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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and biological evaluation of novel hydroquinone dimethyl ethers as potential anticancer and antimicrobial agents

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Abstract The synthesis of two novel series of quinol dimethyl ethers linked to either various functionalities or to some biologically active nitrogenous heterocycles is described. Nine of the newly synthesized quinol dimethyl ethers **5a**, **b**, **9b**, **10a**, **d**, **12a**, **b,** and **13a**, **b** were selected by the NCI and were tested initially at a single high dose (10 μM) in the full NCI 60 cell panel. Four of the screened quinol dimethyl ethers bearing unsubstituted phenylhydrazone **5a**, 4-chlorophenylhydrazone **5b**, 4-chlorophenyl-3-sulfanyl-1,2,4-triazole **9b**, as well as 4-chloroanilino-1,3,4-oxadiazole **12b** moieties satisfied the threshold antitumor screen. 4-Chlorophenylhydrazone **5b** showed very promising results and accordingly was chosen for in vivo antitumor screening. Thus, compound **5b** of the series could be considered as the potential lead for development of novel anticancer agents. In addition, compounds **5a–c**, **6a–c**, **9a–c**, **10a, b, d, e, g, h**, **11a–c**, **12a–c,** and **13a–c** were screened for their in vitro antimicrobial activity. Some of the tested compounds exhibited special high activity comparable to the reference ampicillin against *Pseudomonas aeruginosa* and *Escherichia coli*.

Keywords Quinol dimethyl ethers · Oxadiazoles · Triazoles · Thiadiazoles · Anticancer activity · Antimicrobial activity

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Introduction

Despite immense advances in the field of basic and medicinal research, that have resulted in higher cure rates for a number of malignancies, cancer remains the second leading cause of death after heart disorders in developing as well as advanced countries (Cozzi, 2003; Johnston *et al.*, 2005). Although major advances have been made in the chemotherapeutic management of some patients, discovering new anticancer agents remains critically important. Meanwhile, patients with neoplastic disorders who are subjected to chemotherapeutic treatment are mostly susceptible to microbial infections due to subsequent lack of immunity. Nevertheless, the emergence of some pathogenic bacteria and opportunistic fungi resistant to currently marketed antimicrobial agents pose a serious challenge to the medical community and highlight the urgent demand for the discovery of new, more potent, and selective non-traditional antimicrobial agents (Akbas and Berber, 2005). Therefore, the concept of monotherapy by a single drug with dual utility for those patients suffering from cancer disease accompanied with microbial infections, might be advantageous from both therapeutic and cost-effective

stand points with the hope of minimal side effects and more patient compliance.

Quinol dimethyl ethers derivatives were found to be associated with a variety of chemotherapeutic effects including anticancer (Chaaban *et al.*, 2007; Tu *et al.*, 2010; Ong *et al.*, 1988), antimicrobial (Ong *et al.*, 1988; Chaaban *et al.*, 2006), and antiprotozoal activities (Tapia *et al*., 2003).

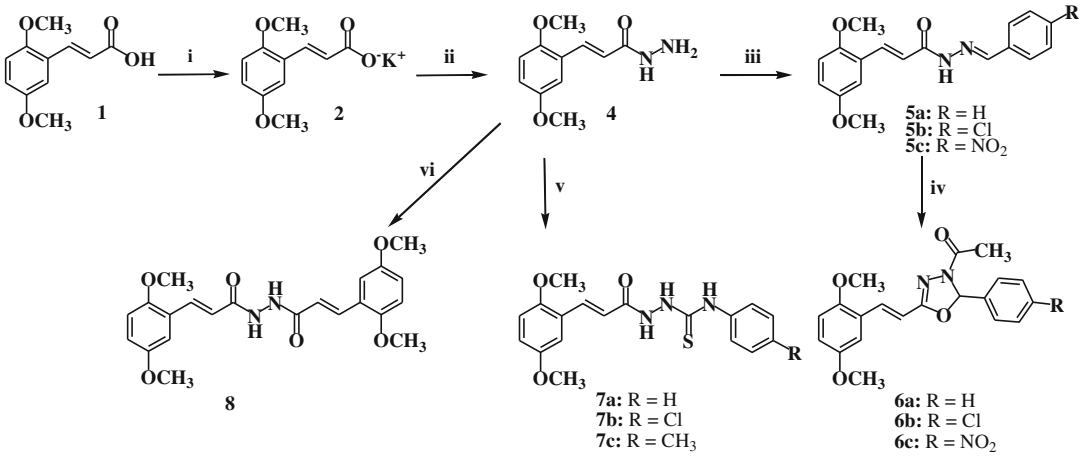
In view of the above-mentioned facts, and inspired by the promising chemotherapeutic activities encountered with some quinol dimethyl ethers reported in our previous publications (Chaaban *et al.*, 2006; Chaaban *et al.*, 2007), we considered it worthwhile to synthesize some novel quinol dimethyl ethers with the hope of discovering more active and selective antimicrobial and/or anticancer agents. It was rationalized to study the effect of incorporating various functionalities which are known to contribute to a variety of chemotherapeutic activities including α,β-unsaturated ketone, acid hydrazide, hydrazone, and thiosemicarbazide groups (Chornous *et al.*, 2001; Kumar *et al.*, 2003; Won *et al.*, 2005; Metwally *et al.*, 2006; Xue *et al.*, 2008; Rostom *et al.*, 2009; Ashour and Abdel Wahab, 2009; Pingaew *et al.*, 2010). In addition, it was considered of interest to incorporate other chemotherapeutically active heterocyclic rings [triazoles (Duran *et al*. 2002; Pintilie *et al.*, 2007; Havaldar and Patil, 2008), oxadiazoles (Rollas *et al.*, 2002; Joshi *et al.*, 2008; Lee *et al.*, 2010), and thiadiazoles (Kilcigil *et al.*, 2005; Matysiak and Opolski, 2006; Kadi *et al.*, 2007)] within the structure. These combinations were suggested in an attempt to investigate the possible synergistic influence of such structure hybridizations on the anticipated activity, hoping to discover a new lead structure which would have a significant antitumor and/or antimicrobial potential at small concentrations. Furthermore, owing to the well-documented chemotherapeutic potential associated with hydroquinones (Barrero *et al.*, 1999; Cordero *et al.*, 2004; Huang *et al.*, 2008; Liu *et al.*, 2010), it seemed interesting to prepare compounds incorporating hydroquinone moiety to explore the influence of such structural assembly on the anticipated biologic activities.

Results and discussion

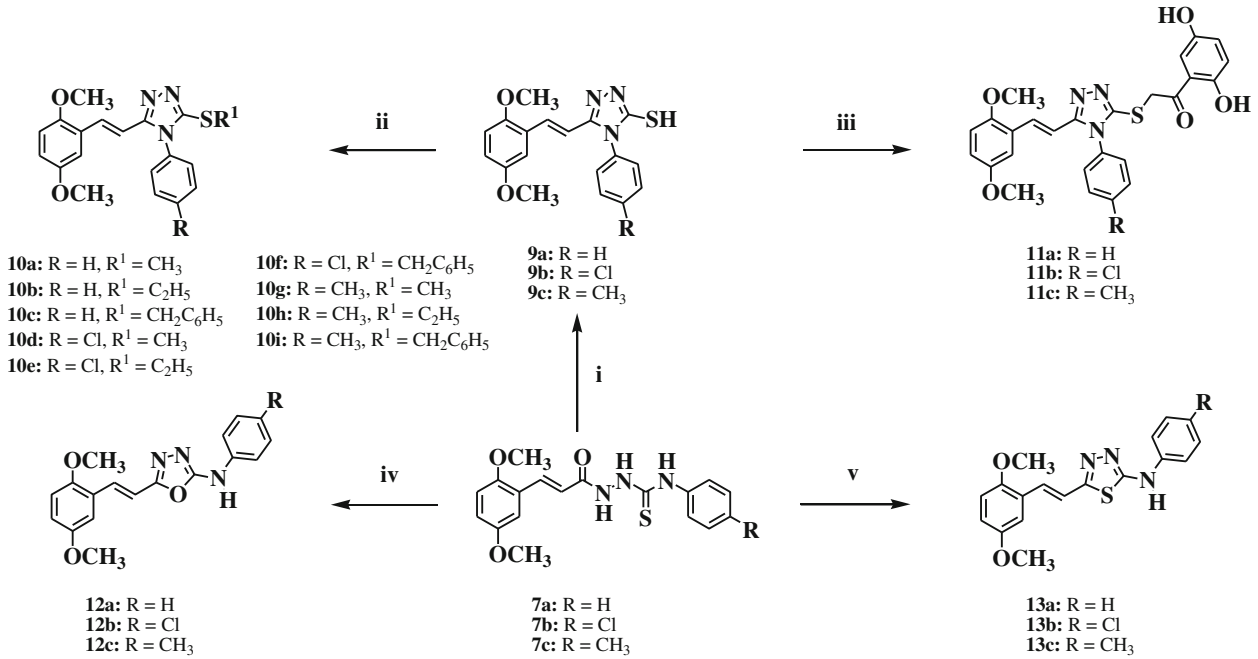
Chemistry

Synthesis of the intermediate and target compounds was performed according to the reactions outlined in Schemes 1 and 2. 3-(2,5-Dimethoxyphenyl)propenoic acid **1** was prepared via Knoevenagel condensation by reacting a solution of 2,5-dimethoxybenzaldehyde with malonic acid in pyridine in the presence of piperidine according to the previously reported reaction conditions (Gribkov *et al.*, 1994). Treating 3-(2,5-dimethoxyphenyl)propenoic acid **1** with 1 M potassium hydroxide solution at room temperature afforded potassium 3-(2,5-dimethoxyphenyl)propenoate **2** which was treated with ethyl chloroformate in chloroform in the presence of pyridine yielding the activated ester **3** which was added to a stirred ice-cooled suspension of 80 % hydrazine hydrate in chloroform according to the reported reaction conditions (Harada and Kondo, 1968) to afford 2,5-dimethoxyphenylpropenoic acid hydrazide **4**. Stirring warm solution of the latter and the appropriately substituted benzaldehyde in absolute ethanol gave rise to substituted hydrazones **5a–c** which were cyclized in acetic anhydride following the reported reaction conditions (Jin *et al.*, 2006; Amr *et al.*, 2008), yielding oxadiazolylethanone derivatives **6a–c**.

