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Original article

5-Isopropylidene-3-ethyl rhodanine induce growth inhibition followed by apoptosis in leukemia cells

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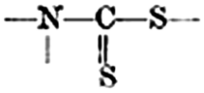
ABSTRACT

5-Isopropylidene-3-ethyl rhodanine **II** was prepared by conventional and Microwave assisted synthesis. For the first time, we found that rhodanine **II** treatment led to cytotoxicity in leukemic cell line, CEM by inducing apoptosis.

1. Introduction

Rhodanine derivatives are attractive compounds due to their biological activities. They are anticonvulsant, antibacterial, antiviral and antidiabetic in nature [1]. Rhodanine derivatives have also been reported as inhibitors of Hepatitis C Virus (HCV) protease [2], uridine diphospho-*N*-acetylmuramate/L-alanine ligase [3], bacterial β-lactamase and Mur ligases [4, 5]. Recently, substituted rhodanines were investigated for tau aggregation inhibitor properties [6]. Rhodanines are classified as nonmutagenic [7] and a long-term study on the clinical effects of the rhodanine-based Epalrestat for antidiabetic demonstrated that it is well tolerated [8]. The use of the rodanine analogs as anti-leukemia agents has also been reported in literatures [9–15]. Rhodanine derivatives were found to have marked mildew-proofing activity. It is interesting to note that the new mildew-proofing agents contain the structure



present in many plant fungicides (tetramethylthiuram disulphide and the salts of dithiocarbamic acid), as well as a carbonyl group conjugated with an ethylenic linkage, found in another class of fungicides [16]. Due to various possibilities of chemical derivatization of the rhodanine ring, rhodanine-based compounds will probably remain a privileged scaffold in drug discovery. Therefore, the synthesis of these compounds is of considerable interest.

The rhodanine moiety has been synthesized by various methods such as addition of isothiocyanate to mercaptoacetic acid followed by acid catalyzed cyclisation, or the reaction of ammonia or primary amines with carbon disulfide and chloroacetic acid in the presence of bases [17,18].

Arylidenerhodanines are frequently identified as potent hits in high throughput screening against various prokaryotic and eukaryotic targets. Condensation of aromatic aldehydes at the nucleophilic C-5 active methylene has been performed using piperidinium benzoate in refluxing toluene or sodium acetate in refluxing glacial acetic acid [17,18]. Recently, Sim et al. [19] reported the synthesis of 5-arylalkylidene rhodanines in 60–82% yields by heating the reactants suspended in toluene at 110 °C for 3 days. Sing et al. [20] reported the condensation of rhodanine with an aldehyde (0.1 mmol) by heating in anhydrous EtOH (200 mL) for 6 h at 80 °C. Zhang Alloum et al. reported the successful synthesis of some 5-arylalkylidene rhodanines on solid inorganic supports in dry media under microwave irradiation [21,22]. Jian-Feng Zhou et al. have reported the synthesis of bis(benzylidene)cycloalkanones by the aldol condensation in an aqueous medium under phase-transfer catalysis and microwave irradiation [23] and synthesis of 5-arylalkylidene rhodanines by the aldol condensation of aromatic aldehydes with rhodanine using tetrabutylammonium bromide (TBAB) as phase-transfer catalyst in an aqueous medium under microwave irradiation.

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Alternatively, the alkylidene rhodanines were prepared by the condensation of 3-3-carboxyethylrhodanines with oxo-compounds unrelated to aromatic aldehydes and tested for possible antimetabolic activity [24]. Rhodanine, Ketone, and NH_4OAc were refluxed in toluene for 3 days to prepare the alkylidene rhodanin derivatives. Obviously, these methods involve long reaction times, high temperatures, use large quantities of organic solvents and some give unsatisfactory yields. Due to these drawbacks the number of available reports on the investigation of alkylidene rhodanines was very few. This prompted us to synthesise few rhodanine derivatives and their corresponding oxygen (2-thio-2,4-oxazolidine derivatives) and nitrogen (thiohydantions) analogs. Besides we show that the newly synthesized isopropylidene rhodanine derivatives induced cytotoxicity in leukemic cells in a dose- and time-dependent manner. The results are encouraging, which makes them a promising starting point for further synthesis and optimization by QSAR studies.

