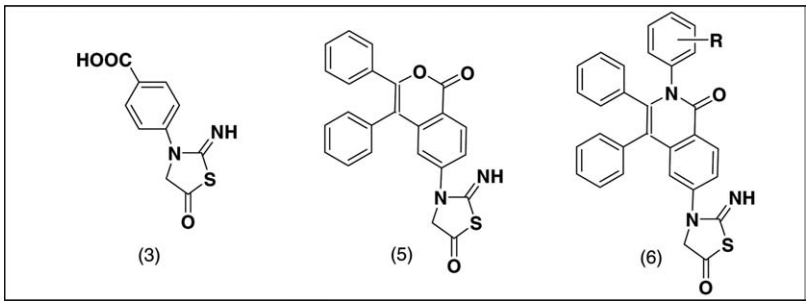
Synthesis and Antibacterial Activity of Isochromene and Isoquinoline Derivative

Vijay V. Dabholkar and Dilip R. Tripathi*

Organic Research Laboratory, Department of Chemistry, K. C. College, Churchgate, Mumbai-400 020, India
*E-mail: diliptripathi1@yahoo.co.in

Received March 6, 2009
DOI 10.1002/jhet.245
Published online 8 February 2011 in Wiley Online Library (wileyonlinelibrary.com).



Synthesis of 4-thioureidobenzoic acid (**2**), which on cyclization with ethyl chloroacetate gave 4-(2-imino-5-oxothiazolidin-3-yl)-benzoic acid (**3**), which on further reacting with benzoin (**4**) in the presence of polyphosphoric acid afforded the synthesis of 2-imino-3-(-1-oxo-3,4-diphenyl-1H-isochromen-6-yl) thiazolidin-5-one (**5**). Compound **5** was then reacted with substituted anilines to give 6-(2-imino-5-oxothiazolidin-3-yl)-3,4-diphenyl-2-(substituted)-isoquinolin-1(2H)-one (6). The structures of the compounds have been elucidated on the basis of IR 1HNMR, 13C NMR, and elemental analysis. Representative samples were screened for their antimicrobial activity against gram-negative bacteria, *E. coli* and *P. aeruginosa* and gram-positive bacteria, *S. aureus*, and *C. diphtheriae* using disc diffusion method.

J. Heterocyclic Chem., **48**, 529 (2011).

INTRODUCTION

Para-aminobenzoic acid (PABA) is a B complex vitamin that is synthesized in the body. PABA is used in the formation of folic acid and the metabolism of protein. It is an antioxidant that helps to protect skin from sunburn and cancer [1]. PABA supplementation appears to increase the ability of some infertile women to become pregnant [2]. PABA is also used in the treatment of rheumatoid arthritis [3].

Thiazoles are amongst the most frequently encountered heterocyclic compounds of biological interest, along with many other applications. Thiazoles have received attention in a range of applications from antibiotics [4] to photosensitizers [5].

The isochromene framework is a frequent structural motif in naturally occurring molecules. For example, flaccidin isolated from the orchids *Dendrobium amoenum* and *Coelogyne flaccida* includes an isochromene framework [6]. A number of compounds isolated from *Cannabis sativa* also can be viewed as isochromene derivatives [7]. The roots and the stem of a tree *Ulmus davidiana* (widespread in Korea) are used in oriental medicines for the treatment of inflammations, edema, and stomach cancer. Natural sesquiterpenoids isolated from *U. davidiana* also incorporate an isochromene fragment in their structure [8]. Hence, there is a continuing interest in the elaboration of new routes to the isochromene ring as well as to new interesting derivatives of this heterocyclic system.

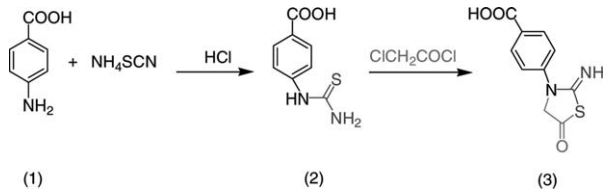
The vast pharmacological activity of simple 4-substituted tetrahydroisoquinolines has generated much interest in their synthesis and in the last years several new naturally occurring compounds of this type have been isolated [9]. For example, the antidepressant drug Nomifensine [10] has a close relationship to the alkaloid Cherylline. Some of the isoquinoline derivatives have proved to be an agonist of dopamine receptors [11].

