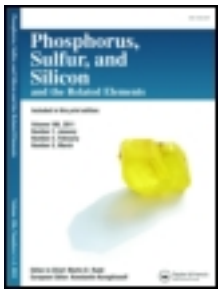


Phosphorus, Sulfur, and Silicon and the Related Elements

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gpss20

SYNTHESIS OF SOME NEW 1,2,4-TRIAZOLO[3,4-b]-THIADIAZOLE DERIVATIVES AS POSSIBLE ANTICANCER AGENTS

K. Subrahmanya Bhat [a] , D. Jagadeesh Prasad [a] , Boja Poojary [a] & B. Shivarama Holla [a]
[a] Department of Chemistry, Mangalore University, Mangalagangotri, India

Version of record first published: 16 Aug 2010.

To cite this article: K. Subrahmanya Bhat, D. Jagadeesh Prasad, Boja Poojary & B. Shivarama Holla (2004): SYNTHESIS OF SOME NEW 1,2,4-TRIAZOLO[3,4-b]-THIADIAZOLE DERIVATIVES AS POSSIBLE ANTICANCER AGENTS, Phosphorus, Sulfur, and Silicon and the Related Elements, 179:8, 1595-1603

To link to this article: http://dx.doi.org/10.1080/10426500490464186

Phosphorus, Sulfur, and Silicon, 179:1595–1603, 2004

ISSN: 1042-6507 print / 1563-5325 online
DOI: 10.1080/10426500490464186

SYNTHESIS OF SOME NEW 1,2,4-TRIAZOLO[3,4-*b*]-THIADIAZOLE DERIVATIVES AS POSSIBLE ANTICANCER AGENTS

K. Subrahmanya Bhat, D. Jagadeesh Prasad, Boja Poojary, and B. Shivarama Holla
Department of Chemistry, Mangalore University, Mangalagangotri, India

(Received July 3, 2003; accepted December 5, 2003)

*3-Substituted-4-amino-5-mercapto-1,2,4-triazoles are versatile synthons for constructing various biologically active heterocycles. Their cyclization with carboxylic acids gives fused five-membered derivatives, whereas with α-bromoketones gives a six-membered heterocycle. In this article we report the synthesis of a series of 1,2,4-triazolo[3,4-b]thiadiazoles **5** starting from 4-amino-3-substituted-5-mercapto-1,2,4-triazoles **3** and fluorobenzoic acids **4** using phosphorous oxychloride as cyclizing agent. Fourteen of the newly synthesized compounds were screened for anticancer properties. Four among them showed in vitro anticancer activity.*

Keywords: Anticancer activity; phosphorus oxychloride; triazolothiadiazoles

INTRODUCTION

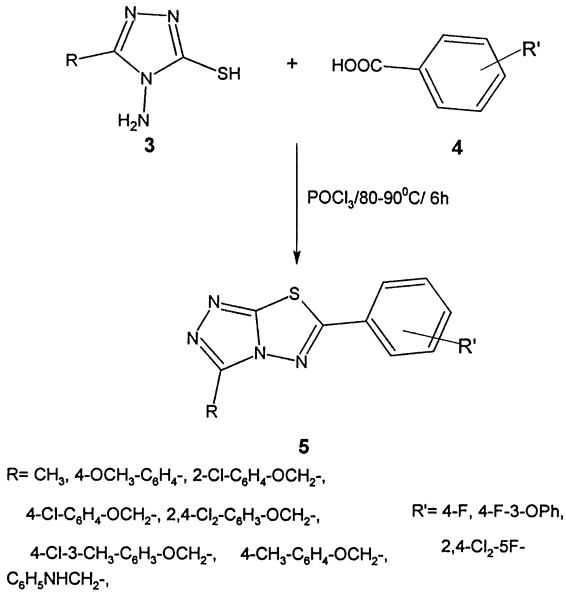
Various 1,2,4-triazole derivatives are found to be associated with diverse pharmacological activities.[1–4] The 1,2,4-triazole nucleus has been incorporated into a wide variety of therapeutically interesting drug candidates, including H_1/H_2 histamine receptor blockers, cholinesterase-active agents, CNS stimulants, antianxiety agents, and sedatives.[5] It was also found that the thiadiazole nucleus, which incorporates a toxophoric N—C—S linkage, exhibits a large number of

