Synthesis of alkyl-5,8-dimethyl-6-phenyl-5,6-dihydropyrazolo[3,4-*f*][1,2,3,5]tetrazepin-4(3*H*)-ones of pharmaceutical interest

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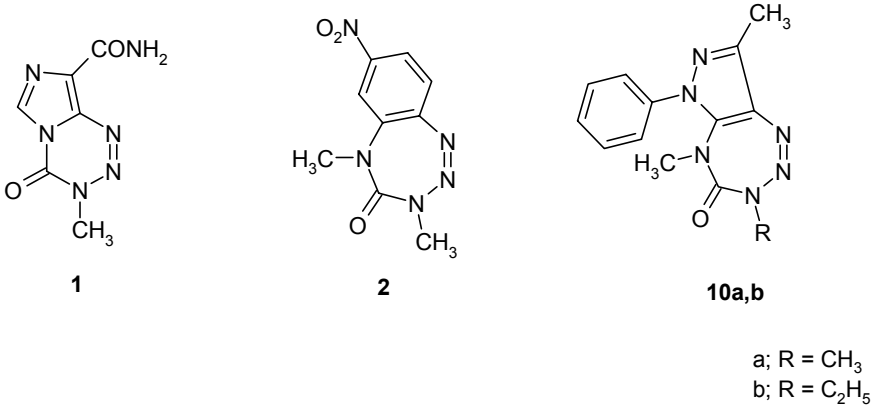
Abstract
The multistep synthesis of two pyrazolo[3,4-*f*][1,2,3,5]tetrazepin-4(3*H*)-one derivatives, a new class of fused 1,2,3,5-tetrazepinones with potential antiproliferative activity, has been carried out. Owing to the instability of the above compounds, the last step of the synthesis was performed at -5/0 °C. The obtained tetrazepinones, when allowed to stand at r.t. for 24 h, afforded quantitatively 1-phenyl-3,6-dimethylpyrazolo [3,4-*d*][1,2,3]triazole.

Keywords: 1,2,3,5-Tetrazepinones, pyrazoles, pyrazolo[3,4-*f*][1,2,3,5]-tetrazepinones, drug resistance, antiproliferative activity

Introduction

The emergence of drug resistance in cancer treatment calls for the availability of new chemotherapeutic agents able to overcome this problem. In 1999 the F.D.A. (USA) approved the DNA-methylating agent temozolomide **1**, principle active of Temodar®, for the treatment of refractory brain tumors.[1-3] Temozolomide leads to the methylation at the O^6-position of guanosine residues in DNA.[4,5] Unfortunately, it also induces high levels of O^6-alkylguanine-DNA alkyltransferase (AGT),[6] a DNA repair protein that removes the guanine methyl group,[7] and as a consequence drug resistance arises. The literature reports the synthesis of some benzo-1,2,3,5-tetrazepin-4(3*H*)-ones which, like temozolomide, contain the N=N-N(CH_3)CO-N atomic sequence.[8-10] Among the above tetrazepinones, compounds **2** was shown to be much more active than temozolomide when tested against a variety of alkylating agent resistant cell lines.[11,12] Taking into account the antiproliferative activity of benzo-1,2,3,5-tetrazepinones and considering that the literature provides very few examples of derivatives containing the tetrazepinone ring fused to a heterocyclic nucleus, we thought it would be of interest to synthesize new derivatives containing the tetrazepinone ring fused to a heterocyclic one, and thereby to gain more insight

into the structure-activity relationship of fused 1,2,3,5-tetrazepinones. Here we describe the attempts for synthesis of pyrazolo[3,4-*f*][1,2,3,5]-tetrazepinone derivatives **10a,b** (see figure 1 and scheme 1).



