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European Journal of Medicinal Chemistry 38 (2003) 759–767
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
www.elsevier.com/locate/ejmech

Short communication

Synthesis characterization and anticancer activity studies on some Mannich bases derived from 1,2,4-triazoles

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Received 6 January 2003; received in revised form 22 April 2003; accepted 22 May 2003

Abstract

A series of 3-substituted 4-[5-(4-methoxy-2-nitrophenyl)-2-furfurylidene] amino-5-mercapto-1,2,4-triazoles (**3**) were synthesized. Aminomethylation of compounds **3** with formaldehyde and various secondary amines furnished Mannich bases **4** and **5**. These compounds were characterized on the basis of IR, ^{1}H-NMR, mass spectral data and elemental analysis. The newly synthesized compounds were screened for their anticancer activity against a panel of 60 cell lines derived from seven cancer types namely, lung, colon, melanoma, renal, ovarian, CNS and leukemia. Some of the compounds were slightly more potent.

Keywords: 1,2,4-Triazoles; Schiff bases; Mannich bases; Anticancer activity

1. Introduction

1,2,4-Triazoles were reported to possess significant antibacterial, antifungal and antihelmintic activities [1–5]. Further, arylfuran-2-carboxaldehyde derivatives were also found to possess antibacterial activity [6]. This gave a great impetus to the search for potential pharmacologically active drugs carrying arylfuran substituents.

Mannich bases were found to possess potent activities such as antibacterial, antifungal, antiviral, antimalarial and CNS depressant [7–10]. Many Mannich bases of 1,2,4-triazoles carrying *N*-methylpiperazine substituent possess protozoacidal and bactericidal activities. Keeping these observations in view and in continuation of our work on the synthesis of biologically active nitrogen and sulphur containing heterocycles [11–14], we report the synthesis of a series of Mannich bases of 3-substituted 4-(5-nitro-2-furfurylidene) amino-1,2,4-triazole-5-thiones (**3**) and their anticancer screening studies.

2. Chemistry

3-Substituted 4-amino-5-mercapto-1,2,4-triazoles **1** were synthesized according to the literature methods [15,16]. 4-Methoxy-2-nitrophenylfurfural (**2**) was synthesized through the Meerwein reaction [17]. Schiff bases **3** were prepared by the condensation of amino-mercaptotriazoles **1** with 4-methoxy-2-nitrophenylfurfural (**2**) in the presence of trace amount of concentrated sulphuric acid. Mannich bases **4** and **5** were prepared by reacting the Schiff bases **3** with secondary amines (morpholine and *N*-methylpiperazine) in presence of formaldehyde in ethanol medium. The structures of compounds **3**–**5** were established on the basis of nitrogen analysis, IR, NMR, and mass spectral data.

3. Results and discussion

The IR spectrum of Mannich base **4d** showed an absorption band at 1600 cm^{-1} indicating that presence of –C=N– in the molecule. The absorption band observed at 1283 cm^{-1} could be attributed to the –C=S functional group. The absorption bands corresponding to the nitro group were seen at 1530 and 1350 cm^{-1},

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0223-5234/03/$ - see front matter © 2003 Published by Éditions scientifiques et médicales Elsevier SAS.
doi:10.1016/S0223-5234(03)00128-4

Table 1
Characterization data of 3-substituted-4-[5-(4-methoxy-2-nitrophenyl)-2-furfurylidene] amino-1,2,4-triazole-5-thiones **3a**–**h**

Compound	R	Melting point (°C)	Yield (%)	Molecular formula	Analysis (%) found (calculated)		
					C	H	N
3a	H	188–190	82	$C_{14}H_{11}N_5O_4S$	48.52 (48.69)	3.12 (3.19)	20.25 (20.28)
3b	CH_3	223–225	84	$C_{15}H_{13}N_5O_4S$	50.34 (50.14)	3.57 (3.62)	19.30 (19.49)
3c	C_2H_5	183–185	76	$C_{16}H_{15}N_5O_4S$	51.61 (51.47)	3.96 (4.02)	18.70 (18.76)
3d	C_3H_7	189–191	88	$C_{17}H_{17}N_5O_4S$	52.83 (52.71)	4.31 (4.39)	17.95 (18.08)
3e	2-ClC_6H_4–OCH_2–	176–178	92	$C_{21}H_{16}ClN_5O_5S$	51.80 (51.95)	3.22 (3.29)	14.35 (14.43)
3f	4-ClC_6H_4–OCH_2–	200–202	90	$C_{21}H_{16}ClN_5O_5S$	51.82 (51.95)	3.17 (3.29)	14.33 (14.43)
3g	2,4-$Cl_2C_6H_3$–OCH_2–	178–180	83	$C_{21}H_{15}Cl_2N_5O_5S$	48.38 (48.55)	2.77 (2.89)	13.39 (13.48)
3h	4Cl,3-$CH_3C_6H_3$–OCH_2–	160–162	80	$C_{22}H_{18}ClN_5O_5S$	52.71 (52.90)	3.49 (3.60)	13.94 (14.02)