The authors are thankful to RSIC, Punjab University, Chandigarh, and RSIC, IIT, Chennai for providing elemental analysis/NMR/mass spectra of the compounds reported herein. KSB is thankful to CSIR, New Delhi for the award of Junior Research Fellowship. We are also thankful to P. M. Akberali of Strides Laboratories, Mangalore for a gift sample of 2,4-dichloro-5-fluoroacetophenone.

Address correspondence to B. Shivarama Holla, Department of Chemistry, Mangalore University, Mangalagangotri 574199, India. E-mail: hollabs@rediffmail.com

biological activities.[6,7] A number of 1,3,4-thiadiazole derivatives posses antibacterial properties comparable with those of sulfonamide drugs.[8] Subsequently, thiadiazole derivatives have found applications as antitumor agents and pesticides.[9–12] The cyclocondensation of 4-amino-3-substituted-5-mercapto-1,2,4-triazole **3** with carboxylic acids **4** using phosphorous oxychloride gives triazolothiadiazoles. Recently, fluorinated heterocycles have attracted attention due to the ability of fluorine to act as polar hydrogen or hydroxyl mimic. Therefore, substitution of hydrogen by fluorine has been a strategy in designing molecules for biological activity studies.[13,14]

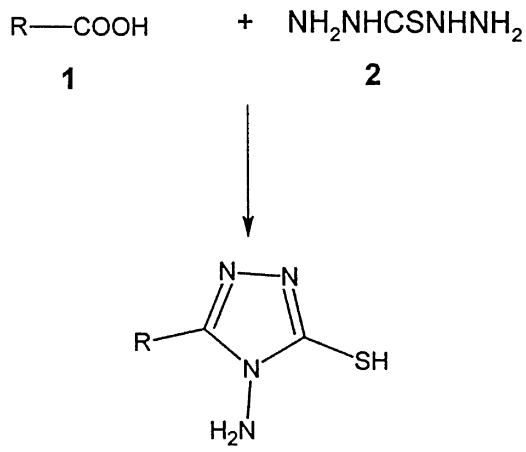
Prompted by the varied biological activities of 1,2,4-triazoles and 1,3,4-thiadiazoles, and in continuation of our work on the synthesis of *N*-bridged heterocycles derived from 1,2,4-triazoles, a series of 1,2,4-triazolo[3,4-*b*]thiadiazoles (Scheme 1) were synthesized. The newly synthesized compounds were characterized by analytical, infrared (IR), ^{1}H nuclear magnetic resonance (NMR), and mass spectral studies. In addition, fourteen of them were screened for their anticancer activities.



SCHEME 1 Synthesis of triazolothiadiazoles (**5**).

RESULTS AND DISCUSSION

The required 4-amino-5-mercapto-1,2,4-triazoles **3** were prepared by the direct fusion of aliphatic/aromatic carboxylic acids **1**, with



R= CH_3, 4-OCH_3-C_6H_4-, 2-Cl-C_6H_4-OCH_2-, 4-Cl-C_6H_4-OCH_2-, 2,4-Cl_2-C_6H_3-OCH_2-, 4-Cl-3-CH_3-C_6H_3-OCH_2-, 4-CH_3-C_6H_4-OCH_2-$C_6H_5NHCH_2$-,

SCHEME 2 Synthesis of 4-amino-3-substituted-5-mercapto-1,2,4-triazoles (**3**).

thiocarbohydrazide **2** (Scheme 2). Then, these triazoles **3** were cyclized with 4-fluoro/4-fluoro-3-phenoxy/2,4-dichloro-5-fluoro benzoic acids **4**, respectively, using phosphorus oxychloride as the cyclizing agent. The new workup procedure reported by Zhang et al. was used.[15] The cyclization of triazoles with various carboxylic acids **4** afforded triazolothiadiazoles **5**. The compounds **5** were confirmed on the basis of elemental analysis, IR, NMR, and mass spectral studies. The characterization data of these compounds **5** are given in Table I.

