1H-[1,2,4]triazol-3-ylmethyl)piperazine (12)

General procedure. To a stirred, cooled (–60 °C) solution of the required azo compound **8** (3.0 mmol) and **7** (0.32 g, 2.0 mmol) in dry CH_2Cl_2 (20 ml) was added dropwise to a solution of $SbCl_5$ (3.0 mmol) in dry CH_2Cl_2 (30 ml). The solution was left with stirring at –60 °C for 1 h, then at 0 °C for 1 h and finally at 23 °C for 10 min, followed by addition of pentane (50 ml). The precipitated solid was dissolved in CH_3CN (40 ml), cooled to 0 °C followed by addition of an aqueous solution of $NaHCO_3$ (2.52 g, 30 mmol in 30 ml of water) and ammonia solution (2 ml). The mixture was stirred at room temperature for 2 h, then the organic solvent was evaporated and the residue was extracted with $CHCl_3$ (3 × 20 ml). The combined organic extracts were dried (Na_2SO_4), filtered and evaporated to dryness and the residue was recrystallized from EtOH or $CHCl_3$-pentane and the oily compounds were purified on SiO_2 column.

1,4-Bis-(1-ethyl-5-methyl-1*H*-[1,2,4]-triazol-3-ylmethyl)piperazine (12a). From **8a** (0.55 g). Yield: 0.55 g, 83%; m.p. 128–131 °C. ^{1}H NMR ($CDCl_3$): δ 4.00 (q, 2H, *J* = 7.0 Hz, CH_2CH_3); 3.53 (s, 2H, CH_2-1′); 2.59 [br s, 4H, 4× (CH_2)]; 2.35 (s, 3H, C_5-Me); 1.36, (t, 3H, CH_2CH_3). ^{13}C NMR ($CDCl_3$): δ 159.0 (C-3); 151.4 (C-5); 55.1 (C-1′); 52.5 (C-piperazine); 43.0 (CH_2CH_3); 14.9 (CH_2CH_3); 11.6 (C_5-*Me*). Anal. calc. for $C_{16}H_{28}N_8$ (332.5): C, 57.81; H, 8.49; N, 33.71. Found: C, 57.60; H, 8.41; N, 33.52. MS: *m/z* (EI) 332 (M^+).

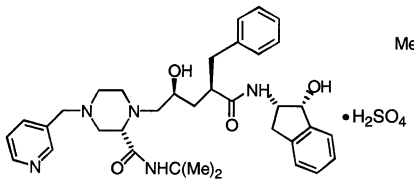
1,4-Bis-(1,5-diethyl-1*H*-[1,2,4]-triazol-3-methyl)piperazine (12b). From **8b** (0.72 g). Yield: 0.57 g, 81%; m.p. 154–155 °C. ^{1}H NMR ($CDCl_3$): δ 4.07–3.99 (m, 4H, 2× *N*-CH_2CH_3); 3.55 (s, 2H, CH_2-1′); 2.62–2.55 [m, 4H, 4× (CH_2)]; 2.37–2.33 (m, 4H, C_5-CH_2CH_3); 1.38–1.30 (m, 12H, 4× CH_2CH_3-triazole). ^{13}C NMR ($CDCl_3$): δ 159.2 (C-3); 151.7 (C-5); 55.9 (C-1′); 50.9 (C-piperazine); 43.0, 42.8 (*N*-CH_2CH_3); 14.9,14.7 (C_5-CH_2CH_3). Anal. calc. for $C_{18}H_{32}N_8$ (350.50): C, 59.97; H, 8.95; N, 31.08. Found: C, 59.76; H, 8.84; N, 30.87. MS: *m/z* (EI) 360 (M^+).

1,4-Bis-(1-isopropyl-5-methyl-1*H*-[1,2,4]-triazol-3-ylmethyl)piperazine (12c). From **8c** (0.48 g). Yield: 0.56 g, 78%; semi-solid. ^{1}H NMR ($CDCl_3$): δ 4.36 [m, 1H, $CH(CH_3)_2$]; 3.51 (s, 2H, CH_2-1′); 2.63 [br s, 4H, 4× (CH_2)]; 2.37 (s, 3H, C_5-Me); 1.44, 1.42 [(2s, 6H, $CH(CH_3)_2$)]. ^{13}C NMR ($CDCl_3$): δ 158.6 (C-3); 150.9 (C-5); 55.1 (C-1′); 51.6 (C-piperazine); 45.7 [$CH(CH_3)_2$]; 22.1 [$CH(CH_3)_2$]; 11.8 (C_5-Me). Anal. calc. for $C_{18}H_{32}N_8$ (360.50): C, 59.97; H, 8.95; N, 31.08. Found: C, 59.72; H, 8.82; N, 30.81. MS: *m/z* (EI) 360 (M^+).