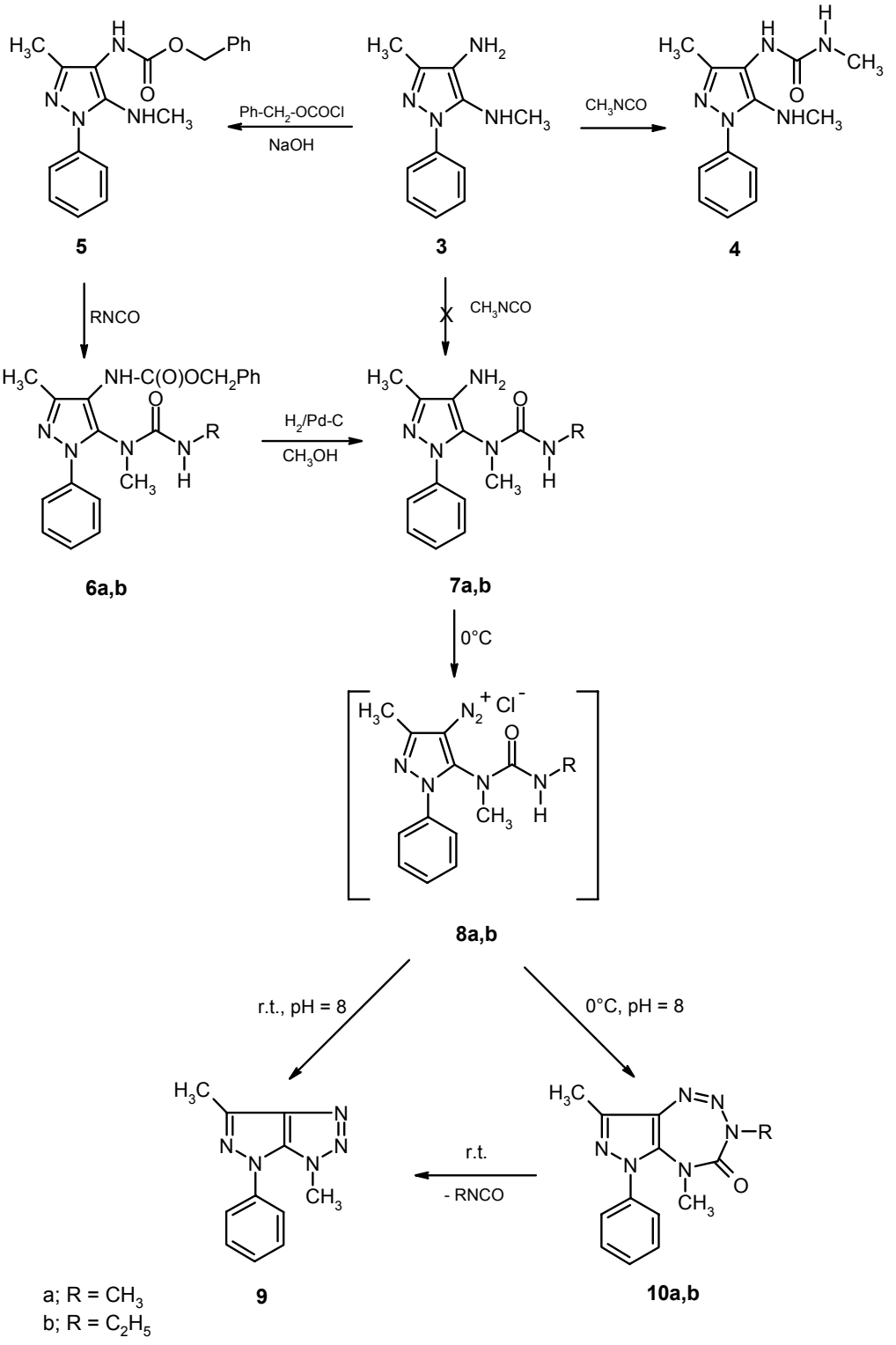
1 **2** **10a,b**

Figure 1

Results and Discussion

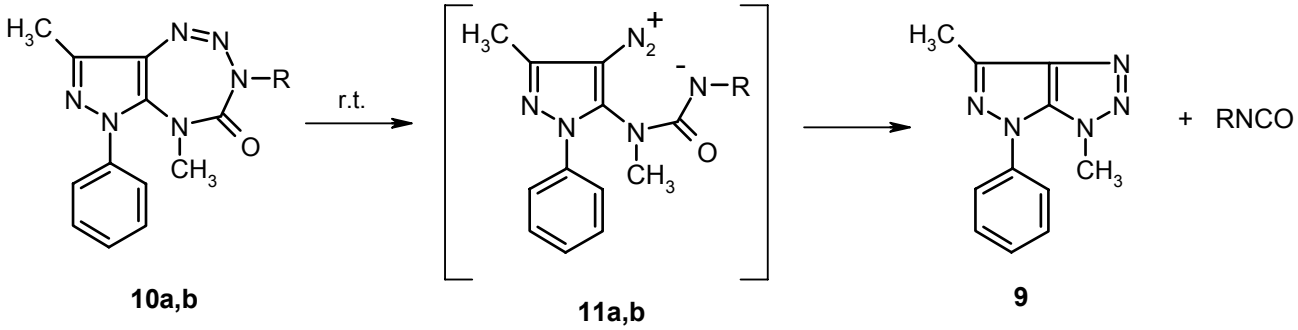
The known starting material **3**, prepared according to literature procedures[13] from 3-methyl-1-phenyl-1*H*-pyrazol-5-amine by a four steps route, was treated with methyl isocyanate in order to obtain the urea derivative **7a** (see scheme 1). However, the structural isomer **4** was instead obtained, thus showing that the pyrazole 4-amino group is more nucleophilic than the 5-methylamino one. At this point the 4-amino group of **3** was protected with a benzyloxycarbonyl group, by treatment with benzyl chloroformate, giving **5** which was then reacted with methyl and ethyl isocyanates. The obtained ureas **6a,b** were deprotected by treatment with hydrogen and 10% Pd-C to give the desired derivatives **7a,b** which, in turn, were diazotized at 0°C with nitrous acid. The diazotization solution of **7a** was adjusted to pH 8 and extracted at r.t. to give a mixture from which a product was separated by column chromatography. We assigned to this compound the structure of the known 1-phenyl -3,6-dimethylpyrazolo[3,4-*d*]triazole **9**[13] on the basis of elemental and spectroscopical data, and realized that compound **10a** lost methyl isocyanate during the work-up. In order to avoid the decomposition of the desired tetrazepinones **10a,b**, the procedure was modified, performing pH adjustment, extraction and evaporation of the extracts at the temperature of -5/0°C. ^{1}H-NMR spectra of extracts registered at 0°C showed that **10a** and **10b** are the unique reaction products formed from **7a** and **7b** respectively. Ring closure was demonstrated by the absence in the NMR spectra of the NH signal of the urea moiety of compounds **7a**,**b**. Both NMR solutions, containing **10a** and **10b,** were allowed to stand at r.t. for 24 h and ^{1}H-NMR spectra were again registered. These spectra showed the identical signals observed in the NMR spectrum of the pyrazolo[3,4-*d*]triazole derivative **9**. The presence of this compound in the solutions was confirmed by TLC, mp and MS measurements. Moreover, each