IR: (KBr, cm^{-1}): **3c**, 3117 and 3050 (NH/SH str.), 2935 (C–H str.), 1603 (C=N str.), 1543 (NO_2 asym), 1380 (NO_2 sym), 1233 (C=S), 1032 (C–S str.). ^{1}H-NMR ($CDCl_3$, 300 MHz): **3b**, δ 13.35 (bs, 1H, SH), 10.48 (s, 1H, –N=CH–), 7.72–7.75 (d, 1H, Ar–H, $J = 8.6$ Hz), 7.46 (d, 1H, Ar–H, $J = 8.6$ Hz), 7.21–7.29 (dd, 1H, Ar–H, $J = 8.6$, 2.4 Hz), 7.17–7.18 (d, 1H, furan-H, $J = 3$ Hz), 6.7–6.71 (d, 1H, furan-H, $J = 3$ Hz), 3.92 (s, 3H, OCH_3), 1.2 (s, 3H, CH_3); **3e**, δ 10.61 (bs, 1H, SH), 10.3 (s, 1H, –N=CH–), 5.3 (s, 2H, $-OCH_2$), 3.91 (s, 3H, OMe), 6.68–6.69 (d, 1H, furan-H, $J = 3.6$ Hz), 7.06–7.07 (d, 1H, furan-H, $J = 3.6$ Hz), 6.95–7.34 (complex multiplet, 5H, Ar–H), 7.66–7.69 (d, 1H, Ar–H, $J = 8$ Hz), 7.73–7.76 (d, 1H, Ar–H, $J = 8$ Hz). Mass: **3a**, *m/z* 345 (M^+, 45%), 244 (4-methoxy-2-nitrophenylfuronitrile, 56%), 101 (3-mercapto-4(H)-1,2,4-triazole, 100%); **3d**, *m/z* 387 (M^+, 2%), 341 ($M^+ - NO_2$, 3%), 244 (*o*-nitro-4-methoxyphenylfuronitrile, 5%), 154 (4-methoxy-2-nitro phenyl cation, 100%), 77 (Ph^+, 43.9%); **3f**, *m/z* 167 (*p*-chlorophenoxy methylacetonitrile, 45%).

respectively. The IR spectral data are given under the Tables 1–3.

In the ^{1}H-NMR spectrum of compound **4d**, the >N–CH_2–N< protons resonated as a singlet at δ 5.1 integrating for two protons. The signal due to the –N=CH– was seen as a singlet at δ 10.42. The two β-protons of the furan ring appeared as two closely spaced doublets at δ 6.67–6.68 ($J = 3.0$ Hz) and δ 7.05–7.07 ($J = 3.0$ Hz), respectively integrating for two protons. A doublet appeared at δ 7.69–7.73 ($J = 7$ Hz) corresponds to one of the aromatic protons, while the remaining aromatic protons appeared as a singlet at δ 7.26 and a doublet of doublet at δ 7.13–7.16 ($J = 1.5$, 1.5 Hz) integrating for three protons. A singlet observed at δ 3.9 was ascribed to methoxy protons. The $-NCH_3$ protons were observed as a singlet at δ 3.7, while a triplet was observed at δ 2.76–2.85 due to $-CH_2-N-CH_2-$ protons. The propyl protons of the triazole ring appeared as a triplet at δ 2.43 ($J = 6$ Hz), a multiplet at δ 2.27 and a triplet at δ 1.27–1.34 ($J = 6$ Hz) integrating for seven

Table 2
Characterization data of 3-substituted-1-(*N*-methyl piperazino)methyl-4-[5-(4-methoxy-2-nitrophenyl)-2-furfurylidene]amino1-2,4-triazole-5-thiones **4a**–**g**

Compound	R	Melting point (°C)	Yield (%)	Molecular formula	Analalysis (%) found (calculated)		
					C	H	N
4a	H	107	68	$C_{20}H_{23}N_7O_4S$	51.34 (52.51)	4.92 (5.03)	21.24 (21.44)
4b	CH_3	83	70	$C_{21}H_{25}N_7O_4S$	53.63 (53.50)	5.24 (5.30)	20.68 (20.80)
4c	C_2H_5	74	60	$C_{22}H_{27}N_7O_4S$	54.60 (54.43)	5.45 (5.56)	20.11 (20.20)
4d	C_3H_7	78	72	$C_{23}H_{29}N_7O_4S$	55.14 (55.31)	5.72 (5.81)	19.50 (19.63)
4e	2-ClC_6H_4–OCH_2–	155	65	$C_{27}H_{22}ClN_7O_5S$	54.55 (54.27)	4.65 (4.69)	16.47 (16.58)
4f	2,4-Cl_2 C_6H_3–OCH_2–	162	68	$C_{27}H_{27}Cl_2N_7O_5S$	51.16 (51.34)	4.20 (4.27)	15.42 (15.53)
4g	4Cl,3-$CH_3C_6H_3$–OCH_2–	159	74	$C_{28}H_{30}ClN_7O_5S$	54.78 (54.99)	4.81 (4.90)	15.88 (16.03)

