Department of Pharmaceutical and Medicinal Chemistry, University College of Pharmaceutical Sciences, Kakatiya University, Warangal, India

# Synthesis of new 2-substituted-[1,3,4]-oxadiazino-[5,6-*b*]-indoles with $H_1$-antihistaminic, antimuscarinic and antimicrobial activity

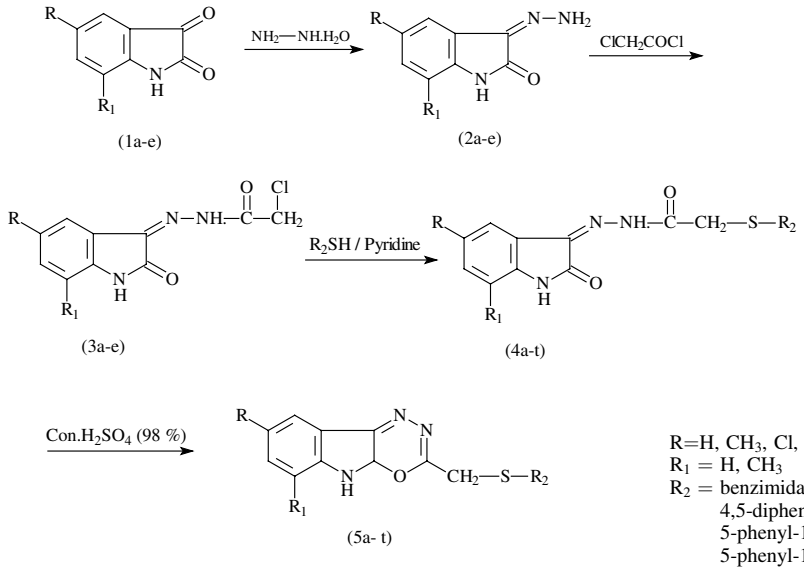
M. AJITHA, K. RAJNARAYANA and M. SARANGAPANI

New 2-substituted-[1,3,4]-oxadiazino-[5,6-*b*]-indoles have been prepared and tested for their antibacterial, antifungal, $H_1$-antihistaminic and antimuscarinic activities. Among them, compounds **5b**, **5d**, **5k** exhibited higher $H_1$-antihistaminic activity than pheniramine maleate. Compounds **5c**, **5d** showed higher antibacterial activity than ampicillin against *Staphylococcus aureus* and *E. coli*, respectively.

## 1. Introduction

Heterocyclic systems possessing an indole moiety exhibit a number of interesting biological activities such as antiviral, antibacterial, antifungal, anti-inflammatory, analgesic, antifertility, diuretic and anticonvulsant activities [1–8]. A lot of work has been carried out on indole derivatives and little work has been done on [1,3,4]oxadiazino-[5,6-*b*]indoles. It is also evident from the literature that benzimidazoles, imidazoles, oxadiazoles and thiadiazoles are equally important in terms of pharmacological activities. Therefore, it seemed promising to synthesize some new [1,3,4]oxadiazino-[5,6-*b*]indoles combining the pharmacologically prominent heterocyclic systems at 2-position through the sulfur linkage and to screen them for antifungal, antibacterial, antimuscarinic, and $H_1$-antihistaminic activities. We present here our results on the design of new 2-substituted-[1,3,4]-oxadiazino-[5,6-*b*]-indoles emphasizing in particular the presence of both benzimidazolyl, 4,5-diphenyl imidazolyl, 5-phenyl−1,3,4-oxadiazolyl and 5-phenyl−1,3,4-thiadiazolyl in one skeleton (**5a–e**, **5f–j**, **5k–o**, **5p–t** Scheme). All the compounds presented here were assayed *in vitro* for their antifungal, antibacterial, $H_1$-antihistaminic, and antimuscarinic activities.