of the above spectra showed signal(s) that we assigned to methyl and ethyl isocyanate respectively (see experimental).



Scheme 1. Synthetic pathway for the formation of tetrazepinones **10a,b**.

When we added little amounts of the above alkyl isocyanates into the respective NMR solutions and spectra were registered again, it was observed the increase of the intensity of signal(s) previously assigned to isocyanates. These data demonstrated that methyl and ethyl isocyanate were eliminated from tetrazepinones **10a,b** respectively during the standing at r.t. of the solutions (see scheme 1). The formation of the triazole derivative **9** could be rationalized by assuming the heterolitic opening of the N_2-N_3 bond of tetrazepinone ring, that would generate a zwitterion species which, in turn, recyclize to triazole, eliminating an alkyl isocyanate molecule[8] (see scheme 2). Owing to their inherent instability, compounds **10a,b** did not give satisfactory elemental analysis data. Moreover, they could not be tested *in vitro* to evaluate their possible antiproliferative activity.



10a,b **11a,b** **9**

a; R = CH_3
b; R = C_2H_5

Scheme 2. Hypothesized mechanistic pathway of the transformation of **10a,b**.

Experimental Section

General Procedures. All melting points were determined on a Büchi 530 capillary melting point apparatus and are uncorrected; IR spectra were recorded with a Perkin Elmer Spectrum RXI FT-IR System spectrophotometer as solid in KBr disc or nujol mull supported on NaCl disks; ^{1}H-NMR spectra were obtained using a Bruker AC-E 250 MHz spectrometer (tetramethylsilane as an internal standard); Mass spectra to 70 eV were obtained using an Autospec Ultima Ortogonal T.O.F.T. (Micromass) spectrometer or a GC-MS Varian Star 3400cx Saturn III spectrometer. Microanalyses (C, H, N) performed in the laboratories of the Dipartimento di Scienze Farmaceutiche, Università degli Studi di Catania, were within ±0.4% of the theoretical values. Extracts containing the tetrazepinones (**10a,b**) were evaporated by an Edwards RV8 rotary vacuum pump.

