HL
(www.heteroletters.org)
Heterocyclic Letters
Vol. 1, No. 1, (2011), 29-33

SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF NEW 3-METHYL-2-PHENYLSPIRO[PYRANO[2,3-f]CHROMONE-8,1'-CYCLOALKAN/8,4'-PIPERIDIN]-4,10-DIONES

Sreenivas Peddolla and David Krupadanam. G. L.*

Department of Chemistry, Osmania University, Hyderabad-500 007, A. P., India
e-mail:*davidkrupa@hotmail.com*

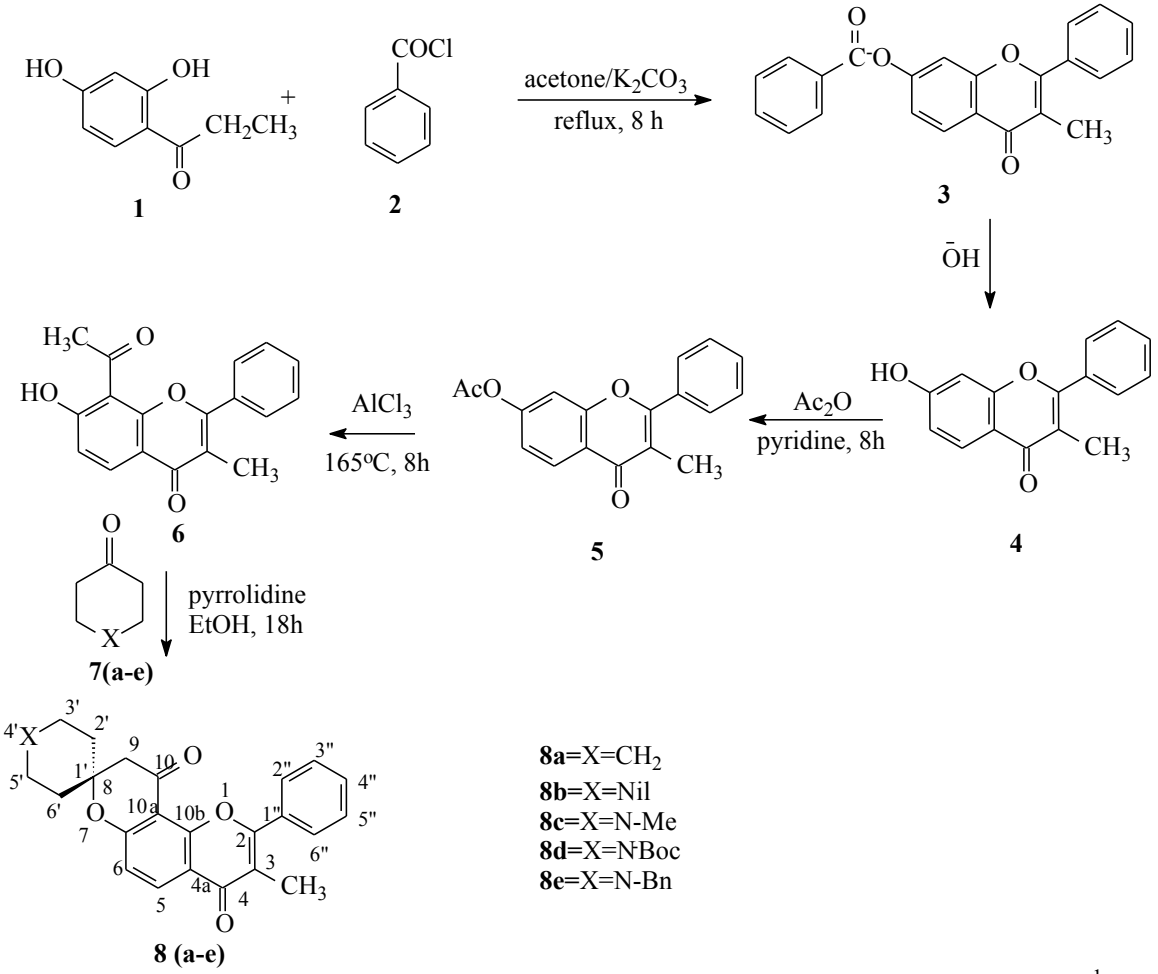
Abstract: 3-Methyl-2-phenylspiro[pyrano[2,3-*f*]chromone-8,1'-cycloalkan/8,4'-piperidin]-4,10-diones **(8a-e)** were synthesized from 8-acetyl-7-hydroxy-3-methylflavone and cycloalkanones/N-substituted piperidones with pyrrolidine as catalyst. All the compounds were tested *in vitro* for their antibacterial activity against Gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus* and Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*. Three compounds **8c, 8d** and **8e** have displayed very good antibacterial activity.

