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Research Paper

Efficient PTSA-catalyzed Synthesis of 1,5-Benzodizepine Derivatives under Mild Conditions and their β –hematin inhibitory activity

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Abstract: Benzodiazepines and its derivatives constitute an important class of heterocyclic compounds which possess a wide range of therapeutic and pharmacological properties. Efficient PTSA-catalyzed synthesis of 1,5-Benzodizepine derivatives under mild conditions was developed and tested for their β –hematin inhibitory activity.

Introduction

Benzodiazepines and its derivatives constitute an important class of heterocyclic compounds which possess a wide range of therapeutic and pharmacological properties [1]. Derivatives of benzodiazepines are widely used as anticonvulsant, antianxiety[2], analgesic[3], sedative[4], antidepressive[5], and hypnotic [6] agents. In the last decade, the area of biological interest of 1,5-benzodiazepines has been extended to several diseases such as cancer, viral infection and cardiovascular disorders[7]. In the last decade, the area of biological interest of 1,5-benzodiazepines has been extended to several diseases such as cancer, viral infection and antiparasitic. The report demonstrates that a peripheral-type benzodiazepine receptor, including the voltage dependent anion channel, is present in the human erythrocyte membrane. This receptor mediates the maxi-anion currents previously described in the erythrocyte membrane. Ligands that block this peripheral-type benzodiazepine receptor reduce membrane transport and conductance in *P falciparum*-infected erythrocytes. These ligands also inhibit in vitro intraerythrocytic growth of *P falciparum*. These channels are obvious targets for selective inhibition in anti-malarial therapies, as well as potential routes for drug delivery in pharmacologic applications [32].

In addition,1,5-benzodiazepines are key intermediates for the synthesis of various fused ring systems such as triazolo-, oxadiazolo-, oxazino- or furanobenzodiazepines [8]. Owing to their

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versatile applications various methods for the synthesis of benzodiazepines have been reported in the literature. These include condensation reactions of *o*-phenylenediamines with α, β-unsaturated carbonyl compounds, with acetophenone in the presence of $BF_3 \cdot Et_2O$, $NaBH_4$, polyphosphoric acid or SiO_2 , $MgO/POCl_3$, $Yb(OTf)_3$, Al_2O_3/P_2O_5 or AcOH under microwave conditions, Amberlyst-15 in the ionic liquid 1-butyl-3-methylimidazolium bromide ([bmim]Br), $CeCl_3 \cdot 7H_2O/NaI$ supported on silica gel, $InBr_3$, $Sc(OTf)_3$, sulfated zirconia, $InCl_3$, CAN, $ZnCl_2$ under thermal conditions, $AgNO_3$. [9-26]. These reactions also occur with various catalysts under solvent free conditions.

Nevertheless, many of these methods suffer from shortcomings such as long reaction times, harsh reaction conditions, low product yields, occurrence of several side products and difficulties in recovery of the products. Moreover, some of the reagents employed are very expensive. Consequently, the search continues for better catalysts in terms of operational simplicity and economic viability to synthesize1, 5-benzodiazepines. In recent years, p-Toluenesulfonic acid (PTSA) has received considerable attention due to its commercial availability and efficient generation of acidic condition in reaction media. It is inexpensive, and has been found to be versatile in functional group transformations. In continuation to our efforts for the development of simple and novel methods for the synthesis of different heterocyclics, we report herein a simple and efficient method for the synthesis of 1,5-benzodiazepines using PTSA as catalyst[27-30]. Drug target heme is still being exploited extensively for designing new antimalarial agents because biochemical drug target heme cannot either be mutated or expressed by the parasite. Based on these observation 1,5-benzodiazepines were synthesised and tested for their β –hematin inhibitory activities. Some of tested compounds have shown moderate β –hematin inhibitory activities

Chemistry

In the first instance, *o*-phenylenediamine (1 equiv.) and acetone (2.5 equiv.) were stirred at ambient temperature in dichloromethane with 20 mol% of PTSA (Scheme 1). The reaction was complete within 1-5 hr. After screening various solvents like methanol, ethanol, isopropanol, ethyl acetate and acetonitrile, we found that the reaction proceeds well in polar solvents, giving slight variations in reaction time and that methanol was the best choice for this reaction. Having successfully performed the reactions of 1,2-phenylenediamine with a wide range of ketones, we focused our attention on examining the reactions of various ketones. Electron withdrawing groups on ketone stimulate the reaction rate, whereas electron releasing groups reduce the reactivity of the ketone. The results revealed that both mono- and disubstituted ketones produce the corresponding benzodiazepines in excellent yields. The results are summarized in Table 1.