Thiosemicarbazide derivatives **7a–c** were prepared by heating a solution of 2,5-dimethoxyphenylpropenoic acid hydrazide **4** in absolute ethanol with an equimolar amount of the appropriate aryl isothiocyanate. It is to be noted down that allowing 2,5-dimethoxyphenylpropenoic acid hydrazide **4** to react with the selected aryl isothiocyanate (phenyl, *p*-chlorophenyl or *p*-tolyl) in absolute ethanol by stirring at room temperature, a totally unexpected one and the same product was obtained, indicating that the reaction has followed a completely different pathway. The structure of the product was proven to be N^1,N^2-bis(2,5-dimethoxyphenylpropenoyl)hydrazine **8** as it contains no sulfur proved by elemental microanalyses. In addition, its ^{1}H NMR spectrum showed two singlets assigned for the four methoxy protons and one deuterium-exchangeable singlet assigned for the two NH protons, besides the ethylenic and aromatic protons at their characteristic chemical shifts. Electron impact mass spectrum showed the molecular ion peak ($M^{\cdot+}$) at *m*/*z* 412 corresponding to $C_{22}H_{24}N_2O_6$ and base peak at *m*/*z* 190. For further confirmation, compound **8** was prepared through stirring an ethanolic solution of the acid hydrazide **4** at room temperature. Suggested mechanism for this unexpected reaction is illustrated in Fig. 1. It involves the attack of the lone pair of electrons of nitrogen of acid hydrazide moiety to the carbonyl of another molecule of acid hydrazide followed by the elimination of a hydrazine moiety. Moreover, sulfanyl triazoles **9a–c** were prepared by heating the appropriate substituted thiosemicarbazides **7a–c** with 1 N aqueous sodium hydroxide. Substituted sulfanyl 1,2,4-triazoles **10a–i** and **11a–c** were synthesized by adding the selected alkyl, aralkyl halide, or 2,5-dihydroxyphenacyl bromide to an ethanolic solution of the appropriate 3-sulfanyltriazole **9a–c** containing sodium ethoxide. On the other hand, 5-substituted anilino-1,3,4-oxadiazoles **12a–c** were prepared by cyclodesulfurization of the appropriate thiosemicarbazides **7a–c** with freshly prepared yellow mercuric oxide. Whereas, 5-substituted anilino-1,3,4-thiadiazoles **13a–c** were prepared either by heating



Scheme 1 Reagents and reaction conditions: *i* CH2(COOH)2, pyridine, piperidine *ii* CHCl3, ClCOOC2H5, NH2NH2.H2O, 0 °C, 12 h *iii* 4-R-C6H4CHO, EtOH, rt., 2 h *iv* Ac2O, reflux, 2 h *v* 4-R-C6H4NCS, EtOH, reflux, 2 h *vi* EtOH, rt., 2 h



Scheme 2 Reagents and reaction conditions: *i* NaOH, reflux, 4 h then HCl *ii* R1X/NaOEt, EtOH, reflux, 2 h *iii* 2,5-(OH)2C6H4COCH2Br, NaOEt, EtOH, reflux, 2 h *iv* yellow HgO, EtOH, reflux, 2 h *v* 10 % H2SO4 in EtOH, reflux, 1 h

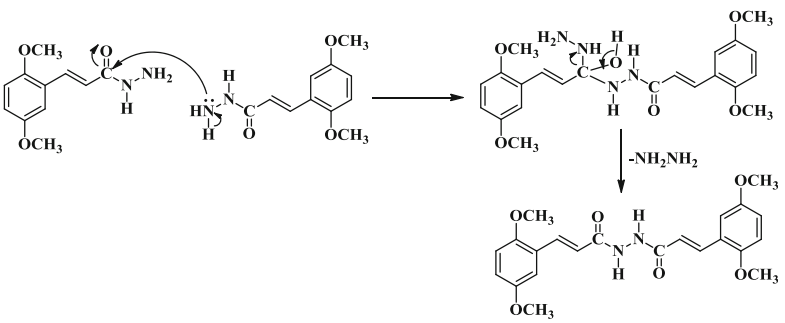


Fig. 1 Suggested mechanism for synthesis of compound **8**

3-(2,5-dimethoxyphenyl)propenoic acid **1** with substituted thiosemicarbazides in phosphorus oxychloride or via dehydration of substituted phenylpropenoyl thiosemicarbazides **7a–c** by boiling their ethanolic solutions in the presence of 10 % sulfuric acid.

Preliminary in vitro anticancer screening

In vitro antitumor activity

Primary in vitro one-dose assay Out of the newly synthesized compounds, nine derivatives **5a**, **b**, **9b**, **10a**, **d**, **12a**, **b**, and **13a**, **b** were selected by the National Cancer Institute (NCI) in vitro disease-oriented human cells screening panel assay to be evaluated for their in vitro antitumor activity. Effective one-dose assay has been added to the NCI 60 cell screen to increase compound throughput and reduce data turn around time to suppliers while maintaining efficient identification of active compounds (Monks *et al.*, 1991; Grever *et al.*, 1992; Boyd and Paull, 1995). All the selected compounds were tested initially at a single high dose (10 μM) in the full NCI 60 cell panel including leukemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate, and breast cancer cell lines. Only compounds which satisfy pre-determined threshold inhibition criteria would progress to the five-dose screen. The data are reported as a mean-graph of the percent growth of treated cells and presented as percentage growth inhibition (GI %) caused by the test compounds. The obtained results showed that four compounds, namely **5a**, **b**, **9b**, and **12b** passed successfully this primary anticancer assay and were consequently carried over to the five-dose screen against a panel of about 60 different tumor cell lines.

In vitro full panel (five-dose) 60-cell line assay

About 60 cell lines of nine tumor subpanels including leukemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate, and breast cancer cell lines were incubated with five concentrations (0.01–100 μM) for each compound and were used to create log concentration—% growth inhibition curves. Three response parameters (GI_{50}, TGI and LC_{50}) were calculated for each cell line. The GI_{50} value (growth inhibitory activity) corresponds to the concentration of the compounds causing 50 % decrease in net cell growth, the TGI value (cytostatic activity) is the concentration of the compounds resulting in total growth inhibition, and the LC_{50} value (cytotoxic activity) is the concentration of the compounds causing net 50 % loss of initial cells at the end of the incubation period (48 h). Subpanel and full panel mean-graph midpoint values (MG-MID) for certain agents are the average of individual real and default GI_{50}, TGI, or LC_{50} values of all cell lines in the subpanel or the full panel, respectively. In the present study, the four active compounds **5a**, **b**, **9b,** and **12b** exhibited potential antitumor activities against most of the tested subpanel tumor cell lines. These compounds showed a distinctive pattern of sensitivity against some individual cell lines (Table 1), as well as a broad spectrum of antitumor activity (Table 2). One-dose screen results of the two hydrazones **5a**, **b** presented in Table 1, revealed that both compounds exhibited significantly high growth inhibition percentages (GI %). Compound **5a** showed promising anticancer activity against ovarian OVCAR-3 and NCI/ADR-RES cell lines with GI % values of 93.98 and 91.49 %, respectively. It also showed lethal effect toward melanoma SK-MEL-2 and MDA-MB-435 as well as lung NCI-H522 tumor cell lines by 1.33, 7.51, and 15.34 %, respectively. Compound **5b** exhibited high activity toward most cell lines especially non-small cell lung NCI-H460, colon HCC-2998, HCT-116 and HT29, ovarian NCI/ADR-RES as well as breast MCF7 with GI % values of 94.81, 97.66, 94.28, 93.51, 96.18, and 91.07 %, respectively. In addition, compound **5b** elicited lethal effect on breast HS 578T and MDA-MB-468 cells, ovarian OVCAR-3 and SK-OV-3, renal RXF 393, melanoma MDA-MB-435 and M14, non-small cell lung NCI-H522, CNS SF-539, and colon COLO 205 cancer cell lines by 1.72, 4.03, 43.38, 4.93, 19.75, 32.37, 34.41, 36.17, 43.21, and 48.92 %, respectively.

Accordingly, both compounds **5a**, **b** were passed for the five-dose assay.

Five-dose screening of compound **5a** revealed that this compound exhibited broad spectrum of activity against most of the tested subpanel tumor cell lines as indicated from its median growth inhibitory concentration (GI_{50}), total growth inhibitory concentration (TGI), and median lethal concentration (LC_{50}) values. GI_{50} values for compound **5a** ranged from 0.283 to 9.66 μM against all leukemia, non-small cell lung (except NCI-H226 cell line), colon, CNS, melanoma, ovarian (except OVCAR-4, OVCAR-5, and SK-OV-3 cell lines), renal (except A498 and TK-10 cells), prostate, and breast cancer cell lines which indicated significant activity. TGI values which ranged from 1.98 to 41.7 μM revealed the high activity of compound **5a** against leukemia HL-60(TB) and RPMI-8226 cells, non-small cell lung HOP-92, NCI-H460 and NCI-H522 cells, colon COLO 205, HCC-2998, HCT-116, KM12 and SW-620 cells, CNS SF-295, SF-539 and SNB-75 cells, melanoma M14, MDA-MB-435, SK-MEL-2, SK-MEL-5 and UACC-257 cells, ovarian NCI/ADR-RES cells, renal CAKI-1, RXF 393, and SN12C cells as well as breast MDA-MB-468 cell lines. LC_{50} values of compound **5a** indicated its high activity against non-small cell lung NCI-H460 as well as colon KM12 tumor cell lines (28.3 and 29.4 μM, respectively).

Furthermore, five-dose testing of compound **5b** indicated that this compound exhibited significantly high potency with

Table 1 In vitro percentage growth inhibition (GI %) caused by the test compounds against some selected tumor cell lines at the single-dose assay

Compound no.	NSC-number	Panel	Subpanel cell lines (growth inhibition percent)
5a	750915/1	Leukemia	CCRF-CEM (44.79), HL-60(TB) (53.65), K-562 (78.77), MOLT-4 (63.47), RPMI-8226 (45.61)
		Non-small cell lung cancer	A549/ATCC (51.64), EKVX (36.04), HOP-62 (32.71), NCI-H23 (34.94), NCI-H460 (86.45), NCI-H522 (115.34)
		Colon cancer	COLO 205 (57.17), HCC-2998 (33.56), HCT-116 (85.84), HCT-15 (58.02), HT29 (79.29), KM12 (68.97), SW-620 (71.26)
		CNS cancer	SF-268 (39.28), SF-295 (51.50), SF-539 (48.21), SNB-19 (52.13), SNB-75 (58.93), U251 (56.48)
		Melanoma	LOX IMVI (57.92), MALME-3 M (40.95), M14 (72.24), MDA-MB-435 (107.51), SK-MEL-2 (101.33), SK-MEL-28 (34.47), SK-MEL-5 (69.19), UACC-257 (48.69), UACC-62 (39.05)
		Ovarian cancer	IGROV1 (61.49), OVCAR-3 (93.98), OVCAR-8 (45.20), NCI/ADR-RES (91.49), SK-OV-3 (40.04)
		Renal cancer	786-0 (45.71), A498 (61.30), ACHN (37.22), CAKI-1 (52.54), RXF 393 (54.58), SN12C (47.88), TK-10 (43.30), UO-31 (43.43)
		Prostate cancer	PC-3 (36.50)
		Breast cancer	MCF7 (82.65), HS 578T (33.54), BT-549 (39.22), T-47D (60.34), MDA-MB-468 (71.94)
5b	750916/1	Leukemia	CCRF-CEM (86.27), HL-60(TB) (79.92), K-562 (85.96), MOLT-4 (73.73), RPMI-8226 (78.69)
		Non-small cell lung cancer	A549/ATCC (72.21), EKVX (56.86), HOP-62 (62.12), NCI-H23 (65.16), NCI-H322 M (58.73), NCI-H460 (94.81), NCI-H522 (136.17)
		Colon cancer	COLO 205 (148.92), HCC-2998 (97.66), HCT-116 (94.28), HCT-15 (70.60), HT29 (93.51), KM12 (81.96), SW-620 (80.06)
		CNS cancer	SF-268 (66.85), SF-295 (68.16), SF-539 (143.21), SNB-19 (77.37), SNB-75 (55.98), U251 (81.64)
		Melanoma	LOX IMVI (63.32), MALME-3 M (45.86), M14 (134.41), MDA-MB-435 (132.37), SK-MEL-2 (77.7), SK-MEL-28 (61.17), SK-MEL-5 (69.44), UACC-257 (47.04), UACC-62 (57.90)
		Ovarian cancer	IGROV1 (82.13), OVCAR-3 (143.38), OVCAR-4 (59.75), OVCAR-5 (71.63), OVCAR-8 (78.96), NCI/ADR-RES (96.18), SK-OV-3 (104.93)
		Renal cancer	786-0 (83.30), A498 (87.46), ACHN (51.56), CAKI-1 (58.25), RXF 393 (119.75), SN12C (53.76), TK-10 (49.01), UO-31 (69.87)
		Prostate cancer	PC-3 (73.78), DU-145 (85.18)
		Breast cancer	MCF7 (91.07), MDA-MB-231/ATCC (71.93), HS 578T (101.72), BT-549 (36.45), T-47D (70.48), MDA-MB-468 (104.03)
9b	752385/1	Leukemia	CCRF-CEM (39.36), HL-60(TB) (95.67), K-562 (77.58), MOLT-4 (47.20), SR (61.74)
		Non-small cell lung cancer	A549/ATCC (33.67), NCI-H23 (51.29), NCI-H460 (61.09)
		Colon cancer	HCC-2998 (54.41), HCT-116 (70.42), HCT-15 (58.79), HT29 (34.61), KM12 (59.06), SW-620 (49.56)
		CNS cancer	SF-295 (68.80), SF-539 (60.50), SNB-19 (48.76), SNB-75 (57.49), U251 (46.74)
		Melanoma	MALME-3 M (70.24), M14 (69.04), MDA-MB-435 (110.26), SK-MEL-5 (67.51), UACC-62 (57.24)
		Ovarian cancer	IGROV1 (59.88), OVCAR-3 (70.57)
		Renal cancer	786-0 (41.08), RXF 393 (48.09), SN12C (33.83), UO-31 (52.87)
		Prostate cancer	PC-3 (41.41)
		Breast cancer	MCF7 (67.99), BT-549 (32.79), MDA-MB-468 (61.17)
10a	752381/1	Renal cancer	UO-31 (44.48)
		Breast cancer	T-47D (41.79)