2. Result and discussion

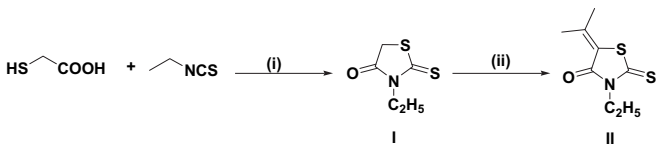
2.1. Synthesis of 5-isopropylidene-3-ethyl rhodanine *II*

A mixture of thioglycollic acid, ethyl isothiocyanate, methanol and water were heated in an oil bath for four hours at 100 °C to yield *N*-ethyl rhodanine **I** (Scheme 1). A mixture of **I**, ammonium malonate and acetone was refluxed in an oil bath at 90 °C for 18 h to yield 5-isopropylidene-3-ethyl rhodanine **II**. In order to develop a greener synthesis the above mixture was treated in a microwave oven at 300 W for 2 min. The yield was comparable to the conventional method but the reaction time was shorter. The compounds were characterized by elemental analysis, UV, IR, NMR and MS data.

2.2. *II* induces cytotoxicity in leukemic cells in a dose- and time-dependent manner

In order to evaluate the cytotoxic effect of 5-isopropylidene rhodanine on growth of leukemic cells, we used trypan blue assay. Leukemic cell line, CEM (T-cell leukemia) was treated with 10, 50, 100 and 250 μM of **II**. Since the compound was dissolved in methanol, the cells with methanol were used as vehicle control. Following addition of compound, cells were counted at intervals of 24 h till the control cells attained stationary phase. Results showed that cell growth was affected with increase in time (Fig. 1). The effect was limited when 10 μM of **II** was used. However, concentrations of 100 and 250 μM resulted in increased cell death (Fig. 1). The IC_{50} of **II** was approximately 40 μM, at 48 h of treatment. These results suggest that **II** induces cytotoxicity in human leukemic cells, in a dose- and time-dependent manner.

The cytotoxicity induced by **II** on proliferation of leukemic cells was further verified using MTT assay. CEM cells treated with 10, 50,100 and 250 μM of **II** were harvested after 24, 48 or 72 hrs and were subjected to MTT assay (Fig. 2). Results showed that cell viability was affected upon treatment with **II** at 50,100 and 250 μM, especially after 48 h. These results further suggest that 5-isopropylidene rhodanine induces the cytotoxicity.



Scheme 1. (i) CH_3OH, H_2O, 100 °C, 4 h; (ii) ammonium malonate, acetone, 90 °C, 18 h.

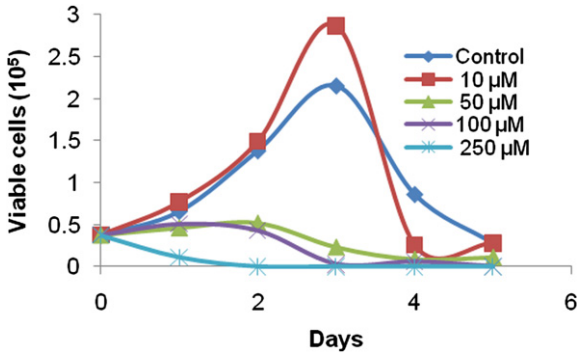


Fig. 1. Cytotoxicity of 5-isopropylidene-3-ethyl rhodanine in leukemic cells. Evaluation of cell viability using trypan blue assay following 5-isopropylidene-3-ethyl rhodanine treatment. CEM cells were treated with 10, 50, 100 and 250 μM of 5-isopropylidene-3-ethyl rhodanine or CH_3OH (vehicle control). Cells were harvested, trypan blue stained and counted every 24 h until it reached stationary phase. The data represented is result of three independent experiments.