1-Methyl-3-[3-methyl-5-(methylamino)-1-phenyl-1*H*-pyrazol-4-yl]urea (4). To a magnetically stirred cold solution (ice bath 0-5°C) of compound **3**[13] (1 mmol) in anhydrous $CHCl_3$ (10 ml), methylisocyanate (0.62 ml, 10 mmol) was added. Stirring was continued overnight at room temperature and then the reaction mixture was evaporated under reduced pressure producing a residue which was crystallized from ethyl acetate to give **4**. Yield 86%; mp 149-151°C; MS (m/z) 259 (M^+); IR (KBr) (cm^{-1}) 3387-3203 (multiple bands, NH), 1672 (CO); ^{1}H-NMR ($CDCl_3$) (δ) 2.16 (3H, s, CH_3); 2.80 (3H, s, CH_3); 2.83 (3H, s, CH_3); 3.53 (1H, br s, exchangeable with D_2O, NH); 5.00 (1H, br s, exchangeable with D_2O, NH); 6.00 (1H, br s, exchangeable with D_2O, NH); 7.35-7.39 (5H, a set of signals, C_6H_5).

Benzyl [3-methyl-5-(methylamino)-1-phenyl-1*H*-pyrazol-4-yl]carbamate (5). To a magnetically stirred cold solution (ice bath 0-5°C) of compound **3**[13] (1 mmol) in 2 ml of 2M aqueous sodium hydroxide/dioxane (1:1) (v/v) benzyl chloroformate (0.157 ml, 1.1 mmol) was added dropwise. Stirring was continued for 1 h and then the reaction suspension was filtered off to obtain the compound (**5**), which was crystallized from ethyl acetate/petroleum ether (b.p. 40-60°C). Yield 87%; mp 95-98°C; MS (m/z) 336 (M^+); IR (nujol) (cm^{-1}) 3521-3246 (multiple bands, NH), 1697 (CO); ^{1}H-NMR ($CDCl_3$) (δ) 2.15 (3H, s, CH_3); 2.71 (3H, s, CH_3); 5.19 (2H, s, CH_2); 6.13 (1H, br s, exchangeable with D_2O, NH); 7.39-7.50 (10H, a set of signals, 2 x C_6H_5).

General procedure for benzyl (3-methyl-5-{[(alkylamino)carbonyl](methyl)amino}-1-phenyl- 1*H*-pyrazol-4-yl)carbamates (6a,b). To a solution of compound **5** (2 mmol) in anhydrous $CHCl_3$ (20 ml), methylisocyanate (1.3 ml, 20 mmol) or ethylisocyanate (2.4 ml, 30 mmol) was added, and the mixture was refluxed for 15 h to obtain compound **6a** or 40 h for compound **6b**. The solutions were concentrated under reduced pressure and the oily residues were crystallized. **6a**: yield 82%; mp 140-141°C [ethyl acetate /petroleum ether (b.p. 40-60 °C)]; MS (m/z): 393 (M^+); IR (KBr) (cm^{-1}) 3386-3198 (multiple bands, NH), 1740 and 1628 (CO); ^{1}H-NMR ($CDCl_3$) (δ) 2.24 (3H, s, CH_3); 2.64 (3H, s, CH_3); 2.80 (3H, s, CH_3); 5.16 (3H, broad signal exchangeable with D_2O for 1H, CH_2 and NH); 6.42 (1H, s, exchangeable with D_2O, NH); 7.35-7.40 (10H, a set of signals, 2 x C_6H_5)**. 6b**: yield 78%; mp 130-131°C (ethyl acetate); MS (m/z) 407 (M^+); IR (KBr) (cm^{-1}) 3396-3211 (multiple bands, NH), 1728 and 1651 (CO); ^{1}H-NMR ($CDCl_3$) (δ) 0.98 (3H, br apparent t, CH_3); 2.25 (3H, s, CH_3); 2.84 (3H, s, CH_3); 3.12 (2H, unresolved signal, CH_2); 5.16 (3H, s, exchangeable with D_2O for 1H, CH_2 and NH); 6.25 (1H, s, NH); 7.36-7.43 (10H, a set of signals, 2 x C_6H_5).