Table 1 continued

Compound no.	NSC-number	Panel	Subpanel cell lines (growth inhibition percent)
10d	752386/1	Leukemia	HL-60(TB) (34.30), K-562 (69.31), SR (58.46)
		Non-small cell lung cancer	A549/ATCC (35.18), EKVX (38.60), NCI-H23 (40.33)
		Colon cancer	HCT-116 (40.23), HCT-15 (41.11), KM12 (46.94)
		CNS cancer	SF-295 (53.20)
		Melanoma	MALME-3 M (55.20), M14 (39.98), MDA-MB-435 (91.27), SK-MEL-5 (46.62), UACC-62 (50.50)
		Ovarian cancer	IGROV1 (38.17)
		Renal cancer	ACHN (32.14), CAKI-1 (35.52), RXF 393 (40.33), UO-31 (60.36)
		Prostate cancer	PC-3 (61.08)
		Breast cancer	MCF7 (53.28), T-47D (57.23), MDA-MB-468 (74.82)
12a	750917/1	Non-small cell lung cancer	HOP-92 (37.50)
12b	752384/1	Non-small cell lung cancer	A549/ATCC (59.77), HOP-62 (64.71), NCI-H226 (43.85), NCI-H322 M (41.40), NCI-H460 (84.49)
		Colon cancer	HCC-2998 (44.64), HCT-116 (78.12), SW-620 (33.32)
		CNS cancer	SF-295 (53.12), SNB-75 (84.25), U251 (58.25)
		Melanoma	LOX IMVI (32.28), MALME-3 M (58.51)
		Ovarian cancer	OVCAR-3 (67.86), OVCAR-4 (116.02), OVCAR-8 (33.31), SK-OV-3 (67.14)
		Renal cancer	786-0 (72.11), ACHN (67.43), RXF 393 (43.71), SN12C (55.07), TK-10 (60.88), UO-31 (95.02)
		Breast cancer	HS 578T (93.09), T-47D (104.56), MDA-MB-468 (33.97)
13a	752382/1	–	–
13b	752383/1	Leukemia	SR (32.12)

Data obtained from NCI in vitro disease-oriented human tumor cell screen at 10 μM concentration

GI_{50} values which ranged from 0.0292 to 10.9 μM against all cell lines except melanoma SK-MEL-2 and UACC-257 cells. TGI values of compound **5b** ranged from 0.0988 to 50.2 μM which indicated high activity against most cell lines. As for the LC_{50}, compound **5b** had high activity against colon HCC-2998 cell line (LC_{50} = 6.52 μM) and moderate activity (LC_{50} = 63.9 μM) against colon COLO 205 cells. Compound **5b** exhibited moderate selectivity against CNS tumor cell lines with selectivity ratio of 4.79, in addition to high selectivity against leukemia, colon, and prostate cancer cell lines with values of 6.95, 15.44, and 7.31, respectively. Hence, compound **5b** was referred to biologic evaluation committee to carry out in vivo hollow fiber assay (HFA). It is to be noted down that *p*-chlorohydrazone **5b** was found to be more potent than the phenyl derivative **5a** due to the presence of chlorine atom which imparts more lipophilic property.

Among the three triazoles, **9b** and **10a**, **d,** screened for antitumor activity, compound **9b** was the most active. It exhibited marked growth inhibition of 95.67 % against leukemia HL-60(TB) cell line as illustrated in Table 1. Compound **9b** also revealed lethal effect toward melanoma MDA-MB-435 by 10.26 %. Accordingly, compound **9b** was selected for the five-dose assay. Compound **9b** exhibited significantly high antitumor activity (GI_{50} values 2.25–9.4 μM) against most tumor cell. In general, alkylation of mercaptotriazoles produced less active compounds.

The *p*-chloroxadiazole derivative **12b** exhibited significant growth inhibition values against some cell lines and accordingly it was passed for five-dose assay. Whereas, its phenyl analog **12a** showed minor activity. Their thiadiazole analogs **13a**, **b** were either devoid of activity (**13a**) or showing minor effect (**13b**).

As illustrated in Table 1, compound **12b** showed pronounced GI % values of 95.02 and 93.09 % against renal UO-31 as well as breast HS 578T cells, respectively. It also elicited lethal activity against breast T-47D as well as ovarian OVCAR-4 cell lines by 4.56 and 16.02 %, respectively.

Compound **12b** exhibited significant high antitumor activity with GI_{50} values 1.87–9.87 μM against non-small cell lung A549/ATCC, HOP-62, NCI-H460 and NCI-H522 cells, colon HCT-116, HT29, CNS SF-268, SF-295, SNB-75 and U251 cells, melanoma LOX IMVI, MALME-3 M, SK-MEL-2 and SK-MEL-5 cells, ovarian cells (except OVCAR-5 cell line), renal cell lines, breast MDA-MB-231/ATCC, HS 578T, and T-47D cell lines.

Table 2 GI_{50}, TGI, and LC_{50} (μM) of five-dose screening of selected compounds

Compound no.	**5a**			**5b**			**9b**			**12b**		
Panel/cell line	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
Leukemia												
CCRF-CEM	3.97	>100	>100	0.228	>100	>100	4.77	>100	>100	>100	>100	>100
HL-60(TB)	2.03	13.7	>100	0.0785	0.483	>100	3.57	>100	>100	>100	>100	>100
K-562	0.488	>100	>100	0.0488	>100	>100	4.95	>100	>100	92.8	>100	>100
MOLT-4	4.45	>100	>100	0.523	>100	>100	7.74	>100	>100	>100	>100	>100
RPMI-8226	2.99	9.96	>100	0.3	15.7	>100	5.22	>100	>100	>100	>100	>100
SR	NT	NT	NT	0.039	16.4	>100	4.56	>100	>100	48.20	>100	>100
Non-small cell lung cancer												
A549/ATCC	4.29	>100	>100	0.242	>100	>100	9.07	>100	>100	3.67	17.8	78.6
EKVX	1.59	59.5	>100	0.816	>100	>100	30.0	>100	>100	40.5	>100	>100
HOP-62	4.95	>100	>100	0.162	50.2	>100	6.48	>100	>100	2.71	7.68	38.9
HOP-92	1.56	13.3	>100	4.43	58.9	>100	3.56	>100	>100	NT	NT	NT
NCI-H226	30.8	>100	>100	0.979	>100	>100	NT	NT	NT	NT	NT	NT
NCI-H23	5.4	87	>100	0.148	2.46	>100	5.88	>100	>100	21.7	>100	>100
NCI-H322M	5.22	>100	>100	0.666	>100	>100	26.9	>100	>100	30.5	>100	>100
NCI-H460	2.08	5.62	28.3	0.119	13.8	>100	4.53	>100	>100	3.77	>100	>100
NCI-H522	1.57	41.7	>100	0.0292	0.382	>100	4.07	>100	>100	7.13	83.7	>100
Colon cancer												
COLO 205	4.8	35.1	>100	0.153	0.595	63.9	12.4	68.4	>100	16.6	>100	>100
HCC-2998	2.35	21.0	94.5	0.139	1.19	6.52	13.3	>100	>100	>100	>100	>100
HCT-116	2.64	10.2	67.2	0.075	3.05	>100	4.05	>100	>100	2.97	13.1	>100
HCT-15	3.62	>100	>100	0.0835	>100	>100	4.39	>100	>100	34.1	>100	>100
HT29	2.1	>100	>100	0.0435	1.52	>100	3.67	>100	>100	9.44	28.3	82.1
KM12	0.834	3.75	29.4	0.0522	8.48	>100	4.02	>100	>100	17.4	>100	>100
SW-620	1.50	6.23	>100	0.0813	>100	>100	5.09	>100	>100	18.3	>100	>100
CNS cancer												
SF-268	7.56	>100	>100	0.718	>100	>100	17.5	>100	>100	7.6	37.9	>100
SF-295	1.00	11.1	>100	0.129	6.44	>100	4.16	>100	>100	6.15	64.5	>100
SF-539	4.42	25.1	>100	0.258	3.16	>100	4.05	97.9	>100	11.8	60.5	>100
SNB-19	4.12	>100	>100	0.34	>100	>100	12.7	>100	>100	13.8	>100	>100
SNB-75	4.39	37.3	>100	0.098	1.17	>100	2.85	>100	>100	2.26	7.94	>100
U251	3.68	>100	>100	0.184	1.49	>100	6.88	>100	>100	3.81	14.3	47.6
Melanoma												
LOX IMVI	4.11	>100	>100	0.254	>100	>100	5.02	>100	>100	7.91	>100	>100
MALME-3M	2.12	>100	>100	NT	>100	>100	70.6	>100	>100	9.87	52.4	>100
M14	2.16	6.77	>100	0.0879	6.7	>100	4.24	>100	>100	20.7	>100	>100
MDA-MB-435	0.283	1.98	>100	0.0311	0.0988	>100	2.83	>100	>100	37.0	>100	>100
SK-MEL-2	2.98	9.32	>100	24.7	>100	>100	4.05	>100	>100	9.66	>100	>100
SK-MEL-28	8.89	>100	>100	0.372	>100	>100	11.1	>100	>100	26.8	>100	>100
SK-MEL-5	2.94	14.6	>100	0.0581	NT	>100	3.58	>100	>100	7.17	>100	>100
UACC-257	5.76	33.6	>100	36.5	>100	>100	>100	>100	>100	21.3	>100	>100
UACC-62	3.55	75.8	>100	0.0680	>100	>100	3.11	>100	>100	16.2	>100	>100
Ovarian cancer												
IGROV1	1.51	>100	>100	0.187	62.9	>100	4.38	>100	>100	7.7	>100	>100
OVCAR-3	3.15	>100	>100	0.0335	0.115	>100	3.34	21.4	>100	3.55	15.9	>100
OVCAR-4	14.3	>100	>100	0.439	>100	>100	20.6	>100	>100	2.44	6.76	>100
OVCAR-5	18.0	>100	>100	10.9	>100	>100	70.3	>100	>100	32.5	>100	>100

