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Laccase Enzyme Catalysed Efficient Synthesis of 3-Substituted-1,2,4-Triazolo(4,3-b)(4,1,2)Benzothiadiazine-8-Ones+

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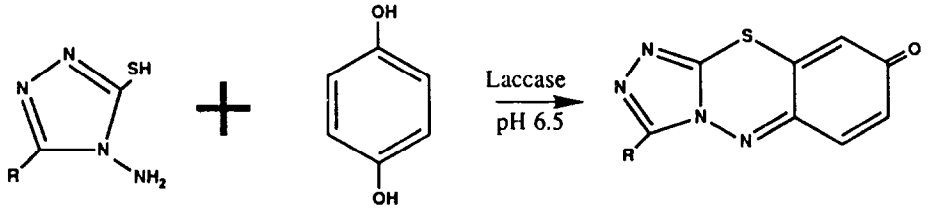
Abstract: Full details of an efficient one step synthesis of 3-substituted(4,3-b)(4,1,2)benzothiadiazine-8-ones (4a-4h) by Laccase enzyme (E.C.1.10.3.2) mediated reaction of various 5-substituted-4-amino-3-mercapto-1,2,4-triazoles (1a-1h) and hydroquinone (2) is described.

Over the past several years the use of enzymes as catalysts on organic synthesis has markedly gained much importance[1a-1b] and the immense potential of enzymes as catalysts in organic synthesis is well documented due to its success in mild conditions of temperature and pH.

s-Triazolocycloalkyl thiadiazines and s-triazolobenzocycloalkyl thiadiazines are known for their antiinflammatory, analgesic, central stimulant, central depressant, antisecretary and sedative depressant activities[2]. Inspite of biological importance, their preparative routes are limited and are rarely dealt with except in a few patents[2]. However, the preparations of s-triazolocycloalkyl thiadiazines and s-thiazolobenzocycloalkyl thiadiazines have been reported from substituted triazoles and halo ketones[3].

In our previous communications we have reported the one pot synthesis of the title compounds[4]. However, the biological importance of s-triazolobenzothiadiazines and our on going studies on synthetic perspectives of enzymes[5], have prompted us to develop a mild and efficient one



(1) (2) (4)

SCHEME -I

+IICT Communication No. 3316

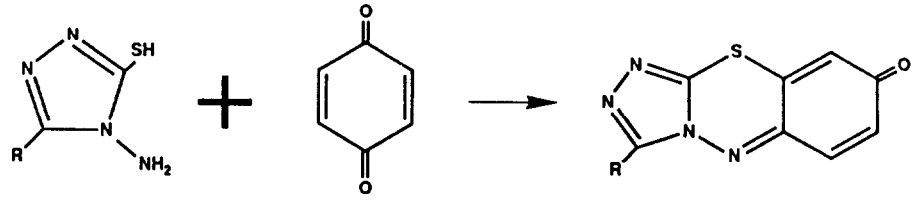
step synthesis of 3-substituted-1,2,4-triazolo (4,3-b)(4,1,2)benzothiadiazine-8-ones (4a-4h) by laccase enzyme (E.C.1.10.3.2) mediated reaction of various 5-substituted-4-amino-3-mercapto-1,2,4-triazoles (1a-h) and hydroquinone (2) in aqueous solution (Scheme-I).

Quinones are synthetically very important intermediates. Many biosyntheses are known to pass through quinoid intermediates[6]. Laccase enzyme catalysed the oxidation of hydroquinones to the corresponding p-benzoquinones efficiently in the presence of molecular oxygen in aqueous solutions[7]. However to the best of our knowledge, no systematic attempt was made to study the laccase enzyme as an oxidising catalyst in organic synthesis, particularly in reactions of the type which we describe here.

Thus in the present communication, we wish to disclose our successful results on the synthesis of various substituted 1,2,4-triazolo(4,3-b)(4,1,2)benzothiadiazine-8-ones (4a-4h) by reaction of p-quinones (3) which are generated in-situ by laccase enzyme catalysed oxidation of hydroquinone(2), and 5-substituted-4-amino-3-mercapto-1,2,4-triazoles (1a-1h) as depicted in the Scheme-II.

Results and Discussion

Several 5- substituted- 4-amino- 3-mercapto- 1,2,4-triazoles (1a-1h) reacted efficiently with p-quinone(3), generated in-situ by laccase enzyme catalysed oxidation of hydroquinone(2), to give (4a-4h) in aqueous solution(Scheme II).