Introduction

Natural and synthetic spirochromanones are reported to have antiarrhythmic[1], antimalarial[2], antihypertensive[3] and free radical scavenging[4] activity. Chromones, flavones and isoflavones are biogenetically closely related heterocyclics reported to have different bioactivity. Marketed flavone drugs include demiflin (coronary vasodilator)[5], flavoxate (diuretic)[6], sylbin (jaundice)[7], flavopyridol (anti cancer)[8] and flavone-8-acetic acid (antineoplastic)[9]. Chromone derived drugs include sorbinil (diabetes)[10], cromakalim (cardiac)[11] and centochroman (contraceptive)[12]. There are no reports of synthesis and antibacterial activity of spirochromanones. Therefore it is considered worthwhile to synthesize 7,8-spirochromanone fused flavones and study their antibacterial activity.

Results and Discussion

Synthesis of 3-methyl-2-phenylspiro[pyrano[2,3-f]chromone-8,1'-cycloalkan/8,4'-piperidin]-4,10-diones (8a-e): The reaction of respropiophenone **(1)** with benzoyl chloride **(2)** in acetone-K_2CO_3 by modified Baker-Venkataraman[13] transformation gave directly 7-benzoyloxy-3-methyl flavone **(3)**. **3** was hydrolysed with 8% methanolic KOH and acidified with dil. HCl to give 7-hydroxy-3-methylflavone **(4)**. **4** on reaction with Ac_2O/pyridine gave 7-O-acetyl flavone **(5)**, which on Fries migration with $AlCl_3$ gave 8-acetyl-7-hydroxy-3-methylflavone **(6)**[14]. Equimolar quantities of 8-acetyl-7-hydroxy-3-methylflavone **(6)** and cycloalkanones/N-substituted piperidones **(7a-e)** in ethanol were stirred for 18 hours at room temperature with pyrrolidine as catalyst[15] to give 3-methyl-2-phenylspiro[pyrano[2,3-f]chromone-8,1'-cycloalkan/8,4'-piperidin]-4,10-diones **(8a-e)**.



In the IR spectrum of **8a,** peaks appeared at 1695 (10-C=O), 1616 (4-C=O). In the ^{1}H -NMR spectrum of **8a**, the newly formed ring protons 9-CH_2 appeared at δ 2.85 as singlet and spirocyclohexane protons (2',3',4',5',6'-CH_2) in the range δ 1.50-1.95 as multiplet. Other peaks appeared at δ 8.21 (d, J=9.0Hz, H-5), 7.16 (d, J=9.0Hz, H-6), phenyl protons at 7.90 (m, H-2",6"), 7.60 (m, H-3",4",5") and 2.10 (s, 3-CH_3). In the ^{13}C-NMR spectrum of **8a**, spiro carbon (C-8) appeared at δ 81.3, and other signal assignments are as follows: 48.8 (C-9), 34.6 (C-2',6'), 25.1 (C-4'), 21.6 (C-3',5'), 188.5 (C-10), 160.3 (C-2), 109.1 (C-3), 177.0 (C-4), 117.8 (C-4a), 133.2 (C-5), 115.9 (C-6), 164.3 (C-6a), 116.6 (C-10a), 155.1 (C-10b), phenyl carbons at 133.1 (C-1"), 128.3 (C-2",6"), 130.2 (C-3",5"), 129.5 (C-4") and 3-CH_3 at δ11.9. In the mass spectrum of **8a**, quasimolecular ion peak appeared at 375 [M+H].