All of the newly synthesized compounds were analyzed satisfactorily for their nitrogen content. The IR spectrum of **5c** showed an absorption band at 1580 cm^{-1}, indicating the presence of C=N in the ring. In the IR spectra of the cyclized products, the absorption bands at 3200–3400 cm^{-1}, and 2300–2400 cm^{-1} attributed to the NH and SH functional groups were absent. This confirmed the involvement of the NH_2 and SH groups of the parent triazole unit in the cyclization. A distinct peak at 1000 cm^{-1} was assigned to C—F stretch. In the IR spectra of the cyclized products, the absorption band corresponding to carbonyl stretching

TABLE I 6-Substituted-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazoles (5a–w)

Compd	R	R′	Mol. formula	Yield (%)	m.p. (°C)	Elemental analysis % N found (cal.)
5a	CH_3	4-F—	$C_{10}H_7FN_4S$	85	170–172	23.78 (23.83)
5b	CH_3	4-F-3-(OC_6H_5)—	$C_{16}H_{11}FN_4OS$	85	156–158	17.10 (17.18)
5c	CH_3	2,4-Cl_2-5-F—	$C_{10}H_5Cl_2FN_4S$	85	154–156	18.52 (18.54)
5d	4-OCH_3—C_6H_4—	4-F—	$C_{16}H_{11}FN_4OS$	80	128–130	17.10 (17.13)
5e	4-OCH_3-C_6H_4—	4-F-3-(OC_6H_5)—	$C_{22}H_{15}FN_4O_2S$	80	138–139	13.35 (13.39)
5f	4-OCH_3—C_6H_4—	2,4-Cl_2-5-F—	$C_{16}H_9Cl_2FN_4OS$	80	178–179	14.12 (14.18)
5g	4-CH_3—C_6H_4—OCH_2—	4-F—	$C_{17}H_{13}FN_4OS$	70	168–170	16.40 (16.47)
5h	4-CH_3—C_6H_4—OCH_2—	4-F-3-(OC_6H_5)—	$C_{23}H_{17}FN_4O_2S$	70	145–148	12.92 (12.96)
5i	4-CH_3—C_6H_4—OCH_2—	2,4-Cl_2-5-F—	$C_{17}H_{11}Cl_2FN_4OS$	70	168–170	13.65 (13.69)
5j	2-Cl—C_6H_4—OCH_2—	4-F—	$C_{16}H_{10}ClFN_4OS$	75	178–180	15.52(15.55)
5k	2-Cl—C_6H_4—OCH_2—	4-F-3-(OC_6H_5)—	$C_{22}H_{14}ClFN_4O_2S$	75	120–122	12.35 (12.38)
5l	2-Cl—C_6H_4—OCH_2—	2,4-Cl_2-5-F—	$C_{16}H_8Cl_3FN_4OS$	75	155–158	12.98 (13.04)
5m	4-Cl—C_6H_4—OCH_2—	4-F-3-(OC_6H_5)—	$C_{22}H_{14}ClFN_4O_2S$	75	122–124	12.35 (12.39)
5n	4-Cl—C_6H_4—OCH_2—	2,4-Cl_2-5-F—	$C_{16}H_8Cl_3FN_4OS$	75	165	—
5o	2,4-Cl_2—C_6H_3—OCH_2—	4-F—	$C_{16}H_9Cl_2FN_4OS$	85	158–160	14.18 (14.21)
5p	2,4-Cl_2—C_6H_3—OCH_2—	4-F-3-(OC_6H_5)—	$C_{22}H_{13}Cl_2FN_4O_2S$	85	138–140	11.48 (11.52)
5q	2,4-Cl_2—C_6H_3—OCH_2—	2,4-Cl_2-5-F—	$C_{16}H_7Cl_4FN_4OS$	85	204–206	12.02 (12.07)
5r	4-Cl-3-CH_3—C_6H_3—OCH_2—	4-F—	$C_{17}H_{12}ClFN_4OS$	70	120–121	14.92 (14.97)
5s	4-Cl-3-CH_3—C_6H_3—OCH_2—	4-F-3-(OC_6H_5)—	$C_{23}H_{16}ClFN_4OS$	70	112–114	12.01 (12.02)
5t	4-Cl-3-CH_3—C_6H_3—OCH_2—	2,4-Cl_2-5-F—	$C_{17}H_{10}Cl_3FN_4OS$	70	168–171	12.6 (12.67)
5u	C_6H_5—NH—CH_2—	4-F—	$C_{16}H_{12}FN_5S$	65	162–164	21.5 (21.54)
5v	C_6H_5—NH—CH_2—	4-F-3-(OC_6H_5)—	$C_{22}H_{16}FN_5OS$	65	108–110	16. 72 (16.78)
5w	C_6H_5—NH—CH_2—	2,4-Cl_2-5-F—	$C_{16}H_{10}Cl_2FN_5S$	65	146–148	17.68 (17.76)