(1) (3) (4)

SCHEME - II

We found that the reaction occurred smoothly in phosphate buffer (pH 6.5) giving rise to quantitative yields of corresponding products. Acetonitrile as co-solvent was found to be helpful in achieving the homogenicity of the reactants (1a-1h). In general the yields of the products have been uniformly good and do not seem to depend on the substitution pattern

Table -1: Laccase catalysed synthesis of 3-substituted-1,2,4-triazolo(4,3-b)(4,1,2)benzothiadiazine-8-ones (4a-4h)

Entry	Compound 4 (R)	Yield %	m.p. 0°C	m/z	IR cm^{-1}	^1H NMR ($CDCl_3$)(δ ppm)
4a	CH_3	90	212	218	1700	7.57(d,1H,J=9.8); 6.75(d,2H); 2.01 (s,3H)
4b	C_2H_5	95	202	232	1710	7.54(d,1H,J=9.8); 6.72(d,2H); 2.8 (q,2H);1.4(t,3H)
4c	Ph	83	198	280	1710	8.12-8.14(2H,m,o-phenyl protons); 7.53-7.58(4H,m,3-phenyl protons & 1 proton of C-6); 6.83(1H of C-7,d, J=9.63); 6.74(1H of C-9,s)
4d	Ph-OCH_3	95	194	310	1700	8.08(2H,d,m- to -OCH_3); 7.02(2H,d, o- to -OCH_3); 7.53(1H of C-6,d,J= 9.9); 6.8(1H of C-7,d,J=9.4); 6.78 (1H of C-9,s); 3.9(3H,s,-OCH_3)
4e	Ph-CH_3	90	200	294	1710	8.2(2H,d,-CH_3); 7.6(3H,d,2 protons o- to methyl & 1 proton of C-6) 6.83 (1H of C-7,d,J=9.63); 6.74(1H of C-9,s); 2.3(3H,s,-CH_3)
4f	Ph-CH_2	95	193	294	1695	7.28-7.39(5H,m, Phenyl protons); 7.46(1H of C-7,d,J=9.69); 6.68(1H, of C-9,s); 4.4(2H,s,-Ph-CH_2 protons)
4g	Cl-Ph	90	220	314	1690	8.1(2H,d,m- to Cl); 7.53(3H,d, o- to Cl & 1H of C-6); 6.83(1H of C-7, d,J=9.63); 6.75(1H of C-9,d)
4h	Br-Ph	90	230	358, 360	1700	8.2(2H,d,m- to Br); 7.6(3H,d,o- to - Br & 1H of C-6); 6.83(1H of C-7,d, J=9.63); 6.75(1H of C-9,d)

of the substrates. TLC and HPLC analysis confirmed the formation of only one product. The optimum pH for the reaction was found to be 6.5 and temperature 30°C.

The reaction of the compounds(1a-1h) listed in Table 1 with in-situ generated (3) by laccase catalysed oxidation of hydroquinone (2) gave almost quantitative yields of products (4a-4h). In conclusion this approach is an efficient and convenient preparative route to compounds 4a-4h under mild conditions.

Experimental

Melting points were determined on open capillaries with mettler FP-51 melting point apparatus and are uncorrected. IR spectrum were recorded in KBr pellets on Perkin Elmer model 710B spectro photometer. ^{1}H NMR spectra were recorded on Brucker 300 MHz or Varian FT-200 MHz instruments in $CDCl_3$ or DMSO-d_6, using TMS as internal standard. Mass spectra were run on VG micromass 7070H. Laccase enzyme (E.C.1.10.3.2) (10,000 units) purchased from Sigma Chemical Company, USA. Substrates 1a-1h were prepared according to reported procedures[8,9,10] as described below. All new compounds gave satisfactory spectral and analytical data.

Synthesis of 5-substituted-4-amino-3-mercapto-1,2,4-triazoles(1a-1h):
The compounds **1a** and **1b** were synthesised by following procedure[8].
A representative procedure for the preparation of **1a** is as follows.

Thiocarbohydrazide[9] (10.4 g, 0.1 mole) was stirred with the acetic acid (20 ml) and heated to boiling for 10 min. Cooling the reaction mixture and dilution with ethylacetate gave the 5-methyl-4-amino-3-mercapto-1,2,4-triazole as a white solid, filtered and recrystallised from 50% aqueous ethanol. Yield 71% mp 205-206°C, m/z 130 (M+); IR (KBr): 3290, 3080, 1635; ^{1}H NMR (DMSO-d_6): δ 13.20(s, 1H), 4.80(s, 1H),1.90(s, 3H).

The compounds **1c-1f** were syhnthesised by following the literature procedure[10]. A representative procedure of 1c is given as follows.
Synthesis of 5-phenyl-4-amino-3-mercapto-1,2,4-triazole(1c):
The representative procedure for the preparation of 1c is as follows:

A suspension of 20 mmoles of the potassium salt of 3-benzoyldithiocarbazate, 40 mmoles of 95% hydrazine, and 2 ml. of water was refluxed with stirring for 1hr. The color of the reaction mixture changed to green, hydrogen sulfide was evolved (lead acetate paper and odor), and a homogeneous solution resulted. Dilution with 100 ml of cold water and acidification with concentrated hydrochloric acid precipitated a white

solid. This product was filtered, washed with 2 x 30 ml. portions of cold water and recrystallized from ethanol. yield 80%. mp. 204-206°C, m/z 192 (M+); IR (KBr): 3300,3100,1630; ^{1}H NMR (DMSO-d_6):δ13.5(s, 1H), 6.90-7.60(m, 5H), 5.60(s, 2H).

The compounds **1g-1h** were synthesised by following the literature procedure[10]. A representative procedure of **1h** is given as follows.