Materials and Methods

All the reactions were monitored by thin layer chromatography over silica gel-G and basic alumina coated TLC plates. The spot on TLC plates were developed by iodine vapours, potassium permagnate spray or Dragendroff spray as the developing agents. The melting points were recorded on an electrically heated melting point apparatus.IR spectra were recorded on Beckman Aculab-10, Perkin Elmer 881 and FTIR 8210 PC, Shimadzu spectrophotometers either on KBr discs or in neat and values are expressed in cm^{-}

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[1].Nuclear Magnetic Resonance (NMR) spectra were recorded on either Bruker Avance DRX-300 MHz or Bruker DRX 200 FT spectrometers using TMS as an internal reference. Chemical shifts (δ in ppm) were reported relative to solvent peak ($CHCl_3$ in $CDCl_3$ at 7.23 ppm and DMSO in DMSO-d_6 at 2.49 ppm) or TMS. Signals were designated as follows: s, singlet; bs, broad singlet; d, doublet; dd, double doublet; t, triplet; m, multiplet.FAB mass spectra were recorded on JEOL SX 102/DA 6000 mass spectrometer using Argon/ Xenon (6 KV, 10 mA) as the FAB gas. EI mass spectra were recorded on JEOL JMS-D-300 spectrometer with the ionization potential of 70 eV and ES mass on Quantro-II, micro mass. Silica gel (60-120mesh) for column chromatography and silica gel (230-400 mesh) for flash column chromatography were used. Room temperature mentioned range between 22-30˚C unless stated otherwise. Anhydrous solvents were prepared as per general procedure mentioned in text book of practical organic chemistry by A.I. Vogel. Common solvents for general use were purchased from E. Merck, Qualigens, Ranbaxy and S. D. Fine Chemicals. Reagents were purchased from Sigma, Aldrich and Across Chemicals.

General procedure for the preparation of 1,5-benzodiazepines

To a stirred solution of *o*-phenylenediamine (1 mmol) in MeOH (1 mL), a ketone (2.5 mmol) and 20 mol% PTSA were added. The reaction mixture was stirred at room temperature until the reaction was complete, as judged by TLC analysis. The reaction mixture was then concentrated and washed with water to give the crude product, which was further purified by flash chromatography on silica gel (eluent: hexane-EtOAc= 5:1).

Compound 3a

Yellow solid, yield: 95%; mp 159-160 ºC; IR (KBr): 3108, 2364, 1589,1460, 1127, 1002 cm^{-1}; ^1H NMR (300MHz, $CDCl_3$) : δppm=7.35 (m, 1H), 7.12 (m,2H), 6.95 (s,2H), 6.90 (m,1H), 6.83 (s, 2H), 3.84 (s,3H), 3.79 (d,9H, J=6.12 MHz), 3.73 (s,6H), 3.31(s,1H), 3.10 (d,1H, J=13.14 MHz), 2.86 (d,1H, J=10.53 MHz) 1.79 (s,3H). ESI-MS: (*m*/*z*): 493 $(M+H)^+$;

Compound 3b

Yellow solid, yield: 92%; mp 121-126 ºC; IR (KBr): 3233, 2364,1670, 1461, 1219, 1025 cm^{-1} ^1H NMR (300MHz, $CDCl_3$) : δppm=7.4 (s,1H), 7.29 (s,2H), 7.13-7.05 (m,3H), 7.02-6.9 (m,1H), 6.85-6.83 (m,1H), 6.79 (d,1H, J=8.4 MHz), 6.68 (d,1H, J=8.4 MHz), 3.86-3.83 (m,9H), 3.68 (s,3H) 3.34 (s,1H), 3.07 (d, 1H, J=13.14 MHz), 2.89 (d, 1H, J=13.17 MHz), 1.75 (s, 3H). ESI-MS: (*m*/*z*): 433 $(M+H)^+$;