IR (KBr, cm^{-1}): **4d**, 2917 (C–H str.), 1600 (C=N), 1530 (NO_2 asym.), 1350 (NO_2 sym.), 1283 (C=S str.), 1033 (C–S str.), 800 (Ar–H str.). ^{1}H-NMR (300 MHz): **4b**, δ 10.5 (s, 1H, –N=CH–), 7.71–7.74 (d, 1H, Ar–H, $J = 8.7$ Hz), 7.27–7.28 (d, 1H, Ar–H, $J = 8.7$ Hz), 7.15–7.18 (dd, 1H, Ar–H, $J = 6$, 2.7 Hz), 7.07–7.09 (d, 1H, furan-H, $J = 3.6$ Hz), 6.68–6.69 (d, 1H, furan-H, $J = 3.6$ Hz), 5.1 (s, 2H, >N–CH_2–N<), 3.91 (s, 3H, OCH_3), 2.87–2.88 (s, 4H, $-CH_2-N-CH_2-$), 2.47–2.57 (s, 4H, $-CH_2-N(Me)-CH_2-$), 3.7 (s, 3H, $-NCH_3$), 1.25 (s, 3H, CH_3); **4d** δ 10.42 (s, 1H, –N=CH–), 7.69–7.73 (d, 1H, Ar–H, $J = 7$ Hz), 7.26 (s, 1H, Ar–H), 7.13–7.16 (dd, 1H, Ar–H, $J = 9.8$, 1.5 Hz), 7.05–7.07 (d, 1H, furan-H, $J = 3$ Hz), 6.67–6.68 (d, 1H, furan-H, $J = 3$ Hz), 5.1 (s, 1H, >N–CH_2–N<), 3.9 (s, 3H, OCH_3), 2.76–2.85 (t, 4H, $-CH_2-N-CH_2-$, $J = 2.4$ Hz), 3.7 (s, 3H, NCH_3), 2.43 (t, 2H, $-CH_2-$, $J = 6$ Hz), 2.27 (m, 6H, CH_2 and $-CH_2-N(Me)-CH_2-$), 1.27–1.34 (t, 3H, CH_3, $J = 6$ Hz); **4f**, δ 10.37 (s, 1H, –N=CH–), 5.25 (s, 2H, $-OCH_2$), 5.15 (s, 2H, >N–CH_2–N<), 3.91 (s, 3H, –OMe), 3.7 (s, 3H, –NMe), 2.92 (s, 4H, $-CH_2-N-CH_2$), 2.55 (s, 4H, $-CH_2-N(Me)-CH_2-$), 6.67–6.68 (d, 1H, furan-H, $J = 3.6$ Hz), 7.1–7.11 (d, 1H, furan-H, $J = 3.6$ Hz), 7.6–7.63 (d, 1H, Ar–H, $J = 8.7$ Hz), 7.29–7.3 (dd, 1H, Ar–H, $J = 8.2$, 2.2 Hz), 7.15–7.27 (m, 4H, Ar–H); **4g**, δ 10.48 (s, 1H, –N=CH–), 7.6–7.63 (d, 1H, Ar–H, $J = 8.6$ Hz), 7.4–7.5 (d, 1H, Ar–H, $J = 8.6$ Hz), 7.2–7.4 (dd, 1H, Ar–H, $J = 7.8$, 2.1 Hz), 7.4–7.5 (d, 1H, Ar–H, $J = 7.8$ Hz), 6.9–6.91 (d, 1H, furan-H, $J = 3$ Hz), 6.83–6.84 (dd, 1H, Ar–H, $J = 6$, 3 Hz), 7.1–7.12 (d, 1H, Ar–H, $J = 3.6$ Hz), 6.64–6.66 (d, 1H, furan-H, $J = 3$ Hz), 5.63 (s, 2H, $-OCH_2$), 5.21 (s, 2H, >N–CH_2–N<), 3.91 (s, 3H, OMe), 2.47 (s, 4H, $-CH_2-N(Me)-CH_2$), 2.89 (s, 4H, $-CH_2-N-CH_2-$), 1.25 (s, 3H, CH_3). Mass: **4a**, *m/z* 113 (*N*-methylpiperazinomethyl cation, 8%), 218 (4-methoxy-2-nitrophenyl cation, 100%).