Table 2 continued

Compound no.	**5a**			**5b**			**9b**			**12b**		
Panel/cell line	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
OVCAR-8	5.44	94.6	>100	0.350	>100	>100	28.4	>100	>100	4.91	21.0	76.4
NCI/ADR-RES	1.46	4.29	>100	0.0391	0.233	90.6	3.63	>100	>100	7.62	98.1	>100
SK-OV-3	12.0	>100	>100	0.149	13.9	>100	9.4	>100	>100	2.79	7.85	60.6
Renal cancer												
786-0	7.0	72.5	>100	0.326	>100	>100	7.17	>100	>100	2.67	7.6	55.3
A498	12.0	>100	>100	0.182	27.5	>100	6.66	>100	>100	1.87	7.76	>100
ACHN	9.66	>100	>100	1.49	>100	>100	23.8	>100	>100	3.29	19.9	>100
CAKI-1	0.443	6.66	>100	0.0879	>100	>100	2.8	NT	>100	6.02	>100	>100
RXF 393	3.9	26.6	>100	0.0469	0.298	>100	3.38	38.7	>100	2.71	7.92	56.1
SN12C	4.17	39.9	>100	0.982	>100	>100	6.07	>100	>100	4.21	>100	>100
TK-10	16.6	>100	>100	0.691	>100	>100	25.8	>100	>100	7.4	83.2	>100
UO-31	5.9	85.5	>100	3.37	>100	>100	2.25	NT	>100	3.13	12.6	78.0
Prostate cancer												
PC-3	5.86	52.1	>100	0.169	>100	>100	8.91	>100	>100	13.3	76.0	>100
DU-145	NT	NT	NT	0.217	8.40	>100	19.1	>100	>100	11.4	36.9	>100
Breast cancer												
MCF7	3.79	>100	>100	0.0743	32.5	>100	4.44	>100	>100	20.7	>100	>100
MDA-MB-231/ATCC	7.24	>100	>100	1.37	>100	>100	14.5	>100	>100	5.23	53.1	>100
HS 578T	9.07	>100	>100	0.284	>100	>100	3.1	>100	>100	2.71	8.01	>100
BT-549	4.05	90.4	>100	1.17	>100	>100	9.38	>100	>100	NT	>100	>100
T-47D	4.86	>100	>100	0.16	>100	>100	4.5	>100	>100	2.54	>100	>100
MDA-MB-468	1.96	6.47	61.6	0.142	0.445	>100	3.51	55.4	>100	16.4	>100	>100

Data obtained from NCI's in vitro disease-oriented human tumor cell screen

NT not tested

Concerning TGI values, compound **12b** showed high activity against non-small cell lung A549/ATCC and HOP-62 cells, colon HCT-116 and HT29 cells, CNS SF-268, SNB-75 and U251, ovarian OVCAR-3, OVCAR-4, OVCAR-8 and SK-OV-3 cells, renal 786-0, A498, ACHN, RXF 393 and UO-31 cells, prostate DU-145 cells as well as breast HS 578T cell lines with TGI values between 6.76 and 37.9 μM. Concerning LC_{50} values, compound **12b** revealed high activity against non-small cell lung HOP-62 and CNS U251 cell lines (LC_{50} values 38.9 and 47.6 μM, respectively). Compound **12b** exhibited moderate selectivity against renal cancer cell lines having a selectivity ratio of 5.51.

Antibacterial and antifungal activities

Twenty-four of the newly synthesized compounds: **5a–c**, **6a–c**, **9a–c**, **10a**, **b**, **d**, **e**, **g**, **h**, **11a–c**, **12a–c,** and **13a–c**, were evaluated for their in vitro antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis* as Gram-positive bacteria, *Escherichia coli* and *Pseudomonas aeruginosa* as Gram-negative bacteria. They were also evaluated for their in vitro antifungal potential against *Candida albicans.* Their inhibition zones by means of the cup-diffusion technique (Jain and Kar, 1971) were measured and further evaluation was carried out to determine their minimum inhibitory concentration (MIC) by the twofold serial dilution method (Scott, 1989) Ampicillin was used as standard antibacterial, while Clotrimazole was used as antifungal reference. Dimethylsulfoxide (DMSO) was used as blank and showed no antimicrobial activity.

Activity of compounds against Gram-positive bacteria: Compounds **9c** and **11c** (MIC = 12.5 μg/ml) showed about 40 % activity of reference antibiotic Ampicillin (MIC = 5 μg/ml) against *S. aureus*. Concerning activity against *B. subtilis*, compounds **9c**, **11b,** and **12a** (MIC **=** 12.5 μg/ml) were equipotent to Ampicillin (MIC = 12.5 μg/ml).

Activity of compounds against Gram-negative bacteria: Regarding activity against *E. coli*, compound **11a** (MIC = 12.5 μg/ml) showed nearly equal activity to reference Ampicillin (MIC = 10 μg/ml). Comparing activity of tested compounds with Ampicillin (MIC **=** 50 μg/ml) against *P. aeruginosa* revealed promising results. Compounds **10 g**, **11a**, **b**, and **12a** (MIC = 12.5 μg/ml) were four times more

active, whereas compounds **6b** and **10a** (MIC = 25 μg/ml) showed twice activity of reference antibiotic. Furthermore, compounds **5a–c**, **6c**, **9b**, **c**, **10d**, **e**, **11c,** and **12c** (MIC = 50 μg/ml) were equipotent to Ampicillin.

Antifungal activity of compounds: Compound **11c** (MIC = 12.5 μg/ml) showed about 40 % activity against *C. albicans* when compared to reference antifungal Clotrimazole (MIC = 5 μg/ml).

According to the MIC, MBC, or MFC specifications of the latest National Committee on Clinical Laboratory Standards (NCCLS), it could be deduced whether the test compound is bactericidal or bacteriostatic to the test organism (French, 2006). Accordingly, concerning the activity against *P. aeruginosa*, compounds **5b**, **c**, **9c**, **10d**, **e**, **11c**, and **12c** were bactericidal (Table 3).

A close examination of the structures of the active compounds presented in Table 3 revealed that the three hydrazone derivatives **5a–c** were equipotent to Ampicillin against *P. aeruginosa*. Besides, the derivative **5b** (R=Cl) showed moderate antifungal activity. Cyclization of the unsubstituted hydrazone **5a** to the corresponding N-acetyl oxadiazole **6a** (R=H) maintained the same activity against *B. subtilis*, decreased the activity against *E. coli* and *P. aeruginosa*, whereas the potency increased against *S. aureus* and *C. albicans*. The 4-chloro cyclized derivative **6b** showed twice the activity of hydrazone **5b** against *P. aeruginosa* and *E. coli*. On the other hand, the 4-nitro derivative **6c** had the same activity of the hydrazone **5c** against Gram-positive and Gram-negative bacteria.

Concerning, the 3-sulfanyl-1,2,4-triazole derivatives **9a–c**, the 4-chloro derivative **9b** was equipotent to Ampicillin against *P. aeruginosa* besides moderate activity against *E. coli*. The 4-tolyl derivative **9c** was the most potent. It exhibited moderate activity against *C. albicans*, half the activity of reference Ampicillin against *S. aureus* and *E. coli*. It also showed same activity of reference against

Table 3 Minimal inhibitory concentrations (MIC) and minimal germicidal concentrations (MBC or MFC) of tested compounds in μg/ml

Compound no.	Gram-positive bacteria				Gram-negative bacteria				Fungi	
	S. aureus		*B. subtilis*		*E. coli*		*P. aeruginosa*		*C. albicans*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC
5a	100	100	50	100	50	50	50	100	100	100
5b	100	100	50	50	50	100	50	50	25	50
5c	100	100	50	50	50	50	50	50	50	50
6a	25	50	50	50	100	100	100	100	50	50
6b	100	100	50	50	25	50	25	50	50	100
6c	100	100	50	100	50	100	50	100	100	100
9a	50	50	50	50	25	50	100	100	100	100
9b	100	100	100	100	50	50	50	100	100	100
9c	12.5	25	12.5	25	25	50	50	50	25	50
10a	50	50	50	50	50	50	25	50	50	50
10b	100	100	100	100	100	100	100	100	50	50
10d	100	100	100	100	100	100	50	50	50	100
10e	100	100	50	100	50	50	50	50	50	50
10g	50	50	50	50	50	50	12.5	25	50	50
10h	100	100	50	100	100	100	100	100	100	100
11a	25	50	25	50	12.5	25	12.5	25	50	50
11b	50	50	12.5	25	25	50	12.5	25	25	50
11c	12.5	25	25	50	25	50	50	50	12.5	25
12a	25	50	12.5	25	50	50	12.5	25	50	50
12b	50	50	100	100	50	50	100	100	50	50
12c	100	100	100	100	50	50	50	50	100	100
13a	50	50	100	100	50	50	100	100	50	50
13b	25	100	100	100	100	100	100	100	25	50
13c	100	100	100	100	100	200	100	100	50	50
A[a]	5	–	12.5	–	10	–	50	–	–	–
C[b]	–	–	–	–	–	–	–	–	5	–

[a] A: Ampicillin trihydrate (Standard broad spectrum antibiotic)

[b] C: Clotrimazole (Standard broad spectrum antifungal agent)

B. subtilis and *P. aeruginosa*. Furthermore, antimicrobial screening of the 5-substituted sulfanyl 1,2,4-triazoles revealed that most compounds exhibited potent antimicrobial activity against *P. aeruginosa*. Compound **10a** (R=H, R^1=CH_3) exhibited twice the activity of reference, while compound **10g** (R, R^1=CH_3) showed four times that of Ampicillin against *P. aeruginosa*. In addition, compounds **10d**, **e** (R=Cl, R^1=CH_3, C_2H_5) were equipotent to Ampicillin against *P. aeruginosa*. Moreover, compound **11a** (R=H) showed nearly similar activity of reference against *E. coli* and four times the activity of reference against *P. aeruginosa*. Compound **11b** (R=Cl) showed similar activity of Ampicillin against *B. subtilis*, about half its activity against *E. coli*, four times the activity against *P. aeruginosa*. Compound **11c** (R=CH_3) was equipotent to Ampicillin against *P. aeruginosa*. Regarding the oxadiazoles **12a–c** tested for antimicrobial activity, the unsubstituted derivative **12a** was equipotent to Ampicillin against *B. subtilis*. Moreover, it was found to be four times more potent than Ampicillin against *P. aeruginosa*. The derivative **12c** (R=CH_3) had similar activity of reference against *P. aeruginosa*. Results showed that among thiadiazoles **13a–c** tested, only the 4-chloro derivative **13b** showed moderate activity against *S. aureus* and *C. albicans*.

Conclusion

Nine of the newly prepared quinol dimethyl ether derivatives **5a**, **b**, **9b**, **10a**, **d**, **12a**, **b**, and **13a**, **b** were selected by the NCI and were tested initially at a single high dose (10 μM) in the full NCI 60-cell panel. Four of the screened compounds which are the unsubstituted phenylhydrazone **5a**, 4-chlorophenylhydrazone **5b**, 4-chlorophenyl-3-sulfanyl-1,2,4-triazole **9b** as well as 4-chloroanilino-1,3,4-oxadiazole **12b** satisfied the threshold inhibition criteria and passed forward for evaluation in the full panel five-dose in vitro antitumor screen. 4-Chlorophenylhydrazone **5b** showed very promising results and accordingly was chosen for in vivo antitumor screening. In addition, 24 of the newly synthesized compounds **5a–c**, **6a–c**, **9a–c**, **10a**, **b**, **d**, **e**, **g**, **h**, **11a–c**, **12a–c,** and **13a–c** were screened for their in vitro antimicrobial activity against five microorganisms, namely *S. aureus* and *B. subtilis*, as representative examples of Gram-positive bacteria, *E. coli* and *P. aeruginosa* as examples of Gram-negative bacteria. They were also evaluated for their in vitro antifungal activity against *C. albicans*. Some of the tested compounds exhibited special high activity comparable to the reference Ampicillin against *P. aeruginosa* and *E. coli*. Some of screened compounds showed potent activity against *B. subtilis,* while others showed a moderate activity against *S. aureus*. On the other hand, most of the tested compounds were found to be inactive against *C. albicans* when compared to the reference Clotrimazole.