**Scheme**




R=H, $CH_3$, Cl, Br
$R_1$ = H, $CH_3$
$R_2$ = benzimidazolyl;
4,5-diphenylimidazolyl;
5-phenyl-1,3,4-oxadiazolyl;
5-phenyl-1,3,4-thiadiazolyl

1a, 2a, 3a: R=H, $R_1$=H
1b, 2b, 3b: R=CH3, $R_1$=H
1c, 2c, 3c: R=Cl, $R_1$=H
1d, 2d, 3d: R=Br, $R_1$=H
1e, 2e, 3e: R=H, $R_1$=$CH_3$
4a, 5a: R=H, $R_1$=H, $R_2$=$C_7H_5N_2$ (benzimidazole)
4b, 5b: R=$CH_3$, $R_1$=H, $R_2$=$C_7H_5N_2$ (benzimidazole)
4c, 5c: R=Cl, $R_1$=H, $R_2$=$C_7H_5N_2$ (benzimidazole)
4d, 5d: R=Br, $R_1$=H, $R_2$=$C_7H_5N_2$ (benzimidazole)
4e, 5e: R=H, $R_1$=$CH_3$, $R_2$=$C_7H_5N_2$ (benzimidazole)
4f, 5f: R=H, $R_1$=H, $R_2$=$C_{15}H_{11}N_2$ (4,5-diphenylimidazole)
4g, 5g: R=$CH_3$, $R_1$=H, $R_2$=$C_{15}H_{11}N_2$ (4,5-diphenylimidazole)
4h, 5h: R=Cl, $R_1$=H, $R_2$= $C_{15}H_{11}N_2$ (4,5-diphenylimidazole)
4i, 5i: R=Br, $R_1$=H, $R_2$=$C_{15}H_{11}N_2$ (4,5-diphenylimidazole)
4j, 5j: R=H, $R_1$=$CH_3$, $R_2$=$C_{15}H_{11}N_2$ (4,5-diphenylimidazole)
4k, 5k: R=H, $R_1$=H, $R_2$=$C_8H_5N_2O$ (5-phenyl-1,3,4-oxadiazole)
4l, 5l: R=$CH_3$, $R_1$=H, $R_2$=$C_8H_5N_2O$ (5-phenyl-1,3,4-oxadiazole)
4m, 5m: R=Cl, $R_1$=H, $R_2$=$C_8H_5N_2O$ (5-phenyl-1,3,4-oxadiazole)
4n, 5n: R=Br, $R_1$=H, $R_2$=$C_8H_5N_2O$ (5-phenyl-1,3,4-oxadiazole)
4o, 5o: R=H, $R_1$=$CH_3$, $R_2$=$C_8H_5N_2O$ (5-phenyl-1,3,4-oxadiazole)
4p, 5p: R=H, $R_1$=H, $R_2$=$C_8H_5N_2S$ (5-phenyl-1,3,4-thiadiazole)
4q, 5q: R=$CH_3$, $R_1$=H, $R_2$=$C_8H_5N_2S$ (5-phenyl-1,3,4-thiadiazole)
4r, 5r: R=Cl, $R_1$=H, $R_2$=$C_8H_5N_2S$ (5-phenyl-1,3,4-thiadiazole)
4s, 5s: R=Br, $R_1$=H, $R_2$=$C_8H_5N_2S$ (5-phenyl-1,3,4-thiadiazole)
4t, 5t: R=H, $R_1$=$CH_3$, $R_2$=$C_8H_5N_2S$ (5-phenyl-1,3,4-thiadiazole)

## 2. Investigations, results and discussion

### 2.1. Synthesis of the compounds

The reaction sequence used in the synthesis of the target compounds **5a**–**5t** is depicted in the Scheme. Isatin hydrazones **2a**–**e** were obtained from an appropriate isatin in alcohol with dropwise addition of hydrazine hydrate [9]. Compounds **3a**–**e** were synthesized by refluxing **2a**–**e** with chloroacetyl chloride in dry benzene under anhydrous conditions using calcium chloride guard tube for 2 h [10]. Isatin-3-[$N^2$-(heteryl-2-thioacetyl)] hydrazones **4a**–**t** were synthesized by refluxing **3a**–**e** with an appropriate heteryl-2-thione (benzimidazole-2-thione [11]; 4,5-diphenyl imidazole-2-thione [12]; 5-phenyl-1,3,4-oxadiazole-2-thione [13]; and 5-phenyl-1,3,4,-thiadiazole-2-thione [14]) in dry pyridine for 30 min. 2-Substituted-[1,3,4]-oxadiazino-[5,6-*b*] indoles **5a**–**t** were synthesized by cyclization of **4a**–**t** with 10 ml of concentrated sulphuric acid. All the newly synthesized compounds were characterized by physical, spectral (IR, PMR) and elemental analysis.