Antibacterial activity

6 and 8a-8e were tested *in vitro* for their antibacterial activity against two Gram-positive bacteria *Bacillus subtilis* MTCC 121 and *Staphylococcus aureus* MTCC 96, and Gram-negative bacteria *Escherichia coli* MTCC 739 and *Pseudomonas aeruginosa* MTCC 2453 using streptomycin as standard. Three compounds **8c, 8d** and **8e** exhibited very good antibacterial activity (expressed in terms of area of inhibition) against both Gram-positive bacteria ranging from 17.6-18.9±0.61-0.84, 16.1-17.4±0.88-0.92, and 15.6-17.5±0.68-0.8 respectively and Gram-negative bacteria ranging from 16.4-17.5±0.82-0.91, 15.5-17.6±0.71-0.86, and 16.1-18.2±0.52-0.63 respectively. **6** showed moderate activity against both Gram positive (11.6-14.4±0.56-0.68) and Gram-negative (10.4-11.2±0.56-0.64) bacteria

respectively. Compounds **8a** and **8b** did not show any activity. Streptomycin showed activity (16.4-19.6±0.86-0.92) against all the above mentioned bacteria.

Conclusions

We described herein an efficient and convenient synthesis of new compounds **8a-8e**. The results on the antibacterial activity are encouraging as 3 out of 6 compounds tested **8c, 8d** and **8e** showed very good antibacterial activity.

Acknowledgements

Authors thanks to Srikanth Reddy, M and Kishore Reddy, T.V of Department of Botany, Osmania University for screening antibacterial activity. One of the authors SP thanks to CSIR, New Delhi for their financial assistance in the form of JRF & SRF.

Experimental Section

All melting points are uncorrected and determined on Polmon digital melting point apparatus (Model No. MP-96). IR spectra were recorded on Schimadzu 435 spectrophotometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded at 300MHz and 75.5MHz on Varian Gemini Unity spectrometer using TMS as internal standard (chemical shifts in δ ppm). The mass spectra were recorded on a VG micro mass 7070-H instrument and LSIMS spectra were recorded on VG AUTOSPEC mass spectrometer.

i) Synthesis of 7-hydroxy-3-methylflavone (4):

A mixture of respropiophenone **(1)** (3.32g, 20 mmol), benzoyl chloride **(2)** (5.1mL, 44 mmol) and anhy.K_2CO_3 (10.0g) in dry acetone was refluxed for 8 h. The acetone was removed from the solution under reduced pressure and the residue was treated with ice-cold water (400mL). The solution was neutralized with dil.HCl, the crude ester 7-benzoyloxy-3-methylflavone **(3)** that separated out was filtered. The crude ester **3** was hydrolyzed with methanolic KOH (8%, 100mL) by refluxing for half an hour. The methanol was distilled under reduced pressure and the reaction mass was treated with ice cold water (400ml) and filtered. The filtrate was neutralized with cold dil. HCl to obtain a solid and filtered. The separated product was washed with saturated sodium bicarbonate solution, dried and recrystallised from methanol to give 7-hydroxy-3-methylflavone **(4)** (4.1g, 81% yield) as light brown color needles. mp. 276 °C (lit. mp 278 °C)[13] .