The pharmacophoric activity of thiazoles, isochromene, and isoquinoline with the PABA as the starting material prompted the design and synthesis of 2-imino-3-(-1-oxo-3,4-diphenyl-1H-isochromen-6-yl)thiazolidin-5-one (**5**) and 6-(2-imino-5-oxothiazolidin-3-yl)-3,4-diphenyl-2-(substituted)-isoquinolin-1(2H)-one (**6**).

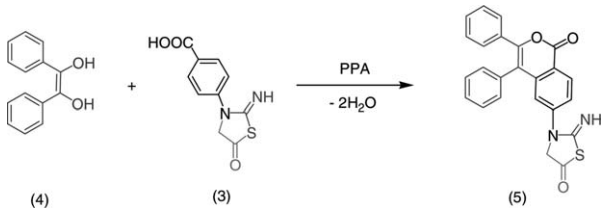
RESULTS AND DISCUSSION

PABA was treated with ammonium thiocynate in the presence of concentrated hydrochloric acid to form the thiocarbamide, 4-thioureidobenzoic acid (**2**), which on cyclization in the presence of pyridine with chloroacetyl

Scheme 1



Scheme 2



chloride forms 4-(2-imino-5-oxothiazolidin-3-yl)-benzoic acid (**3**). Compound **3** on condensation with benzoin (4), in the presence of a dehydrating agent like polyphosphoric acid yielded 2-imino-3-(-1-oxo-3,4-diphenyl-1H-isochromen-6-yl)thiazolidin-5-one (**5**). Compound **5** on further reaction with substituted anilines in the presence of anhydrous pyridine gives 6-(2-imino-5-oxothiazolidin-3-yl)-3,4-diphenyl-2-(substituted)-isoquinolin-1(2H)-one (**6**).

Antimicrobial and antifungal activities. Representative compounds were evaluated for their antibacterial activity against gram-negative bacteria, *E. coli* and *P. aeruginosa* and gram-positive bacteria, *S. aureus* and *C. diphtheriae* using disc diffusion method [12,13]. The zone of inhibition was measured in mm and the activity was compared with standard drug ampicillin trihydrate.

EXPERIMENTAL

Melting points of all synthesized compounds were determined in open capillary tubes on an electro thermal apparatus and are uncorrected. The purity of the compounds was monitored by thin layer chromatography on silica gel coated aluminum plates (Merck) as adsorbent and UV light as visualizing agent. 1H NMR spectra were recorded on Varian 500 MHz NMR spectrophotometer using DMSO-d6 as solvent and TMS as an internal standard (chemical shifts in δ ppm). C, H, N estimation was recorded on Carlo Erba 1108 (CHN) Elemental Analyzer.

General procedure for the synthesis of *thiocarbamide* (2). Thiocarbamides required for the reaction was prepared by the method as described by Joshua [14]. Aromatic amine (0.1 mol), ammonium thiocyanate (0.1 mol) and conc. hydrochloric acid (15 cm^3) were taken in 10 cm^3 water in a conical flask and heated on a wire gauze till turbidity appeared. It was then poured onto crushed ice and the solid obtained was recrystallized from hot water (Scheme 1).

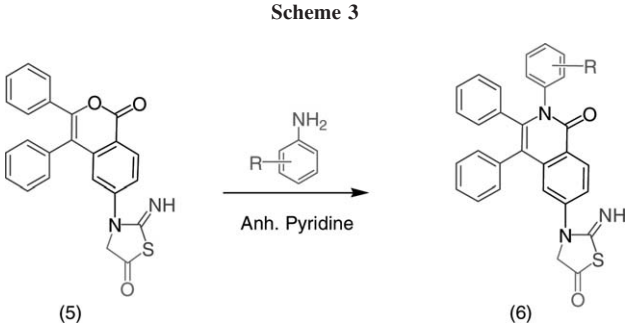
Preparation of thiocarbamide (*4-thioureidobenzoic acid*) (2). PABA (0.1 mol, 13.7 g), ammonium thiocynate (0.1 mol, 7.6 g) and conc. hydrochloric acid (15 cm^3) and 10 cm^3 of water was taken in a conical flask and heated on wire gauze till turbidity appeared. It was then poured onto crushed ice and the solid separated out was recrystallized from alcohol to give 4-thioureidobenzoic acid, yield 75%, m.p. > 300°C. IR (KBr): 3310 (—NH_2), 3100 (—NH), 1710 (—C=O), 1410 (—C=S).