2,2′-(Piperazin-1,4-ylmethyl)-bis-(6,7,8,9-tetrahydro-5*H*-[1,2,4]-triazolo[1,5-a])azepine (12d). From **8d** (0.71 g). Yield: 0.77 g, 86%; semi-solid. ^{1}H NMR ($CDCl_3$): δ 4.16 [m, 2H, CH_2-10); 3.52 (s, 2H, CH_2-1′); 2.86 (m, 2H, CH_2-6); 2.34–2.25 [m, 4H, 4× (CH_2)]; 1.81 (m, 2H, CH_2-8); 1.67 (m, 2H, CH_2-9); 1.56 (m, 2H, CH_2-7). ^{13}C NMR ($CDCl_3$): δ 157.6 (C-3); 157.5 (C-5); 54.9 (C-1′), 51.0 (C-piperazine); 50.6 (C-10); 30.1 (C-8); 27.2 (C-6); 22.7 (C-7). Anal calc. for $C_{20}H_{32}N_8$ (384.52): C, 62.47; H, 8.39; N, 29.14. Found: C, 62.27; H, 8.18; N, 29.03. MS: *m/z* (FAB) 385 (MH^+); 407 (MNa^+).

1-(1,5-Dimethyl-1*H*-[1,2,4-]-triazol-3-ylmethyl)-4-methyl-piperazine (14a). From **8a** (0.55 g) and **13** (0.28 g, 2.00 mmol). Yield: 0.31 g, 75%; oil. ^{1}H NMR ($CDCl_3$): δ 3.67 (s, 3H, *N*-*Me*-triazole); 3.46 (s, 2H, CH_2-1′); 2.57–2.52 [m, 4H, 4× (CH_2)]; 2.39 (s, 3H, C_5-Me); 2.32 (s, 3H, *N*-*Me*-piperazine) ^{13}C NMR ($CDCl_3$): δ 158.8 (C-3); 152.2 (C-5); 54.5 (C-1′); 52.5, 50.9 (*C*-piperazine); 50.9 (C-1′); 45.6 (*N*-*Me*-piperazine); 34.8 (*N*-*Me*-triazole); 11.6 (C_5-*Me*). MS: *m/z* (FAB) ($C_{10}H_{19}N_5$) 210 (MH^+); 232 (MNa^+).

1-(1,5-Diethyl-1*H*-[1,2,4-]triazol-3-ylmethyl)-4-methyl-piperazine (14b). From **8b** (0.50 g, 3.0 mmol) and **13** (0.28 g, 2.0 mmol). Yield: 0.33 g, 70%; oil. ^{1}H NMR ($CDCl_3$): δ 4.00 (q, 2H, *J* = 7.0 Hz, *N*-CH_2CH_3-triazole); 3.52 (s, 2H, CH_2-1′); 2.60–2.51 [m, 4H, 4× (CH_2)]; 2.36 (q, 2H, *J* = 7.1 Hz, *N*-CH_2CH_3-piperazine); 2.34 (s, 3H, *N*-*Me*-piperazine); 1.36, 1.32 (2t, 6H, 2× CH_2CH_3-triazole). ^{13}C NMR ($CDCl_3$): δ 158.8 (C-3); 151.5 (C-5); 55.0 (C-1′); 52.3, 51.3 (*C*-piperazine); 51.4 (C-1′); 45.6 (*N*-*Me*-piperazine); 43.0 (*N*-CH_2CH_3); 14.8 (C_5-CH_2CH_3); 11.6 (*N*-CH_2CH_3); 11.5 (C_5-CH_2CH_3). MS: *m/z* (FAB) ($C_{12}H_{23}N_5$) 238 (MH^+).



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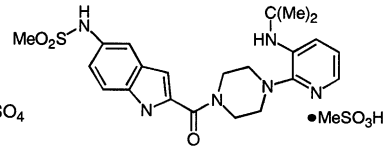

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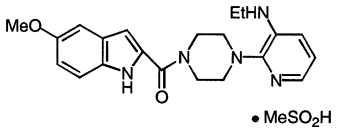

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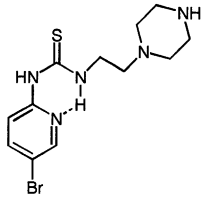

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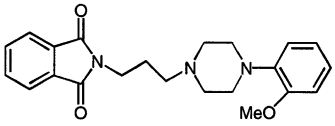
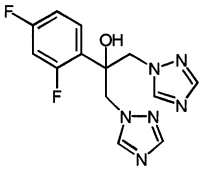


5. NAN-190



6. Fluconazole

2-(4-Methyl-piperazin-1-ylmethyl)-6,7,8,9-tetrahydro-5*H*-[1,2,4]-triazolo[1,5-a]azepine (14c). From **8c** (0.48 g) and **13** (0.28 g, 2.0 mmol). Yield: 0.37 g; 75%; oil. ^{1}H NMR ($CDCl_3$): δ 4.13 [m, 2H, CH_2-10); 3.48 (s, 2H, CH_2-1′); 2.83 (m, 2H, CH_2-6); 2.59–2.54 [m, 4H, 4× (CH_2)]; 1.80 (m, 2H, CH_2-8); 1.73 (m, 2H, CH_2-9); 1.64 (m, 2H, CH_2-7). ^{13}C NMR ($CDCl_3$): δ 157.7 (C-3); 157.4 (C-5); 54.9 (C-1′); 52.5, 51.5 (C-piperazine); 50.8 (C-10); 36.0 (*N-Me*-piperazine); 30.1 (C-8); 27.1 (C-6); 22.8 (C-7). MS: *m/z* (FAB) ($C_{13}H_{23}N_5$) (250) (MH^+); 272 ($MNa^{+)}$.

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