### 2.2. Antibacterial and antifungal assays

Antibacterial and antifungal activity screening was carried out using the cup plate method [15]. Test organisms used were the bacteria: *Bacilus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus macarances* and the fungi: *Pencillium minioluteum, Fusarium solani.*

Since the compounds were poorly water soluble, they were dissolved in propylene glycol. In order to ensure that the solvent had no effect on bacterial growth, an inoculated control test was performed with only propylene glycol at the same dilutions used in our experiment and found inactive in culture media. The compound suspensions were added at the desired concentration into nutrient agar medium for bacteria and potato-dextrose agar medium for fungi. After solidification, 1 µl of the final suspension of $10^8$ bacteria or $10^5$ fungi/ml were applied with a multipoint inoculator. Cultures were incubated for 24 h at 37 °C for bacteria and 48 h at 25 °C for fungi. Ampicillin and clotrimazole were used as reference compounds. The lowest concentration of compounds that completely inhibited growth was considered to be the minimum inhibitory concentration (MIC) expressed in µg/ml. MIC was the mean of three measurements, results are presented in Table 1.

### 2.3. $H_1$-Antihistaminic and antimuscarinic activity

The title compounds were screened for $H_1$-antihistaminic activity on guinea pig ileum and antimuscarinic activity on rat jejunum by standard methods [16–17]. Then the $IC_{50}$ values of all the test compounds were recorded and compared with that of the standard drugs. The compounds with benzimidazolyl (**5b** and **5d**) and oxadiazolyl (**5k**) substituents showed the highest $H_1$-antihistaminic activity and were more potent than pheniramine maleate. Compounds **5a**–**t** showed very low antimuscarinic activity as compared to atropine sulphate. Compounds with benzimidazolyl moiety (**5c** and **5d**) showed better antibacterial activity against *Staphylococcus aureus* and *Echerichia coli* respectively than ampicillin. Compounds **5a**–**t** showed lower antifungal activity as compared to clotrimazole. The compounds with benzimidazolyl substituent were found to be comparatively more potent. The chloro and bromo substituents on the indole nucleus of the compounds enhanced $H_1$-antihistaminic and antimicrobial activities.

**Table 1: Physical and spectral data for 2-substituted-[1,3,4]-oxadiazino-[5,6-b]-indoles**

| Compd. | R | $R_1$ | $R_2$ | Mol. formula | M.p. (°C) | UV ($\lambda_{max}$, mm) $CHCl_3$ | IR (KBr) ($cm^{-1}$) C=O | Mass spectra/$H^1$ NMR |
|---|---|---|---|---|---|---|---|---|
| **5a** | H | H | benzimidazolyl | $C_{17}H_{11}N_5OS$ | 243 | 324.1 | 1688 | 358 (NH), 7.3 (S, 1 H, NH at 2), 7.1–7.5 (m, 4 H, $C_6$, $H_4$ at 2), 4.1 (s, 3 H, $CH_3$ at 2), 1.3 (s, 3 H, $CH_3$ at 6) |
| **5b** | $CH_3$ | H | | $C_{18}H_{13}N_5OS$ | 232 | 320.7 | 1675 | |
| **5c** | Cl | H | | $C_{17}H_{10}N_5OSCl$ | 188 | 324.5 | 1684 | |
| **5d** | Br | H | | $C_{17}H_{10}N_5OSBr$ | 210 | 317.5 | 1679 | |
| **5e** | H | $CH_3$ | | $C_{18}H_{13}N_5OS$ | 231 | 315.0 | 1688 | |
| **5f** | H | H | 4,5-diphenyl-imidazolyl | $C_{25}H_{17}N_5O$ | 273 | 305.0 | 1705 | 342 (NH), 2.3 (S, 1 H, NH at 2), 5.1–5.5 (m, 4 H, $C_6$, $H_4$ at 2), 3.4 (s, 3 H, $CH_3$ at 2), 1.8 (s, 3 H, $CH_3$ at 6) |
| **5g** | $CH_3$ | H | | $C_{26}H_{19}N_5O$ | 265 | – | 1705 | |
| **5h** | Cl | H | | $C_{25}H_{16}N_5OCl$ | 270 | – | 1715 | |
| **5i** | Br | H | | $C_{25}H_{16}N_5OBr$ | 272 | 336.4 | 1674 | |
| **5j** | H | $CH_3$ | | $C_{26}H_{19}N_5O$ | 266 | – | 1683 | |
| **5k** | H | H | 5-phenyl-1,3,4-oxadiazolyl | $C_{18}H_{11}N_5O_2$ | 235 | 356.5 | 1687 | 331 (NH), 3.3 (S, 1 H, NH at 2), 3.1–3.5 (m, 4 H, $C_6$, $H_4$ at 2), 2.4 (s, 3 H, $CH_3$ at 2), 1.2 (s, 3 H, $CH_3$ at 6) |
| **5l** | $CH_3$ | H | | $C_{19}H_{13}N_5O_2$ | 263 | 323.2 | 1705 | |
| **5m** | Cl | H | | $C_{18}H_{10}N_5O_2Cl$ | 258 | – | 1682 | |
| **5n** | Br | H | | $C_{18}H_{10}N_5O_2Br$ | 260 | – | 1685 | |
| **5o** | H | $CH_3$ | | $C_{19}H_{13}N_5O_2$ | 261 | 316.5 | 1665 | |
| **5p** | H | H | 5-phenyl-1,3,4-thiadiazolyl | $C_{18}H_{11}N_5OS$ | 237 | 321.6 | 1710 | 321 (NH), 1.3 (S, 1 H, NH at 2), 4.1–4.3 (m, 4 H, $C_6$, $H_4$ at 2), 1.5 (s, 3 H, $CH_3$ at 2), 1.8 (s, 3 H, $CH_3$ at 6) |
| **5q** | $CH_3$ | H | | $C_{19}H_{13}N_5OS$ | 266 | – | 1655 | |
| **5r** | Cl | H | | $C_{18}H_{10}N_5OSCl$ | 283 | 341.0 | 1660 | |
| **5s** | Br | H | | $C_{18}H_{10}N_5OSBr$ | 285 | – | 1705 | |
| **5t** | H | $CH_3$ | | $C_{19}H_{13}N_5OS$ | 268 | – | 1685 | |