IR (KBr): 3167 cm^{-1}(OH), 1623 cm^{-1}(C=O); ^{1}H NMR ($CDCl_3$): δ 7.95 (d, J=9.0Hz, H-5), 7.59 (m, H-2',6'), 7.48 (m, H-3',4',5'), 6.85 (dd, J=9.0Hz, 2.1Hz, H-6), 6.76 (d, J=2.1Hz, H-8), 2.06 (s, 3-CH_3):^{13}C NMR ($CDCl_3$+DMSO-d_6)(75.5MHz): δ 175.2 (C-4, C=O) 161.7 (C-7), 158.0 (C-8a), 155.9 (C-2), 131.5 (C-1'), 128.2 (C-4'), 127.0 (C-3', 5'), 126.6 (C-2', 6'), 124.9 (C-5), 114.2 (C-4a), 113.5 (C-6), 112.8 (C-3), 100.2 (C-8), 9.6 (3-CH_3). FAB MS: *m/z* 253$[M+H]^+$

ii) Synthesis of 7-O-acetyl-3-methylflavone (5):

To a stirred solution of 7-hydroxy-3-methylflavone **(4)** (2.53g, 10mmol) and acetic anhydride (1.5mL, 15mmol) was added pyridine (0.5mL) and further stirred at room temperature for overnight. After completion of the reaction, it was poured into crushed ice and the solid that separated was filtered to give 7-O-acetyl-3-methylflavone **(5)** (2.85g, 96%).

mp. 192°C : IR (cm^{-1}): 1684 (1"-C=O), 1646 (4-C=O). ^{1}H NMR ($CDCl_3$): δ 8.32 (d, J=9.2 Hz, H-5), 7.65 (m, H-2', 6'), 7.57 (m, H-3', 4', 5'), 7.17 (d, J=9.2 Hz, H-6),) 2.40 (s, H-7-OAc), 2.12 (s, 3-CH_3); ^{13}C NMR ($CDCl_3$): 187.9 (C-1"), 177.0 (C-4), 163.5 (C-7), 160.4 (C-8a), 154.2 (C-2), 133.3 (C-5), 132.7 (C-1'), 130.3 (C-3', 5'), 129.3 (C-4'), 128.2 (C-2', 6'), 117.7 (C-4a), 116.6 (C-8), 115.7 (C-6), 109.0 (C-3), 33.0 (O=C-$\underline{C}H_3$), 11.7 (3-CH_3); ESIMS: *m/z* 295 [M+H].

iii) Synthesis of 8-acetyl-7-hydroxy-3-methylflavone (6):

A mixture of 7-O-acetyl-3-methylflavone **(5)** (2.94g, 10mmol) and anhy.$AlCl_3$ (2.64g, 20mmol) heated at 165^oC for 8 hours, then added 50% aq. HCl to the reaction mixture under ice cooling, extracted with ethyl acetate (2x20mL), dried, concentrated and purified by column chromatography using chloroform to give 8-acetyl-7-hydroxy-3-methylflavone **(6)** (2.14g, 72%).

mp 148°C ; IR (cm^{-1}): 1694 (1"-C=O), 1634 (4-C=O); ^1H NMR ($CDCl_3$) δ 13.93 (s, 7-OH), 8.38 (d, J=9.2 Hz, H-5), 7.62 (m, H-2",6"), 7.55 (m, H-3",4",5"), 7.02 (d, J=9.2 Hz, H-6),), 2.75 (s, H-8-OAc), 2.18 (s, 3-CH_3); ^{13}C NMR ($CDCl_3$): δ 203.1 (C=O of 8-Ac), 177.0 (C-4), 163.5 (C-7), 160.4 (C-2), 154.2 (C-8a), 133.3 (C-5), 132.7 (C-1"), 130.3 (C-3",5"), 129.3 (C-4"), 128.2 (C-2",6"), 117.7 (C-4a), 116.6, 116.1 (C-6), 108.0 (C-3), 32.5 (O=C-$\underline{C}H_3$), 11.8 (2-CH_3); ESIMS: *m/z* 295 [M+H].