General procedure for 1-(4-amino-3-methyl-1-phenyl-1*H*-pyrazol-5-yl)-3-alkyl-1-methylureas (7a,b). To a solution of compound **6a,b** (0.76 mmol) in methanol (35 ml) 10% Pd-C as a catalyst (30 mg) was added. The mixture was left under hydrogenation in a Parr apparatus at 50 psi for 20 h. After this time the suspension was filtered, and the filtrate was concentrated under reduced pressure to give an oily residue which was crystallized. **7a**: yield 62%; mp 183-186°C (ethyl acetate); MS (m/z) 259 (M^+); IR (KBr) (cm^{-1}) 3399-3230 (NH_2 e NH), 1654 (CO); ^{1}H-NMR ($CDCl_3$) (δ) 2.28 (3H, s, CH_3); 2.81 (3H, d, J = 4.39 Hz, CH_3); 2.95 (3H, s, CH_3); 3.60 (2H, broad signal, exchangeable with D_2O, NH_2); 4.97 (1H, br apparent q, exchangeable with D_2O, NH); 7.27-7.43 (5H, a set of signals, C_6H_5). **7b**: yield 58%; mp 138-140°C (ethyl acetate);

MS (m/z) 273 (M^+); IR (KBr) (cm^{-1}) 3412-3220 (multiple bands, NH_2 e NH), 1661 (CO); ^{1}H-NMR ($CDCl_3$) (δ) 1.04 (3H, t, J = 6.77, CH_3); 2.27 (3H, s, CH_3); 2.94 (2H, s, exchangeable with D_2O, NH_2); 2.96 (3H, s, CH_3); 3.25 (2H, m, CH_2); 4.86 (1H, br apparent t, NH); 7.24-7.41 (5H, a set of signals, C_6H_5).

Trasformation of 1-(4-amino-3-methyl-1-phenyl-1*H*-pyrazol-5-yl)-1,3-dimethylurea (7a) in 3,6-dimethyl-4-phenyl-3,4-dihydropyrazolo[3,4-*d*][1,2,3]triazole (9).[13] To a magnetically stirred cold solution (ice bath 0°C) of compound **7a** (0.8 mmol) in 2N aqueous hydrochloric acid (2.5 ml), a solution of 20% aqueous sodium nitrite (0.28 ml) was added dropwise. Stirring was continued for 1 h at 0°C and after this time the reaction mixture was extracted with dichloromethane (3 x 5 ml). The water phase was treated with a saturated solution of $NaHCO_3$ until pH 8 and then the reaction mixture was extracted with dichloromethane (5 x 7 ml), and the combined extracts were dried (Na_2SO_4) and concentrated under reduced pressure. The oily residue was chromatographed following the Flash Chromatography procedure[14]: external diameter of the column 4 cm, silica gel (32-63 μm), eluent ethyl acetate/petroleum ether (b.p. 40-60 °C) (6:4 V/V) (600 ml). Fractions 7-9 (each 50 ml) were collected and evaporated to give pure **9** as a colorless solid (150 mg), yield 40%; mp 159-160°C (ethanol) (literature mp 166-167°C[13]); MS (m/z) 213 (M^+); ^{1}H-NMR ($CDCl_3$) (δ) 2.57 (3H, s, C-CH_3); 4.14 (3H, s, N-CH_3); 7.30-7.52 (5H, a set of signals, C_6H_5).

General procedure for 3-Alkyl-5,8-dimethyl-6-phenyl-5,6-dihydropyrazolo[3,4-*f*][1,2,3,5] tetrazepin-4(3*H*)-ones (10a,b). To a magnetically stirred cold solution (-5°C) of compound **7a,b** (0.8 mmol) in 2N aqueous hydrochloric acid (2.5 ml) a solution of 20% aqueous sodium nitrite (0.28 ml) was added dropwise, keeping the temperature at -5/0°C. Stirring was continued at the above temperature for 1 h after which the reaction mixture was extracted with cold dichloromethane (-5°C) (3 x 5 ml). The water phase was treated at -5/0°C with a saturated solution of $NaHCO_3$ until pH 8 and then was extracted with cold dichloromethane (-5°C) (5 x 7 ml). The combined extracts were dried (Na_2SO_4) and the solvent was evaporated under reduced pressure (rotary vacuum pump) at 0°C to give a practically pure clear oil. **10a**: yield 78%; MS (m/z) 213 (M^+ -CH_3NCO); ^{1}H-NMR ($CDCl_3$) (δ) 2.42 (3H, s, C-CH_3); 2.81 (3H, s, N-CH_3); 3.44 (3H, s, N-CH_3); 7.48 (5H, br s, C_6H_5). **10b**: yield 83%; MS (m/z) 213 (M^+-C_2H_5NCO); ^{1}H-NMR ($CDCl_3$) (δ) 1.34 (3H, t, J=7.17 Hz, CH_2-$\underline{CH_3}$); 2.43 (3H, s, C-CH_3); 2.80 (3H, s, N-CH_3); 3.94 (2H, q, J=7.17 Hz, $\underline{CH_2}$-CH_3); 7.42-7.53 (5H, a set of signals, C_6H_5).