Table 3
Characterization data of 3-substituted 1-(morpholinomethyl)-4-[5-(4-methoxy-2-nitrophenyl)-2-furfurylidene]amino-1,2,4-triazole-5-thiones **5a–g**

Compound	R	Melting point (°C)	Yield (%)	Molecular formula	Analysis (%) found (calculated)		
					C	H	N
5a	H	77	72	$C_{19}H_{20}N_6O_5S$	51.28 (51.35)	4.44 (4.50)	18.86 (18.91)
5b	CH_3	68	68	$C_{20}H_{22}N_6O_5S$	52.26 (52.40)	4.75 (4.80)	18.49 (18.58)
5c	C_2H_5	89	60	$C_{21}H_{24}N_6O_5S$	53.22 (53.38)	5.00 (5.08)	17.73 (17.79)
5d	C_3H_7	73	65	$C_{22}H_{26}N_6O_5S$	54.25 (54.32)	5.26 (5.34)	17.30 (17.28)
5e	2-ClC_6H_4–OCH_2–	123	69	$C_{26}H_{25}ClN_6O_6S$	53.26 (53.42)	4.19 (4.28)	14.28 (14.38)
5f	2,4-$Cl_2C_6H_3$–OCH_2–	158	75	$C_{26}H_{24}Cl_2N_6O_6S$	50.26 (50.40)	3.76 (3.87)	13.48 (13.59)
5g	4Cl,3-$CH_3C_6H_3$–OCH_2–	143	80	$C_{27}H_{27}ClN_6O_6S$	54.31 (54.18)	4.39 (4.50)	13.96 (14.04)

[1]H-NMR (300 MHz): **5b**, δ 10.6 (s, 1H, –N=CH–), 7.7–7.8 (d, 1H, Ar–H, $J = 8.6$ Hz), 7.2–7.3 (d, 1H, Ar–H, $J = 8.6$ Hz), 7.1–7.2 (dd, 1H, Ar–H, $J = 6$, 1.5 Hz), 7.05 (d, 1H, furan-H, $J = 3$ Hz), 6.7 (d, 1H, furan-H, $J = 3$ Hz), 5.05 (s, 2H, >N–CH_2–N<), 3.9 (s, 3H, OCH_3), 3.6–3.7 (t, 4H, –CH_2–O–CH_2–, $J = 6$ Hz), 2.8 (t, 4H, CH_2–N–CH_2, $J = 5$ Hz), 1.4 (s, 3H, CH_3); **5c**, δ 10.5 (s, 1H, –N=CH–), 7.7 (d, 1H, Ar–H, $J = 7$ Hz), 7.3 (d, 1H, Ar–H, $J = 7$ Hz), 7.2 (dd, 1H, Ar–H, $J = 6$, 1.5 Hz), 7.1 (d, 1H, furan-H, $J = 3$ Hz), 6.7 (d, 1H, furan-H, $J = 3$ Hz), 5.05 (s, 1H, >N–CH_2–N<), 3.9 (s, 1H, OCH_3), 3.7 (t, 4H, –CH_2–O–CH_2–, $J = 6$ Hz), 2.8 (t, 4H, –CH_2–N–CH_2–, $J = 6$ Hz), 1.7–1.9 (t, 2H, CH_2, $J = 6$ Hz), 1.8 (q, 2H, CH_2, $J = 6.5$ Hz), 1.1 (t, 3H, CH_3, $J = 6$ Hz). Mass: **5b**, *m/z* 100 (morpholinomethyl cation, 100%); **5c**, *m/z* 441 ($M^+ - OCH_3$, 5%), 457 ($M^+ - CH_3$, 3%), 100 (morpholinomethyl cation, 50%), 154 (4-methoxy-2-nitrophenyl cation, 100%).

protons. The NMR spectral data are given in Tables 1–3.

In the mass spectrum of compound **3d**, the molecular ion peak appeared at *m/z* 387 consistent with the molecular formula $C_{17}H_{17}N_5O_4S$. The peak appeared at *m/z* 244 was due to the formation of 4-methoxy-2-nitrophenylfuronitrile, while the peak observed at *m/z* 154 as base peak was accounted for the formation of 4-methoxy-2-nitrophenyl cation.

In the mass spectrum of Mannich base **5c**, a peak observed at *m/z* 100 was explained due to the formation of morpholinomethyl cation thus confirming the formation of Mannich bases **5**. The peak appeared at *m/z* 154 as base peak was accounted for the formation of 4-methoxy-2-nitrophenyl cation. The mass spectral data of Mannich bases **4** and **5** are given in Tables 1–3.

4. Pharmacology

All the newly synthesized compounds were screened for their anticancer activity at NIH, Bethesda, Maryland, USA under the Drug Discovery Programme of NCI according to procedure suggested by Boyd[18] in a primary three cell line-one dose anticancer assay against NCI–H 460 (lung), MCF 7 (breast) and SF 268 (CNS). In the current protocol each cell line is inoculated on a pre-incubated microtiter plate. The test agents are added at a single concentration and the culture is incubated for 48 h. End point determinations are made with Sulforhodamine B, a protein binding dye. Results for each test agents are reported as the percent growth of the tested cells when treated with the untreated control cells. Compounds which reduce the growth of any one of the cell lines to 32% or less (negative numbers indicate cell kill) are passed on for evaluation in the full penal of 60 cell lines over a 5-log dose range. In the present screening program (Table 4), four compounds were found to be inactive while the compounds **3a**, **3b**, **3e–g**, **4a**, **4e**, **5e–g** possessed growth percentage to less than 32% are regarded as active compounds. These compounds were then passed on for evaluation in the full panel of 60 cell lines derived from seven cancer types namely, lung, colon, melanoma, renal, ovarian, CNS and leukemia.