Compound 3c-

Yellow solid, yield: 83%; mp 171-178 ºC; IR (KBr): 3333, 2853, 1638, 1478, 1216, 763 cm^{-1}1H NMR (300MHz, $CDCl_3$): δppm=7.47-7.15 (m, 10H), 7.08-7.05 (m, 2H), 6.83 (d, 1H, J=7.02 MHz), 3.42 (s, 1H), 3.09 (d, 1H, J=13.17 MHz), 2.91 (d, 1H, J=13.23 MHz), 1.73 (s, 3H). ESI-MS: (*m*/*z*): 471$(M+H)^+$;

Compound 3d

Yellow solid, yield: 87%; mp 135-140 ºC; IR (KBr): 3020, 2363, 1640, 1216, 764 zcm^{-1}1H NMR (300MHz, DMSO) : δ ppm= 7.69 (d, J = 8.7 Hz, 2H), 7.49-7.39 (m, 5H), 7.26-7.09 (m, 6H), 6.90 (t, J = 12.6 Hz, 1H), 2.28 (s, 2H), 1.69 (s, 3H) ESI-MS: (*m*/*z*): 382 $(M+H)^+$;

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Compound 3e

Yellow solid, yield: 91%; mp 197-200 ºC; IR (KBr): 3451, 1638, 1218, 765, cm^{-1}1H NMR (300MHz, $CDCl_3$) : δppm=7.5-7.50 (m, 4H), 7.3-7.27 (m, 1H), 7.08-7.04 (m, 2H), 6.92-6.81 (m, 5H), 3.40 (s, 1H), 3.09 (d, 1H, J=13.2 MHz), 2.9 (d, 1H, J=13.17 MHz) 1.74 (s, 3H). ESI-MS: (*m*/z): 349 $(M+H)^+$;

Inhibition of β-hematin formation assay –

Male swiss mice, weighing 15–20 g were inoculated with $1X10^5$ *P. yoelii* infected RBCs. Blood of infected animal at~50% parasitemia was collected by cardiac puncture in 2.0% citrate buffer and centrifuged at 3000 rpm for 10 min at 4^0C. The plasma was used in assay of β hematin formation. The assay mixture contained 100 mM sodium acetate buffer pH (5.1), 50 µL plasma, 100 µM hemin as the substrate and 1–10 µg compound/drug in a total volume of 1.0 mL. The control tube contained all reagents except compound. The reaction mixture in triplicate was incubated at 37°C for 16 h in a rotary shaker. The reaction was stopped by centrifugation at10,000 rpm for 10 min at 30° C. The pellet was suspended in 100 mM Tris–HCl buffer pH (7.4) containing 2.5% SDS. The pellet obtained after centrifugation was washed thrice with distilled water (TDW) to remove free hemin attached to β-hematin. The pellet was solubilized in 50 µL of 2 N NaOH and volume was made up to 1.0 mL with TDW. Absorbance was measured at 400 nm [31-34].

Inhibition of β-hematin formation : -

Most of the synthesized compounds showed moderate β –hematin inhibitory activities (IC_{50} > 3.5 µg/ml) not better than the reference drug chloroquine (IC_{50} 3.65 µg/ml) (Table 2).Compound 3a-3c have shown moderate inhibitory activity against β-hematin formation.

Conclusions

In summary, we have disclosed an efficient and economic method for the synthesis of 1,5-benzodiazepines. We also demonstrated the electronic effects on the reaction of various substitutions on the ketone. Electron withdrawing groups on the ketone stimulate the reaction rate, whereas electron releasing groups reduce the reactivity of the ketone. Simple workup and easy isolation under mild reaction conditions are the best features of the present methodology. Most of synthesized compounds were shown moderate activity against β-hematin formation. 1,5-benzodiazepines can be new lead in antimalarial chemotherapy.