Experimental

Chemistry

Melting points were determined in open glass capillaries using a Griffin melting point apparatus and are all uncorrected. Infrared spectra (IR) were recorded, using KBr disks, using a Perkin-Elmer 1430 Infrared spectrophotometer. Nuclear magnetic resonance (^{1}H NMR) were determined using a Jeol NMR 500 MHZ spectrophotometer, in the Faculty of Science, Alexandria University and are reported as δ values (ppm) relative to tetramethylsilane (TMS) as an internal reference. The type of signal was indicated by one of the following letters: s = singlet, d = doublet, t = triplet, q = quartet, and m = multiplet. Mass spectra were run on a Finnigan mass spectrometer model SSQ/7000 (70 eV) or on a gas chromatograph/mass spectrometer Shimadzu GCMS-Qp2010 Plus (70 eV), in the Faculty of Science, Cairo University. Elemental microanalyses were performed at the microanalytical unit, Faculty of Pharmacy, Alexandria University, at the microanalytical unit, Faculty of Science, Cairo University or at Inspectorate International Ltd., Jubail technical centre, Jubail laboratory, Saudi Arabia. Reaction progress was monitored by thin-layer chromatography (TLC) on silica gel (60 GF254, Merck) using glass plates and the spots were visualized by exposure to iodine vapor or to UV-lamp at λ 254 nm for few seconds.

2,5-Dimethoxycinnamic acid (**1**)

A mixture of malonic acid (12.49 g, 120 mmol) and 2,5-dimethoxybenzaldehyde (9.97 g, 60 mmol) was dissolved in pyridine (24 ml) at 50 °C. Piperidine (0.9 ml) was then added and the mixture was heated at 80–85 °C for 2 h followed by refluxing for additional 3 h. The solution was cooled, then poured into water, and acidified slowly using concentrated hydrochloric acid (3 ml) to pH 5. The formed yellow precipitate was filtered, washed with cold water, air dried, and finally crystallized from ethanol giving 2,5-dimethoxycinnamic acid **1** as yellow crystals, m.p. 148–150 °C; yield 11.75 g (94 %).

Potassium 3-(2,5-dimethoxyphenyl)propenoate (**2**)

A solution of 1 M potassium hydroxide (53 ml) was added to 3-(2,5-dimethoxyphenyl)propenoic acid **1** (11.04 g, 53 mmol) while stirring over a period of 30 min. The resulting solution was evaporated to dryness to yield

potassium 3-(2,5-dimethoxyphenyl)propenoate **2** which was used for the following step.

3-(2,5-Dimethoxyphenyl)propenoic acid hydrazide (**4**) (Ichiro *et al*. 2006)

A suspension of potassium 3-(2,5-dimethoxyphenyl)propenoate **2** (4.93 g, 20 mmol), pyridine (0.04 ml), ethyl chloroformate (1.91 ml, 20 mmol) in chloroform (18 ml) was added rapidly to a well-stirred, ice-cooled suspension of 80 % hydrazine hydrate (1.87 ml, 60 mmol) in chloroform (18 ml). After standing at 0 °C for an overnight, the reaction mixture was clarified by filtration. The clear filtrate was successively washed with saturated sodium bicarbonate solution (40 ml) and water (40 ml). The chloroformic solution was dried over anhydrous sodium sulfate then evaporated till dryness; the crude residue of the acid hydrazide was crystallized as yellow crystals. Physicochemical data are listed in Table 4 and ^{1}H NMR spectral data are listed in Table 5. IR (KBr, υ/cm): 3279, 3204 (NH_2, υNH), 1660 (C=O), 1550 (δNH), 1220, 1045 (C–O–C). MS, *m/z* (relative abundance %): 223 (19.4) ($M^{\cdot+}$+1), 222 (12.9) ($M^{\cdot+}$), 161 (38.7), 135 (16.1), 111 (19.4), 110 (19.4), 101 (12.9), 97 (22.6), 93 (29), 88 (16.1), 87 (22.6), 85 (22.6), 84 (25.8), 83 (19.4), 76 (38.7), 74 (29), 73 (25.8), 70 (19.4), 69 (45.2), 67 (16.1), 61 (12.9), 57 (100), 56 (35.5), 55 (32.3).

N^1-3-(2,5-Dimethoxyphenyl)propenoyl-N^2-4-substituted benzylidene hydrazines (**5a–c**)

A solution of the selected substituted benzaldehyde (5 mmol) in absolute ethanol (5 ml) was gradually added with stirring to a warm solution of the acid hydrazide **4** (1.11 g, 5 mmol) in absolute ethanol (10 ml). The reaction mixture was stirred at 40 °C for 2 h and then set aside for an overnight in refrigerator for complete precipitation. The formed yellow precipitates were filtered, washed with cold ethanol, air dried, and crystallized. Physicochemical data are listed in Table 4 and ^{1}H NMR spectral data are listed in Table 5. IR (KBr, υ/cm): 3222–3100 (υNH), 1703–1651 (C=O), 1622–1621 (C=N), 1577–1541 (δNH), 1237–1218, 1048–1044 (C–O–C). MS, *m/z* (relative abundance %) of compound **5a:** 310 (8) ($M^{\cdot+}$), 192 (10.3), 191 (100), 176 (64.4), 133 (13.4), 105 (19), 104 (11.4), 103 (16.2), 92 (15.9), 91 (13.8), 89 (20.5), 78 (11.4), 77 (34.9), 65 (27.6), 64 (10.6), 63 (23.1), 55 (10.3), 51 (32.5), 50.3 (17). MS, *m/z* (relative abundance %) of compound **5b:** 346 (2.8) ($M^{\cdot+}$+2), 344 (4.3) ($M^{\cdot+}$), 192 (18.8), 191 (100), 176 (52.8), 161 (15.7), 148 (21), 133 (26.2), 119 (11.1), 118 (14.2), 105 (25), 103 (14.5), 99 (11.4), 92 (14.2), 91 (19.8), 90 (26.2), 89 (62.7), 79 (14.5), 78 (12.7), 77 (48.5), 76 (13.9), 75 (20.4), 74 (10.2), 65 (11.7), 64 (10.2), 63 (46.9), 62 (20.7), 54 (11.7), 53 (11.4), 51 (39.8), 50 (25). MS, *m/z* (relative abundance %) of compound **5c:** 355 (8.7) ($M^{\cdot+}$), 192 (11.1), 191 (100), 176 (39.3), 148 (13.9), 133 (12.8), 105 (12.7), 91 (14.5), 89 (13.3), 77 (18.4), 63 (17.2), 51 (18.6).

1-[2-Aryl-5-(2,5-dimethoxystyryl)-2,3-dihydro-1,3,4-oxadiazol-3-yl]ethanones (**6a–c**)

A mixture of the appropriate hydrazone **5a–c** (1 mmol) in acetic anhydride (5 ml) was heated under reflux for 2 h. The reaction mixture was then concentrated to half its volume, set aside to attain room temperature then gradually poured onto stirred crushed ice to decompose the excess unreacted acetic anhydride and left in refrigerator overnight. The separated yellow products were filtered, washed with water, air dried, and crystallized. Physicochemical data are listed in Table 4 and ^{1}H NMR spectral data are listed in Table 5. IR (KBr, υ/cm): 1666–1656 (C=O), 1607–1580 (C=N), 1219–1218, 1041–1038 (C–O–C). MS, *m/z* (relative abundance %) of compound **6a:** 353 (5.2) ($M^{\cdot+}$+1), 352 (28.1) ($M^{\cdot+}$), 279 (35.8), 191 (100), 176 (40.5), 175 (19.3), 174 (10.5), 161 (14.6), 148 (11.3), 133 (24), 105 (22.9), 103 (10.2), 91 (13.8), 90 (15.4), 89 (23.4), 77 (29.8), 76 (11), 63 (19.3), 51 (24). MS, *m/z* (relative abundance %) of compound **6c:** 397 (9.8) ($M^{\cdot+}$), 324 (32.7), 192 (15.2), 191 (100), 176 (50.4), 175 (14.5), 161 (10), 148 (14.6), 133 (15.3), 105 (14.7), 89 (14.3), 77 (20.5), 63 (17), 51 (13.1).

4-Aryl-1-[3-(2,5-dimethoxyphenyl)propenoyl] thiosemicarbazides (**7a–c**)

A solution of the selected aryl isothiocyanate (5 mmol) in absolute ethanol (5 ml) was gradually added to a well-stirred solution of an equimolar amount of the substituted acid hydrazide **4** (1.11 g, 5 mmol) in absolute ethanol (10 ml). The reaction mixture was heated under reflux for 2 h, cooled, water was added, and kept aside overnight for complete precipitation. The formed yellow precipitates were filtered, washed with water, air dried, and crystallized. Physicochemical data are listed in Table 4 and ^{1}H NMR spectral data are listed in Table 5. IR (KBr, υ/cm): 3209–3160 (υNH), 1664–1630 (C=O), 1574–1547 (δNH), 1574–1547, 1289–1285, 1184–1173, 987–970 (N–C=S thioamide I, II, III, IV bands), 1224–1220, 1050–1044 (C–O–C). MS, *m/z* (relative abundance %) of compound **7a**: 355 (11.9) ($M^{\cdot+}$−2), 191 (90.5), 177 (14.3), 176 (21.4), 135 (47.6), 120 (14.3), 94 (11.9), 93 (21.4), 91 (19), 77 (100), 76 (11.9), 75 (19), 74 (19), 65 (14.3), 64 (28.6), 63 (26.2), 62 (26.2), 61 (21.4), 56 (11.9), 55 (21.4), 54 (14.3), 52 (14.3), 51 (90.5), 50 (50).

Table 4 Physicochemical data of compounds **4–13**

Compound no.	R	R^1	Yield (%)	M.P. °C Cryst. sol.	Analyses %		
					El.	Calcd.	Found
4	–	–	58	144–146 benzene	C	59.45	59.38
					H	6.35	6.53
					N	12.60	12.55
5a	H	–	71	154–156 methanol	C	69.66	69.50
					H	5.85	5.66
					N	9.03	8.96
5b	Cl	–	77	188–190 ethanol	C	62.70	62.88
					H	4.97	4.96
					N	8.12	7.88
5c	NO_2	–	78	224–226 ethanol	C	60.84	61.28
					H	4.82	4.22
					N	11.83	11.79
6a	H	–	89	132–134 ethanol/water (9:1)	C	68.17	67.96
					H	5.72	6.06
					N	7.95	7.91
6b	Cl	–	86	166–172 ethanol/water (9:1)	C	62.10	61.96
					H	4.95	4.84
					N	7.24	7.25
6c	NO_2	–	89	82–84 ethanol/water (9:1)	C	60.45	60.70
					H	4.82	4.71
					N	10.57	10.46
7a	H	–	70	164–166 ethanol/water (9:1)	C	60.49	60.71
					H	5.36	5.04
					N	11.76	11.47
7b	Cl	–	92	172–174 ethanol	C	55.17	55.96
					H	4.63	4.52
					N	10.72	10.63
7c	CH_3	–	66	242–244 methanol	C	61.44	61.56
					H	5.70	6.00
					N	11.31	11.64
8	–	–	78	244–246 ethanol	C	64.07	63.90
					H	5.87	5.53
					N	6.79	7.01
9a	H	–	93	244–246 acetonitrile	C	63.70	63.68
					H	5.05	4.66
					N	12.38	12.19
9b	Cl	–	88	232–234 methanol	C	57.83	58.20
					H	4.31	4.60
					N	11.24	11.29
9c	CH_3	–	66	236–238 methanol	C	64.57	64.80
					H	5.42	5.20
					N	11.89	11.65
10a	H	CH_3	68	144–146 ethanol/water (9:1)	C	64.57	64.68
					H	5.42	5.54
					N	11.89	12.24
10b	H	CH_2CH_3	77	102–104 *n*-hexane	C	65.37	65.72
					H	5.76	5.55
					N	11.44	11.55