To further check the cytotoxic effect of **II**, LDH release in the cell culture suspension was measured using LDH assay (Fig. 3). The methanol treated cells were used as vehicle control and **II** treated cells showed LDH release suggesting cytotoxic effect on CEM cells. The membrane of CEM cells are disrupted due to cytotoxic effect of **II** causing release of LDH (Lactate dehydrogenase) into the supernatant. Results showed time-dependent increase in the LDH release upon treatment with the **II**, further confirming the above results (Fig. 3).

2.3. Cell cycle analysis by flow cytometry

We performed FACS analysis to determine the effect of **II** on cell cycle progression. CEM cells were stained with ethidium bromide after 72 h of treatment with **II** (10, 50, 100 and 250 μM) and subjected to FACS. Histogram of control cells showed a standard cell cycle pattern, which includes G1 and G2 separated by S phase (Fig. 4). The subG1 phase (mostly dead cells) was not prominent. Interestingly, upon addition of **II**, a concentration dependent change was observed in the cell cycle pattern (Fig. 4) leading to accumulation of cells in subG1 and decline of G2/M, G1 and S phases indicating apoptosis.

Results showed dose-dependent accumulation of subG1 population of cells, which is a hall mark of apoptosis (Fig. 4). These findings indicate that at the ranges of concentration studied, the anti-proliferative effect of **II** on leukemic cells could be attributed primarily to the induction of apoptosis, with less or no contribution

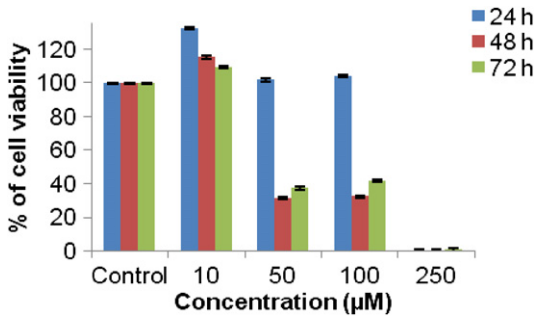


Fig. 2. Determination of % cell viability by MTT assay in CEM cells. Cells were cultured with 10, 50, 100 and 250 μM of 5-isopropylidene-3-ethyl rhodanine or vehicle control for 24, 48 and 72 h. The percentage of cell viability was calculated considering methanol treated (control) cells as 100% and plotted with representation of error bars.

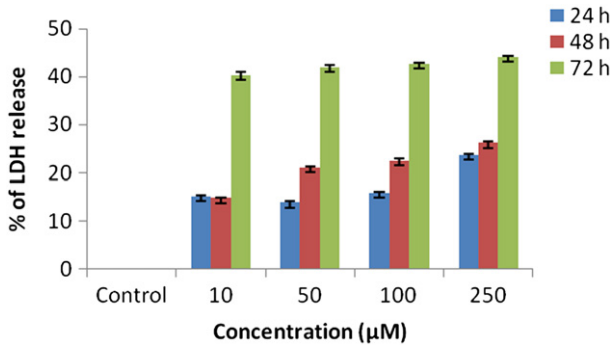


Fig. 3. Measurement of LDH release following treatment with 5-isopropylidene-3-ethyl rhodanine. After the exposure of CEM cells with 5-isopropylidene-3-ethyl rhodanine at different concentrations (10, 50, 100 and 250 μM) for 24, 48 and 72 h, the release of LDH was measured at 490 nm. Results are presented as percentage of LDH release by subtracting the control values from treated ones. The data presented is result of three independent experiments and error bars are indicated.

from cell cycle arrest. Thus, the results obtained, confirmed our earlier observation of **II** induced cytotoxicity.

2.4. Discussion

Leukemia, being one of the most threatening hematological malignant cancers, has been found to be very sensitive to anti-cancer chemotherapeutic agents, which either interfere with cell cycle or cause apoptosis [25]. This factor intrigues scientists to look for more specific and effective chemical drugs against it. Leukemic cells lines serve as useful tools to study factors and processes associated with their differentiation and apoptosis pathways [26]. Hence, cytotoxic effects of **II** on CEM cells (a T-cell leukemic cell line derived from T-cell leukemia patient) is studied.