**Table 2: Pharmacological data of 2-substituted-[1,3,4]-oxadiazino-[5,6-b]-indoles**

| Compd. | Recry. Solv (EtOH) Yield(%) | $H_1$-anti histaminic activity* | Anti muscarinic activity* | Anti-fungal activity** | | Anti-bacterial activity** | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | *F. solani* | *P. minioleuteum* | *B. subtilis* | *B. macerences* | *E. coli* | *S. aureus* |
| **5a** | 47 | – | – | 10 | 11 | 6 | 8 | 9 | 10 |
| **5b** | 54 | 275[a] | 1652.8 | 12 | 11 | 10 | 5 | 11 | 8 |
| **5c** | 46 | 690 | 621.7 | 7 | 13 | 11 | 10 | 18[a] | 23[a] |
| **5d** | 58 | 470[a] | 550 | 7 | 13 | 13 | 12 | 22[a] | 10 |
| **5e** | 69 | – | – | 12 | 8 | 12 | 6 | 8 | 9 |
| **5f** | 45 | 950 | – | 10 | 10 | 7 | 9 | 10 | 8 |
| **5g** | 39 | – | 535.3 | – | 10 | 10 | 7 | 12 | 8 |
| **5h** | 46 | – | 497 | 12 | 12 | 12 | 12 | 10 | 6 |
| **5i** | 43 | 640 | 1085 | 10 | 16 | 11 | 9 | 9 | 8 |
| **5j** | 53 | 746 | – | 12 | 13 | 9 | 6 | 10 | – |
| **5k** | 35 | 364[a] | 835 | – | 11 | 10 | 8 | 8 | 6 |
| **5l** | 61 | – | 756 | 8 | 10 | 10 | 8 | 9 | 8 |
| **5m** | 49 | 878 | 635 | – | 14 | 13 | 9 | 10 | 12 |
| **5n** | 58 | – | – | 12 | 12 | 14 | 7 | 8 | 12 |
| **5o** | 47 | 830 | 620 | 10 | 13 | 8 | 8 | 9 | 8 |
| **5p** | 56 | – | – | 9 | 10 | 8 | – | – | 8 |
| **5q** | 58 | 900 | 635 | 8 | 10 | 8 | – | – | 10 |
| **5r** | 56 | 821 | 765.8 | 10 | 14 | 12 | – | 12 | 8 |
| **5s** | 45 | 910 | 654 | 13 | 16 | 12 | – | 14 | 10 |
| **5t** | 39 | – | – | 14 | 11 | 12 | 8 | 12 | 9 |
| Pheniramine maleate | | 720 | – | – | – | – | – | – | – |
| Atropine sulfate | | – | 30 | – | – | – | – | – | – |
| Clotrimazole | | – | – | 22 | 20 | – | – | – | – |
| Ampicillin | | – | – | – | – | 19 | 18 | 15 | 18 |