Table 4 continued

Compound no.	R	R^1	Yield (%)	M.P. °C Cryst. sol.	Analyses %		
					El.	Calcd.	Found
10c	H	$CH_2C_6H_5$	66	194–196 methylene chloride/pet. ether (1:5)	C	69.91	69.72
					H	5.40	5.19
					N	9.78	9.56
10d	Cl	CH_3	79	158–160 methanol	C	58.83	58.51
					H	4.68	4.68
					N	10.83	10.80
10e	Cl	CH_2CH_3	50	146–148 benzene	C	59.77	60.59
					H	5.02	5.66
					N	10.46	11.00
10f	Cl	$CH_2C_6H_5$	82	224–226 methylene chloride/pet. ether (1:5)	C	64.72	64.07
					H	4.78	5.21
					N	9.06	8.40
10g	CH_3	CH_3	91	164–166 methanol	C	65.37	65.71
					H	5.76	6.00
					N	11.44	11.72
					C	66.12	66.25
10h	CH_3	CH_2CH_3	70	109–111 methanol	H	6.08	6.38
					N	11.01	10.95
10i	CH_3	$CH_2C_6H_5$	80	166–168 methylene chloride/pet. ether (1:5)	C	70.40	70.24
					H	5.68	5.54
					N	9.47	9.70
11a	H	–	80	254–256[a] dioxane	C	63.79	64.00
					H	4.74	4.34
					N	8.58	8.66
11b	Cl	–	69	240–242[a] dioxane	C	59.60	59.44
					H	4.23	4.01
					N	8.02	8.01
11c	CH_3	–	60	270–272[a] dioxane	C	64.40	64.72
					H	5.00	4.88
					N	8.34	8.60
12a	H	–	71	178–180 methanol	C	66.86	66.62
					H	5.30	5.61
					N	13.00	12.85
12b	Cl	–	63	216–218 ethanol	C	60.42	60.88
					H	4.51	4.52
					N	11.74	11.83
12c	CH_3	–	91	186–188 ethanol	C	67.64	67.50
					H	5.68	5.54
					N	12.46	12.73
13a	H	–	83[b] (60)[c]	232–234 ethanol	C	63.70	64.00
					H	5.05	5.07
					N	12.38	12.43
13b	Cl	–	62[b] (58)[c]	236–238 ethanol	C	57.83	57.62
					H	4.31	4.12
					N	11.24	11.06
13c	CH_3	–	59[b] (55)[c]	210–212 ethanol	C	64.57	64.81
					H	5.42	5.09
					N	11.89	11.99

[a] Melting with decomposition

[b] Method A

[c] Method B

Table 5 ^{1}H NMR spectral data for selected compounds

Compound no.	^{1}H NMR
4*	NH_2 (under DMSO); 3.70, 3.75 (two s, each 3H, $2OCH_3$); 6.52 (d, 1H, $J = 16.05$ Hz, CH–CO); 6.90–7.00 (m, 2H, Ar–$C_{3,4}$–H); 7.21 (d, 1H, $J = 3.05$ Hz, Ar–C_6–H); 7.77 (d, 1H, $J = 16.05$ Hz, CH = CH–CO); 12.33 (s, 1H, NH, D_2O-exchangeable)
5a*	3.71, 3.74, 3.80, 3.81 (four s, 6H, OCH_3 *cis* and *trans* conformers), 6.75, 7.60 (two d, 1H, $J = 16.05$ Hz, = CH–CO *cis* and *trans* conformers), 6.90–7.02 (m, 2H, dimethoxyphenyl–$C_{3,4}$–H); 7.11 (d, 1H, $J = 2.3$ Hz, dimethoxyphenyl–C_6–H); 7.35–7.44 (m, 3H, phenyl–$C_{3,4,5}$–H); 7.70 (d, 2H, $J = 6.9$ Hz, phenyl–$C_{2,6}$–H), 7.79, 7.87 (two d, 1H, $J = 16.05$ Hz, CH=CH–CO *cis* and *trans* conformers); 8.03, 8.21 (two s, 1H, CH=N *cis* and *trans* conformers) 11.50, 11.68 (two s, 1H, NH, D_2O-exchangeable *cis* and *trans* conformers)
5b*	3.80, 3.83, 3.85, 3.87 (four s, 6H, OCH_3 *cis* and *trans* conformers), 6.94–7.04 (m, 2H, dimethoxyphenyl–$C_{3,4}$–H); 7.20 (d, 1H, $J = 3.8$ Hz, dimethoxyphenyl–C_6–H); 7.39 (d, 2H, $J = 8.4$ Hz, *p*-chlorophenyl–$C_{2,6}$–H); 7.65 (d, 2H, $J = 8.4$ Hz, *p*-chlorophenyl–$C_{3,5}$–H), 7.52, 7.57 (two d, 1H, $J = 8.4$, 16.05 Hz,=CH–CO *cis* and *trans* conformers), 7.83, 8.18 (two d, 1H, $J = 16.05$ Hz, CH=CH–CO *cis* and *trans* conformers); 7.95, 7.98 (two s, 1H, CH=N *cis* and *trans* conformers) 9.98, 10.25 (two s, 1H, NH, D_2O-exchangeable *cis* and *trans* conformers)
5c*	3.72, 3.75, 3.81, 3.82 (four s, 6H, OCH_3 *cis* and *trans* conformers), 6.95–7.03 (m, 2H, dimethoxyphenyl–$C_{3,4}$–H); 7.12 (d, 1H, $J = 3.1$ Hz, dimethoxyphenyl–C_6–H); 7.96 (d, 2H, $J = 8.4$ Hz, *p*-nitrophenyl–$C_{2,6}$–H); 8.26 (d, 2H, $J = 8.4$ Hz, *p*-nitrophenyl–$C_{3,5}$–H), 6.76, 7.60 (two d, 1H, $J = 8.4$, 16.05 Hz, =CH–CO *cis* and *trans* conformers), 7.80, 7.88 (two d, 1H, $J = 16.05$ Hz, CH=CH–CO *cis* and *trans* conformers); 8.12, 8.30 (two s, 1H, CH=N *cis* and *trans* conformers), 11.78, 11.95 (two s, 1H, NH, D_2O-exchangeable *cis* and *trans* conformers)
6b*	2.17 (s, 3H, $COCH_3$); 3.71, 3.74 (two s, each 3H, OCH_3); 6.91 (dd, 1H, $J = 9.2$, 3.05 Hz, dimethoxyphenyl–C_4–H); 6.96 (d, 1H, $J = 9.2$ Hz, dimethoxyphenyl–C_3–H); 7.00 (d, 1H, $J = 16.05$ Hz, phenyl–CH=CH); 7.07 (s, 1H, oxadiazoline–C_5–H); 7.30 (d, 1H, $J = 3.05$ Hz, dimethoxyphenyl–C_6–H); 7.42 (d, 1H, $J = 16.05$ Hz, CH=CH); 7.44 (d, 2H, $J = 8.4$ Hz, *p*-chlorophenyl–$C_{2,6}$–H); 7.49 (d, 2H, $J = 8.4$ Hz, *p*-chlorophenyl–$C_{3,5}$–H)
6c**	2.30 (s, 3H, $COCH_3$); 3.79, 3.84 (two s, each 3H, OCH_3); 6.75 (d, 1H, $J = 16.05$ Hz, phenyl–CH=CH); 6.85 (d, 1H, $J = 9.2$ Hz, dimethoxyphenyl–C_3–H); 6.90 (dd, 1H, $J = 9.2$, 3.05 Hz, dimethoxyphenyl–C_4–H); 7.02 (d, 1H, $J = 3.05$ Hz, dimethoxyphenyl–C_6–H); 7.06 (s, 1H, oxadiazoline–C_5–H); 7.59 (d, 1H, $J = 16.05$ Hz, CH=CH); 7.67 (d, 2H, $J = 8.4$ Hz, *p*-nitrophenyl–$C_{2,6}$–H); 8.26 (d, 2H, $J = 8.4$ Hz, *p*-nitrophenyl–$C_{3,5}$–H)
7a*	3.71, 3.79 (two s, each 3H, OCH_3); 6.68 (d, 1H, $J = 14.5$ Hz, =CH–CO); 6.95 (dd, 1H, $J = 9.15$, 3.05 Hz, dimethoxyphenyl–C_4–H); 7.00 (d, 1H, $J = 9.15$ Hz, dimethoxyphenyl–C_3–H); 7.06 (d, 1H, $J = 3.05$ Hz, dimethoxyphenyl–C_6–H); 7.11 (t, 1H, $J = 7.95$ Hz, phenyl–C_4–H); 7.29 (t, 2H, $J = 7.95$ Hz, phenyl–$C_{3,5}$–H); 7.38–7.48 (m, 2H, phenyl–$C_{2,6}$–H); 7.68 (d, 1H, $J = 15.85$ Hz, CH=CH–CO); 9.70, 10.12 (two s, 3H, NH, D_2O-exchangeable)
7b*	3.70, 3.78 (two s, each 3H, OCH_3); 6.68 (d, 1H, $J = 14.5$ Hz, =CH–CO); 6.90–7.10 (m, 3H, dimethoxyphenyl–$C_{3,4,6}$–H); 7.34 (d, 2H, $J = 8.4$ Hz, *p*-chlorophenyl–$C_{2,6}$–H); 7.46 (d, 2H, $J = 8.4$ Hz, *p*-chlorophenyl–$C_{3,5}$–H); 7.69 (d, 1H, $J = 16.05$ Hz, CH=CH–CO); 9.77, 10.12 (two s, 3H, NH, D_2O-exchangeable)
8*	3.71, 3.79 (two s, 12H, $4OCH_3$); 6.80 (d, 2H, $J = 16.05$ Hz, CH–CO); 6.94 (dd, 2H, $J = 9.15$, 3.05 Hz, Ar–C_4–H); 7.00 (d, 2H, $J = 9.15$ Hz, Ar–C_3–H); 7.08 (d, 2H, $J = 3.05$ Hz, Ar–C_6–H); 7.68 (d, 2H, $J = 16.05$ Hz, CH=CH–CO); 10.43 (s, 2H, 2NH, D_2O-exchangeable)
9a*	3.60, 3.63 (two s, each 3H, OCH_3); 6.59 (d, 1H, $J = 16.8$ Hz, phenyl–CH=CH); 6.85 (dd, 1H, $J = 9.2$, 3.05 Hz, dimethoxyphenyl–C_4–H); 6.88 (d, 1H, $J = 9.2$ Hz, dimethoxyphenyl–C_3–H); 6.97 (d, 1H, $J = 3.05$ Hz, dimethoxyphenyl–C_6–H); 7.36 (d, 1H, $J = 16.8$ Hz, CH=CH); 7.41 (d, 2H, $J = 6.9$ Hz, phenyl–$C_{2,6}$–H); 7.51–7.61 (m, 3H, phenyl–$C_{3,4,5}$–H); 14.01 (s, 1H, SH, D_2O-exchangeable)
9c*	2.38 (s, 3H, C_6H_4–CH_3); 3.62, 3.64 (two s, each 3H, OCH_3); 6.57 (d, 1H, $J = 16.8$ Hz, phenyl–CH=CH); 6.86 (dd, 1H, $J = 9.15$, 3.05 Hz, dimethoxyphenyl–C_4–H); 6.89 (d, 1H, $J = 9.15$ Hz, dimethoxyphenyl–C_3–H); 6.98 (d, 1H, $J = 3.05$ Hz, dimethoxyphenyl–C_6–H); 7.27 (d, 2H, $J = 8.4$ Hz, *p*-tolyl–$C_{3,5}$–H); 7.36 (d, 1H, $J = 16.8$ Hz, CH=CH); 7.37 (d, 2H, $J = 8.4$ Hz, *p*-tolyl–$C_{2,6}$–H); 13.96 (s, 1H, SH, D_2O-exchangeable)
10a*	2.56 (s, 3H, S–CH_3); 3.62, 3.64 (two s, each 3H, OCH_3); 6.71 (d, 1H, $J = 16.05$ Hz, phenyl–CH=CH); 6.82 (dd, 1H, $J = 9.2$, 3.05 Hz, dimethoxyphenyl–C_4–H); 6.88 (d, 1H, $J = 9.2$ Hz, dimethoxyphenyl–C_3–H); 6.99 (d, 1H, $J = 3.05$ Hz, dimethoxyphenyl–C_6–H); 7.42–7.47 (m, 2H, phenyl–$C_{2,6}$–H); 7.48 (d, 1H, $J = 16.05$ Hz, CH=CH); 7.57–7.63 (m, 3H, phenyl–$C_{3,4,5}$–H)
10b*	1.26 (t, 3H, $J = 7.2$ Hz, SCH_2CH_3); 3.07 (q, 2H, $J = 7.2$ Hz, SCH_2CH_3); 3.63, 3.64 (two s, each 3H, OCH_3); 6.72 (d, 1H, $J = 16.05$ Hz, phenyl–CH=CH); 6.84 (dd, 1H, $J = 9.2$, 3.05 Hz, dimethoxyphenyl–C_4–H); 6.90 (d, 1H, $J = 9.2$ Hz, dimethoxyphenyl–C_3–H); 6.99 (d, 1H, $J = 3.05$ Hz, dimethoxyphenyl–C_6–H); 7.41–7.45 (m, 2H, phenyl–$C_{2,6}$–H); 7.49 (d, 1H, $J = 16.05$ Hz, CH=CH); 7.58–7.63 (m, 3H, phenyl–$C_{3,4,5}$–H)
10e*	1.25 (t, 3H, $J = 7.2$ Hz, SCH_2CH_3); 3.06 (q, 2H, $J = 7.2$ Hz, SCH_2CH_3); 3.66 (s, 6H, OCH_3); 6.72 (d, 1H, $J = 16.05$ Hz, phenyl–CH=CH); 6.85 (dd, 1H, $J = 9.2$, 3.05 Hz, dimethoxyphenyl–C_4–H); 6.89 (d, 1H, $J = 9.2$ Hz, dimethoxyphenyl–C_3–H); 7.06 (d, 1H, $J = 3.05$ Hz, dimethoxyphenyl–C_6–H); 7.49 (d, 1H, $J = 16.05$ Hz, CH=CH); 7.50 (d, 2H, $J = 8.4$ Hz, *p*-chlorophenyl–$C_{2,6}$–H); 7.68 (d, 2H, $J = 8.4$ Hz, *p*-chlorophenyl–$C_{3,5}$–H)