iv) General procedure for the synthesis of 3-methyl-2-phenylspiro[pyrano[2,3-f] chromone-8,1'-cycloalkan/8, 4'-piperidin]-4,10-diones (8a-e):

To a stirred solution of 8-acetyl-7-hydroxy-3-methylflavone **(6)** (2.94 g, 10mmol) dissolved in ethanol (50mL) and pyrrolidine (1mmol) was added and stirred at rt for 15 minutes then cycloalkanone/N-substituted piperidone **(7a-e)** was added and stirred for 18 hours at rt. The reaction monitored by TLC. After the completion of the reaction distill out the ethanol, pour in to crushed ice, solid that separated was filtered and chromatographed over silica gel to give corresponding products **(8b-8e).**

3-Methyl-2-phenylspiro[pyrano[2,3-f]chromone-8,1'-cyclohexan]-4,10-dione (8a): mp 186 °C; yield 83%; IR (cm^{-1}): 1695(10-C=O), 1616(4-C=O); ^1H NMR ($CDCl_3$) δ 8.21(d, J=9.0 Hz, H-5), 7.90 (m, H-2", 6"), 7.60 (m, H-3", 4", 5"), 7.16 (d, J=9.0 Hz, H-6), 2.85 (s, 9-CH_2), 2.10 (s, 3-CH_3), 1.50-1.95 (m, H-2', 3', 4', 5', 6'); ^{13}C NMR ($CDCl_3$) : δ 188.5 (C=O, C-10), 177.0 (C=O), 164.3 (C-6a), 160.3 (C-2), 155.1 (C-10b), 133.2 (C-5), 133.1 (C-1"), 130.2 (C-3", 5"), 129.5 (C-4"), 128.3(C-2", 6"), 117.8 (C-4a), 116.6 (C-10a), 115.9 (C-6), 109.1 (C-3), 81.3 (C -8), 48.8 (C-9), 34.6 (C-2', 6'), 25.1 (C-4'), 21.6 (C-3', 5'), 11.9 (3-Methyl); ESIMS: *m/z* 375 [M+H].

3-Methyl-2-phenylspiro[pyrano[2,3-f]chromone-8,1'-cyclopentan]-4,10-dione (8b): mp. 221 °C; yield 80%; IR (cm^{-1}): 1697(10-C=O), 1633(4-C=O); ^1H NMR ($CDCl_3$): δ 8.18 (d, J=9.0 Hz, H-5), 7.85 (m, H-2", 6"), 7.58 (m, H-3", 4", 5"), 7.16 (d, J=9.0 Hz, H-6), 2.95 (s, H-9), 2.10 (s, 3-CH_3), 1.70-1.98 (m, 8H); ^{13}C NMR ($CDCl_3$, 75 MHz) : 188.4 (C-10), 176.9 (C-4), 164.8 (C-6a), 160.3 (C-2), 155.0 (C-10b), 132.8 (C-5), 130.2 (C-1"), 129.4 (C-3", 5"), 128.2 (C-2", 4", 6"), 117.8 (C-4a), 116.4 (10a), 116.0 (C-6), 109.2 (C-3), 90.1 (C-8), 47.6 (C-9), 37.3 (C-2', 5'), 23.9 (C-3', 4'), 11.8 (C-3-Me). ESIMS: *m/z* 361 [M+H].