The following compounds exhibited anticancer properties.

Compound **3a** with NSC code no. 724643 was active against six cancer cell lines: leukemia: HL-60(TB), K-562, MMOLT-4; renal cancer: A498, TK-10; breast cancer: MDA-MB-435 at concentration less than 20 μM.

Compound **3b** with NSC code no. 724644 was active against a single cancer cell line, non-small cell lung cancer: HOP-92, at concentration less than 20 μM.

Compound **3e** with NSC code no. 724646 was active against eight cancer cell lines: non-small cell lung cancer: NCI-H23; colon cancer: HCT-116; melanoma: LOX IMVI; ovarian cancer: OVCAR-3; renal cancer: A498, RXF 393, TK-10; breast cancer: MCF7 at concentration less than 20 μM.

Compound **3f** with NSC code no. 724645 was active against 31 cancer cell lines: leukemia: CCRF-CEM, HL-60(TB), K-562, MMOLT-4; non-small cell lung cancer: A549/ATCC, EKVX, HOP-92, NCI-H226, NCI-H23, NCI-H460; colon cancer: HCC-2998, HCT-116, HCT-15; CNS cancer: SF-268, SF-539, U251; melanoma: MALME-3M, SK-MEL-28, UACC-257, UACC-62; ovarian cancer: SK-OV-3; renal cancer: 786-O, ACHN, RXF 393, SN12C, TK-10; prostate cancer: PC-3, breast cancer: MCF7, NCI/ADR-RES, MDA-MB-231/ATCC, BT-549 at concentration less than 20 μM.

Table 4
Sixty cell line in vitro anticancer screening data (GI_{50}, μM) of compounds

Cell line	Compound no.			
	3a	**3b**	**3e**	**3f**
	NSC 724643	NSC 724644	NSC 724646	NSC724645
Leukemia				
CCRF-CEM	26.1	100	23.1	17.5
HL-60(TB)	14.5	100	22.4	14.8
K-562	16.9	100	34.1	11.6
MMOLT-4	16.3	100	–	16.5
RPMI-8226	22.5	100	24.9	–
SR	–	100	34.8	–
Non-small cell lung cancer				
A549/ATCC	36.2	100	30.4	11.6
EKVX	75.0	100	–	12.4
HOP-62	62.2	46.0	26.7	22.6
HOP-92	–	12.4	–	15.1
NCI-H226	–	–	20.1	13.5
NCI-H23	39.3	100	18.5	13.8
NCI-H322M	43.0	100	21.5	20.0
NCI-H460	36.2	100	23.7	13.0
NCI-H522	40.2	100	49.0	25.7
Colon cancer				
COLO 205	45.6	100	40.2	20.1
HCC-2998	38.9	100	–	16.5
HCT-116	29.6	100	17.3	16.8
HCT-15	26.0	100	26.5	16.0
HT29	32.6	100	41.1	21.1
KM12	27.2	100	20.9	81.2
SW-620	29.8	100	–	–
CNS cancer				
SF-268	29.1	47.5	32.3	16.6
SF-295	36.0	36.3	22.6	–
SF-539	40.9	39.4	–	18.6
SNB-19	63.2	100	42.2	20.1
SNB-75	–	–	100	37.4
U251	–	–	22.0	15.5
Melanoma				
LOX IMVI	–	–	17.6	–
MALME-3M	23.8	54.1	–	14.1
M14	49.0	100	26.6	32.6
SK-MEL-28	48.6	100	55.0	17.3
UACC-62	41.1	100	–	17.8
UACC-257	–	100	28.4	15.9
Ovarian cancer				
IGROV1	39.6	100	52.7	–
OVCAR-3	33.9	100	17.7	–
OVCAR-4	82.9	100	45.4	28.7
OVCAR-5	97.0	100	44.9	24.7
OVCAR-8	38.2	84.0	21.3	24.6
SK-OV-3	45.5	100	35.4	19.9
Renal cancer				
786-0	66.2	91.1	28.4	18.4
A498	13.5	100	14.4	–
ACHN	50.7	93.0	25.6	17.2
CAKI-1	32.6	100	24.6	39.6
RXF 393	32.2	32.8	15.5	12.7
SN12C	59.1	100	26.4	19.9
TK-10	18.0	69.1	13.2	14.6
UO-31	54.4	100	31.7	33.5
Prostate cancer				
PC-3	35.7	100	23.6	12.2
DU-145	80.5	100	–	–

Table 4 (*Continued*)

Cell line	Compound no.			
	3a NSC 724643	**3b** NSC 724644	**3e** NSC 724646	**3f** NSC724645
Breast cancer				
MCF7	25.4	100	18.7	16.9
NCI/ADR-RES	30.5	100	22.6	15.8
MDA-MB-231/ATCC	–	–	46.7	14.2
HS 578T	30.6	100	32.5	47.7
MDA-MB-435	19.8	100	20.0	25.9
BT-549	30.1	100	–	13.6
T-47D	51.8	100	21.8	95.7