Trasformation of tetrazepinones (10a,b) in 3,6-dimethyl-4-phenyl-3,4-dihydropyrazolo[3,4-*d*] [1,2,3]triazole (9).[13] The NMR solutions of tetrazepinones **10a,b** were allowed to stand at r.t. for 24 h. After this time ^{1}H-NMR spectra were registered. Both the spectra showed the signals (δ) for the triazole derivative **9**: 2.57 (3H, s, CH_3); 4.14 (3H, s, CH_3). Moreover, they showed the signal(s) (δ) for the eliminated alkyl isocyanates[15]: (from **10a)**: 3.02 (3H, s, CH_3); (from **10b**): 1.28 (3H, t, CH_3), 3.33 (2H, q, CH_2). When a little amount of alkyl isocyanate was added in the respective NMR solution and the ^{1}H-NMR spectra were registered again, the isocyanates signal(s) were intensified. At this point each of the NMR solutions was evaporated under vacuum and the solid residue obtained resulted be identical to **9** (TLC, mp, MS).

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References

1. Reilly, S. M.; Newlands, E. S.; Glaser, M. G.; Brampton, M.; Rice Edwards, J. M.; Illingworth, R. D.; Richards P. G.; Colquhoun C.; Lewis P.; Stevens, M. F. G. *Eur. J. Cancer* **1993**, *29A*, 940.
2. Friedman, H. S.; Kerby, T.; Calvert, H. *Clin. Cancer Res.* **2000**, *6*, 2585.
3. Batchelor, T. *The Lancet* **2000**, *355*, 1115.
4. Newlands, E. S.; Stevens, M. F. G.; Wedge, S. R.; Wheelhouse, R. T.; Brock, C. *Cancer Treat. Rev*. **1997**, *23*, 35.
5. Taverna, P.; Catalano, C. V.; Citti, L.; Bonfanti, M.; D'Incalci, M. *Anticancer Drugs* **1992**, *aug 3(4)*, 401.
6. Friedman, H. S.; Kerby, T.; Calvert, H. *Clin. Cancer Res*. **2000**, *3*, 2585.
7. D'Incalci, M.; Citti, L.; Taverna, P.; Catalano, C. V. *Cancer Treat. Rev*. **1988**, *15*, 279.
8. Jean-Claude, B. J.; Just, G. *Heterocycles* **1998**, *48*, 1347.
9. Dumont-Hornebeck, B.; Strube, Y. N.; Vasilescu, D.; Jean-Claude B. J. *Bioorg. Med. Chem. Lett.* **2000***, 10,* 2325.
10. Jean-Claude, B. J.; Damian, S.; Damian, Z.; Do Khan, L.; Chan, T. H.; Leyland-Jones, B. *Proc. Am. Assoc. Cancer Res.* **1994**, *35*, 402.
11. Jean-Claude, B. J.; Mustafa, A.; Watson, A. J.; Damian, Z.; Vasilescu, D.; Chan, T. H.; Leyland-Jones, B. *J. Pharm. Exper. Ther.* **1999**, *288*, 484.
12. Jean-Claude, B. J.; Mustafa, A.; Damian, Z.; De Marte, J.; Vasilescu, D. E.; Yen, R.; Chan, T. H.; Leyland-Jones, B. *Biochem. Pharm.* **1999**, *57*, 753.
13. Gonzales, E.; Sarlin, R.; Elguero, J. *J. Het. Chem*. **1975**, *12*, 279.
14. Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem*. **1978**, *43*, 2923.
15. Click on www.aist.go.jp/RIODB/SDBS/ and search methyl isocyanate or ethyl isocyanate.