1',3-Dimethyl-2-phenylspiro[pyrano[2,3-f]chromone-8,4'-piperidin]-4,10-dione (8c): mp. 192 °C; yield 79%; IR (cm^{-1}): 1693 (10-C=O), 1626 (4-C=O); ^1H NMR ($CDCl_3$): δ 8.22 (d, J=9.0 Hz, H-5), 7.90 (m, 2H, H-2", 6"), 7.60 (m, H-3", 4", 5"), 7.15 (d, J=9.0 Hz, H-6), 2.88 (s, 9-CH_2), 2.30 (m, H_a-2', 6'), 2.20 (s, N-CH_3), 2.10 (s, 3- CH_3), 1.98 (m, H_e-2', 6'), 1.96 (m, H_e-3', 5'), 1.86 (m, H_e-3', 5'); ^{13}C NMR($CDCl_3$): 187.8 (C-10), 176.6 (C-4), 163.6 (C-6a), 160.0 (C-2), 154.7 (C-10b), 132.9 (C-5), 132.7 (C-1"), 130.0 (C-3", 5"), 129.3 (C-4"), 128.1 (C-2", 6"), 117.6 (C-4a), 116.4 (C-10a), 115.6 (C-6), 108.9 (C-3), 78.5 (C-8), 50.5 (C-2', 6'), 48.3 (C-9), 45.9 (N-Me), 33.9 (C-3', 5'), 11.6 (3-CH_3); ESIMS: *m/z* 390 [M+H].

1'-*tert*-butyloxycarbonyl-3-methyl-2-phenylspiro[pyrano[2,3-f]chromone-8,4'-piperidin]-4,10-dione(8d): mp. 216 °C; yield 79%; IR (cm^{-1}): 1696 (10-C=O), 1622 (4-C=O); ^1H NMR ($CDCl_3$): δ 8.39 (d, J=9.0 Hz, H-5), 7.89 (m, H-2", 6"), 7.55 (m, H-3", 4", 5"), 7.02 (d, J=9.0 Hz, H-6), 3.90 (m, H_e-2', 6'), 3.22 (m, H_a-2', 6') 2.79 (s, 9-CH_2), 2.25 (s, 3-CH_3), 2.04 (m, H_e-3', 5'), 1.65 (m, H_a-3', 5'), 1.25 (s, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$) : 187.9 (C-10), 177.0 (C-4), 163.5 (C-6a), 160.4 (C-2), 154.8 (N-C=O), 154.2 (C-10b), 133.3 (C-5), 132.7 (C-1"), 130.3 (C-3", 5"), 129.3 (C-4"), 128.2 (C-2", 6"), 117.7 (C-4a),

116.6 (C-10a), 115.7 (C-6), 109.0 (C-3),79.6 (C(CH$_3$)$_3$), 79.1 (C-8), 48.4 (C-9), 39.3 (C-2', 6'), 33.7 (C-3', 5'), 28.3 (C(CH$_3$)$_3$), 11.7 (2-CH$_3$); ESIMS: *m/z* 476 [M+H].

1'-Benzyl-3-methyl-2-phenylspiro[pyrano[2,3-f]chromone-8,4'-piperidin]-4,10-dione (8e): mp. 142 °C; yield 81%; IR (cm^{-1}): 1694 (10-C=O), 1633 (4-C=O); ^{1}H NMR (CDCl$_3$, 200 MHz): δ 8.21 (d, J=9.0 Hz, H-5), 7.89 (m, 2H, H-2", 6"), 7.59 (m, 3H, H-3", 4", 5") 7.25 (m, 5H, N-CH$_2$-Ph), 7.12 (d, J=9.0 Hz, H-6), 3.50 (s, 2H, N-CH$_2$-Ph), 2.79 (s), 2.58 (m, 2H, H$_e$-2',6'), 2.25(m, 2H, H$_a$-2',6'), 2.10 (s, 3-CH$_3$), 2.04 (m, 2H, H$_e$-3',5'), 1.65 (m, 2H, H$_a$-3',5'); ^{13}C NMR (CDCl$_3$) : 187.9 (C-10), 176.7 (C-4), 163.6 (C-6a), 160.1 (C-2), 154.7 (C-10b), 137.7 (C-5), 132.9 (C-1'"), 132.6 (C-1"), 130.0, 128.6, 127.0 (benzyl), 129.2 (C-3",5"), 128.5 (C-4"), 126.9 (C-2",6"), 111.5 (C-4a), 116.3 (C-10a), 115.7 (C-6), 108.8 (C-3), 79.0 (C-8), 62.6 (N-CH$_2$), 52.7 (C-2', 6'), 48.3 (C-9), 33.9 (C-3', 5'), 11.6 (3-CH$_3$); ESIMS: *m/z* 465 [M+H].