In the present study, we find that **II** is capable of inducing cytotoxic activity and induction of apoptosis in CEM. Although **II** induced cytotoxicity in these cells, the IC_{50} value was about 40 μM after 72 h, which is higher than many synthetic compounds. In fact, previous reports showed that similar doses were also used for other compounds by Roy et al. [27] and Kumar et al. [28]. IC_{50} values suggest that **II** exhibited good cytotoxicity, showed remarkable suppression on the CEM cell growth as the concentration and time increases in dose dependent manner. Hence, our results showed that **II** affected growth of leukemic cells in a time- and dose-dependent manner. Generally, cell death could be either through necrosis or apoptosis. The former process is associated with relatively large damage to the surrounding tissue. The latter is associated with controlled elimination of cancer cells. Thus for the possible treatment of cancer, cytotoxic compounds should preferentially act via apoptosis. To address the question whether **II** induces cell death via apoptosis, we performed FACS analysis to determine the effect on cell cycle progression. Appearance of Sub-G1 peak accumulation in present study as indicated by FACS analysis is a hall mark of apoptosis (Fig. 4) and characteristic of apoptotic cell behaviour, which is not for the case of necrosis [29]. However, we could not find any evidence for cell cycle arrest. FACS analysis showed that **II** interferes with cell division leading to inhibition of cell proliferation ultimately leading to cell death. These findings indicate that at the ranges of concentration studied, the anti-proliferative effect of **II** on leukemic cells could be attributed primarily to the induction of apoptosis, with less or no contribution from cell cycle arrest.

3. Experimental protocols

3.1. General procedures

All compounds prepared were characterized by ^{1}H NMR, IR and elemental analyses and are described in the experimental section. Melting points were determined in a XT-5 digital melting point instrument and are uncorrected. IR spectra were recorded on a Nicolet Avatar 360 FT-IR spectrometer. ^{1}H NMR spectra were measured at 400 MHz on a Bruker-400 spectrometer using TMS as internal standard and $CDCl_3$ as solvent. MS spectra were obtained on a Shimadzu LCMS instrument. Elemental analyses were determined using a Perkin–Elmer 240C Elemental Analyzer.

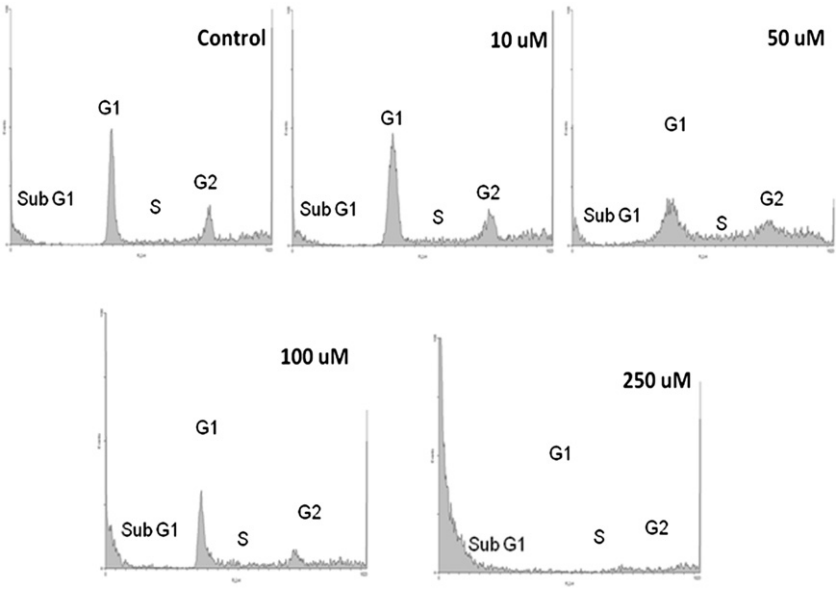


Fig. 4. Cell cycle analysis of leukemic cells following 5-isopropylidene-3-ethyl rhodanine treatment. CEM cells were treated with 5-isopropylidene-3-ethyl rhodanine (10, 50, 100 and 250 μM) for 72 h, harvested and stained with ethidium bromide and quantified by flow cytometry. In all cases the histograms show the percentage of cells in the G_0/G1, G1, S and G_2/M phase of the cell cycle. For each sample 10,000 cells were used for sorting.