IR (KBr, ν_{cm-1}): **5a**: 3050 (Ar—CH str.), 2980 (CH_3 str.), 1608/1492 (C═N str.), 1175 (C—F str.). ^{1}H NMR δ ($CDCl_3$): **5b**: 7–7.65 (m, Aromatic, 12H), 3.86 (s, CH_3, 3H).

Mass (% abundance): **5b**: 326 (M^+, 30%), 243 (5%), 231 (85%), 211 (24%), 169 (10%), 131 (22%), 77 (64%), 69 (100%), 51 (42%). IR (KBr, ν_{cm-1}): **5c**: 3100 (CH str. Aromatic), 1580 (C═N str.), 1440 (C—N str.), 1080 (C—F str.), 880 (C—Cl str.), ^{1}H NMR δ ($CDCl_3$): 5c: 7.93–7.96 (d, Ar H, IH, $J = 9.08$), 7.79–7.82 (d, Ar H, 1H, $J = 8.6$), 7.65–7.67 (d, Ar $_{H—F\,meta}$, 1H, 6.47), 7.54–7.52 (d, Ar $_{H—F\,meta}$, $J = 5.87$).

frequency around 1700–1750 cm^{-1} was absent, which again gave a conclusive evidence for the cyclization.

The formation of cyclized products **5** were further supported by recording the ^1H NMR spectra of a few selected compounds. Thus, in the ^1H NMR spectra of triazolothiadiazoles, the characteristic downfield signal at δ 13.4 attributed to the —N=C—SH moiety was absent. A sharp signal at δ 5.4 attributable to the N—NH_2 group in the parent triazoles was also absent in the cyclized product. In the ^1H NMR spectrum of **5b**, a singlet that appeared at δ 2.77 was attributed to the methyl protons. The remaining 8 aromatic protons resonated as multiplets in the range δ 7–7.64. In the ^1H NMR spectrum of **5c**, a singlet that appeared at δ 2.82 was attributed to the methyl protons. The remaining aromatic protons appeared as doublet of doublets due to ortho and meta coupling with fluorine in the range δ 7.6–7.96 integrating for two protons. In the ^1H NMR spectrum of **5k**, a singlet was observed at δ 5.6 due to the presence of OCH_2 protons. The remaining protons resonated as multiplets in the aromatic region δ 6.92–7.61 integrating for 12 protons.