4-(2-Imino-5-oxothiazolidin-3-yl)-benzoic acid (3). In a 100 cm^3 round bottom flask 4-thioureidobenzoic acid (**2**) (0.01 mol, 1.96 g) was weighed and added (0.01 mol, 0.80 mL) of chloroacetyl chloride. This mixture was refluxed for about 2 h monitored the reaction progress by TLC. After completion of the reaction it was poured onto crushed ice and neutralized with dilute NaOH solution. The precipitate separated was filtered and washed with excess water (Scheme 1). The solid was recrystallized from alcohol, yield 78%, m.p. 242°C. IR (KBr): 3160 (=NH), 1710 (—C=O), 1680 (—C=O), 1415 (—C=S), 875 (—C—S—C—).

2-Imino-3-(-1-oxo-3,4-diphenyl-1H-isochromen-6-yl)thiazolidin-5-one (5). A mixture of 4-(2-imino-5-oxothiazolidin-3-yl)-benzoic acid (**3**) (0.01 mol, 2.36 g) and benzoin (desyl alcohol) (**4**) (0.01 mol, 2.12 g) in polyphosphoric acid (10 mL) [18 parts by weight of P_2O_5 and 10 parts by weight of H_3PO_4] was heated at 100°C for 5 h. During heating the contents were occasionally stirred. Subsequently, the reaction mixture was poured into ice cold water (100 mL) and left as such for 1 h. A solid separated out, which was filtered off and washed initially with 10% aqueous sodium bicarbonate solution (50 mL) and finally with excess water (checked with litmus paper for the completed removal of acid) (Scheme 2). The solid thus obtained was dried under vacuum and recrystallized from acetonitrile:methanol (1:1), yield 68%, m.p. 85°C. IR (KBr): 3090 (=NH), 1710 (—C=O), 1680 (—C=O), 1430 (—C=S), 850 (—C—S—C—). 1H NMR; 3.62 (s, 1H, NH), 4.11 (s, 1H, CH_2), 6.5–8.2 (m, 13H, Ar-C). 13C NMR; 68.2 (CH_2), 96.5–140.1 (C=C & Ar-C), 155.3 (C=N), 161.5 & 166.1 (2x C=O). Elemental Analysis: Actual, C; 69.90, H; 3.88, N; 3.40. Observed, C; 69.97, H; 3.92, N; 3.46

General procedure for the synthesis of *6-(2-imino-5-oxothiazolidin-3-yl)-3,4-diphenyl-2-(substituted)-isoquinolin-1(2H)-one* (6). The synthesis was reported by Pandey [15]. Compound **6** was synthesized by heating under reflux a mixture of 2-imino-3-(-1-oxo-3,4-diphenyl-1H-isochromen-6-yl)thiazolidin-5-one (**5**) (0.001 mol) and substituted aromatic amine (0.001 mol) in anhydrous pyridine (20 mL) for 6 h. The solution was cooled to RT and acidified with dil. HCl (50 mL). A solid separated out, which was filtered off and washed with water successively (4 × 25 mL) (Scheme 3).

Scheme 3



6-(2-Imino-5-oxothiazolidin-3-yl)-3,4-diphenyl-2-(m-tolyl)-isoquinolin-1(2H)-one (6a). Compound **6a** was synthesized by heating under reflux, a mixture of 2-imino-3-(-1-oxo-3,4-diphenyl-1H-isochromen-6-yl)thiazolidin-5-one (**5**) (0.001 mol, 0.41 g) and substituted aromatic amine (0.001 mol) in anhydrous pyridine (20 mL) for 6 h. The solution was cooled to RT and acidified with dil. HCl (50 mL). The solid separated out was filtered off and washed with water successively (4 × 25 mL). The solid thus obtained was dried under vacuum and recrystallized from absolute alcohol, yield 72%, m.p. 210°C. IR (KBr): 3060 (=NH), 1690 (—C=O), 1610 (—C=O), 1450 (—C=S), 860 (—C—S—C—). 1H NMR; 2.37 (s, 3H, CH_3), 3.78 (s, 1H, NH), 4.30 (s, 2H, CH_2), 6.5–8.0 (m, 17H, Ar-H). 13C NMR; 17.7 (CH_3), 68.2 (CH_2), 96.5–140.1 (C=C & Ar-C), 155.3 (C=N), 161.5 & 166.1 (2x C=O). Elemental Analysis: Actual, C; 74.25, H; 4.59, N; 8.38. Observed, C; 74.19, H; 4.50, N; 8.44.