*3.1.1. Preparation of N-ethyl rhodanine **I***

A mixture of thioglycollic acid (9.2 g, 0.1 mol), ethyl isothiocyanate (10.45 g, 0.12 mol), methanol (80 ml) and water (1000 ml) were taken in a 3 lit capacity flask and was heated in an oil bath for 4 h at 100 °C. After 4 h, the reaction mixture was cooled and lower oily layer, which was slight yellow in colour, was separated by decantation and cooled strongly in a freezing mixture. The oily layer got solidified. The solid was extracted with ether, dried and distilled out at low pressure to yield **I**. Yield 72%, mp 38 °C. IR (KBr, ν, cm^{-1}): 1544 (C–N), 1720. MS: APCI (Positive mode) *m/z*: 161.99 $[M+H]^+$. ^1H NMR ($CDCl_3$, 400 MHz) δ: 1.21 (t, 3H, $J = 7.1$ Hz), 3.76 (m, 2H), 3.95 (q, 2H, $J = 7.0$ Hz). Anal. Calcd. for $C_5H_7NOS_2$: C, 37.24; H, 4.38; N, 8.69. Found: C, 37.32; H, 4.43; N, 8.58.

*3.1.2. Preparation of 5-isopropylidene-3-ethyl rhodanine **II***

A mixture of *N*-ethyl rhodanine (2.4 g, 0.15 mol), ammonium malonate (4.4 g, 0.035 mol) and acetone (13.8 ml, 0.25 mol) was refluxed in an oil bath at 90 °C for 18 h. After reflux it was cooled and the separated solid was filtered. It was recrystallised from methanol to yield pale yellow needles **II**. Yield 62%, m.p. 54 °C. λ_{max} (MeOH) 342 nm. IR (KBr,ν, cm^{-1}): 1600 (C=C), 1720 (C=O). MS: APCI (Positive mode) *m/z*: 202.02 $[M+H]^+$. ^1H NMR ($CDCl_3$, 400 MHz) δ: 1.23 (t, 3H, $J = 7.1$ Hz), 2.03 (s, 3H), 2.46 (s, 3H), 4.15 (q, 2H, $J = 7.0$ Hz). ^{13}C NMR ($CDCl_3$) δ: 11.7, 17.0, 17.2, 37.6, 116, 147, 164 and 192. Anal. Calcd. for $C_8H_{11}NOS_2$: C, 47.73; H, 5.51; N, 6.96. Found: C, 47.71; H, 5.48; N, 6.99.

3.1.3. Microwave assisted synthesis

A mixture of *N*-ethyl rhodanine (2.4 g, 0.15 mole), ammonium malonate (4.4 g, 0.035 mol) and acetone (13.8 ml, 0.25 mol) was heated to 160 °C in a microwave oven at 300 W for 2 min. After heating it was cooled and the separated solid was recrystallised from methanol to yield pale yellow needles **II**. The physical data were identical.

3.1.4. Cell culture

Human leukemia cell line CEM was purchased from National Center for Cell Science, Pune, India. Cells were grown in RPMI 1640 medium (Gibco) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Sigma) and 100 mg/L pencillin-streptomycin at 37 °C in a humidified atmosphere with 5% CO_2. Cells were diluted at a ratio of 1:5 every 2–3 days.

3.1.5. Compound

The compound **II** used in the present study is synthetic compound dissolved in methanol. The maximum concentration of methanol used in the experiments was equal to the same amount used as vehicle control. The final concentration of MeOH used in control was 0.8% which was equivalent to the maximum concentration of methanol used in the experiments. In all the experiments described herein compound was added after 24 h of cell culture.