In the mass spectrum of compound **5b**, an intense molecular ion peak was observed at *m/z* 326. An intense peak observed at *m/z* 231 may have been due to the formation of 4-fluoro-3-(phenoxy)-thiobenzoyl cation. The base peak observed at *m/z* 69 may have been due to the formation of stable fluorocyclobutadienyl cation. In the mass spectrum of compound **5d**, molecular ion peak was observed at *m/z* 326. The fragmentation pattern is similar to that of compound **5b**, giving *m/z* values at 231, 139, and 69. The spectral data for some of the compounds are given under Table I.

BIOLOGICAL STUDIES

Anticancer Activity

Fourteen of the newly synthesized compounds were evaluated for their antitumor activity following the NCI screening program.[16–18] They were first evaluated in a 3-cell line, one-dose preliminary anticancer assay. The results of this assay are reported in Table II. Compounds **5k**, **5m**, **5p**, and **5u** were considered active.

When these compounds were passed on for evaluation against a full panel of 60 human cell lines, they showed variable antitumor activities against most of the tested subpanel tumor cell lines at the GI50 level. The subpanel tumor cell lines' median growth inhibitory concentration (the average sensitivity of each subpanel towards each of the test compounds) and the full panel mean graph midpoint (MG-MID;

TABLE II The 3-Cell Line, One-Dose Primary Anticancer Assay Results

Compd.	Growth percentage (LUNG) NCI-H 460	(Breast) MCF-7	(CNS) SF-268	Activity
5a	103	86	97	Inactive
5b	97	95	100	Inactive
5c	104	124	98	Inactive
5d	52	37	54	Inactive
5e	46	53	62	Inactive
5j	67	63	60	Inactive
5k	6	24	26	Active
5m	3	19	50	Active
5o	76	97	106	Inactive
5p	16	45	39	Active
5q	117	100	99	Inactive
5t	107	120	99	Inactive
5v	107	121	84	Inactive
5u	11	17	88	Active

Samples are tested at 1.00×10^{-04} Molar concentrations.
Growth percentages less than 32 are considered as active.

the average of all cell lines towards each of the test compounds) are reported in Table III. Compound **5k** is active against lung cancer HOP-92 with a GI50 value of 15.2 μg/ml. Compound **5m** is highly active against leukemia MOLT-4, ovarian cancer OVCAR-3 and prostate cancer PC-3 with a GI50 value of 3.51, 8.76, and 0.0735 μg/ml, respectively. Compound **5p** is highly active against leukemia MOLT-4, lung cancer HOP-92, CNS cancer SF-295, ovarian cancer OVCAR-3 and prostate cancer PC-3 with a GI50 value of 7.08, 0.25, 6.2, 0.7, and 8.94 μg/ml, respectively. Compound **5u** is active against leukemia RPMT-8226, lung cancer NCI-H322M, and colon cancer HCC-2998, with a GI50 value of 6.99, 6.22, and 0.58 μg/ml, respectively.

The ratio obtained by dividing the compounds full panel MG-MID (μM) by its individual subpanel MG-MID (μM) is considered as a measure of compound selectivity. Ratios between 3 and 6 refer to moderate selectivity, ratios >6 indicate high selectivity towards corresponding cell line, and compounds not meeting either of these criteria are considered nonselective.[19] All the active compounds in the present study prove to be nonselective against any of the 9 tumor subpanels tested. However, regarding the selectivity of the tested compounds against the highly affected individual tumor cell lines, compound **5m** exhibits high selectivity towards prostate cancer PC-3 at the GI50 level with a selectivity

TABLE III Subpanel Tumor Cell Lines Median Growth Inhibitory Concentration (GI50) and Full-Panel Mean Graph Midpoint (MG-MID)