Antibacterial study

The antibacterial activity was determined by disc diffusion method[16]. The compounds dissolved in DMSO (1 mg/ml) in which the Whatman paper No. 1 paper disks (6 mm diameter) impregnated, dried under sterile flow box and put on an agar plates inoculated on nutrient agar plates previously seeded with test bacteria. The plates were incubated at 37°C for bacteria (18-24 hours) and examined for the zones of inhibition. Streptomycin used as positive and negative control. The organisms *Bacillus subtilis* MTCC 121, *Staphylococcus aureus* MTCC 96, *Escherichia coli* MTCC 739, *Pseudomonas aeruginosa* MTCC 2453 used for antimicrobial assays. The experiment was performed in triplicates.

References

1. J. M. Elliott, H. G. Selnick, D. A. Claremon, J. J. Baldwin, S. A. Buhrow, J. W. Butcher, C. N. Habecker, S.W. King, J. J. Lynch, B. T. Philips, G. S. Ponticello, E. M. Radzilowski, D. C. Remy, R. B. Stein, J. I. White, M. B. Young, *J. Med. Chem*. **35**, 3973-3976 (1992).
2. (i) G. W. Quin, H. C. Chen, H. C. Wang, M. K. Qian, *Huaxue Xuebao*, **39**, 83-85 (1981). (ii) R. Xu, J. K.Snyder, K. Nakanishi, *J. Am. Chem. Soc*. **106**, 734-736 (1984).
3. R. Bergmann, R. Gericke, *J. Med. Chem.* **33**, 492-504 (1990).
4. V. Panteleon, P. Marakos, N. Pouli, E. Mikros, I. Andreadou, *Chem. Pharm. Bull*. **51**, 522-529 (2003).
5. P. D. Re, L. Sagramora, V. Mancini, P. Valenti, L. Cima, *J. Med. Chem*. **13**, 527-531 (1970).
6. I. Setniker, A. Cova, M. Magistretti, *J. Arzneimittal-Forch*, **25**, 1916-1917 (1975).
7. M. Bandopadhyay, N. P. Paradeshi, T. R. Sheshadri, *Indian J. Chem*. **10**, 808-809 (1972).
8. M. Lopez-Lazaro, *Current Med. Chem.; Anti-Cancer Agents,* **2**, 691-714 (2002).
9. R. K. Y. Zee-Cheng, C. C. Cheng, *Drugs of the Future*, **12**, 123-125 (1987).
10. A. C. Lipinski, E. C. Aldinger, A.T. Beyer, J. Border, F.D. Burdi, L. B. Bussolotti, B. P. Inskeep, W. T. Siegel, *J. Med. Chem*. **35**, 2169-2177 (1992).
11. S, K. Atwal, J. G. Grover, Z. S. Ahmed, N. F. Ferrora. N. T. Harper, S. Kim, G. P. Sleph, S. Dzwonczyk, D. A. Russel, S. Moreland, R. J. Mecullough, E. D. Normandin, *J. Med. Chem*. **36**, 3971-3974 (1993).
12. V. P. Kamboj, S. Ray, B. N. Dhawan, *Drugs Today* **28**, 227-232 (1992).
13. D. S. Bapat, K. Venkataraman, *Indian Acad. Sci*. **21B**, 214-217 (1955).
14. J. V. R. Sarma, G. Srimannarayana, N. V. Subba Rao, *Indian J. Chem*. **13**, 228-232 (1975).
15. H. J. Kabbe, *Synthesis*, 886-889 (1978).
16. R. Y. Wu, *Bot Bull Acad Sin*, **25,** 111–123 (1984).