Table 5 continued

Compound no.	^{1}H NMR
11b*	3.66 (s, 6H, OCH_3); 4.80 (s, 2H, S–CH_2); 6.73 (d, 1H, $J = 16.05$ Hz, phenyl–CH=CH); 6.81 (d, 1H, $J = 8.4$ Hz, dihydroxyphenyl–C_3–H); 6.85 (dd, 1H, $J = 8.4$, 3.05 Hz, dimethoxyphenyl–C_4–H); 6.91 (d, 1H, $J = 8.4$ Hz, dimethoxyphenyl–C_3–H); 6.92–6.96 (m, 1H, dihydroxyphenyl–C_4–H); 7.07 (d, 1H, $J = 3.05$ Hz, dimethoxyphenyl–C_6–H); 7.11 (d, 1H, $J = 3.05$ Hz, dihydroxyphenyl–C_6–H); 7.53 (d, 2H, $J = 8.4$ Hz, *p*-chlorophenyl–$C_{2,6}$–H); 7.69 (d, 2H, $J = 8.4$ Hz, *p*-chlorophenyl–$C_{3,5}$–H); 7.48 (d, 1H, $J = 16.05$ Hz, CH=CH); 9.18, 10.52 (two s, each 1H, 2OH, D_2O-exchangeable)
11c*	2.40 (s, 3H, C_6H_4–CH_3); 3.64, 3.65 (two s, each 3H, OCH_3); 4.79 (s, 2H, S–CH_2); 6.69 (d, 1H, $J = 16.05$ Hz, phenyl–CH=CH); 6.71 (d, 1H, $J = 16.05$ Hz, phenyl–CH=CH); 6.81 (d, 1H, $J = 9.15$ Hz, dihydroxyphenyl–C_3–H); 6.85 (dd, 1H, $J = 9.15$, 3.05 Hz, dimethoxyphenyl–C_4–H); 6.90 (d, 1H, $J = 9.15$ Hz, dimethoxyphenyl–C_3–H); 6.94 (dd, 1H, $J = 9.15$, 3.05 Hz, dihydroxyphenyl–C_4–H); 7.00 (d, 1H, $J = 3.05$ Hz, dimethoxyphenyl–C_6–H); 7.12 (d, 1H, $J = 3.05$ Hz, dihydroxyphenyl–C_6–H); 7.33 (d, 2H, $J = 8.4$ Hz, *p*-tolyl–$C_{3,5}$–H); 7.41 (d, 2H, $J = 8.4$ Hz, *p*-tolyl–$C_{2,6}$–H). 7.48 (d, 1H, $J = 16.05$ Hz, CH=CH); 9.17, 10.54 (two s, each 1H, 2OH, D_2O-exchangeable)
12a*	3.80, 3.85 (two s, each 3H, OCH_3); 6.85 (d, 1H, $J = 9.15$ Hz, dimethoxyphenyl–C_3–H); 6.88 (dd, 1H, $J = 9.15$, 3.05 Hz, dimethoxyphenyl–C_4–H); 7.03 (d, 1H, $J = 3.05$ Hz, dimethoxyphenyl–C_6–H); 7.08 (d, 1H, $J = 16.05$ Hz, phenyl–CH=CH); 7.11 (t, 1H, $J = 7.65$ Hz, phenyl–C_4–H); 7.39 (t, 2H, $J = 7.65$ Hz, phenyl–$C_{3,5}$–H); 7.56 (d, 2H, $J = 7.65$ Hz, phenyl–$C_{2,6}$–H); 7.60 (d, 1H, $J = 16.05$ Hz, CH=CH); 9.73 (s, 1H, NH, D_2O-exchangeable)
12c**	2.22 (s, 3H, C_6H_4–CH_3); 3.73, 3.78 (two s, each 3H, OCH_3); 6.91 (dd, 1H, $J = 9.15$, 3.05 Hz, dimethoxyphenyl–C_4–H); 6.98 (d, 1H, $J = 9.15$ Hz, dimethoxyphenyl–C_3–H); 7.12 (d, 2H, $J = 7.65$ Hz, *p*-tolyl–$C_{2,6}$–H); 7.21 (d, 1H, $J = 16.05$ Hz, phenyl–CH=CH); 7.34 (d, 1H, $J = 3.05$ Hz, dimethoxyphenyl–C_6–H); 7.46 (d, 2H, $J = 7.65$ Hz, *p*-tolyl–$C_{3,5}$–H); 7.51 (d, 1H, $J = 16.05$ Hz, CH=CH); 10.53 (s, 1H, NH, D_2O-exchangeable)
13a*	3.72, 3.79 (two s, each 3H, OCH_3); 6.81–7.00 (m, 3H, dimethoxyphenyl–$C_{3,4,6}$–H); 7.28 (d, 1H, $J = 16.05$ Hz, phenyl–CH=CH), 7.33 (t, 3H, $J = 7.65$ Hz, phenyl–$C_{3,4,5}$–H); 7.49 (d, 1H, $J = 16.05$ Hz CH=CH); 7.60 (d, 2H, $J = 7.65$ Hz, phenyl–$C_{2,6}$–H); 10.50 (s, 1H, NH, D_2O-exchangeable)
13b*	3.72, 3.79 (two s, each 3H, OCH_3); 6.88 (dd, 1H, $J = 9.2$, 3.05 Hz, dimethoxyphenyl–C_4–H); 6.97 (d, 1H, $J = 9.2$ Hz, dimethoxyphenyl–C_3–H); 7.27 (d, 1H, $J = 3.05$ Hz, dimethoxyphenyl–C_6–H); 7.29 (d, 1H, $J = 16.05$ Hz, phenyl–CH=CH), 7.38 (d, 2H, $J = 9.15$ Hz, *p*-chlorophenyl–$C_{2,6}$–H); 7.50 (d, 1H, $J = 16.05$ Hz CH=CH); 7.65 (d, 2H, $J = 9.15$ Hz, *p*-chlorophenyl–$C_{3,5}$–H), 10.64 (s, 1H, NH, D_2O-exchangeable)

* DMSO-d6 δppm

** CDCl3 δppm

N^1,N^2-Bis(2,5-dimethoxyphenylpropenoyl)hydrazine (**8**)

This unexpected compound **8** was prepared by stirring a solution of the acid hydrazide **4** (1.11 g, 5 mmol) in absolute ethanol (15 ml) at room temperature for 2 h. The formed yellow precipitate was filtered, washed with cold ethanol, air dried, and crystallized. Physicochemical data are listed in Table 4 and ^{1}H NMR spectral data are listed in Table 5. IR (KBr, υ/cm): 3165 (υNH), 1630 (C=O), 1576 (δNH), 1221, 1039 (C–O–C). MS, *m/z* (relative abundance %): 413 (28.1) ($M^{\cdot+}$+1), 412 (12.5) ($M^{\cdot+}$), 382 (12.5), 381 (25), 222 (37.5), 221 (15.6), 201 (25), 194 (18.8), 192 (21.9), 190 (100), 161 (43.8), 144 (15.6), 137 (12.5), 135 (56.3), 127 (28.1), 121 (62.5), 115 (37.5), 114 (15.6), 110 (15.6), 109 (43.8), 77 (12.5), 76 (12.5), 70 (31.3), 68 (15.6), 59 (37.5), 58 (12.5), 54 (25), 50 (37.5).

4-Aryl-5-(2,5-dimethoxystyryl)-3-sulfanyl-4H-1,2,4-triazoles (**9a–c**)

A solution of the appropriate thiosemicarbazide derivative **7a–c** (1 mmol) in 1 N aqueous sodium hydroxide solution (20 ml) was heated under reflux for 4 h. The reaction mixture was filtered while hot, cooled to attain room temperature, acidified with dilute hydrochloric acid to pH 5, and kept in the refrigerator overnight. The formed precipitates were filtered, washed with water, air dried, and crystallized as yellow crystals. Physicochemical data are listed in Table 4 and ^{1}H NMR spectral data are listed in Table 5. IR (KBr, υ/cm): 2928–2901 (SH), 1631–1630 (C=N), 1218–1213, 1050–1046 (C–O–C). MS, *m/z* (relative abundance %) of compound **9a:** 340 (8) ($M^{\cdot+}$+1), 339 (25.8) ($M^{\cdot+}$), 309 (24.5), 308 (77.3), 162 (11), 118 (12.9), 105 (11.7), 103 (22.7), 96 (10.4), 92 (12.9), 91 (21.5), 90 (12.9), 89 (31.3), 78 (19), 77 (100), 76 (25.2), 75 (26.4), 74 (11.7), 65 (31.9), 64 (19.6), 63 (28.2), 62 (17.8), 60 (27.6), 54 (17.2), 53 (12.3), 52 (12.3), 51 (90.2), 50 (44.2).