Subpanel tumor cell line	**5k**	**5m**	**5p**	**5u**
Leukamia	>100	15.98	15.85	>100
Lung cancer	30.29	18.77	17.68	31.20
Colon cancer	>100	27.95	26.28	25.43
CNS cancer	27.92	27.3	18.73	32.35
Melanoma	>100	25.5	23.30	28.61
Ovarian cancer	41.76	23.31	18.08	43.42
Renal cancer	>100	15.07	16.86	>100
Prostate cancer	30.8	13.82	23.67	43.8
Breast cancer	36.19	16.85	23.78	34.11
MG-MID	34.67	17.38	15.85	30.2

MG-MID, average sensitivity of each subpanel and of all cell lines towards each of the test compounds.

ratio 236.46. On the other hand, compound **5p** shows selectivity against lung cancer HOP-92 subpanel tumor cell line at GI50 level with a selectivity ratio of 63.4. The selectivity ratios for a particular cell line to other compounds are given in Table IV.

The activity of the tested compounds could be correlated to the structural variations. The compounds **5k**, **5m**, and **5p** which contained 4-fluoro-3-phenoxyphenyl moiety exhibited antitumor activities. It is also interesting to note that compound **5u**, with anilinomethyl substituent on triazole ring and 4-fluorophenyl group on thiadiazole ring, is also active. These compounds raise the possibility of their further derivatization in order to explore scope and limitations of their activities.

TABLE IV The most Affected Cell Lines and the Corresponding GI50 and Sensitivity Towards Each of the Active Compounds

Compd.	Most affected cell line	GI50	MG-MID	Sensitivity ratio
5k	Lung cancer HOP-92	15.2 μg/ml	34.67	2.28
5m	Leukemia MOLT-4	3.51 μg/ml	17.38	4.95
	Ovarian cancer OVCAR-3	8.76 μg/ml		1.98
	Prostate cancer PC-3	0.0735 μg/ml		236.46
5p	Leukemia MOLT-4	7.08 μg/ml	15.85	2.24
	Lung cancer HOP-92	0.25 μg/ml		63.4
	CNS cancer SF-295	6.2 μg/ml		2.56
	Ovarian cancer OVCAR-3	0.7 μg/ml		22.6
	Prostate cancer PC-3	8.94 μg/ml		1.77
5u	Leukemia RPMT-8226	6.99 μg/ml	30.2	4.32
	Lung cancer NCI-H322M	6.22 μg/ml		4.86
	Colon cancer HCC-2998	0.58 μg/ml		52.07

EXPERIMENTAL

Melting points were determined in open capillary tubes and were uncorrected. IR spectra were recorded in KBr pellets on a Perkin-Elemer 157 IR spectrophotometer. ^{1}H NMR spectra were recorded in DMSO-d_6 on EM-390 (300 MHz) NMR spectrometer. Mass spectra were recorded on MASPEC low resolution instrument operating at 70 eV. The purity of compounds was checked by thin layer chromatography (TLC) on silica gel plates using a hexane: ethylacetate (10:1) solvent system, and iodine was used as a visualizing agent.

4-Amino-5-mercapto-1,2,4-triazoles (3)

A mixture of carboxylic acid **1** (0.01 mol) and thiocarbohydrazide **2** (0.01 mol) contained in a round-bottomed flask was heated on a mantle until the contents melted. The mixture was maintained at this temperature for 15–20 min (Scheme 2). The product obtained on cooling was treated with sodium bicarbonate solution to dissolve the unreacted carboxylic acid if any. It was then washed with water and collected by filtration. The product was recrystallized from a mixture of dioxane and ethanol to afford the title compounds **3**. Their physical data are in agreement with the literature values.[20]

6-Substituted-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazoles (5a--w)