* Dose at which 50% inhibition observed $IC_{50}$ μg/ml
** Zone of inhibition in mm
a = Statistically significant at $P < 0.05$

## 3. Experimental

All reagents used were purchased from Sd.Fine Chemicals Company (Mumbai, India). Melting points were determined in an open capillaries on a Gallenkamp apparatus (Sanyo Gallenkamp, Loughborough, UK) and were uncorrected. UV spectra ($\lambda_{max}$ $CHCl_3$, $H_2O$) were recorded on a Perkin Elmer spectrophotometer (Perkin Elmer, Rotkreuz, Switzerland). IR spectra (KBr, $Cm^{-1}$) were recorded on a Perkin Elmer spectrophotometer (577 model). $H^1$ NMR spectra were recorded on a Bruker WM-400 spectrometer (in δ ppm) (Bruker, Flavoil, Switzerland) using TMS as internal standard. MS were recorded on a Jeol D-300 (EI/CI) spectrometer (Jeol, Tokyo, Japan). Elemental analysis were performed on a Carlo Erba 1108 elemental analyzer (Heraeus, Hanau, Germany).

### *3.1. Isatin hydrazones 2a–e*

To a vigorously stirred solution of an appropriate isatin **1a–e** [18] (4.5 g, 0.01 ml) in alcohol (20 ml) at room temperature, hydrazine hydrate (99%, 0.015 mol) was added dropwise. The reaction mixture was warmed on water bath for 10 min, and kept in refrigerator for 3 h. The resultant yellow crystalline solid was filtered, washed repeatedly with small portions of cold water and finally with a small quantity of cold methanol. The precipitated solid was filtered, dried and crystallized from methanol to give pure product **2a–e** [9]. The data of compounds produced was compared with the data available in the literature.

### *3.2. Isatin-3-[$N^2$-(chloroacetyl)] hydrazones 3a–e*

A solution of **2a–e** (3.2 g, 0.01 mol) was heated under reflux with chloro acetyl chloride (0.01 mol) in dry benzene under anhydrous conditions using calcium chloride guard-tube for 2 h. The product thus separated was filtered washed with small portions of benzene (20 ml). After cooling the solid obtained was filterd, dried and recrystallized from acetone to yield a yellow crystalline product. The compounds were characterized by physical and spectral data. For instance, compound **3b** (R=$CH_3$, $R_1$=H) was obtained in such a reaction. Yield: 3 g (90%) of product m.p: 268 ºC and spectral data of compound UV($CHCl_3$): 303.6 nm ($\lambda_{max}$); IR (KBr): 3210 (NH), 1690 (C=O, lactam), 1655 (C=O, acid hydrazide), 1610 (C=N) $cm^{-1}$. PMR Spectrum DMSO-d6/TMS/500 mHz), δ ppm: 4.81 (S, 2 H, $-CH_2$, $-CO-$) 6.8–7.5 (m, 4 H, Ar-H), 9.1 (–NH, acid hydrazide), 11.3 (NH, lactam).

### *3.3. Isatin-3-[$N^2$-(heteryl-2-thioacetyl)] hydrazones 4a–t*

A mixture of **3a–e** [10] (2.5 g, 0.01 mol) and heteryl-2-thione (2.5 g, 0.01 mol) in dry pyridine (8 ml) was refluxed for 30 min, then the reaction mixture was poured into crushed ice and added dilute hydrochloric acid to neutralize the pyridine. The precipitated solid was filtered, dried and crystallized from ethanol to give pure products **4a–t**. The compounds obtained were characterized by physical and spectral data. For example, yield of the compound **4b** (R=$CH_3$, $R_1$=H, $R_2$ = benzimidazol-2-thione) was 2.5 g (65%); m.p. 223 °C and the spectral data UV($CHCl_3$): 312 nm ($\lambda_{max}$); IR(KBr): 3440 (NH, imidazole), 3159 (NH, indole), 1720 (NH–CO), 1688 (C=O, indole), 1621 (C=N), 1598 (C=N)$cm^{-1}$. PMR spectrum (in DMSO-D6, δ ppm: 12.7 (S, 1 H, CONH), 11.2 (S, 1 H, NH indole), 6.9–7.5 (m, 15 H, Aromatic including NH of imidazole), 4.4 (S, 2 H, $CH_2-S$).