4-Aryl-3-(2,5-dimethoxystyryl)-5-substituted sulfanyl-4H-1,2,4-triazoles (**10a–i**)

The appropriate sulfanyltriazole **9a–c** (1 mmol) was dissolved in an ethanolic solution of sodium ethoxide prepared by dissolving sodium metal (23 mg, 0.001 g atom) in

absolute ethanol (15 ml). The appropriate alkyl halide (2 mmol) or aralkyl halide (1 mmol) was then gradually added to the solution. The reaction mixture was heated under reflux for 2 h, concentrated, cooled, diluted with water, and allowed to stand for an overnight. The obtained precipitates were filtered, washed with water, air dried, and crystallized. Physicochemical data are listed in Table 4 and ^{1}H NMR spectral data are listed in Table 5. IR (KBr, υ/cm): 1636–1625 (C=N), 1289–1265, 1178–1159 (C–S–C), 1225–1208, 1050–1017 (C–O–C). MS, *m/z* (relative abundance %) of compound **10i:** 444 (5.2) ($M^{\cdot+}$+1), 443 (15.2) ($M^{\cdot+}$), 442 (7.3), 414 (8.6), 413 (20.2), 412 (59.8), 411 (20.8), 322 (9.9), 321 (15.6), 320 (31), 319 (13.5), 250 (5.3), 221 (16.1), 220 (5.7), 181 (9.3), 174 (5.2), 149 (8.5), 148 (11), 133 (10.5), 131 (5.2), 123 (5.5), 105 (9.9), 103 (5.6), 92 (10.9), 91 (100), 90 (9.9), 89 (14.3), 78 (5), 77 (9.2), 65 (41.3), 64 (16.9), 63 (8.6), 51 (6.6).

2-{4-Aryl-5-[2-(2,5-dimethoxyphenyl)vinyl]-4H-1,2,4-triazol-3-yl-sulfanyl}-1-(2,5-dihydroxyphenyl) ethanones (**11a–c**)

The selected sulfanyltriazole **9a–c** (3 mmol) was dissolved in an ethanolic solution of sodium ethoxide prepared by dissolving sodium metal (69 mg, 0.003 g atom) in absolute ethanol (35 ml). To this solution a solution of 2,5-dihydroxyphenacyl bromide (0.69 g, 3 mmol) in absolute ethanol (10 ml) was gradually added and the reaction mixture was heated under reflux for 2 h, concentrated, cooled, diluted with water and refrigerated overnight. The precipitates obtained were filtered, washed with water, air dried, and crystallized as yellow precipitates. Physicochemical data are listed in Table 4 and ^{1}H NMR spectral data are listed in Table 5. IR (KBr, υ/cm): 3450 (OH), 1648–1639 (C=O), 1587–1584 (C=N), 1279, 1171–1169 (C–S–C), 1234–1231, 1049–1048 (C–O–C). MS, *m/z* (relative abundance %) of compound **11b:** 525 (2.8) ($M^{\cdot+}$+2), 523 (4.7) ($M^{\cdot+}$), 375 (12.6), 374 (10.7), 373 (37.8), 344 (39), 343 (28.4), 342 (100), 341 (12.8), 174 (29), 169 (13.7), 159 (10.8), 158 (10.1), 152 (20.5), 151 (13.9), 150 (51.4), 149 (15.7), 148 (10.5), 146 (14.5), 145 (11.4), 143 (18), 138 (7.8), 137 (36.8), 133 (13), 132 (10.3), 125 (11.6), 121 (35.1), 118 (15.2), 117 (10.9), 116 (12.2), 114 (10.6), 113 (19.1), 112 (16.5), 111 (44.5), 108 (10.1), 105 (15.4), 103 (19), 102 (14.9), 99 (11.5), 93 (13.1), 92 (24.7), 91 (18.6), 90 (15.9), 89 (23.4), 77 (17.4), 76 (10.1), 75 (22.4).

5-(4-Substituted anilino)-2-(2,5-dimethoxystyryl)-1,3,4-oxadiazoles (**12a–c**)

To a suspension of the selected thiosemicarbazide **7a–c** (1 mmol) in absolute ethanol (10 ml) dry freshly prepared yellow mercuric oxide (0.26 g, 1.2 mmol) was added and the mixture was heated under reflux for 2 h. The reaction mixture was filtered while hot to separate the precipitated black mercuric sulfide and the precipitate was washed with ethanol (2 × 10 ml). The combined filtrate and washings were then evaporated to dryness and the remaining residue was crystallized. Physicochemical data are listed in Table 4 and ^{1}H NMR spectral data are listed in Table 5. IR (KBr, υ/cm): 3335–3333 (υNH), 1627–1615 (C=N), 1582–1580 (δNH), 1233–1228, 1049–1042 (C–O–C). MS, *m/z* (relative abundance %) of compound **12a:** 323 (12.9) ($M^{\cdot+}$), 293 (17), 292 (100), 189 (10.5), 174 (16.4), 146 (14.1), 133 (10.5), 120 (13.1), 118 (16.8), 105 (11.6), 103 (14), 102 (11.6), 92 (25.4), 91 (20.4), 89 (22.7), 78 (11.9), 77 (97.4), 76 (15.2), 65 (35.6), 64 (14.7), 63 (28.7), 62 (10.7), 53 (12.1), 52 (12.6), 51 (77), 50 (18.4).

5-(4-Substituted anilino)-2-(2,5-dimethoxystyryl)-1,3,4-thiadiazoles (**13a–c**)

Method A

To a mixture of 3-(2,5-dimethoxyphenyl)propenoic acid **1** (0.42 g, 2 mmol) and the appropriate thiosemicarbazide derivative (2 mmol), phosphorus oxychloride (5 ml) was gradually added with continuous cooling and stirring. The reaction mixture was then heated under reflux for 30 min, concentrated to a smaller volume, cooled, and poured onto ice-cold water while stirring. Reaction mixture was then neutralized with sodium carbonate solution and the formed yellow precipitates were filtered, thoroughly washed with water, air dried, and crystallized.

Method B

A solution of the thiosemicarbazide derivative **7a–c** (2 mmol) in absolute ethanol (30 ml) containing concentrated sulfuric acid (3 ml) was heated under reflux for 1 h. The reaction mixture was then concentrated, cooled, and poured onto crushed ice with shaking. The resulting sticky yellow residues were triturated with water. The obtained precipitates were filtered, washed with water, air dried, and crystallized. Physicochemical data are listed in Table 4 and ^{1}H NMR spectral data are listed in Table 5. IR (KBr, υ/cm): 3250–3222 (υNH), 1610–1603 (C=N), 1565–1560 (δNH), 1288–1257, 1179–1093 (C–S–C), 1222–1216, 1035–1030 (C–O–C). MS, *m/z* (relative abundance %) of compound **13b:** 375 (7.8) ($M^{\cdot+}$+2), 373 (22.8) ($M^{\cdot+}$), 344 (44.4), 343 (27.2), 342 (100), 299 (12.9), 191 (20.6), 175 (10.9), 174 (11.4), 171 (11.2), 152 (10.6), 111 (10), 89 (11.6), 77 (12.6), 75 (14.2).

Biology

Anticancer screening

In vitro antitumor activity

Preliminary in vitro one-dose antitumor screening Out of the newly synthesized compounds, nine derivatives, namely **5a**, **b**, **9b**, **10a**, **d**, **12a**, **b**, and **13a**, **b**, were selected by the National Cancer Institute (NCI) in vitro disease-oriented human cells screening panel assay to be evaluated for their in vitro anticancer activity. Primary in vitro one-dose anticancer assay was performed using the full NCI 60-cell panel in accordance with the current protocol of the Drug Evaluation Branch, NCI, Bethesda (Monks *et al*., 1991; Grever *et al*., 1992; Boyd and Paull, 1995). These cell lines were incubated with one concentration (10 μM) for each tested compound. A 48-h continuous drug exposure protocol was used and a sulphorhodamine B (SRB) protein assay was employed to estimate cell viability or growth. Four compounds, namely **5a**, **b**, **9b**, and **12b**, passed this primary anticancer assay and consequently were carried over to the five-dose screen against a panel of about 60 different tumor cell lines.

Full in vitro five-dose antitumor assay Compounds **5a**, **b**, **9b,** and **12b** were subjected to the NCI in vitro disease-oriented human cells screening panel assay to screen their antitumor activities. About 60 cell lines of nine tumor subpanels including leukemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate, and breast cancer cell lines were utilized. The human tumor cell lines of the cancer screening panel were grown in RPMI-1640 medium containing 5 % fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells were inoculated into 96-well microtiter plates in 100 ml at plating densities ranging from 5,000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37 °C, 5 % CO_2, 95 % air, and 100 % relative humidity for 24 h before the addition of experimental drugs. After 24 h, two plates of each cell line were fixed in situ with TCA to represent a measurement of the cell population for each cell line at the time of drug addition. Experimental drugs were solubilized in dimethyl sulphoxide at 400-fold desired final maximum test concentration and stored frozen before use. At the time of drug addition, an aliquot of frozen concentrate was thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 mg/ml Gentamicin. Additional four-, ten-fold- or 1/2 log serial dilutions were made to provide a total of five drug concentrations plus control. Aliquots of 100 ml of these different drug dilutions were added to the appropriate microtiter wells already containing 100 ml of medium resulting in the required final drug concentrations. Following drug addition, the plates were incubated for an additional 48 h at 37 °C, 5 % CO_2, 95 % air, and 100 % relative humidity. For adherent cells, the assay was terminated by the addition of cold TCA. Cells were fixed in situ by the gentle addition of 50 ml of cold 50 % (w/v) TCA (final concentration, 10 % TCA) and incubated for 60 min at 4 °C. The supernatant was discarded and the plates were washed five times with tap water and air dried. Sulphorhodamine B (SRB) solution (100 ml) at 0.4 % (w/v) in 1 % acetic acid was added to each well and plates were incubated for 10 min at room temperature. After staining, unbound dye was removed by washing five times with 1 % acetic acid and the plates were air dried. Bound stain was subsequently solubilized with 10 mM trizma base and the absorbance was read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology was the same except that the assay was terminated by fixing settled cells at the bottom of the wells by gently adding 50 ml of 80 % TCA (final concentration, 16 % TCA). Three response parameters (GI_{50}, TGI, and LC_{50}) were calculated for each cell line (Monks *et al*., 1991; Grever *et al*., 1992; Boyd and Paull, 1995). The results are presented in Table 2.

Antimicrobial screening

Inhibition-zone measurements

All the selected compounds were evaluated by the agar cup diffusion technique (Scott *et al*., 1989) using a 1 mg/ml solution in DMSO. The test organisms were *S. aureus* (DSM 1104) and *B. subtilis* (ATCC 6633) as Gram-positive bacteria; *E. coli* (ATCC 11775) and *P. aeruginosa* (ATCC 10145) as Gram-negative bacteria. *C. albicans* (DSM 70014) was also used as a representative for fungi. Each 100 ml of sterile molten agar (at 45 °C) received 1 ml of 6-h broth culture and then the seeded agar was poured into sterile Petri dishes. Cups (8-mm diameter) were cut in the agar. Each cup received 0.1 ml of the 1 mg/ml solution of the test compounds. The plates were then incubated at 37 °C for 24 h or, in the case of *C. albicans*, for 48 h. A control using DMSO without the test compound was included for each organism. Ampicillin was used as standard antibacterial, while clotrimazole was used as antifungal reference.

Minimal inhibitory concentration (MIC) measurement

The minimal inhibitory concentrations (MIC) of all the selected compounds were measured by the twofold serial broth dilution method (Scott *et al*., 1989). The test organisms were grown in their suitable broth: 24 h for bacteria

and 48 h for fungi at 37 °C. Twofold serial dilutions of solutions of the test compounds were prepared using 200, 100, 50, 25, and 12.5 μg/ml. The tubes were then inoculated with the test organisms; each 5 ml received 0.1 ml of the above inoculum and were incubated at 37 °C for 48 h. Then, the tubes were observed for the presence or absence of microbial growth. The MIC values of the prepared compounds are listed in Table 3.

Minimal germicidal concentration (MBC or MFC) measurement

MIC tests were extended to measure the MBC or MFC as follows:

A loopfull from the tube not showing visible growth (MIC) was spread over a quarter of Muller–Hinton agar plate. After incubation, 24 h for bacteria and 48 h for fungi, the plates were examined for growth. Again, the tube containing the lowest concentration of the test compound that failed to yield growth on subculture plates was judged to contain the MBC/MFC of that compound for the respective test organism (Table 3).

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