To a mixture of suitably substituted triazoles **3** (2 mmol) and fluoro/4-fluoro-3-phenoxy/2,4-dichloro-5-fluoro benzoic acid **4** (2 mmol), phosphorus oxychloride (10 ml) was added and the contents were heated under reflux for 6 h in a water bath (Scheme 1). Excess of phosphorus oxychloride was then distilled off, and the residue was poured onto crushed ice and stirred well. It was then washed with sodium bicarbonate solution (5%) and the resulting solid product was washed with water and recrystallized from ethanol:dioxane mixture. The physicochemical data for the synthesized compounds are given in Table I. The prepared compounds were evaluated for their anticancer activities using the National Cancer Institute (NCI) in vitro anticancer screening assay.[17–19]

REFERENCES

[1] T. Hirota, T. Yamamoto, and T. Nakayama, *J. Heterocycl. Chem.*, **28,** 257 (1991).
[2] H. L. Yale and J. J. Piala, *J. Med. Chem.*, **9,** 42 (1966).

[3] N. D. Heindel and J. R. Reid, *J. Heterocycl. Chem.*, **17,** 1087 (1980).
[4] J. Sandstrom, *Advances in Heterocyclic Chemistry*, **9,** 165 (1968).
[5] A. Mohsen, M. E. Omar, and A. Wafa, *J. Heterocycl. Chem.*, **23,** 1339 (1986).
[6] a) Lubrizol Corp., *US Patent* 4,246,126 (1981); b) Lubrizol Corp., *Chem. Abstr.*, **94,** 142505h (1981).
[7] Q. Bano, N. Tiwari, S. Giri, and Nizamuddin, *Ind. J. Chem.*, **31B,** 467 (1992) and references cited therein.
[8] B. S. Holla, R. Gonsalves, and S. Shenoy, *Farmaco*, **53,** 574 (1998).
[9] B. S. Holla, B. S. Rao, R. Gonsalves, B. K. Sarojini, and K. Shridhara, *Farmaco*, **57,** 693 (2002).
[10] B. S. Holla, K. N. Poojary, B. S. Rao, and M. K. Shivananda, *Eur. J. Med. Chem.*, **37,** 511 (2002) and references cited therein.
[11] B. S. Holla, R. Gonsalves, B. K. Sarojini, and S. Shenoy, *Ind. J. Chem.*, **40B,** 475 (2001).
[12] Z. Y. Zhang and S. X. Wen, *Heterocycles*, **48,** 561 (1998).
[13] a) O. H. David and S. R. Henry, *Chem. Commun.*, **45,** 645 (1997); b) B. S. Holla, K. S. Bhat, and N. S. Shetty, *Phosphorus, Sulfur, and Silicon*, **178,** 2193 (2003).
[14] a) R. Filler and Y. Kobayashi, *Biomedicinal Aspects of Fluorine Chemistry* (Elsevier, Amsterdam, 1982); b) M. Hudlicky, *Chemistry of Organic Fluorine Compounds*, Vol. **II** (American Chemical Society, Washington DC, 1995), p. 979.
[15] L. X. Zhang, A. J. Zhang, X. X. Chen, X. X. Lei, X. Y. Nan, D. Y. Chen, and Z. Y. Zhang, *Molecules*, **7,** 681 (2002).
[16] M. R. Grever, S. A. Schepartz, and B. A. Chabner, *Seminars Oncol.*, **19,** 622 (1992).
[17] M. R. Boyd and K. D. Paull, *Drug Rev. Res.*, **34,** 91 (1995).
[18] E. M. Acton, V. L. Narayanan, P. A. Risbood, R. H. Shoemaker, D. T. Vistica, and M. R. Boyd, *J. Med. Chem.*, **37,** 2185 (1994).
[19] K. D. Paull, R. Shoemaker, L. Hodes, A. Monks, D. A. Scudiero, L. Rubinstein, J. Plowman, and M. R. Boyd, *J. Natl. Cancer Inst.*, **81,** 1088 (1989).
[20] B. S. Holla, R. Gonsalves, and B. K. Sarojini, *Ind. J. Chem.*, **36B,** 943 (1997).