Synthesis of 5-p-bromophenyl-4-amino-3-mercapto-1,2,4-triazole(1h):

A solution of 30 mmoles of 5-p-bromophenyl-2-mercapto-1,3,4-oxadiazole[11], 20 ml. of water and 16 g. of 95% hydrazine was refluxed for 4 hrs, diluted with 200 ml. of cold water, acidified by the dropwise addition of concentrated hydrochloric acid, and filtered. The solid was washed with a minimum of cold water and recrystallized from 50% aqueous ethanol. yield 60%. mp.173-175°C, m/z 271 (M+); IR (KBr): 3300, 3100, 1635; ^{1}H NMR (DMSO-d_6): δ 13.8 (s, 1H), 7.80 (d, 2H), 7.20 (d, 2H), 5.60 (s, 1H).

General procedure for the preparation of 3-substituted-1,2,4-triazolo (4,3-b)(4,1,2)-benzothiadiazine-8-ones (4a-4H):

A representative procedure illustrated for compounds **4a** is as follows:

To a magnetically stirred solution of a mixture of 5-methyl-4-amino-3-mercapto-1,2,4-triazole (**1a**, 1.30 g, 0.01 mole) and 1.1 g (0.01 mole) of hydroquinone (**2**) in a 50 ml of phosphate buffer (pH 6.5): acetonitrile mixture (3:1), laccase enzyme (0.002 g, 10,000 units) was added and change in color of the contents from colourless to deep reddish brown was noticed. After, stirring for 12 hrs the reaction mixture was extracted with chloroform (2x50 ml) and combined solvents extracts were dried over anhydrous sodium sulphate. The solvent was removed under reduced pressure and residue was recrystallised from methanol to give 1.96 g (90%) of **4a**. mp 212°C, m/z 218 (M+); IR (KBr): 1700, 1625, 1605; ^{1}H NMR ($CDCl_3$): δ 7.57 (d, 1H J=9.8 Hz); 6.75 (d, 2H); 2.01 (s, 3H).

Compounds **4b-4h** were exactly made according to general procedure described above starting from **1b-1h**. The yields and spectral details of all the products **4a-4h** are given in **Table-1**.

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REFERENCES

1. Jones, J.B."Enzymes in Organic Chemistry", Porter, R and Clark, S., Pitman Press, London, 1985, p3; b) Butts, S. and Roberts, S.M., "Chemistry in Britan", 1987, p 137; c) Whitisides, G.M. and Wong,

C.H., **Angew.Chem., Int.Ed.Engl., 24,** 617 (1985); d) Simon, H., Bader, J., Gunther, H., Neumann, S. and Thomas, J.,**Angew.Chem. Int.Ed.Engl., 24,** 539 (1985); e) Yamada, H. and Shimizu, S., **Angew.Chem.,Int.Ed.Engl., 27,** 622 (1988); f) Wong, C.H., **Science., 244,** 1145 (1989).

2. Radha Rani, B. Ph.D., Thesis 1989, Osmania University, Hyderabad, India.
3. a) Albrecht, W.L. and Sweet, F.W., **U S Patent,** 3.954.983; C.A. **85,** 78172h (1976); b) Albrecht, W.L. **U S Patent,** 3.954.984; C.A. **85,** 78173h (1976); c) Albrecht, W.L. and Jones, W.D., **U S Patent,** 3,954,981; C.A. **85,** 177510v (1976); d) Albrecht, W.L., Jones, W.D. and Sweet, F.W., **J.Heterocyclic Chem., 15(2),** 209, (1978).
4. Radha Rani, B. Rahman, M.F. and Bhalerao, U.T., **Org.Pre.Proce.Int., 23(2),** 157 (1991).
5. a) Bhalerao U.T., Muralikrishna, C. and Pandey, G. **Synth.Commun.,19,** 1303 (1989); b) Pandey, G., Muralikrishna C. and Bhalerao U.T., **Tetrahedron, 45,** 6687 (1989); c) Pandey, G., Muralikrishna C. and Bhalerao U.T., **Tetrahedron Lett., 31,** 3771 (1990); d) Bhalerao U.T., Muralikrishna, C. and Pandey, G. J.C.S.Chem.Commun.,, 1176 (1992).
6. a) Taylor, W.I. and Buttersby, A.R., "Oxidative Coupling Phenols", Marcel Dekker Pbn., New York, 1967; b) Scott, A.I., "Quarterly Reviews", 1965, I.P. 19; c) King, T.E. "Oxidases and Related Redox Systems". Barnes and Noble, New York, 1965.
7. Yakushiji, E. **Proc.Imp.Acad.,** (Tokyo), **16,** 39 (1940).
8. Potts, K.T. and Huseby, R.M. **J.Org.Chem., 31,** 3528 (1966).
9. Audrieth, L.F., Scott, E.S. and Kippur, P.S., **J.Org.Chem., 19,** 733 (1954)
10. Reid, J.R. and Heindel, N.D., **J.Heterocyclic Chem., 13,** 925 (1976).
11. Hoggarth, E. **J.Chem.Soc.,** 4811, 1952.

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