Cell line	Compound no.			
	3g NSC 724647	**4b** NSC 724655	**4e** NSC 724656	**5e** NSC 724651
Leukemia				
CCRF-CEM	16.3	22.0	21.7	18.7
HL-60(TB)	21.5	14.9	32.8	17.0
K-562	19.3	16.2	–	18.2
MMOLT-4	14.0	20.5	–	19.3
RPMI-8226	11.7	20.6	–	15.2
SR	–	–	–	–
Non-small cell lung cancer				
A549/ATCC	19.5	60.8	14.1	27.4
EKVX	48.9	100	21.5	54.9
HOP-62	19.8	32.9	21.7	30.0
HOP-92	–	–	21.0	21.6
NCI-H226	25.2	–	15.6	–
NCI-H23	16.1	26.1	16.3	22.9
NCI-H322M	18.0	38.3	23.5	21.0
NCI-H460	18.0	29.3	14.7	19.8
NCI-H522	29.7	65.0	29.5	38.9
Colon cancer				
COLO 205	32.9	52.5	24.4	36.6
HCC-2998	–	34.7	14.5	24.1
HCT-116	17.5	21.7	16.1	19.6
HCT-15	28.6	28.7	17.4	28.2
HT29	34.2	28.0	23.5	49.9
KM12	15.9	17.7	11.9	17.1
SW-620	30.7	100	–	27.4
CNS cancer				
SF-268	23.1	28.2	17.5	24.0
SF-295	18.0	18.3	–	38.1
SF-539	25.7	54.4	19.7	28.6
SNB-19	28.4	58.3	27.1	36.2
SNB-75	–	–	20.3	–
U251	22.0	–	13.2	26.1
Melanoma				
LOX IMVI	–	–	–	
MALME-3M	19.9	44.6	20.9	18.0
M14	16.0	42.4	17.7	22.3
SK-MEL-2	–	–	31.6	–
SK-MEL-28	30.3	50.0	24.5	46.0
UACC-257	–	–	–	–
UACC-62	34.7	55.4	19.2	32.0
SK-MEL-5	–	–	–	–
Ovarian cancer				
IGROV1	38.7	63.4	–	59.5
OVCAR-3	16.1	16.2	–	16.7
OVCAR-4	28.9	79.1	–	35.1
OVCAR-5	21.3	62.6	21.6	55.3
OVCAR-8	29.7	–	17.3	39.4
SK-OV-3	23.6	45.0	30.6	25.7

Table 4 (*Continued*)

Cell line	Compound no.			
	3a NSC 724643	**3b** NSC 724644	**3e** NSC 724646	**3f** NSC724645
Renal cancer				
786-0	29.0	39.4	17.4	34.4
A498	16.8	–	–	–
ACHN	45.3	69.4	23.1	33.6
CAKI-1	17.3	24.4	51.9	21.3
RXF 393	16.6	30.2	10.4	22.3
SN12C	24.2	100	17.9	32.0
TK-10	16.8	24.3	12.9	14.9
UO-31	43.9	100	30.1	37.1
Prostate cancer				
PC-3	19.4	24.9	15.3	20.7
DU-145	38.3	100	–	34.1
Breast cancer				
MCF7	15.9	30.4	12.9	19.4
NCI/ADR-RES	19.8	21.6	18.5	21.3
MDA-MB-231/ATCC	–	–	14.6	–
HS 578T	40.8	–	38.7	33.9
MDA-MB-435	18.1	14.2	18.3	17.1
BT-549	21.9	100	17.2	14.3
T-47D	44.0	45.6	22.1	53.9

Cell line	Compound no.	
	5f NSC 724652	**5g** NSC 724653
Leukemia		
CCRF-CEM	16.2	19.3
HL-60(TB)	19.1	24.1
K-562	18.2	20.4
MMOLT-4	19.4	21.8
RPMI-8226	12.7	12.5
SR	27.7	26.3
Non-small cell lung cancer		
A549/ATCC	24.1	19.3
EKVX	100	26.4
HOP-62	35.9	38.6
HOP-92	82.9	–
NCI-H226	–	100
NCI-H23	22.5	20.2
NCI-H322M	25.1	24.6
NCI-H460	25.6	18.5
NCI-H522	45.1	25.5
Colon cancer		
COLO 205	30.8	39.8
HCC-2998	22.7	16.5
HCT-116	16.5	17.6
HCT-15	26.9	30.7
HT29	42.0	21.6
KM12	16.7	29.1
SW-620	–	19.9
CNS cancer		
SF-268	28.6	33.8
SF-295	17.4	18.6
SF-539	18.8	22.0
SNB-19	28.7	41.2
SNB-75	–	–
U251	–	30.5
Melanoma		
LOX IMVI	–	–
MALME-3M	24.6	17.9

Table 4 (*Continued*)