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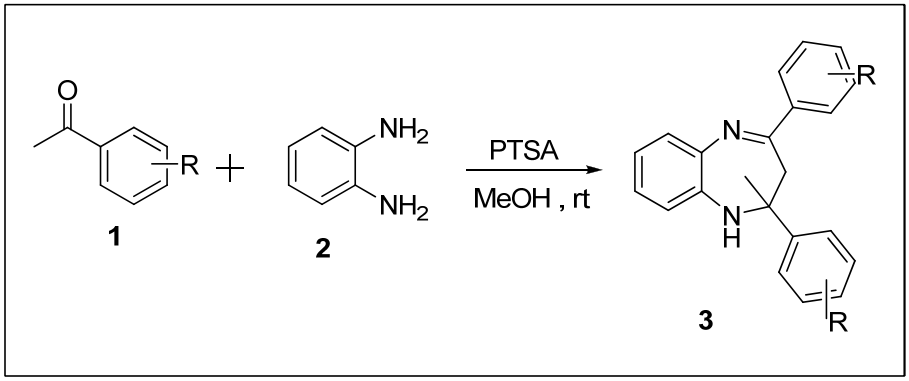
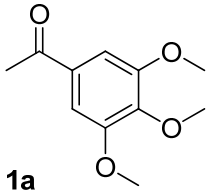
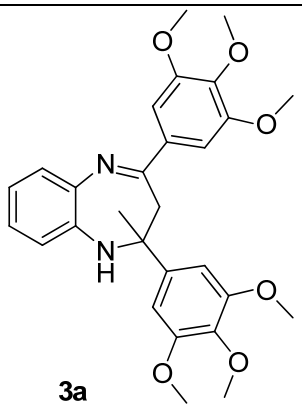
Scheme 1. Synthesis of 1,5-benzodiazepines



Reagents and Reaction conditions- *o*-phenylenediamine (1equiv.), different acetophenone (2.5 equiv.), MeOH, rt, 2-5 hr

Table 1- Compounds of scheme 1

S.No.	Acetophenone(1)	Product (3)	Time (hrs)	yield%
1.	 1a	 3a	1	95

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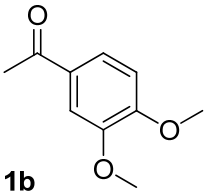
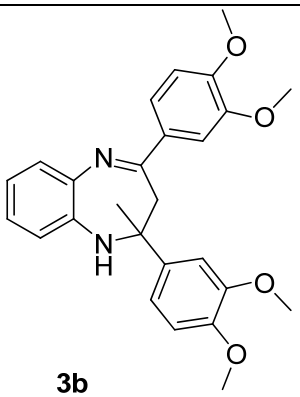
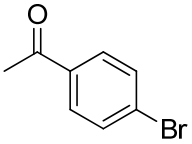
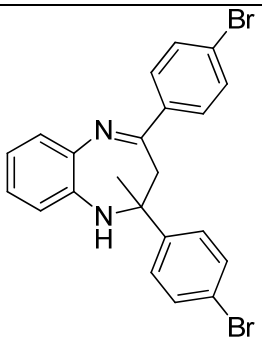
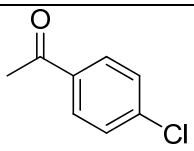
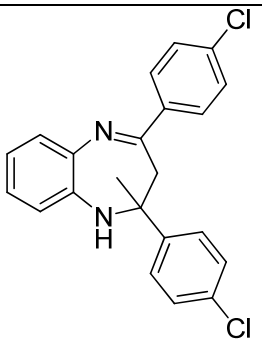
2	 **1b**	 **3b**	**1.5**	**92**
3	 **1c**	 **3c**	**4**	**83**
4	 **1d**	 **3d**	**4**	**87**

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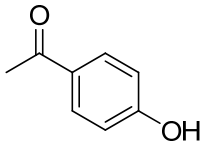
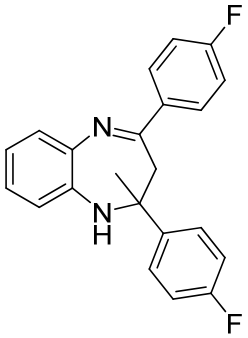
5	 **1e**	 **3e**	**5**	**91**

Table 2. Inhibition of hemozoin formation

Compounds	Inhibition of β-hematin formation IC_{50} (μg/mL)
3a	15.50
3b	13.60
3c	13.80
3d	14.10
3e	13.90
CQ	3.65

Data are the mean of three different experiments in triplicate. The IC_{50} represents the concentration of compound that inhibit β-hematin formation by 50%.

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