3.1.6. Cell viability by trypan blue exclusion

The effect of compound **II** on the viability of CEM cells was determined by trypan blue dye exclusion assay [30]. Briefly, the cells were plated at a density of 1×10^5 in six-well plates followed by addition of different concentrations of compound (10, 50, 100 and 250 μm in methanol) or vehicle alone. After incubation for every 24 h, cells were collected and diluted in equal volume of media and mixed with 20 μl of trypan blue. Cells were counted under the microscope using hemocytometer.

3.1.7. Cell proliferation by MTT assay

The growth inhibitory effect of the compound **II** towards the CEM cells were measured by means of MTT (3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay [31]. The cleavage and conversion of the soluble yellowish MTT to the insoluble purple formazan by active mitochondrial dehydrogenase of living cells has been used to develop an assay system alternative to other assays for measurement of cell proliferation. Harvested cells were seeded in to 96-well plate (1×10^5 cell/ml) with varying concentrations of the compound **II** (10, 50, 100 and 250 μM) and incubated. For every 24 h, the cells were collected and MTT assay was performed. Four hours to the end of incubation, 10 μl of MTT solution (5 mg/ml in PBS) was added to each well containing fresh and cultured medium. At the end, the insoluble formazan produced was dissolved in solution containing 10% SDS and 50% DMF (left for 2 h at 37 °C in dark conditions) and optical density (OD) was read against reagent blank with multi well scanning spectrophotometer (ELISA reader, Model Expert 96, Asys Hitchech, Austria) at a wavelength of 570 nm. Absorbance is a function of concentration of converted dye. The OD value of study groups was divided by the OD value of untreated control and presented as percentage of control (as 100%).

3.1.8. LDH release assay

Release of lactate dehydrogenase (LDH) is an indicator of membrane integrity and hence cell injury. LDH assay was performed to assess the LDH release to the media following treatment with the compound 5-isopropylidene rhodanine (10, 50,100 and 250 μM) on CEM cells for 24, 48 and 72 h and it was measured using standard protocols [32,33]. The intracellular LDH was determined after lysing the cells by rapid freezing and thawing in liquid nitrogen. The LDH release was measured at an absorbance of 490 nm. The percentage of LDH release was calculated as: (LDH activity in media)/(LDH activity in media + intracellular LDH activity) X100%. Results are presented as percentage of LDH release substracting the control values from treated ones.

3.1.9. Cell cycle analysis

CEM cells were cultured and treated with different concentrations of compound **II**. Cells were harvested after 72 h. The cells were processed, stained with ethidium bromide (Sigma, USA) and subjected to flow cytometry (FACScan, BD Biosciences, USA) using CellQuest Pro software using excitation 488 nm laser and emission at 560/670 nm. A minimum of 10,000 cells were acquired per sample and histograms were analyzed using WinMDI 2.8 software.

References

[1] (a) Y. Ohishi, T. Mukai, M. Nagahara, M. Yajima, N. Kajikawa, K. Miyahara, T. Takano, Chem. Pharm. Bull. 38 (7) (1990) 1911;
(b) Y. Momose, K. Meguro, H. Ikeda, C. Hatanaka, S. Oi, T. Sohda, Chem. Pharm. Bull. 39 (6) (1991) 1440.
[2] K. Sudo, Y. Matsumoto, M. Matsushima, M. Fujiwara, K. Konno, K. Shimotohno, S. Shigeta, T. Yokota, Biochem. Biophys. Res. Commun. 238 (1997) 643.
[3] M.M. Sim, S.B. Ng, A.D. Buss, S.C. Crasta, K.L. Goh, S.K. Lee, Bioorg. Med. Chem. Lett. 12 (4) (2002) 697.
[4] R. Frlan., A. Kovac, D. Blanot, S. Gobec, S. Pecar, A. Obreza, Molecules 13 (2008) 11.
[5] K. Kristan, M. Kotnik, M. Oblak, U.J. Urleb, Biomol. Screen 14 (4) (2009) 412–418.
[6] B. Bulic, M. Pickhardt, I. Khilstunova, J. Biernat, E.M. Mandelkow, E. Mandelkow, H. Waldmann, Angew. Chem. Int. Ed. 46 (2007) 1–6.
[7] E. Zeiger, B. Anderson, S. Haworth, T. Lawlor, W. Mortelmans, Speck Environ. Mutagen 9 (1987) 1–110.
[8] N. Hotta, Diabetes Care 29 (2006) 1538–1544.
[9] P. Villain-Guillot, M. Gualtieri, L. Bastide, F. Roquet, J. Martinez, M. Amblard, M. Pugniere, J. Leonetti, J. Med. Chem. 50 (2007) 4195–4204.
[10] M. Kawakami, K. Koya, T. Ukai, N. Tatsuta, A. Ikegawa, K. Ogawa, T. Shishido, L. B. Chen, J. Med. Chem. 41 (1998) 130–142.
[11] H.T.Y. Fahmy, A.A. Bekhit, Pharmazie 57 (12) (2002) 800–803.
[12] D. Havrylyuk, B. Zimenkovsky, R. Lesyk, Phosphorous, Sulfur, and Silicon and the Related Elements 184 (2009) 638–650.
[13] H. Wang, D.I. Hammoudeh, A. Viacava Follis, B.E. Reese, J.S. Lazo, S.J. Metallo, E. V. Prochownikl, Mol. Cancer Ther. 6 (2007) 2399.