Cell line	Compound no.			
	3a	**3b**	**3e**	**3f**
	NSC 724643	NSC 724644	NSC 724646	NSC724645
M14	26.0	25.7		
SK-MEL-2	–	–		
SK-MEL-28	27.7	20.5		
UACC-62	87.6	30.8		
Ovarian cancer				
IGROV1	–	40.3		
OVCAR-3	14.6	17.3		
OVCAR-4	21.7	31.9		
OVCAR-5	38.8	33.2		
OVCAR-8	–	33.5		
SK-OV-3	10.8	34.4		
Renal cancer				
786-0	32.5	32.5		
A498	–	12.4		
ACHN	36.1	39.1		
CAKI-1	22.7	32.6		
RXF 393	24.3	19.1		
SN12C	24.5	21.3		
TK-10	14.1	16.5		
UO-31	42.7	28.8		
Prostate cancer				
PC-3	18.6	17.7		
DU-145	100	38.0		
Breast cancer				
MCF7	20.7	100		
NCI/ADR-RES	21.1	27.6		
MDA-MB-231/ATCC	18.6	–		
HS 578T	–	37.8		
MDA-MB-435	–	21.0		
BT-549	13.2	34.6		
T-47D	38.6	44.1		

Compound **3g** with NSC code no. 724647 was active against 21 cancer cell lines: leukemia: CCRF-CEM, K-562, MMOLT-4, RPMI-8226; non-small cell lung cancer: A549/ATCC, HOP-62, NCI-H23, NCI-H322M, NCI-H460; colon cancer: HCT-116, KM12; CNS cancer: SF-295; melanoma: MALME-3M, M14; ovarian cancer: OVCAR-3, renal cancer: A498, CAKI-1, RXF 393, TK-10, prostate cancer: PC-3; breast cancer: MCF7, NCI/ADR-RES, MDA-MB-435 at concentration less than 20 μM.

Compound **4b** with NSC code no. 724655 was active against six cancer cell lines: leukemia: HL-60(TB), K-526; colon cancer: KM12; CNS cancer: SF-295; ovarian cancer: OVCAR-3; breast cancer: MDA-MB-435 at concentration less than 20 μM.

Compound **4e** with NSC code no. 724656 was active against 24 cancer cell lines: non-small cell lung cancer: A549/ATCC, NCI-H226, NCI-H23, NCI-H460; colon cancer: HCC-2998, HCT-116, HCT-15, KM12; CNS cancer: SF-268, SF-539, SNB-75, U251; melanoma: M-14, UACC-62; ovarian cancer: OVCAR-8; renal cancer: 786-O, RXF 393, SN12C, TK-10; prostate cancer: PC-3; breast cancer: MCF7, NCI/ADR-RES, MDA-MB-231/ATCC, MDA-MB-435, BT-549 at concentration less than 20 μM.

Compound **5e** with NSC code no. 724651 was active against 14 cancer cell lines: leukemia: CCRF-CEM, HL-60(TB), K-562, MMOLT-4, RPMI-8226; non-small cell lung cancer: NCI-H460; colon cancer: HCT-116, KM12; melanoma: MALME-3M; ovarian cancer: OVCAR-3; renal cancer: TK-10; breast cancer: MCF7, MDA-MB-435, BT-549 at concentration less than 20 μM.

Compound **5f** with NSC code no. 724652 was active against 16 cancer cell lines: leukemia: CCRF-CEM, HL-60(TB), K-526, MMOLT-4, RPMI-8226; non-small cell lung cancer: EKVX; colon cancer: HCT-116, KM12; CNS cancer: SF-295, SF-539; ovarian cancer: OVCAR-3, SK-OV-3; renal cancer: TK-10; prostate cancer: PC-3, DU-145; breast cancer: BT-549 at concentration less than 20 μM.

Compound **5g** with NSC code no. 724653 was active against 14 cancer cell lines: leukemia: CCRF-CEM, RPMI-8226; non-small cell lung cancer: A549/ATCC, NCI-H460; colon cancer: HCC-2998, HCT-116, SW-620; CNS cancer: SF-295; melanoma: MALME-3M; ovarian cancer: OVCAR-3; renal cancer: A498, RXF

393, TK-10; prostate cancer: PC-3 at concentration less than 20 μM.

5. Conclusions

Compounds **3a**, **3b**, **3e–g**, **4b**, **4e**, **5e–g** were found to be slightly more potent then the rest of the compounds. All the compounds showed very weak potency, in the range 10–100 μM.