### *3.4. 2-Substituted [1,3,4]-oxadizino-[5,6-b]-indoles 5a–t*

Compounds **4a–t** (2.8 g, 0.1 mol) were dissolved in 10 ml of concentrated sulphuric acid. The reaction mixture was kept aside for 4 h and was then poured into crushed ice and neutralized with ammonia solution (10%). The solid obtained was filtered, dried and recrystallized from ethanol. The compounds obtained were characterized by physical and spectral data. For example compound **5b** (R=$CH_3$, $R_1$=H, $R_2$ = benzimidazol-2-thione): Yield: 2 g (75%); m.p. 273 °C and spectral data exhibited UV ($CHCl_3$): 324 nm ($\lambda_{max}$); IR (KBr): 3428 (NH, imidazole), 1623(C=N), 1034 (C–O–C) $cm^{-1}$. PMR spectrum (in DMSO-D6, δ ppm): 7.6–7.4 (m, 15 H, Aromatic including NH of imidazole), 4.1 (S, 2 H, $CH_2-S$). Compounds **5a–e**, **5f–j**, **5k–o**, and **5p–t** were prepared similarly.

Acknowledgements: The authors are thankful to U.G.C., New Delhi for the financial assistance. Authors are also grateful to Dr. Reddy's Research Foundation, Hyderabad. for providing I.R and PMR spectral analysis of the compounds.

**References**

1 Bauer, D. J.; Sadler, P. W.: Br. J. Pharmacol. **15**, 101 (1960)
2 Anusha, D.; Varinder, K.; Singh, P.: Ind. J. Pharm. Sci. **55,** 129 (1993)
3 Abdel, R. M.; Gendy, Z. E.; Mohmoud, M. B.: Ind. J. Chem. **29**, 352 (1990)
4 Ayalp, A.; Neibioglu, D.: Pak. J. Pharmacol. **6**, 1 (1989)
5 Debat, J.; Ger, O.: C. A. **72,** 697 (1970)

6 Krishna, C. J.; Renuka, J.; Pooran, C.; Saroj, G. J.: Ind. Chem. Soc. **760**, 51 (1983)
7 Lal, B.; Singh, P.; Bahaduri, A. P.; Kas, K.: Ind. J. Chem. **13**, 898 (1975)
8 Popp, F. D.; Pajouhesh, H.: J. Pharm. Sci. **71**, 1052 (1982)
9 Buu-Hoi, N. P.; Guettier.: Bull. Soc. Chim. 1, 586 (1946)
10 Sarangapani, M.; Malla Reddy, V.: Indian Drugs **36(6)**, 357 (1999)
11 Van Allan, J. A.; Deacon, B. D.: Organic Synthesis Collective **6**, 569 (1963)
12 Purushotham, E.; Rajashekharan.; Pillani. V. N.: Ind. J. Chem. **29**, 18 (1990)
13 Shivaram, H. B.; Narayana, P. K.; Balakrishna, K.; Gowda, V. P.: Ind. J. Heterocyclic Chem. **5**, 273 (1996)
14 Himatkar, V. P.; Fernandes, P. S.; Kavitha, A. V.: Ind. J. Chem. **29**, 135 (1990)
15 British Pharmacopoeia. Pharmaceutical Press, London **4**, 796 (1953)
16 Turner, R. A.: Screening Methods in pharmacology **27**, 43 (1995)
17 Turner, R. A.: Screening Methods in pharmacology **27**, 142( 1995)
18 Marvel, C. S.; Heirs, G. S.: Organic synthesis collective **1**, 327 (1941)

Received May 24, 2002
Accepted August 14, 2002

Dr. M. Sarangapani
Associate Professor
Department of Pharmaceutical
and Medicinal Chemistry
University College of
Pharmaceutical Sciences
Kakatiya University
Warangal – 506009
India
mandasarangapani@yahoo.co.in