6-(2-Imino-5-oxothiazolidin-3-yl)-2,3,4-triphenylisoquinolin-1(2H)-one (6b). IR (KBr): 3060 (=NH), 1710 (—C=O), 1630 (—C=O), 1460 (—C=S), 850 (—C—S—C—). 1H NMR; 3.62 (s, 1H, NH), 4.31 (s, 1H, CH_2), 6.8–7.8 (m, 18H, Ar-H). 13C NMR; 65.2 (CH_2), 111.9–144.1 (C=C & Ar-C), 158.1 (C=N), 163.4 & 169.1 (2x C=O). Elemental Analysis: Actual, C; 73.92, H; 4.31, N; 8.62. Observed, C; 73.85, H; 4.39, N; 8.68.

6-(2-Imino-5-oxothiazolidin-3-yl)-3,4-diphenyl-2-(2-chlorophenyl)-isoquinolin-1(2H)-one (6c). IR (KBr): 3060 (=NH), 1690 (—C=O), 1610 (—C=O), 1480 (—C=S), 850 (—C—S—C—), 750 (C—Cl). 1H NMR; 3.71 (s, 1H, NH), 4.40 (s, 1H, CH_2), 7.0–8.2 (m, 17H, Ar-H). 13C NMR; 69.7 (CH_2), 104.2–147.3 (C=C & Ar-C), 151.5 (C=N), 161.7 & 168.3 (2x C=O). Elemental Analysis: Actual, C; 69.03, H; 3.84, N; 8.05. Observed, C; 69.11, H; 3.91, N; 8.11

6-(2-Imino-5-oxothiazolidin-3-yl)-3,4-diphenyl-2-(2-carboxilic acid)-isoquinolin-1(2H)-one (6d). IR (KBr): 3080 (=NH), 1690 (—C=O), 1610 (—C=O), 1460 (—C=S), 850 (—C—S—C—). 1H NMR; 3.69 (s, 1H, NH), 4.40 (s, 1H, CH_2), 7.15–7.89 (m, 17H, Ar-H), 8.85 (s, 1H, OH). 13C NMR; 69.1 (CH_2), 96.1–147.3 (C=C & Ar-C), 157.7 (C=N), 168.1 & 170.1 (2x C=O), 175.2 (C=O). Elemental Analysis: Actual, C; 70.06, H; 3.95, N; 7.91. Observed, C; 70.11, H; 3.87, N; 7.83.

6-(2-Imino-5-oxothiazolidin-3-yl)-3,4-diphenyl-2-(4-methoxyphenyl)-isoquinolin-1(2H)-one (6e). IR (KBr): 3050 (=NH), 2850 (OCH_3), 1690 (—C=O), 1610 (—C=O), 1460 (—C=S), 850 (—C—S—C—). 1H NMR; 3.71 (s, 1H, NH), 3.89 (s, 3H, OCH_3), 4.24 (s, 2H, CH_2), 6.72–8.34 (m, 17H, Ar-H). 13C NMR; 55.6 (OCH3), 69.4 (CH_2), 112.2–144.1 (C=C & Ar-C), 151.2 (C—OCH_3), 161.3 & 168.4 (2x C=O), 179.3 (C=N). Elemental Analysis: Actual, C; 71.95, H; 4.45, N; 8.12. Observed, C; 71.88, H; 4.38, N; 8.08.

Experimental procedure for antibacterial testing. Make suspension of the desired cultures in saline with the help of nichrome loop. The turbidity achieved should be 0.5 macfarland standard. Within 15 min after adjusting the turbidity of the inoculum suspension, dip a sterile nontoxic swab into the adjusting suspension. Rotate the swab several times, pressing firmly on the inside wall of the tube above the flute level to remove excess inoculums from the swab.

Inoculate the dried surface of MH plate by streaking the swab over the entire sterile agar surface. Repeat this two more times. Place the plate top and allow standing for 3–5 min, but not longer than 15 min, for any excess surface moisture to be absorbed before applying the antibiotic disc. There should be a confluent lawn of growth when done properly. If only isolated colonies grow, the inoculums were too light and the test should be repeated.

Table 1

Antibacterial activity.

	Gram positive		Gram negative	
Compounds	*S. aureus*	*C. diphtheria*	*P. aeruginosa*	*E. coli*
5	15	17	14	11
6a	21	23	19	20
6b	16	17	13	11
6c	23	25	20	19
6d	22	23	21	19
6e	21	22	18	20
Ampicillin trihydrate (standard)	26	28	22	21
DMSO	0	0	0	0

Concentration selected was 100 μg/mL and DMSO was used as the solvent.