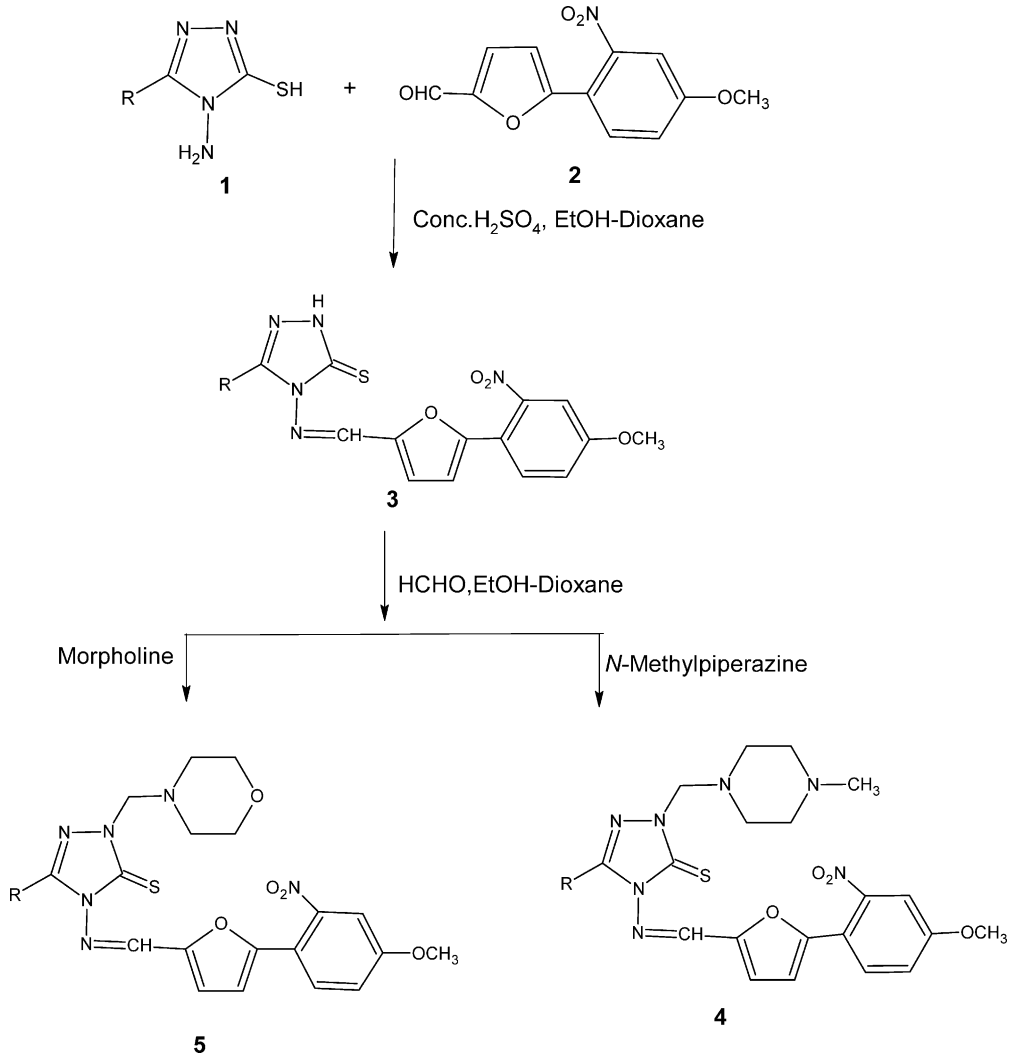
6. Experimental

6.1. Chemistry

Melting points were determined by capillary method and are uncorrected. The IR spectra (in KBr pellets) were recorded on a Shimadzu FT-IR 157 spectrophotometer. ^{1}H-NMR spectra were recorded either on a Perkin–Elmer EM-300 MHz or on a Bruker WH-200 MHz spectrometer using TMS as an internal standard. The mass spectra were recorded on a JEOL JMS-D 300 spectrometer operating at 70 eV. The purity of the compounds was checked by thin layer chromatography (TLC) on silica gel plates.

6.1.1. 3-Substituted 4-[5-(4-methoxy-2-nitrophenylfurfural)-2-furfurylidene]amino-5-mercapto 1,2,4-triazoles (3)

To a suspension of 4-methoxy-2-nitrophenylfurfuraldehyde (**2**; 10 mmol) in ethanol–dioxane (2:1), an equimolar amount of the corresponding amino mercapto triazole **1** was added. The suspension was heated until a clear solution was obtained. Then a few drops of concentrated sulphuric acid were added and the solution was heated under reflux for 3–4 h on a water-bath. The precipitated solid was filtered off and recrystallized from a mixture of ethanol and dioxane (2:1) to yield the title compounds **3**.



Scheme 1.

*6.1.2. 1-Aminomethyl-3-substituted-4-[5-(4-methoxy-2-nitrophenyl)-2-furfurylidene]-amino-1,2,4-triazole-5-thiones (**4** and **5**)*

The Schiff bases **3** (10 mmol) were dissolved in a mixture of ethanol and dioxane (2:1). Then formaldehyde (40% 1.5 mL) and secondary amine (10 mmol) in ethanol were introduced to this solution. The mixture was stirred for 1–2 h and kept overnight at room temperature. The solid separated was collected by filtration and recrystallized from a mixture of ethanol and dioxane (2:1) to yield the title compounds **4** and **5** (Scheme 1).

Acknowledgements

The authors are grateful to the Head, RSIC, CDRI Lucknow and the Director, RSIC, Panjab University, Chandigarh for providing microanalysis, IR, NMR and mass spectral data. The authors are also thankful to Dr V.L. Narayanan, NIH, Bethesda, USA for anticancer screening studies of compounds reported herein.

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