[14] C. Xing, L. Wang, X. Tang, Y.Y. Sham, Bioorg Med. Chem. 15 (5) (2007) 2167–2176.
[15] L. Wang, F. Kong, C.L. Kokoski, D.W. Andrews, C. Xeng, Bioorg. Med. Chem. Lett. 18 (2008) 236–240.
[16] C. Frances Brown, C. Bradsher, Nature 168 (1951) 171–172.
[17] F.C. Brown, Chem. Rev. 61 (5) (1961) 463.
[18] S.P. Singh, S.S. Parmar, K.V. Raman, I. Stenberg, Chem. Rev. 81 (2) (1981) 175.
[19] C.L. Lee, M.M. Sim, Tetrahedron Lett. 41 (30) (2000) 5729.
[20] W.T. Sing, C.L. Lee, S.L. Yeo, S.P. Lim, M.M. Sim, Bioorg. Med. Chem. Lett. 11 (2) (2001) 91.
[21] M. Zhang, C.D. Wang, S.L. Yu, Z.B. Tian, L. Zhang, Chem. Chin. Univ. 15 (1994) pp. 1647, Chem. Abstr. 122 (1995) 213992z.
[22] A.B. Alloum, S. Bakkas, K. Bougrin, M. Soufiaoui, J. New Chem. 22 (1998) 809.
[23] J.F. Zhou, Org. Prep. Proced. Int. 37 (2005) 75.
[24] B.M. Turkevich, Chem. Heterocyclic Compd. 2 (5) (1966) 535–537.
[25] R. Bieker, C. Lerchenmuffer, J. Wehmeyer, H.L. Serve, R.M. Mesters, T. Buchner, E. Berdel, Oncol. Rep. 10 (2003) 915–920.
[26] J.F. Keur, C.M. Winterford, B.V. Harmon, Cancer 73 (1994) 2013–2026.
[27] H.K. Roy, et al., FEBS Lett. 496 (2, 3) (2001) 143–146.
[28] A. Kumar, et al., Invest New Drugs 26 (5) (2008) 425–435.
[29] I. Nicoletti, G. Migliorati, M.C. Pagliacci, F. Grignani, C. Riccardi, J. Immunol. Methods 139 (1991) 271–279.
[30] M.S. Shahabuddin, M. Nambiar, B. Choudhary, G.M. Advirao, S.C. Raghavan, Invest. New Drugs Jan 14 (2009) [Epub ahead of print].
[31] Kishore K. Chiruvella, Vijayalakshmi Kari, Bibha Choudhary, Mridula Nambiar, Rama Gopal Ghanta, Sathees C. Raghavan, FEBS Lett. 582 (2008) 4066–4076.
[32] C.V. Kavitha, M. Nambiar, C.S. Anandakumar, B. Choudhary, K. Muniyappa, K. S. Rangappa, S.C. Raghavan, Biochem. Pharmacol. 77 (3) (2009) 348–363.