Disc (4 mm diameter) were prepared from Wattmann filter paper no.41 and were used after autoclaving them at 121 psi then for 15 min. It was then dried in hot air oven. The compounds were tested at the concentration of 100 μg/mL using dimethylsulphoxide (DMSO) as the diluent. Now dip the disc in the appropriate dilution and place them evenly on the surface of agar plate either by using sterile forceps or the dispensing apparatus, no more than five disc on a 100 mm plate. A blank DMSO disc should also be placed on the plate to see if DMSO is interfering with the zone of inhibition. The disc should not be moved, once it has come in contact with the agar surface as some of the compound diffuses almost instantaneously. Invert the plate and place them in an incubator at 35°C with in 15 min after the disc are applied. Plates should be incubated aerobically. After 16–18 h of incubation examine each plate. The zone of inhibition was measured in mm and was compared with the reference standard antibiotics namely Ampicilline trihydrate drugs 100 μg/mL. Zone of inhibition were determined and the results of such studies are summarized in Table 1.

Compound **6a** and **6e** with a methyl and methoxy substituent, respectively showed moderate activity against gram positive and gram negative organisms, whereas compound **6b** with no substituent showed very low activity.

Highest degree of activity against gram negative micro organisms, *S. aureus* and *C. diphtheria* was shown by **6c** with a chloro substituent, and **6d** with a carboxylic acid substituent showed good activity against gram positive microorganisms, *P. aeruginosa* and *E. coli*.

The substituted, carboxylic and chloro derivatives of the title compound were found to be the lead antimicrobial agents.

Acknowledgments. The authors are grateful to the Principal Ms. Manju J. Nichani and Management of K.C. College, Mumbai for providing necessary facilities and to the Head, Department of Microbiology for antimicrobial studies. Authors are also thankful to the Director, Institute of Science, Mumbai (India) for providing spectral analyses.

REFERENCES AND NOTES

[1] Willis, I.; Kligman, A. M. Arch Dermatol 1970, 102, 405.

[2] Sieve, B. F. South Med Surg 1942, 104, 135.

[3] Wiesel, L. L.; Barritt, A. S.; Stumpe, W. M. Am J Med Sci 1951, 222, 243.

[4] Hoshino, K.; Ishida, H.; Omovskaya, O.; Dudley, M.; Rleger, R.; Watkins, W. J.; Zhang, J. Z.; Renau, T. E.; Lee, V. J.; Ota, T.; Nakayama, K.; Ishida, Y.; Otsuka, M.; Kawato, H. Japan Kokai Tokkyo Koho, CODEN: JKXXAF JP 2002322054 A2 20021108: Japan, 2002; Hoshino, K.; Ishida, H.; Omovskaya, O.; Dudley, M.; Rleger, R.; Watkins, W. J.; Zhang, J. Z.; Renau, T. E.; Lee, V. J.; Ota, T.; Nakayama, K.; Ishida, Y.; Otsuka, M.; Kawato, H. Chem Abstr 2002, 137, 346134.

[5] Shimada, Y. Japan Kokai Tokkyo Koho, CODEN:JXXAF JP 02113070 A2 19900425: Japan, 1990; Shimada, Y. Chem Abstr 1990, 113, 99405.

[6] Majumder, P. L.; Guha, S.; Sen, S. Phytochemistry 1999, 52, 1365.

[7] Papadakis, D. P.; Salemink, C. A.; Alikaridis, F. J.; Kephalas, T. A. Tetrahedron 1983, 39, 2223.

[8] Kim, J. P.; Kim, W. G.; Koshino, H.; Jung, J.; Yoo, I. D. Phytochemistry 1996, 43, 425.

[9] Kobayashi, S.; Tokumoto, T. J Chem Soc Chem Commun 1984, 1043.

[10] Heptner, W.; Schacht, U.; Biochem Pharmacol 1974, 23, 3413.

[11] Dandzidge, P. A.; Kaiser, C.; Brenner, M.; Gaitanopoulos, D.; Davis, L. D.; Webb, R. L.; Foley, J. J.; Sarau, H. M. J Med Chem 1984, 27, 28.

[12] Cruickshank, R.; Duguid, J. P.; Marmion, B. P. Medicinal Microbiology, 12th ed.; Churchill Livingstone: London, 1975; Vol. 11.

[13] Arthington-Skaggs, B. A.; Motley, M.; Morrison, C. J. J Clin Microbiol 2000, 38, 2254.

[14] Joshua, C. P. J Org Chem 1963, 28, 1293.

[15] Singh, V. K.; Pandey, V. K. Indian J Chem Sect B 2006, 45, 2745.

[16] Application of polyphosphoric acid (PPA) arises from the fact that it is a mild reagent, even though a strongly dehydrating agent. It does not usually bring about charring or polymerization, most probably because it is a poor proton source.